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FULL PAPER

Design, synthesis, and biological evaluation of new 1,4-diarylazetidin-2-one derivatives (β-lactams) as selective cyclooxygenase-2 inhibitors

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Abstract

A new series of 1,4-diarylazetidin-2-one derivatives (β-lactams) were designed and synthesized to evaluate their biological activities as selective cyclooxygenase-2 (COX-2) inhibitors. In vitro COX-1 and COX-2 inhibition studies showed that all compounds were selective inhibitors of the COX-2 isozyme with IC_{50} values in the 0.05–0.11 μM range, and COX-2 selectivity indexes in the range of 170–703.7. Among the synthesized β-lactams, 3-methoxy-4-(4-(methylsulfonyl)phenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**4j**) possessing trimethoxy groups at the N-1 phenyl ring exhibited the highest COX-2 inhibitory selectivity and potency, even more potent than the reference drug celecoxib. The analgesic activity of the synthesized compounds was also determined using the formalin test. Compound **4f** displayed the best analgesic activity among the synthesized molecules. Molecular modeling studies indicated that the methylsulfonyl pharmacophore group can be inserted into the secondary pocket of the COX-2 active site for interactions with Arg^{513}. The structure–activity data acquired indicate that the β-lactam ring moiety constitutes a suitable scaffold to design new 1,4-diarylazetidin-2-ones with selective COX-2 inhibitory activity.

KEYWORDS
1,4-diarylazetidin-2-ones, antinociceptive activity, cyclooxygenase-2 inhibition, docking studies, formalin test, molecular modeling, β-lactams

1 | INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used medications. Through their anti-inflammatory, antipyretic, and analgesic activities, they appear as a preferred treatment in various inflammatory diseases such as arthritis, rheumatisms, as well as relieving the pains of daily life. Considering that NSAIDs inhibit both isoforms of cyclooxygenase (COX; constitutive COX-1, responsible for cytoprotective effects; inducible COX-2, responsible for inflammatory effects), they can have side effects associated with GI ulcers and renal function suppression.[1,2] Thus, it was thought that more selective COX-2 inhibitors would have reduced side effects. On this basis, a number of selective COX-2 inhibitors were developed as safer NSAIDs with improved gastric safety profile.[3] In addition, COX-2 inhibitors have been shown to reduce the risk of several cancer types, such as breast, colon, lung, and prostate cancers[4–7] and neurodegenerative diseases such as Parkinson[8] and Alzheimer's[9] diseases. Selective COX-2 inhibitors such as celecoxib and rofecoxib (COXIBs) mainly belong to a class of tricyclics that possess two vicinal diaryl moieties attached to a five- or six-membered cyclic scaffold containing a characteristic methanesulfonyl, sulfonamido, or azido group on one of the aryl rings that plays an important role on COX-2 selectivity.[10–23] However, the market removal of some COXIBs such as rofecoxib and valdecoxib

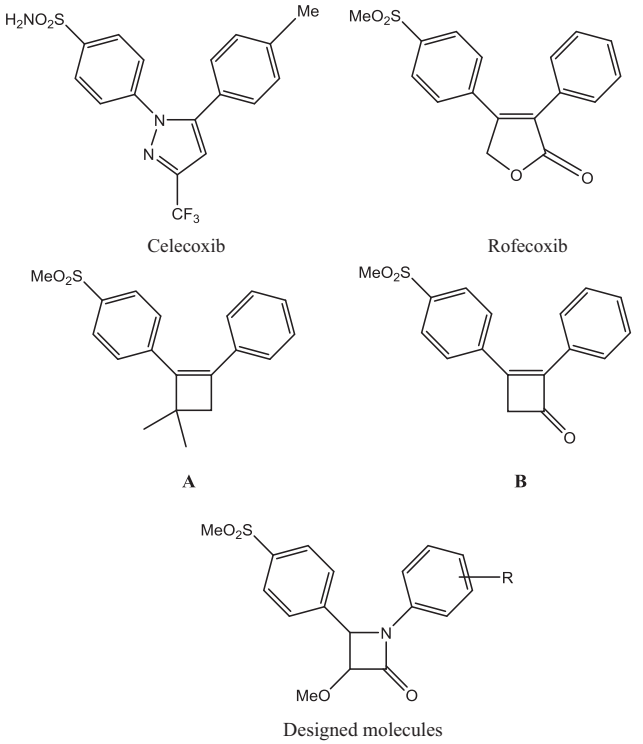


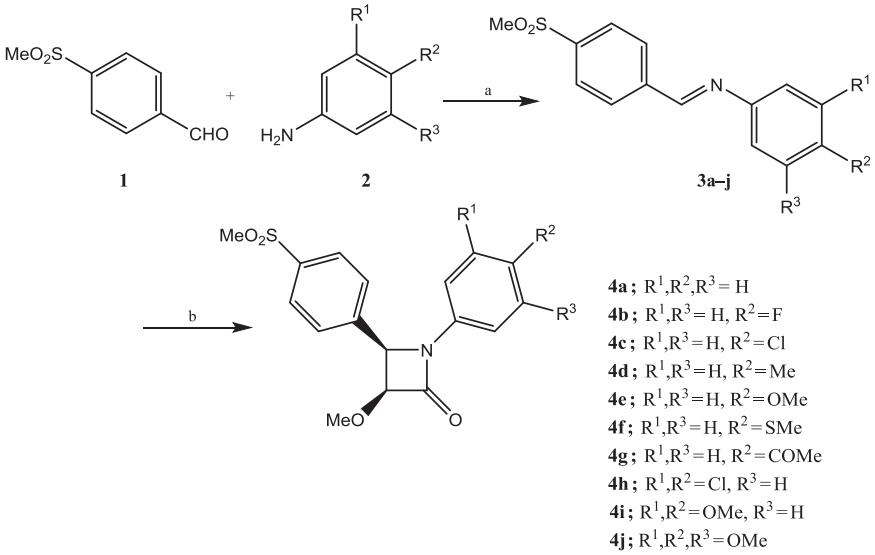
FIGURE 1 Chemical structures of celecoxib, rofecoxib, lead compounds (**A** and **B**), and our designed molecules

due to increased risk of heart attack and cardiovascular toxicity[24] encourages the medicinal chemists to develop new scaffolds for COX-2 inhibitory activities with improved safety profiles. For this reason, novel scaffolds with high selectivity for COX-2 inhibition need to be found and evaluated for their biological activities. In this regard, ring contraction to smaller carbocycles such as cyclobutenes leads to potent COX-2 inhibitors (compounds: **A** and **B**; Figure 1). Accordingly, compounds with a cyclobutene central ring show IC_{50} values for COX-1 of 0.12 (**A**) and >5 μM (**B**), for COX-2, 0.002 (**A**) and 0.11 μM (**B**).[25] Therefore, it is interesting to synthesize new COX-2 inhibitors having the 1,4-diarylazetidin-2-one (β-lactam ring) scaffold as a 4-membered heterocyclic central ring to evaluate their COX-2 inhibitory activities. The β-lactam ring has been widely used as an active moiety for designing antibiotics,[26] antitumors,[27] thrombin inhibitors,[28] antihyperglycemic,[29] anti-HIV,[30] and analgesic agents[31] due to its safety and activity. As part of our research program, aimed at discovering new selective COX-2 inhibitors, we focused our attention on the synthesis, COX inhibitory, analgesic activity and some molecular modeling studies of 1,4-diaryl-β-lactams possessing a methylsulfonyl COX-2 pharmacophore at the *para* position of the C-4 phenyl ring and different substituents at the N-1 phenyl ring. The rationale for the design of these compounds was based on the application of the β-lactam moiety as a new scaffold for developing new selective COX-2 inhibitors.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

Due to the importance of β-lactams, several synthetic methods have been developed for the preparation of the β-lactam ring.[32] The Staudinger reaction is regarded as the most fundamental and versatile method for the synthesis of the β-lactam ring and is used for the synthesis of a large number of β-lactams.[33] This reaction is known as the [2 + 2] cycloaddition of ketenes to imines. The ketenes are generally formed in situ by the reaction of acyl halides with tertiary amines (such as triethylamine, TEA). Due to low stability and high toxicity of acyl halides, as the ketene precursors, many reagents such as *p*-toluenesulfonyl chloride, ethyl chloroformate, triphosgene, and Vilsmeier reagent are used to generate ketenes in situ from acids.[34] In this study, *p*-toluenesulfonyl chloride (tosyl chloride) was used as the acid activator to synthesize 1,4-diarylazetidin-2-ones from different imine derivatives and methoxy acetic acid in the presence of trimethylamine (Scheme 1).[34,35]



SCHEME 1 Synthesis route for the target compounds **4a–f**. Reagents and conditions: (a) Dry DMF, 25°C, 24 hr; (b) dry DMF, TEA, methoxy acetic acid, TsCl, 25°C, 24 hr. DMF, dimethylformamide; TEA, triethylamine

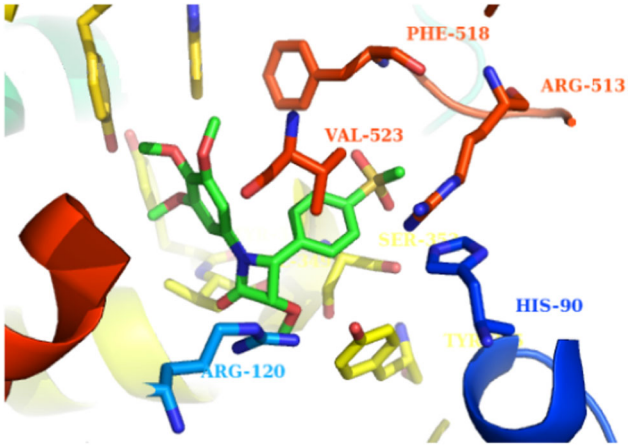


FIGURE 2 Docking of 3-methoxy-4-(4-(methylsulfonyl)phenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one **4j** (in green) in the active site of murine COX-2. Hydrogen atoms have been removed to improve clarity

The stereochemistry of products from the Staudinger reaction depends on numerous factors, including the reaction conditions, the order of addition of the reagents, and the substituents present on the imine intermediate. On this basis, we used a modified Staudinger reaction that leads to β-lactam with *cis* selectivity ($J_{3,4}$ > 4.0 Hz for the *cis* and $J_{3,4}$ < 3.0 Hz for the *trans* stereoisomers).[36]

2.2 | Molecular modeling

The binding interactions of the most selective COX-2 inhibitor (**4j**) within the COX-2 binding site were investigated. The most stable enzyme–ligand complex of 3-methoxy-4-(4-(methylsulfonyl)-phenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**4j**) possessing a methysulfonyl COX-2 pharmacophore group within the COX-2 binding site (Figure 2) shows that the *para*-SO_2Me substituent inserts into the secondary pocket present in COX-2 (Arg^{513}, Phe^{518}, and Val^{523}). One of the O-atoms of the SO_2Me moiety forms a hydrogen binding with the NH of Arg^{513} (distance = 3.3 Å), whereas the other O-atom is close to the NH of Phe^{518} (distance = 3.8 Å). The oxygen atom of the methoxy group of C-3 is also close to the N*H* of Arg^{120} (distance = 2.5 Å) and, therefore, can form a hydrogen bond with this amino acid. In addition, the C═O of the β-lactam scaffold is almost close to the NH of Ser^{353} (distance = 4.7 Å), which may form a hydrogen-bonding interaction. It was interesting to note that the methoxy groups of N-1 phenyl ring formed hydrogen bonds with hydroxyl groups (OH) of Tyr^{348} (distance = 3.1 Å), Tyr^{385} (distance = 2.7 Å) and NH of indole ring of Trp^{387} (distance = 4.3 Å). Figures 3 and 4 show that (**4j**) and SC-558 (co-crystallized inhibitor) were superimposed tightly in COX-2 active site and both had similar interactions with active site amino acids. Additional docking studies were performed to show the superimposition of all synthesized compounds and SC-558, which showed that SO_2Me moiety of all designed compounds was inserted in the secondary pocket of the COX-2 active site. The molecular docking scores of synthesized

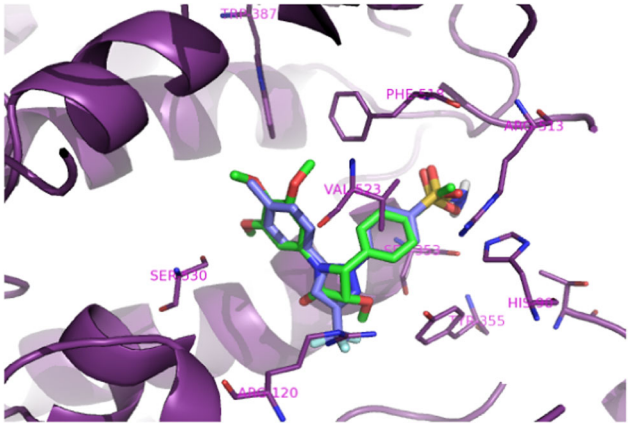


FIGURE 3 Superimposition of SC-558 (blue) and **4j** (green) with COX-2

compounds were also represented in Table 1. Overall, most of the compounds with (3*S*,4*R*) stereochemistry showed higher docking scores than (4*S*,3*R*) stereoisomers.

2.3 | Biological activity

A group of 1,4-diarylazetidin-2-ones (**4a–j**) containing SO_2Me at the *para* position of the C-4 phenyl ring and a variety of substituents at N-1 phenyl ring were synthesized to investigate the structure–activity relationship (SAR) of these compounds. Accordingly, all compounds were potent and selective inhibitors of the COX-2 isozyme with IC_{50} values in the highly potent 0.050–0.108 μM range, and COX-2 selectivity indices in the 124.7–319.8 range (Table 2). The IC_{50} values determined for the in vitro inhibition of COX-1 and COX-2 indicated that the nature and size of substituents on the N-1 phenyl ring influenced both selectivity and potency for COX-2 inhibitory activity. Our results showed that compounds having methoxy groups (such as **4e**, **4i**, and **4j**) were more potent and selective COX-2 inhibitors compared with other analogs. This may be explained by the hydrogen

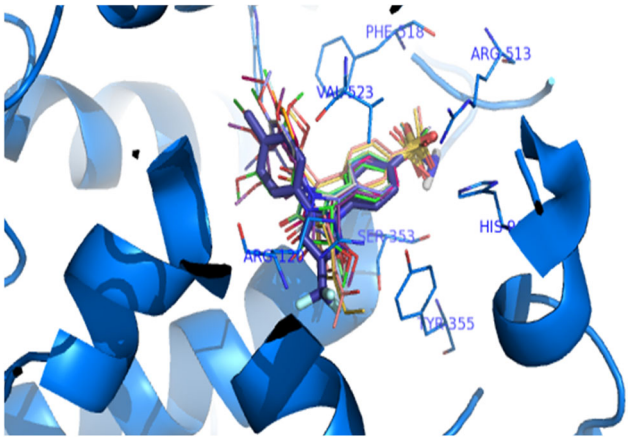
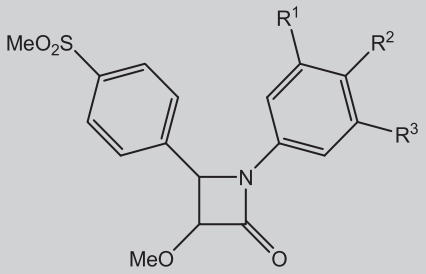


FIGURE 4 Three-dimensional superimposed representations of SC-558 and the synthesized compounds with COX-2

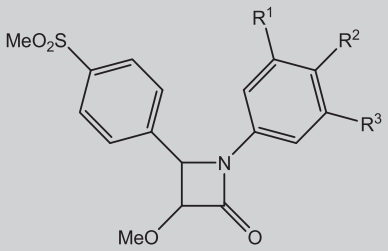
TABLE 1 Docking scores for compounds **4a–j**



Compound	R^1	R^2	R^3	Affinity (kcal/mol; 3*S*,4*R*)	Affinity (kcal/mol; 3*R*,4*S*)
4a	H	H	H	−7.1	−6.7
4b	H	F	H	−7.3	−7.2
4c	H	Cl	H	−7.0	−6.8
4d	H	Me	H	−7.2	−5.9
4e	H	OMe	H	−5.9	−6.0
4f	H	SMe	H	−5.1	−4.4
4g	H	COMe	H	−6.4	−6.9
4h	H	Cl	Cl	−4.8	−5.5
4i	H	OMe	OMe	−4.4	−3.0
4j	OMe	OMe	OMe	−4.0	−3.3

binding of methoxy groups with COX-2 active site amino acids. Subsequently, compounds with mono- or di-Cl or SMe substituent (**4c**, **4f**, and **4h**) showed less selectivity and potency for COX-2 isozyme, which may be explained by steric parameters for interaction with COX-2 active binding site. In addition, suitable substituents such as F (**4b**) or COMe (**4g**) at the *para* position of the N-1 phenyl ring also increased both selectivity and potency for COX-2 inhibitory activity. It seems that F and COMe would have facilitated charge-transfer interaction due to their electron-withdrawing properties. According to our results, the ability of the substituent at the N-1 phenyl ring for hydrogen binding formation and also charge-transfer interaction may be important for COX-2 inhibitory activity in these series of compounds. In accordance with our results, 3-methoxy-4-(4-(methylsulfonyl)phenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**4j**) was the most potent and selective (IC_{50} = 0.058 μM, SI = 240.7) COX-2 inhibitor among the synthesized compounds. The ability of synthesized compounds for in vivo analgesic activity, which was evaluated by the formalin test, is also presented in Table 3. A significant reduction in the AUC of pain score was seen in groups treated with **4b**, **4c**, **4d**, and **4f** ($p < 0.001$), **4a** and **4g** ($p < 0.01$), **4e** and **4j** ($p < 0.05$) compared with the control group. However, neither **4h** nor **4i** could change the AUC of pain score compared with the control group. Therefore, it can be assumed that disubstituted compounds or compounds with bulky substituents were not active enough in analgesic activity evaluation. Among active analgesic compounds, it seems that electron-donating groups having

TABLE 2 In vitro COX-1 and COX-2 enzyme inhibition assay data



Compound	R^1	R^2	R^3	IC_{50} (μM)[a] COX-1	IC_{50} (μM)[a] COX-2	Selectivity index (SI)[b]
4a	H	H	H	11.80	0.089	132.5
4b	H	F	H	13.82	0.065	212.5
4c	H	Cl	H	12.98	0.083	156.4
4d	H	Me	H	12.28	0.076	186.2
4e	H	OMe	H	12.84	0.063	203.8
4f	H	SMe	H	13.47	0.108	124.7
4g	H	COMe	H	13.26	0.061	217.4
4h	H	Cl	Cl	14.23	0.097	146.7
4i	H	OMe	OMe	13.96	0.058	240.7
4j	OMe	OMe	OMe	15.99	0.050	319.8
Celecoxib				24.30	0.06	405

[a]Values are means of two determinations acquired using an ovine COX-1/COX-2 assay kit and the deviation from the mean is <10% of the mean value.
[b]In vitro COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

TABLE 3 Effects of pretreatment with celecoxib or new compounds on pain-related behaviors of rats in the formalin test

Compound	AUC of pain score (mean ± SEM)
Control	84.19 ± 4.52
4a	52.95 ± 7.76**
4b	48.58 ± 5.13***
4c	51.75 ± 4.02***
4d	42.73 ± 2.07***
4e	57.90 ± 2.30*
4f	34.73 ± 3.33***
4g	50.77 ± 6.84**
4h	98.29 ± 2.11
4i	100.30 ± 1.31
4j	56.82 ± 2.65*
Celecoxib	48.63 ± 10.83***

Note: Data are shown as mean ± SEM ($N = 6$).
Abbreviations: AUC, area-under-the-curve; SEM, standard error of the mean.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$—significant difference compared with the control group.

good lipophilicity, such as Me and SMe, could lead to better analgesic effects, whereas electron-withdrawing groups, like F, Cl, and COMe, decreased analgesic effects. Overall, these findings are in good agreement with the in vitro results. However, in some cases, there was not a meaningful correlation between in vitro and in vivo results. This may be explained by the physiochemical and pharmacokinetic parameters of these compounds, which can influence their in vivo activities.

3 | CONCLUSIONS

A series of β-lactam analogs of **4a–j** were synthesized and evaluated for their COX inhibitory activity using an enzyme chemiluminescent assay. Our results indicated that 3-methoxy-4-(4-(methylsulfonyl)-phenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one **4j** was the most potent ($IC_{50} = 0.05$ μM), and selective (SI = 319.8), COX-2 inhibitor among the synthesized compounds, which may be due to better interaction with the COX-2 active site. It was more potent than celecoxib ($IC_{50} = 0.06$ μM; SI = 405) in terms of COX-2 inhibitory activity but showed less selectivity. The analgesic activity of the synthesized compounds was also determined using the formalin test. According to our results, 3-methoxy-4-(4-(methylsulfonyl)phenyl)-1-(4-(methylthio)phenyl)azetidin-2-one (**4f**) displayed the best analgesic activity among the synthesized molecules.

Our results indicated that (i) β-lactam ring is a suitable scaffold (template) to design COX-1/-2 inhibitors, (ii) COX-1/-2 inhibition is sensitive to the substituent of N-1 phenyl ring, (iii) small electron-donating groups such as Me and SMe could lead to better analgesic effects.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

All chemicals and solvents used in this study were purchased from Merck AG and Aldrich Chemical. Melting points (mps) were determined with a Thomas–Hoover capillary apparatus. Infrared (IR) spectra were acquired using a Perkin Elmer Model 1420 spectrometer. A Bruker FT-500 MHz instrument (Bruker Biosciences) was used to acquire ^{1}H-NMR spectra with TMS as an internal standard. Chloroform-D and dimethyl sulfoxide (DMSO)-D_6 were used as solvents. Coupling constant (J) values are estimated in hertz (Hz) and spin multiples are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). The mass spectral measurements were performed on a 6410 Agilent LC-MS triple quadrupole mass spectrometer (LC-MS) with an electrospray ionization (ESI) interface. Microanalyses, determined for C and H, were within ±0.4% of theoretical values.

The original spectra are provided as Supporting Information, as are the InChI codes of the investigated compounds together with some biological activity data.

4.1.2 | General method for imine formation

A mixture of 4-(methylsulfonyl)benzaldehyde **1** (20 mmol) and appropriate amine **2** (20 mmol) in dry DMF (dimethylformamide; 5 ml) in the presence of molecular sieve was stirred under an argon atmosphere at 25°C for 48 hr to obtain imine **3**. After this time, water (50 ml) was added and the resulting solution was left to stand until a solid product crystallized. The resulting imine was recrystallized from ethanol (yield: 53–72%).

*N-(4-(Methylsulfonyl)benzylidene)aniline (**3a**)*
Yield, 75%; white crystalline powder; mp: 130.5–131.5°C; IR (KBr): ν (cm^{-1}) 1,624 (C=N), 1,315, and 1,115 (SO_2); LC-MS (ESI) m/z: 260.1 (M+1, 100); Anal. calcd. for $C_{14}H_{13}NO_2S$: C, 64.84; H, 5.05; N, 5.40. Found: C, 64.62; H, 4.84; N, 5.69.

*4-Fluoro-N-(4-(methylsulfonyl)benzylidene)aniline (**3b**)*
Yield, 75%; off-white crystalline powder; mp: 140–141°C; IR (KBr): ν (cm^{-1}) 1,632 (C=N), 1,312, and 1,150 (SO_2); LC-MS (ESI) m/z: 278.1 (M+1, 100); Anal. calcd. for $C_{14}H_{12}FNO_2S$: C, 60.63; H, 4.36; N, 5.05. Found: C, 60.34; H, 4.24; N, 5.29.

*4-Chloro-N-(4-(methylsulfonyl)benzylidene)aniline (**3c**)*
Yield, 80%; white crystalline powder; mp: 147–148°C; IR (KBr): ν (cm^{-1}) 1,635 (C=N), 1,322, and 1,145 (SO_2); LC-MS (ESI) m/z: 294.1 (M+1, 100); Anal. calcd. for $C_{14}H_{12}ClNO_2S$: C, 57.24; H, 4.12; N, 4.77. Found: C, 57.34; H, 4.29; N, 4.51.

*Methyl-N-(4-(methylsulfonyl)benzylidene)aniline (**3d**)*

Yield, 81%; pale yellow crystalline powder; mp: 184–185°C; IR (KBr): ν (cm^{-1}) 1,632 (C=N), 1,318, and 1,160 (SO_2); LC-MS (ESI) *m/z*: 274.1 (M+1, 100); Anal. calcd. for $C_{15}H_{15}NO_2S$: C, 65.91; H, 5.53; N, 5.12. Found: C, 65.77; H, 5.24; N, 5.01.

*4-Methoxy-N-(4-(methylsulfonyl)benzylidene)aniline (**3e**)*

Yield, 82%; off-white crystalline powder; mp: 216–217°C; IR (KBr): ν (cm^{-1}) 1,629 (C=N), 1,310, and 1,157 (SO_2); LC-MS (ESI) *m/z*: 290.1 (M+1, 100); Anal. calcd. for $C_{15}H_{15}NO_3S$: C, 62.26; H, 5.22; N, 4.84. Found: C, 62.39; H, 5.01; N, 5.03.

*4-Methylthio-N-(4-(methylsulfonyl)benzylidene)aniline (**3f**)*

Yield, 91%; off-white crystalline powder; mp: 240°C; IR (KBr): ν (cm^{-1}) 1,631 (C=N), 1,310, and 1,148 (SO_2); LC-MS (ESI) *m/z*: 306.1 (M+1, 100); Anal. calcd. for $C_{15}H_{15}NO_2S_2$: C, 58.99; H, 4.95; N, 4.59. Found: C, 59.22; H, 4.77; N, 4.33.

*4-Acetyl-N-(4-(methylsulfonyl)benzylidene)aniline (**3g**)*

Yield, 75%; white crystalline powder; mp: 188–189°C; IR (KBr): ν (cm^{-1}) 1,634 (C=N), 1,312, and 1,150 (SO_2); LC-MS (ESI) *m/z*: 302.1 (M+1, 100); Anal. calcd. for $C_{16}H_{15}NO_3S$: C, 63.77; H, 5.02; N, 4.65. Found: C, 63.99; H, 4.88; N, 4.87.

*3,4-Dichloro-N-(4-(methylsulfonyl)benzylidene)aniline (**3h**)*

Yield, 80%; white crystalline powder; mp: 179–180°C; IR (KBr): ν (cm^{-1}) 1,630 (C=N), 1,310, and 1,140 (SO_2); LC-MS (ESI) *m/z*: 328.0 (M+1, 100); Anal. calcd. for $C_{14}H_{11}Cl_2NO_2S$: C, 65.35; H, 4.31; N, 5.44. Found: C, 65.21; H, 4.51; N, 5.77.

*3,4-Dimethoxy-N-(4-(methylsulfonyl)benzylidene)aniline (**3i**)*

Yield, 74%; yellow crystalline powder; mp: 157–158°C; IR (KBr): ν (cm^{-1}) 1,632 (C=N), 1,313, and 1,144 (SO_2); LC-MS (ESI) *m/z*: 320.1 (M+1, 100); Anal. calcd. for $C_{16}H_{17}NO_4S$: C, 60.17; H, 5.36; N, 4.39. Found: C, 60.35; H, 5.55; N, 4.55.

*3,4,5-Trimethoxy-N-(4-(methylsulfonyl)benzylidene)aniline (**3j**)*

Yield, 83%; yellow crystalline powder; mp: 179–180°C; IR (KBr): ν (cm^{-1}) 1,628 (C=N), 1,310, and 1,158 (SO_2); LC-MS (ESI) *m/z*: 320.1 (M+1, 100); Anal. calcd. for $C_{17}H_{19}NO_5S$: C, 61.24; H, 5.74; N, 4.20. Found: C, 61.39; H, 5.50; N, 4.44.

4.1.3 | General procedure for the preparation of β-lactams (4a–j)

Imine **3** (30 mmol) was dissolved in dry DMF (5 ml) and TEA (100 mmol), tosyl chloride (30 mmol) and methoxy acetic acid (30 mmol) were added and stirred at room temperature for 24 hr. After this time, water was added and the resulting precipitate was filtered and recrystallized in ethanol (yield: 53–72%). The *cis* and *trans* stereochemistries of 2-azetidinones were concluded from coupling constants of H-3 and H-4 ($J_{3,4}$ > 4.0 Hz for the cis and $J_{3,4}$ < 3.0 Hz for the *trans* stereoisomer).

*3-Methoxy-4-(4-(methylsulfonyl)phenyl)-1-phenylazetidin-2-one (**4a**)*

Yield; 65%; off-white crystalline powder; mp: 187.5–188.5°C; IR (KBr): ν (cm^{-1}) 1,750 (C=O), 1,312, and 1,153 (SO_2); ^{1}H-NMR ($CDCl_3$): δ ppm 3.12 (s, 3H, SO_2CH_3), 3.32 (s, 3H, OCH_3), 4.92 (d, 1H, CH, J = 5.0 Hz), 5.3 (d, 1H, CH, J = 5.0 Hz), 7.14–7.15 (m, 1H, phenyl H_4), 7.30–7.32 (m, 4H, phenyl H_2–H_6), 7.64 (d, 2H, 4-methylsulfonylphenyl H_2 and H_6, J = 8.3 Hz), and 8.0 (d, 2H, 4-methylsulfonylphenyl H_3 and H_5, J = 8.3 Hz); ^{13}C-NMR ($CDCl_3$): δ ppm 44.39, 58.85, 60.92, 84.88, 117.30, 124.85, 127.70, 128.89, 129.32, 136.57, 140.05, 140.76, and 163.88; LC-MS (ESI) *m/z*: 332.1 (M+1, 100); Anal. calcd. for $C_{17}H_{17}NO_4S$: C, 61.61; H, 5.17; N, 4.23. Found: C, 61.47; H, 4.98; N, 3.97.

*1-(4-Fluorophenyl)-3-methoxy-4-(4-(methylsulfonyl)phenyl)azetidin-2-one (**4b**)*

Yield: 53%; brown crystalline powder; mp: 136.5–138.5°C; IR (KBr): ν (cm^{-1}) 1,743 (C=O), 1,309, and 1,150 (SO_2); ^{1}H-NMR ($CDCl_3$): δ ppm 3.14 (s, 3H, SO_2CH_3), 3.32 (s, 3H, OCH_3), 4.93 (d, 1H, CH, J = 4.9 Hz), 5.32 (d, 1H, CH, J = 4.9 Hz), 7.28–7.32 (m, 4H, 4-fluorophenyl H_2–H_6), 8.09 (d, 2H, 4-methylsulfonylphenyl H_2 and H_6, J = 8.4 Hz), and 8.13 (d, 2H, 4-methylsulfonylphenyl H_3 and H_5, J = 8.4 Hz); ^{13}C-NMR ($CDCl_3$): δ ppm 44.46, 58.88, 61.15, 85.09, 116.23, 122.53, 127.87, 128.89, 129.42, 140.75, 142.45, 157.45, and 163.59; LC-MS (ESI) *m/z*: 350.1 (M+1, 100); Anal. calcd. for $C_{17}H_{16}FNO_4S$: C, 58.44; H, 4.62; N, 4.01. Found: C, 58.71; H, 4.50; N, 4.25.

*1-(4-Chlorophenyl)-3-methoxy-4-(4-(methylsulfonyl)phenyl)azetidin-2-one (**4c**)*

Yield, 65%; white crystalline powder; mp: 229–231°C; IR (KBr): ν (cm^{-1}) 1,755 (C=O), 1,322, and 1,164 (SO_2); ^{1}H-NMR ($CDCl_3$): δ ppm 3.12 (s, 3H, SO_2CH_3), 3.31 (s, 3H, OCH_3), 4.93 (d, 1H, CH, J = 4.9 Hz), 5.32 (d, 1H, CH, J = 4.9 Hz), 7.23–7.30 (m, 4H, 4-Chlorophenyl H_2–H_6), 7.61 (d, 2H, 4-methylsulfonylphenyl H_2 and H_6, J = 8.2 Hz), and 7.90 (d, 2H, 4-methylsulfonylphenyl H_3 and H_5, J = 8.2 Hz); ^{13}C-NMR ($CDCl_3$): δ ppm 44.39, 58.92, 61.06, 85.10, 118.52, 127.80, 128.86, 130.02, 135.06. 139.56, 140.99, and 163.77; LC-MS (ESI) *m/z*: 366.1 (M+1, 100); Anal. calcd. for $C_{17}H_{16}ClNO_4S$: C, 55.81; H, 4.41; N, 3.83. Found: C, 60.05; H, 4.74; N, 4.12.

*3-Methoxy-4-(4-(methylsulfonyl)phenyl)-1-(p-tolyl)azetidin-2-one (**4d**)*

Yield, 69%; white crystalline powder; mp: 197–198°C; IR (KBr): ν (cm^{-1}) 1,754 (C=O), 1,320, and 1,163 (SO_2); ^{1}H-NMR ($CDCl_3$): δ ppm 2.32 (s, 3H, CH_3), 3.11 (s, 3H, SO_2CH_3), 3.30 (s, 3H, OCH_3), 4.91 (d, 1H, CH, J = 4.9 Hz), 5.32 (d, 1H, CH, J = 4.9 Hz), 7.11 (d, 2H, 4-methylphenyl H_2 and H_6, J = 8.4 Hz), 7.20 (d, 2H, 4-methylphenyl H_3 and H_5, J = 8.4 Hz), 7.62 (d, 2H, 4-methylsulfonylphenyl H_2 and H_6, J = 8.3 Hz), and 7.99 (d, 2H, 4-methylsulfonylphenyl H_3 and H_5, J = 8.3 Hz); ^{13}C-NMR ($CDCl_3$): δ ppm 20.92, 44.40, 58.81, 60.89, 84.90, 117.24, 127.66, 128.89, 129.79, 134.13, 134.60, 140.21, 140.69, and 163.61; LC-MS (ESI) *m/z*: 346.1 (M+1, 100); Anal. calcd. for $C_{18}H_{19}NO_4S$: C, 62.59; H, 5.54; N, 4.05. Found: C, 62.88; H, 5.81; N, 4.19.

*3-Methoxy-1-(4-methoxyphenyl)-4-(4-(methylsulfonyl)phenyl)-azetidin-2-one (**4e**)*

Yield, 70%; light brown crystalline powder; mp: 170–171°C; IR (KBr): ν (cm^{-1}) 1,750 (C═O), 1,324, and 1,164 (SO_2); ^{1}H-NMR ($CDCl_3$): δ ppm 3.12 (s, 3H, SO_2CH_3), 3.30 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 4.91 (d, 1H, CH, J = 4.9 Hz), 5.30 (d, 1H, CH, J = 4.9 Hz), 6.84 (d, 2H, 4-methoxyphenyl H_3 and H_5, J = 6.9 Hz), 7.25 (d, 2H, 4-methoxyphenyl H_2 and H_6, J = 6.9 Hz), 7.62 (d, 2H, 4-methylsulfonylphenyl H_2 and H_6, J = 8.3 Hz), and 7.9 (d, 2H, 4-methylsulfonylphenyl H_3 and H_5, J = 8.3 Hz); ^{13}C-NMR ($CDCl_3$): δ ppm 44.39, 55.45, 58.80. 61.04, 84.98, 114.50, 118.63, 127.67, 129.17, 130.03, 140.22, 140.72, 156.64, and 163.28; LC-MS (ESI) *m/z*: 362.1 (M+1, 100); Anal. calcd. for $C_{18}H_{19}NO_5S$: C, 59.82; H, 5.30; N, 3.97. Found: C, 60.05; H, 5.49; N, 4.21.

*3-Methoxy-4-(4-(methylsulfonyl)phenyl)-1-(4-(methylthio)phenyl)-azetidin-2-one (**4f**)*

Yield, 72%; light yellow crystalline powder; mp: 169–171°C; IR (KBr): ν (cm^{-1}) 1,743 (C═O), 1,317, and 1,157 (SO_2); ^{1}H-NMR ($CDCl_3$): δ ppm 2.47 (s, 3H, SCH_3), 3.12 (s, 3H, SO_2CH_3), 3.31 (s, 3H, OCH_3), 4.92 (d, 1H, CH, J = 4.9 Hz), 5.32 (d, 1H, CH, J = 4.9 Hz), 7.19–7.30 (dd, 4H, 4-methylthiophenyl H_2–H_6), 7.62 (d, 2H, 4-methylsulfonylphenyl H_2 and H_6, J = 8.2 Hz), and 7.9 (d, 2H, 4-methylsulfonylphenyl H_3 and H_5, J = 8.23 Hz); ^{13}C-NMR ($CDCl_3$): δ ppm 16.03, 44,39, 58.85, 60.95, 84.98, 117.86, 121.69, 127.84, 128.88, 134.00, 134.66, 139.91, 140.81, and 163.63; LC-MS (ESI) *m/z*: 378.1 (M+1, 100); Anal. calcd. for $C_{18}H_{19}NO_4S_2$: C, 57.27; H, 5.07; N, 3.71. Found: C, 57.45; H, 5.41; N, 3.52.

*1-(4-Acetylphenyl)-3-methoxy-4-(4-(methylsulfonyl)phenyl)azetidin-2-one (**4g**)*

Yield, 67%; light yellow crystalline powder; mp: 170–171°C; IR (KBr): ν (cm^{-1}) 1,740 (C═O), 1,668 (C═O), 1,379, and 1,147 (SO_2); ^{1}H-NMR ($CDCl_3$): δ ppm 2.58 (s, 3H, COMe), 3.12 (s, 3H, SO_2CH_3), 3.37 (s, 3H, OCH_3), 4.97 (d, 1H, CH, J = 5.1 Hz), 5.40 (d, 1H, CH, J = 5.1 Hz), 7.37 (d, 2H, 4-acetylphenyl H_2 and H_6, J = 8.3 Hz), 7.62 (d, 2H, 4-methylsulfonylphenyl H_2 and H_6, J = 8.7 Hz), 7.93 (d, 2H, 4-methylsulfonylphenyl H_3 and H_5, J = 8.3 Hz), and 8.01 (d, 2H, 4-acetylphenyl H_3 and H_5, J = 8.7 Hz); ^{13}C-NMR ($CDCl_3$): δ ppm 26.09, 44.38, 58.98, 61.13, 85.10, 120.89, 127.94, 128.85, 129.93, 133.39, 139.37, 140.22, 141.03, 164.21, and 196.55; LC-MS (ESI) *m/z*: 374.1 (M+1, 100); Anal. calcd. for $C_{19}H_{19}NO_5S$: C, 61.11; H, 5.13; N, 3.75. Found: C, 61.38; H, 5.42; N, 4.00.

*1-(3,4-Dichlorophenyl)-3-methoxy-4-(4-(methylsulfonyl)phenyl)-azetidin-2-one (**4h**)*

Yield, 70%; white crystalline powder; mp 165–167°C; IR (KBr): ν (cm^{-1}) 1,748 (C═O), 1,383, and 1,151 (SO_2); ^{1}H-NMR ($CDCl_3$): δ ppm 3.12 (s, 3H, SO_2CH_3), 3.30 (s, 3H, OCH_3), 4.94 (d, 1H, CH, J = 5.0 Hz), 5.33 (d, 1H, CH, J = 5.0 Hz), 7.09–7.11 (dd, 1H, 3,4-dichlorophenyl H_6 J = 8.7 Hz, J = 2.4 Hz), 7.36 (d, 1H, 3,4-dichlorophenyl H_5, J = 8.7 Hz), 7.49 (d, 1H, 3,4-dichlorophenyl H_2, J = 2.4 Hz), 7.60 (d, 2H, 4-methylsulfonylphenyl H_2 and H_6, J = 8.3 Hz), and 8.01 (d, 2H, 4-methylsulfonylphenyl H_3 and H_5, J = 8.3 Hz); ^{13}C-NMR ($CDCl_3$): δ ppm 44.39, 58.97, 61.18, 85.23, 116.47, 119.08, 122.72, 127.94, 128.82, 129.68, 131.01, 135.79, 139.11, 140.22, and 163.90; LC-MS (ESI) *m/z*: 400.1 (M+1, 100); Anal. calcd. for $C_{17}H_{15}Cl_2NO_4S$: C, 61.99; H, 4.59; N, 4.25. Found: C, 70.19; H, 4.28; N, 4.53.

*1-(3,4-Dimethoxyphenyl)-3-methoxy-4-(4-(methylsulfonyl)phenyl)-azetidin-2-one (**4i**)*

Yield, 58%; yellow crystalline powder; mp: 154–155°C; IR (KBr): ν (cm^{-1}) 1,750 (C═O), 1,312, and 1,141 (SO_2); ^{1}H-NMR ($CDCl_3$): δ ppm, 3.12 (s, 3H, SO_2CH_3), 3.13 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.91 (d, 1H, CH, J = 4.8 Hz), 5.30 (d, 1H, CH, J = 4.8 Hz), 6.95 (m, 2H, 3,4-dimethoxyphenyl H_5 and H_6), 6.98 (s, 1H, 3,4-dimethoxyphenyl H_2), 7.63 (d, 2H, 4-methylsulfonylphenyl H_2 and H_6, J = 8.1 Hz), and 8.01 (d, 2H, 4-methylsulfonylphenyl H_3 and H_5, J = 8.09 Hz); ^{13}C-NMR ($CDCl_3$): δ ppm 44.42, 56.12, 56.36, 58.53, 61.05, 84.88, 109.37, 112.32, 114.96, 127.72, 128.15, 135.12, 139.05, 145.58, 148.77, 151.05, and 163.79; LC-MS (ESI) *m/z*: 392.1 (M+1, 100); Anal. calcd. for $C_{19}H_{21}NO_6S$: C, 58.30; H, 5.41; N, 3.58. Found: C, 58.02; H, 5.11; N, 3.22.

*3-Methoxy-4-(4-(methylsulfonyl)phenyl)-1-(3,4,5-trimethoxyphenyl)-azetidin-2-one (**4j**)*

Yield, 66%; yellow crystalline powder; mp: 156–157°C; IR (KBr): ν (cm^{-1}) 1,750 (C═O), 1,303, and 1,146 (SO_2); ^{1}H-NMR ($CDCl_3$): δ ppm 3.12 (s, 3H, SO_2CH_3), 3.14 (s, 3H, OCH_3), 3.7 (s, 6H, 3,4,5-trimethoxyphenyl H_3 and H_5), 3.81 (s, 3H, 3,4,5-trimethoxyphenyl H_4), 4.91 (d, 1H, CH, J = 4.9 Hz), 5.31 (d, 1H, CH, J = 4.9 Hz), 6.55 (s, 2H, 3,4,5-trimethoxyphenyl H_2 and H_6), 7.64 (d, 2H, 4-methylsulfonylphenyl H_2 and H_6, J = 8.3 Hz), and 8.01 (d, 2H, 4-methylsulfonylphenyl H_3 and H_5, J = 8.3 Hz); ^{13}C-NMR ($CDCl_3$): δ ppm 44.46, 56.18, 56.43, 58.84, 61.04, 84.78, 115.11, 126.13, 127.83, 128.19, 129.35, 130.97, 137.77, 141.19, 142.17, 150.05, and 163.71; LC-MS (ESI) *m/z*: 422.1 (M+1, 100); Anal. calcd. for $C_{20}H_{23}NO_7S$: C, 57.00; H, 5.50; N, 3.32. Found: C, 56.77; H, 5.79; N, 3.35.

4.2 | Molecular modeling

Docking studies were performed using AutoDock software version 3.0. The coordinates of the X-ray crystal structure of the selective COX-2 inhibitor SC-558 bound to the murine COX-2 enzyme were obtained from the RCSB Protein Data Bank (1cx2) and hydrogens were added. The ligand molecules were constructed using the Builder module and were energy-minimized for 1,000 iterations reaching a convergence of 0.01 kcal/mol Å. The energy-minimized ligands were superimposed on SC-558 in the PDB file 1cx2 after which SC-558 was deleted. The purpose of docking is to search for favorable binding configurations between the small flexible ligands and the rigid protein. Protein residues with atoms greater than 7.5 Å from the docking box were removed for efficiency. These docked structures were very similar to the minimized structures obtained initially. The

quality of the docked structures was evaluated by measuring the intermolecular energy of the ligand–enzyme assembly.[37,38]

4.3 | Biological assays

4.3.1 | In vitro COX inhibition assays

The assay was performed using an enzyme chemiluminescent kit (Cayman Chemical, MI) according to our previously reported method.[39] The Cayman chemical chemiluminescent COX (ovine) inhibitor screening assay utilizes the heme-catalyzed hydroperoxidase activity of ovine cyclooxygenases to generate luminescence in the presence of a cyclic naphthalene hydrazide and the substrate arachidonic acid. Arachidonate-induced luminescence was shown to be an index of real-time catalytic activity and demonstrated the turnover inactivation of the enzyme. Inhibition of COX activity, measured by luminescence, by a variety of selective and nonselective inhibitors showed potencies similar to those observed with other in vitro and whole-cell methods.

4.3.2 | In vivo evaluation of the compound analgesic effects

Formalin test was used to evaluate the analgesic effects of compounds as described by Dubuisson and Dennis (the formalin test is a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats).[40] Drugs were dissolved in DMSO and were administered by intraperitoneal (ip) injection at the dose of 40 mg/kg.[41] The volume of injection was 10 ml/kg. Control group received vehicle (DMSO). One group received celecoxib (40 mg/kg) as a standard treatment. Thirty minutes after drug administration, formalin 5% (50 µl) was injected into the dorsal surface of the left hind paw, and the rats were placed individually in acrylic chambers (30 × 30 × 30 cm) and continuously observed for 60 min. Pain-related behaviors were quantified as 0 = normal weight-bearing on the injected paw, 1 = limping during locomotion or resting the paw lightly on the floor, 2 = elevation of the injected paw so that at most the nails touch the floor, and 3 = licking, biting, or shaking the injected paw. The area-under-the-curve (AUC) for pain score against time plot was measured and compared between groups.

4.3.3 | Statistical analysis

Results were shown as mean ± standard error of the mean. Statistical analysis was done using Prism 6 (GraphPad Software Inc.). One-way analysis of variance followed by Bonferroni's multiple comparison test was used to compare AUCs of pain scores between groups. $p < 0.05$ was considered as statistically significant.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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