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Oxazolo[4,5-b]pyridine-Based Piperazinamides as GSK-3β Inhibitors with Potential for Attenuating Inflammation and Suppression of Pro-Inflammatory Mediators

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Recent studies reveal that glycogen synthase kinase-3β (GSK-3β) acts as a pro-inflammatory enzyme, and by inhibiting this kinase, inflammation can be controlled. In this regard, a series of 17 piperazine-linked oxazolo[4,5-b]pyridine-based derivatives was synthesized and evaluated for *in vitro* GSK-3β inhibitory and *in vivo* anti-inflammatory activity. The compounds **7d**, **7e**, **7g**, and **7c** displayed the best GSK-3β inhibitory activity among all the synthesized compounds, with corresponding IC_{50} values of 0.34, 0.39, 0.47, and 0.53 μM. Among the compounds **7d**, **7e**, **7g**, and **7c** examined for *in vivo* anti-inflammatory activity in the rat paw edema model, compound **7d** exhibited maximum inhibition, reducing the paw volume by 62.79 and 65.91% at 3 and 5 h post-carrageenan administration, respectively, in comparison to indomethacin (76.74% at 3 h and 79.54% at 5 h after carrageenan administration). Furthermore, these compounds (**7d**, **7e**, **7g**, and **7c**) were also found to substantially inhibit pro-inflammatory mediators, i.e., TNF-α, IL-1β, and IL-6, *ex vivo* in comparison to indomethacin and did not pose any gastric ulceration risk, indicating the potential of this oxazolopyridine scaffold for the development of GSK-3β inhibitors and their application as anti-inflammatory agents.

Keywords: Amide / Inflammation / Kinase inhibition / Oxazolopyridines

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Introduction

Glycogen synthase kinase-3 (GSK-3) is a serine/threonine kinase prevalent in two major mammalian isoforms, GSK-3α (MW 51 kDa) and GSK-3β (MW 47 kDa). GSK-3 modulates a diversity of physiological processes such as insulin signaling, glycogen metabolism, neuronal functions, cellular proliferation, apoptosis, and embryonic development. This multi-functional kinase, however, if deregulated, is reported a culprit in various diseases, including diabetes, Alzheimer's disease (AD) and other CNS disorders, chronic inflammatory disorders, malaria, and cancer [1, 2]. GSK-3 is thus targeted for the development of therapeutic agents against these diseases.

GSK-3β is also an important positive mediator of inflammation possessing capacity to modulate the inflammatory response in both innate and adaptive immune cells. GSK-3β augments the production of various pro-inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor (TNF), while concurrently suppressing the production of the anti-inflammatory cytokines such as interleukin-10 (IL-10). Conversely, the application of GSK-3β

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inhibitors like lithium chloride, SB216763, TDZD8, and CHIR99021 have been reported to reduce the effect by inhibiting the production of pro-inflammatory cytokines and increasing anti-inflammatory cytokine production (Fig. 1) [3–7]. Toll-like receptor (TLR)-dependent activation of the PI3K-Akt signaling pathway has been found to downregulate GSK-3 activity [8, 9]. Inhibition of GSK-3β modulates the inflammatory response by inducing down-regulation of NF-κB-mediated transcription (without affecting IκB degradation and nuclear translocation of NF-κB) [10], which is responsible for gene expression and production of various pro-inflammatory mediators like TNF-α, IL-1β, iNOS, IL-6, etc. [3, 8, 11–13]. Attenuation of GSK-3 activity bolsters cAMP response element-binding protein (CREB) to sequester coactivator CREB-binding protein (CBP) from NF-κB, leading to suppression of NF-κB/CBP-mediated activation of pro-inflammatory cytokine gene transcription [3, 12]. Inhibition of GSK-3β thus controls inflammation and protects the host against inflammatory-mediated pathology and death in animal models. Remarkably, the *in vivo* administration of GSK-3β inhibitors has been reported to suppress inflammation in various animal models including TNF-α administration, endotoxemia, experimental colitis, type II collagen-induced arthritis, ovalbumin-induced asthma, carrageenan-induced

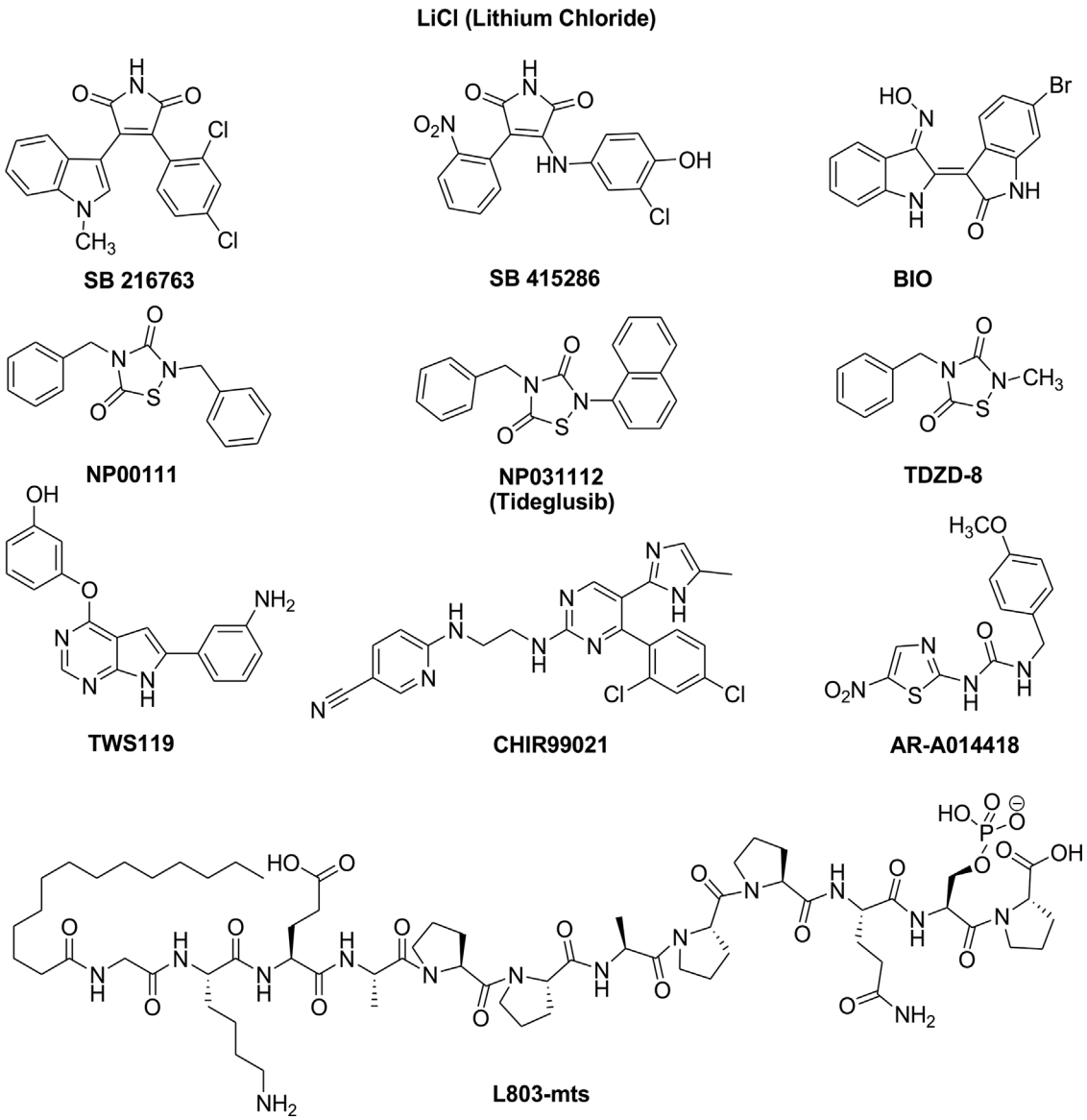


Figure 1. Some known GSK-3β inhibitors with reported anti-inflammatory potential.

lung injury, and experimental autoimmune encephalomyelitis [3, 5, 11–22]. This association between GSK-3β and inflammation further increases the scope for development of GSK-3β inhibitors for the treatment of inflammatory disorders in a broad range of conditions.

In continuation of our search for GSK-3 inhibitors, we focused our attention on oxazolo-pyridines. Oxazolo-pyridines have been found to be relatively potent anti-inflammatory, anti-analgesic, anti-pyretic, anti-leishmanial, anti-cancerous, and anti-diabetic agents. They hold potential value for a broad spectrum of conditions where one or more of the symptoms of inflammation, fever, and pain are manifested including rheumatoid arthritis, osteoarthritis, gout, infectious arthritis, rheumatic fever, ultraviolet erythema, atopic dermatitis, contact dermatitis, seborrheic dermatitis, diaper rash, and inflammatory conditions of the ocular system [23–28]. Oxazolo-pyridines have also been reported as potent GSK-3β inhibitors [29, 30]. So far not much work has been done on this scaffold and there is ample scope for the development of more potent inhibitors from this ligand. In view of the pharmacological potential of hitherto relatively unexplored oxazolo[4,5-b]pyridine scaffold and the privileged nature of piperazine linkage, we synthesized novel piperazine-linked oxazolo[4,5-b]pyridine based derivatives as GSK-3β inhibitors. A series of 17 compounds has been synthesized, and assessed for *in vitro* GSK-3β inhibition. Given the potential role of GSK-3β inhibition in controlling inflammation, inhibitors of GSK-3β are thus believed to hold a significant potential for the suppression of inflammatory conditions. The synthesized compounds exhibiting significant GSK-3β inhibition have been therefore evaluated for *in vivo* anti-inflammatory activity by paw-edema method, and the compounds showing prominent anti-inflammatory profile have been examined for their effect on pro-inflammatory mediators, like TNF-α, IL-1β, and IL-6 using *ex vivo* assays. The molecular docking studies of the active compounds have also been performed against GSK-3β to understand the molecular interactions of these compounds with the GSK-3β site. Since the anti-inflammatory compounds are known to pose a side effect of gastric ulceration, the active compounds have also been checked for their gastric ulceration risk.

Results and discussion

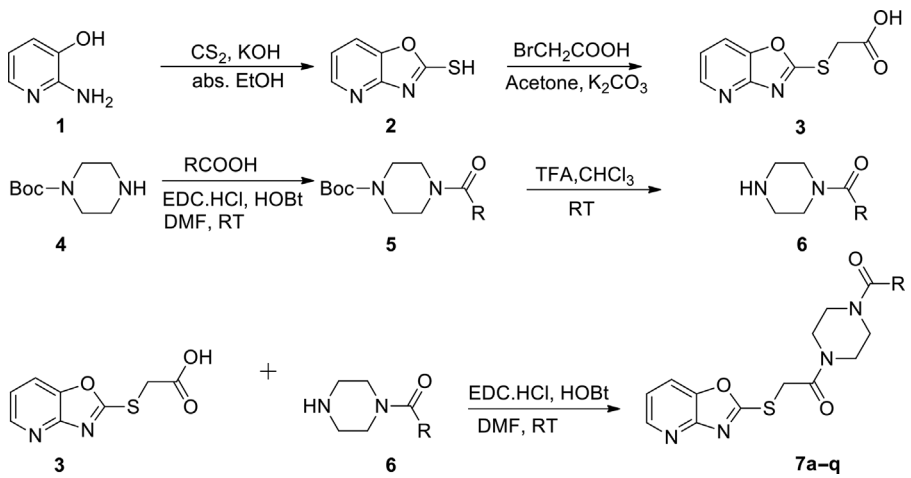
Chemistry

The compounds have been synthesized according to Scheme 1. 2-Amino-3-hydroxy pyridine **1** on refluxing with carbon disulphide and potassium hydroxide in absolute ethanol yielded 2-mercapto oxazolo[4,5-b]pyridine **2**, which on treatment with bromoacetic acid in the presence of potassium carbonate in acetone formed oxazolo[4,5-b]pyridine-2-mercaptoacetic acid **3**. On the other hand, *N*-Boc piperazine **4** was coupled with various acids using EDC.HCl as a coupling agent, to form various *N*-Boc piperazinamides **5** which when deprotected with TFA and chloroform mixture gave the requisite piperazinamides **6**. Intermediates **3** and **6** were then coupled by EDC.HCl in the presence of HOBt to afford the target compounds **7a–q**, as shown in Table 1. The target compound structures have been established by ^{1}H NMR, ^{13}C NMR, IR and mass spectral techniques.

Biological activity

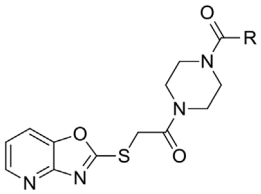
In vitro GSK-3β inhibitory activity

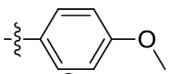
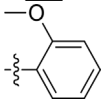
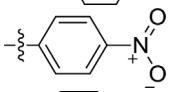
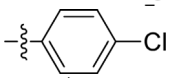
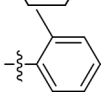
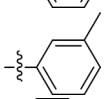
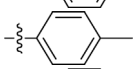
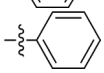
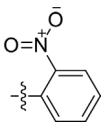
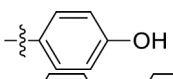
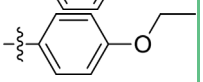
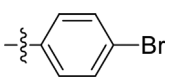
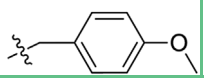
All the synthesized compounds were screened for *in vitro* GSK-3β inhibition potential by Kinase-Glo™ luminescent method [31, 32]. The results as depicted in Table 1 indicate that the compounds inhibited GSK-3β in the submicromolar to micromolar range. Compounds **7d**, **7e**, **7g**, and **7c** were most active among all the synthesized compounds with corresponding IC_{50} values of 0.34, 0.39, 0.47, and 0.53 μM. Staurosporine, taken as standard, exhibited an IC_{50} value of



Scheme 1. Synthetic approach for novel oxazolo[4,5-b]pyridine-based piperazinamides.

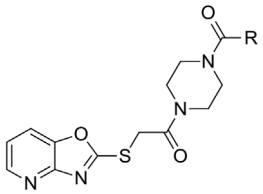
Table 1. Oxazolo[4,5-b]pyridine-based derivatives and their GSK-3β inhibitory activity.

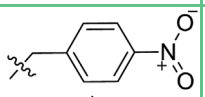
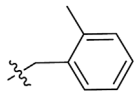
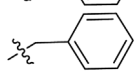


Compound	Compound (R)	GSK-3β Inh. IC_{50} (μM) ± SEM[a)]
7a		5.79 ± 0.17
7b		18.12 ± 0.78
7c		**0.53 ± 0.03**
7d		**0.34 ± 0.02**
7e		**0.39 ± 0.03**
7f		4.82 ± 0.06
7g		**0.47 ± 0.05**
7h		9.05 ± 0.12
7i		15.32 ± 0.16
7j		0.72 ± 0.08
7k		16.25 ± 0.94
7l		12.43 ± 0.47
7m		0.91 ± 0.15
7n		15.12 ± 0.57

(*Continued*)

Table 1. (*Continued*)



Compound	Compound (R)	GSK-3β Inh. IC_{50} (μM) ± SEM[a)]
7o		19.31 ± 1.03
7p		2.34 ± 0.09
7q		18.90 ± 0.87
Staurosporine		**0.04 ± 0.01**

[a]SEM, Standard error of the mean.
Numbers in bold indicate the activity of the most active compounds.

0.04 μM. Compound **7j** also displayed moderate inhibition in single-digit micromolar range while the remaining compounds exhibited relatively weak inhibition.

From the *in vitro* GSK-3β inhibition results, it can be seen that the benzyl derivatives demonstrated lower activity in comparison to their phenyl counterparts. Furthermore, among the phenyl derivatives, *para*-hydroxy substituent exhibited better activity than *para*-methoxy substituent which in turn imparted more activity than *para*-ethoxy substituent, implying decrease in activity with increase in size.

In vivo anti-inflammatory activity

The synthesized compounds **7d**, **7e**, **7g**, and **7c**, displaying potential GSK-3β inhibitory activity were also evaluated for their *in vivo* anti-inflammatory activity by carrageenan-induced hind-paw edema model. Indomethacin, an anti-inflammatory drug, was taken as a standard. SB216763, a known GSK-3β inhibitor, reported to exert anti-inflammatory effect in various *in vitro* and *in vivo* models, was also used as the reference. The results shown in Table 2 indicated that all the tested compounds displayed a time-dependent increase in anti-inflammatory activity after 3 and 5 h post-carrageenan administration, by ameliorating inflamed rat paw edema. The compounds **7d** and **7e** exhibited significantly controlled inflammation, reducing the paw volume by 62.79 and 60.46% inhibition at 3 h and, 65.91 and 63.64% inhibition,

Table 2. ***In vivo*** **anti-inflammatory activity of oxazolo[4,5-b]pyridine-based derivatives.**

Group	Change in paw edema volume (mL) after drug treatment (±SEM)		% Inhibition	
	3 h	5 h	3 h	5 h
Control	1.016 ± 0.101	1.033 ± 0.088	–	–
Indomethacin	0.467 ± 0.056***	0.483 ± 0.048***	76.74	79.54
SB216763	0.567 ± 0.076**	0.550 ± 0.084**	67.44	70.45
7c	0.650 ± 0.043**	0.633 ± 0.076**	51.16	54.55
7d	0.583 ± 0.048**	0.567 ± 0.099**	62.79	65.91
7e	0.617 ± 0.060**	0.600 ± 0.073**	60.46	63.64
7g	0.600 ± 0.073**	0.583 ± 0.079**	58.14	61.36

Data are analyzed by one-way ANOVA followed by Dunnett's t-test and expressed as mean ± SEM from six observations; ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$.

respectively, at 5 h post-carrageenan administration relative to indomethacin (76.74% at 3 h and 79.54% at 5 h after carrageenan administration). Compounds **7g** and **7c** also showed a fairly good activity, reducing the paw volume by 58.14 and 51.16% after 3 h interval and 61.36 and 54.55%, respectively, after 5 h interval. SB216763 exhibited inhibition of 67.44% at 3 h and 70.45% at 5 h post-carrageenan treatment in comparison to indomethacin.

On comparison of these results with GSK-3β inhibitory data, it was found that the anti-inflammatory profile of the tested compounds **7d**, **7e**, **7g**, and **7c** was observed to be in agreement with their observed GSK-3β inhibition activities. The compounds, therefore, exhibited good correlation between GSK-3β inhibition and anti-inflammatory property, suggesting the role GSK-3β inhibitors can play against inflammation and inflammatory associated conditions.

*Effects of compounds **7d, 7e, 7g,** and **7c** on pro-inflammatory mediator levels*

The inflammatory process is characterized by the generation of large volumes of the pro-inflammatory mediators like TNF-α, IL-1β, and IL-6, which have a central role in the perpetuation of chronic inflammation and tissue damage during progression of inflammatory disorder. GSK-3β upregulates the production of these pro-inflammatory mediators, thereby positively modulates the inflammation. The suppression of these molecules has been reported to result in a reduction of disease severity [33]. The compounds showing potent activity against both GSK-3β as well as *in vivo* inflammation were therefore further evaluated to study their effect on pro-inflammatory mediators, TNF-α, IL-1β, and IL-6. A significant increase in the level of these pro-inflammatory markers was observed in carrageenan-induced inflammation group in comparison to control, as evident from a higher concentration of TNF-α, IL-1β, and IL-6 in carrageenan-injected rat paws relative to indomethacin and SB216763-treated groups. The treatment with selected conjugates **7d**, **7e**, **7g**, and **7c** suppressed the elevation in the level of IL-1β, and TNF-α significantly when compared to carrageenan group, with **7d** possessing the highest potential (Fig. 2).

Ulcerogenic risk evaluation

The compounds possessing potential anti-inflammatory activity were also studied for their gastric ulcerogenic risk. As compared to indomethacin, SB216763 and the active compounds **7d**, **7e**, **7g**, and **7c** did not cause any gastric ulceration at even three times higher dose than used for anti-inflammatory activity (Fig. 3).

Molecular docking studies

In order to understand binding interactions of the synthesized compounds, docking studies of the active compounds (**7d**, **7e**, **7g**, and **7c**) were performed against GSK-3β protein (PDB: 1Q3D). Staurosporine was taken as the reference. As depicted in Table 3, compound **7d** shows the highest glide score of −6.29 in comparison to staurosporine with glide score of −10.01. It forms H-bond with LYS-85 and water residue (Fig. 4). These interactions might account for its observed GSK-3β inhibitory activity *in vitro*.

Conclusion

A series of seventeen piperazine-linked oxazolo[4,5-b]pyridine-based derivatives has been synthesized and screened for *in vitro* GSK-3β inhibitory activity. The compounds **7d**, **7e**, **7g**, and **7c** displayed highest activity among all the synthesized compounds with corresponding IC_{50} values of 0.34, 0.39, 0.47, and 0.53 μM. Out of compounds **7d**, **7e**, **7g**, and **7c** examined for *in vivo* anti-inflammatory activity in rat-paw edema model, compound **7d** exhibited maximum inhibition of 65.91% at 5 h post-carrageenan administration in comparison to indomethacin (79.54%). Furthermore, these compounds (**7d**, **7e**, **7g**, and **7c**) were also found to inhibit the pro-inflammatory mediators, viz., TNF-α, IL-1β, and IL-6 *ex vivo* substantially in comparison to indomethacin, and did not pose any gastric ulceration risk, indicating the potential of this

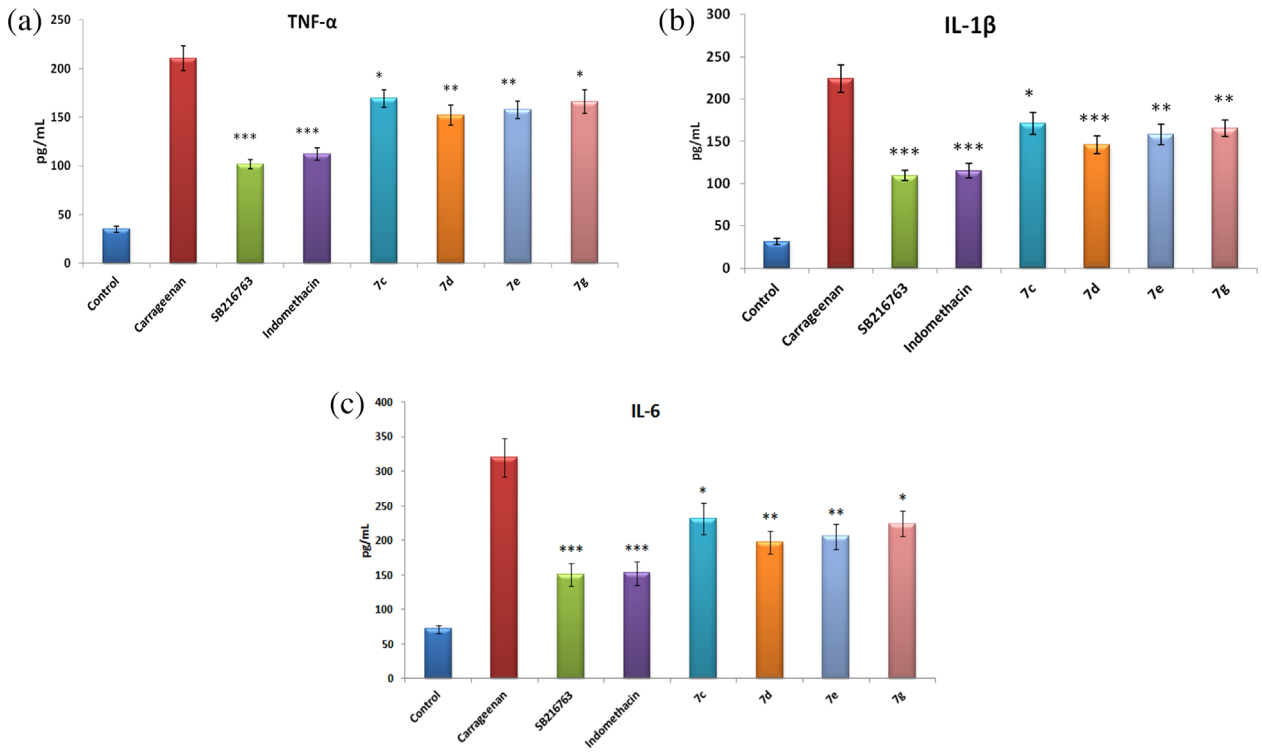


Figure 2. Effects of test compounds, indomethacin and SB216763 on inflammatory mediators was measured in rat paw homogenate. (a) TNF-α, (b) IL-1β, and (c) IL-6. Data are expressed as mean ± SEM of six rats. $^{**}p < 0.01$, $^{***}p < 0.001$ when compared with the carrageenan group.

oxazolopyridine scaffold for the development of GSK-3β inhibitors and their application as anti-inflammatory agents.

Experimental

Chemistry

General

The chemicals and reagents employed in this study were purchased from Sigma–Aldrich, Merck (India), and Spectrochem. Melting points were measured using Electro-thermal IA 9100 apparatus (Shimadzu, Japan) and are uncorrected; IR spectra were recorded on a Bruker spectrophotometer (USA), NMR spectra were determined on a Bruker (300 and 400 MHz) spectrometer in DMSO-d_6 and the chemical shifts are expressed as ppm against TMS as internal reference. Mass spectra were recorded at 70 eV (EI Ms-QP 1000EX, Shimadzu, Japan) or by a Waters UPLC-TQD (ESI-MS). Column chromatography was performed on Merck silica gel 60 (particle size 0.06–0.20 mm). Elemental analysis was carried out using Elemental Vario EL III elemental analyzer and data are reported in % standard. All the compounds synthesized in the current study are novel and were confirmed from spectral data.

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

*Synthesis of 2-mercapto-1,3-oxazolopyridine (**2**)*

A mixture of 1.8 g (32.7 mmol) of KOH, 2.1 mL of CS_2 and 50 mL of dry alcohol was taken in 100 mL R.B. flask and refluxed for 30 min. Then 3 g (27.2 mmol) of 2-amino-3-hydroxy-pyridine (**1**) was added to the reaction mixture and refluxing was continued for 10 h. The progress of reaction was monitored continuously by TLC. The reaction mixture was then concentrated, cooled, poured in to a beaker containing crushed ice and stirred for few minutes and then filtered. A yellow-colored solid was formed which was dried. It was then dissolved in ethyl acetate and filtered to remove insoluble impurities. The ethyl acetate fraction was concentrated to obtain the pure compound.

*Synthesis of 1,3-oxazolopyridine-2-mercapto-acetic acid (**3**)*

A mixture of 2 g (13.07 mmol) of 2-mercapto-1,3-oxazolopyridine (**3**), 2.6 mL (19.6 mmol) of TEA and around 40–50 mL THF was taken in a 100 mL R.B. flask and refluxed for half an hour. Then 2.92 g (19.6 mmol) of bromoacetic acid was added and

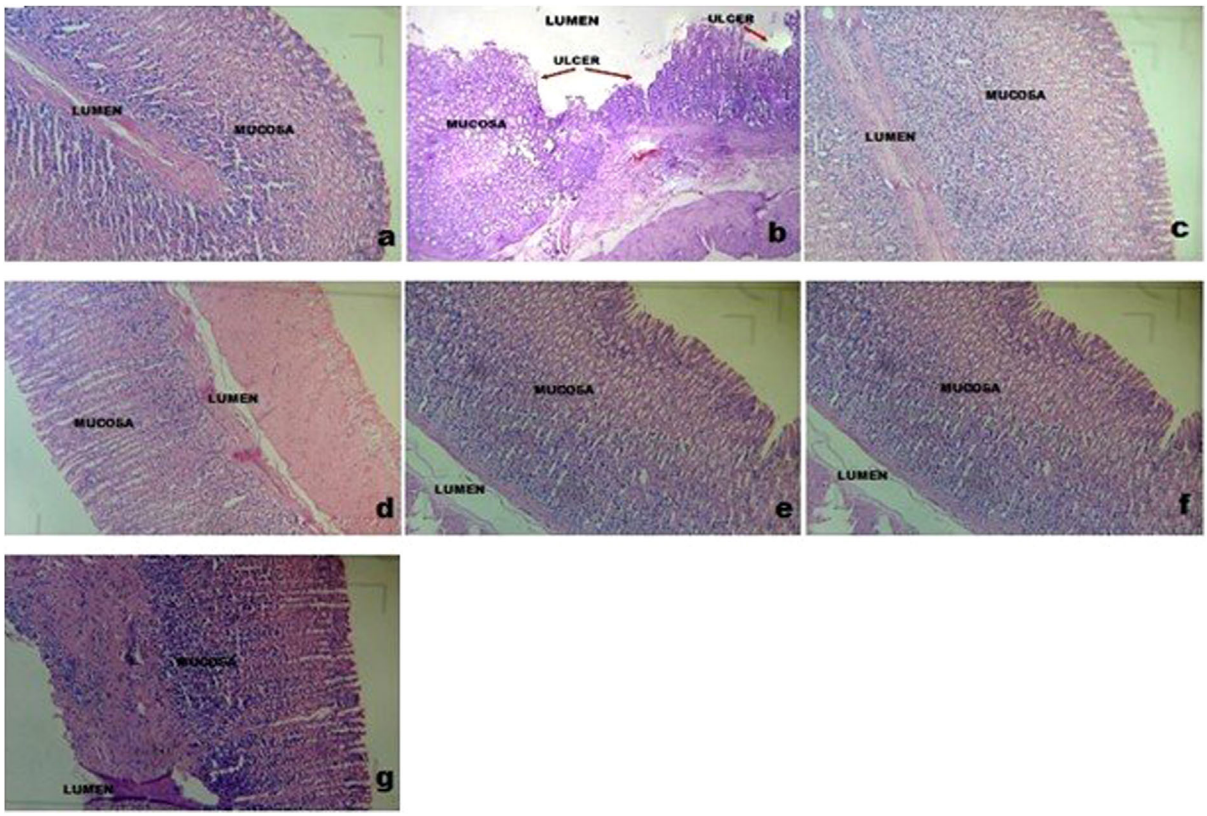


Figure 3. Histopathology report of ulcerogenic risk potential of active compounds. (a) Control, 10×; (b) SB216763, 10×; (c) indomethacin, 10×; (d) **7c**, 10×; (e) **7d**, 10×; (f) **7e**, 10×; (g) **7g**, 10×.

refluxing was continued for 8 h. The progress of the reaction mixture was monitored continuously by TLC. On completion of the reaction, the reaction mixture was concentrated, poured on crushed ice and 2 drops of conc. H_2SO_4 were added, white-colored precipitate was formed which was filtered, dried, and used as such for the next step.

*Synthesis of the N-Boc piperazinamides (**5**)*
Appropriate aryl acids or arylalkyl acids (1 eq) were dissolved in dry DMF. EDC.HCl (1.5 eq) and HOBt (catalytic amount) were added and stirring was continued for 30 min at room temperature followed by addition of *N*-Boc piperazine (**4**) (1.2 eq). The reaction mixture was allowed to stir for 8–10 h with regular monitoring by TLC. After completion of the reaction, the reaction mixture was poured onto crushed ice and the solid obtained was filtered and air dried. The compounds were then purified in ethyl acetate to afford the desired intermediates.

Table 3. ***In silico*** **docking scores with respect to GSK-3β protein target (PDBID: 1Q3D).**

Compound code	Docking score
7d	−6.29
7e	−6.25
7g	−6.24
7c	−6.24
Standard (staurosporine)	−10.01

*Synthesis of the piperazinamides (**6**)*
The *N*-Boc piperazinamides (**5**) were deprotected by adding to it a mixture of TFA and chloroform (2:3) and stirred in a magnetic stirrer for 8 h. On completion of the reaction, the reaction mixture was concentrated to near dryness. The crude residue was packed onto a silica gel column and the desired intermediates (**6**) were eluted with methanol/chloroform (3:7).

*Synthesis of the target compounds **7a–q***
1,3-Oxazolopyridine-2-mercapto-acetic acid (**3**) (1 eq) was dissolved in dry DMF. EDC.HCl (1.5 eq) and HOBt (catalytic amount) were added and stirring was continued for 30 min at room temperature followed by addition of piperazinamides (**6**) (1.2 eq). The reaction mixture was allowed to stir for 8–10 h with regular monitoring by TLC. After completion of the reaction,

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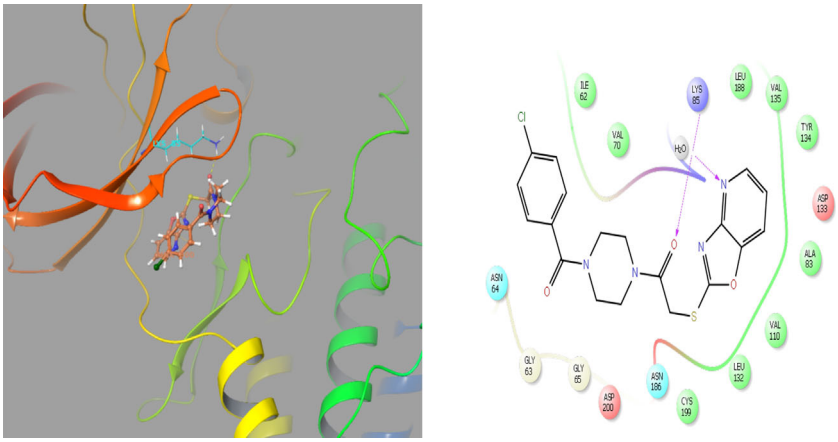

Figure 4. Docking poses and interaction of compound **7d** with respect to the GSK-3β protein.

the reaction mixture was poured onto crushed ice and the solid obtained was filtered and air dried. The compounds were then purified in methanol to afford the target compounds (**7a–q**).

*1-[4-(4-Methoxybenzoyl)piperazin-1-yl]-2-{[1,3]-oxazolo[4,5-b]pyridin-2-ylsulfanyl}ethan-1-one (**7a**)*
Creamish powder; yield: 82%; m.p. 179–180°C; IR (KBr): ν (cm^{-1}) 3418, 3396, 3013, 2850, 1697, 1681, 1600, 1493, 1296, 1247, 1164, 1116, 1010; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.44–3.58 (m, 8H), 3.74 (s, 3H), 4.58 (s, 2H); 6.94 (d, 2H, J = 8.8 Hz), 7.29 (dd, 1H, J = 8.0 and 4.8 Hz), 7.35 (d, 2H, J = 8.4 Hz), 8.02 (dd, 1H, J = 8.0 and 1.2 Hz), 8.37 (dd, 1H, J = 4.8 and 1.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 36.66, 41.71, 45.31, 55.24, 113.65, 118.02, 119.56, 127.44, 129.16, 143.51, 145.80, 155.24, 160.30, 164.77, 168.39, 169.20; MS (ESI) *m/z*: 413 $(M+1)^+$. Anal. calcd. for $C_{20}H_{20}N_4O_4S$: C, 58.24; H, 4.89; N, 13.58; O, 15.52; S, 7.77%. Found: C, 58.29; H, 4.86; N, 13.55; S, 7.78%. The ^{13}C NMR, ^{1}H NMR, and ESI-MS spectra of **7a** are provided as Supporting Information.

*1-[4-(2-Methoxybenzoyl)piperazin-1-yl]-2-{[1,3]-oxazolo [4,5-b]-pyridin-2-ylsulfanyl}ethan-1-one (**7b**)*
Creamish powder; yield: 77%; m.p. 161–163°C; IR (KBr): ν (cm^{-1}) 3430, 3386, 3012, 2848, 1696, 1683, 1598, 1495, 1293, 1251, 1161, 1118, 1010; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.42–3.55 (m, 8H), 3.74 (s, 3H), 4.46 (s, 2H), 7.01–7.07 (m, 2H), 7.21–7.25 (m, 1H), 7.29 (dd, 1H, J = 8.0 and 4.8 Hz), 7.42–7.45 (m, 1H), 7.74–7.76 (m, 1H), 8.32 (dd, 1H, J = 4.8 and 1.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 36.74, 42.26, 45.76, 55.05, 113.71, 118.01, 120.56, 122.59, 126.45, 129.96, 130.73, 143.68, 145.80, 155.34, 160.30, 164.75, 166.59, 168.21; MS (ESI) *m/z*: 413 $(M+1)^+$. Anal. calcd. for $C_{20}H_{20}N_4O_4S$: C, 58.24; H, 4.89; N, 13.58; O, 15.52; S, 7.77%. Found: C, 58.26; H, 4.84; N, 13.56; S, 7.76%.

*1-(4-(4-Nitrobenzoyl)piperazin-1-yl)-2-(oxazolo[4,5-b]-pyridin-2-ylthio)ethanone (**7c**)*
White powder; yield: 83%; m.p. 182–184°C; IR (KBr): ν (cm^{-1}) 3420, 3377, 3017, 2855, 1701, 1688, 1601, 1493, 1294, 1246, 1164, 1130; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.19–3.55 (m, 8H), 4.47 (s, 2H), 7.12 (dd, 1H, J = 8.0 and 5.2 Hz), 7.50 (d, 2H, J = 8.4 Hz), 7.86 (d, 1H, J = 8.0 Hz), 8.07–8.09 (m, 2H), 8.20–8.22 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.03, 42.11, 45.71, 118.49, 120.06, 124.25, 128.88, 142.42, 144.02, 146.30, 148.38, 155.73, 165.34, 167.85, 168.85; MS (ESI) *m/z*: 428 $(M+1)^+$. Anal. calcd. for $C_{19}H_{17}N_5O_5S$: C, 53.39; H, 4.01; N, 16.38; O, 18.72; S, 7.50%. Found: C, 53.35; H, 4.03; N, 16.36; S, 7.48%.

*1-(4-(4-Chlorobenzoyl)piperazin-1-yl)-2-(oxazolo[4,5-b]-pyridin-2-ylthio)ethanone (**7d**)*
White powder; yield: 83%; m.p. 161–162°C; IR (KBr): ν (cm^{-1}) 3410, 3379, 3011, 2844, 1696, 1681, 1592, 1485, 1289, 1244, 1161, 1109; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.35–3.69 (m, 8H), 4.47 (s, 2H), 6.87 (dd, 1H, J = 8.0 and 5.2 Hz), 7.16–7.20 (m, 1H), 7.29–7.31 (m, 1H), 7.38–7.40 (m, 1H), 7.48 (d, 1H, J = 8.4 Hz), 7.75 (d, 1H, J = 8.4 Hz), 7.80 (dd, 1H, J = 5.6 and 1.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.01, 42.24, 45.72, 113.67, 117.65, 122.57, 127.82, 128.56, 135.85, 136.76, 145.83, 148.52, 163.58, 168.38, 169.41; MS (ESI) *m/z*: 417 $(M+1)^+$. Anal. calcd. for $C_{19}H_{17}ClN_4O_3S$: C, 54.74; H, 4.11; Cl, 8.50; N, 13.44; O, 11.51; S, 7.69%. Found: C, 54.71; H, 4.06; N, 13.42; S, 7.68%.

*1-(4-(2-Methylbenzoyl)piperazin-1-yl)-2-(oxazolo[4,5-b]-pyridin-2-ylthio)ethanone (**7e**)*
White powder; yield: 71%; m.p. 141–143°C; IR (KBr): ν (cm^{-1}) 3415, 3391, 3009, 2847, 1694, 1683, 1597, 1491, 1292, 1249, 1157, 1103; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.38 (s, 3H), 3.38–3.59 (m, 8H), 4.58 (s, 2H), 7.27–7.31 (m, 3H), 7.72–7.75 (m, 2H), 7.76 (dd, 1H, J = 5.2 and 1.2 Hz), 8.02 (dd, 1H, J = 8.0 and 1.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.69, 37.05, 41.06, 46.14, 117.65, 122.56, 126.71, 128.60, 129.86, 130.71, 136.92, 138.34, 145.81, 148.52, 160.27, 164. 58, 166.55, 168.24; MS (ESI) *m/z*: 397 $(M+1)^+$. Anal. calcd. for $C_{20}H_{20}N_4O_3S$: C, 60.59; H, 5.08; N, 14.13; O, 12.11; S, 8.09%; S, 7.69%. Found: C, 60.63; H, 5.05; N, 14.15; S, 8.06%.

*1-(4-(3-Methylbenzoyl)piperazin-1-yl)-2-(oxazolo[4,5-b]-pyridin-2-ylthio)ethanone (**7f**)*
White powder; yield: 74%; m.p. 152–154°C; IR (KBr): ν (cm^{-1}) 3440, 3375, 3014, 2865, 1697, 1691, 1593, 1495, 1296, 1247,

1151, 1110; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.37 (s, 3H), 3.32–3.63 (m, 8H), 4.56 (s, 2H), 7.26–7.31 (m, 3H), 7.71–7.74 (m, 2H), 7.76 (dd, 1H, J = 5.2 and 1.2 Hz), 8.03 (dd, 1H, J = 8.0 and 1.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 18.82, 37.01, 42.24, 45.74, 118.07, 122.06, 125.67, 128.60, 129.82, 130.11, 135.89, 138.33, 145.82, 148.46, 160.74, 164.57, 166.91, 168.22; MS (ESI) *m/z*: 397 $(M+)^+$. Anal. calcd. for $C_{20}H_{20}N_4O_3S$: C, 60.59; H, 5.08; N, 14.13; O, 12.11; S, 8.09%; S, 7.69%. Found: C, 60.63; H, 5.10; N, 14.11; S, 8.10%.

*1-(4-(4-Methylbenzoyl)piperazin-1-yl)-2-(oxazolo[4,5-b]-pyridin-2-ylthio)ethanone (**7g**)*
White powder; yield: 74%; m.p. 157–159°C; IR (KBr): ν (cm^{-1}) 3430, 3386, 3015, 2847, 1697, 1681, 1596, 1487, 1288, 1246, 1161, 1105; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.37 (s, 3H), 3.51–3.61 (m, 8H), 4.65 (s, 2H), 7.30–7.33 (m, 3H), 7.75–7.79 (m, 3H), 8.38 (dd, 1H, J = 8.0 and 1.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 18.79, 36.87, 41.04, 46.76, 118.48, 121.97, 126.59, 127.10, 129.63, 131.02, 136.37, 137.98, 145.82, 148.43, 160.84, 163.95, 166.76, 168.17; MS (ESI) *m/z*: 397 $(M+1)^+$. Anal. calcd. for $C_{20}H_{20}N_4O_3S$: C, 60.59; H, 5.08; N, 14.13; O, 12.11; S, 8.09%; S, 7.69%. Found: C, 60.59; H, 5.08; N, 14.13; S, 8.09%.

*1-(4-Benzoylpiperazin-1-yl)-2-(oxazolo[4,5-b]-pyridin-2-ylthio)ethanone (**7h**)*
White powder; yield: 73%; m.p. 145–147°C; IR (KBr): ν (cm^{-1}) 3430, 3386, 3015, 2851, 1696, 1674, 1589, 1499, 1283, 1247, 1154, 1103; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.52–3.61 (m, 8H), 4.65 (s, 2H), 7.32–7.35 (m, 1H), 7.37–7.40 (m, 2H), 7.43–7.49 (m, 1H), 7.82 (dd, 1H, J = 8.0 and 1.2 Hz), 7.86–7.91 (m, 2H), 8.38 (dd, 1H, J = 4.8 and 1.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 36.79, 42.25, 45.83, 116.64, 121.56, 128.17, 129.76, 130.21, 135.82, 144.97, 148.14, 160.13, 163.71, 165.10, 168.36; MS (ESI) *m/z*: 383 $(M+1)^+$. Anal. calcd. for $C_{19}H_{18}N_4O_3S$: C, 59.67; H, 4.74; N, 14.65; O, 12.55; S, 8.38%. Found: C, 59.71; H, 4.72; N, 14.66; S, 8.39%.

*1-(4-(2-Nitrobenzoyl)piperazin-1-yl)-2-(oxazolo[4,5-b]-pyridin-2-ylthio)ethanone (**7i**)*
White powder; yield: 77%; m.p. 135–137°C; IR (KBr): ν (cm^{-1}) 3426, 3391, 3012, 2847, 1693, 1681, 1589, 1497, 1281, 1251, 1147, 1110; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.43–3.55 (m, 8H), 4.64 (s, 2H), 7.26 (dd, 1H, J = 8.0 and 5.2 Hz), 7.76–7.77 (m, 1H), 7.80–7.86 (m, 2H), 8.07–8.09 (m, 1H), 8.25–8.28 (m, 1H), 8.42–8.46 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.02, 42.12, 45.71, 118.49, 120.46, 123.58, 126.37, 131.08, 133.03, 137.20, 144.32, 146.72, 148.56, 155.03, 165.13, 166.71, 169.12; MS (ESI) *m/z*: 428 $(M+1)^+$. Anal. calcd. for $C_{19}H_{17}N_5O_5S$: C, 53.39; H, 4.01; N, 16.38; O, 18.72; S, 7.50%. Found: C, 53.43; H, 4.02; N, 16.36; S, 7.48%.

*1-(4-(4-Hydroxybenzoyl)piperazin-1-yl)-2-(oxazolo[4,5-b]-pyridin-2-ylthio)ethanone (**7j**)*
White powder; yield: 80%; m.p. 165–167°C; IR (KBr): ν (cm^{-1}) 3521, 3430, 3373, 3011, 2847, 1693, 1681, 1586, 1503, 1279, 1246, 1145, 1106; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.27–3.40 (m, 8H), 4.42 (s, 2H), 6.58 (d, 2H, J = 8.8 Hz), 7.06–7.14 (m, 3H), 7.86 (dd, 1H, J = 8.0 and 1.2 Hz), 8.20 (dd, 1H, J = 4.8 and 1.2 Hz), 9.65 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 36.04, 42.26, 45.34, 115.41, 118.50, 120.06, 126.22, 129.83, 144.03, 146.30, 155.74, 159.36, 165.29, 168.87, 170.05; MS (ESI) *m/z*: 399 $(M+1)^+$. Anal. calcd. for $C_{19}H_{18}N_4O_4S$: C, 57.27; H, 4.55; N, 14.06; O, 16.06; S, 8.05%. Found: C, 57.23; H, 4.56; N, 14.04; S, 8.08%.

*1-(4-(4-Ethoxybenzoyl)piperazin-1-yl)-2-(oxazolo[4,5-b]-pyridin-2-ylthio)ethanone (**7k**)*
White powder; yield: 81%; m.p. 163–165°C; IR (KBr): ν (cm^{-1}) 3432, 3393, 3035, 2846, 1697, 1683, 1595, 1493, 1291, 1244, 1147, 1118, 1030; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.11 (t, 3H, J = 6.8 Hz), 3.27–3.41 (m, 8H), 3.84 (q, 2H, J = 6.8 Hz), 4.42 (s, 2H), 6.75 (d, 2H, J = 8.4 Hz), 7.10–7.18 (m, 3H), 7.86 (dd, 1H, J = 8.0 and 1.2 Hz), 8.20 (dd, 1H, J = 4.8 and 1.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.02, 37.06, 42.23, 45.81, 63.71, 114.57, 118.48, 120.04, 127.80, 129.65, 144.02, 146.29, 155.75, 160.11, 165.29, 168.86, 169.74; MS (ESI) *m/z*: 427 $(M+1)^+$. Anal. calcd. for $C_{21}H_{22}N_4O_4S$: C, 59.14; H, 5.20; N, 13.14; O, 15.01; S, 7.52%. Found: C, 59.11; H, 5.17; N, 13.15; S, 7.52%.

*1-(4-(4-Bromobenzoyl)piperazin-1-yl)-2-(oxazolo[4,5-b]-pyridin-2-ylthio)ethanone (**7l**)*
White powder; yield: 72%; m.p. 160–162°C; IR (KBr): ν (cm^{-1}) 3445, 3391, 3030, 2847, 1699, 1681, 1593, 1503, 1291, 1247, 1139, 1119; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.58–3.67 (m, 8H), 4.66 (s, 2H), 7.33–7.37 (m, 1H), 7.48–7.55 (m, 4H), 8.08 (d, 1H, J = 7.8 Hz), 8.43–8.45 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.08, 42.10, 45.73, 115.51, 118.47, 120.04, 129.01, 129.54, 134.86, 144.02, 146.28, 155.74, 165.30, 168.72, 168.86; MS (ESI) *m/z*: 461 $(M+1)^+$. Anal. calcd. for $C_{19}H_{17}BrN_4O_3S$: C, 49.47; H, 3.71; Br, 17.32; N, 12.14; O, 10.40; S, 6.95%. Found: C, 49.43; H, 3.72; N, 12.15; S, 6.96%.

*2-(4-Chlorophenyl)-1-(4-(2-(oxazolo[4,5-b]-pyridin-2-ylthio)acetyl)piperazin-1-yl)ethanone (**7m**)*
White powder; yield: 76%; m.p. 138–139°C; IR (KBr): ν (cm^{-1}) 3435, 3390, 3029, 2833, 1697, 1683, 1593, 1496, 1281, 1246, 1151, 1118; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.49–3.58 (m, 8H), 3.72 (m, 2H), 4.65 (s, 2H), 7.27–7.31 (m, 1H), 7.52–7.57 (m, 4H), 8.03 (d, 1H, J = 8.0 Hz), 8.21–8.24 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.07, 42.21, 43.65, 45.76, 117.58, 121.93, 129.61, 130.15, 133.90, 135.34, 144.64, 146.71, 159.45, 163.14, 168.27, 169.12; MS (ESI) *m/z*: 431 $(M+1)^+$. Anal. calcd. for $C_{20}H_{19}ClN_4O_3S$: C, 55.75; H, 4.44; Cl, 8.23; N, 13.00; O, 11.14; S, 7.44%. Found: C, 55.78; H, 4.45; N, 13.01; S, 7.43%.

*2-(4-Methoxyphenyl)-1-(4-(2-(oxazolo[4,5-b]-pyridin-2-ylthio)acetyl)piperazin-1-yl)ethanone (**7n**)*
White powder; yield: 76%; m.p. 182–184°C; IR (KBr): ν (cm^{-1}) 3433, 3373, 3040, 2841, 1693, 1681, 1595, 1503, 1291, 1253, 1146, 1119, 1037; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.46–3.62 (m, 8H), 3.73 (s, 2H), 3.76 (s, 3H), 4.64 (s, 2H), 7.03 (d, 2H, J = 8.4 Hz), 7.32 (dd, 1H, J = 8.0 and 4.8 Hz), 7.36 (d, 2H,

$J = 8.4$ Hz), 8.04 (dd, 1H, $J = 8.0$ and 1.2 Hz), 8.32 (dd, 1H, $J = 4.8$ and 1.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.07, 42.21, 43.65, 45.76, 55.81, 113.79, 118.22, 122.43, 127.64, 129.96, 143.78, 145.89, 155.65, 160.13, 163.79, 168.25, 169.36; MS (ESI) *m/z*: 427 $(M+1)^+$. Anal. calcd. for $C_{21}H_{22}N_4O_4S$: C, 59.14; H, 5.20; N, 13.14; O, 15.01; S, 7.52%. Found: C, 59.17; H, 5.21; N, 13.13; S, 7.51%.

*2-(4-Nitrophenyl)-1-(4-(2-(oxazolo[4,5-b]-pyridin-2-ylthio) acetyl)piperazin-1-yl)ethanone (**7o**)*
White powder; yield: 75%; m.p. 178–180°C; IR (KBr): ν (cm^{-1}) 3434, 3391, 3018, 2846, 1693, 1681, 1593, 1493, 1301, 1248, 1145, 1122; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.54–3.63 (m, 8H), 3.75 (s, 2H), 4.58 (s, 2H), 7.13 (dd, 1H, $J = 8.0$ and 5.2 Hz), 7.47 (d, 2H, $J = 8.4$ Hz), 7.80 (d, 1H, $J = 8.0$ Hz), 8.02–8.05 (m, 2H), 8.21–8.24 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.11, 42.31, 43.56, 45.69, 118.54, 120.35, 124.71, 129.02, 141.86, 144.32, 146.38, 148.17, 155.76, 164.82, 168.57, 169.64; MS (ESI) *m/z*: 442 $(M+1)^+$. Anal. calcd. for $C_{20}H_{19}N_5O_5S$: C, 54.41; H, 4.34; N, 15.86; O, 18.12; S, 7.26%. Found: C, 54.38; H, 4.32; N, 15.86; S, 7.23%.

*2-(Oxazolo[4,5-b]pyridin-2-ylthio)-1-(4-(2-(o-tolyl)acetyl)-piperazin-1-yl)ethanone (**7p**)*
White powder; yield: 75%; m.p. 153–155°C; IR (KBr): ν (cm^{-1}) 3441, 3392, 3018, 2845, 1693, 1669, 1593, 1493, 1279, 1239, 1151, 1123; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.21 (s, 3H), 3.52–3.62 (m, 8H), 3.73 (s, 2H), 4.65 (s, 2H), 7.10–7.15 (m, 4H), 7.35 (dd, 1H, $J = 8.1$ and 4.8 Hz), 8.08 (dd, 1H, $J = 8.1$ and 0.9 Hz), 8.44 (dd, 1H, $J = 4.8$ and 1.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 36.05, 117.06, 118.36, 121.52, 122.25, 126.68, 131.63, 137.81, 141.27, 143.32, 147.15, 153.26; MS (ESI) *m/z*: 411 $(M+1)^+$. Anal. calcd. for $C_{21}H_{22}N_4O_3S$: C, 61.44; H, 5.40; N, 13.65; O, 11.69; S, 7.81%. Found: C, 61.47; H, 5.38; N, 13.66; S, 7.80%.

*2-(Oxazolo[4,5-b]pyridin-2-ylthio)-1-(4-(2-phenylacetyl)-piperazin-1-yl)ethanone (**7q**)*
Creamish white powder; yield: 77%; m.p. 133–135°C; IR (KBr): ν (cm^{-1}) 3430, 3386, 3015, 2851, 1696, 1674, 1589, 1499, 1283, 1247, 1154, 1103; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.49–3.57 (m, 8H), 3.74 (s, 2H), 4.67 (s, 2H), 7.08–7.11 (m, 1H), 7.22–7.29 (m, 3H), 7.37–7.40 (m, 2H), 7.80 (dd, 1H, $J = 8.1$ and 0.9 Hz), 8.36 (dd, 1H, $J = 4.8$ and 1.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.15, 42.47, 43.32, 46.24, 118.12, 120.97, 127.67, 128.45, 129.83, 135.62, 144.74, 147.97, 159.23, 163.52, 165.61, 168.49; MS (ESI) *m/z*: 397 $(M+1)^+$. Anal. calcd. for $C_{20}H_{20}N_4O_3S$: C, 60.59; H, 5.08; N, 14.13; O, 12.11; S, 8.09%. Found: C, 60.65; H, 5.04; N, 14.12; S, 8.10%.

Pharmacology

Chemicals

Human recombinant GSK-3β and pre-phosphorylated polypeptide substrate glycogen synthase-2 (GS-2) were purchased from Merck-Millipore Corporation (India). Kinase-Glo Luminescent Kinase Assay (catalog no. V6713) was obtained from Promega Corporation (Madison, WI). Indomethacin, carrageenan, carboxymethylcellulose, SB216763, and staurosporine were purchased from Sigma–Aldrich Chemicals Pvt. Limited, Bangalore, India.

GSK-3β inhibitory activity

The compound library was assessed for their GSK-3β inhibition as per the Kinase-Glo assay method of Baki et al. [31]. This assay determines the activity of the kinase by quantifying the amount of ATP which remains in solution following a kinase reaction. In a typical assay, 10 μL of test compound of different concentrations (dissolved in dimethyl sulfoxide (DMSO) and diluted with assay buffer) and 10 μL (20 ng) of enzyme were added to each well followed by 20 μL of assay buffer containing substrate and ATP to obtain a concentration of 25 μM substrate and 1 μM ATP per well. The final DMSO concentration in the reaction mixture was kept less than 1%. After incubation at 30°C for 30 min, the enzymatic reaction was quenched with 40 μL of Kinase-Glo reagent. Luminescence was recorded after 10 min using an Infinite F200® PRO multimode reader (Tecan). The activity is proportional to the difference of the total and consumed ATP. The activity was maximum in the absence of inhibitor and was used to calculate the inhibitory activities of test compounds.

Animals

Wistar rats of either sex (150–200 g) were acquired from Central Animal House, Hamdard University, New Delhi. Rats were kept in the Central Animal House of Hamdard University in colony cages at an ambient temperature of 25 ± 2°C and relative humidity 45–55% with 12 h light/dark cycles after initial acclimatization for about 1 week. They were provided free access to standard rodent pellet diet and water *ad libitum*. Fourteen hours preceding the start of the experiment, the animals were sent to lab and fed only with water *ad libitum*. The experimental study was conducted in accordance with guidelines of the Institutional Animal Ethics Committee of the University, Jamia Hamdard (Hamdard University), New Delhi, India.

Anti-inflammatory activity

The synthesized compounds were evaluated for their *in vivo* anti-inflammatory activity using carrageenan-induced hind paw edema method. The rat paw edema was induced by subplantar injection of 0.1 mL of 1% freshly prepared carrageenan suspension in normal saline into the right hind paw of rats. The standard anti-inflammatory drug, indomethacin (0.05 mmol/kg) was taken as positive control while as negative control was given as equivalent volume of 0.9% saline solution. GSK-3β inhibitor SB216763 (0.05 mmol/kg) was also administered to one group orally. The test groups were administered synthesized compounds at a dosage equimolar to the positive control. The positive control, negative control, SB216763, and test compounds were administered orally to respective groups 1 h preceding the carrageenan treatment. The paw volumes were measured in

two time points at an interval of 3 and 5 h post-carrageenan treatment by using plethysmometer [34].

Effects of active compounds on production of carrageenan-induced pro-inflammatory mediators

Wistar albino rats weighing 150–200 g were used. Rats were fasted for 18 h before i.p. dosing with the test compound. The standard drug indomethacin (10 μM) and GSK-3β inhibitor SB216763 (10 μM) were given orally as a positive control. The control group was administered orally with 0.9% of 0.1 mL of saline solution only. The test groups were administered orally with the synthesized compounds at equimolar dosage of the standard drug, 2 h before an intra-planter injection of carrageenan into rat paw. Following dosing of drugs, rats were injected with 0.2 mL of 1% freshly prepared carrageenan in normal saline. Five hours later, the blood was collected from the tail vein. Blood was centrifuged for 10 min at 3000 rpm to isolate the serum. Serum levels of TNF-α, IL-1β, IL-6, and COX-2 in control and experimental rats were measured by ELISA using a commercially available diagnostic kit (Invitrogen, Carlsbad, CA). The results were expressed as pg/mL. COX-2 levels were also measured by ELISA. The results were expressed as IU/dL.

Ulcerogenic activity

The compounds exhibiting potent anti-inflammatory activity were studied further for their gastric ulcerogenic risk [35]. This was performed at threefold higher dose as compared to that used for anti-inflammatory activity. Each group consisted of three animals which were later sacrificed 5 h after oral drug administration.

Docking studies

The *in silico* docking studies were carried out along with *in vitro* studies, using Schrodinger Software (Maestro Ver 10). The ligands were virtually prepared by Lig-Prep module (Ver 3.3). The protein was prepared by Protein Preparation Wizard through Maestro Interface (Ver 10). The binding pocket was generated by selecting a grid with respect to reference ligand staurosporine. The docking was carried using Glide Module (Ver 6.6) with Extra Precision (XP) mode. The docked molecules were ranked with respect to the Glide score.

Statistical analysis

Results are expressed as the mean ± SEM, and different groups were compared using one-way analysis of variance (ANOVA) followed by Tukey-Kramer test for multiple comparisons.

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