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Effect of the mimic structure on the molecular recognition properties of molecularly imprinted polymers for ochratoxin A prepared by a fragmental approach

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ABSTRACT

In this work the fragmental approach was used to prepare several molecularly imprinted ethylene dimethacrylate-co-methacrylic acid polymers with molecular recognition towards the mycotoxin ochratoxin A, with the aim of searching for simpler mimic templates than the well-known N-(4-chloro-1-hydroxy-2-naphthoylamido)-(L)-phenylalanine. The screening for binding of two different kinds of ochratoxin-related molecules was performed by HPLC analysis. Ochratoxin A and the mimic templates were eluted in acetonitrile - acetic acid (0.1% v/v) and the imprinting factor was measured for all the ligands on all the columns packed with the imprinted polymers. The experimental results show that changes to the amino acidic sub-structure or the presence/absence of a chlorine atom in position 4 on the naphthalene ring system does not affect the molecular recognition of ochratoxin A by the resulting imprinted polymer. On the contrary, the presence of the bulky naphthalene ring system in the mimic template seems to be necessary to preserve the molecular recognition of ochratoxin. This binding behavior was found to be compatible with *in silico* simulations of the complexation between some of the mimic templates and molecules of methacrylic acid. The use of the mimic template N-(1-hydroxy-2-naphthoylamido)-(L)-phenylalanine seems to represent a synthetically simple approach to the preparation of imprinted polymers with molecular recognition properties towards ochratoxin A.

1. INTRODUCTION

One of the main critical points associated with the use of molecularly imprinted polymers (MIPs) for analytical applications is related to the residual template not being completely removed from the polymeric matrix and slow leakage during its use. Such template loss (“bleeding”) is usually detected at very low levels and it represents a significant source of interferences and systematic errors.

Up to today, an efficient strategy to overcome this drawback has been the use of an analyte mimic of the template molecule in the so-called “template mimic” approach [1]. It consists of the use as a template of a structural analogue of the molecule which is of analytical interest.

When the analogue is difficult to synthesize, expensive, or simply not available as a commercial product, it could be convenient to use commercially available substances that are less strictly related to the target analyte, but having a more limited molecular recognition effect [2-5]. In this case, in fact, the structural differences between the analyte and the mimic template become significant, and the similarity between molecules remains confined to the overall molecular shape and the preservation of substituents able to form non-covalent interactions with the binding sites.

In the past, several examples of MIPs binding the mycotoxin Ochratoxin A (OTA, **1**) have been published [6-8], and this mycotoxin has been frequently considered as target analyte for MISPE of complex samples [9-15]. When taken into consideration, the problem of polymer bleeding has been successfully addressed by using the rationally designed mimic N-(4-chloro-1-hydroxy-2-naphthoylamido)-(L)-phenylalanine (CHNA-Phe, **3**) as a template molecule [6]. Notwithstanding its success, the use of CHNA-Phe as a mimic poses the problem of its synthesis. In fact, the main precursor of this template, 4-chloro-1-hydroxy-2-naphthoic acid (CHNA), is not commercially available, and should be prepared from 1-hydroxy-2-naphthoic acid by regioselective chlorination.

In this work, with the aim of finding simpler mimic compounds and obtaining insights into the effect of the mimic template structure on the OTA recognition by imprinted polymers, we considered several molecules related to CHNA-Phe and obtained them by progressive truncation of the amino acid substructure or elimination of substituents on the naphthoyl

ring system, thus determining the effect on the molecular recognition behavior of progressively simpler template molecules designed via a fragmental approach.

2. MATERIALS AND METHODS

2.1. Materials

Ochratoxin A, 97% purity grade, was obtained from Fermentek (Jerusalem, Israel). 4-Chlorosalicylic acid, ethylene dimethacrylate (EDMA), N-hydroxysuccinimide, 1-hydroxy-2-naphthoic acid, methacrylic acid (MAA), 2-naphthoic acid, salicylic acid and sulfonyl chloride were from Sigma-Aldrich-Fluka (Milan, Italy). Acetic acid, L-alanine, 2,2'-azobis-(2-methylpropionitrile) (AIBN), N,N-dicyclohexylcarbodiimide, glycine, L-phenylalanine and all the organic solvents were from VWR International (Milan, Italy). 4-Chloro-1-hydroxy-2-naphthoic acid was prepared from the commercially available 1-hydroxy-2-naphthoic acid in according to the procedure previously reported in literature [6].

Ethanol-free chloroform used as porogen solvent was obtained from commercial HPLC grade chloroform by distillation. Polymerization inhibitors in monomers were removed by clean-up on activated alumina columns.

The HPLC-MS/MS apparatus was an Accela High Speed LC (1250 quaternary solvent delivery pump, thermostated autosampler provided with a 25 μ l injection system) interfaced with an ion-trap LCQ Fleet mass spectrometer (ESI mode) and a data acquisition system Xcalibur 2.0 from ThermoScientific (Milan, Italy). The HPLC column was a 50 \times 2.1 mm Hypersil Gold reverse-phase C18 from ThermoScientific (Milan, Italy). Ligand stock solutions were prepared dissolving 2.5 mg of substance in 1.0 ml of acetonitrile and storing in the dark at -20 $^{\circ}$ C.

2.2. Synthesis of OTA analogs

The synthesis of the OTA analogs reported in figure 1 was developed utilizing the reaction between a primary amine and the N-hydroxysuccinimidyl derivative of a carboxylic acid in according to the procedure previously reported in literature with minor modifications [6]. For example, here the synthesis of N-(4-chloro-1-hydroxy-2-naphthoylamido)-(L)-phenylalanine (CHNA-Phe, **3**) is reported. In a 250 ml round-bottom flask containing 500 mg

(2.25 mmoles) of CHNA dissolved into 50 ml of ice-cold anhydrous tetrahydrofuran were added in sequence 271 mg (2.36 mmoles) of N-hydroxysuccinimide and 487 mg (2.36 mmoles) of freshly crystallized N,N-dicyclohexylcarbodiimide. The mixture was stirred for two hours at 4 °C, the N,N-dicyclohexylurea formed was separated by filtration in a G4 Buchner, and then rapidly added drop by drop under vigorous stirring in a solution of 1.12 g (6.75 mmoles) of L-phenylalanine dissolved in 50 ml of sodium hydrogencarbonate 0.1 M. The reaction mixture was stirred at room temperature overnight, then evaporated in a rotavapor. The residue was acidified to pH 2 with 0.1 M aqueous hydrogen chloride, dispersed under sonication into 50 ml of ethylacetate and washed three times with 50 ml of acidified water. The organic layer was dried over anhydrous sodium sulphate and evaporated under a stream of air. The raw product obtained was recrystallized twice in absolute ethanol, giving the product as a white powder (648 mg, 78% yield), deemed pure by HPLC-MS with mobile phase MeCN - AcOH, 99+1 v/v (eluent A) and H₂O - AcOH, 99+1 v/v (eluent B) in A+B gradient from 1+9 (1 min) to 9+1 (6 min), then isocratic to 7 min.

Mass spectra: CHNA-Phe, **3**: 368 (molecular ion), 324 (M-COOH), 220 (M-C₆H₅CH₂COOH), 177 (C₁₀H₅ClOH); CHNA-Ala, **4**: 292 (molecular ion), 248 (M-COOH), 177 (C₁₀H₅ClOH); CHNA-Gly, **5**: 278 (molecular ion), 234 (M-COOH), 177 (C₁₀H₅ClOH); HNA-Phe, **6**: 334 (molecular ion), 290 (M-COOH), 186 (M-C₆H₅CH₂COOH), 143 (C₁₀H₆OH); ClSA-Phe, **7** 318 (molecular ion), 274 (M-COOH), 170 (M-C₆H₅CH₂COOH), 126 (C₆H₅ClOH); SA-Phe, **8**: 284 (molecular ion), 240 (M-COOH), 136 (M-C₆H₅CH₂COOH).

¹H NMR (δ/ppm, CDCl₃, 250 MHz): CHNA-Phe, **3**: 11.8 (1H, s, COOH), 8.5 (1H, d, naphtalenic H), 8.2 (1H, d, CONH), 7.8 (4H, d, naphtalenic H), 7.3 (1H, s, naphtalenic H), 6.8 (2H, d, aromatic H), 5.2 (1H, s, aromatic OH), 4.8 (1H, t, CHCOOH); CHNA-Ala, **4**: 11.7 (1H, s, COOH), 8.5 (1H, d, naphtalenic H), 8.2 (1H, d, CONH), 7.8 (4H, d, naphtalenic H), 7.3 (1H, s, naphtalenic H), 4.8 (1H, q, CHCOOH); 1.4 (3H, d, CH₃); CHNA-Gly, **5**: 11.7 (1H, s, COOH), 8.5 (1H, d, naphtalenic H), 8.2 (1H, d, CONH), 7.8 (1H, d, naphtalenic H), 7.3 (1H, s, naphtalenic H), 3.8 (2H, d, CH₂COOH); HNA-Phe, **6**: 11.8 (1H, s, COOH), 8.3 (1H, d, naphtalenic H), 8.1 (1H, d, naphtalenic H), 7.9 (2H, d, naphtalenic H), 7.6 (2H, t, naphtalenic H), 8.2 (1H, d, CONH), 6.8 (2H, d, aromatic H), 5.2 (1H, s, aromatic OH), 4.9 (1H, t, CHCOOH); ClSA-Phe, **7**: 11.9 (1H, s, COOH), 8.2 (1H, d, CONH), 7.6 (1H, d, aromatic

H), 7.4 (2H, t, aromatic H), 7.3 (2H, d, aromatic H), 7.0 (1H, d, aromatic H), 5.2 (1H, s, aromatic OH), 4.9 (1H, t, CHCOOH); SA-Phe, **8**: 11.9 (1H, s, COOH), 8.2 (1H, d, CONH), 7.6 (1H, d, aromatic H), 7.6 (1H, t, aromatic H), 7.4 (2H, t, aromatic H), 7.3 (2H, d, aromatic H), 7.2 (1H, t, aromatic H), 7.0 (1H, d, aromatic H), 5.2 (1H, s, aromatic OH), 4.9 (1H, t, CHCOOH).

2.3. Synthesis of molecularly imprinted polymers

The imprinted polymers were prepared according to a method previously reported in literature with minor modifications [6]. In 10 ml thick wall borosilicate test tubes, solutions with molar ratio template:functional monomer:cross-linker 1:6:54 were prepared by dissolving 0.27 mmoles of OTA-mimic in 4.8 ml of anhydrous chloroform. Then, 0.162 ml (1.62 mmoles) of MAA, 2.85 ml of EDMA (14.6 mmoles) and 0.051 g of AIBN were added. The mixtures were purged with nitrogen and sonicated in an ultrasonic bath for 10 min, sealed, and left to polymerize overnight at 60 °C. 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. The washed polymers were dried under vacuum at 70 °C for 2 h and stored in a desiccator. Blank polymers (NIPs) were prepared and treated in the same manner, omitting the template.

2.4. Liquid Chromatography

Adequate amounts of polymer were suspended in a 1+1 v/v ethanol-water mixture, and the slurry was packed in 3.9x100-mm stainless-steel HPLC columns. The packing of the stationary phases was performed by gradually adding the slurry of the polymer to the column and eluting it with 1+1 (v/v) ethanol-water at constant pressure of 20 MPa. The packed columns were washed at 1 ml/min with 9+1 (v/v) ethanol-acetic acid until a stable baseline was reached (254 nm).

Packed columns were equilibrated at a flow rate of 1 ml/min with 40 ml of acetonitrile-acetic acid 0.1% (v/v); then, 5 µl of stock solution of ligand diluted to 50 µg/ml with the

mobile phase were injected and eluted at 1 ml/min recording absorbance at 344 nm. Each elution was repeated three times to assure chromatogram reproducibility. Column void volumes were measured for each mobile-phase formulation by eluting 5 μ l of acetone 0.1% (v/v) in acetonitrile, and the absorbance was recorded at 343 nm (332 nm with OTA, 304 nm with ClSA-Phe and SA-Phe). The capacity factor (k) was calculated as $(t-t_0)/t_0$, where t is the retention time of the eluted ligand and t_0 is the retention time corresponding to the column void volume. The imprinting factor (IF) is defined as an index of the imprinting efficacy respect to a NIP prepared in the same conditions. It was calculated as k_{MIP}/k_{NIP} , where k_{MIP} is the capacity factor of a ligand eluted on the imprinted column and k_{NIP} is the capacity factor of a ligand eluted on the NIP.

2.5. Computer simulation

The computer used to simulate monomer-template interactions was a Intel Core2 double CPU 3.00 GHz, 5 GB of RAM, running a Windows 7 operative system with the molecular graphic software HyperChem 8.08 (Hypercube Inc., Waterloo, Canada). Molecular models of templates and methacrylic acid were separately optimized by using a semiempirical quantum method (RM1) and assembled together. Then, a simulated annealing process was applied to optimize the arrangement of the resulting supramolecular structures. Annealing conditions were fixed as 300K considering the dynamic equilibrium reached after 2000 fs, with step of 0.1 fs. At the end of the annealing process, the position of methacrylic acid around the template was optimized again to reach a minimum of potential energy.

3. RESULTS AND DISCUSSION

The successful mimic template CHNA-Phe was rationally designed to preserve the general structure of the target analyte OTA. In fact, a comparison between OTA and this mimic showed almost complete overlapping of the two molecules, with a high degree of similarity not only structurally, but also as solvent accessible surfaces, electrostatic potential surfaces and lipophilic/hydrophilic surfaces [6], while a polymer prepared with the same mimic, but with completely different functional monomers showed the same recognition properties towards OTA [7], whereas a polymer prepared with OTA as a template recognized

the mimic well [9].

Therefore, the rational design of novel mimics for OTA will meet the same criteria used for Phe-CHNA, including the chirality of the amino acidic sub-structure and the planarity of naphthalene rings. Moreover, to assure an efficient imprinting effect, the several distinct potential points of interaction with functional monomers the α -carboxyl of L-phenylalanine, the amide bridge and the phenolic hydroxyl should be retained. These criteria, though valid, seem to be somewhat restrictive, because it is not possible to change most of the molecular structure of the reference mimic without the risk of losing OTA recognition in the resulting imprinted polymer. As a consequence, we decided to study two different kinds of mimic molecules: OTA-analogs with amino acids other than L-phenylalanine, obtained by truncation of the amino acid substructure (type-1 mimics: CHNA-Ala, CHNA-Gly), and OTA-analogs obtained by the subtraction of an aromatic ring or chlorine to CHNA substructure (type-2 mimics: HNA-Phe, CHNA-CISA, CHNA-SA).

3.1. Type-1 mimic templates

When type-1 mimics are used to prepare the imprinted polymers, the experimental results reported in table 1 show that OTA is better retained by the imprinted columns than the template itself. This fact represents further evidence of what we had previously observed regarding a polymer imprinted with CHNA-Phe: OTA was recognized better than the template and most of the analogs examined, both in acetonitrile and in chloroform [6]. This fact may be related to the different strength of nonspecific interactions between the polymer and ligand. In fact, examining the capacity factors measured on the non-imprinted column, it is possible to observe that this polymer retains OTA much more than the mimics. This confirms the generally accepted view that - to the net of non-specific interactions - a polymer imprinted with a mimic tends to bind this molecule better than the target. Interestingly, all the polymers prepared with type-1 mimics recognize the OTA molecule quite well. It should be considered that a decrease of the molecular complexity from the bulky phenylalanine to the small glycine means that the solvent accessible surface, that can be considered as an appropriate marker of molecular complexity for molecules involved in ligand-receptor interactions, changes from 529 Å² of CHNA-Phe to 439 Å² of CHNA-Gly,

through 456 Å² of the intermediate CHNA-Ala, with an overall decrease of 17%. Notwithstanding, OTA recognition by the imprinted polymers prepared with CHNA-Ala- or CHNA-Gly is not reduced when compared to CHNA-Phe. In fact, the imprinting factor for OTA is IF=6.83 measured onto the polymer imprinted with CHNA-Phe, IF=8.00 for CHNA-Ala and IF=7.71 for CHNA-Gly-imprinted polymers. This data shows that not only is a bulky amino acid side chain not essential for an efficient mimic template of OTA, but that a template is able to induce the formation of an imprinted polymer with binding properties towards a slightly larger target molecule, clearly confirming what had been previously reported in literature for polymers imprinted with carbamates [16] or mimics of micotoxins other than OTA, such as citrinin [4], mycophenolic acid [5], and domoic acid [17].

3.2. Type-2 mimic templates

When type-2 mimics are used to prepare the imprinted polymers the experimental results reported in table 2 confirm what was observed for polymers prepared with type-1 mimic templates. In fact, OTA is better retained by the imprinted columns than the template itself in terms of capacity factor, but it is less recognized than the mimic templates in terms of imprinting factor. Moreover, mimic templates are better recognized by their corresponding imprinted polymer than the other mimics. Beside this, there is a marked difference respect to imprinted polymers obtained by using type-1 mimic templates: the polymers prepared with type-1 mimic templates (base on naphtalenic ring) show a marked capacity to retain both OTA and related molecules, while polymers prepared by using templates based on the benzene ring (CISA-Phe and SA-Phe) retain these ligands to a lesser extent. This show how the presence of a bulky naphtalene substructure in the mimic template will determine good recognition and retention of OTA by a molecularly imprinted polymer. Contrarily, the imprinted polymer prepared by using HNA-Phe as a template can be assimilated to a type-1 mimic: the presence/absence of a chlorine on the aromatic nucleus does not seem to be decisive in determining the binding capacity of the resulting imprinted polymer towards OTA.

3.3. Interpretation of the binding results.

The experimental results obtained using polymers prepared with type-1 or type-2 template mimics can be interpreted as a whole in terms of binding site structure. We simulated the non-covalent interaction between three templates, CHNA-Phe, CHNA-Gly and ClSA-Phe, and methacrylic acid molecules in order to gain some insights into the three-dimensional structure of the resulting non-covalent complex. From the images of these complexes reported in figures 2 (CHNA-Phe / MAA complex), 3 (CHNA-Gly / MAA complex) and 4 (ClSA-Phe / complex) it is possible to see that the prevailing non-covalent interaction is due to cyclic hydrogen bond between the two residues of methacrylic acid and, respectively, the amino acidic α -carboxyl of the templates and the sub-structure defined by the amidic carbonyl and the phenolic hydroxyl. Moreover, as effects of the torsion angle centered on the α -carbon of the amino acid, the templates have the amino acidic substituent lying on a plane perpendicular to the isocoumarinic structure of OTA, oriented in a direction opposite to the hydrogen bond between the methacrylic acid residue and the amino acidic α -carboxyl. Thus, it is plausible that a binding site obtained by imprinting with type-1 templates will very poorly recognize ligands with different amino acidic substituents protruding outside its cavity, thus explaining why CHNA-Phe, CHNA-Ala and CHNA-Gly are recognized in the same manner by polymers prepared with these templates. For the same reason, the polymer imprinted with HNA-Phe behaves in the same way; the lack of chlorine atom in the template will not affect the molecular recognition properties of the polymer itself, as this substituent is positioned on the side of the molecule opposed to the position of the cyclic hydrogen bonds which stabilize the binding site-ligand complex. As regards the binding behavior of the polymer imprinted with ClSA-Phe, the poor recognition of OTA by this polymer (and by the related polymer imprinted with SA-Phe) can be explained by the reduced accessible surface of the template compared to the target molecule; the loss of the large naphthalenic system, that is substituted by a smaller benzene ring, causes the formation of binding sites which are too small to fully accommodate the OTA molecules during the rebinding, with a related loss of molecular recognition.

4. CONCLUSIONS

In this work we used the fragmental approach to search for simpler mimic templates than

CHNA-Phe for the molecular imprinting of OTA. The screening for binding of two different kinds of OTA-related molecules, nominally type-1 mimics (OTA-analogs with amino acids other than L-phenylalanine, obtained by the truncation of the amino acid substructure) and type-2 mimics (OTA-analogs obtained by the subtraction of an aromatic ring or chlorine to CHNA substructure), shows that changes to the amino acidic sub-structure or the presence/absence of a chlorine atom in position 4 on the naphthalene ring system does not affect the molecular recognition of OTA by the resulting imprinted polymer.

In conclusion, considering that the synthesis of the intermediate CHNA by regioselective chlorination represents one of the main drawbacks in preparing molecularly imprinted polymers by using an efficient mimic template of OTA, the alternative use of the mimic template HNA-Phe, available in high yield from the direct reaction between the cheap and commercially available L-phenylalanine and 1-hydroxy-2-naphthoic acid, seems to represent the most viable approach.

REFERENCES

- [1] L.I. Andersson, A. Paprica, T. Arvidsson, *Chromatographia*, 46 (1997) 57-62.
- [2] J.L. Urraca, M.D. Marazuela, E.R. Merino, G. Orellana, M.C. Moreno-Bondi, *J. Chromatogr. A*, 1116 (2006) 127-134.
- [3] M. Appell, D.F. Kendra, E.K. Kim, C.M. Maragos, *Food Addit. Contam.*, 24 (2007) 43-52.
- [4] B.-Y. Guo, S.-X. Wang, B.-Z. Ren, X. Li, F. Qin, F., J.-Z. Li, *J. Sep. Sci.*, 33 (2010) 1156-1160.
- [5] D. De Smet, V. Kodeck, P. Dubruel, C. Van Peteghem, E. Schacht, S. De Saeger, *J. Chromatogr. A*, 1218 (2011) 1122-1130.
- [6] C. Baggiani, G. Giraudi, A. Vanni, *Bioseparation*, 10 (2002) 389-394.
- [7] J. Jodlbauer, M.M. Maier, W. Lindner, *J. Chromatogr. A*, 945 (2002) 45-63.
- [8] N.W. Turner, E.V. Piletska, K. Karim, M. Whitcombe, M. Malecha, N. Magan, C. Baggiani, S.A. Piletsky, *Biosens. Bioelectron.*, 20 (2004) 1060-1067.
- [9] N.M. Maier, G. Buttinger, S. Welhartizki, E. Gavioli, W. Lindner, *J. Chromatogr. B*, 804 (2004) 103-111.
- [10] S.N. Zhou, E.P.C. Lai, J.D. Miller, *Anal. Bioanal. Chem.*, 378 (2004) 1903-1906.
- [11] J.C.C. Yu, S. Krushkova, E.P.C. Lai, E. Dabek-Zlotorzynska, *Anal. Bioanal. Chem.*, 382 (2005) 1534-1540.
- [12] J.C.C. Yu, E.P.C. Lai, *React. Funct. Polym.*, 63 (2005) 171-176.
- [13] J.C.C. Yu, E.P.C. Lai, *React. Funct. Polym.*, 66 (2006) 702-711.
- [14] J.C.C. Yu, E.P.C. Lai, *Food Chem.*, 105 (2007) 301-310.
- [15] W.H. Ali, D. Derrien, F. Alix, C. Perollier, O. Lepine, S. Bayouhd, F. Chapuis-Hugon, V. Pichon, *J. Chromatogr. A*, 1217 (2010) 6668-6673.
- [16] C. Baggiani, L. Anfossi, P. Baravalle, C. Giovannoli, C. Tozzi, G. Giraudi, *Anal. Chim. Acta.*, 531 (2005) 199-207.
- [17] W.H. Zhou, X.C. Guo, H.Q. Zhao, S.X. Wu, H.H. Yang, X.R. Wang, *Talanta*, 84 (2011) 777-782.

Table 1: capacity factors (k) and imprinting factors (IF) measured in imprinted and not-imprinted polymers for ochratoxin A and type-1 mimic templates.

	NIP	MIP CHNA-Phe		MIP CHNA-Ala		MIP CHNA-Gly	
	k	k	IF	k	IF	k	IF
OTA	1.08	7.26	6.73	8.62	8.00	8.31	7.71
CHNA- Phe	0.35	6.44	18.42	6.16	17.62	5.29	15.14
CHNA-Ala	0.56	4.66	8.27	7.28	12.93	5.42	9.63
CHNA-Gly	0.74	3.89	5.27	4.68	6.35	6.86	9.30

Table 2: capacity factors (k) and imprinting factors (IF) measured in imprinted and not-imprinted polymers for ochratoxin A and type-2 mimic templates.

	NIP	MIP HNA-Phe		MIP CISA-Phe		MIP SA-Phe	
	k	k	IF	k	IF	k	IF
OTA	1.08	7.24	6.72	3.65	2.66	2.66	2.47
HNA- Phe	0.34	5.74	16.89	1.15	3.38	1.14	3.37
CISA-Phe	0.30	3.10	10.34	3.96	13.21	1.97	6.58
SA-Phe	0.26	0.73	2.81	1.21	4.67	3.48	13.37

LEGEND OF FIGURES

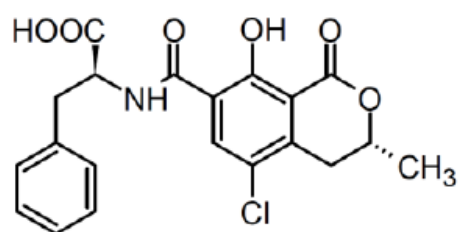
Figure 1. Molecular structure of ochratoxin A, 4-chloro-1-hydroxy-2-naphtoic acid and related mimic templates.

Figure 2. Sketch of an hypothetical imprinted binding site accommodating the template CHNA-Phe. Cyclic hydrogen bonds between functional monomers and template molecule are represented by dotted lines.

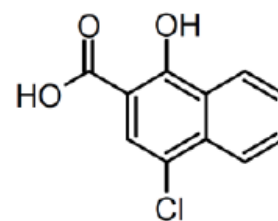
Figure 3. Sketch of an hypothetical imprinted binding site accommodating the template Gly-Phe. Cyclic hydrogen bonds between functional monomers and template molecule are represented by dotted lines.

Figure 4. Sketch of an hypothetical imprinted binding site accommodating the template CLSA-Phe. Cyclic hydrogen bonds between functional monomers and template molecule are represented by dotted lines.

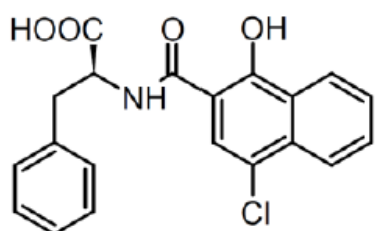
Figure 1



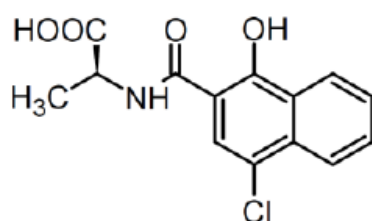
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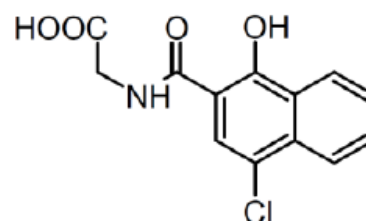
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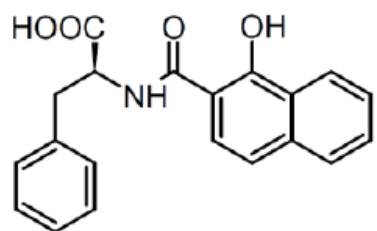
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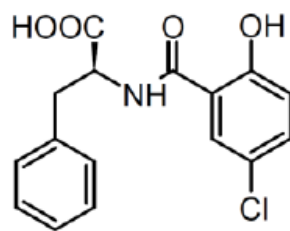
CHNA-Ala, 4



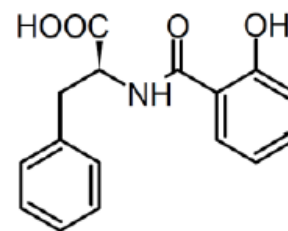
CHNA-Gly, 5



HNA-Phe, 6



ClSA-Phe, 7



SA-Phe, 8

Figure 2

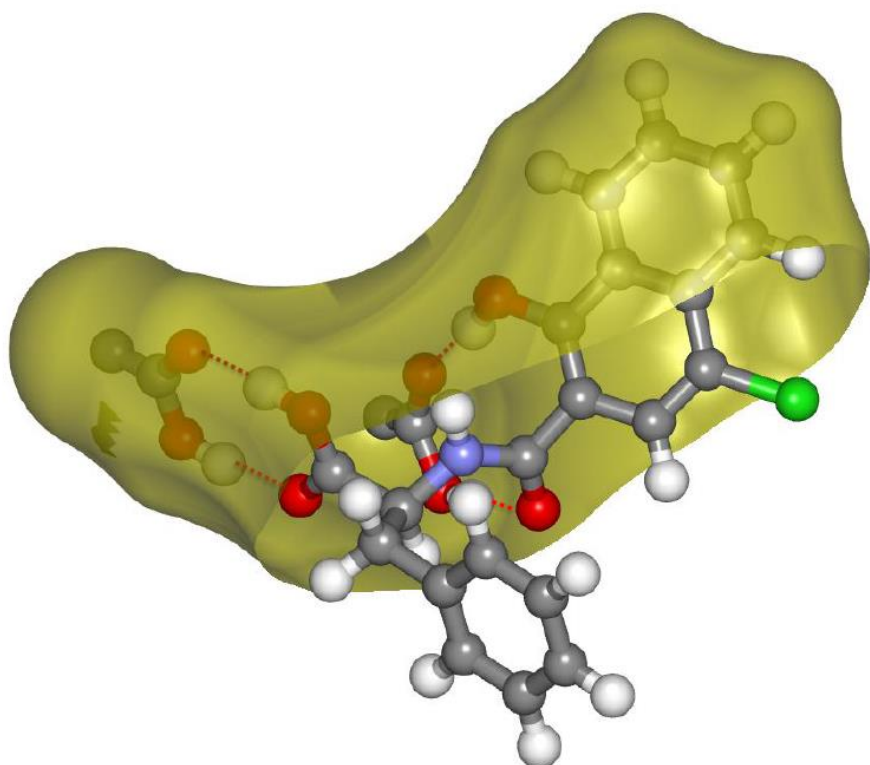


Figure 3

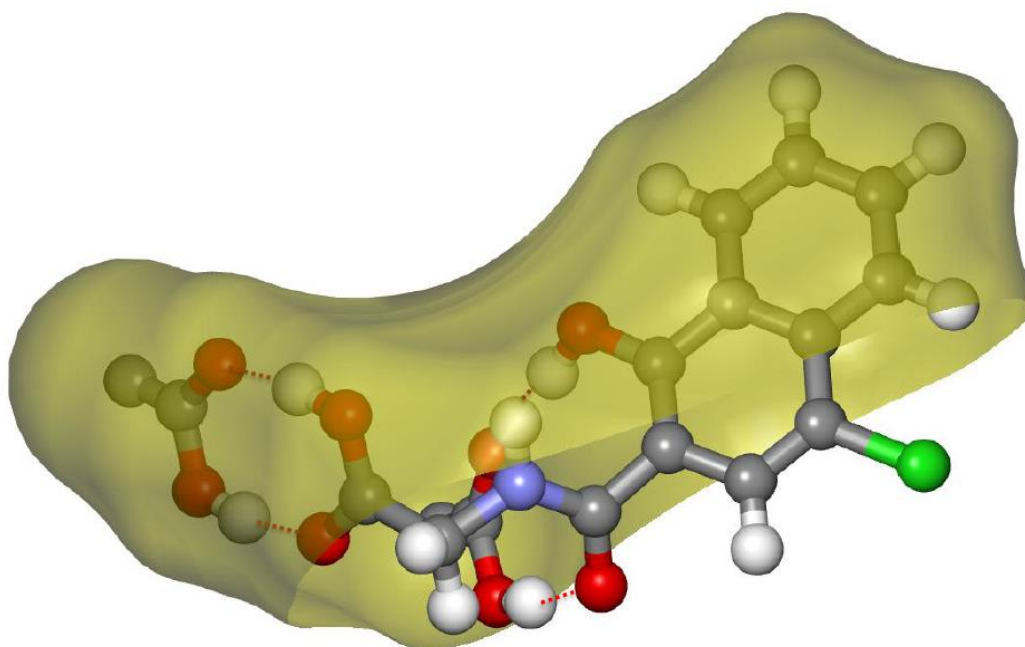


Figure 4

