Food and Chemical Toxicology 131 (2019) 110556

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

In silico study of the mechanism of action, pharmacokinetic and toxicological properties of some *N*-methylanthranilates and their analogs

Lorane Izabel da Silva Hage-Melim[a,b], João Gabriel Curtolo Poiani[b], Carlos Henrique Tomich de Paula da Silva[b], Fábio Boylan[c,*]

[a] Laboratory of Pharmaceutical and Medicinal Chemistry, Federal University of Amapá, Amapá, Brazil
[b] Computational Laboratory of Pharmaceutical Chemistry, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil
[c] School of Pharmacy and Pharmaceutical Sciences, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin 2, Ireland

ARTICLE INFO

Keywords:
N-methylanthranilic acid esters
Anti-inflammatory/Antinociceptive
Anxiolytic
Antidepressant
Anti-allergic
ADME/Tox

ABSTRACT

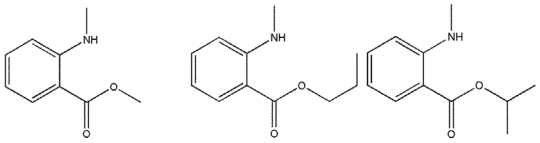
The *in silico* evaluation for the three previously synthesized compounds (Methyl (MMA), propyl (PMA) and isopropyl (IMA) *N*-methylanthranilate), MMA and IMA originally found in the leaf essential oil of *Choisya ternata,* provided a very good confirmation for the *in vivo* pharmacological results obtained with such compounds for a number of pharmacological targets. This manuscript dealt with their assessment in six pharmacological targets to understanding anti-inflammatory, antinociceptive, anxiolytic, antidepressant and anti-allergic activities using docking molecular as well as their pharmacokinetics and toxicological parameters prediction. The compound IMA seems to be the best one when all the combined parameters are put together. Interestingly this compound presented the best *in vivo* profile in previous studies by the group. Derivatives of the three original molecules were proposed. Overall the second modification (5-[2-(methoxycarbonyl)anilino]pentanoic acid, 5-[2-(propoxycarbonyl)anilino]pentanoic acid and 5-(2-{[(propan-2-yl)oxy]carbonyl}anilino)pentanoic acid) of all three original molecules was the one that achieved highest score in molecular docking and a better combination of the other parameters. Further research as in the obtaining of such derivatives via synthesis and their *in vivo* testing to confirm their higher pharmacological potential is currently on the way.

1. Introduction

The evaluation of *Choisya ternata* Kunth (Rutaceae) leaves essential oil allowed for the identification of methyl *N*-methylanthranilate (MMA) and isopropyl *N*-methylanthranilate (IMA) as minor constituents (Radulović et al., 2011). IMA was described for the first time in nature and to allow its unequivocal structure elucidation, gram quantities of it were synthesized together with MMA and their propyl derivative (PMA) (Radulović et al., 2011). These volatile anthranilate derivatives were submitted to a variety of pharmacological tests showing promising results as antinociceptive (Pinheiro et al., 2014; Radulović et al., 2011), anti-inflammatory (Pinheiro et al., 2015), gastro-, hepato- and nephroprotective (Radulović et al., 2013a, 2013b, 2015, 2017), anxiolytic and anti-depressant, as well as an increase on diazepam-induced sleep time (Radulović et al., 2013c). Also, the toxicity of MMA and IMA has been investigated in a model of acute toxicity (Pinheiro et al., 2014; SCCS, 2011) showing no signs of toxicity. This was confirmed through the application of IMA and MMA on rat liver tissue and assessment of its morphology and function by means of standard biochemical and histopathological analyses (Radulović et al., 2017). There is still no experimental data in relation to the toxicity of PMA. In relation to pharmacokinetics there are no studies on absorption and distribution of such compounds. However Radulović et al. have studied in depth the metabolism and urinary excretion of IMA and MMA (Radulović et al., 2017). No information was obtained for the derivative PMA. In order to further provide an understanding to the pharmacological properties of these three anthranilic acid derivatives and to gain more first-hand information about PMA and other possible derivatives, a set of *in silico* experiments were performed. Methyl (MMA), propyl (PMA) and isopropyl (IMA) *N*-methylanthranilate molecules were investigated for their mechanism of action and pharmacokinetic and toxicological properties. In order to improve these predictions, some derivatives have been proposed based on the original structure of the three template molecules.

* Corresponding author.
E-mail address: fabio.boylan@tcd.ie (F. Boylan).

https://doi.org/10.1016/j.fct.2019.06.003
Received 10 January 2019; Received in revised form 31 May 2019; Accepted 1 June 2019
Available online 07 June 2019



2. Material and methods

2.1. Studied molecules

The methyl (MMA), propyl (PMA) and isopropyl (IMA) *N*-methylanthranilates and some derivatives were used in this study. The derivatives were proposed using two programs: ChemSketch (ACD, 2015) for the design of molecules and Discovery Studio Visualizer (Acclerys, 2007) for the pre-optimization of the same.

2.2. Molecular docking

For the docking study, we downloaded files deposited in the Protein Data Bank (PDB) from the Research Collaboratory for Structural Bioinformatics (Li et al., 2008; Sandy and Butler, 2009; Orlando and Malkowski, 2016) with the coordinates of the crystallographic structures of therapeutic targets Cyclooxygenase-1 (COX-1) (PDB ID: 3N8X, resolution: 2.75 Å) complexed with the nimesulide inhibitor; Cyclooxygenase-2 (COX-2) (PDB ID: 5IKQ, resolution: 2.41 Å) complexed with meclofenamic acid and prostacyclin; Transient receptor potential cation channel subfamily V member 1 (TRPV1) (PDB ID: 5IS0, resolution: 3.43 Å) complexed with capsazepine; Gamma-aminobutyric acid receptor subunit beta-3 (GABA) (PDB ID: 4COF, resolution: 2.97 Å); Serotonin transporter (SERT) (PDB ID: 5I71, resolution: 3.15 Å) complexed with s-citalopram; and Histamine H1 receptor (HRH1) (PDB ID: 3RZE, resolution: 3.1 Å) complexed with depexin.

To perform the molecular docking, we added hydrogen atoms and removed water molecules from the enzymes. The inhibitors that were complexed with each therapeutic target were extracted. Prior to performing the docking simulation, we validated our results by calculating the Root-mean-square deviation (RMSD) between the experimental and the conformation of the ligand that yielded the best pose after docking. To calculate the docking, we used the following coordinates: COX-1: x = −21.43; y = −50.79 and z = 1.42; COX-2: x = 22.83; y = 51.56 and z = 17.81; TRPV1: x = 139.19; y = 109.16 and z = 102.08; GABA: x = −20.76; y = −19.24 and z = 127.55; SERT: x = −32.02; y = −19.74 and z = 2.07; and HRH: x = 16.66; y = 35.97 and z = 22.31.

To identify the interactions between the compounds and the therapeutic targets, it was necessary to identify the amino acids making up the binding site: COX-1: Arg120, Tyr355, Ile523 and Ser530; COX-2: Tyr385 and Ser530 (Borges et al., 2018); TRPV1: Tyr511, Ser 512, Met 514, Leu515, Leu518, Phe 543, Leu547, Thr550, and Asn551 (Kim et al., 2018); GABAa: Asp 43, Tyr62, Gln 64, Tyr97, Leu99, Met 115, Tyr157, Phe200 and Tyr205 (Hoerbelt et al., 2016); SERT: Ala 96, Asp98, Trp 103, Tyr175, Tyr176, Asn 177, Ala 331, Phe335, Ser 439 and Glu 493 (Malikowska et al., 2017); HRH1: Asp107, Thr112, Ile115, Trp158, Asn198, Phe424, Trp428 and Phe432 (Shimamura et al., 2011).

2.3. Prediction of the pharmacokinetic (ADME) and toxicological (TOX) properties

The prediction of pharmacokinetic properties, absorption, distribution, metabolism and excretion (ADME), was performed through QikProp software. The *in silico* toxicity assessment was performed using the DEREK software (Ridings et al., 1996), developed and marketed by LHASA UK (School of Chemistry, University of Leeds, UK) and Harvard University (Boston, MA, USA). Toxicological properties that could be predicted were mutagenicity, carcinogenicity, nephrotoxicity, hepatotoxicity, skin sensitization, corrosivity, among others (Dearden et al., 1997).

3. Results and discussion

3.1. Molecular docking

Molecular docking is a computational method currently widely used in drug discovery. The aim of docking is to identify the mode of interaction of the molecules under study, in this case of MMA, PMA, IMA and their derivatives at the binding site of the enzyme or receptor through interactions and to predict the binding affinity between the protein-binding complexes (Gupta and Mohan, 2014). The GOLD (Genetic Optimization for Ligand Docking) software uses the genetic algorithm for purposes of flexible docking experiments of ligands into protein binding sites. The GOLD software was used to investigate the modes of interaction between the studied compounds and therapeutic targets (Chandak et al., 2014).

The RMSD value indicates the accuracy of the docking poses calculated by the GOLD docking algorithm compared with the experimentally poses of a compound bound to a biological target. A RMSD less than 2 Å is considered to be successful (i.e. to have justified validity) (Cole et al., 2005). In this study, the best RMSD values obtained with the targets COX-1, COX-2, TRPV1, GABA, SERT e HRH1 were 0.605 Å; 0.504 Å; 1.514 Å; 0.775 Å; 0.947 Å e 0.717 Å, respectively.

Then, we performed docking between the therapeutic targets and the compounds MMA, PMA and IMA. We selected the docking results that yielded the highest Goldscore for each therapeutic target (Table 1 - Supplemental material).

3.1.1. Anti-inflammatory action

Prostaglandins derive from arachidonic acid (AA) in a reaction catalyzed by COX, which can exist as COX-1 and COX-2. AA upon neopathological stimuli is released from the cell membrane. Inhibitors of this enzyme will interfere in this reaction and then the disease process begins. Recently, involvements of COX-1 in cancer and inflammation were firmly established (Vitale et al., 2016).

In this study, the interactions between the synthesized compounds and the COX-1 and COX-2 therapeutic targets for *in silico* evaluation of the anti-inflammatory activity were estimated. With the therapeutic target COX-1, the molecular docking of the MMA, PMA and IMA presented Goldscore of 49.44; 57.20 and 56.18, respectively. With COX-1, MMA had eight interactions, two hydrogen bonds, with the amino acids Arg120 and Tyr355, and six hydrophobic interactions, with the amino acids Val116, Leu352, Ile523, Ala527 and Leu531. PMA presented seven interactions, being a hydrogen bond, with the amino acid Arg120 and six hydrophobic interactions, with the amino acids Val116, Val349, Ile523, Leu352, Tyr355 and Leu 359. IMA presented eight interactions, being a hydrogen bond, with amino acid Arg120 and seven hydrophobic interactions, with the amino acids Val116, Val349, Leu352, Tyr355, Ile523, Ala527 and Leu531 (Fig. 1 and Table 1 - Supplemental material).

With COX-2, MMA presented nine interactions, two hydrogen bonds, with the amino acids Tyr385 and Ser530, one of the Pi-Sulfur type, with the amino acid Met522, and six hydrophobic interactions, with the amino acids Val 344, Tyr348, Val349, Tyr385, Trp387 and Gly526/Ala527. PMA had five interactions, one of Pi-Sulfur type, with amino acid Met522 and four hydrophobic interactions, with the amino acids Tyr355, Trp387, Val 523 and Gly526/Ala527. IMA presented seven interactions, one of Pi-Sulfur type, with amino acid Met522 and six hydrophobic interactions, with amino acids Val349, Leu352, Trp387, Gly526 and Ala527 (Fig. 2 and Table 1 - Supplemental material).

In a study by Gouda et al. (2016), *N*-(4-bromophenyl)-7-cyano-6-(4-

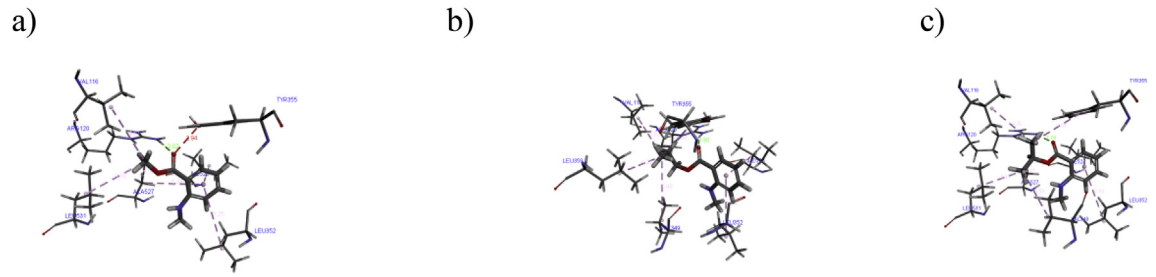


Fig. 1. Docking mode of studied compounds into COX-1 (PDB ID: 3N8X). a) MMA b) PMA and c) IMA.

methylphenylsulfonamido)-2,3-dihydro-1*H*-pyrrolizine-5-carboxamide and (*R,S*)-*N*-(4-bromophenyl)-7-cycno-6-(2-(4-isobutylphenyl)propanamido)-2,3-dihydro-1*H*-pyrrolizine-5-carboxamide exhibited high binding affinities into COX-1 enzyme. Both of them exhibited one hydrogen bond between their pyrrolizin-2-sulfonamide and pyrrolizin-2-carboxamide and phenolic OH of Tyr355 and NH2 of Arg120, respectively. For COX-2, 6-(4-methylphenylsulfonamido)-2,3-dihydro-1*H*-pyrrolizine conserved four hydrogen bonds between its sulfonamide and aniline NH moieties and NH_2 of Arg120, phenolic OH of Tyr355, and carbonyl group of Leu352.

In a study by Świątek et al. (2017), 2-[1-oxo-1-(4-phenylpiperazin-1-yl) butan-2-yl]-4,6-dimethylisothiazolo[5,4-b]pyridin-3(2*H*)-one interacts with COX-1 by Arg120 and Ser353 and with COX-2 by Tyr355, Arg120 and Ser353. 2-{3-oxo-3-[4-(3-chlorophenyl)piperazin-1-yl] propyl}-1,2-benzisothiazol-3(2*H*)-one interacts with COX-1 by Arg 120 and with COX-2 by Ser353 and Tyr355.

In vivo studies performed by Pinheiro et al. (2015), revealed that MMA, PMA and IMA presented significant anti-inflammatory activity. In the molecular docking studies performed here, all molecules showed interactions with the targets evaluated and by the number of interactions, Goldscore value, interaction with key amino acids for activity, it is suggested that MMA is the compound that has the best anti-inflammatory action.

3.1.2. Antinociceptive activity

Transient receptor potential (TRP) ion channels form a notable family of cation channels related to the ability of an organisms to identify noxious mechanical, thermal, and chemical stimuli that signals the perception of pain, taste, and changes in temperature (Darré and Domene, 2015).

The transient receptor potential cation channel subfamily V member 1, also known as the capsaicin receptor or vanilloid receptor 1 (TRPV1) is the main representative of a subfamily of thermosensitive TRP channels that enable somatosensory cells to detect temperature changes in the environment, being activated by pernicious high temperatures. As a result of this feature, the TRPV1 channel plays an essential role in the molecular mechanisms responsible for injury-related hyperalgesia and pain hypersensitivity (Julius, 2013).

In this study, the interactions between the MMA, PMA and IMA and the TRPV1 target were evaluated. MMA had nine interactions, five hydrogen bonds, with the amino acids Tyr554, Arg557, Ala566 and Gln 700, and four hydrophobic interactions, with Leu553, Arg557, Ala566 and Val 567. PMA presented four interactions, two hydrogen bonds, with the amino acids Thr550 and Asn551, and two hydrophobic interactions, with Leu553 and Ala566. IMA presented five interactions, two hydrogen bonds, with the amino acids Thr550 and Asn551, and three hydrophobic interactions, with Leu515, Leu553 and Ile569 (Fig. 3 and Table 1 - Supplemental material).

Darré and Domene (2015) demonstrated the main interactions between capsaicin and TPRV1 include Tyr511, which appears to form a hydrogen bond with the amide oxygen of capsaicin, and also contributes to the hydrophobic cluster formed by Leu515, Ile 573, Phe587, and Leu 669 that anchors the vanillyl ring.

Kim et al. (2018) showed the interaction of 2-(3,5-dihalo-4-aminophenyl)acetamide analogues into TRPV1. The 2,6-dichloroaniline group was involved in the hydrophobic interactions with Val 508, Tyr511, Leu515, Tyr555, Ile 564, Tyr565, and Ile569. The amide group participated in the hydrogen bonding with Tyr511. The 6-trifluoromethylpyridine group formed the hydrophobic interactions with Thr550 and Tyr554, along with Phe587 and Phe 591 from the adjacent monomer. Additionally, the 4-methylpiperidine ring oriented itself towards the upper region of the binding site and made hydrophobic interactions with Leu518 and Leu547.

Results obtained by Pinheiro et al. (2014) indicate that the *in vivo* antinociceptive effect of MMA, PMA and IMA involves, at least in part, the glutamatergic system. In addition to the results obtained in the capsaicin-induced nociception test it is possible that they could produce antinociception by regulating TRPV1 receptors, thus contributing to the modulation of nociceptive transmission. In this study, all compounds showed interactions with TRPV1, but interactions were identified in greater numbers with the key amino acids of the binding site between PMA and IMA with the target under study. This leads to the belief that chain extension helps in the interaction of the molecule with TRPV1.

3.1.3. Anxiolytic activity

The γ-aminobutyric acid type A receptors ($GABA_A$Rs) are the principal mediators of rapid inhibitory synaptic transmission in the human brain. A decrease in $GABA_A$R signaling triggers hyperactive neurological manifestations such as insomnia, anxiety and epilepsy (Miller and Aricescu, 2014).

In this study, the compounds MMA, PMA and IMA were evaluated with possible interaction with the target GABA. For the MMA, eight

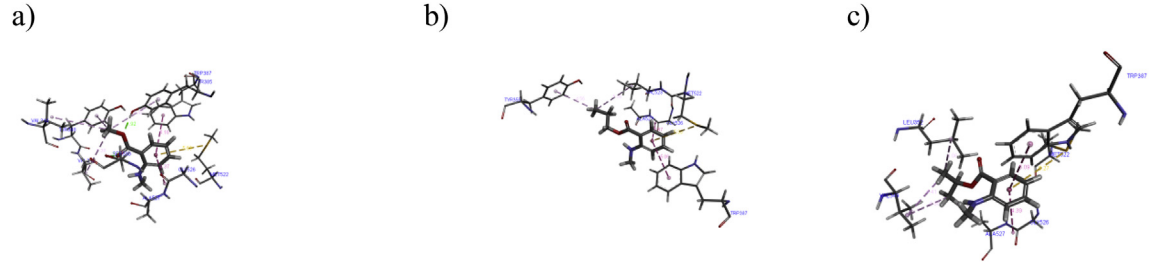


Fig. 2. Docking mode of studied compounds into COX-2 (PDB ID: 5IKQ). a) MAN b) PAN e c) ISOAN.

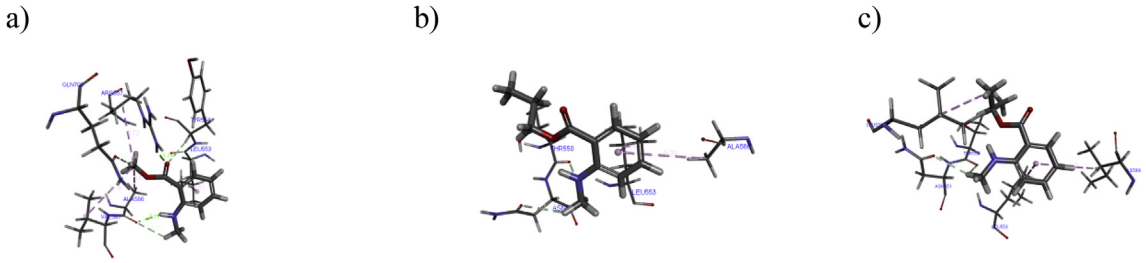


Fig. 3. Docking mode of studied compounds into TRPV1 (PDB ID: 5IS0). a) MMA b) PMA and c) IMA.

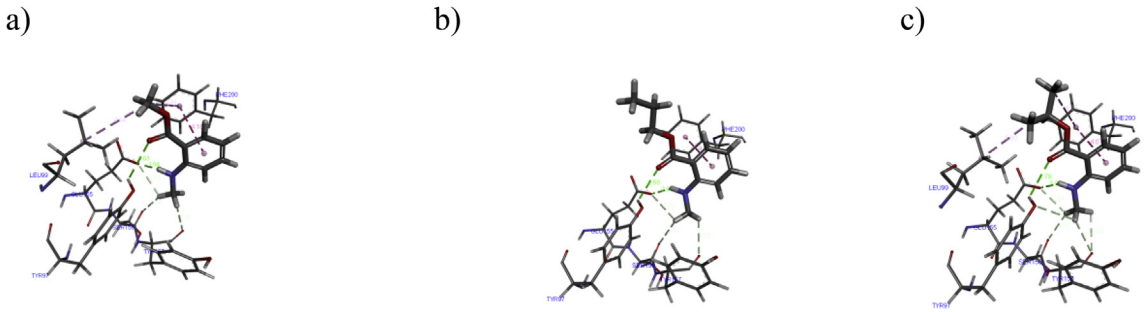


Fig. 4. Docking mode of studied compounds into GABAa (PDB ID: 4COF). a) MMA b) PMA and c) IMA.

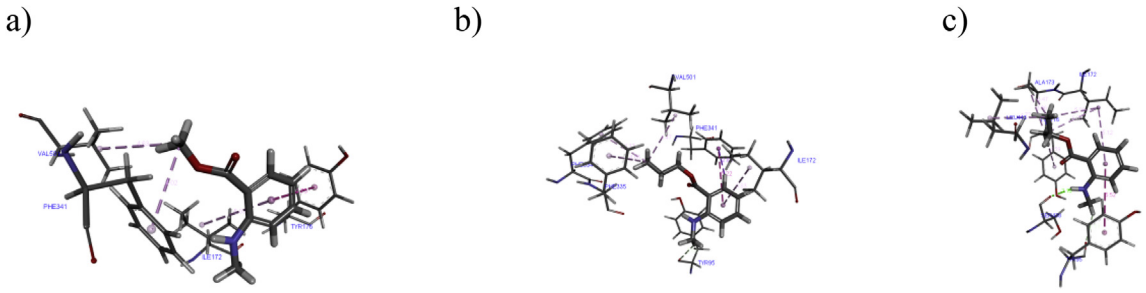


Fig. 5. Docking mode of studied compounds into SERT (PDB ID: 5I71). a) MMA b) PMA and c) IMA.

interactions were observed, five hydrogen bonds, with the amino acids Tyr97, Glu155, Ser156 and Tyr157, and three hydrophobic interactions, with Leu99 and Phe200. PMA had six interactions, five hydrogen bonds, with the amino acids Tyr97, Glu155, Ser156 and Tyr157, and a hydrophobic interaction, with Phe200. IMA presented ten interactions, seven hydrogen bonds, with the amino acids Tyr97, Glu155, Ser156 and Tyr157 and three hydrophobic interactions, with Leu99 and Phe200 (Fig. 4 and Table 1 - Supplemental material).

A study by Miller and Aricescu looking at interactions between benzamidine and GABA showed that the benzamidine benzyl ring is stacked between the side chains of Phe200 and Tyr62, whereas its amidinium group forms hydrogen bonds with the Glu155 side chain and backbone carbonyls of Ser156 and Tyr157, and putative cation-p interactions with the Tyr157 and Tyr205 aromatic rings (Miller and Aricescu, 2014).

In a study by Radulović et al. (2015), MMA and IMA did not induce sleep in mice but significantly prolonged the diazepam-induced sleep, in a dose-dependent way, suggesting an interaction with the GABA receptor complex. In our study, all the molecules showed interaction with GABAa, however the IMA was the one that presented satisfactory GoldScore and greater number of interactions with the target.

3.1.4. Antidepressant activity

A 'broad-spectrum' antidepressant approach able to inhibit serotonin, noradrenaline and/or dopamine transporters is what clinical evidence and recent research on mono-amino-based therapies have been pointing out to. The majority of the antidepressants currently used in clinics act as inhibitors of one or more mono-amino transporters (Iversen, 2000). Primarily situated in presynaptic membranes, they are mainly dopamine, noradrenaline and serotonin transporters (DAT, NET and SERT, respectively). Recently, structural information about DAT, SERT and NET has been the subject of intensive research (Malikowska et al., 2017).

In our study, molecular docking was performed between MMA, PMA and IMA and the therapeutic target SERT. MMA presented four hydrophobic interactions, with the amino acids Ile172, Tyr176, Phe341 and Val501. PMA presented seven interactions, one hydrogen bond, with the amino acid Tyr95, and six hydrophobic interactions, with the amino acids Ile172, Phe 334, Phe335, Phe341 and Val501. IMA presented ten interactions, being two hydrogen bonds, with the amino acids Tyr95 and Ser 348, and eight hydrophobic interactions, being Tyr95, Ile172, Ala173, Tyr176 and Leu443 (Fig. 5 and Table 1 - Supplemental material).

In a study by Szabó et al. (2018), the trifluoromethyl group attached to one of the aromatic rings of fluoxetine is located in the hydrophobic part of the binding pocket formed by Ile 168, Ala 169, Ile172, Ala173, Gly 442 and Leu443 residues into SERT. The other aromatic ring is positioned close to Val501, Phe335, Phe341, Ile172 and Tyr175 and is mostly buried in the hydrophobic pocket. The flexible chain is well positioned to form hydrogen bonds with several acceptors, for example Asp98 side chain and Phe335 backbone NH.

In a study by Radulović et al. (2013c), MMA and IMA presented antidepressant-like effects, but the results suggested a different mode of action of the anthranilates to the one of imipramine, which acts by

inhibition of serotonin and norepinephrine reuptake within synaptic clefts in the central nervous system. In our study, MMA, PMA and IMA presented interactions with SERT, with MMA being less intense and IMA with higher number of interactions.

3.1.5. Anti-allergic property

Histamine is a biogenic amine and an important mediator in various physiological and pathophysiological conditions such as arousal state, allergy and inflammation (Schwartz et al., 1991). Histamine exerts its effects through the activation of four distinct histamine receptors (H1, H2, H3 and H4) all belonging to the G-protein-coupled-receptor (GPCR) superfamily. In type I hypersensitivity allergic reactions, H1R is activated by histamine released from mast cells, which are stimulated by various antigens (Simons, 2004). Many studies have been performed to develop H1R antagonists, known generally as antihistamines.

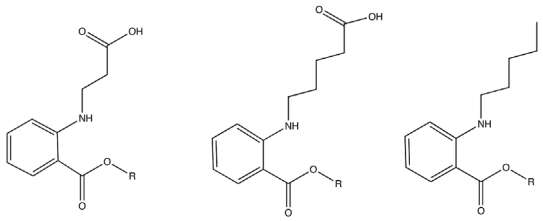
In our study, the compounds MMA, PMA and IMA were evaluated in order to identify interaction with HRH1. MMA presented nine interactions, four hydrogen bonds, with the amino acids Asp107, Ser111 and Tyr431, and five hydrophobic interactions, with Trp158, Trp428, Tyr431, Phe435 and Ile 454. PMA presented nine interactions: two hydrogen bonds, with amino acids Ser111 and Asn198, and seven hydrophobic interactions, with Tyr108, Trp158, Phe424, Trp428 and Phe432. IMA had ten interactions, all being hydrophobic interactions, with the amino acids Tyr108, Ile115, Trp158, Phe199, Phe424, Trp428 and Phe432 (Fig. 6 and Table 1 - Supplemental material).

Asp107, a strictly conserved residue in aminergic receptors, forms an anchor salt bridge with the amine moiety of the ligand. This interaction has been reported to be essential for the binding of HRH1 antagonists as well as agonists in mutational studies (BruysterS et al., 2004). In a molecular docking study by Sader et al. (2017), fexofenadine interacts with amino acid residues Asp107, Tyr108, Arg 176, Lys 179, Phe199, Trp428, Phe432 and Phe435.

In our study, PMA and IMA presented higher Goldscore values and a large number of interactions with HRH1, but the MMA molecule was the only one that presented interaction with Aps107, essential for activity.

3.2. Proposed derivatives

With the results obtained in the calculation of molecular docking, it was possible to observe some important regions that the ligands were unable to interact with for the used targets. To address this, some modifications were proposed in the molecules in order to increase their activity. In this sense, the carbon chain bound to the nitrogen atom was increased and in the first two modifications of each molecule this chain was finalized with a carboxyl functional group.



R= Me (MMA derivatives)
R= Pr (PMA derivatives)
R= iPr (IMA derivatives)

Then, the molecular docking was performed between all proposed modifications in the molecules and the studied targets using the same parameters used for the original molecules. All proposed modifications presented higher Goldscore than the original molecules. It is believed that the modifications aided in improving the quality of the interactions. In general, the second modification for all original molecules (derivative 2) was the one that presented the best results for all targets as can be seen in Table 2 - Supplemental material and Figs. 7–12. This find probably occurred due to the increase in the chain and the presence of a carboxyl functional group at the end of the chain, which enabled the molecules to make more hydrophobic interactions as well as more hydrogen bonding (donor and acceptor) with the targets studied.

To exemplify the best fit of the templates and modifications performed, Fig. 13 shows the MMA, PMA and IMA molecules and their respective derivatives into COX-1 binding site (PDB ID: 3N8X).

3.3. Prediction of pharmacokinetic and toxicological properties

In the process of drug development it is important to predict the pharmacological and toxicological properties of the candidates as early as possible, since this information will define the capacity of the molecule to become or not a drug. Predictions of pharmacokinetic and toxicological properties of selected compounds were performed (Tables 1 and 2, respectively).

The prediction of pharmacokinetic properties: absorption, distribution, metabolism and excretion (ADME) was performed through QikProp software, which estimates a range of relevant pharmaceutical properties of organic molecules through predictions with 3D molecular structure, providing fast and accurate results. Average values obtained for 95% of the known drugs were used as reference (Jorgensen, 2004).

QikProp calculates more than twenty descriptors, among them the percentage of human oral absorption (%HOA), albumin binding, blood-brain barrier permeability (logBB), Caco-2 and MDCK cell permeability (Caco-2), Number of violations of Jorgensen's rule of three (Rule of 3) and Number of violations of Lipinski's rule of five (RO5), among others (Schrödinger, 2011).

In predicting human oral gastrointestinal absorption, molecules with HOA > 80% have high and < 25% have poor absorption (Schrödinger, 2011). The molecules MMA, MMA derivative 3, PMA, PMA derivative 3, IMA, IMA derivatives 2 and 3 are likely to be highly absorbed by the intestine while the others are likely to have

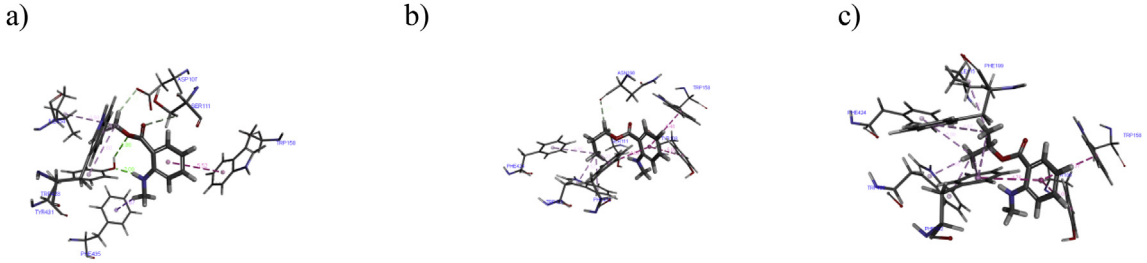


Fig. 6. Docking mode of studied compounds into HRH1 (PDB ID: 3RZE). a) MMA b) PMA and c) IMA.

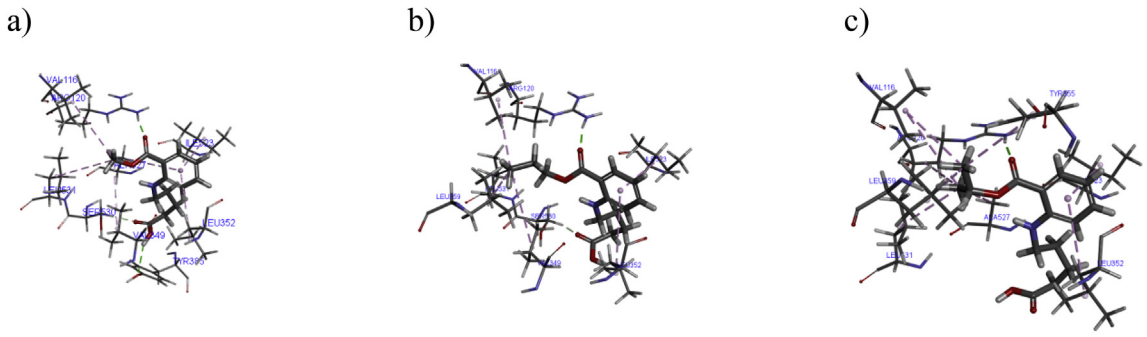


Fig. 7. Docking mode of studied compounds into COX-1 (PDB ID: 3N8X). a) MMA derivative 2, b) PMA derivative 2 and c) IMA derivative 2.

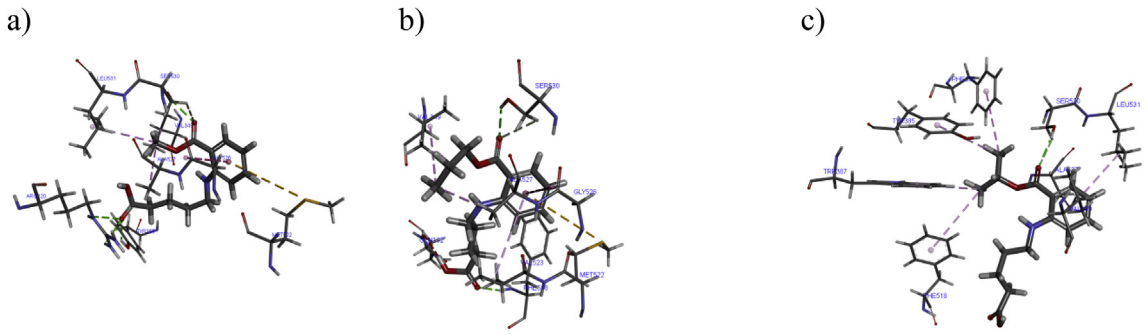


Fig. 8. Docking mode of studied compounds into COX-2 (PDB ID: 5IKQ). a) MMA derivative 2, b) PMA derivative 2 and c) IMA derivative 2.

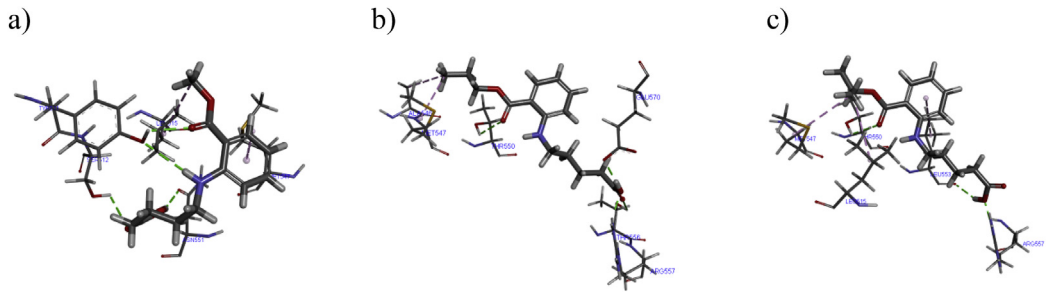


Fig. 9. Docking mode of studied compounds into TRPV1 (PDB ID: 5IS0). a) MMA derivative 2, b) PMA derivative 2 and c) IMA derivative 2.

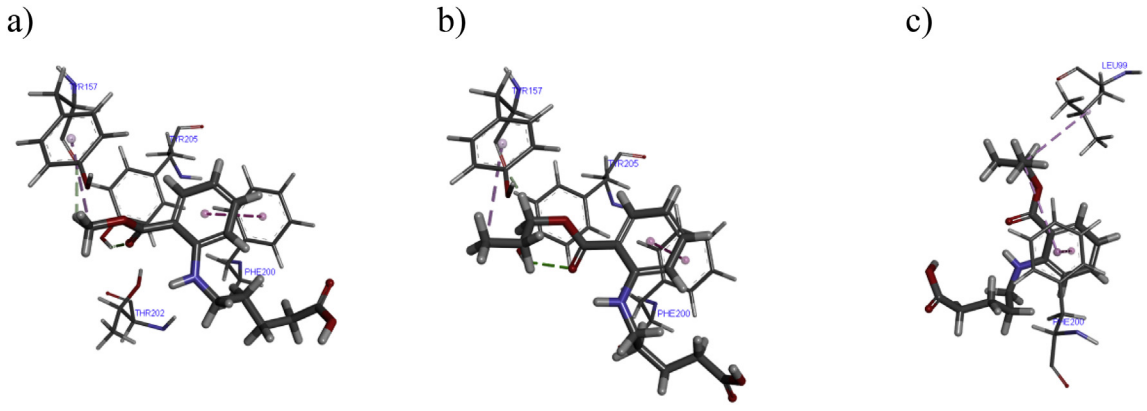


Fig. 10. Docking mode of studied compounds into GABAa (PDB ID: 4COF). a) MMA derivative 2, b) PMA derivative 2 and c) IMA derivative 2.

intermediate absorption.

In the permeability analysis of Caco-2 and MDCK cells, drug candidates with permeability of less than 25 nm/s indicate low permeability, whereas values above 500 nm/s indicate high permeability (Schrödinger, 2011). The molecules MMA, MMA derivative 3, PMA, PMA derivative 3, IMA, IMA derivative 3 are likely to have high permeability while the others are likely to have moderate permeability.

The blood-brain barrier is a natural structure protecting the organism, selectively allowing the access of chemical structures to the central nervous system. Drugs with LogBB = −3.0 – 1.2 are able to cross the blood-brain barrier (BBB) (Schrödinger, 2011). Therefore, all molecules are likely to cross the BBB. However, the evaluation of this

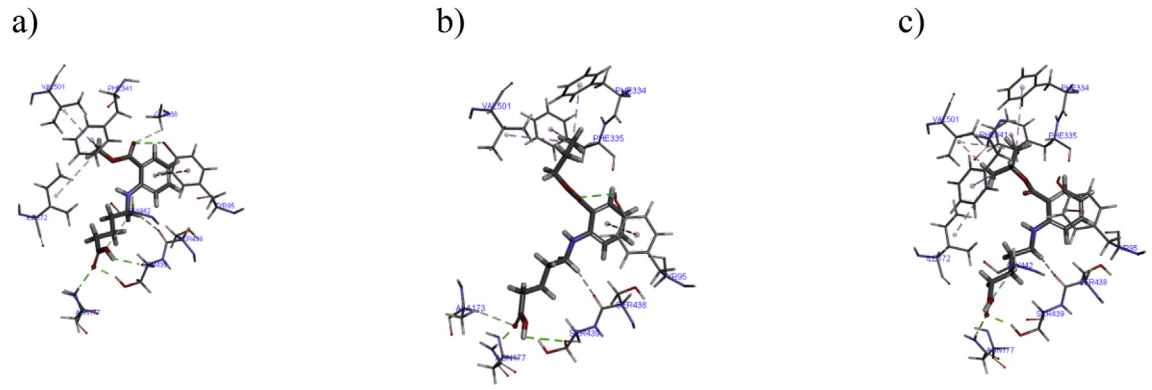


Fig. 11. Docking mode of studied compounds into SERT (PDB ID: 5I71). a) MMA derivative 2, b) PMA derivative 2 and c) IMA derivative 2.

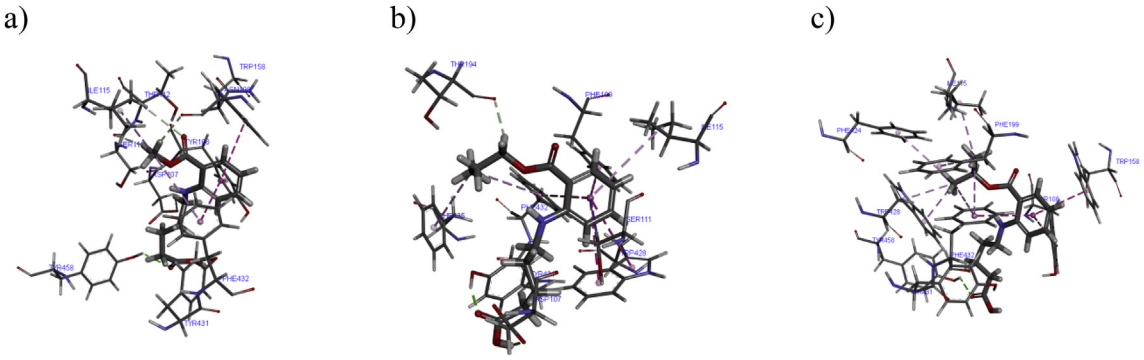


Fig. 12. Docking mode of studied compounds into HRH1 (PDB ID: 3RZE). a) MMA derivative 2, b) PMA derivative 2 and c) IMA derivative 2.

criterion must be in conjunction with the target to be studied, because if this target is located in the CNS the results are satisfactory, otherwise, this good permeability may favour the appearance of side effects.

The RO5 establishes criteria such as: molecular weight less than 500; lipophilicity of the ligands (Log P) of less than 5; the number of donor groups of hydrogen atoms less than 5; the number of acceptor groups of hydrogen atoms less than 10. Compounds that satisfy these rules are considered to have drug-like properties (Lipinski et al., 1997).

According to Mohan et al. (2007), the Qikprop software predicts the reference value in relation to the number of violations within Lipinski's RO5. Thus, the lower the number of violations, the greater the possibility that the molecule will be used for therapeutic purposes, and the number of 1 (one) violation will be the maximum considered. All molecules did not present any violations of the RO5.

In relation to the toxicological properties, computational tools can predict the toxic potential of the most diverse molecules, considerably impacting the discovery and development of new drugs, and toxicity is one of the fundamental parameters for the continuation of the research (Muster et al., 2008).

DEREK has a system that predicts from the qualitative point of view

Table 1
Pharmacokinetic properties of selected molecules.

MOLECULE	STARS	HOA%	Caco-2 (nm/sec)	MDCK (nm/sec)	logBB	RO5
MMA	2	93.014	1,237.68	622.942	-0.354	0
MMA - DERIVATIVE 1	0	72.884	102.555	53.672	-0.918	0
MMA - DERIVATIVE 2	0	77.73	108.495	57.04	-1.076	0
MMA - DERIVATIVE 3	1	100	4,237.14	2,355.81	-0.142	0
PMA	2	100	2,880.32	1,552.21	-0.172	0
PMA - DERIVATIVE 1	0	74.69	64.543	32.537	-1.413	0
PMA - DERIVATIVE 2	0	79.464	66.968	33.86	-1.608	0
PMA - DERIVATIVE 3	1	100	2,865.76	1,543.73	-0.516	0
IMA	2	100	3,376.38	1,843.07	-0.026	0
IMA - DERIVATIVE 1	0	77.751	108.012	56.766	-1.035	0
IMA - DERIVATIVE 2	0	82.424	113.006	59.608	-1.238	0
IMA - DERIVATIVE 3	1	100	4,150.14	2,303.58	-0.254	0

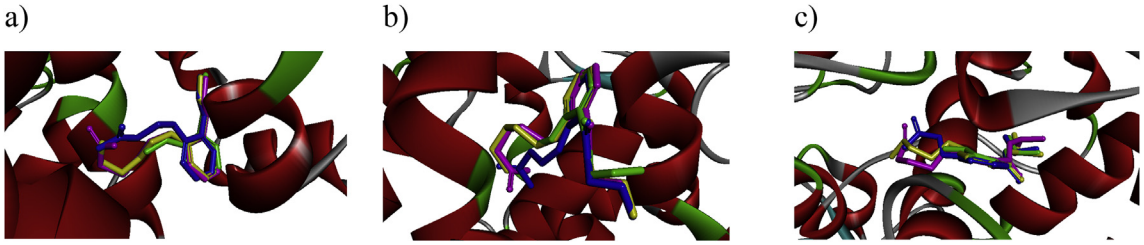


Fig. 13. Docking mode of studied compounds into COX-1 (PDB ID: 3N8X). a) MMA (green), MMA derivative 1 (blue), MMA derivative 2 (magenta), MMA derivative 3 (yellow); b) PMA (green), PMA derivative 1 (blue), PMA derivative 2 (magenta), PMA derivative 3 (yellow); and c) IMA (green), IMA derivative 1 (blue), IMA derivative 2 (magenta), IMA derivative 3 (yellow). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Toxicological properties of selected molecules.

Moelcule	Carcinogenicity	Mutagenicity	Skin sensitization human	Hepatotoxicity human
MMA	No alerts	No alerts	Plausible Aromatic 2nd amine	Plausible Precursor of aniline
MMA- DERIVATIVE 1	No alerts	No alerts	No alerts	No alerts
MMA- DERIVATIVE 2	No alerts	No alerts	No alerts	Plausible Short chain fatty acid
MMA- DERIVATIVE 3	No alerts	No alerts	No alerts	No alerts
PMA			Plausible Aromatic 2nd amine	No alerts
PMA - DERIVATIVE 1	No alerts	No alerts	No alerts	No alerts
PMA - DERIVATIVE 2	No alerts	No alerts	No alerts	Plausible Short chain fatty acid
PMA - DERIVATIVE 3	No alerts	No alerts	No alerts	No alerts
IMA	No alerts	No alerts	Plausible Aromatic 2nd amine	No alerts
IMA - DERIVATIVE 1	No alerts	No alerts	No alerts	No alerts
IMA - DERIVATIVE 2	No alerts	No alerts	No alerts	Plausible Short chain fatty acid
IMA - DERIVATIVE 3	No alerts	No alerts	No alerts	No alerts

and the search for 2D similarity of the molecule or its fragments in a database of fragments with known toxicological properties (Mohan et al., 2007). Thus, the software alerts are generated about the possible toxic action of the investigated chemical compounds, being able to interpret toxicophoric substructures present in the compounds as possible inducers of certain types of toxicity, such as mutagenicity, carcinogenicity, skin sensitization, irritation, reproductive effects, neurotoxicity (Ridings et al., 1996; Cariello et al., 2002).

The DEREK software indicates whether a specific toxic response can occur but does not provide a quantitative estimate of the prediction. Such predictions are made by considering the presence of alkyl halides, aldehydes, α, β unsaturated compounds, aromatic amines, phenols, hydroquinones, isothiazolinones, alkyl sulfonates and aromatic nitro groups in the test molecules (Estrada et al., 2004).

In this study, MMA, PMA and IMA molecules presented an alert for skin sensitization in humans as something that could happen due to the presence of aromatic primary or secondary amine. Hepatotoxicity in humans was predicted to MMA, MMA derivative 2, PMA derivative 2 and IMA derivative 2 due to the presence of a precursor of aniline in MMA and short chain fatty acid for the last 3 molecules. It is important to note that the MMA molecule showed no toxicity in the studies conducted by Radulović et al. (2013a, 2017), however, the *in silico* results have only pointed out to the possibility, without confirming toxicity. The others did not present alerts for skin sensitization in humans and hepatotoxicity in humans. None of the tested molecules show alerts for carcinogenicity and mutagenicity.

With the obtained results, MMA was the best molecule for the anti-inflammatory and anti-allergic activities. Among the derivatives of this molecule, the second modification was the one that presented better results in comparison to the template MMA. Among all the tested targets, PMA presented better results for antinociceptive and antidepressant activities, being its derivative 3 superior for antinociceptive activity while the derivative 2 showed to be better for antidepressant activity. IMA presented the best results for antinociceptive, anxiolytic and antidepressant activities. Its derivative 1 presented superior results for antinociceptive activity while derivative 2 showed to be better for antidepressant activity. Despite obtaining upper Goldscore for all modifications proposed, none of them was able to obtain a higher number of interactions with the target GABAa, being IMA still the one to possess the better anxiolytic activity. For the prediction of ADME properties, all compounds presented satisfactory results. Prediction of toxicity showed some warnings however *in vivo* studies conducted so far pointed out towards the safety aspects of IMA and MMA.

4. Conclusions

Overall, IMA was the compound, among the original ones evaluated *in silico* that presented the highest GOLDSCORE together with a combination of good pharmacokinetic and toxicological parameters. After proposed modifications to the original structures, it appeared that the derivative 2 of the three previous molecules would be the ones combining the most attractive properties to be potentially used as medicines. At the moment the derivative 2 of the original molecules is being synthesized in order to allow for their *in vivo* evaluation.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.06.003.

References

Accelrys Discovery Studio. V. 4.0. T.C., San Diego, CA 92121 USA.
version 11.0. ACD/ChemSketch. Advanced Chemistry Development, Inc., Toronto, ON, Canada. www.acdlabs.com.
Borges, R.S., Lima, E.S., Keita, H., Ferreira, I.M., Fernandes, C.P., Cruz, R.A.S., Duarte, J.L., Velázquez-Moyado, J., Ortiz, B.L.S., Castro, A.N., Ferreira, J.V., Hage-Melim, L.I.S., Carvalho, J.C.T., 2018. Anti-inflammatory and antialgic actions of a nanoemulsion of Rosmarinus officinalis L. essential oil and a molecular docking study of its major chemical constituents. Inflammopharmacology 26, 183–195.
BruysterS, M., Pertz, H.H., Teunissen, A., Bakker, R.A., Gillard, M., Chatelain, P., Schunack, W., Timmerman, H., Leurs, R., 2004. Mutational analysis of the histamine H1-receptor binding pocket of histaprodifens. Eur. J. Pharmacol. 487, 55–63.
Cariello, N.F., Wilson, J.D., Britt, B.H., Wedd, D.J., Burlinson, B., Gombar, V., 2002. Comparison of the computer programs DEREK and TOPKAT to predict bacterial mutagenicity. Deductive estimate of risk from existing knowledge. Toxicity prediction by komputer assisted technology. Mutagenesis 17, 321–329.
Chandak, N., Kumar, P., Kaushik, P., Varshney, P., Sharma, C., Kaushik, D., Jain, S., Aneja, K.R., Sharma, P.K., 2014. Dual evaluation of some novel 2-amino-substituted coumarinylthiazoles as anti-inflammatory–antimicrobial agents and their docking studies with COX-1/COX-2 active sites. J. Enzym. Inhib. Med. Chem. 29, 476–484.
Cole, J.C., Murray, C.W., Nissink, J.W., Taylor, R.D., Taylor, R., 2005. Comparing protein–ligand docking programs is difficult. Proteins 60, 325–332.
Darré, L., Domene, C., 2015. Binding of capsaicin to the TRPV1 ion channel. Mol. Pharm. 12, 4454–4465.
Dearden, J.C., Barratt, M.D., Benigni, R., Bristol, D.W., Combes, R.D., Cronin, M., Judson, T.D., Payne, P.N., Richard, A.M., Tichy, M., Worth, A.P., Yourick, J.J., 1997. The development and validation of expert systems for predicting toxicity. ATLA 25, 223–252.

Estrada, E., Patlewicz, G., Gutierrez, Y., 2004. From knowledge generation to knowledge archive. A general strategy using TOPS-MODE with DEREK to formulate new alerts for skin sensitization. J. Chem. Inf. Comput. Sci. 44, 688–698.
Gouda, A.M., Ali, H.I., Almalki, W.H., Azim, M.A., Abourehab, M.A., Abdelazeem, A.H., 2016. Design, synthesis, and biological evaluation of some novel pyrrolizine derivatives as COX inhibitors with anti-inflammatory/analgesic activities and low ulcerogenic liability. Molecules 21, E201.
Gupta, S., Mohan, C.G., 2014. Dual binding site and selective acetylcholinesterase inhibitors derived from integrated pharmacophore models and sequential virtual screening. BioMed Res. Int. 2014, 21.
Hoerbelt, P., Ramerstorfer, J., Ernst, M., Sieghart, W., Thomson, J.L., Hough, L.B., Fleck, M.W., 2016. Mutagenesis and computational docking studies support the existence of a histamine binding site at the extracellular β3 + β3- interface of homooligomeric β3 GABAA receptors. Neuropharmacology 108, 252–263.
Iversen, L., 2000. Neurotransmitter transporters: fruitful targets for CNS drug discovery. Mol. Psychiatry 5, 357–362.
Jorgensen, W.L., 2004. The many roles of computation in drug discovery. Science 303, 1813–1818.
Julius, D., 2013. TRP channels and pain. Annu. Rev. Cell Dev. Biol. 29, 355–384.
Kim, C., Ann, J., Lee, S., Kim, E., Choi, S., Blumberg, P.M., Frank-Foltyn, R., Bahrenberg, G., Stockhausen, H., Christoph, T., Lee, J., 2018. 4-Aminophenyl acetamides and propanamides as potent transient receptor potential vanilloid 1 (TRPV1) ligands. Bioorg. Med. Chem. 26, 4509–4517.
Li, Y.-C., Chiang, C.-W., Yeh, H.-C., Hsu, P.-Y., Whitby, F.G., Wang, L.-H., Chan, N.-L., 2008. Structures of prostacyclin synthase and its complexes with substrate analog and inhibitor reveal a ligand-specific heme conformation change. J. Biol. Chem. 283, 2917–2926.
Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J., 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. 23, 3–25.
Malikowska, N., Fijałkowski, Ł., Nowaczyk, A., Popik, P., Sałat, K., 2017. Antidepressant-like activity of venlafaxine and clonidine in mice exposed to single prolonged stress - a model of post-traumatic stress disorder. Pharmacodynamic and molecular docking studies. Brain Res. 1673, 1–10.
Miller, P.S., Aricescu, A.R., 2014. Crystal structure of a human GABAA Receptor. Nature 512, 270–275.
Mohan, C.G., Gandhi, T., Garg, D., Shinde, R., 2007. Computer-assisted methods in chemical toxicity prediction. Mini Rev. Med. Chem. 7, 499–507.
Muster, W., Breidenbach, A., Fischer, H., Kirchner, S., Müller, L., Pähler, A., 2008. Computational toxicology in drug development. Drug Discov. Today 13, 303–310.
Orlando, B.J., Malkowski, M.G., 2016. Substrate-selective inhibition of cyclooxygeanse-2 by fenamic acid derivatives is dependent on peroxide tone. J. Biol. Chem. 291, 15069–15081.
Pinheiro, M.M., Miltojević, A.B., Radulović, N.S., Abdul-Wahab, I.R., Boylan, F., Fernandes, P.D., 2015. Anti-inflammatory activity of Choisya ternata Kunth essential oil, ternanthranin, and its two synthetic analogs (methyl and propyl N-methylanthranilates). PLoS One 10, 1–21.
Pinheiro, M.M., Radulović, N.S., Miltojević, A.B., Boylan, F., Dias Fernandes, P., 2014. Antinociceptive esters of N-methylanthranilic acid: mechanism of action in heat-mediated pain. Eur. J. Pharmacol. 727, 106–114.
Radulović, N.S., Miltojević, A.B., McDermott, M., Waldren, S., Parnell, J.A., Pinheiro, M.M.G., Fernandes, P.D., Menezes, F.S., 2011. Identification of a new antinociceptive alkaloid isopropyl N-methylanthranilate from the essential oil of *Choisya ternata* Kunth. J. Ethnopharmacol. 135, 610–619.
Radulović, N.S., Jovanović, I., Ilić, I.R., Randjelović, P.J., Stojanović, N.M., Miltojević, A.B., 2013a. Methyl and isopropyl N-methylanthranilates attenuate diclofenac- and ethanol-induced gastric lesions in rats. Life Sci. 93, 840–846.
Radulović, N.S., et al., 2013b. Influence of methyl and isopropyl N-methylantranilates on carbon tetrachloride-induced changes in rat liver morphology and function. F.U. Phy. Chem. Technol. 11, 67–73.
Radulović, N.S., Miltojević, A.B., Randjelović, P.J., Stojanović, N.M., Boylan, F., 2013c. Effects of methyl and isopropyl N-methylanthranilates from *Choisya ternate* Kunth (rutaceae) on experimental anxiety and depression in mice. Phytother Res. 27, 1334–1338.
Radulović, N.S., Randjelović, P.J., Stojanović, N.M., Ilić, I.R., Miltojević, A.B., Stojković, M.B., Ilić, M., 2015. Effect of two esters of N-methylanthranilic acid fromRutaceae species on impaired kidney morphology and function in rats caused by CCl4. Life Sci. 135, 110–117.
Radulović, N.S., Miltojević, A.B., Stojanović, N.M., Randjelović, P.J., 2017. Distinct urinary metabolite profiles of two pharmacologically active N-methylanthranilates: three approaches to xenobiotic metabolite Identification. Food Chem. Toxicol. 109, 341e355.
Ridings, J.E., Barratt, M.D., Cary, R., Earnshaw, C.G., Eggington, C.E., Ellis, M.K., Judson, P.N., Langowski, J.J., Marchant, C.A., Payne, M.P., Watson, W.P., Yih, T.D., 1996. Computer prediction of possible toxic action from chemical structure: an update on the DEREK system. Toxicology 106, 267–279.
Sader, S., Cai, J., Muller, A.C.G., Wu, C., 2017. Can human allergy drug fexofenadine, an antagonist of histamine (H1) receptor, be used to treat dog and cat? Homology modeling, docking and molecular dynamic Simulation of three H1 receptors in complex with fexofenadine. J. Mol. Graph. Model. 75, 106–116.
Sandy, M., Butler, A., 2009. Microbial iron acquisition: marine and terrestrial siderophores. Chem. Rev. 109, 4580–4595.
SCCS (Scientific Committee on Consumer Safety), 2011. Opinion on Methyl *N*-Methylanthranilate (Phototoxicity Only). https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_075.pdf, Accessed date: 5 April 2019.
Schrödinger, 2011. QikProp, Version 4.4. LLC.
Schwartz, J.C., Arrang, J.M., Garbarg, M., Pollard, H., Ruat, M., 1991. Histaminergic transmission in the mammalian brain. Physiol. Rev. 71, 1–51.
Shimamura, T., Shiroishi, M., WeyanD, S., Tsujimoto, H., Winter, G., Katritch, V., Abagyan, R., Cherezov, V., Liu, W., Han, G.W., Kobayashi, T., Stevens, R.C., Iwata, S., 2011. Structure of the human histamine H1 receptor complex with doxepin. Nature 475, 65–70.
Simons, F.E., 2004. Advances in H1-antihistamines. N. Engl. J. Med. 351, 2203–2217.
Świątek, P., Strzelecka, M., Urniaz, R., Gębczak, K., Gębarowski, T., Gąsiorowski, K., Malinka, W., 2017. Synthesis, COX-1/2 inhibition activities and molecular docking study of isothiazolopyridine derivatives. Bioorg. Med. Chem. 25, 316–326.
Szabó, L., Mile, V., Kiss, D.J., Kovács, K., Földes, T., Németh, T., Tóth, T., Homlok, R., Balogh, G.T., Takács, E., Wojnárovits, L., 2018. Applicability evaluation of advanced processes for elimination of neurophysiological activity of antidepressant fluoxetine. Chemosphere 193, 489–497.
Vitale, P., Panella, A., Scilimati, A., Perrone, M.G., 2016. COX-1 inhibitors: beyond structure toward therapy. Med. Res. Rev. 36, 641–671.