Medicinal Chemistry Research (2023) 32:495–505
https://doi.org/10.1007/s00044-023-03022-0

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Novel Benzo[4,5]imidazo[1,2-*a*]pyrimidine derivatives as selective Cyclooxygenase-2 Inhibitors: Design, synthesis, docking studies, and biological evaluation

Maryam Bayanati[1,2] · Mona Khoramjouy[3] · Mehrdad Faizi[3] · Mahsa Azami Movahed[1] · Mohammad Mahboubi-Rabbani[1,4] · Afshin Zarghi[1]

Received: 19 October 2022 / Accepted: 12 January 2023 / Published online: 23 January 2023

Abstract

The present study was aimed at the synthesis and evaluation of a new series of benzo[4,5]imidazo[1,2-*a*]pyrimidine having a methylsulfonyl group as COX-2 (cyclooxygenase-2) inhibitor pharmacophore. Molecular modeling studies were performed using the Autodock program, and the results demonstrated that methylsulfonyl pharmacophore was adequately placed into the COX-2 active site. The in vitro and in vivo COX-2 inhibitory effects were also evaluated. In the in vitro assay, all newly synthesized compounds showed moderate to good selectivity for the inhibition of the COX-2 enzyme. However, compound 2-(4-(methylsulfonyl) phenyl)-4-phenylbenzo[4,5]imidazo[1,2-*a*]pyrimidine (**5a**) showed the highest COX-2 inhibitory effect (IC_{50}: 0.05 μM) even more than celecoxib as the reference drug (IC_{50}: 0.06 μM). For the in vivo study, the writing reflex test was used, and the results indicated that all synthesized compounds had well dose-dependent anti-nociceptive activity. The in vivo evaluation also showed that compound 2-(4-(methylsulfonyl)phenyl)-4-(*p*-tolyl)benzo[4,5]imidazo[1,2-*a*]pyrimidine (**5d**) had the highest activity in the writing reflex test (ED_{50}: 5.75 mg/kg). In addition, the cytotoxicity effects of the synthesized compounds were tested on MCF-7 breast cancer cells, and all compounds showed considerable inhibitory results.

Keywords Benzo[4,5]imidazo[1,2-*a*]pyrimidine · Cyclooxygenase-2 · Writing test · MCF-7 · Anti-cancer

Introduction

Many mediators have interfered in the inflammatory process, such as cytokines, cell adhesion molecules, and autacoids, including prostaglandins (PGs). The pain and tissue blood flow principally increase in the presence of PGs, especially PGE_2 [1]. PGs are biosynthesized by cyclooxygenase (COX) enzyme, so COX enzyme inhibitors such as nonsteroidal anti-inflammatory drugs (NSAIDs) decrease inflammation. The COX isoforms that have been discovered are COX-1, COX-2, and COX-3 [2]. COX-1 and COX-2 are more important than COX-3, and NSAIDs effects are attributed to the inhibition of these isoforms [3]. The COX-1 isoform has a constitutive role and expresses PGs responsible for maintaining physiological functions such as gastric mucosa protection and vascular homeostasis. In contrast, COX-2 is an inducible enzyme and over-expressed in response to the release of several pro-inflammatory mediators. The COX-2 isoform is over-expressing in the inflammatory sites and introduces the PGs produced in pathologic conditions [4]. However, NSAIDs as anti-inflammatory drugs not only inhibit COX-2 but also these drugs inhibit COX-1 isoform and cause many side effects, such as gastrointestinal problems associated with the inhibition of COX-1 as a protector of the gastric mucosa. To prevent the side effects

✉ Afshin Zarghi
zarghi@sbmu.ac.ir

1 Department of Pharmaceutical Chemistry, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran

2 Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran

3 Department of Pharmacology and Toxicology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran

4 Department of Pharmaceutical Chemistry, School of Pharmacy, Iran University of Medical Sciences, Tehran, Iran

of NSAIDs, the difference between COX-1 and COX-2 isoforms should be identified to design the compounds with high selectivity for inhibition COX-2 enzyme [5, 6]. In general, the active site of these isoforms are very similar, but the significant difference between these isoforms is a second pocket in the binding site of the COX-2 isoform due to the replacement of the bulk isoluesin523 amino acid in COX-1 by valine at the binding site of COX-2. So, the large compounds place properly into the COX-2 active site while they do not in the COX-1 active site [7]. Therefore, compounds can be designed that are more desirable to COX-2 than COX-1 and do not show the side effects of this isoform inhibition. The COX-2 role is investigated in diverse diseases, such as Alzheimer's disease [8–10], several types of cancer [11–15], and Parkinson's disease [16]. Also, the upregulation of COX-2 in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been studied [17]. Some well-known COX-2 inhibitors, like Rofecoxib, have been withdrawn from the market due to cardiovascular effects [18]. Therefore, the introduction of new COX-2 inhibitors with high potency and low side effects is desirable. Diarylheterocycles are abundantly present in the structure of selective COX-2 inhibitors. The hetero or carbocyclic ring systems or acylic structures constitute the central core of this group and consist of two aryl substitutions. One of the two phenyl rings carries a pharmacophore group that replaces the secondary hydrophobic pocket at the COX-2 active site and is responsible for selectivity. According to recent studies, the COX-2 inhibitors with tricyclic rings as central skeletons are introduced [19–21]. Benzimidazole rings have been appeared as the prominent scaffold in medicinal chemistry and also used as the central core in COX-2 inhibitors [22–28]. Our previous work introduced a new potent and selective COX-2 inhibitors class with tricyclic benzimidazole central core (A, Fig. 1) [21]. In this regard, we designed a new series of imidazo[1,2-*a*] pyrimidine compounds with a tricyclic central core. To design these compounds, we hybrid molecules comprising benzimidazole and pyrimidine ring as new COX-2 inhibitors. Pyrimidine rings are interesting structures widely used in the design of COX-2 inhibitors and have been shown anti-cancer effects (B, Fig. 1) [29–34]. Based upon this, we designed, synthesized, and evaluated a series of novel 2,4-diphenylbenzo[4,5]imidazo[1,2-*a*]pyrimidine derivatives with high potency and selectivity. The newly synthesized compounds were employed to assess their ability to inhibit COX-1 and COX-2 enzymes. Also, the anti-cancer effects of these series were assayed on the MCF-7 breast cancer cell line. In addition, an in vivo assay of these compounds has also been performed and showed promising results.

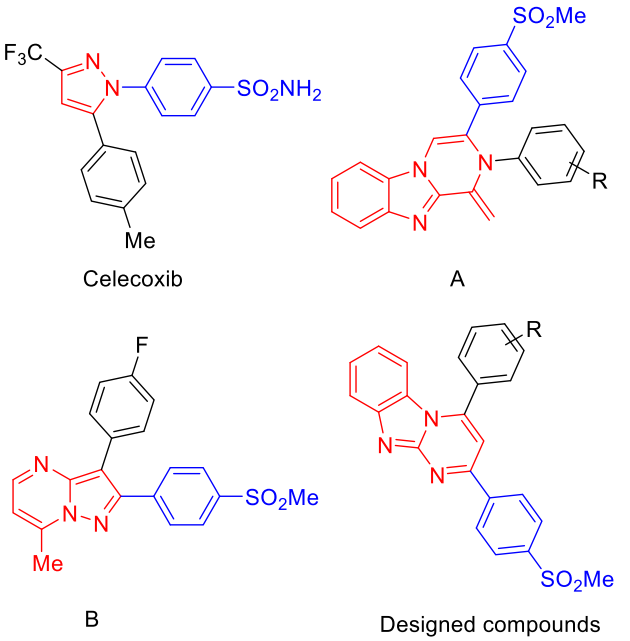


Fig. 1 COX-2 inhibitors containing tricyclic benzimidazole central core (**A**), pyrimidine ring (**B**), and designed compounds

Results and discussion

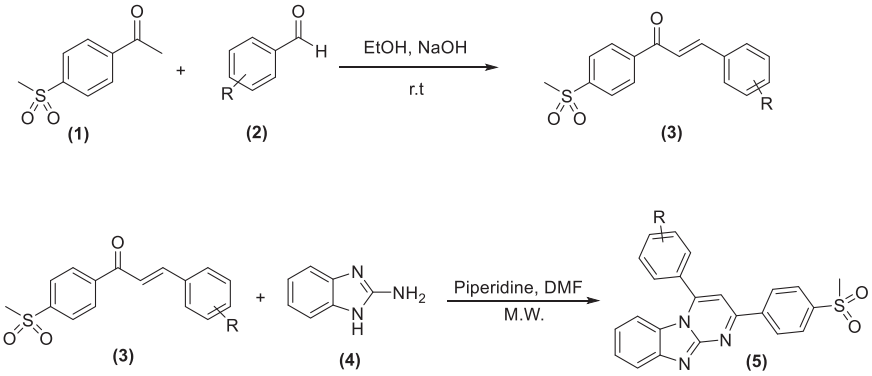
Chemistry

The synthetic procedure of the target molecules is described in Scheme 1. Accordingly, the synthesis of this series was carried out by reaction of two main precursors: substituted chalcones and 2-aminobenzimidazole. To synthesize related chalcones, at first, 4'-(methylsulfonyl) acetophenone **(1)** was synthesized from thioanisole as starting substance in two steps reaction as explained in our previous work [35]. Accordingly, first 4'-(methylthio)acetophenone was obtained from the reaction of thioanisole with acetyl chloride in the presence of $AlCl_3$ and acetyl chloride in chloroform at room temperature. Then, oxidation step was performed in THF/water as the solvent, and 4'-(methylsulfonyl)acetophenone was produced using Oxone reagent. The 4'-(methylsulfonyl)acetophenone was treated with different aldehyde derivatives at room temperature in an alcoholic NaOH solution to give desired chalcones **(3)** [36]. Finally, 2-aminobenzimidazole **(4)** reacted with substituted chalcones **(3)** in DMF as solvent under microwave irradiation and the presence of a catalytic amount of piperidine to produce 2,4-diphenylbenzo[4,5]imidazo[1,2-*a*]pyrimidine derivatives [37] (**5a-o**).

Molecular docking study

A docking study was performed to investigate the probable interaction between 2,4-diphenylbenzo[4,5]imidazo[1,2-*a*]

Scheme 1 The synthesis procedure of target benzo[4,5]imidazo[1,2-*a*]pyrimidine derivatives



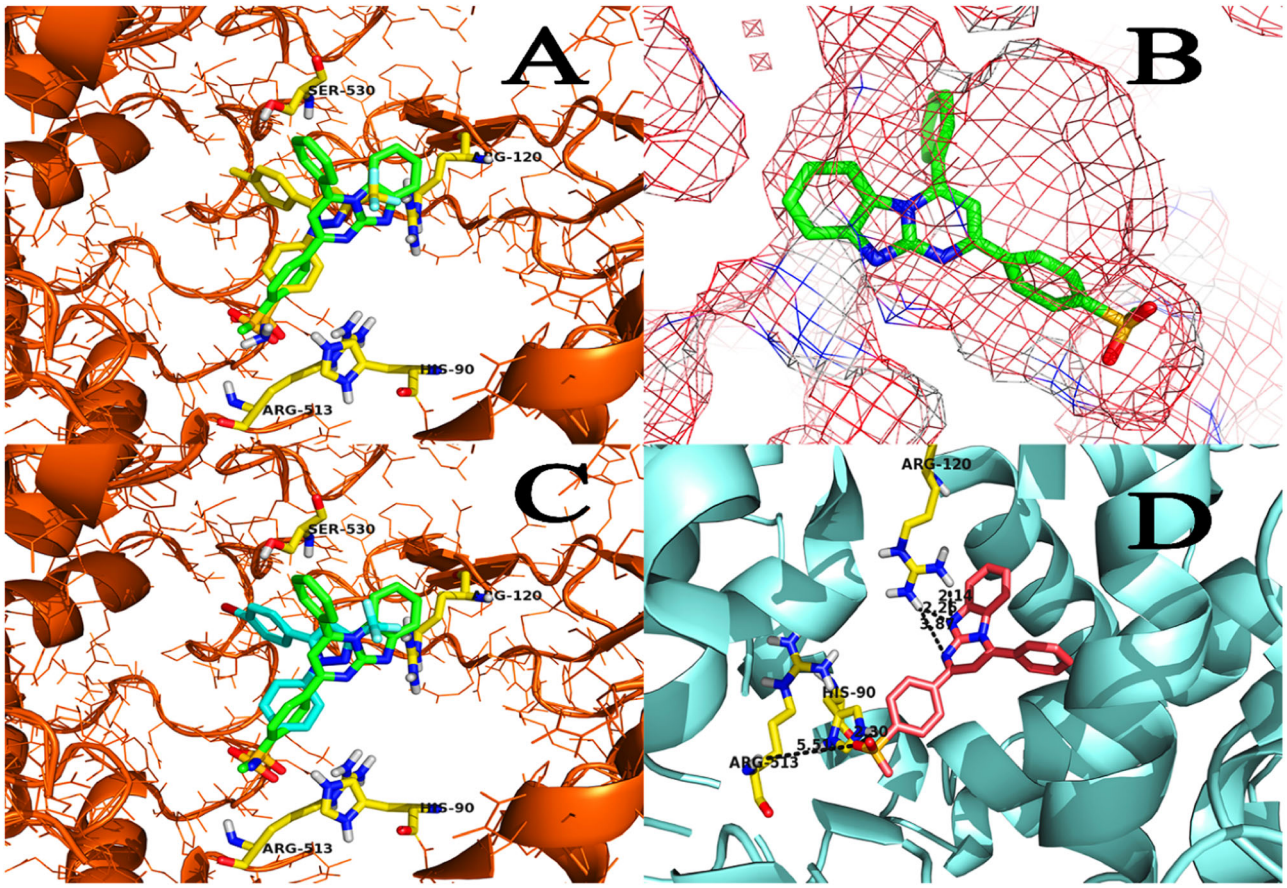


Fig. 2 A model of an efficient compound (**5a**) in the COX-2 isoform active site: **A** Celecoxib and **5a** superimposition (**B**) placement of SO2Me as pharmacophore group in the secondary pocket, (**C**) Sc-558 and **5a** superimposition, **D** The most important interactions distance. PyMol created pictures

pyrimidine derivatives and the COX-2 active site. This study shows the interaction of compounds and active site, and partly explains the reasons for the existence of different derivatives in potency and selectivity. Generally, the SO_2Me group of all derivatives was inserted in the secondary pocket of the COX-2 enzyme that is responsible for the selectivity. Also, the superimposition of compound **5a** with celecoxib confirmed the correct placement of the pharmacophore group in this hydrophobic pocket. The oxygen atoms of the SO_2Me group in compound **5a** have formed hydrogen bonds with NH of His-90 (distance: 2.30 Å) and NH of Arg-513 (distance: 5.53 Å). Also, hydrogen bonds between N-atoms of 2,4-diphenylbenzo[4,5]imidazo[1,2-*a*]pyrimidine structure and Arg-120 (distances: 3.87, 2.25, and 2.14 Å) were observed Fig. 2.

Table 1 The COX-1 and COX-2 enzyme inhibition assay

Compound	R	IC_{50} in μM		Selectivity Index (SI)
		Ovine COX-1	Human recombinant COX-2	
5a	H	6.1	0.05	122
5b	2-Me	48.3	> 100	ND
5c	3-Me	24.0	> 100	ND
5d	4-Me	40.8	0.68	60
5e	2-OMe	6.9	0.06	115
5f	3-OMe	> 100	> 100	ND
5g	4-OMe	> 100	5.4	> 18.5
5h	3,4-diOMe	13.9	> 100	ND
5i	3,4,5-triOMe	16.3	0.58	28.1
5j	2-F	14.0	0.28	50
5k	3-F	> 100	5.8	17.2
5l	4-F	38.8	0.69	99.4
5m	2-Cl	15.2	> 100	ND
5n	3-Cl	10.4	0.24	43.3
5o	4-Cl	17.8	> 100	ND
Celecoxib		24.6	0.06	410

ND Not determined

Biological evaluations

In vitro cyclooxygenase activity

A new series of 2,4-diphenylbenzo[4,5]imidazo[1,2-*a*]pyrimidine with SO_2Me pharmacophore group at the *para*-position of the C-2 phenyl ring and C-4 phenyl ring having various substituents were synthesized, and the COX-2 inhibitory activity of these compounds was determined. COX-2 inhibition assay was performed accordingly to our previously reported method using a fluorescent enzyme kit (Table 1) [38]. The COX-2 inhibition results indicated that compound **5a** had the highest potency, even more than celecoxib as a known drug, and also showed the highest selectivity among all compounds. These results demonstrated that the presence of any substitution, especially on the *para*- and *meta*- position of the C-1 phenyl ring, reduces the efficiency and selectivity of COX-2 inhibitory activity. This effect may be attributed to the presence of steric hindrance around these positions. According to these results, compounds with *ortho*- methoxy or fluoro substituents showed considerable in vitro activities, apparently due to the formation of hydrogen bonds with the COX-2 active site. The higher potency and selectivity of compound **5e** compared with compound **5j** may be explained by the presence of a hydrogen acceptor area at this position.

In vivo anti-inflammatory assay

The anti-inflammatory of synthesized compounds possessing high potency and selectivity on the COX-2 enzyme was also screened in mice. The in vivo acetic acid-induced writhing test is widely used as a non-selective model for determining pain [39, 40]. The results of the acetic acid-induced writhing test for the investigation of the nociceptive responses are reported in Table 2. As shown in Table 2, all compounds had high efficiency in decreasing inflammation. Moreover, some compounds have been shown to have better effects compared to celecoxib as, a well-known anti-inflammatory drug. Compound **5a** had a high potency and selectivity on COX-2 inhibition as well as an anti-inflammatory effect. However, compound **5d** showed the highest efficacy in the in vivo test, which may be attributed to the pharmacokinetic properties. These results indicated that the compounds with hydrophilic substitution are more effective than compounds having hydrophobic substitution.

In vitro anti-cancer study

The synthesized compounds have been screened against the MCF-7 breast cancer cell line. As shown in Table 2, all compounds demonstrated considerable anti-proliferative activities against the MCF-7 cell line compared with

Table 2 The anti-inflammation activities of target compounds

Compound	Dose (mg/kg)	Response (n) Mean ± SD	Response (n) Mean ± SEM	*p*-value summary	Estimated $ED_{50\ (mg/kg)}$ (95% Confidence Interval)
Control	0 mg/kg	48.87 ± 4.75	48.87 ± 0.59	-	-
5a	5 mg/kg	25 ± 1.58	25 ± 0.20	****	8.65 (7.22–10.34)
	10 mg/kg	20.75 ± 3.07	20.75 ± 0.38	****	
	20 mg/kg	17.25 ± 2.95	17.25 ± 0.37	****	
	40 mg/kg	12.63 ± 2.64	12.63 ± 0.33	****	
	80 mg/kg	10.00 ± 1.50	10.00 ± 0.19	****	
5d	5 mg/kg	18.63 ± 5.87	18.63 ± 0.73	****	5.75 (4.15–7.79)
	10 mg/kg	17.88 ± 4.28	17.88 ± 0.54	****	
	20 mg/kg	14.75 ± 4.47	14.75 ± 0.56	****	
	40 mg/kg	13.25 ± 2.59	13.25 ± 0.32	****	
	80 mg/kg	10.25 ± 2.73	10.25 ± 0.34	****	
5e	5 mg/kg	38.38 ± 1.32	38.38 ± 0.16	****	28.99 (26.07–32.25)
	10 mg/kg	36.63 ± 1.41	36.63 ± 0.18	****	
	20 mg/kg	28.25 ± 3.99	28.25 ± 0.50	****	
	40 mg/kg	19.13 ± 3.76	19.13 ± 0.47	****	
	80 mg/kg	15 ± 1.58	15 ± 0.20	****	
5g	10 mg/kg	36.63 ± 1.32	36.63 ± 0.16	****	32.09 (28.21–36.53)
	20 mg/kg	29.13 ± 6.27	29.13 ± 0.78	****	
	40 mg/kg	21.88 ± 3.37	21.88 ± 0.42	****	
	80 mg/kg	13.63 ± 1.32	13.63 ± 0.16	****	
5i	10 mg/kg	39.25 ± 2.38	39.25 ± 0.30	***	40.06 (35.75–44.95)
	20 mg/kg	30.87 ± 3.82	30.87 ± 0.48	****	
	40 mg/kg	24.62 ± 3.04	24.62 ± 0.38	****	
	80 mg/kg	15.87 ± 3.18	15.87 ± 0.40	****	
5j	5 mg/kg	33.75 ± 1.39	33.75 ± 0.17	****	15.34 (13.14–17.89)
	10 mg/kg	25.88 ± 2.98	25.88 ± 0.37	****	
	20 mg/kg	19.88 ± 3.06	19.88 ± 0.38	****	
	40 mg/kg	16.75 ± 3.11	16.75 ± 0.39	****	
	80 mg/kg	13.38 ± 2.45	13.38 ± 0.31	****	
5k	10 mg/kg	32.38 ± 2.34	32.38 ± 0.29	****	22.80 (20.03–25.92)
	20 mg/kg	25.38 ± 3.43	25.38 ± 0.43	****	
	40 mg/kg	17.63 ± 4.79	17.63 ± 0.60	****	
	80 mg/kg	11.88 ± 3.18	11.88 ± 0.40	****	
5l	10 mg/kg	34.25 ± 1.71	34.25 ± 0.21	****	25.26 (22.90–27.91)
	20 mg/kg	26.13 ± 2.67	26.13 ± 0.33	****	
	40 mg/kg	19.25 ± 4.32	19.25 ± 0.54	****	
	80 mg/kg	11.75 ± 1.48	11.75 ± 0.18	****	
5n	5 mg/kg	28.50 ± 4.72	28.50 ± 0.59	****	12.11 (9.77–14.96)
	10 mg/kg	23.50 ± 3.20	23.50 ± 0.40	****	
	20 mg/kg	19.88 ± 4.11	19.88 ± 0.51	****	
	40 mg/kg	15.75 ± 4.35	15.75 ± 0.54	****	
	80 mg/kg	11.50 ± 3.77	11.40 ± 0.47	****	
Celecoxib	10 mg/kg	33.62 ± 1.79	33.62 ± 0.22	****	20.21 (18.05–22.60)
	20 mg/kg	26.37 ± 2.86	26.37 ± 0.35	****	
	40 mg/kg	13.75 ± 2.72	13.75 ± 0.34	****	
	80 mg/kg	7.37 ± 2.39	7.37 ± 0.29	****	

***$p < 0.001$, ****$p < 0.0001$

Table 3 The cytotoxic effects of synthesized compounds

% Inhibition (10 μM)	*R*	Compound
53.33	H	**5a**
76.93	2-Me	**5b**
76.00	3-Me	**5c**
78.20	4-Me	**5d**
74.27	2-OMe	**5e**
75.40	3-OMe	**5f**
79.60	4-OMe	**5g**
79.33	3,4-diOMe	**5h**
77.93	3,4,5-triOMe	**5i**
76.73	2-F	**5j**
58.73	3-F	**5k**
56.67	4-F	**5l**
51.27	2-Cl	**5m**
72.73	3-Cl	**5n**
72.33	4-Cl	**5o**
75.80	Celecoxib	

celecoxib as a reference, probably due to their COX-2 and PGs inhibitory effects Table 3.

Conclusion

This study introduced a new series of benzo[4,5]imidazo[1,2-*a*]pyrimidine derivatives as selective COX-2 inhibitors. The biological evaluation results, including COX-2 inhibition activities, in vivo anti-inflammatory and anti-cancer effects on the MCF-7 breast cancer cell line, indicated that the majority of the synthesized compounds had selectivity for COX-2 inhibitory activity and demonstrated considerable anti-proliferative activities against the MCF-7 cell line. These results indicated that 2,4-diphenylbenzo[4,5]imidazo[1,2-*a*]pyrimidine possessing SO_2Me pharmacophore group is a suitable structure having a tricyclic central core for designing new selective COX-2 inhibitors.

Materials and methods

Experimental

All chemicals and solvents were commercially available and prepared from Aldrich Chemical Co., Acros Co., and Merck AG Chemical Co., and any further purification was not used. The MicroSYNTH (Milestone company) was used for the synthesis of final derivatives. Through Thomas–Hoover capillary instrument, melting points were measured. Using a Perkin Elmer Model 1420 spectrometer on the υmax (cm^{-1}) scale, all IR spectra were recorded in a KBr disk. All the ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker FT-500 MHz instrument (Bruker Biosciences, USA) with internal reference trimethylsilane (TMS). High-resolution mass spectra were recorded on a 6410 Agilent LC-MS triple quadrupole mass spectrometer (LC-MS) with an electrospray ionization (ESI) interface. The absorbance was determined using a spectrophotometer ELISA reader (Infinite® M200, TECAN Austria GmbH, Grodig, Austria).

General procedure for the synthesis of chalcones (3)

The synthesis of target compounds begins with the production of substituted chalcone. Synthesis of related chalcones was accomplished by the treatment of 4'-(methylsulfonyl)acetophenone **1** (1 mmol) with aryl aldehyde **2** (1 mmol) and 10 wt % sodium hydroxide solution (3 mL) in ethanol; and for 30 min, continue stirring at room temperature. With TLC, the completion progress of the reaction was monitored, and after finishing starting substances, the precipitates were filtered and rinsed with cold ethanol; finally, the pure products were obtained.

General synthesis procedure of 2,4-diphenylbenzo[4,5]imidazo[1,2-*a*]pyrimidine derivatives (5a-o)

2-Aminobenzimidazole (1.5 mmol) was added to DMF (3 ml) as a solvent, and then a catalytic amount of piperidine (10% mmol) was added to this mixture. Then, substituted chalcone was added (1 mmol), and the reaction was accomplished under microwave irradiation at power 450 W for 10 min. For the purity process, methanol (2 ml) was added to the mixture, and after 10 min, the precipitate was filtered and washed with cold methanol. The product was purified by column chromatography on silica gel with dichloromethane:methanol (respectively 8:1).

2-(4-(Methylsulfonyl)phenyl)-4-phenylbenzo[4,5]imidazo[1,2-*a*]pyrimidine (5a)

Yellow solid, 80%; M. P. 221 °C; IR (KBr) υ_{max}1170, 1329 (SO_2) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.06 (s, 3H, SO_2CH_3), 6.66 (d, $J = 7.6$ Hz, 1H, benzimidazole H_7), 7.01 (t, 1H, benzimidazole H_6), 7.24 (s, 1H, pyrimidine ring), 7.43 (br, 1H, benzimidazole H_5), 7.62–7.69 (m, 5H, phenyl), 7.93 (d, $J = 7.6$ Hz, 1H, benzimidazole H_4), 8.03 (d, $J = 7.6$ Hz, 2H, 4-methylsulfonylphenyl H_2 & H_6), 8.42 (d, $J = 7.6$ Hz, 2H, 4-methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 43.47, 104.25, 113.73, 119.41, 120.83, 125.45, 126.25, 126.97, 127.27, 127.66, 128.54,

129.48, 130.37, 131.12, 140.59, 141.37, 149.14, 157.70. HRMS (ESI^+) m/z calc for $C_{23}H_{17}N_3O_2S$ 399.11, found 400 (M + H); Anal. Calcd. for $C_{23}H_{17}N_3O_2S$: C, 69.16; H, 4.29; N, 10.52. Found: C, 69.02; H, 4.50; N, 10.71.

2-(4-(Methylsulfonyl)phenyl)-4-(*o*-tolyl)benzo[4,5] imidazo[1,2-*a*]pyrimidine (5b)

Yellow solid, 83%; M. P. 278 °C; IR (KBr) υ_{max} 1170, 1328 (SO_2) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2 2.44 (s, 3H, CH_3), 3.04 (s, 3H, SO_2CH_3), 6.67 (d, 1H, $J = 8.4$ Hz, benzimidazole H_7), 6.99 (t, 1H, benzimidazole H_6), 7.21 (s, 1H, pyrimidine ring), 7.38–7.50 (m, 5H, 2-methylphenyl & benzimidazole H_5), 7.89 (d, $J = 8.4$ Hz, 1H, benzimidazole H_4), 8.00 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_2 & H_6), 8.40 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.52, 43.43, 104.15, 113.80, 119.26, 120.74, 124.24, 125.38, 126.22, 126.90, 127.61, 127.68, 128.36, 131.04, 138.59, 140.58, 141.30, 144.35, 149.37, 150.43, 157.63; HRMS (ESI^+) m/z calc for $C_{24}H_{19}N_3O_2S$ 414.12, found 414 (M + H); Anal. Calcd. for $C_{24}H_{19}N_3O_2S$: C, 69.71; H, 4.63; N, 10.16. Found: C, 69.95; H, 4.82; N, 10.29.

2-(4-(Methylsulfonyl)phenyl)-4-(*m*-tolyl)benzo[4,5] imidazo[1,2-*a*]pyrimidine (5c)

Yellow solid, 79%; M. P. 270 °C; IR (KBr) υ_{max} 1162, 1323 (SO_2) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.44 (s, 3H, CH_3), 3.04 (s, 3H, SO_2CH_3), 6.67 (d, $J = 8.4$ Hz, 1H, benzimidazole H_7), 6.99 (t, 1H, benzimidazole H_6), 7.21 (s, 1H, pyrimidine ring), 7.38–7.52 (m, 5H, 3-methylphenyl & benzimidazole H_5), 7.88 (d, $J = 8$ Hz, 1H, benzimidazole H_4), 7.99 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_2 & H_6), 8.39 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (100 MHz, $CDCl_3$) 20.52, 43.43, 104.16, 113.81, 119.25, 120.73, 124.24, 125.37, 126.22, 126.90, 127.60, 127.68, 128.35, 130.98, 138.59, 140.57, 141.30, 144.34, 149.37, 150.43, 157.63; HRMS (ESI^+) m/z calc for $C_{24}H_{19}N_3O_2S$ 413.12, found 414 (M + H); Anal. Calcd. for $C_{24}H_{19}N_3O_2S$: C, 69.71; H, 4.63; N, 10.16. Found: C, 69.97; H, 4.78; N, 10.11.

2-(4-(Methylsulfonyl)phenyl)-4-(*p*-tolyl)benzo[4,5] imidazo[1,2-*a*]pyrimidine (5d)

Yellow solid, 79%, M. P. 265 °C; IR (KBr) υ_{max} 1167, 1328 (SO_2) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.50 (s, 3H,CH_3), 3.04 (s, 3H, SO_2CH_3), 6.73 (d, $J = 8.4$ Hz, 1H, benzimidazole H_7), 6.99 (t, 1H, benzimidazole H_6), 7.18 (s, 1H, pyrimidine ring), 7.37-7.42 (m, 3H, 4-methylphenyl H_3 & H_5 & benzimidazole H_5), 7.49 (d, $J = 8$ Hz, 2H, 4-methylphenyl H_2 & H_6), 7.88 (d, $J = 8$ Hz, 1H, benzimidazole H_4), 7.99 (d, $J = 8$ Hz, 2H, 4-methylsulfonylphenyl H_2 & H_6), 8.38 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.73, 44.48, 105.36, 114.92, 120.27, 121.71, 126.43, 127.29, 127.94, 128.19, 128.64, 129.22, 130.17, 141.63, 141.85, 142.33, 145.38, 150.47, 151.53, 158.67; HRMS (ESI^+) m/z calc for $C_{24}H_{19}N_3O_2S$ 413.12, found 414 (M + H); Anal. Calcd. for $C_{24}H_{19}N_3O_2S$: C, 69.71; H, 4.63; N, 10.16. Found: C, 69.55; H, 4.40; N, 10.31.

4-(2-Methoxyphenyl)-2-(4-(methylsulfonyl)phenyl) benzo[4,5]imidazo[1,2-*a*]pyrimidine (5e)

Yellow solid, 80%; M. P. 273 °C; IR (KBr) υ_{max} 1152, 1310 (SO_2) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.059 (s, 3H, SO_2CH_3), 3.58 (s, 3H, OCH_3), 6.65 (d, $J = 8.4$ Hz, 1H, benzimidazole H_7), 7.40 (t, 1H, benzimidazole H_6), 7.10 (d, $J = 8.4$ Hz, 1H, 2-methoxyphenyl H_3), 7.18–7.22 (m, 1H, 2-methoxyphenyl H_5), 7.31 (s, 1H, pyrimidine ring), 7.42-7.46 (m, 2H, 2-methoxyphenyl H_4 & H_6), 7.66 (t, 1H, benzimidazole H_6), 7.96 (d, $J = 8.4$ Hz, 1H, benzimidazole H_4), 8.03 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_2 & H_6), 8.45 (d, 2H, 4-methylsulfonylphenyl H_3 & H_5 $J = 8.4$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 44.50, 55.65, 96.12, 106.18, 111.33, 114.07, 119.83, 121.01, 121.54, 122.22, 126.63, 127.57, 127.99, 128.82, 130.27, 133.23, 141.60, 142.47, 148.23, 157.30; HRMS (ESI^+) m/z calc for $C_{24}H_{19}N_3O_3S$ 429.12, found 430 (M + H); Anal. Calcd. for $C_{24}H_{19}N_3O_3S$: C, 67.12; H, 4.46; N, 9.78. Found: C, 67.33; H, 4.22; N, 9.51.

4-(3-Methoxyphenyl)-2-(4-(methylsulfonyl)phenyl) benzo[4,5]imidazo[1,2-*a*]pyrimidine (5f)

Yellow solid, 83%; M. P. 251 °C; IR (KBr) υ_{max} 1157, 1324 (SO_2) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.05 (s, 3H, SO_2CH_3), 3.83 (s, 3H, O CH_3), 6.72 (d, $J = 8.4$ Hz, 1H, benzimidazole H_7), 7.02 (t, 1H, benzimidazole H_6), 7.10 (s, 1H, 3-methoxyphenyl H_2), 7.20 (t, 2H, 3-methoxyphenyl H_4 & H_6), 7.24 (s, 1H, pyrimidine ring), 7.41 (t, 1H, benzimidazole H_5), 7.53 (t, 1H, 3-methoxyphenyl H_5), 7.90 (d, $J = 8$ Hz, 1H, benzimidazole H_4), 8.00 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_2 & H_6), 8.40 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (100 MHz, $CDCl_3$): 43.43, 54.63, 104.17, 112.58, 113.85, 116.00, 119.24, 119.30, 120.90, 125.49, 126.11, 126.93, 127.64, 129.79, 132.14, 140.52, 141.38, 144.19, 148.94, 150.30, 157.70, 159.26. HRMS (ESI^+) m/z calc for $C_{24}H_{19}N_3O_3S$ 429.12, found 430 (M + H); Anal. Calcd. for $C_{24}H_{19}N_3O_3S$: C, 67.12; H, 4.46; N, 9.78. Found: C, 67.01; H, 4.29; N, 9.59.

4-(4-Methoxyphenyl)-2-(4-(methylsulfonyl)phenyl) benzo[4,5]imidazo[1,2-*a*]pyrimidine (5g)

Yellow solid, 84%; M. P. 275 °C; IR (KBr) υ_{max} 1152, 1310 (SO_2) cm^{-1}; ^1H NMR NMR (400 MHz, $CDCl_3$) δ 3.05 (s, 3H, SO_2CH_3), 3.92 (s, 3H, OCH_3), 6.82 (d, $J = 8.8$ Hz, 1H, benzimidazole H_7), 7.03 (t, 1H, benzimidazole H_6), 7.12 (d, $J = 8.4$ Hz, 2H, 4-methoxyphenyl H_3 & H_5), 7.22 (s, 1H, pyrimidine ring), 7.42 (t, 1H, benzimidazole H_5), 7.55 (d, $J = 8.4$ Hz, 2H, 4-methoxyphenyl H_2 & H_6), 7.92 (d, $J = 8$ Hz, 1H, benzimidazole H_4), 8.01 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_2 & H_6), 8.41 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 44.49, 55.66, 105.69, 114.89, 114.94, 120.15, 121.84, 124.13, 126.60, 127.23, 127.98, 128.71, 129.89, 141.56, 142.43, 150.40, 151.40, 158.91, 181.96; HRMS (ESI^+) m/z calc for $C_{24}H_{19}N_3O_3S$ 429.12, found 430 (M + H); Anal. Calcd. for $C_{24}H_{19}N_3O_3S$: C, 67.12; H, 4.46; N, 9.78. Found: C, 66.99; H, 4.60; N, 9.89.

4-(3,4-Dimethoxyphenyl)-2-(4-(methylsulfonyl) phenyl)benzo[4,5]imidazo[1,2-*a*]pyrimidine (5h)

Yellow solid, 87%; M. P. 249 °C; IR (KBr) υ_{max} 1157, 1314 (SO_2) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.06 (s, 3H, SO_2CH_3), 3.84 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 6.83 (d, $J = 8.4$ Hz, 1H, benzimidazole H_7), 7.04–7.10 (m, 3H, benzimidazole H_6, 3,4-dimethoxyphenyl H_2 & H_5), 7.20 (d, $J = 7.6$ Hz, 1H, 4-methoxyphenyl H_6), 7.26 (s, 1H, pyrimidine ring), 7.44 (t, 1H, benzimidazole H_5), 7.94 (d, $J = 8$ Hz, 1H, benzimidazole H_4), 8.02 (d, $J = 8$ Hz, 2H, 4-methylsulfonylphenyl H_2 & H_6), 8.42 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 43.44, 55.18, 55.26, 104.63, 109.86. 110.58. 113.96, 119.10, 120.27, 120.89, 123.18, 125.64, 126.12, 126.96, 127.70, 140.50, 141.45, 148.62, 149.23, 150.29, 150.42, 157.95; HRMS (ESI^+) m/z calc for $C_{25}H_{21}N_3O_4S$ 459.12, found 460 (M + H); Anal. Calcd. for $C_{25}H_{21}N_3O_4S$: C, 65.35; H, 4.61; N, 9.14. Found: C,65.77; H, 4.81; N, 9.01.

4-(3,4,5-Trimethoxyphenyl)-2-(4-(methylsulfonyl) phenyl)benzo[4,5]imidazo[1,2-*a*] pyrimidine (5i)

Yellow solid, 85%; M. P. 225 °C; IR (KBr) υ_{max} 1157, 1322 (SO_2) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.04 (s, 3H, SO_2CH_3), 3.82 (s, 6H, OCH_3), 3.96 (s, 3H, OCH_3), 6.73 (d, $J = 8.4$ Hz, 1H, benzimidazole H_7), 7.08 (t, 1H, benzimidazole H_6), 7.28 (s, 1H, pyrimidine ring), 7.34 (s, 1H, 3,4,5-trimethoxyphenyl H_2), 7.36 (s, 1H, 3,4,5-trimethoxyphenyl H_6), 7.44 (t, 1H, benzimidazole H_5), 7.88 (d, $J = 7.6$ Hz, 1H, benzimidazole H_4), 7.97 (d, $J = 7.68$ Hz, 2H, 4-methylsulfonylphenyl H_2 & H_6), 8.34 (d, $J = 7.88$ Hz, 2H, 4-methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 44.40, 56.52, 61.43, 65.69, 105.35, 114.92, 115.82, 120.09, 121.79, 126.70, 127.08, 127.96, 128.67, 132.36, 140.23, 141.50, 142.41, 150.09, 151.47, 154.16, 158.87, 169.07; HRMS (ESI^+) m/z calc for $C_{26}H_{23}N_3O_5S$ 489.12, found 490 (M + H); Anal. Calcd. for $C_{26}H_{23}N_3O_5S$: C, 63.79; H, 4.74; N, 8.58. Found: C, 63.52; H, 4.49; N, 8.42.

4-(2-Flourophenyl)-2-(4-(methylsulfonyl)phenyl) benzo[4,5]imidazo[1,2-*a*]pyrimidine (5j)

Yellow solid, 81%; M. P. 254 °C; IR (KBr) υ_{max} 1154, 1317 (SO_2) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.04 (s, 3H, SO_2CH_3), 6.66 (d, 1H, $J = 8.4$ Hz, benzimidazole H_7), 7.05 (t, 1H, benzimidazole H_6), 7.30 (s, 1H, pyrimidine ring), 7.33 (t, 1H, 2-flourophenyl H_5), 7.43 (m, 2H, 2-flourophenyl H_3 & H_4), 7.60 (t, 1H, benzimidazole H_5), 7.69 (m, 1H, 2-flourophenyl H_6), 7.91 (d, $J = 8$ Hz, 1H, benzimidazole H_4), 8.00 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_2 & H_6), 8.40 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 44.48, 106.24, 113.71, 116.73, 120.43, 122.43, 125.53, 126.64, 127.36, 128.00, 128.71, 130.60, 133.76, 141.40, 142.48, 144.40, 145.20, 151.10, 158.53, 161.03; HRMS (ESI^+) m/z calc for $C_{23}H_{16}FN_3O_2S$ 417.12, found 418 (M + H); Anal. Calcd. for $C_{23}H_{16}FN_3O_2S$: C, 66.17; H, 3.86; N, 10.07. Found: C, 66.29; H, 4.01; N, 10.02.

4-(3-Flourophenyl)-2-(4-(methylsulfonyl)phenyl) benzo[4,5]imidazo[1,2-*a*]pyrimidine (5k)

Yellow solid, 81%; M. P. 290 °C; IR (KBr) υ_{max} 1157, 1316 (SO_2) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.13 (s, 3H, SO_2CH_3), 6.76 (d, $J = 8.4$ Hz, 1H, benzimidazole H_7), 7.11 (t, 1H, benzimidazole H_6), 7.27 (s, 1H, pyrimidine ring), 7.32 (s, 1H, 3-flourophenyl H_2), 7.41–7.74 (m, 4H, 3-flourophenyl H_4 & H_5 & H_6, benzimidazole H_5), 8.01 (d, $J = 8$ Hz, 1H, benzimidazole H_4), 8.11 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_2 & H_6), 8.49 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 44.48, 105.40, 114.50, 115.71, 115.94, 118.51, 118.72, 120.53, 122.23, 124.21, 124.25, 126.74, 126.99, 128.06, 128.71, 131.56, 131.64, 141.37, 142.59, 148.51, 151.58, 158.80, 161.73; HRMS (ESI^+) m/z calc for $C_{23}H_{16}FN_3O_2S$ 417.12, found 418 (M + H); Anal. Calcd. for $C_{23}H_{16}FN_3O_2S$: C, 66.17; H, 3.86; N, 10.07. Found: C, 66.01; H, 3.68; N, 10.20.

4-(4-Flourophenyl)-2-(4-(methylsulfonyl)phenyl) benzo[4,5]imidazo[1,2-*a*]pyrimidine (5l)

Yellow solid, 81%; M. P. 290 °C; IR (KBr) υ_{max} 1165, 1325 (SO_2) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.06 (s, 3H,

SO_2CH_3), 6.69 (d, $J = 8.4$ Hz, 1H, benzimidazole H_7), 7.06 (t, 1H, benzimidazole H_6), 7.23 (s, 1H, pyrimidine ring), 7.34 (t, 2H, 4-flourophenyl H_3 & H_5), 7.45 (t, 1H, benzimidazole H_5), 7.61–7.64 (m, 2H, 4-flourophenyl H_2 & H_6), 7.95 (d, $J = 8.4$ Hz, 1H, benzimidazole H_4), 8.41 (d, $J = 8$ Hz, 2H, 4-methylsulfonylphenyl H_2 & H_6), 8.43 (d, $J = 8$ Hz, 2H, 4-methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 43.44, 104.51, 113.47, 115.84, 116.05, 119.51, 121.03, 125.63, 127.00, 127.66, 129.51, 129.60, 140.62, 141.72, 148.29, 151.40, 159.25, 161.99; HRMS (ESI^+) m/z calc for $C_{23}H_{16}FN_3O_2S$ 417.12, found 418 (M + H); Anal. Calcd. for $C_{23}H_{16}FN_3O_2S$: C, 66.17; H, 3.86; N, 10.07. Found: C, 66.40; H, 3.99; N, 10.21.

4-(2-Chlorophenyl)-2-(4-(methylsulfonyl)phenyl) benzo[4,5]imidazo[1,2-*a*]pyrimidine (5m)

Yellow solid, 81%; M. P. 290 °C; IR (KBr) υ_{max} 1165, 1326 (SO_2) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.04 (s, 3H, SO_2CH_3), 6.42 (d, $J = 8.4$ Hz, 1H, benzimidazole H_7), 7.02 (t, 1H, benzimidazole H_6), 7.28 (s, 1H, pyrimidine ring), 7.42 (t, 1H, benzimidazole H_5), 7.56–7.65 (m, 4H, 2-chlorophenyl), 7.90 (d, $J = 8$ Hz, 1H, benzimidazole H_4), 8.00 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_2 & H_6), 8.41 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 44.48, 105.45, 113.65, 120.37, 122.53, 126.62, 127.19, 127.98, 128.09, 128.72, 130.48, 131.24, 132.75, 133.61, 141.46, 142.46, 145.27, 147.05, 150.97, 154.97, 154.16, 158.62; HRMS (ESI^+) m/z calc for $C_{23}H_{16}ClN_3O_2S$ 432.12, found 433 (M + H); Anal. Calcd. for $C_{23}H_{16}ClN_3O_2S$: C, 63.67; H, 3.72; N, 9.68. Found: C, 63.76; H, 3.99; N, 9.83.

4-(3-Chlorophenyl)-2-(4-(methylsulfonyl)phenyl) benzo[4,5]imidazo[1,2-*a*]pyrimidine (5n)

Yellow solid, 81%; M. P. 290 °C; IR (KBr) υ_{max} 1164, 1323 (SO_2) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.14 (s, 3H, SO_2CH_3), 6.78 (d, $J = 8.8$ Hz, 1H, benzimidazole H_7), 7.14 (t, 1H, benzimidazole H_6), 7.26 (s, 1H, pyrimidine ring), 7.33 (s, 1H, 3-chlorophenyl H_2), 7.53–7.38 (m, 4H, 2-chlorophenyl H_4 & H_5 & H_6 & benzimidazole H_5), 8.03 (d, $J = 8.4$ Hz, 1H, benzimidazole H_4), 8.12 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_2 & H_6), 8.50 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 44.48, 105.74, 114.52, 119.62, 120.54, 122.54, 126.52, 128.09, 128.51, 128.75, 130.96, 131.66, 133.68, 136.06, 139.89, 142.66, 148.66, 156.88, 162.72; HRMS (ESI^+) m/z calc for $C_{23}H_{16}ClN_3O_2S$ 432.12, found 433 (M + H); Anal. Calcd. for $C_{23}H_1Cl_6N_3O_2S$: C, 63.67; H, 3.72; N, 9.68. Found: C, 63.80; H, 3.99; N, 9.84.

4-(4-Chlorophenyl)-2-(4-(methylsulfonyl)phenyl) benzo[4,5]imidazo[1,2-*a*]pyrimidine (5p)

Yellow solid, 81%; M. P. 290 °C; IR (KBr) υ_{max} 1152, 1312 (SO_2) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.13 (s, 3H, SO_2CH_3), 6.80 (d, $J = 8.4$ Hz, 1H, benzimidazole H_7), 7.13 (t, 1H, benzimidazole H_6), 7.28 (s, 1H, pyrimidine ring), 7.51 (t, 1H, benzimidazole H_5), 7.65 (d, $J = 8.8$ Hz, 2H, 4-chlorophenyl H_2 & H_6), 7.70 (d, $J = 8.8$ Hz, 2H, 4-chlorophenyl H_3 & H_5), 7.99 (d, $J = 8.4$ Hz, 1H, benzimidazole H_4), 8.10 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_2 & H_6), 8.48 (d, $J = 8.4$ Hz, 2H, 4-methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 44.48, 105.42, 114.55, 120.59, 122.10, 126.66, 127.09, 128.03, 128.67, 129.80, 129.97, 130.46, 137.81, 141.43, 142.53, 145.37, 148.91, 151.35, 158.68; HRMS (ESI^+) m/z calc for $C_{23}H_{16}ClN_3O_2S$ 432.12, found 433 (M + H); Anal. Calcd. for $C_{23}H_{16}ClN_3O_2S$: C, 63.67; H, 3.72; N, 9.68. Found: C, 63.58; H, 3.45; N, 9.54.

Molecular modeling

AutoDock software version 4.0 was used to find the suitable placement of ligands in the active site and investigation important interactions between flexible ligands and the rigid COX-2 enzyme. A high-resolution PDB format file of the 3D crystallized enzyme was retrieved from the protein data bank server with PDB code 6COX. To prepare the enzyme for ducking, the SC-588 molecule as internal ligand and water molecules were first removed from this protein. The next steps were adding Kollman charges and, in the following, merging non-polar hydrogens. Finally, AutoDock 4 was used to gain the PDBQT format of the enzyme. To achieve the conformation with minimized energy, HyperChem 8.0 program through the MM + method was used, and then with AutoDock tools, the PDBQT format of ligands was obtained. A grid box with dimensions 40*40*40 around the active site of protein was built. Docking run was put to 100, and the Lamarckian genetic search algorithm was used. For more efficiency, the ligands-enzyme interactions with distances greater than 7.0 Å were removed. At last, for each compound, the structure with lower energy was chosen as the best conformation [41, 42].

Biological assays

In vitro cyclooxygenase inhibition evaluations

The in vitro COX inhibition assay was carried out with a Fluorescent enzyme kit (Cayman Chemical, MI, USA) (37). This Fluorescent inhibitor screening kit utilizes the production of fluorescence using the heme-catalyzed peroxidase activity of cyclooxygenase (COX-1: ovine, COX-2:

human recombinant). This assay is based on the generation of resorufin as a fluorescent compound in the reaction PGG_2 with ADHP (10-acetyl-3,7-dihydroxyphenoxazine). High potency compounds are determined due to the less intensity of fluorescence arising from resorufin [43].

In vivo anti-inflammatory assay

This experiment used male NMRI mice (18–22 g), obtained from the Animal House of School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran. They were housed in eight-mouse cages with a standard diet (temperature: 22 ± 2 °C, light-dark cycle: 12 h, humidity: 45–55%). All novel compounds in the vehicle (DMSO) were dispersed and injected at the doses of 5, 10, 20, and 40 mg/kg body weight of mice intraperitoneally (i.p.). This experiment, based on a protocol approved by the "Animal Experimentation Ethics Committee" of the University of Shahid Beheshti Medical Sciences, was performed, and each animal only once was used. For investigation of the nociceptive responses in mice, the acetic acid-induced writhing test was used. Thirty minutes after administration of different doses of the novel compounds, an acetic acid solution of 1% to each animal was injected to cause abdominal constriction, a typical writhing response, and used celecoxib and vehicle as a positive and negative control, respectively. The number of writhing responses during a 30 min period by observation was counted. The mean ± SEM of eight mice for each group was represented. The estimated ED_{50} values were calculated with a 95% confidence interval by using Graph Pad Prism software by nonlinear regression analysis of the log dose-response curve.

In vitro anti-cancer assay

Anti-cancer effects of these compounds against MCF-7, breast cancer cell line, were evaluated based on the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide)) assay.

MCF-7 cancerous cell line was prepared from the Iranian Biological Resource Center (IBRC), Tehran, Iran. The cancerous cells were seeded in 96-well culture plates with the RPMI1640 medium under a high-humidity atmosphere with 5% CO_2 at 37 °C. One day after, the culture medium present in the wells was replaced with a medium containing celecoxib as a reference compound, synthesized compounds, and RPMI culture medium as a control. Under the above situation, the cells for 72 h were allowed to incubate. Then 10 µL MTT was added, and incubation was continued for 4 h at 37 °C. After 4 h, the supernatant was removed, and cells for 20 min at 37 °C were exposed to 100 µL DMSO. The spectrophotometer plate reader determined the absorbance of cells at 570 nm [44].

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

References

1. Khare A, Trivedi S, Rajak H, Pawar R, Patil U, Singour P. Hansch analysis of novel pyrimidine derivatives as highly potent and specific COX-2 inhibitors. Med Chem Res. 2012;21:672–80. https://doi.org/10.1007/s00044-011-9566-8
2. Zarghi A, Arfaei S. Selective COX-2 inhibitors: a review of their structure-activity relationships. Iran J Pharm Res. 2011;10:655. https://doi.org/10.22037/ijpr.2011.1047
3. Makar KW, Poole EM, Resler AJ, Seufert B, Curtin K, Kleinstein SE, et al. COX-1 (PTGS1) and COX-2 (PTGS2) polymorphisms, NSAID interactions, and risk of colon and rectal cancers in two independent populations. Cancer Causes Control. 2013;24:2059–75. https://doi.org/10.1007/s10552-013-0282-1
4. Willoughby DA, Moore AR, Colville-Nash PR. COX-1, COX-2, and COX-3 and the future treatment of chronic inflammatory disease. Lancet. 2000;355:646–8. https://doi.org/10.1016/S0140-6736(99)12031-2
5. Vane JR, Botting RM. Mechanism of action of nonsteroidal anti-inflammatory drugs. Am J Med. 1998;104:2S–8S. https://doi.org/10.1016/S0002-9343(97)00203-9
6. Zeilhofer HU. Prostanoids in nociception and pain. Biochem Pharm. 2007;73:165–74. https://doi.org/10.1016/j.bcp.2006.07.037
7. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: Structural, cellular, and molecular biology. Annu Rev Biochem. 2000;69:145–82. https://doi.org/10.1146/annurev.biochem.69.1.145
8. Aisen PS. Evaluation of selective COX-2 inhibitors for the treatment of Alzheimer's disease. J Pain Symptom Manag. 2002;23:S35–S40. https://doi.org/10.1016/S0885-3924(02)00374-3
9. Trepanier CH, Milgram NW. Neuroinflammation in Alzheimer's disease: Are NSAIDs and selective COX-2 inhibitors the next line of therapy. J Alzheimers Dis. 2010;21:1089–99. https://doi.org/10.3233/JAD-2010-090667
10. Ho L, Purohit D, Haroutunian V, Luterman JD, Willis F, Naslund J, et al. Neuronal cyclooxygenase 2 expression in the hippocampal formation as a function of the clinical progression of Alzheimer disease. Arch Neurol. 2001;58:487–92. https://doi.org/10.1001/archneur.58.3.487
11. Zhang Z, Ghosh A, Connolly PJ, King P, Wilde T, Wang J, et al. Gut-Restricted Selective Cyclooxygenase-2 (COX-2) Inhibitors for Chemoprevention of Colorectal Cancer. J Med Chem. 2021;64:11570–96. https://doi.org/10.1021/acs.jmedchem.1c00890
12. Alaaeddine RA, Elzahhar PA, AlZaim I, Abou-Kheir W, Belal AS, El-Yazbi AF. The Emerging Role of COX-2, 15-LOX and PPARγ in Metabolic Diseases and Cancer: An Introduction to Novel Multi-target Directed Ligands (MTDLs). Curr Med Chem. 2021;28:2260–300. https://doi.org/10.2174/0929867327999200820173853
13. Berbecka M, Forma A, Baj J, Furtak-Niczyporuk M, Maciejewski R, Sitarz R. A Systematic Review of the Cyclooxygenase-2 (COX-2) Expression in Rectal Cancer Patients Treated with Preoperative Radiotherapy or Radiochemotherapy. J Clin Med. 2021;10:4443 https://doi.org/10.3390/jcm10194443
14. Mahboubi Rabbani SMI, Zarghi A. Selective COX-2 inhibitors as anti-cancer agents: a patent review (2014-2018). Expert Opin Ther Pat. 2019;29:407–27. https://doi.org/10.1080/13543776.2019.1623880

15. Mirian M, Zarghi A, Sadeghi S, Tabaraki P, Tavallaee M, Dadrass O, et al. Synthesis and cytotoxic evaluation of some novel sulfonamidederivativesagainst a few human cancer cells. Iran J Pharm Res. 2011;10:741 https://doi.org/10.22037/ijpr.2011.980
16. Yan H, Zhao H, Kang Y, Ji X, Zhang T, Wang Y, et al. Parecoxib alleviates the motor behavioral decline of aged rats by ameliorating mitochondrial dysfunction in the substantia nigra via COX-2/PGE2 pathway inhibition. Neuropharmacology. 2021:108627. https://doi.org/10.1016/j.neuropharm.2021.108627
17. Chen JS, Alfajaro MM, Chow RD, Wei J, Filler RB, Eisenbarth SC, et al. Nonsteroidal anti-inflammatory drugs dampen the cytokine and antibody response to SARS-CoV-2 infection. J Virol. 2021;95:e00014–21. https://doi.org/10.1128/JVI.00014-21
18. Pannunzio A, Coluccia M. Cyclooxygenase-1 (COX-1) and COX-1 inhibitors in cancer: a review of oncology and medicinal chemistry literature. Pharmaceuticals. 2018;11:101 https://doi.org/10.3390/ph11040101
19. Irfan M. Selective cyclooxygenase-2 inhibitors: A review of recent chemical scaffolds with promising anti-inflammatory and COX-2 inhibitory activities. Med Chem Res. 2020;29:809–30. https://doi.org/10.1007/s00044-020-02528-1
20. Biava M. Introduction to COX inhibitors. Future Med Chem. 2018;10:1737-40. https://doi.org/10.4155/fmc-2018-0159
21. Azami Movahed M, Daraei B, Shahosseini S, Esfahanizadeh M, Zarghi A. Design, synthesis, and biological evaluation of new pyrazino[1, 2-a] benzimidazole derivatives as selective cyclooxygenase (COX-2) inhibitors. Arch Pharm (Weinh, Ger). 2019;352:1800265 https://doi.org/10.1002/ardp.201800265
22. Carvalho LC, Ribeiro D, Seixas RS, Silva AM, Nave M, Martins AC, et al. Synthesis and evaluation of new benzimidazole-based COX inhibitors: a naproxen-like interaction detected by STD-NMR. RSC Adv. 2015;5:49098–109. https://doi.org/10.1039/C5RA04984A
23. Keri RS, Hiremathad A, Budagumpi S, Nagaraja BM. Comprehensive review in current developments of benzimidazole-based medicinal chemistry. Chem Biol Drug Des. 2015;86:19–65. https://doi.org/10.1111/cbdd.12462
24. Kaur S, Minhas R, Kaur B, Bansal G. Design, synthesis and evaluation of benzimidazole-NsCOXis hybrids for the treatment of Alzheimer's disease. 2021. https://doi.org/10.21203/rs.3.rs-230559/v1
25. Veerasamy R, Roy A, Karunakaran R, Rajak H. Structure–Activity relationship analysis of Benzimidazoles as emerging anti-inflammatory agents: An overview. Pharmaceuticals. 2021;14:663 https://doi.org/10.3390/ph14070663
26. Rathore A, A Siddiqui A, Ali A, Shahar Yar M. Synthesis and evaluation of benzimidazole derivatives as selective COX-2 inhibitors. Med Chem. 2015;11:188–99. https://doi.org/10.2174/1573406410666140815121613
27. Zarghi A, Reihanfard H, Arfaei S, Daraei B, Hedayati M. Design and synthesis of new 1, 2-diaryl-4, 5, 6, 7-tetrahydro-1H-benzo [d] imidazoles as selective cyclooxygenase (COX-2) inhibitors. Med Chem Res. 2012;21:1869–75. https://doi.org/10.1007/s00044-011-9709-y
28. Babbar R, Swikriti, Arora S. A comprehensive review on therapeutic potential of Benzimidazole: A miracle scaffold. J Pharm Technol, Res Manag. 2020;8:23–9. https://doi.org/10.15415/jptrm.2020.81004
29. Tietz O, Kaur J, Bhardwaj A, Wuest FR. Pyrimidine-based fluorescent COX-2 inhibitors: synthesis and biological evaluation. Org Biomol Chem. 2016;14:7250–7. https://doi.org/10.1039/C6OB00493H
30. Sanad SM, Mekky AE. New pyrido [3′, 2′: 4, 5] thieno [3, 2-d] pyrimidin-4 (3 H)-one hybrids linked to arene units: synthesis of potential MRSA, VRE, and COX-2 inhibitors. Can J Chem. 2021;99:1–10. https://doi.org/10.1139/cjc-2021-0121
31. Almansa C, de Arriba AF, Cavalcanti FL, Gómez LA, Miralles A, Merlos M, et al. Synthesis and SAR of a new series of COX-2-selective inhibitors: pyrazolo [1, 5-a] pyrimidines. J Med Chem. 2001;44:350–61. https://doi.org/10.1021/jm0009383
32. Beswick PJ, Blackaby AP, Bountra C, Brown T, Browning K, Campbell IB, et al. Identification and optimisation of a novel series of pyrimidine based cyclooxygenase-2 (COX-2) inhibitors. Utilisation of a biotransformation approach. Bioorg Med Chem Lett. 2009;19:4509–14. https://doi.org/10.1016/j.bmcl.2009.02.089
33. ur Rashid H, Martines MAU, Duarte AP, Jorge J, Rasool S, Muhammad R, et al. Research developments in the syntheses, anti-inflammatory activities and structure–activity relationships of pyrimidines. RSC Adv. 2021;11:6060–98. https://doi.org/10.1039/D0RA10657G
34. Aeluri R, Alla M, Polepalli S, Jain N. Synthesis and antiproliferative activity of imidazo [1, 2-a] pyrimidine Mannich bases. Eur J Med Chem. 2015;100:18–23. https://doi.org/10.1016/j.ejmech.2015.05.037
35. Azami Movahed M, Daraei B, Zarghi A. Synthesis and biological evaluation of new imidazo [1, 2-a] pyridine derivatives as selective COX-2 inhibitors. Lett Drug Des Disco. 2016;13:793–9. https://doi.org/10.2174/1570180813666160613090944
36. Farzaneh S, Shahhosseini S, Arefi H, Daraei B, Esfahanizadeh M, Zarghi A. Design, Synthesis and Biological Evaluation of New 1, 3-diphenyl-3-(phenylamino) propan-1-ones as Selective Cyclooxygenase (COX-2) Inhibitors. Med Chem. 2018;14:652–9. https://doi.org/10.2174/1573406414666180525133221
37. Reddy MV, Reddy GCS, Lien NTK, Kim DW, Jeong YT JT An efficient and green synthesis of benzo [4, 5] imidazo [1, 2-a] pyrimidines using highly active and stable poly acrylic acid-supported layered double hydroxides. 2017;73:1317–23. https://doi.org/10.1016/j.tet.2017.01.037
38. Zarghi A, Najafnia L, Daraee B, Dadrass OG, Hedayati M. Synthesis of 2, 3-diaryl-1, 3-thiazolidine-4-one derivatives as selective cyclooxygenase (COX-2) inhibitors. Bioorg Med Chem Lett. 2007;17:5634–7. https://doi.org/10.1016/j.bmcl.2007.07.084
39. Koster R. Editor Acetic acid for analgesic screening. Fed Proc. 1959
40. Singh S, Majumdar D. Analgesic activity of Ocimum sanctum and its possible mechanism of action. Int J Pharmacogn. 1995;33:188–92. https://doi.org/10.3109/13880209509065361
41. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem. 1998;19:1639–62. 10.1002/(SICI)1096-987X(19981115)19:14<1639::AID-JCC10>3.0.CO;2-B
42. Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY, et al. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. Nature. 1996;384:644 https://doi.org/10.1038/384644a0
43. COX Fluorescent Inhibitor Screening Assay Kit [Available from: https://www.caymanchem.com/pdfs/700100.pdf
44. Ahmaditaba MA, Shahosseini S, Daraei B, Zarghi A, Houshdar Tehrani MHDesign. Synthesis, and biological evaluation of new peptide analogues as selective COX-2 Inhibitors. Arch Pharm (Weinh, Ger). 2017;350:1700158 https://doi.org/10.1002/ardp.201700158