Design, synthesis, and biological evaluation of new 2-(4-(methylsulfonyl)phenyl)-N-phenylimidazo[1,2-a]pyridin-3-amine as selective COX-2 inhibitors

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Research Article

Keywords: selective COX-2 inhibitors, synthesis, imidazo[1,2-a]pyridine, antinociceptive activity, molecular modeling

Posted Date: December 6th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2317701/v1

Version of Record: A version of this preprint was published at Medicinal Chemistry Research on March 2nd, 2023. See the published version at https://doi.org/10.1007/s00044-023-03041-x.

Abstract

Cyclooxygenase (COX), which plays a role in converting arachidonic acid to inflammatory mediators, could be inhibited by non-steroidal anti-inflammatory drugs (NSAIDs). Although potent NSAIDs are available for the treatment of pain, fever, and inflammation, some side effects such as gastrointestinal ulcers limit the use of these medications. During recent years, selective COX-2 inhibitors with a lower incidence of adverse effects attained an important position in medicinal chemistry. In order to introduce some new potent COX-2 inhibitors, a new series of 2-(4-(methylsulfonyl)phenyl)-*N*-phenylimidazo[1,2-*a*]pyridin-3-amines was designed, synthesized, and evaluated. The designed compounds were synthesized through multistep reactions. Enzyme inhibition assay and formalin test were performed to evaluate the activity of these compounds. The docking studies demonstrated SO_2Me pharmacophore was inserted into the secondary pocket of COX-2, and other parts of docked molecules were positioned as well as a crystallographic ligand in the COX-2 active site. Among these compounds, 8-methyl-2-(4-(methylsulfonyl)phenyl)-*N*-(*p*-tolyl)imidazo[1,2-*a*]pyridin-3-amine (**5n**) exhibited the most potency and selectivity against COX-2 (IC_{50}= 0.07 μM, SI= 508.6). The antinociceptive activity assessment via the formalin test showed that nine derivatives (**5a, 5d, 5h, 5i, 5k, 5q, 5r**, **5s**, and **5t**) possessed significant activity compared with the control group with a *p*-value less than 0.05.

1. Introduction

Arachidonic acid, the precursor of prostanoids, is metabolized to PGH_2 by cyclooxygenase enzyme (COX) in a two-step process. The synthesized PGH_2 is converted to prostaglandins and other prostanoids by variable synthase enzymes. Prostanoids play a role in many inflammatory processes. Therefore, COX is the key enzyme of the arachidonic acid cascade, and COX inhibition is a helpful way to reduce inflammation, pain, and fever caused by prostaglandins. Cyclooxygenase exists in three isoforms, COX-1, COX-2, and COX-3. COX-1 participates in physiological functions, whereas COX-2 mediates pathological processes [1]. Much less is known about COX-3, which is expressed in the cerebral cortex and cardiac tissue and regulates fever and pain [2]. Non-steroidal anti-inflammatory drugs (NSAIDs) demonstrate their anti-inflammatory effects by inhibiting both COX-1 and COX-2 isoforms, which cause to miss out advantages of COX-1 functions such as stomach protection and renal hemodynamics. Hence, non-selective inhibition of the COX enzyme leads to side effects such as gastrointestinal ulcers, kidney injuries, *etc*.

In contrast to NSAIDs, selective COX-2 inhibitors diminish undesirable COX-2 inflammatory mediators without interrupting COX-1 housekeeping functions. Besides, COX-2 overexpression was reported in different diseases such as many cancers (breast, colorectal, and prostate cancer), neurodegenerative diseases like amyotrophic lateral sclerosis (ALS), Alzheimer's, and Parkinson's disease [3–6]. These findings suggest that COX-2 could be one of the therapeutic targets in these pathophysiological disorders [7]. Accordingly, plenty of studies proved that COX-2 inhibition plays a beneficial role in the treatment of

such diseases. Owing to a wide variety of applications, the discovery of potent COX-2 inhibitors with a safe profile of adverse effects is noticeable.

There is a wide variety of COX-2 inhibitors. Generally, these compounds contain two vicinal phenyl rings on a central system which can be carbo/heterocyclic (tricyclics) or acyclic [8]. A pharmacophore group such as methanesulfonyl, sulfonamide, or azido at the *para*-position of one of the phenyl rings plays an important role in COX-2 selectivity [9]. These substituents could insert into the secondary pocket that exists in the COX-2 isozyme. This pocket includes three crucial amino acids: Arg513, His90, and Val 523. The pharmacophore group of COX-2 inhibitors forms hydrogen bonding with essential amino acids after inserting into the secondary pocket, which leads to selective inhibition of COX-2.

Different types of central heterocyclic or carbocyclic ring systems, such as 4-, 5- and 6-membered rings and fused bicyclic, tricyclic, and spiro ring systems, are seen as a central core of COX-2 inhibitors [10–17]. Acyclic COX-2 inhibitors (non-tricyclics) contain a two-membered (olefins) or three-membered (chalcones) chain structure, which is the essential point for sub-classification of these compounds [17–22]. In addition, in order to discover novel templates of COX-2 inhibitors, many researchers introduced small peptide analogs of COX-2 inhibitors [23, 24]. Furthermore, conjugating COX-2 inhibitors with some moieties such as nitric oxide-releasing and ferrocene is one strategy to afford molecules with reduced side effects and more parallel biological effects [25, 26]. Some hybrid molecules named COX/LOX inhibitors provide improved anti-inflammatory, cardiovascular, and gastrointestinal safety profiles [27, 28].

According to the literature, a class of 1,1-diphenyl-2-(4-methylsulfonylphenyl)-2-alkyl-1-ethenes was prepared and evaluated among acyclic compounds. In this group, selectivity and COX-2 inhibitory potency depend on 2-alkyl chain length; the namely n-butyl substituent (Fig. 1-A) exhibited high potency and selectivity even better than celecoxib (IC_{50} = 0.014 μM, selectivity index > 7142) [29]. Furthermore, some 1,3-diarylurea derivatives possessing a SO_2Me pharmacophore at the *para*-position of the N-1 phenyl ring were synthesized as selective COX-2 inhibitors. In this group, the nature of *para*-substituents on the N-3 phenyl ring affect potency and selectivity against COX-2 enzyme; as an example, 1-(4-methylsulfonylpheny)-3-(4-methoxyphenyl) urea (Fig. 1-B) with a hydrogen acceptor group such as methoxy was the most potent and selective among the synthesized compounds (IC_{50} = 0.11 μM, and selectivity index = 203.6) [30].

Imidazo[1,2-*a*]pyridine is one of the most popular bicyclic heterocyclic pharmacophores due to its broad spectrum of biological activities such as anticancer, anticonvulsant, hypnotic, antimycobacterial, antimicrobial, antiviral, analgesic, and antidiabetic are known as a privileged scaffold in medicinal chemistry [31–33]. Based on the literature review and structure-activity relationship (SAR) of COX-2 inhibitors, in our previous study, imidazo[1,2-*a*]pyridine scaffold was chosen for COX-2 inhibitory activity; we reported a new series of 2-(4-(methylsulfonyl)phenyl)imidazo[1,2-*a*]pyridine with different substituents on C-3 of the central ring. The results of *in vitro* studies indicated that COX-2 inhibitory activity was impressed by the nature and size of mannich base on C-3 of imidazo[1,2-*a*]pyridine ring. Compound with morpholine ring at C-3 showed the highest potency and selectivity (Fig. 1-C) (IC_{50} = 0.07 μM, selectivity

index = 217.1) [34]. To design more efficient and selective COX-2 inhibitors rationally, we modified the previous 2-(4-(methylsulfonyl)phenyl)imidazo[1,2-*a*]pyridine derivatives. Accordingly, the mannich base group at C-3 of the central ring was substituted with the phenylamino group. Consequently, the present study described some novel 2-(4-(methylsulfonyl)phenyl)-*N*-phenylimidazo[1,2-*a*]pyridin-3-amine derivatives in order to evaluate *xin vitro* COX-1/COX-2 inhibition and *in vivo* analgesic activities.

2. Results And Discussion

2.1. Chemistry

The target 2-(4-(methylsulfonyl)phenyl)-*N*-phenylimidazo[1,2-*a*]pyridin-3-amine derivatives were synthesized via the route outlined in Scheme 1.

Initially, α-bromo-4-(methylsulfonyl)acetophenone **1** was prepared according to the literature procedure [34]. The α-bromo-4-(methylsulfonyl)acetophenone **1** and appropriate 4-substituted aniline **2** in the presence of $NaHCO_3$ in anhydrous MeOH were reacted to afford 1-(4-(methylsulfonyl)phenyl)-2-(phenylamino)ethan-1-ones **(3a-e)** [35]. Condensation of **3** with different 2-aminopyridines in *i*-PrOH at 80°C gave final 2-(4-(methylsulfonyl)phenyl)-*N*-phenylimidazo[1,2-*a*]pyridin-3-amine derivatives (**5a-t)** in good yields [36].

2.2. Biological evaluation

2.2.1. *In vitro* cyclooxygenase (COX) inhibition assays

The inhibitory activities of novel 2-(4-(methylsulfonyl)phenyl)-*N*-phenylimidazo[1,2-*a*]pyridin-3-amines against COX-1 and COX-2 were evaluated by *in vitro* assay. As shown in Table 1, all compounds were selective COX-2 inhibitors with selectivity indices of 42.3-508.6 and COX-2 IC_{50} values of 0.07–0.39 μM. Compound 8-methyl-2-(4-(methylsulfonyl)phenyl)-*N*-(*p*-tolyl)imidazo[1,2-*a*]pyridin-3-amine **(5n)** with COX-2 IC_{50} value of 0.07 μM and selectivity index of 508.6 exhibited highest inhibitory potency and selectivity.

Structurally the synthesized compounds **5a–t** could be categorized into five groups based on the type of substituent at the *para* position of phenylamino ring: hydrogen, 4-fluoro, 4-chloro, 4-methyl, and 4-methoxy derivatives (**5a-d**, **5e-h**, **5i-l**, **5m-p**, and **5q-t**) to evaluate steric, electronic and hydrophobic effects on activities. In each group, hydrogen was replaced with a methyl substituent at different positions of the imidazopyridine ring to examine hydrophobic and steric parameters around this ring.

As shown in Table 1, replacing fluorine at the *para* position of phenylamino improved both potency and selectivity compared to other groups. It may be explained by the ability of fluorine to form hydrogen bonding with amino acids of the active site. Among compounds having 4-F and 4-OMe (in F and OMe groups), derivatives having methyl on imidazopyridine ring (**5f**, **5g**, **5h**, **5r**, **5s**, and **5t**) and derivatives without methyl on imidazopyridine ring (**5e** and **5q**) exhibited the highest and the lowest COX-2 IC_{50} and selectivity index respectively in each group. It seems that methyl substituent on imidazopyridine ring

leads to better interaction of F and OMe on phenylamino ring due to the more appropriate orientation of these molecules into the active site of COX-2.

In this series, introducing a suitable substituent, especially on C-8 of imidazo[1,2-*a*]pyridine ring, enhances selectivity on COX-2 isozyme (except in fluoro and chloro group). This may be explained by steric hindrance during the interaction of the molecule with COX-1.

These results indicated that the 2-phenyl-*N*-phenylimidazo[1,2-*a*]pyridin-3-amine structure was a suitable scaffold for COX-1/2 inhibition, and adding SO_2Me pharmacophore at the *para* position of C-2 phenyl ring enhanced COX-2 potency and selectivity.

Table 1
The COX-1 and COX-2 enzyme inhibition assay and analgesic effects of test compounds

Group	Compound	R_1	R_2	IC_{50} in μM		Selectivity Index (SI)	AUC of pain score (mean and 95% confidence interval)	p-value
				COX-1	COX-2			
Hydrogen	5a	H	H	18.1	0.07	258.6	27.93 (17.36–38.49)***	< 0.001
	5b	H	8-Me	35.6	0.08	445	84.38 (29.06–139.7)	> 0.05
	5c	H	7-Me	55.1	ND	ND	110.40 (93.48–127.3)	> 0.05
	5d	H	5-Me	24.6	0.07	351.4	78.41 (51.63–105.2)*	< 0.05
Fluoro	5e	F	H	23.8	0.1	238.0	97.23 (80.96–113.5)	> 0.05
	5f	F	8-Me	31.5	0.07	450.0	105.50 (84.51–126.4)	> 0.05
	5g	F	7-Me	33.6	0.08	420.0	93.70 (77.07–110.3)	> 0.05
	5h	F	5-Me	40.2	0.08	502.5	84.18 (65.63–102.7)*	< 0.05
Chloro	5i	Cl	H	34.3	0.07	490	87.44 (72.24–102.6)*	< 0.05
	5j	Cl	8-Me	29.4	0.14	210	98.90 (69.43–128.4)	> 0.05
	5k	Cl	7-Me	ND	0.08	ND	71.03 (41.28–100.8)**	< 0.01
	5l	Cl	5-Me	27.1	0.08	338.7	102.40 (76.88–127.9)	> 0.05
Methyl	5m	Me	H	23.1	0.10	231.0	74.71 (64.95–84.47)	> 0.05
	5n	Me	8-Me	35.6	0.07	508.6	71.87 (60.52–83.22)	> 0.05
	5o	Me	7-Me	33.9	0.07	484.3	77.77 (70.59–84.95)	> 0.05
	5p	Me	5-Me	19.6	0.12	163.3	71.01 (64.46–77.56)*	< 0.05

Methoxy	**5q**	OMe	H	16.5	0.39	42.3	65.62 (55.73–75.61)*	< 0.05
	5r	OMe	8-Me	30.0	0.08	375.0	43.80 (36.29–51.31)***	< 0.001
	5s	OMe	7-Me	29.7	0.10	297	51.27 (45.29–57.25)***	< 0.001
	5t	OMe	5-Me	21.1	0.09	234.4	52.79 (46.87–58.71)***	< 0.001
	Celecoxib			24.3	0.06	405	64.29 (50.81–77.78)***	< 0.001
	Control			-	-	-	107.90 (97.29–118.5)	-

[a]Values are means of two determinations acquired using an ovine/human recombinant 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}). *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significant difference compared with the control group (n = 6)

2.2.2. *In vivo* evaluation of analgesic effects

The *in vivo* formalin test was performed to assess the analgesic activity of synthesized compounds. The result was compared with celecoxib as the reference drug. The results have been summarized in Table 2. A significant reduction in the AUC of pain score was shown in groups treated with **5a**, **5r**, **5s** and **5t** ($p <$ 0.001), **5k** ($p < 0.01$) and **5d**, **5h**, **5i**, **5q** ($p < 0.05$) compared with the control group. There was at least one efficient compound in each category. In OMe substituted compounds, all the compounds (**5q**, **5r**, **5s**, and **5t**) were active enough in reducing the AUC of pain score ($p < 0.05$). Surprisingly, even compound **5q** that presented poor COX-2 inhibition compared to other potent compounds showed significant antinociceptive activity. It seems some pharmacokinetic factors affected differently *in vitro* and *in vivo* activities of this molecule.

2.3. Molecular modeling studies

The docking poses of two potent and selective compounds depicted the interaction of the SO_2Me group with essential amino acids of the COX-2 secondary pocket. As shown in Fig. 2, the oxygen atoms of SO_2Me of each molecule can form hydrogen bonds with NH of Arg513, His90, and Phe518. The imidazopyridine moiety of molecules forms hydrogen bonds with NH of Arg120 within nitrogen atoms of the ring. Further, fluoro substituent of **5h** interacts with NH of Gly526. These docking studies show that hydrophobic side chains of Trp387, Leu531, Val 349, Leu384, Tyr385, and Met522 residues surround hydrophobic moieties of molecules such as phenyl or pyridine rings which may undergo hydrophobic interactions. The docking results also revealed that **5h** and **5n** positions in the COX-2 active site provided

a suitable orientation, and these molecules were perfectly superimposed on **Sc-558**, a selective inhibitor in complex with COX-2 (Fig. 3).

3. Conclusion

In conclusion, a series of 2-(4-(methylsulfonyl)phenyl)-*N*-phenylimidazo[1,2-*a*]pyridin-3-amine having different substituents at the *para* position of the *N*-phenyl ring were introduced as COX-2 inhibitors. Most of the compounds showed high potencies and selectivity indices against COX-2 isozyme. The 8-methyl-2-(4-(methylsulfonyl)phenyl)-*N*-(*p*-tolyl)imidazo[1,2-*a*]pyridin-3-amine (**5n**) was the most active compound in enzyme inhibition assay, even more selective than reference drug celecoxib (S.I.= 508). The formalin test indicated that *N*-(4-methoxyphenyl)-8-methyl-2-(4-(methylsulfonyl)phenyl)imidazo[1,2-*a*]pyridin-3-amine (**5r**) displayed the highest analgesic activity. In addition, compounds **5a**, **5s**, and **5t** with significant reductions in AUC of pain scores were also promising compounds in the formalin test.

4. Materials And Methods

4.1. General methods

All chemicals handled in the preparations were purchased from Merck and/or Sigma-Aldrich. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker FT-400 MHz instrument (Brucker Biosciences, USA) using Chloroform-D and DMSO-d_6 as solvents and tetramethylsilane (TMS) as an internal standard. Melting points were determined with a Thomas–Hoover capillary apparatus. Infrared spectra were obtained using a Perkin Elmer Model 1420 spectrometer. The mass spectral measurements were observed on a 6410 Agilent LC-MS triple quadrupole mass spectrometer (LC-MS) with an electrospray ionization (ESI) interface. Microanalyses determined for C, H and N were within ± 0.4% of the theoretical values.

4.2. Chemical synthesis

4.2.1. General procedure for the synthesis of 1-(4-(methylsulfonyl)phenyl)-2-(phenylamino)ethan-1-ones (3)

A mixture of α-bromo-4-(methylsulfonyl)acetophenone **1** (7 mmol) and 4-substituted-aniline **2** (7 mmol) in anhydrous methanol (10 ml) and in the presence of two equivalents of sodium hydrogen carbonate was stirred at room temperature for 16 hours. The reaction mixture was filtered out and washed with cold MeOH and water. The crude was used in the next step without any purification.

1-(4-(methylsulfonyl)phenyl)-2-(phenylamino)ethan-1-one (**3a**)

Yield, 91%; Yellow powder; mp: 156–158 ^{o}C; IR (KBr disk): vcm^{-1} 1155, 1296 (SO_2), 1700 (C = O), 3396 (NH); LC-MS (ESI) m/z: 288 ($[M-H]^-$, 100).

2-((4-fluorophenyl)amino)-1-(4-(methylsulfonyl)phenyl)ethan-1-one (**3b**)

Yield, 89%; Yellow powder; mp: 166–168 ^{o}C; IR (KBr disk): vcm^{-1} 1142, 1309 (SO_2), 1692 (C = O), 3364 (NH); LC-MS (ESI) m/z: 306 ([M-H]$^{-}$, 100).

2-((4-chlorophenyl)amino)-1-(4-(methylsulfonyl)phenyl)ethan-1-one (**3c**)

Yield, 87%; Yellow powder; mp: 171–173 ^{o}C; IR (KBr disk): vcm^{-1} 1142, 1308 (SO_2), 1692 (C = O), 3355 (NH); LC-MS (ESI) m/z: 322 ([M-H]$^{-}$, 100).

1-(4-(methylsulfonyl)phenyl)-2-(*p*-tolylamino)ethan-1-one (**3d**)

Yield, 86%; Yellow powder; mp: 153–154 ^{o}C; IR (KBr disk): vcm^{-1} 1152, 1297 (SO_2), 1683 (C = O), 3390 (NH); LC-MS (ESI) m/z: 302 ([M-H]$^{-}$, 100).

2-((4-methoxyphenyl)amino)-1-(4-(methylsulfonyl)phenyl)ethan-1-one (**3e**)

Yield, 81%; dark yellow powder; mp: 149–151 ^{o}C; IR (KBr disk): vcm^{-1} 1154, 1297 (SO_2), 1675 (C = O), 3361 (NH); LC-MS (ESI) m/z: 318 ([M-H]$^{-}$, 100).

General procedure for the synthesis of 2-(4-(methylsulfonyl)phenyl)-N-phenylimidazo[1,2-a]pyridin-3-amine derivatives (5 *)*

An appropriate derivative of **3** (1.73 mmol), 2-aminopyridine derivatives (1.73 mmol), ZnI_2 (0.52 mmol), 4 Å MS (850 mg), and *i*-PrOH (8.5 ml) were added, and the mixture was stirred at 80 ^{o}C. After completing the reaction, the mixture was cooled to room temperature and then filtered and washed with water and cool *i*-PrOH to obtain 20 different derivatives.

2-(4-(Methylsulfonyl)phenyl)-*N*-phenylimidazo[1,2-*a*]pyridin-3-amine (**5a**)

Yield, 79%; yellow powder; mp: 189 ^{o}C (decomposed); IR (KBr disk): vcm^{-1} 1158, 1299 (SO_2), 1682 (C = N), 3404 (NH); ^{1}H NMR (DMSO-d_6): δ ppm 3.25 (s, 3H, SO_2Me), 6.55–6.57 (d, 2H, J = 7.6 Hz, phenyl H_2 & H_6), 6.72–6.75 (t, 1H, phenyl H_4), 6.89–6.92 (t, 1H, imidazopyridine H_6), 6.98–7.02 (t, 2H, J = 7.6 Hz, phenyl H_3 & H_5), 7.24–7.27 (t, 1H, imidazopyridine H_7), 7.42–7.44 (d, 1H, imidazopyridine H_8), 7.64–7.66 (d, 1H, imidazopyridine H_5), 7.98-8.00 (d, 2H, J = 8.4 Hz, methylsulfonylphenyl H_2 & H_6), 8.11–8.13 (d, 2H, J = 8.4 Hz, methylsulfonylphenyl H_3 & H_5), 8.16 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ ppm 43.57, 113.26, 113.49, 117.67, 117.84, 119.32, 123.80, 127.25, 127.67, 127.97, 129.31, 129.44. 130.09, 140.50, 144.72, 146.77; LC-MS (ESI) m/z: 364 ([M + H]$^{+}$, 100); Anal. Calcd. For $C_{20}H_{17}N_3O_2S$: C, 66.10; H, 4.72; N, 11.56. Found: C, 65.88; H, 4.74; N, 11.68.

8-Methyl-2-(4-(methylsulfonyl)phenyl)-*N*-phenylimidazo[1,2-*a*]pyridin-3-amine (**5b**)

Yield, 71%; yellow powder; mp:261–263 °C; IR (KBr disk): vcm^{-1} 1158, 1269 (SO_2), 1631 (C = N), 3373 (NH); ^1H NMR ($CDCl_3$): δ ppm 2.62 (s, 3H, CH_3), 2.96 (s, 3H, SO_2Me), 5.61 (s, 1H, NH), 6.52–6.54 (d, 2H, *J* = 7.6 Hz, phenyl H_2 & H_6), 6.65–6.69 (t, 1H, *J* = 6.8 Hz phenyl H_4), 6.80–6.84 (t, 1H, *J* = 7.2 Hz, imidazopyridine H_6), 7.00-7.02 (d, 1H, *J* = 6.8 Hz, imidazopyridine H_7), 7.13–7.17 (t, 2H, *J* = 7.6 Hz, phenyl H_3 & H_5), 7.67–7.68 (d, 1H, *J* = 6.8 Hz, imidazopyridine H_5), 7.83–7.85 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H_2 & H_6), 8.17–8.19 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (DMSO-d_6): δ ppm 16.65, 43.99, 113.20, 113.48, 119.25, 121.29, 121.56, 124.76, 127.23, 127.40, 127.72, 130.07, 135.61, 139.18, 139.57, 142.85, 145.71; LC-MS (ESI) m/z: 378 ($[M + H]^+$, 100); Anal. Calcd. For $C_{21}H_{19}N_3O_2S$: C, 66.82; H, 5.07; N, 11.13. Found: C, 66.76; H, 4.96; N, 11.24.

7-Methyl-2-(4-(methylsulfonyl)phenyl)-*N*-phenylimidazo[1,2-*a*]pyridin-3-amine (**5c**)

Yield, 78%; yellow powder; mp: 239–241 °C; IR (KBr disk): vcm^{-1} 1162, 1317 (SO_2), 1661 (C = N), 3235 (NH); ^1H NMR (DMSO-d_6): δ ppm 2.38 (s, 3H, CH_3), 3.20 (s, 3H, SO_2Me), 6.50–6.52 (d, 2H, *J* = 7.6 Hz, phenyl H_2 & H_6), 6.72–6.75 (t, 1H, *J* = 7.2 Hz, phenyl H_4), 6.79–6.8 (d, 1H, *J* = 6.8 Hz, imidazopyridine H_6), 7.12–7.16 (t, 2H, *J* = 7.6 Hz, phenyl H_3 & H_5), 7.43 (s, 1H, imidazopyridine H_8), 7.85–7.87 (d, 1H, *J* = 6.8 Hz imidazopyridine H_5), 7.92–7.94 (d, 2H, *J* = 8.0 Hz, methylsulfonylphenyl H_2 & H_6), 8.27–8.29 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H_3 & H_5), 8.32 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ ppm 21.32, 43.99, 113.45, 115.70, 116.10, 119.23, 120.59, 123.05, 127.14, 127.69, 130.06, 135.72, 136.89, 139.23, 139.50, 142.96, 145.75; LC-MS (ESI) m/z: 378 ($[M + H]^+$, 100); Anal. Calcd. For $C_{21}H_{19}N_3O_2S$: C, 66.82; H, 5.07; N, 11.13. Found: C, 66. 69; H, 5.11; N, 11.19.

5-Methyl-2-(4-(methylsulfonyl)phenyl)-*N*-phenylimidazo[1,2-*a*]pyridin-3-amine (**5d**)

Yield, 72%; yellow powder; mp: 210 °C (decomposed); IR (KBr disk): vcm^{-1} 1162, 1326 (SO_2), 1668 (C = N), 3361 (NH); ^1H NMR (DMSO-d_6): δ ppm 2.66 (s, 3H, CH_3), 3.21 (s, 3H, SO_2Me), 6.65–6.67 (d, 2H, *J* = 6.8 Hz, phenyl H_2 & H_6), 6.67–6.72 (t, 1H, *J* = 7.6 Hz, phenyl H_4), 6.80–6.82 (d, 1H, *J* = 6.8 Hz, imidazopyridine H_6), 7.13–7.17 (t, 2H, *J* = 7.2 Hz, phenyl H_3 & H_5), 7.23–7.25 (t, 1H, *J* = 7.2 Hz, imidazopyridine H_7), 7.49–7.51 (d, 1H, *J* = 8.8 Hz, imidazopyridine H_8), 7.91–7.93 (d, 2H, *J* = 8.8 Hz, methylsulfonylphenyl H_2 & H_6), 7.96–7.98 (d, 1H, *J* = 8.8 Hz, imidazopyridine H_8) 8.22 (s, 1H, NH), 8.30–8.32 (d, 2H, *J* = 8.8 Hz, methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (DMSO-d_6): δ ppm 18.25, 43.91, 113.24, 114.26, 116.09, 118.78, 121.66, 126.57, 127.38, 127.62, 130.23, 136.66, 137.77, 139.10, 139.71, 144.29, 148.15; LC-MS (ESI) m/z: 378 ($[M + H]^+$, 100); Anal. Calcd. For $C_{21}H_{19}N_3O_2S$: C, 66.82; H, 5.07; N, 11.13. Found: C, 66.91; H, 5.09; N, 11.02.

N-(4-Fluorophenyl)-2-(4-(methylsulfonyl)phenyl)imidazo[1,2-*a*]pyridin-3-amine (**5e**)

Yield, 56%; cream powder; mp: 238 °C (decomposed); IR (KBr disk): vcm^{-1} 1170, 1330 (SO_2), 1648 (C = N), 3263 (NH); ^1H NMR (DMSO-d_6): δ ppm 3.22 (s, 3H, SO_2Me), 6.50–6.54 (m, 2H, phenyl H_2 & H_6), 6.95–7.02

(m, 3H, phenyl H_3 & H_5, imidazopyridine H_6), 7.34–7.38 (t, 1H, *J* = 7.2 Hz, imidazopyridine H_7), 7.66–7.68 (d, 1H, *J* = 9.2 Hz, imidazopyridine H_8), 7.94–7.96 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H_2 & H_6), 8.00-8.02 (d, 1H, *J* = 6.8 Hz, imidazopyridine H_5), 8.29–8.31 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H_3 & H_5), 8.36 (s, 1H, NH); ^{13}C NMR (DMSO-*d*$_6$): δ ppm 43.97, 113.26, 114.48, 114.56, 116.47, 116.70, 117.94, 121.17, 123.78, 126.36, 127.24, 127.76, 136.05, 138.99, 139.73, 142.08, 142.57, 155.17, 157.49; LC-MS (ESI) m/z: 382 ($[M + H]^+$, 100); Anal. Calcd. For $C_{20}H_{16}FN_3O_2S$: C, 62.98; H, 4.23; N, 11.02. Found: C, 63.15; H, 4.21; N, 11.08.

N-(4-Fluorophenyl)-8-methyl-2-(4-(methylsulfonyl)phenyl)imidazo[1,2-*a*]pyridin-3-amine (**5f**)

Yield, 47%; white powder; mp: 224–226 °C; IR (KBr disk): vcm^{-1} 1144, 1308 (SO_2), 1621 (C = N), 3212 (NH); ^{1}H NMR (DMSO-*d*$_6$): δ ppm 2.59 (s, 3H, CH_3), 3.22 (s, 3H, SO_2Me), 6.50–6.53 (m, 2H, phenyl H_2 & H_6), 6.86–6.89 (t, 1H, *J* = 6.8 Hz, imidazopyridine H_6), 6.97–7.01 (t, 2H, *J* = 8.8 Hz, phenyl H_3 & H_5), 7.16–7.18 (d, 1H, *J* = 6.8 Hz, imidazopyridine H_7), 7.84–7.86 (d, 1H, *J* = 6.8 Hz, imidazopyridine H_5), 7.95–7.97 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H_2 & H_6), 8.30–8.32 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H_3 & H_5), 8.35 (s, 1H, NH); ^{13}C NMR (DMSO): δ ppm 16.63, 44.00, 113.25, 114.47, 114.54, 116.44, 116.67, 121.54, 124.79, 127.22, 127.42, 127.75, 135.59, 139.13, 139.60, 142.17, 142.87, 155.13, 157.46; LC-MS (ESI) m/z: 396 ($[M + H]^+$, 100); Anal. Calcd. For $C_{21}H_{18}FN_3O_2S$: C, 63.78; H, 4.59; N, 10.63. Found: C, 63.61; H, 4.62; N, 10.57.

N-(4-Fluorophenyl)-7-methyl-2-(4-(methylsulfonyl)phenyl)imidazo[1,2-*a*]pyridin-3-amine (**5g**)

Yield, 52%; yellow powder; mp: 238–240 °C; IR (KBr disk): vcm^{-1} 1152, 1310 (SO_2), 1645 (C = N), 3215 (NH); ^{1}H NMR (DMSO-*d*$_6$): δ ppm 2.38 (s, 3H, CH_3), 3.21 (s, 3H, SO_2Me), 6.48–6.52 (m, 2 H, phenyl H_2 & H_6), 6.80–6.81 (d, 1H, *J* = 6.8 Hz, imidazopyridine H_6), 6.97–7.01 (t, 2H, *J* = 8.8 Hz, phenyl H_3 & H_5), 7.43 (s, 1H, imidazopyridine H_8), 7.87-7,89 (d, 1H, *J* = 7.2 Hz, imidazopyridine H_5), 7.93–7.95 (d, 2H, *J* = 8.8 Hz, methylsulfonylphenyl H_2 & H_6), 8.26–8.28 (d, 2H, *J* = 8.8 Hz, methylsulfonylphenyl H_3 & H_5), 8.30 (s, 1H, NH); ^{13}C NMR (DMSO-*d*$_6$): δ ppm 21.31, 43.99, 114.41, 114.49, 115.76, 116.11, 116.44, 116.66, 120.74, 123.02, 127.11, 127.71, 135.69, 136.93, 139.16, 139.54, 142.21, 142.96, 155.12, 157.44; LC-MS (ESI) m/z: 396 ($[M + H]^+$, 100); Anal. Calcd. For $C_{21}H_{18}FN_3O_2S$: C, 63.78; H, 4.59; N, 10.63. Found: C, 63.88; H, 4.55; N, 10.66.

N-(4-Fluorophenyl)-5-methyl-2-(4-(methylsulfonyl)phenyl)imidazo[1,2-*a*]pyridin-3-amine (**5h**)

Yield, 60%; white powder; mp: 230 °C (decomposed); IR (KBr disk): vcm^{-1} 1153, 1315 (SO_2), 1648 (C = N), 3343 (NH); ^{1}H NMR (DMSO-*d*$_6$): δ ppm 2.68 (s, 3H, CH_3), 3.21 (s, 3H, SO_2Me), 6.47–6.49 (m, 2H, phenyl H_2 & H_6), 6.85–6.87 (d, 1H, *J* = 6.8 Hz, imidazopyridine H_6), 6.97–7.01 (t, 2H, *J* = 8.8 Hz, phenyl H_3 & H_5), 7.23–7.25 (t, 1H, *J* = 8.4 Hz, imidazopyridine H_7), 7.50–7.52 (d, 1H, *J* = 8.8 Hz, imidazopyridine H_8), 7.93–7.95 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H_2 & H_6), 8.10–8.12 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl

H_3 & H_5), 8.33 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ ppm 18.83, 43.92, 114.25, 116.08, 116.56, 116.78, 121.96, 126.53, 127.37, 127.63, 130.11, 136.69, 137.72, 139.09, 139.70, 141.21, 144.28, 154.71, 157.03; LC-MS (ESI) m/z: 396 ([M + H]$^+$, 100); Anal. Calcd. For $C_{21}H_{18}FN_3O_2S$: C, 63.78; H, 4.59; N, 10.63. Found: C, 63.59; H, 4.63; N, 10.71.

N-(4-Chlorophenyl)-2-(4-(methylsulfonyl)phenyl)imidazo[1,2-*a*]pyridin-3-amine (**5i**)

Yield, 55%; yellow powder; mp: 226–228 °C; IR (KBr disk): vcm^{-1} 1154, 1307 (SO_2), 1669 (C = N), 3333 (NH); ^{1}H NMR (DMSO-d_6): δ ppm 3.22 (s, 3H, SO_2Me), 6.53–6.55 (d, 2H, J = 8.4 Hz, phenyl H_2 & H_6), 6.96–6.99 (t, 1H, J = 6.8 Hz, imidazopyridine H_6), 7.18–7.20 (d, 2H, J = 8.8 Hz phenyl H_3 & H_5), 7.34–7.38 (t, 1H, J = 8.4 Hz, imidazopyridine H_7), 7.67–7.69 (d, 1H, J = 8.8 Hz, imidazopyridine H_8), 7.95–7.97 (d, 2H, J = 8.4 Hz, methylsulfonylphenyl H_2 & H_6), 8.00-8.02 (d, 1H, J = 6.8 Hz, imidazopyridine H_5), 8.28–8.30 (d, 2H, J = 8.4 Hz, methylsulfonylphenyl H_3 & H_5), 8.54 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ ppm 43.97, 113.36, 115.08, 117.96, 120.47, 122.81, 123.77, 126.45, 127.25, 127.80, 129.86, 136.15, 138.90, 139.80, 142.66, 144.64; LC-MS (ESI) m/z: 398 ([M + H]$^+$, 100), 400 (M + 3, 32%); Anal. Calcd. For $C_{20}H_{16}ClN_3O_2S$: C, 60.38; H, 4.05; N, 10.56. Found: C, 60.45; H, 4.01; N, 10.58.

N-(4-Chlorophenyl)-8-methyl-2-(4-(methylsulfonyl)phenyl)imidazo[1,2-*a*]pyridin-3-amine (**5j**)

Yield, 59%; creamy-yellowish powder; mp: 199–201 °C; IR (KBr disk): vcm^{-1} 1141, 1304 (SO_2), 1670 (C = N), 3354 (NH); ^{1}H NMR (DMSO-d_6): δ ppm 2.58 (s, 3H, CH_3), 3.22 (s, 3H, SO_2Me), 6.51–6.53 (d, 2H, J = 8.4 Hz, phenyl H_2 & H_6), 6.86–6.89 (t, 1H, J = 6.8 Hz, imidazopyridine H_6), 7.17–7.19 (m, 3H, imidazopyridine H_7, phenyl H_3 & H_5), 7.84–7.86 (d, 1H, J = 6.4 Hz, imidazopyridine H_5), 7.95–7.97 (d, 2H, J = 8.4 Hz, methylsulfonylphenyl H_2 & H_6), 8.28–8.31 (d, 2H, J = 8.4 Hz, methylsulfonylphenyl H_3 & H_5), 8.54 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ ppm 16.64, 44.00, 113.34, 115.06, 120.85, 121.51, 122.74, 124.88, 127.23, 127.46, 127.78, 129.84, 135.68, 139.03, 139.67, 142.96, 144.73; LC-MS (ESI) m/z: 412 ([M + H]$^+$, 100), 414 ([M + H + 2]$^+$, 32%); Anal. Calcd. For $C_{21}H_{18}ClN_3O_2S$: C, 61.24; H, 4.40; N, 10.20. Found: C, 61.01; H, 4.44; N, 10.30.

N-(4-Chlorophenyl)-7-methyl-2-(4-(methylsulfonyl)phenyl)imidazo[1,2-*a*]pyridin-3-amine (**5k**)

Yield, 71%; yellow powder; mp: 238 °C (decomposed); IR (KBr disk): vcm^{-1} 1150, 1299 (SO_2), 1670 (C = N), 3201 (NH); ^{1}H NMR (DMSO-d_6): δ ppm 2.38 (s, 3H, CH_3), 3.21 (s, 3H, SO_2Me), 6.50–6.52 (d, 2H, J = 8.4 Hz, phenyl H_2 & H_6), 6.80–6.82 (d, 1 H, J = 6.8 Hz, imidazopyridine H_6), 7.17–7.19 (d, 2H, J = 8.8 Hz, phenyl H_3 & H_5), 7.44 (s, 1H, imidazopyridine H_8), 8.87–7.89 (d, 1H, J = 6.8 Hz, imidazopyridine H_5), 7.93–7.95 (d, 2H, J = 8.4 Hz, methylsulfonylphenyl H_2 & H_6), 8.25–8.27 (d, 2H, J = 8.4 Hz, methylsulfonylphenyl H_3 & H_5), 8.50 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ ppm 21.31, 43.99, 115.02, 115.85, 116.13, 120.04, 122.71, 123.01, 127.12, 127.75, 129.84, 135.78, 137.04, 139.06, 139.60, 143.05, 144.77; LC-MS (ESI) m/z:

412 ([M + H]$^+$, 414 ([M + H + 2]$^+$, 32%); Anal. Calcd. For $C_{21}H_{18}ClN_3O_2S$: C, 61.24; H, 4.40; N, 10.20. Found: C, 61.20; H, 4.37; N, 10.26.

N-(4-Chlorophenyl)-5-methyl-2-(4-(methylsulfonyl)phenyl)imidazo[1,2-*a*]pyridin-3-amine (**5l**)

Yield, 73%; yellow powder; mp: 249 °C (decomposed); IR (KBr disk): vcm^{-1} 1148, 1316 (SO_2), 1655 (C = N), 3372 (NH); ^{1}H NMR (DMSO-d_6): δ ppm 2.65 (s, 3H, CH_3), 3.21 (s, 3H, SO_2Me), 6.49–6.51 (d, 2H, *J* = 8.4 Hz, phenyl H_2 & H_6), 6.67–6.69 (d, 1H, *J* = 6.8 Hz, imidazopyridine H_6), 7.18–7.26 (m, 3H, phenyl H_3 & H_5, imidazopyridine H_7), 7.49–7.52 (d, 2 H, *J* = 8.8 Hz, imidazopyridine H_8), 7.92–7.94 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H_2 & H_6), 8.09–8.11 (d, 1H, *J* = 8.4 Hz,), 8.27–8.29 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H_3 & H_5), 8.40 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ ppm 18.81, 43.90, 114.41, 116.14, 121.14, 122.28, 126.69, 127.34, 127.69, 129.35, 130.03, 136.56, 137.76, 138.92, 139.82, 144.37, 147.07; LC-MS (ESI) m/z: 412 ([M + H]$^+$, 100), ([M + H + 2]$^+$, 32%); Anal. Calcd. For $C_{21}H_{18}ClN_3O_2S$: C, 61.24; H, 4.40; N, 10.20. Found: C, 61.16; H, 4.43; N, 10.16.

2-(4-(Methylsulfonyl)phenyl)-*N*-(*p*-tolyl)imidazo[1,2-*a*]pyridin-3-amine (**5m**)

Yield, 63%; yellow powder; mp: 198–200 °C; IR (KBr disk): vcm^{-1} 1153, 1313 (SO_2), 1635 (C = N), 3335 (NH); ^{1}H NMR (DMSO-d_6): δ ppm 2.15 (s, 3H, CH_3), 3.21 (s, 3H, SO_2Me), 6.42–6.44 (d, 2H, *J* = 8.0 Hz, phenyl H_3 & H_5), 6.93–6.97 (m, 3H, imidazopyridine H_6, phenyl H_2 & H_6), 7.33–7.37 (t, 1H, *J* = 7.6 Hz, imidazopyridine H_7), 7.65–7.67 (d, 1H, *J* = 9.2 Hz, imidazopyridine H_8), 7.93–7.97 (m, 3H, methylsulfonylphenyl H_2 & H_6, imidazopyridine H_5), 8.21 (s, 1H, NH), 8.29–8.31 (d, 1H, *J* = 8.4 Hz, methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (DMSO-d_6): δ ppm 20.55, 43.97, 113.13, 113.z51, 117.89, 121.42, 123.79, 126.25, 127.23, 127.72, 127.90, 130.51, 135.99, 139.11, 139.63, 142.48, 143.18; LC-MS (ESI) m/z: 378 ([M + H]$^+$, 100); Anal. Calcd. For $C_{21}H_{19}N_3O_2S$: C, 66.82; H, 5.07; N, 11.13. Found: C, 66.98; H, 5.11; N, 11.19.

8-Methyl-2-(4-(methylsulfonyl)phenyl)-*N*-(*p*-tolyl)imidazo[1,2-*a*]pyridin-3-amine (**5n**)

Yield, 59%; dark yellow powder; mp: 200–202 °C; IR (KBr disk): vcm^{-1} 1156, 1315 (SO_2), 1632 (C = N), 3370 (NH); ^{1}H NMR (DMSO-d_6): δ ppm 2.15 (s, 3H, 4-CH_3), 2.58 (s, 3H, 8-CH_3), 3.21 (s, 3H, SO_2Me), 6.41–6.43 (d, 2H, *J* = 8.0 Hz, phenyl H_3 & H_5), 6.84–6.87 (t, 1H, *J* = 6.8 Hz, imidazopyridine H_6), 6.94–6.96 (d, 2H, *J* = 8.0 Hz, phenyl H_2 & H_6), 7.14–7.16 (d, 1H, *J* = 6.8 Hz, imidazopyridine H_7), 7.80–7.82 (d, 1H, *J* = 6.8 Hz, imidazopyridine H_5), 7.93–7.95 (d, 1H, *J* = 8.4 Hz, methylsulfonylphenyl H_2 & H_6), 8.20 (s, 1H, NH), 8.30–8.32 (d, 1H, *J* = 8.4 Hz, methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (DMSO-d_6): δ ppm 16.64, 20.55, 44.01, 113.10, 113.50, 121.55, 121.79, 124.68, 127.22, 127.36, 127.69, 127.83, 130.48, 135.52, 139.25, 139.49, 142.78, 143.27; LC-MS (ESI) m/z: 392 ([M + H]$^+$, 100); Anal. Calcd. For $C_{22}H_{21}N_3O_2S$: C, 67.50; H, 5.41; N, 10.73. Found: C, 67.41; H, 4.39; N, 10.78.

7-Methyl-2-(4-(methylsulfonyl)phenyl)-*N*-(*p*-tolyl)imidazo[1,2-*a*]pyridin-3-amine (**5o**)

Yield, 61%; creamy powder; mp: 274 ^{o}C (decomposed); IR (KBr disk): vcm^{-1} 1158, 1320 (SO_2), 1648 (C = N), 3224 (NH); ^{1}H NMR (DMSO-d_6): δ ppm 2.16 (s, 3H, 4-CH_3), 2.38 (s, 3H, 7-CH_3), 3.20 (s, 3H, SO_2Me), 6.40–6.42 (d, 2H, *J* = 8.0 Hz, phenyl H_3 & H_5), 6.78–6.80 (d, 1H, *J* = 6.8 Hz, imidazopyridine H_6), 6.94–6.96 (d, 2H, *J* = 8.4 Hz, phenyl H_2 & H_6), 7.42 (s, 1H, imidazopyridine H_8), 7.83–7.84 (d, 1H, *J* = 6.8 Hz, imidazopyridine H_5), 7.91–7.93 (d, 1H, *J* = 8.4 Hz, methylsulfonylphenyl H_2 & H_6), 8.15 (s, 1H, NH), 8.26–8.28 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (DMSO-d_6): δ ppm 19.47, 20.23, 42.91, 112.38, 114.53, 114.99, 119.91, 121.95, 126.02, 126.58, 126.73, 129.39, 134.53, 135.71, 138.21, 138.35, 141.80, 142.23; LC-MS (ESI) m/z: 392 ([M + H]$^{+}$, 100); Anal. Calcd. For $C_{22}H_{21}N_3O_2S$: C, 67.50; H, 5.41; N, 10.73. Found: C, 67.64; H, 5.42; N, 10.66.

5-Methyl-2-(4-(methylsulfonyl)phenyl)-*N*-(*p*-tolyl)imidazo[1,2-*a*]pyridin-3-amine (**5p**)

Yield, 63%; yellow powder; mp: 330 ^{o}C (decomposed); IR (KBr disk): vcm^{-1} 1156, 1321 (SO_2), 1648 (C = N), 3390 (NH); ^{1}H NMR (DMSO-d_6): δ ppm 2.15 (s, 3H, CH_3), 2.66 (s, 3H, CH_3), 3.20 (s, 3H, SO_2Me), 6.42–6.44 (d, 2H, *J* = 8.0 Hz, phenyl H_3 & H_5), 6.64–6.66 (d, 1H, *J* = 6.8 Hz,imidazopyridine H_6), 6.94–6.96 (d, 2H, *J* = 8.0 Hz, phenyl H_2 & H_6), 7.20–7.24 (t, 1H, *J* = 7.2 Hz, imidazopyridine H_7), 7.48–7.50 (d, 1H, *J* = 9.6 Hz, imidazopyridine H_8), 7.89–7.92 (d, 1H, *J* = 8.4 Hz, methylsulfonylphenyl H_2 & H_6), 8.04 (s, 1H, NH), 8.29–8.31 (d, 1H, *J* = 8.4 Hz, methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (DMSO-d_6): δ ppm 18.82, 20.53, 43.81, 114.19, 116.06, 117.43, 122.06, 126.50, 127.23, 127.35, 127.59, 128.10, 130.67, 136.69, 137.68, 139.66, 144.26, 145.81; LC-MS (ESI) m/z: 392 ([M + H]$^{+}$, 100); Anal. Calcd. For $C_{22}H_{21}N_3O_2S$: C, 67.50; H, 5.41; N, 10.73. Found: 67.59; H, 5.38; N, 10.75.

N-(4-Methoxyphenyl)-2-(4-(methylsulfonyl)phenyl)imidazo[1,2-*a*]pyridin-3-amine (**5q**)

Yield, 38%; dark yellow powder; mp: 103–105 ^{o}C; IR (KBr disk): vcm^{-1} 1160, 1320 (SO_2), 1639 (C = N), 33230 (NH); ^{1}H NMR ($CDCl_3$): δ ppm 3.22 (s, 3H, SO_2Me), 3.63 (s, 3H, OCH_3), 6.46–6.48 (d, 2H, *J* = 8.8 Hz, phenyl H_3 & H_5), 6.76–6.78 (d, 2H, *J* = 9.2 Hz, phenyl H_2 & H_6), 6.93–6.97 (t, 1H, *J* = 6.8 Hz, imidazopyridine H_6), 7.32–7.36 (t, 1H, *J* = 8.0 Hz, imidazopyridine H_7), 7.64–7.67 (d, 1H, *J* = 9.2 Hz, imidazopyridine H_8), 7.93–7.96 (d, 1H, *J* = 8.8 Hz, methylsulfonylphenyl H_2 & H_6), 7.97–7.99 (d, 1H, *J* = 6.8 Hz, imidazopyridine H_5), 8.07 (s, 1H, NH), 8.31–8.33 (d, 1H, *J* = 8.8 Hz, methylsulfonylphenyl H_3 & H_5); ^{13}C NMR ($CDCl_3$): δ ppm 43.98, 55.67, 113.09, 114.48, 117.89, 121.89, 123.81, 126.21, 127.22, 127.71, 135.90, 139.15, 139.60, 142.42, 153.04; LC-MS (ESI) m/z: 394 ([M + H]$^{+}$, 100); Anal. Calcd. For $C_{21}H_{19}N_3O_3S$: C, 64.11; H, 4.87; N, 10.68. Found: C, 63.93; H, 4.89; N, 10.76.

N-(4-Methoxyphenyl)-8-methyl-2-(4-(methylsulfonyl)phenyl)imidazo[1,2-*a*]pyridin-3-amine (**5r**)

Yield, 41%; white-creamy powder; mp: 229–231 ^{o}C; IR (KBr disk): vcm^{-1} 1151, 1314 (SO_2), 1627 (C = N), 3252 (NH); ^{1}H NMR (DMSO-*d_6*): δ ppm 2.57 (s, 3H, CH_3), 3.21 (s, 3H, SO_2Me), 3.62 (s, 3H, OCH_3), 6.45–6.48 (d, 2H, *J* = 8.8 Hz, phenyl H_3 & H_5), 6.75–6.77 (d, 2H, *J* = 8.8 Hz, phenyl H_2 & H_6), 6.84–6.87 (t, 1H, *J* = 7.2 Hz, imidazopyridine H_6), 7.14–7.16 (d, 1H, *J* = 6.8 Hz, imidazopyridine H_7), 7.82–7.84 (d, 1H, *J* = 6.8 Hz, imidazopyridine H_8), 7.94–7.96 (d, 1H, *J* = 8.4 Hz, methylsulfonylphenyl H_2 & H_6), 8.07 (s, 1H, NH), 8.32–8.34 (d, 1H, *J* = 8.8 Hz, methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (DMSO-*d_6*): δ ppm 16.64, 44.01, 55.67, 113.07, 114.47, 115.54, 121.57, 122.27, 124.64, 127.21, 127.70, 135.43, 139.28, 139.46, 142,73, 153.00; LC-MS (ESI) m/z: 408 ([M + H]$^{+}$, 100); Anal. Calcd. For $C_{22}H_{21}N_3O_3S$: C, 64.85; H, 5.19; N, 10.31. Found: C, 65.01; H, 5.15; N, 10.25.

N-(4-Methoxyphenyl)-7-methyl-2-(4-(methylsulfonyl)phenyl)imidazo[1,2-*a*]pyridin-3-amine (**5s**)

Yield, 44%; white powder; mp: 138–140 ^{o}C; IR (KBr disk): vcm^{-1} 1155, 1307 (SO_2), 1648 (C = N), 3227 (NH); ^{1}H NMR (DMSO-*d_6*): δ ppm 2.37 (s, 1H, CH_3), 3.21 (s, 3H, SO_2Me), 3.63 (s, 3H, OCH_3), 6.44–6.47 (d, 2H, *J* = 8.8 Hz, phenyl H_3 & H_5), 6.75–6.77 (m, 3H, phenyl H_2 & H_6, imidazopyridine H_6), 7.41 (s, 1H, imidazopyridine H_8), 7.84–7.86 (d, 1H, *J* = 7.2 Hz, imidazopyridine H_8), 7.92–7.94 (d, 1H, *J* = 8.4 Hz, methylsulfonylphenyl H_2 & H_6), 8.03 (s, 1H, NH), 8.28–8.30 (d, 1H, *J* = 8.4 Hz, methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (DMSO-*d_6*): δ ppm 21.31, 44.00, 55.67, 114.42, 115.55, 116.06, 121.47, 123.05, 127.10, 127.67, 135.54, 136.75, 139.33, 139.40, 142.83, 152.99; LC-MS (ESI) m/z: 408 ([M + H]$^{+}$, 100); Anal. Calcd. For $C_{22}H_{21}N_3O_3S$: C, 64.85; H, 5.19; N, 10.31. Found: C, 64.98; H, 5.14; N, 10.24.

N-(4-Methoxyphenyl)-5-methyl-2-(4-(methylsulfonyl)phenyl)imidazo[1,2-*a*]pyridin-3-amine (**5t**)

Yield, 50%; yellow-orange powder; mp: 156 ^{o}C (decomposed); IR (KBr disk): vcm^{-1} 1152, 1313 (SO_2), 1641 (C = N), 3365 (NH); ^{1}H NMR (DMSO-*d_6*): δ ppm 2.68 (s, 1H, CH_3), 3.21 (s, 3H, SO_2Me), 3.62 (s, 3H, OCH_3), 6.37–6.39 (d, 2H, *J* = 8.4 Hz, phenyl H_3 & H_5), 6.64–6.65 (d, 1H, *J* = 6.8 Hz, imidazopyridine H_6), 6.76–6.78 (d, 2H, *J* = 8.4 Hz, phenyl H_2 & H_6), 7.20–7.23 (t, 1H, *J* = 7.2 Hz, imidazopyridine H_7), 7.47–7.49 (d, 1H, *J* = 8.8 Hz, imidazopyridine H_8), 7.90–7.92 (m, 3H, methylsulfonylphenyl H_2 & H_6, NH), 8.31–8.33 (d, 1H, *J* = 8.0 Hz, methylsulfonylphenyl H_3 & H_5); ^{13}C NMR (DMSO-*d_6*): δ ppm 18.86, 43.93, 55.61, 114.03, 114.15, 114.92, 115.70, 116.06, 122.48, 126.47, 127.36, 127.59, 136.72, 137.66, 139.20, 139.62, 141.93, 144.18, 152.50; LC-MS (ESI) m/z: 408 ([M + H]$^{+}$, 100); Anal. Calcd. For $C_{22}H_{21}N_3O_3S$: C, 64.85; H, 5.19; N, 10.31. Found: C, 64.76; H, 5.22; N, 10.35.

4.3. Molecular modeling and docking studies

The docking studies between designed compounds and COX-2 isozyme were carried out by the AutoDock Vina program [37]. This procedure was accomplished after ligand and enzyme preparation. In brief, the 3D structure of murine COX-2 (ID: 6COX) was obtained from RCSB Protein Data Bank [38]. After eliminating the crystallized ligand and water molecules, polar hydrogens and Kollman charges were

added to the protein. The 3D structures of two potent and selective derivatives were created and energetically minimized in HyperChem 8.0 software by the MM^+ method. Then, the Gasteiger charges were added to ligands. Finally, the pdbqt files of ligands and enzyme, which were used in docking created with AutoDock tools. Acquired configurations resulting from docking were searched to find suitable and efficient interactions with enzyme.

4.4. Biological assay

4.4.1. In vitro cyclooxygenase (COX) inhibition assays

This assay was performed using a COX fluorescent inhibitor screening assay kit (Cayman Chemical, MI, USA). The Cayman COX (ovine COX-1/ human recombinant COX-2) inhibitor screening assay utilizes the peroxidase component of COXs. In this assay, the reaction between PGG_2 and ADHP (10-acetyl-3,7-dihydroxyphenoxazine) produces the highly fluorescent compound resorufin. Resorufin fluorescence can be analyzed with a 530–540 nm excitation wavelength and an emission wavelength of 585–595 nm [39]. Consequently, higher inhibition of the COX enzyme leads to lower resorufin production, which means less fluorescence intensity.

4.4.2. In vivo evaluation of compound analgesic effects

4.4.2.1. Animals and reference drug

Analgesic effects of compounds were evaluated using formalin test in rats [40]. Male Wistar rats (Pasteur Institute, Iran), weighing 110–150 g, were used. Rats were housed in a temperature-controlled condition (25 ± 2°C) and 12 h light/dark cycle and free access to food and water except during the experiment. Animals were randomly divided into groups for each test compound (N = 6), and each rat was used only once during the experiments.

4.4.2.2. Formalin test

The basis of this pain assessment is the subcutaneous injection of formalin 5% into the paw and then monitoring of animal's pain-related behavior in test and control groups. Synthesized compounds or celecoxib (Sigma-Aldrich, Germany) were dissolved in DMSO and were administered by intraperitoneal (i.p.) injection (40 mg/kg, Volume of injection 1 ml/kg) 30 min before the test. The control group received DMSO (1 ml/kg) 30 min before the test. Formalin 5% (40 µL) was injected into the dorsal surface of the left hind paw, and the rats were placed individually in plexiglass chambers (30 x 30 x 30 cm) and continuously observed for 60 min. Pain-related behaviors were quantified according to the following numerical scale: 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 as described by Dubuisson and Dennis. The area under the curve (AUC) for pain score against time plot was measured and compared between groups.

*4.4.2.3. Statistical analysis of the data*Results were shown as mean and 95% confidence interval. Statistical analysis was done using Prism 6 (GraphPad Software Inc.). One-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test was used to compare AUCs of pain scores between groups. The $p < 0.05$ was regarded as statistically significant.

Declarations

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Acknowledgment

The authors acknowledge the support from the Research Deputy of School of Pharmacy, Shahid Beheshti University of Medical Sciences under grant number 20940.

Conflicts of interest

The authors declare no conflict of interest.

Ethical Approval

The project was found to be in accordance to the ethical principles and the national norms and standards for conducting Medical Research in Iran (ethics code: IR.SBMU.PHARMACY.REC.1401.135).

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Figures

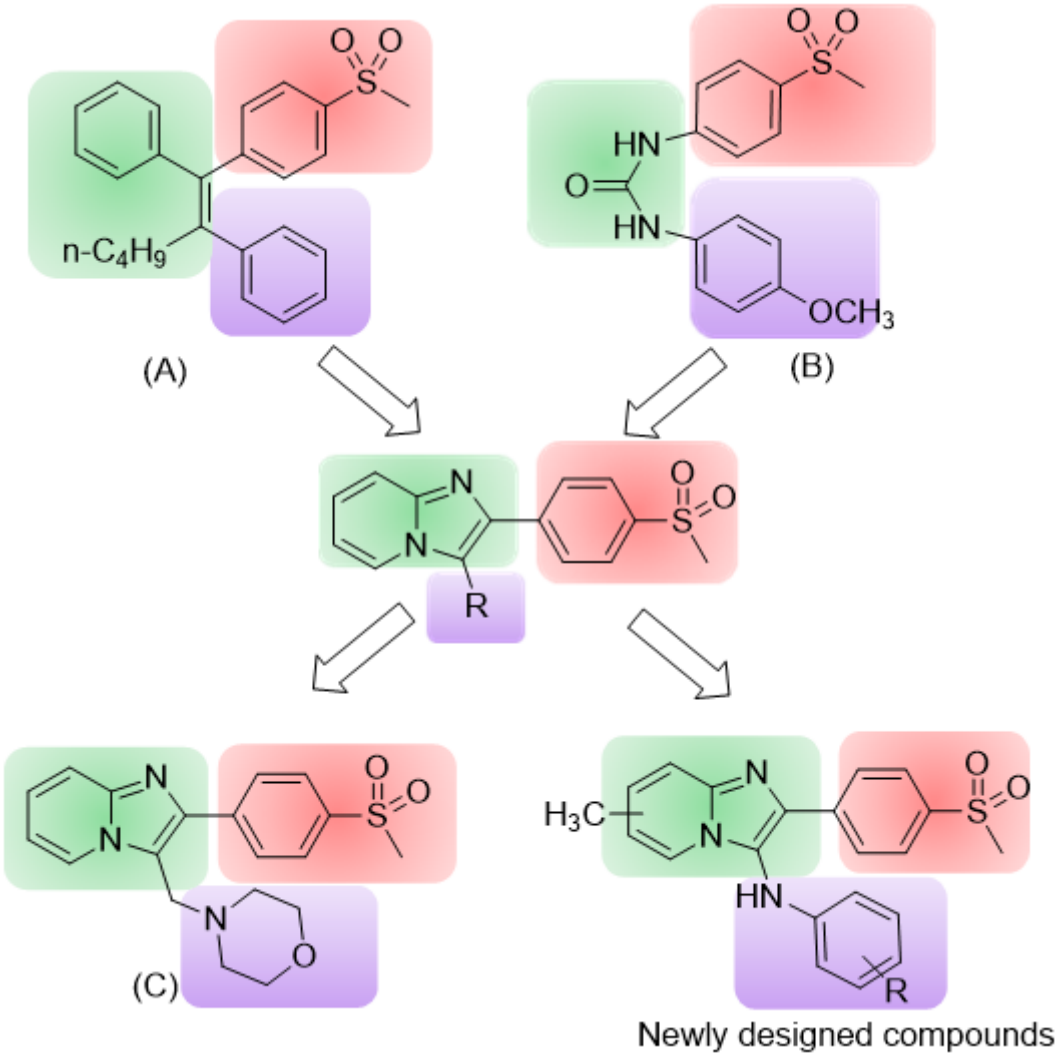

Figure 1

Chemical structures of three reported COX-2 inhibitors as lead compounds (**A**, **B**, and **C**) and the designed molecules

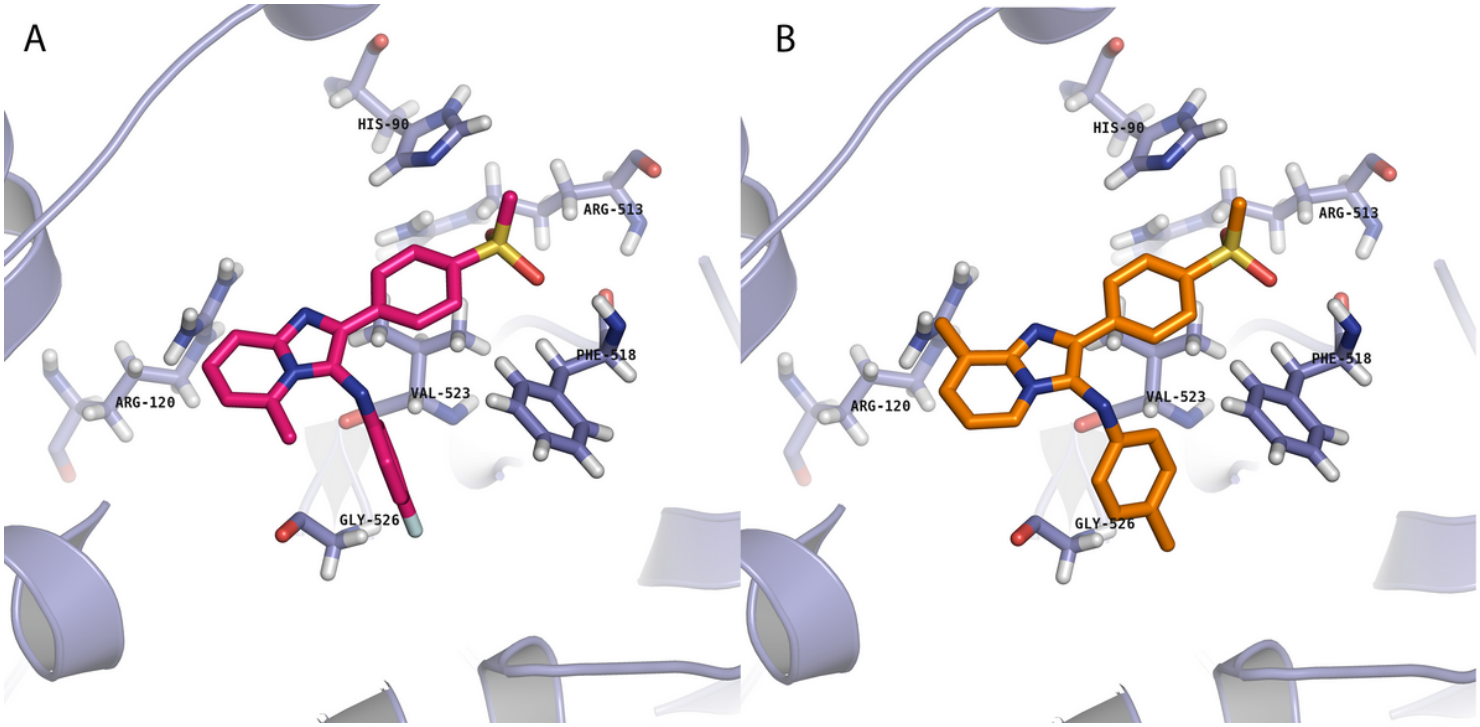

Figure 2

Binding model of compound **5h** (A), **5n**(B) in COX-2 (6COX) active site

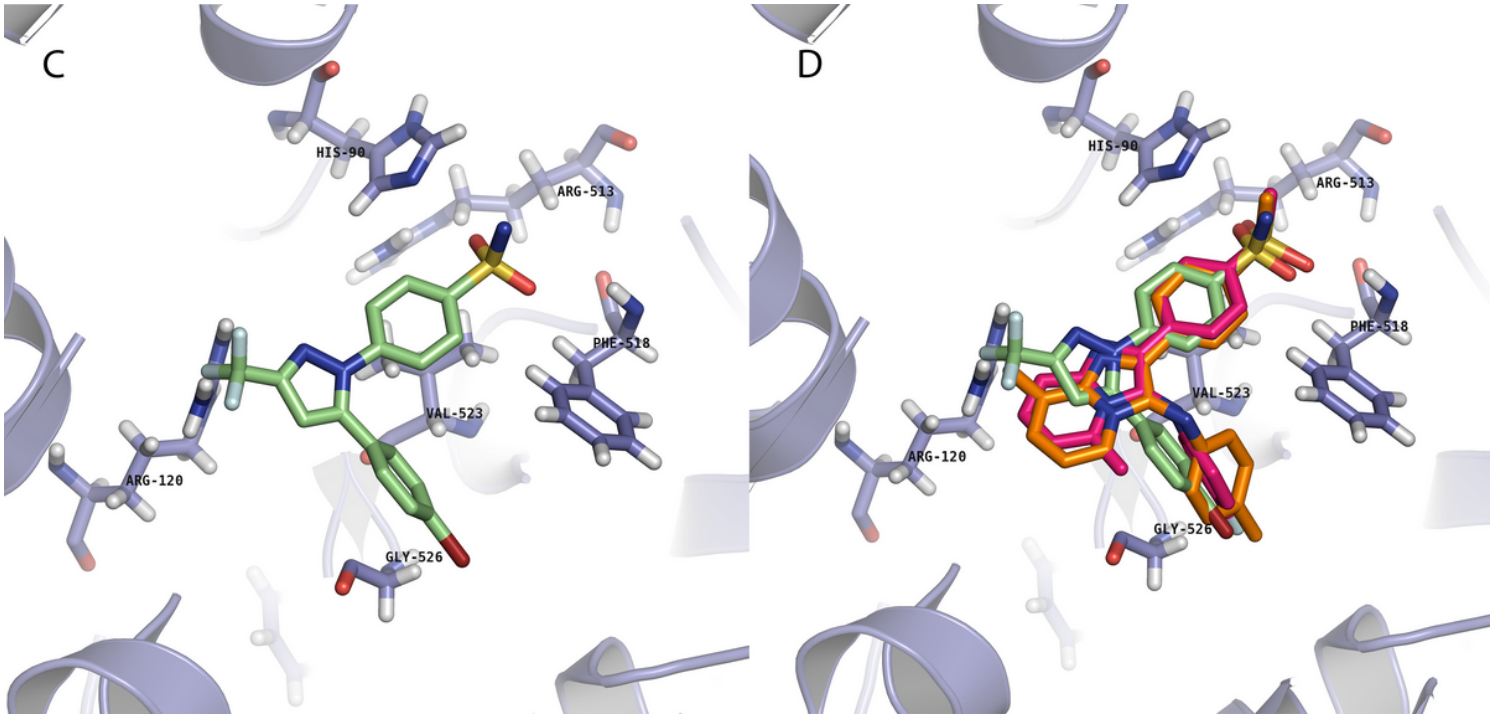

Figure 3

Binding model of compound **Sc-558** (C) and superimposition of **5h** and **5n** on **Sc-558** (D)

Supplementary Files

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