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ORIGINAL ARTICLE

Multicomponent reaction for synthesis, molecular docking, and anti-inflammatory evaluation of novel indole-thiazole hybrid derivatives

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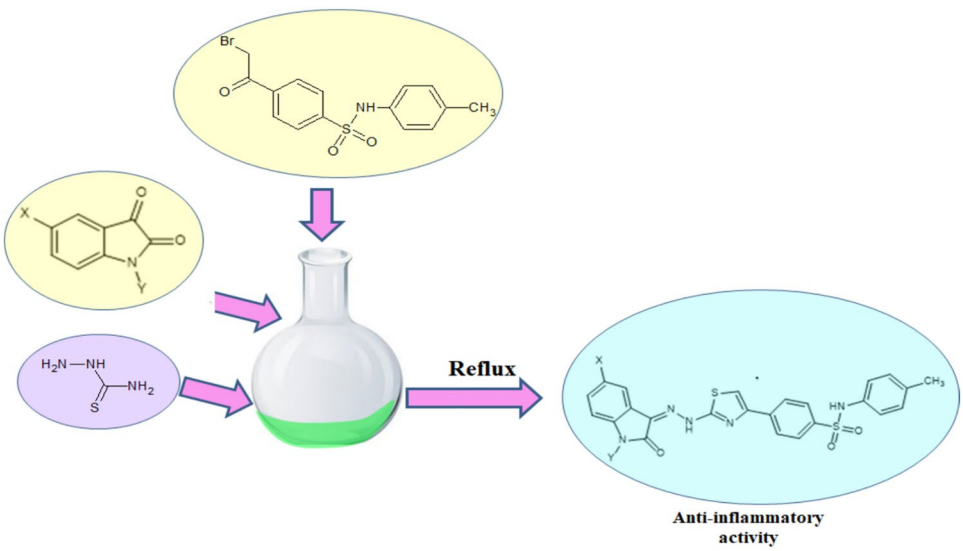
Abstract

In this article, novel thiazol-indolin-2-one derivatives **4a–f** have been synthesized via treatment of thiosemicarbazide (**1**) with some isatin derivative **2a–f** and *N*-(4-(2-bromoacetyl)phenyl)-4-tolyl-sulfonamide (**3)** under reflux in ethanol in the presence of triethyl amine (TEA). The structures of new products were elucidated by elemental and spectral analyses. Moreover, all compounds were investigated for their in vivo anti-inflammatory activity using celecoxib as a reference drug. The target compound **4b** was the most active anti-inflammatory candidate and exhibited higher edema inhibition (EI = 38.50%) than that recorded by celecoxib (EI = 34.58%) after 3 h. Furthermore, the most active compounds **4b** and **4f** were subjected to a molecular docking study inside COX-2 enzyme to show their binding interactions**.** Both compounds **4b** and **4f** showed good fitting into COX-2 binding site with docking energy scores − 11.45 kcal/mol and − 10.48 kcal/mol, respectively which indicated that compound **4b** revealed the most promising and effective anti-inflammatory potential.

Graphical abstract



Keywords Indole derivatives · Thiazole · Multicomponent reaction · Anti-inflammatory activity · Histopathological examination

Extended author information available on the last page of the article

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Introduction

Inflammation is a biological reaction to a disturbance in tissue homeostasis and body defense chemicals in which cells penetrate the affected tissue causing increasing blood flow, vascular permeability, and vasodilatation [1, 2]. Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used medications for relieving pain and inflammation by inhibiting Cyclooxygenase (COX) enzymes [3, 4]. The constitutive COX-1 performs numerous physiological activities as protecting gastric mucosa, vascular homeostasis and platelet aggregation, while the other isoform, the inducible COX-2 is concerned with prostaglandins that promote inflammation and modulate pain [5–7].

The use of traditional NSAIDs as aspirin, indomethacin and phenazone causes gastrointestinal side effects due to the inhibition of both COX isoforms [8–10]. Selective COX-2 inhibitor medications as celecoxib, valdecoxib, and rofecoxib have been prepared to avoid the side effects produced by traditional NSAIDs [11, 12]. Unfortunately, rofecoxib and valdecoxib were taken off the market due to their cardiovascular side effects including myocardial infarction and the occurrence of high blood pressure [13–15]. So, there is a great demand for selective COX-2 inhibitors with diminished side effects. Indole is one of the most widely used scaffolds in a broad range of anti-inflammatory agents [16–18]. Many research investigations have focused on indole-based NSAIDs such as indomethacin (**I**) to enhance their COX-2 selectivity and decrease the ulcerogenic adverse effects that linked to their strong COX-1 selectivity and drugs acidic properties [19–21]. Knaus and co-workers synthesized a new set of indole derivatives substituted at N-1 and C-3 [22]. From the prepared indole derivatives, compound **II** was the most selective (SI > 312) and potent (COX-2 IC_{50} = 0.32 μM) COX-2 inhibitor. In 2021, new indole derivatives having thiosemicarbazone moiety were prepared and screened for their anti-inflammatory effect using carrageenan-induced paw edema assay [23]. Compound **III** recorded superior COX-2 selectivity (SI = 23.06) than displayed by celecoxib (SI = 11.88).

Thiazole is a five-membered heterocyclic ring [24–28] with many pharmacological utilities as anticancer [29, 30], antioxidant [31, 32], antimicrobial [33, 34], antidiabetic [35], anthelmintic [36] and anti-inflammatory [37, 38]. For example, the thiazole derivative **IV** significantly inhibited edema (60.82%) in carrageenan-induced edema compared with indomethacin (53.21%) [39]. Also, thiazole derivative **V** recorded comparable edema inhibition (EI = 87%) to that registered by indomethacin (EI = 91%) after 4 h [40].

Considering the aforesaid data and as an extension and development of our previous studies [41–50], we present the design and construction of novel indole-thiazole hybrids and biologically screened for their anti-inflammatory effect. This work aims to get new compounds with selective COX-2 inhibition, favorable anti-inflammatory potential and minimized gastric side effects. This aim has been achieved by hybridization of the privileged indole ring with the thiazole nucleus in one chemical entity (Fig. 1).

Result and discussion

Chemistry

In this work, treatment of thiosemicarbazide (**1**) with isatin derivatives **2a–f** and 4-(bromoacetyl)-*N*-(4-methylphenyl) benzenesulfonamide (**3**) via three-component reaction under reflux in ethanol/tiethylamine (TEA). Firstly, compound **1** was allowed to react with some isatin derivatives **2a–f** namely; isatin (**2a**), 5-chloro-isatin (**2b**), 5-nitro-isatin (**2c**), *N*-methyl-isatin (**2d**), 5-chloro-*N*-methyl-isatin (**2e**), 5-nitro-*N*-methyl-isatin (**2f**), then compound **3** was added until the

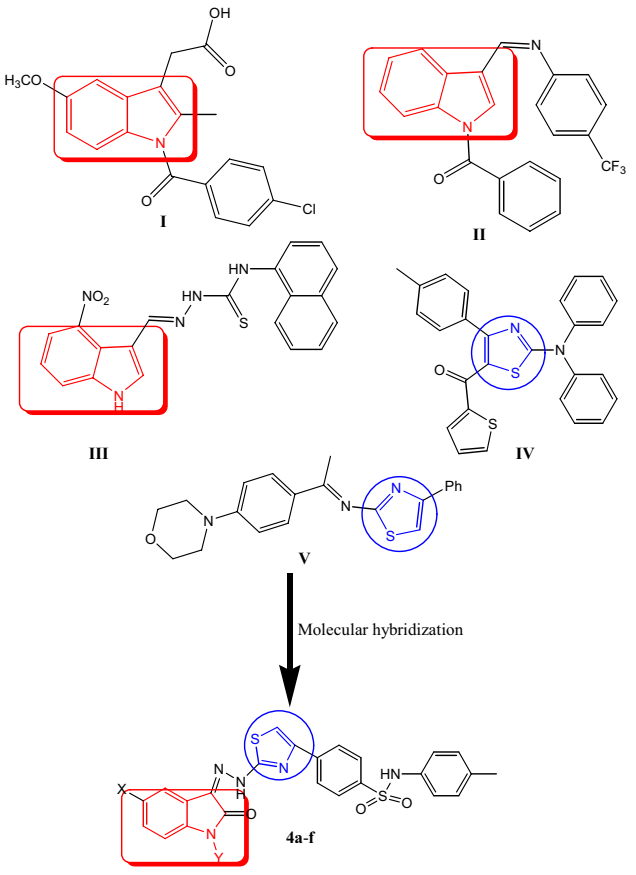
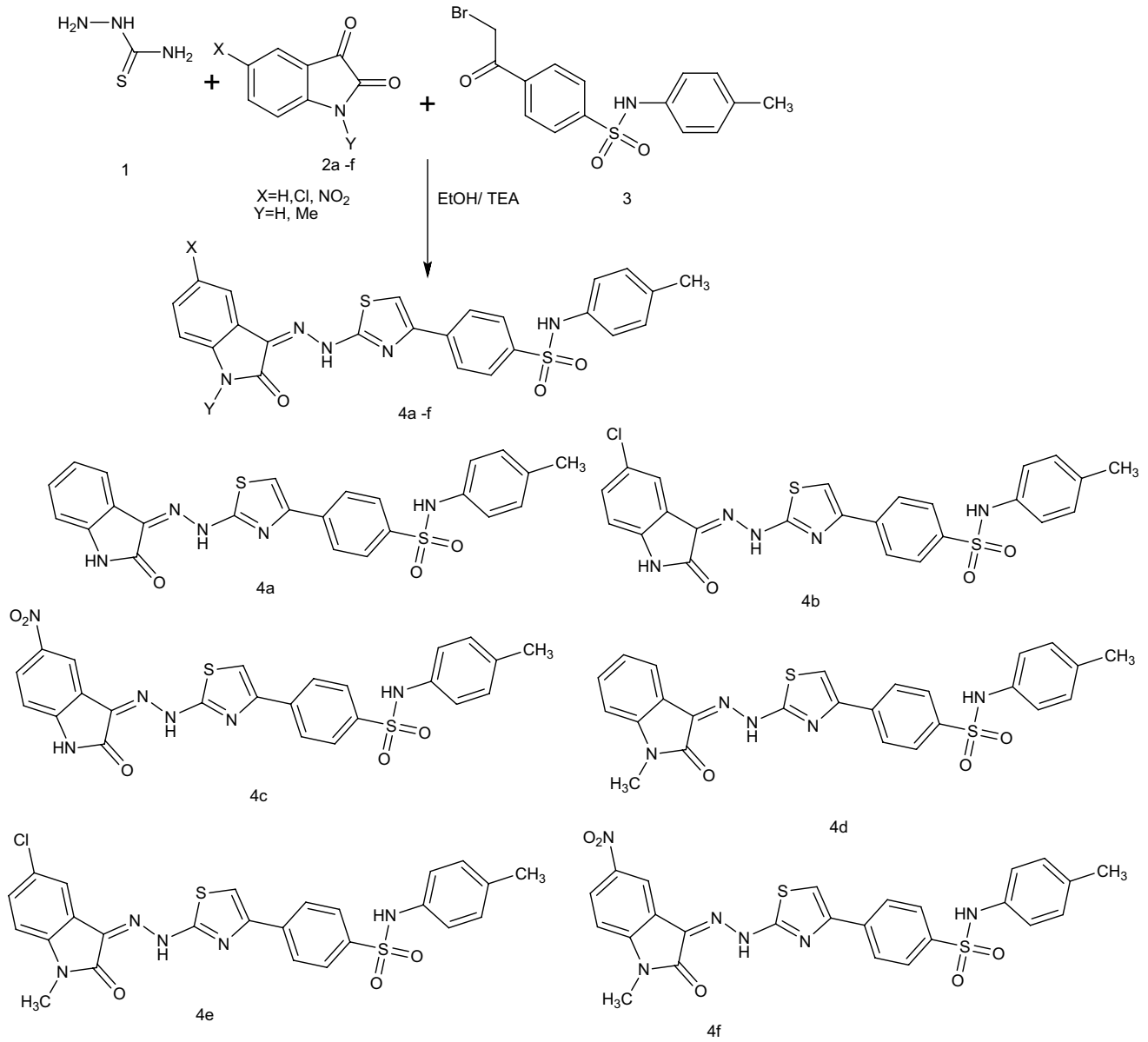


Fig. 1 Examples of some reported indoles (**I–III**) and thiazoles (**IV, V**) and the rationale for the design of target compounds **4a–f**



Scheme 1. Synthesis of indole-thiazole hybrid derivatives **4a–f**

reaction completed (TLC), to obtain thiazol-indolin-2-one derivatives **4a–f** (Scheme 1).

The chemical structure of compounds **4a–f** was determined using different elemental and spectroscopic analyses such as ^{1}H-NMR, ^{13}CNMR, as well as infrared spectroscopy.

Their infrared spectra revealed the existence of new bands in the range 3363–3124 cm^{-1} corresponding to NH groups. ^{1}H NMR spectra showed, as well as the aromatic signals, new singlet signals in the region δ 13.30–9.03 ppm for NH groups. The N-CH_3 proton in compounds **4d–f** appeared as a singlet signal in the range δ 3.73–3.84 ppm, respectively. Moreover, their ^{13}C NMR spectra matched the accurate chemical structure which showed the carbonyl groups in the range 190.6–161.2 ppm and the N-CH_3 groups in compounds **4d–f** appeared in the region δ 34.5–30.6 ppm, and the CH_3 groups at range δ 22.4–21.3 ppm, respectively. Furthermore, elemental analyses of thiazoles **4a–f** provided the correct structure of the new products (cf. experimental).

Biological screening

Anti-inflammatory activity

The anti-inflammaory potential of indol-3-ylidenehydrazino-1,3-thiazole derivatives **4a–f** was estimated applying the carrageenan-induced rat paw edema method using

Table 1 Anti-inflammatory potential of test compounds (4a-f) using celecoxib reference drug

Compound	Diameter inflammation (mm)			% Edema inhibition		
	1 h	3 h	5 h	1 h	3 h	5 h
Control	3.90 ± 0.14	3.90 ± 0.14	3.90 ± 0.14	–	–	–
Carrageenan	5.76 ± 0.08	7.20 ± 0.08	7.91 ± 0.15	–	–	–
Celecoxib	4.09 ± 0.06	4.71 ± 0.18	4.01 ± 0.14	28.99	34.58	49.30
4a	5.70 ± 0.14	5.90 ± 0.12	6.90 ± 0.15	01.04	18.05	12.76
4b	4.90 ± 0.51	4.46 ± 0.23	4.30 ± 0.33	12.72	38.50	45.63
4c	5.53 ± 0.08	5.52 ± 0.09	4.81 ± 0.23	03.99	23.33	39.19
4d	5.60 ± 0.96	6.09 ± 0.22	6.03 ± 0.59	02.77	15.41	23.76
4e	5.74 ± 0.23	6.79 ± 0.19	7.22 ± 0.23	0.34	5.69	8.72
4f	5.36 ± 0.30	4.90 ± 0.20	4.60 ± 0.06	6.94	31.94	41.84

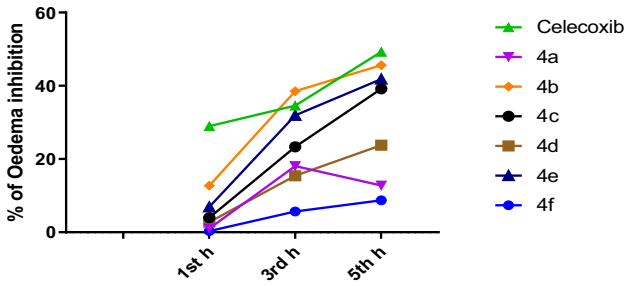


Fig. 2 The % of edema inhibition in response to test compounds

celecoxib as a standard. Each target compound was taken immediately before inflammation induction by carrageenan injection. The anti-inflammatory potential was recorded according to paw volume changes after 1, 3 and 5 h as displayed in Table 1. The obtained outcomes disclosed that 4-{2-[2-(5-chloro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene) hydrazino]-1,3-thiazol-4-yl}-*N*-phenyl-4-tolylsulfonamide (**4b**) was the most active candidate with edema inhibition percent equal to 12.72–45.63%. Furthermore, this compound **4b** showed higher edema inhibition (EI = 38.50%) than that exhibited by celecoxib (EI = 34.58%) after 3 h. Moreover, compound **4f** revealed comparable edema inhibition (3 h; EI = 31.94%, 5 h; EI = 41.84%) to that recorded by celecoxib (3 h; EI = 34.58%, 5 h; EI = 49.30%). In addition, within 1*H*-indole derivatives **4a–c**, the 5-chloroindole derivative (**4b**) was the most active anti-inflammatory candidate (3 h; EI = 38.50%, 5 h; EI = 45.63%) followed by 5-nitro analogue (**4c**) (3 h; EI = 23.33%, 5 h; EI = 39.19%) while compound **4a** with no substitution at positions 1 and 5 of indole moiety exhibited the least anti-inflammatory activity (3 h; EI = 18.05%, 5 h; EI = 12.76%). In case of *N*-methylindole derivatives (**4d–f**), the 5-nitroindole candidate (**4f**) showed the highest edema inhibition percent (3 h; EI = 31.94%, 5 h; EI = 41.84%) followed by compound **4d** (3 h; EI = 15.41%, 5 h; EI = 23.76%) then the 5-chloro analogue (**4e**) (3 h; EI = 5.69%, 5 h; EI = 8.72%). From the recorded data in (Table 1 and Fig. 2), it is clear that compound **4b** has the most promising anti-inflammatory potential in comparison with celecoxib.

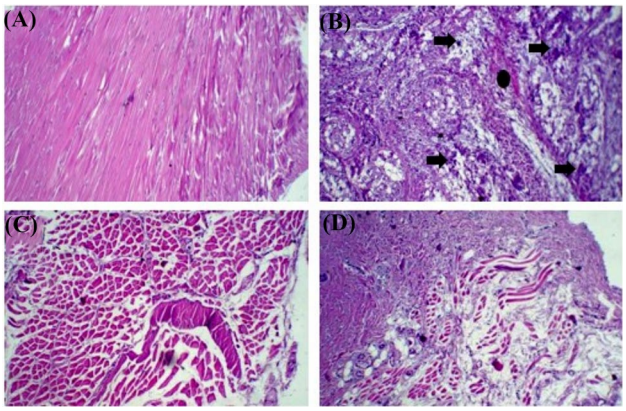


Fig. 3 Microscopic examination of the impact of 4b on paw tissues following carrageenan injection. **A** control group with normal paw tissue, **B** carrageenan group disclosed an acute inflammation (black arrow) with edema (black star), **C** Celecoxib group with remarkable attenuation of edema and neutrophils migration, **D** compound **4b** treated group with significant attenuation of inflammation and edema. Sections stained with H&E, (×200)

Histological investigation

The impact of compound **4b** on paw tissue after carrageenan injection, comparing the results to those observed with indomethacin. It has been found that compound **4b** is the most effective of the tested products. As shown in Fig. 3, the paw tissues of control rats (2, A) are not inflamed. The carrageenan model demonstrated neutrophil migration in addition to acute inflammation (black arrow) and hemorrhagic edema (black star) (Fig. 2B). Nevertheless, the rats given Celecoxib showed a notable decrease in inflammation Fig. 2C. Figure 2D demonstrated the impact of test chemical **4b** on paw tissue inflammation, demonstrating a notable reduction in inflammatory cells and edema.

Table 2 I*n vitro* inhibitory effects of compounds **4b** and **4f** on COX-1 and COX-2 enzymes

Compounds	COX-1 IC_{50} (μM)	COX-2 IC_{50} (μM)	SI
4b	21.97 ± 1.56	8.26 ± 0.77	2.65
4f	99.50 ± 3.34	23.03 ± 0.75	4.32
Indomethacin	0.52 ± 0.06	0.77 ± 0.041	0.67
Celecoxib	7.11 ± 0.28	1.60 ± 0.036	4.44

In vitro COX inhibition screening

Using a colorimetric enzyme immunoassay (EIA), the most active candidates **4b** and **4f** inhibitory efficacy against COX-1/COX-2 enzymes was ascertained. For each hybrid, the effectiveness was measured using serial dilutions to determine the concentration that inhibited 50% of the enzyme (IC_{50}) (100, 10, 1, and 0.1 M). Additionally, for COX-1/COX-2, selectivity indexes (SI values) against COX-2 were computed and compared with reference drugs celecoxib and indomethacin.

The results obtained from the **4b** and **4f** hybrids are displayed in (Table 2), which indicates that both compounds **4b** and **4f** exhibited higher COX-2 inhibitory effect than COX-1 enzyme.

Compound **4b** was the most potent COX-2 inhibitor (IC_{50} = 8.26 μM), while **4f** was the most COX-2 selective (SI = 4.32) compared with indomethacin (COX-2 IC_{50} = 0.77 μM, SI = 0.67) and celecoxib (COX-2 IC_{50} = 1.60 μM, SI = 4.44). Furthermore, both **4b** (SI = 2.65) and **4f** (SI = 4.32) were more COX-2 selective than indomethacin (SI = 0.67). In addition, the 5-nitroindole candidate (**4f**) exhibited comparable selectivity index (SI = 4.32) to that recorded by celecoxib (SI = 4.44).

Histological evaluation of ulcers

Most NSAIDs have been found to induce stomach ulcers. Finding innovative anti-inflammatory medicines that helps to avoid this problem is crucial because the unfavorable gastrointestinal adverse reactions of current anti-inflammatory therapies is a major disadvantage Thus, to find any gastroprotective effects of the most potent anti-inflammatory compounds **4b** and **4f**, we investigated how hybrids affected rat stomach ulcers. Figure 4 clearly depicts the changes in the histology of the stomachs of rats. While the indomethacin-treated group (Fig. 4B) showed an interfered with glandular mucosal layer with numerous focal ulcerative zones, marked by the bringing of the epithelial lining and an accumulation of necrotic tissue, the control group (Fig. 4A) showed a normal gastric mucosa without any indication of erosion or inflammation. Furthermore, after administration of both compounds **4b** and **4f** (Fig. 4C, D) the glandular mucosa and submucosa in the several regions investigated in the stomach mucosa exhibited largely intact histological structure without any defects. More information from a new study with long term treatment is required to clarify the gastric effects of these compounds at long term of usage.

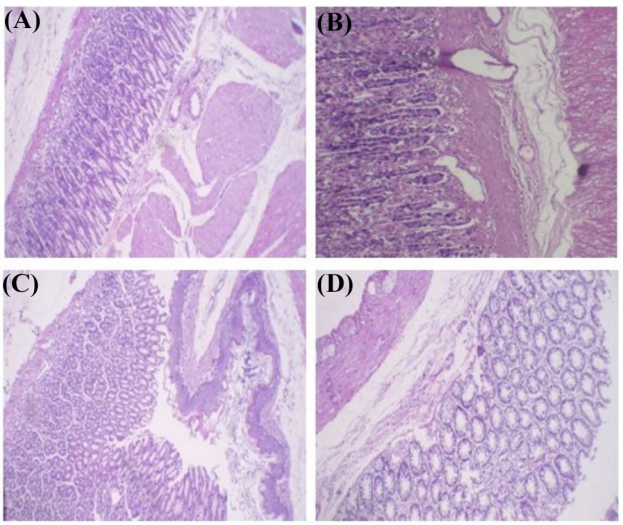


Fig. 4 Macroscopical evaluation effect of compounds **4b** and **4f** on the integrity of gastric mucosal membranes. **A** control group. **B** Indomethacin-induced peptic ulcer. **C 4b** treated group, **D 4f** treated group. (H&E stain) magnification power × 20

Inflammation induced by LPS in the RAW 264.7 cell line

The efficacy of synthesized compounds and their cytotoxicity on LPS-induced RAW 264.7 cells were evaluated at different concentrations by using the MTT assay. As seen in Fig. 5, as compared to LPS-induced macrophages, none of the two compounds were able to stop the growth of macrophages at 5 μM.

Structure–activity relationship

From the anti-inflammatory activity of the newly prepared compounds, some relations between their structure and activity could be concluded as outlined in Fig. 6.

In case of 1*H*-indole derivatives **4a-c**, the presence of electron-withdrawing groups (Cl, NO_2) at C-5 of indole moiety markedly improved the in *vivo* anti-inflammatory potential. This is obvious upon comparing the anti-inflammatory activity of compound **4b** (3 h; EI = 38.50%, 5 h; EI = 45.63%) and **4c** (3 h; EI = 23.33%, 5 h; EI = 39.19%) with unsubstituted analog **4a** (3 h; EI = 18.05%, 5 h; EI = 12.76%). In addition, methylation of N-1 of indole derivatives attenuated the anti-inflammatory potential (except **4c)** as observed in methylation of **4b** (3 h; EI = 38.50%, 5 h; EI = 45.63%) to **4e** (3 h; EI = 5.69%, 5 h; EI = 8.72%). While, methylation

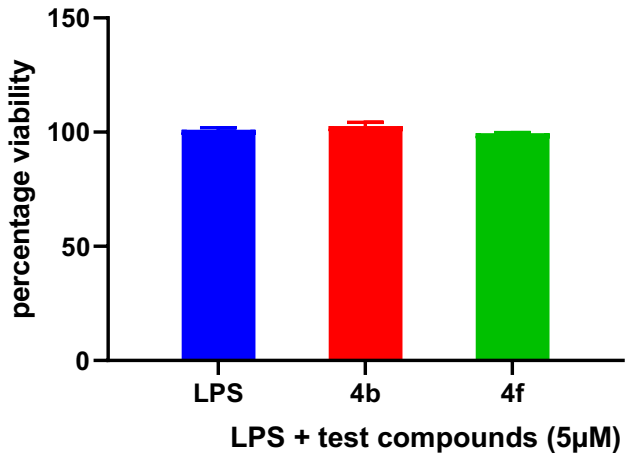


Fig. 5 Cytotoxic evaluation of **4b** and **4f** hybrids in RAW 264.7 cells stimulated with lipopolysaccharides (LPS). The MTT assay was used to measure cell viability. The data is shown as mean ± SD (n = 3). Relative to the LPS-induced cells, the statistical analysis showed no significant differences ($p > 0.05$)

of 5-nitroindole derivative (**4c**) (3 h; EI = 23.33%, 5 h; EI = 39.19%) to 5-nitro-*N*-methylindole derivative **4f** (3 h; EI = 31.94%, 5 h; EI = 41.84%) enhanced the anti-inflammatory potential.

Molecular docking study

To gain insights into the fundamental mechanism of action of newly prepared indol-3-ylidenehydrazino-1,3-thiazole derivatives, molecular docking of the most active candidates (**4b** and **4f**) was conducted inside COX-2 active region. The results of docking including docking score (Kcal/mol), types of interactions and the binded amino acids are listed in Table 3 and Figs. 7 and 8.

Compound **4b** revealed good binding within COX-2 with binding energy score = -11.45 kcal/mol. Conventional hydrogen bonds with ARG376, TRP139, ASP229 and GLY235 amino acids were detected (Fig. 7). In addition, this compound **4b** displayed other Pi-Cation interactions with LYS333, Pi-Alkyl binding with LEU145, PRO538; Amide-Pi Stacked with LEU224 and Van der Waals interactions with SER143 and ASN375 (Fig. 7).

Furthermore, compound **4f** exhibited three hydrogen bonding interactions with ARG44, CYS41 and GLY135 amino acids with a binding energy score equal to − 10.48 kcal/mol. also, other binding interactions were registered as Pi-Alkyl binding with LYS468, PRO153 and ALA156; Amide-Pi binding with VAL155 and Van der Waals interaction with ASN34, ALA156 and ARG469 (Fig. 8).

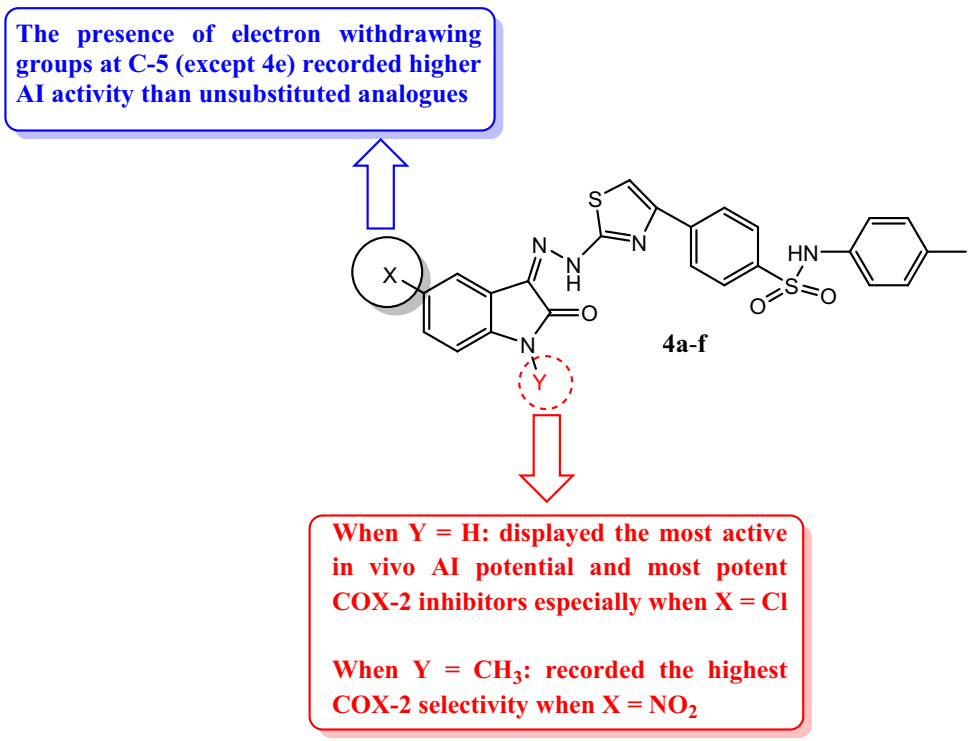


Fig. 6 SAR study of in vivo anti-inflammatory activity of target compounds **4a–f**

Table 3 Outcomes of docking study for target candidates **4b** and **4f** inside COX-2 enzyme

Compound no	Docking score (Kcal/mol)	Number of bonds	Type of interactions	Amino acids	Function group
4b	− 11.45	11	H-bond	ARG376	SO_2
			H-bond	TRP139	=N
			H-bond	ASP229	NH
			H-bond	GLY235	Indole NH
			Amide-Pi	LEU224	Thiazole
			Pi-Cation	LYS333	Indole
			Pi-Cation	LYS333	Indole
			Pi-Alkyl	LEU145	Phenyl
			Pi-Alkyl	PRO538	Methyl
			Van der Waal	SER143	=N
			Van der Waal	ASN375	Phenyl
2f	− 10.48	13	H-bond	CYS41	NH
			H-bond	ARG44	SO_2
			H-bond	GLY135	NO_2
			Pi-Alkyl	LYS468	Phenyl
			Pi-Alkyl	LYS468	Methyl
			Pi-Alkyl	CYS36	Thiazole
			Pi-Alkyl	PRO153	Thiazole
			Pi-Alkyl	ALA156	Indole
			Pi-Alky	ALA156	Indole
			Amide-Pi	VAL155	Indole
			Van der Waal	ARG469	SO_2
			Van der Waal	ALA156	NO_2
			Van der Waal	ASN34	Indole

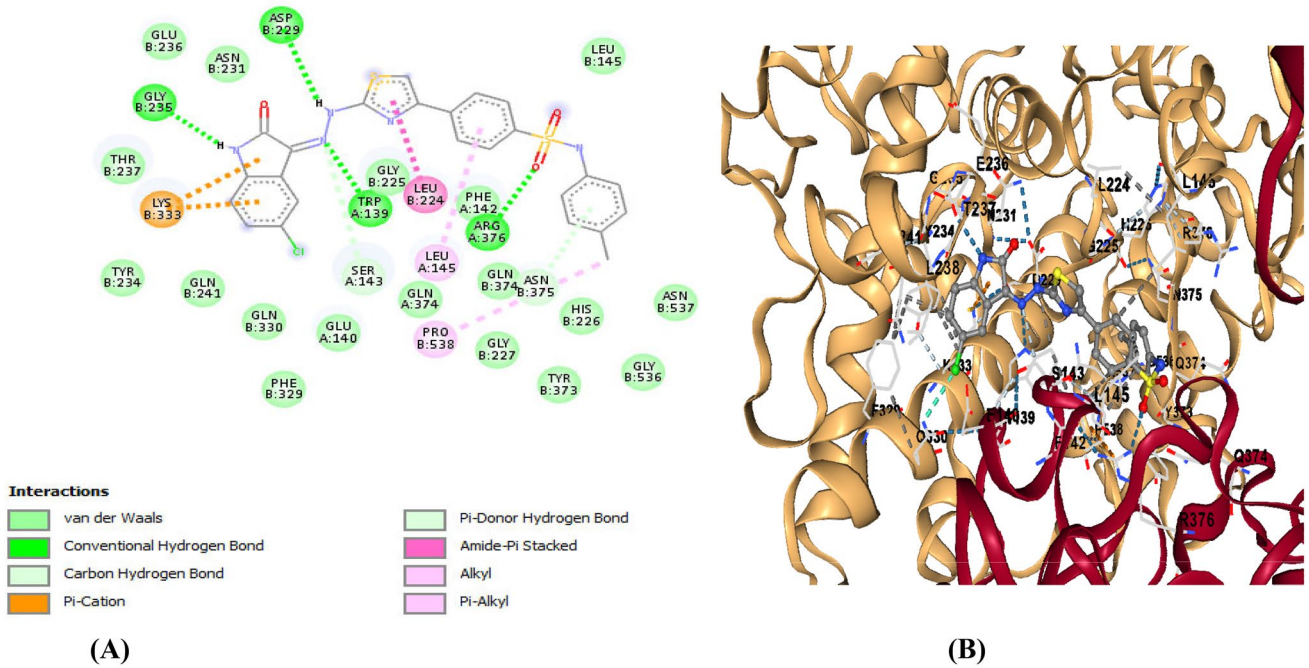


Fig. 7 The proposed binding mode of compound **4b** within COX-2 enzyme. **A** 2D binding form, **B** 3D binding form

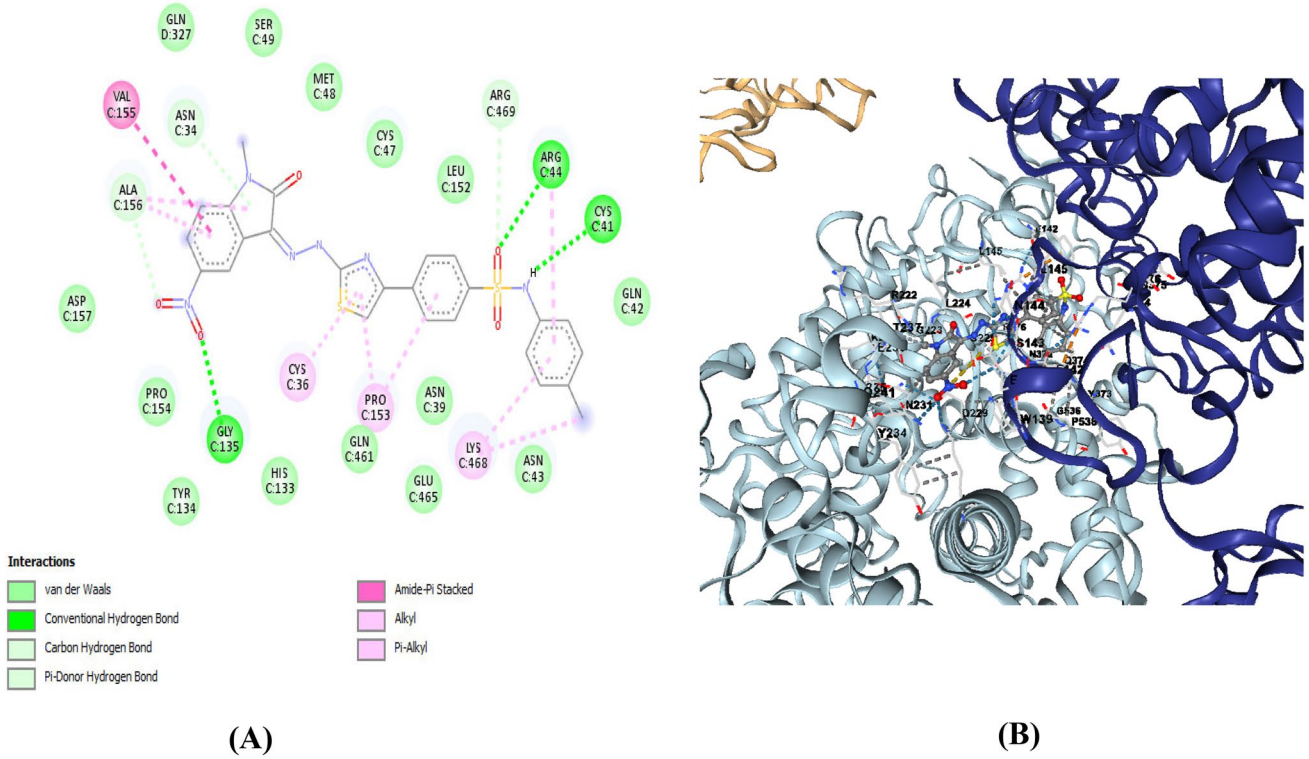


Fig. 8 The proposed binding mode of compound **4f** within COX-2 enzyme. **A** 2D binding form, **B** 3D binding form

Experimental

Chemistry

All melting points were recorded on Melt-Temp II melting point apparatus. IR spectra were measured as KBr pellets on a Shimadzu DR-8001 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker at 400 MHz and 100 MHz using TMS as an internal reference, DMSO-d_6 as solvent. The elemental analyses were carried out on a Perkin-Elmer 240C Micro analyzer. All reactions were monitored by thin layer chromatography (TLC) using precoated plates of silica gel G/UV-254 of 0.25 mm thickness (Merck 60F254), with R_f value 0.58–64 (eluent solvent, Hexane/EtOH, 5:1).

General Procedure for the Synthesis of thiazole derivatives 4a–f

An equimolar mixture of thiosemicarbazide (**1**) (0.01 mol, 0.91 g) with the appropriate isatin derivative (**2a–f**) (0.01 mol) was allowed to reflux in ethanol (20 ml., 5 drops TEA). Then 4-(bromoacetyl)-*N*-(4-methylphenyl)benzenesulfonamide (**3**) (0.01 mol, 0.35 g) was added to the reaction mixture, and refluxing was continued for 20–30 min until the reaction was complete (TLC) to afford the corresponding thiazole derivatives (**4a–f**). The reaction mixture was allowed to cool to room temperature and the solid precipitate was filtrated, and recrystallized from ethanol.

4-{2-[2-(2-Oxo-1,2-dihydro-1H-indol-3-ylidene) hydrazino]-1,3-thiazol-4-yl}-*N*-phenyl-4-tolyl sulfonamide (4a)

Yield, 85%; Mp 335–337 °C; R_f value = 0.59; IR cm^{-1}: 3363, 3178, 3120 (3NH), 3064 (C–$H_{arom.}$), 1682 (C=O), ^{1}H NMR δ 13.30 (br,1H, NH), 11.20 (s, 1H, NH), 10.29 (s, 1H, NH), 7.76–6.85 (m, 13H, $CH_{arom.}$), 2.33 (s, 3H, -CH_3); ^{13}C NMR; 166.5 (C=O), 150.9, 143.8, 141.7, 137.9, 137.0, 132.5, 130.9, 130.3, 130.2, 130.1, 127.1, 125.0, 122.9, 120.4, 120.2, 120.1, 111.5, 106.4, 21.3; Anal. Calcd. For $C_{24}H_{19}N_5O_3S_2$ (489.56) C (58.88%), H (3.91%), N (14.31%), S (13.10%); Found C (58.95%), H (3.98%), N (14.26%), S (13.16%).

4-{2-[2-(5-Chloro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)hydrazino]-1,3-thiazol-4-yl}-*N*-phenyl-tolylsulfonamide (4b)

Yield, 88%; Mp 341–343 °C; R_f value = 0.58; IR cm^{-1}: 3308, 3124 (3NH), 3061 (CH- arom.), 1671 (C=O), ^{1}H NMR δ: 13.24 (s,1H, NH), 11.26 (s, 1H, NH), 10.29 (s, 1H, NH), 8.39–6.81 (m, 12H, $CH_{arom.}$), 2.33 (s, 3H, -CH_3); ^{13}C

NMR δ: 163.3 (C=O), 150.9, 143.9, 143.8, 141.0, 140.2, 138.7, 137.9, 137.0, 131.3, 130.2, 129.9, 127.1, 127.0, 127.0, 125.8, 121.8, 120.4, 120.1, 119.4, 21.3; Anal. Calcd. For $C_{24}H_{18}ClN_5O_3S_2$ (524.01) C (55.01%), H (3.46%), N (13.36%), S (12.24%); Found; C (54.98%), H (3.39%), N (13.29%), S (12.18%); MS: *m/z*: 524 $[M]^+$.

4-{2-[2-(5-Nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)hydrazino]-1,3-thiazol-4-yl}-*N*-phenyltolysulfonamide (4c)

Yield, 78%; Mp 312–314 °C; R_f value = 0.60; IR cm^{-1}: 3247- 3154 (3NH), 3085 (C–$H_{arom.}$), 1654 (C=O), ^{1}H NMR *δ:* 10.28 (s,1H, NH), 9.14 (s, 1H, NH), 8.64–7.11 (m, 13H, CH_{arom} + $NH_.$), 2.32 (s, 3H, CH_3), ^{13}C NMR δ: 163.5 (C=O), 146.6, 146.2, 144.9, 134.1, 133.5, 133.0, 131.5, 130.0, 130.7, 130.3, 128.9, 128.7, 128.6, 127.2, 123.8, 123.6, 123.4, 22.4; Anal. Calcd. For $C_{24}H_{18}N_6O_5S_2$ (534.56); C (53.92%), H (3.39%), N (15.72%), S (12.00%) Found: C (53.86%), H (3.46%), N (15.65%), S (12.06%); MS: *m/z*: 534 $[M]^+$.

(4-(2-(2-(1-methyl-2-oxoindolin-3-ylidene)hydrazinyl)thiazol-4-yl)-*N*-(p-tolyl) benzene sulfonamide (4d)

Yield, 82%; Mp 328–330 °C; R_f value = 0.64; IR cm^{-1}: 3217, 3161 (2NH), 3078 (C–$H_{arom.}$), 1658 (C=O), ^{1}H NMR *δ* 13.16 (br,1H, NH), 10.45 (s, 1H, NH), 7.75–7.02 (m, 13H, $CH_{arom.}$), 3.73 (s, 3H, N-CH_3), 2.30 (s, 3H, CH_3); ^{13}C NMR δ: 161.2 (C=O), 152..4, 143.8, 142.1, 138.7, 137.1, 133.0, 131.2, 130.2, 129.8,128.9, 127.2, 123.3, 122.2, 120.5, 119.5, 119.3, 115.4, 34.5, 21.3; Anal. Calcd. For $C_{25}H_{21}N_5O_3S_2$ (503.36); C (59.62%), H (4.20%), N (13.91%), S (12.73%) Found; C (59.69%), H (4.16%), N (13.84%), S (12.85%); MS: *m/z*: 503 $[M]^+$.

4-{2-[2-(5-Chloro-2-oxo-1,2-dihydro-*N*-methyl-3H-indol-3-ylidene)hydrazino]-1,3-thiazol-4-yl}-N-phenyl-4 tolylsulfonamide (4e)

Yield, 92%; Mp 330–335 °C; R_f value = 0.62; IR cm^{-1}: 3190, 3167 (2NH), 3047 (C–$H_{arom.}$), 1661 (C=O), ^{1}H NMR *δ* 12.27 (s,1H, NH), 10.33 (s, 1H, NH), 7.79–6.99 (m, 12H, $CH_{arom.}$), 3.75 (s, 3H, N-CH_3), 2.33 (s, 3H, CH_3), ^{13}C NMR δ (ppm): 190.6 (C=O), 146.6, 146.5, 140.5, 137.3, 134.2, 132.8, 131.8, 131.1, 130.8, 129.0, 128.6, 128.5, 127.5, 126.0, 123.3, 122.7, 122.3,121.8, 118.7, 31.6, 21.6; Anal. Calcd. For $C_{25}H_{20}ClN_5O_3S_2$(538.04) C (55.81%), H (3.75%), N (13.02%), S (11.92%) Found C (55.86%), H (3.83%), N (12.97%), S (11.86%); MS: *m/z*: 538 $[M]^+$.

4-{2-[2-(5-Nitro-2-oxo-*N*-methyl-3H-indol-3-ylidene) hydrazino]-1,3-thiazol-4-yl}-*N*-phenyl-4-tolylsulfonamide (4f)

Yield, 86%; Mp 305–307 °C; R_f value = 0.60; IR cm^{-1}: 3251, 3126 (2NH), 3085 (C–$H_{arom.}$), 1654 (C=O), ^{1}H NMR *δ* 9.18 (s,1H, NH), 9.03 (s, 1H, NH), 8.64–7.11 (m, 12H, $CH_{arom.}$), 3.84 (s, 3H, N-CH_3), 2.34 (s, 3H, CH_3), ^{13}C NMR δ: 162.3 (C=O), 154.2, 153.0, 151.0, 150.3, 146.3, 133.2, 132.7, 132.6, 132.3, 131.7, 130.7, 129.8, 129.7, 129.3, 128.6, 124.4, 123.0, 30.6, 22.0; Anal. Calcd. For $C_{25}H_{20}N_6O_5S_2$ (548.59); C (54.73%), H (3.67%), N (15.32%), S (11.69%) Found: C (54.69%), H (3.73%), N (15.26%), S (11.78%). MS: *m/z*: 548 $[M]^+$.

Biological evaluation

In vivo anti-inflammatory activity

For the in vivo assessment of the test compounds' anti-inflammatory activity, male Wister rats weighing 180 ± 10 g each were employed, with celecoxib serving as the reference drug. All animals had to acclimate to the criteria set by the Institutional Animals Ethics Committee (IAEC) of the Faculty of Science at Sohag University for at least one week prior to the investigations (permit No;). For this in vivo evaluation, 40 adult male Westar rats (n = 4) were randomly assigned. The selected agents were suspended in 1% newly prepared carboxy methyl cellulose (CMC) prior to being administered orally by gavage. Following a sub plantar injection of 100 µL of freshly prepared carrageenan gel (1% distilled water) into each rat's left hind paw, changes in paw thickness were observed [46]. Rats were administered test compounds orally via gavage one hour before the injection of carrageenan. Paw thickness was measured one, three, and five hours after the development of inflammation. The tested compound's effects were quantified as a percentage of edema inhibition. The anti-inflammatory potential is expressed as a percentage suppression of paw edema and quantified [52].

Histopathological analysis of the tissues in the paws

Prior to being embedded in paraffin, the tissues from the paws were stored in a 10% formalin-neutral buffer. Hematoxylin and eosin (H&E) were used to stain the slides after thin sections of 5–6 µm were cut using a microtome. The slides that were made with a light microscope exhibit pathological changes in them.

In vitro COX inhibition Screening

By utilizing the appropriate Human enzyme immune assay (ELIA) kits (Cayman Chemical, USA) and adhering to the

manufacturer's instructions, the inhibitory activities of the test hybrids **4b** and **4f** versus COX-1, and COX-2 enzymes were assessed. The results were expressed as an IC_{50} value (μM). The data for studies conducted in triplicate are shown as $IC_{50} \pm SD$. As positive controls, we employed the well-known COX-1, and COX-2 inhibitors indomethacin and celecoxib [53].

Histological evaluation of ulcers

To assess the ulcerogenic potential of different hybrids, twenty-four adult male Albino Wistar rats weighing 180 ± 20 g were divided into four groups. Water was supplied to the animals as needed throughout their overnight fast before treatment. The chosen hybrids were given orally at a dose of 50 mg/kg, whereas the positive control was indomethacin (30 mg/kg suspension in 1% CMC orally). Four hours later, the rats were slaughtered, and to assess the extent of gastrointestinal injury, their stomachs were promptly removed, cleaned with ice-cold saline, and preserved in 10% formalin saline [54]. The stomach tissues from each group were thinly sliced and prepared into slides, which were then stained with hematoxylin and eosin (H&E) for microscopic examination [55].

Assessment of the cytotoxicity of selected hybrids on RAW cells

RAW 264.7 cells were seeded at 5000 cells per well in a 96-well plate and incubated for 24 h. Following this, the cells were exposed to the investigated hybrids (**4b** and **4f**) at five concentrations (5, 10, 20, 40, and 80 μM) for two hours before being stimulated with 1 μg/mL lipopolysaccharide (LPS) for 48 h. The cytotoxic activity of the produced hybrids was determined using the MTT assay. The hybrids were used at subtoxic concentrations to the cells in all subsequent tests to prevent any cytotoxic influence [56,57].

Docking study

The crystal structure of COX-2 was downloaded from Protein Data Bank (PDB:1CX2) and the molecular docking was performed following our previously reported work [52].

Statistical analysis

The obtained data were statistically analyzed using GraphPad Prism version 9, and the mean values and standard deviations (mean ± SD) were presented as a result. The significance of mean differences was evaluated using the Tukey–Kramer test and one-way analysis of variance (ANOVA), with p-values of less than 0.05 being considered statistically significant.

Conclusion

New series of novel indole-thiazole hybrids derivatives **4a–f** were synthesized via multi-components of thiosemicarbazide with some isatine derivatives **a–f** and *N*-(4-(2-bromoacetyl)phenyl)-4-methylbenzenesulfonamide (**3**) under reflux in ethanol. The chemical structures of novel compounds were elucidated by elemental and spectral analyses. All new compounds have been screened for their anti-inflammatory activity using celecoxib as a reference drug. It has been found that compound **4b** (3 h; EI = 38.50%, 5 h; EI = 45.63%) has the most promising and effective anti-inflammatory potential. Furthermore, molecular docking study of compounds **4b** and **4f** displayed that these compounds fitted into the COX-2 binding site with good docking energy scores.

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Author contribution All authors wrote the main manuscript text and reviewed the manuscript.

Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval This study has been carried on rats and the authors take personal responsibility for knowingtheir statutory responsibility under the Animal (Scientific Procedures) Act 1986, under the acceptance of Committee for Scientific Research Ethics (CSRE), code no. (CSRE-23-24)-Sohag University.

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