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Synthesis and Cyclooxygenase-2 Inhibitory activity Evaluation of Some Pyridazine Derivatives

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ABSTRACT

This work aimed to discover safe and effective pyridazine-based cyclooxygenase-2 (COX-2) inhibitors. Thirty-three pyridazine-based compounds (compounds 1 to 33) were designed. The *in silico* studies were conducted to predict their toxicity, docking scores (DS), pharmacokinetic parameters, and drug-likeliness properties compared to celecoxib. Based on the safety and efficacy data obtained by *in silico* studies, four compounds (7, 12, 16 and 24) were synthesized, and the spectral analysis confirmed their chemical structures. Additionally, the *in vitro* COX-2 inhibitory activity of these four compounds was evaluated. Eleven compounds were predicted as non-toxic compounds. The DS of four compounds, 7 (DS = -9.72 kcal/mol), 12 (DS = -10.48 kcal/mol), 16 (DS = -9.71 kcal/mol), and 24 (DS = -9.46 kcal/mol), was better than celecoxib (DS = -9.15). These compounds (7, 12, 16, and 24) also demonstrated better oral absorption (83.53% each) than celecoxib (79.20%) in addition to their promising drug-likeliness properties. The compounds 7 (101.23%; $p<0.05$), 12 (109.56%; $p<0.05$), 16 (108.25%; $p<0.05$), and 24 (103.90%; $p<0.05$) also exhibited superior COX-2 inhibition to celecoxib (100%; $p<0.05$). Compounds 7, 12, 16 and 24 are useful lead compounds in developing drugs for various diseases in which high levels of COX-2 are implicated.

Keywords: Pyridazine, Benzothiazole, *In silico* studies, Synthesis, COX-2 inhibition.

INTRODUCTION

Cyclooxygenase-2 (COX-2), an inducible enzyme, is produced during the inflammation of the body cells[1]. The untreated inflammation may progress to many diseases, including disability-causing diseases (different types of arthritis and Alzheimer's disease), cancer, pancreatitis, hepatitis, atherosclerosis, CNS diseases (epilepsy and depression), asthma, irritable bowel disease, and kidney injury[2]. The commonly used non-steroidal anti-inflammatory drugs (NSAID) block COX-1 and COX-2 enzymes. COX-1, a constitutive cell enzyme as opposed to COX-2, supports the maintenance of

kidney functions, platelet aggregation, and gastric mucosa activities. The adverse effects of NSAIDs (gastric ulcers and kidney malfunctions) are mostly caused by COX-1 inhibition[1-3]. As a result, the COX-2 enzyme inhibitors (celecoxib and rofecoxib) were created[4]. However, celecoxib and rofecoxib have recently demonstrated cardiac toxicity effects[5]. These effects make it imperative to develop better COX-2 inhibitors.

Pyridazine-based compounds demonstrate diverse biological activities[6-12], including COX-2 inhibitory activity[1,2]. Emorfazone (Pentoil) Fig.1 is a clinically used pyridazine-based anti-inflammatory agent and is claimed to lack ulcerogenic effects associated with traditional NSAIDs[13].

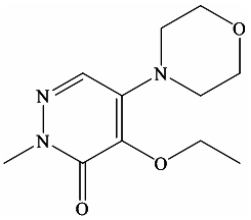


Fig. 1. Chemical structure of emorfazone

The Zomipirac-based pyridazine derivatives have also displayed potent COX-2 inhibition[2]. The potential for multiple structural modifications in the pyridazine ring and literature confirming the COX-2 inhibitory activity of the pyridazine nucleus makes the pyridazine-based compounds a suitable framework for creating non-ulcerogenic COX-2 inhibitors[14]. Accordingly, this study was planned to discover safe and effective COX-2 inhibitors.

EXPERIMENTAL

General

Sigma Aldrich (USA) provided the analytical-grade chemicals utilized in this study. The Gallenkamp melting point apparatus, Shimadzu 440 spectrophotometer (for generating FTIR data), Varian Gemini 125/500 MHz spectrophotometer (for recording ^{13}C-NMR and ^{1}H-NMR data, respectively), and a 70 eV GCMS/QP 1000 Ex mass spectrophotometer (for obtaining the mass spectra) were used to obtain the spectral data of the synthesized compounds.

Design of the compounds

Thirty-three compounds were designed using ChemDraw (version 21) software Fig. 2. The reaction between the intermediates disclosed in the United States Patent Number US4052395A and the commercially available 2-hydrazineylbenzo[d] thiazole served as the basis for designing these compounds[15].

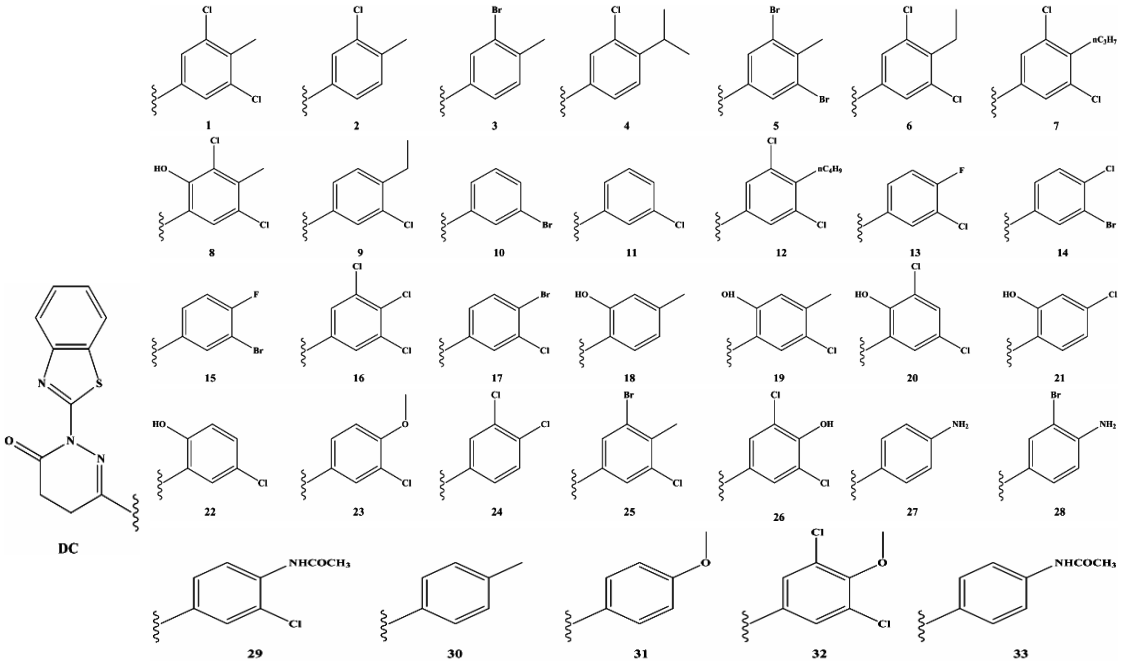


Fig. 2. Chemical structures of the designed compounds (DC)

Toxicity prediction

All thirty-three DCs (compounds 1 to 33) were assessed for their toxicity properties employing the ProTox-II web server[16]. The Mol-Files of the DC were created with the ChemDraw software. These Mol-Files were opened with the notepad, and the contents were copied and pasted into the ProTox-II web server. The start button was pressed to obtain the toxicity data of the compounds Table 1.

Molecular docking

The Molecular Operating Environment software (MOE) (2019.0102 version, Chemical Computing Group Inc., Canada) was utilized for this study. The COX-2 protein (PDB ID: 5-KIR) was utilized for this purpose, employing celecoxib as a standard[1,2,13]. The 5-KIR protein was uploaded into the software, and the Quickprep button was pressed to obtain the purified and ready-to-use 5-KIR protein for docking. The MDB files of compounds (7, 8, 12, 14, 16, 17, 19, 20, 21, 22, 24, and celecoxib) were created. The docking was started utilizing ready-to-use 5-KIR proteins and the MDB files of the compounds, and the docking scores (DS in kcal/mol) and root mean square deviation (RMSD) of each docked compound were noted Table 2.

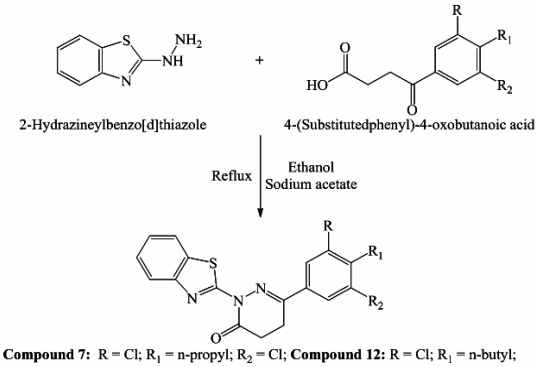
Prediction of the pharmacokinetic parameters

The pharmacokinetic parameters were predicted by Swiss-ADME software[16,17]. The Mole-Files of the compounds were inserted in the software with the import button, the run button was pressed, and the data was recorded Table 2. The % absorption of the compounds was also calculated utilizing the following formula 3.

%Absorption = 109-(0.345xTPSA)

Synthesis of compounds 7,12,16 and 24

An equimolar mixture of 4-(3,5-dichloro-4-propylphenyl)-4-oxobutanoic acid (0.01 mole) and 2-hydrazineylbenzo[d]thiazole (0.01 mole) was refluxed in ethanol (50 mL) in the presence of a catalytic amount of sodium acetate for two hours. A precipitate was obtained, which was filtered and recrystallized from ethanol to obtain compound 7. This method was also used to synthesize compounds 12, 16 and 24 by replacing 4-(3,5-dichloro-4-propylphenyl)-4-oxobutanoic acid with 4-(4-butyl-3,5-dichlorophenyl)-4-oxobutanoic acid, 4-oxo-4-(3,4,5-trichlorophenyl) butanoic acid, and 4-(3,4-dichlorophenyl)-4-oxobutanoic acid, respectively Scheme 1. Table 3 provides the physical and spectral information for compounds 7, 12, 16 and 24.



Compound 7: R = Cl; R_1 = n-propyl; R_2 = Cl; **Compound 12:** R = Cl; R_1 = n-butyl; R_2 = Cl; **Compound 16:** R = R_1 = R_3 = Cl; **Compound 24:** R = R_1 = Cl; R_2 = H

Scheme 1. Synthesis of compounds 7, 12, 16, and 24

***In vitro* COX-2 inhibitory activity**

The COX-1/COX-2 inhibitory activity of the compounds 7, 12, 16, 24, celecoxib, and indomethacin was carried out by the 10-fold dilution method (1-10^{-}4 µg/mL) employing Cayman's human COX-1/COX-2 kit (560131, Ann Arbor, MI, USA)[1-3]. The reagent preparation and experiment execution followed the supplier's instructions. The previous publication also provides a brief procedure of this experiment and calculations of the IC_{50} values for COX-1/COX-2 inhibition by regression analysis[1-3]. The selectivity index (SI=IC_{50} for COX-1/IC_{50} for COX-2) was also calculated with the obtained data Table 4.

Statistical analysis

The experimental data were statistically analyzed using the SPSS software (version 20, Chicago, IL, USA). Results are statistically significant if p<0.05 (N=3; Mean±SD) is achieved.

RESULTS

The toxicity study of the thirty-three DC was assessed with the ProTox II software[16]. The results revealed that nineteen compounds were hepatotoxic, six showed carcinogenic properties, and three demonstrated both hepatotoxic and carcinogenic behavior. Overall, twenty-two DCs displayed either hepatotoxic or carcinogenic properties. Eleven DCs were found to be non-toxic Table 1.

Table 1: Predicted toxicity data of the non-toxic DC

Compound	Oral LD_{50} (mg/kg)	Oral toxicity class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
Celecoxib	1400	4	No	Yes	No	No	No
7	1000	4	No	No	No	No	No
8	1000	4	No	No	No	No	No
12	1000	4	No	No	No	No	No
14	1000	4	No	No	No	No	No
16	1000	4	No	No	No	No	No
17	1000	4	No	No	No	No	No
19	1000	4	No	No	No	No	No
20	1000	4	No	No	No	No	No
21	1000	4	No	No	No	No	No
22	1000	4	No	No	No	No	No
24	1000	4	No	No	No	No	No

The eleven compounds were selected for their docking and Swiss-ADME analyses Table 2[1,2,13,17]. The molecular docking study of eleven compounds for COX-2 protein (PDB ID: 5-KIR) revealed that compounds 7 (DS = -9.72 kcal/mol), 12 (DS = -10.48 kcal/mol), 16 (DS = -9.71 kcal/mol), and 24 (DS = -9.46 kcal/mol) demonstrated better DS than celecoxib (DS = -9.15). Other compounds exhibited low docking scores concerning celecoxib. The RMSD values of the compounds were <1.5, representing good binding of the compounds with the target site[16]. Compounds 7, 12, 16 and 24 had better absorption (83.53% each) than celecoxib (79.20%). The compounds 14 and 17 also had better absorption than celecoxib but a lower docking score than celecoxib. Other compounds had less absorption and DS than celecoxib. None of the compounds was permeant to the blood-brain barrier, except compound 24. None of the compounds was an inhibitor of CYP2D6 or a substrate for P-gp. Compounds 19, 20, 21 and 22 tended to inhibit CYP3A4. All the compounds qualified the Lipinski rule of drug-likeliness.

Based on the predicted toxicity, molecular docking, and the Swiss-ADME data, compounds 7, 12, 16 and 24 were selected for their synthesis Scheme 1. The compounds' spectral data corresponded to the assigned structures of 7, 12, 16, and 24 Table 3.

Compounds 7, 12, 16, and 24 were evaluated for their COX-1 and COX-2 inhibition against indomethacin (non-specific COX inhibitor) and celecoxib (COX-2 inhibitor) (Table 4)[1-3]. The results of Table 4 indicate that compounds 7, 12, 16, and 24 were more effective COX-2 inhibitors than celecoxib but less effective COX-1 inhibitors than indomethacin Figure 3.

Table 2: The DS, pharmacokinetic parameters, and drug-likeness properties of the compounds

					Pharmacokinetics						
Compounds	DS (kcal/mol)	RMSD (Å)	LogP (o/w)	TPSA ($Å^2$)	GI absorption	BBB permeant	P-gp substrate	CYP2D6 inhibitor	CYP3A4 inhibitor	Drug-likeness (Lipinski rule)	Calculated% absorption
Celecoxib	-9.15	0.88	3.40	86.36	High	No	No	No	No	Yes	79.20
7	-9.72	1.19	5.36	73.80	High	No	No	No	No	Yes	83.53
8	-7.62	1.44	4.32	94.03	High	No	No	No	No	Yes	76.55
12	-10.48	1.19	5.70	73.80	High	No	No	No	No	Yes	83.53
14	-7.10	0.59	4.46	73.80	High	No	No	No	No	Yes	83.53
16	-9.71	1.07	4.87	73.80	High	No	No	No	No	Yes	83.53
17	-7.96	0.98	4.46	73.80	High	No	No	No	No	Yes	83.53
19	-7.77	1.12	3.82	94.03	High	No	No	No	Yes	Yes	76.55
20	-7.26	0.82	3.98	94.03	High	No	No	No	Yes	Yes	76.55
21	-6.65	1.26	3.52	94.03	High	No	No	No	Yes	Yes	76.55
22	-7.24	0.77	3.46	94.03	High	No	No	No	Yes	Yes	76.55
24	-9.46	1.06	4.38	73.80	High	Yes	No	No	No	Yes	83.53

TPSA = Topological polar surface area

Table 3: Characterization data of compounds 7, 12, 16, and 24

Compound (MF; MW; M.P.; R_f values*; FTIR in KBr, ν in cm^{-1})	^{1}H-NMR(DMSO-d_6, 500 MHz, δ in ppm)	^{13}C-NMR (DMSO-d_6, 125 MHz, δ in ppm)	Mass (m/z)
7($C_{20}H_{17}Cl_2N_3OS$;418; 188-190°C; 0.73; 1660 (C=O), 1570 (C=N), 1522 (C=C) and 1113 (C–S))	0.95 (t, 3H, -CH_3), 1.62 (m, 2H, -CH_2-CH_3), 2.42 (t, 2H, methylene of C4-pyridazine), 2.62 (t, 2H, x pyridazine), 2.62 (t, 2H, -CH_2-CH_2-CH_3), 2.90 (t, 2H, methylene of C5-pyridazine), 7.50-7.52 (dd, 2H, Ar-H), 7.75 (s, 2H, Ar-H), 8.14-8.17 (dd, 2H, Ar-H)	11.6 (-CH_3), 21.0 (-CH_2-CH_3), 22.3 (C4, pyridazine), 25.6 (-CH_2-CH_2-CH_3), 30.3 (C5, pyridazine), 117.2, 120.7, 123.4, 124.2, 126.3 (2C), 129.7, 133.0, 133.8 (2C), 140.8, 145.4, 152.1, 167.0 (C=O, pyridazine), 173.4 (C2, benzothiazole)	418 (M^+, 100%), 419 (M^++1) 420 (M^++2), 284, 231, 188, 135
12($C_{21}H_{19}Cl_2N_3OS$; 432; 176-178°C; 0.71; 1661 (C=O), 1571 (C=N), 1521 (C=C), 1111 (C–S))	0.88 (t, 3H, -CH_3), 1.31 (m, 2H, -CH_2-CH_3), 1.51 (m, 2H, -CH_2 -CH_2-CH_3), 2.42 (t, 2H, methylene of C4-pyridazine), 2.61 (t, 2H, -CH_2 -CH_2-CH_2-CH_3), 2.90 (t, 2H, methylene of C5-pyridazine), 7.50-7.53 (dd, 2H, Ar-H), 7.76 (s, 2H, Ar-H), 8.12-8.17 (dd, 2H, Ar-H)	12.1 (-CH_3), 20.3 (-CH_2-CH_3), 22.4 (C5, pyridazine), 23.2 (-CH_2-CH_2-CH_2-CH_3), 28.3 (-CH_2-CH_2-CH_2-CH_3), 31.4 (C4, pyridazine), 111.2, 120.7, 123.4, 124.2, 126.0 (2C), 129.8, 133.0, 133.9 (2C), 141.4, 145.4, 152.1, 167.0 (C=O, pyridazine), 173.4 (C2, benzothiazole)	432 (M^+, 100%), 433 (M^++1), 434 431 (M^++2), 284, 231, 202, 135
16($C_{17}H_{10}Cl_3N_3OS$; 410; 192-194°C; 0.74; 1665 (C=O), 1573 (C=N), 1525 (C=C), 1115 (C–S))	2.42 (t, 2H, methylene of C4-pyridazine), 2.91 (t, 2H, methylene of C5-pyridazine), 7.72 (s, 2H, Ar-H), 7.50-7.53 (dd, 2H, , Ar-H) 8.12-8.16 (dd, 2H, Ar-H)	22.3 (C5, pyridazine), 30.3 (C4, pyridazine), 117.2, 120.7, 123.4, 124.2, 127.6 (2C), 129.7, 133.8, 134.5, 141.4 (2C), 145.4, 152.1, 167.0 (C=O, pyridazine), 173.4 (C2, benzothiazole)	410 (M^+, 100%), 411 (M^++1), 412 (M^++1), 284, 231, 179, 13524
24($C_{17}H_{11}Cl_2N_3OS$; 376; 181-183oC; 0.78; 1663 (C=O), 1572 (C=N), 1522 (C=C), 1114 (C–S))	2.41 (t, 2H, methylene of C4-pyridazine), 2.90 (t, 2H, methylene of C5-pyridazine), 7.50-7.52 (dd, 2H, Ar-H), 7.67 (d, 1H, , Ar-H) 7.84-7.87 (dd, 2H, Ar-H), 8.12-8.16 (dd, 2H, Ar-H)	22.3 (C5, pyridazine), 30.3 (C4, pyridazine), 117.2, 120.7, 123.4, 124.2, 125.2, 129.2 (2C), 129.7, 132.4 (2C), 134.6, 145.4, 152.1, 167.0 (C=O, pyridazine), 173.4 (C2, benzothiazole)	376 (M^+, 100%), 377 (M^++1) 378 (M^++2), 284, 231, 145, 135

MF: Molecular formula; MW: Molecular weight; M.P.: Melting point; *R_f values in a benzene and acetone mixture (8:2)

Table 4: *In vitro* COX-1/COX-2 inhibitory activity of 7, 12, 16, and 24

Compounds	COX-1 (IC_{50}, nM*)	%COX-1 inhibition	COX-2 (IC_{50}, nM*)	%COX-2 inhibition	SI	%SI
7	360.5 ± 0.40	59.36	17.88 ± 0.18	101.23	20.16	109.62
12	365.30 ± 0.28	58.58	16.52 ± 0.25	109.56	22.11	120.22
16	380.10 ± 0.15	56.30	16.72 ± 0.38	108.25	22.73	123.59
24	376.35 ± 0.40	56.86	17.42 ± 0.50	103.90	21.60	117.45
Celecoxib	333.0 ± 0.22	64.26	18.10 ± 0.51	100	18.39	100
Indomethacin	214.0 ± 0.14	100	69.10 ± 0,32	26.19	3.09	16.80

*p<0.05; SI=Selectivity index

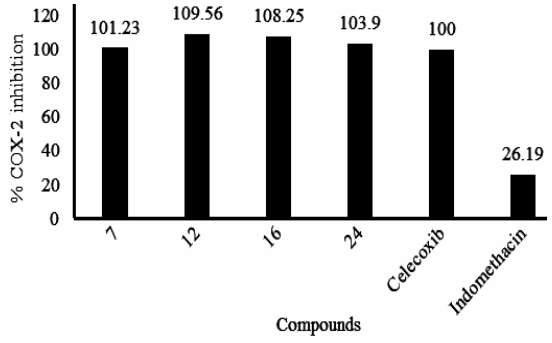


Fig. 3. COX-2 inhibitory activity of 7, 12, 16, 24, celecoxib, and indomethacin

DISCUSSION

This study focused on discovering effective and safe pyridazine-based COX-2 inhibitors for treating disorders linked to elevated COX-2 levels. We designed thirty-three pyridazine-based compounds and performed various experiments (*in silico* studies, molecular docking, synthesis, and COX-2 inhibition activity). The *in silico* toxicity data of the DC revealed similar LD_{50} (1000 mg/kg) and toxicity class (class 4) Table 1. This is due to their structural similarities[18]. It is also imperative to note that celecoxib displayed carcinogenic behavior,

but eleven compounds were non-toxic Table 1. The eleven non-toxic compounds were subjected to their molecular docking study. In the molecular docking study, the larger negative value for the DS and an RMSD<1.5 specifies a compound's greater affinity and good binding of the compounds with COX-2, respectively[1,2]. The docking study revealed that compounds 7 (DS = -9.72 kcal/mol), 12 (DS = -10.48 kcal/mol), 16 (DS = -9.71 kcal/mol), and 24 (DS = -9.46 kcal/mol) were more potent than celecoxib (DS = -9.15 kcal/mol) in inhibiting the COX-2 enzyme Table 2. Accordingly, compounds 7, 12, 16, and 24 were synthesized, and their chemical structures were confirmed by spectral analysis Table 3.

The *in vitro* COX-2 inhibitory activity of compounds 7 ($R = R_2 = Cl$; R_1 = n-propyl; COX-2 inhibition = 101.23%), 12 ($R = R_2 = Cl$; R_1 = n-butyl; COX-2 inhibition = 109.56%), 16 ($R = R_1 = R_2 = Cl$; COX-2 inhibition = 108.25%), and 24 ($R = R_1 = Cl$; $R_2 = H$; COX-2 inhibition = 103.90%) was better than celecoxib (COX-2 inhibition = 100%) Table 4. This phenomenon aligned with the molecular docking data Table 2. It is known that the *n*-butyl group (compound 12) is more lipophilic than the n-propyl group (compound 7), and trichloro substituents (compound 16) provide higher lipophilicity to compounds than dichloro substituents (compound 24)[19,20,21]. The benzothiazole ring is a lipophilic ring, which may also contribute to the lipophilic character of DC[22]. This understanding is also evident from the Log P data (an indicator of the compound's lipophilicity) that compounds 7, 12, 16, and 24 have greater lipophilicity than celecoxib Table 1. This effect reveals that higher lipophilicity is required for DC's potent COX-2 inhibitory activity. This understanding also aligns with previous studies suggesting that lipophilic compounds are better COX-2 inhibitors[23].

The data of Table 2 also reflects that compounds 7, 12, 16, and 24 were not an inhibitor of CYP2D6/CYP3A4 or a substrate for P-gp, implying that these compounds may not pose metabolism-related drug interactions with other medicines[24]. These compounds also qualified Lipinski's rule of drug-likeliness, suggesting the possibility of their development as a drug molecule[1,13,17]. The compounds 7, 12, 16, and 24 inhibit the COX-1 enzyme to a lesser extent than celecoxib and indomethacin Table 4, signifying their better safety profile concerning the ulcerogenic effects associated with traditional NSAIDs[1,2]. Compounds 7, 12, 16, and 24 may be useful as lead compounds in developing drugs for various diseases (including some disability-causing diseases) in which high levels of COX-2 are implicated. Except for compound 24, none of the compounds could pass through the blood-brain barrier Table 2. This fact implies compound 24 and its derivative may be more useful in developing anti-inflammatory agents for certain CNS diseases like epilepsy, Alzheimer's disease, and depression Figure 4.

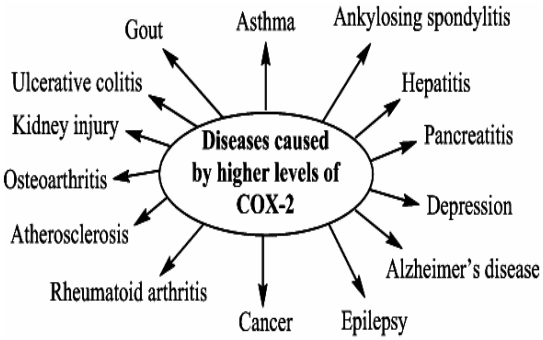


Fig. 4. Diseases instigated by elevated COX-2 levels 2

Many pyridazine-based pharmacodynamic agents have been developed to treat various diseases like hypertension (indolidan and bemoradan), congestive heart failure (levosimendan and pimobendane), depression (minaprine), PDE3-associated illness (imazodan and zardaverine) and cancer (Olaparib)[2,7]. Accordingly, compounds 7, 12, 16, and 24 may further be assessed to check their efficacy against these diseases. The authors believe additional research is necessary to confirm these theories about compounds 7, 12, 16, and 24. Further, the chemical structures of our designed compounds can be altered easily and serve as a new template for developing safe, effective, and potent COX-2 inhibitors.

CONCLUSION

Four compounds (7, 12, 16, and 24) displayed in silico study-based non-toxic properties, appreciable pharmacokinetic parameters, and drug-likeliness assets. These compounds were also more effective than celecoxib at blocking COX-2 activity. The chemical structures of the DC can be altered easily and serve as a new template for developing safe, effective, and potent COX-2 inhibitors. Accordingly, compounds 7, 12, 16 and 24 and their derivatives may be useful in developing drugs against diseases demonstrating high levels

of COX-2 enzyme. However, more research is advised to confirm these potential implications for our molecules.

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Conflict of interest

No conflict of interest is associated with this work.

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