Molecularly imprinted polymers for corticosteroids: analysis of binding selectivity

UNIVERSITÀ DEGLI STUDI DI TORINO

MOLECULARLY IMPRINTED POLYMERS FOR CORTICOSTEROIDS: ANALYSIS OF BINDING SELECTIVITY

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Abstract

In this work we prepared a library of cortisol-imprinted polymers via a sequential approach by combining 10 different functional monomers and 5 porogen solvents. The best combinations of functional monomers, cross-linkers and porogen solvents in terms of cortisol binding were used to prepare three imprinted polymers — polyacrylamide-*co*-ethylene dimethacrylate (porogen: chloroform), poly-4-vinylpyridine-*co*-ethylene dimethacrylate (porogen: chloroform) and polyacrylamide-*co*-ethylene dimethacrylate (porogen: acetonitrile) — whose selectivity towards 10 synthetic corticosteroids and 4 natural steroidal hormones was tested. The experimental results obtained show how different combinations of functional monomers, cross-linkers and porogen solvents produce cortisol-imprinted polymers with very different selectivity patterns, and that a careful optimization of the pre-polymerization mixtures makes it possible to increase the number of target steroids recognized by the resulting imprinted polymer. Moreover, through the use of a Free-Wilson analysis of the binding selectivity, it has been possible to obtain insights on the steroidal structural motifs able to increase or decrease the molecular recognition of corticosteroids by the imprinted polymers.

Keywords: molecularly imprinted polymer, corticosteroids, cortisol, molecular recognition, selectivity, Free-Wilson analysis

1 – Introduction

The improper or illegal use of corticosteroidal hormones as veterinary drugs may result in unwanted residues in food products derived from livestock breeding (Courtheyn et al., 2002). To protect consumers' health, the European Union has made the use of corticosteroidal hormones in livestock breeding and aquaculture illegal (Commission Regulation, 1990). At the same time, maximum residue limits for these compounds in specific food matrices have been established (Council Directive, 1996a), and surveillance for the presence of residues of these drugs in food-producing animals and foods has been regulated (Council Directive, 1996b).

Confirmatory analysis requires affordable instrumental methods. Thus, as corticosteroids are not volatile substances, direct gas chromatography/mass spectrometry (GC/MS) analysis of biological samples should be ruled out. The liquid chromatography/mass spectrometry (LC/MS) approach seems to be the most affordable approach (Deventer et al., 2006; Mazzarino and Botre, 2006; Thevis and Schänzer, 2007). However, the direct detection of target corticosteroids in complex biological matrices can be a difficult task, and sample clean-up treatments are frequently necessary before performing the instrumental analysis. With the aim of simplifying the clean-up step, in recent years there has been a significant increase in the use of highly selective solid phases for the extraction of analytes in complex samples. Besides the widespread use of immunoaffinity sorbents (Pichon et al., 2002), extraction based on molecularly imprinted polymers — the so called "molecularly imprinted solid phase extraction" (MISPE) technique — has been proposed as a very efficient and innovative approach. In fact, this technique is particularly suitable for clean-up applications where analyte selectivity in the presence of very complex samples represents the main problem (Tamayo et al., 2007; Kloskowski et al., 2009; Laskov and Jandera, 2009).

To extract synthetic corticosteroids from biological samples using the MISPE approach efficiently, an imprinted polymer should be able to selectively recognize (as the main analytical target) not a single, well defined substance, but a quite broad class of drugs, characterized by the presence of several different substituents (halogen atoms, methyl and hydroxy functional groups, double bonds) placed typically in positions 6, 9, 16, 17 or 21 on the same corticosteroidal skeleton. Thus, because of almost similar molecular structures, it is necessary to use a corticosteroid representative of the whole class of molecules as a template molecule. At the same time, the chosen template should not represent a significant analytical target in itself because of the so called "bleeding effect" typical of most of the imprinted polymers (Ellwanger et al., 2001), where the slow release of residual template molecules during the extraction process could contaminate samples potentially containing corticosteroids of analytical concern with template molecules.

In past years several papers have been published dealing with molecular imprinting of corticosteroids such as cortisol and corticosterone (Ramström et al., 1996; Ramström et al.,

1998; Baggiani et al., 2000), but no attempts were made to obtain molecularly imprinted polymers with group selectivity. In this work, we report the development of molecularly imprinted polymers with selective binding properties towards several synthetic corticosteroids. Cortisol is a steroid hormone present in the corporeal fluids of mammals, and is not routinely searched for when attempting to detect food contamination. It was chosen as a template molecule as it represents a natural corticosteroid very similar to most of the synthetic corticosteroids which are an interesting target for analytical determinations. Thus, it could represent a good mimic template to prepare corticosteroid-selective imprinted polymers.

2 – Experimental

2.1 – Materials

2,2-Dimethoxy-2-phenylacetophenone (DMPA), all the functional monomers (acrylic acid, AA; allylamine, ALA; acrylamide, AM; N,N-diethylaminoethylmethacrylate, DEAEM; N,N-dimethylaminoethylmethacrylate, DMAEM; ethyleneglycole methacrylate phosphate, EMP; 2-hydroxyethylmethacrylate, HEMA; methacrylic acid, MAA; N-vinylpyrrolidone, NVP; styrene, ST; 4-vinylpyridine, 4VP; see figure 1-SI, supplemental information, for structures), cross-linkers (divinylbenzene, DVB; ethylene dimethacrylate, EDMA; glycerol dimethacrylate, GDMA; pentaerithrytole tetraacrylate, PETA; pentaerithrytole triacrylate, PETRA; triacryloylhexahydro-s-triazine, TAT; trimethylolpropane trimethacrylate, TRIM; see figure 2-SI, supplemental information, for structures) and steroids (beclomethasone, BECL; betamethasone, BETA; cortisone, CONE; cortisol, CORT; corticosterone, CSTONE; dexamethasone, DEXA; fludrocortisone, FLUD; flumetasone, FLONE; fluorometholone, FMET; 6α-methylprednisolone, MPRED; prednisolone, PRED; progesterone, PROG; prednisone, PRONE; testosterone, TEST; triamcinolone, TRIA; see figure 1 for structures) used in this work were from Sigma–Aldrich–Fluka (Milan, Italy). Acetic acid and all the organic solvents were from VWR International (Milan, Italy).

Polymerization inhibitors in monomers were removed by cleanup on activated alumina columns. Steroid stock solutions were prepared by dissolving 20.0 mg of substance in 4.00 ml of acetonitrile and stored in the dark at -20 °C. All the solvents were of HPLC quality, other chemicals were of analytical grade.

The high-performance liquid chromatography apparatus (L-6200 constant-flow binary pump, L-4250 UV-Vis detector, Rheodyne 7100 six-port injection valve provided with 5ml injection loop, D7000 data acquisition system) was from Merck-Hitachi (Darmstadt, Germany).

2.2 – Synthesis of molecularly imprinted polymers

The imprinted polymers were prepared in according to a method previously reported in literature (Baggiani et al., 2000), with minor modifications. In 5 ml thick wall borosilicate glass vials, solutions with molar ratio template : functional monomer : cross-linker 1+3+27 were

prepared by dissolving 50.0 mg (0.138 mmoles) of cortisol in 1.50 ml of anhydrous porogenic solvent. Then, 0.414 mmoles of functional monomer, 3.72 mmoles of cross-linker and 5 mg (19.5 μmoles) of DMPA were added. DMPA was used as photoinitiator because of its insensibility to the presence of oxigen in the polymerization mixture (Mijangos et al., 2006), thus avoiding the degassing step. The composition of each of the imprinted polymers are reported in table 1. The vials were sonicated in an ultrasonic bath for 10 minutes, sealed, and the mixtures were photopolymerised at 4 °C for 30 hours using a 200 W medium-pressure mercury lamp. The bulk polymers obtained were broken with a steel spatula, grounded in a mechanical mortar and mechanically wet-sieved to 15–38 μm. Then, the template was extracted by packing the polymers in polypropylene SPE columns and exhaustively washing with acetic acid – methanol 1+9 (v/v) till no template was detectable by HPLC analysis of eluate. No efforts were made to measure the amount of template molecule recovered. The washed polymers were dried under vacuum at 70 °C for 2 hours and stored in a desiccator. Blank polymers were prepared in the same experimental conditions by omitting the template.

2.3 – Equilibrium binding assay

About 10 mg of imprinted or non-imprinted polymers were exactly weighed in 3 ml flat bottom glass vials, suspended in 500 μl of acetonitrile and sonicated for 10 minutes. Then, 500 μl of 40 μg/ml steroid solution in acetonitrile was added and the vials were incubated for 24 hours at 21 °C under continuous agitation on a rocking table. The vials were centrifuged at 3500 rpm for 20 minutes and 500 μl aliquots were sampled, filtered through 0.22 μm polypropylene filters and transferred into HPLC autosampler vials. To evaluate the reproducibility of the binding assay, each partition was repeated three times and the amount of free steroid was evaluated as the average of the single values measured.

Reverse phase HPLC analysis was used for quantification of the free steroid. Chromatographic separation was performed on a monolithic octadecyl-silica column (Chromolith Performance RP-18, 100 mm x 4.6 mm, VWR International). The detection wavelength was 254 nm. The mobile phase consisted of acetonitrile–water 6+4 (v/v). The mobile phase flow-rate was set to 1.00 ml/min. Reference standard solutions for steroids of concentration 0.1, 0.25, 0.5, 1, 2.5, 5 and 10 μg/ml were analysed three times consecutively and peak areas were plotted against concentration. A calibration curve was drawn using weighed linear regression (weight = 1/conc.).

The amount of steroid bound to the polymers was calculated by subtracting the concentration of free steroid from the known initial concentration. The net bound steroid quantity was calculated as the amount of steroid bound to the imprinted polymer subtracted from the amount of steroid bound to the related non-imprinted polymer. The selectivity factor (α) was defined as an index of polymer selectivity towards steroids related to the template molecule. It

was calculated as $B_{steroid}/B_{cortisol}$, where B is the net steroid bound.

2.4 - Free-Wilson analysis

All statistical calculations were performed using SigmaStat 3.0 software (Systat, Richmond, CA, USA). To develop affordable regression models, a backward stepwise linear regression approach was used to select multicollinearity-free subsets of molecular descriptors with a minimal number of independent variables. In this approach, all of the variables comprising a descriptor subset were entered into a multiple linear regression equation. Then, the variables that contributed the least to the regression (those where the values of the F-test of significance were lower than a certain value) were removed progressively from the equation. After each variable was removed, the F-value of each removed variable was checked again, and any variables with an F-value above a certain value were re-entered into the equation. The process was iterated until removing or adding variables did not improve the prediction of the dependent variable. Finally, multiple linear regression models were calculated using the variables selected previously.

3 - Results and discussion

3.1 – Selection of the best binding polymer

The synthesis of a successful imprinted polymer depends on many experimental variables influencing the polymerization process as well as the subsequent template molecular recognition. While the effect of several physical factors — such as temperature of polymerization, type of radical initiation and polymerization time — seems to be firmly established (Piletska et al., 2009), the optimal chemical composition of the pre-polymerization mixture is still matter of empirical knowledge. In fact, in spite of several successful attempts to rationalize the molecular recognition properties of imprinted polymers by computational methods (Nicholls et al., 2009), at present, the trial-and-error approach is the most preferred.

As concerns molecular imprinting of corticosteroids, published papers describe the use of methacrylic acid as a functional monomer, ethylene dimethacrylate as cross-linker and tetrahydrofurane or chloroform as porogenic solvents (Ramström et al., 1996; Ramström et al., 1998; Baggiani et al., 2000). However, there are no plausible reasons to exclude out of hand several other monomers and solvents in the preparation of corticosteroid-imprinted polymers. Unfortunately, the complete screening of the binding properties of polymers prepared in accordance with all the possible combinations of monomers and solvents involves the preparation of large and rapidly expanding combinatorial libraries of imprinted (and, naturally, non-imprinted) polymers. Considering the monomers and the solvents used in this work (11 functional monomers, 7 cross-linkers, 5 solvents, see table 1), an exhaustive experimental screening of all those possible combinations is not practical, as it requires the preparation, work-up and screening of 770 different polymers.

To avoid this drawback, we planned to screen a reduced number of polymers, developing a three-step approach. Firstly, we prepared 11 imprinted polymers where the functional monomer was systematically changed (table 1, entries MIP01 – MIP11), while the nature of the cross-linker (EDMA) and porogenic solvent (chloroform) was taken from a successful, previously published, polymer formulation (Baggiani et al., 2000). After the functional monomer corresponding to the imprinted polymer with the best binding properties towards the template molecule was identified, in the 2nd step this functional monomer (AM) and the porogenic solvent (chloroform) were taken as they were, while the effect of the cross-linker was considered by preparing 6 imprinted polymers in which the cross-linker was systematically changed (table 1, entries MIP12 – MIP17). In the 3rd and final step the functional monomer and the cross-linker combination corresponding to imprinted polymers with good binding properties were taken as they were and 4 imprinted polymers where prepared by changing the porogenic solvent (table 1, entries MIP18 – MIP21).

The results reported in table 1 show that, as far as the functional monomers are concerned, it is very difficult to extrapolate the binding behaviour of the resulting imprinted polymer from the structure and the properties of the functional monomer used to prepare it. Different monomers sharing good hydrogen bond acceptor and donor properties behave in different manners. In fact, polymers containing acrylamide (AM) or 4-vinylpyridine (4VP) show the best binding properties, while acrylic acid results in one of the last efficient functional monomers and the polymer prepared with methacrylic acid (MAA) — which is one of the most commonly used functional monomers and is reported in literature for several successful corticosteroid imprintings (Ramström et al., 1996; Ramström et al., 1998; Baggiani et al., 2000) — shows a less marked binding ability and is comparable with many other functional polymers considered in this study. Surprisingly, ethyleneglycole methacrylate phosphate (EMP) and 2-hydroxyethyl methacrylate (HEMA) — two monomers described as very efficient in molecular imprinting of steroids (Sreenivasan, 1998; Kugimiya et al., 2001) — did not behave better than methacrylic acid, while tertiary amines (DEAEM and DMAEM) and N-vinylpyrrolidone (NVP) do not show any binding difference between imprinted and non-imprinted polymers.

Contrarily, the effect of cross-linker structure on the template rebinding is not completely unexpected. In fact, the most commonly used monomer in molecular imprinting — ethylene dimethacrylate (EDMA) — turned out to be the best cross-linker selected, with a net binding markedly better than other apparently promising monomers, such as trimethylolpropane trimethacrylate (TRIM) and divinylbenzene (DVB). In this case, the cross-linker polarity and flexibility do not seem to be factors relevant in the binding performances, while TRIM — a cross-linker currently considered as a valid alternative to EDMA — behaves far less better.

As regards the nature of the porogenic solvent, acetonitrile (polar and weak hydrogen bond acceptor) shows itself to be more efficient than N,N-dimethylformamide and tetrahydrofurane

(polar and hydrogen bond acceptors) in generating a polymer with molecular recognition properties towards cortisol. However, it should be taken into account that rebinding was measured in acetonitrile, and thus, as a consequence of the well known "porogen memory effect" (Spivak et al., 1997; Yoshizako et al., 1998), it is not strange that a polymer prepared in the same solvent will show better binding properties. Finally, it should be considered that, surprisingly, toluene (non-polar and non-hydrogen bond forming) turned out to be completely inefficient in generating a polymer with binding properties towards the target molecule, whilist chloroform (non-polar and weak hydrogen bond donor) turned out to be a very efficient solvent, comparable to acetonitrile.

3.2 – Analysis of binding selectivity

The screening approach described in the previous section resulted in three combinations of functional monomers, cross-linkers and porogenic solvents with a good net cortisol binding: MIP03, MIP11 and MIP18. The main goal of this paper consists in the identification of an imprinted polymer with good recognition properties towards several cortisol-related steroids. Thus, the selectivity towards 12 cortisol-related and 2 non-related (progesterone and testosterone) steroids was measured for all these polymers.

From the results reported in table 2 it is clear that different functional monomers and porogenic solvents produced polymers with different selectivity properties. In fact, it is possible to observe that while MIP18 recognized well (α>0.7) 8 out of 12 template-related steroids, MIP03 and MIP11 recognized only 1 and 4 out of 12, respectively. On the contrary, as regards the number of poorly recognized cortisol-related steroids (α<0.3), MIP11 showed recognition for all the cortisol-related steroids considered, whilst MIP18 did not recognize 2 out of 12 steroids and MIP03 showed itself to be the least efficient polymer, with 8 out of 12 steroids not recognized. Moreover, as far as the recognition of steroids not strictly related to cortisol (progesterone and testosterone) is concerned, the polymers showed significant selectivity towards cortisol-related steroids, as only MIP11 showed a limited recognition for testosterone, and nothing significant for progesterone.

In the attempt to explain these selectivity patterns in terms of molecular structural motifs conditioning the molecular recognition we performed a Free-Wilson analysis of the results reported in table 2. The Free-Wilson analysis consists is a multivariate approach where the presence/absence of a given substituent on a molecular skeleton common to a set of homogenous molecules can be directly related to the correspondent binding behaviour. This approach is based on the assumption that each substituent makes an additive and constant contribution to the binding behaviour regardless of substituent variation in the rest of the molecule. Thus, when performing a multiple linear regression on the binding data vs. a "substituent matrix" (whose values are set to 1 when a substituent is present on a given molecule, and 0 otherwise — see supplemental information, table 1-SI) it is possible to obtain a

linear equation whose coefficients can be related, in terms of sign and magnitude, to the effect of a given substituent in molecular recognition (Kubinyi, 1993).

The analysis of the selectivity data reported in table 2 produced a set of multiple linear regression models relating the MIP selectivity to the presence of certain substituents on the steroidal ring:

α(MIP03) = (0.201±0.0879) – (0.440±0.105) $\Delta^{1,2}$ - (0.359±0.122) 11C=O + (0.569±0.131) 17αOH

R^2 = 0.691, SEE=0.152, n=15, F=8.184, P=0.004, PRESS=0.810

α(MIP11) = (0.428±0.0858) – (0.536±0.109) $\Delta^{1,2}$ + (0.282±0.124) 6αMe + (0.309±0.124) 16βMe + (0.599±0.121) 17αOH

R^2 = 0.778, SEE=0.149, n=15, F=8.752, P=0.003, PRESS=0.655

α(MIP18) = (0.184±0.0718) – (0.526±0.0813) $\Delta^{1,2}$ + (0.225±0.0935) 6αMe + (0.297±0.0783) 11βOH + (0.544±0.0981) 17αOH

R^2 = 0.877, SEE=0.116, n=15, F=17.758, P<0.001, PRESS=0.248

The equations obtained were statistically significant, with values of multiple correlation coefficient (R^2), F-test of significance, standard estimated error (SEE), significance level of the model (P) and predicted residual error sum of squares (PRESS) acceptable for a qualitative analysis of the models. Moreover, the plots of calculated vs. experimental selectivity (supplemental information, figure 3-SI) showed a reasonable ability of the models to correlate the presence of certain substituents on the steroidal skeleton with the dependent variables, while the plots of residuals vs. calculated α values (supplemental information, figure 4-SI) showed no major outliers in the descriptor dataset, and the plots of Cook's distances (supplemental information, figure 5-SI) showed that all of the elements in the dataset had nearly the same influence on the regression coefficients.

As concernis the coefficients of the regression equations, it is possible to observe that the presence of a double bond in position $\Delta^{1,2}$ on the steroidal A-ring produces a significant decrease of recognition (coefficient $\Delta^{1,2}$<0). This can be explained by considering that the template molecule has no such structural feature, while the main part of the synthetic corticosteroids considered in this work has it. Thus, as the double bond in position $\Delta^{1,2}$ forces the A-ring into a planar configuration different from the configuration of the A-ring for cortisol (see figure 2), this feature can be considered one of the main discriminating structural differences of steric nature between template and target molecules.

As cortisol and most target molecules present 3 hydroxyls in position 11β, 17α and 21 (except for fluorometholone), suitable for hydrogen bonding with functional monomers, it is reasonable to expect that the presence of these molecular features reinforce the molecular recognition behaviour. In fact, this is verified for all the equations, where there are positive regression coefficients corresponding to the presence of hydroxyls in position 11β (MIP18) and 17α (MIP03, MIP11 and MIP18). Moreover, the presence of a keto instead of a hydroxyl group in position 11 weakens the molecular recognition, but only for MIP03.

Finally, as regards the effect of methyl or halogen substituents, the additional presence of a methyl group in position 6α compared to cortisol reinforces the molecular recognition for MIP11 and MIP18, while the presence of a methyl substituent in position 16β reinforces the molecular recognition, but only for MIP11. On the other hand, the presence of a halogen substituent in position 9 does not seem to influence the molecular recognition.

4 - Conclusions

The experimental results obtained in this work show that different combinations of functional monomers, cross-linkers and porogen solvents produce cortisol-imprinted polymers with very different selectivity patterns, and that a careful optimization of the pre-polymerization mixtures makes possible to increase the number of target steroids recognized by the resulting imprinted polymer. Through the use of a quantitative structure-activity relationship approach, it has been possible to correlate the binding selectivity of the imprinted polymers with the molecular features of the examined steroids, obtaining insights on the steroidal structural motifs able to increase or decrease the molecular recognition of corticosteroids by the imprinted polymers. In particular, concerning the use of cortisol as mimic template, the Free-Wilson analysis indicates that the absence of a double bond in position $\Delta^{1,2}$ on the steroidal ring A decreases the molecular recognition towards many synthetic corticosteroids. Thus, it is reasonable that a "modified" cortisol, i.e. $\Delta^{1,2}$-dehydrocortisol, should be a better template. Studies are ongoing.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version.

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Tables

Table 1: composition and net cortisol binding of imprinted polymers

polymer	functional monomer	cross-linker	porogen	net cortisol binding, μg/g
MIP01	AA	EDMA	$CHCl_3$	2.23
MIP02	ALA	EDMA	$CHCl_3$	6.56
MIP03	AM	EDMA	$CHCl_3$	16.02
MIP04	DEAEM	EDMA	$CHCl_3$	0.00
MIP05	DMAEM	EDMA	$CHCl_3$	0.00
MIP06	EMP	EDMA	$CHCl_3$	4.70
MIP07	HEMA	EDMA	$CHCl_3$	4.94
MIP08	MAA	EDMA	$CHCl_3$	7.09
MIP09	NVP	EDMA	$CHCl_3$	0.00
MIP10	ST	EDMA	$CHCl_3$	6.89
MIP11	4VP	EDMA	$CHCl_3$	11.59
MIP12	AM	DVB	$CHCl_3$	7.68
MIP13	AM	TAT	$CHCl_3$	0.00
MIP14	AM	GDMA	$CHCl_3$	5.67
MIP15	AM	PETA	$CHCl_3$	2.00
MIP16	AM	PETRA	$CHCl_3$	2.37
MIP17	AM	TRIM	$CHCl_3$	1.00
MIP18	AM	EDMA	MeCN	17.43
MIP19	AM	EDMA	DMF	0.65
MIP20	AM	EDMA	THF	0.00
MIP21	AM	EDMA	toluene	1.00

Table 2: selectivity factor (α) measured for 14 cortisol-related steroids. MIP03: polyacrylamide-*co*-ethylene dimethacrylate (porogen: chloroform); MIP11: poly-4-vinylpyridine-*co*-ethylene dimethacrylate (porogen: chloroform); MIP18: polyacrylamide-*co*-ethylene dimethacrylate (porogen: acetonitrile). Bold: steroids well recognized by the polymer(α>0.7). Underlined: steroids poorly recognized by the polymer (α<0.3).

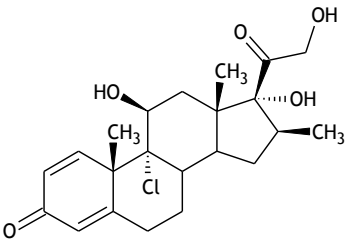
	MIP03	MIP11	MIP18
beclomethasone	0.40	**0.95**	**1.06**
betamethasone	0.27	0.65	**0.71**
cortisone	0.23	**0.96**	**1.06**
cortisol (template)	**1.00**	**1.00**	**1.00**
corticosterone	0.30	0.51	**0.78**
dexamethasone	0.29	0.56	0.64
fludrocortisone	**0.72**	**1.12**	**1.46**
flumetasone	0.21	0.55	0.64
fluorometholone	0.39	0.60	**0.92**
6α-methylprednisolone	0.53	**0.94**	**1.13**
prednisolone	0.13	0.46	0.43
progesterone	0.13	0.23	0.14
prednisone	0.16	0.33	0.35
testosterone	0.18	0.55	0.28
triamcinolone	0.24	0.56	**0.71**

Legend of figures

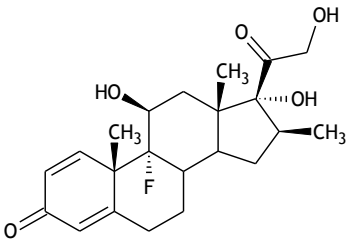
Figure 1: steroids used in this work. BECL: beclomethasone; BETA: betamethasone; CONE: cortisone; CORT: cortisol (template); CSTONE: corticosterone; DEXA: dexamethasone; FLUD: fludrocortisone; FLONE: flumetasone; FMET: fluorometholone; MPRED: 6α-methylprednisolone; PRED: prednisolone; PROG: progesterone; PRONE: prednisone; TEST: testosterone; TRIA: triamcinolone

Figure 2: comparison of steroidal ring A structure in absence (left image) and presence (right image) of a double bond in position 1,2

Figure 1



BECL



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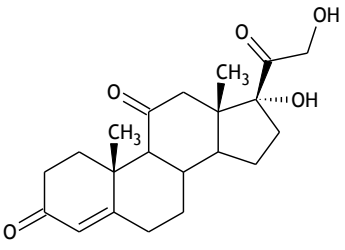
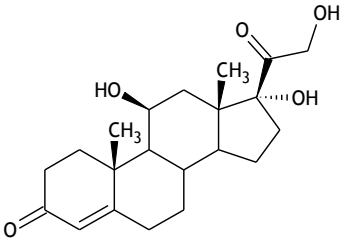


CONE



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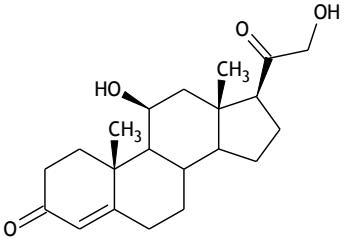

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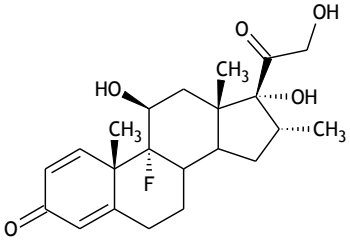

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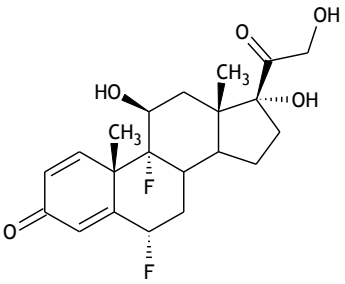

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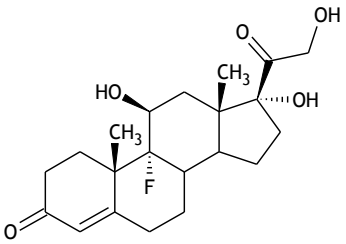

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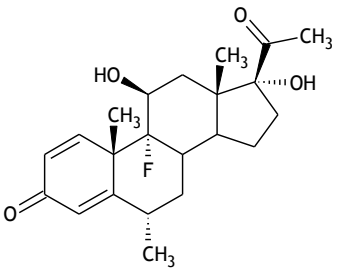

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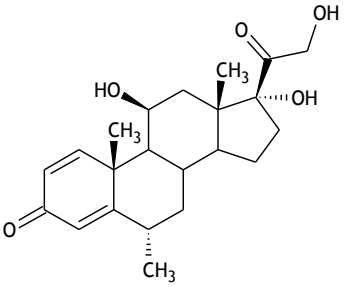

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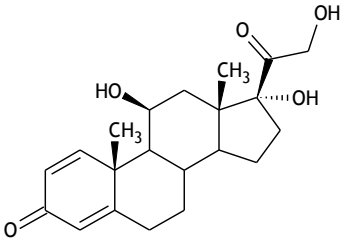

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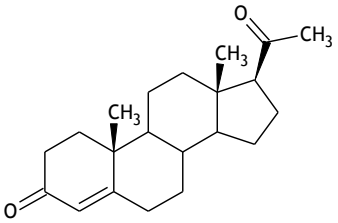
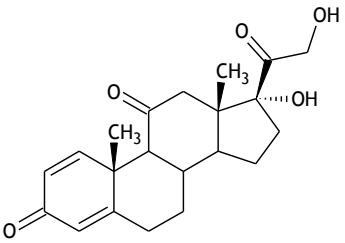


PROG



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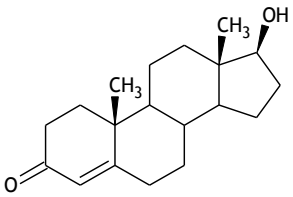
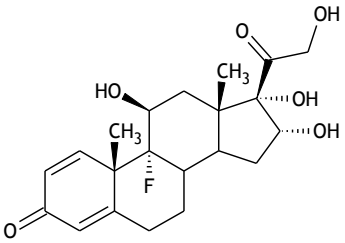


TEST



TRIA

Figure 2