Molecular Docking, Drug-Likeness Analysis, *In Silico* Pharmacokinetics, and Toxicity Studies of *p*-Nitrophenyl Hydrazones as Anti-inflammatory Compounds against COX-2, 5-LOX, and H$^+$/K$^+$ ATPase

Sodeeq Babalola[1,*]   Nosakhare Igie[2]   Isaiah Odeyemi[2]

[1] Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria
[2] Department of Chemistry and Biochemistry, The University of Texas at Dallas, Richardson, Texas, United States

Address for correspondence Sodeeq Babalola, B. Sc. Chemistry, Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Samaru, Zaria, Kaduna State 810107, Nigeria (e-mail: wale.babalola91@gmail.com).

Abstract

Nonsteroidal anti-inflammatory drugs (NSAIDs) and coxibs are traditional medicines for the treatment of inflammation, yet associated with serious side effects. Hence, the need for discovering novel compounds with valuable clinical benefits is of great importance. In this study, 18 derivatives of *p*-nitrophenyl hydrazones were docked against COX-2, 5-LOX, and H$^+$/K$^+$ ATPase, followed by predicting their drug-likeness and absorption, distribution, metabolism, and excretion (ADME) properties. From the docking analysis, 1-(4-nitrophenyl)-2-[(3,4,5-trimethoxyphenyl)methylidene]hydrazine (**3**), 4-hydroxy-2-methyl-6-[(2-(4-nitrophenyl)hydraz-1-ylidene)methyl]thiochroman-1,1-dioxide (**6**), 4-methoxy-2-methyl-6-[(2-(4-nitrophenyl)hydraz-1-ylidene)methyl]thiochroman-1,1-dioxide (**8**), 2-methyl-6-[(2-(4-nitrophenyl)hydraz-1-ylidene)methyl]-4-(trifluoromethyl)thiochroman-1,1-dioxide (**11**), 4-[(2-(4-nitrophenyl)hydraz-1-ylidene)methyl]benzenesulfonamide (**13**), 4-[(2-(4-nitrophenyl)hydraz-1-ylidene)methyl]-3-(trifluoromethyl)benzenesulfonamide (**14**), 5-methyl-6-[4-[(2-(4-nitrophenyl)hydraz-1-ylidene)methyl]phenyl]-2,3,4,5-tetrahydropyridazin-3-ol (**16**), and 5-methyl-6-[4-[(2-(4-nitrophenyl)hydraz-1-ylidene)methyl]phenyl]-4,5-dihydropyridazin-3(2*H*)-one (**17**) showed promise as potent multi-target inhibitors of COX-2, 5-LOX, and H$^+$/K$^+$ ATPase. These compounds are less COX-2 selective than the control (celecoxib). "Drug-likeness" analysis passed Lipinski's, Egan's, Veber's, Muegge's, and Ghose's rules. The compounds also passed Pfizer and GSK rules, as well as golden triangle's rule for identification of potent and metabolically stable drugs. The pharmacokinetic profiles of the compounds were excellent, safe, and compliant with their potential anti-inflammatory activity. The results of the study can be used for future optimization of those derivatives for better molecular interactions against COX-2, 5-LOX, and H$^+$/K$^+$ ATPase, and inflammation-effective inhibition.

Keywords
► *p*-nitrophenyl hydrazones
► anti-inflammatory
► molecular docking
► docking simulation
► pharmacokinetics
► drug-likeness

Introduction

Inflammation is the body's complicated biochemical response to damaging stimuli including irritants, infections, damaged cells, etc. It is the organism's preventive attempt to start the healing process and eliminate harmful stimuli.[1] It can be triggered by pathogens (bacteria, fungi, and viruses), trauma (shock or burns), toxic substances (pollutants), and immune system responses (hypersensitivity).[2] Nonsteroidal anti-inflammatory drugs (NSAIDs) are the traditional medicine for the management of inflammation, yet associated with gastric toxicity. Long-term usage of NSAIDs has been linked to gastrointestinal (GI) ulcers, bleeding, and nephrotoxicity. The carboxylic acid moiety in most NSAIDs leads to local irritation and reduces the synthesis of tissue prostaglandin, which weakens the homeostatic role of cytoprotective prostaglandins in supporting GI health and balance.[3]

Gastric and duodenal ulcers are frequent GI tract illnesses with significant clinical incidence rates and the potential for serious upper GI hemorrhage. Lowering the output of acid favors ulcer healing.[4] Considering the important role of H^+/K^+ ATPase in gastric acid production, H^+/K^+ ATPase inhibition is the primary method for the treatment of GI acid-related diseases.[4] Among the H^+/K^+ ATPase inhibitors, proton pump inhibitors (PPIs) were extensive drugs in reducing acid in the stomach,[4] and often co-administered with NSAIDs to overcome NSAID-induced GI events in ulcer treatment.

The activity of cyclooxygenases (COX-1/COX-2) and lipoxygenase (5-LOX) can be suppressed during NSAID treatment.[1] Most GI adverse effects can be due to COX-1 inhibition. Highly selective inhibitors of COX-2 can even generate cardiovascular side effects. In contrast, the cardiac problems can be alleviated by LOX inhibitors. Co-inhibition of COX and 5-LOX potentially reduces side effects on the cardiovascular and GI tract while retaining the primary activity of COX-1/2 inhibitors.[5] Therefore, targeting COX-1,2/5-LOX may be a promising strategy to discover more effective drugs with less or no adverse effects.

Hydrazone is a pharmacophoric moiety in inhibiting COX and LOX enzymes with better safety and efficacy.[6,7] Benzothiazole hydrazones have been reported as potent inhibitors of the H^+/K^+ ATPase enzyme and COX-2 enzyme.[4] In this work, novel derivatives of hydrazones were designed. Their potential as multi-target inhibitors of H^+/K^+ ATPase, COX-2, and 5-LOX are further investigated.

Following the promising activity of *p*-nitrophenyl hydrazones against tumor necrosis factor-α in a reported patent which described their therapeutic effect against chronic inflammatory diseases,[8] the current work designed 18 *p*-nitrophenyl hydrazones using structure–activity relationship (SAR). (*E*)-4-(1-(2-(4-nitrophenyl)hydrazineylidene)ethyl)aniline was used as a lead compound which indicated potent inhibition of the enzyme with an IC_{50} of $1.2\ E^{-04}$.[8]

Our SAR study suggested that: (1) the presence of two aryl groups linked together by a hydrazone moiety bridge is required for anti-inflammatory activity. (2) The anti-inflammatory activity of hydrazones could be increased by the presence of at least one nitro group on either hydrazine ring or aldehyde ring (3) The structure of the designed therapeutic drug can be expressed by $Ar_1R_1C=N\text{-}NR_2Ar_2$ (wherein $R_1 = H$, C_1–C_6 alkyl group; $R_2 = H$, C_1–C_6 alkyl group). (4) The presence of electron-withdrawing groups on Ar_1 (aldehyde ring) and electron-donating group on Ar_2 (hydrazine ring) increases anti-inflammatory activity. (5) The presence of an electron-donating group on Ar_1 and Ar_2 increases antiulcer activity. (6) The presence of both electron-withdrawing and electron-donating groups on Ar_1 and electron-donating group on Ar_2 decreases the anti-inflammatory activity. (7) When Ar_1 is a benzene ring, there is good anti-inflammatory activity. (8) Replacement of Ar_1 with a 2 to 6 long aliphatic branched or straight chains decreases anti-inflammatory and antiulcer activities. (9) Replacement of Ar_1 with heterocycles such as indole, pyridine, furan, thiophene, and pyrrole decreases anti-inflammatory and antiulcer activities. Our work suggested that the designed compounds have shown potential as anti-inflammatory agents, and their interactions with ATPase and LOX-5 are promising indications that they will be devoid of adverse effects largely associated with NSAIDs and coxibs.

Materials and Methods

Protein Crystal Structure and Ligand Collection

Three-dimensional (3D) crystal structures of the proteins; COX-2 co-crystalized with celecoxib, human 5-LOX, and human H^+/K^+ ATPase were obtained from the RCSB Protein Data Bank (RCSB PDB) with PDB ID; 3LN1, 3O8Y, and 6JXH, respectively. Celecoxib, zileuton, and omeprazole were used as the reference drugs, and their structures were retrieved from PubMed and PDB.

Molecular Docking Using Autodock Vina

Docking simulation was performed with Autodock Vina script using bash commands in the Cygwin run time environment. This study involves the docking simulation of 18 *p*-nitrophenyl hydrazone derivatives within the binding site of COX-2, 5-LOX, and H^+/K^+ ATPase. The docking procedure was performed in a flexible docking mode, which creates conformations for each input ligand automatically. The produced ligand poses were subjected to a series of hierarchical filters to asses ligand's interaction with the receptor. This approach penalizes steric conflicts while recognizing favorable hydrogen bonding hydrophobic and metal-ligation interactions. After the simulation was completed, the binding energies of the ligands were ranked using Excel. Each ligand pose (conformation) was viewed in UCSF chimera 1.11.2 (www.cgl.ucsf.edu/chimera); the most favorable complexes formed were viewed in the discovery studio software (https://discover.3ds.com/discovery-studio-visualizer-download) wherein various interactions between the ligands and the receptors were elucidated in a two-dimensional (2D) format.

Drug-Likeness, *In Silico* Pharmacokinetics, and Toxicity Studies

The drug-likeness studies, *in silico* pharmacokinetics, and toxicity studies were evaluated on ADMETlab 2.0 (https://

admetmesh.scbdd.com) and Protox-II web servers (https://tox-new.charite.de). The ADMETlab 2.0 was used to evaluate detailed parameters of drug-likeness, as well as absorption, distribution, metabolism, and excretion (ADME), which are the pharmacokinetic properties. The toxicity studies were conducted on Protox-II and ADMETlab 2.0 web servers. The data were statistically analyzed using a two-way analysis of variance (ANOVA) with replication, residual error test, and Tukey's multiple comparison test.

Results

Docking Simulation

The binding energies of the best poses for each compound with COX-2, 5-LOX, and H$^+$/K$^+$ ATPase are summarized in ►**Table 1**. It is worth mentioning that all the highly selective COX-2 inhibitors (so-called "coxibs"), including rofecoxib, valdecoxib, etoricoxib, parecoxib, celecoxib, meloxicam, and lumiracoxib, are associated with serious toxicity, and have been withdrawn from the market by the United States Food and Drug Administration (FDA) except for celecoxib. However, celecoxib remains in the market with stringent warnings. Thus, the designed multi-target compounds with binding affinity for COX-2 but less selectivity than celecoxib may ameliorate the toxicities associated with NSAIDs and celecoxib. Interestingly, our data showed that all the compounds have lower docking scores than that of celecoxib (-12.6 kcal).

Zileuton is a selective and specific 5-LOX inhibitor. Interestingly, binding energies of all the designed compounds (except for compound **1**) at the 5-LOX active site were better than that of zileuton. Also, all the compounds had higher affinity for the active site of H$^+$/K$^+$ ATPase in comparison to omeprazole. Exception to the observation was the compounds **1**, **3**, **4**, and **5** that had lower but comparable binding affinity within the active site of the enzyme.

►**Table 2** summarizes hydrogen bond interactions of each compound in the active site. Our data also showed that all the compounds are competitive inhibitors of COX-2 enzyme. The interactions of the designed compounds within COX-2 active site were examined. These were compared to the interactions of celecoxib within the active site of COX-2 enzyme. Compounds **3** and **16** formed hydrogen bond interactions with Ser516, which is a NSAID key interaction for anti-inflammatory activity[9] suggesting their anti-inflammatory activity potential. However, like celecoxib, most of the compounds exhibited strong interactions with the active site of COX-2. It is worth noting that compounds **3**, **13**, and **16** had the best interactions with the COX-2 active site. Compounds **12**–**16** showed high affinity for COX-2 binding. Of all the designed compounds, compound **3** indicated superior binding interactions contributing 5, 4, and 5 hydrogen bonding to COX-2, 5-LOX, and ATPase. 2D and 3D illustrations of interactions of compound **3**, celecoxib, zileuton, and omeprazole with COX-2, 5-LOX, and the H$^+$/K$^+$ ATPase active site are included in the Supporting Information (►**Figs. S1**–**S12 [online only]**).

Only compound **10** is a competitive inhibitor of 5-LOX. Compounds **2**, **7**–**9**, **11**, **14**, **16**, and **17** bind at three of the five binding sites of zileuton. Compounds **1**, **3**, **6**, **10**, and **18** bind at two of the binding sites of zileuton. Compounds **4**, **5**, and **13** bind at one of the five binding sites of zileuton. All of these compounds also bind at 1 to 3 sites within the active sites where zileuton does not bind. Compound **15** does not bind at any binding site of zileuton. All the *p*-nitrophenyl hydrazones bind at the same binding site within the active site of 5-LOX at their most favorable binding energy, indicating that the *para*-nitro group confers site-directing or selectivity. Exceptions to this are compounds **12** and **17** which bind at different binding sites. However, the two compounds bind at the nitro group-directing site at their other poses where other *p*-nitrophenyl hydrazones bind.

The binding analysis revealed that all the compounds are competitive inhibitors of H$^+$/K$^+$ ATPase except for compounds **1**, **2**, **9**, **12**, and **18**. The halogens in compounds **1**, **2**, and **12** are site-directing as these compounds bind at the same binding within the active site. However, this is not observed with compounds **11** and **14**, which are competitive inhibitors. It can be said that binding of compounds **11** and **14** within the active site is directed by the sulphonyl (SO$_2$) moiety rather than the substituted halogens. Compounds **9** and **18** are also noncompetitive inhibitors because they did not bind where omeprazole binds within the active site.

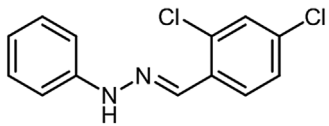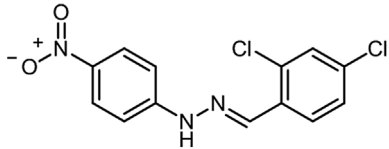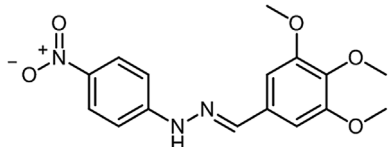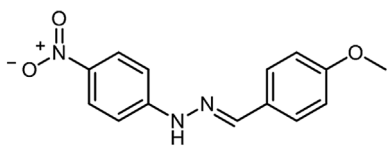### Drug-Likeness, Physicochemical Properties, and Medicinal Chemistry Friendliness
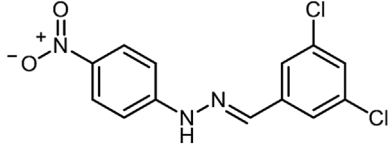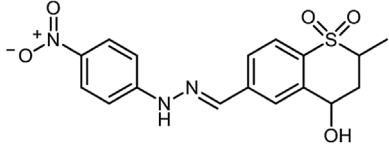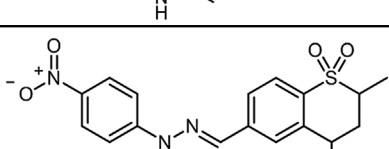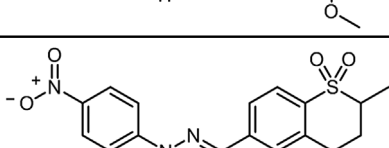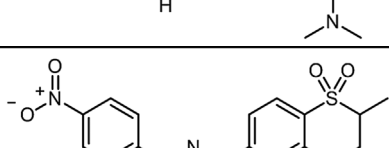
Compounds passing Lipinski's rule of five, Veber's rule, and Egan's rule are considered orally bioavailable. Lipinski's rule of five considers the parameters of molecular weight (MW) $\leq$ 500 Da, number of H-bond acceptors (nHA) $\leq$ 5, number of H-bond donors (nHD) $\leq$ 10, and logP $\leq$ 5, while the Veber's rule considers the number of rotatable bonds (nRot) $\leq$ 10 and the topological polar surface area (TPSA) $\leq$ 140 Å^2. Egan's rule parameters includes logP $\leq$ 5.88 and TPSA $\leq$ 131.6 Å^2, Ghose's rule parameters includes 160 $\leq$ MW $\leq$ 480, -0.4 $\leq$ logP $\leq$ 5.6, 40 $\leq$ molar refractivity $\leq$ 130, 20 $\leq$ atoms $\leq$ 70, Muegge's rule parameters includes 200 $\leq$ MW $\leq$ 600, -2 $\leq$ logP $\leq$ 5, number of rings (nRing) $\leq$ 7, number of carbon atoms $>$ 4, number of heteroatom (nHet) $>$ 1, nRot $\leq$ 15, nHA $\leq$ 10, nHD $\leq$ 5, and golden triangle rule for metabolically stable compounds considers 200 $\leq$ MW $\leq$ 50, -2 $\leq$ logD $\leq$ 5.

Our data suggested that most of the compounds were identified as druggable structures, and only compounds with two or more parameters violating the rules are considered orally not bioavailable. ►**Table 3** suggests that all the compounds conformed to the above rules, therefore, are drug-like compounds.

Solubility of the compounds in water was considered as logS values with all compounds being -4.00 and -7.00, other than **3** and **4**, suggesting a poor solubility of the compounds in water. Except for compound **12** with high logD equal to 4.12, all oth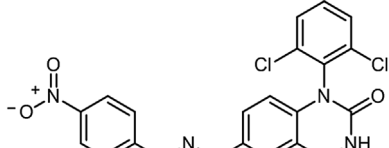er designed compounds have logP at physiological pH 7.4 below the threshold of 4.0 mol/L, indicating that they are soluble at the physiological pH 7.4.
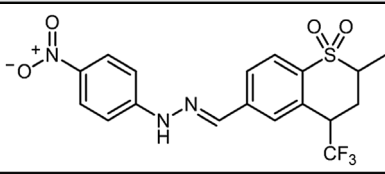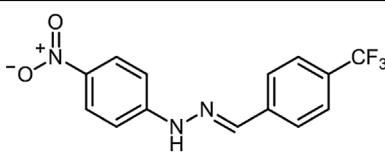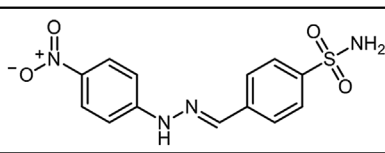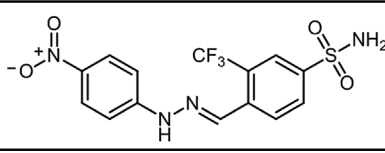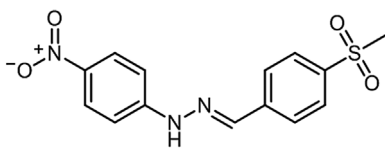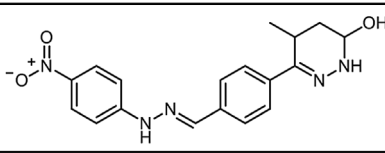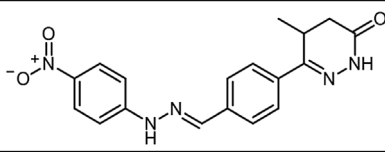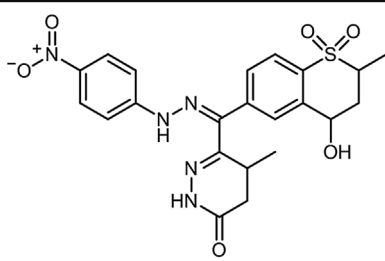
Quantitative estimate of drug-likeness (QED) is a measure of drug-likeness based on the concept of desirability. QED is calculated by integrating the outputs of the desirability

Table 1 Binding energy (kcal/mol)

Compound	Structure	COX-2	5-LOX	H+/K+ ATPase
1		−8.4	−5.3	−6.5
2		−8.9	−7.2	−7.3
3		−7.6	−6.9	−6.8
4		−8.4	−7.5	−6.7
5		−8.9	−7.7	−6.9
6		−7.8	−7.2	−8.0
7		−8.1	−8.1	−8.7
8		−8.0	−6.7	−7.8
9		−7.9	−6.8	−7.7
10		−7.9	−6.8	−8.0

(*Continued*)

Table 1 (*Continued*)

Compound	Structure	COX-2	5-LOX	H$^+$/K$^+$ ATPase
11		−8.1	−7.6	−8.6
12		−9.5	−8.8	−8.0
13		−9.5	−8.1	−7.3
14		−9.6	−7.3	−7.5
15		−9.0	−7.9	−7.2
16		−9.7	−7.7	−7.8
17		−8.8	−7.4	−7.9
18		−8.8	−8.0	−8.3
Celecoxib		−12.6		
Zileuton			−6.2	
Omeprazole				−7.0

functions based on eight drug-likeness-related properties, including MW, logP, nHA, nHD, PSA, nRot, the number of aromatic rings (nAr), and the number of alerts for undesirable functional groups. The synthetic accessibility scores are within 6, and the fraction of sp³-hybridized carbon score (Fsp3) is below the 0.42 minimum suitable threshold. ►**Table 4** shows that QED scores of most compounds fall between 0.64 to 0.48, which is a bit below the threshold score of 0.67 for attractive compounds. Compounds **1**, **2**, **4**, **5**, **10**, **12**, **13**, and **15** with QED scores ≥0.49 are considered to be above the meniscus for unattractive compounds.

MCE-18 stands for medicinal chemistry evolution in 2018,[10] it is usually used to assess the novelty and lead potential of pharmacologically relevant molecules.

Table 2 Hydrogen bond interactions

Compound	No. of hydrogen bonding for COX-2 (residues)	No. of hydrogen bonding for 5-LOX (residues)	No. of hydrogen bonding for H$^+$/K$^+$ ATPase (residues)
1	0	1 (Trp605)	0
2	2 (Arg499, Phe504)	2 (Gln15, Arg401)	1 (Thr152)
3	5 (Ser516, Val335, Ile503, Phe504)	4 (Ser14, Asp170, Tyr383, Phe402)	5 (Thr134, Cys813, Glu900, Gln924)
4	0	3 (Tyr81,Tyr383, Phe402)	3 (Thr134, Cys813)
5	2 (His75, Phe504)	3 (Ser14, Arg401)	2 (Gln924, Tyr928)
6	2 (His75, Thr79)	3 (Asp170, Arg401, Gln611)	4 (Arg328, Tyr802, Cys813, Ile814)
7	1 (His75)	1 (Arg401)	3 (Ile814, Asn989)
8	2 (His75, Gln178)	3 (Gly174, Asn180, Arg401)	4 (Ile814, Gln924, Tyr928, Asn989)
9	1 (His75)	4 (Asp170, Ser171, Arg401, Asn613)	2 (Gln104, Gln159)
10	1 (Asn567)	2 (Asp422, Arg596)	1 (Ile814)
11	2 (His75, Gln178)	4 (Asp170, Ser171, Arg401, Gln611)	5 (Asn138, Arg328, Tyr802, Cys813, Ile814)
12	2 (Ile503, Phe504)	3 (His373, Ala424, Asn425)	1 (Thr152)
13	4 (Gln178, Ser339, Arg499, Phe504)	5 (Ser14, Lys83, Tyr383, Asp166, Arg401)	5 (Thr134, Asp137, Asn138, Arg328, Tyr925)
14	4 (Gln178, Ser339, Arg499, Phe504)	3 (Lys83, Ser171, Gln611)	7 (Thr134, Asp137, Arg328, Ile814, Asn989)
15	2 (His75, Arg499)	2 (Lys83, Arg401)	4 (Gln127, Asn138, Tyr925, Asn989)
16	4 (Arg106, Gln178, Phe504, Ser516)	2 (Gln15, Tyr383)	4 (Thr138, Asp137, Ile814, Asn989)
17	2 (His75, Gln178)	5 (Lys83, Asp170, Ser171, Arg401)	3 (Ile814, Tyr928, Asn989)
18	2 (Asn567, Val568)	4 (Tyr558, Asp559, Gln609)	0
Celecoxib	3 (Gln178, Arg499, Phe504)		
Zileuton		2 (His195, Phe197)	
Omeprazole			2 (Asp137, Ile814)

Compounds **6** and **8–11** are ranked as compounds with high structural similarity to the compounds disclosed in patent records, whereas compound **18** with an MCE-18 score of 86.79 is considered a novel scaffold with a strong drug-like structure.

Pan-assay interference compounds (PAINS) are undesirable hits and are often filtered off from compound libraries. As shown in ►**Table 4**, PAINS values of all the compounds were zero, indicating that there is no PAINS substructure incorporated in the compounds. The designed compounds gave zero alerts for PAINS; therefore, they are more suitable for drug discovery bioassays and have drug-like potentials.

The alarm NMR rule is also used to identify potentially reactive or promiscuous compounds. Like PAINS filters, it cannot distinguish between bad or innocent suspects including covalent inhibitors. Thus, reactivity alerts from alarm NMR can be useful as a good indicator of possible phase I and II metabolic reactions of compounds. ►**Table 4** shows that the number of alerts was 2 to 5, representing the number of labile groups including the nitro group's possible reduction *in vivo*, amine group's oxidation *in vivo*, and the aromatic substitutions that can be influenced by the substituents on the ring within each compound.

The Bristol-Myers Squibb (BMS) rule is used to filter undesirable reactive compounds and reagents that could cause serious toxicities. Our data showed that all the compounds gave zero alerts for the BMS rule, suggesting that none of the compounds contain undesirable reactive substructure(s). All the compounds also gave zero alerts for the Chelator rule, indicating that none of the compounds are polydentate ligands.

ADME Predictions

Absorption

Examples of experimental screens are represented by the human colon adenocarcinoma cell lines (Caco-2) and MDCK (Madin-Darby canine kidney cell line) screening approaches

Table 3 Physicochemical properties of the designed compounds

Compound	MW	nHA	nHD	nRot	nRing	nHet	TPSA (Å)	logS	logP	logD
1	264	2	1	3	2	4	24.39	−4.83	4.55	3.64
2	309	5	1	4	2	7	70.21	−4.85	4.24	3.60
3	331	8	1	7	2	8	97.90	−3.87	2.94	3.53
4	271	6	1	5	2	6	79.44	−3.74	3.18	3.69
5	309	5	1	4	2	7	70.21	−4.85	4.31	3.35
6	375	8	2	4	3	9	121.90	−4.67	3.25	1.99
7	455	8	2	5	4	10	99.87	−7.00	5.00	3.27
8	389	8	1	5	3	9	110.90	−5.95	3.61	2.98
9	402	8	1	5	3	9	104.91	−5.87	3.63	2.57
10	384	8	1	4	3	9	125.46	−6.55	3.65	2.42
11	427	7	1	5	3	11	101.67	−6.69	4.36	3.58
12	309	5	1	5	2	8	67.53	−6.59	4.72	4.12
13	320	8	3	5	2	9	127.69	−5.28	3.13	2.18
14	388	8	3	6	2	12	127.69	−5.80	3.79	2.98
15	319	7	1	5	2	8	101.67	−5.28	3.37	2.55
16	353	8	3	5	3	8	112.15	−4.86	3.79	3.26
17	351	8	3	6	3	8	112.95	−4.11	2.61	2.80
18	485	11	4	6	4	12	167.32	−4.15	1.84	1.61
Piroxicam	316	6	1	2	3	7	96.43	−4.67	1.30	0.05
Celecoxib	381	5	2	4	3	9	77.98	−4.87	3.47	3.40

Table 4 Drug-likeness and medicinal chemistry friendliness

Compounds	QED	SA	Fsp3	MCE-18	PAINS	Alarm NMR rule	BMS rule	Chelator rule
1	0.64	1.80	0	10.00	0	2	0	0
2	0.52	2.04	0	12.00	0	4	0	0
3	0.48	2.07	0.19	13.00	0	5	0	0
4	0.52	1.85	0.07	11.00	0	5	0	0
5	0.52	2.12	0	12.00	0	4	0	0
6	0.48	3.57	0.24	65.71	0	5	0	0
7	0.29	2.65	0.05	48.09	0	4	0	0
8	0.48	3.63	0.28	65.22	0	5	0	0
9	0.47	3.64	0.32	68.04	0	5	0	0
10	0.49	3.75	0.22	65.46	0	5	0	0
11	0.45	3.75	0.28	75.00	0	5	0	0
12	0.53	2.05	0.07	14.00	0	4	0	0
13	0.49	2.05	0.00	14.00	0	5	0	0
14	0.46	2.41	0.07	18.00	0	5	0	0
15	0.52	2.02	0.07	14.00	0	5	0	0
16	0.44	3.51	0.22	55.64	0	4	0	0
17	0.47	2.44	0.11	17.00	0	3	0	0
18	0.30	4.28	0.27	86.79	0	4	0	0
Piroxicam	0.78	3.78	0.13	58.24	0	5	0	0
Celecoxib	0.75	2.14	0.12	22.00	0	1	0	0

Table 5 Absorption

Compound	Caco-2	MDCK ($\times 10^{-6}$)	Pgp-inhi.	Pgp-subs.	HIA	F30%	F20%	F10%
1	−4.28	12	0.004	0.032	0.003	0.976	0.062	0.55
2	−4.33	146	0.002	0.004	0.004	0.004	0.001	0.55
3	−4.61	55	0.024	0.016	0.008	0.028	0.003	0.55
4	−4.41	201	0	0.028	0.010	0.016	0.002	0.55
5	−4.37	139	0.004	0.008	0.006	0.004	0.001	0.55
6	−5.51	65	0.001	0.083	0.025	0.739	0.002	0.55
7	−4.60	125	0.308	0.010	0.009	0.002	0.002	0.55
8	−4.86	136	0.002	0.025	0.010	0.061	0.002	0.55
9	−5.76	73	0	0.061	0.009	0.002	0.002	0.55
10	−5.08	220	0	0.005	0.009	0.001	0.002	0.55
11	−4.73	143	0.010	0.004	0.007	0.003	0.001	0.55
12	−4.42	159	0.001	0.015	0.007	0.002	0.001	0.55
13	−5.24	201	0.001	0.010	0.013	0.002	0.002	0.55
14	−4.83	171	0.001	0.016	0.007	0.002	0.002	0.55
15	−5.03	116	0.001	0.008	0.014	0.003	0.002	0.55
16	−5.12	3	0	0.171	0.028	0.824	0.014	0.55
17	−5.05	45	0.077	0.031	0.017	0.001	0.001	0.55
18	−6.00	14	0.014	0.731	0.093	0.011	0.002	0.55
Piroxicam	−6.05	18	0.082	0.002	0.010	0.003	0.002	0.56
Celecoxib	−4.77	23	0.084	0.005	0.003	0.001	0.002	0.55

Note: Empirical decision for P-gp, HIA, and F: 0–0.3, excellent; 0.3–0.7, good; 0.7–1.0, poor.

to assess membrane permeability to evaluate human oral absorption in drug discovery.[11] As shown in ►**Table 5**, Caco-2 permeability values of compounds **6**, **9**, **13**, and **18** were less than −5.15 cm/s minimum,[11] however, higher than −6.05 cm/s for piroxicam. The rest of the compounds are regarded as compounds with proper Caco-2 permeability with their permeability value greater than −5.15 cm/s.

MDCK permeability values of compounds **1**, **16**, **18**, and piroxicam were apparently moderate with permeability coefficient (*Papp*) values ranging between 2×10^{-6} and 20×10^{-6} cm/s. MDCK permeability values of the other compounds were greater than 20×10^{-6} cm/s, suggesting high passive permeability of the compounds

Compounds **3**, **7**, **11**, and **17** indicated a stronger substrate affinity of P-glycoprotein (P-gp) than inhibition of the enzyme with scores less than 0.30. This was also observed with celecoxib and piroxicam being stronger substrates than inhibitors of the P-gp enzyme. Compound **18** is a weak or nonsubstrate of P-gp with a score of 0.73 according to the results in ►**Table 5**. All other compounds are more potent inhibitors of P-gp than their P-gp substrate tendencies.

The human intestinal absorption (HIA) model predicts HIA after oral administration. Our data showed that all the compounds had HIA scores below 0.01, and this indicates that they are non-HIA+, i.e., their HIA far exceeds 30%. Compound **1** has best HIA profile according to the results

in ►**Table 5**, which is equal to HIA of celecoxib. Compounds **3**, **6**, **7**, **9**–**12**, and **14** had better HIA compared with piroxicam, while compounds **4** and **8** had HIA equal to that of piroxicam.

The 30% bioavailability (F30%) values of most compounds were excellent (quite below 0.1), suggesting that 30% of each of these compounds is orally bioavailable, except for compounds **1**, **6**, and **16** with F30% scores being 0.976, 0.739, and 0.824, respectively. All the compounds demonstrated excellent F20% and good F10% scores, suggesting that 20% and 10% of each of these compounds are orally bioavailable. Taken together, all the compounds demonstrated good human absorption and the extent of oral bioavailability suggesting that a significant percent of the compounds will reach systemic circulation and target sites.

Distribution

As shown in ►**Table 6**, the plasma protein binding (PPB) for the designed compounds was found to be between 91.28 and 101.00%, which is a bit more than the maximum earmarked (90%) for proper PPB.[11] Compound **18** had PPB (91.28%) closest to the 90% threshold. It has been noticed that many clinically successful drugs exhibit high PPB. For example, celecoxib had a PPB value of 94.96%, which is greater than 90% indicating that there is no fast and hard rule with PPB. Furthermore, this claim is also supported by the documented statistics of drugs approved by FDA.[12]

Table 6 Distribution

Compound	PPB (%)	Volume distribution (L/kg)	BBB	F_u (%)
1	100.52	2.731	0.198	0.82
2	100.80	1.779	0.107	0.61
3	98.97	0.714	0.14	1.55
4	99.25	0.732	0.228	0.74
5	101.00	2.085	0.072	0.55
6	98.46	0.361	0.025	1.23
7	100.50	1.198	0.424	0.74
8	99.30	0.534	0.045	0.80
9	97.53	1.066	0.495	2.23
10	98.77	0.444	0.012	1.14
11	100.50	0.942	0.333	0.38
12	99.99	1.310	0.28	0.30
13	98.37	0.492	0.016	0.97
14	99.00	0.597	0.181	0.54
15	98.42	0.305	0.034	1.21
16	98.30	1.146	0.887	0.83
17	92.04	1.021	0.401	7.21
18	91.28	0.380	0.028	11.05
Piroxicam	73.60	0.340	0.967	27.15
Celecoxib	94.96	1.105	0.586	5.01

Abbreviations: PPB, plasma protein binding; BBB, blood–brain barrier; F_u, fraction unbound.
Note: Empirical decision for BBB: 0–0.3, excellent; 0.3–0.7, good; 0.7–1.0, poor.

Volume distribution (Vd) values of all the designed compounds were between 0.305 and 2.731 L/kg, which fall within the proper Vd range 0.04–20 L/kg threshold[11] and are similar to those of reference drugs: piroxicam (0.340 L/kg) and celecoxib (1.105 L/kg).

The blood–brain barrier (BBB) has been identified as a dynamic interface that maintains optimal conditions for neuronal and glial activity by controlling the flow of chemicals between the blood and the brain. Neurodegenerative diseases (such as Alzheimer's disease and multiple sclerosis), stroke and traumatic brain damage, infectious processes, and inflammatory pain are all thought to be linked to the BBB. As a result of BBB failure in various diseases, transport and permeability may be hindered.[13] ►**Table 6** shows that BBB permeabilities of most of the compounds were less than 0.6 scores, suggesting that their permeability is in excellent category. However, BBB permeability of compound **16** was 0.887, suggesting that the compound may not be able to pass through BBB.

The fraction unbound or plasma free drug fraction (F_u) for most of the compounds was less than 5% minimum except for compounds **17** and **18** with F_u being 7.21% and 11.05% F_u, respectively. This demonstrates that most of the compounds

have a low fraction unbound as a consequence of high PPB. Generally, the lower the PPB, the higher the F_u. In the absence of transporters, the concentration of free drug is the same on both sides of the biological membrane at a steady state, and also the free drug or unbound concentration at the site of action is the species that exert pharmacological activity such as *in vivo* efficacy and toxicity, according to the free drug hypothesis. This may be true for the designed hydrazones because of their high passive permeability and probable quick rate of permeation, which accelerate the pace of attaining equilibrium across membranes.

Metabolism

Cytochrome P450 (CYP) enzyme have wide substrate specificity high polymorphism, and they are key determinants in drug–drug interactions.[14] Metabolisms of the designed compounds were predicted by assessing their affinity to metabolizing enzymes (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4), and the results are summarized in ►**Table 7**. Generally, compounds with scores between 0.0 and 0.3 are strong inhibitors/substrates of the CYP-metabolizing enzymes, scores between 0.4 and 0.7 denote moderate–weak inhibitor/substrates of CYP, and scores between 0.8 and 1.0 denote non-inhibitors/substrates of CYP enzymes.

Enzyme CYP1A2 generally metabolizes aromatic amines and heterocyclic compounds. Our data showed that compounds **9**, **18**, and piroxicam are strong inhibitors of CYP1A2 with scores of 0.27, 0.01, and 0.17; however, weak or non-substrates of the enzyme with scores of 0.93, 0.55, and 0.62, respectively, suggesting that CYP1A2 may not metabolize compounds **9** and **18**. Compounds **2**, **5**, **6**, **10**, **12**, **13**, and **15**–**17** are substrates of CYP1A2 with high affinity. All other compounds and celecoxib are weak inhibitors and substrates of the metabolizing enzyme. This means that they may not interact with the enzyme CYP1A2.

Compounds **1**, **2**, **4**, **5**, **7**, **10**, **12**, and **15** are strong substrates of CYP2C19, while compounds **6**, **10**, **13**, **14**, **16**, and piroxicam are strong inhibitors and substrates of CYP2C19. However, celecoxib and other designed *p*-nitro-phenyl hydrazones are weak inhibitors and substrates of the metabolizing enzyme CYP2C19.

Compounds **3**, **13**, **15**, and piroxicam are strong inhibitors of the CYP2C9-metabolizing enzyme. Compounds **2**, **4**–**6**, **8**–**10**, **12**, **14**, **16**, and **18** are moderate to weak inhibitors of CYP2C9. Celecoxib and other compounds are noninhibitors of the metabolizing enzyme. Also, all the designed compounds and the reference drugs have no substrate affinities/tendencies for CYP2C9.

Compounds **3**, **4**, **6**–**18**, and celecoxib are strong inhibitors of the CYP2D6-metabolizing enzyme. Piroxicam is a strong inhibitor and substrate of CYP2D6. Compounds **2** and **5** are moderate–weak inhibitors of the metabolizing enzyme. Compounds **6**, **8**, **14**, **15**, **17**, and celecoxib are moderate–weak substrates of CYP2D6. Compounds **1**–**5**, **7**, **9**, **11**, **12**, and **16** are nonsubstrates of CYP2D9.

Compound **1**, **2**, **5**–**9**, **13**, **14**, piroxicam, and celecoxib are strong inhibitors of CYP3A4. The rest of the compounds are weak or noninhibitors of CYP3A4. Likewise, compounds **1**, **2**,

Table 7 Metabolism

Compound	CYP1A2		CYP2C19		CYP2C9		CYP2D6		CYP3A4	
	Inhib.	Subs.	Inhib.	Subs.	Inhib.	Subs.	Inhib.	Subs.	Inhib.	Subs.
1	0.99	0.47	0.95	0.12	0.81	0.84	0.55	0.76	0.20	0.26
2	0.95	0.21	0.91	0.09	0.67	0.92	0.51	0.87	0.27	0.30
3	0.54	0.97	0.60	0.66	0.37	0.84	0.02	0.88	0.58	0.73
4	0.87	0.68	0.57	0.22	0.43	0.94	0.24	0.90	0.61	0.51
5	0.97	0.20	0.88	0.09	0.56	0.77	0.58	0.84	0.26	0.16
6	0.34	0.11	0.38	0.41	0.39	0.95	0.10	0.56	0.21	0.42
7	0.44	0.64	0.92	0.27	0.90	0.94	0.31	0.83	0.39	0.92
8	0.54	0.55	0.48	0.67	0.48	0.92	0.20	0.67	0.33	0.58
9	0.27	0.93	0.47	0.70	0.44	0.93	0.24	0.90	0.26	0.86
10	0.44	0.13	0.38	0.07	0.53	0.96	0.17	0.22	0.43	0.50
11	0.72	0.59	0.94	0.48	0.93	0.98	0.41	0.75	0.60	0.77
12	0.91	0.18	0.81	0.08	0.64	0.91	0.28	0.85	0.44	0.22
13	0.46	0.11	0.10	0.07	0.11	0.85	0.14	0.34	0.32	0.48
14	0.82	0.59	0.28	0.13	0.49	0.88	0.21	0.47	0.21	0.25
15	0.70	0.12	0.40	0.11	0.17	0.90	0.14	0.60	0.45	0.74
16	0.76	0.12	0.34	0.08	0.44	0.87	0.03	0.78	0.41	0.31
17	0.64	0.33	0.88	0.06	0.79	0.90	0.40	0.51	0.83	0.15
18	0.01	0.55	0.23	0.28	0.58	0.98	0.03	0.16	0.72	0.84
Piroxicam	0.17	0.62	0.13	0.20	0.10	0.95	0.22	0.14	0.20	0.58
Celecoxib	0.84	0.6	0.71	0.62	0.86	0.70	0.052	0.60	0.15	0.85

Abbreviations: Inhib., inhibitors; Subs., substrate.
Note: Empirical decision: 0–0.3, excellent; 0.3–0.7, good; 0.7–1.0, poor.

5, **12**, **14**, **16**, and **17** are strong substrates of CYP3A4. The rest of the compounds as displayed in ►**Table 7** and are weak or noninhibitors of the metabolizing enzyme.

Excretion

The clearance and half-life of the compounds were predicted. As shown in ►**Table 8**, compounds **1**, **3**, and **4** had moderate clearance with clearance prediction between 5 and 15 mL/min/kg, while compounds **2** and **5–18** had low clearance with the clearance prediction less than 5 mL/min/kg. The half-life values of compounds **3** and piroxicam were moderate, which may be greater than 3 hours, while the half-life of compound **16** was poor, which may be less than 3 hours. The half-life values of celecoxib and other designed hydrazones were predicted to be excellent, and therefore may be greater than 3 hours.

Toxicity

The results for different toxicity endpoints for the designed compounds are shown in ►**Tables 9** to **14**. For the proper analysis of their toxicities, some approved anti-inflammatory drugs including piroxicam, celecoxib, and aspirin (also known as acetylsalicylic acid [ASA]) are used as references. Also, a cardioprotective hydrazone drug levosimendan

(LSD) and a simple aromatic hydrazone (SAH) were used as references for the inherent compound class toxicities. The structures of the compounds were simply drawn using ChemDraw tools and were analyzed using the toxicity analysis platform Protox-II. The percentage of accuracy prediction and average percentage of similarity of each compound compared with the datasets of the models used are also estimated in the results in ►**Table 9**. Our data showed that compounds **6–11**, **14–18**, piroxicam, celecoxib, and LSD are safe to be formulated as ophthalmic drugs. However, other designed compounds and ASA are predicted to be either eye irritant or eye corrosion, and may not be formulated as an ophthalmic dosage form.

LD_{50} (mg/kg) is a measure of agent toxicity, with the value of 50–300 being class III, 300–2,000 being class IV, and 2,000–5,000 being class V according to the toxic class of the Globally Harmonized System of classification of labeling of chemicals. As shown in ►**Table 10**, compounds **12–15** and ASA are categorized as class III, suggesting that they are toxic if swallowed. Compounds **1–5**, **7**, **8**, **16–18**, ASA, LSD, SAH, piroxicam, and celecoxib are categorized as class IV, suggesting that they may be harmful if swallowed. Compounds **6**, **10**, and **11** are categorized as class V, suggesting that they may be harmful if swallowed.[13]

Table 8 Excretion

Compound	Clearance (mL/min/kg)	Half-life ($T_{1/2}$)
1	5.697	0.152
2	3.852	0.092
3	6.340	0.383
4	5.638	0.161
5	3.913	0.124
6	0.809	0.090
7	1.585	0.087
8	1.100	0.050
9	2.189	0.053
10	0.543	0.079
11	1.433	0.019
12	4.148	0.049
13	0.980	0.101
14	1.100	0.046
15	0.509	0.067
16	2.060	0.717
17	4.539	0.197
18	1.420	0.099
Piroxicam	1.033	0.561
Celecoxib	0.992	0.029

Note: Empirical decision for $T_{1/2}$: 0–0.3, excellent; 0.3–0.7, good; 0.7–1.0: poor.

Prediction for human hepatotoxicity (H-HT)/drug-induced liver injury (DILI), carcinogenic, immunotoxic, mutagenic, and cytotoxic potentials for candidates was also conducted.

Data from safety profiles of H-HT/DILI (►**Table 10**) showed that among the designed compounds, **1–7**, **12**, and **16** were within excellent safety, **8–11**, **13**, and **18** were good safety, however, compounds **11**, **14**, **15**, and **17** may be hepatotoxic. It is worth mentioning that the score of safety profiles of H-HT/DILI of celecoxib was 0.64, indicating a possibly considerable hepatotoxicity risk of the compound.

Compounds **6**, **7**, **9–11**, **14**, and **18** were noncarcinogenic (inactive). However, most of the designed compounds including piroxicam, celecoxib, LSD, and SAH carry low risk of carcinogenicity (active) with probability between 0.53 and 0.72 (►**Table 10**). The carcinogenicity may be due to the presence of primary and secondary amine functional groups in their structures. Because primary and secondary amine groups of a drug may react with sodium nitrite to yield *N*-nitroso compounds. A typical instance is the development of hemorrhagic liver tumors in rats due to the formation of dimethylnitrosamine from aminopyrine and nitrite in rat stomach.[15] Piroxicam, celecoxib, and LSD are also predicted to be carcinogenic for the same reason. Although ASA was found to be noncarcinogenic with 0.81 probability, it has been reported that its sodium salt (sodium salicylate) is carcinogenic.[15]

Immunotoxicity of NSAIDs has been reported.[16] Similar lethality for ASA, diclofenac, and sulindac was also documented.[17] Our data suggested that compounds **3**, **6**, **9**, **11**, and **14** were predicted to be immunotoxic (active) with probability between 0.53 and 0.92. The other compounds and reference drugs were nonimmunotoxic (inactive) with probabilities between 0.69 and 0.92 for the designed hydrazones, 0.69 for piroxicam, 0.99 for celecoxib, and 0.99 for ASA.

NSAIDs including indomethacin, oxyphenbutazone, and methyl salicylate are mutagenic in the Ames test.[18] Our data showed that most of the compounds, including SAH and LSD, were predicted as mutagenic (►**Table 10**). However, piroxicam, celecoxib, and ASA were nonmutagenic with probabilities of 0.71, 0.75, and 0.95, respectively.

NSAIDs has direct cytotoxicity (apoptosis and necrosis).[19] Oral and intravenous administration of NSAIDs lead to the development of gastric lesions, and this may be not only associated with COX inhibition but also with the COX-independent direct cytotoxic effect of NSAIDs. Interestingly, nearly all the designed hydrazones are noncytotoxic according to the results in ►**Table 10**. Exceptions are compounds **6** and **8**, which have been predicted to be cytotoxic.

The predicted human ether-a-go-go related gene (hERG) blocker for all compounds was 0.01 to 0.38, suggesting an excellent safety profile (►**Table 11**). The predicted oral acute toxicity (OAT) of all compounds in rats or mice were in the range of 0.02 to 0.40, indicating excellent to good safety score of a dose of >500 mg/kg, except for compound **17**, celecoxib, LSD, and ASA with scores between 0.76 and 0.89, suggesting that these compounds may exhibit OAT in mice or rats with dose ≤500 mg/kg.

Dermatological toxicity of NSAIDs is well documented.[20] The FDA maximum recommended daily dose (FDAMDD) provides an estimate of the toxic dose threshold of chemicals in humans. Our data suggested that the predicted FDAMDD values for most of the compounds and the reference drug LSD were less than 0.011 mmol/kg-bw/day. However, for compounds **1**, **4**, **12**, **17**, and celecoxib, their FDAMDD equals 0.011 mmol/kg-bw/day, and for compound **3**, piroxicam, SAH, and ASA, their FDAMDD values were greater than 0.011 mmol/kg-bw/day.

All the reference NSAIDs had nonsensitive scores except for ASA with a moderate skin sensitivity score of 0.51. Our data suggested that compounds **1–5**, **7**, and SAH are sensitive to skin and may not be formulated for topical formulations. However, compounds **10**, **15–17**, ASA, and LSD may be tolerant as skin topical medicine. Compounds **6**, **8**, **9**, **11–14**, **18**, piroxicam, and celecoxib may be well tolerated by the skin.

Allergic and pseudo-allergic reactions of NSAIDs could lead to urticaria/angioedema including anaphylactic shock and asthma.[21–23] Our data showed that compounds **2–4**, **9–12**, **14**, **17**, piroxicam, and LSD are predicted to cause respiratory toxicity. Compounds **1**, **6**, **8**, **13**, **15**, **18**, ASA, and SAH are predicted as nonrespiratory toxic compounds with excellent safety profiles, whereas compounds **5**, **7**, and celecoxib are predicted to be moderately safe compounds. It worth noting that although respiratory toxicity of ASA was

Table 9 Organ toxicity continued

Compounds	Eye corrosion	Eye irritation	Prediction-accuracy (%)	Average similarity (%)
1	0.610	0.99	70.97	87.77
2	0.470	0.99	69.26	70.27
3	0.220	0.82	68.07	63.74
4	0.770	0.99	69.26	70.57
5	0.780	0.99	68.07	67.42
6	0.003	0.13	54.26	46.30
7	0.003	0.01	54.26	43.93
8	0.003	0.06	54.26	45.06
9	0.003	0.02	54.26	45.53
10	0.003	0.04	54.26	48.42
11	0.003	0.01	54.26	46.53
12	0.790	0.99	68.07	61.99
13	0.003	0.92	67.38	56.53
14	0.003	0.57	54.26	48.40
15	0.004	0.32	67.38	56.53
16	0.004	0.35	67.38	52.64
17	0.003	0.03	54.26	48.99
18	0.003	0.01	23.00	36.84
Piroxicam	0.020	0.003	54.26	47.16
Celecoxib	0.003	0.08	54.26	48.08
ASA	0.010	0.99	100.00	100.00
LSD	0.010	0.30	69.26	73.69
SAH	0.960	1.00	70.97	82.53

Abbreviations: ASA, acetylsalicylic acid; LSD, levosimendan; SAH, simple aromatic hydrazine.
Note: Empirical decision for eye corrosion/irritation: 0–0.3: excellent safe; 0.3–0.7: good safe; 0.7–1.0: poor safe.

predicted to be nontoxic with 0.27, ASA-exacerbated respiratory disease (formerly known as ASA-induced asthma or ASA-intolerant asthma) has also been reported.[23]

Toxicity Pathway

Nuclear Receptor Pathway Toxicity
The designed hydrazones were evaluated for their possible interactions with the nuclear receptors that may result in off-target toxicity. Data from ►**Table 12** showed that the designed hydrazones may not interact with most of the nuclear receptors except for the aryl hydrocarbon receptor (NR-AhR) and estrogen receptor (NR-ER). Compounds **1**–**4** and SAH may interact with NR-AhR with probabilities of 0.52–0.73. Compounds **1**, **4**, and SAH may also interact with NR-ER with probabilities of 0.52, 0.64, and 0.67, respectively. Interactions between AhR and NF-kB pathways in the lung strongly suggest the importance of this cross-talk in diseases such as lung carcinogenesis, inflammation of the lung, and asthma.[24] As such, the interaction of the stated compounds with the NR-AhR may not lead to toxicity.

Stress Response Pathway Toxicity
As shown in ►**Table 13**, all the designed hydrazones were inactive with SR-ARE, SR-ATAD5, SR-HSE, and SR-p53. Compounds **1**–**6** and **12**–**15** are predicted to interact with stress response mitochondrial membrane potential (SR-MMP) with probabilities as indicated in ►**Table 13**. The role of MMP in "mito-inflammation" has been well documented.[25] Therefore, the possible interaction of the designed compounds with MMP may not lead to toxicity.

Environmental Toxicity
The prediction results for environmental toxicity are listed in ►**Table 14**. Our data showed that bioconcentration factor (BCF) values of compounds **1**–**3** and **5** were 3.000 to 3.700 log10(L/kg), which is equivalent to 1,000 to 5,000 L/kg categorized as bioaccumulative by the United States Environmental Protection Agency (U.S. EPA) under the Toxic Substances Control Act (TSCA).[26] Other designed hydrazones and reference drugs had BCF [log10 (L/kg)] value below 3.000, which corresponds to 1,000 L/kg categorized as nonbioaccumulative by the U.S. EPA under the TSCA. Compounds **1**, **2**, and **5** have BCF [log10(L/kg)] values of 3.404, 3.429, and 3.345 respectively, which are below

Table 10 Organ toxicity continued

Compound	LD$_{50}$ (mg/kg)	Toxic class	H-HT/DILI	Carcinogenic	Immunotoxic	Mutagenic	Cytotoxic
1	1,000	4	0.03	0.58 (A)	0.92 (I)	0.58 (I)	0.81 (I)
2	1,000	4	0.05	0.50 (A)	0.64 (I)	0.57 (A)	0.68 (I)
3	1,800	4	0.27	0.58 (A)	0.96 (A)	0.58 (A)	0.72 (I)
4	1,500	4	0.13	0.67 (A)	0.76 (I)	0.85 (A)	0.72 (I)
5	800	4	0.09	0.57(A)	0.81 (I)	0.73 (A)	0.66 (I)
6	3,200	5	0.28	0.52 (I)	0.55 (A)	0.69 (A)	0.74 (I)
7	1,450	4	0.21	0.56 (I)	0.56 (I)	0.50 (I)	0.54 (A)
8	1,450	4	0.63	0.52 (A)	0.50 (I)	0.78 (A)	0.54 (A)
9	2,000	4	0.54	0.50 (I)	0.92 (A)	0.62 (A)	0.60 (I)
10	3,200	5	0.58	0.53 (I)	0.81 (I)	0.66 (A)	0.66 (I)
11	3,200	5	0.96	0.54 (I)	0.79 (A)	0.64 (A)	0.74 (I)
12	220	3	0.11	0.53 (A)	0.93 (I)	0.54 (I)	0.61 (I)
13	250	3	0.43	0.59 (A)	0.99 (I)	0.58 (I)	0.75 (I)
14	250	3	0.74	0.50 (I)	0.53 (A)	0.55 (I)	0.82 (I)
15	250	3	0.75	0.56 (A)	0.88 (I)	0.54 (I)	0.77 (I)
16	1,000	4	0.03	0.68 (A)	0.66 (I)	0.72 (A)	0.72 (I)
17	1,000	4	0.86	0.72 (A)	0.77 (I)	0.73 (I)	0.68 (I)
18	600	4	0.52	0.52 (I)	0.74 (I)	0.68 (A)	0.65 (I)
Piroxicam	480	4	0.25	0.71 (A)	0.69 (I)	0.71 (I)	0.65 (I)
Celecoxib	1,400	4	0.64	0.56 (A)	0.99 (I)	0.75 (I)	0.91 (I)
ASA	250	3	0.26	0.80 (I)	0.99 (I)	0.97 (I)	0.94 (I)
LSD	507	4	0.10	0.66 (A)	0.99 (I)	0.50 (A)	0.78 (I)
SAH	1,250	4	0.02	0.73 (A)	0.99 (I)	0.91 (A)	0.82 (I)

Abbreviations: ASA, acetylsalicylic acid; LSD, levosimendan; SAH, simple aromatic hydrazine; A, active; I, inactive.
Note: Empirical decision for H-HT/DILI: 0–0.3, excellent; 0.3–0.7, good; 0.7–1.0, poor.

the 3.700 log10(L/kg) threshold by Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) threshold for very bioaccumulative chemicals, while BCF values of other designed hydrazones and reference drugs are below the 3.300 log10(L/kg) REACH threshold for bioaccumulative chemicals.

The IGC$_{50}$ (50% inhibitory growth concentration) values of most compounds for the population growth endpoint of *Tetrahymena pyriformis* were higher than those of the reference drugs, indicating less toxicity when compared with the reference drugs.

Likewise, data from acute toxicity (96-hour LC$_{50}$ [LC$_{50}$FM]) of the compounds to the fathead minnow indicated that LC$_{50}$FM values of compounds **10** and **16** are the highest among all the designed compounds, indicating they are the safest. Compounds **2**, **5**, **7**, **10**, and **12** are less toxic compared with LSD. Compound **11** had comparable LC$_{50}$FM to LSD. Compounds **1**, **3**, **4**, **8**, **9**, and **14** are more toxic than LSD but less toxic than celecoxib. Compounds **6** and **13** have comparable LC$_{50}$FM to celecoxib. Compounds **15**, **17**, and **18** are more toxic compared with LSD and celecoxib but are safer than piroxicam and ASA.

Acute toxicity of compounds to *Daphnia magna* (LC$_{50}$DM) suggested that compounds **1–5** and **10–14** had LC$_{50}$DM values greater than 6.000 −log10[(mg/L)/(1,000 × MW)], which is comparable to that of celecoxib. This implies that these compounds are as safe as celecoxib but nonetheless safer than piroxicam, ASA, and LSD with LC$_{50}$DM values being 4.382, 2.997, and 5.408 −log10[(mg/L)/(1,000 × MW)], respectively. Compounds **6–9** and **15–17** had LC$_{50}$DM values which are safer than those of piroxicam and ASA, and are safer or comparable to LSD LC$_{50}$DM. Compound **18** had an LC$_{50}$DM value of 4.653, which is safer than that of piroxicam (4.382) and ASA (2.997) but less safe compared with celecoxib (6.252) and LSD (5.408). Taken together, all the designed hydrazones are apparently benign to the aquatic environment. A preprint of these results has been previously reported.[27]

Discussion

The COX-2 enzyme is a biological target used for discovery of wide range of anti-inflammatory drugs as it is responsible for the biosynthesis of prostaglandins which are mediators of inflammation. Hence, in this study, a docking study was

Table 11 Organ toxicity continued

Compound	hERG blocker	OAT	FDAMDD	Skin sensitivity	Respiratory toxicity
1	0.02	0.04	0.67	0.89	0.02
2	0.14	0.03	0.74	0.92	0.81
3	0.22	0.03	0.18	0.94	0.80
4	0.20	0.02	0.64	0.95	0.86
5	0.16	0.02	0.81	0.89	0.69
6	0.04	0.05	0.94	0.25	0.03
7	0.34	0.04	0.85	0.79	0.65
8	0.19	0.03	0.93	0.29	0.03
9	0.38	0.12	0.95	0.19	0.86
10	0.01	0.22	0.98	0.42	0.96
11	0.03	0.03	0.96	0.09	0.78
12	0.07	0.02	0.57	0.36	0.93
13	0.10	0.12	0.93	0.12	0.19
14	0.03	0.08	0.95	0.08	0.94
15	0.21	0.04	0.86	0.59	0.05
16	0.11	0.28	0.85	0.53	0.96
17	0.16	0.89	0.63	0.61	0.94
18	0.04	0.25	0.94	0.13	0.14
Piroxicam	0.02	0.40	0.21	0.08	0.77
Celecoxib	0.11	0.77	0.68	0.01	0.58
ASA	0.02	0.76	0.01	0.51	0.27
LSD	0.03	0.89	0.87	0.42	0.99
SAH	0.01	0.02	0.34	0.95	0.06

Abbreviations: ASA, acetylsalicylic acid; FDAMDD, FDA maximum recommended daily dose; hERG, human ether-a-go-go related gene; LSD, levosimendan; OAT, oral acute toxicity; SAH, simple aromatic hydrazine.
Note: Empirical decision: 0–0.3, excellent; 0.3–0.7, good; 0.7–1.0, poor.

accomplished to explore the possible binding conformers for the newly designed hydrazones into the COX-2 active site to predict their binding mode and explain their possible anti-inflammatory activity. It is noteworthy to know that all NSAIDs except ASA are reversible inhibitors of COX enzymes. ASA covalently modifies both COX-1 and COX-2 through acetylation of Ser530 and Ser516 respectively.[9] The hydrogen bond interaction of compounds **3**, **13**, **14**, and **16** with Ser530 and Ser516 suggests their potential anti-inflammatory activity.

5-LOX is responsible for the metabolism of arachidonic acid for the biosynthesis of leukotrienes which are potent proinflammatory mediators. Leukotrienes, the metabolites of 5-LOX enzyme are highly associated with hypersensitivity and allergic reactions including asthma, airway edema, bronchospasm, etc. Leukotrienes have been implicated in NSAID-induced cardiovascular and hypersensitivity side effects. Therefore, COX/5-LOX inhibitors are potential new drugs for the treatment of inflammation. Notably, zileuton is the only approved and marketed 5-LOX inhibitor, yet it is associated with drawbacks, for example, low potency and poor pharmacokinetic

profiles including rapid clearance and short half-life. In an *in vivo* anti-inflammatory study in mice using a carrageenan-induced paw edema model, compounds **2–5** demonstrated longer acting characteristics compared with piroxicam and celecoxib at doses of 10, 30, and 50 mg/kg.[28] Especially, compound **3** with a binding energy of −7.6 indicated superior activity compared with celecoxib after 3, 4.5, and 6 hours of inflammation induction at 10, 30, and 50 mg/kg doses, respectively.[28] Interestingly, compound **3** was endowed as an inhibitor for COX/5-LOX (►**Table 1**), and has considerable potency and good ADME predictions (►**Tables 5–8**). Compound **3** may be a potential lead compound for further *in vivo* anti-inflammatory evaluations, which is consistent with the reported study.

H^+/K^+ ATPase catalyzes the last step of gastric acid secretion. GI side effects of NSAIDs are associated with over-secretion of gastric acid due to inhibition of biosynthesis of cytoprotective prostaglandin responsible for the production of GI protective mucus. Omeprazole covalently interacts with Cys813 and Cys892. Lansoprazole reacts with Cys813 and Cys321. Also, pantoprazole and

Table 12 Nuclear receptor pathway toxicity

Compound	NR-AR	NR-AR-LBD	NR-AhR	NR-Ar	NR-ER	NR-ER-LBD	NR-PPAR-γ
1	0.91 (I)	0.98 (I)	0.73 (A)	0.55 (I)	0.52 (A)	0.77 (I)	0.96 (I)
2	0.98 (I)	0.89 (I)	0.52 (A)	0.68 (I)	0.75 (I)	0.73 (I)	0.93 (I)
3	0.85 (I)	0.99 (I)	0.60 (A)	0.75 (I)	0.55 (I)	0.86 (I)	0.97 (I)
4	0.69 (I)	0.99 (I)	0.54 (A)	0.61 (I)	0.64 (A)	0.94 (I)	0.97 (I)
5	0.99 (I)	0.97 (I)	0.59 (I)	0.69 (I)	0.71 (I)	0.78 (I)	0.93 (I)
6	0.97 (I)	0.97 (I)	0.82 (I)	0.93 (I)	0.86 (I)	0.89 (I)	0.96 (I)
7	0.93 (I)	0.98 (I)	0.61 (I)	0.86 (I)	0.89 (I)	0.91 (I)	0.95 (I)
8	0.90 (I)	0.97 (I)	0.81 (I)	0.86 (I)	0.87 (I)	0.87 (I)	0.96 (I)
9	0.94 (I)	0.96 (I)	0.85 (I)	0.87 (I)	0.86 (I)	0.83 (I)	0.96 (I)
10	0.98 (I)	0.98 (I)	0.79 (I)	0.93 (I)	0.88 (I)	0.76 (I)	0.95 (I)
11	0.97 (I)	0.98 (1)	0.79 (I)	0.89 (1)	0.88 (I)	0.82 (I)	0.95 (I)
12	0.98 (I)	0.99 (I)	0.62 (I)	0.79 (I)	0.65 (I)	0.83 (I)	0.92 (I)
13	0.99 (I)	0.98 (I)	0.97 (I)	0.97 (I)	0.94 (I)	0.97 (I)	0.98 (I)
14	0.95 (I)	0.97 (I)	0.97 (I)	0.90 (I)	0.84 (I)	0.95 (I)	0.96 (I)
15	0.97 (I)	0.99 (I)	0.89 (I)	0.95 (I)	0.90 (I)	0.95 (I)	0.97 (I)
16	0.93 (I)	0.98 (I)	0.65 (I)	0.90 (I)	0.85 (I)	0.95 (I)	0.95 (I)
17	0.93 (I)	0.99 (I)	0.64 (I)	0.90 (I)	0.86 (I)	0.96 (I)	0.96 (I)
18	0.93 (I)	0.98 (I)	0.89 (I)	0.96 (I)	0.90 (I)	0.95 (I)	0.95 (I)
Piroxicam	0.97 (I)	0.99 (I)	0.87 (I)	0.92 (I)	0.90 (I)	0.97 (I)	0.95 (I)
Celecoxib	1.00 (I)	0.99 (I)	0.99 (I)	0.93 (I)	1.00 (A)	0.98 (I)	0.98 (I)
ASA	0.99 (I)	1.00 (I)	0.99 (I)	1.00 (I)	0.98 (I)	0.99 (I)	0.99 (I)
LSD	0.98 (I)	0.98 (I)	0.66 (I)	0.93 (I)	0.88 (I)	0.99 (I)	0.98 (I)
SAH	0.99 (I)	1.00 (I)	0.66 (A)	0.84 (I)	0.67 (A)	0.96 (I)	0.91 (I)

Abbreviations: ASA, acetylsalicylic acid; LSD, levosimendan; SAH, simple aromatic hydrazine; A, active; I, inactive.

tenatoprazole covalently interact with both Cys813 and Cys822. Covalent interaction of pantoprazole and tenatoprazole with Cys822 confers a longer duration of action and irreversibility.[29] The longer half-live of the designed *p*-nitrophenyl hydrazones may confer therapeutic advantage over the PPIs. In this study, the interaction of compounds **1**, **2**, **3**, **4**, **6**, and **11** with essential amino acids Cys813 and Cys822 in the H$^+$/K$^+$ ATPase active (►**Table 2**) also indicated their potential to efficiently inhibit the proton pump enzyme. Also, these also positioned the compounds to be optimized as reversible covalent inhibitors of the proton pump enzyme.

Inspired, all the hydrazone derivatives showed excellent pharmacokinetic profile (►**Tables 5–8**) with apparent safety profiles (►**Tables 9–14**). As for the observed disparities between Caco-2 and MDCK permeability, it can be explained by the P-gp inhibition and substrate affinities of the compounds. Drugs that are P-gp substrates usually have disparities in their Caco-2 and MDCK permeability. Examples include vinblastine, a P-gp substrate having low permeability in the Caco-2 model but high permeability in the MDCK model. Prazosin is another P-gp substrate that had medium permeability in the Caco-2 model but high permeability in the MDCK model. Also, quinidine, a P-gp substrate, had high permeability in the Caco-2 model but medium permeability in the MDCK model. Although most of the compounds had high passive permeability in both models, however, their permeability disparities can be linked to the P-gp efflux activity. This is the case with compounds **3** and **17** with lower apparent *Papp* values compared with compounds **7** and **11**, which are also P-gp substrates with high affinity.

The MDCK *in vitro* permeability model is sensitive to P-gp efflux activity, while the Caco-2 *in vivo* model is not. This P-gp efflux activity effect is more pronounced for compounds with high passive permeability ($>20 \times 10^{-6}$ cm/s). The effect of P-gp activity on its substrates' permeability has been reported by Jin et al,[30] who revealed that there was a substantial increase in permeability of P-gp substrates in the MDCK model when cyclosporin A (a P-gp inhibitor) was added. However, there was no observable difference in permeability of P-gp substrates in the Caco-2 model when cyclosporin A was added. This explained why compound **17** had low Caco-2 permeability and high MDCK apparent permeability. It also explained why most of the designed hydrazones are strong inhibitors of P-gp and had high apparent *Papp* values.

High PPB contributes to longer half-life of the compounds. PPB statistics for pharmaceuticals authorized by the U.S. FDA

Table 13 Stress response pathway toxicity

Compound	SR-ARE	SR-ATAD5	SR-HSE	SR-MMP	SR-p53
1	0.76 (I)	0.60 (I)	0.76 (I)	0.69 (A)	0.77 (I)
2	0.82 (I)	0.82 (I)	0.82 (I)	0.61 (A)	0.75 (I)
3	0.90 (I)	0.68 (I)	0.90 (I)	0.64 (A)	0.88 (I)
4	0.94 (I)	0.56 (I)	0.94 (I)	0.54 (A)	0.77 (I)
5	0.84 (I)	0.88 (I)	0.84 (I)	0.73 (A)	0.78 (I)
6	0.94 (I)	0.85 (I)	0.94 (I)	0.51 (A)	0.88 (I)
7	0.93 (I)	0.80 (I)	0.93 (I)	0.60 (I)	0.78 (I)
8	0.94 (I)	0.85 (I)	0.94 (I)	0.53 (I)	0.89 (I)
9	0.90 (I)	0.85 (I)	0.90 (I)	0.56 (I)	0.90 (I)
10	0.91 (I)	0.80 (I)	0.91 (I)	0.53 (I)	0.90 (I)
11	0.93 (I)	0.83 (I)	0.93 (I)	0.50 (I)	0.91 (I)
12	0.87 (I)	0.90 (I)	0.87 (I)	0.70 (A)	0.83 (I)
13	0.97 (I)	0.99 (I)	0.97 (I)	0.63 (A)	0.96 (I)
14	0.96 (I)	0.97 (I)	0.96 (I)	0.52 (A)	0.91 (I)
15	0.97 (I)	0.96 (I)	0.97 (I)	0.59 (A)	0.95 (I)
16	0.89 (I)	0.88 (I)	0.89 (I)	0.67 (I)	0.76 (I)
17	0.91 (I)	0.88 (I)	0.91 (I)	0.61 (I)	0.72 (I)
18	0.91 (I)	0.92 (I)	0.91 (I)	0.58 (I)	0.91 (I)
Piroxicam	0.97 (I)	0.95 (I)	0.97 (I)	0.58 (I)	0.87 (I)
Celecoxib	0.99 (I)	0.98 (I)	0.99 (I)	0.87 (I)	0.95 (I)
ASA	0.99 (I)	0.99 (I)	0.99 (I)	0.97 (I)	0.98 (I)
LSD	0.98 (I)	0.98 (I)	0.98 (I)	0.88 (I)	1.00 (A)
SAH	0.89 (I)	0.61 (I)	0.89 (I)	0.51 (I)	0.94 (I)

Abbreviations: ASA, acetylsalicylic acid; LSD, levosimendan; SAH, simple aromatic hydrazine; A, active; I, inactive.

Table 14 Environmental toxicity

Compound	BCF [log10(L/kg)]	IGC$_{50}$	LC$_{50}$FM	LC$_{50}$DM
1	3.404	4.980	5.682	6.003
2	3.429	5.052	5.998	6.291
3	3.027	4.279	5.382	6.472
4	2.903	4.637	5.730	6.329
5	3.345	5.049	6.000	6.242
6	0.539	4.313	5.249	5.473
7	2.536	4.651	5.984	5.924
8	1.058	4.574	5.509	5.853
9	0.816	4.692	5.563	5.778
10	0.843	4.933	6.785	6.231
11	0.944	4.917	5.849	6.042
12	2.775	4.738	5.935	6.484
13	1.110	4.504	5.286	6.067
14	1.492	4.690	5.563	6.519
15	0.503	3.987	4.937	5.297
16	1.472	4.885	6.511	5.968
17	0.956	4.463	4.879	5.436
18	0.420	4.157	4.385	4.653
Piroxicam	0.359	3.991	3.521	4.382
Celecoxib	1.559	4.585	5.332	6.252
ASA	0.217	2.661	3.748	2.997
LSD	0.735	4.790	5.900	5.408
SAH	2.728	4.443	5.202	5.487

Abbreviation: ASA, acetylsalicylic acid; IGC$_{50}$, concentration of a substance in water in mg/L that could cause 50% growth inhibition to *Tetrahymena pyriformis* after 48 hours; LC$_{50}$FM, concentration of a substance in water in mg/L that could cause 50% of fathead minnow to die after 96 hours; LC$_{50}$DM, the concentration of the designed hydrazones in water in mg/L that could cause 50% of *Daphnia magna* to die after 48 hours; LSD, levosimendan; SAH, simple aromatic hydrazine.
Note: Unit for IGC$_{50}$, LC$_{50}$FM, and LC$_{50}$DM is $-\log10[(mg/L)/(1{,}000 \times MW)]$.

from 2003 to 2013 show that 45% of newly approved drugs had a PPB of >95%, while 24% have a PPB of >99%. Thus, compounds with a PPB greater than 99% find importance in drug design.[12] As shown in ►**Table 6**, an increase in PPB while a decrease in F$_u$ in comparison to the control drugs were observed. However, *in vivo* in reference to Smith et al,[31] the unbound concentration does not depend on PPB after oral administration. This may be due to the fact that the *in vivo* exposure of the therapeutic target to the concentration of the free drug, as measured by the AUC$_u$, the exposure or measurement of the quantity of unbound drug in the body, is independent of the F$_u$ for most orally administered drugs. The total AUC (AUC$_{total}$) bound plus unbound decreases as the F$_u$ increases owing to an increase in the clearance.

Conclusion

This docking analysis has revealed that compounds **3**, **6**, **8**, **11**, **13**, **14**, **16**, and **17** indicated promise as potent multi-target inhibitors of COX-2, 5-LOX, and H$^+$/K$^+$ ATPase with potential anti-inflammatory activity devoid of adverse effects of NSAIDs. These compounds demonstrate plausible pharmacokinetic profiles with apparent safety profiles.

Supporting Information

The docking conformation, crystal conformation, and the corresponding RMSDs for celecoxib/zileuton/omeprazole, as well as 2D and 3D illustrations of interactions of compound **3**/celecoxib/zileuton/omeprazole with COX-2, 5-LOX, and the H$^+$/K$^+$ ATPase active site are included in the Supporting Information (►**Figs. S1–S12** [online only]).

Conflict of Interest
The authors declare no conflict of interest.

References

1 Abdelgawad MA, Labib MB, Abdel-Latif M. Pyrazole-hydrazone derivatives as anti-inflammatory agents: design, synthesis, biological evaluation, COX-1,2/5-LOX inhibition and docking study. Bioorg Chem 2017;74:212–220

2 Ashley NT, Weil ZM, Nelson RJ. Inflammation: mechanisms, costs, and natural variation. Annu Rev Ecol Evol Syst 2012;43:385–406

3 Gökçe M, Utku S, Küpeli E. Synthesis and analgesic and anti-inflammatory activities 6-substituted-3(2H)-pyridazinone-2-acetyl-2-(p-substituted/nonsubstituted benzal)hydrazone derivatives. Eur J Med Chem 2009;44(09):3760–3764

4 Wang SM, Zha GF, Rakesh KP, et al. Synthesis of benzo[*d*]thiazole-hydrazone analogues: molecular docking and SAR studies of potential H+/K+ ATPase inhibitors and anti-inflammatory agents. MedChemComm 2017;8(06):1173–1189

5 Nguyen HT, Vu TY, Chandi V, Polimati H, Tatipamula VB. Dual COX and 5-LOX inhibition by clerodane diterpenes from seeds of Polyalthia longifolia (Sonn.) Thwaites. Sci Rep 2020;10(01):15965

6 Kumar N, Chauhan L. Analgesic and anti-inflammatory potential of hydrazones. J Chem Pharm Res 2014;6:916–934

7 Asif M, Husain A. Analgesic, anti-inflammatory, and antiplatelet profile of hydrazones containing synthetic molecules. J Appl Chem 2013;2013:247203

8 Hiroyuki Y, Yuichiro S, Masato F. Chronic inflammatory disease therapeutic agent. JP Patent 2012046453A. March, 2012

9 Kulkarni RG, Achaiah G, Sastry GN. Novel targets for antiinflammatory and antiarthritic agents. Curr Pharm Des 2006;12(19): 2437–2454

10 Ivanenkov YA, Zagribelnyy BA, Aladinskiy VA. Are we opening the door to a new era of medicinal chemistry or being collapsed to a chemical singularity? J Med Chem 2019;62(22):10026–10043

11 Xiong G, Wu Z, Yi J, et al. ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties. Nucleic Acids Res 2021;49(W1):W5–W14

12 Liu X, Wright M, Hop CE. Rational use of plasma protein and tissue binding data in drug design. J Med Chem 2014;57(20): 8238–8248

13 Cecchelli R, Berezowski V, Lundquist S, et al. Modelling of the blood-brain barrier in drug discovery and development. Nat Rev Drug Discov 2007;6(08):650–661

14 Stanley LA. Part VI: Basic animal anatomy and physiology. In: Badal Mccreath S, Delgoda R, eds. Pharmacognosy. 1st ed. Amsterdam: Elsevier; 2017:528–545

15 Brambilla G, Martelli A. Genotoxicity and carcinogenicity studies of analgesics, anti-inflammatory drugs and antipyretics. Pharmacol Res 2009;60(01):1–17

16 Yoshioka S, Ohno N, Miura T, Adachi Y, Yadomae T. Immunotoxicity of soluble beta-glucans induced by indomethacin treatment. FEMS Immunol Med Microbiol 1998;21(03):171–179

17 Takahashi H, Ohno N, Adachi Y, Yadomae T. Association of immunological disorders in lethal side effect of NSAIDs on beta-glucan-administered mice. FEMS Immunol Med Microbiol 2001;31(01):1–14

18 Kuboyama N, Fujii A. Mutagenicity of analgesics, their derivatives, and anti-inflammatory drugs with S-9 mix of several animal species. J Nihon Univ Sch Dent 1992;34(03):183–195

19 Tomisato W, Tsutsumi S, Rokutan K, Tsuchiya T, Mizushima T. NSAIDs induce both necrosis and apoptosis in guinea pig gastric mucosal cells in primary culture. Am J Physiol Gastrointest Liver Physiol 2001;281(04):G1092–G1100

20 Reuter H, Tarr Gs. Review of the safety of nonsteroidal anti-inflammatory drugs and selective cyclo-oxygenase-2 inhibitors. S Afr Fam Pract 2019;57:18–22

21 Cryer B, Dubois A. The advent of highly selective inhibitors of cyclooxygenase–a review. Prostaglandins Other Lipid Mediat 1998;56(5–6):341–361

22 Stevenson DD, Sanchez-Borges M, Szczeklik A. Classification of allergic and pseudoallergic reactions to drugs that inhibit cyclooxygenase enzymes. Ann Allergy Asthma Immunol 2001;87(03):177–180

23 Namazy JA, Simon RA. Sensitivity to nonsteroidal anti-inflammatory drugs. Ann Allergy Asthma Immunol 2002;89(06):542–550, quiz 550, 605

24 Tian Y, Ouyang N, Thomas PE. Regulation of xenobiotic sensor PXR and AhR by NF-B and its roles in xenobiotic detoxification and inflammation-associated carcinogenesis. In: McQueen CA, ed. Comprehensive Toxicology. 2nd ed. Amsterdam: Elsevier Science; 2010:579–585

25 Patergnani S, Vitto VAM, Pinton P, Rimessi A. Mitochondrial stress responses and "mito-inflammation" in cystic fibrosis. Front Pharmacol 2020;11:581114

26 Environmental Protection Agency. Persistent bioaccumulative toxic (PBT) chemicals; lowering of reporting thresholds for certain PBT chemicals; addition of certain PBT chemicals; community right-to-know toxic chemical reporting. Fed Regist 1999;64 (209):58666–58753

27 Babalola S, Igie N, Odeyemi I. Structure-based discovery of multitarget directed anti-inflammatory p-nitrophenyl hydrazones; molecular docking, drug-likeness, in-silico pharmacokinetics, and toxicity studies. ChemRxiv. Cambridge: Cambridge Open Engage; 2022

28 Babalola S, Igie N, Odeyemi I, Idris AY, Sanni YM, Hamza AN. In-vivo anti-inflammatory activity studies of some *p*-nitrophenyl hydrazones. Discovery (Read) 2022;58(319):698–706

29 Shin JM, Munson K, Vagin O, Sachs G. The gastric HK-ATPase: structure, function, and inhibition. Pflugers Arch 2009;457(03):609–622

30 Jin X, Luong TL, Reese N, et al. Comparison of MDCK-MDR1 and Caco-2 cell based permeability assays for anti-malarial drug screening and drug investigations. J Pharmacol Toxicol Methods 2014;70(02):188–194

31 Smith DA, Di L, Kerns EH. The effect of plasma protein binding on in vivo efficacy: misconceptions in drug discovery. Nat Rev Drug Discov 2010;9(12):929–939