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New benzimidazothiazole derivatives as anti-inflammatory, antitumor active agents: Synthesis, in-vitro and in-vivo screening and molecular modeling studies

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ABSTRACT

A new series of benzimidazothiazole derivatives has been synthesized. The structure of the products was confirmed by spectroscopic techniques such as IR, NMR and mass spectroscopy. The tested compounds were evaluated for their anti-inflammatory activity either *in vitro* through the COX enzyme inhibition assay, or *in vivo* through carrageenan paw edema technique. Results revealed that compound **25** and **29** represented the most active ones among the entire series with % inhibition 72.19, 72.07 for COX-1, and 87.46, 87.38 for COX-2, respectively. Interestingly, all synthesized compounds exhibited IC_{50} values less than both reference drugs celecoxib and naproxen, indicating their superior potency. For compound 25, it showed about 340 and 198 times more potent than celecoxib and naproxen respectively as COX-1 inhibitor (IC_{50} value 0.044 vs. 15.000 and 8.700 μM), and 10 and 115 times more potent than the same drugs as COX-2 inhibitor (IC_{50} value 4.52 vs. 40.00 and 520.00 nM). The antitumor activity of the products was also evaluated and the results obtained are consistent with those obtained by the anti-inflammatory screening where compounds **25** and **29** proved to be the most active ones among the other compounds with %GI ranging from 31.5 to 62.5% and they exhibited the lowest IC_{50} values as well. The ADMET analysis of the tested compounds was also performed in addition to the molecular modeling studies that included flexible alignment, surface and electrostatic maps in addition to the Lipinisk's rule of five.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) refer to those agents that are used to cease pain and inflammation through their ability to inhibit the cyclooxygenase enzyme. Numerous advances have been taken in the development of new and optimum agents with high potency and decreased major side effects that appear by the prolonged use of such drugs. The action of such drugs is simplified through the inhibition of cyclooxygenase enzyme which is responsible for induction of pain and inflammation [1]. Three COX isoforms have been identified: COX-1, COX-2, and COX-3. COX-1 is important for the production of prostaglandins involved in normal cellular activity including protection of gastric mucosa, whereas COX-2 is considered an inducible isozyme producing prostaglandins at inflammatory sites. Therefore, another approach to eliminate NSAID-associated gastric side effects is to use selective COX-2 inhibitors. Different chemical classes were developed during these agents design and development. Naproxen belongs to propionic acid derivatives that are used as non-selective NSAIDs to control pain, chronic inflammation, joint swelling and arthritis symptoms [2–5]. Despite efficacy as anti-inflammatory agents, the induction of GIT ulceration is the common challenge in further development of new agents. Furthermore, the development of distress, hemorrhage and iron-deficiency anemia was found to be due to the presence of free carboxylic group in these agents [6,7]. It was concluded that the induction of ulceration and GIT distress developed as a result of two major pathways; firstly the direct action of the COOH group that leads to increase of the gastric acidity and secondly the indirect action through the inhibition of prostaglandin formation whose main role is the protection of the gastric mucosa against increased acidity [7]. Blocking and masking of this group was adopted and applied in

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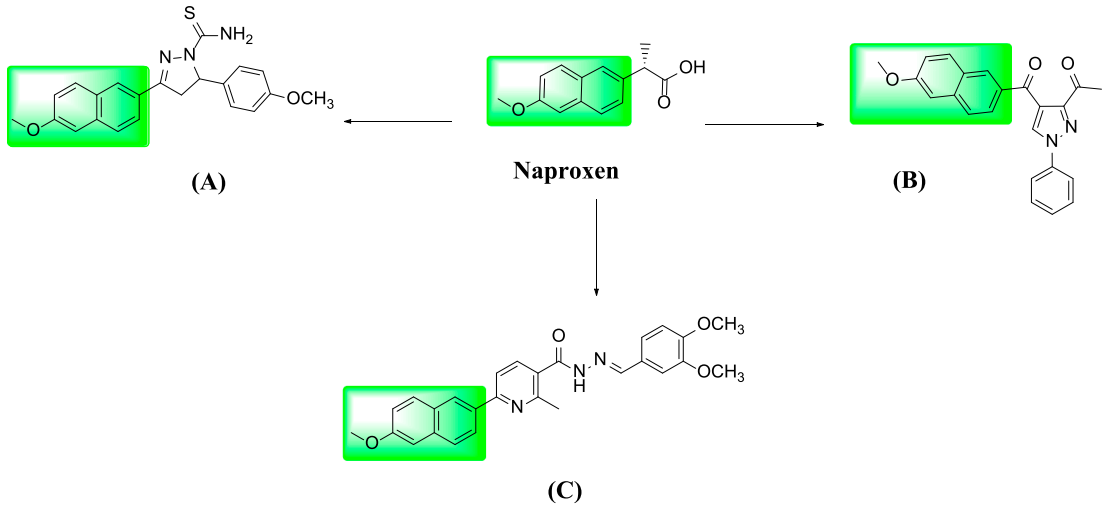


Fig. 1. Naproxen and active derivatives from the literature.

previous research (general structures A-C) and resulted in the production of very active and potent agent when compared to naproxen [8] (Fig. 1).

Tilomisole (WY-18,251) is an experimental drug, belonging to the benzimidazothiazole derivatives and characterized by having antiinflammatory activity [9,10]. It still has the free carboxylic group that was proved to be responsible for the major side effects as previously mentioned. The major aim of the present research is to design and develop new compounds containing the benzimidazothiazole system but devoid from the free carboxylic group, while encompassing other moieties instead to promote the action of the synthesized compounds as potential anti-inflammatory agents. For example, the pyridine moiety that is present in etoricoxib – a recent selective COX-2 inhibitor- has been incorporated with the above mentioned, with the aim to improve the selectivity of the synthesized compounds towards COX-2 enzyme (Fig. 2).

On the other hand, literature revealed that the administration of NSAIDs especially selective COX-2 inhibitors is associated with low risk of cancer development, particularly colorectal, adenomatous polyps and to some extend other types of cancer. This was explained by the ability of such drugs to restore normal apoptosis in human adenomatous colorectal polyps and in other cancer cells that have lost the adenomatous polyposis coli gene function [11].

From the above mentioned data, series of benzimidazothiazole derivatives have been synthesized, their structures were confirmed by different spectroscopic techniques (^{1}H NMR, ^{13}C NMR, IR, Mass spectroscopy). The target compounds were evaluated for their anti-inflammatory either *in vitro* through COX-1/COX-2 inhibition assay or *in vivo* through carrageenan paw-edema technique. In addition, they were evaluated as antitumor agents against selected cell lines namely Hepatocellular carcinoma (HEPG-2), Mammary gland breast cancer (MCF-7), and Colorectal carcinoma (HCT-116). The molecular modeling studies were performed through calculation of Lipiniski' rule of five, ADMET analysis, flexible alignment and electrostatic maps of the most active compounds obtained.

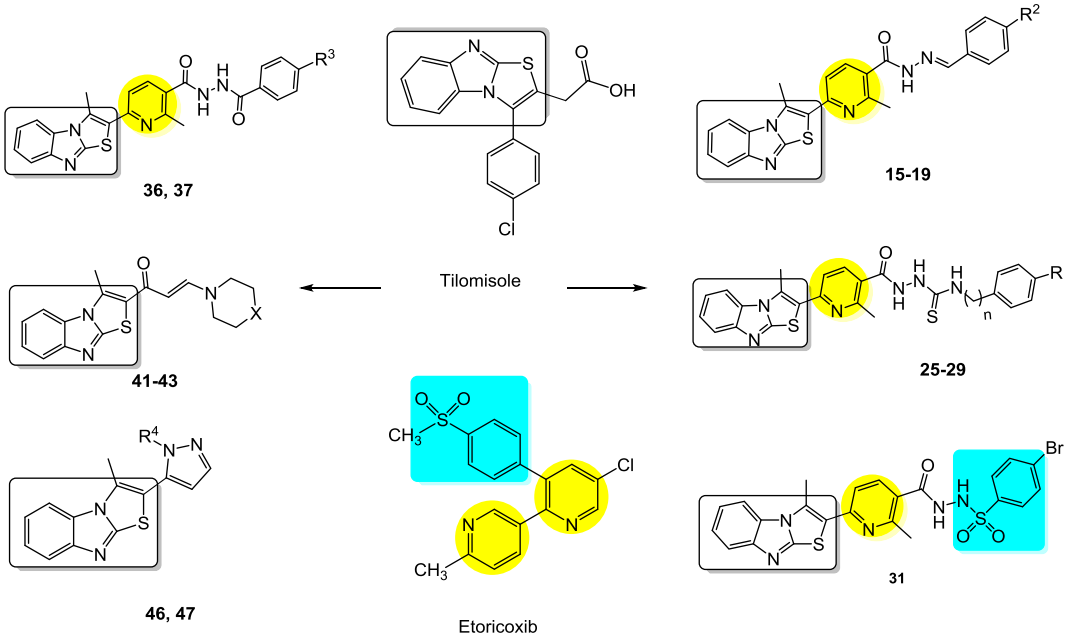
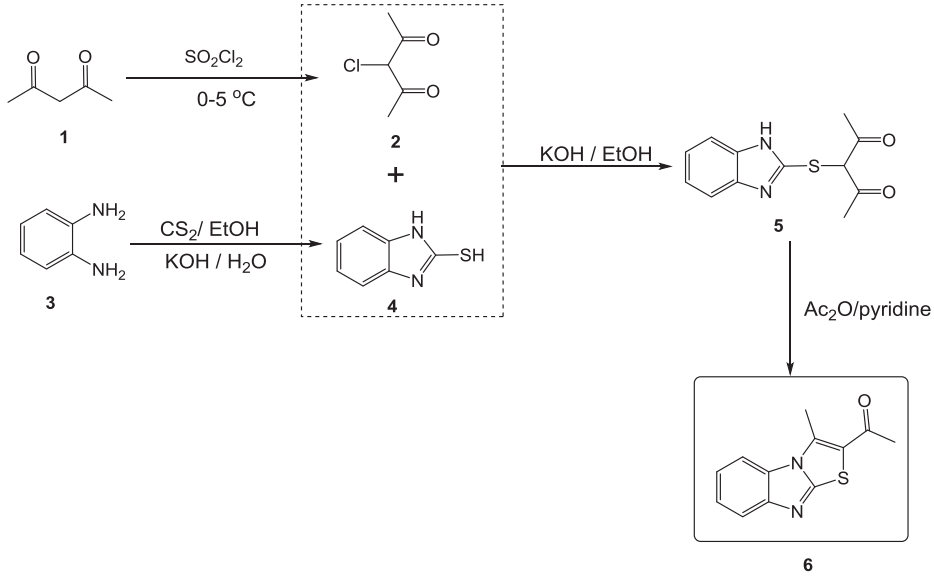


Fig. 2. Correlation between Tilomisole, Etoricoxib and the target compounds.



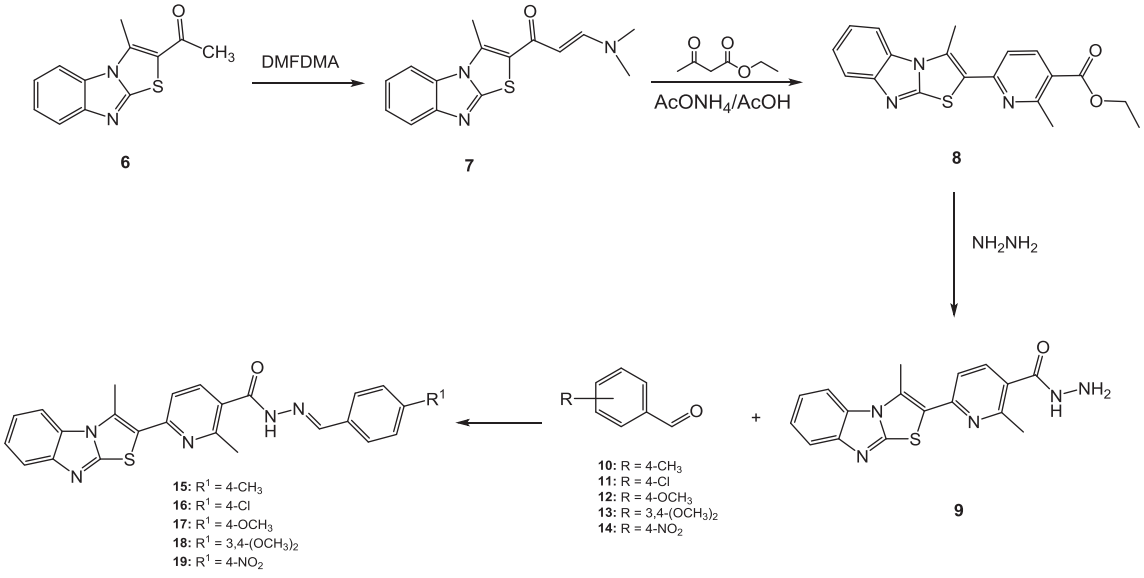
Scheme 1. Synthesis of compound 6.

2. Results and discussion

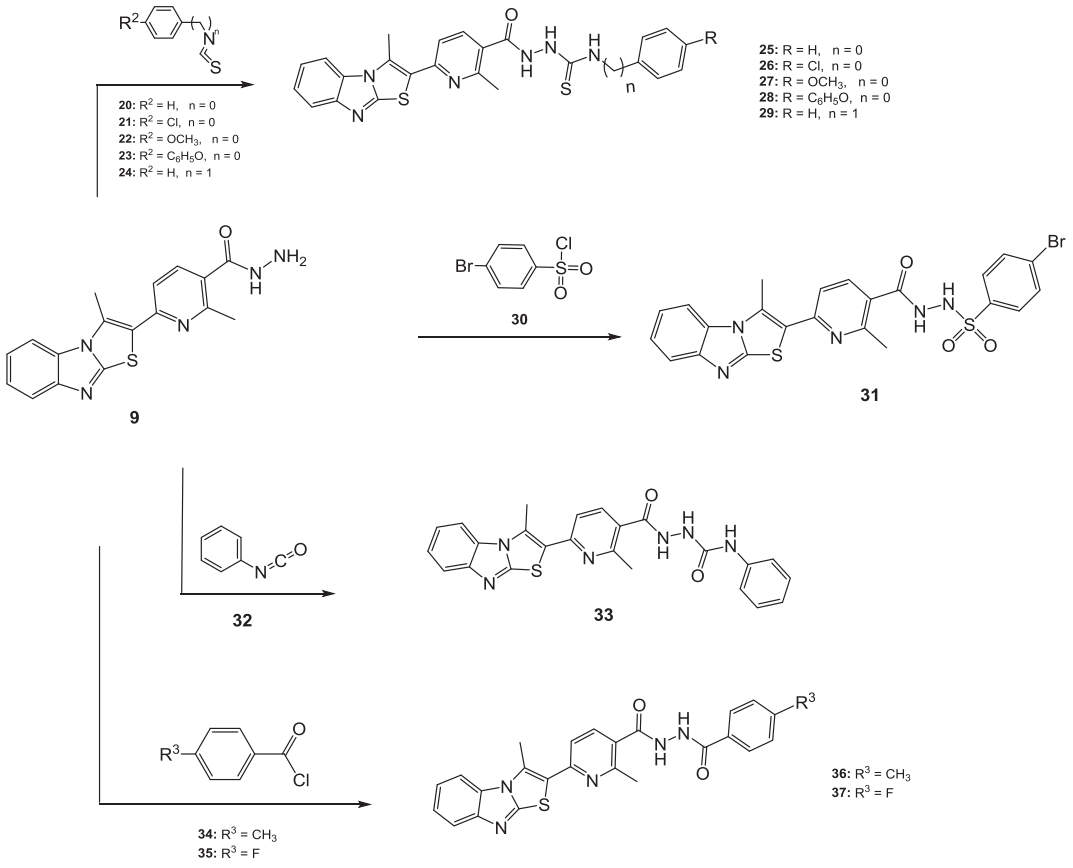
2.1. Chemistry

The synthetic strategy of the target compounds is illustrated in Schemes 1–4. The synthesis of the starting agent 1-(3-methylbenzo[4,5] imidazo[2,1-*b*]thiazol-2-yl)ethan-1-one **(6)** is illustrated in Scheme 1 where 3-chloropentane-2,4-dione **2** reacted with 1*H*-benzo[*d*]imidazole-2-thiol **4** in alcoholic solution of potassium hydroxide to produce 3-((1*H*-benzo[*d*]imidazol-2-yl)thio)pentane-2,4-dione **5.** The latter compound underwent cyclization in acetic anhydride containing catalytic amount of pyridine to produce that starting agent **6.** Scheme 2 showed the synthesis of the target compounds **15–19** through the interaction of **6** with dimethylformamide dimethylacetal (DMFDMA) to produce the enaminone **7** which reacted with ethyl acetoacetate in presence of acetic acid/ammonium acetate mixture to produce ethyl 2-methyl-6-(3-methylbenzo[4,5]imidazo[2,1-b]thiazol-2-yl)nicotinate **8.** The hydrazide form of compound **8** is formed through its reaction with excess hydrazine hydrate to obtain the key compound **9.** Upon reaction of compound **9** with substituted aldehydes **10–14**, schiff's bases **15–19** were produced. The spectroscopic data obtained confirmed the production of these compounds, for example the ^{1}H NMR spectra of compound **15** illustrated the appearance of the CH_3 protons of the corresponding toluladehyde that appeared as a singlet at δ = 1.92, in addition to the singlet peaks corresponding to the methoxy groups of compounds **17** and **18** that appeared at δ 3.82, 3.74 ppm respectively.

Scheme 3 showed the synthesis of groups of derivatives obtained by the interaction of the key compound **9** with a variety of agents. Compounds **25–29** were obtained through the interaction of **9** with substituted phenyl isothiocyanate **20–24** in ethanol. The structures of these compounds were confirmed by the spectral NMR data that showed the appearance of new peaks such as the methoxy group of compound **27** at δ 3.77 ppm and the appearance of benzylic-H of compound **29** at δ 4.78 ppm. The suphonamide derivative **31** was obtained through the interaction of compound **9** with 4-bromobenzenesulfonyl chloride **30** in pyridine. Similaily, the urea derivative **33** is obtained by the reaction of **9** with phenyl isocyanate **32** under the same reaction conditions. Both structures were confirmed by spectroscopic data of ^{1}H NMR, ^{13}C NMR



Scheme 2. Synthesis of compounds 15–19.



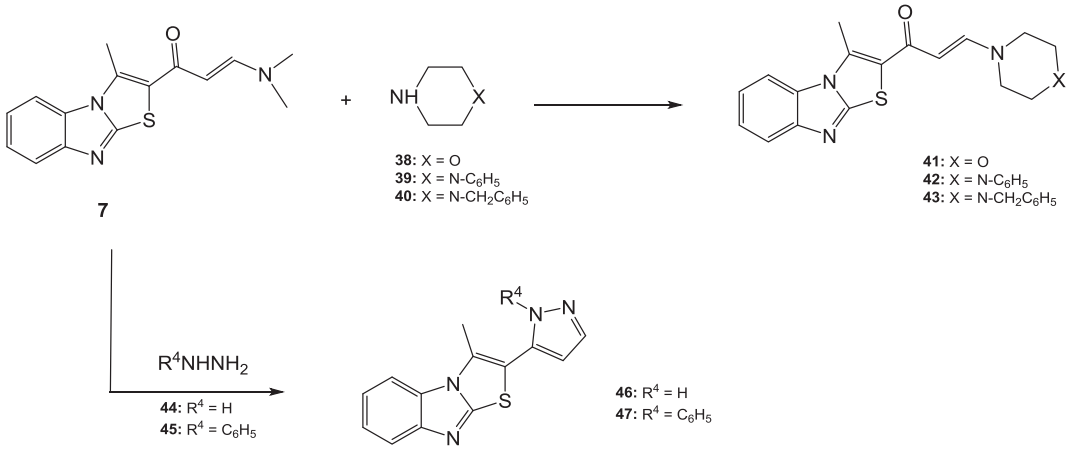
Scheme 3. Synthesis of compounds 25–29, 31, 33, 36, and 37.

and mass spectroscopy. On the other hand, compounds **36** and **37** were prepared through the reaction of **9** with either 4-tolyl chloride **34** or 4-fluorobenzoyl chloride **35** respectively. The appearance of the peak of CH_3 protons at δ 3.14 ppm confirmed the structure of the produced compound **36**. Finally, the synthesis of compounds **41–43**, **46** and **47** is illustrated in Scheme 4. The enaminone **7** reacted with secondary amine **38–40** to produce compounds **41–43**, or reacted with hydrazines **44** and **45** to produce compounds **46** and **47.** The appearance of the doublet peaks in ^{1}H NMR of compounds **41–43** in the aromatic region confirmed the synthesis of such compounds; in addition the appearance of two aromatic doublet peaks corresponding to 2 protons indicated the formation of compounds **46** and **47.**

2.2. Biological screening

2.2.1. In vivo anti-inflammatory activity

The anti-inflammatory activity was performed to determine the percentage inhibition (I%) of edema for each tested compound 4 h after the induction of inflammation using *in vivo* rat carrageenan-induced foot paw edema model reported previously [12–14]. The reference drugs celecoxib and naproxen were used as positive controls in the study; however, the significant decreasing in the activity of all



Scheme 4. Synthesis of compounds 41–43, 46, and 47.

Table 1
The percentage inhibition of COX-1 and COX-2 of the target compounds.

Comp.	% inhibition (I%)	%I COX-1	%I COX-2	IC_{50} (μM) (COX-1)	IC_{50} (nM) (COX-2)	Selectivity Index (SI) (COX-2/COX-1)
15	74.3	69.21	87.02	0.0201	99.50	4.95
16	59.8	68.97	85.35	0.1540	19.62	0.127
17	64.9	62.06	80.51	0.1910	67.28	0.35
18	75.3	63.64	81.66	0.0301	138.3	4.59
19	72.8	65.21	79.41	0.0155	94.32	6.09
25	79.5	72.19	87.46	0.0440	4.520	0.10
26	67.8	63.91	81.85	0.2410	63.59	0.26
27	62.4	66.78	83.86	0.2830	126.5	0.45
28	78.1	69.94	85.99	0.1670	13.36	0.08
29	75.9	72.07	87.38	0.0750	16.02	0.21
31	78.7	64.07	81.96	0.2130	45.83	0.22
33	49.3	47.88	69.30	1.4470	178.14	0.40
36	51.2	55.12	75.25	0.4760	349.00	0.73
37	70.9	64.03	81.93	0.2880	105.8	0.37
41	72.4	72.43	83.42	0.0237	56.50	2.38
42	69.2	58.00	77.46	0.3877	124.34	0.32
43	65.3	62.64	80.93	0.2409	80.40	0.33
46	70.0	71.68	87.13	0.0997	33.20	0.33
47	73.4	70.16	86.14	0.0113	35.20	3.11
Celecoxib	89.0 ± 1.6	2.20	61.40	15.000	40.00	0.002
Naproxen		94.90	71.50	8.700	520.0	0.06

compounds was noticed after 6 h (Table 1). According to the provided data, the thiourea derivatives **25–29** exhibited the highest values of I% of edema produced with values ranging from 62.4 to 79.5%, in which compound **25** had the highest value among this series with 79.5% inhibition of edema. Schiff's bases represented the series with lower values with range 59.8–75.3% inhibition. On the other hand, compounds **33** and **36** exhibited the least active compounds among all series with I % values of 49.3 and 51.2% respectively.

2.2.2. In vitro cyclooxygenase inhibitory activity

Percentage of inhibition (I%) of COX-1 and COX-2 of tested compounds at a concentration of 5.0 μM were illustrated in Table 1. The obtained data revealed that the tested compounds exhibited % inhibition against COX-2 enzyme more than COX-1 with selectivity index (COX-2/COX-1) ranging from 1.21 to 1.45. It was also noticed that compound **25** exhibited the highest % inhibition values against both COX-1 and COX-2 among the entire tested compounds and this result is consistent with the *in vivo* experiments in which this compound exerted the highest % edema inhibition value. Compounds **33** and **36** that had the lower % inhibition in the *in vivo* experiments, also proved to be the least active compounds with %I for COX-1 and COX-2 values of 47.88, 69.30 and 55.12, 75.25 respectively.

2.2.3. Determination of IC_{50} of the target compounds

The IC_{50} (for both COX-1 in μM and COX-2 in nM) of the synthesized compounds was determined using Cayman colorimetric COX (ovine) inhibitor screening assay kit and the calculations were performed as per the kit guidelines. It was noticed that in case of COX-1, all the tested compounds had IC_{50} less than both reference drugs celecoxib and naproxen indicating their potency (Table 1). For compound **25** (the most active among the entire compounds), it exhibited IC_{50} value about 340 times more potent than celecoxib and 198 times more potent than naproxen as COX-1 inhibitor. Furthermore, IC_{50} obtained against COX-2 is calculated in nM and many compounds had very low IC_{50} value compared with the reference drug such as **25** (10 times more potent than celecoxib and 115 times more potent than naproxen), in addition to compounds **28, 29** and **16** with IC_{50} values of 13.36, 16.02 and 19.62 nM respectively. Although compounds **33** and **36** were the least active among all series but they also exerted IC_{50} values against COX-1 and COX-2 less than those obtained from the reference drugs [12–14].

The LD_{50} of the tested compounds was evaluated by the use of different routes of administration in both mice and rat models (Table 2). The products were administered in different concentrations using the intraperitoneal (IP), Oral, intravenous (IV), and subcutaneous (SC) routes in mice, while they were administered using the IP and oral routes only in rats. It was revealed that the tested compounds exhibited high values of LD_{50} when the compounds were administered IP or orally in mice indicating higher safety of these derivatives over the reference drug naproxen, while most of them exhibited very low values using the IV, SC in mice and IP, and oral in rats (Table 2).

2.3. Molecular modeling study

The molecular modeling study was conducted using "Molecular Operating Environment" (MOE) system version 2009.11. The lowest energy conformers for the most active **25** and the least active **36** were calculated and obtained (Fig. 3). For more understanding the difference in the biological activity, surface mapping calculations were conducted using the mentioned program [15–18]. A surface map was constructed for compound **25,** the most active compound among the products against the least active one **36**, which pointed to the extra hydrogen bonding and the distribution of the hydrophobic region in compound **25** that could be responsible for the increased activity of this compound over compound **36** (Fig. 4).

2.3.1. Flexible alignment

Ligand-based active site alignment is a major adopted technique for the structural analysis of protein–ligand complexes, where a good alignment requires small strain energy of each molecule with a similar shape and overlapping of aromatic rings [16]. We have studied the similarity between the 3D structures of the most active compound **25** and **naproxen** using flexible alignment employing MOE/MMFF94 flexible alignment by automatized superposition of the compounds under investigation. A top scored alignment along with the least strain energy is shown in Fig. 5, where there is a good alignment between compound **25** and **naproxen** explaining and emphasizing its activity which is compatible with experimental data.

Additionally, the electrostatic maps have been obtained for both compounds **25** and **36**, the red regions represented the hydrogen bond acceptors and hydrophilic regions while the white regions represented the lipophilic regions (Fig. 6).

2.3.2. Docking

Docking study has been started from the selection of the X-ray

Table 2
LD_{50} (Lethal dose that kills 50% of the population) of the tested compounds in mice and rat.

Comp. No.	Mouse								Rat			
	I.P.[a]		Oral		I.V.[b]		S.C.[c]		I.P.		Oral	
	mg/kg	R[d]	mg/kg	R	mg/kg	R	mg/kg	R	mg/kg	R	mg/kg	R
15	540	0.46	560	0.62	80	0.66	82	0.47	46	0.26	32	0.25
16	580	0.47	710	0.81	83	0.66	83	0.47	69	0.23	29	0.23
17	570	0.45	560	0.62	84	0.64	82	0.47	48	0.26	34	0.25
18	570	0.43	560	0.60	81	0.64	77	0.44	67	0.21	35	0.22
19	560	0.49	660	0.73	85	0.62	98	0.46	52	0.29	28	0.20
25	660	0.54	530	0.77	80	0.55	400	0.45	100	0.13	110	0.08
26	850	0.36	570	0.77	130	0.49	480	0.45	330	0.14	140	0.08
27	770	0.53	560	0.78	88	0.55	540	0.43	85	0.15	130	0.08
28	720	0.51	560	0.78	64	0.56	410	0.43	58	0.07	56	0.29
29	620	0.49	480	0.80	85	0.48	360	0.44	79	0.08	96	0.08
31	1300	0.36	1200	0.63	220	0.46	910	0.20	76	0.21	110	0.24
33	810	0.41	610	0.73	83	0.51	590	0.42	200	0.12	120	0.15
36	830	0.39	550	0.77	110	0.44	250	0.26	160	0.11	57	0.15
37	1100	0.37	470	0.74	130	0.42	340	0.25	120	0.09	38	0.15
41	260	0.46	1100	0.80	62	0.71	150	0.51	320	0.20	160	0.35
42	200	0.37	1200	0.71	32	0.60	160	0.50	280	0.26	140	0.31
43	200	0.43	870	0.75	45	0.66	100	0.48	100	0.16	62	0.27
46	660	0.38	570	0.78	100	0.51	190	0.37	180	0.30	74	0.27
47	830	0.52	630	0.75	55	0.61	200	0.58	93	0.30	81	0.32
Naproxen	350	0.63	590	0.81	330	0.72	580	0.88	250	0.68	560	0.78

[a]Intraperitoneal Route, [b]Intravenous Route, [c]Subcutaneous Route, [d]Reability

structure. The X-ray structures of COX-2 were taken from PDB database www.rcsb.org (PDB code 3N8Z) [19]. In order to confirm the adequacy of the docking procedure, docking of co-crystallized ligands was carried out into COX-2 complex (Fig. 7). From the mentioned figure, it was observed the interaction of the most active compound **25** with the active site of the enzyme, in which the interaction occurs through two hydrogen bonds with Tyr 130, in addition to the hydrophobic interaction of the pyridine ring with Arg 466 and Glu 44.

2.4. Lipinski rule of five

The parameters of Lipinski's rule of five of all the synthesized compounds in addition to the reference drug naproxen are illustrated in Table 3.. It is mainly used to evaluate and determine the biological activity and the bioavailability of the tested compounds. It describes the different pharmacokinetic parameters of the compounds under investigation. Taking a closer look to the data provided, it was observed that most of the compounds except compounds **19** and **25–29** exhibited total polar surface area (TPSA) less than 140 $Å^2$ indicating the good oral bioavailability of such compounds, compounds **41–43**, **46** and **47** exhibited TPSA values close to that of the reference drug naproxen. Furthermore, concerning the cLog P that refers to the calculated lipophilicity of the compounds, it was also noticed that the target compounds exerted Log P values very close and nearly equal to that of the reference agent.

2.5. ADMET study

The pharmacokinetic properties of the synthesized compounds were calculated theoretically by the aid of online application PreADME https://preadmet.bmdrc.kr. The results obtained revealed that all the tested compounds exerted excellent absorption through the intestine with HIA values exceeding 93.41% (Table 4). It was also noticed that these compounds exhibited good BBB penetration even when compared

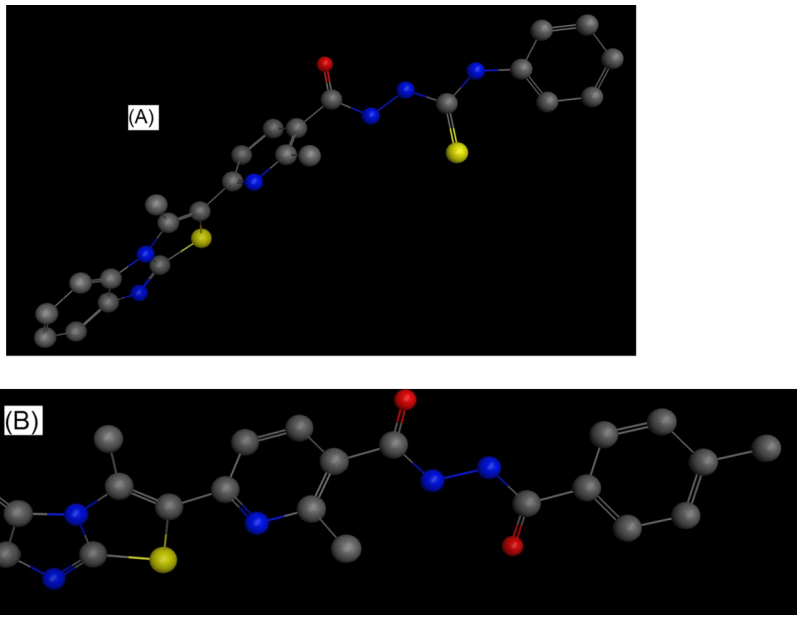


Fig. 3. Lowest energy conformers of most active compounds 25 (A) and least inactive compounds 36 (B) with balls and cylinders rendering.

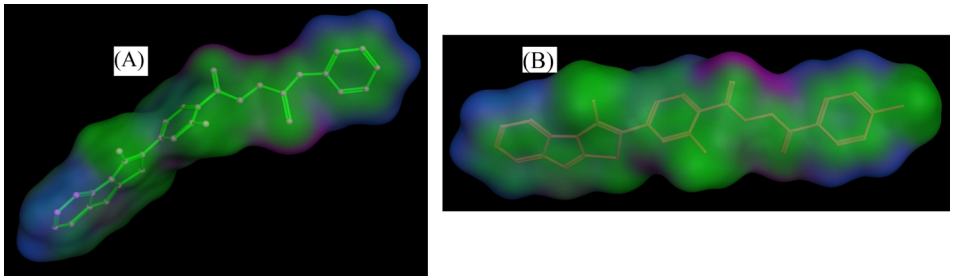


Fig. 4. Surface map for the most active compound 25 (A); the least active compound 36 (B). Pink: hydrogen bond, blue: mild polar, green: hydrophobic region.

with the reference drug naproxen. They also exerted good plasma protein binding affinity similar to naproxen. The only derivative that has CYP inhibitory effect is compound **15** otherwise all the other compounds cause no inhibition to such metabolizing enzymes. It was also noticed that most of the compounds had negative carcinogenic activity on both mice and rats, except for compounds **29** and **37** that had positive results. All the tested compounds including the reference drug had mutagenic tendency (Table 4).

2.6. In-vitro anticancer screening

Most of the synthesized products were subjected to *in vitro* anti-tumor screening using the MTT assay method [20,21]. Three types of cancer cells were used; Hepatocellular carcinoma (HEPG-2), Mammary gland breast cancer (MCF-7) and Colorectal carcinoma (HCT-116). The cell lines were obtained from ATCC *via* Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. Doxorubicin was used as a standard anticancer drug for comparison. Average GI% was calculated using 10 μM concentrations, cytotoxicity (IC_{50}, μM) that caused loss of 50% inhibition was evaluated and they are shown in Table 5. Most of the tested compounds exhibited moderate GI%, compounds **25, 27** and **29** represented the most active products among the tested compounds, they revealed GI% value against the three cell lines ranging from 31.5 to 62.4%, they also exhibited the lowest IC_{50} values 33.49, 23.05, 20.36 respectively. On the other hand, compounds **36** and **37** represented the least active compounds with GI% ranging 10.3–29.6, and the highest IC_{50} values of 97.88 and 69.20 respectively. The data obtained from the antitumor screening is consistent with those of the anti-inflammatory in which compound **25** is the most active one among the entire compounds and compound **36** represented the least active one (Table 5).

3. Structure activity relationship

By taking a closer look to the *in-vitro* and *in vivo* anti-inflammatory screening of the compounds under investigations, it was revealed that the thiourea derivatives **25** and **29** represented the most active compounds among the tested ones with IC_{50} values of 4.52 and 16.02 nM respectively, these values were equal to 1/10 and 1/2 values of that of celcoxib, while their isocyanate analogues **33** and **36** represented the least active ones with IC_{50} values of 178.14 and 349.00 nM. On the other hand, the antitumor screening of the compounds represented similar data, in which compounds **25** and **29** are the most potent and compounds **36** and **37** are the least active among the products under investigation. The mentioned data indicated the importance of the thiourea moiety for activity contrast for the isocyanate ones.

4. Conclusion

Benzimidazothiazole derivatives have been designed and synthesized; they were evaluated for their anti-inflammatory and antitumor activities. Compounds **25** and **29** proved to be the most active agents among the entire derivatives. They had the highest % inhibition of edema, highest % inhibition of both COX enzymes, the highest GI% against the tested tumor cells and finally the lowest IC_{50}. Naproxen and celecoxib were used as standard drugs in the anti-inflammatory screening, while doxorubicin was used as positive control for the antitumor screening. ADMET analysis of the synthesized compounds was evaluated, molecular modeling studies including flexible alignment, electrostatic and surface maps were obtained for the active compounds.

5. Experimental section

The synthesis of the designed compounds was performed in Faculty of Pharmacy, Mansoura University, Mansoura, Egypt. The antitumor screening was performed in the department of pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt. The anti-inflammatory screening and enzyme assay was conducted in Department of Pharmacology and Toxicology, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt. Molecular modeling experiments were performed using 'Molecular Operating Environment' software on Core i7 workstation. Melting points (°C) were determined on Mettler FP80 melting point apparatus and are uncorrected. Microanalyses were performed on a Perkin-Elmer 240 elemental analyzer. All of the new compounds were analyzed for C, H and N and agreed with the proposed structures within ± 0.4% of the theoretical values. ^{1}H, ^{13}C NMR were recorded on a Joel 500 MHz FT spectrometer and Brucker 400 MHz spectrometer; chemical shifts are expressed in δ ppm with reference to

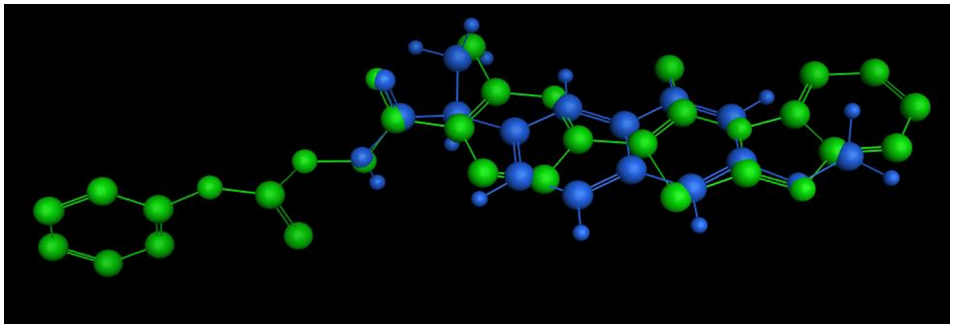


Fig. 5. Flexible alignment of the most active compound 25 (green) and naproxen (blue).

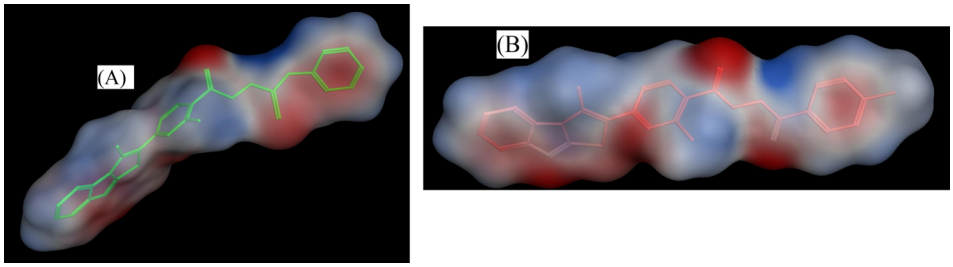


Fig. 6. The electrostatic map of the most active compound 25 (A), and the least active one 36 (B).

TMS. Mass spectral (MS) data were obtained on a Perkin Elmer, Clarus 600 GC/MS and Joel JMS-AX 500 mass spectrometers. Thin layer chromatography was performed on pre-coated (0.25 mm) silica gel GF_{254} plates (E. Merck, Germany), compounds were detected with 254 nm UV lamp. Silica gel (60–230 mesh) was employed for routine column chromatography separations. All the fine chemicals and reagents used were purchased from Aldrich Chemicals Co, USA. Compounds **6**, **7** and **41** were previously reported [22,23]

5.1. Chemistry

5.1.1. *Ethyl 3-methyl-6-(3-methylbenzo[4,5]imidazo[2,1-b]thiazol-2-yl)picolinate (8)*

A mixture of compound **7** (0.57 g, 0.002 mol), ethyl acetoacetate (0.325 g, 0.002 mol), ammonium acetate (1.23 g, 0.016 mol), in glacial acetic acid (30 ml) was refluxed for 6 hrs. The reaction was then poured onto ice-water. The precipitate formed was filtered, washed with water, and crystallized from acetic acid to obtain the required product. Yield %: 69.3, mp: 185–90 °C, $C_{19}H_{17}N_3O_2S$, **^{1}H NMR (500 MHz, DMSo-d_6);** δ 1.34 (t, 3H, J = 14.5 Hz, CH_2CH_3), 2.91 (s, 3H, CH_3), 3.13 (s, 3H, CH_3), 4.34 (q, 2H, J = 21.5 Hz, CH_2CH_3), 7.29 (t, 1H, J = 15.5 Hz, Ar-H), 7.38 (t, 1H, J = 14.5 Hz, Ar-H), 7.70 (d, 1H, J = 8.0 Hz, Ar-H), 7.81 (d, 1H, J = 8.5 Hz, Ar-H), 8.09 (d, 1H, J = 8.0 Hz, Ar-H), 8.29 (d, 1H, J = 8.5 Hz, Ar-H). **^{13}C NMR** δ 14.0, 14.1, 24.5, 61.2, 112.3, 118.5, 118.6, 121.1, 121.3, 123.3, 123.8, 130.4, 131.2, 139.5, 147.7, 152.0, 154.3, 164.0, 165.5.

5.1.2. *3-Methyl-6-(3-methylbenzo[4,5]imidazo[2,1-b]thiazol-2-yl) picolinohydrazide (9)*

A mixture of compound **8** (1 gm) and excess hydrazine hydrate (10 ml) was refluxed for 8 hrs, the reaction mixture was poured onto ice-water, and the precipitate formed was filtered, washed with iced water, crystallized form aqueous ethanol to obtain compound **9**. Yield %: 89.4, mp: 258–62 °C, $C_{17}H_{15}N_5OS$, **^{1}H NMR (400 MHz, DMSo-d_6);** δ 2.59 (s, 3H, CH_3), 3.10 (s, 3H, CH_3), 4.57 (s, 2H, NH_2), 7.30 (t, 1H, J = 14.0 Hz, Ar-H), 7.38 (t, 1H, J = 14.0 Hz, Ar-H), 7.72 (t, 2H, J = 12.0 Hz, Ar-H), 7.83 (d, 1H, J = 8.5 Hz, Ar-H), 8.09 (d, 1H, J = 8.0 Hz, Ar-H), 9.68 (s, 1H, NH). **^{13}C NMR** δ 14.3, 23.2, 112.6, 118.8, 119.0, 121.4, 122.0, 124.0, 129.5, 130.2, 130.8, 137.0, 148.2, 150.5, 154.8, 156.5, 167.2. **MS** *m/z* (%): 337 (15.4, M^+).

Table 3
Calculated parameters of Lipinski's rule of five for the synthesized compounds.

Comp. no	Parameters					
	cLog P[a]	TPSA[b]	MW[c]	*n*HBA[d]	*n*HBD[e]	*n*RB[f]
15	5.263	97.47	439.53	6	1	4
16	5.608	97.47	459.95	6	1	4
17	4.964	106.7	455.53	7	1	5
18	4.972	115.93	485.56	8	1	6
19	3.358	146.3	470.50	9	1	5
25	3.358	141.26	472.59	7	3	6
26	5.467	141.26	507.03	7	3	6
27	4.831	150.49	502.61	8	3	7
28	6.615	150.49	564.68	8	3	8
29	4.767	141.26	486.61	7	3	7
31	4.577	139.66	556.46	8	2	5
33	4.658	126.24	456.52	8	3	4
36	4.574	114.21	455.53	7	2	4
37	4.405	114.21	459.50	7	2	4
41	2.746	72.66	327.40	5	0	3
42	4.236	66.67	402.51	5	0	4
43	4.498	66.67	416.54	5	0	5
46	3.211	71.8	254.31	4	1	1
47	4.673	60.42	330.41	4	0	2
Naproxen	5.263	46.53	230.26	3	1	3

[a] Calculated Lipophillicity.
[b] Total Polar Surface Area.
[c] Molecular Weight.
[d] Number of Hydrogen Bond Acceptor.
[e] Number of Hydrogen Bond Donor.
[f] Number of Rotatable Bond.

5.1.3. *(E)-N'-Substituted benzylidene-3-methyl-6-(3-methylbenzo [4,5]imidazo[2,1-b]thiazol-2-yl)picolinohydrazides (15–19)*

Ethanolic solution (40 ml) of compound **9** (1.2 g, 0.0035 mol), the appropriate benzaldehyde derivatives **10–14** and catalytic amount of

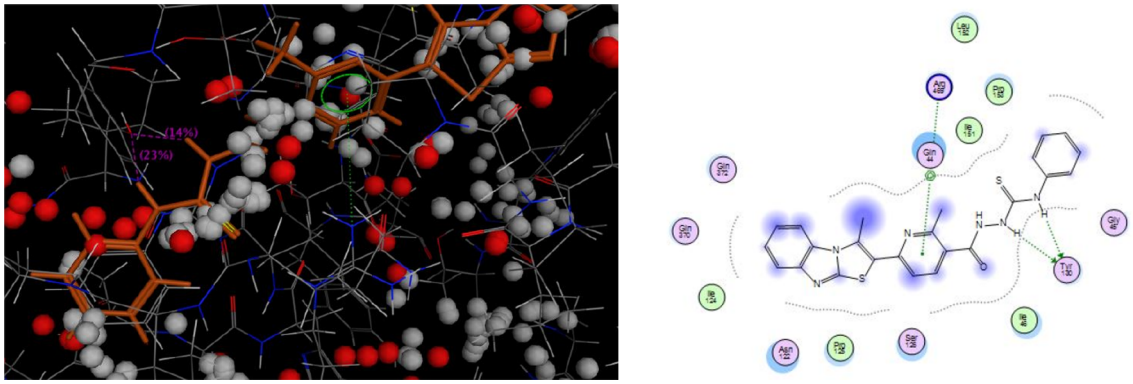


Fig. 7. Compound 25 (the most active) in binding site of COX-2.

Table 4
ADMET profile for the target products compounds and the control drug naproxen.

Comp.	HIA	PPB	BBB	MDCK	CYP Inhibition	AMES Toxicity	Carcinogenecity	
							Mouse	Rat
15	100.0	ND	1.499	204.40	Inhibitor	Mutagen	Negative	Negative
16	97.26	96.26	0.022	0.0465	Non-Inhibitor	Mutagen	Negative	Negative
17	96.85	97.28	0.029	0.0487	Non-Inhibitor	Mutagen	Negative	Negative
18	96.95	94.74	0.068	0.0478	Non-Inhibitor	Mutagen	Negative	Negative
19	97.35	94.33	0.047	0.0468	Non-Inhibitor	Mutagen	Negative	Negative
25	95.79	100.0	0.102	0.0936	Non-Inhibitor	Mutagen	Positive	Negative
26	96.94	97.24	1.402	0.8132	Non-Inhibitor	Mutagen	Positive	Negative
27	95.47	98.93	0.056	0.0528	Non-Inhibitor	Mutagen	Positive	Negative
28	96.76	97.48	0.146	0.1258	Non-Inhibitor	Mutagen	Positive	Negative
29	95.94	99.05	0.066	0.0739	Non-Inhibitor	Mutagen	Positive	Positive
31	96.39	96.13	0.011	0.0182	Non-Inhibitor	Mutagen	Positive	Negative
33	94.48	97.98	0.042	0.1051	Non-Inhibitor	Mutagen	Positive	Negative
36	95.88	95.50	0.018	0.0472	Non-Inhibitor	Mutagen	Positive	Negative
37	95.78	93.54	0.015	0.0487	Non-Inhibitor	Mutagen	Positive	Positive
41	98.01	72.81	0.192	54.150	Non-Inhibitor	Mutagen	Negative	Negative
42	97.65	91.79	0.074	0.9025	Non-Inhibitor	Mutagen	Negative	Negative
43	97.67	81.09	0.070	0.3469	Non-Inhibitor	Mutagen	Negative	Negative
46	93.41	83.21	1.321	49.234	Non-Inhibitor	Mutagen	Negative	Negative
47	97.54	94.01	3.409	7.6172	Non-Inhibitor	Mutagen	Negative	Positive
Naproxen	98.08	93.95	0.042	185.87	Non-Inhibitor	Mutagen	Negative	Negative

HIA: Human Intestinal Absorption, **PPB**: Plasma Protein Binding, **BBB**: Blood Brain Barrier, **MDCK**: Madin Darby Canine Kidney.

glacial acetic acid (1 ml) was refluxed for 2–4 hrs. After the reaction completion, the solvent was removed under vacuum, and the solid formed was collected and crystallized from aqueous ethanol to obtained compounds **15–19**. **15:** Yield%: 46.3, mp: 274–7 °C, $C_{25}H_{21}N_5OS$, **^{1}H NMR (400 MHz, DMSo-d$_6$);** δ 2.37 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 3.12 (s, 3H, CH_3), 7.18 (d, 1H, J = 8.0 Hz, Ar-H), 7.29 (d, 1H, J = 7.5 Hz, Ar-H), 7.33 (s, 1H, N═CH), 7.40 (t, 2H, J = 15.0 Hz, Ar-H), 7.65 (d, 1H, J = 8.0 Hz, Ar-H), 7.72 (d, 1H, J = 8.0 Hz, Ar-H), 7.80 (d, 1H, J = 7.5 Hz, Ar-H), 7.91 (d, 1H, J = 8.0 Hz, Ar-H), 8.03 (d, 1H, J = 8.0 Hz, Ar-H), 8.10 (d, 1H, J = 8.5 Hz, Ar-H), 8.29 (s, 1H, NH). **^{13}C NMR δ** 14.3, 21.5, 23.2, 112.6, 118.5, 121.4, 122.1, 124.1, 127.2, 129.3, 130.0, 130.8, 131.6, 137.4, 140.3, 140.7, 145.3, 148.2, 150.3, 151.0, 154.8, 155.3, 156.7, 163.9, 169.8. **MS** *m/z* (%): 439 (17.6, M^+). **16:** Yield%: 51.2, mp: 252–5 °C, $C_{24}H_{18}ClN_5OS$, **^{1}H NMR (500 MHz, DMSo-d$_6$);** δ 2.63 (s, 3H, CH_3), 3.11 (s, 3H, CH_3), 7.28 (t, 1H, J = 15.5 Hz, Ar-H), 7.37 (t, 1H, J = 16.0 Hz, Ar-H), 7.43 (d, 1H, J = 6.5 Hz, Ar-H), 7.53 (d, 1H, J = 8.0 Hz, Ar-H), 7.70 (d, 1H, J = 8.0 Hz, Ar-H), 7.77 (d, 2H, J = 8.5 Hz, Ar-H), 7.80 (s, 1H, N═CH), 7.89 (d, 1H, J = 8.5 Hz, Ar-H), 8.02 (d, 1H, J = 8.0 Hz, Ar-H), 8.09 (d, 1H, J = 8.0 Hz, Ar-H), 8.31 (s, 1H, NH). **^{13}C NMR δ** 13.9, 22.8, 56.0, 110.7, 112.1, 118.4, 118.5, 128.8, 129.0, 137.0, 146.7, 147.8, 150.5, 156.3, 160.7, 163.6, 169.5. **MS** *m/z* (%): 459 (11.4, M^+). **17:** Yield%: 39.7, mp: 226–8 °C, $C_{25}H_{21}N_5O_2S$, **^{1}H NMR (400 MHz, DMSo-d$_6$);** δ 2.94 (s, 3H, CH_3), 3.12 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 6.92 (d, 1H, J = 8.5 Hz, Ar-H), 7.04 (d, 2H, J = 9.0 Hz, Ar-H), 7.29 (t, 1H, J = 15.5 Hz, Ar-H), 7.38 (t, 1H, J = 14.5 Hz, Ar-H), 7.71 (d, 1H, J = 12.5 Hz, Ar-H), 7.77 (d, 1H, J = 13.5 Hz, Ar-H), 7.90 (d, 1H, J = 8.0 Hz, Ar-H), 8.01 (d, 1H, J = 8.0 Hz, Ar-H), 8.07 (d, 1H, J = 12.5 Hz, Ar-H), 8.27 (s, 1H, N═CH), 11.86 (s, 1H, NH). **^{13}C NMR δ** 13.9, 22.8, 55.3, 112.2, 114.4, 118.4, 118.6, 121.0, 121.4, 124.0, 128.3, 128.9, 129.0, 130.0, 130.4, 136.9, 147.8, 148.0, 154.3, 156.3, 161.0, 163.3, 169.2. **MS** *m/z* (%):455 (25.7, M^+). **18:** Yield%: 56.7, mp: 241–5 °C, $C_{26}H_{23}N_5O_3S$, **^{1}H NMR (400 MHz, DMSo-d$_6$);** δ 2.64 (s, 3H, CH_3), 3.11 (s, 3H, CH_3), 3.74 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 6.98 (s, 1H, Ar-H), 7.04 (d, 1H, J = 8.5 Hz, Ar-H), 7.22 (d, 1H, J = 8.0 Hz, Ar-H), 7.29 (t, 1H, J = 15.0 Hz, Ar-H), 7.38 (t, 1H, J = 15.0 Hz, Ar-H), 7.70 (d, 1H, J = 8.5 Hz, Ar-H), 7.78 (d, 1H, J = 7.5 Hz, Ar-H), 8.00 (d, 1H, J = 8.0 Hz, Ar-H), 8.07 (d, 1H, J = 8.0 Hz, Ar-H), 8.24 (s, 1H, N═CH), 11.88 (s, 1H, NH). **^{13}C NMR δ** 13.8, 22.8, 55.5, 55.6, 108.2, 108.6, 111.4, 111.6, 112.2, 118.4, 118.6, 121.0, 122.1, 136.9, 143.9, 147.8, 148.2, 149.1, 163.3, 169.3. **MS** *m/z* (%): 485 (17.4, M^+). **19:** Yield%: 57, mp: 250–5 °C,$C_{24}H_{18}N_6O_3S$, **^{1}H NMR (400 MHz, DMSo-**

Table 5
In Vitro antitumor activity (IC_{50}, μM) of the target compounds.

Comp. No	Average Growth Inhibition% of cells (GI%, 10 μM)			In Vitro Cytotoxicity (IC_{50}, μM)		
	HePG-2	HCT-116	MCF-7	HePG-2	HCT-116	MCF-7
15	23.8	20.9	25.3	46.22±3.1	59.33±3.0	38.38±2.9
16	19.4	14.8	29.5	69.48±3.9	75.29±4.0	42.29±3.1
17	21.3	17.8	22.4	36.69±2.7	43.80±2.8	23.56±2.1
18	17.5	14.5	26.9	74.66±4.2	86.74±4.5	61.39±3.7
25	31.5	32.7	47.4	41.23±2.9	37.28±2.5	21.96±1.9
27	51.7	45.2	62.4	24.69±2.1	28.67±1.9	15.78±1.6
29	43.7	37.5	51.4	22.31±1.8	25.94±1.7	12.84±1.4
36	10.3	9.3	18.2	>100	>100	93.66±5.5
37	18.0	15.9	29.6	76.21±4.6	80.90±4.3	50.48±3.3
42	21.6	22.9	36.5	51.38±3.4	64.82±3.2	31.23±2.6
46	27.1	22.6	36.8	46.94±3.2	54.47±2.9	33.14±2.8
Doxorubicin	71.7	68.6	73.1	4.50±0.2	5.23±0.3	4.17±0.2
Naproxen	2.50	2.30	1.64	>100	>100	>100

IC50 (μM): 1–10 (very strong). 11–20 (strong). 21–50 (moderate). 51–100 (weak), > 100 (non-cytotoxic).

d_6); δ 2.83 (s, 3H, CH_3), 2.91 (s, 3H, CH_3), 7.30 (t, 1H, J = 15.0 Hz, Ar-H), 7.75(d, 1H, J = 8.5 Hz, Ar-H), 7.85 (t, 1H, J = 15 Hz, Ar-H), 8.02(d, 2H, J = 8.0 Hz, Ar-H), 8.13 (d, 1H, J = 8.0 Hz, Ar-H), 8.18 (d, 1H, J = 8.0 Hz, Ar-H), 8.24 (d, 1H, J = 8.0 Hz, Ar-H), 8.42 (s, 1H, N═CH), 11.88 (s, 1H, NH). **^{13}C NMR δ** 13.6, 23.1,111.8, 118.2, 119.2, 121.0, 127.7, 128.2, 136.8, 145.7, 145.7, 163.3, 169.3

5.1.4. 2-(2-Methyl-6-(3-methylbenzo[4,5]imidazo[2,1-b]thiazol-2-yl)nicotinoyl)-N-Substituted (un)phenylhydrazine-1-carbothioamides (25–28).

A solution of compound **9** (1.2 g, 0.0035 mol), and the appropriate phenyl isothiocyantes **20–23** (0.0035 mol) in ethanol (30 ml) was refluxed for 6–8 hrs. The solvent was then evaporated under vacuum, and the solid formed was collected and crystallized from ethanol to obtain compounds **25–28**. **25:** Yield%: 47.5, mp: 240–3 °C, $C_{24}H_{20}N_6OS_2$, **IR (cm^{-1}):** 735 (C—S), 1234 (C═S), 1649 (C═O), 3256 (NH), 3448 (NH). **^{1}H NMR (500 MHz, DMSo-d_6);** δ 2.66 (s, 3H, CH_3), 3.10 (s, 3H, CH_3), 7.18 (t, 1H, J = 15.0 Hz, Ar-H), 7.29 (t, 1H, J = 15.5 Hz, Ar-H), 7.34–7.39 (m, 6H, Ar-H), 7.45 (brs, 1H, NH), 7.70 (d, 1H, J = 8.5 Hz, Ar-H), 7.80 (d, 1H, J = 8.0 Hz, Ar-H), 8.09 (d, 1H, J = 8.0 Hz, Ar-H), 9.79 (s, 1H, NH), 10.52 (s, 1H, NH). **^{13}C NMR δ** 14.00, 23.2, 56.0, 61.1, 112.2, 112.3, 118.5, 121.0, 121.5, 123.6, 137.3, 139.8, 147.8, 150.6, 152.1, 154.3, 165.5. **MS** *m/z* (%): 472 (10.9, M^+). **26:** Yield%: 36.8, mp: 245–9 °C, $C_{24}H_{19}ClN_6OS_2$, **IR (cm^{-1}):** 736 (C—S), 1233 (C═S), 1642 (C═O), 3222 (NH), 3451(NH). **^{1}H NMR (400 MHz, DMSo-d_6);** δ 2.68 (s, 3H, CH_3), 3.13 (s, 3H, CH_3), 7.31 (t, 2H, J = 6.8 Hz, Ar-H), 7.37–7.44 (m, 5H, Ar-H), 7.52 (brs, 1H, NH), 7.71 (d, 1H, J = 7.2 Hz, Ar-H), 7.80 (d, 1H, J = 7.6 Hz, Ar-H), 8.09 (d, 1H, J = 7.2 Hz, Ar-H), 9.92 (s, 1H, NH), 10.57 (s, 1H. NH). **MS** *m/z* (%): 506 (0.01, M^+), 507 (0.01, M^{+1}), 509 (0.01, M^{+3}). **27:** Yield%: 56.4, mp: 170–4 °C, $C_{25}H_{22}N_6O_2S_2$, **IR(cm^{-1}):** 733 (C—S), 1250 (C═S), 1677 (C═O), 3281 (NH), 3450(NH). **^{1}H NMR (400 MHz, DMSo-d_6);** δ 2.67 (s, 3H, CH_3), 3.11 (s, 3H, CH_3), 3.77 (s, 3H, OCH_3), 6.95 (d, 2H, J = 8.4 Hz, Ar-H), 7.30 (d, 3H, J = 7.2 Hz, Ar-H), 7.38 (t, 2H, J = 15.2 Hz, Ar-H), 7.71 (d, 1H, J = 8.0 Hz, Ar-H), 7.78 (d, 1H, J = 8.0 Hz, Ar-H), 8.08 (d, 1H, J = 8.0 Hz, Ar-H), 8.18 (brs, 1H, NH), 9.74 (s, 1H, NH), 10.51 (s, 1H, NH). **^{13}C NMR δ** 14.4, 19.0, 23.6, 55.7, 56.5, 112.6, 113.9, 118.5, 119.0, 121.4, 122.0, 124.1, 128.1, 130.5, 130.8, 132.4, 137.8, 148.2, 151.0, 154.7, 157.4, 167.4. **MS** *m/z* (%): 502 (35.1, M^+). **28:** Yield%: 64.2, mp: 220–3 °C, $C_{30}H_{24}N_6O_2S_2$, **IR(cm^{-1}):** 742 (C—S), 1261 (C═S), 1654 (C═O), 3259 (NH), 3451 (NH). **^{1}H NMR (500 MHz, DMSo-d_6);** δ 2.66 (s, 3H, CH_3), 3.11 (s, 3H, CH_3), 7.01 (d, 4H, J = 6.8 Hz, Ar-H), 7.13 (t, 1H, J = 15.2 Hz, Ar-H), 7.29 (t, 1H, J = 7.5 Hz, Ar-H), 7.36–7.40 (m, 5H, Ar-H), 7.70 (d, 2H, J = 8.0 Hz, Ar-H),7.79 (d, 1H, J = 8.0 Hz, Ar-H), 8.08 (d, 1H, J = 9.0 Hz, Ar-H), 8.18 (brs, 1H, NH), 9.79 (brs, 1H, NH), 10.52 (brs, 1H, NH). **^{13}C NMR δ** 13.9, 23.1, 56.0, 61.1, 112.2, 112.3, 118.1, 118.4, 118.5, 120.9, 121.4, 123.4, 123.6, 130.1, 137.3, 139.4, 147.8, 150.6, 154.3, 156.9. **MS** *m/z* (%): 564 (17.3, M^+).

5.1.5. N-Benzyl-2-(2-methyl-6-(3-methylbenzo[4,5]imidazo[2,1-b]thiazol-2-yl)nicotinoyl) hydrazine-1-carbothioamide (29)

A solution of compound **9** (1.2 g, 0.0035 mol), and benzyl isothiocyanate **24** (0.52 g, 0.0035 mol) in ethanol (30 ml) was refluxed for 7 hrs, then continued as under compounds **25–28**. Yield%: 68.9, mp: 232–5 °C, $C_{25}H_{22}N_6OS_2$, **IR(cm^{-1}):** 736(C—S), 1264(C═S), 1678(C═O), 3450(NH). **^{1}H NMR (500 MHz, DMSo-d_6);** δ 2.63 (s, 3H, CH_3), 3.10 (s, 3H, CH_3), 4.78 (s, 2H, benzylic-H), 7.22–7.32 (m, 6H, Ar-H), 7.37 (t, 1H, J = 16.0 Hz, Ar-H), 7.69 (d, 1H, J = 8.0 Hz, Ar-H), 7.77 (d, 1H, J = 8.0 Hz, Ar-H), 8.08 (d, 1H, J = 8.5 Hz, Ar-H), 8.13 (d, 1H, J = 8.0 Hz, Ar-H), 8.69 (s, 1H, NH), 9.55 (s, 1H, NH), 10.39 (s, 1H, NH). **^{13}C NMR δ** 14.00, 21.4, 45.4, 61.1, 110.8, 112.7, 120.6, 121.3, 122.0, 124.7, 125.7, 126.4, 127.3, 128.6, 131.4, 136.2, 140.9, 143.7, 149.0, 150.1, 151.3, 154.3, 165.5. **MS** *m/z* (%): 578 (0.07, M^+).

5.1.6. 4-Bromo-N'-(2-methyl-6-(3-methylbenzo[4,5]imidazo[2,1-b]thiazol-2-yl)nicotinoyl) benzenesulfonohydrazide (31)

An equimolar mixture of compound **9** (1.1 g, 0.0032 mol), and 4-bromobenzene sulphonyl chloride **30** (0.81 g, 0.0032 mol) in pyridine (5 ml) was stirred at room temperature for 24 hrs. The reaction mixture was then poured onto ice-water. The solid formed was then collected, washed with water, crystallized from aqueous ethanol to obtain compound **31.** Yield%: 35.4, mp: 220–4 °C, $C_{23}H_{18}BrN_5O_3S_2$, **^{1}H NMR (400 MHz, DMSo-d_6);** δ 1.96 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 6.94 (t, 1H, J = 15.5 Hz, Ar-H), 7.01 (t, 1H, J = 14.0 Hz, Ar-H), 7.31 (d, 3H, J = 6.8 Hz, Ar-H), 7.40 (d, 4H, J = 12.4 Hz, Ar-H), 7.69 (d, 1H, J = 7.6 Hz, Ar-H), 9.98 (s, 1H, NH), 10.36 (s, 1H, NH). **^{13}C NMR δ** 14.3, 22.8, 113.3, 117.6, 118.8, 122.6, 124.0, 125.2, 127.6, 128.1, 128.2, 129.9, 130.3, 131.0, 131.1, 132.6, 137.5, 138.8, 143.8, 150.4, 154.1, 156.7, 166.5. **MS** *m/z* (%):556 (22.8, M^+).

5.1.7. 2-(2-Methyl-6-(3-methylbenzo[4,5]imidazo[2,1-b]thiazol-2-yl)nicotinoyl)-N-phenyl hydrazine-1-carboxamide (33)

An equimolar mixture of compound **9** (1.1 g, 0.0032 mol), and phenyl isocyanate **32** (0.43 g, 0.0032 mol) in pyridine (5 ml) was stirred at room temperature for 24 hrs, then continued as under **31** Yield%: 42.1, mp: 184–6 °C, $C_{24}H_{20}N_6O_2S$, **^{1}H NMR (400 MHz, DMSo-d_6);** δ 2.67 (s, 3H, CH_3), 3.12 (s, 3H, CH_3), 6.98 (t, 1H, J = 14.5 Hz, Ar-H), 7.29 (t, 3H, J = 15.2 Hz, Ar-H), 7.38 (t, 1H, J = 15.2 Hz, Ar-H), 7.50 (d, 2H, J = 8.0 Hz, Ar-H), 7.71 (d, 1H, J = 8.0 Hz, Ar-H), 7.78 (d, 1H, J = 8.0 Hz, Ar-H), 7.99 (d, 1H, J = 8.0 Hz, Ar-H), 8.09 (d, 1H, J = 8.0 Hz, Ar-H), 8.31 (s, 1H, NH), 8.96 (s, 1H, NH), 10.26 (s, 1H, NH). **^{13}C NMR δ** 14.3, 23.3, 112.6, 118.6, 118.8, 119.0, 121.4, 121.9, 122.5, 124.1, 128.7, 129.2, 129.3, 130.5, 130.8, 137.4, 140.1, 148.2, 151.0, 154.8, 155.8, 156.8, 167.9. **MS** *m/z* (%): 456 (3.8, M^+).

5.1.8. 2-Methyl-6-(3-methylbenzo[4,5]imidazo[2,1-b]thiazol-2-yl)-N'-(4-substituted benzoyl) nicotinohydrazide (36, 37)

A mixture of compound **9** (1.1 g, 0.0032 mol), and substituted benzoyl chloride **34, 35** (0.0032 mol) in pyridine (5 ml) was stirred at room temperature for 24 hrs, then continued as under **31**. **36:** Yield%: 46.1, mp: > 300 °C , $C_{25}H_{21}N_5O_2S$, **^{1}H NMR (400 MHz, DMSo-d_6);** δ 2.40 (s, 3H, CH_3), 2.71 (s, 3H, CH_3), 3.14 (s, 3H, CH_3), 7.29–7.42 (m, 4H, Ar-H), 7.70 (d, 1H, J = 12.8 Hz, Ar-H), 7.82 (d, 1H, J = 8.0 Hz, Ar-H), 7.86 (d, 2H, J = 8.0 Hz, Ar-H), 7.97 (d, 1H, J = 8.0 Hz, Ar-H), 8.11 (d, 1H, J = 8.0 Hz, Ar-H), 10.47 (s, 1H, NH), 10.57 (s, 1H, NH). **^{13}C NMR δ** 14.4, 21.5, 23.2, 112.7, 118.9, 119.0, 121.5, 122.0, 128.0, 128.9, 129.6, 130.0, 130.6, 130.8, 137.3, 142.5, 148.1, 151.0, 154.8, 156.7, 166.1, 167.4. **MS** *m/z* (%): 455 (11.0, M^+). **37:** Yield%: 39.4, mp: > 300 °C, $C_{24}H_{18}FN_5O_2S$, **^{1}H NMR (400 MHz, DMSo-d_6);** δ 2.70 (s, 3H, CH_3), 3.14 (s, 3H, CH_3), 7.32 (t, 1H, J = 15.2 Hz, Ar-H), 7.40 (t, 3H, J = 15.6 Hz, Ar-H), 7.73 (d, 1H, J = 8.0 Hz, Ar-H), 7.82 (d, 1H, J = 8.0 Hz, Ar-H), 7.98 (d, 1H, J = 8.0 Hz, Ar-H), 8.03 (t, 2H, J = 8.0 Hz, Ar-H), 8.11 (d, 1H, J = 8.0 Hz, Ar-H), 10.54 (s, 1H, NH), 10.71 (s, 1H, NH). **^{13}C NMR δ** 14.4, 23.2, 112.7, 116.0, 116.2, 118.9, 119.0, 121.6, 122.1, 124.2, 128.8, 129.2, 129.3, 130.6, 130.8, 137.3, 147.7, 151.0, 154.7, 156.7, 163.5, 165.2, 166.0, 167.4. **MS** *m/z* (%): 459 (22.1, M^+).

5.1.9. (E)-1-(3-Methylbenzo[4,5]imidazo[2,1-b]thiazol-2-yl)-3-(4-substituted piperazin-1-yl)prop-2-en-1-ones (42, 43)

An equimolar mixture of enaminone **7** (1.2 g, 0.0035 mol), and phenyl piprazine derivatives **39, 40** (0.0038 mol) in ethanol (10 ml) was refluxed for 12 hrs. The reaction was then poured in ice water, the solid formed was filtered, washed with water, crystallized from aqueous ethanol to obtain compounds **42** and **43**. **42:** Yield%: 64.7, mp: 120–4, $C_{23}H_{22}N_4OS$, **^{1}H NMR (400 MHz, $CDCl_3$-d_6);**δ3.19 (s, 3H, CH_3), 3.30 (t, 4H, J = 10.4 Hz, piperazine-H), 3.62 (br s, 4H, J = 10.4 Hz, piperazine-H), 5.57 (d, 1H, J = 12.4 Hz, CH═CH),6.95–699 (m, 3H, Ar-H), 7.27–7.35 (m, 3H, Ar-H), 7.42 (t, 1H, J = 15.6 Hz, Ar-H), 7.81 (t, 2H, J = 8.0 Hz, Ar-H), 7.89 (d, 1H, J = 8.4 Hz, CH═CH). **^{13}C-NMRδ** 14.1,

31.0, 89.1, 93.9, 111.6, 117.0, 119.3, 121.1,121.2, 121.9, 124.1, 129.4, 130.5, 134.9, , 148.8, 150.6, 152.6, 154.7, 181.5. ^{15}N NMR: δ 0.57, 123.2, 200, 399.4. **MS** *m/z* (%):402 (17.3, M^+). **43:** Yield%: 45.1, mp: 160–6, $C_{24}H_{24}N_4OS$, **^{1}H NMR (400 MHz, $CDCl_3$-d_6);** δ 2.56 (t, 4H, J = 12.0 Hz, piperazine-H), 3.18 (s, 3H, CH_3), 3.46(brs, piperazine-H), 3.59 (s, 2H, benzylic-H), 5.50 (d, 1H, J = 12.0 Hz, CH═CH),7.27–7.37 (m, 6H, Ar-H), 7.41 (t, 1H, J = 16.0 Hz, Ar-H), 7.78 (d, 1H, J = 12.0 Hz, Ar-H), 7.80 (d, 1H, J = 12.0 Hz, Ar-H), 7.90 (d, 1H, J = 8.0 Hz, CH═CH). **^{13}C-NMR**δ14.0, , 45.4, 45.7, 51.7, 53.6, 62.7, 93.4, 111.5, 119.3, 121.1, 122.1, 124.0, 127.5, 128.5, 129.1, 130.5, 134.4, 137.2, 148.7, 152.8, 154.7, 181.4.MS *m/z* (%):416 (10.2, M^+).

3-Methyl-2-(1H-substituted (un)pyrazol-3-yl)benzo[4,5]imidazo [2,1-b]thiazoles (46, 47)

A mixture of enaminone **7** (1.2 gm, 0.0035 mol), and substituted hydrazine (0.0037 mol) in absolute ethanol (10 ml) was refluxed for 5 h. The reaction mixture was followed up by TLC, the reaction was then poured into ice water. The formed solid was filtered, washed with water obtaining crystallized from aqueous ethanol to obtain compounds **46** and **47**. **46:** Yield%: 55.7, mp: 240–6, $C_{13}H_{10}N_4S$, **^{1}H NMR (500 MHz, DMSo-d_6);** δ 2.96 (s, 3H, CH_3), 6.70 (s, 2H, pyrazole-H), 7.25 (t, 1H, J = 16.5 Hz, Ar-H),7.33 (t, 1H, J = 15.5 Hz, Ar-H), 7.68 (d, 1H, J = 8.0 Hz, Ar-H), 7.93 (s, 1H, NH), 8.02 (d, 1H, J = 8.0 Hz, Ar-H). **^{13}C NMR** δ 13.4, 103.6, 112.1, 115.2, 118.9, 121.2, 123.5, 126.5, 130.8, 131.0, 143.1, 148.1, 154.4. **MS** *m/z* (%):254 (15.17, M^+), 255 (2.91, M^{+1}), 256 (0.76, M^{+2}). **47:** Yield%: 51.9, mp: 150–4, $C_{19}H_{14}N_4S$, **^{1}H NMR (400 MHz, $CDCl_3$-d_6);** δ 2.43 (s, 3H, CH_3), 6.67 (d, 1H, J = 1.6 Hz, Ar-H), 7.24 (d, 1H, J = 7.6 Hz, Ar-H), 7.28 (d, 1H, J = 2.8 Hz, Ar-H), 7.36–7.45 (m, 6H, Ar-H), 7.72 (d, 1H, J = 8.4 Hz, Ar-H), 7.81 (d, 1H, J = 8.8 Hz, Ar-H). **^{13}C NMR** δ 12.8, 109.6, 110.7, 111.2, 119.4, 121.1, 123.8, 124.4, 128.1, 128.9, 129.4, 130.3, 132.3, 139.3, 140.7, 147.9, 155.5. **MS** *m/z* (%):330 (95.9, M^+), 331 (23.85, M^{+1}), 332 (5.21, M^{+2}), 333 (1.02, M^{+3}).

5.2. In vivo anti-inflammatory activity

Male Sprague Dawley rats weighing 250 g were purchased from local source and kept at room temperature (22 ± 2 °C) in a light-controlled room with an alternating 12 h light/dark cycle. They were fasted with free access to water at least 16 h prior to experiments. The tested compounds were prepared as suspension in vehicle (0.5% methyl cellulose) and celecoxib was used as a standard drug. The positive control group animals received the reference drug while the negative control received only the vehicle. The anti-inflammatory activity was evaluated using *in vivo* rat carrageenan-induced foot paw edema model reported previously [21–23]. Edema was produced by injecting 0.25 ml of a solution of 1% l-carrageenan in the hind paw. The rats were injected intraperitoneally with 1 ml suspension in 0.5% methyl cellulose of the tested compounds and reference drug. The percentage edema (E %) at each time point and the percentage inhibition of edema (I%) of each group were calculated as follows

$$\text{Oedema(E\%)} = \frac{PT_t - PT_0}{PT_0}$$

$$\text{Inhibition(I\%)} = \left(1 - \frac{E_D}{E_C}\right) \times 100$$

where PT_0 and PT_t are the paw thickness (mm) before and after carrageenan injection respectively, and E_D and E_C are the mean percentage edema of the treated and control group respectively. Values reported as mean ± S.E.M., significant differences were calculated using ANOVA [12–14].

5.3. In vitro cyclooxygenase inhibitory assay

The *in vitro* ability of test compounds and celecoxib to inhibit the COX-1 and COX-2 isozymes was carried out using Cayman colorimetric COX (ovine) inhibitor screening assay kit (Catalog no. 701070) supplied by Cayman chemicals, USA. The calculations were performed as per the kit guidelines.

5.4. Determination of the IC_{50} of the target compounds

IC_{50} (Concentration at which there was 50% inhibition of COX-1) of the target compounds were determined by the aid of Cayman colorimetric COX (ovine) inhibitor screening assay kit through the determination of the average absorbance of all the samples. Subtract the absorbance of the background wells from the initial activity sample, then divide by the 100% initial activity sample, and multiply by 100 to give the percent inhibition. Graph the percent inhibition and determine the IC_{50} value by using the three results obtained.

5.5. Molecular modeling studies

The investigated compounds were subjected to molecular modeling experiments using 'Molecular Operating Environment' software (MOE of Chemical Computing Group Inc., on a Core i7 workstation). The molecules were built using the Builder module of MOE. Their geometry was optimized by using the MMFF94x force field. The Molecular Mechanics (MM) calculations in vacuo, dipole bond option for electrostatics, Polake–Ribiere algorithm, and Root Mean Square Deviation (RMSD) gradient of 0.01 kcal/mol conformational searching in torsional space was performed using the multi-conformer method. Energy minima for the above compounds were determined by a semi-empirical method AM1 (as implemented in MOE, 2009.10). Lowest energy conformer of each new analogue 'global-minima' was used for modeling [15–18].

5.6. In vitro cancer screening

The cell lines tested were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100 µg/ml streptomycin at 37 °C in a 5% CO_2 incubator. The cell lines were seeded in a 96-well plate at a density of 1.0×10^4 cells/well. at 37 °C for 48 h under 5% CO_2. After incubation the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5 mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100 µl is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800, USA). The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) × 100 [20,21].

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