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Ergot alkaloids: From witchcraft till *in silico* analysis. Multi-receptor analysis of ergotamine metabolites

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ABSTRACT

The term Ergot is referred to the sclerotium of ascomycetes – a protective kernel produced during resting stage of some fungi – which replaces seeds of susceptible cereals and plants intended for human and animal diet. It contains various composition of tryptophan-derived toxins defined ergot alkaloids. Since sclerotia can be harvested and milled together with cereals, they represent a source of food and feed contamination after breakage and spreading of mycotoxins into the various milling fractions. The effects of ergot alkaloids, including those adverse for human health, have been known since the Middle Ages. Nevertheless, as recently stated by the European Food Safety Authority, further information is needed on metabolism and target receptors-binding of common alkaloids in food. Unfortunately, the experimental investigation is challenging due to the high costs in terms of time and money. This study was thus aimed at assessing whether the *in silico* modeling can be an effective tool to investigate the interaction between multiple serotonin receptors and a wide set of ergotamine metabolites, including experimentally detected molecules and predicted derivatives. Validated models provided precious insights about the effects exerted by metabolic modifications on the receptor–ligand interaction. Such structural information may be useful to support the design of further experimental analysis.

1. Introduction

Ergot is the sclerotium of parasitic ascomycetes belonging to the *Claviceps* genus that replaces the seeds of susceptible host plants – traditionally rye and triticale, but also wheat, barley, millet and oats – and thus it can be harvested together with cereals [1]. Besides lipids (40% in weight), sclerotia contain a range of tryptophan-derived toxins, known as "ergot alkaloids" (EAs). The total amount and the pattern of EAs significantly vary between fungus strain, geographic regions and host plants [2]. Ergots can be a relevant source of food and feed contamination as a consequence of the processing, mostly referable to grinding processes, of ergot-containing cereals through the spreading of alkaloids after sclerotia breakage [3]. EAs have long been known as toxic compounds with a broad spectrum of adverse effects on human and animal health leading mainly to the ergotism disease. The first well documented outbreak of ergotism dates back in the Middle Age – which faded into witchcraft and folklore. Currently, it has been recognized the occurrence of two illness forms, namely gangrenous ergotism and convulsive ergotism [4]. The former is responsible for several effects on the central nervous system in the form of spasms, hallucinations and epileptic fits. The latter implies circulation disorders, and it damages the poorly blood supplied parts of the body, such as fingers or toes. In the worst cases, the loss of damaged parts may occur due to tissue necrosis [5]. Interestingly, it is

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commonly believed that the cognitive effects of ergotism were at the base of several social troubles in the past, such as the witch trials of Salem and the arising of mystic religious movements [6]. Nowadays it is known that EAs action is mainly mediated by the interaction with α-adrenergic, dopaminergic and serotonergic receptors classes [4].

As recently stated by the European Food Safety Authority (EFSA) [7], additional data on toxicokinetic and toxicodynamic, including metabolism, of relevant EAs are required. In particular, deeper studies about the comparative target neurotransmitter receptor-binding activity are needed. Furthermore, data on human metabolism of relevant EAs in food and the toxicodynamic of their metabolites are poor so far, even if paramount for the in-depth understanding of EAs action in living organisms. De facto, up to date only few hydroxylated metabolites were identified under *in vitro* conditions [8]. Moreover, even if it is commonly believed that hepatic metabolites maintain the activity of parent compounds, this hypothesis is actually based on data carried out on a unique metabolite of dihydroergotamine [9]. As the experimental investigation is hardly demanding in terms of costs and time, the *in silico* modeling may offer a fundamental support in selecting lead compounds to be tested in workbench trials. Within this framework, as a proof of concept, this paper is focused on the interaction between some serotonin receptors and the alkaloid ergotamine, which is one of the most abundant ergopeptine alkaloid occurring in food and feed [7,10], alongside a number of human metabolites. Ergotamine consists of a natural D-lysergic acid (the ergoline ring) linked to a tricyclic peptide by a peptide bond (Fig. 1). The presence of a chiral center at C-8 determines the equilibrium between R and S epimers (-ine and -inine forms, respectively), but the S isomer is considered devoid of activity and only the -ine form is of biological relevance [11]. Therefore, the present work mainly aimed at assessing whether a straightforward computational procedure – whose reliability to evaluate the bioactivity of small molecules was already proved [12–15] – could be used to investigate the interaction of ergoline-related compounds with 5-hydroxy-triptamine receptors 2A, 2B and 2C (*i.e.* 5HT-2A, 5TH-2B and 5HT-2C, respectively). Then, it was proposed the activity profiling of both putative and experimentally detected ergotamine metabolites in respect of such receptors. The work-flow mainly involved: (i) the homology modeling of 5HT-2A and 5HT-2C models on the basis of the crystallographic structure of 5HT-2B; (ii) the analysis of sequences and pocket anatomy; (iii) the training procedure on 5HT-2A, 5HT-2B and 5HT-2C models by using *ad hoc* training-set; (iv) the assessment of procedural performance by comparing results of GOLD–HINT coupling with those obtained by using other benchmark software; (v) the screening of queries-set on validated models.

Whereas the effects on the binding to receptors exerted by structural changes on the ergoline ring have been already accounted (*e.g.* ref. [16,17]), to the best of our knowledge, the present paper addressed for the first time the effect of metabolic modifications toward serotonergic activity of such a wide range of ergotamine's human metabolites. Specifically, structural insights were collected on the effect exerted by such modifications on the molecular initiating event underlying serotonergic action. Such structural information may be useful to support the design of further experimental trials.

2. Material and methods

2.1. Computational procedure and software

2.1.1. Homology modeling

The crystallographic structure of human 5HT-2B receptor (PDB code 4IB4 [18]) was the template for the homology modeling of 5HT-2A and 5HT-2C receptors. Among the various receptors, they have been chosen due to the highest sequence identity respect to 5HT-2B. The fusion BRILL domain was removed and only transmembrane domain was taken into account. All co-crystallized molecules, with the only exception of ergotamine, were removed. The Modeler software, version 9.10, was used [19] and the option to include the ligand ergotamine into the binding site was chosen. Then, each model was relaxed by performing a mild local minimization (250 iterations using Powell algorithm) within 5 Å around the ligand. Local pairwise alignments were conducted by using the on-line tool EMBOSS-Water Pairwise Sequence Alignment (http://www.ebi.ac.uk) and Smith–Waterman algorithm was chosen.

2.1.2. Molecular modeling

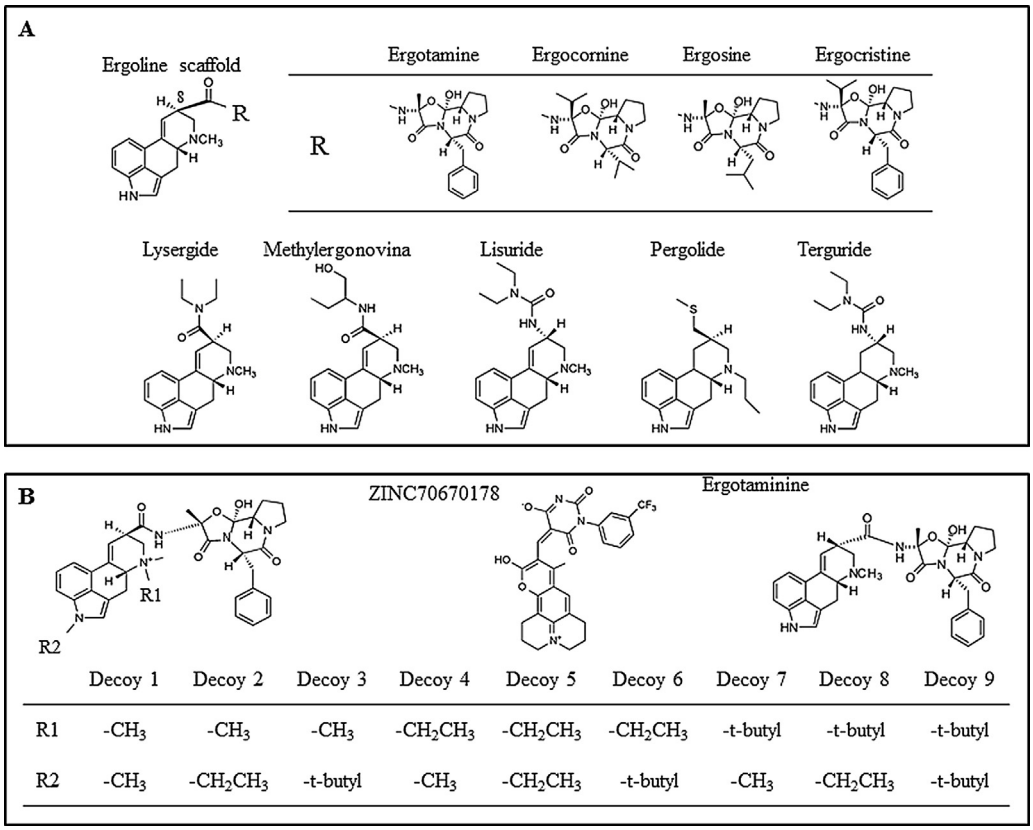
All protein structures and ligands were processed by using the software Sybyl, version 8.1 (www.tripos.com). All atoms were checked for atom- and bond-type assignments. Amino- and carboxyl-terminal groups were set as protonated and deprotonated, respectively. Hydrogen atoms were computationally added to the protein and energy-minimized using the Powell algorithm whit a coverage gradient of $\leq 0.5\,\text{kcal}\,(\text{mol}\,\text{Å})^{-1}$ and a maximum of 1500 cycles.

2.1.3. Pharmacophore models

The ligand binding site was defined by using the Flapsite tool of the FLAP (Fingerprint for Ligand And Protein) software developed by Molecular Discovery Ltd (http://www.moldiscovery.com) [20], while the GRID algorithm [21] was used to investigate the corresponding pharmacophoric space. The DRY probe was used to describe the potential hydrophobic interactions, while the sp2 carbonyl oxygen (O) and the neutral flat amino (N1) probes were used to describe the hydrogen bond acceptor and donor capacity of the target, respectively. All images were obtained using the software PyMol version 1.7 (http://www.pymol.org).

2.1.4. Docking simulations and re-scoring procedures

The coupling of GOLD, to perform docking simulations, and HINT software, as re-scoring function, has been already proved to be effectively able to evaluate the bioactivity of small molecules [12–15]. The docking simulations of compounds were performed with the GOLD version 5.1 (CCDC; Cambridge, UK; http://www.ccd.cam.ac.uk). All crystallographic waters and ligands were removed and 50 poses for each compound were generated. No constraints



	Decoy 1	Decoy 2	Decoy 3	Decoy 4	Decoy 5	Decoy 6	Decoy 7	Decoy 8	Decoy 9
R1	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_2CH_3$	$-CH_2CH_3$	$-CH_2CH_3$	-t-butyl	-t-butyl	-t-butyl
R2	$-CH_3$	$-CH_2CH_3$	-t-butyl	$-CH_3$	$-CH_2CH_3$	-t-butyl	$-CH_3$	$-CH_2CH_3$	-t-butyl

Fig. 1. Training set compounds.

were set up, and the explorable space was defined in a radius of 10 Å from the centroid of the binding pocket. For each GOLD docking search, a maximum number of 100,000 operations were performed on a population of 100 individuals with a selection pressure of 1.1. Operator weights for crossover, mutation, and migration were set to 95, 95, and 10, respectively. The number of islands was set to 5 and the niche to 2. The hydrogen bond distance was set to 2.5 Å and the vdW linear cut-off to 4.0. Ligand flexibility options "flip pyramidal N", "flip amide bonds", and "flip ring corners" were allowed. Each best scored pose according to GOLD scoring function was re-scored by HINT. The software HINT (Hydrophatic INTeraction) [22] was used as the re-scoring function on the basis of previous studies attesting the higher reliability of HINT scoring with respect to other scoring functions, and its successful use in the search for ligands for other targets, as well as in the estimation of ligand binding free energies. More in details, the score provides the evaluation of thermodynamic benefits of protein–ligand interaction, and therefore low/negative scores indicate not appreciable protein–ligand interactions [13–15,23–27]. GOLD uses a Lamarckian genetic algorithm and scores may slightly change from run to run. Therefore, in order to exclude a non-causative categorization of compounds, we conducted simulations in triplicate and the mean values are reported.

2.1.5. Docking simulations with AutoDock Vina

AutoDock Vina version 1.1.2 was used [28]. The explorable space was defined in a box having 10 Å side lengths from the centroid of the binding site. The maximum number of binding mode to generate was set at 25 and the maximum energy difference between the best binding mode and the worst one displayed was set at 9.

2.1.6. Metabolites identifications

Phase I metabolites and glycosylated derivatives of ergotamine were computed by using the two benchmark software MetaSite (http://moldiscovery.com) [29,30] and MetaPrint2D-React [31], respectively. In both predictions human metabolism was accounted and only first generation metabolites were collected.

2.2. Data collection

Data were collected considering three well-defined sets – a training set and two query sets – composed as reported below.

2.2.1. Training set

Molecular docking is widely used to predict novel active compounds or to decipher the mode of action of ligands *versus* a given protein. Currently, the capability to distinguish true ligands from false ligands

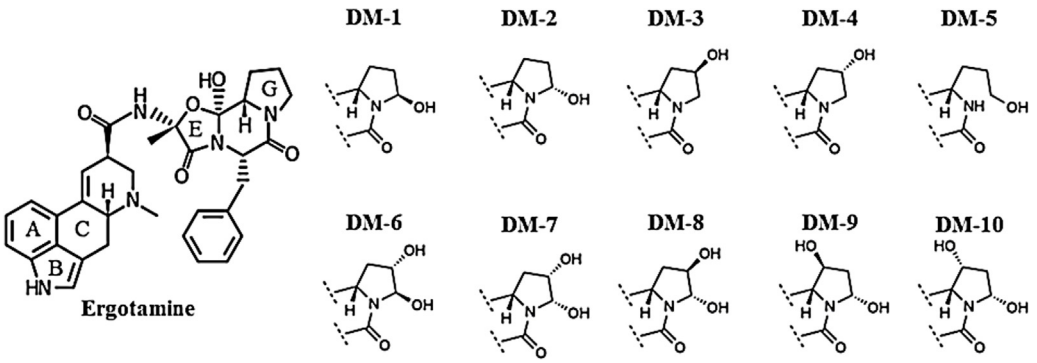


Fig. 2. Experimentally determined metabolites.

(*i.e.* decoys) is a solid check to assess the reliability of docking analysis and the efficacy of scoring functions [32]. More in details, the capability to maximize the total amount of true positives and true negatives, avoiding false positives and false negatives, is the final endpoint of each *in silico* model. Specifically, in the matter of upstream screening of potential health-damaging compounds, false negatives should be strictly avoided. In this work the most of true ligands derived from repositories of GPCR Decoy Database (GDD) [33]. In addition, the previously described ligands ergocornine, ergocristine and ergosine were also included (Fig. 1A). All ligands were checked for the activity through PubChem-BioAssay database (http://pubchem.ncbi.nlm.nih.gov). With respect to decoys set, the procedure herein proposed applies much better when intraclass comparison is performed, as stated by recent findings [34]. Therefore, the employment of decoys structurally related to ergopeptine ligands is

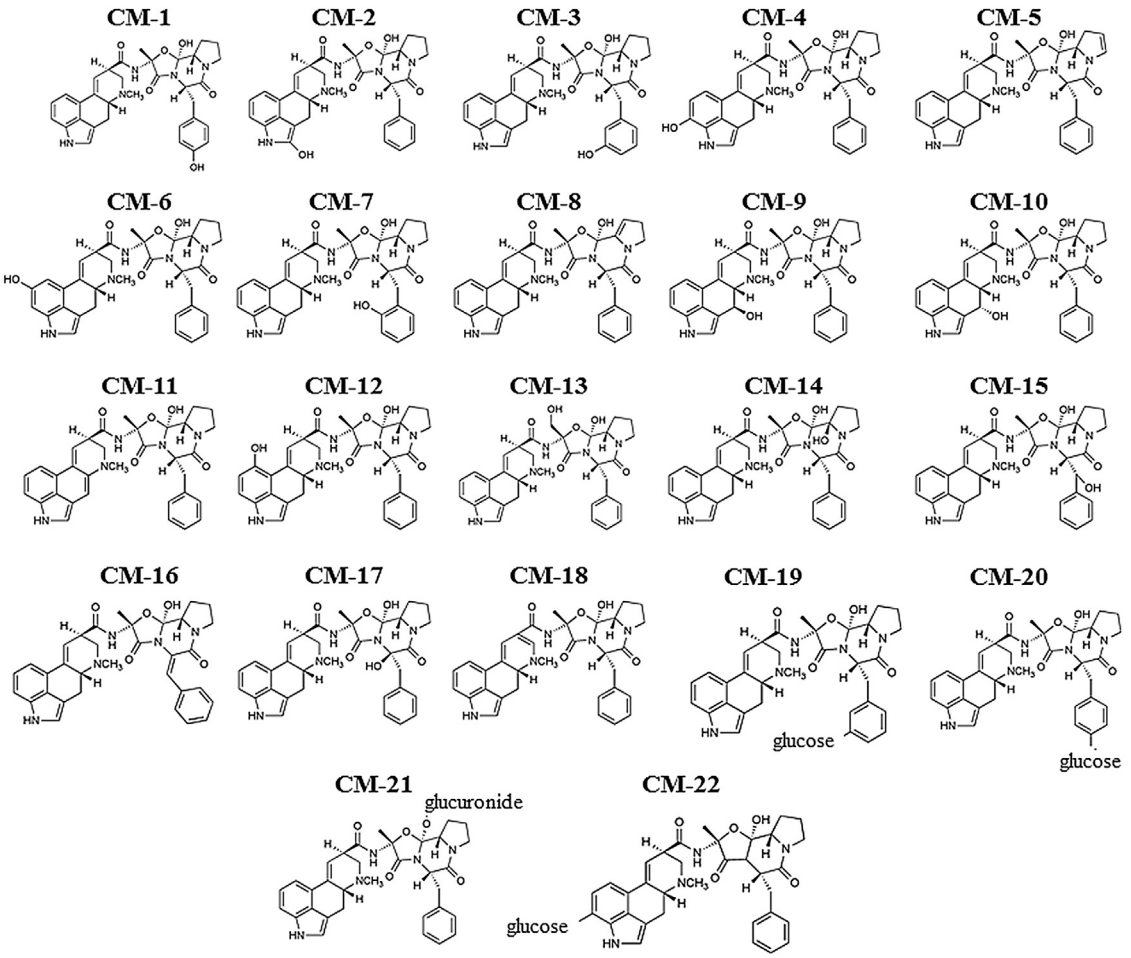


Fig. 3. Computed metabolites.

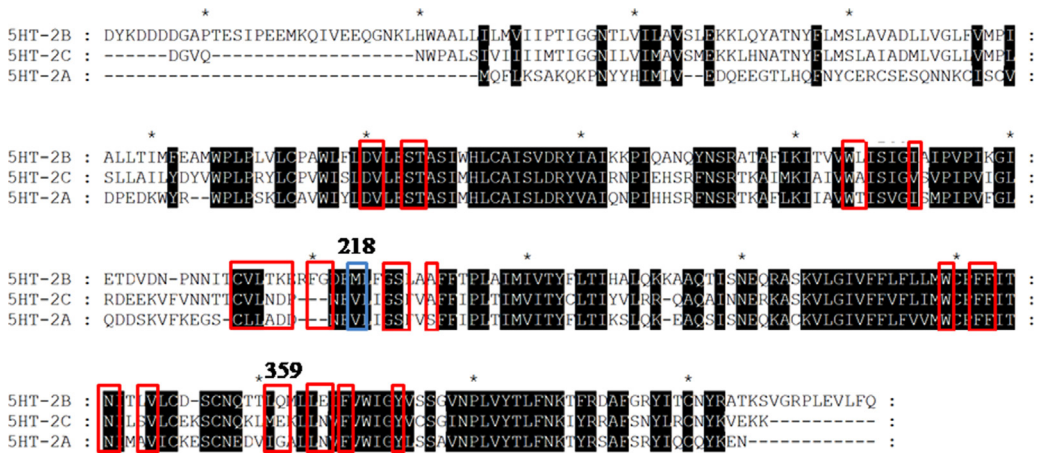


Fig. 4. Sequence alignment of 5HT-2A, 5HT-2B and 5HT-2C. Conserved residues are labeled in black, while boxes indicate residues composing the binding site. Blue box indicates M218V.

probative for procedural reliability. Unlikely, to the best of our knowledge, there are no data in the literature concerning the lack of binding between ergopeptine alkaloids and receptors under analysis (with the only exception of -inine forms, as aforementioned). For these reasons, the employment of knowledge-based virtual decoys was chosen [35]. Specifically, single-point mutagenesis experiments on serotonin receptors revealed the residues involved in ergotamine binding, and identified which lacking contacts are responsible for the loss of interaction [36]. Such information was thus used to modify ergotamine scaffold in a semi-combinatorial fashion, in order to disrupt the key contacts. In addition to ergotaminine, the most structurally related compound found in DUD database [37] – that is an example of public repository of decoys – was also included (Fig. 1B).

2.2.2. Experimentally determined query set

A total of 10 ergotamine metabolites arising from *in vitro* experiments were collected from the literature [8]. The set mainly consisted of hydroxylated metabolites on the ring G, with the only exception of one molecule bearing such ring opened (Fig. 2).

2.2.3. Computed query set

Data on human metabolism of ergotamine are scarce so far. Albeit the few results found in the literature provided precious contributions to the research, they cannot be considered exhaustive. Thus, in the aim to more exhaustively evaluate the effects of metabolism, a series of putative conjugates and phase-I metabolites were collected (Fig. 3). Although the simulation returned all the mono-hydroxylated compounds already included in the experimentally determined set, those were not further considered.

3. Results

3.1. Sequence analysis and pocket anatomy

Overall the models of 5HT-2A and 5HT-2C respectively showed 60% and 61% of sequence identity with 5HT-2B, and 71% between themselves. At the level of ligand binding site – defined in a radius of 5 Å around the ligand and comprising 30 residues – 5HT-2A and 5HT-2C showed 66.7% and 70% of residues conservation respect to 5HT-2B, whilst they had 74% of residues conservation between themselves (Fig. 4). Overall the pharmacophoric analysis of binding sites revealed quite similar anatomies and comparable physico-chemical characteristics since all pockets shared a marked hydrophobic environment with few polar patches, mainly favorable to receive hydrogen-bond donor groups (Fig. 5). Nevertheless, some single-point mutations determined slight changes mainly in the form of local shape resizing (further information are reported below).

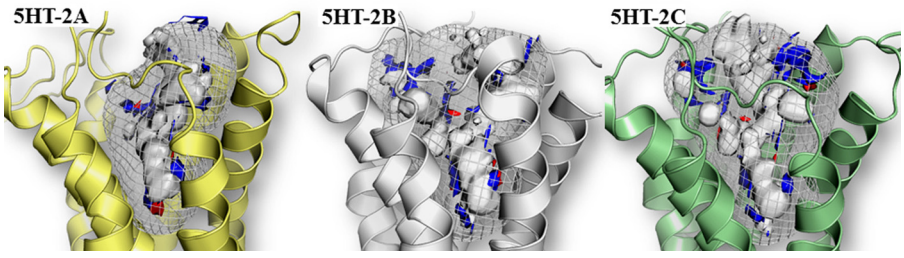


Fig. 5. The anatomy of binding sites. The shape of the binding sites is represented in mesh. The white, red and blue contours represent the favorable binding site regions for placing, respectively, hydrophobic, hydrogen-bond-acceptor, and hydrogen-bond-donor groups.

Table 1
Training set results and thresholds definitions.

Compounds		5HT-2A	5HT-2B	5HT-2C
Ligands	Ergocornine	130	234	−801*
	Ergocristine	459	661	−1.11E+04*
	Ergosine	482	234	387
	Ergotamine	1004	820	1117
	Lisuride	372	230	282
	Lysergide	858	973	1083
	Methylergonovine	487	463	412
	Pergolide	861	364	450
	Terguride	348	643	1221
Decoys	Decoy 01	−6075	−1046	−1454
	Decoy 02	−6499	−9.08E+04	−1.22E+05
	Decoy 03	−2938	−2827	−2059
	Decoy 04	−30.04E+05	−3.21E+04	−5066
	Decoy 05	−828	−2.27E+04	−1384
	Decoy 06	−10.01E+04	−1.06E+07	−3421
	Decoy 07	−1356	−4105	−877
	Decoy 08	−4.69E+07	−1.24E+08	−4.34E+04
	Decoy 09	−4.61E+11	−2.92E+09	−2261
	ZINC79670178	−653	−464	−194
	Ergotaminine	−5.78E+04	−8.17e+07	−9.04E+04
	Threshold	130	230	Rejected

* Non congruent records invalidating 5HT-2C model.

3.2. Training procedure

The training procedure mainly consisted into the empirical setting of the HINT thresholds in order to categorize ligands and decoys into two clusters. As reported in Table 1, consistent cut-off scores were provided for 5HT-2A and 5HT-2B (130 and 230 HINT scores, respectively). Accordingly, only interactions recording scores above the cut-off value were considered. On the contrary, 5HT-2C model was rejected due to the presence of two false negatives (namely, ergocornine and ergocristine).

3.3. Validation of procedural performance

The consistence of procedural performance on 5HT-2A and 5HT-2B models was assessed by comparing results of GOLD–HINT coupling with those obtained by using the native GOLD scoring function GOLDScore and one of the most used benchmark docking software, namely AutoDock Vina. Neither of them provided consistent results, and thus the case-specific efficacy of GOLD–HINT coupling was confirmed (Table 2, Fig. 6). In addition also the geometric reliability was established since the procedure was able to well reproduce the crystallographic pose of ergotamine within 5HT-2B model (with an RMSD of 0.3 Å; Fig. 7).

3.4. Results of ergotamine metabolites

All the results of detected and calculated metabolites are summarized in Table 3. All metabolites predicted as able to interact with 5HT-2A and 5HT-2B occupied the pocket in a fashion resembling the crystallographic pose of ergotamine (Fig. 7), and in accordance to previous findings of ergot derivatives [17]. Since the architecture of binding between ergotamine and 5HT-receptors has been already described elsewhere [18,36], it was not treated here again. Rather, we discussed solely the effects exerted by metabolic modifications – implying that the main interactions were conserved – emphasizing inactivating effects and model-specific patterns.

3.4.1. Experimentally determined metabolites

Concerning the interaction with 5HT-2A, hydroxylation of the ergotamine G ring variably affects protein–ligand interaction. In particular, the top of the pocket showed a rigid alternation of polar and hydrophobic patches. The mono-hydroxylated compounds DM-1, DM-2, DM-3, DM-4, DM-5 and the di-hyroxylated compound DM-10 better satisfied such pharmacophoric requirements. Conversely, the rest of the di-hydroxylated compounds (*i.e.* DM-6, DM-7, DM-8 and DM-9) were predicted as unable to interact with the model mainly due to the improper distribution of hydrophilic groups within the hydrophobic environment. A completely different pattern was observed for 5HT-2B, since only DM-3 was predicted as able to interact. This can be explained by the localized reduction of available volume due to the G359Q mutation that does not allow a proper positioning of hydroxyl groups (Fig. 8A).

3.4.2. Computed metabolites

None of the conjugated metabolites was predicted as able to interact with the two models, mainly as a result of steric hindrance. The rest of metabolites, with the exception of CM-11 and CM-17, were predicted as able to interact with 5HT-2A model. The lack of interactions was respectively caused by the ergoline moiety's structural stiffening (as a consequence of carbon–carbon bond reduction) which neglected the pocket requirements (CM-11), and by hydrophobic-polar interferences due to the improper positioning of the hydroxyl group (CM-17). A quite different pattern was globally observed for 5HT-2B where the putatively inactive compounds were, in addition

Table 2
GOLD scores and AutoDock Vina scores of training set within 5HT-2A and -2B models.

Compounds	5HT-2A		5HT-2B	
	GOLD Score	AutoDock Vina	GOLD score	AutoDock Vina
Ergocornine	51	−10.7	65	−10.6
Ergocristine	60	−9.5	75	−10.0
Ergosine	75	−11.6	71	−10.3
Ergotamine	78	−11.1	80	−11.3
Lisuride	46	−8.2	60	−8.0
Lysergide	54	−10.5	54	−10.0
Methylergonovine	71	−10.5	69	−9.8
Pergolide	56	−9.1	50	−8.7
Terguride	37	−8.3	42	−8.5
Decoy 01	23	−0.4	55	−1.3
Decoy 02	51	1.7	46	−7.3
Decoy 03	30	13.9	12	2.4
Decoy 04	52	−2.5	50	−6.5
Decoy 05	29	−3.2	32	−7.1
Decoy 06	23	6.1	6	−6.6
Decoy 07	30	5.4	35	1.0
Decoy 08	−1	4.0	12	−3.1
Decoiy 09	0	11.4	2	1.5
ZINC79670178	51	−10.5	49	−10.6
Ergotaminine	47	−7.8	45	−9.5

Table 3
Query sets results.

Compound		5HT-2A		5HT-2B	
		HINT score	Expected interaction	HINT score	Expected interaction
Experimentally detected	DM-1	669	Positive	−598	Negative
	DM-2	390	Positive	−253	Negative
	DM-3	414	Positive	700	Positive
	DM-4	227	Positive	154	Negative
	DM-5	234	Positive	3	Negative
	DM-6	65	Negative	−184	Negative
	DM-7	−272	Negative	−327	Negative
	DM-8	46	Negative	−258	Negative
	DM-9	37	Negative	−387	Negative
	DM-10	326	Positive	−807	Negative
Computational prediction	CM-1	505	Positive	165	Negative
	CM-2	659	Positive	268	Positive
	CM-3	309	Positive	269	Positive
	CM-4	401	Positive	457	Positive
	CM-5	1113	Positive	783	Positive
	CM-6	602	Positive	371	Positive
	CM-7	688	Positive	383	Positive
	CM-8	976	Positive	811	Positive
	CM-9	534	Positive	−285	Negative
	CM-10	643	Positive	372	Positive
	CM-11	118	Negative	−908	Negative
	CM-12	361	Positive	−430	Negative
	CM-13	497	Positive	463	Positive
	CM-14	287	Positive	622	Positive
	CM-15	418	Positive	458	Positive
	CM-16	458	Positive	395	Positive
	CM-17	−976	Negative	167	Negative
	CM-18	740	Positive	657	Positive
	CM-19	−3.5E+03	Negative	−5487	Negative
	CM-20	−7205	Negative	−2786	Negative
	CM-21	−4913	Negative	−2E+05	Negative
	CM-22	−7260	Negative	−4703	Negative

to CM-11 and CM-17, CM-1, CM-9 and CM-12. CM-9 and CM-12 shared hydroxylation at the level of ergoline moiety which occupied the buried portion of the pocket. Therefore, the local shape reorganization between 5HT-2A and 5HT-2B at the bottom of the pocket, due to A225S and M218V mutations, may partially explain these results (Fig. 8B). On the contrary, CM-1 – that held para-hydroxylation on phenylalanine side chain – was predicted as inactive due to pocket shape resizing and pharmacophoric switch caused by D147K mutation at the top of the pocket (Fig. 8C).

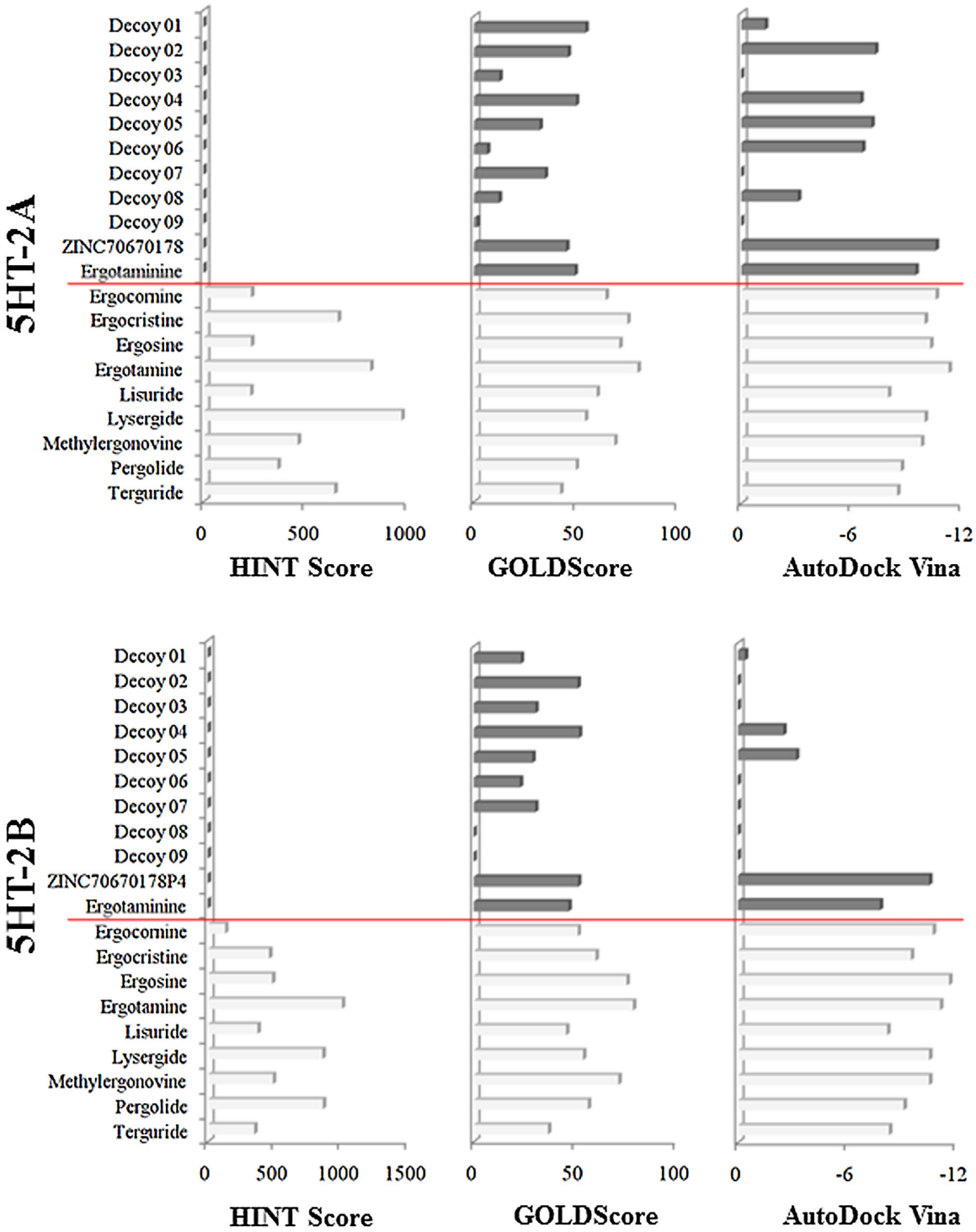


Fig. 6. Validation of computational procedure. Rescoring procedure by using the software HINT allowed the proper clustering of compounds. Red lines separate ligands (white bars) from decoys (gray bars).

Taken together these findings outline the existence of several metabolic forms possibly able to interact with serotonin receptors. Therefore the hierarchical screening of the entire set of metabolites may allow to proceed with experimental trials exclusively on noteworthy compounds. Also, the computed metabolites might be actually accounted for the targeted detection in samples, thus concurring to further define the metabolic pattern of ergotamine likely responsible for serotonergic action.

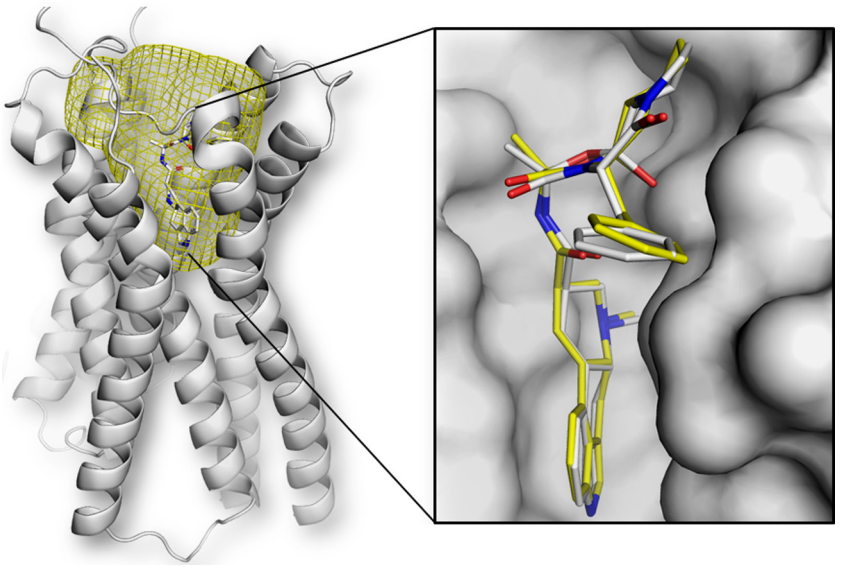


Fig. 7. Binding mode of ergotamine within 5HT-2B receptor. The protein is represented in ribbon, the binding pocket in yellow mesh and the ligand in sticks. The comparison between crystallographic data (white colored) and computed pose (yellow colored) is detailed in the side box.

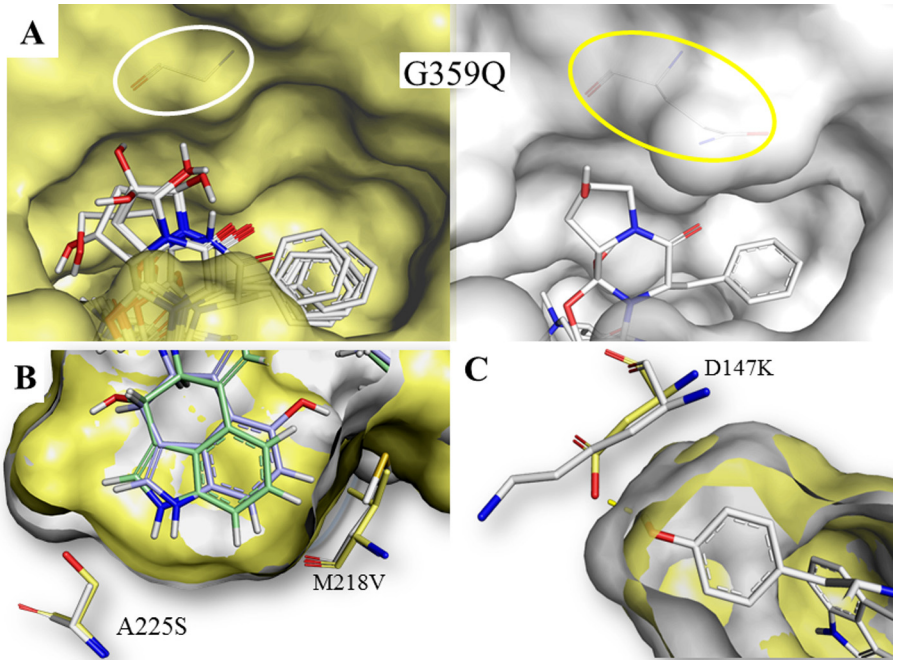


Fig. 8. Structural basis of structure-specific interaction patterns. The binding sites are represented in surface while ligands and amino acids are represented in sticks. (A) G ring sub-site resizing between 5HT-2A (yellow surface on the left) and 5HT-2B (white surface on the right) due to G359Q mutation (highlighted by colored rings). The ligands predicted as able to interact with the respective models are reported. (B) Shape resizing at the bottom of the pocket (highlighted by blue ring) between 5HT-2A (yellow surface) and 5HT-2B (white surface). Ligands showing differential interaction are reported (CM-9 is colored in green, and CM-12 is colored in blue, respectively). In 5HT-2B model, the ergoline moiety of both compounds is too close to pocket sides thus preventing favorable interactions. (C) Effect of D147K mutation on the interaction of CM-1 with 5HT-2A (yellow surface) and 5HT-2B (white surface). The yellow dotted line indicates hydrogen bond. The presence of lysine-to-aspartate substitution prevents hydrophobic-polar interferences and gains further interaction.

4. Discussion

Nowadays the presence of EAs in food and feed is of growing concern and further data on toxicodynamics and toxicokinetics, including metabolism, of the most common EAs are needed, as recently stated by EFSA [7]. The interaction with some neurotransmitter receptors (including serotonin receptors) is a well-known mode of action leading to a series of adverse effects on human health. It is likely that EAs, as xenobiotics, undergo massive metabolic changes by hosts detoxifying mechanisms, and their metabolites may exert a certain bioactivity as

well. Nevertheless, neither extensive studies on human metabolism of EAs occurring in food (*e.g.* ergotamine), nor the effect of metabolism on parent compound activity, are systematically under study so far, also on account of the efforts required for the *a priori* multi-receptors analysis of metabolome activity. Actually, the existence of truly detoxified forms and still active metabolites is largely overlooked, and, consequently, the lack of knowledge in respect to the consequences of metabolic modifications may lead to a deceptive scenario for risk assessment. Within this framework, it has been assessed herein whether an *in silico* analysis may be an effective choice to analyze the interaction between some members of serotonin receptors class and ergotamine metabolites. The main goal was to provide a fast and straightforward procedure to support and drive the design of smart trials to support hazard characterization. In this respect, it should be kept in mind that quantum chemical approaches – which effectively address the calculation of the Gibbs free energy alongside geometrical aspects of complex formation – have been successfully applied to the rational design of ergoline-based chemicals [38], also against 5HT-2A [17]. Nonetheless, in the case of virtual screening of wide datasets of compounds, the use of such methods may be challenging and demanding, especially in terms of hardware requirements. For this reason, the use of a validated molecular mechanic approach based on docking–rescoring coupling – which takes advantage of an empirical scoring function – was a less demanding and easy to implement choice. Hence, since the EA-serotonin receptor interaction is at the basis of the molecular initiating event of EAs action, and 5HT-2B three-dimensional structure was available in Protein Data Bank, a 3D molecular modeling approach based on docking simulations and re-scoring procedure has been successfully used.

Validating procedures assessed the reliability of 5HT-2A and 5HT-2B models to be applied to a set of ergotamine metabolites. Notably, such set comprised both experimentally detected compounds and purely computed metabolites. The study was mainly aimed at investigating how metabolism can plausibly affect the bioactivity. According to previous studies, the activity of hydroxylated metabolites was confirmed, although the output suggests certain receptor specificity. The reduction of available volume due to G359Q mutation at the level of the sub-site for G ring might reduce the interaction with 5HT-2B for the most of hydroxylated metabolites in the G ring. In this regard, one has to keep in mind that, when homology relationship subsists, sequence and structure similarities are key conditions at the basis of functional inference of proteins [39]. Therefore the residues conservation in key locus for protein function plausibly determines functional conservation. For this reason, albeit 5HT-2C model was rejected, it can be hypothesized a behavior similar to 5HT-2B since at such locus both held comparable bulky side-chains (*i.e.* glutamate and asparagine, respectively). On the contrary, at the level of tolerance of modifications on ergoline moiety, it can be supposed a 5HT-2A-like behavior due to the occurrence of the same M218V mutation (Fig. 4).

With respect to conjugated derivatives, none of the glycosylated compounds seemed to be able to interact with 5HT-2A and 5HT-2B models, supporting thus no bioactivity. On the basis of the conservation of the overall anatomy of the pocket, we could postulate the lack of interaction with 5HT-2C, as well. Being the detoxification of contaminated food and feed a paramount task [40], this finding can be transferred to that context, taking into consideration that any conjugation of a polar and bulky group that originates from technological treatment of raw materials or from metabolic transformations may significantly reduce the serotonergic action of EAs. Notably, no significant contributions to the interaction were charged on modifications on G ring. Those were rather tolerated when the reduction of interferences was possible. This finding might be taken into account to conceive inactivating modifications. Contrariwise several of computed modifications brought further interactions. In particular CM-3, CM-4, CM-6, CM-7, CM-13 gained further hydrogen-bonds with both models, while CM-2 with 5HT-2B only (data not shown). For the rest of metabolites predicted as able to interact, it is concluded that modifications did not interfere in a large extent to the pockets, thus allowing the interaction with the binding site.

Strikingly, it is worthy of note that more than two-thirds of metabolites herein considered were predicted as active. Therefore, taken altogether, our findings ultimately suggest the need for further data on serotonergic activity of ergotamine's metabolites, which might actually elude in some extent, at least, the phase I detoxifying pathway.

5. Conclusions

Summarizing, in the present paper the *in silico* modeling proved to be an effective choice to investigate the interaction of ergopeptine alkaloids derivatives with multiple serotonergic receptors. Moreover, to the best of our knowledge, such a wide array of human metabolites of ergotamine has been taken into consideration for the first time. Models for 5HT-2A, 5HT-2B and 5HT-2C were provided, and albeit the last was not validated, it was possible to outline possible guidelines for future experiments on the basis of sequence conservation and structural analogies. This work actually represents a knowledge-based, straightforward and cost effective choice to provide the priority setting of compounds before bench analysis, as the lack of commercially available compounds prevents from systematic trials on multiple serotonergic receptors. On the condition that a sufficient system of background knowledge subsists for design and assessing the model – so as to define the domain of applicability unequivocally – this approach may be extended to any toxicant of interest in food toxicology, offering thus a useful strategy to support hazard identification at an early stage.

Conflict of interest statement

The authors declare no conflict of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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