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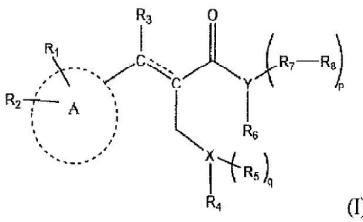
(54) Title: NLRP3 INFLAMMASOME-INHIBITING COMPOUNDS AND THE USE THEREOF

(57) Abstract: The invention relates to compounds of general formula (I), having inhibitory activity against the NLRP3 inflammasome. Said compounds are useful in the prevention and/or treatment of diseases and/or disorders mediated by the NLRP3 inflammasome.



(I)

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NLRP3 INFLAMMASOME-INHIBITING COMPOUNDS AND THE USE THEREOF

Technical field of invention

The invention relates to NLRP3 inflammasome-inhibiting compounds which are particularly useful in the prevention and/or treatment of diseases and/or disorders mediated by the NLRP3 inflammasome.

Prior art

The discovery that the immune system and the inflammatory processes correlated with its chronic activation underlie a huge number of disorders which account for the highest global morbidity and mortality figures was one of the major medical discoveries of the last twenty years [Netea et al. 2017, Slavich 2015, Bennett, et al. 2018].

Chronic inflammatory disorders are now recognised as the main cause of death worldwide, over 50% of deaths being attributable to disorders accompanied by various inflammatory states (acute or chronic), such as acute myocardial ischaemia, stroke, type 2 diabetes, chronic kidney failure, non-alcoholic steatohepatitis (NASH), numerous autoimmune and neurodegenerative diseases, and some forms of cancer [Straub and Schradin 2016, Furman et al. 2019, GBD 2017 Causes of Death Collaborators 2018].

Inflammation is a process activated by the host's immune system in response to stimuli recognised as harmful, such as the presence of irritants, pathogens and the products thereof, and in response to excessive cell death. Inflammasomes are intracellular complexes which act as "sensors" of the innate immune system and perform the role of main promoters of the inflammatory response by triggering a cascade of events that cause secretion of pro-inflammatory cytokines interleukin (IL)-1beta (interleukin-1β or IL-1β) and IL-18, and cell death by pyroptosis. The NLRP3 inflammasome is the most widely

studied of the inflammasomes, because it is involved in numerous pathological processes.

The NLRP3 inflammasome is a multiprotein complex consisting of protein NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) which assembles in the cytosol with ASC protein (apoptosis-associated speck-like protein containing a CARD) and procaspase-1, forming an oligomeric aggregate called an inflammasome, which is capable of causing autoproteolysis of procaspase-1, generating the active form of the protease called caspase-1. The latter then cleaves pro-IL-1beta and pro-IL-18 generating IL-1beta and IL-18, which cause a powerful inflammatory response. Moreover, the caspase-1 activated by NLRP3 cleaves the protein gasdermin-D, forming gasdermin-N, which latter forms pores in the cell membrane that lead to cell death by the process known as pyroptosis [Groslambert and Py 2018, He et al. 2016] and to the release of proinflammatory material into the extracellular space.

Abnormal activation and hyperactivation of NLRP3 are undoubtedly involved in numerous acute and chronic inflammatory disorders [Mangan et al. 2018].

The physiological role of NLRP3 is not yet fully understood, and no mutations inactivating gene *nlrp3* have been described to date. Conversely, mutations activating in gene *nlrp3* (*NALP3* or *CIAS-1*) generate an NLRP3 protein which is continuously activated. Said mutations are the etiological factor of a set of autoinflammatory disorders known as cryopyrinopathies (CAPS) [Booshehri and Hoffman 2019, Mortimer et al. 2016].

Data obtained from studies of animal models and supported by studies on patients demonstrate that activation of NLRP3 leads to a chronic inflammatory state that can cause, accompany and promote numerous pathological processes which have a major impact on public health [Fusco et al. 2020]. The main disorders correlated with increased, uncontrolled activation of NLRP3 are: (i) metabolic and cardiovascular disorders such as atherosclerosis [Jing and Fu 2019], type 2 diabetes [Lee et al. 2013], inflammation induced

by obesity and insulin resistance [Vandanmagsar et al. 2011, Yin et al. 2014], myocardial ischaemia [Wang et al. 2014, Toldo and Abbate 2018], stroke [Ren et al. 2018.]; (ii) chronic inflammatory disorders that seriously affect various organs and tissues in a progressively degenerative manner. Among said disorders, uncontrolled activation of NLRP3 has been found in inflammatory bowel diseases such as Crohn's disease and ulcerating colitis [Liu et al. 2017, Zhen and Zhang 2019], various forms of arthritis, including rheumatoid arthritis [Vande Walle et al. 2014, Guo et al. 2018], systemic lupus erythematosus, Sjögren's syndrome, ankylosing spondylitis, systemic sclerosis [Li et al 2020], gout [Martinon et al. 2006, Szekanecz et al. 2019], non-alcoholic steatohepatitis (NASII) and hepatic fibrosis [Boaru et al. 2012, Mridha et al 2017, Wu et al. 2017]; (iii) inflammatory disorders of the airways [Primiano et al. 2016, Theofani et al. 2019], including the severe inflammatory pulmonary complications correlated with Sars-CoV-2 infection (COVID-19) [Freeman and Swartz 2020]; (iv) neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, head injury [Heneka et al. 2018], and multiple sclerosis [Malhotra et al. 2020]; (v) other disorders, such as sepsis [Cornelius et al. 2020], sterile corneal inflammation [Shimizu et al. 2019] and some myelodysplastic syndromes [Basiorka et al. 2016, Ratajczak et al. 2020], have been correlated with hyperactivation of the inflammasome.

These clinical and experimental observations demonstrate that the NLRP3 inflammasome is an extremely interesting target for the discovery of new medicaments for the treatment of disorders for which an optimum treatment is not yet available [Chauhan et al. 2020].

Biopharmaceuticals that block IL-1beta (anakinra, canakinumab and rilonacept) are already present on the market and used to treat various inflammatory disorders. Anakinra is approved for the treatment of cryopyrinopathies, rheumatoid arthritis, colchicine-

resistant Familial Mediterranean Fever (FMF) and Schnitzler syndrome. Canakinumab is approved for the treatment of cryopyrinopathies, Familial Mediterranean Fever (FMF), tumour necrosis factor receptor-associated periodic syndrome (TRAPS), hyperimmunoglobulinaemia D syndrome/mevalonate kinase deficiency (HIDS/MKD), idiopathic juvenile arthritis, gouty arthritis and adult Still's disease. The CANTOS clinical trial (NCT01327846) also demonstrated significant activity in reducing secondary cardiovascular events and strokes and preventing mortality due to cardiovascular events (-31%) in patients at cardiovascular risk. The CANTOS study also demonstrated a 77% reduction in lung cancer deaths after treatment with canakinumab. Rilonacept is approved by the FDA for treatment of cryopyrinopathies (Familial Mediterranean Fever (FMF) and Muckle-Wells syndrome (MWS). Although it is effective, the clinical use of IL-1beta blockers involves some recognised, problematic limitations, which have not yet been resolved. The main limitations of treatment based on the use of IL-1beta blockers are as follows:

1) total blocking of the effects of IL-1beta, obtained with the blockers on the market, makes patients more liable to infection. Clinical treatment with anakinra and canakinumab has demonstrated an increased risk of infections of the upper airways and urinary tract caused by *Escherichia coli* and *Streptococci*. Other significant side effects associated with IL-1beta inhibitor treatment are neutropenia and thrombocytopenia.

2) In view of the limited stability and non-ideal pharmacokinetics of biological IL-1beta blockers, their clinical use requires said medicaments to be administered by injection, usually in hospital. This considerably limits patient compliance. The frequency of administration can range from daily (anakinra) to weekly (rilonacept) or every eight weeks (canakinumab), and the treatment is lifelong.

3) IL-1beta blocker treatment is expensive.

4) IL-1beta blockers do not block the inflammatory response mediated by IL-18 or cell death by pyroptosis, which amplifies and supports the inflammatory response.

The development of synthetic molecules able to inhibit activation of the inflammatory signalling pathway by directly inhibiting the NLRP3 inflammasome would overcome the limitations of the treatments currently available, and increase the number of disorders treatable with said medicaments. In particular, selective inhibition of the NLRP3 inflammasome would block the signalling pathway leading to release of IL-1beta upstream, thus reducing the secretion of said inflammatory cytokine. The generalised inhibition of IL-1beta which is obtained with the blockers currently on the market gives rise to immunosuppression and increased risk of infection. Inhibiting the NLRP3 inflammasome would enable other inflammasomes (AIM2, NLRC4, NLRP1) present in the cells of the innate immune system to produce IL-1beta. This would minimise the risks of severe immunosuppression and infection.

Small molecules able to inhibit NLRP3 could have physicochemical properties allowing their oral administration, thus greatly simplifying the therapeutic dosage regimen and increasing patient compliance.

Moreover, the cost of a treatment with synthetic NLRP3 inhibitors would be lower than the cost of treatment with biological IL-1beta blockers, leading to a considerable saving for the national health service.

Unlike IL-1beta blockers, NLRP3 inhibitors can also block the inflammatory responses due to IL-18 secretion and cell death by pyroptosis, which amplifies and supports the inflammatory process, releasing "cell debris" (Danger-Associated Molecular Patterns; DAMP) and other pro-inflammatory mediators by means of cell lysis. As a result of said molecular mechanism, the number of disorders treatable with an NLRP3 inhibitor is undoubtedly higher than that of the disorders treatable with a simple IL-1beta inhibitor.

This benefit of selective NLRP3 inhibitors has been demonstrated in a study of NLRP3 knock-out mice, which demonstrated that there was no greater risk of infection than for wild-type mice. Moreover, the mice lacking NLRP3 did not exhibit any metabolic defects, thus demonstrating the safety of the therapeutic strategy [Youm et al. 2013].

Although some molecules able to inhibit the activation or assembly of the NLRP3 inflammasome more or less selectively have been discovered, there are still no NLRP3 inflammasome inhibitors on the market [Zahid et al. 2019, Bertinaria et al. 2019]. The molecule whose development is at the most advanced stage is dapansutrile (OLT-1177; Olatec Therapeutics LLC, New York, NY, USA). Said NLRP3 inhibitor is currently in phase 2a of development, and is undergoing a clinical trial for the treatment of gouty arthritis (EUDRACT number: 2016-000943-14). The results published to date are favourable, indicating good safety, tolerability and activity [Kluck et al. 2020]. The company Inflazome (Inflazome UK Ltd, Cambridge, UK) is developing inzomelid and somalix, NLRP3 inhibitors which are both in phase 1 of clinical development (WO 2016/131098, US 10,538,487 and EP 3.259,253). Said molecules derive from modulation of molecule MCC950, the reference NLRP3 inhibitor currently used in pharmacological studies.

There is consequently still a need to identify compounds able to inhibit the NLRP3 inflammasome which are useful in the prevention and/or treatment of diseases and/or disorders mediated by the NLRP3 inflammasome.

List and brief description of figures

Figure 1 shows the effect of INF176 treatment (1-20 μM) on (A) pyroptosis of human macrophages stimulated with LPS/ATP; (B) release of IL-1beta from human macrophages stimulated with LPS/ATP. *$p < 0.05$ vs ATP. Statistics: Student's t-test.

Figure 2 shows the effect of INF176 treatment at the dose of 25 and 50 mg/kg on

(A) variation in body weight and (B) spleen weight. *p<0.05 vs CTR (control) and [a]p<0.05 vs DSS. Statistics: ANOVA analysis followed by Tukey's post hoc test.

Figure 3 shows the effect of INF176 treatment at the dose of 25 and 50 mg/kg on: (A) colon length, (B) disease activity index (DAI), (C) myeloperoxidase (MPO) levels in colon, and (D) interleukin-1beta (IL-1β) levels in colon. *p<0.05 vs CTR (control) and [a]p<0.05 vs DSS. Statistics: ANOVA analysis of compounds, followed by Tukey's post hoc test.

Figure 4 shows the effect of INF176 treatment at the dose of 50 mg/kg on: (A) escape latency, (B) number of crossings in quadrant, (C) number of entries into quadrant, (D) p-tau protein expression in brain tissues, (E) β amyloid 1-42 (Aβ1-42) protein levels in brain tissues. [a]p<0.05 vs SAMR1 (control), *p<0.05 vs SAMP8. Statistics: ANOVA analysis followed by Tukey's post hoc test.

Figure 5 shows the effect of INF176 treatment at the dose of 50 mg/kg on: (A) number of pellets expelled in 1 hour, (B) colon contractions elicited by electrical stimuli, (B) cholinergic colon contractions elicited by electrical stimuli, (B) tachykininergic colon contractions elicited by electrical stimuli. [a]p<0.05 vs SAMR1 (control), *p<0.05 vs SAMP8. Statistics: ANOVA analysis followed by Tukey's post hoc test.

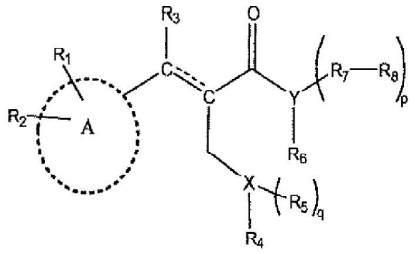
Figure 6 shows the effect of INF176 treatment at the dose of 50 mg/kg on interleukin-1beta (IL-1β) levels in the colon. Statistics: ANOVA analysis followed by Tukey's post hoc test.

Figure 7 shows the chromatogram of the HPLC analysis conducted to determine the stability of INF177 in PBS in the presence of glutathione 10x.

Figure 8 shows the chromatogram of the HPLC analysis conducted to determine the stability of INF177 in PBS in the presence of cysteamine 10x.

Summary of the invention

The object of the present invention is compounds of general formula (I), and the corresponding sub-formulae:



wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, q, p, X and Y are as defined below,

and their enantiomers, diastereomers, rotamers or mixtures thereof;

and the pharmaceutically acceptable salts or solvates thereof.

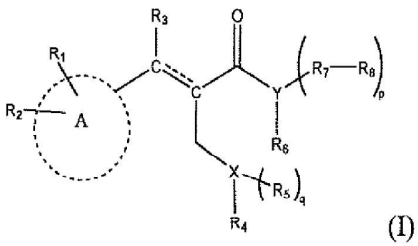
The invention also relates to compositions containing at least one compound of general formula (I), (Ia), (Ib) or (Ic) as defined below, and at least one pharmaceutically acceptable excipient or carrier.

Further objects of the invention are compounds of general formula (I) for use as a medicament, in particular to inhibit the NLRP3 inflammasome.

According to a further aspect, the invention relates to compounds of general formula (I) for use in the prevention and/or treatment of inflammatory, autoimmune, neurodegenerative, cardiovascular, metabolic and neoplastic diseases and/or disorders.

Detailed description of the invention

The object of the present invention is compounds of general formula (I):



(I)

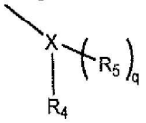
wherein:

A is a C_3-C_{10}-cycloalkyl, preferably monocyclic or bicyclic C_5-C_{10}-cycloalkyl; 5- to 10-membered, saturated or partly saturated, monocyclic or bicyclic heterocycle; monocyclic or bicyclic C_6-C_{10}-aryl; 5- to 10-membered monocyclic or bicyclic heteroaryl; A is preferably a 5 or 6-membered, saturated or partly saturated, monocyclic heterocycle, or a 9 or 10-membered, saturated or partly saturated, bicyclic heterocycle; or a monocyclic C_5-C_6-aryl, or a bicyclic C_9-C_{10}-aryl; or a 5 or 6-membered monocyclic heteroaryl or a 9 or 10-membered bicyclic heteroaryl; wherein the heteroatom is preferably N or O; more preferably A is phenyl, naphthyl, furanyl or indolyl, and most preferably A is phenyl;

R_1 and R_2, which are the same or different, can occupy any position on A, and can be hydrogen; halogen such as F, Cl, Br or I; linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_4-alkyl; linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_4-alkoxy; a nitro group; nitrile; a substituted or unsubstituted amido group; a substituted or unsubstituted amino group; a substituted or unsubstituted ester group; a trifluoromethyl group; R_1 and R_2 are preferably hydrogen, halogen such as F, Cl, Br or I, linear or branched C_1-C_4-alkyl, linear or branched C_1-C_4-alkoxy, a nitro group; R_1 and R_2 are more preferably hydrogen, chloro, bromo, methyl, methoxy or a nitro group; most preferably R_1 is hydrogen and R_2 is chloro; R_1 or R_2 is preferably in the 2 position when A is phenyl;

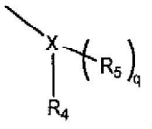
 can be a single bond or a double bond;

R_3 can be -H, -OH, -OR_9 or -O(CO)R_9, wherein R_9 can be hydrogen, a linear or branched C_1-C_4-alkyl, substituted or unsubstituted, saturated or unsaturated; R_3 is preferably hydrogen or –OH; R_3 is more preferably hydrogen;

in the  group X can be N, O, S, S(O) or SO_2;

R_4 can be a linear or branched, substituted or unsubstituted, saturated or unsaturated C_{1-4} alkyl group; monocyclic or bicyclic C_3-C_{10}-cycloalkyl, substituted or unsubstituted, preferably a C_3-C_6-cycloalkyl; monocyclic or bicyclic C_6-C_{14}-aryl, substituted or unsubstituted, preferably a C_6-C_{10}-aryl, more preferably a C_5-C_6-aryl; 5- to 10-membered heterocycle, saturated or partly saturated, monocyclic or bicyclic, substituted or unsubstituted, preferably a C_5-C_6- heterocycle; monocyclic or polycyclic 5- to 14-membered heteroaryl, preferably monocyclic or bicyclic, substituted or unsubstituted, preferably a C_5-C_6-heteroaryl; R_4 is preferably monocyclic or bicyclic C_6-C_{10}-aryl, substituted or unsubstituted, or C_3-C_6-cycloalkyl, substituted or unsubstituted; more preferably, R_4 is cyclohexyl or phenyl;

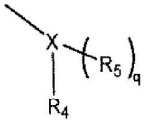
q can be 0 (zero) or 1; when q is equal to 1, X is N and R_5 is hydrogen; a linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_4-alkyl group; monocyclic or bicyclic C_3-C_{10}-cycloalkyl;

alternatively, the  group can be an amino-acid residue wherein:

- X is an N, S or O atom of the side chain of an amino acid, preferably natural, selected from serine; tyrosine; threonine; lysine; cysteine; q is zero (R_5 is therefore not present) and R_4 is the remainder of the amino acid which can be protected or unprotected on the NH_2 and/or COOH terminal groups; in a preferred aspect the terminal NH_2 group is acetylated; in a preferred aspect, the amino-acid residue is N-acetylcysteine or N-Boc cysteine methyl ester; or

- X is the N atom of the terminal amino group bonded to the stereogenic carbon atom in alpha of a preferably natural, protected or unprotected, amino acid, selected from alanine, arginine, asparagine, aspartic acid, cysteine, glycine, glutamic acid, glutamine, histidine,

isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine; q is equal to 1, R_5 is hydrogen; and R_4 represents the remainder of the amino-acid structure, protected or unprotected, for example acetylated on the N atom of the side chain or esterified with a linear or branched C_1-C_4-alkyl group, preferably methyl, on the terminal carboxyl group;

alternatively when, in the  group, X is N, R_4 and R_5 can be joined to form a saturated, partly saturated or unsaturated, monocyclic or bicyclic C_3-C_{10}-heterocyclic ring with the N atom; R_4 and R_5 preferably form a monocyclic C_3-C_6-heterocyclic ring with the N atom; more preferably, R_4 and R_5 form a piperidine or pyrrolidine ring with the N atom; most preferably, R_4 and R_5 form a pyrrolidine ring with the N atom;

Y can be selected from O, N and S; is preferably O or N; and is more preferably N;

when Y is an oxygen or sulfur atom, in the -(R_7-R_8)p group p is equal to zero and R_6 can be hydrogen, a linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_8-alkyl group; a monocyclic or bicyclic C_3-C_{10}-cycloalkyl; a substituted or unsubstituted arylalkyl; a 6- to 14-membered monocyclic or bicyclic heteroaryl; R_6 is preferably hydrogen or a linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_4-alkyl group; R_6 is more preferably a linear or branched, saturated, unsubstituted C_1-C_4-alkyl group; R_6 is most preferably ethyl;

when Y is a nitrogen atom, p is equal to 1, R_6 and R_7, which are the same or different, are selected from hydrogen, a linear or branched, saturated or unsaturated, substituted or unsubstituted C_1-C_4-alkyl group; a substituted or unsubstituted aryl, arylalkyl or heteroaryl group; preferably, it can be a substituted phenylalkyl group; more preferably $-(CH_2)_2$-phenyl-SO_2NH_2; most preferably R_6 is hydrogen and R_7 is $-(CH_2)_2$-phenyl-

SO_2NH_2;

R_8 can be selected from H, COOH, $COOR_9$, $C(O)R_9$, CN, $CONH(R_9)$, $S(O)NHR_9$ and $S(O)_2NHR_9$, wherein R_9 is as defined above;

alternatively, R_6 and R_7 can be joined to form a 3- to 8-membered heterocyclic ring;

R_8 is as defined above;

and their enantiomers, diastereomers, rotamers or mixtures thereof;

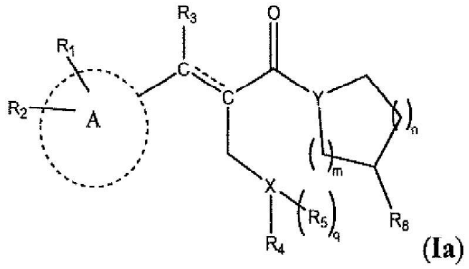
and the pharmaceutically acceptable salts or solvates thereof;

for use as a medicament, in particular for use in the prevention and/or treatment of NLRP3 inflammasome-mediated diseases and/or disorders.

According to one embodiment of the invention,  is a single bond and A, R_1, R_2, R_3. R_4, R_5, R_6, R_7, R_8, q, p, X and Y are as defined above.

According to a further embodiment, when  is a double bond, X is N or O, A is phenyl, R_1 is in the 2 position and is preferably a halogen, more preferably chloro, R_2, R_3. R_4, R_5, R_6, R_7, R_8, q, p and Y are as defined above.

According to a preferred aspect, the compounds for use according to the invention have general formula (Ia), when R_6 and R_7 form a ring:



(Ia)

wherein

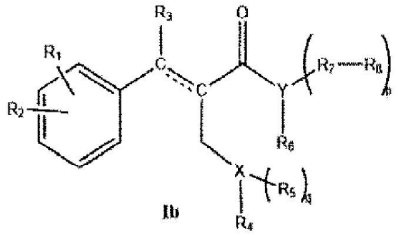
A, R_1, R_2, R_3. R_4, R_5, R_8, q and X are as defined above;

n and m, which are the same or different, can be 0 (zero) or an integer between 1 and 3; when n and m are equal to zero, a three-membered cycle is generated between C-

R_8, Y, and the remaining -CH_2- group; when n and m, which are different from one another, are 0 (zero) or 1, a 4-membered ring is formed; or n and m, which are the same or different, can be 1, 2 or 3 forming 5- to 9-, preferably 5- to 8-membered rings; according to a preferred aspect, m is 2 and n is 1; according to a more preferred aspect, n is 2 and m can be 1 or 2; most preferably, n is 2 and m is 1;

preferably, when Y is N, R_6 and R_7 can be joined to form a 3- to 6-membered monocyclic substituted heterocyclic ring with the N atom; more preferably, R_6 and R_7 form a substituted piperidine or pyrrolidine ring with the N atom; most preferably, R_6 and R_7 form, with the N atom, a piperidine ring substituted in the 3 or 4 position.

According to a further preferred aspect the compounds for use according to the invention have general formula (Ib):



Ib

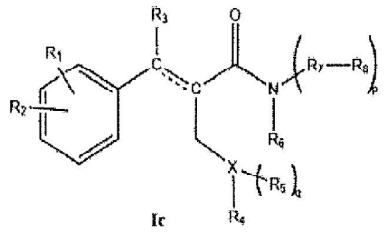
wherein

R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, q, p and X are as defined above,

their enantiomers, diastereomers, rotamers or mixtures thereof;

and the pharmaceutically acceptable salts or solvates thereof.

According to a particularly preferred aspect the compounds for use according to the invention wherein Y is N have general formula (Ic):



Ic

wherein

R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, q, p and X are as defined above,

their enantiomers, diastereomers, rotamers or mixtures thereof;

and the pharmaceutically acceptable salts or solvates thereof.

According to the present invention, "C_1-C_8-alkyl" represents a linear or branched, saturated or unsaturated alkyl chain containing 1 to 8 carbon atoms. "C_1-C_4-alkyl" represents a linear or branched alkyl chain containing 1 to 4 carbon atoms, which may be saturated, such as methyl, ethyl, propyl, isopropyl, butyl, sec-butyl or tert-butyl, or unsaturated, such as ethenyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, ethynyl, 1-propynyl, 2-propynyl, 1-butynyl, 2-butynyl or 3-butynyl, preferably ethynyl, 1-propynyl, 2-propynyl, 1-butynyl, 2-butynyl or 3-butynyl. The "C_1-C_8-alkyl" or "C_1-C_4-alkyl" group can be substituted by a halogen (Cl, F, Br, I), OH, cyano group, nitro group, amino group or C_1-C_4-alkyl-amino, C_1-C_4-alkyl, C_1-C_4-alkoxy, C_1-C_4-allyl, C_1-C_4-haloalkyl, C_1-C_4-haloalkoxy.

"C_1-C_4-alkoxy" represents a linear or branched, saturated or unsaturated alkyl radical containing 1 to 4 carbon atoms, bonded to an oxygen atom. The "C_1-C_4-alkoxy" group can be substituted by C_1-C_4-alkyl.

"C_3-C_{10}-cycloalkyl" represents a saturated or partly saturated hydrocarbon ring containing 3 to 10 carbon atoms, which is monocyclic, preferably cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl, or bicyclic, preferably decalin or tetralin. "C_5-C_{10}-cycloalkyl" represents a saturated or partly saturated hydrocarbon ring containing 5 to 10 carbon atoms, which is monocyclic, preferably cyclopentyl, cyclohexyl or cycloheptyl, or bicyclic, preferably decalin or tetralin. "C_3-C_6-cycloalkyl" represents a saturated or partly saturated hydrocarbon ring containing 3 to 6 carbon atoms, which is monocyclic, preferably cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl. The "C_3-C_{10}-alkyl" group can be substituted by a halogen (Cl, F, Br, I), OH, cyano group, nitro group,

amino group or C_1-C_4-alkyl-amino, C_1-C_4-alkyl, C_1-C_4-alkoxy, C_1-C_4-allyl, C_1-C_4-haloalkyl, C_1-C_4-haloalkoxy.

"C_6-C_{10}-aryl" represents a monocyclic or bicyclic or tricyclic aromatic ring having 6 to 10 carbon atoms, preferably monocyclic or bicyclic, and is more preferably phenyl or naphthyl; most preferably it is phenyl.

"C_6-C_{10}-aryl" represents a monocyclic or bicyclic aromatic ring having 6 to 10 carbon atoms.

"5- to 10-membered heterocycle" represents a saturated or partly saturated, monocyclic or bicyclic ring containing one or more heteroatoms selected from nitrogen, oxygen and sulfur; the heterocycle preferably contains at least one nitrogen atom.

"Monocyclic or bicyclic 5- to 10-membered heteroaryl" represents a monocyclic or polycyclic aromatic ring containing one or more heteroatoms selected from nitrogen, oxygen and sulfur. Preferably, the monocyclic heteroaryl ring contains at least one nitrogen atom. More preferably it is a monocyclic heteroaryl, for example selected from thiophene, furan, pyrrole, oxazole, isoxazole, thiadiazole, oxadiazole, imidazole and pyrimidine. Alternatively, it is a bicyclic heteroaryl, such as indole.

The bicyclic systems can be "condensed ring systems", "bridged ring systems" or "bicyclic spiro-ring systems".

The term "halogen" refers to fluorine, chlorine, bromine and iodine.

According to the invention, an aryl, heteroaryl or arylalkyl group, such as a phenylalkyl group, can be substituted with a halogen (Cl, F, Br, I), OH, cyano group, nitro group, amino group or C_1-C_4-alkyl-amino, C_1-C_4-alkyl, C_1-C_4-alkoxy, C_1-C_4-allyl, C_1-C_4-haloalkyl, C_1-C_4-haloalkoxy defined according to the invention.

An amido, amino or ester group can be substituted with C_1-C_4-alkyl.

According to the present invention,  represents that the two carbon atoms

which join A to the carbonyl group can be bonded via a single or double bond to form a saturated or unsaturated chain. When the two carbon atoms are joined by a double bond, the substituents present on the double bond can have either the E (trans) or Z (cis) configuration. When the two carbon atoms are joined by a single bond, the substituents can have any spatial arrangement.

The compounds according to the invention which have one or more stereogenic (asymmetrical) carbon atoms can exist as stereoisomers (optical isomers), i.e. as enantiomers or diastereomers or mixtures thereof. According to the present invention, the compounds can take the form of optically pure enantiomers; pure diastereomers; mixtures of enantiomers; mixtures of diastereomers; racemic mixtures, racemates, or racemate mixtures of enantiomers. Moreover, according to the present invention, the compounds can take the form of conformational isomers or rotamers.

The amino acids are in their D or L configuration.

According to the present invention, a "protecting group" can be selected from those listed in Peter G.M. Wuts, Theodora W. Greene *"Greene's Protective Groups in Organic Synthesis"*, Fourth Edition, 2007 John Wiley & Sons Inc., pp. 533-646 and pp. 696-926, and Isidro-Llobet A., Alvarez M. Albericio F. "Amino Acid-Protecting Groups", Chem. Soc. Rev. 2009, *109,* 2455–2504; amino protecting groups are, for example, tert-butyl-oxy-carbonyl (Boc) or acetyl, and terminal carboxyl protecting groups are, for example, methyl, ethyl, tert-butyl or benzyl.

The compounds according to the present invention can be converted to the corresponding pharmaceutically acceptable salts by reacting with the corresponding organic or inorganic acids, or organic or inorganic bases, or with amino acids such as lysine or arginine.

Examples of pharmaceutically acceptable inorganic acids or inorganic bases are

hydrochloric, hydrobromic, sulphuric, phosphoric and nitric acid, sodium hydroxide, potassium hydroxide and calcium hydroxide.

Examples of pharmaceutically acceptable organic acids or organic bases are oxalic, tartaric, maleic, succinic, citric, fumaric, acetic, methanesulphonic, benzoic, carbonic and pamoic acid, tris-(2-hydroxymethyl)-aminomethane (tromethamine) and sodium methylate.

It has now surprisingly been found that the compounds of general formula (I), (Ia), (Ib) and (Ic) as defined above are useful in the prevention and/or treatment of NLRP3 inflammasome-mediated diseases and/or disorders.

As shown in the Examples, the experiments conducted demonstrate that the compounds according to the invention possess inhibitory activity towards the NLRP3 inflammasome. Such activity makes the compounds according to the invention useful in the prevention and/or treatment of diseases and/or disorders wherein activation of the NLRP3 inflammasome contributes to the onset and/or progression of said diseases or said disorders.

The object of the present invention is the use of a compound of general formula (I), (Ia), (Ib) or (Ic) as defined above as a medicament, in particular to inhibit the NLRP3 inflammasome. Inhibiting the NLRP3 inflammasome means reducing the activity of the inflammasome, and in particular the ability of the NLRP3 inflammasome to produce IL-1beta.

According to a further aspect, the invention relates to compounds of general formula (I), and the corresponding sub-formulae (Ia), (Ib) and (Ic), as defined above, for use in the prevention and/or treatment of inflammatory, autoimmune, neurodegenerative, cardiovascular, metabolic and neoplastic diseases and/or disorders.

According to a preferred aspect, the compounds according to the invention are

useful in the prevention and/or treatment of inflammation associated with autoimmune, neurodegenerative, cardiovascular, metabolic and neoplastic diseases and/or disorders.

Moreover, the compounds according to the invention are useful in the prevention and/or treatment of diseases and/or disorders, or the inflammation associated therewith, such as:

- cryopyrin-associated periodic syndromes (CAPS) which comprise familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and chronic infantile neurological cutaneous and articular syndrome (CINCA), also known as neonatal-onset multisystem inflammatory disease (NOMID);

- asthma, chronic or acute inflammatory arthritis, osteoarthritis, rheumatoid arthritis, acute or chronic joint disease, psoriasis, sterile corneal inflammation, systemic sclerosis, ankylosing spondylitis, sepsis, chronic inflammatory bowel diseases, irritable bowel syndrome, inflammation induced by viral infections (such as those caused by the SARS-CoV-2 (COVID-19) virus;

- Alzheimer's disease, multiple sclerosis, Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and correlated symptoms (such as gastrointestinal disorders);

- cardiovascular diseases (such as hypertension, myocardial infarction, diabetic cardiomyopathy, atherosclerosis, pericarditis and ischaemia);

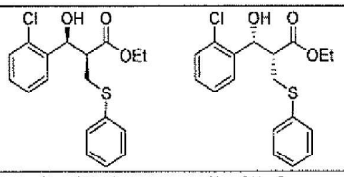
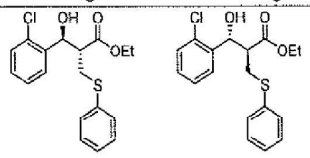
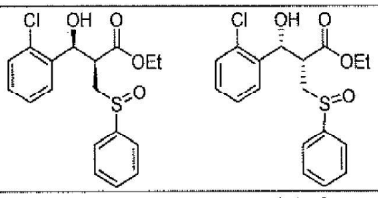
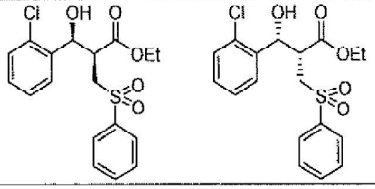
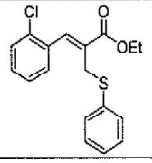
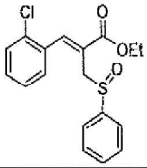
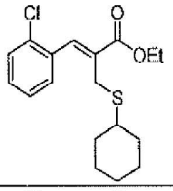
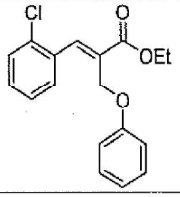
- non-alcoholic steatohepatitis (NASH), liver disease and correlated disorders such as hepatic fibrosis;

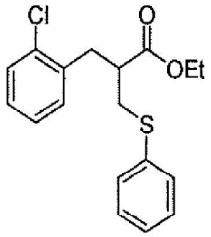
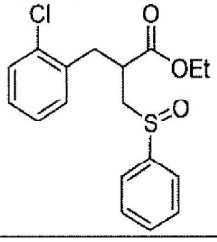
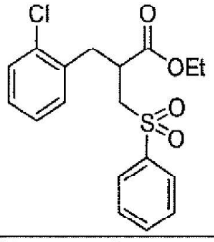
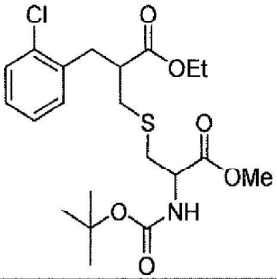
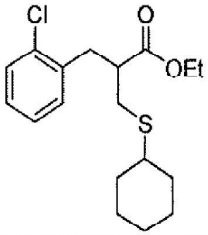
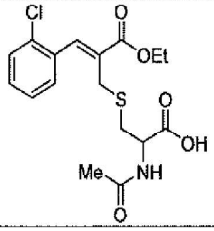
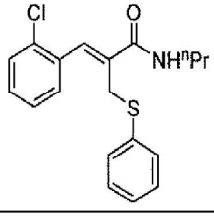
- obesity, type I / type II diabetes, kidney disease and correlated disorders (such as gastrointestinal disorders);

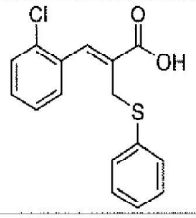
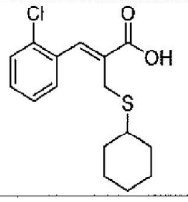
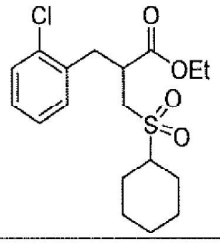
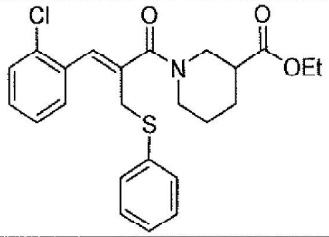
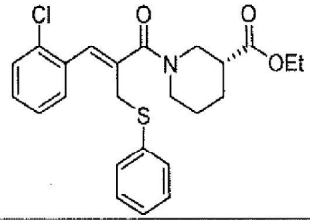
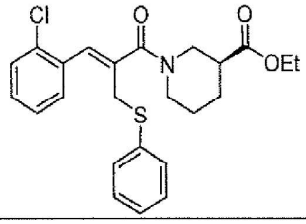
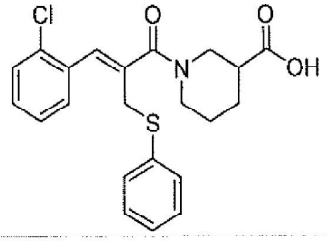
- tumours (such as stomach cancer, head/neck cancer, lung cancer, melanoma), myelodysplastic syndromes.

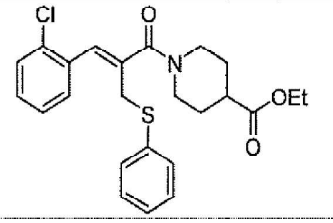
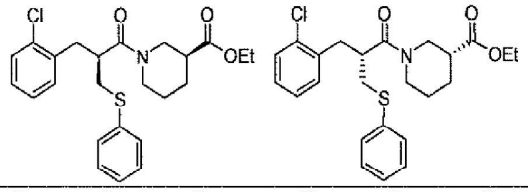
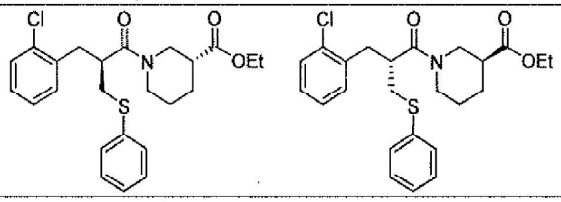
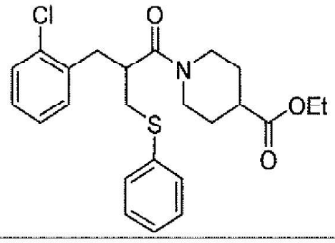
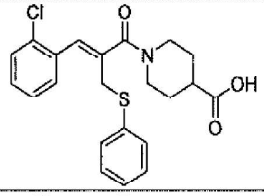
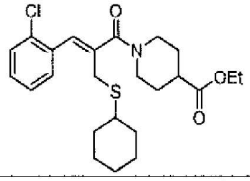
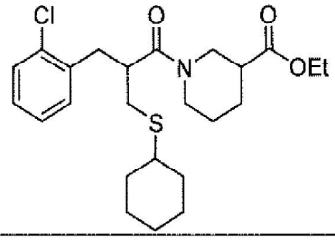
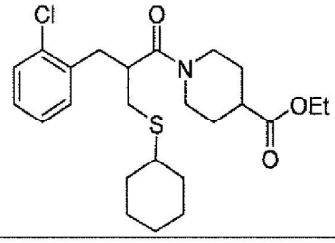
The preferred compounds for use according to the invention are listed in Table 1a:

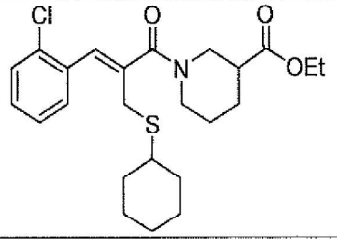
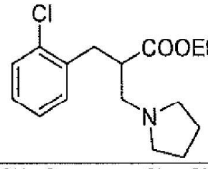
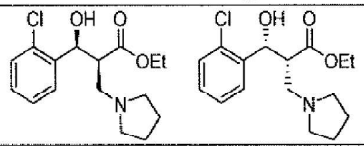
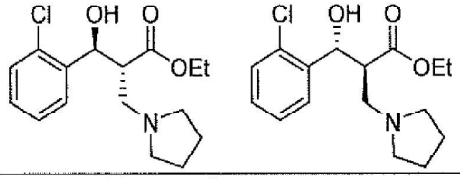
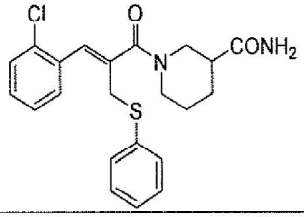
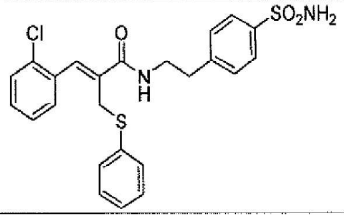
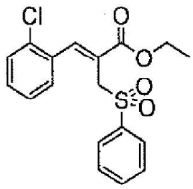
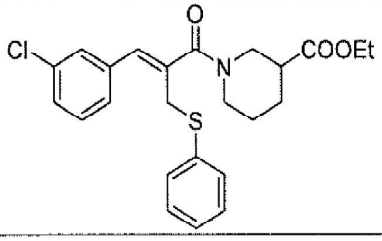
Table 1a

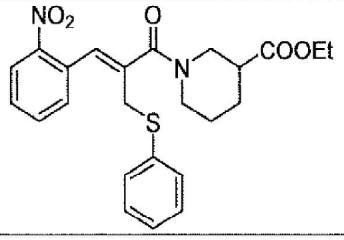
Compound	Structure	Pyroptosis inhibition (% ± SEM)[a]
INF38s		23.5 ± 4.2
INF38a		26.9 ± 4.9
INF44		< 10
INF45		45.8 ± 6.7
INF42		69.1 ± 2.2
INF50		41.1 ± 2.5
INF56		41.6 ± 3.9
INF57		34.5 ± 0.3

Compound	Structure	Pyroptosis inhibition (% ± SEM)[a]
INF43		51.0 ±5.6
INF48		33.6 ± 1.2
INF49		50.6 ± 5.8
INF55		< 10
INF110		< 10
INF85		21.3 ± 4.6
INF82		19.0 ± 4.1

Compound	Structure	Pyroptosis inhibition (% ± SEM)[a]
INF80		< 10
INF86		< 10
INF111		< 10
INF176		45.20 ± 7.2
INF202		40.9 ± 1.2
INF203		14.4 ± 4.3
INF177		20.1 ± 9.8

Compound	Structure	Pyroptosis inhibition (% ± SEM)[a]
INF180		13.8 ± 7.1
INF184		< 10
INF185		< 10
INF186		< 10
INF187		< 10
INF188		< 10
INF192		62.2 ± 9.78
INF193		< 10

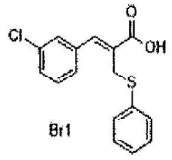
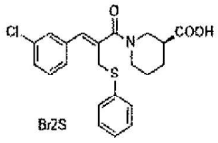
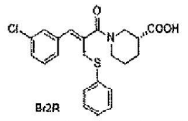
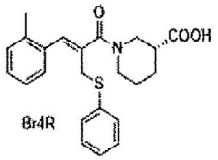
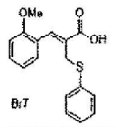
Compound	Structure	Pyroptosis inhibition (% ± SEM)[a]
INF194		< 10
INF61		41.7±5.2
INF37 syn		32.2±5.7
INF37 anti		21.1±2.9
INF219		13.0 ± 8.2
INF220		73.9 ± 13.9
INF51		38.9 ± 4.5
INF230		39.0 ± 15.4

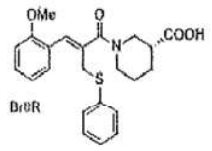
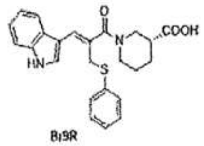
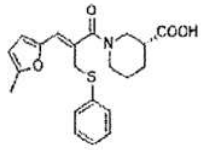
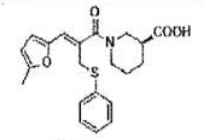
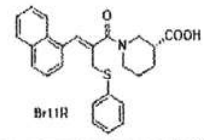
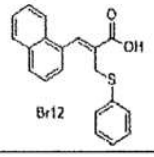
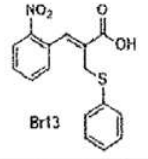
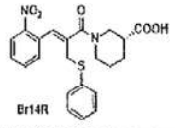
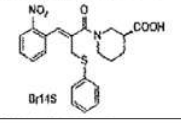
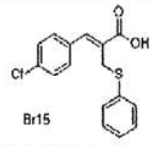
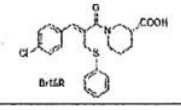
Compound	Structure	Pyroptosis inhibition (% ± SEM)[a]
INF231		32.6 ± 19.8

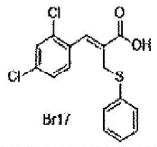
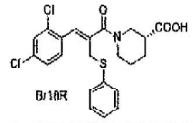
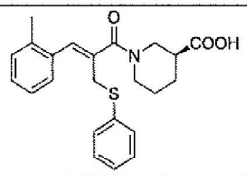
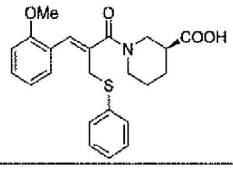
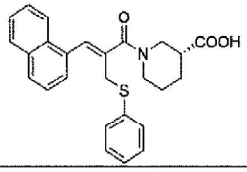
According to a further preferred aspect, the compounds for use according to the invention are selected from INF37syn, INF37anti, INF38s, INF38a, INF42, INF43. INF45, INF49, INF56, INF57, INF61, INF82, INF85, INF176, INF177, INF180, INF202, INF203. INF192, INF219 and INF220, more preferably from INF43. INF49, INF56, INF57, INF61, INF85, INF176, INF177, INF192, INF219 and INF220.

Further preferred compounds according to the invention are listed in Table 1b:

Table 1b

Compound	Structure
Br1	
Br2S	
Br2R	
Br4R	
Br7	

Compound	Structure
Br8R	
Br9R	
Br10R	
Br10S	
Br11R	
Br12	
Br13	
Br14R	
Br14S	
Br15	
Br16R	

Compound	Structure
Br17	
Br18R	
Br4S	
Br8S	
Br11S	

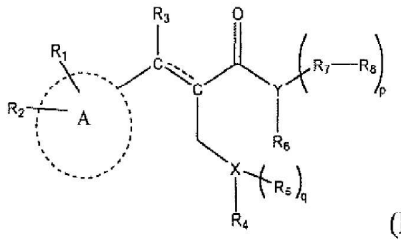
The compounds according to the invention are useful in treatment methods comprising administration of said compounds in a therapeutically effective amount to an individual in need thereof for the prevention and/or treatment of diseases and/or disorders as defined above.

The compounds according to the invention of formula (I) can be used in combination with other therapeutic agents such as anti-inflammatories, non-steroidal anti-inflammatory drugs (NSAIDs), biological, anti-diabetic, anti-Alzheimer, anti-Parkinson or anti-sclerosis medicaments, to achieve greater therapeutic efficacy, a reduction in the amount of medicament administered to the patient, and therefore a lower incidence of associated adverse effects.

The invention also relates to compositions containing at least one compound of general formula (I), (Ia), (Ib) or (Ic), and at least one pharmaceutically acceptable excipient or carrier.

The daily dose of active ingredient administered can be a single dose or an effective amount divided into multiple doses to be administered, for example, in the course of a day. The dosage regimen and frequency of administration for treatment of the disorders described above with the compound according to the invention and/or with the pharmaceutical compositions according to the present invention will be selected on the basis of a variety of factors including, for example, the patient's age, body weight, sex and medical conditions and the severity of the disease, the administration route, pharmacological factors, and any concomitant treatment with other medicaments. In some cases, dosage levels lower or higher than said range, and/or more frequent doses, can be used, obviously at the discretion of the doctor and depending on the stage of the disease.

A preferred aspect of the invention is compounds of general formula (I):



(I)

wherein:

A is a C_3-C_{10}-cycloalkyl, preferably monocyclic or bicyclic C_5-C_{10}-cycloalkyl; 5- to 10-membered, saturated or partly saturated, monocyclic or bicyclic heterocycle; monocyclic or bicyclic C_6-C_{10}-aryl; 5- to 10-membered monocyclic or bicyclic heteroaryl; A is preferably a 5 or 6-membered, saturated or partly saturated, monocyclic heterocycle, or a 9 or 10-membered, saturated or partly saturated, bicyclic heterocycle; or a monocyclic C_5-C_6-aryl, or a bicyclic C_9-C_{10}-aryl; or a 5 or 6-membered monocyclic heteroaryl or a 9 or 10-membered bicyclic heteroaryl; wherein the heteroatom is preferably N or O; more preferably A is phenyl, naphthyl, furanyl or indolyl, and most preferably A is phenyl;

R_1 and R_2, which are the same or different, can occupy any position on A, and can be hydrogen; halogen such as F, Cl, Br or I; linear or branched, substituted or unsubstituted,

saturated or unsaturated C_1-C_4-alkyl; linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_4-alkoxy; a nitro group; nitrile; a substituted or unsubstituted amido group; a substituted or unsubstituted amino group; a substituted or unsubstituted ester group; a trifluoromethyl group; R_1 and R_2 are preferably hydrogen, halogen such as F, Cl, Br or I, linear or branched C_1-C_4-alkyl, linear or branched C_1-C_4-alkoxy, a nitro group; R_1 and R_2 are more preferably hydrogen, chloro, bromo, methyl, methoxy, a nitro group; most preferably R_1 is hydrogen and R_2 is chloro;

wherein at least one of R_1 and R_2 is other than hydrogen when A is phenyl,

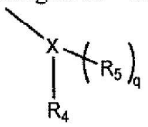
wherein at least one of R_1 and R_2 is other than hydrogen when X is SO_2,

wherein at least one of R_1 and R_2 is preferably other than H and is in the 2 position when A is phenyl, and R_6 and R_7 do not form a ring and the other R_1 or R_2 can occupy any other position on A,

R_1 or R_2 is preferably a halogen such as F, Cl, Br or I, is in the 2 position when A is phenyl, and is more preferably Cl;

 can be a single bond or a double bond;

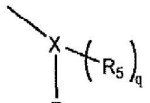
R_3 can be -H, -OH, -OR_9 or -O(CO)R_9, wherein R_9 can be hydrogen, a linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_4-alkyl; R_3 is preferably hydrogen or –OH; R_3 is more preferably hydrogen;

in the  group, X can be N, O, S, S(O) or SO_2, or can be O, S, S(O) or SO_2 when Y is O;

R_4 can be a linear or branched, substituted or unsubstituted, saturated or unsaturated C_{1-4} alkyl group; monocyclic or bicyclic C_3-C_{10}-cycloalkyl; substituted or unsubstituted, preferably a C_3-C_6-cycloalkyl; monocyclic or bicyclic C_6-C_{14}-aryl, substituted or unsubstituted, preferably a C_6-C_{10}-aryl, more preferably a C_5-C_6-aryl; 5- to 10-membered

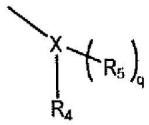
heterocycle, saturated or partly saturated, monocyclic or bicyclic, substituted or unsubstituted, preferably a C_5-C_6- heterocycle; monocyclic or polycyclic 5- to 14-membered heteroaryl, preferably monocyclic or bicyclic, substituted or unsubstituted, preferably a C_5-C_6-heteroaryl; R_4 is preferably monocyclic or bicyclic C_6-C_{10}-aryl, substituted or unsubstituted, or C_3-C_6-cycloalkyl, substituted or unsubstituted; more preferably, R_4 is cyclohexyl or phenyl;

q can be 0 (zero) or 1; when q is equal to 1, X is N and R_5 is hydrogen; a linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_4-alkyl group; monocyclic or bicyclic C_3-C_{10}-cycloalkyl;

alternatively, the  group can be an amino-acid residue wherein:

- X is an N, S or O atom, or an S or O atom when Y is O, of the side chain of an amino acid, preferably natural, selected from serine; tyrosine; threonine; lysine; cysteine; q is zero (R_5 is therefore not present) and R_4 is the remainder of the amino acid which can be protected or unprotected on the NH_2 and/or COOH terminal groups; in a preferred aspect, the terminal NH_2 group is acetylated; in a preferred aspect, the amino-acid residue is N-acetylcysteine or N-Boc cysteine methyl ester; or

- X is the N atom of the terminal amino group bonded to the stereogenic carbon atom in alpha of a preferably natural, protected or unprotected, amino acid, selected from alanine, arginine, asparagine, aspartic acid, cysteine, glycine, glutamic acid, glutamine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine; q is equal to 1, R_5 is hydrogen; and R_4 represents the remainder of the amino-acid structure, protected or unprotected, for example acetylated on the N atom of the side chain or esterified with a linear or branched C_1-C_4-alkyl group, preferably methyl, on the terminal carboxyl group;

alternatively when, in the  group, X is N, Y is other than O, R_4 and R_5 can be joined to form a monocyclic or bicyclic, saturated, partly saturated or unsaturated C_3-C_{10}-heterocyclic ring with the N atom; R_4 and R_5 preferably form a monocyclic C_3-C_6-heterocyclic ring with the N atom; more preferably, R_4 and R_5 form a piperidine or pyrrolidine ring with the N atom; most preferably R_4 and R_5 form a pyrrolidine ring with the N atom;

Y can be selected from O, N and S; is preferably O or N; and is more preferably N;

when Y is an oxygen or sulfur atom, in the -(R_7-R_8)p group p is equal to zero and R_6 can be hydrogen, a linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_8-alkyl group; a monocyclic or bicyclic C_3-C_{10}-cycloalkyl; a substituted or unsubstituted arylalkyl; a 6- to 14-membered monocyclic or bicyclic heteroaryl; R_6 is preferably hydrogen or a linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_4-alkyl group; R_6 is more preferably a linear or branched, saturated, unsubstituted C_1-C_4-alkyl group; R_6 is most preferably ethyl;

when Y is a nitrogen atom, p is equal to 1, R_6 and R_7, which are the same or different, are selected from hydrogen, a linear or branched, saturated or unsaturated, substituted or unsubstituted C_1-C_4-alkyl group; a substituted or unsubstituted aryl, arylalkyl or heteroaryl group; they can preferably be a substituted phenylalkyl group; more preferably –$(CH_2)_2$-phenyl-SO_2NH_2; most preferably R_6 is hydrogen and R_7 is –$(CH_2)_2$-phenyl-SO_2NH_2;

R_8 can be selected from H, COOH, $COOR_9$, $C(O)R_9$, CN, $CONH(R_9)$, $S(O)NHR_9$ and $S(O)_2NHR_9$, wherein R_9 is as defined above;

alternatively, R_6 and R_7 can be joined to form a 3- to 8-membered heterocyclic ring; R_8 is as defined above;

and their enantiomers, diastereomers, rotamers or mixtures thereof;

and the pharmaceutically acceptable salts or solvates thereof;

According to one aspect of the invention, in the compounds of formula (I), when R_6 and R_7 do not form a ring, in the compounds of formula (Ib) and formula (Ic) as defined below:

- when A is phenyl, R_1 and R_2 are as defined above,  is a double bond, R_3 is H or OH, Y is O, X is S, q and p are zero, R_4 is methyl, R_6 is hydrogen, a linear or branched, substituted or unsubstituted, saturated or unsaturated C_3-C_8-alkyl group; a monocyclic or bicyclic C_3-C_{10}-cycloalkyl; a substituted or unsubstituted arylalkyl; a 6- to 14-membered monocyclic or bicyclic heteroaryl; R_6 is preferably hydrogen or a linear or branched, substituted or unsubstituted, saturated or unsaturated C_3-C_6-alkyl group; and/or
- when A is phenyl, naphthyl or thiophene, R_1 and R_2 are as defined above, R_3 is H, Y is O, X is SO_2, q and p are zero, R_4 is phenyl, ethyl or methyl, 4-chlorophenyl, 4-toluene, R_6 is methyl or ethyl,  is a single bond; and/or
- when A is phenyl or naphthyl, R_1 and R_2 are as defined above,  is a double bond, R_3 is H, Y is O, X is O, q and p are zero, R_4 is methyl, R_6 is hydrogen, methyl, a linear or branched, substituted or unsubstituted, saturated or unsaturated C_3-C_8-alkyl group; a monocyclic or bicyclic C_3-C_{10}-cycloalkyl; a substituted or unsubstituted arylalkyl; a 6- to 14-membered monocyclic or bicyclic heteroaryl; R_6 is preferably hydrogen or a linear or branched, substituted or unsubstituted, saturated or unsaturated C_3-C_6-alkyl group;
- when A is phenyl, R_1 and R_2 are as defined above,  is a double bond, R_3 is H, Y is O, X is O, q and p are zero, R_4 is phenyl, R_6 is methyl, ethyl, a linear or

branched, substituted or unsubstituted, saturated or unsaturated C_3-C_8-alkyl group; a monocyclic or bicyclic C_3-C_{10}-cycloalkyl; a substituted or unsubstituted arylalkyl; a 6- to 14-membered monocyclic or bicyclic heteroaryl; R_6 is preferably hydrogen or a linear or branched, substituted or unsubstituted, saturated or unsaturated C_3-C_6-alkyl group;

- when A is phenyl, R_1 and R_2 are as defined above, Y is O and X is N, p is zero, R_6 is methyl, q is one,  is preferably a double bond, and is more preferably a single bond or a double bond; R_4 and R_5 are joined to form a monocyclic or bicyclic, saturated, partly saturated or unsaturated C_3-C_{10}-heterocyclic ring with the N atom; R_4 and R_5 preferably form a monocyclic C_3-C_6-heterocyclic ring with the N atom; more preferably, R_4 and R_5 form a piperidine or pyrrolidine ring with the N atom; most preferably R_4 and R_5 form a pyrrolidine ring with the N atom.

According to a further aspect of the invention:

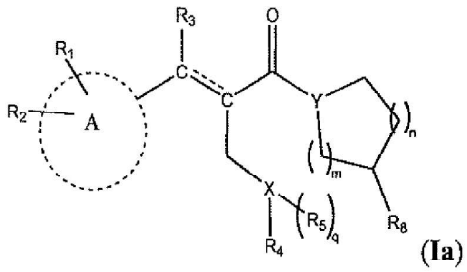
- when A is phenyl,  is a double bond, R_3 is hydrogen, Y is O, R_6 is methyl, q and p are zero and X is sulfur, R_4 is other than methyl, ethyl, n-propyl, tert-butyl, cyclohexyl, phenyl, 4-chlorophenyl, 4-bromophenyl, 3-chlorophenyl, 3-bromophenyl, 2-bromophenyl, benzyl;
- when A is 4-methoxyphenyl,  is a double bond, R_3 is hydrogen, Y is O, R_6 is methyl, q and p are zero and X is sulfur, R_4 is other than methyl, ethyl, phenyl;
- when A is 2-chlorophenyl,  is a double bond, R_3 is hydrogen, Y is O, R_6 is methyl, q and p are zero and X is sulfur, R_4 is other than phenyl;
- when A is 3-chlorophenyl,  is a double bond, R_3 is hydrogen, Y is O, R_6 is methyl, q and p are zero and X is sulfur, R_4 is other than methyl;

- when A is 4-chlorophenyl,  is a double bond, R_3 is hydrogen, Y is O, R_6 is methyl, q and p are zero and X is sulfur, R_4 is other than methyl, ethyl, phenyl, 4-chlorophenyl, 4-bromophenyl, 3-chlorophenyl, 3-bromophenyl, 2-bromophenyl, benzyl;
- when A is 4-methylphenyl,  is a double bond, R_3 is hydrogen, Y is O, R_6 is methyl, q and p are zero and X is sulfur, R_4 is other than methyl, phenyl, 4-chlorophenyl, 4-bromophenyl, 3-chlorophenyl, 3-bromophenyl;
- when A is 2-nitrophenyl,  is a double bond, R_3 is hydrogen, Y is O, R_6 is methyl, q and p are zero and X is sulfur, R_4 is other than methyl and phenyl;
- when A is 4-isopropyl-phenyl or 4-methoxycarbonylphenyl,  is a double bond, R_3 is hydrogen, Y is O, R_6 is tert-butyl, q and p are zero and X is sulfur, R_4 is other than phenyl;
- when A is 4-methoxyphenyl,  is a double bond, R_3 is hydrogen, Y is O, R_6 is hydrogen, q and p are zero and X is sulfur, R_4 is other than phenyl;
- when A is 2-chlorophenyl, 4-nitrophenyl, 3-bromo-phenyl, 4-methyl-phenyl or 2-thienyl,  is a double bond, R_3 is hydrogen, Y is O, R_6 is methyl or ethyl, q and p are zero and X is SO_2, R_4 is other than phenyl;
- when A is phenyl, 4-chlorophenyl, 4-methoxyphenyl, 3.4-dimethoxyphenyl, 3-methylphenyl, 1-naphthyl, 2-furyl or 4-bromo-phenyl,  is a double bond, R_3 is hydrogen, Y is O, X is SO_2, R_4 is phenyl and q and p are zero, R_6 is other than methyl;
- when A is phenyl,  is a double bond, R_3 is hydrogen, Y is O, X is SO_2, R_4 is

4-methylphenyl and q and p are zero, R_6 is other than methyl;

- when A is 4-bromophenyl, ⁻⁻⁻ is a double bond, R_3 is hydrogen, Y is O, X is SO_2, R_4 is 4-methylphenyl, 4-chlorophenyl, methyl or ethyl, q and p are zero, R_6 is other than methyl;
- when A is 4-bromophenyl, ⁻⁻⁻ is a double bond, R_3 is hydrogen, Y is O, X is S, R_4 is ethyl, q and p are zero, R_6 is other than methyl;
- when A is 4-fluorophenyl, 4-trifluoromethyl-phenyl, 4-cyanophenyl or 2,4-dichlorophenyl, ⁻⁻⁻ is a double bond, R_3 is hydrogen, Y is O, X is SO_2, R_4 is phenyl and q and p are zero, R_6 is other than ethyl.

When R_6 and R_7 form a ring, the compounds have formula (Ia):



(Ia)

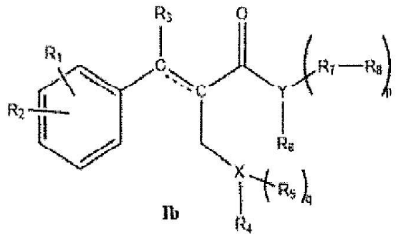
wherein

A, R_1, R_2, R_3, R_4, R_5, R_8, q, and X are as defined above;

n and m, which are the same or different, can be 0 (zero) or an integer between 1 and 3; when n and m are equal to zero, a three-membered cycle is generated between C-R_8, Y, and the remaining -CH_2- group; when n and m, which are different from one another, are 0 (zero) or 1, a 4-membered ring is formed; or n and m, which are the same or different, can be 1, 2 or 3 forming 5- to 9-, preferably 5- to 8-membered rings; according to a preferred aspect, m is 2 and n is 1; according to a more preferred aspect, n is 2 and m can be 1 or 2; most preferably, n is 2 and m is 1;

preferably, when Y is N, R_6 and R_7 can be joined to form a 3- to 6-membered monocyclic substituted heterocyclic ring with the N atom; more preferably, R_6 and R_7 form a substituted piperidine or pyrrolidine ring with the N atom; most preferably, R_6 and R_7 form, with the N atom, a piperidine ring substituted in the 3 or 4 position.

A preferred aspect of the invention is compounds of general formula (Ib):



Ib

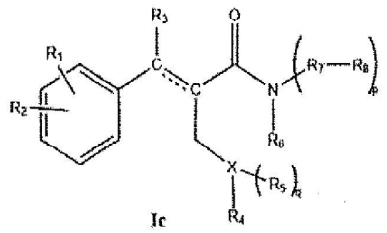
wherein

R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, q, p, X and Y are as defined above,

their enantiomers, diastereomers, rotamers or mixtures thereof;

and the pharmaceutically acceptable salts or solvates thereof.

Particularly preferred are compounds wherein Y is N of general formula (Ic):



Ic

wherein

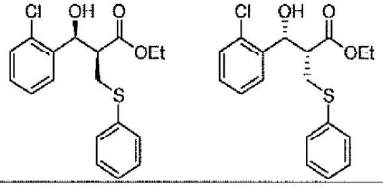
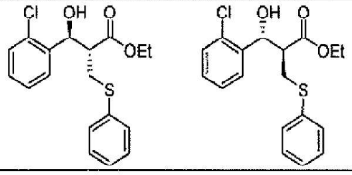
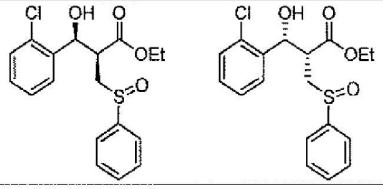
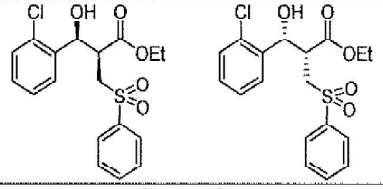
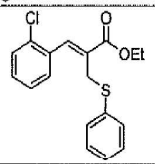
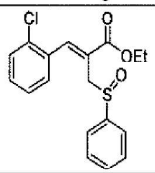
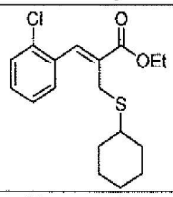
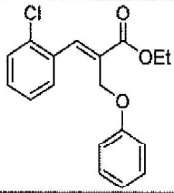
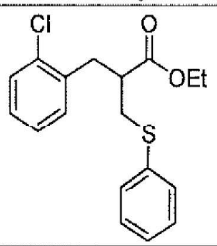
R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, q, p and X are as defined above,

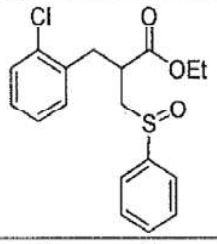
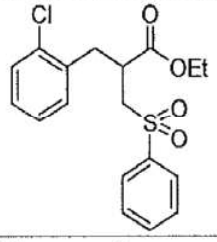
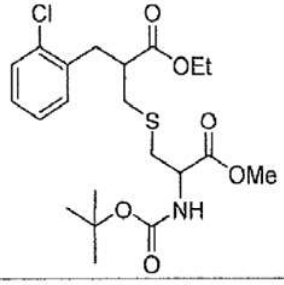
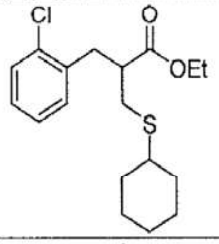
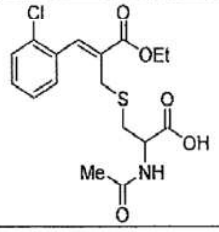
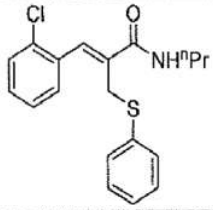
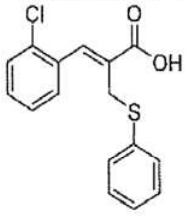
their enantiomers, diastereomers, rotamers or mixtures thereof;

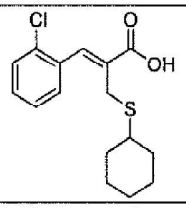
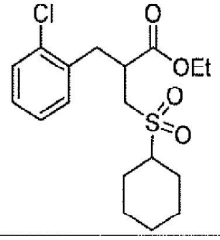
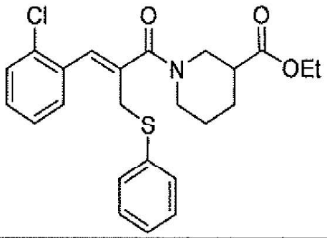
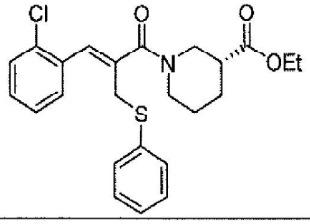
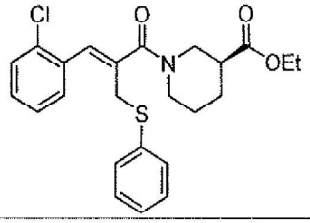
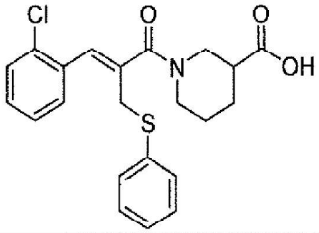
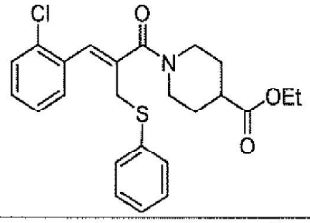
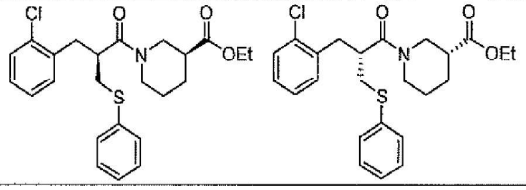
and the pharmaceutically acceptable salts or solvates thereof.

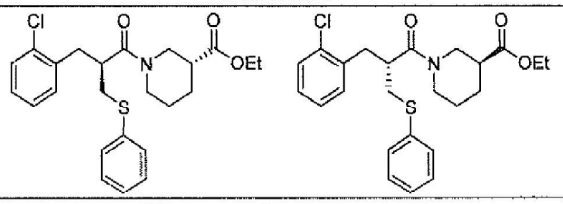
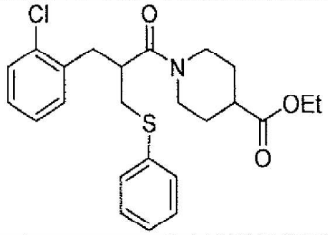
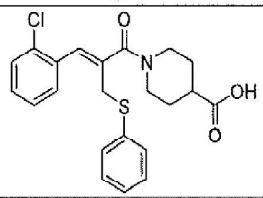
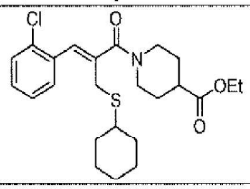
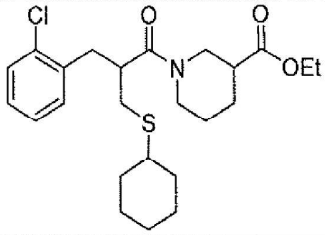
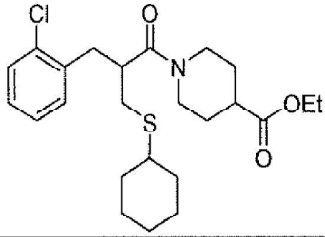
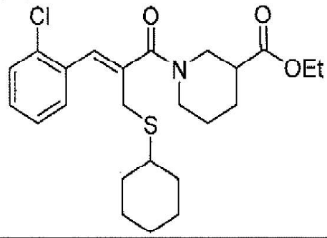
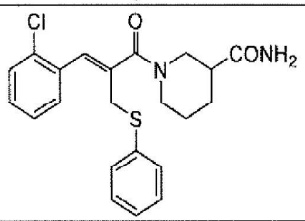
The compounds of general formula (I), (Ia), (Ib) or (Ic) are preferably selected from those listed in Table 2a:

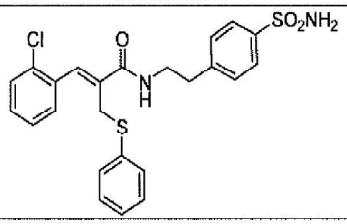
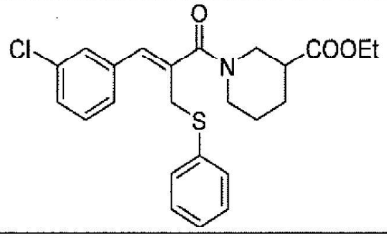
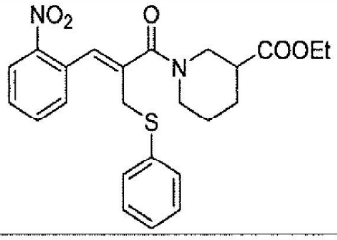
Table 2a

Compound	Structure
INF38s	
INF38a	
INF44	
INF45	
INF42	
INF50	
INF56	
INF57	
INF43	

Compound	Structure
INF48	
INF49	
INF55	
INF110	
INF85	
INF82	
INF80	

Compound	Structure
INF86	
INF111	
INF176	
INF202	
INF203	
INF177	
INF180	
INF184	

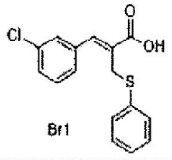
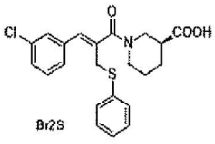
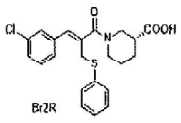
Compound	Structure
INF185	
INF186	
INF187	
INF188	
INF192	
INF193	
INF194	
INF219	

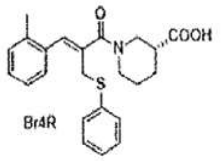
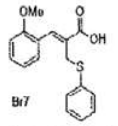
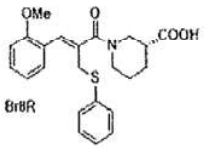
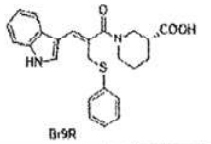
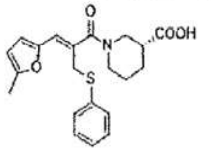
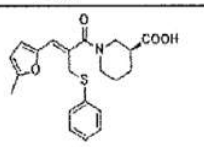
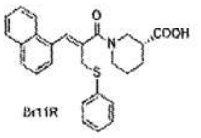
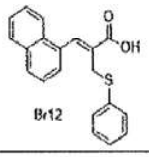
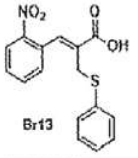
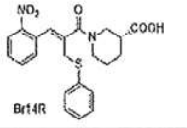
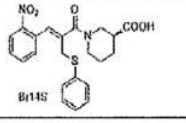
Compound	Structure
INF220	
INF230	
INF231	

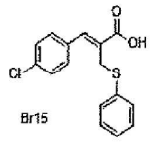
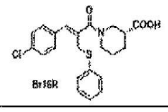
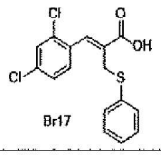
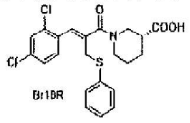
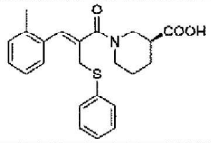
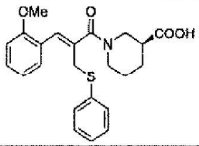
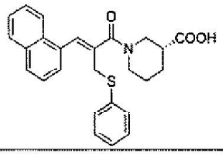
According to a further preferred aspect, the compounds according to the invention are selected from INF38s, INF38a, INF42, INF43. INF45, INF49, INF56, INF57, INF82, INF85, INF176, INF177, INF180, INF202, INF203. INF192, INF219 and INF220, more preferably from INF43. INF49, INF56, INF57, INF85, INF176, INF177, INF192, INF219 and INF220.

Further preferred compounds according to the invention are listed in Table 2b.

Table 2b

Compound	Structure
Br1	
Br2S	
Br2R	

Compound	Structure
Br4R	
Br7	
Br8R	
Br9R	
Br10R	
Br10S	
Br11R	
Br12	
Br13	
Br14R	
Br14S	

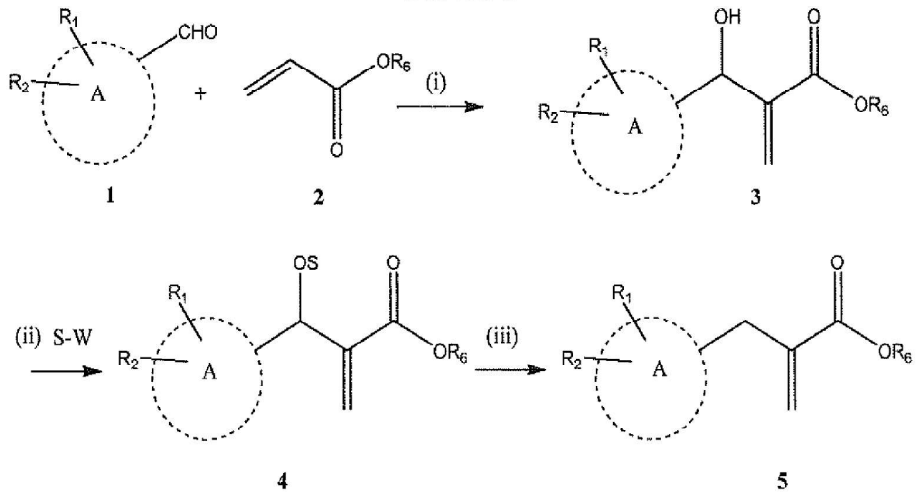
Compound	Structure
Br15	
Br16R	
Br17	
Br18R	
Br4S	
Br8S	
Br11S	

The compounds according to the invention can be administered orally, parenterally, topically or by injection, for example by intra-articular injection.

The compounds according to the present invention can be obtained as described in the synthesis schemes set out below.

The compounds of general formula (I) can be synthesised according to Schemes 1-7 below.

Scheme 1

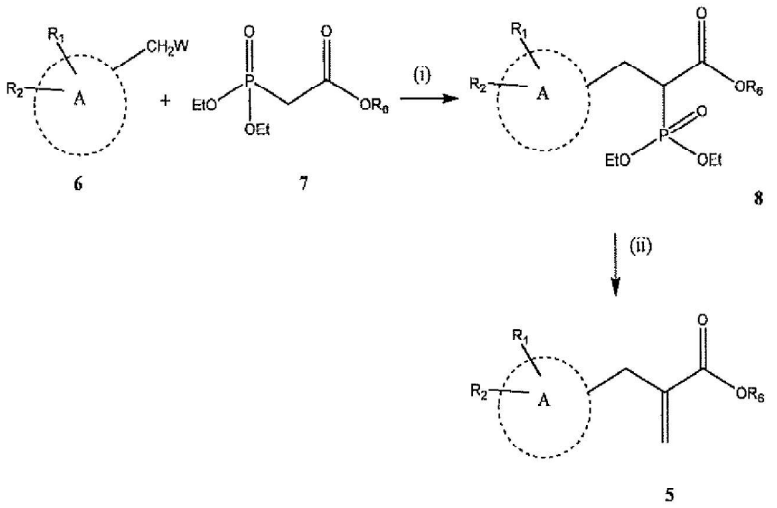


The reaction required to obtain the compounds of formula **3** can be conducted with a suitably substituted aldehyde (**1**), which is reacted according to a Morita-Baylis-Hillman (MBH) reaction using one of the procedures reported in the literature, such as in Min Shi, Fei-Jun Wang, Mei-Xin Zhao and Yin Wei "The Chemistry of the Morita–Baylis–Hillman Reaction", RSC Catalysis Series No. 8, 2011, Published by the Royal Society of Chemistry. In step (i), a compound of formula **1** is reacted with an α,β-unsaturated ester (**2**) in the presence of a base such as 1,4-diazabicyclo [2.2.2] octane (DABCO), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), triethylamine (Et_3N) or diisopropylethylamine (DIPEA), or in the presence of a phosphine such as triphenylphosphine, tritolylphosphine or tributylphosphine in a solvent selected from acetonitrile, tetrahydrofuran (THF), dichloromethane, methanol, ethanol, 2-propanol, butanol, water or mixtures thereof, at a temperature ranging between -40°C and +200°C for a time ranging between a few minutes and 30 days, as indicated in Scheme 1, to obtain the intermediates of formula **3**.

The intermediate of formula **3** is then converted, in step (ii), to compounds of formula **4**, by treatment with an electrophilic agent S-W, wherein W is a leaving group as defined in Smith M.B. and March J. "*Advanced Organic Chemistry*" 5th ed. 2001, Wiley & Sons, p. 449. By way of example, electrophilic agents such as acetic anhydride,

trifluoroacetic anhydride and trifluoromethanesulphonic anhydride can be used. The reaction is conducted in a solvent such as dichloromethane, THF or 1,4-dioxane in the presence or absence of a base for a time ranging between a few minutes and 24 hours. The preferred bases for the reaction are 4-dimethylaminopyridine (DMAP) or DBU. Compound **4** is then converted (step iii) to compound **5** by reduction with a reducing agent such as sodium borohydride, sodium cyanoborohydride or sodium triacetoxyborohydride in a solvent such as THF, diethyl ether, methanol, water or mixtures thereof. A catalyst such as DABCO can be added to the reaction mixture. The reaction is conducted at temperatures ranging between -20 and +100°C for a time ranging between a few minutes and 24 hours.

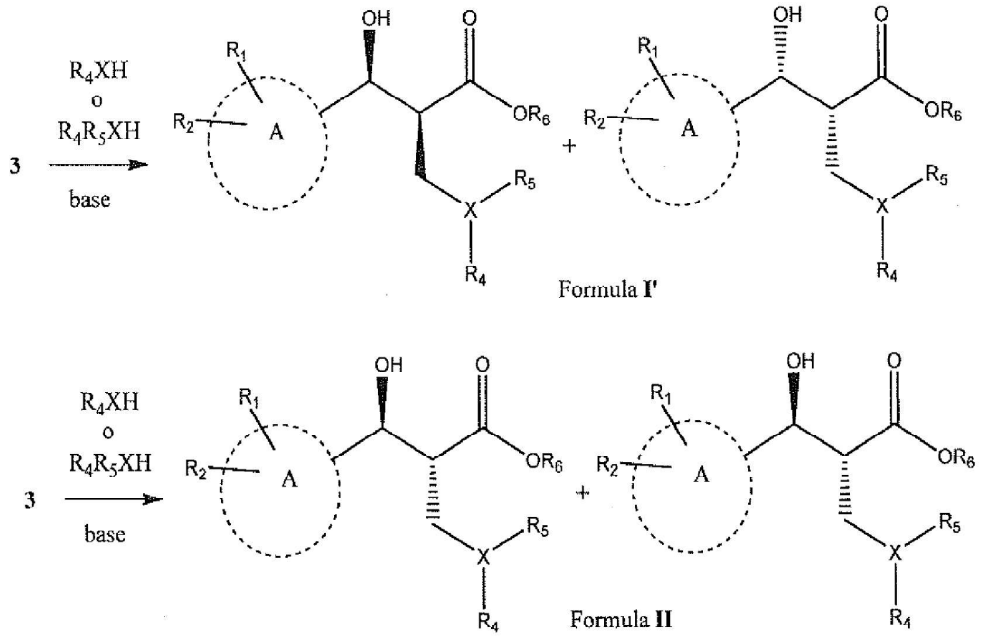
Scheme 2



Alternatively, the compounds of formula **5** can be obtained, as described in Scheme 2, by reacting a compound of formula **6**, wherein W is a leaving group as defined in Smith M.B. and March J. "*Advanced Organic Chemistry*" 5th ed. 2001, Wiley & Sons, p. 449. W is preferably represented by a halogen atom. The compound of formula **6** is reacted with a suitably substituted phosphonoacetate (**7**) in the presence of a base such as sodium hydride, sodium amide or potassium *tert*-butoxide, in a suitable solvent such as dimethylformamide (DMF), dimethylsulphoxide (DMSO), THF or 1,4-dioxane, at a temperature ranging between -40°C and +200°C for a time ranging between a few minutes

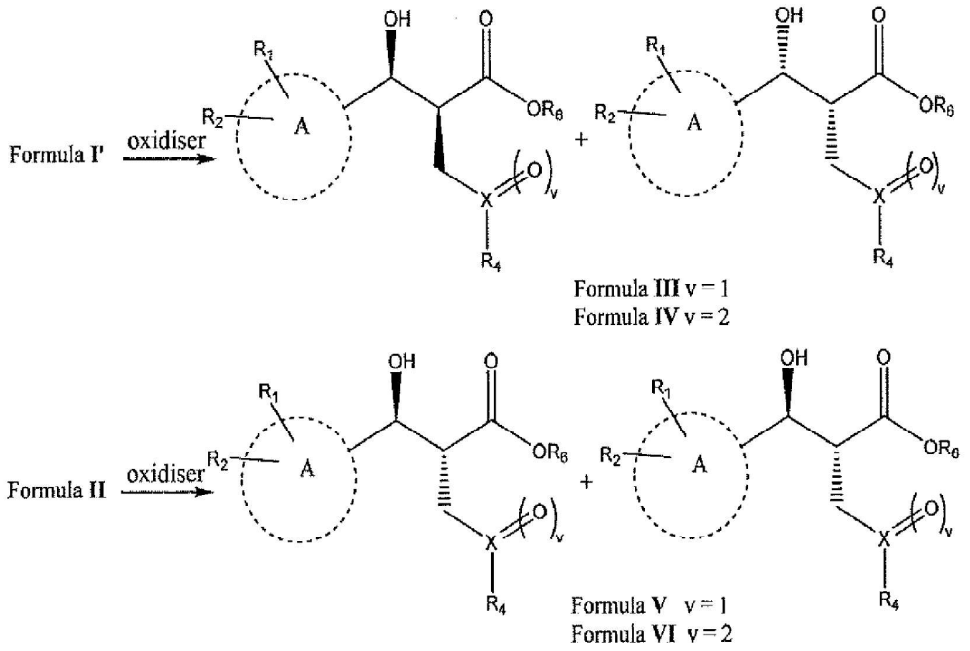
and 72 hours. The resulting intermediate **8** is purified by silica gel chromatography and reacted with an excess of paraformaldehyde in the presence of a base such as K_2CO_3, Na_2CO_3 or Cs_2CO_3 in water at a temperature ranging between 0 and +100°C for a time ranging between a few minutes and 72 hours to obtain the compound of formula **5**.

Scheme 3



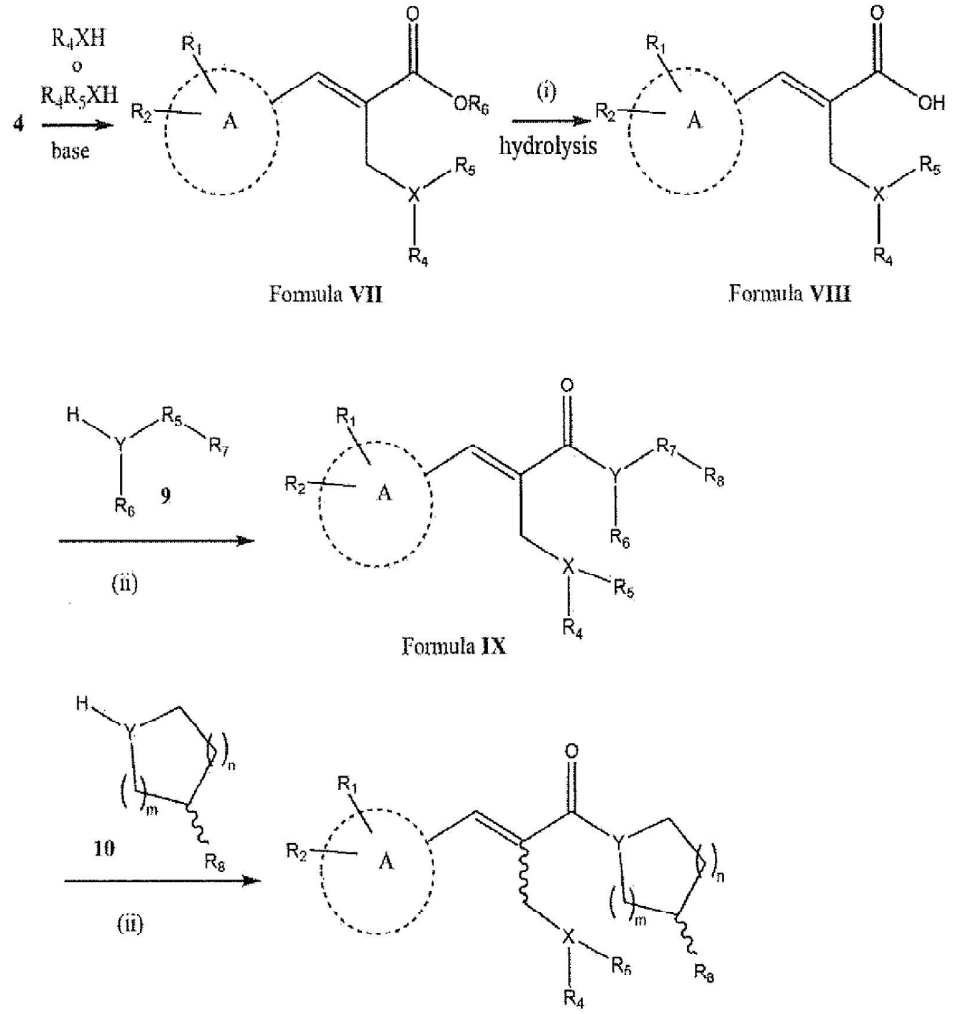
Compound **3** can be converted to the products of formulae **I'** and **II** by reaction with a suitable nucleophile R_4XH or R_4R_5XH in a basic medium, as illustrated in Scheme 3. The reaction is conducted in a solvent such as THF, dichloromethane, acetonitrile, DMF or DMSO in the presence of a base such as DABCO, DBU, Et_3N, DIPEA, potassium *tert*-butoxide or sodium hydride, and in an inert gas atmosphere such as nitrogen or argon. The reaction is conducted at a temperature ranging between -20 and +180°C, preferably at room temperature. The reaction provides a mixture of products which can contain various stereoisomeric forms of the desired products. The products of formulae **I'** and **II** are separated by preparative silica gel chromatography.

Scheme 4



The compounds of formulae **I'** and **II** can be oxidised using suitable oxidation reagents known to the skilled person, such as those described in Burke S.D and Danheiser R.L. eds. "Handbook of Reagents for Organic Synthesis – Oxidizing and Reducing Agents", John Wiley and Sons Ltd 1999 pages 15-518 and the references cited. Of the preferred reagents, *meta*-chloroperoxybenzoic acid (mCPBA), hydrogen peroxide, ammonium persulphate and potassium peroxymonosulphate (oxone) can be used in an organic solvent such as dichloromethane or acetic acid, or in water or in mixtures thereof. The reaction times can range from a few minutes to 72 hours. The reaction can be conducted at temperatures ranging between -78 and +180°C. Compounds having formulae **III-VI** are thus obtained, after chromatographic purification.

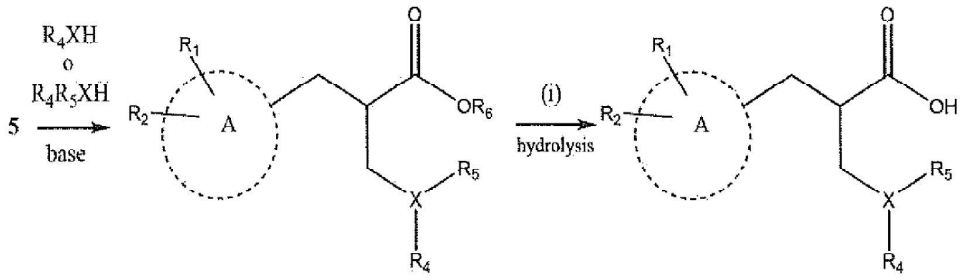
Scheme 5



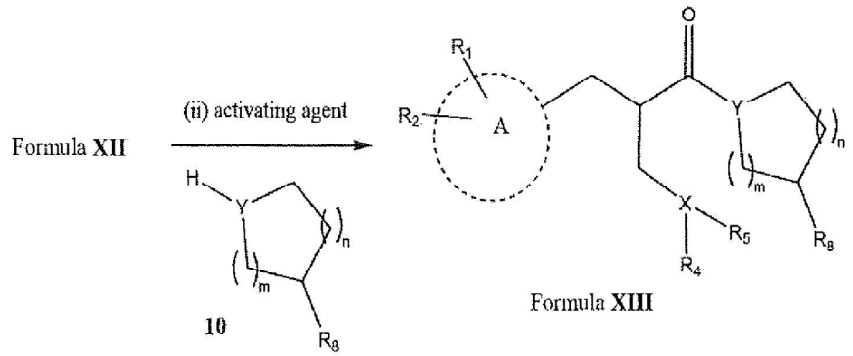
The synthesis of the compounds of formulae **VII-X** is described in Scheme 5. Compound of formula **4** is reacted with a suitable nucleophile R_4XH or R_4R_5XH in a basic medium as illustrated in Scheme 5. The reaction is conducted in a solvent such as THF, dichloromethane, acetonitrile, DMF or DMSO in the presence of a base such as DABCO, DBU, Et_3N, DIPEA, potassium *tert*-butoxide or sodium hydride, and in an inert gas atmosphere such as nitrogen or argon. The reaction is conducted at a temperature ranging between -20 and +180°C, preferably at room temperature, to obtain the compounds having formula **VII**. Said compounds then undergo hydrolysis in a basic medium (i) using a base such as sodium hydroxide, potassium hydroxide or lithium hydroxide in a solvent such as water, THF, 1,4-dioxane or mixtures thereof at temperatures ranging between -20 and +

100°C for a time ranging between a few minutes and 72 hours, to obtain the compounds of formula **VIII**. Alternatively, hydrolysis (i) can be conducted by treating a compound of formula **VII** with an acid such as trifluoroacetic acid, hydrochloric acid, hydrobromic acid or methanesulphonic acid in a solvent such as dichloromethane, 1,4-dioxane, water or mixtures thereof, at temperatures ranging between -20 and +100°C for a time ranging from a few minutes to 72 hours. Compounds having formula **VIII** are thus obtained. Reaction (ii) described in Scheme 5 can be conducted by treating a compound of formula **VIII** with a suitable activating agent or coupler selected, for example, from those described in Pearson A.J. and Roush W.J. editors, "Handbook of Reagents for Organic Synthesis – Activating Agents and Protecting Groups", John Wiley and Sons Ltd. 1999, pp. 1-482 and references cited. The preferred activating reagents are thionyl chloride, 2-(1*H*-benzotriazol-1-yl)-1,1,3.3-tetramethyluronium hexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBt), 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazole[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU), benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBoP), carbonyldiimidazole (CDI), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), dicyclohexylcarbodiimide (DCC) and N-hydroxysuccinimide (NHS), used alone or mixed in a suitable solvent such as dichloromethane, DMF, DMSO, acetonitrile or THF, or in mixtures thereof. The reaction can be conducted in the presence of a suitable base such as Et_3N, DIPEA or DMAP for a time ranging between a few minutes and 3 hours at temperatures ranging between -20 and +120°C. Compounds of formula **9** or **10** are added to the solution of the activated compound of formula **VIII**, and the mixture is left under stirring at a temperature ranging between -20 and +120°C for a time ranging between a few minutes and 90 hours. Compounds having formulae **IX** and **X** are thus obtained.

Scheme 6



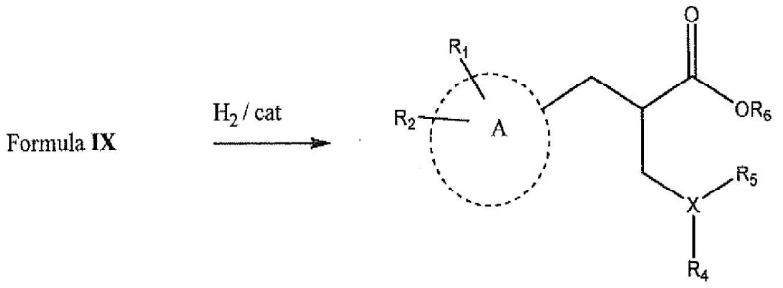
Formula **XI** Formula **XII**



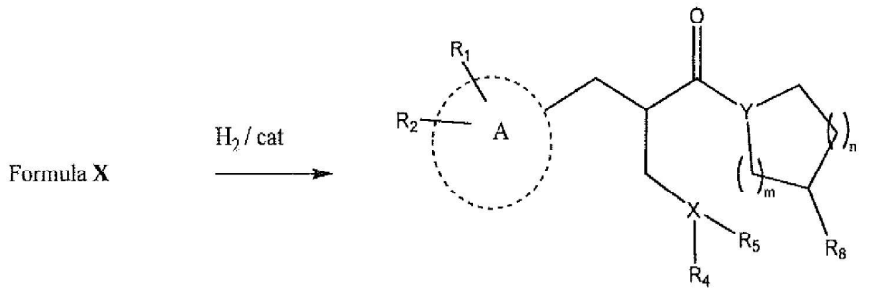
Compounds of formulae **XI-XIII** are synthesised as described in Scheme 6. A compound of formula **5** is reacted with a suitable nucleophile R_4XH or R_4R_5XH in a basic medium as illustrated in Scheme 6. The reaction is conducted in a solvent such as THF, dichloromethane, acetonitrile, DMF or DMSO in the presence of a base such as DABCO, DBU, Et_3N, DIPEA, potassium *tert*-butoxide or sodium hydride, and in an inert gas atmosphere such as nitrogen or argon. The reaction is conducted at a temperature ranging between -20 and +180°C, preferably at a temperature ranging between 20 and 40°C. The reaction provides a mixture of products which can contain various stereoisomeric forms of the desired products of formula **XI** which, where necessary, are separated by preparative silica gel chromatography. Compounds of formula **XI** then undergo hydrolysis in a basic medium using a base such as sodium hydroxide, potassium hydroxide or lithium hydroxide in a solvent such as water, THF, 1,4-dioxane or mixtures thereof at temperatures ranging between -20 and +100°C for a time ranging between a few minutes and 72 hours, to obtain compounds of formula **XII**. Alternatively, hydrolysis (i) can be conducted by treating a

compound of formula **XI** with an acid such as trifluoroacetic acid, hydrochloric acid, hydrobromic acid or methanesulphonic acid in a solvent such as dichloromethane, 1,4-dioxane, ethyl acetate, water or mixtures thereof, at temperatures ranging between -20 and +100°C for a time ranging from a few minutes to 72 hours. Reaction (ii) described in Scheme 6 can be conducted by treating a compound of formula **XIII** with a suitable activating agent or coupler selected, for example, from those described in Pearson A.J. and Roush W.J. editors, "Handbook of Reagents for Organic Synthesis – Activating Agents and Protecting Groups", John Wiley and Sons Ltd. 1999, pp. 1-482 and references cited. The preferred activating reagents are thionyl chloride, 2-(1*H*-benzotriazol-1-yl)-1,1,3.3-tetramethyluronium hexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBt), 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazole[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU), benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBoP), carbonyldiimidazole (CDI), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), dicyclohexylcarbodiimide (DCC) and N-hydroxysuccinimide (NHS), used alone or mixed in a suitable solvent such as dichloromethane, DMF, DMSO, acetonitrile or THF, or in mixtures thereof. The reaction can be conducted in the presence of a suitable base such as Et_3N, DIPEA or DMAP for a time ranging between a few minutes and 3 hours at temperatures ranging between -20 and +120°C. Compounds of formula **10** are added to the solution of the activated compound of formula **XII**, and the mixture is left under stirring at a temperature ranging between -20 and +120°C for a time ranging between a few minutes and 90 hours. Compounds having formula **XIII** are thus obtained.

Scheme 7



Formula **XI**



Formula **XIII**

Compounds of formulae **XI** - **XIII** can also be synthesised by catalytic reduction of the compounds of formulae **IX** and **X**, as illustrated in Scheme 7. In said procedure, the reaction is conducted by dissolving the compound of formula **IX** or **X** in a solvent such a methanol, ethanol, 2-propanol, n-butanol, ethyl acetate, THF, 1,4-dioxane or mixtures thereof, and a suitable catalyst is added such as Pd supported on carbon, Pt supported on carbon, PtO_2 or, in general, the suitable substances described in Burke S.D and Danheiser R.L. eds., "Handbook of Reagents for Organic Synthesis – Oxidizing and Reducing Agents", John Wiley and Sons Ltd 1999, pages 15-518 and references cited. The mixture is placed under vigorous stirring in H_2 gas atmosphere at a pressure ranging between 1 and 50 bars and at a temperature ranging between 0°C and 120°C for a time ranging between a few minutes and 72 hours. Compounds having formulae **XI** and **XIII** are thus obtained. To obtain compounds of formula **XII**, compounds of formula **XI** undergo hydrolysis according to the same procedures as described above in Scheme 6 step (i).

The examples below further illustrate the invention.

Examples

Synthesis examples

Materials and methods

All reactions were monitored by thin-layer chromatography (TLC) on Merck 60 F254 plates (0.25 mm), which were detected with UV light and/or by spraying a solution of $KMnO_4$ (0.5 g in 100 mL of 0.1N NaOH) and bromocresol green (0.04 g in 100 mL of EtOH, then treated with 1N NaOH). The flash chromatography purifications used Merck silica gel with 60 mesh particles. Commercially available reagents and solvents were used without further purifications.

The ^1H and ^{13}C spectra were recorded on a Jeol ECZ 600 M30, at 600 and 150 MHz respectively. The coupling constants (J) are expressed in Hertz (Hz), and the chemical shift values (δ) are supplied in ppm relative to the deuterated solvent used as internal standard.

The abbreviations used to describe plurality are: s=singlet, d=doublet, m=multiplet, dd= doublet of doublets; and the abbreviations used to identify the protons are: Ar*H*=aromatic protons, Pip*H*=piperidine protons.

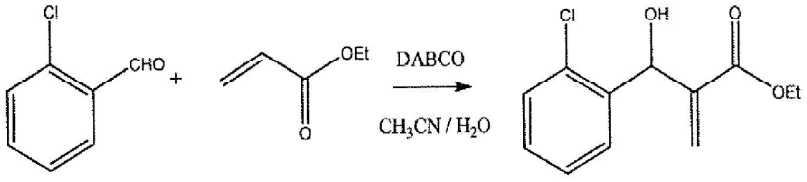
The low resolution ESI mass spectra were recorded on a Micromass Quattro Micro TP API (Waters Corporation, Milford, MA, USA) equipped with an ESI source.

The purity of the final products was determined by reverse-phase HPLC (RP-HPLC). The tests were performed with an HP1100 chromatography system (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump (G1311A), a membrane degasser (G1379A), and a diode-array detector (DAD) (G1315B) integrated into the HP1100 system. The analysis data were processed with the HP ChemStation system (Agilent Technologies). The analysis column used was a LiChrosper 100 C18-e (250x4.6 mm, 5 μm) (Merck KGaA, 64271 Darmstadt, Germany) using the eluent indicated for each compound. All the compounds were solubilised in the mobile phase at a

concentration of about 0.1 mg/mL, and injected through a 20 µL loop. The retention times (t_R) were obtained with a flow rate of 1.0 mL/min, and the effluent was monitored at two wavelengths (226 and 254 nm) and calibrated on the reference at 800 nm. The purity of the compounds was calculated as the percentage ratio between the main peak areas and those of any impurities at the two wavelengths, also using DAD purity analysis of the chromatographic peak. The actual purity value and the eluent used for the elution are reported for each compound at the characterisation stage. The melting points (mp) were determined in a glass capillary using a Büchi 540 melting point measuring apparatus.

Further abbreviations used are: 40-70°C petroleum ether (PE), ethyl acetate (EtOAc), diethyl ether (Et_2O), methanol (MeOH), tetrahydrofuran (THF), dimethylformamide (DMF), dimethylsulphoxide (DMSO), retention factor (Rf), retention time (t_R), mass spectrometry (MS), nuclear magnetic resonance (NMR), acetic anhydride (Ac_2O), minute (min).

Example 1 - Synthesis of ethyl 2-((2-chlorophenyl)(hydroxy)methyl)acrylate (3a)

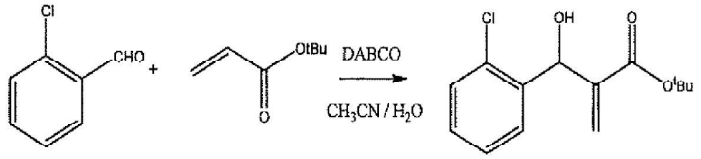


3a

Ethyl acrylate (9.3 mL; 84.89 mmol) and water (54 mL) are added to a solution of 2-chlorobenzaldehyde (4.00 g; 28.46 mmol) in CH_3CN (9.3 mL). DABCO (3.2 g; 28.46 mmol) is then added to the mixture, and the reaction is left under stirring for 7 days at 20°C. The mixture is diluted with CH_2Cl_2 (30 mL) and extracted with 1N HCl (3 x 30 mL) and an NaCl saturated solution (30 mL), then dried (Na_2SO_4), and the solvent evaporated under low pressure. The residue is purified by flash chromatography on silica gel column,

eluting with a PE/EtOAc 9/1 mixture. The compound is obtained as a colourless oil (5.42 g; yield 79%). CI-MS (isobutane) m/z: 241-243 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ 7.60–7.12 (m, 4H, Ar*H*); 6.33 (s, 1H, C=C*H*); 5.97 (s, 1H, C*H*OH); 5.60 (s, 1H, C=C*H*); 4.20 (q, *J* = 7.1 Hz, 2H, C*H*$_2$CH$_3$); 3.44 (s, 1H, O*H*); 1.25 (t, 3H, CH$_2$C*H*$_3$); ^{13}C-NMR ($CDCl_3$): δ 166.5; 140.9; 138.4; 132.8; 129.4; 129.0; 128.2; 127.0; 126.6; 69.2; 61.1; 14.0.

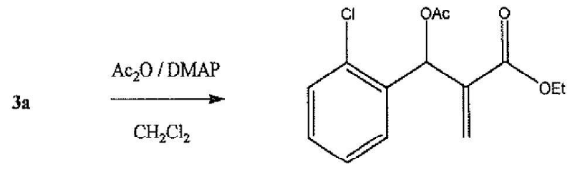
Example 2 - Synthesis of *tert*-butyl 2-((2-chlorophenyl)(hydroxy)-methyl)acrylate (3b)



3b

Tert-butyl acrylate (13.22 mL; 91.06 mmol), H_2O (10 mL) and DABCO (3.19 g; 28.46 mmol) are added to a solution of 2 chloro-benzaldehyde (3.20 mL; 28.46 mmol) in CH_3CN (90 mL). The reaction mixture is left under magnetic stirring at 20°C for 7 days. The solvent is evaporated under low pressure, and the residue is taken up with CH_2Cl_2 (25 mL) and washed with 1N HCl (3 x 25 mL) and NaCl saturated solution (30 mL), then dried (Na_2SO_4), and the solvent evaporated under low pressure. The crude compound is purified by flash chromatography on silica gel using PE/EtOAc 9/1 as eluent. **3b** is obtained as a pale yellow oil (2.9 g; yield 38%). MS (ESI) m/z: 269-271 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ 7.51 (dd, J=7.7. 1.6 Hz, 1H, ArH); 7.35 (dd, J=7.9, 1.2 Hz, 1H, ArH); 7.28 (d, J=7.6, 1.1 Hz, 1H, ArH); 7.25–7.23 (d, 1H, ArH); 6.25 (s, 1H, C=CH); 5.93 (s, 1H, OH-CH); 5.53 (s, 1H, C=CH); 1.43 (s, 9H, CH_3); 1.25 (s, 1H, OH).

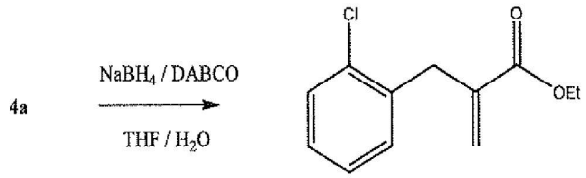
Example 3 - Synthesis of ethyl 2-(acetoxy(2-chlorophenyl)methyl)acrylate (4a)



4a

Acetic anhydride (0.509 g; 4.99 mmol) dissolved in CH_2Cl_2 (10 mL) is added slowly over a period of 1 hour to a solution of **3a** (1.00 g; 4.16 mmol) and DMAP (102 mg, 0.831 mmol) in CH_2Cl_2 (10 mL) at 0°C, maintaining the mixture under stirring at 20°C. The reaction mixture is extracted with water (15 mL) and $NaHCO_3$ 10% w/v (3 x 30 mL), then with a NaCl saturated solution (30 mL). The organic phase is dried (Na_2SO_4) and the solvent is evaporated under low pressure. The residue is purified by flash chromatography on silica gel using a PE/EtOAc 9/1 mixture as eluent. Compound **4a** is obtained as a colourless oil (0.783 g; yield 67%). CI-MS (isobutane) m/z: 283-285 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.47-7.26 (m, 4H, Ar*H*); 7.06 (s, 1H, C*H*); 6.47 (s, 1H, C=C*H*); 5.63 (s, 1H, C=C*H*); 4.19 (q, J = 7.1 Hz, 2H, CH_2CH_3); 2.12 (s, 3H, CH_3); 1.23 (t, J = 7.1 Hz, 3H, CH_2CH_3).

Example 4 - Synthesis of ethyl 2-(2-chlorobenzyl)acrylate (5a) (PROCEDURE A)

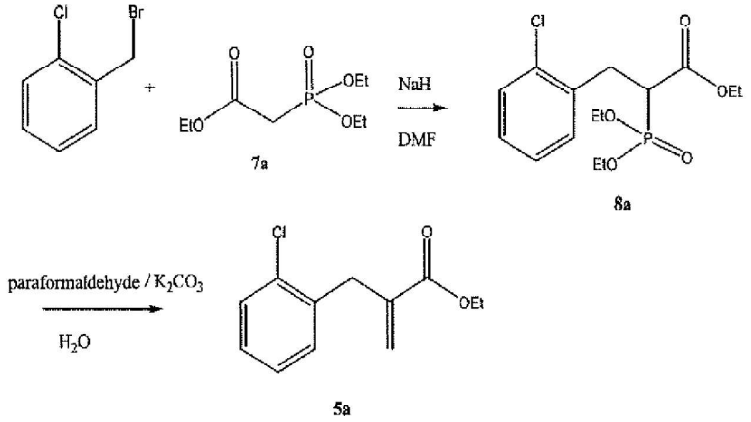


5a

$NaBH_4$ (0.129 g; 3.42 mmol) and DABCO (0.384 g; 3.42 mmol) are added in succession to a solution of **4a** (0.968 g; 3.42 mmol) in THF/H_2O 1/1 (40 mL), maintained under an inert atmosphere (N_2). The reaction is left under stirring for 1 hour. The mixture

is diluted with water (20 mL), extracted with EtOAc (3 x 60 mL) and dried (Na_2SO_4), and the solvent is evaporated under low pressure. The residue is purified by flash chromatography on silica gel using a PE/EtOAc 95/5 mixture as eluent; compound **5a** is obtained as a colourless oil (0.649 g; yield 84%). CI-MS (isobutane) m/z: 225-227 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.55-6.99 (m, 4H, Ar*H*); 6.27 (d, 1H, C=C*H*); 5.33 (d, 2H, C=C*H*); 4.22 (q, *J* = 7.1 Hz, 2H, CH_2CH_3); 3.76 (s, 2H, Ph-CH_2); 1.29 (t, J = 7.1 Hz, 3H, CH_2CH_3).

Example 5 - Synthesis of ethyl 2-(2-chlorobenzyl)acrylate (5a) (PROCEDURE B)

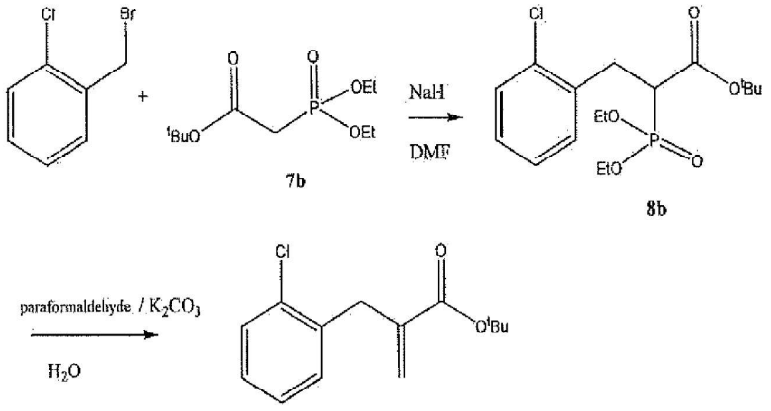


60% NaH in mineral oil (0.820 g; 20.5 mmol) is added to a solution of ethyl diethoxyphosphorylacetate (3.93 g; 17.6 mmol) in anhydrous DMF (30 mL) placed at 0°C in an inert atmosphere (N_2). 2-chlorobenzyl bromide (3.00 g; 14.6 mmol) is added after 2 hours, and the reaction is left under stirring in an inert atmosphere (N_2) for 16 hours. H_2O (15 mL) is added to the reaction, and the mixture is extracted with EtOAc (2 x 15 mL), washed with a NaCl saturated solution (15 mL) and dried (Na_2SO_4), and the solvent is removed under low pressure. The residue is purified by flash chromatography on silica gel using a PE/EtOAc 7/3 mixture to elute the unreacted 2-chlorobenzyl bromide, and then using PE/EtOAc 1/1 to obtain 3.42 g of compound **8a** (yield 67%). Intermediate **8a** is not further characterised, but is used directly in the next step.

A solution of K_2CO_3 (4.00 g; 29.0 mmol) in H_2O (60 mL) is added to a solution of

intermediate **8a** (3.36 g; 9.66 mmol) and paraformaldehyde (1.91 g; 0.064 mol) in H_2O (60 mL), and the reaction is left under stirring for 16 hours at 90°C. The mixture is extracted with EtOAc (2 x 20 mL), washed with a NaCl saturated solution (20 mL) and dried (Na_2SO_4), and the solvent is removed under low pressure. The residue is purified by flash chromatography on silica gel using a PE/EtOAc 7/3 mixture to obtain 1.63 g of compound **5a** (yield 75%). The characterisation of this compound is identical to that of the compound obtained by Procedure A.

Example 6 - Synthesis of *tert*-butyl 2-(2-chlorobenzyl) acrylate (5b)

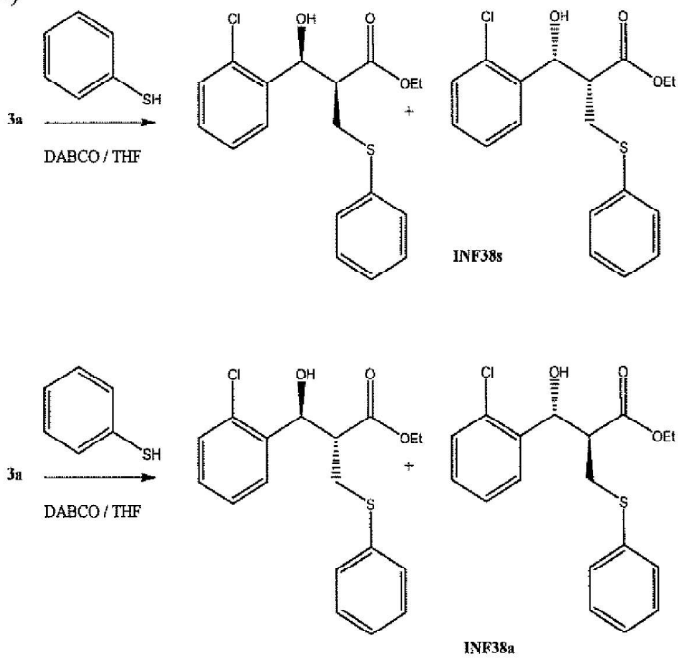


60% NaH in mineral oil (1.23 g; 30.7 mmol) is added to a solution of *tert*-butyldiethylphosphonoacetate (6.17 mL; 26.3 mmol) in anhydrous DMF (40 mL) maintained at 0°C under an inert atmosphere (N_2) . The reaction mixture is stirred for 2.5 hours at 25°C; 2-chlorobenzyl bromide (2.84 mL; 21.9 mmol) is then added drop by drop at 0°C, and the solution is left under magnetic stirring for 2 hours at 25°C. The reaction mixture is cooled to 0°C, and water (20 mL) is added. After 16 hours the solvent is evaporated under low pressure. The crude compound is solubilised in diethyl ether (30 mL) and washed with H_2O (2 x 10 mL) and an NaCl saturated solution (10 mL), then dried (Na_2SO_4) and concentrated under low pressure to give *tert*-butyl 3-(2-chlorophenyl)-2-(diethyloxyphosphoryl)propanoate (**8b**) (8.20 g; yield 99%) as a white solid, which is used

in the next step without further purification.

A solution of K_2CO_3 (8.62 g; 62.4 mmol) in H_2O (60 mL) is added to a solution of **8b** (8.20 g; 21.9 mmol) and paraformaldehyde (5.25 mL; 175 mmol) in H_2O (80 mL). The reaction mixture is heated at 90°C for 16 hours. The mixture is cooled to room temperature and extracted with EtOAc (3 x 40 mL). The organic phase is washed with a NaCl saturated solution (15 mL) and dried (Na_2SO_4), and the solvent is evaporated under low pressure. The crude compound is purified by flash chromatography on silica gel using PE/EtOAc 95/5 as eluent. Compound **5b** is obtained (4.98 g; yield 90%) as a colourless oil. MS (ESI) m/z: 275-277 $[M+Na]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.36–7.15 (m, 4H, ArH); 6.17 (s, 1H, C=C*H*H); 5.25 (m, 1H, C=C*H*H); 3.71 (s, 2H, CH_2); 1.45 (s, 9H, CH_3). ^{13}C-NMR ($CDCl_3$): δ, 166.1; 139.9; 136.9; 134.5; 130.9; 129.6; 127.8; 126.8; 126.0; 80.9; 35.5; 28.0.

Example 7 - Synthesis of the compounds of formula INF38 (GENERAL PROCEDURE)



DABCO (0.190 g; 1.69 mmol) and thiophenol (0.103 mL; 0.997 mmol) are added to a solution of **3a** (0.200 g; 0.831 mmol) in distilled THF (15 mL), maintained under an inert atmosphere (N_2), and the reaction is placed under stirring at 20°C for 2.5 hours. The

mixture is extracted with CH_2Cl_2 (3 x 15 mL), 1N HCl (25 mL) and H_2O (25 mL). The organic phase is dried (Na_2SO_4) and the solvent is evaporated under low pressure. The crude compound is purified by flash chromatography on silica gel, eluting with PE/EtOAc 95/5 then with PE/EtOAc 9/1. The syn diastereomer **INF38s** (65%) and the anti diastereomer **INF38a** (11%) are isolated in this way.

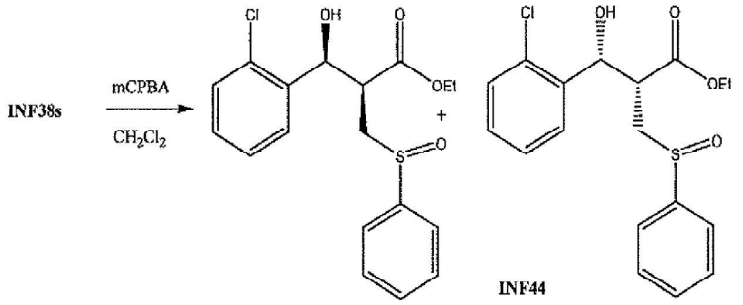
Ethyl(2S,3S)-3-(2-chlorophenyl)-3-hydroxy-2-(phenylsulphanylmethyl)-propanoate and **ethyl(2R,3R)-3-(2-chlorophenyl)-3-hydroxy-2-(phenylsulphanylmethyl)propanoate (INF38s).**

CI-MS (isobutane) m/z: 351-353 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.37–6.96 (m, 9H, Ar*H*); 5.42 (t, *J* = 3.0 Hz, 1H, C*H*OH); 4.12 (q, *J* = 7.2 Hz, 2H, CH_2CH_3); 3.38–2.92 (m, 3H aliphatic and O*H*); 1.19 (t, *J* = 7.1 Hz, 3H, CH_2CH_3). ^{13}C-NMR ($CDCl_3$): δ, 173.6; 137.7; 135.5; 131.7; 129.6; 129.02; 128.98; 128.8; 128.2; 126.9; 126.0; 70.6; 61.3; 49.3; 29.8; 14.0. Cytotoxicity (MTT assay): IC_{50} > 100 μM.

Ethyl(2S,3S)-3-(2-chlorophenyl)-3-hydroxy-2-(phenylsulphanylmethyl)-propanoate and **ethyl(2S,3R)-3-(2-chlorophenyl)-3-hydroxy-2-(phenylsulphanylmethyl)propanoate (INF38a).**

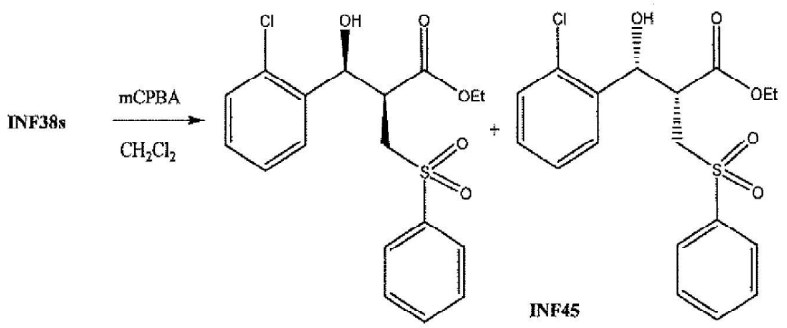
CI-MS (isobutane) m/z 351-353 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.44–7.07 (m, 9H, Ar*H*); 5.42–5.27 (dd, *J* = 7.8 Hz, 1H, C*H*OH), 4.09 (q, *J* = 7.1 Hz, 2H, CH_2CH_3); 3.58 (d, *J* = 7.9 Hz, 1H, O*H*); 3.39–3.25 (m, 1H, C*H*CH_2); 3.22-3.07 (m, 2H, CH_2S); 1.06 (t, *J* = 7.1 Hz, 3H, CH_2CH_3). ^{13}C-NMR ($CDCl_3$): δ, 173.4; 138.9; 135.1; 131.9; 129.9; 129.5; 129.0; 127.3; 127.0; 126.6; 70.8; 61.1; 50.2; 33.5; 14.0. Cytotoxicity (MTT assay): IC_{50} > 100 μM.

Example 8 – Synthesis of ethyl 3-(2-chlorophenyl)-3-hydroxy-2-((phenylsulphinyl)methyl)propanoate (INF44)



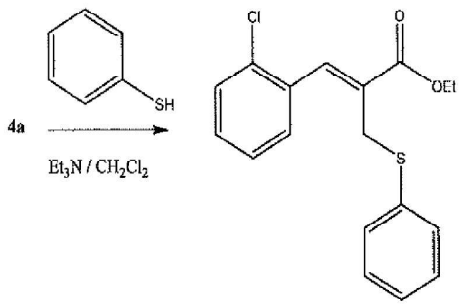
75% mCPBA (0.085 g; 0.371 mmol) is added to a solution of **INF38s** (0.130 g; 0.371 mmol) in CH_2Cl_2 (10 mL). The reaction mixture is left under magnetic stirring at 20°C for 18 hours. The reaction mixture is extracted with a 10% w/v solution of NaOH (3 x 20 mL) and NaCl saturated solution (20 mL), the organic phase is dried (Na_2SO_4), and the solvent is removed under low pressure. The crude compound is purified by flash chromatography on silica gel column using a PE/EtOAc 7/3 mixture as eluent. Compound **INF44** is obtained as a colourless oil (0.092 g; yield 68%). CI-MS (isobutane) m/z: 367-369 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.54–7.11 (m, 9H, ArH); 5.49 (d, J = 2.8 Hz, 1H, C*H*OH); 4.19 (q, J = 7.1 Hz, 2H, CH_2CH_3); 4.14–4.00 (m, 1H, C*H*COOEt); 3.78 (s, 1H, OH); 3.41–3.16 (m, 2H, CH_2SO); 1.18 (t, J = 7.0 Hz, 3H, CH_2CH_3). Cytotoxicity (MTT assay): IC_{50} > 100 µM.

Example 9 – Synthesis of ethyl 3-(2-chlorophenyl)-3-hydroxy-2-((phenylsulphonyl)methyl)propanoate (INF45)



75% mCPBA acid (0.211 g; 0.918 mmol) is added to a solution of **INF38s** (0.103 g; 0.306 mmol) in CH_2Cl_2 (10 mL). The reaction mixture is left under magnetic stirring at 20°C for 18 hours. The reaction mixture is extracted with a 10% w/v solution of NaOH (3 x 20 mL) and with NaCl saturated solution (20 mL). The organic phase is dried (Na_2SO_4), and the solvent is removed under low pressure. The crude compound is purified by flash chromatography on silica gel column using a PE/EtOAc 7/3 mixture as eluent. Compound **INF45** is obtained as a white solid (0.098 g; yield: 82%). CI-MS (isobutane) m/z: 383-385 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.70-7.02 (m, 9H, Ar*H*); 5.30 (d, J = 3.8 Hz, 1H, C*H*OH); 4.03 (q, *J* = 7.1 Hz, 2H, CH_2CH$_3$); 3.75 (dt, *J* = 10.2; 4.2 Hz, 1H, C*H*COOEt); 3.32-3.06 (m, 2H, CH_2SO$_2$); 1.17 (t, 3H, *J* = 7.1 Hz, CH$_2$CH_3); ^{13}C-NMR ($CDCl_3$): δ, 172.0; 138.6; 137.32; 137.29; 134.1; 134.0; 131.9; 130.5; 130.24; 130.20; 129.8; 129.6; 128.6, 128.3; 127.4; 70.9; 62.2; 52.7; 45.2; 45.2; 14.3. Mp: 78.8-83.1 °C. Cytotoxicity (MTT assay): IC_{50} > 100 μM.

Example 10 – Synthesis of ethyl (Z)-3-(2-chlorophenyl)-2-((phenylthio)methyl)acrylate (INF42)

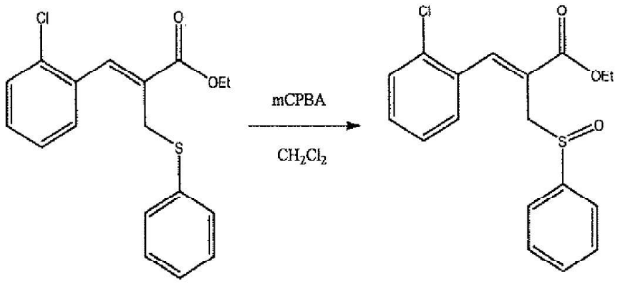


INF42

Thiophenol (0.240 mL; 2.33 mmol) and triethylamine (0.355 mL; 2.55 mmol) are added to a solution of **4a** (0.60 g; 2.12 mmol) in CH_2Cl_2 (7.5 mL) maintained in an inert atmosphere (N_2). The reaction is left under vigorous stirring for 30 minutes at 20°C. The reaction mixture is then diluted with H_2O (5 mL) and extracted with 1N HCl (3 x 20 mL)

and NaCl saturated solution (20 mL). The organic phase is dried (Na_2SO_4), and the solvent is removed under low pressure. The residue is purified by flash chromatography on silica gel column, eluting with pure PE and then with a PE/EtOAc 95/5 mixture. Two fractions are obtained, the first containing the mixture of the two isomers (E/Z) and the second containing the pure Z isomer (**INF42**; 0.260 g; yield: 37%). CI-MS (isobutane) m/z: 333-335 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.80 (s, 1H, C=C*H*); 7.34-7.14 (m, 9H, Ar*H*); 4.29 (q, J = 7.1 Hz, 2H, CH_2CH$_3$); 3.91 (s, 2H, CH_2S); 1.33 (t, J = 7.1 Hz, 3H, CH$_2$CH_3). Cytotoxicity (MTT assay): IC_{50} > 100 μM.

Example 11 – Synthesis of (Z)-ethyl 3-(2-chlorophenyl)-2-((phenylsulphinyl)methyl)acrylate (INF50)

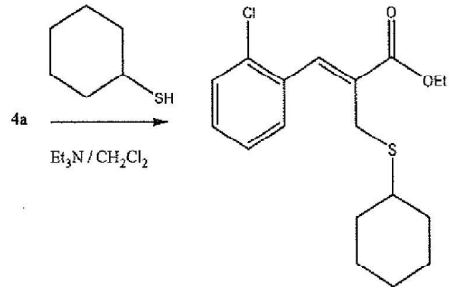


INF42 INF50

75% mCPBA (0.105 g; 0.449 mmol) is added to a solution of **INF42** (0.150 g; 0.449 mmol) in CH_2Cl_2 (10 mL), and the reaction mixture is left under magnetic stirring at 20°C for 18 hours. The reaction mixture is extracted with a 10% w/v solution of NaOH (3 x 20 mL) and NaCl saturated solution (20 mL), then dried (Na_2SO_4), and the solvent is removed under low pressure. The crude compound is purified by flash chromatography on silica gel column using a PE/EtOAc 9/1 mixture as eluent. Compound **INF50** is obtained as a colourless oil (0.156 g; yield: 88%). CI-MS (isobutane) m/z: 349-351 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ, 8.08 (s, 1H, C=C*H*); 7.82–7.18 (m, 9H, Ar*H*); 4.36–4.11 (m, 2H, CH_2CH$_3$); 3.91 (m, 2H, CH_2SO); 1.30 (t, *J* = 7.1 Hz, 3H, CH$_2$CH_3); ^{13}C-NMR ($CDCl_3$): δ, 166.6;

144.2; 143.4; 134.4; 132.9; 131.7; 131.3; 130.9; 129.9; 129.6; 127.3; 124.8; 124.5; 62.1; 57.3; 14.6. Cytotoxicity (MTT assay): IC_{50} 9.7 ± 0.2 μM.

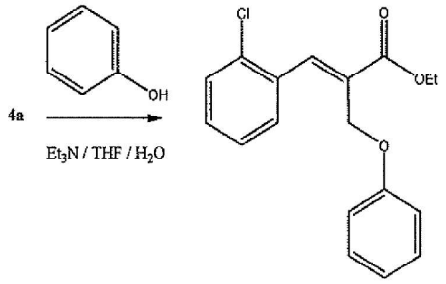
Example 12 – Synthesis of (Z)-ethyl 3-(2-chlorophenyl)-2-((cyclohexylthio)methyl)acrylate (INF56)



INF56

Triethylamine (0.037 mL; 0.265 mmol) and cyclohexylmercaptan (0.027 mL; 0.230 mmol) are added to a solution of **4a** (0.050 g; 0.177 mmol) in DMF (5 mL), and the mixture is left under vigorous stirring at 60°C under an inert atmosphere (N_2) for 18 hours. The reaction is treated with H_2O (30 mL) and EtOAc (50 mL), the phases are separated, and the organic phase is further washed with H_2O (3 x 60 mL). The organic phase is dried (Na_2SO_4) and the solvent is evaporated under low pressure. The residue is purified by flash chromatography on silica gel column using a PE/EtOAc 98/2 mixture as eluent. The compound **INF56** (0.0234 g; yield 39%) is obtained as a pale yellow oil which solidifies with time. CI-MS (isobutane) m/z: 339-341 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.76 (s, 1H, C=C*H*); 7.61–7.14 (m, 4H, Ar*H*); 4.32 (q, J = 7.0 Hz, 2H, CH_2CH_3); 3.53 (s, 2H, CH_2S); 1.86-1.47 (m, 5H, *H* cyclohexyl); 1.37 (t, J = 7.1 Hz, 3H, CH_2CH_3); ^{13}C-NMR ($CDCl_3$): δ, 167.4; 136.9; 134.6; 134.2; 132.4; 130.9; 130.2; 130.0; 127.1; 61.7; 44.2; 33.8; 26.9; 26.5; 26.2; 14.7. Mp: 49.4-53.2 °C. Cytotoxicity (MTT assay): IC_{50} > 100 μM.

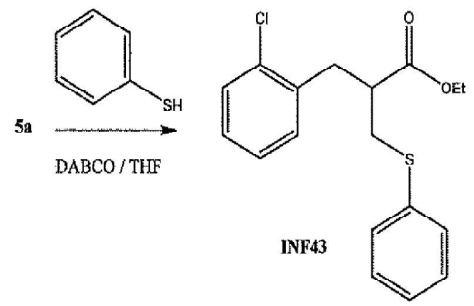
Example 13 – Synthesis of (E)-ethyl 3-(2-chlorophenyl)-2-(phenoxymethyl)acrylate (57)



INF57

K_2CO_3 (0.137 g; 0.991 mmol) and phenol (0.080 g; 0.851 mmol) are added to a solution of **5a** (0.200 g; 0.707 mmol) in THF/H_2O 1/3 (20 mL). The reaction mixture is left under vigorous stirring at 80°C for 18 hours. After 18 hours, a 2% w/v solution of NaOH (10 mL) is added and the mixture is extracted with EtOAc (3 x 25 mL), dried (Na_2SO_4), and the solvent removed under low pressure. The crude compound is purified by flash chromatography on silica gel column using a PE/EtOAc 9/1 mixture as eluent. **INF57** is thus obtained as a white amorphous semisolid (0.104 g; yield: 48%). CI-MS (isobutane) m/z: 316-318 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.88 (s, 1H, C=C*H*); 7.44-7.02 (m, 9H, Ar*H*); 4.61 (s, 2H, CH_2O); 4.32 (q, J = 7.1 Hz, 2H, CH_2CH$_3$); 1.37 (t, J = 7.1 Hz, 3H, CH$_2$CH_3). Cytotoxicity (MTT assay): IC_{50} > 100 μM.

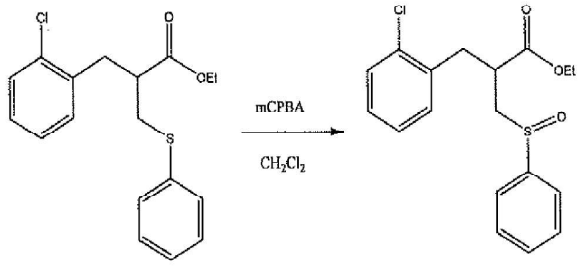
Example 14 – Synthesis of ethyl 2-(2-chlorobenzyl)-3-(phenylthio)propanoate (INF43)



INF43

DABCO (0.326 g; 2.91 mmol) and thiophenol (0.358 mL; 3.49 mmol) are added to a solution of **5a** (0.350 g; 1.45 mmol) in THF (15 mL) in an inert atmosphere (N_2), and the reaction is left under magnetic stirring for 4 hours. The mixture is diluted with CH_2Cl_2 (15 mL) and extracted with 1N HCl (3 x 30 mL) and NaCl saturated solution (30 mL), dried (Na_2SO_4), and the solvent removed under low pressure. The residue is purified by flash chromatography on silica gel column using a PE/EtOAc 98/2 mixture as eluent. Compound **INF43** is obtained as a colourless oil (0.247 g; yield: 50%). CI-MS (isobutane) m/z: 335-337 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.44-7.07 (m, 9H, Ar*H*); 4.03 (q, J = 7.1 Hz, 2H, CH_2CH_3); 3.37-2.92 (m, 5H, *H* aliphatic); 1.12 (t, J = 7.1 Hz, 3H, CH_2CH_3). ^{13}C-NMR ($CDCl_3$): δ, 174.0; 136.6; 136.0; 134.6; 131,7; 130.2; 130.0; 129.4; 128.6; 127.2; 126.8; 61.1; 45.9; 36.1, 35.9; 14.5. Cytotoxicity (MTT assay): IC_{50} > 100 μM.

Example 15 – Synthesis of ethyl 2-(2-chlorobenzyl)-3-(phenylsulphinyl)propanoate (INF48)

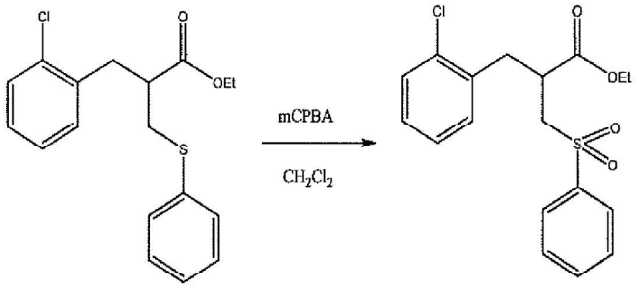


INF43 INF48

75% mCPBA (0.067 g; 0.300 mmol) is added to a solution of **INF43** (0.100 g; 0.300 mmol) in CH_2Cl_2 (10 mL), and the reaction mixture is left under magnetic stirring at 20°C for 18 hours. The solution is extracted with 1% w/v NaOH (3 x 20 mL) and NaCl saturated solution (20 mL), dried (Na_2SO_4), and the solvent evaporated under low pressure. The crude compound is purified by flash chromatography on silica gel column using a PE/EtOAc 8/2 mixture as eluent. Compound **INF48** is obtained as a colourless oil (0.087 g; yield: 83%). CI-MS (isobutane) m/z: 351-353 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.66–7.12

(m, 9H, Ar*H*); 4.29 (q, J = 7.1 Hz, 2H, CH_2CH$_3$); 3.34-2.81 (m, 5H, *H* aliph); 1.33 (t, J = 7.1 Hz, 3H, CH$_2$CH_3). ^{13}C-NMR (CDCl$_3$): δ, 173.2; 144.2; 143.6; 134.6; 131.4; 130.1; 130.1; 129.7; 129.6; 128.8; 127.3; 124.6; 124.3; 61.6; 58.6; 40.3; 36.1; 14.4. Cytotoxicity (MTT assay): IC_{50} > 35.1 ± 10.1 µM.

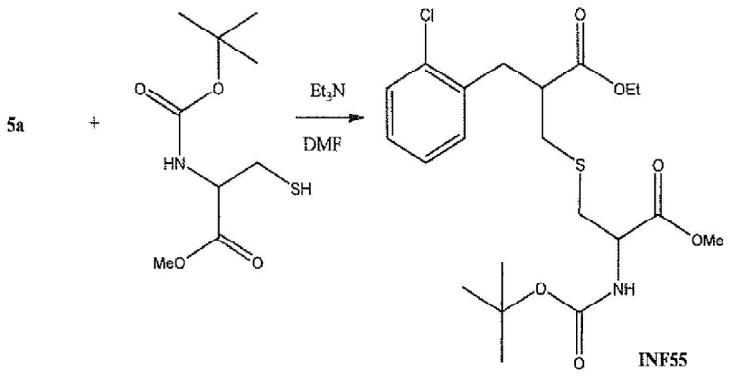
Example 16 – Synthesis of ethyl 2-(2-chlorobenzyl)-3-(phenylsulphonyl)-propanoate (INF49)



INF43 INF49

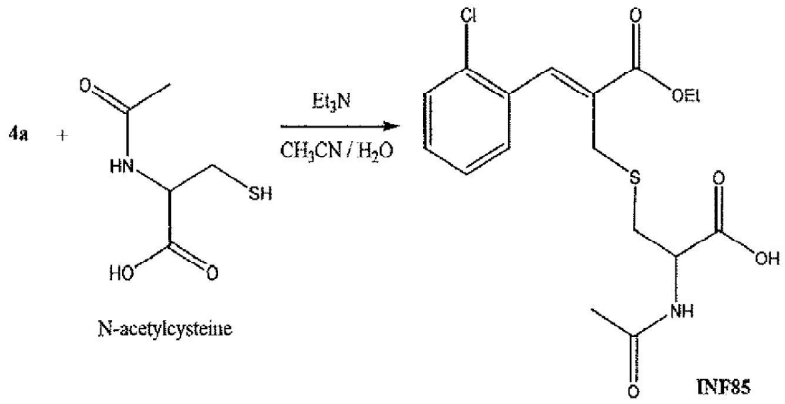
75% mCPBA (0.207 g; 0.898 mmol) is added to a solution of **INF43** (0.100 g; 0.300 mmol) in CH_2Cl_2 (10 mL), and the reaction is left under magnetic stirring at 20°C for 18 hours. The solution is diluted with water (10 mL) and extracted with a 10% w/v solution of $NaHCO_3$ (3 x 20 mL) and NaCl saturated solution (20 mL), dried (Na_2SO_4) and the solvent removed under low pressure. The crude compound is purified by flash chromatography on silica gel column using a PE/EtOAc 8/2 mixture as eluent. Compound **INF49** is obtained as a colourless oil (0.078 g; yield: 72%). CI-MS (isobutane) m/z: 367-369 $[M+1]^+$; ^{1}H-NMR (CDCl$_3$): δ, 7.95–6.98 (m, 9H, Ar*H*); 3.94 (q, *J* = 7.1 Hz, 2H, CH_2CH$_3$); 3.75 (dd, *J* = 14.2; 9.8 Hz, 1H, Ph-C*H*); 3.27 (ddd, *J* = 17.7; 7.9; 2.7 Hz, 1H, C*H*COOEt); 3.14 (dd, *J* = 14.3; 2.8 Hz, 1H, Ph-C*H*); 3.08–2.92 (m, 2H, CH_2SO_2); 1.09 (t, *J* = 7.1 Hz, 3H, CH$_2$CH_3); ^{13}C-NMR (CDCl$_3$): δ, 172.3; 138.7; 134.5; 134.2; 133.8; 131.1; 129.8; 129.2; 128.6; 128.2; 126.9: 61.3; 56.7; 40.2; 36.1; 13.9. Cytotoxicity (MTT assay): IC_{50} > 100 µM.

Example 17 – Synthesis of ethyl 3-((2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)thio)-2-(2-chlorobenzyl)propanoate (INF55)



5a (0.053 g; 0.236 mmol) is added to a solution of methyl (*tert*-butoxycarbonyl)cysteinate (0.072 g; 0.07 mmol) and triethylamine (0.0427 mL; 0.307 mmol) in DMF (3 mL), maintained under an inert atmosphere (N_2). The reaction is placed at 60°C for 18 hours. The reaction mixture is diluted with 0.1N HCl (10 mL), then extracted with EtOAc (3 x 10 mL), dried (Na_2SO_4), and the solvent removed under low pressure. The crude compound is purified by flash chromatography on silica gel column using a PE/EtOAc 9/1 mixture as eluent; compound **INF55** is thus obtained (0.0454 g; yield: 42%). CI-MS (isobutane) m/z: 459-461 $[M+1]^+$; ^{1}H-NMR (DMSO): δ, 7.44–7.06 (m, 4H, Ar*H*); 5.38 (s, 1H, N*H*); 4.52 (s, 1H, C*H*NH); 4.08 (q, J = 7.0 Hz, 2H, CH_2CH_3); 3.74 (s, 3H, $COOCH_3$); 3.16–2.58 (m, 7H, *H* aliph); 1.45 (s, 9H, $C(CH_3)_3$); 1.14 (t, J = 7.1 Hz, 3H, CH_2CH_3); ^{13}C-NMR ($CDCl_3$): δ, 174.0; 171.8; 136.5; 134.6; 131.6; 130.0; 128.6; 127.1; 77.9; 77.4; 77.0; 61.2; 53.6; 53.0; 46.2; 46.0; 36.0; 35.6; 34.8; 28.6; 14.5. Cytotoxicity (MTT assay): IC_{50} > 100 μM.

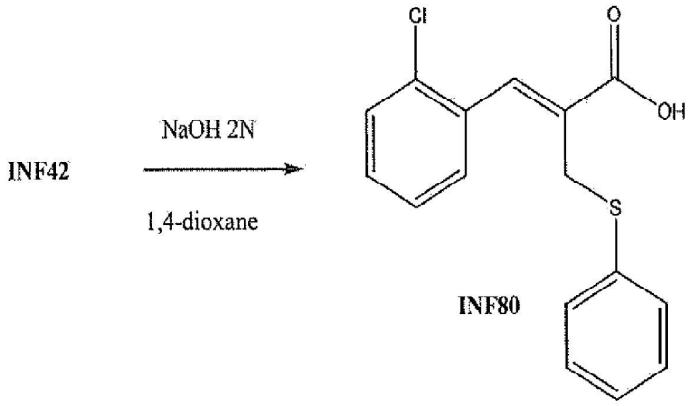
Example 18 – Synthesis of (S,Z)-2-acetamido-4-((3-(2-chlorophenyl)-2-(ethoxycarbonyl)allyl)thio)butanoic acid (INF85)



Triethylamine (0.190 mL; 1.85 mmol) and N-acetylcysteine (0.302 mg; 1.85 mmol) are added to a solution of **4a** (0.209 g; 0.740 mmol) in CH_3CN/H_2O 2/1 (6 mL), maintained under an inert atmosphere (N_2), and the reaction mixture is left under stirring at 20°C for 16 hours. 0.1N NaOH (10 mL) is added to the reaction mixture, which is extracted with EtOAc (25 mL). The aqueous phase is acidified with 2N HCl and extracted with EtOAc (3 x 30 mL). The organic phases are washed with a NaCl saturated solution (25 mL), dried (Na_2SO_4), and evaporated under low pressure. The crude compound is purified by flash chromatography on silica gel column, eluting with CH_2Cl_2/EtOAc 1/1 (+0.1% HCOOH) to provide **INF85** as a colourless oil (0.099 g; yield: 35%). Negative ESI/MS m/z: 384-386 $[M-H]^-$; ^1H NMR ($CDCl_3$): δ, 8.38 (br, 1H, COOH); 7.83 (s, 1H, C=CH); 7.57-7.20 (m, 4H, ArH); 7.02 (d, *J*= 7.3 Hz, NH); 4.99-4.67 (m, 1H, CH); 4.33 (q, *J*= 7.1 Hz, CH_2CH_3); 3.57 (q, J= 12.1 Hz, CH_2S); 3.17-2.87 (m, 2H, $CHCH_2S$); 2.07 (s, 3H, CH_3); 1.37 (t, *J*=7.1 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 173.3; 172.5; 167.5, 138.7; 134.5; 133.5; 130.9; 130.6; 130.1; 127.4; 62.1; 52.5; 34.8; 30.1; 26.6; 22.7; 14.2. Cytotoxicity (MTT assay): IC_{50} > 100 μM.

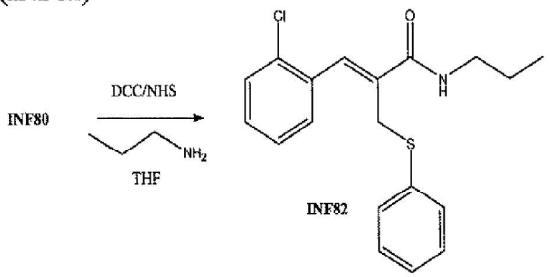
Example 19 – Synthesis of (Z)-3-(2-chlorophenyl)-2-

((phenylthio)methyl)acrylic acid (INF80)



Compound **INF42** (0.828 g; 2.49 mmol) is dissolved in 1,4-dioxane (10 mL), and 2N NaOH (10 mL) is added. The reaction mixture is placed under stirring at 20°C for 16 hours, and then acidified to pH=1 with 2N HCl (10 mL) and extracted with EtOAc (3 x 50 mL). The organic phases are washed with a NaCl saturated solution, dried (Na_2SO_4), and evaporated under low pressure to obtain compound **INF80** as a cream-coloured solid (0.759 g; yield: 88%). Mp: 97.6-99.9°C; Negative ESI/MS m/z: 303-305 $[M-H]^-$; ^1H NMR ($CDCl_3$): δ, 12.36 (br, 1H, COOH); 7.98 (s, 1H, C=CH); 7.39-7.20 (m, 9H, ArH); 3.93 (s, 2H, CH_2S); ^{13}C NMR ($CDCl_3$): δ 172.6; 140.2; 135.3; 134.4; 133.0; 131.2; 130.3; 130.1; 129.7; 129.6; 128.9; 127.0; 126.7; 32.0. Cytotoxicity (MTT assay): IC_{50} 92.8 ± 1.6 μM.

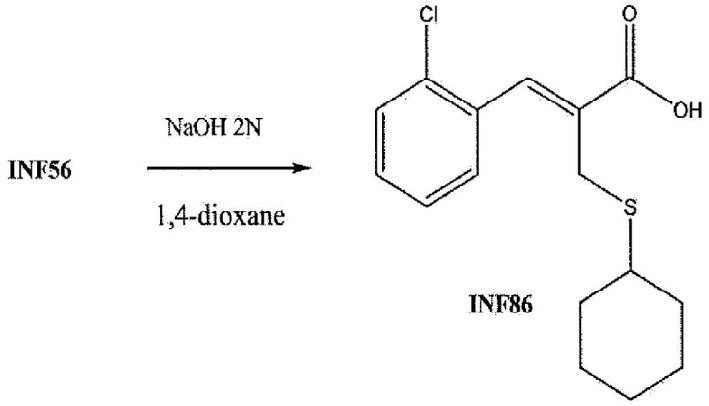
Example 20 – Synthesis of (Z)-3-(2-chlorophenyl)-2-((phenylthio)methyl)-N-propylacrylamide (INF82)



Compound **INF80** (0.222 g; 0.731 mmol) is dissolved in THF (5 mL), and DCC (0.151 g; 0.731 mmol) and NHS (0.0841 g; 0.731 mmol) are added to the resulting solution,

maintained at 0°C. The mixture is placed under magnetic stirring at 0°C for 10 minutes, and then at 20°C for 2 hours. Propylamine (0.120 mL; 1.426 mmol) is then added to the reaction mixture, and the reaction is stirred at 20°C for 16 hours. The resulting suspension is filtered, the filtrate diluted with 2N HCl (10 mL), and extracted with EtOAc (3 x 25 mL). The combined organic phases are washed with a NaCl saturated solution, dried (Na_2SO_4), and evaporated under low pressure. The crude compound is purified by flash chromatography on silica gel column, using CH_2Cl_2/EtOAc 98/2 as eluent to provide **INF82** as a colourless oil (0.131 g; yield: 52%). Positive ESI/MS m/z: 346-348 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.47 (s, 1H, C=CH); 7.39-7.26 (m, 9H, ArH); 6.42 (s, 1H, NH); 3.93 (s, 2H, CH_2S); 3.35 (q, J=6.5 Hz, 2H, CH_2); 1.70-1.52 (m, 2H, CH_2); 0.97 (t, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ, 168.3; 135.3; 134.8; 134.6; 133.9; 133.3; 130.9; 130.5; 130.0; 129.9; 129.4; 127.4; 127.0; 42.1; 32.9; 23.2; 11.9. Cytotoxicity (MTT assay): IC_{50} = 63.3 ± 1.3 µM.

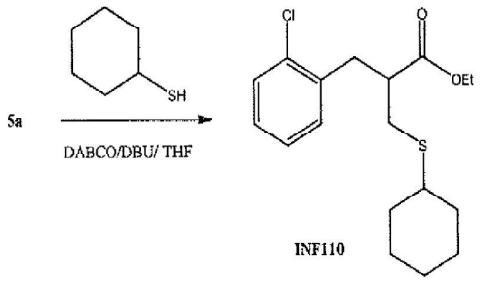
Example 21 – Synthesis of (Z)-3-(2-chlorophenyl)-2-((cyclohexylthio)methyl)acrylic acid (INF86)



A solution of 2N NaOH (3 mL) is added to a solution of compound **INF56** (0.0401 g; 0.118 mmol) in 1,4-dioxane (3 mL), and the reaction mixture is left under stirring at 20°C for 16 hours. The mixture is acidified to pH=1 with 2N HCl (10 mL), and then extracted with EtOAc (3 x 50 mL). The combined organic phases are washed with a NaCl

saturated solution (20 mL), dried (Na_2SO_4), and the solvent is evaporated under low pressure to obtain **INF86** as a colourless oil (0.0367 g; yield: 95%). Negative ESI/MS: 309-311 $[M-H]^-$; ^{1}H-NMR ($CDCl_3$): δ, 10.42 (br, 1H, COOH); 7.97 (s, 1H, C=CH); 7.65-7.28 (m, 4H, ArH); 3.58 (s, 2H, CH_2S); 2.60-2.58 (m, 1H, CH); 1.84-1.21 (m, 10H, cyclohexyl); ^{13}C-NMR ($CDCl_3$): δ, 172.4; 139.0; 134.3; 133.5; 130.9; 130.5; 130.2; 129.7; 126.8; 43.9; 33.3; 26.2; 26.1; 25.8. Cytotoxicity (MTT assay): IC_{50} > 100 μM.

Example 22 – Synthesis of ethyl 2-(2-chlorobenzyl)-3-(cyclohexylthio)propanoate (INF110)

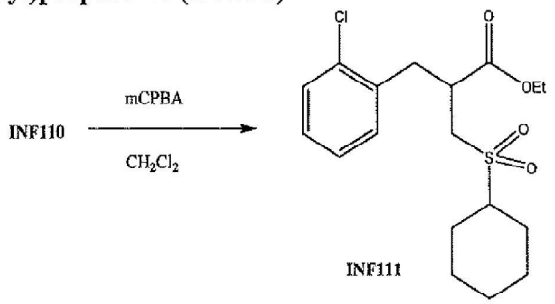


INF110

DABCO (0.628 g; 5.60 mmol), DBU (0.835 mL; 5.60 mmol) and cyclohexanethiol (0.822 mL; 6.72 mmol) are added in succession to a solution of **5a** (0.630 g; 2.80 mmol) in anhydrous THF (20 mL), maintained at 20°C in an inert atmosphere (N_2). The reaction mixture is kept under stirring at 20°C for 4 hours. The mixture is diluted with CH_2Cl_2 (15 mL) and treated with 1N HCl (25 mL), and the two phases are separated. The aqueous phase is further extracted with CH_2Cl_2 (3 x 15 mL), and the combined organic phases are washed with a NaCl saturated solution (30 mL), dried (Na_2SO_4), and evaporated under low pressure. The resulting crude compound is purified by flash chromatography on silica gel column using PE/EtOAC 97/3 as eluent, followed by PE/EtOAC 95/5, to provide **INF110** as a colourless oil (0.247 g; yield: 51%). MS/ESI m/z: 341-343 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.40 – 6.98 (m, 4H, ArH); 4.00 (q, 1H, J = 7.1 Hz, CH_2); 3.17 – 2.85 (m, 3H); 2.83 – 2.47 (m, 3H); 1.85 (dd, 2H, J = 9.6; 8.3 Hz); 1.68 (dd, 2H, J = 9.1; 6.7 Hz); 1.53-1.43 (m,

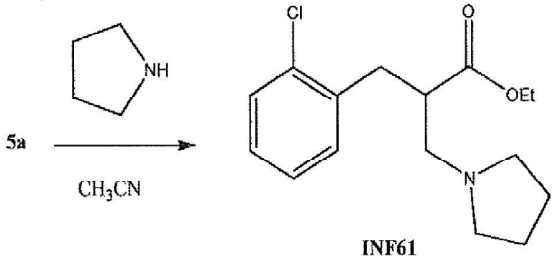
1H); 1.36 – 1.15 (m, 5H); 1.06 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$): δ, 174.4; 136.8; 134.5; 131.7; 129.9; 128.5; 127.1; 61.0; 46.5; 44.2; 36.2; 34.0; 32.1; 26.4; 26.2; 14.5. Cytotoxicity (MTT assay): IC_{50} 66.0 ± 1.4 μM.

Example 23 – Synthesis of ethyl 2-(2-chlorobenzyl)-3-(cyclohexylsulphonyl)propanoate (INF111)



75% mCPBA (0.890 g; 3.87 mmol) is added to a solution of compound **INF110** (0.438 g; 1.29 mmol) in CH_2Cl_2 (15 mL), and the reaction mixture is left under stirring at 20°C for 16 hours. The mixture is diluted with CH_2Cl_2 (15 mL), and extracted with a 10% solution of $NaHCO_3$ (3 x 15 mL). The organic phase is washed with a NaCl saturated solution (20 mL) and dried (Na_2SO_4), and the solvent is evaporated under low pressure. The resulting crude compound is purified by flash chromatography on silica gel column using PE/EtOAC 8/2 as eluent, to provide **INF111** as a colourless oil (0.409 g; yield: 85%). MS/ESI m/z: 373-375 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.38 – 7.21 (m, 1H); 7.17 – 7.03 (m, 3H); 4.12 – 3.96 (m, 2H, CH_2); 3.55 – 3.24 (m, 2H); 3.15 – 2.81 (m, 3H); 2.79 – 2.58 (m, 1H); 2.18 – 1.57 (m, 6H); 1.47 – 1.13 (m, 4H); 1.05 (t, 3H, J = 7.2 Hz, CH_3). ^{13}C-NMR ($CDCl_3$): δ, 173.2; 135.3; 134.7. 131.7; 130.2; 129.1; 127.4; 61.9; 61.8; 50.4; 39.8; 36.4; 25.4; 25.3; 25.2; 14.3. Cytotoxicity (MTT assay): IC_{50} 98.1 ± 4.8 μM.

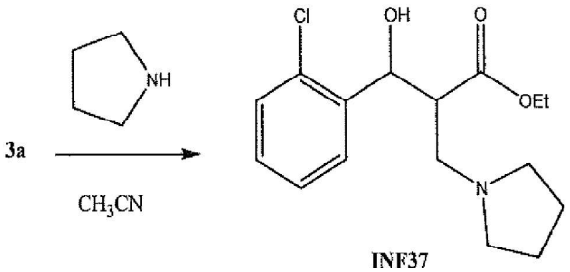
Example 24 – Synthesis of ethyl 2-(2-chlorobenzyl)-3-(pyrrolidine-1-yl)propanoate (INF61)



INF61

Pyrrolidine is added to a solution of **5a** (0.838 g; 3.730 mmol) in CH_3CN (10 mL), and the reaction mixture is left under stirring at 20°C for 72 hours. The solvent is evaporated under low pressure and the crude residue obtained is purified by flash chromatography on silica gel column, eluting with CH_2Cl_2/MeOH 98/2 to provide **INF61** as a pale yellow oil (0.836 g; yield: 80%). CI-MS (isobutane) m/z: 296-298 $[M+1]^+$; ^{1}H-NMR (DMSO-D6): δ, 7.32-7.10 (m, 4H, Ar-H); 4.05-3.98 (m, 2H, OCH_2CH_3); 3.12-2.81 (m, 5H, CH_2CHCH_2N); 2.58-2.49 (m, 4H, Pyr-H); 1.72 (m, 4H, Pyr-H); 1.05 (t, 3H, J = 7.2 Hz, CH_3). ^{13}C-NMR ($CDCl_3$): δ, 175.1; 137.4; 131.6; 129.8; 128.2; 127.0; 60.6; 58.6; 54.5; 45.9; 35.0; 30.1; 24.0; 14.5. Cytotoxicity (MTT assay): IC_{50} > 100 μM.

Example 25 – Synthesis of ethyl 3-(2-chlorophenyl)-3-hydroxy-2-(pyrrolidin-1-ylmethyl)propanoate (INF37)



INF37

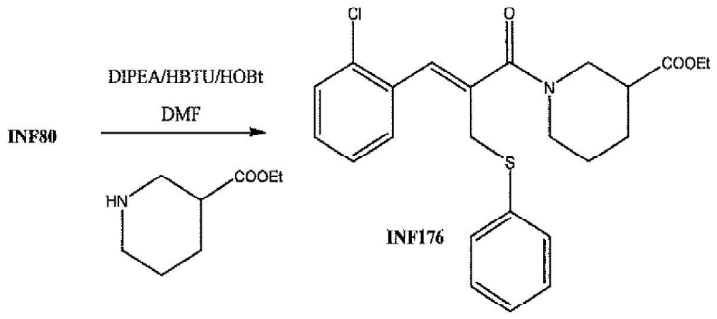
Pyrrolidine (0.103 g; 1.45 mmol) is added to a solution of **3a** (0.264 g; 1.10 mmol) in CH_3CN (2 mL), and the reaction mixture is left under stirring at 20°C for 24 hours. The solvent is evaporated under low pressure and the crude residue is taken up with CH_2Cl_2 (25

mL), washed with H_2O (2 x 20 mL) and dried (Na_2SO_4). The resulting oily residue is purified by flash chromatography on silica gel column, eluting with CH_2Cl_2/MeOH 99/1 (+ 0.1% Et_3N) to provide **INF37** as a pale green oil (0.246 g; yield: 72%). Two fractions are obtained, identified as:

INF37 (syn). CI-MS (isobutane) m/z: 312-314 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.63 (d, J = 7.4 Hz, 1H, Ar-H); 7.40-7.17 (m, 3H, Ar-H); 5.57 (d, J = 13.2 Hz, C*H*OH); 3.96 (m, 2H, OC*H*$_2$CH$_3$); 3.36-3.29 (m, 1H, C*H*CH$_2$N); 3.05-2.98 (m, 1H, CHC*H*HN); 2.82-2.61 (m, 5H, CHCH*H*N, 4 Pyr-H); 1.80 (m, 4H, Pyr-H); 1.01 (t, 3H, J = 7.1 Hz, CH$_3$). ^{13}C-NMR ($CDCl_3$): δ, 171.4; 139.6, 132.5; 129.3; 128.8; 128.6; 126.9; 73.7; 60.6; 56.9; 51.8; 49.1; 23.4; 13.7. Cytotoxicity (MTT assay): IC_{50} > 100 μM.

INF37 (anti). CI-MS (isobutane) m/z: 312-314 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.50 (d, J = 8.5 Hz, 1H, Ar-H); 7.41-7.00 (m, 3H, Ar-H); 5.42 (d, J = 17.2 Hz, C*H*OH); 3.98 (m, 2H, OC*H*$_2$CH$_3$); 3.41-3.21 (m, 1H, C*H*CH$_2$N); 3.16-3.01 (m, 1H, CHC*H*HN); 3.03-2.86 (m, 1H, CHCH*H*N); 2.83-2.62 (m, 4H, Pyr-H); 1.83 (m, 4H, Pyr-H); 1.03 (t, 3H, J = 7.2 Hz, CH$_3$). ^{13}C-NMR ($CDCl_3$): δ, 172.7; 139.6; 131.7; 129.3; 128.8; 127.9; 126.8; 70.1; 60.7; 55.0; 54.4; 48.6; 23.5; 13.9. Cytotoxicity (MTT assay): IC_{50} > 100 μM.

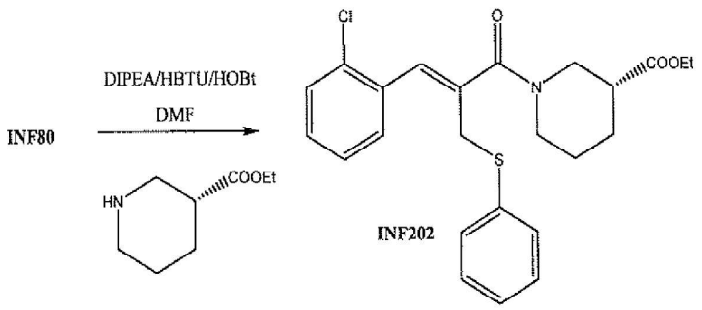
Example 26 – Synthesis of ethyl (Z)-1-(3-(2-chlorophenyl)-2-((phenylthio)methyl)acryloyl)piperidine-3-carboxylate (INF176)



DIPEA (0.111 mL; 0.66 mmol), HOBt (4.43 mg; 0.03 mmol) and HBTU (0.187 g; 0.49 mmol) are added to a solution of **INF80** (0.100 g; 0.33 mmol) in DMF (5 mL), and

ethyl nipecotate (0.050 mL; 0.33 mmol) is added after 30 minutes. The reaction mixture is left to react under magnetic stirring for 18 hours at 20°C. The mixture is diluted with 15 mL of diethyl ether and the organic phase is washed with 1N HCl (3 x 15 mL) and NaCl saturated solution (15 mL), and dried (Na_2SO_4). The solvent is evaporated under low pressure, and the resulting residue is purified by flash chromatography on silica gel column, eluting with PE/EtOAc 9/1 followed by PE/EtOAc 8/2. **INF176** is obtained as a pale yellow oil (0.100 g; yield: 69%). The purity (HPLC) is 96%; eluent CH_3CN/H_2O + 0.1% CF_3COOH, 70/30; flow rate 1.0 mL/min; t_R= 8.558. Rf= 0.75. (PE/EtOAc/MeOH 7.5:2:0.5); MS (ESI) m/z: 444-446 $[M+H]^+$; ^{1}H-NMR (DMSO-D6, 80 °C): δ, 7.47–7.46 (m, 1H, Ar-*H*); 7.35-7.33 (m, 3H, Ar-*H*); 7.16-7.08 (m, 5H, Ar-*H*); 6.61 (s, 1H, C=C*H*); 4.20-4.18 (m, 1H, C*H*-pip); 4.04 (q, J= 7.1 Hz; 2H, O-CH_2); 3.91-3.86 (m, 2H, S-CH_2); 3.08-2.93 (m, 2H, N-CH_{2pip}); 2.44 (m, 2H, CH_{2pip}); 1.96-1.94 (m, 1H, CH_{2pip}); 1.66–1.57 (m, 2H, CH_{2pip}); 1.41-1.39 (m, 1H, CH_{2pip}); 1.23 (t, J= 7.1 Hz; 3H, CH_3). ^{13}C-NMR ($CDCl_3$): δ, 172.6; 169.6; 135.3; 134.4; 133.4; 130.5; 129.65; 129.58; 129.2; 129.0; 128.5; 128.4, 126.8; 126.0; 60.7; 48.2*; 43.9; 41.3; 31.8; 27.7*; 24.8*; 14.3. *peaks doubled due to the presence of rotational isomers (rotamers). Cytotoxicity (MTT assay): IC_{50} 94.2 ± 6.9 μM.

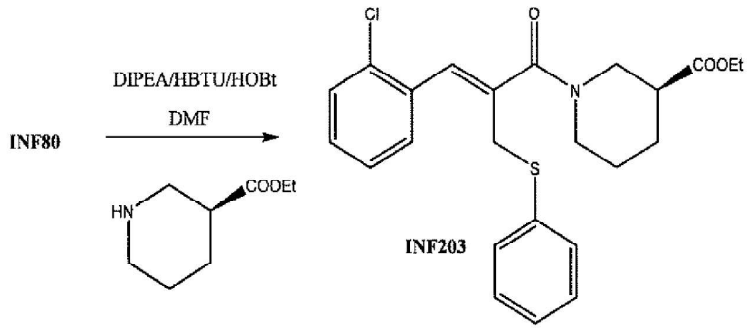
Example 27 – Synthesis of ethyl (R,Z)-1-(3-(2-chlorophenyl)-2-((phenylthio)methyl)acryloyl)piperidine-3-carboxylic acid (INF202)



Compound **INF202** is obtained by following the same procedure as described in

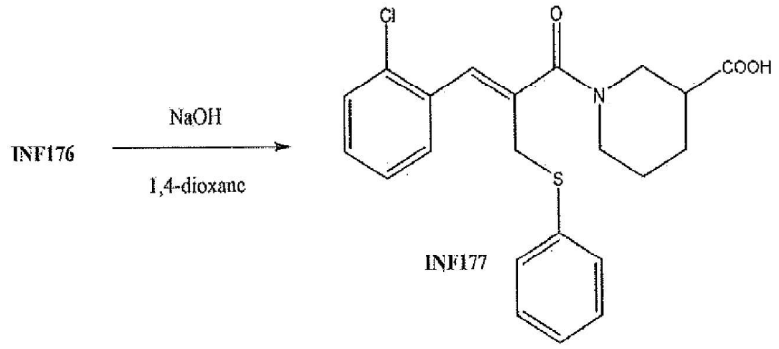
Example 26 for **INF176,** using **INF80** (0.08 g; 0.262 mmol), DIPEA (0.088 mL; 0.525 mmol), HOBt (4.00 mg; 0.03 mmol), HBTU (0.149 g; 0.393 mmol) and (R)-ethyl nipecotate (0.044 mL; 0.288 mmol). **INF202** is obtained (0.100 g; yield: 88%). ESI/MS m/z: 444-446 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.41–7.38 (m, 1H, Ar-*H*); 7.31 (m, 1H, Ar-*H*); 7.26 (m, 2H, Ar-*H*); 7.15–7.06 (m, 5H, Ar-*H*); 6.58 (s, 1H, C=C*H*); 4.49 (m, 2H, O-CH_2); 3.92 (dd, *J*=23.9; 7.3 Hz, 2H, N-CH_2); 3.08–2.93 (m, 2H, S-CH_2); 2.61 (m, 2H, N-CH_{2pip}); 2.41 (dd, *J*=49.7; 24.3 Hz, 1H, C*H*); 1.80–1.66 (m, 2H, CH_{2pip}); 1.63–1.40 (m, 2H, CH_{2pip}); 1.23 (m, 3H).

Example 28 – Synthesis of ethyl (S,Z)-1-(3-(2-chlorophenyl)-2-((phenylthio)methyl)acryloyl)piperidine-3-carboxylate (INF203)



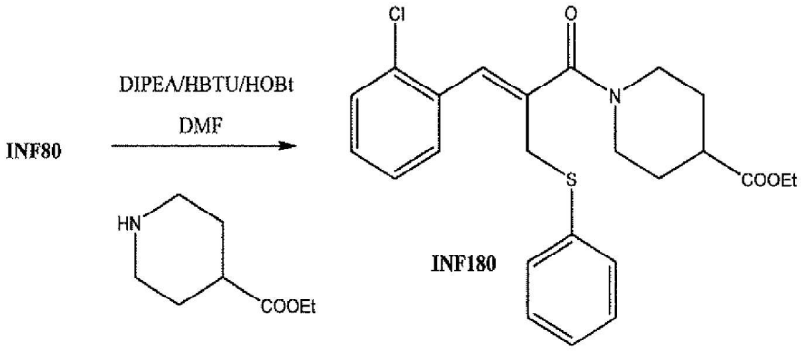
Compound **INF203** is obtained by following the same procedure as described in Example 25 for INF176, using INF80 (0.08 g; 0.262 mmol), DIPEA (0.088 mL; 0.525 mmol), HOBt (4.00 mg; 0.03 mmol), HBTU (0.149 g; 0.393 mmol) and (S)-ethyl nipecotate (0.044 mL; 0.288 mmol). **INF203** is obtained (0.086 g; yield: 74%). MS (ESI) m/z: 444-446 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.41–7.38 (m, 1H, Ar-*H*); 7.31 (m, 1H, Ar-*H*); 7.26 (m, 2H, Ar-*H*); 7.15–7.06 (m, 5H, Ar-*H*); 6.58 (s, 1H, C=C*H*); 4.49 (m, 2H, O-CH_2); 3.92 (dd, *J*=23.9; 7.3 Hz, 2H, N-CH_2); 3.08–2.93 (m, 2H, S-CH_2); 2.61 (m, 2H, N-CH_{2pip}); 2.41 (dd, *J*=49.7; 24.3 Hz, 1H, C*H*); 1.80–1.66 (m, 2H, CH_{2pip}); 1.63–1.40 (m, 2H, CH_{2pip}); 1.23 (m, 3H).

Example 29 – Synthesis of (Z)-1-(3-(2-chlorophenyl)-2-((phenylthio)methyl)acryloyl)piperidine-3-carboxylic acid (INF177)



2.5M NaOH (0.500 mL) is added to a solution of **INF176** (0.047 g; 0.110 mmol) in dioxane (1 mL), and the reaction is laced under stirring at 20°C for 18 hours. When said time has elapsed, 1N HCl (5 mL) and H_2O (5 mL) are added, and the reaction mixture is extracted with CH_2Cl_2 (3 x 15 mL) and NaCl saturated solution (15 mL), and dried (Na_2SO_4). The solvent is evaporated under low pressure, and the crude compound is purified by flash chromatography on silica gel column, eluting with CH_2Cl_2/MeOH 95/5. **INF177** is obtained as a rubbery solid (0.042 g; yield: 96%). Purity (HPLC): 98% eluent CH_3CN/H_2O + 0.1% CF_3COOH, 60/40; flow rate 1.0 mL/min; t_R= 7.303. Rf= 0.2 (DCM/MeOH 95/5); Negative ESI/MS m/z: 414-416 $[M-H]^-$; ^{1}H-NMR (CD_3OD): δ, 7.70 (s, 1H, Ar*H*); 7.47 (d, *J*=12.1 Hz, 2H, Ar*H*); 7.34 (d, *J*=12.4 Hz, 2H, Ar*H*); 7.28–7.17 (m, 4H, Ar*H*); 7.09 (s, 1H, C=C*H*); 4.07 (s, 1H, C*H*); 3.68 (s, 2H, S-CH_2); 3.62 (t, *J*=5.9 Hz, 3H, Pip-*H*); 3.41-3.15 (m, 2H, Pip-*H*); 2.29-1.87 (m, 3H, Pip-*H*). ^{13}C-NMR (CD_3OD): δ, 178.0; 170.2; 134.0; 133.2; 131.8; 130.4; 129.5; 129.4; 129.3; 129.1; 128.9; 128.6; 126.7; 125.9; 39.4; 31.8; 31.6; 29.6; 27.1; 22.6. Cytotoxicity (MTT assay): IC_{50} > 100 µM.

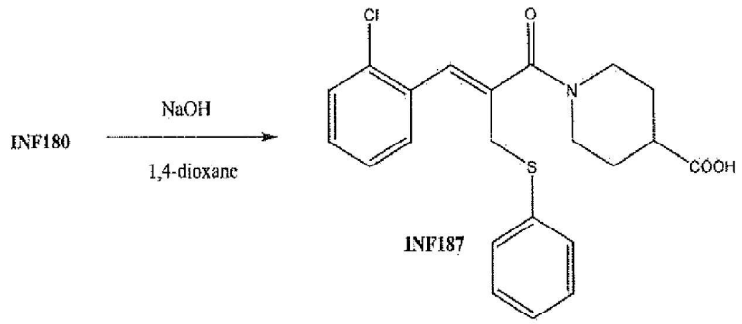
Example 30 – Synthesis of ethyl (Z)-1-(3-(2-chlorophenyl)-2-((phenylthio)methyl)acryloyl)piperidine-4-carboxylate (INF180)



DIPEA (0.322 mL; 1.90 mmol), HOBt (0.013 g; 0.095 mmol) and HBTU (0.539 g; 1.42 mmol) are added to a solution of **INF80** (0.289 g; 0.95 mmol) in DMF (15 mL), and ethyl isonipecotate (0.146 mL; 0.95 mmol) is added after 30 minutes. The reaction mixture is left to react under magnetic stirring for 16 hours at 20°C. The reaction mixture is diluted with 15 mL of diethyl ether, and the organic phase is washed with 1N HCl (3 x 15 mL) and NaCl saturated solution (15 mL), dried (Na_2SO_4), and the solvent evaporated under low pressure. The resulting crude compound is purified by flash chromatography on silica gel column, eluting with PE/EtOAc 8/2. **INF180** is obtained as a rubbery solid (0.218 g; yield: 52%). Purity (HPLC): 97%; eluent CH_3CN/H_2O + 0.1% CF_3COOH, 70/30; flow rate 1.0 mL/min; t_R= 7.947. Rf= 0.7 (PE/EtOAc/MeOH 7:2:1); MS (ESI) m/z: 444-446 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.41–7.38 (m, 1H, Ar-*H*); 7.36–7.33 (m, 1H, Ar-*H*); 7.28 (d, *J*=3.5 Hz, 1H, Ar-*H*); 7.26 (d, *J*=12.3 Hz, 1H, Ar-*H*); 7.13 (t, *J*=7.2 Hz, 2H, Ar-*H*); 7.10–7.07 (m, 1H, Ar-*H*); 7.05 (d, *J*=7.9 Hz, 2H, Ar-*H*); 6.59 (s, 1H, C=C*H*); 4.16–4.08 (m, 3H, H_{pip}); 4.03 (s, 2H, H_{pip}); 3.00 (s, 2H, S-CH_2), 2.55–2.45 (m, 1H, C*H*); 1.99–1.85 (m, 2H, H_{pip}); 1.80 (s, 1H, H_{pip}); 1.67 (s, 2H, H_{pip}); 1.25 (dd, *J*=9.5; 4.7 Hz, 3H, CH_3). ^{13}C-NMR ($CDCl_3$): δ, 174.3; 169.9; 135.4; 134.41; 134.36; 133.50; 130.58; 129.7; 129.6; 129.0; 128.6; 128.5; 126.9; 126.2; 60.7; 47.0; 41.1; 32, 8; 27. 9; 14.3. Cytotoxicity (MTT assay): IC_{50} > 100

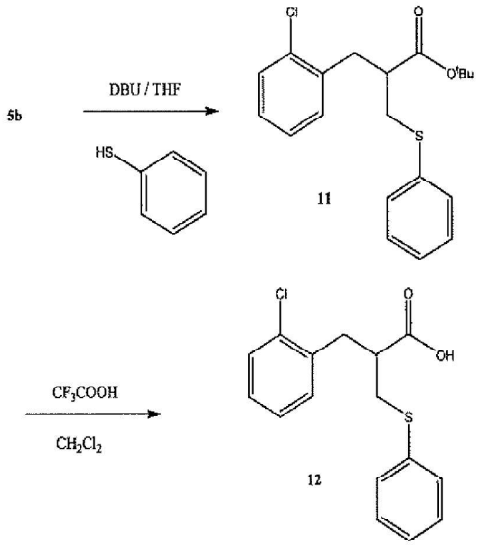
μM.

Example 31 – Synthesis of (Z)-1-(3-(2-chlorophenyl)-2-((phenylthio)methyl)acryloyl)piperidine-4-carboxylic acid (INF187)



2.5M NaOH (1.14 mL) is added to a solution of **INF180** (0.107 g; 0.24 mmol) in dioxane (2 mL), and the reaction is conducted at 20°C for 18 hours under stirring. The reaction mixture is diluted with 1N HCl (5 mL) and H_2O (5 mL), and extracted with CH_2Cl_2 (3 x 15 mL) and NaCl saturated solution (15 mL), dried (Na_2SO_4), and the solvent evaporated under low pressure. The resulting crude compound is purified by flash chromatography on silica gel column, eluting with CH_2Cl_2/MeOH 95/5. **INF187** is obtained as a pale yellow oil (0.062 g; yield: 62%). Purity (HPLC): 98.6%; eluent CH_3CN/H_2O + 0.1% CF_3COOH, 60/40; flow rate 1.0 mL/min; t_R= 6.416. Rf= 0.3 (DCM/MeOH 95/5); Negative ESI/MS m/z: 414-416 $[M-H]^-$; ^{1}H-NMR (CD_3OD): δ, 8.81 (t, *J*=8.5 Hz, 1H, Ar*H*); 8.74–8.69 (m, 3H, Ar*H*); 8.51–8.44 (m, 3H, Ar*H*); 8.42–8.32 (m, 2H, Ar*H*); 7.97 (s, 1H, C=C*H*); 5.69 (d, *J*=10.7 Hz, 1H, C*H*); 5.35 (d, *J*=13.1 Hz, 3H, Pip-*H*); 4.64 (s, 2H, S-CH_2); 4.33–3.62 (m, 1H, Pip-*H*); 3.35–2.87 (m, 4H, Pip-*H*). ^{13}C-NMR (CD_3OD): δ, 176.8; 170.3; 134.9; 134.2; 134.0; 133.2; 130.4; 129.7; 129.4; 129.3; 128.8; 128.6; 126.8; 126.0; 41.3; 40.6; 30.9; 27.7. Cytotoxicity (MTT assay): IC_{50} > 100 μM.

Example 32 – Synthesis of 2-(2-chlorobenzyl)-3-(phenylthio) propanoic acid (12)

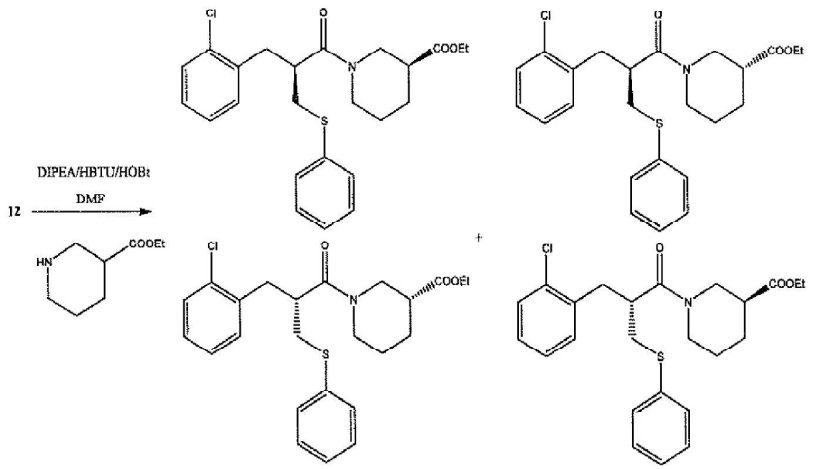


DBU (1.21 mL; 8.07 mmol) and thiophenol (0.988 mL; 9.69 mmol) are added to a solution of **5b** (1.02 g; 4.04 mmol) in THF (42 mL), maintained under an inert atmosphere (N_2). The reaction mixture is left under magnetic stirring for 4 hours at 20°C. The solvent is evaporated under low pressure, the resulting residue is taken up with CH_2Cl_2 (10 mL), and the organic phase is washed with 1N HCl (3 x 20mL) and NaCl saturated solution (15 mL), dried (Na_2SO_4), and the solvent evaporated under low pressure. The resulting crude compound is purified by flash chromatography on silica gel column, eluting with PE/DCM 8/2. The intermediate *tert*-butyl 2-(2-chlorobenzyl)-3-(phenylthio) propanoate (**11**) is obtained as a colourless oil (1.12 g; yield: 77%). Rf=0.43 (PE/EtOAc 95/5); ESI/MS m/z: 363-365 $[M+H]^+$. Intermediate **11**, characterised by mass spectrometry, is used directly in the next step.

CF_3COOH (6 mL; 78.4 mmol) is added to a solution of **11** (1.08 g; 2.99 mmol) in CH_2Cl_2 (60 mL), and the reaction mixture is left under magnetic stirring at 16°C for 20 hours. The organic phase is washed with H_2O (2 x 15 mL) and NaCl saturated solution (15 mL), and dried (Na_2SO_4). After evaporation of the solvent under low pressure, 2-(2-

chlorobenzyl)-3-(phenylthio) propanoic acid (**12**) is obtained (0.812 g; yield 89%) as pure compound. Negative ESI/MS m/z: 305-307 $[M-H]^-$; ^{1}H-NMR ($CDCl_3$): δ, 8.45 (s, 1H, OH); 6.93–6.90 (m, 1H, Ar-*H*); 6.87 (t, *J*=1.7 Hz, 1H, Ar-*H*), 6.85 (t, *J*=1.6 Hz, 1H, Ar-*H*); 6.83 (d, *J*=1.7 Hz, 1H, Ar-*H*); 6.81 (dd, *J*=8.1; 1.7 Hz, 1H, Ar-*H*); 6.79–6.76 (m, 1H, Ar-*H*); 6.76 (t, *J*=1.7 Hz, 1H, Ar-*H*); 6.75 (s, 1H, Ar-*H*); 6.75–6.72 (m, 1H, Ar-*H*); 2.81–2.76 (m, 1H, C*H*); 2.69 (dd, *J*=9.2; 4.6 Hz, 2H, CH_2); 2.68–2.64 (m, 2H, CH_2). ^{13}C-NMR ($CDCl_3$): δ, 179.5; 135.8; 135.3; 134.37; 131.41; 130.2; 129.9; 129.1; 128.5; 127.0; 126.8; 45.4; 35.4; 35.2.

Example 33 – Synthesis of ethyl 1-(2-(2-chlorobenzyl)-3-(phenylthio)propanoyl)piperidine-3-carboxylate (INF184 and INF185)



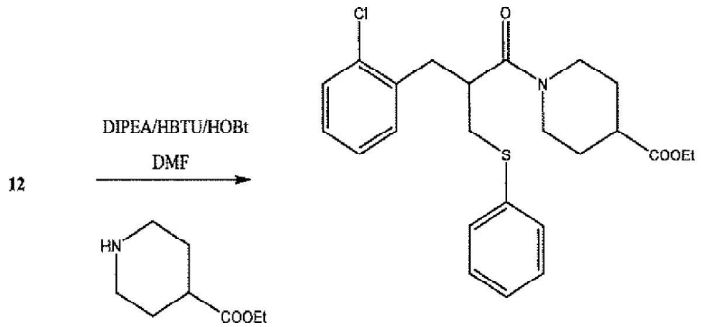
DIPEA (0.163 mL; 0.96 mmol), HOBt (6.47 mg; 0.05 mmol) and HBTU (0.273 g; 0.72 mmol) are added to a solution of **12** (0.146 g; 0.48 mmol) in DMF (7.3 mL). Ethyl nipecotate (0.075 mL; 0.48 mmol) is added after 30 minutes. The reaction mixture is left under magnetic stirring for 18 hours at 20°C. The mixture is diluted with 15 mL of diethyl ether, and the organic phase is washed with 1N HCl (3 x 15 mL) and NaCl saturated solution (15 mL), and dried (Na_2SO_4). The solvent is evaporated under low pressure, and the crude compound is purified by flash chromatography on silica gel column, eluting with

PE/EtOAc 8.5/1.5. A mixture of **INF184** and **INF185** is obtained as a yellow oil (68 mg; yield: 32%).

The mixture of isomers is isolated in two different aliquots; each of which is more concentrated than one of the two diastereomers. The 1st aliquot consists of 8% isomer **INF184** and 92% **INF185**. The 2nd aliquot consists of 86% isomer **INF184** and 14% **INF185**; eluent CH_3CN/H_2O + 0.1% CF_3COOH, 65/35; flow rate 1.0 mL/min. The retention times are 17.440 minutes (**INF184**) and 18.405 minutes (**INF185**) respectively.

INF184 + **INF185** mixture of isomers: ^{1}H-NMR ($CDCl_3$): δ, 7.33–7.22 (m, 7H, Ar*H*); 7.22–7.09 (m, 11H, Ar*H*); 4.58 (m, 2H, C*H*$_2$); 4.14–3.96 (m, 4H, C*H*$_2$); 3.64 (d, *J*=13.5 Hz, 2H, C*H*$_2$); 3.46 (m, 2H, C*H*$_2$); 3.32 (m, 2H, C*H*); 3.17–3.01 (m, 4H, C*H*$_2$); 3.02–2.90 (m, 2H, C*H*); 2.67–2.55 (m, 2H, C*H*$_2$); 2.41–2.26 (m, 2H, C*H*$_2$); 2.22–2.14 (m, 2H, C*H*$_2$); 2.07–1.90 (m, 2H, C*H*$_2$); 1.78–1.59 (m, 2H, C*H*$_2$); 1.47–1.28 (m, 4H, C*H*$_2$); 1.25–1.18 (m, 6H, C*H*$_3$). ^{13}C-NMR ($CDCl_3$): δ, 173.41; 172.94, 172.37; 172.22; 136.85; 136.47; 136.15; 136.10; 134;35; 134.24; 132.47; 132.00; 129.87; 129.83; 129.34; 129.27; 128.68; 128.64; 127.25; 127.17; 126.9; 126.44; 126.11; 60.94; 60.83; 47.50; 46.41; 44.22; 42.83; 42.33; 41.64; 40.18; 40.13; 37.73; 37.59; 36.62; 35.34; 27.87; 27.51; 24.88; 24.78; 14.50; 14.47. Cytotoxicity (MTT assay): IC_{50} 75.4 ± 2.6 μM.

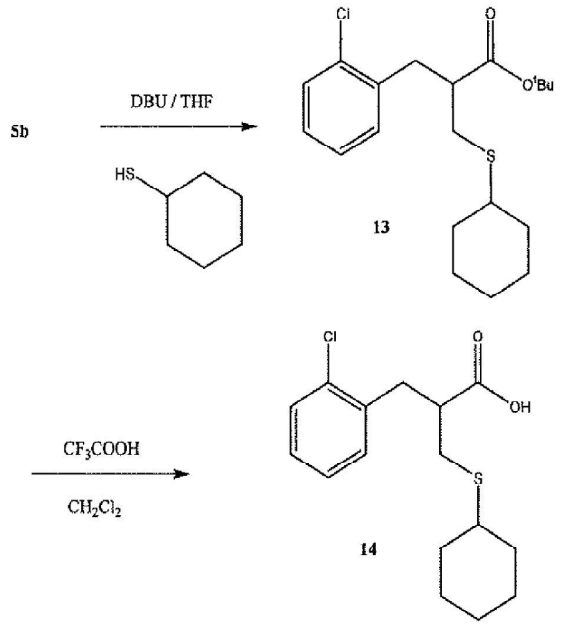
Example 34 – Synthesis of ethyl 1-(2-(2-chlorobenzyl)-3-(phenylthio)propanoyl)piperidine-4-carboxylate (INF186)



INF186

DIPEA (0.157 mL; 0.92 mmol), HOBt (6.21 mg; 0.05 mmol) and HBTU (0.261 g; 0.69 mmol) are added to a solution of **12** (0.140 g; 0.46 mmol) in DMF (7.00 mL). After 30 minutes under stirring at 20°C, ethyl isonipecotate (0.070 mL; 0.46 mmol) is added, and the mixture is left under stirring at 20°C for 18 hours. The mixture is diluted with diethyl ether (15 mL), and the organic phase is washed with 1N HCl (3 x 15 mL) and NaCl saturated solution (15 mL), and dried (Na_2SO_4). The solvent is evaporated under low pressure, and the crude residue is purified by flash chromatography on silica gel column, eluting with DCM/EtOAc 98/2); **INF186** is thus obtained as a pale yellow oil (0.126 g; yield: 62%). Purity (HPLC) >99%; eluent CH_3CN/H_2O + 0.1% CF_3COOH, 60/40; flow rate 1.0 mL/min; t_R= 16.019. Rf= 0.23 (DCM/EtOAc 98/2); MS (ESI) m/z: 446-448 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.86–6.66 (m, 9H, Ar*H*); 4.44–4.00 (m, 2H, CH_2); 3.59–3.25 (m, 2H, CH_2); 3.30–2.91 (m, 2H, CH_2); 2.88–2.44 (m, 1H, C*H*); 2.25 (dd, J = 12.2; 9.6 Hz, 1H, C*H*); 2.16 (s, 6H, CH_2); 1.77–1.30 (m, 2H, CH_2); 1.26–1.08 (m, 3H, CH_3). ^{13}C-NMR ($CDCl_3$): δ, 174.0; 171.9; 135.9; 132.2; 132.0; 129.7; 129.1; 129.0; 128.9; 127.2; 127.0; 126.2; 60.7; 45.3; 41.6; 39.9; 37.4; 36.2; 27.9; 14.3. Cytotoxicity (MTT assay): IC_{50} 94.6 ± 4.0 μM.

Example 35 – Synthesis of 2-(2-chlorobenzyl)-3-(cyclohexylthio) propanoic acid (14)

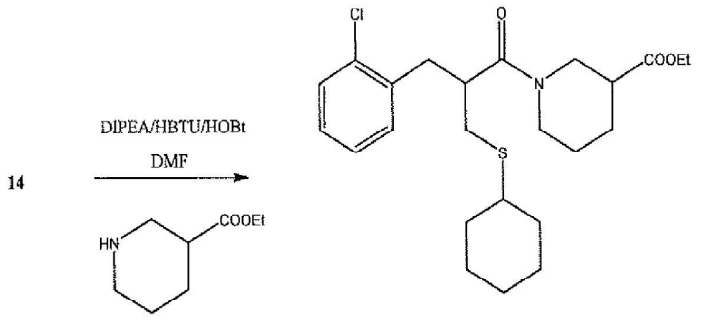


DBU (2.18 mL; 14.7 mmol) and cyclohexanethiol (1.16 mL; 11.8 mmol) are added to a solution of **5b** (1.06 g; 4.19 mmol) in CH_2Cl_2 (11 mL), maintained under an inert atmosphere (N_2); the reaction mixture is left under magnetic stirring at 20°C for 18 hours. The reaction mixture is treated with H_2O (15 mL) and CH_2Cl_2 (15 mL), and the phases are separated. The organic phase is washed with 1N HCl (3 x 15 mL) and NaCl saturated solution (15 mL), and dried (Na_2SO_4). The solvent is evaporated under low pressure, and the crude residue is purified by flash chromatography on silica gel column, eluting with PE/DCM 8/2. The intermediate *tert*-butyl 2-(2-chlorobenzyl)-3-(cyclohexylthio) propanoate is thus obtained (**13**; 0.544 g; yield: 35%). Rf= 0.6 (PE/DCM 7/3); MS (ESI) m/z: 369-371 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.33 (d, *J*= 3.6 Hz, 1H, Ar*H*); 7.26–7.20 (m, 1H, Ar*H*); 7.14 (t, *J*= 7.3 Hz, 2H, Ar*H*); 3.08–3.01 (m, 1H, CH_2-C*H*-CH_2); 2.97–2.86 (m, 2H, CH_2); 2.79 (dd, *J*= 12.5; 8.0 Hz, 1H, S-C*H*); 2.69–2.58 (m, 2H, CH_2); 1.91 (d, *J*= 12.9 Hz, 2H,CH_2); 1.73 (s, 2H, CH_2); 1.59 (d, *J*= 10.6 Hz, 2H); 1.33 (s, 9H, CH_3); 1.30–1.20 (m, 4H, cyclohexyl-*H*). ^{13}C-NMR ($CDCl_3$): δ, 173.6; 137.0; 134.5; 131.8; 129.9; 128.3;

126.9; 81.2; 47.0; 44.1; 36.3; 33.9; 32.4; 28.3; 26.4; 26.2.

CF_3COOH (3 mL; 39.6 mmol) is added to a solution of **13** (0.522 g; 1.42 mmol) in CH_2Cl_2 (30 mL), and the reaction mixture is left under magnetic stirring at 20°C for 16 hours. The organic phase is washed with 1N HCl (2 x 15 mL) and NaCl saturated solution (15 mL), and dried (Na_2SO_4). The solvent is evaporated under low pressure and the crude residue is purified by flash chromatography on silica gel column, eluting with PE/EtOAc 7/3) to obtain **14** as a yellow oil (0.348 g; yield 79%). Negative ESI/MS m/z: 311-313 [M-H]$^-$; ^{1}H-NMR ($CDCl_3$): δ, 8.52 (s, 1H, OH); 7.34 (dd, *J*= 5.5; 3.6 Hz, 1H, Ar-*H*); 7.26–7.21 (m, 1H, Ar-*H*); 7.17 (dd, *J*= 5.6; 3.5 Hz, 2H, Ar-*H*); 3.14–3.06 (m, 2H CH-CH_2); 3.03 (dd, *J*= 13.4; 6.2 Hz, 1H, C*H*); 2.82 (dd, *J*= 13.2; 7.6 Hz, 1H, CH-CH_b); 2.71 (dd, *J*= 13.1; 4.7 Hz, 1H CH-CH_a); 2.63 (s, 1H, S-C*H*); 1.94–1.81 (m, 2H, cyclohexyl-*H*); 1.73 (s, 2H, cyclohexyl-*H*); 1.58 (d, *J*= 10.3 Hz, 2H, cyclohexyl-*H*); 1.31-1.16 (m, 4H, cyclohexyl-*H*). ^{13}C-NMR ($CDCl_3$): δ, 179.7; 136.2; 134.3; 131.5; 129.8; 128.4; 127.0; 46.0; 44.0; 35.4; 33.6; 31.3; 26.2; 25.9.

Example 36 – Synthesis of ethyl 1-(2-(2-chlorobenzyl)-3-(cyclohexylthio)propanoyl)piperidine-3-carboxylate (INF192)

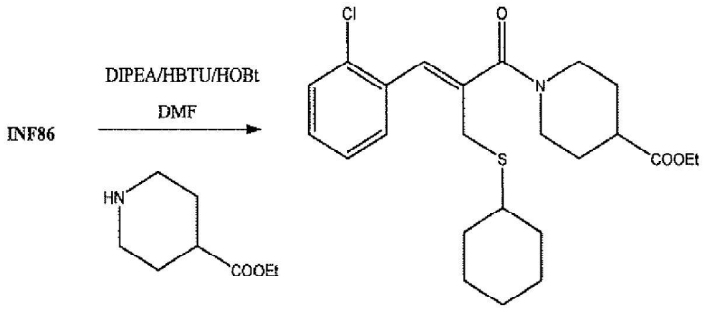


INF192

DIPEA (0.359 mL; 2.11 mmol), HOBt (0.014 g; 0.11 mmol) and HBTU (0.599 g; 1.58 mmol) are added to a solution of **14** (0.330 g; 1.06 mmol) in DMF (16 mL). After 30 minutes under stirring at 20°C, ethyl nipecotate (0.164 mL; 1.06 mmol) is added, and the

mixture is left under stirring at 20°C for 18 hours. The mixture is diluted with diethyl ether (15 mL), and the organic phase is washed with 1N HCl (3 x 15 mL) and NaCl saturated solution (15 mL), and dried (Na_2SO_4). The solvent is evaporated under low pressure and the crude residue is purified by flash chromatography on silica gel column, eluting with PE/EtOAc 9/1), followed by PE/EtOAc 8/2; **INF192** is thus obtained as a pale yellow oil (0.279 g; yield: 59%). Purity (HPLC) >99%; eluent CH_3CN/H_2O + 0.1% CF_3COOH, 80/20; flow rate 1.0 mL/min; t_R=13.237. Rf= 0.33 (PE/EtOAc 8/2); MS (ESI) m/z: 452-454 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.32 (t, *J*= 6.8 Hz, 1H, Ar*H*); 7.23 (d, *J*= 32.3 Hz, 1H, Ar*H*); 7.15 (d, *J*= 3.0 Hz, 2H, Ar*H*); 4.77 4.36 (m, 1H, C*H*); 4.11 (s, 2H, CH_2); 4.02–3.59 (m, 1H, C*H*); 3.41 (t, *J*= 37.6 Hz, 1H, C*H*); 3.24–2.82 (m, 4H, CH_2); 2.76–2.27 (m, 5H, CH_2); 2.18–1.99 (m, 1H); 1.90 (dd, *J*= 46.7; 33.2 Hz, 2H, CH_2); 1.84–1.64 (m, 3H, CH_2); 1.57 (t, *J*= 25.2 Hz, 2H, CH_2); 1.53–1.38 (m, 2H, CH_2); 1.30–1.27 (m, 3H, CH_2); 1.25 (d, *J*= 6.8 Hz, 3H, CH_3). ^{13}C-NMR ($CDCl_3$): δ, 173.2; 172.4; 136.9; 134.2; 132.3; 131.8; 128.3; 126.9; 60.8; 47.9; 46.4; 44.5; 41.5; 37.7; 33.9; 33.6; 33.4; 27.2; 26.2; 25.9; 24.3; 14.3. Cytotoxicity (MTT assay): IC_{50} 59.8 ± 0.7 μM.

Example 37 – Synthesis of ethyl (Z)-1-(3-(2-chlorophenyl)-2-((cyclohexylthio)methyl)acryloyl)piperidine-4-carboxylate (INF188)

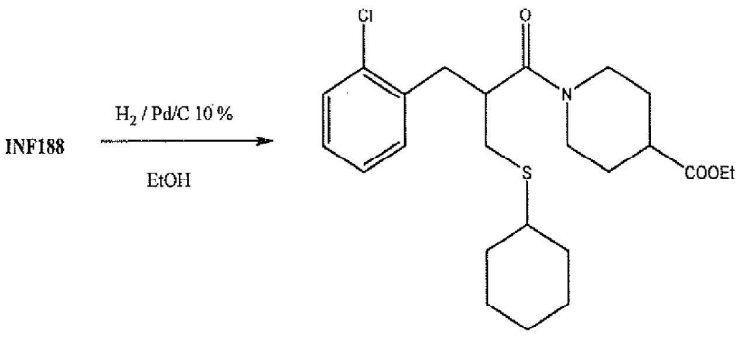


INF188

DIPEA (0.213 mL; 1.26 mmol), HOBt (8.48 mg; 0.06 mmol) and HBTU (0.357 g; 0.94 mmol) are added to a solution of **INF86** (0.195 g; 0.63 mmol) in DMF (9.5 mL), and

after 30 minutes under stirring at 20°C, ethyl isonipecotate (0.096 mL; 0.63 mmol) is added. The reaction mixture is left to react under magnetic stirring for 18 hours at 20°C. The mixture is diluted with diethyl ether (15 mL), and the organic phase is washed with 1N HCl (3 x 15 mL) and NaCl saturated solution (15 mL), and dried (Na_2SO_4). The solvent is evaporated under low pressure and the crude residue is purified by flash chromatography on silica gel column, eluting with PE/EtOAc 8.5/1.5); **INF188** is thus obtained as a pale yellow oil (0.199 g; yield 70%). Purity (HPLC): 96%; eluent CH_3CN/H_2O + 0.1% CF_3COOH, 70/30; flow rate 1.0 mL/min; t_R=17.767. Rf= 0.5 (PE/EtOAc/MeOH 8/1.5/0.5); MS (ESI) m/z: 450-452 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.42–7.38 (m, 3H, Ar-*H*); 7.38–7.34 (m, 3H, Ar-*H*); 7.29 (dd, *J*= 7.4; 1.1 Hz, 1H, Ar-*H*); 7.19 (td, *J*= 7.7; 1.6 Hz, 1H, Ar-*H*); 6;56 (s, 2H, C=C*H*); 5.56–5.06 (m, 3H, C*H*$_2$); 4.16 (q, *J*= 7.1 Hz, 5H, C*H*$_2$); 3.58 (s, 4H, S-C*H*$_2$); 3.20 (m, 2H, C*H*$_{pip}$); 2.59 (m, 4H, C*H*$_2$); 2.50–2.44 (m, 4H, C*H*$_2$); 2.00 (d, *J*= 11.6 Hz, 4H, C*H*$_2$); 1.91 (m, 6H, C*H*$_3$); 1.67 (s, 4H, C*H*$_2$); 1.54 (m, 4H, C*H*$_2$); 1.25 (m, 14H, C*H*$_2$). Cytotoxicity (MTT assay): IC_{50} 58.6 ± 5.3 μM.

Example 38 – Synthesis of ethyl 1-(2-(2-chlorobenzyl)-3-(cyclohexylthio)propanoyl)piperidine-4-carboxylate (INF193)



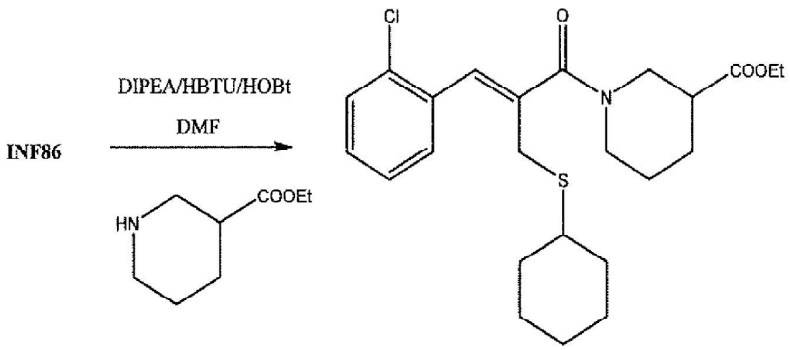
INF193

10% Pd/C (2.37 mg) is added to a solution of **INF188** (0.100 g; 0.22 mmol) in absolute EtOH (2.6 mL), and the mixture is placed under H_2 atmosphere at the pressure of

1 bar. The reaction mixture is left under stirring at 20°C for 18 hours. The catalyst is filtered through celite, and the filtrate is evaporated under low pressure to obtain **INF193** as a yellow oil (0.090 g; yield 90%).

Purity (HPLC): 91%; eluent CH_3CN/H_2O + 0.1% CF_3COOH, 70/30; flow rate 1.0 mL/min; t_R=18.238. Rf= 0.2 (PE/EtOAc 9/1); MS (ESI) m/z: 452-454 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.34–7.29 (m, 2H, Ar*H*); 7.22–7.17 (m, 1H, Ar*H*); 7.17–7.14 (m, 2H, Ar*H*); 7.14 (dd, J= 3.0; 0.8 Hz, 2H, Ar*H*); 7.12–7.10 (m, 1H, Ar*H*); 4.76–4.35 (m, 2H, C*H*$_2$); 4.20–4.04 (m, 4H, C*H*$_2$); 4.01–3.59 (m, 2H, C*H*); 3.52–3.28 (m, 2H, C*H*); 3.20–2.99 (m, 2H, C*H*); 2.99–2.80 (m, 5H, C*H*$_2$); 2.73–2.57 (m, 5H, C*H*$_2$); 2.54–2.28 (m, 3H, C*H*$_2$); 2.15–1.83 (m, 5H, C*H*$_2$); 1.81–1.62 (m, 7H, C*H*$_2$); 1.61–1.37 (m, 7H, C*H*$_2$); 1.27 (dd, *J*= 13.2; 6.1 Hz, 7H, C*H*$_2$); 1.25–1.21 (m, 8H, C*H*$_2$); 1.05–0.95 (m, 1H, C*H*$_2$). Cytotoxicity (MTT assay): IC_{50} 77.5 ± 7.3 μM.

Example 39 – Synthesis of ethyl (Z)-1-(3-(2-chlorophenyl)-2-((cyclohexylthio)methyl)acryloyl)piperidine-3-carboxylate (INF194)

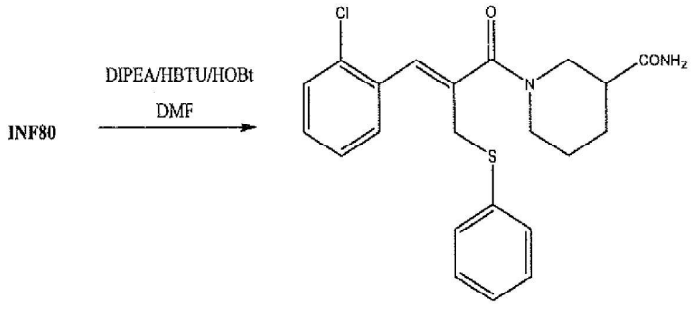


INF194

DIPEA (0.062 mL; 0.36 mmol), HOBt (2.43 mg; 0.02 mmol) and HBTU (0.102 g; 0.27 mmol) are added to a solution of **INF86** (0.056 g; 0.18 mmol) in DMF (3 mL). After 30 minutes' stirring at 20°C, ethyl nipecotate (0.028 mL; 0.18 mmol) is added to the mixture, and the reaction mixture is left under stirring for 16 hours at 20°C. The mixture is diluted with diethyl ether (15 mL), and the organic phase is washed with 1N HCl (3 x 15

mL) and NaCl saturated solution (15 mL), and dried (Na_2SO_4). The solvent is evaporated under low pressure, and the crude residue is purified by flash chromatography on silica gel column, eluting with PE/EtOAc 8/2. **INF194** is thus obtained as a rubbery solid (0.052 g; yield 64%). Purity (HPLC): 89%; eluent CH_3CN/H_2O + 0.1% CF_3COOH, 80/20; flow rate 1.0 mL/min; t_R= 11.690. Rf= 0.5 (PE/EtOAc/MeOH 8/1.5/0.5); MS (ESI) m/z: 450-452 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.41 (dd, *J*=7.6; 1.6 Hz, 1H, Ar-*H*); 7.31–7.25 (m, 3H, Ar-*H*); 6.59 (s, 1H, C=C*H*); 4.65 (s, 2H, CH_2); 4.19 (m, 3H, CH_2); 3.62 (t, *J*= 14.2 Hz, 2H, CH_2); 3.19 (m, 2H, CH_2); 2.64 (m, 2H, CH_2); 2.45 (s, 1H, CH_2); 2.28–2.11 (m, 1H, S-C*H*), 1.88–1.82 (m, 1H, C*H*); 1.61 (d, *J*= 5.5 Hz, 3H, CH_2); 1.52 (d, *J*= 16.4 Hz, 2H, CH_2); 1.26 (t, *J*= 7.0 Hz, 3H, CH_3); 1.12 (m, 5H, CH_2). ^{13}C-NMR ($CDCl_3$): δ, 173.0; 171.4; 134.9; 134.4; 134.0; 131.0; 129.8; 129.7; 127.8; 127.2; 61.1; 47.9; 45.6; 43.0; 41.6; 35.4; 33.5; 27.9; 26.4; 26.0; 22.2; 14.6. Cytotoxicity (MTT assay): IC_{50} 78.3 ± 10.2 μM.

Example 40 – Synthesis of (Z)-1-(3-(2-chlorophenyl)-2-((phenylthio)methyl)acryloyl)piperidine-3-carboxamide (INF219)

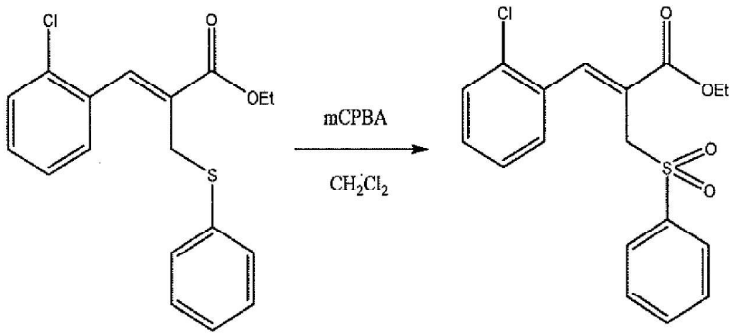


INF219

DIPEA (0.177 mL; 1.065 mmol), HOBt (0.005 g; 0.034 mmol) and HBTU (0.202 g; 0.532 mmol) are added to a solution of **INF80** (0.108 g ; 0.335 mmol) in DMF (3 mL). The mixture is placed under stirring at 20°C for 30 minutes; piperidine-3-carboxamide (0.390 g; 0.390 mmol) is then added, and the mixture is left under stirring at 20°C for 16 hours. The solvent is evaporated under low pressure, and the resulting residue is treated with a 10% w/v

solution of $NaHCO_3$ (15 mL) and extracted with CH_2Cl_2 (3 x 10 mL). The combined organic phases are washed with a NaCl saturated solution (15 mL), dried (Na_2SO_4), and the solvent evaporated under low pressure. The crude compound is purified by flash chromatography on silica gel column, eluting with CH_2Cl_8/EtOAc 8/2 to provide **INF219** (0.110 g; yield 75%) as a white solid. Mp: 60.5 – 61.4 °C; MS (ESI) m/z: 415-417 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$) δ, 7.37 (s, 1H); 7.28-7.24 (m, 3H); 7.09-6.99 (m, 5H); 6.58 (s, 1H); 5.87 (s, 1H); 3.99-3.98 (m, 3H); 3.64-3.10 (m, 2H); 2.60-2.44 (m, 1H); 1.97-1.95 (m, 1H); 1.83-1.67 (m, 1H); 1.46 (d, 2H, J = 48.5 Hz); ^{13}C-NMR ($CDCl_3$) δ, 175.1; 170.1; 135.0; 134.1; 133.8; 133.1; 130.3; 129.5; 129.4; 129.0; 128.8; 128.1; 126.7; 125.9; 48.1; 44.1; 41.7; 31.4; 27.2; 24.6. Cytotoxicity (MTT assay): IC_{50} > 100 μM.

Example 41 – Synthesis of ethyl (Z)-3-(2-chlorophenyl)-2-((phenylsulphonyl)methyl)acrylate (INF51)

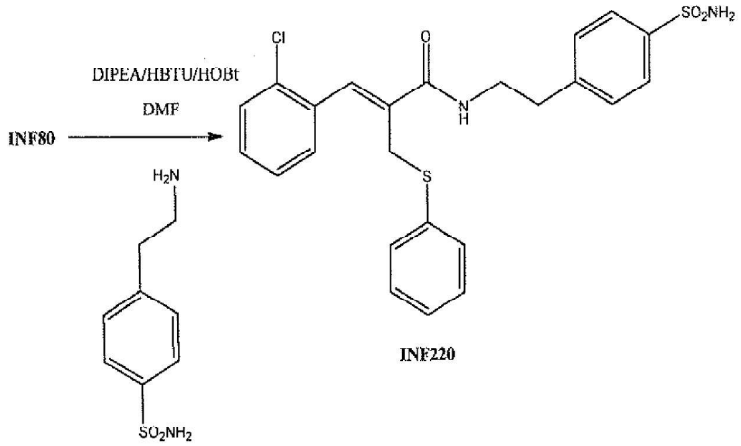


INF42 **INF51**

75% mCPBA (0.301 g; 1.35 mmol) is added to a solution of **INF42** (0.150 g; 0.449 mmol) in CH_2Cl_2 (10 mL), and the reaction mixture is left under magnetic stirring at 20°C for 18 hours. The reaction mixture is extracted with a 10% w/v solution of NaOH (3 x 20 mL) and NaCl saturated solution (20 mL), dried (Na_2SO_4), and the solvent is removed under low pressure. The crude compound is purified by flash chromatography on silica gel column using a PE/EtOAc 8:2 mixture as eluent. Compound **INF51** is obtained as a

colourless oil (0.149 g; yield 91%). CI-MS (isobutane) m/z: 365-367 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ, 8.01 (s, 1H, C=C*H*), 7.89–7.20 (m, 9H, Ar*H*); 4.39 (s, 2H, CH_2SO_2); 4.09 (q, J = 7.1 Hz, 2H, CH_2CH_3); 1.25 (t, J = 7.1 Hz, 3H, CH_2CH_3). ^{13}C-NMR ($CDCl_3$): δ, 166.3; 143.2; 139.7; 134.4; 134.2; 132.7; 131.0; 130.3; 130.1; 129.5; 128.8; 127.4; 123.8; 62.1; 55.2; 14.4. Cytotoxicity (MTT assay): IC_{50} 20.1 ± 14.3 μM.

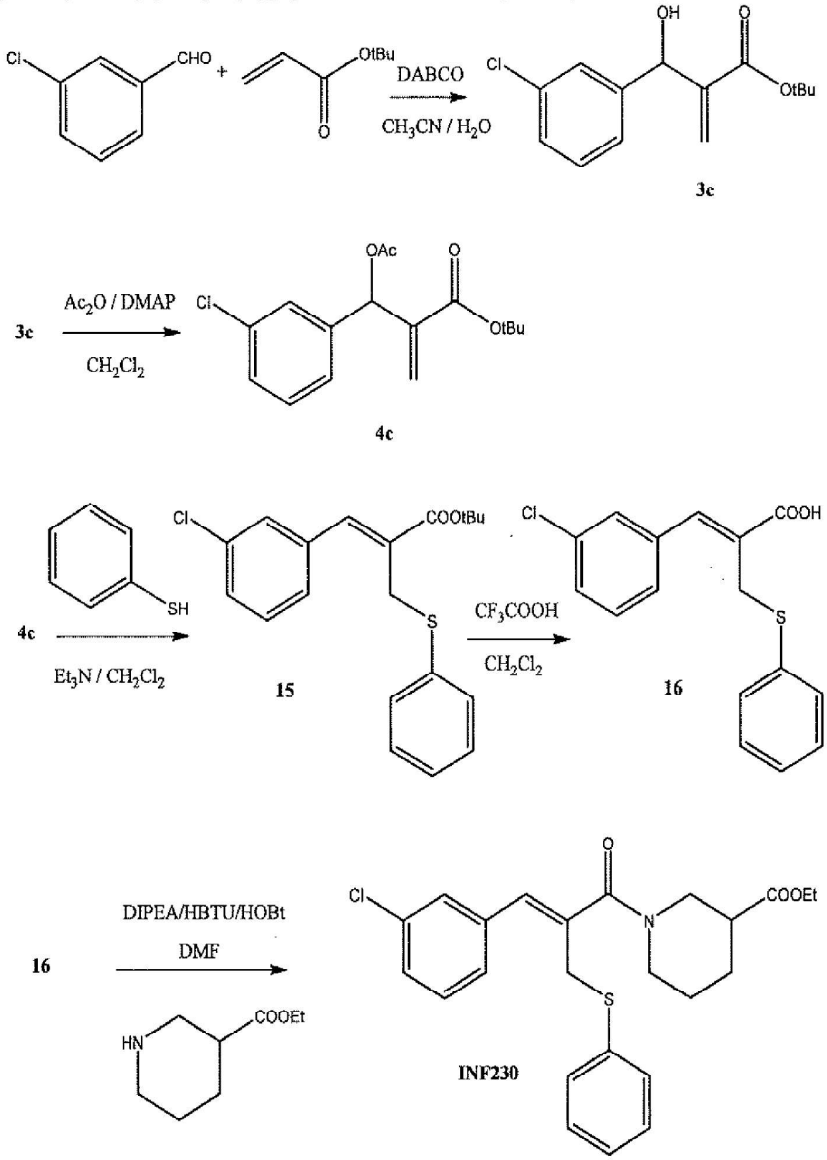
Example 42 – Synthesis of (Z)-3-(2-chlorophenyl)-2-((phenylthio)methyl)-N-(4-sulphamoylphenethyl)acrylamide (INF220)



DIPEA (0.111 mL; 0.656 mmol), HOBt (0.0044 g; 0.033 mmol) and HBTU (0.187 g; 0.492 mmol) are added to a solution of *INF80* (0.100 g; 0.328 mmol) in DMF (5 mL). The mixture is placed under stirring at 20°C for 30 minutes; 4-(2-aminoethyl)benzenesulphonamide (0.066 g; 0.328 mmol) is then added, and the mixture is left under stirring at 20°C for 5 hours. The solvent is evaporated under low pressure, and the resulting residue is diluted with diethyl ether (20 mL) and washed with 1N HCl (2 x 15 mL), then with H_2O (2 x 15 mL). The organic phase is washed with a NaCl saturated solution (15 mL) and dried (Na_2SO_4), and the solvent is evaporated under low pressure. The crude compound is purified by flash chromatography on silica gel column, eluting with PE/EtOAc/MeOH 7/2/1 to provide **INF220** (0.080 g; yield 50%) as a pale yellow semisolid. MS (ESI) m/z: 487-489 $[M+H]^+$; ^{1}H-NMR (DMSO-D6) δ, 7.74 (d, 2 H, J= 8.3 Hz, ArH);

7.51 (d, 1H, J= 7.9 Hz, ArH); 7.43 (d, 2H, J= 8.3 Hz, ArH); 7.37 (m, 1H, ArH); 7.29-7.19 (m, 9H, ArH e C*H*=C); 3.89 (s, 2H, CH_2S); 3.43 (t, 2H, J= 7.1 Hz, NCH_2CH$_2$); 2.88 (t, 2H, J= 7.1 Hz, NCH$_2$CH_2); ^{13}C-NMR ($CDCl_3$) δ, 168.1; 144.4; 140.5; 134.8; 134.2; 133.9; 133.5; 133.2; 130.5; 130.1; 129.9; 129.7 (2 overlapping peaks); 129.2; 127.2; 126.8; 40.8; 35.4; 32.3. Cytotoxicity (MTT assay): IC_{50} > 62.5 ± 4.2 μM.

Example 43 – Synthesis of ethyl (Z)-1-(3-(3-chlorophenyl)-2-((phenylthio)methyl)acryloyl)piperidine-3-carboxylate (INF230)



t-Butyl acrylate (15 mL; 103.3 mmol) and water (5 mL) are added to a solution of 3-chlorobenzaldehyde (4.84 g; 34.4 mmol) in CH_3CN (45 mL). DABCO (3.7 g; 34.4

mmol) is then added to the mixture, and the reaction is left under stirring for 7 days at 20°C. The mixture is diluted with CH_2Cl_2 (30 mL) and extracted with 1N HCl (3 x 30 mL) and NaCl saturated solution (30 mL), then dried (Na_2SO_4), and the solvent evaporated under low pressure. The residue is purified by flash chromatography on silica gel column, eluting with a PE/acetone 8/2 mixture. t-butyl 2-((3-chlorophenyl)(hydroxy)methyl)acrylate (3c) is obtained as a colourless oil (4.23 g; yield 46%).

Acetic anhydride (2.09 g; 20.41 mmol) dissolved in CH_2Cl_2 (20 mL) is added slowly over a period of 1 hour to a solution of 3c (4.23 g; 15.7 mmol) and DMAP (380 mg, 3.14 mmol) in CH_2Cl_2 (20 mL), maintaining the mixture under stirring at 20°C. The reaction mixture is extracted with water (30 mL) and 10% w/v $NaHCO_3$ (3 x 30 mL), then with a NaCl saturated solution (30 mL). The organic phase is dried (Na_2SO_4), and the solvent is evaporated under low pressure. The residue is purified by flash chromatography on silica gel using a PE/EtOAc 95/5 mixture as eluent. t-butyl 2-(acetoxy(3-chlorophenyl)methyl)acrylate (4c) is obtained as a colourless oil (3.5 g; yield: 72%).

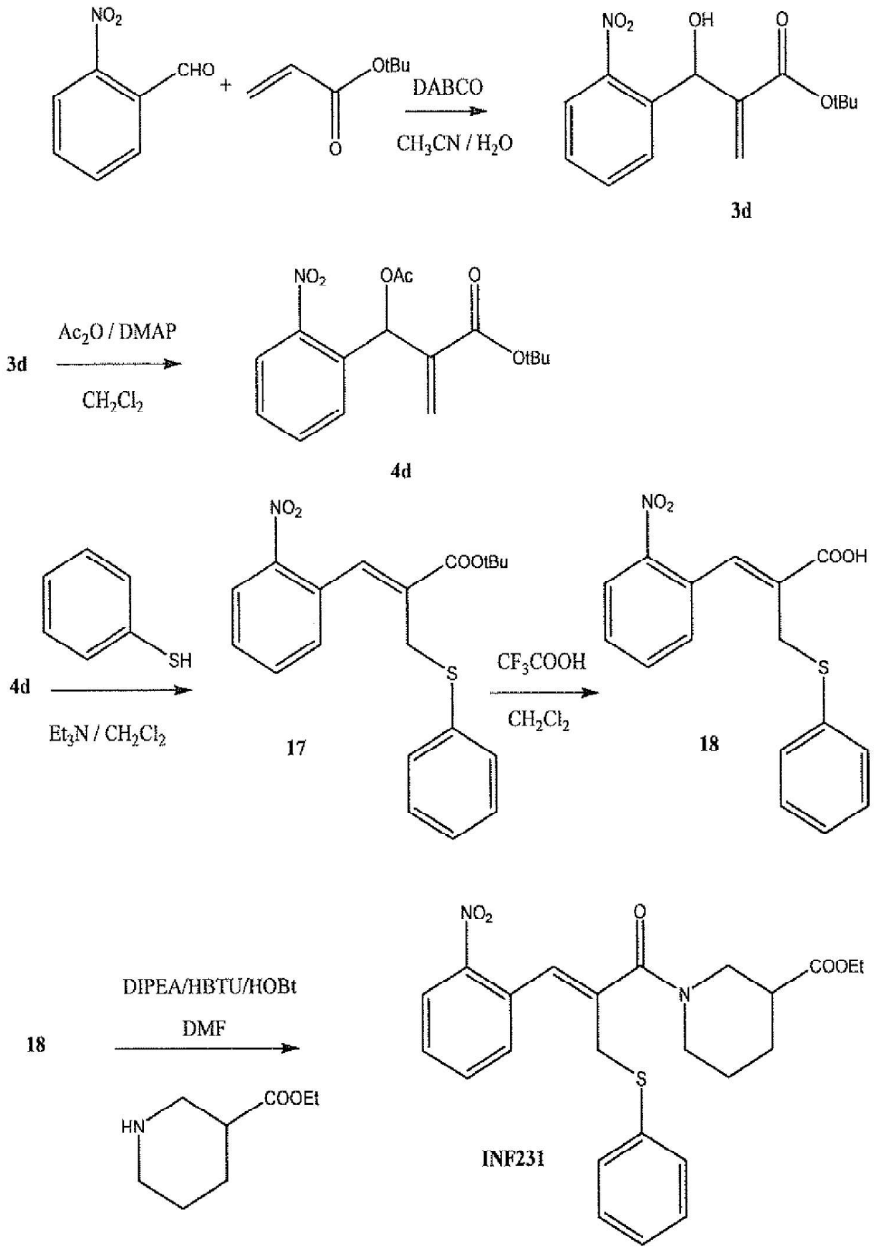
Thiophenol (1.15 mL; 11.3 mmol) and triethylamine (1.88 mL; 13.5 mmol) are added to a solution of 4c (3.5 g; 11.3 mmol) in CH_2Cl_2 (50 mL), maintained in an inert atmosphere (N_2). The reaction is left under vigorous stirring for 30 minutes at 20°C. The reaction mixture is then diluted with H_2O (20 mL) and extracted with 1N HCl (3 x 40 mL) and NaCl saturated solution (30 mL). The organic phase is dried (Na_2SO_4), and the solvent is removed under low pressure. The residue is purified by flash chromatography on silica gel column, eluting with PE/DCM 8/2 and then increasing the percentage of DCM in the mixture to PE/EtOAc 6/4; t-butyl (Z)-3-(3-chlorophenyl)-2-((phenylthio)methyl)acrylate (15) is obtained; 2.9 g; yield 71%.

Compound 15 (0.828 g; 2.49 mmol) is dissolved in a mixture of TFA in 10% DCM

(25 mL). The reaction mixture is placed under stirring at 20°C for 16 hours, and then diluted with H_2O (30 mL) and extracted with DCM (3 x 40 mL). The organic phases are washed with a NaCl saturated solution and dried (Na_2SO_4), and the crude solid is recrystallised from acetonitrile to obtain (Z)-3-(3-chlorophenyl)-2-((phenylthio)methyl)acrylic acid (16) as a white solid (1.6 g; yield: 65%).

DIPEA (0.559 mL; 3.28 mmol), HOBt (22 mg; 0.164 mmol) and HBTU (0.933 g; 2.46 mmol) are added to a solution of 16 (0.500 g; 1.64 mmol) in DMF (10 mL), and ethyl nipecotate (0.255 mL; 1.64 mmol) is added after 30 minutes. The reaction mixture is left to react under magnetic stirring for 18 hours at 20°C. The mixture is diluted with 15 mL of diethyl ether, and the organic phase is washed with 1N HCl (3 x 15 mL) and NaCl saturated solution (15 mL), and dried (Na_2SO_4). The solvent is evaporated under low pressure, and the resulting residue is purified by flash chromatography on silica gel column, eluting with PE/EtOAc 8/2. Ethyl (Z)-1-(3-(3-chlorophenyl)-2-((phenylthio)methyl)acryloyl)piperidine-3-carboxylate (**INF230**) is obtained as a pale yellow oil (0.301 g; yield: 41%). MS (ESI) m/z: 466-468 $[M+Na]^+$; ^{1}H-NMR ($CDCl_3$): δ, 7.41–7.26 (m, 2H, Ar-*H*); 7.25-7.22 (m, 1H, Ar-*H*); 7.21-7.12 (m, 5H, Ar-*H*); 6.49 (s, 1H, C=C*H*); 4.61-4.47 (m, 1H, C*H*-pip); 4.04 (q, J= 7.1 Hz; 2H, O-CH_2); 3.93-3.83 (m, 2H, S-CH_2); 3.08-2.94 (m, 2H, N-CH_{2pip}); 2.44 (m, 2H, CH_{2pip}); 2.09-1.95 (m, 1H, CH_{2pip}); 1.74–1.57 (m, 2H, CH_{2pip}); 1.41-1.39 (m, 1H, CH_{2pip}); 1.24 (t, J= 7.1 Hz; 3H, CH_3).

Example 44 – Synthesis of ethyl (Z)-1-(3-(2-nitrophenyl)-2-((phenylthio)methyl)acryloyl)piperidine-3-carboxylate (INF231)



t-Butyl acrylate (9.3 mL; 84.89 mmol) and water (5 mL) are added to a solution of 2-nitrobenzaldehyde (5.20 g; 34.4 mmol) in CH_3CN (45 mL). DABCO (3.86 g; 34.4 mmol) is then added to the mixture, and the reaction is left under stirring for 4 days at 20°C. The mixture is diluted with CH_2Cl_2 (30 mL) and extracted with 1N HCl (3 x 40 mL) and NaCl

saturated solution (30 mL), then dried (Na_2SO_4), and the solvent evaporated under low pressure. The residue is purified by flash chromatography on silica gel column, eluting with a DCM/EtOAc 9/1 mixture. t-butyl 2-((2-nitrophenyl)(hydroxy)methyl)acrylate (3d) is obtained as a colourless oil (5.76 g; yield 60%).

Acetic anhydride (2.73 g; 26.53 mmol) dissolved in CH_2Cl_2 (20 mL) is added slowly over a period of 1 hour to a solution of 3d (5.70 g; 20.41 mmol) and DMAP (0.50 mg, 4.08 mmol) in CH_2Cl_2 (70 mL) at 0°C, maintaining the mixture under stirring at 20°C. The reaction mixture is extracted with water (30 mL) and 10% w/v $NaHCO_3$ (3 x 30 mL), then with a NaCl saturated solution (30 mL). The organic phase is dried (Na_2SO_4), and the solvent is evaporated under low pressure. The residue is purified by flash chromatography on silica gel using a PE/EtOAc 9/1 mixture as eluent. t-butyl 2-(acetoxy(2-nitrophenyl)methyl)acrylate (4d) is obtained as a colourless oil (2.35 g; yield 36%).

Thiophenol (0.750 mL; 7.31 mmol) and triethylamine (1.26 mL; 8.77 mmol) are added to a solution of 4d (2.35 g; 7.31 mmol) in CH_2Cl_2 (50 mL), maintained in an inert atmosphere (N_2). The reaction is left under vigorous stirring for 30 minutes at 20°C. The reaction mixture is then diluted with H_2O (30 mL) and extracted with 1N HCl (3 x 40 mL) and NaCl saturated solution (20 mL). The organic phase is dried (Na_2SO_4), and the solvent is removed under low pressure. The residue is purified by flash chromatography on silica gel column, eluting with a PE/EtOAc 9/1 mixture to provide t-butyl (Z)-3-(2-nitrophenyl)-2-((phenylthio)methyl)acrylate (17) as a pale yellow oil (1.4 g; yield: 52%).

Compound 17 (0.828 g; 2.49 mmol) is dissolved in a mixture of TFA in 10% DCM (25 mL). The reaction mixture is placed under stirring at 20°C for 16 hours, and then diluted with H_2O (30 mL) and extracted with dichloromethane (3 x 40 mL). The organic phases are washed with a NaCl saturated solution, dried (Na_2SO_4), and evaporated under low pressure to obtain (Z)-3-(2-nitrophenyl)-2-((phenylthio)methyl)acrylic acid (18) as a

cream-coloured solid (0.944 g; yield 79%).

DIPEA (0.510 mL; 1.98 mmol), HOBt (18 mg; 0.15 mmol) and HBTU (0.850 g; 2.24 mmol) are added to a solution of 18 (0.472 g; 1.49 mmol) in DMF (10 mL), and ethyl nipecotate (0.231 mL; 01.49 mmol) is added after 30 minutes. The reaction mixture is left to react under magnetic stirring for 18 hours at 20°C. The mixture is diluted with 15 mL of diethyl ether, and the organic phase is washed with 1N HCl (3 x 15 mL) and NaCl saturated solution (15 mL), and dried (Na_2SO_4). The solvent is evaporated under low pressure, and the resulting residue is purified by flash chromatography on silica gel column, eluting with PE/EtOAc 6/4 followed by PE/EtOAc 1/1. Ethyl (Z)-1-(3-(2-nitrophenyl)-2-((phenylthio)methyl)acryloyl)piperidine-3-carboxylate (**INF231**) is obtained as a pale yellow oil (0.316 g; yield: 47%). MS (ESI) m/z: 477 $[M+Na]^+$; ^{1}H-NMR ($CDCl_3$): δ, 8.17–8.12 (m, 1H, Ar-*H*); 7.65-7.41 (m, 3H, Ar-*H*); 7.08-7.01 (m, 5H, Ar-*H*); 6.82 (s, 1H, C=C*H*); 4.59-4.42 (m, 1H, C*H*-pip); 4.18-4.05 (m; 2H, O-CH_2); 3.91-3.86 (m, 2H, S-CH_2); 3.21-2.93 (m, 2H, N-CH_{2pip}); 2.53-2-44 (m, 2H, CH_{2pip}); 1.98-1.92 (m, 1H, CH_{2pip}); 1.67–1.55 (m, 2H, CH_{2pip}); 1.43-1.37 (m, 1H, CH_{2pip}); 1.25 (t, J= 7.2 Hz; 3H, CH_3).

Example 45 – Stability

The compound INF177 was incubated at the concentration of 100 μM in PBS pH 7.4 (0.1% DMSO) at 37°C, in the absence and in the presence of excess glutathione or cysteamine (10x). The reaction mixture underwent repeated HPLC analyses over 24 hours.

The HPLC analysis was conducted with an HP 1200 chromatography system (Agilent Technologies, Palo Alto, CA, USA) consisting of an integrated quaternary pump (model G1311A), degasser (model G1322A), UV MWD detector (model G1365D) and fluorescence detector (model G1321A). The data were processed with the HP ChemStation system program (Agilent Technologies).

The analyses were conducted using the ZORBAX SB-Phenyl column (250×4.6mm,

5 μm; Agilent) as stationary phase and CH_3CN/H_2O (+0.1% HCOOH), 70/30 (v/v), flow rate = 1.0 mL/min, as mobile phase, injecting 20 μL of sample (Rheodyne, Cotati, CA); the chromatograms were acquired at the wavelengths of 234 and 250 nm.

As demonstrated by the chromatograms shown in Figures 7 and 8, no appreciable reactivity was observed in either condition:

- % compound > 98% (after 4 hours with glutathione and after 2.5 hours with cysteamine),
- the % compound does not fall below 96%, even when the time is increased to 24 hours.

Bioassays

Materials and methods - Preparation and treatment of cells

THP-1 cells, a monocytic human cell line derived from the peripheral blood of a male patient suffering from acute monocytic leukaemia (www.atcc.org), were cultured in RPMI 1640 medium (Aurogene, Rome, Italy), with the addition of foetal bovine serum (10%, Aurogene), L-glutamine (2 mM, Aurogene), penicillin (100 IU/ml, Aurogene) and streptomycin (100 mg/ml, Aurogene). The medium was changed every 2-3 days, and the cells were maintained in an incubator at 37°C, with 5% CO_2, and with suitable humidity.

The cells were seeded in 48-well plates (90.000 cells/well), and differentiated with phorbol 12-myristate 13-acetate (PMA – 50 nM, 24 hours; Sigma-Aldrich). On the next day, the differentiated THP-1s were washed twice with the balanced saline solution phosphate-buffered saline (PBS), and stimulated with lipopolysaccharide (LPS) (10 μg/mL, 4 hours; Sigma-Aldrich), prepared in serum-free medium. After 4 hours the cells were incubated with the compounds at the concentration of 10 μM for 1 hour, operating in triplicate; INF176 and INF177 were tested at three different concentrations: 1, 10 and 20 μM. Finally, the cells were stimulated with ATP 5 mm for 1.5 hours. The supernatants

were then harvested for the subsequent analyses.

Example 46 - Lactate dehydrogenase (LDH) release

LDH release into the supernatant obtained as described above was quantified using the CytoTox 96 nonradioactive cytotoxicity assay (Promega Corporation, Madison, MI, USA), a colorimetric assay wherein LDH activity is measured with an NADH-dependent enzymatic reaction:

$$\text{lactate} + NAD^+ \rightarrow \text{pyruvate} + NADH, \text{ catalysed by LDH}$$

$$NADH + \text{iodonitrotetrazolium} \rightarrow NAD^+ + \text{formazan, catalysed by diaphorase.}$$

Briefly, an equal volume of CytoTox Reagent was added to the supernatant and, after 30 minutes' incubation, the reaction was stopped by adding the stop solution. The formazan concentration was determined by measuring absorbance, using a microplate reader (Victor X4 – EnSight, PerkinElmer, Waltham, MA, USA), at $\lambda = 490$ nm. Cell death was expressed according to the manufacturer's instructions.

Example 47 - IL-1β release

IL-1β release into the supernatant of cells treated as described above was determined with the Human IL-1 beta Uncoated ELISA kit (Invitrogen, Waltham, MA, USA), according to the manufacturer's instructions.

Briefly, on the first day the 96-well plate (Nunc Immuno plate, Thermofisher) was coated with anti-IL-1β capture antibody, included in the kit, and left to incubate for 16 hours at 4°C, under stirring. On the next day, after washing the wells with PBS buffer + 0.05% Tween-20 and incubating with a saturated solution for 1 hour at room temperature, the standard protein or samples were added to each well, and the plate was incubated overnight at 4°C, under stirring. The next day, after suitable washes with PBS + 0.05% Tween-20, the anti-IL-1β biotinylate secondary antibody, included in the kit, was added; after one hour, the plate was washed, and avidin conjugated with the enzyme horseradish

peroxidase (HRP) for 30 minutes at room temperature was added, followed by the substrate tetramethylbenzidine (TMB) for 15 minutes. The reaction was stopped with a 2N solution of H_2SO_4.

The interleukin levels were determined by measuring absorbance at λ= 450 nm, using a microplate reader (Victor X4 - EnSight).

Example 48 - Cytotoxicity (MTT assay)

The THP-1 cells were seeded in a 96-well plate (15.000 cells/well) and incubated with the test compounds at four different concentrations (0.1, 1, 10 and 100 μM), operating in triplicate. The cells were placed in an incubator at 37°C, with 5% CO_2, and cell viability was measured after 72 hours' incubation, using the MTT assay. This is a colorimetric assay, based on conversion of water-soluble 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich) to a violet-coloured insoluble formazan, by the mitochondrial dehydrogenase present in the live cells. The formazan is solubilised in acidified isopropanol, and the concentration is determined by measuring absorbance with a microplate reader (Victor X4 – Ensight, PerkinElmer) at λ= 570 nm. Viability was expressed as a percentage of the viability of the control cells, which were either untreated or treated with the carrier. The IC_{50} was calculated with the Graph Pad Prism software.

Example 49 – Anti-pyroptotic activity and inhibition of IL-1beta release

The compound INF176 was tested to measure its ability to inhibit NLRP3-dependent cell pyroptosis in human macrophages, using the experimental protocol previously published [Cocco et al. 2017].

The THP-1 cells were differentiated into macrophages by treating them with PMA 50 nM (24 hours) and then with LPS (10 μg/mL) for 4 hours. The cells were treated with the compound (10 μM) for 1 hour. Pyroptosis was induced by treatment with ATP 5 mm.

After 90 minutes, cell death was evaluated by measuring the LDH level in the cell supernatant using the *CytoTox 96 Non-Radioactive Cytotoxicity Assay* (Promega Corporation, Madison, MI, USA).

Compound INF176 is able to inhibit NLRP3-dependent cell pyroptosis induced by LPS/ATP in a dose-dependent manner, with inhibition of 25.7 ± 5.9 – 58.7 ± 7.6% in the range of concentrations tested (Figure 1A). INF176 also inhibits IL-1beta release from human macrophages stimulated with pro-inflammatory substances such as LPS/ATP. Said effect is also dose-dependent, with inhibition of 35 ± 1.2% at the maximum concentration tested (Figure 1B).

Example 50 – Anti-inflammatory activity in colitis

Studies conducted *in vivo* on an experimental murine model of colitis induced by dextran sodium sulphate (DSS) demonstrated a good level of efficacy of INF176 in counteracting intestinal inflammation. In particular, oral administration (p.o.) of INF176 at the doses of 25 mg/kg/day and 50 mg/kg/day gave rise to an improvement in the systemic and tissue parameters associated with colitis (Figures 2-3). In particular, INF176 counteracts weight loss and increased spleen weight in the animal, and significantly reduces the disease activity index (DAI), the levels of interleukin-1beta and myeloperoxidase (index of degree of infiltration of inflammatory cells) in colon tissues (Figures 2-3).

It should be noted that administration of INF176 improves slowing of colon transit in SAMP8 animals (Figure 5A). Moreover, *in vitro* studies demonstrate that INF176 significantly improves the colon contractions elicited by electrical stimuli, significantly enhancing both cholinergic and tachykininergic colon contractions (Figures 5B, 5C and 5D). Treatment of SAMP8 animals with INF176 also reduces the increase in IL-1β levels in the colon (Figure 6).

Example 51 – Activity in the treatment of neurodegenerative disorders

Compound INF176 was tested *in vivo* in an animal model of mice with spontaneous accelerated senescence (SAMP8), used as a model of neurodegenerative disorders such as mild cognitive impairment (MCI), which in most cases evolve to Alzheimer's disease (AD).

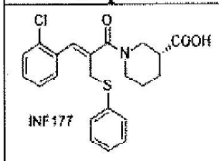
Chronic administration (2 months) of compound INF176 (50 mg/kg/day p.o.) to SAMP8 animals, starting in the earliest stages of the disease, before the appearance of the first symptoms, significantly counteracts cognitive decline (evaluated with the Morris test and expressed as escape latency, number of crossings in the quadrant, and number of entries into the quadrant) and significantly reduces expression of p-tau protein (considered to be a disease marker) in brain tissues, with results comparable to donepezil (DON), a medicament currently approved and used for the treatment of AD (Figures 4A, 4B and 4C).

Example 52 – Molecular docking studies

The induced-fit docking (IFD) [Sherman et al.] protocol included in the Maestro software suite (release 2022-1, Schröedinger Inc.) was used for the molecular docking studies. The structure of NLRP3 was obtained from the Protein Data Bank (PDB ID:7ALV), and processed in Maestro. The hydrogen atoms were added with PropKa 3.0, assuming a pH of 7.4. The residue of Ala228 was selected as centre of the docking cavity, and the radius of the cavity was set to 20 Å. In the first step of the IFD protocol, 10 initial poses were used with softened-potential docking, and subsequently refined with the Prime package, to accommodate the ligand by reorienting the side chains of the residues around the ligand. Ligands characterised within 30 Kcal/mol of the minimum energy structure were processed with a last round of docking and scoring with Glide. The ligands were then again subjected to docking in the binding site, and the score was allocated with the Extra Precision (XP) scoring function in Glide [Friesner et al.]. The results of the studies

conducted are set out in Table 3:

Table 3

Compound	DOCKING SCORE	XP GScore	glide gscore	glide emodel
 INF177_R	-8.54	-8.54	-8.54	-85.34
Br1	-8.83	-8.83	-8.83	-54.61
Br2S	-12.19	-12.19	-12.19	-77.79
Br2R	-9.30	-9.30	-9.30	-79.98
Br4R	-8.79	-8.79	-8.79	-67.23
Br7	-8.76	-8.76	-8.76	-56.36
Br8R	-7.42	-7.42	-7.42	-73.78
Br9R	-10.11	-10.11	-10.11	-83.40
Br10R	-11.45	-11.45	-11.45	-74.10
Br10S	-11.19	-11.19	-11.19	-65.00
Br11R	-8.79	-8.79	-8.79	-82.68
Br12	-8.63	-8.63	-8.63	-67.34
INF80	-9.21	-9.21	-9.21	-55.21
Br13	-8.13	-8.13	-8.13	-65.27
Br14R	-8.48	-8.48	-8.48	-76.94
Br14S	-8.78	-8.78	-8.78	-72.19
Br15	-7.17	-7.17	-7.17	-51.13
Br16R	-9.73	-9.73	-9.73	-77.59
Br17	-9.24	-9.24	-9.24	-63.13
Br18R	-9.86	-9.86	-9.86	-86.47
Br4S	-10.70	-10.70	-10.70	-66.78
Br8S	-8.89	-8.89	-8.89	-79.95
Br11S	-9.95	-9.95	-9.95	-92.30

The studies demonstrate that INF177, metabolite of INF176, which proved active in the tests conducted according to examples 49, 50 and 51 reported above, is characterised by a docking score of -8.54, representative of the bonding capacity of the NLRP3 protein in the NACHT domain (PDB ID:7ALV). The compounds exemplified in the table have docking scores equivalent to those of INF177, which are therefore predictive of the efficacy of said compounds.

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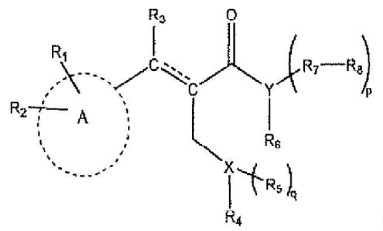
CLAIMS

1. Compounds of general formula (I):



(I)

wherein

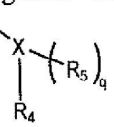
A is a C_3-C_{10}-cycloalkyl, preferably monocyclic or bicyclic C_5-C_{10}-cycloalkyl; 5- to 10-membered, monocyclic or bicyclic, saturated or partly saturated heterocycle; monocyclic or bicyclic C_6-C_{10}-aryl; 5- to 10-membered monocyclic or bicyclic heteroaryl; A is preferably a 5 or 6-membered, saturated or partly saturated, monocyclic heterocycle, or a 9 or 10-membered, saturated or partly saturated, bicyclic heterocycle; or a monocyclic C_5-C_6-aryl, or a bicyclic C_9-C_{10}-aryl; or a 5 or 6-membered monocyclic heteroaryl or a 9 or 10-membered bicyclic heteroaryl; wherein the heteroatom is preferably N or O; more preferably A is phenyl, naphthyl, furanyl or indolyl, and most preferably A is phenyl;

R_1 and R_2, which are the same or different, can occupy any position on A, and can be hydrogen; halogen such as F, Cl, Br or I; linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_4-alkyl; linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_4-alkoxy; a nitro group; nitrile; a substituted or unsubstituted amido group; a substituted or unsubstituted amino group; a substituted or unsubstituted ester group; a trifluoromethyl group; R_1 and R_2 are preferably hydrogen, halogen such as F, Cl, Br or I, linear or branched C_1-C_4-alkyl, linear or branched C_1-C_4-alkoxy, a nitro group; R_1 and R_2 are more preferably hydrogen, chloro, bromo, methyl, methoxy or a nitro group; most preferably R_1 is hydrogen and R_2 is chloro; R_1 or R_2 is preferably in the 2

position when A is phenyl;

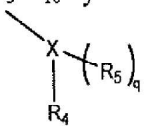
 is a single bond or a double bond;

R_3 is selected from -H, -OH, -OR_9 and -O(CO)R_9, wherein R_9 can be hydrogen, a linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_4-alkyl; R_3 is preferably hydrogen or –OH; R_3 is more preferably hydrogen;

in the  group, X is selected from N, O, S, S(O) and SO_2;

R_4 can be a linear or branched, substituted or unsubstituted, saturated or unsaturated C_{1-4} alkyl group; monocyclic or bicyclic C_3-C_{10}-cycloalkyl; substituted or unsubstituted, preferably a C_3-C_6-cycloalkyl; monocyclic or bicyclic C_6-C_{14}-aryl, substituted or unsubstituted, preferably a C_6-C_{10}-aryl, more preferably a C_5-C_6-aryl; 5- to 10-membered heterocycle, saturated or partly saturated, monocyclic or bicyclic, substituted or unsubstituted, preferably a C_5-C_6-heterocycle; monocyclic or polycyclic 5- to 14-membered heteroaryl, preferably monocyclic or bicyclic, substituted or unsubstituted, preferably a C_5-C_6-heteroaryl; R_4 is preferably monocyclic or bicyclic C_6-C_{10}-aryl, substituted or unsubstituted, or C_3-C_6-cycloalkyl, substituted or unsubstituted; more preferably, R_4 is cyclohexyl or phenyl;

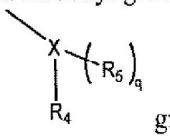
q is 0 (zero) or 1; when q is equal to 1, X is N and R_5 is hydrogen; a linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_4-alkyl group; monocyclic or bicyclic C_3-C_{10}-cycloalkyl;

alternatively, the  group can be an amino-acid residue wherein:

- X is an N, S or O atom of the side chain of an amino acid, preferably natural, selected from serine; tyrosine; threonine; lysine; cysteine; q is zero and R_4 is the remainder of the amino acid optionally protected on the NH_2 and/or COOH terminal groups; the terminal

NH_2 group is preferably acetylated; the amino-acid residue is preferably N-acetylcysteine or N-Boc cysteine methyl ester; or

- X is the N atom of the terminal amino group bonded to the stereogenic carbon atom in alpha of a preferably natural, optionally protected amino acid, selected from alanine, arginine, asparagine, aspartic acid, cysteine, glycine, glutamic acid, glutamine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine; q is equal to 1, R_5 is hydrogen; and R_4 represents the remainder of the amino-acid structure, optionally protected, for example acetylated on the N atom of the side chain or esterified with a linear or branched C_1-C_4-alkyl group, preferably methyl, on the terminal carboxyl group;

alternatively when, in the  group, X is N, R_4 and R_5 are joined to form a saturated, partly saturated or unsaturated, monocyclic or bicyclic C_3-C_{10}-heterocyclic ring with the N atom; R_4 and R_5 preferably form a monocyclic C_3-C_6-heterocyclic ring with the N atom; more preferably, R_4 and R_5 form a piperidine or pyrrolidine ring with the N atom; most preferably, R_4 and R_5 form a pyrrolidine ring with the N atom;

Y is selected from O, N and S; is preferably O or N; and is more preferably N;

when Y is an oxygen or sulfur atom, in the -(R_7-R_8)p group p is equal to zero and R_6 is selected from hydrogen, a linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_8-alkyl group; a monocyclic or bicyclic C_3-C_{10}-cycloalkyl; a substituted or unsubstituted arylalkyl; a 6- to 14-membered monocyclic or bicyclic heteroaryl; R_6 is preferably hydrogen or a linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_4-alkyl group; R_6 is more preferably a linear or branched, saturated, unsubstituted C_1-C_4-alkyl group; R_6 is most preferably ethyl;

when Y is a nitrogen atom, p is equal to 1, R_6 and R_7, which are the same or

different, are selected from hydrogen, a linear or branched, saturated or unsaturated, substituted or unsubstituted C_1-C_4-alkyl group; a substituted or unsubstituted aryl, arylalkyl or heteroaryl group; and are preferably a substituted phenylalkyl group; more preferably –$(CH_2)_2$-phenyl-SO_2NH_2; most preferably R_6 is hydrogen and R_7 is –$(CH_2)_2$-phenyl-SO_2NH_2;

R_8 is selected from H, COOH, $COOR_9$, $C(O)R_9$, CN, $CONH(R_9)$, $S(O)NHR_9$ and $S(O)_2NHR_9$, wherein R_9 is as defined above;

alternatively, R_6 and R_7 are joined to form a 3- to 8-membered heterocyclic ring;

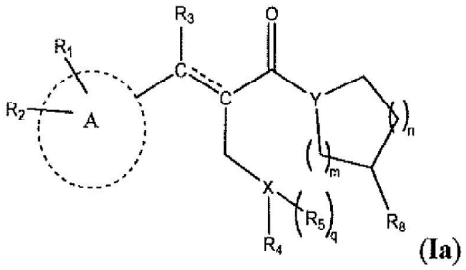
R_8 is as defined above;

and their enantiomers, diastereomers, rotamers or mixtures thereof;

and the pharmaceutically acceptable salts or solvates thereof;

for use in the prevention and/or treatment of diseases and/or disorders mediated by the NLRP3 inflammasome.

2. Compounds for use according to claim 1, having general formula (Ia):



(Ia)

wherein

A, R_1, R_2, R_3, R_4, R_5, R_8, q, X and Y are as defined in claim 1,

n and m, which are the same or different, are 0 (zero) or an integer between 1 and 3; preferably m is 2 and n is 1; more preferably n is 2 and m is 1 or 2; most preferably, n is 2 and m is 1;

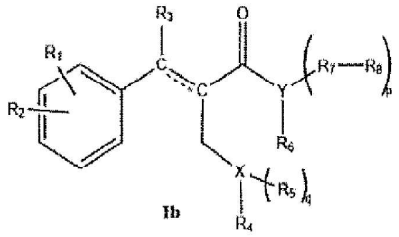
preferably, when Y is N, R_6 and R_7 are joined to form a 3- to 6-membered

monocyclic substituted heterocyclic ring with the N atom; more preferably, R_6 and R_7 form a substituted piperidine or pyrrolidine ring with the N atom; most preferably, R_6 and R_7 form, with the N atom, a piperidine ring substituted in the 3 or 4 position;

and their enantiomers, diastereomers, rotamers or mixtures thereof;

and the pharmaceutically acceptable salts or solvates thereof.

3. Compounds for use according to claim 1, having general formula (Ib):



Ib

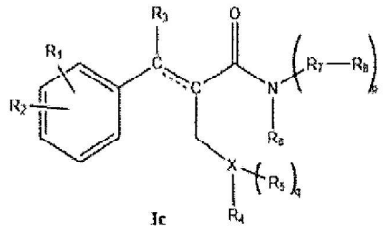
wherein

R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, q, p, X and Y are as defined in claim 1,

and their enantiomers, diastereomers, rotamers or mixtures thereof;

and the pharmaceutically acceptable salts or solvates thereof.

4. Compounds for use according to claim 1, having general formula (Ic):



Ic

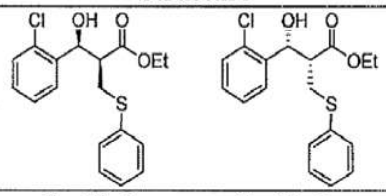
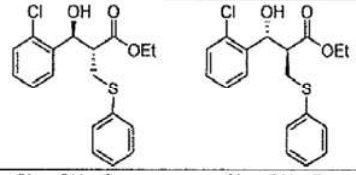
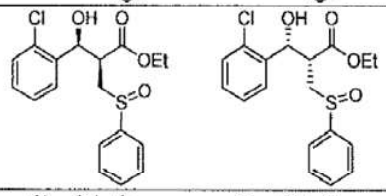
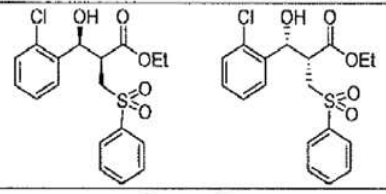
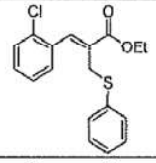
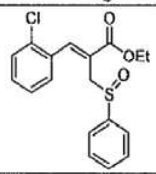
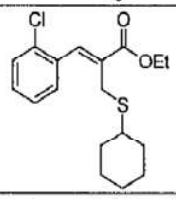
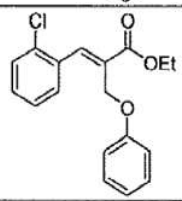
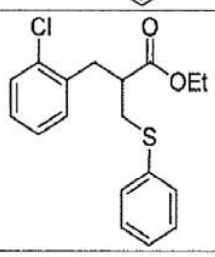
wherein

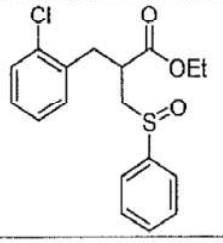
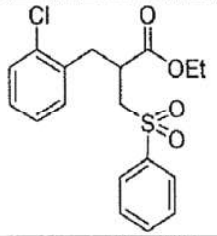
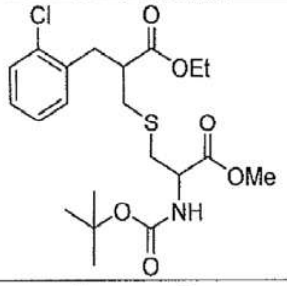
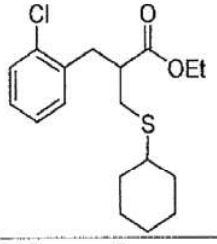
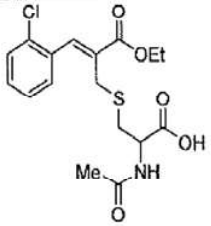
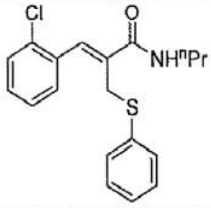
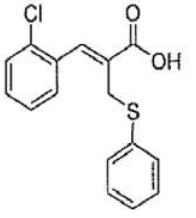
R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, q, p and X are as defined in claim 1,

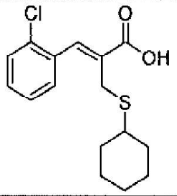
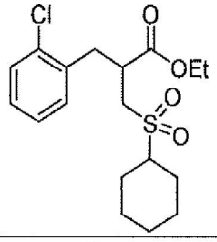
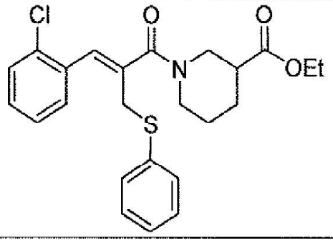
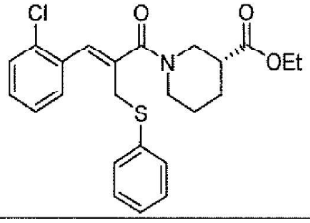
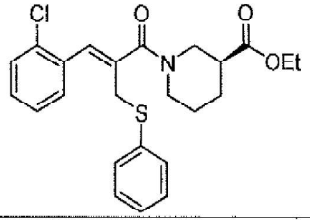
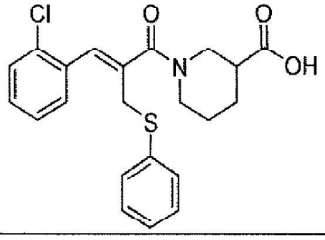
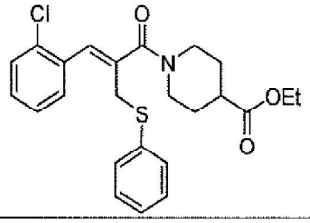
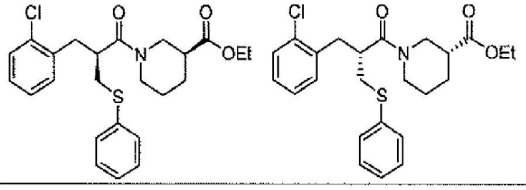
and their enantiomers, diastereomers, rotamers or mixtures thereof;

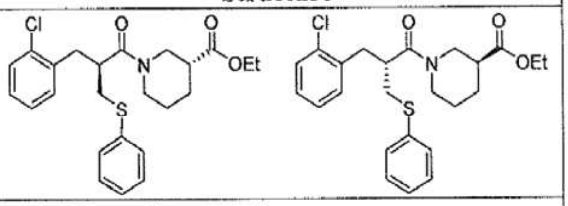
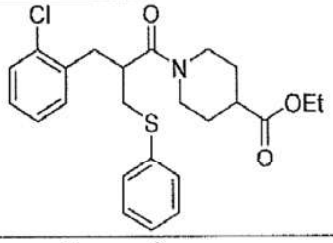
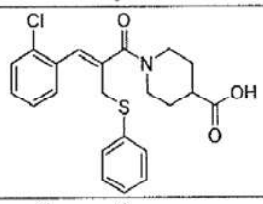
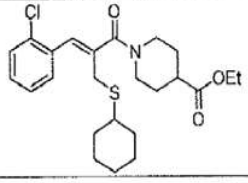
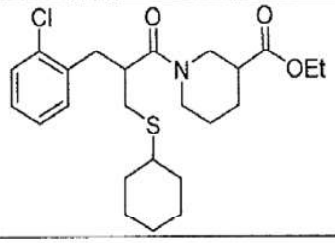
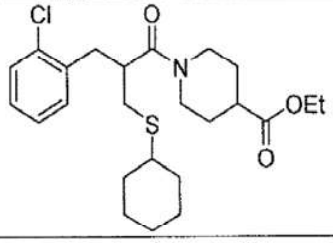
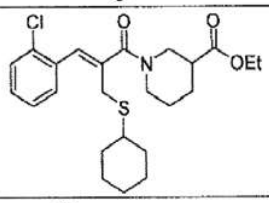
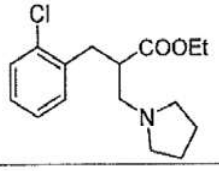
and the pharmaceutically acceptable salts or solvates thereof.

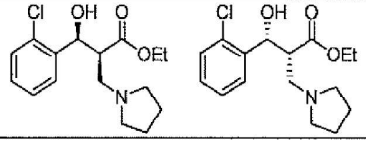
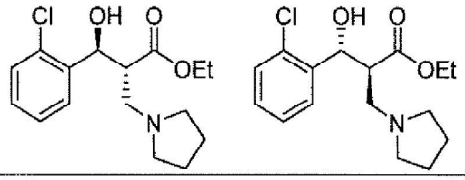
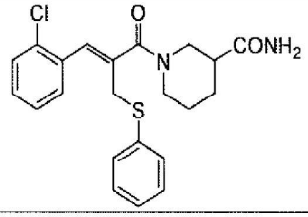
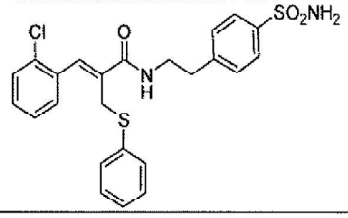
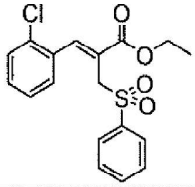
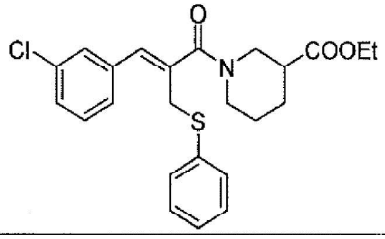
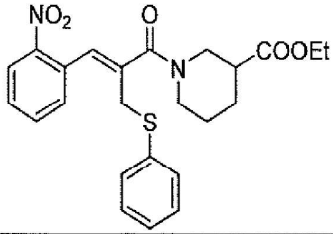
5. Compounds for use according to claims 1-4, selected from:

Compound	Structure
INF38s	
INF38a	
INF44	
INF45	
INF42	
INF50	
INF56	
INF57	
INF43	

Compound	Structure
INF48	
INF49	
INF55	
INF110	
INF85	
INF82	
INF80	

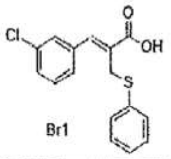
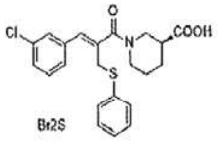
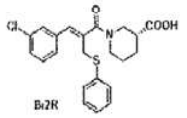
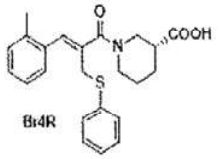
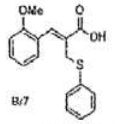
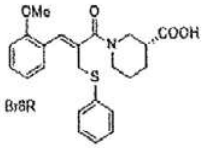
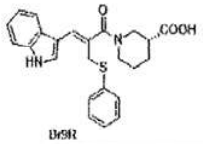
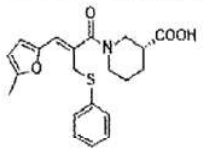
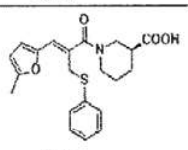
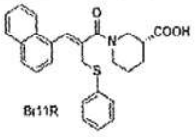
Compound	Structure
INF86	
INF111	
INF176	
INF202	
INF203	
INF177	
INF180	
INF184	

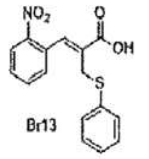
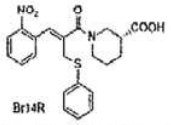
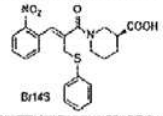
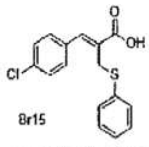
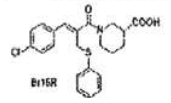
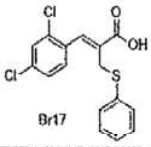
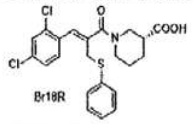
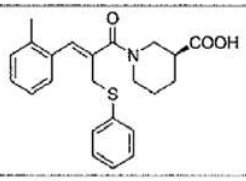
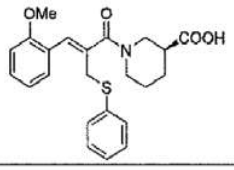
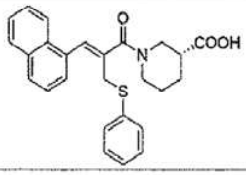
Compound	Structure
INF185	
INF186	
INF187	
INF188	
INF192	
INF193	
INF194	
INF61	

Compound	Structure
INF37 syn	
INF37 anti	
INF219	
INF220	
INF51	
INF230	
INF231	

6. Compounds for use according to claims 1-4, selected from:

5

Compound	Structure
Br1	
Br2S	
Br2R	
Br4R	
Br7	
Br8R	
Br9R	
Br10R	
Br10S	
Br11R	

Compound	Structure
Br12	
Br13	
Br14R	
Br14S	
Br15	
Br16R	
Br17	
Br18R	
Br4S	
Br8S	
Br11S	

7. Compounds for use according to claims 1-6, as an NLRP3 inflammasome-

inhibiting medicament.

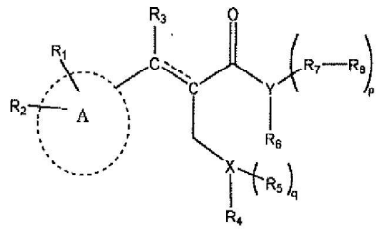
8. Compounds for use according to claims 1-6, in the prevention and/or treatment of inflammatory, autoimmune, neurodegenerative, cardiovascular, metabolic and neoplastic diseases and/or disorders.

9. Compounds for use according to claim 8, wherein the diseases and/or disorders are selected from:

- cryopyrin-associated periodic syndromes (CAPS) which comprise familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and chronic infantile neurological cutaneous and articular syndrome (CINCA), also known as neonatal-onset multisystem inflammatory disease (NOMID);
- asthma, chronic or acute inflammatory arthritis, osteoarthritis, rheumatoid arthritis, acute or chronic joint disease, psoriasis, sterile corneal inflammation, systemic sclerosis, ankylosing spondylitis, sepsis, chronic inflammatory bowel diseases, irritable bowel syndrome, inflammation induced by viral infections (such as those caused by the SARS-CoV-2 (COVID-19) virus);
- Alzheimer's disease, multiple sclerosis, Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and correlated symptoms (such as gastrointestinal disorders);
- cardiovascular diseases (such as hypertension, myocardial infarction, diabetic cardiomyopathy, atherosclerosis, pericarditis and ischaemia);
- non-alcoholic steatohepatitis (NASH), liver disease and correlated disorders such as hepatic fibrosis;
- obesity, type I diabetes, type II diabetes, kidney disease and correlated disorders (such as gastrointestinal disorders);
- tumours (such as stomach cancer, head/neck cancer, lung cancer, melanoma),

myelodysplastic syndromes.

10. Compounds of general formula (I):



(I)

wherein

A is a C_3-C_{10}-cycloalkyl, preferably monocyclic or bicyclic C_5-C_{10}-cycloalkyl; 5- to 10-membered, saturated or partly saturated, monocyclic or bicyclic heterocycle; monocyclic or bicyclic C_6-C_{10}-aryl; 5- to 10-membered monocyclic or bicyclic heteroaryl; A is preferably a 5 or 6-membered, saturated or partly saturated monocyclic heterocycle, or a 9 or 10-membered, saturated or partly saturated bicyclic heterocycle; or a monocyclic C_5-C_6-aryl, or a bicyclic C_9-C_{10}-aryl; or a 5 or 6-membered monocyclic heteroaryl or a 9 or 10-membered bicyclic heteroaryl; wherein the heteroatom is preferably N or O; more preferably A is phenyl, naphthyl, furanyl or indolyl, and most preferably A is phenyl;

R_1 and R_2, which are the same or different, can occupy any position on A, and can be hydrogen; halogen such as F, Cl, Br or I; linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_4-alkyl; linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_4-alkoxy; a nitro group; nitrile; a substituted or unsubstituted amido group; a substituted or unsubstituted amino group; a substituted or unsubstituted ester group; a trifluoromethyl group; R_1 and R_2 are preferably hydrogen, halogen such as F, Cl, Br or I, linear or branched C_1-C_4-alkyl, linear or branched C_1-C_4-alkoxy, a nitro group; R_1 and R_2 are more preferably hydrogen, chloro, bromo, methyl, methoxy or a nitro group; most preferably R_1 is hydrogen and R_2 is chloro;

wherein at least one of R_1 and R_2 is other than hydrogen when A is phenyl,

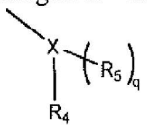
wherein at least one of R_1 and R_2 is other than hydrogen when X is SO_2,

wherein at least one of R_1 and R_2 is preferably other than H and is in the 2 position when A is phenyl, and R_6 and R_7 do not form a ring, and the other R_1 or R_2 can occupy any other position on A,

R_1 or R_2 is preferably a halogen such as F, Cl, Br or I, is in the 2 position when A is phenyl, and is more preferably Cl;

 is a single bond or a double bond;

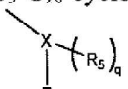
R_3 is selected from -H, -OH, -OR_9 and -O(CO)R_9, wherein R_9 is hydrogen or a linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_4-alkyl; R_3 is preferably hydrogen or –OH; R_3 is more preferably hydrogen;

in the  group, X is selected from N, O, S, S(O) and SO_2, or can be O, S, S(O) or SO_2 when Y is O;

R_4 can be a linear or branched, substituted or unsubstituted, saturated or unsaturated C_{1-4} alkyl group; monocyclic or bicyclic C_3-C_{10}-cycloalkyl; substituted or unsubstituted, preferably a C_3-C_6-cycloalkyl; monocyclic or bicyclic C_6-C_{14}-aryl, substituted or unsubstituted, preferably a C_6-C_{10}-aryl, more preferably a C_5-C_6-aryl; 5- to 10-membered heterocycle, saturated or partly saturated, monocyclic or bicyclic, substituted or unsubstituted, preferably a C_5-C_6-heterocycle; monocyclic or polycyclic 5- to 14-membered heteroaryl, preferably monocyclic or bicyclic, substituted or unsubstituted, preferably a C_5-C_6-heteroaryl; R_4 is preferably monocyclic or bicyclic C_6-C_{10}-aryl, substituted or unsubstituted, or C_3-C_6-cycloalkyl, substituted or unsubstituted; more preferably, R_4 is cyclohexyl or phenyl;

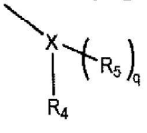
q is 0 (zero) or 1; when q is equal to 1, X is N and R_5 is hydrogen; a linear or

branched, substituted or unsubstituted, saturated or unsaturated C_1-C_4-alkyl group; monocyclic or bicyclic C_3-C_{10}-cycloalkyl;

alternatively, the  group is an amino-acid residue wherein:

- X is an N, S or O atom, or an S or O atom when Y is O, of the side chain of an amino acid, preferably natural, selected from serine; tyrosine; threonine; lysine; cysteine; q is zero and R_4 is the remainder of the amino acid optionally protected on the terminal NH_2 and/or COOH groups; the terminal NH_2 group is preferably acetylated; the amino-acid residue is preferably N-acetylcysteine or N-Boc cysteine methyl ester; or

- X is the N atom of the terminal amino group bonded to the stereogenic carbon atom in alpha of a preferably natural, optionally protected amino acid, selected from alanine, arginine, asparagine, aspartic acid, cysteine, glycine, glutamic acid, glutamine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine; q is equal to 1, R_5 is hydrogen; and R_4 represents the remainder of the amino-acid structure, optionally protected, for example acetylated on the N atom of the side chain or esterified with a linear or branched C_1-C_4-alkyl group, preferably methyl, on the terminal carboxyl group;

alternatively when, in the  group, X is N, Y is other than O, R_4 and R_5 are joined to form a monocyclic or bicyclic, saturated, partly saturated or unsaturated C_3-C_{10}-heterocyclic ring with the N atom; R_4 and R_5 preferably form a monocyclic C_3-C_6-heterocyclic ring with the N atom; more preferably, R_4 and R_5 form a piperidine or pyrrolidine ring with the N atom; most preferably, R_4 and R_5 form a pyrrolidine ring with the N atom;

Y is selected from O, N and S; is preferably O or N; and is more preferably N;

when Y is an oxygen or sulfur atom, in the -(R_7-R_8)p group p is equal to zero and

R_6 is selected from hydrogen, a linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_8-alkyl group; a monocyclic or bicyclic C_3-C_{10}-cycloalkyl; a substituted or unsubstituted arylalkyl; a 6- to 14-membered monocyclic or bicyclic heteroaryl; R_6 is preferably hydrogen or a linear or branched, substituted or unsubstituted, saturated or unsaturated C_1-C_4-alkyl group; R_6 is more preferably a linear or branched, saturated, unsubstituted C_1-C_4-alkyl group; R_6 is most preferably ethyl;

when Y is a nitrogen atom, p is equal to 1, R_6 and R_7, which are the same or different, are selected from hydrogen, a linear or branched, saturated or unsaturated, substituted or unsubstituted C_1-C_4-alkyl group; a substituted or unsubstituted aryl, arylalkyl or heteroaryl group; are preferably a substituted phenylalkyl group; more preferably –$(CH_2)_2$-phenyl-SO_2NH_2; most preferably R_6 is hydrogen and R_7 is –$(CH_2)_2$-phenyl-SO_2NH_2;

R_8 is selected from H, COOH, $COOR_9$, $C(O)R_9$, CN, $CONH(R_9)$, $S(O)NHR_9$ and $S(O)_2NHR_9$, wherein R_9 is as defined above;

alternatively, R_6 and R_7 are joined to form a 3- to 8-membered heterocyclic ring;

R_8 is as defined above;

and their enantiomers, diastereomers, rotamers or mixtures thereof;

and the pharmaceutically acceptable salts or solvates thereof;

wherein

in compounds of formula (I), when R_6 and R_7 do not form a ring:

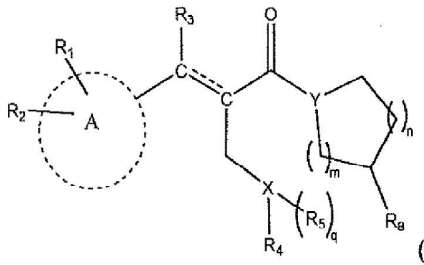
- when A is phenyl, R_1 and R_2 are as defined above,  is a double bond, R_3 is H or OH, Y is O, X is S, q and p are zero, R_4 is methyl, R_6 is hydrogen, a linear or branched, substituted or unsubstituted, saturated or unsaturated C_3-C_8-alkyl group; a monocyclic or bicyclic C_3-C_{10}-cycloalkyl; a substituted or unsubstituted arylalkyl; a 6- to 14-membered monocyclic or bicyclic heteroaryl; R_6 is preferably

hydrogen or a linear or branched, substituted or unsubstituted, saturated or unsaturated C_3-C_6-alkyl group; and/or

- when A is phenyl, naphthyl or thiophene, R_1 and R_2 are as defined above, R_3 is H, Y is O, X is SO_2, q and p are zero, R_4 is phenyl, ethyl or methyl, 4-chlorophenyl, 4-toluene, R_6 is methyl or ethyl,  is a single bond; and/or
- when A is phenyl or naphthyl, R_1 and R_2 are as defined above,  is a double bond, R_3 is H, Y is O, X is O, q and p are zero, R_4 is methyl, R_6 is hydrogen, methyl, a linear or branched, substituted or unsubstituted, saturated or unsaturated C_3-C_8-alkyl group; a monocyclic or bicyclic C_3-C_{10}-cycloalkyl; a substituted or unsubstituted arylalkyl; a 6- to 14-membered monocyclic or bicyclic heteroaryl; R_6 is preferably hydrogen or a linear or branched, substituted or unsubstituted, saturated or unsaturated C_3-C_6-alkyl group; and/or
- when A is phenyl, R_1 and R_2 are as defined above,  is a double bond, R_3 is H, Y is O, X is O, q and p are zero, R_4 is phenyl, R_6 is methyl, ethyl, a linear or branched, substituted or unsubstituted, saturated or unsaturated C_3-C_8-alkyl group; a monocyclic or bicyclic C_3-C_{10}-cycloalkyl; a substituted or unsubstituted arylalkyl; a 6- to 14-membered monocyclic or bicyclic heteroaryl; R_6 is preferably hydrogen or a linear or branched, substituted or unsubstituted, saturated or unsaturated C_3-C_6-alkyl group; and/or
- when A is phenyl, R_1 and R_2 are as defined above, Y is O and X is N, p is zero, R_6 is methyl, q is one,  is preferably a double bond, and is more preferably a single bond or a double bond; R_4 and R_5 are joined to form a monocyclic or bicyclic, saturated, partly saturated or unsaturated C_3-C_{10}-heterocyclic ring with the N atom; R_4 and R_5 preferably form a monocyclic C_3-C_6-heterocyclic ring with the N atom;

more preferably, R_4 and R_5 form a piperidine or pyrrolidine ring with the N atom; most preferably R_4 and R_5 form a pyrrolidine ring with the N atom.

11. Compounds according to claim 10 having general formula (Ia):



(Ia)

wherein

A, R_1, R_2, R_3, R_4, R_5, R_8, q, X and Y are as defined in claim 10,

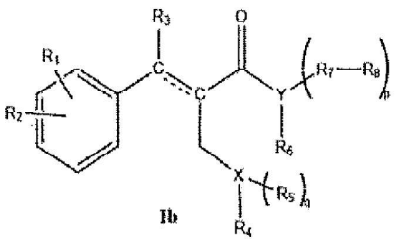
n and m, which are the same or different, are 0 (zero) or an integer between 1 and 3; preferably m is 2 and n is 1; more preferably n is 2 and m is 1 or 2; most preferably, n is 2 and m is 1;

preferably, when Y is N, R_6 and R_7 are joined to form a 3- to 6-membered monocyclic substituted heterocyclic ring with the N atom; more preferably, R_6 and R_7 form a substituted piperidine or pyrrolidine ring with the N atom; most preferably, R_6 and R_7 form, with the N atom, a piperidine ring substituted in the 3 or 4 position;

and their enantiomers, diastereomers, rotamers or mixtures thereof;

and the pharmaceutically acceptable salts or solvates thereof.

12. Compounds according to claim 10 having general formula (Ib):



Ib

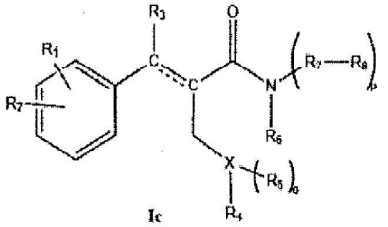
wherein

R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, q, p, X and Y are as defined in claim 10,

and their enantiomers, diastereomers, rotamers or mixtures thereof;

and the pharmaceutically acceptable salts or solvates thereof.

13. Compounds according to claim 10 having general formula (Ic):



Ic

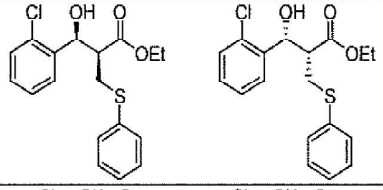
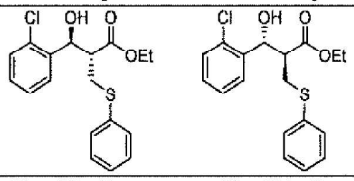
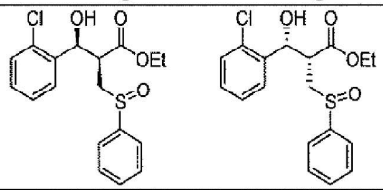
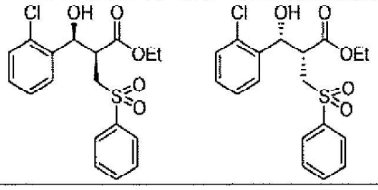
wherein

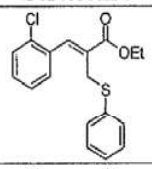
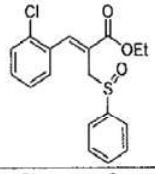
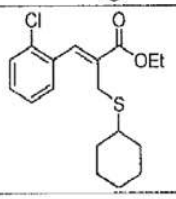
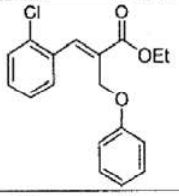
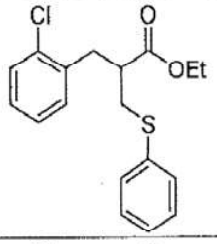
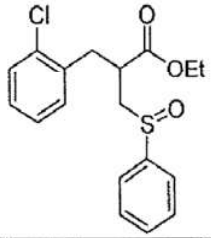
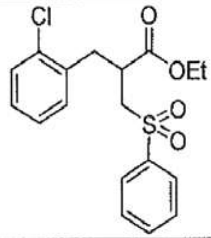
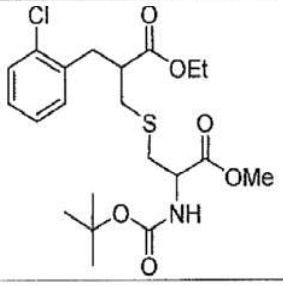
R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, q, p and X are as defined in claim 10,

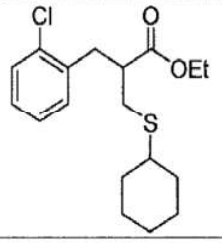
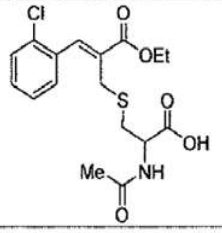
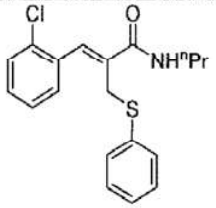
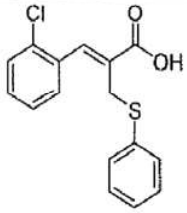
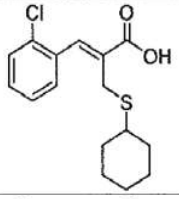
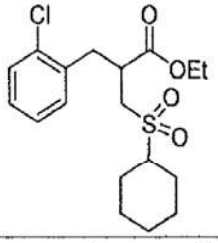
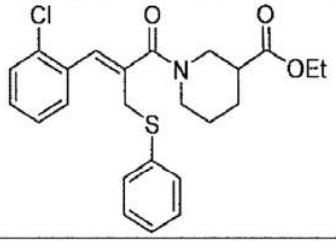
and their enantiomers, diastereomers, rotamers or mixtures thereof;

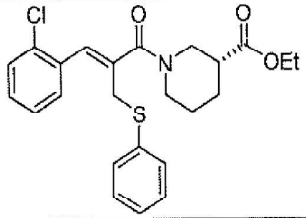
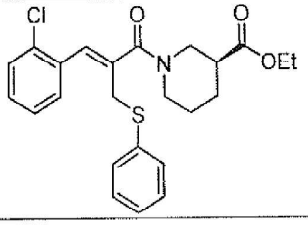
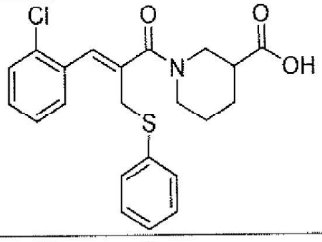
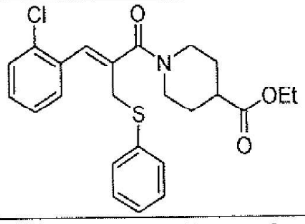
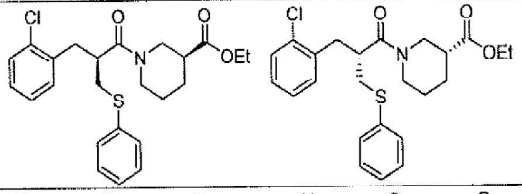
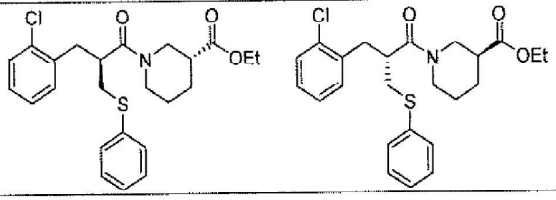
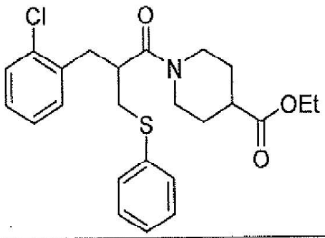
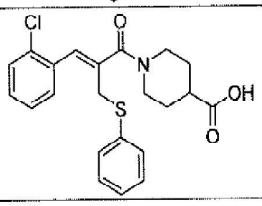
and the pharmaceutically acceptable salts or solvates thereof.

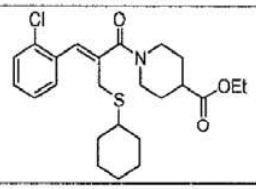
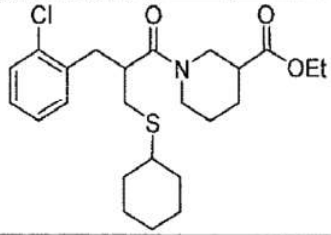
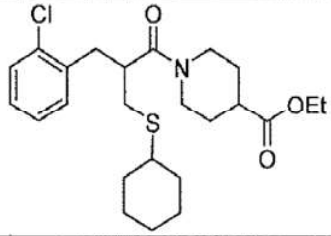
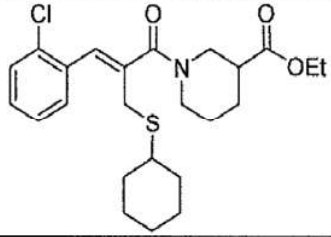
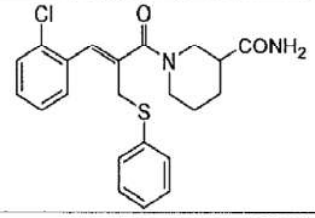
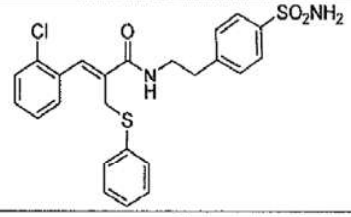
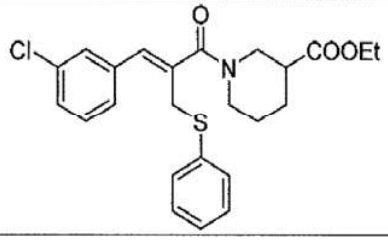
14. Compounds according to claims 10-13, selected from:

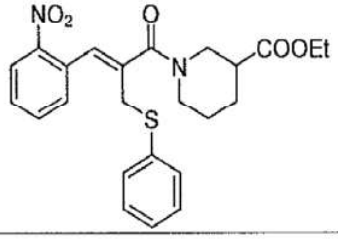
Compound	Structure
INF38s	
INF38a	
INF44	
INF45	

Compound	Structure
INF42	
INF50	
INF56	
INF57	
INF43	
INF48	
INF49	
INF55	

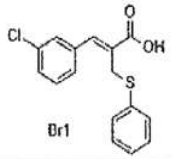
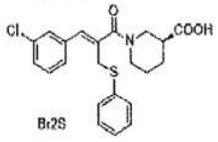
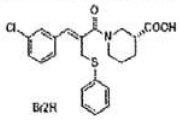
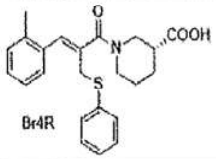
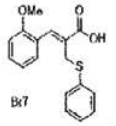
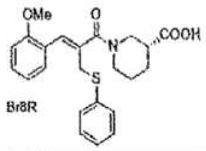
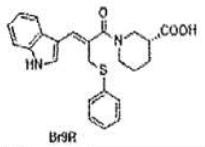
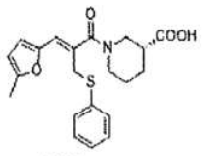
Compound	Structure
INF110	
INF85	
INF82	
INF80	
INF86	
INF111	
INF176	

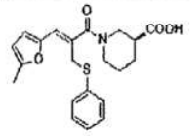
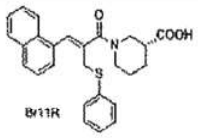
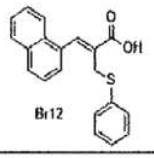
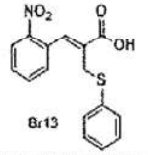
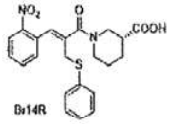
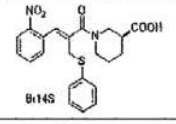
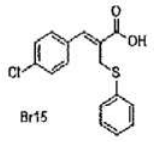
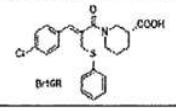
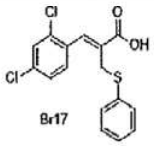
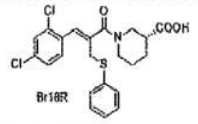
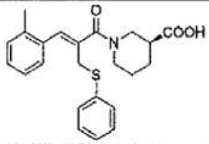
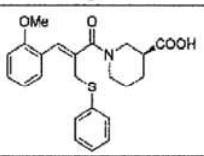
Compound	Structure
INF202	
INF203	
INF177	
INF180	
INF184	
INF185	
INF186	
INF187	

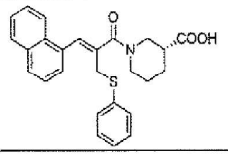
Compound	Structure
INF188	
INF192	
INF193	
INF194	
INF219	
INF220	
INF230	

Compound	Structure
INF231	

15. Compounds according to claims 10-13. selected from:

Compound	Structure
Br1	
Br2S	
Br2R	
Br4R	
Br7	
Br8R	
Br9R	
Br10R	

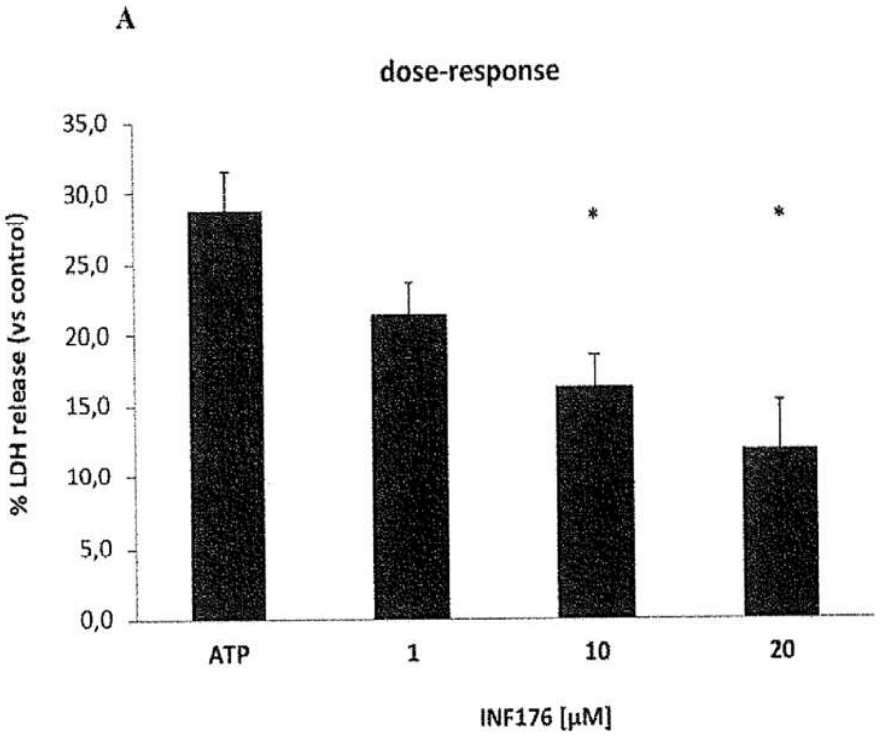
Compound	Structure
Br10S	
Br11R	
Br12	
Br13	
Br14R	
Br14S	
Br15	
Br16R	
Br17	
Br18R	
Br4S	
Br8S	

Compound	Structure
Br11S	

16. Compounds according to claims 10-15, for use as a medicament.

17. Pharmaceutical composition comprising at least one compound according to claims 10-15, and at least one pharmaceutically acceptable excipient.

Figure 1


A
dose-response
% LDH release (vs control)
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
*
*
ATP
1
10
20
INF176 [µM]

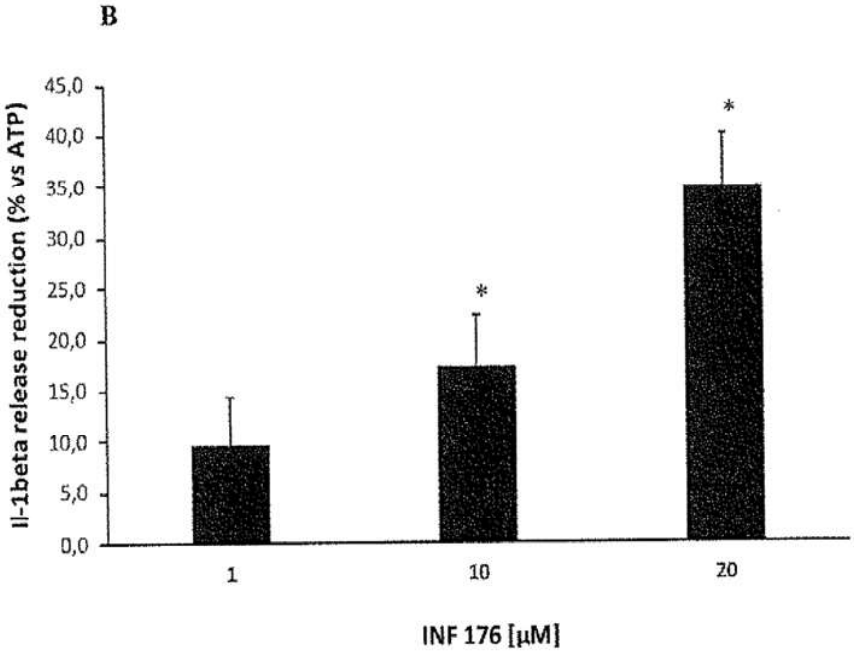
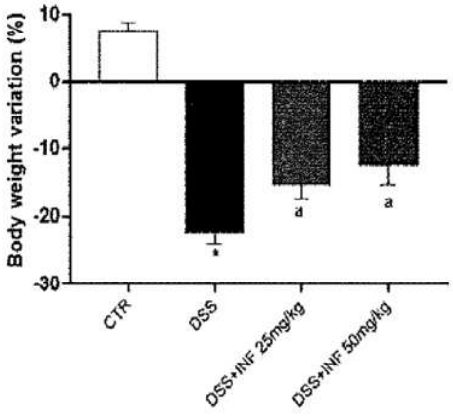

B
Il-1beta release reduction (% vs ATP)
45,0
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
*
*
1
10
20
INF 176 [µM]

Figure 2

A


Body weight variation (%)
10
0
-10
-20
-30
*
a
a
CTR
DSS
DSS+INF 25mg/kg
DSS+INF 50mg/kg

B

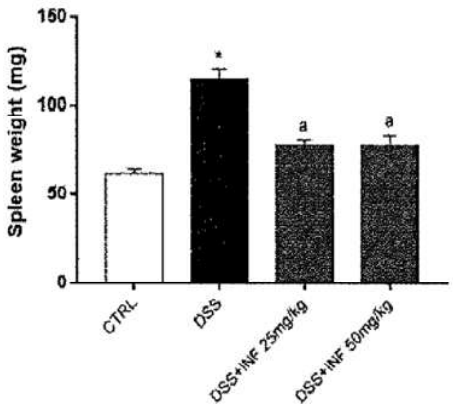

Spleen weight (mg)
150
100
50
0
*
a
a
CTRL
DSS
DSS+INF 25mg/kg
DSS+INF 50mg/kg

Figure 3

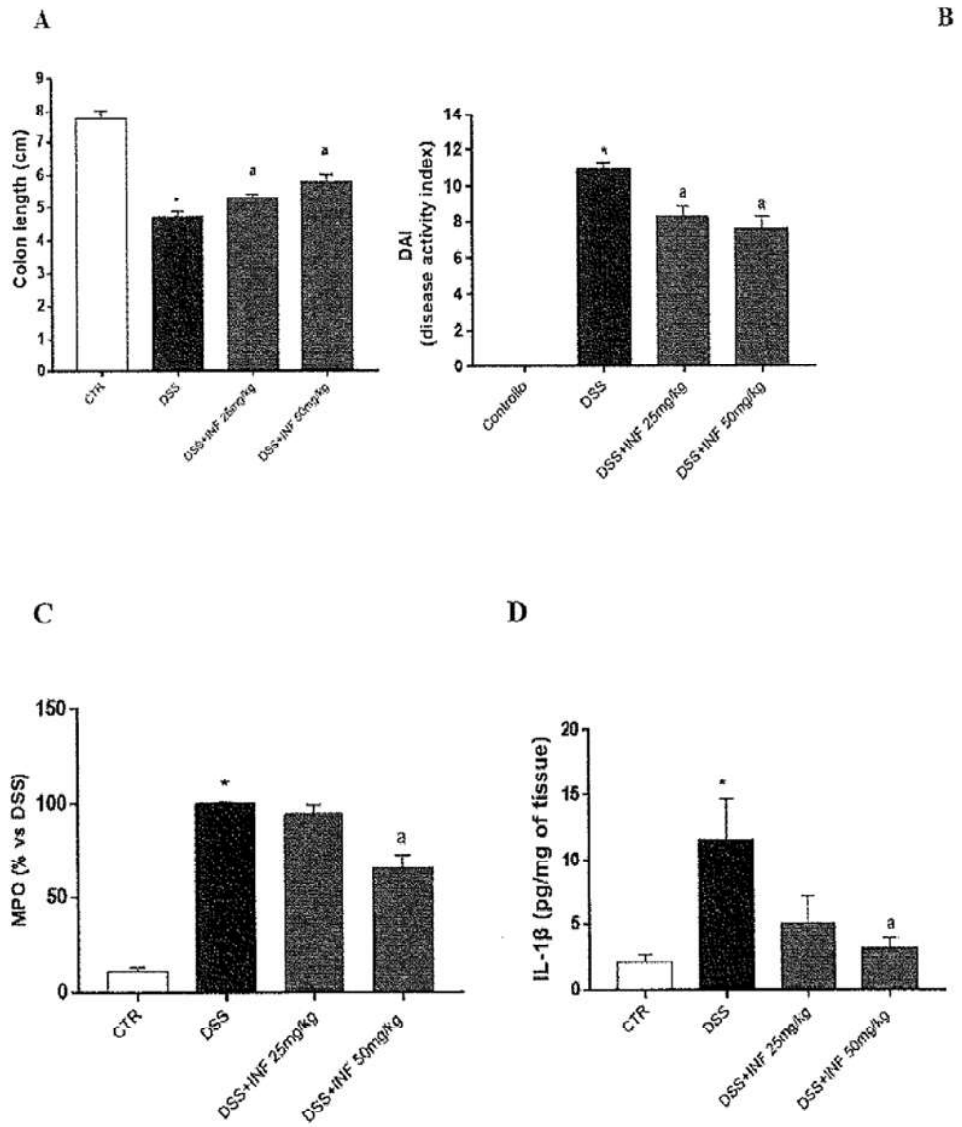

A
B
Colon length (cm)
CTR
DSS
DSS+INF 25mg/kg
DSS+INF 50mg/kg
DAI (disease activity index)
Controllo
DSS
DSS+INF 25mg/kg
DSS+INF 50mg/kg
C
D
MPO (% vs DSS)
CTR
DSS
DSS+INF 25mg/kg
DSS+INF 50mg/kg
IL-1β (pg/mg of tissue)
CTR
DSS
DSS+INF 25mg/kg
DSS+INF 50mg/kg

Figure 4

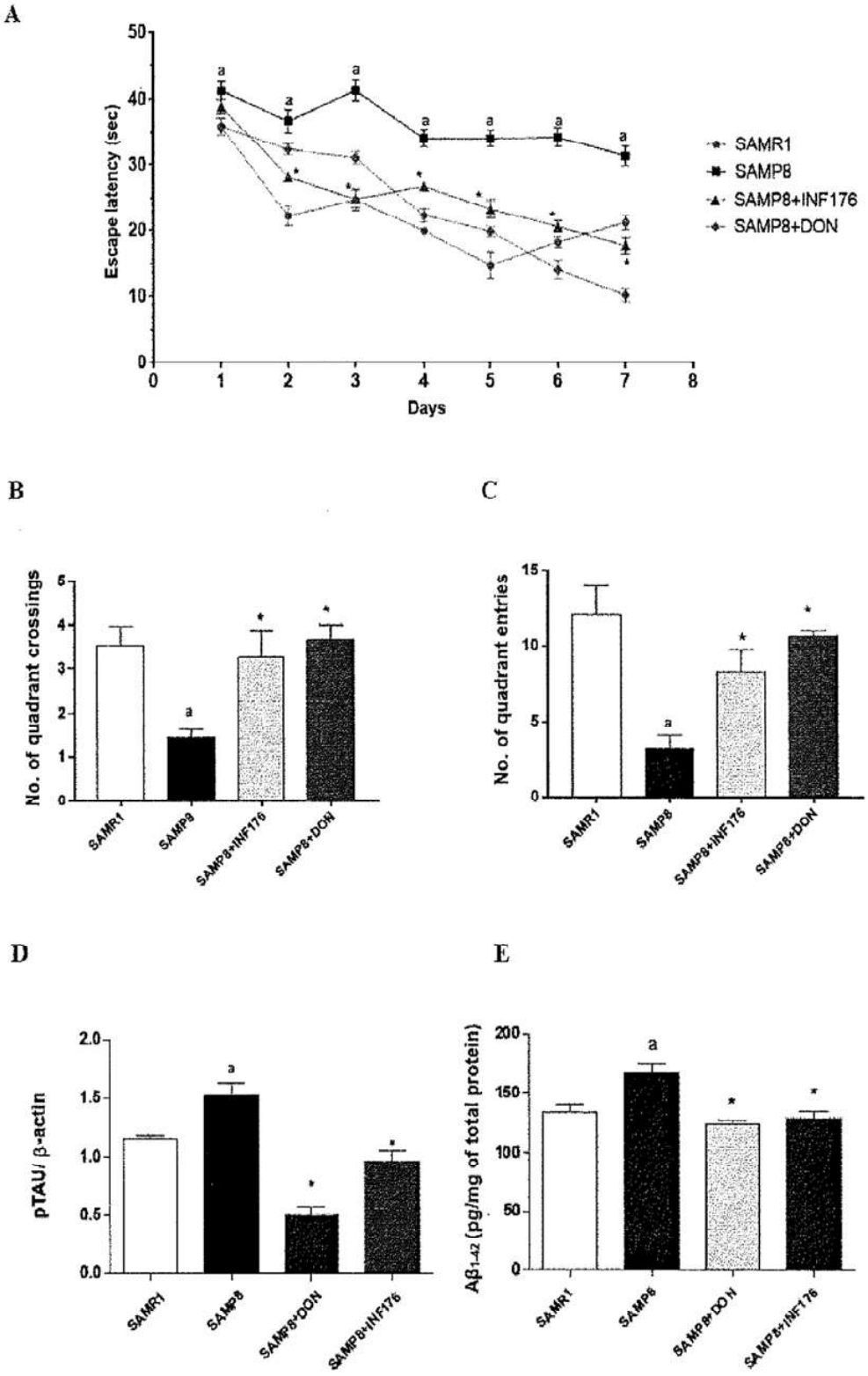

A
Escape latency (sec)
Days
SAMR1
SAMP8
SAMP8+INF176
SAMP8+DON
B
No. of quadrant crossings
C
No. of quadrant entries
D
pTAU/ β-actin
E
Aβ1-42 (pg/mg of total protein)

Figure 5

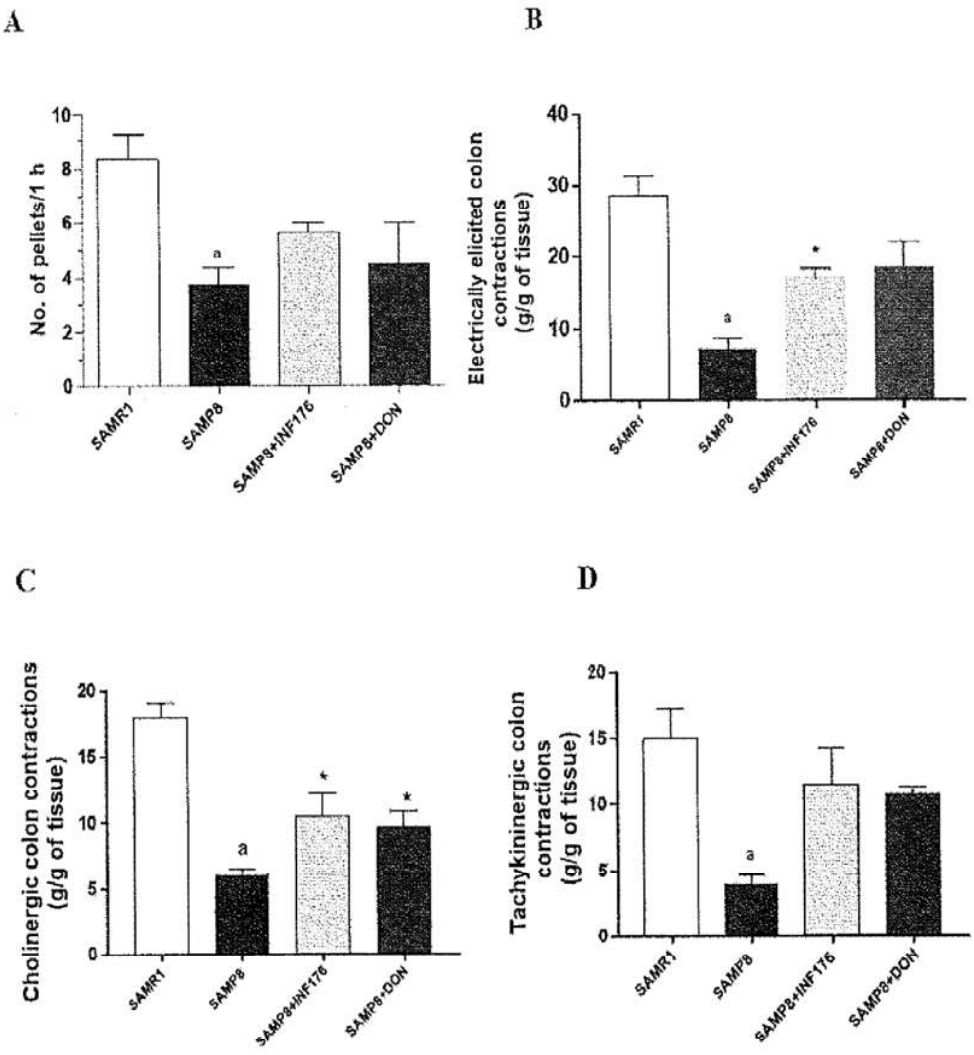

A
No. of pellets/1 h
10
8
6
4
2
0
a
SAMR1
SAMP8
SAMP8+INF176
SAMP8+DON
B
Electrically elicited colon contractions (g/g of tissue)
40
30
20
10
0
a
*
SAMR1
SAMP8
SAMP8+INF176
SAMP8+DON
C
Cholinergic colon contractions (g/g of tissue)
20
15
10
5
0
a
*
*
SAMR1
SAMP8
SAMP8+INF176
SAMP8+DON
D
Tachykininergic colon contractions (g/g of tissue)
20
15
10
5
0
a
SAMR1
SAMP8
SAMP8+INF176
SAMP8+DON

Figure 6

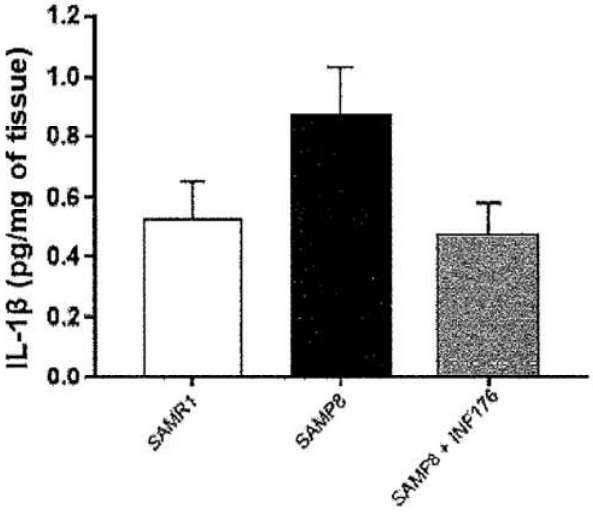

IL-1β (pg/mg of tissue)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
SAMR1
SAMP8
SAMP8 + INF176

Figure 7

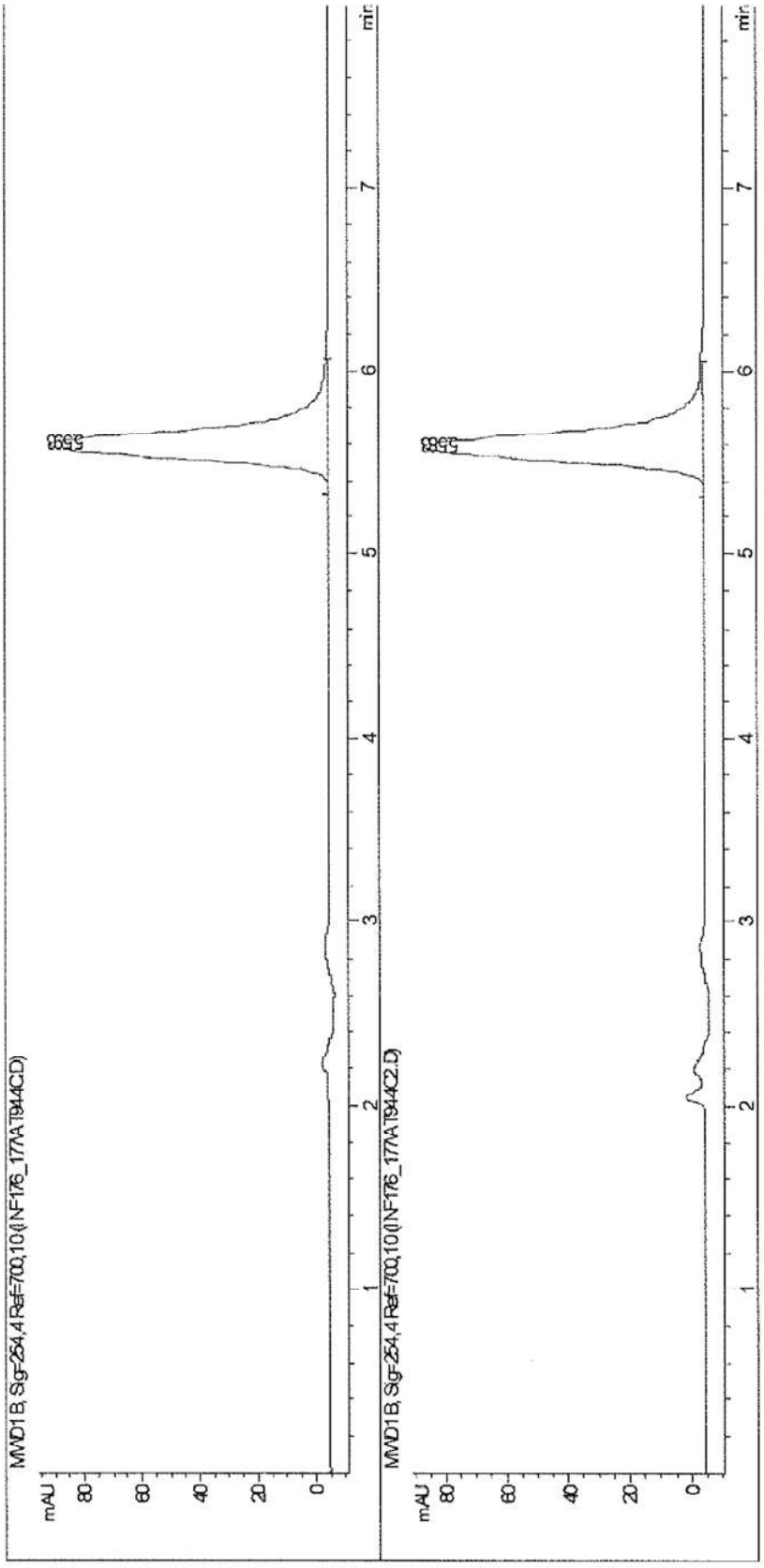
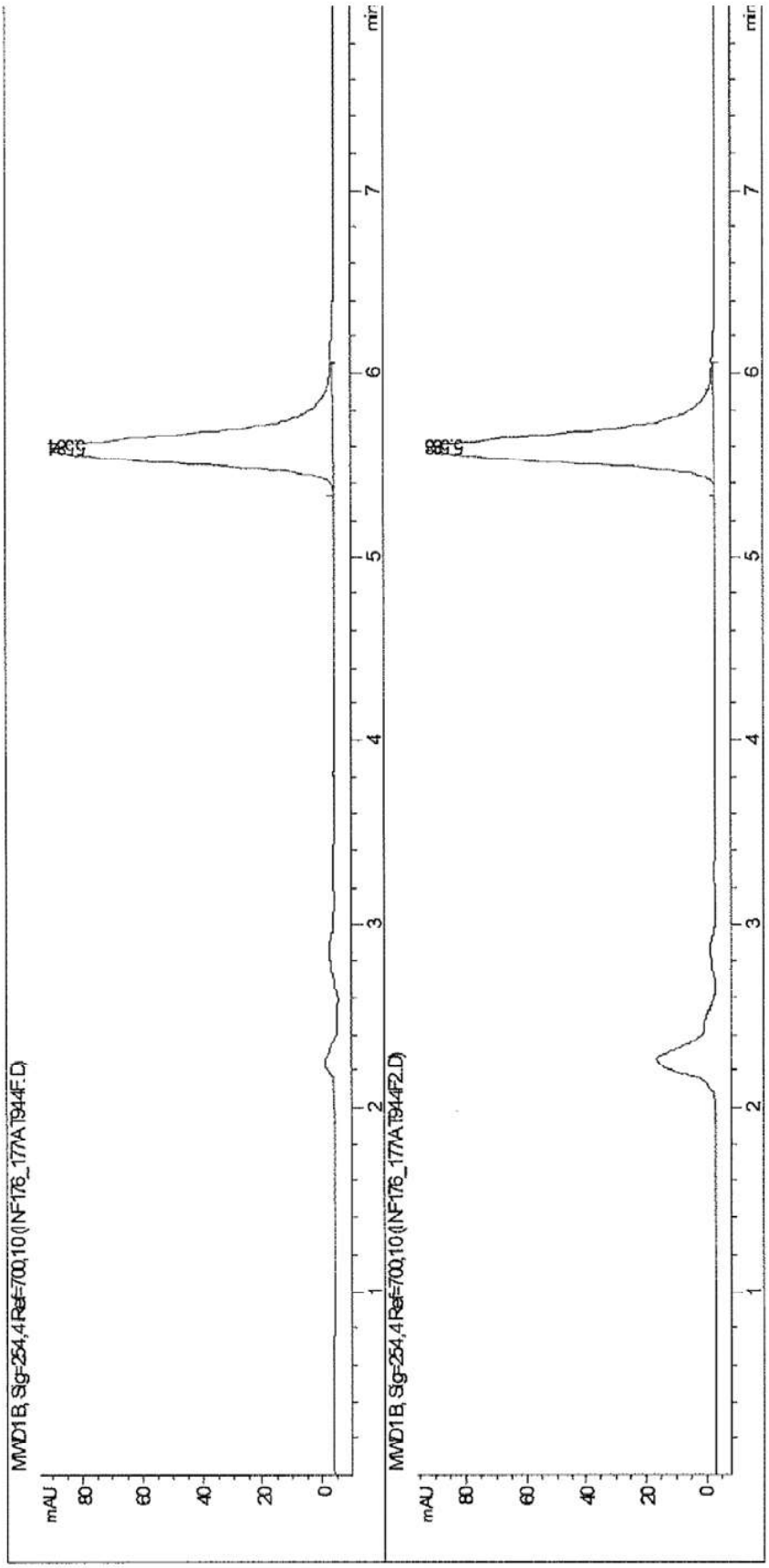

MWD1 B, Sig=254,4 Ref=700,10 (INF176_17ATB44CD)
mAU
5.530
MWD1 B, Sig=254,4 Ref=700,10 (INF176_17ATB44C2.D)
mAU
5.528

Figure 8


MWD1 B, Sig=254,4 Ref=700,10 (INF176_177A7944F.D)
mAU
MWD1 B, Sig=254,4 Ref=700,10 (INF176_177A7944F2.D)
mAU

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2022/054072

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D211/14 C07D207/06 A61K31/4422 A61P37/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CARVALHO BERNARDO BASBAUM PORTINHO DE PUGA ET AL: "On the development of a nucleophilic methylthiolation methodology", ORGANIC & BIOMOLECULAR CHEMISTRY , vol. 18, no. 28 22 July 2020 (2020-07-22), pages 5420-5426, XP055884619, ISSN: 1477-0520, DOI: 10.1039/D0OB01149E Retrieved from the Internet: URL:https://pubs.rsc.org/en/content/articlepdf/2020/ob/d0ob01149e Schemes 3-5 and scheme 6 at page 5423 Provisos exclude many compounds disclosed therein ----- -/--	10,12

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 July 2022	27/07/2022

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Goss, Ilaria

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2022/054072

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-13 (partially)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	XIE PEIZHONG ET AL: "Water-promoted C-S bond formation reactions", NATURE COMMUNICATIONS , vol. 9, no. 1 1 December 2018 (2018-12-01), XP055884626, DOI: 10.1038/s41467-018-03698-8 Retrieved from the Internet: URL:https://www.nature.com/articles/s41467-018-03698-8.pdf Not all compounds disclosed are excluded by the provisos; table 1 -----	10,12
X	PEREIRA ADRIANE A. ET AL: "Methylsulfenylation of Electrophilic Carbon Atoms: Reaction Development, Scope, and Mechanism", EUROPEAN JOURNAL OF ORGANIC CHEMISTRY , vol. 2017, no. 12 27 January 2017 (2017-01-27), pages 1578-1582, XP055884631, DE ISSN: 1434-193X, DOI: 10.1002/ejoc.201601613 Retrieved from the Internet: URL:https://api.wiley.com/onlinelibrary/tdm/v1/articles/10.1002%2Fejoc.201601613 compounds 19,28,29 -----	10,12
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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2022/054072

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	SHANMUGAM P ET AL: "Studies on montmorillonite K10-microwave assisted isomerisation of Baylis-Hillman adduct. Synthesis of E-trisubstituted alkenes and synthetic application to lignan core structures by vinyl radical cyclization", TETRAHEDRON, ELSEVIER SIENCE PUBLISHERS, AMSTERDAM, NL, vol. 60, no. 41, 4 October 2004 (2004-10-04), pages 9283-9295, XP004567088, ISSN: 0040-4020, DOI: 10.1016/J.TET.2004.07.067 Compounds of the series 5, whenever Z is CO2Et; page 9285 -----	10,12
X	RICHTER H ET AL: "Polymer Bound 3-Hydroxy-2-methylidenepropionic Acids. A Template for Multiple Core Structure Libraries", THE JOURNAL OF ORGANIC CHEMISTRY, AMERICAN CHEMICAL SOCIETY, vol. 64, no. 4, 1 January 1999 (1999-01-01), pages 1362-1365, XP003019289, ISSN: 0022-3263, DOI: 10.1021/JO981582U compounds 2,5,6,7 ----- -/--	10,12

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X	MATTHEW S. J. MANGAN ET AL: "Targeting the NLRP3 inflammasome in inflammatory diseases", NATURE REVIEWS DRUG DISCOVERY, vol. 17, no. 8, 20 July 2018 (2018-07-20), pages 588-606, XP055682104, GB ISSN: 1474-1776, DOI: 10.1038/nrd.2018.97 cited in the application the whole document Table 2, page 602 INF39 Table 2, page 601 OLT1177, referred to by the applicant	1-17
A	WO 2016/131098 A1 (UNIV QUEENSLAND [AU] ET AL.) 25 August 2016 (2016-08-25) cited in the application the whole document claims 18-57	1-17
A	BERTINARIA MASSIMO ET AL: "Development of covalent NLRP3 inflammasome inhibitors: Chemistry and biological activity", ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, ACADEMIC PRESS, US, vol. 670, 16 November 2018 (2018-11-16), pages 116-139, XP085797274, ISSN: 0003-9861, DOI: 10.1016/J.ABB.2018.11.013 [retrieved on 2018-11-16] cited in the application the whole document De novo design of NLRP3 inhibitors, page 124, b) scaffold Fig.8: product 10 and INF58	1-17
T	HE YUAN ET AL: "Mechanism and Regulation of NLRP3 Inflammasome Activation", TRENDS IN BIOCHEMICAL SCIENCES, vol. 41, no. 12, 2 July 2020 (2020-07-02), pages 1012-1021, XP029819150, ISSN: 0968-0004, DOI: 10.1016/J.TIBS.2016.09.002	1-9

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 1-13(partially)

The present independent claims 1 and 6 relate to an extremely large number of possible compounds and compounds for medical use. Support and disclosure are to be found, however, for only a very small proportion of the compounds claimed. In this respect, applicant's attention is drawn to the fact that all compounds provided show structural common features such as A meaning only 2-chlorophenyl and the definition of the cyclic elements being equally very limited compared to the definitions given in the claims.

Furthermore only one compound has been provided and tested, namely INF176 which is better represented by general formula (Ia). Considering the very less structural support and the very restricted support in terms of activity data, the generalization made in order to arrive at either claim 1 or claim 6 does not seem a reasonable disclosure of a plausible effect (shown for only 1 compound) at all and needs a consistent revision.

Non-compliance with the substantive provisions is such that a meaningful search of the whole claimed subject-matter of the claim could not be carried out. The extent of the search was consequently limited.

The search of claims 1 and 6 was restricted to those claimed compounds and compounds for medical use which appear to be supported and to a generalisation of their structural formulae.

The search has been carried out by limiting the general formula (I) according to claim 1 to the last definition of A (phenyl) and the last two preferred definitions given for R1 and R2 also relating to the point of attachment.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) PCT declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2022/054072

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