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Design, synthesis and biological evaluation of novel diphenylthiazole-based cyclooxygenase inhibitors as potential anticancer agents

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications as analgesics and antipyretics. Currently, there is a growing interest in their antitumor activity and their ability to reduce the risk and mortality of several cancers. While several studies revealed the ability of NSAIDs to induce apoptosis and inhibit angiogenesis in cancer cells, their exact anticancer mechanism is not fully understood. However, both cyclooxygenase (COX)-dependent and -independent pathways were reported to have a role. In an attempt to develop new anticancer agents, a series of diphenylthiazole substituted thiazolidinone derivatives was synthesized and evaluated for their anticancer activity against a panel of cancer cell lines. Additionally, the inhibitory activity of the synthesized derivatives against COX enzymes was investigated as a potential mechanism for the anticancer activity. Cytotoxicity assay results showed that compounds **15b** and **16b** were the most potent anticancer agents with half maximal inhibitory concentrations (IC_{50}) between **8.88** and **19.25** μM against five different human cancer cell lines. Interestingly, COX inhibition assay results were in agreement with that of the cytotoxicity assays where the most potent anticancer compounds showed good COX-2 inhibition comparable to that of celecoxib. Further support to our results were gained by the docking studies which suggested the ability of compound **15b** to bind into COX-2 enzyme with low energy scores. Collectively, these results demonstrated the promising activity of the newly designed compounds as leads for subsequent development into potential anticancer agents.

1. Introduction

Cancer remains one of the major leading causes of mortality all over the world. Although the extensive studies and the wide variety of the existing anticancer agents, the cancer treatment remains an aggravating and challenging problem. Therefore, development of novel anticancer drugs with better therapeutic profile and less side effects to combat this tenacious disease is still in demand. During the past few years, cyclooxygenases (COX-1 and COX-2), key enzymes catalyze the rate-limiting step in prostaglandins biosynthesis, were introduced as novel targets for cancer treatment [1,2]. Currently, there is an increasing body of evidence stating that targeting COX enzymes, especially COX-2 isoform, is an effective approach for the prevention or treatment of various types of cancers. Several studies have showed that COX-2 is overexpressed in solid malignancies such as colon, prostate, and breast [3]. A significant relationship between over-expression of COX-2 and survival of patients with various cancers has been reported in retrospective studies [4]. Moreover, it was reported that the administration of NSAIDs such as sulindac and salicylates can induce apoptosis in cultured HT-29 human colon cancer cells [4]. It was also proposed that these effects may occur by both COX-dependent and COX-independent mechanisms [5]. Another mechanism by which NSAIDs may exert their anticancer effects involves the suppression of angiogenesis. In addition, COX-2 was found to induce

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pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and produce prostaglandins that have both autocrine and paracrine effects on the proliferation and the migration of endothelial cells *in vitro* [6]. Conversely, there are some other compelling evidence suggesting COX-2 independent mechanisms that might be involved [7,8]. Two NSAIDs, sulindac (**1**) and celecoxib (**2**), Fig. 1, have been found to inhibit the growth of adenomatous colorectal polyps and cause regression of existing polyps in patients with familial adenomatous polyposis (FAP) [2,9,10]. Thus, celecoxib has been recently approved as a chemopreventive in colon cancer and presently investigated as a chemotherapeutic for other advanced cancers. Notably, indomethacin showed an inhibitory effect on human colorectal cancer through apoptosis induction and G1 arrest [11]. It was also found that it suppresses angiogenesis via the inhibition of mitogen activated protein kinase activity (MAPK) [12]. From a chemical point of view, diphenylheterocycle has been recognized earlier as a promising backbone for COX inhibitors design. It is found in several drugs such as celecoxib and valedoxib and their relevant structural analogues which are used as potent anti-inflammatory and analgesic agents [13]. Another example for diphenylheterocycle based-COX inhibitors is a series of diarylthiazoles (**3**) which was prepared and showed high affinity for COX enzymes, preferentially for COX-2 subtype, Fig. 1 [14].

On the other hand, thiazolidinone and thiazolidinedione (TZD) are important and frequently used scaffolds in drug design and discovery that possess a huge variety of biological activities including an anticancer effect [15–17]. Numerous compounds bearing the TZD moiety such as the PI3K inhibitor, GSK1059615 (**4**) [18] and thiazolidinone-based derivatives (**5** and **6**) have been recognized as potential anticancer agents, Fig. 2 [19]. A current paradigm proved that 4-thiazolidinone derivatives exhibit their anticancer activity through acting on different antitumor biotargets such as tumor necrosis factor TNFα [20], antiapoptotic biocomplex Bcl-XL-BH3 [21], many types of phosphatases [22–24] and integrin $\alpha_v\beta_3$ receptor [25].

Based on the aforementioned data and the existing need for developing superior anticancer agents, we herein describe our efforts to design and synthesize novel hybrid molecules that could be used as potential anticancer agents with more potent pharmacological profile and less toxicity. In doing so, we combined the thiazolidinone skeleton with the diphenylthiazole as a COX-scaffold using the tethering technique in drug discovery, Fig. 3. Considering the earlier displayed reports on the biological activity of both diphenylthiazole derivatives and thiazolidinone moiety, we could infer that the newly designed compounds would exhibit good antitumor activity via, at least in part, inhibiting COX enzymes.

2. Results and discussion

2.1. Chemistry

The chemistry used for preparing the novel target compounds was straightforward and the general synthetic pathways are depicted in Schemes 1 and 2. Benzoin was reacted with thionyl chloride in pyridine to afford desyl chloride **8** which was readily condensed with thiourea in absolute ethanol using a previously reported method to give the unsubstituted starting material **10a**, 4,5-diphenylthiazol-2-amine, in high yield [26]. In order to prepare the sulfonamide derivative **10b**, the desyl chloride **8** was firstly subjected to a chlorosulfonation reaction using excess amount from chlorosulfonic acid in methylene chloride at room temperature for 24 h to form the sulfonyl chloride derivative which was subsequently aminated with NH_4OH in THF to afford the respective sulfonamide substituted desyl chloride **9**. The cyclization of derivative **9** was carried out as mentioned earlier with thiourea to afford the sulfonamide derivative **10b**. The intermediates **11a** and **11b** were obtained via reaction of the starting materials **10a** and **10b** with chloroacetyl chloride in methylene chloride and triethylamine at 0 °C. The heterocyclization of the intermediates **11a** and **11b** using ammonium isothiocyanate in refluxing ethanol efficiently furnished 2-((4,5-Diphenylthiazol-2-yl)imino)thiazolidin-4-one **12a** and 4-(2-((4-Oxothiazolidin-2-ylidene)amino)-4-phenylthiazol-5-yl)benzenesulfonamide **12b** respectively, Scheme 1. The mechanism that has been proposed for the formation of thiazolidine-4-one derivatives **12a** and **12b** was shown in Fig. 4 as previously reported [27]. Consequently, the final compounds **13–16** were obtained in excellent yields using Knoevenagel condensation of the intermediates **12a** and **12b** with the appropriate aldehyde in toluene in the presence of ammonium acetate. It is noteworthy that the methine proton mostly appeared downfield as a singlet with chemical shift equal to more than 7.00 ppm due to the reported deshielding effect attributed to the adjacent carbonyl group owing to the presence of the Knoevenagel reaction products as only Z isomers [28]. All the newly synthesized compounds (Table 1) were characterized and confirmed by elemental analysis and various spectral data (^{1}H NMR, ^{13}C NMR and HRmass).

2.2. Pharmacological screening

2.2.1. Anticancer activity

The newly synthesized compounds were screened *in vitro* for their anti-proliferative activity against five cancer cell lines; HCT-116 (human colon cancer), Caco-2 (human colon carcinoma), MCF-7 (human breast carcinoma), DU-145 (human prostate carcinoma, epithelial-like cell line) and PC-3 (human Prostate Carcinoma). The results were expressed in terms of IC_{50} values (the concentration that caused a 50% inhibition) where the well-known anticancer agent doxorubicin was used as positive control. It was observed from the results (Table 2) that compounds **12a** was inactive and **12b** showed the least potency with IC_{50} values of more than 140.6 μM against the all used cancer cell lines however, **12b** bearing a sulfonamide group exhibited slightly increased activity. This observation confirms that the benzylidene moiety at the 5-position of the 4-thiazolidinone is essential for the biological activity including anticancer effect based on the well-established previous postulate [16,17,29,30]. Subsequently, extensive SAR studies were conducted to show how the substitution at 5-position with various substituted benzylidene or other moieties affected

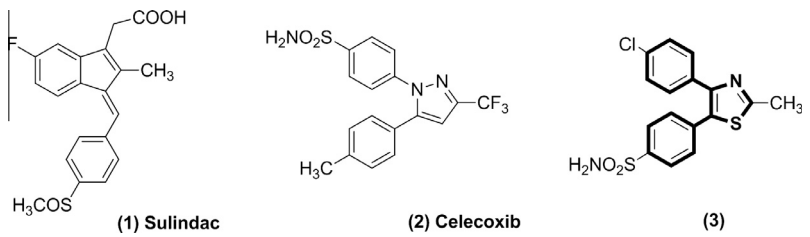


Fig. 1. Chemical structures of some reported NSAIDs with anticancer activity.

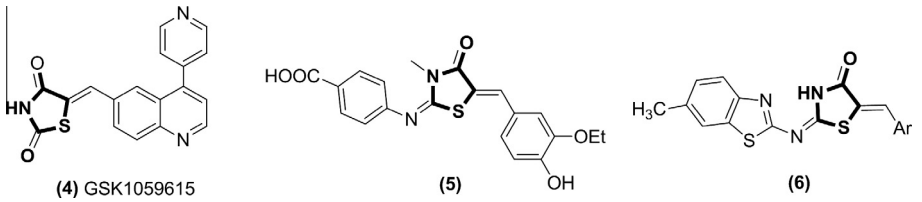


Fig. 2. Chemical structures of some thiazolidinone and thiazolidinedione derivatives with anticancer activity.

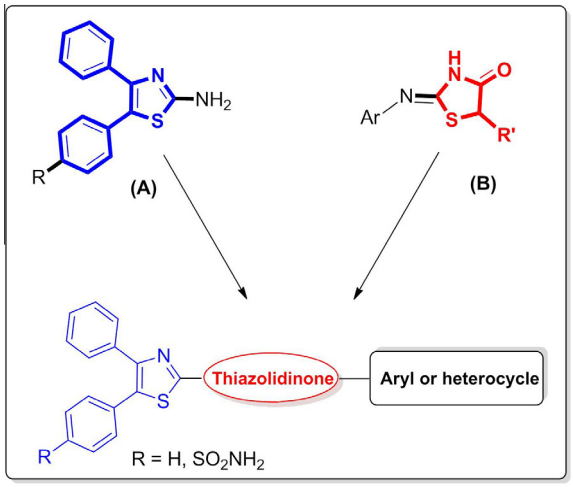
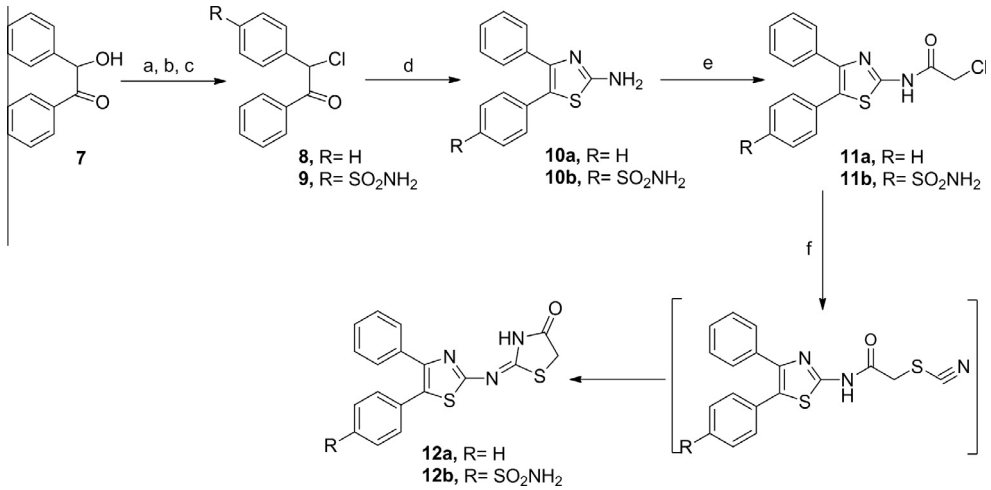


Fig. 3. Design strategy of the newly synthesized compounds.

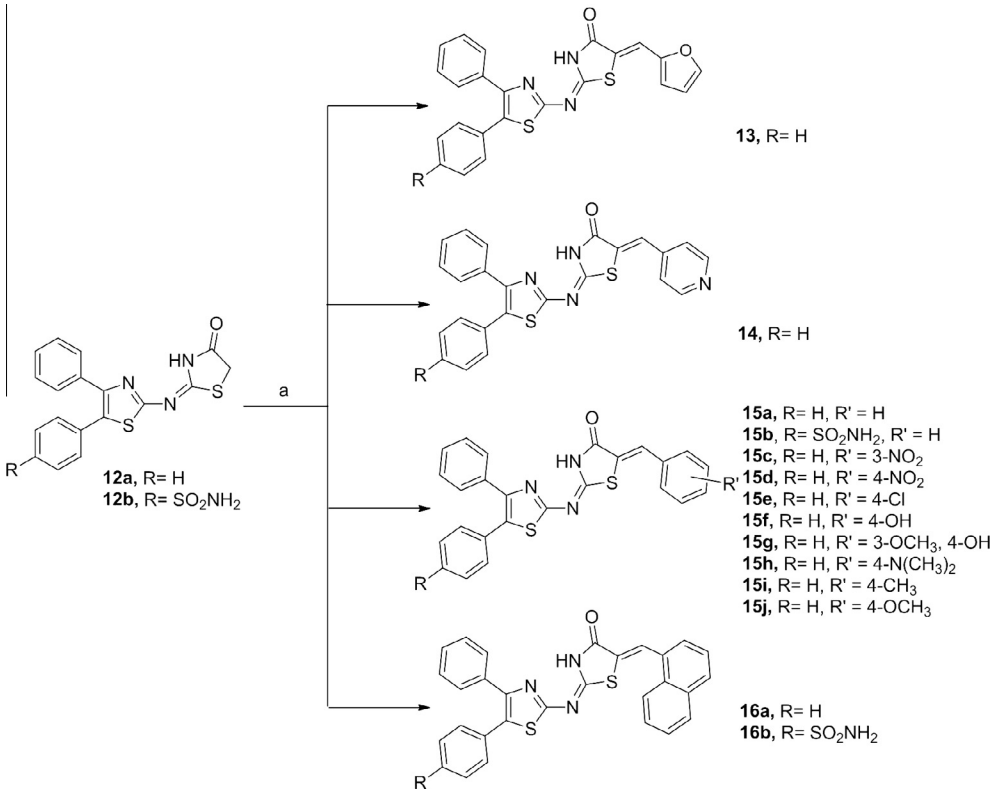
the antiproliferative activities. In doing so, the extension of the chemical structure of compound **12a** with furan-2-ylmethylene moiety in **13**, resulted in an observed increase in the anticancer activity nearly 53–62%. Furthermore, the replacement of the five-membered furan ring with pyridine ring in compound **14** revealed an increased activity with IC_{50} values between 23.68 and 62.16 μM. Compound **15a** with an unsubstituted benzylidene moiety showed comparable anticancer activity to the furyl derivatives **13**. However, a marked increase in the anticancer activity of **15a** was observed on substitution with the COX-2 directing sulfamoyl group as in compound **15b** with IC_{50} values between 12.04 and 19.25 μM suggesting a possible critical role for the COX enzyme as a molecular mechanism for the anticancer activities of the newly synthesized compounds. In addition, an increase in the antiproliferative activity was observed by substitution the 5-benzylidene moiety with electron withdrawing groups as in compounds **15c** and **15d** with exception of the 4-chloro analogue **15e**. The effect of using electron withdrawing groups on the activity of compound **15a** was nearly equal to that effect of the sulfamoyl group as in **15b**. It can be seen that the position of the substituent on the 5-benzylidene affects the anticancer activity. *The meta*-nitro derivative **15c** exerted slightly higher anticancer activity compared to its *para*-analog **15d**. Similarly, the substitution of 5-benzylidene moiety with electron donating groups resulted in an increase in the anticancer activity, but this increase in anticancer activity due to the electron donating groups in **15f-j** is slightly less than that of the electron withdrawing groups. Finally, the replacement of the 5-benzylidene moiety with the bulky 5-(naphthalen-1-ylmethylene) moiety in **16a** revealed an increase in the anticancer activities. These results demonstrate that the effect of the substitution on the anticancer activity is mainly sterically more than electronically. Interestingly, compound **16b** with a sulfamoyl group was the most active compound in the series with IC_{50} values in the range 8.88–17.02 μM. Overall, the antiproliferative activity of our compounds was essentially affected by the presence of a substituted 5-benzylidene or more bulky moieties in addition to a sulfonamide group on the diphenylthiazole system.

2.2.2. COX inhibition assay

It is well known that COX enzymes, especially COX-2, are overexpressed in many types of tumors such as colon cancer, breast cancer, and melanoma [31–33]. This strong association existing between COX enzymes and cancer make it a valid target for prevention and treatment of cancer. In an attempt to investigate the molecular mechanism of the new diphenylthiazole derivatives as



Scheme 1. [a] Reaction reagents and conditions: (a) $SOCl_2$, pyridine, 1 h; (b) chlorosulfonic acid, CH_2Cl_2, 0 °C, stirring, overnight; (c) NH_4OH, THF, rt, 2 h; (d) thiourea, EtOH, reflux, 2 h; (e) $ClCH_2COCl$, TEA, CH_2Cl_2, rt, 5 h; (f) NH_4SCN, acetone.



Scheme 2. [a] Reaction reagents and conditions: (a) Aldehyde, CH_3COONH_4, toluene, reflux, 3 h.

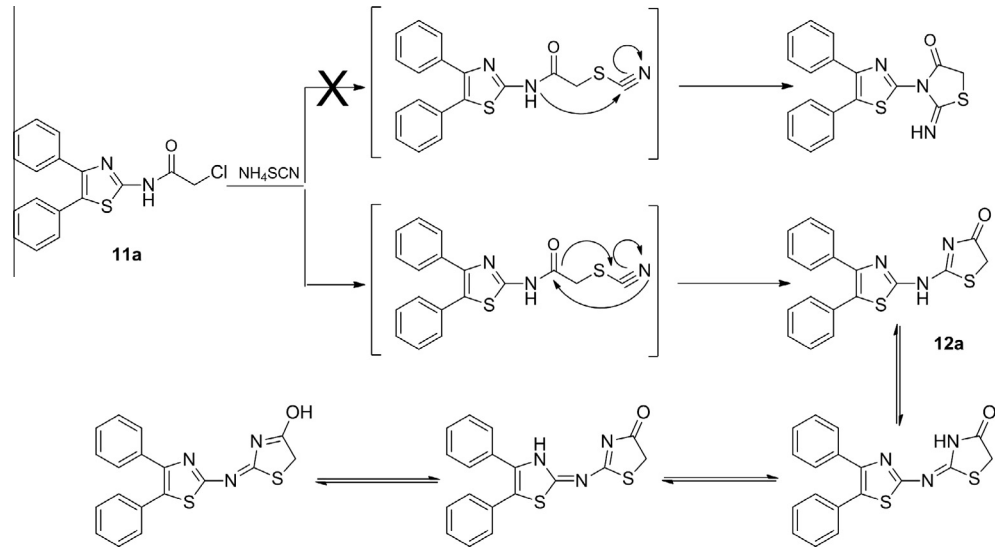
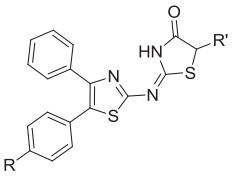


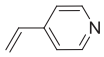
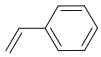
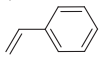
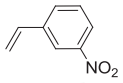
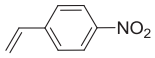
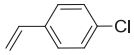
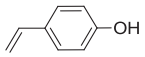
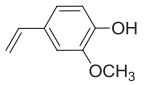
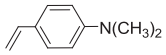
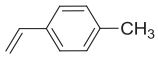
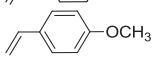
Fig. 4. The proposed mechanism for the formation of thiazolidine-4-one ring and all possible tautomers.

antiproliferative agents, COX inhibition assay was performed at concentration of 10 μM and 0.5 μM for COX-1 and COX-2 respectively, using celecoxib as a positive control. The results were expressed in terms of inhibition percentages and presented in Fig. 5. Obviously, most of the compounds, except **12b** and **15h**, showed moderate to high inhibitory activities compared to celecoxib with different selectivity profiles. Compound **12b** showed a weak inhibitory activity against both COX subtypes which is owing to the absence of the substituted 5-benzylidene moiety while a reverse trend of relatively increased activities was observed with the rest of compounds except compound **15h**. The replacement of the furan ring in **13** with pyridine in **14** resulted in a marked increase in the inhibitory activity and selectivity for COX-1 without any change in the inhibition percentage of COX-2. Moreover, compounds **15g** and **15i** bearing electron donating groups were the most active among the substituted benzylidene derivatives with high COX-2 selectivity.

On the other hand, compounds **15c**, **15d** and **15e** with electron withdrawing groups showed good inhibitory activity against both COX subtypes with slight preference to COX-1. It was clear from the results that the electronic effect on the COX inhibitory activity was nearly similar but it affects the selectivity profiles. In addition,

Table 1
List of the newly synthesized diphenylthiazole derivatives (**12–16**) with molecular weights, melting points and yields.



Comp.	R	R′	Molecular formula	Mol. Wt.	M.P. (°C)	Yield (%)
12a	H	H	$C_{18}H_{13}N_3OS_2$	351.45	240–242	67
12b	SO_2NH_2	H	$C_{18}H_{14}N_4O_3S_3$	430.52	146–148	51
13	H		$C_{23}H_{15}N_3O_2S_2$	429.51	277–278	81
14	H		$C_{24}H_{16}N_4OS_2$	440.54	218–220	77
15a	H		$C_{25}H_{17}N_3OS_2$	439.55	253–254	74
15b	SO_2NH_2		$C_{25}H_{18}N_4O_3S_3$	518.63	112–114	59
15c	H		$C_{25}H_{16}N_4O_3S_2$	484.55	261–262	72
15d	H		$C_{25}H_{16}N_4O_3S_2$	484.55	275–277	74
15e	H		$C_{25}H_{16}ClN_3OS_2$	474.00	272–275	73
15f	H		$C_{25}H_{17}N_3O_2S_2$	455.55	283–285	71
15g	H		$C_{26}H_{19}N_3O_3S_2$	485.58	213–215	77
15h	H		$C_{27}H_{22}N_4OS_2$	482.62	272–274	70
15i	H		$C_{26}H_{19}N_3OS_2$	453.58	263–265	78
15j	H		$C_{26}H_{19}N_3O_2S_2$	469.58	231–233	76
16a	H		$C_{29}H_{19}N_3OS_2$	489.61	241–244	73
16b	SO_2NH_2		$C_{29}H_{20}N_4O_3S_3$	568.69	165–166	54

compound **16a** with the bulky naphthyl moiety exhibited good inhibitory activity against COX-1 and COX-2 similar to compounds **15**.

It is well established that COX-2 active site is larger than that of COX-1 and it is characterized by the presence of a side pocket allowing accommodation of bulky compounds bearing additional groups such as sulfonamide or methylsulfone [34]. Based on this fact, it was found that compounds **15b** and **16b** bearing a sulfonamide moiety exhibited good inhibitory activities with high selectivity towards COX-2. Generally, it can be concluded that both free sulfamoyl and the increase in the molecular volume by addition of aryl-methylene moiety play the major role in increasing inhibitory activities against COX subtypes and they enhance the COX-2 selectivity.

Comparing the COX inhibition results with that of the anticancer screening assay, there was a consistency between both results where compounds that showed high COX inhibitory activity, in particular with high COX-2 selectivity, as in compounds **15b** and **16b** were the most active antiproliferative agents while the compounds **12b**, **13**, **15a** and **15h** with weak COX inhibitory activities showed the lowest anticancer activity. Based on these findings, it could be concluded that there is a strong correlation between the COX inhibition and the anticancer activity which confirms our suggestion of COX inhibition as a possible molecular mechanism for our compounds as anticancer agents beside other mechanisms that should be further investigated.

2.3. Molecular Docking Study

In order to understand the structural basis for the COX-2 activity, selectivity and the proper binding mode of the newly synthesized diphenylthiazole ligands, two compounds, **12b** and **15b**, were selected to be docked into the active sites of COX-2 using LIGANDFIT embedded in Discovery Studio software [35]. The 3D crystal structure of COX-2 complexed with a cocrystallized inhibitor (PDB code: **6COX**) was selected for this study. It was reported that the substitution of Ile523 in COX-1 with the less bulky Val523 in COX-2 creates an additional polar side pocket and

Table 2
IC_{50} values for the newly synthesized compounds on five cancerous cell lines; HCT-116, Caco-2, MCF-7, DU-145, PC-3 cell lines.

Comp.	IC_{50} (μM)				
	HCT-116	Caco-2	MCF-7	DU-145	PC-3
12a	NA	NA	NA	NA	NA
12b	140.6	151.7	157.62	230.14	294.52
13	71.78	75.48	79.18	83.62	95.46
14	23.68	30.34	33.3	51.06	62.16
15a	82.07	91.20	82.35	86.21	95.14
15b	12.04	13.28	16.50	19.25	14.36
15c	12.20	11.84	15.09	16.51	18.30
15d	14.06	15.54	20.72	20.72	20.9
15e	29.10	30.25	29.50	41.29	48.28
15f	21.01	24.12	29.12	26.3	34.2
15g	30.34	39.22	42.18	54.76	78.44
15h	75.48	82.88	104.34	68.82	113.96
15i	20.58	24.36	25.01	32.54	35.98
15j	20.10	24.13	21.15	40.25	39.87
16a	19.36	21.54	19.58	21.98	35.32
16b	8.88	11.10	17.02	15.90	12.58
Doxorubicin	0.97	NA	1.18	0.89	1.01

Cells were treated with the test compounds or vehicle for 48 h. Data were reported as mean ± S.D. (n = 6).
Five human cancer cell lines were used; HCT-116 (human colon cancer), Caco-2 (human colon carcinoma), MCF-7 (human breast carcinoma), DU-145 (human prostate carcinoma, epithelial-like cell line) and PC-3 (*human Prostate Carcinoma*). Doxorubicin for was used as a positive control in the anticancer screening assay. NA means Not Active.

increases the volume of the COX-2 active site that makes it accommodate more bulky structures [34]. The presence of such side pocket allows additional interactions with amino acids such as Arg513, replaced by a His513 in COX-1, and His90. The structure of traditional COX-2 inhibitors exploits binding with Arg513 in the COX-2 side-pocket, often via methylsulfone or sulfonamide groups, to accomplish their COX-2 selectivity over COX-1.

In this regard, we firstly docked one potent and COX-2 selective compound, **15b**, within the active binding site of COX-2. Obviously, from Fig. 6(B), compound **15b** assumed binding mode and interactions similar to the cocrystallized ligand, **S-58701**, 1-phenylsulfonamide-3-trifluoromethyl-5-p-bromophenylpyrazole, in which positioning the sulfonamide moiety of **15b** within the side additional pocket of COX-2 and forming H-bond with the NH of Arg513 residue was similar to fitting of same moiety of **S-58701** into the same side pocket in addition to forming another H-bond with NHs of His90 residues, Fig. 6(C). Moreover, the thiazolidinone ring tends to form additional two H-bonds with Arg-120. On the other hand, the benzylidene moiety at the 5-position of the 4-thiazolidinone seems to protrude outside the binding pocket forming hydrophobic aromatic stacking interactions with Tyr355 and Tyr115 residues, Fig. 6(A). In addition, the other phenyl ring of the Y-shaped diphenylthiazole core was hydrophobically stacked within a hydrophobic pouch composed of the hydrophobic side chains of Tyr345, Val349, Tyr385 and Trp387 which correlates well with the position of the bromophenyl fragment of **S-58701** into the same pocket, Fig. 6(A).

On the contrary, the docking of the inactive compound **12b** within the COX-2 active pocket revealed that this compound assumed a different binding pattern compared to the cocrystallized ligand, **S-58701**, Fig. 7(C), where the 4-thiazolidinone ring was deeply embedded inside the side pocket instead of the sulfonamide group, Fig. 7(B). Clearly from Fig. 7(A), sulfonamide moiety was

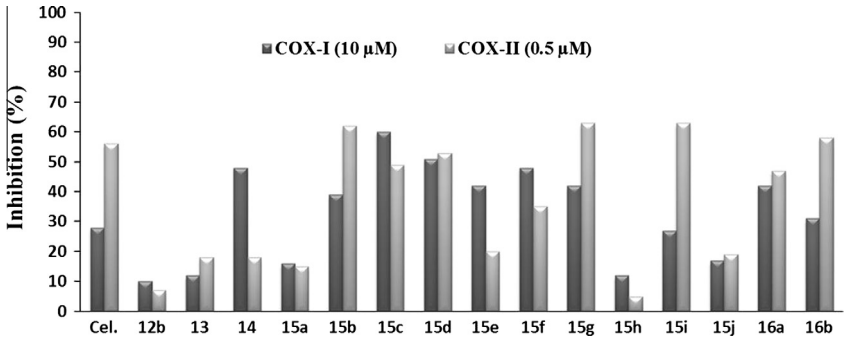


Fig. 5. The *in vitro* COX inhibition percentages of the newly synthesized compounds.

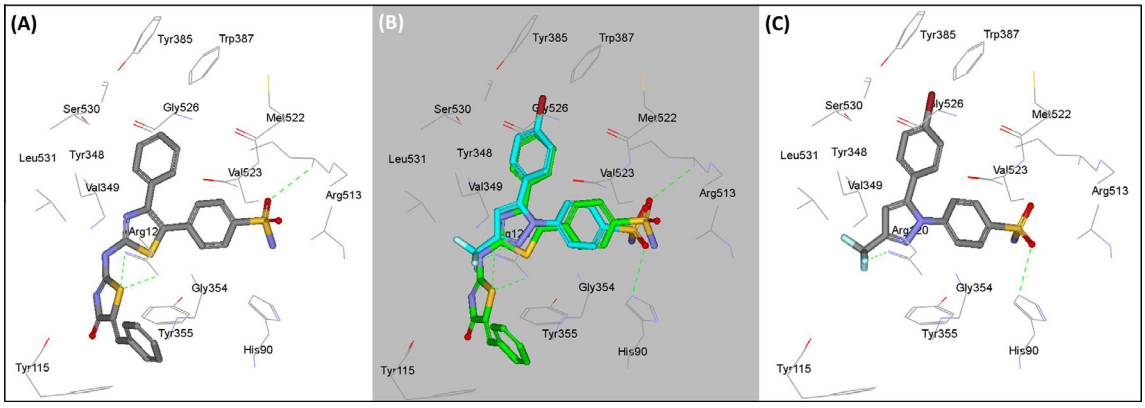


Fig. 6. (A) Docking and binding mode of compound **15b** within COX-2 active site (PDB code: **6COX**). (B) The superposition of the docked poses **15b** (green) and the co-crystallized **S-58701** (cyan) within active site of COX-2. (C) Docking and binding mode of co-crystallized **S-58701** into the same COX-2 binding pocket. Hydrogen bonds are represented by dashed green lines. All hydrogens are removed for the purposes of clarity.

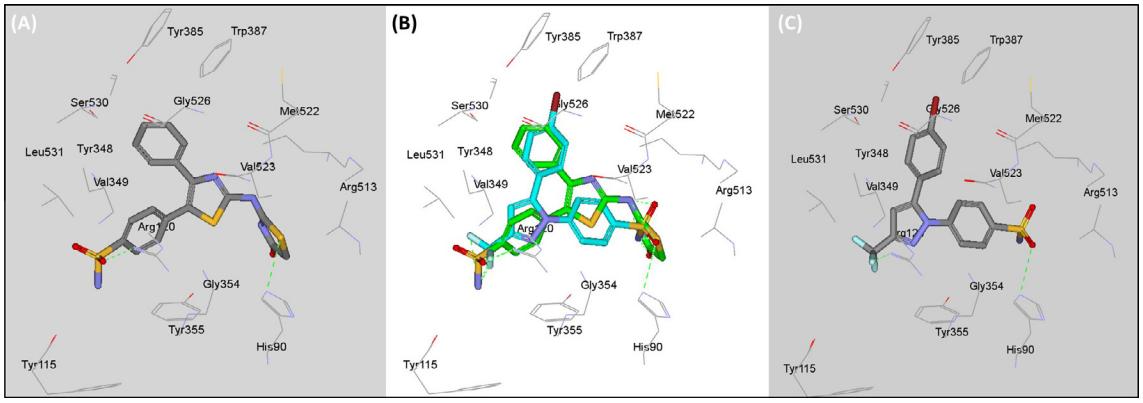


Fig. 7. (A) Docking and binding mode of compound **12b** within COX-2 active site (PDB code: **6COX**). (B) The superposition of the docked poses **12b** (green) and the co-crystallized **S-58701** (cyan) within active site of COX-2. (C) Docking and binding mode of co-crystallized **S-58701** into the same COX-2 binding pocket. Hydrogen bonds are represented by dashed green lines. All hydrogens are removed for the purposes of clarity.

hydrogen-bonded to Arg-120 residue and the 4-thiazolidinone ring formed another H-bond with His90 within the additional side corridor. The different disposition of compound **12b** within COX-2 active site could be the reason for the inferior bioactivity which reflected its low antiproliferative activity, Fig. 7(B).

3. Conclusions

A series of diphenylthiazole substituted thiazolidinone derivatives was synthesized and evaluated as anticancer agents against a panel of cancer cell lines. The COX inhibition assay was performed in order to investigate the molecular mechanism of their anticancer potential. The results showed that compounds **15b** and **16b** were the most potent anticancer agents with IC_{50} values between **8.88** and **14.36** μM against the five human cell lines (HCT-116, Caco-2, MCF-7, DU-145 and PC-3). Interestingly, the COX inhibition assay results were consistent with that of the cell viability assay where the potent anticancer compounds showed good COX-2 inhibition percentages (up to 63%) comparable to celecoxib at a concentration of 0.5 μM. Combining the results of the cell viability assay and COX inhibition, we can conclude that a strong relationship exists between the anticancer activity and the COX inhibitory activity of these compounds, and we can suggest that COX inhibition may contribute, at least in part, to the mechanism of their anticancer activity. The docking studies also support the results concluded from the COX assay and the anti-proliferation screening. It was clear that our ligands that bearing a benzylidene moiety at the 5-position of the 4-thiazolidinone in addition to a sulfonamide group have high binding affinities for COX-2 enzyme, which was reflected in their *in vitro* activity. In conclusion, these results demonstrate that our compounds are good leads for subsequent development into potential anticancer agents. Further experiments are needed to unveil additional possible molecular mechanisms involved in the anticancer activity of our compounds and to evaluate their chemotherapeutic potential in more details.

4. Experimental protocols

4.1. Chemistry

Chemical reagents and solvents were obtained from commercial sources. Solvents are dried by standard methods when necessary. Melting points (m.p.) were uncorrected and were carried out by open capillary tube method using IA 9100MK-Digital Melting Point Apparatus. Microanalyses were carried out at the microanalytical Center, Faculty of Science, Cairo University. Infrared spectra were made on BRUKER Vector 22 (Japan), infrared spectrophotometers and were expressed in wavenumber (cm^{-1}) using potassium bromide disc. The proton magnetic resonance ^1H NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer at 400 MHz and BRUKER APX400 spectrometer at 400 MHz in the specified solvent, chemical shifts were reported on the δ scale and were related to that of the solvent and J values are given in Hz. ^{13}C NMR spectra were obtained on a Bruker APX400 at 100 MHz at the faculty of pharmacy, Beni-Suef University. Mass spectra were recorded on Fennigan MAT, SSQ 7000, Mass spectrometer, at 70 eV (EI) at the microanalytical Center, Faculty of Science, Cairo University. The high resolution mass spectra (HRMS) were recorded on a Waters Micromass Q-Tof Micro mass spectrometer with a lock of spray source. Thin layer chromatography, was done using Macherey–Nagel Alugram Sil G/UV254 silica gel plates and methylene choride-methanol (9.5:0.5) as the eluting system.

2-Chloro-1,2-diphenylethanone ***(8)*** [26]. A mixture of benzoin (10 g, 47 mmol) and pyridine (5.7 ml) was heated until a solution was obtained, then cooled in an ice bath until solid. The mass obtained was coarsely ground and thionyl chloride (7.5 g, 63 mmol) was added slowly with vigorous stirring and cooling in an ice bath. After about an hour, water was added and the solid is coarsely ground, filtered and washed several times with water. The crude product was dried by suction and left overnight over calcium chloride until full dryness. The white powder obtained was recrystallized from ethanol to give colorless crystals of 2-chloro-1,2-diphenylethanone **8** (79%). MS (EI) m/z 231.15 (M^++1).

4-(1-Chloro-2-oxo-2-phenylethyl)benzenesulfonamide ***(9).*** Chlorosulfonic acid (4.0 mL, 60 mmol) was carefully added to a solution of 2-chloro-1,2-diphenylethanone **8** (2.3 g, 10 mmol) in CH_2Cl_2 (20 ml) at 0 °C. After about 30 min, the ice bath was removed and the resulting solution was stirred at room temperature for 24 h. The mixture was thereafter poured on ice/water, and extracted with CH_2Cl_2 (3 × 40 ml). The combined organic extracts were washed with saturated aqueous $NaHCO_3$, brine and dried over Na_2SO_4. The solvent was removed in vacuo and the obtained crude sulfonyl chloride product was used immediately for the next step. To a solution of the crude sulfonyl chloride (1 g, 3 mmol) in THF (20 mL) at room temperature, an excess of NH_4OH (5 ml of 30% w/v) was added dropwise with stirring. The reaction was allowed to proceed for 2 h. The reaction mixture was quenched with water (100 ml) and extracted with ethyl acetate (3 × 40 ml). The organic extract was washed with water and dried over Na_2SO_4. The solvent was removed in vacuo, and the residue was purified by flash column chromatography (SiO_2) using dichloromethane/methanol (9.5: 0.5) as eluent to give compound **9** (43%) as a yellow

solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.60 (s, 2H, $SO_2N\underline{H}_2$), 6.24 (s, 1H), 7.23–8.14 (m, 9H). MS (EI) m/z 310.32 (M^++1).

4,5-Diphenylthiazol-2-amine ***(10a).*** [26]. A solution of 2-Chloro-1,2-diphenylethanone, **8** (10 g, 41.5 mmol) in ethanol (40 ml) and thiourea (3.5 g, 45 mmol) were refluxed for 2 h. The solvent was removed under vacuum and the solid product was soaked in 100 mL of 30% NaOH at 50 °C for 5 h. The crude product was filtered and washed with water to neutrality and then was recrystallized from 95% ethanol to obtain a yellow solid (75%) of compound **10a**. MS (EI) m/z 252.00 (M^+).

4-(2-amino-4-phenylthiazol-5-yl)benzenesulfonamide ***(10b).*** Yellow solid, yield 41%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.98 (s, 2H, $SO_2N\underline{H}_2$), 7.27–7.90 (m, 9H). ^{13}C NMR (101 MHz, DMSO): δ 170.83, 145.07, 132.44, 129.80, 129.30, 129.11, 128.93, 128.72, 126.53, 124.42, 117.70. MS (EI) m/z 332.27 (M^++1).

2-Chloro-N-(4,5-diphenylthiazol-2-yl)acetamide ***(11a).*** [36]. To a solution of compound **10a** (5 g, 19.8 mmol) and triethylamine (6 g, 59.5 mmol) in CH_2Cl_2 at 0 °C, chloroacetyl chloride (2.9 g, 25.7 mmol) was added. The reaction mixture was stirred for 1 h at 0 °C then for 5 h at room temperature. The organic layer was then separated and evaporated in vacuo. The residue obtained was purified by flash column chromatography (SiO_2) using dichloromethane/methanol (9.5: 0.5) as eluent to give brown solid (62%) of 2-chloro-N-(4,5-diphenylthiazol-2-yl)acetamide **11a**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.45 (s, 2H, $C\underline{H}_2$), 7.29–7.42 (m, 10H), 10.48 (s, 1H, N$\underline{H}$). MS (EI) m/z 328.05 (M^+).

2-Chloro-N-(4-phenyl-5-(4-sulfamoylphenyl)thiazol-2-yl)acetamide ***(11b).*** Brown solid, yield 53%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.99 (s, 2H, $SO_2N\underline{H}_2$), 4.96 (s, 2H, $C\underline{H}_2$), 7.34–7.94 (m, 9H), 12.12 (s, 1H, N$\underline{H}$). MS (EI) m/z 407.15 (M^+).

2-((4,5-Diphenylthiazol-2-yl)imino)thiazolidin-4-one ***(12a).*** A mixture of 2-chloro-N-(4,5-diphenylthiazol-2-yl)acetamide, **11a** (3 g, 9.1 mmol) and ammonium thiocyanate (1.4 g, 18.2 mmol) was refluxed for 5 h in acetone (40 ml). After the reaction was completed, the mixture was cooled. The precipitate was collected by filtration, washed with water and recrystallized from ethanol/acetone mixture to afford compound **6a** as a light brown solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.03 (s, 2H, $C\underline{H}_2$), 7.29–7.49 (m, 10H), 12.17 (s, 1H, N$\underline{H}$). ^{13}C NMR (101 MHz, DMSO): δ 174.57 (C=O), 167.25, 163.87, 146.08, 134.76, 132.01, 129.82, 129.48, 128.86, 128.79, 128.74, 128.36, 35.53 ($\underline{C}H_2$). HRMS calcd. for $C_{18}H_{13}N_3OS_2$ $[M+H]^+$ 351.0500, found 352.0615. Anal. Calcd for: $C_{18}H_{13}N_3OS_2$: C, 61.52; H, 3.73; N, 11.96. Found: C, 61.15; H, 3.48; N, 12.03.

4-(2-((4-Oxothiazolidin-2-ylidene)amino)-4-phenylthiazol-5-yl) benzenesulfonamide ***(12b).*** Dark orange solid, ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.05 (s, 2H, $SO_2N\underline{H}_2$), 4.20 (s, 2H, $C\underline{H}_2$), 7.24–7.90 (m, 9H), 12.12 (s, 1H, N$\underline{H}$). ^{13}C NMR (101 MHz, DMSO): δ 174.29 (C=O), 168.14, 162.49, 145.65, 137.00, 136.47, 130.11, 129.11, 129.00, 128.94, 127.51, 126.73, 126.54, 34.83 (CH_2). HRMS calcd. for $C_{18}H_{14}N_4O_3S_3$ $[M+H]^+$ 430.0228, found 431.0377. Anal. Calcd for: $C_{18}H_{14}N_4O_3S_3$: C, 50.22; H, 3.28; N, 13.01. Found: C, 50.15; H, 3.65; N, 13.27.

General procedure A*. Synthesis of compounds* ***(13, 14, 15a-j and 16a-b).*** *2-((4,5-diphenylthiazol-2-yl)imino)-5-(furan-2-ylmethylene)thiazolidin-4-one* ***(13).*** A mixture of compound **12a** (0.5 g, 1.57 mmol) and the 2-furaldehyde (0.15 g, 1.57 mmol) was refluxed with ammonium acetate (0.25 g, 3.20 mmol) in 20 mL anhydrous toluene. The reaction mixture was refluxed for 3 h and then the solvent was evaporated. The residue was dissolved in water with acidified with conc. HCl. The product was extracted into ethyl acetate, dried over sodium sulfate and evaporated under vacuum to obtain the purified target compound **13** as greenish yellow solid. ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 6.79 (d, J = 3.50 Hz, 1H), 7.11 (t, J = 4.26, 1H), 7.33–7.43 (m, 8H), 7.57 (d, J = 6.88 Hz, 3H), 8.11 (s, 1H), 12.66 (s, 1H, N$\underline{H}$). ^{13}C NMR (101 MHz, DMSO): δ 167.23 (C=O), 166.66, 157.76, 157.71, 150.23, 147.91, 146.44, 134.63, 131.89, 129.89, 129.52, 129.01, 128.97, 128.77, 128.51, 122.11, 119.04, 118.93, 114.10. HRMS calcd. for $C_{23}H_{15}N_3O_2S_2$ $[M+H]^+$ 429.0606, found 430.0760. Anal. Calcd for: $C_{23}H_{15}N_3O_2S_2$: C, 64.32; H, 3.52; N, 9.78. Found: C, 64.51; H, 3.36; N, 10.07.

2-((4,5-Diphenylthiazol-2-yl)imino)-5-(pyridin-4-ylmethylene) thiazolidin-4-one ***(14).*** General Procedure A, orange solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.28–7.33 (m, 4H), 7.34–7.41 (m, 6H), 7.48 (d, J = 6.49 Hz, 2H), 7.54 (d, J = 4.01 Hz, 1H), 8.02 (d, J = 4.29 Hz, 1H), 8.71 (d, J = 8.20 Hz, 1H), 12.17 (s, 1H, N$\underline{H}$). ^{13}C NMR (101 MHz, DMSO): δ 174.62 (C=O), 167.23, 163.93, 150.84, 146.07, 145.30, 136.79, 134.75, 132.01, 129.87, 129.82, 129.48, 129.35, 128.85, 128.79, 128.75, 128.36, 119.87. HRMS calcd. for $C_{24}H_{16}N_4OS_2$ $[M+H]^+$ 440.0766, found 441.0809. Anal. Calcd for: $C_{24}H_{16}N_4OS_2$: C, 65.43; H, 3.66; N, 12.72. Found: C, 65.62; H, 3.57; N, 12.49.

5-Benzylidene-2-((4,5-diphenylthiazol-2-yl)imino)thiazolidin-4-one ***(15a).*** General Procedure A, yellow solid. ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 7.34–7.41 (m, 8H), 7.51–7.61 (m, 5H), 7.71 (d, J = 7.58 Hz, 2H), 7.76 (s, 1H), 12.80 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO): δ 170.03 (C=O), 166.49, 157.68, 156.80, 154.12, 146.36, 143.80, 134.59, 134.02, 132.54, 130.79, 130.59, 129.88, 129.75, 129.55, 128.92, 128.74, 128.60, 125.44. HRMS calcd for $C_{25}H_{17}N_3OS_2$ $[M+H]^+$ 439.0813, found 440.0977. Anal. Calcd for: $C_{25}H_{17}N_3OS_2$: C, 68.31; H, 3.90; N, 9.56. Found: C, 68.11; H, 3.59; N, 9.46.

4-(2-((E)-((Z)-5-Benzylidene-4-oxothiazolidin-2-ylidene)amino)-4-phenylthiazol-5-yl)benzenesulfonamide ***(15b).*** General Procedure A, brown solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.20 (s, 2H, $SO_2N\underline{H}_2$), 7.27–7.60 (m, 14H), 8.05 (s, 1H), 10.02 (s, 1H, N$\underline{H}$). ^{13}C NMR (101 MHz, DMSO): δ 172.53 (C=O), 163.13, 161.26, 137.73, 132.89, 130.00, 129.84, 129.65, 129.45, 129.35, 129.14, 129.07, 128.93, 128.66, 128.29, 128.07, 127.07, 126.72, 125.77. HRMS calcd. for $C_{25}H_{18}N_4O_3S_3$ $[M+H]^+$ 518.0541, found 519.0609. Anal. Calcd for: $C_{25}H_{18}N_4O_3S_3$: C, 57.90; H, 3.50; N, 10.80. Found: C, 57.61; H, 3.26; N, 11.07.

2-((4,5-Diphenylthiazol-2-yl)imino)-5-(3-nitrobenzylidene)thiazolidin-4-one ***(15c).*** General Procedure A, orange solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.34–7.60 (m, 9H), 7.85–7.93 (m, 3H), 8.15–8.34 (m, 2H), 8.53 (d, J = 12.07 Hz, 1H), 8.96 (s, 1H, N$\underline{H}$). ^{13}C NMR (101 MHz, DMSO): δ 167.22 (C=O), 148.71, 148.42, 147.68, 135.56, 134.63, 134.63, 131.83, 131.32, 131.07, 129.91, 129.86, 129.58, 129.12, 128.96, 128.75, 124.87, 123.45, 122.62, 118.49, 113.04. HRMS calcd. for $C_{25}H_{16}N_4O_3S_2$ $[M+H]^+$ 484.0664, found 485.0715. Anal. Calcd for: $C_{25}H_{16}N_4O_3S_2$: C, 61.97; H, 3.33; N, 11.56. Found: C, 62.15; H, 3.61; N, 11.29.

2-((4,5-Diphenylthiazol-2-yl)imino)-5-(4-nitrobenzylidene)thiazolidin-4-one ***(15d).*** General Procedure A, dark orange solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.31–7.49 (m, 11H), 7.77–7.86 (m, 2H), 8.28–8.35 (m, 2H), 10.15 (s, 1H, N$\underline{H}$). ^{13}C NMR (101 MHz, DMSO): δ 170.58 (C=O), 156.04, 148.50, 144.31, 135.13, 132.31, 129.75, 129.39, 129.05, 128.96, 128.92, 128.89, 128.72, 128.49, 128.34, 128.17, 125.94, 117.00, 112.73. HRMS calcd. for $C_{25}H_{16}N_4O_3S_2$ $[M+H]^+$ 484.0664, found 485.0505. Anal. Calcd for: $C_{25}H_{16}N_4O_3S_2$: C, 61.97; H, 3.33; N, 11.56. Found: C, 62.13; H, 3.51; N, 11.75.

5-(4-Chlorobenzylidene)-2-((4,5-diphenylthiazol-2-yl)imino)thiazolidin-4-one ***(15e).*** General Procedure A, yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.29–7.93 (m, 15H), 12.16 (s, 1H, N$\underline{H}$). ^{13}C NMR (101 MHz, DMSO): δ 182.87 (C=O), 156.75, 154.77, 132.47, 132.16, 131.39, 130.90, 130.61, 130.17, 129.91, 129.60, 129.01, 128.62, 128.23, 127.77, 127.65, 127.45, 127.35, 126.25. HRMS calcd. for $C_{25}H_{16}ClN_3OS_2$ $[M+H]^+$ 473.0423, found 474.0557. Anal. Calcd for: $C_{25}H_{16}ClN_3OS_2$,C, 63.35; H, 3.40; N, 8.87. Found: C, 63.47; H, 3.73; N, 8.96.

2-((4,5-Diphenylthiazol-2-yl)imino)-5-(4-hydroxybenzylidene) thiazolidin-4-one ***(15f).*** General Procedure A, dark yellow solid. ^{1}H

NMR (400 MHz, DMSO-d_6): δ 4.01 (s, 1H, OH), 6.93 (d, J = 8.23 Hz, 2H), 7.32–7.37 (m, 8H), 7.52 (d, J = 9.14 Hz, 4H), 7.63 (s, 1H), 12.62 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO): δ 167.57 (C=O), 166.57, 160.22, 157.08, 146.20, 134.70, 133.03, 132.93, 131.90, 129.84, 129.43, 129.35, 128.99, 128.91, 128.76, 128.44, 124.97, 121.02, 116.67. HRMS calcd. for $C_{25}H_{17}N_3O_2S_2$ $[M+H]^+$ 455.0762, found 456.0769. Anal. Calcd for: $C_{25}H_{17}N_3O_2S_2$: C, 56.91; H, 3.76; N, 9.22. Found: C, 56.64; H, 3.53; N, 8.97.

2-((4,5-Diphenylthiazol-2-yl)imino)-5-(4-hydroxy-3-methoxybenzylidene)thiazolidin-4-one ***(15g).*** General Procedure A, dark Yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.78 (s, 3H, CH_3), 4.02 (s, 1H, OH), 6.93 (s, 1H), 7.18–7.65 (m, 13H), 12.64 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO): δ 167.56 (C=O), 166.65, 157.39, 156.77, 149.86, 148.45, 146.52, 134.77, 133.43, 131.88, 129.82, 129.47, 128.92, 128.77, 128.48, 126.29, 125.22, 120.74, 116.52, 113.15, 99.98, 55.88. HRMS calcd. for $C_{26}H_{19}N_3O_3S_2$ $[M+H]^+$ 485.0868, found 486.0891. Anal. Calcd for: $C_{26}H_{19}N_3O_3S_2$: C, 64.31; H, 3.94; N, 8.65. Found: C, 64.67; H, 4.12; N, 8.97.

5-(4-(Dimethylamino)benzylidene)-2-((4,5-diphenylthiazol-2-yl)imino)thiazolidin-4-one ***(15h).*** General Procedure A, orange solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.03 (s, 6H, $2CH_3$), 6.82 (d, J = 8.52 Hz, 2H), 7.29–7.61 (m, 13H), 12.54 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO): δ 166.98 (C=O), 161.41, 156.49, 147.63, 134.61, 132.66, 129.87, 129.56, 129.53, 129.03, 128.96, 128.80, 128.74, 128.56, 127.50, 127.49, 124.59, 119.86, 115.36, 46.25 ($2CH_3$). HRMS calcd. for $C_{27}H_{22}N_4OS_2$ $[M+H]^+$ 482.1235, found 483.1408. Anal. Calcd for: $C_{27}H_{22}N_4OS_2$: C, 67.19; H, 4.59; N, 11.61. Found: C, 67.34; H, 4.79; N, 11.36.

2-((4,5-Diphenylthiazol-2-yl)imino)-5-(4-methylbenzylidene)thiazolidin-4-one ***(15i).*** General Procedure A, shiny yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.55 (s, 3H, CH_3), 7.14 (d, J = 12.42 Hz, 2H), 7.34–7.66 (m, 12H), 7.71 (s, 1H), 12.70 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO): δ 174.41 (C=O), 167.02, 163.71, 150.62, 145.85, 145.08, 136.58, 134.53, 131.80, 129.61, 129.26, 129.14, 128.64, 128.58, 128.54, 128.44, 128.14, 123.02, 119.65, 25.31. HRMS calcd. for $C_{26}H_{19}N_3OS_2$ $[M+H]^+$ 453.0970, found 454.1008. Anal. Calcd for: $C_{26}H_{19}N_3OS_2$: C, 68.85; H, 4.22, N, 9.26. Found: C, 68.71; H, 3.99 N, 9.67.

2-((4,5-Diphenylthiazol-2-yl)imino)-5-(4-methoxybenzylidene)thiazolidin-4-one ***(15j).*** General Procedure A, yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.85 (s, 3H, OCH_3), 7.14 (d, J = 8.42 Hz, 2H), 7.32–7.41 (m, 8H), 7.56 (d, J = 7.56 Hz, 2H), 7.65 (d, J = 8.42 Hz, 2H), 7.71 (s, 1H), 12.60–12.71 (s, 1H). ^{13}C NMR (101 MHz, DMSO): δ 170.58 (C=O), 156.04, 148.50, 144.31, 135.13, 132.31, 129.75, 129.39, 129.14, 129.05, 128.96, 128.92, 128.89, 128.72, 128.49, 128.34, 125.94, 117.00, 112.73, 53.50 (OCH_3). HRMS calcd. for $C_{26}H_{19}N_3O_2S_2$ $[M+H]^+$ 469.1954, found 470.1141. Anal. Calcd for: $C_{26}H_{19}N_3O_2S_2$: C, 66.50; H, 4.08; N, 8.95. Found: C, 66.64; H, 4.38; N, 9.09.

2-((4,5-Diphenylthiazol-2-yl)imino)-5-(naphthalen-1-ylmethylene)thiazolidin-4-one ***(16a).*** General Procedure A, dark orange solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.23–7.49 (m, 10H), 7.60–7.83 (m, 4H), 7.98–8.13 (m, 3H), 8.35 (d, J = 19.74 Hz, 1H), 12.77 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO): δ 168.14 (C=O), 165.45, 151.03, 150.06, 138.35, 133.80, 132.00, 129.86, 129.84, 129.52, 129.49, 129.34, 129.05, 128.88, 128.87, 128.83, 128.80, 128.74, 128.69, 128.47, 128.37, 127.30, 125.88, 122.85, 100.00. HRMS Calcd for
$C_{29}H_{19}N_3OS_2$ $[M+H]^+$ 489.0970, found 490.1122. Anal. Calcd for: $C_{29}H_{19}N_3OS_2$: C, 71.14; H, 3.91; N, 8.58. Found: C, 71.39; H, 3.78; N, 8.76.

4-(2-((E)-((Z)-5-(Naphthalen-1-ylmethylene)-4-oxothiazolidin-2-ylidene)amino)-4-phenylthiazol-5-yl)benzenesulfonamide ***(16b).*** General Procedure A, light brown solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.12 (s, 2H, SO_2NH_2), 7.23–7.49 (m, 10H), 7.60–7.83 (m, 2H), 7.98–8.12 (m, 3H), 8.13–8.38 (m, 2H), 12.71 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO): δ 167.83 (C=O), 165.13, 150.71, 149.75, 138.03, 133.48, 131.69, 129.91, 129.55, 129.53, 129.20, 129.18, 129.03, 128.74, 128.57, 128.56, 128.52, 128.49, 128.43, 128.38, 128.15, 128.06, 126.99, 125.57, 122.54. HRMS calcd. for $C_{29}H_{20}N_4O_3S_3$ $[M+H]^+$ 568.0698, found 569.0667. Anal. Calcd for: $C_{29}H_{20}N_4O_3S_3$: C, 61.25; H, 3.54; N, 9.85. Found: C, 61.30; H, 3.71; N, 10.12.

4.2. Pharmacological screening

4.2.1. Anticancer activity

4.2.1.1. Cell culture. The human cancer cell lines HCT-116, Caco-2, MCF-7, DU-145 and PC-3 were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and cultured in Dulbecco's modified Eagle's medium/F12 medium (DMEM/F-12), DMEM or RPMI-1640 media (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Gibco) according to ATCC recommendation. All the cell lines were cultured at 37 °C in a humidified incubator containing 5% CO_2.

4.2.1.2. Cell viability assay. All the synthesized diphenylthiazole substituted thiazolidinone derivatives were evaluated *in vitro* for their cytotoxic activity against five different cancer cell lines, HCT-116, Caco-2, MCF-7, DU-145 and PC-3, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay as mentioned before [37,38]. Briefly, cancer cells were seeded in 96-well plates for 24 h, and treated with test compounds in 5% FBS-supplemented media for 72 h. Controls received DMSO (vehicle) at a concentration equal to that in drug-treated cells. After treatment, cells were incubated again in the same media containing 0.5 mg/mL MTT at 37 °C for 2 h. Reduced MTT was solubilized in DMSO (200 μL/well) for determination of absorbance at 570 nm using a microplate reader, Table 2.

4.2.2. Colorimetric COX-1/COX-2 inhibition assay

The *in vitro* inhibition of COX-1/COX-2 was measured using colorimetric COX (ovine) Inhibitor Screening Assay Kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions and as mentioned before [39]. For COX-1 reaction, 10 μM of celecoxib or test compounds were used while for COX-2, only 0.5 μM of celecoxib or test compounds were used. After the addition of arachidonic acid and incubation, the absorbance was measured at 590 nm using plate reader, Fig. 5.

4.3. Molecular Docking Study

The binding sites were generated from the co-crystallized ligand, **S-58701**, within COX-2 protein structure (PDB code: 6COX). Selected two ligands, **12b** and **15b**, were energy minimized using CHARMm ForceField and then docked into the former prepared proteins active sites using LIGANDFIT imbedded into Discovery Studio Software [35] with the following docking protocol: (i) number of Monte Carlo search trials = 30,000, search step for torsions with polar hydrogens = 30°. (ii) The Root Mean Square Difference (RMS) threshold for ligand-to-binding site shape match was set to 2.0 employing a maximum of 1.0 binding site partitions and 1.0 site partition seed. (iii) The interaction energies were assessed employing Consistent Force Field (CFF) force field with a non-bonded cutoff distance of 10.0 Å and distance-dependent dielectric. An energy grid extending 3.0 Å from the binding site was implemented. (iv) Rigid body ligand minimization parameters were: 10 iterations of steepest descend (SD) minimization followed by 20 Broyden–Fletcher–Goldfarb–Shanno (BFGS) iterations applied to every successful orientation of the docked ligand. (v) A maximum of 10 diverse docked conformations/poses of optimal interaction energies were saved. (vi) The saved conformers/ poses

were further energy-minimized within the binding site for a maximum of 1000 rigid-body iterations.

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