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Evaluation of *N*-benzoylthiourea derivatives as possible analgesic agents by predicting their physicochemical and pharmacokinetic properties, toxicity, and analgesic activity

Suko Hardjono[1,*], **Siswandono**[1], **and Rina Andayani**[2]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Airlangga University, Campus B Jalan Dharmawangsa Dalam, Surabaya 60282, Indonesia

[2]Department of Pharmacy, Faculty of Medicine, Hang Tuah University, Jalan Arief Rachman Hakim 150, Surabaya 60111, Indonesia

*Corresponding author: suko-h@ff.unair.ac.id

ABSTRACT This study aimed to predict the physicochemical properties, pharmacokinetic properties (ADME), toxicity, and analgesic activity of 30 compounds of *N*-benzoylthiourea derivatives that are potential analgesic drugs. One of the mechanisms of action of *N*-benzoylthiourea derivatives is the inhibition of the cyclooxygenase-2 (COX-2) isoenzyme. An in silico test was performed by docking a compound that would predict its activity with the target COX-2 isoenzyme, PDB ID: 1PXX, using the MVD (Molegro Virtual Docker) program. The result of the docking was a form of energy bond indicated by the value of the rerank score (RS), where compounds that had lower RS values were predicted to have a higher activity. The pkCSM and Protox online tools were used to predict various physicochemical properties. Based on the RS values, the *N*-benzoylthiourea derivatives can be predicted to have lower analgesic activity than diclofenac, the reference ligand. Three of the *N*-benzoylthiourea derivatives—*N*-(2,4-*bis*-trifluoromethyl)-benzoylthiourea, *N*-(3,5-*bis*-trifluoromethyl)benzoylthiourea, and *N*-(3-trifluoromethoxy)-benzoylthiourea—had RS values of -90.82, -94.73, and -92.76, respectively, suggesting that these compounds were predicted to have analgesic activity relatively similar to diclofenac (RS value = -95.16). Furthermore, the majority of the *N*-benzoylthiourea derivatives were predicted to have good pharmacokinetic properties (ADME), and cause relatively low toxicity.

KEYWORDS ADME; analgesic activity; molecular modeling; *N*-benzoylthiourea; toxicity

1. Introduction

Drug design is an attempt to develop existing drugs. Drug design is often described as a systematic elaboration process for further developing existing drugs with the aim of obtaining new drugs with better activity and reducing or eliminating the side effects that exist through molecular manipulation. Molecular manipulation or structural modification is the introduction of a number of parent compounds, identify structures and test their biological activity (Siswandono 2014). The structural changes of a compound will alter the physicochemical properties of the compound, including lipophilic, electronic and steric properties, and this may cause changes in the biological activity of the compound (Hardjono et al. 2016).

To be more effective and efficient in conducting structural modifications, before synthesized compound required an effort to predict the physical properties of the molecule chemical compound, the pharmacokinetic properties (ADME), toxicity, and know the description of drug interactions with the receptor. The method that is now being developed is molecular modeling (Schlick 2010). Molecular modeling is widely used in chemistry and computational biology to study the molecular nature of biological molecule drug compounds and receptors and to understand drug action at the molecular and atomic level, through a drug interaction process simulation receptor (docking) with the aid of a computer. This technique is called the in silico test which has a very important role in the field of medicinal chemistry in order to design, discover and optimize bioactive compounds in the process of drug development (Hinchliffe 2008; Siswandono 2016).

The in silico test is performed by performing a molecular docking that will predict its activity with the selected target cell. Docking is an attempt to harmonize between the ligand which is a small molecule into the target cell which is a large protein molecule (Jensen 2007). The results of in silico test in the form of bond energy value or rerank score (RS). The bond energy indicates the amount of energy needed to form a bond between the ligand and

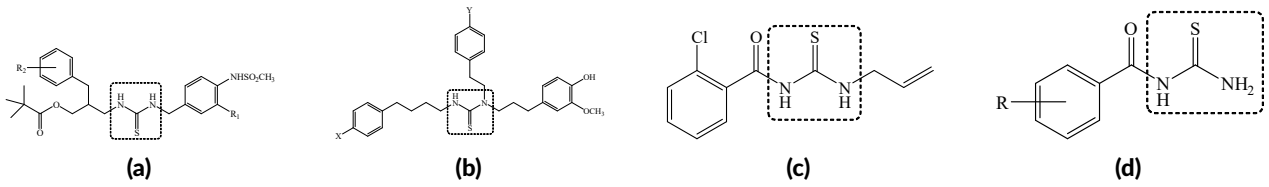


FIGURE 1 Compounds with thiourea pharmacophore: (a) *N*-(3-acyloxy-2-benzylpropyl)-*N*'-[4-(methylsulfonylamino)benzyl]thiourea analogues; (b) 1-(3-(4-hydroxy-3-methoxyphenyl)propyl-1-phenethyl-3-(-4-phenylbuthyl)thiourea derivatives; (c) 1-allyl-3-(2-chlorobenzoyl)-thiourea; (d) *N*-benzoylthiourea derivatives.

the receptor. The lower the bonding energy means the bond is more stable. The more stable the ligand bond with the receptor it can be predicted that the activity is getting higher (Hardjono 2012).

The thiourea group has been noted for its correlation with analgesic activity in a number of reports. For example, novel *N*-(3-acyloxy-2-benzylpropyl)-*N*'-[4-(methylsulfonylamino)benzyl]thiourea analogues (Figure 1a) have been reported for their potent analgesic activity (Lee et al. 2002, 2004). Another thiourea derivatives as *N*,*N*',*N*'-trisubstituted thiourea derivatives (Figure 1b) have been reported as new efficient analgesic agents by antagonistic effects against vanilloid receptor (Park et al. 2003). Shalas et al. (2016) reported that 1-allyl-3-(2-chloro-benzoyl)thiourea (Figure 1c) have a good analgesic activity. Budiati et al. (2010) evaluated benzoylurea derivatives for analgesic activity in mice (*Mus musculus*) and showed a better analgesic activity compared to Na-diclofenac. The compound of *N*-benzoylthiourea derivatives (Figure 1d) has same thiourea pharmacophore as the previous compounds.

In this study, the in silico test of thirty *N*-benzoylthiourea derivatives was used to predict its analgesic activity against cyclooxygenase-2 (COX-2) isoenzyme. The first enzyme in the prostanoid synthetic pathway is PG endoperoxide G/H synthase, which is officially called cyclooxygenase or COX. There are two distinct COX isoforms, COX-1 and COX-2. COX-1, expressed constitutively in most cells, is considered the dominant, but not exclusive, source of prostanoids for housekeeping functions, such as cytoprotection of the gastric epithelium. COX-2, in contrast, is upregulated by cytokines, shear stress, and growth factors and is the principle source of prostanoid formation in inflammation and cancer. The inhibition of cyclooxygenase-2 (COX-2) is thought to largely mediate the antipyretic, analgesic, and anti-inflammatory actions of non-steroidal anti-inflammatory drugs (NSAIDs), while the simultaneous inhibition of cyclooxygenase-1 (COX-1) largely but not exclusively accounts for unwanted adverse effects in the GI tract (Brunton et al. 2011).

In this study, as selective COX-2 isoenzyme receptors PDB ID: 1PXX, containing DIF_701, a ligand (Rowlinson et al. 2003). The results of the in silico test were the RS values of N-benzoylthiourea and derivatives thereof. From all of RS values obtained will be determined the compound derivative of *N*-benzoylthiourea which has the lowest RS value or predicted to have the highest analgesic activity. Thomsen and Christensen (2006) have examined the accuracy of the molecular docking method over other methods. MolDock in Molegro Virtual Docker program has the best accuracy, as described in Table 1.

To obtain *N*-benzoylthiourea derivative compounds theoretically, can be synthesized with benzoyl chloride derivative base material and react with thiourea in the basic condition. Therefore, the determination of 30 derived compounds under study based on the availability of benzoyl chloride derived compounds in the market, so that later more ease in the process of synthesis.

2. Materials and methods

2.1. Tools

Lenovo computers, Windows 10 operating system, 64 bit, Intel Core i5-7200U processor, CPU 250 GHz 270 GHz, 8.00 GB RAM.

2.2. Programs

Chem Bio Draw Version 11 (CambridgeSoft), a licensed software; Chem Bio 3D Version 11 (CambridgeSoft), a licensed software; Molegro Virtual Docker 5.5 (Molegro ApS), a licensed software; SMILES Translator, pkCSM, and Protox are free online tools.

2.3. Methodology

2.3.1. Preparation of target protein (COX-2 isoenzyme)

The structure of the COX-2 isoenzyme molecule can be downloaded via the protein data bank site (http://www.rcsb.org/pdb/home/home.do). In this study, COX-2 isoenzyme with PDB ID 1PXX was selected as a target protein, because it contains a ligand diclofenac

TABLE 1 Comparison of the docking accuracy and average of RMSD values of MolDock, Glide, and Surflex.

Method	Docking accuracy (%)	Average RMSD (all cases) (A)	Average RMSD (A) (RMSD, 2.0 A)
MolDock	87.01	1.38	0.90
Glide	81.82	1.38	0.74
Surflex	75.32	1.86	0.91

(DIF_701). The ligand contains a carboxylic group (-COOH) that acts as a pharmacophore in the ligand-receptor interaction process, and theN-benzoylthiourea derivative compounds contain a similar group, ie –$CSNH_2$, which is also expected to function as pharmacophore (Rowlinson et al. 2003; Shalas et al. 2016).

2.3.2. *Prediction of the physicochemical, pharmacokinetic, and toxicity properties of the compounds*

Predicted physicochemical properties such as molecular weight (MW), the logarithm of octanol/water partition coefficient (LogP), number of rotatable bonds (Torsion), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), and polar surface activity (PSA) are performed by the pkCSM online tool. Prediction nature of pharmacokinetics (ADME: absorption, distribution, metabolism, and excretion) and toxicity of the *N*-benzoylthiourea derivatives also be done by a pkCSM online tool (Pires et al. 2015; Saeed et al. 2017). First, the molecular structure of 30 derivatives of *N*-benzoylthiourea and the reference compound diclofenac 2-D drawn by Chem Bio Draw Version 11 program, then copied to the Chem Bio 3D Version 11 program to make the 3-D structure, then stored in a file.sdf or .pdb. Second, the 3-D structure of 30 compounds of *N*-benzoylthiourea derivatives and diclofenac is translated into SMILES format by SMILES Translator online available from https://cactus.nci.nih.gov/translate/. SMILES format of these compounds were processed using the pkCSM online tool, available from http://biosig.unimelb.edu.au/pkcsm/prediction, to predict ADME and toxicity of the compounds. To predict toxicity (LD_{50}) per oral on rodent and toxicity classification of compounds based on globally harmonized system (GSH), Protox online tool (available from http://tox.charite.de/tox/) was used (Pires et al. 2015; Ruswanto et al. 2017).

2.3.3. *Molecular docking*

Compounds that will dock were drawn into their 2-D molecular structures with the Chem Bio Draw v.11, then were copied to the Bio Chem 3D v.11 to create a 3-D structure. Having measured the minimum energy is then stored in .mol2 (SYBYL2). After stored then done the process of docking to COX-2 isoenzyme PDB ID.1PXX by the Molegro Virtual Docker version 5.5. The results obtained are the value of root-mean-square deviation (RMSD) and rerank score (RS), which is the energy required in the ligand-receptor interaction process, and from that value can be predicted the analgesic activity of the compound through COX-2 isoenzyme inhibitor (Siswandono 2016).

3. Results and discussion

3.1. *The chemical structures*

The chemical structures of the *N*-benzoylthiourea derivatives, along with diclofenac as the reference compound, are shown in Figure 2. Thirty groups (R) were substituted on *N*-benzoylthiourea compound to obtain 30 new compounds of *N*-benzoylthiourea derivatives (Table 2).

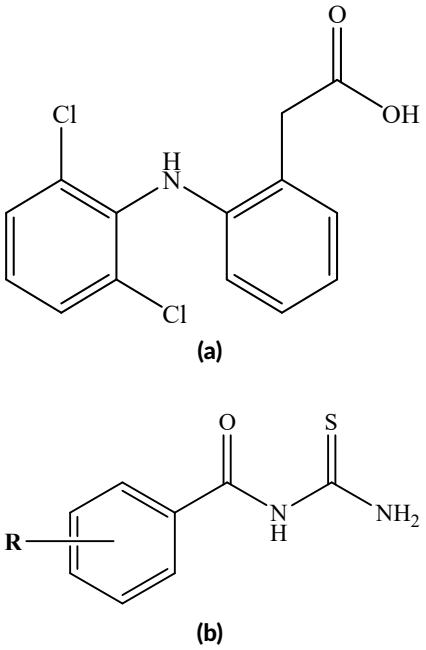


FIGURE 2 The chemical structure of diclofenac (a) and *N*-benzoylthiourea derivatives (b).

3.2. *Prediction of the physicochemical and pharmacokinetic properties*

The prediction of the in silico values of the physicochemical properties of *N*-benzoylthiourea and diclofenac as the reference compound can be seen in Table 3. The In silico test produces the predictions of the molecular weight, logaritmic octanol/water partition coefficient, rotatable bond, hydrogen bond acceptors, hydrogen bond donors and polar surface activity.

Lipinski et al. (1997) analyzed 2,245 drugs from the World Drugs Index database and concluded that the compound is more likely to have poor absorption or permeability if the molecular weight exceeds 500, the calculated octanol/water partition coefficient (logP) exceeds +5, there are more than 5 H-bond donors (HBD) expressed as the sum of O–H and N–H groups, and there are more than 10 H-bond acceptors (HBA) expressed as the sum of N and O atoms. The above analysis is called the Lipinski rules of five because all values are multiples of five. The molecular weight values of *N*-benzoylthiourea derivatives ranged from 180.23 to 316.23 (<500), the range of the value of log the octanol/water partition coefficient (log P) from 0.53 to 2.70 (<5), the amount of HBD = 2 (≤ 5), and the amount of HBA ranged from 2 to 6 (<10) (Table 3). Based on these results, all derivatives of the compound meet the Lipinski rules of five requirements so it can be predicted that the compounds will be easily absorbed and have high permeability.

TABLE 2 The chemical structures of *N*-benzoylthiourea derivatives and diclofenac (DIF_701) as the reference compound.

Compound no.	Position	R	Compound name
1	4	H	*N*-benzoylthiourea
2	2	CH_3	*N*-(2-methyl)benzoylthiourea
3	3	CH_3	*N*-(3-methyl)benzoylthiourea
4	4	CH_3	*N*-(4-methyl)benzoylthiourea
5	3,4	2 (CH_3)	*N*-(3,4-dimethyl)benzoylthiourea
6	4	$C(CH_3)_3$	*N*-(4-*tert*-butyl)benzoylthiourea
7	4	OCH_3	*N*-(4-methoxy)benzoylthiourea
8	4	$OCH_2CH_2CH_3$	*N*-(4-propoxy)benzoylthiourea
9	4	$N(CH_3)_2$	*N*-(4-dimethylamino)benzoylthiourea
10	4	CN	*N*-(4-cyano)benzoylthiourea
11	4	$N{=}N\text{-}C_6H_5$	*N*-(4-phenylazo)benzoylthiourea
12	3	F	*N*-(3-fluoro)benzoylthiourea
13	4	F	*N*-(4-fluoro)benzoylthiourea
14	2	Cl	*N*-(2-chloro)benzoylthiourea
15	3	Cl	*N*-(3-chloro)benzoylthiourea
16	4	Cl	*N*-(4-chloro)benzoylthiourea
17	3,4	2 (Cl)	*N*-(3,4-dichloro)benzoylthiourea
18	2,4	2 (Cl)	*N*-(2,4-dichloro)benzoylthiourea
19	3,5	2 (Cl)	*N*-(3,5-dichloro)benzoylthiourea
20	3	Br	*N*-(3-bromo)benzoylthiourea
21	4	Br	*N*-(4-bromo)benzoylthiourea
22	4	I	*N*-(4-iodo)benzoylthiourea
23	4	CF_3	*N*-(4-trifluoromethyl)benzoylthiourea
24	2,4	2 (CF_3)	*N*-(2,4-*bis*-trifluoromethyl)benzoylthiourea
25	3,5	2 (CF_3)	*N*-(3,5-*bis*-trifluoromethyl)benzoylthiourea
26	3	OCF_3	*N*-(3-trifluoromethoxy)benzoylthiourea
27	4	OCF_3	*N*-(4-trifluoromethoxy)benzoylthiourea
28	3	NO_2	*N*-(3-nitro)benzoylthiourea
29	4	NO_2	*N*-(4-nitro)benzoylthiourea
30	3,5	2 (NO_2)	*N*-(3,5-dinitro)benzoylthiourea
31	Reference Compound		Diclofenac

3.3. Prediction of the ADMET properties and toxicity

Prediction in silico values of ADMET properties and toxicity of *N*-benzoylthiourea and diclofenac as reference compound can be seen in Table 4. According to Chander et al. (2017), the compound is said to have a good absorption when its absorption value is over 80% and considered to be poorly absorbed if the value is less than 30% (Pires et al. 2015). The intestine is normally the primary site for absorption of a drug from an orally administered solution. The intestinal absorption method is built to predict the proportion of compounds that were absorbed through the human small intestine. From Table 4 it can be seen that the intestinal absorption (human) value of most of the *N*-benzoylthiourea derivatives range from 69.73 to 94.37%, so it is predictable that almost all derivatives of the compound will be absorbed very well in the intestine. Only the number 10, 28, 29, and 30 compounds had a value of < 80%, which is predicted to be moderately absorbed.

Skin permeability is an important consideration for improving drug efficacy, particularly of interest for the development of transdermal drug delivery. According to Pires et al. (2015), a compound is considered to have a relatively low skin permeability if it has a logKp > -2.5 cm/h. From Table 4 it can be seen that the skin permeability (logKp) value of *N*-benzoylthiourea derivatives range from -2.67 to -3.19 cm/h (< -2.5), so it can be predicted that all the derivatives of the compound have good skin permeability.

The Caco-2 cell line is composed of human epithelial colorectal adenocarcinoma cells. The Caco-2 monolayer of cells is widely used as an in vitro model of the human intestinal mucosa to predict the absorption of orally administered drugs by measuring logarithm of the apparent

TABLE 3 Predicted in silico values of physicochemical properties of *N*-benzoylthiourea derivatives and diclofenac using pkCSM online tool.

Compound no.	MW	LogP	Torsion	HBA	HBD	PSA (A^2)	Lipinski rule of 5 requirements
1	180.23	0.66	2	2	2	75.57	Yes
2	194.26	0.97	2	2	2	81.94	Yes
3	194.26	0.97	2	2	2	81.94	Yes
4	194.26	0.97	2	2	2	81.94	Yes
5	208.29	1.28	2	2	2	88.30	Yes
6	236.34	1.96	3	2	2	101.03	Yes
7	210.26	0.67	3	3	2	87.05	Yes
8	238.31	1.45	5	3	2	94.06	Yes
9	223.30	0.73	3	3	2	121.08	Yes
10	205.24	0.53	3	3	2	86.33	Yes
11	284.34	3.08	4	4	2	121.08	Yes
12	198.22	0.80	2	2	2	79.74	Yes
13	198.22	0.80	2	2	2	79.74	Yes
14	214.68	1.31	2	2	2	85.88	Yes
15	214.68	1.31	2	2	2	85.88	Yes
16	214.68	1.31	2	2	2	85.88	Yes
17	249.12	1.97	2	2	2	96.18	Yes
18	249.12	1.97	2	2	2	96.18	Yes
19	249.12	1.97	2	2	2	96.18	Yes
20	259.13	1.42	2	2	2	89.44	Yes
21	259.13	1.42	2	2	2	89.44	Yes
22	306.13	1.26	2	2	2	94.83	Yes
23	248.23	1.68	3	2	2	94.43	Yes
24	316.23	2.70	4	2	2	113.29	Yes
25	316.23	2.70	4	2	2	113.29	Yes
26	264.23	1.56	4	3	2	99.55	Yes
27	264.23	1.56	4	3	2	99.55	Yes
28	225.23	0.57	3	4	2	90.22	Yes
29	225.23	0.57	3	4	2	90.22	Yes
30	270.23	0.48	4	6	2	104.88	Yes
31	296.15	4.36	4	2	2	120.33	Yes

MW = molecular weight; LogP = the logaritmic octanol/water partition coefficient; Torsion = rotatable bond; HBA = hydrogen bond acceptors; HBD = hydrogen bond donors; PSA = polar surface activity.

permeability coefficient (log Papp; log cm/s). According to Pires et al. (2015), a compound is considered to have a high Caco-2 permeability if it has a Papp > 8 x 10^6 cm/s. For the pkCSM predictive model, high Caco-2 permeability would translate into predicted log Papp values > 0.90 cm/s. As Table 4 shows, the value of Caco-2 permeability (log Papp) of the *N*-benzoylthiourea derivatives ranged from -0.31 to 1.39 cm/s, with 23 compounds having logPapp> 0.9 cm/s, so it can be predicted that the compounds have a high Caco-2 permeability. Eight *N*-benzoylthiourea derivatives, compounds number 7, 8, 10, 26, 27, 28, 29 and 30 had log Papp< 0.9 cm/s, so it is predicted that these have a low Caco-2 permeability.

The volume of distribution (VDss) is the theoretical volume that the total dose of a drug would need to be uniformly distributed to give the same concentration that is observed in blood plasma. The higher the (VDss) is, the more of a drug that is distributed in tissue rather than plasma. This predictive model was built using the calculated steady-state volume of distribution (VDss) in humans, which is given as the log L/kg. According to Pires et al. (2015), VDss is considered low if it is below 0.71 L/kg (log VDss < -0.15) and high if it is above 2.81 L/kg (log VDss > 0.45). From Table 4 it can be seen that the value of VDss of *N*-benzoylthiourea derivatives ranged from -0.14 to -0,78, with only one compound having a VDss value of < -0.15 (number 6). Therefore, it can be predicted that almost all derivatives of these compounds can be distributed evenly to give the same concentration as in blood plasma.

TABLE 4 Prediction in silico of pharmacokinetic properties (ADME), and toxicity of *N*-benzoylthiourea derivatives and diclofenac using pkCSM and Protox online tool.

Compound no.	Absorption				Distribution		Metabolism				Excretion		Toxicity		
	Intestinal absorption (human, %)	Skin permeability (log Kp, cm/h)	Caco-2 permeability (log Papp; log cm/s)	VDss (human, log L/kg)	BBB permeability (logBB)	CNS permeability (logPs)	CYP2D6 substrate	CYP3A4 substrate	CYP2D6 inhibitor	CYP3A4 inhibitor	Total Clearance (log ml/min/kg)	Renal OCT2 substrate	Hepato-toxicity	LD_{50}*(mg/kg)	Class*
1	79.50	-2.91	1.20	-0.20	-0.22	-2.44	No	No	No	No	-0.19	No	No	960	4
2	79.58	-2.88	1.22	-0.17	-0.20	-2.37	No	No	No	No	-0.23	No	No	1950	4
3	79.58	-2.88	1.22	-0.17	-0.20	-2.37	No	No	No	No	-0.24	No	No	1950	4
4	79.58	-2.88	1.22	-0.17	-0.20	-2.37	No	No	No	No	-0.25	No	No	1950	4
5	79.67	-2.85	1.24	-0.15	-0.19	-2.30	No	No	No	No	-0.39	No	No	3000	5
6	94.26	-2.67	1.30	-0.14	0.12	-2.09	No	No	No	No	-0.30	No	No	1950	4
7	80.06	-3.19	0.27	-0.33	-0.23	-2.07	No	No	No	No	-0.16	No	No	1990	4
8	94.37	-3.08	0.39	0.22	-0.18	-2.61	No	No	No	No	-0.19	No	No	1990	4
9	82.29	-3.00	1.21	-0.18	-0.17	-2.53	No	No	No	No	-0.22	No	No	2885	5
10	74.67	-3.19	0.26	-0.30	-0.09	-2.05	No	No	No	No	-0.23	No	No	1950	4
11	91.65	-2.99	1.04	-0.40	-0.31	-2.62	No	No	No	No	-0.27	No	No	2947	5
12	83.47	-3.05	1.21	-0.30	-0.21	-2.48	No	No	No	No	-0.28	No	No	1950	4
13	83.47	-3.05	1.21	-0.30	-0.21	-2.48	No	No	No	No	-0.34	No	No	1500	4
14	83.36	-2.82	1.25	-0.24	-0.23	-2.33	No	No	No	No	-0.05	No	No	920	4
15	83.36	-2.82	1.25	-0.24	-0.23	-2.33	No	No	No	No	-0.26	No	No	1450	4
16	83.36	-2.82	1.25	-0.24	-0.23	-2.33	No	No	No	No	-0.32	No	No	1250	4
17	91.89	-2.75	1.29	-0.28	0.07	-2.21	No	No	No	No	-0.13	No	No	1250	4
18	91.89	-2.75	1.29	-0.28	0.07	-2.21	No	No	No	No	-0.12	No	No	1250	4
19	91.89	-2.75	1.29	-0.28	0.07	-2.21	No	No	No	No	-0.28	No	No	1450	4
20	93.49	-2.81	1.25	-0.23	-0.24	-2.31	No	No	No	No	-0.28	No	No	1950	4
21	93.49	-2.81	1.25	-0.23	-0.24	-2.31	No	No	No	No	-0.35	No	No	960	4
22	83.19	-2.84	1.24	-0.22	-0.25	-2.34	No	No	No	No	-0.63	No	No	960	4
23	91.94	-2.83	1.29	-0.35	0.08	-2.29	No	No	No	No	-0.27	No	Yes	818	4
24	88.67	-2.82	1.39	-0.50	0.09	-2.22	No	No	No	No	-0.07	No	No	1950	4
25	88.67	-2.82	1.39	-0.50	0.09	-2.22	No	No	No	No	-0.16	No	Yes	1170	4
26	91.73	-3.04	0.31	-0.51	-0.19	-2.49	No	No	No	No	-0.21	No	No	2000	4
27	91.73	-3.04	-0.31	-0.51	-0.19	-2.49	No	No	No	No	-0.28	No	No	1990	4
28	72.71	-2.81	-0.18	-0.49	-0.48	-2.63	No	No	No	No	-0.11	No	No	2800	5
29	72.71	-2.81	-0.18	-0.49	-0.48	-2.63	No	No	No	No	-0.17	No	No	1530	4
30	69.73	-2.80	0.12	-0.78	-1.01	-2.82	No	No	No	No	0.13	No	No	2800	5
31	89.40	-2.65	1.39	-0.68	-0.11	-1.83	No	No	No	No	0.30	No	No	53	3

VDSS = steady state of volume distribution; BBB = blood-brain barrier; CNS = central nervous system; CYP2D6 = cytochrome P2D6; CYP3A4 = cytochrome 3A4; Renal OCT2 = renal organic cation transporter 2; LD = lethal dose (oral on a rodent).

The brain is protected from exogenous compounds by the blood-brain barrier (BBB). The ability of a drug to cross into the brain is an important parameter to consider to help reduce side effects and toxicities or to improve the efficacy of drugs whose pharmacological activity is with in the brain. Blood-brain permeability is measured in vivo in animals models as logBB, the logarithmic ratio of brain to plasma drug concentrations. According to Pires et al. (2015), for a given compound, a logBB > 0.3 considered to readily cross the blood-brain barrier while molecules with logBB < -1 are poorly distributed to the brain. From Table 4 it can be seen that the logBB value of the *N*-benzoylthiourea derivatives range from -1.01 to 0.12, which means greater than -1, so it can be predicted that all the derivatives of the compound a reable to penetrate the blood-brain barrier moderately.

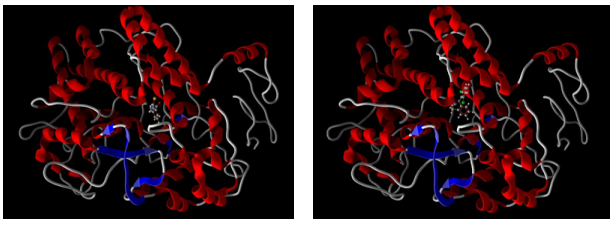
TABLE 5 Predicted RMSD and rerank score values of analgesic activity of *N*-benzoylthiourea derivatives and diclofenac by docking with Molegro Virtual Docker.

Compound no.	RMSD	Rerank score
1	0.028	-67.34 ± 0.88
2	0.289	-71.00 ± 1.81
3	0.007	-71.95 ± 0.36
4	2.207	-70.14 ± 1.06
5	0.007	-76.05 ± 0.02
6	0.26	-81.99 ± 0.41
7	2.012	-72.95 ± 1.03
8	1.725	-82.87 ± 1.40
9	0.113	-77.63 ± 0.13
10	1.454	-73.43 ± 1.79
11	0.131	-86.36 ± 3.28
12	2.278	-70.42 ± 0.00
13	0.015	-69.88 ± 0.03
14	0.027	-69.81 ± 0.48
15	2.385	-70.23 ± 0.80
16	2.401	-69.39 ± 0.42
17	0.171	-73.86 ± 0.04
18	0.054	-72.68 ± 0.01
19	0.085	-73.40 ± 0.21
20	1.401	-70.54 ± 1.46
21	0.006	-68.48 ± 0.02
22	0.318	-69.27 ± 0.99
23	0.122	-80.88 ± 1.50
24	0.019	-90.82 ± 1.91
25	0.576	-94.73 ± 0.80
26	1.343	-92.76 ± 0.03
27	1.709	-82.22 ± 0.34
28	3.947	-78.52 ± 1.06
29	2.396	-74.99 ± 1.60
30	0.275	-89.36 ± 0.29
31	1.093	-95.16 ± 0.98

Measuring blood-brain permeability can difficult with confounding factors. The permeability of the blood-surface area of the central nervous system (CNS) permeability (logPS) is a more direct measurement. It is obtained from in situ brain perfusions with the compound directly injected into the carotid artery. This lacks the systemic distribution effects which may distort brain penetration. According to Pires et al. (2015), a compound with logPS > -2 is considered to penetrate the CNS, while those with logPS < -3 are considered as unable to penetrate the CNS. From Table 4 it can be seen that the logPS value of *N*-benzoylthiourea derivatives range from -2.05 to -2.82, so it can be predicted that all the derivatives of the compound are able to penetrate the CNS moderately.

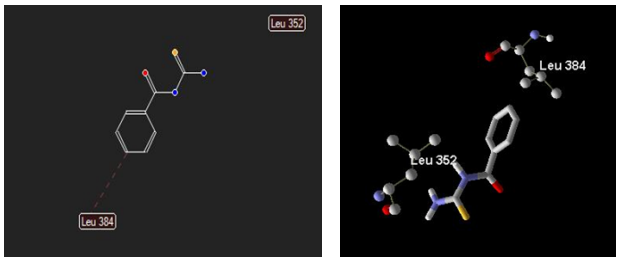
Cytochrome P450 is an important detoxification enzyme in the body, mainly found in the liver. It oxidizes xenobiotics to facilitate their excretion. Many drugs are deactivated by the cytochrome P450's, and some can be activated by it. Inhibitors of this enzyme, such as grape fruit juice, can affect drug metabolism and are contraindicated. The cytochrome P450's are responsible for the metabolism of many drugs. However, inhibitors of the P450's can dramatically alter the pharmacokinetics of these drugs. It is therefore important to assess whether a given compound is likely to be a cytochrome P450 substrate. The two main isoforms responsible for drug metabolism are P2D6 cytochrome (CYP2D6) and P3A4 cytochrome (CYP3A4). From Table 4 it can be seen that all *N*-benzoylthiourea derivatives do not affect or inhibit the enzymes CYP2D6 and CYP3A4, so it can be predicted that all of the derivatives in the body tend to be metabolized by the P450 enzyme.

Organic cation transporter 2 is a renal uptake transporter that plays an important role in disposition and renal clearance of drugs and endogenous compounds. OCT2 substrates also have the potential for adverse interactions with coadministered OCT2 inhibitors. Assessing a candidate potential to be transported by OCT2 provides useful information regarding not only its clearance but potential contraindications. From Table 4 it can be seen that all of the *N*-benzoylthiourea derivatives do not affect the OCT2 substrate, so it can be predicted that the derived compound is not an OCT2 substrate.



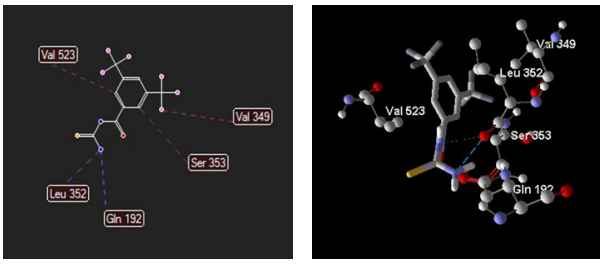
(a) (b)

FIGURE 3 The 3-D description of the COX-2 isoenzyme in the backbone form with *N*-benzoylthiourea (a) and diclofenac (b) as the reference compound.



(a) (b)

FIGURE 4 2-D and 3-D representation of the interaction between *N*-benzoylthiourea and amino acid residues of COX-2 isoenzyme PDB ID: 1PXX.

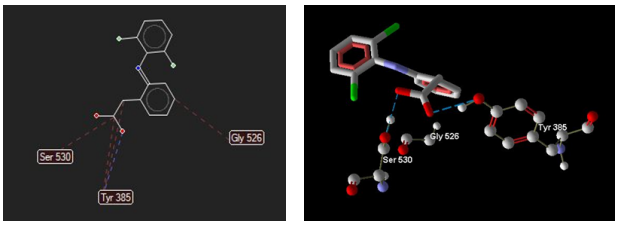


(a) (b)

FIGURE 5 2-D and 3-D representation of the interaction between *N*-(3,5- *bis*-trifluoromethyl)-benzoylthiourea and amino acid residues of COX-2 isoenzyme PDB ID: 1PXX.

Drug clearance is measured by the proportionality constant CLTOT and occurs primarily as a combination of hepatic clearance (metabolism in the liver and biliary clearance) and renal clearance (excretion via the kidneys). It is related to bioavailability and is important for determining dosing rates to achieve steady-state concentrations. The larger of the CLTOT value of the compound, the faster the excretion process. From Table 4 it can be seen that the log CLTOT value of *N*-benzoyl-thiourea derivatives ranges from -0.63 to 0.13 mL/min/kg, and from those values can be predicted the rate of excretion of the compound.

Drug-induced liver injury is a major safety concern for drug development and a significant cause of drug attrition. A compound was classed as hepatotoxic if it had at least one pathological or physiological liver event which is strongly associated with the disrupted normal function of the liver. From Table 4 it can be seen that almost all of *N*-benzoylthiourea derivatives are not hepatotoxic, there are only two compounds (number 23 and 25) predicted to induce hepatotoxic effects.



(a) (b)

FIGURE 6 2-D and 3-D representation of the interaction between diclofenac and amino acid residues of CO-2 isoenzyme PDB ID: 1PXX.

It is important to consider the toxic potency of a potential compound. The lethal dosage values (LD_{50}) are a standard measurement of acute toxicity used to assess the relative toxicity of different molecules. The LD_{50} is the amount of a compound given all at once that causes the death of 50% of a group of test animals. To complement the toxicity prediction of *N*-benzoylthiourea derivatives there was an oral acutetoxicity in silico test on a rodent (LD_{50}) and an acute toxicity classification of compounds based on Globally Harmonized System (GSH), using the Protox online tool. From Table 4 it can be seen that the prediction of LD textsubscript50 values on the rodents of *N*-benzoylthiourea derivatives range from 818 to 3000 mg/kg, it means that includes toxicity classes varying from 4–5 GSH tables which mean compounds have harmful effects if swallowed (LD_{50} = > 300 to ≤ 2000 mg/kg) until it is may beharmful effects if swallowed (LD_{50} = > 2000 to ≤ 5000 mg/kg) (United Nations 2005).

There are three *N*-benzoylthiourea derivatives belonging to the category 5 GSH, i.e. compound number 5, 9, 11, 28 and 30. All of *N*-benzoylthiourea derivatives more safety than diclofenac (LD_{50} = 53 mg/kg, toxicity class = 3).

3.4. Prediction of the analgesic activity

The results of in silico docking test using the Molegro Virtual Docker, between *N*-benzoyl-thiourea derivative compounds and diclofenac with COX-2 isoenzyme PDB ID: 1PXX can be seen in Table 5.

From Table 5 it can be seen that the RS values of the *N*-benzoylthiourea derivative compounds range from -67.34 to -94.73 kcal/mol, and from those values the compound activity can be predicted. The RS value of diclofenac was -95.16 kcal/mol, which means all

TABLE 6 The amino acids of COX-2 isoenzymes involved in interaction with *N*-benzoylthiourea, *N*-(3,5-*bis*-trifluoromethyl)benzoylthiourea, and diclofenac.

No.	Compound	Hydrogen Bond	Steric Interaction
1.	*N*-benzoylthiourea	-	Leu 384; Leu 352
2.	*N*-(3,5-*bis*-trifluoromethyl)-benzoylthiourea	Gln 192; Leu 352	Ser 353; Val 523; Val 349
3.	Diclofenac	Tyr 385; Ser 530	Ser 530; Tyr 385 (2); Gly 526

the investigated compounds were predicted to have a potential affinity fortarget protein. Compound number 24, 25, and 26 [*N*-(2,4-*bis*-trifluoromethyl)benzoylthiourea, *N*-(3,5-*bis*-trifluoromethyl)benzoylthiourea and *N*-(3-trifluoromethoxy)benzoylthiourea] has the bond energy or RS value of -90.82; -94.73, and -92.76, respectively, which means the compounds are predicted to have the relative same analgesic activity with diclofenac. Almost of the compounds have RMSD value < 2.5, the only compound number 28 has an RMSD value of 3.947.

The 3-D description of the COX-2 isoenzyme in the backbone form with *N*N-benzoylthiourea and diclofenac as reference compound can be seen in Figure 3. The 2-D and 3-D representation of the interaction between *N*-benzoylthiourea, *N*-(3,5-*bis*-trifluoromethyl)benzoylthiourea and diclofenac with COX-2 isoenzyme PDB ID: 1PXX can be seen in Figure 4, 5, and 6. The amino acids of COX-2 isoenzymes involved in interaction with *N*-benzoylthiourea, *N*-(3,5-*bis*-trifluoromethyl)benzoylthiourea, and diclofenac can be seen in Table 6. From Table 6 it can be concluded that the bond between *N*-(3,5-*bis*-trifluoromethyl)-benzoylthiourea with 1PXX as the target of COX-2 isoenzyme is the most stable, since it is supported by the presence of a hydrogen bond with amino acids: Leu 352 and Gln 192, and a steric interaction with the amino acids:Ser 353, Val 523, and Val 349.

4. Conclusions

Based on the results of this molecular modeling study it can be concluded that compound number 24, 25, and 26 [*N*-(2,4-*bis*-trifluoromethyl)benzoylthiourea, *N*-(3,5-*bis*-trifluoromethyl)benzoylthiourea and *N*-(3-trifluoromethoxy)benzoylthiourea] is most feasible to be synthesized and tested in vitro and in vivo activity, as it is predicted to be absorbed very well in the intestine, has good skin permeability, can be distributed evenly to give the same concentration as blood plasma, able to penetrate blood-brain barrier moderately, tend to be metabolized by P450 enzyme, has relatively low toxicity, and predicted to have higher analgesic activity among the 30 *N*-benzoylthiourea derivatives studied. However, it should be noted because the predicted compound number 25 can cause hepatotoxicity effects.

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Authors' contributions

SH was responsible for the prediction of pharmacokinetic properties and all data analysis, S the prediction of physicochemical properties and anagesic activity (docking), and RA the prediction of toxicity. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare no competing interest.

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