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Design, synthesis and biological evaluation of pyridine acyl sulfonamide derivatives as novel COX-2 inhibitors

Xiang Lu †, Hui Zhang †, Xi Li, Guo Chen, Qing-Shan Li, Yin Luo, Ban-Feng Ruan, Xian-Wei Chen, Hai-Liang Zhu *

State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, PR China

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ABSTRACT

A series of pyridine acyl sulfonamide derivatives (**1–24**) have been designed and synthesized and their biological activities were also evaluated as potential cyclooxygenase-2 (COX-2) inhibitors. Among all the compounds, compound **23** displayed the most potent COX-2 inhibitory activity with an IC_{50} of 0.8 μM. Antitumor and anti-inflammatory assays indicated that compound **23** owned high antiproliferative activity against B16-F10, HepG2 and MCF-7 cancer cell lines as well as COX-2-derived prostaglandin E_2 (PGE_2) inhibitory activity of murine macrophage RAW 264.7 cell line with IC_{50} values of 2.8, 1.2, 1.8 and 0.15 μM, respectively. Docking simulation was performed to position compound **23** into the COX-2 active site to determine the probable binding model.

1. Introduction

Since the early 1990s, it has been reported that cyclooxygenases (COXs) are responsible for the production of prostaglandins H2, which is a precursor for the biosynthesis of prostaglandins, thromboxanes and prostacyclins.[1] COXs exist in two distinct isoforms, a constitutive form cyclooxygenase-1 (COX-1) and an inducible form cyclooxygenase-2 (COX-2). The COX-1 is a housekeeping enzyme expressed in resting cells of most tissues involved in protection of gastric mucosa, platelet aggregation and renal blood flow. Whereas the COX-2 is rapidly induced in response to a variety of pro-inflammatory stimuli such as tumor necrosis factor-a (TNF-α), interleukines and growth factors, and plays an important role in pain, oncogenesis, various acute and chronic inflammatory symptoms.[2–6] Therefore, molecules that selective inhibition of COX-2 enzymatic activity would be of significant therapeutic value.[1]

Classical nonsteroidal anti-inflammatory drugs (NSAIDs) have been widely used in the treatment of acute and chronic inflammation states. However, all these drugs cause untoward side effects related to COX-1 inhibition, among which gastrointestinal irritation leading to ulcers and bleeding is the most common.[7] This promising concept of COX-2 selective inhibition led to the development of a large number of new molecules such as celecoxib, valdecoxib and nimesulide. Among these structures, sulfonamide is a promising skeleton that has shown the COX-2 selective inhibition, but minimize the risk of unwanted side effects.[8] On the other hand, synthesis of the pyridine ring system and its derivatives occupy an important place in the realm of synthetic pharmaceutical chemistry, due to their therapeutic and pharmacological properties. They have emerged as integral backbones of over 7000 existing drugs. The pyridine ring is also an integral part of anticancer and anti-inflammatory agents. Among them, some compounds showed their remarkable effects on COX-2 inhibitor potency in vitro, according to previous researches.[9,10] It encouraged us to continuously screen new pyridine acyl sulfonamide derivatives as potential COX-2 inhibitory agents. The two combined substructures, pyridine ring and sulfonamide, might exhibit synergistic effect in antitumor and anti-inflammatory activities. The objectives of present work are (1) to synthesize new pyridine acyl sulfonamide derivatives; (2) to evaluate their anticancer, anti-inflammatory and anticyclooxygenases activities; (3) to explore the binding modes of these compounds at the active site of COX-2 by docking simulations.

2. Results and discussion

2.1. Chemistry

The synthesis route of compounds **1–24** followed the general pathway outlined in Scheme 1. Coupling pyridinecarboxylic acid, substituted benzenesulfonamide amides, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) and 4-dimethylaminopyridine (DMAP) were dissolved in CH_2Cl_2 and refluxed to give the desired compounds **1–24**. Among these compounds, **6–10**, **12–14**, **16–18** and **20–24** are reported for the first time.

* Corresponding author. Tel.: +86 25 8359 2572; fax: +86 25 8359 2672.
E-mail address: zhuhl@nju.edu.cn (H.-L. Zhu).
† These two authors equally contributed to this paper.

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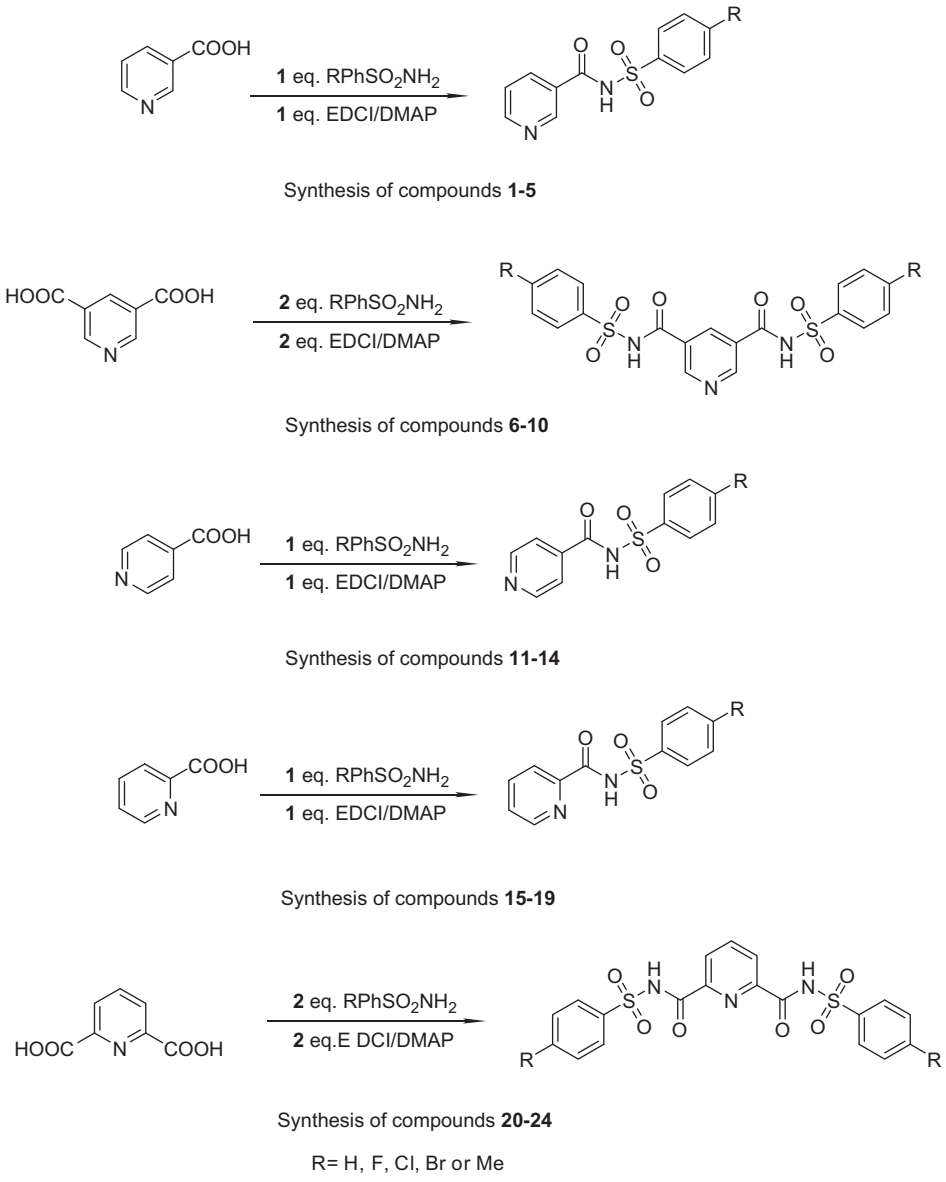

Scheme 1. Synthesis route of compounds **1–24**. Reagents and conditions: EDCI/DMAP, CH_2Cl_2, reflux, 8–10 h.

These compounds gave satisfactory elementary analytical and spectroscopic data. ^{1}H NMR and ESI MS spectra were consistent with the assigned structures. Furthermore, the crystal structure of compound **7** was determined by single crystal X-ray diffraction analysis and the crystal data are presented in Table 1 and Figure 1.

2.2. Biological evaluation

The data of the anti-inflammatory activities of the pyridine acyl sulfonamide derivatives (**1–24**) were summarized in Table 2. It can be seen that most of the synthesized compounds exhibited remarkable and selective inhibitory activities against the COX-2 isozyme, but weak to COX-1. Among these compounds, **23** showed the most potent COX-2 inhibitory activity (IC_{50} = 0.8 μM). The results of western blotting assay further confirmed that compound **23** with the concentration above 0.75 μM could obviously inhibit lipopolysaccharide (LPS)-induced COX-2 expression in murine macrophage RAW 264.7 cell line (Fig. 3).

Table 1
Crystallographic data and structure refinements for compound **7**

Compound	**7**
Empirical formula	$C_{19}H_{13}F_2N_3O_8S_2$
Formula weight	513.46
Crystal system	Monoclinic
Space group	C_2/c
a (Å)	22.700(5)
b (Å)	8.4010(17)
c (Å)	10.970(2)
α (°)	90
β (°)	91.36(3)
γ (°)	90
V (Å^3)	2091.4(7)
Z	4
D_c/g cm^{-3}	1.631
F(000)	1048.0
Reflections collected/unique	2609/2002
Absorption coefficient (mm^{-1})	0.328
R_1; wR_2 [$I > 2\sigma(I)$]	0.0777/0.2292
R_1; wR_2 (all data)	0.0634/0.2094
GF	1.099

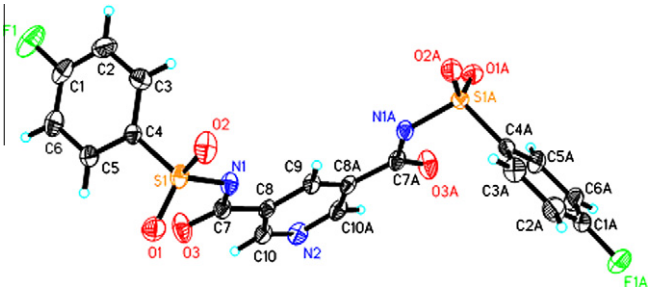

Figure 1. Crystal structure diagrams of compound **7**. H atoms are shown as small spheres of arbitrary radii.

Table 2
Inhibition (IC_{50}) of COX-1, PGE_2 and COX-2 in lipopolysaccharide (LPS)-activated murine macrophage RAW 264.7 cells

Compound	R1	IC_{50} (μM)		
		COX-1	PGE_2	COX-2
1	H	>50	9.5	15.8
2	F	>50	8.6	11.7
3	Cl	35.8	3.2	6.6
4	Br	40.5	2.8	4.9
5	Me	>50	8.8	18.2
6	H	36.9	2.6	7.9
7	F	40.2	1.9	5.6
8	Cl	29.8	1.7	3.4
9	Br	42.5	1.2	2.8
10	Me	>50	2.2	8.5
11	F	>50	9.2	12.1
12	Cl	41.2	8.9	12.6
13	Br	38.0	5.5	7.8
14	Me	>50	9.8	19.4
15	H	>50	4.5	8.8
16	F	43.8	3.3	6.3
17	Cl	39.7	2.8	6.0
18	Br	>50	1.9	3.6
19	Me	>50	5.9	8.0
20	H	35.8	1.6	3.7
21	F	40.3	1.1	2.6
22	Cl	41.5	0.87	1.9
23	Br	35.6	0.15	0.8
24	Me	39.8	2.1	5.6
Celecoxib		27.5	0.10	0.12

Prostaglandin E_2 (PGE_2) is one of the principal mediators of inflammation, which is biosynthesised via COX-2 enzymes.[11] As shown in Table 2, compounds **23** was found to be promising inhibit PGE_2 with an IC_{50} value of 0.15 μM, which was comparable to that of celecoxib (IC_{50} = 0.10 μM). These data suggested that pyridine acyl sulfonamide derivatives inhibited the inflammatory activity via the COX-2 pathway and were devoid of toxicity due to the absence of COX-1 inhibition which is maintenance of normal physiological functions.[12–15]

Furthermore, being associated with inflammation, it is well documented that COX-2 is especially overexpressed in many human cancer entities such as melanoma, hepatic carcinoma, breast cancer and colorectal cancer, which is assumed to play an important role in carcinogenesis by stimulating angiogenesis, tissue invasion, metastasis and apoptosis inhibition.[6,16,17] Herein, compounds **1–24** were evaluated for their anticancer activities against the three cancer cell lines including melanoma cell line (B16-F10), human breast cancer cell line (MCF-7) and liver cancer cell line (HepG2). The results were summarized in Table 3. It was observed that pyridine acyl sulfonamide derivatives (**1–24**) have been found to show good inhibitory activities displaying IC_{50} values between 1.2 and 20.2 μM. Among them, compound **23** displayed the most potent inhibitory activity (IC_{50} = 1.2 μM for HepG2, 2.8 μM for B16-F10, and 1.8 μM for MCF-7) better than that of the positive control celecoxib.

Table 3
Inhibition (IC_{50}) of B16-F10, MCF-7 and HepG2 cells proliferation

Compound	R1	IC_{50} (μM)		
		B16-F10	MCF-7	HepG2
1	H	14.5	11.5	10.4
2	F	13.8	10.6	9.0
3	Cl	11.9	10.1	8.6
4	Br	9.7	8.3	6.7
5	Me	18.5	15.5	12.3
6	H	11.3	10.6	9.2
7	F	8.8	7.9	6.2
8	Cl	10.6	4.2	5.4
9	Br	7.2	4.1	3.3
10	Me	9.2	10.8	10.0
11	F	13.8	13.9	12.4
12	Cl	12.4	10.1	7.9
13	Br	8.8	8.7	6.3
14	Me	16.2	14.1	20.2
15	H	14.2	10.6	9.9
16	F	10.9	11.0	8.5
17	Cl	9.4	9.8	8.1
18	Br	8.5	6.8	5.0
19	Me	16.7	11.8	12.1
20	H	10.7	9.3	5.3
21	F	10.0	4.6	3.7
22	Cl	6.2	4.2	3.8
23	Br	2.8	1.8	1.2
24	Me	12.2	6.2	4.2
Celecoxib		85.6	40.8	95.5

Subsequently SAR studies were performed to determine how the substituents of the subunits affected the COX-2 inhibitory activities. Compounds **1–24** with *para* substituted on phenyl ring exhibited significant COX-2 inhibitory activities in order of Br > Cl > F > Me. The results demonstrated that an electron-withdrawing halogen group may be helpful to improve COX-2 inhibitory activities while electron donating methyl substituted may show some relatively negative effects. The results also indicated that in the case of constant phenyl ring substituents, change of various kinds of pyridine carboxylic acids skeletons could also affect the activities of these compounds. Compounds with 2-pyridinecarboxylic acid skeleton (**15–19**) showed more potent activities than those with nicotinic acid (**1–5**) and isonicotinic acid (**11–14**) skeletons. Moreover, several symmetrically substituted derivatives with 2,6-pyridinedicarboxylic acid and pyridine-3,5-dicarboxylic acid skeleton (**6–10, 20–24**) displayed more potent than the corresponding nonsymmetrically substituted derivatives (**1–5, 15–19**). Molecular symmetry, as a broad structural property, is frequently present in many drugs.[18] The biological activities of the designed compounds suggested that molecular symmetry might be a valid approach to obtain potent new COX-2 inhibitory agents.

Molecular docking was performed to study the biding model of compound **23** with COX-2 based on the crystal structure of COX-2 protein (PDB code: 1cx2). The binding model of compounds **23** and catalytic site of COX-2 are depicted in Figure 2. It was showed that the amide group and the oxygen of carboxyl group of compound **23** exhibited hydrogen bond with Leu 352 and Arg 513 in COX-2 (distance = 2.76 and 2.30 Å, respectively). In addition, the oxygen atoms of the sulfonamide system formed two hydrogen bonds with the amino hydrogen of Phe518 and Arg530 (length: Arg530 N–H···O = 1.88 Å and Phe518 N–H···O = 2.33 Å). Overall, these results can provide a good explanation for the high potency of compound **23** towards COX-2 protein.

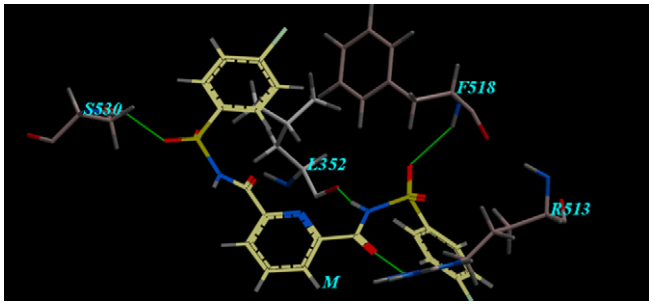

Figure 2. Binding mode of compound **23** with COX-2; ligand is represented as wire, the interacting residues are depicted as stick and ball. The H-bond (green) is displayed as line.

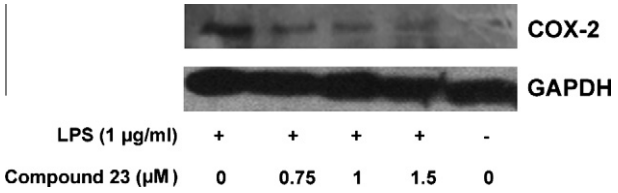

Figure 3. Inhibitory effect of compound **23** on COX-2 protein expression. RAW 264.7 cells (5×10^5 cells) were incubated in 12 well culture plate for 24 h, and then treated with compounds and LPS (1 μg/ml) for 18 h. After incubation, cells were lysed, and protein was applied on SDS–polyacrylamide gel. The level of COX-2 protein expression was examined by western blotting analysis.

3. Conclusions

In summary, we have prepared a series of pyridine acyl sulfonamide derivatives (**1–24**) and evaluated their biological activities. All of these compounds can inhibit the growth of three cancer cell lines. Compound **23** were most exciting, as it inhibited the cancer cell lines more potently than standard anticancer agents (celecoxib). Moreover promising anti-inflammatory activities were also obtained by those compounds. Bioavailability and toxicity test revealed the compounds to be nontoxic with drug properties. The results of this study may find a leading compound (**23**) toward the development of new therapeutic agent to fight against cancer and inflammation.

4. Experimental

4.1. Chemistry

The synthesis of pyridine acyl sulfonamide derivatives was followed the general reaction pathway outlined in Scheme 1. Compounds **1–24** were synthesized by coupling substituted benzenesulfonamide with nicotinic acid, isonicotinic acid, picolinic acid, pyridine-2,6-dicarboxylic acid or pyridine-3,5-dicarboxylic acid, using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI), 4-dimethylaminopyridine (DMAP) as condensing agent. The mixture was refluxed in anhydrous CH_2Cl_2 for 8–10 h. The products were extracted with ethyl acetate. The extract was washed successively with HCl, $NaHCO_3$ and water, dried over Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography using petroleum ether and ethyl acetate (1:1).

4.2. Spectral properties of intermediate compounds

4.2.1. *N*-(Phenylsulfonyl)nicotinamide (1)

White powders, mp: 242–244 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.53– 7.56 (m, 1H), 7.63–7.66 (m, 2H), 7.72 (t, J = 7.5 Hz, 1H), 8.00–8.02 (m, 2H), 8.23–8.25 (m, 1H), 8.76–8.78 (m, 1H), 9.00 (m, 1H). ESI-MS: 263.0 ($C_{12}H_{11}N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_{10}N_2O_3S$: C, 54.95; H, 3.84; N, 10.68. Found: C, 54.74; H, 3.85; N, 10.72.

4.2.2. *N*-(4-Fluorophenylsulfonyl)nicotinamide (2)

White powders, mp: 245–246 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.46–7.49 (m, 2H), 7.56–7.58 (m, 1H), 8.06–8.09 (m, 2H), 8.26–8.28 (m, 1H), 8.77–8.79 (m, 1H), 9.01 (d, J = 1.6 Hz, 1H). ESI-MS: 281.0 ($C_{12}H_{10}FN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_9FN_2O_3S$: C, 51.42; H, 3.24; N, 9.99. Found: C, 51.24; H, 3.25; N, 9.95.

4.2.3. *N*-(4-Chlorophenylsulfonyl)nicotinamide (3)

White powders, mp: 241–243 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.58–7.62 (m, 1H), 7.84 (d, J = 8.6 Hz, 2H), 7.93 (d, J = 9.1 Hz, 2H), 8.29–8.33 (m, 1H), 8.78–8.81 (m, 1H), 9.02 (d, J = 2.0 Hz, 1H). ESI-MS: 298.1 ($C_{12}H_{10}ClN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_9ClN_2O_3S$: C, 48.57; H, 3.06; N, 9.44. Found: C, 48.31; H, 3.31; N, 9.22.

4.2.4. *N*-(4-Bromophenylsulfonyl)nicotinamide (4)

White powders, mp: 255–356 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.51–7.61 (m, 1H), 7.82–7.85 (m, 2H), 7.90–7.93 (m, 2H), 8.28–8.32 (m, 1H), 8.77–8.79 (m, 1H), 9.01 (d, J = 2.2 Hz, 1H). ESI-MS: 343.0 ($C_{12}H_{10}BrN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_9BrN_2O_3S$: C, 42.24; H, 2.66; N, 8.21. Found: C, 42.37; H, 2.67; N, 8.23.

4.2.5. *N*-Tosylnicotinamide (5)

White powders, mp: 225–227 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.40 (s, 3H), 7.44 (d, J = 8.0 Hz, 2H), 7.52–7.57 (m, 1H), 7.90 (d, J = 8.2 Hz, 2H), 8.23 (d, J = 8.0 Hz, 1H), 8.77–8.79 (m, 1H), 8.99 (d, J = 1.8 Hz, 1H). ESI-MS: 277.1 ($C_{13}H_{13}N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{12}N_2O_3S$: C, 56.51; H, 4.38; N, 10.14. Found: C, 56.38; H, 4.39; N, 10.10.

4.2.6. N^3,N^5-Bis(phenylsulfonyl)pyridine-3,5-dicarboxamide (6)

White powders, mp: 290–292 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.62–7.67 (t, J = 7.5 Hz, 4H), 7.70–7.75 (t, J = 7.1 Hz, 2H), 8.00 (d, J = 7.2 Hz, 4H), 8.68 (t, J = 2.1 Hz, 1H), 9.12 (d, J = 2.0 Hz, 2H). ESI-MS: 446.0 ($C_{19}H_{16}N_3O_6S_2$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{15}N_3O_6S_2$: C, 51.23; H, 3.39; N, 9.43. Found: C, 51.29; H, 3.47; N, 9.40.

4.2.7. N^3,N^5-Bis(4-fluorophenylsulfonyl)pyridine-3,5-dicarboxamide (7)

White powders, mp: 302–304 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.48 (t, J = 8.72 Hz, 4H), 7.95 (s, 2H), 8.07–8.10 (m, 4H), 8.68 (s, 1H), 9.13 (d, J = 1.8 Hz, 2H). ESI-MS: 482.0 ($C_{19}H_{14}F_2N_3O_6S_2$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{13}F_2N_3O_6S_2$: C, 47.40; H, 2.72; N, 8.73. Found: C, 47.35; H, 2.73; N, 8.75.

4.2.8. N^3, N^5-Bis(4-chlorophenylsulfonyl)pyridine-3,5-dicarboxamide (8)

White powders, mp: 305–307 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.69–7.70(dd, $J1$ = 2.0, $J2$ = 6.9, 4H), 7.97–7.99 (dd, $J1$ = 2.0, $J2$ = 6.9, 4H), 8.66 (t, J = 2.1 Hz, 1H), 9.10 (d, J = 2.1 Hz, 2H). ESI-MS: 515.9($C_{19}H_{14}Cl_2N_3O_6S_2$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{13}Cl_2N_3O_6S_2$: C, 47.37; H, 2.55; N, 8.17. Found: C, 47.31; H, 2.56; N, 8.15.

4.2.9. N^3,N^5-Bis(4-bromophenylsulfonyl)pyridine-3,5-dicarboxamide (9)

White powders, mp: 309–311 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.87 (d, J = 8.7 Hz, 4H), 7.93 (d, J = 8.7 Hz, 4H), 8.69 (t, J = 2.1 Hz, 1H), 9.13 (d, J = 2.1 Hz, 2H). ESI-MS: 604.8 ($C_{19}H_{14}Br_2N_3O_6S_2$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{13}Br_2N_3O_6S_2$: C, 37.83; H, 2.17; N, 6.97. Found: C, 37.72; H, 2.16; N, 6.95.

4.2.10. N^3,N^5-Ditosylpyridine-3,5-dicarboxamide (10)

White powders, mp: 303–305 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.40–2.50(m, 6H), 7.44 (d, J = 8.2 Hz, 4H), 7.89 (d, J = 8.4 Hz, 4H), 8.66 (t, J = 2.0 Hz, 1H), 9.10 (t, J = 2.0 Hz, 2H), 9.12 (d, J = 2.0 Hz, 2H). ESI-MS: 474.0 ($C_{21}H_{20}Br_2N_3O_6S_2$, $[M+H]^+$). Anal. Calcd for $C_{21}H_{19}Br_2N_3O_6S_2$: C, 53.27; H, 4.04; N, 8.87. Found: C, 53.24; H, 4.03; N, 8.85.

4.2.11. *N*-(4-Fluorophenylsulfonyl)isonicotinamide (11)

White powders, mp: 221–222 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.42–7.46 (t, J = 8.8 Hz, 2H), 7.86 (d, J = 6.1 Hz, 2H), 8.03–8.06 (m, 2H), 8.77–8.78 (d, J = 4.6, 2H). ESI-MS: 281.0 ($C_{12}H_{10}FN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_9FN_2O_3S$: C, 51.42; H, 3.24; N, 9.99. Found: C, 51.48; H, 3.27; N, 9.96.

4.2.12. *N*-(4-Chlorophenylsulfonyl)isonicotinamide (12)

White powders, mp: 235–238 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.65–7.67 (d, J = 8.6 Hz, 2H), 7.91–7.98 (m, 4H), 8.79 (d, J = 6.0 Hz, 2H). ESI-MS: 297.0 ($C_{12}H_{10}ClN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_9ClN_2O_3S$: C, 48.57; H, 3.06; N, 9.44. Found: C, 48.47; H, 3.07; N, 9.41.

4.2.13. *N*-(4-Bromophenylsulfonyl)isonicotinamide (13)

White powders, mp: 246–248 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.78–7.81 (d, J = 8.6 Hz, 2H), 7.87–7.92 (t, J = 8.1 Hz, 4H), 8.79 (d, J = 6.2 Hz, 2H). ESI-MS: 340.9 ($C_{12}H_{10}BrN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_9BrN_2O_3S$: C, 42.24; H, 2.66; N, 8.21. Found: C, 42.42; H, 2.67; N, 8.23.

4.2.14. *N*-Tosylisonicotinamide (14)

White powders, mp: 230–232 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.50 (s, 3H), 7.42–7.45 (d, J = 8.4 Hz, 2H), 7.77–7.79 (m, 2H), 7.87–7.90 (d, J = 8.4 Hz, 2H), 8.74–8.76 (m, 2H). ESI-MS: 277.9 ($C_{13}H_{13}N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{12}N_2O_3S$: C, 56.51; H, 4.38; N, 10.14. Found: C, 56.70; H, 4.36; N, 10.18.

4.2.15. *N*-(Phenylsulfonyl)picolinamide (15)

White powders, mp: 221–213 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.62- 7.65 (t, J = 7.8 Hz, 2H), 7.70–7.72 (m, 2H), 7.97 (d, J = 7.8 Hz, 1H), 8.02–8.05 (t, J = 7.9 Hz, 3H), 8.71 (d, J = 4.7 Hz, 1H), 12.34 (s, 1H). ESI-MS: 263.0 ($C_{12}H_{11}N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_{10}N_2O_3S$: C, 54.95; H, 3.84; N, 10.68. Found: C, 54.75; H, 3.86; N, 10.65.

4.2.16. *N*-(4-Fluorophenylsulfonyl)picolinamide (16)

White powders, mp: 225–228 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.46–7.49 (m, 2H), 7.71–7.74 (m, 1H), 8.00 (d, J = 7.8 HZ, 1H), 8.04–8.07 (m, 1H), 8.09–8.12 (m, 2H), 8.71 (d, J = 4.1,1H). ESI-MS: 280.6 ($C_{12}H_{10}FN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_9FN_2O_3S$: C, 51.42; H, 3.24; N, 9.99. Found: C, 51.46; H, 3.23; N, 9.96.

4.2.17. *N*-(4-Chlorophenylsulfonyl)picolinamide (17)

White powders, mp: 245–247 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.70–7.75 (m, 3H), 8.00–8.04 (m, 3H), 8.04–8.09 (m, 1H), 8.71 (d, J = 4.7 Hz, 1H). ESI-MS: 297.0 ($C_{12}H_{10}ClN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_9ClN_2O_3S$: C, 48.57; H, 3.06; N, 9.44. Found: C, 48.54; H, 3.07; N, 9.47.

4.2.18. *N*-(4-Bromophenylsulfonyl)picolinamide (18)

White powders, mp: 255–256 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.72–7.75 (m, 1H), 7.84–7.86 (m, 2H), 7.94–7.96 (m, 2H), 8.01 (d, J = 7.7 Hz, 1H), 8.06–8.09 (m, 1H), 8.71 (d, J = 4.2 Hz, 1H). ESI-MS: 340.9 ($C_{12}H_{10}BrN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_9BrN_2O_3S$: C, 42.24; H, 2.66; N, 8.21. Found: C, 42.28; H, 2.65; N, 8.19.

4.2.19. *N*-Tosylpicolinamide (19)

White powders, mp: 220–222 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.50 (s, 3H), 7.43 (d, J = 8.0 Hz, 2H), 7.68–7.71 (m, 1H), 7.91–7.93 (d, J = 8.3 Hz, 1H), 8.00–8.04 (m, 1H), 8.69–8.71 (m, 1H), 12.18 (s, 1H). ESI-MS: 278.5 ($C_{13}H_{13}N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{12}N_2O_3S$: C, 56.51; H, 4.38; N, 10.14. Found: C, 56.30; H, 4.40; N, 10.12.

4.2.20. N^2,N^6-Bis(phenylsulfonyl)pyridine-2,6-dicarboxamide(20)

White powders, mp: 313–315 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.66–7.70 (t, J = 7.4 Hz, 4H), 7.75–7.79 (m, 2H), 8.08–8.11 (d, J = 7.1 Hz, 4H), 8.17–8.25(m, 3H), 12.99 (s, 2H). ESI-MS: 446.9 ($C_{19}H_{16}N_3O_6S_2$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{15}N_3O_6S_2$: C, 51.23; H, 3.39; N,9.43. Found: C,51.43; H,3.40; N, 9.45.

4.2.21. N^2,N^6-Bis(4-fluorophenylsulfonyl)pyridine-2,6-dicarboxamide (21)

White powders, mp: 315–317 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.49–7.55 (t, J = 8.8 Hz, 4H), 8.15–8.19 (m, 4H), 8.21–8.26(m, 3H), 12.97 (s, 2H). ESI-MS: 482.0 ($C_{19}H_{14}F_2N_3O_6S_2$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{13}F_2N_3O_6S_2$: C, 47.40; H, 2.72; N, 8.73. Found: C, 47.32; H, 2.71; N, 8.75.

4.2.22. N^2,N^6-Bis(4-chlorophenylsulfonyl)pyridine-2,6-dicarboxamide (22)

White powders, mp: 325–326 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.88–7.91 (d, J = 8.6 Hz, 4H), 7.99–8.03 (d, J = 8.6 Hz 4H), 8.22–8.27 (m, 3H), 12.99 (s, 2H). ESI-MS: 515.0($C_{19}H_{14}Cl_2N_3O_6S_2$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{13}Cl_2N_3O_6S_2$: C, 44.37; H, 2.55; N, 8.17. Found: C, 44.32; H, 2.54; N, 8.19.

4.2.23. N^2,N^6-Bis(4-bromophenylsulfonyl)pyridine-2,6-dicarboxamide (23)

White powders, mp: 330–332 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.75–7.78 (d, J = 8.6 Hz, 4H), 8.08–8.11 (d, J = 8.6 Hz 4H), 8.22–8.26 (m, 3H), 12.99 (s, 2H). ESI-MS: 604.8($C_{19}H_{14}Br_2N_3O_6S_2$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{13}Br_2N_3O_6S_2$: C, 37.83; H, 2.17; N, 6.97. Found: C, 37.88; H, 2.16; N, 6.99.

4.2.24. N_2,N_6-Ditosylpyridine-2,6-dicarboxamide (24)

White powders, mp: 310–312 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.41 (s, 6H), 7.46–7.49 (d, J = 8.4 Hz, 4H), 7.96 (d, J = 8.4 Hz, 4H), 8.20–8.24(m, 3H), 12.89 (s, 2H). ESI-MS: 474.0 ($C_{21}H_{20}N_3O_6S_2$, $[M+H]^+$). Anal. Calcd for $C_{21}H_{19}N_3O_6S_2$: C, 53.27; H, 4.04; N, 8.87. Found: C, 53.18; H, 4.03; N, 8.89.

4.3. Crystal structure determination

Crystal structure of compound **7** were determined on a Nonius CAD4 diffractometer equipped with graphite-monochromated Mo K_α (λ = 0.71073 Å) radiation. The structure was solved by direct methods and refined on F^2 by full-matrix least-squares methods using SHELX-97. All the nonhydrogen atoms were refined anisotropically. All the hydrogen atoms were placed in calculated positions and were assigned fixed isotropic thermal parameters at 1.2 times the equivalent isotropic U of the atoms to which they are attached and allowed to ride on their respective parent atoms. The contributions of these hydrogen atoms were included in the structure-factors calculations.

4.4. Anticancer assay

The antiproliferative activity of the prepared compounds was determined using a standard (MTT)-based colorimetric assay.[19] Briefly, HepG2, B16-F10 and MCF-7 cell lines were seeded at a

density of 2×10^4 cells/ml in 96-well microtiter plates. After 24 h, exponentially growing cells were exposed to the compounds (**1–24**) at final concentrations ranging from 0.1 to 100 μM. After 48 h, cell survival was determined by the addition of an MTT solution (0.5 mg/mL MTT in DMEM cell culture medium). After 4 h incubation, the optical absorbance was measured at 570 nm on an ELISA microplate reader. In all experiments three replicate wells were used for each drug concentration. Each assay was carried out at least three times.

4.5. Molecular modeling (docking) studies

Molecular docking of compounds into the three dimensional COX-2 complex structure (PDB code: 1cx2) was carried out using the Molsoft ICM-Pro software package (version 3.5-0a).

4.6. In vitro cyclooxygenase (COX) inhibition assays

The ability of the test compounds **1–24** to inhibit COX-1 and COX-2 was determined using chemiluminescent enzyme assays kit (Cayman Chemical, Ann Arbor, MI, USA) according to previously reported method.[20]

4.7. Anti-inflammatory assay

The inhibitory effects of samples on PGE_2 expression were evaluated in lipopolysaccharide (LPS)-activated murine macrophage RAW 264.7 cells, using a method modified from that previously reported.[21]

4.8. Western immunoblot analysis

RAW 264.7 cells were pretreated with compounds **23** at the concentrations of 0.75, 1 and 1.5 μM for 15 min before treatment with 1 μg/mL LPS for 18 h and examining the expression of COX-2 protein. Cells were lysed with lysis buffer. Western immunoblot analysis was performed using a method described by Cheenpracha, et al.[22]

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