Synthesis and biological evaluation of 2-(4-methylsulfonyl phenyl) indole derivatives: Multi-target compounds with dual antimicrobial and anti-inflammatory activities

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Abstract
Three series of 2-(4-methylsulfonylphenyl) indole derivatives have been designed and synthesized.
The synthesized compounds were evaluated for their antimicrobial, COX inhibitory and anti-inflammatory activities. Compound 7g was identified to be the most potent antibacterial candidate against strains of MRSA, E. coli, K. pneumoniae, P. aeruginosa, and A. baumannii, respectively with safe therapeutic dose. Compounds 7a-k, 8a-c and 9a-c showed good anti-inflammatory activity with high selectivity toward COX-2 in comparison with reference drugs indomethacin and celecoxib. Compounds 9a-c were found to release moderate amounts of NO to decrease the side effects associated with selective COX-2 inhibitors. A molecular modeling study for compounds 7b, 7h, and 7i into COX-2 active site correlated with results of in vitro COX-2 inhibition assays.

1. Introduction
Bacterial resistance reached a threatening level due to the misuse of antibiotics thus searching for new antimicrobial agents is a very important issue [1]. Furthermore, administration of multiple drugs to relieve inflammation associated with a bacterial infection may have some secondary health problems and may increase adverse effects [2]. Unfortunately, there are few drugs possessed these two activities in a single compound. Therefore, there are continuous trails to develop a monotherapy against inflammation due to microbial infection (dual antimicrobial / anti-inflammatory agent) with minimal adverse effects and high safety margin [3].
The nonsteroidal anti-inflammatory drugs (NSAIDs) used as the main treatment for pain, fever, and inflammation through inhibition of cyclooxygenase (COX) enzymes [4–6]. Selective COX–2 inhibitor drugs like valdecoxib I, celecoxib II and rofecoxib III relief inflammation without any gastric side effects due to selective inhibition of inducible COX-2 isozyme [7] (Fig. 1). Despite of less gastric irritation of selective COX–2 inhibitors, they showed some cardiovascular problems such as myocardial infarction and high blood pressure [8, 9], leading to the withdrawal of both rofecoxib and valdecoxib from market [10]. The explanation for cardiovascular problems may due to inhibition of vasodilatory prostacyclin (PGI₂) and an increase in the level of platelet activator thromboxane A₂ (TxA₂) [11].
Nitric oxide (NO) showed vasodilator activity and inhibition of platelets aggregation [12]. Accordingly, attachment of NO donor moiety to selective COX-2 inhibitors may be beneficial to overcome the cardiovascular side effects [13, 14].

[Insert Figure 1 about here]

A lot of biologically aryl hydrazone derivatives with antimicrobial activity [15–17] such as nitrofurantoin IV [18, 19]. Additionally, indole-based indomethacin V is a potent NSAIDs used for the treatment of inflammatory diseases such as rheumatoid arthritis and osteoarthritis [20] but due to high selectivity for COX-1 inhibition and its acidic nature, it had a clear ulcerogenic effect [21].

Herein, we aimed to make molecular hybridization of the indole part of indomethacin with p-methylsulfonyl phenyl part of selective COX-2 inhibitors to fit the general structure of coxibs [presence of a diaryl heterocycle bearing one sulfonamide (SO₂NH₂) or methylsulfonyl (SO₂CH₃) group] [22] and keep in mind presence of different substitutions at position 3 in indole with the hope to get compounds with dual antimicrobial / anti-inflammatory activity (Fig. 2).

[Insert Figure 2 about here]

2. Results And Discussion

Chemistry

The compounds were synthesized through a series of reactions illustrated in scheme 1, 2. The reaction of p-methylsulfonyl acetophenone (3) with 4-un/substituted phenylhydrazine HCl under Fischer indole synthesis conditions yielded indole derivatives (5a-c) that are converted to indole-3-carbaldehyde derivatives (6a-c) by Vilsmeir Haack's formylation reaction using POCl₃ and DMF (Scheme 1).

IR spectra for compounds 6a-c showed significant bands at 3205-3320 cm⁻¹ of indole NH, 1657-1670 cm⁻¹ of C=O and 1150, 1300 cm⁻¹ of SO₂. ¹H NMR spectra showed a signal at δ 10.00 - 10.04 ppm of aldehydic proton (H-C=O), 3.17-3.21 ppm of SO₂CH₃ and 12.92-12.62 ppm of indole NH which is D₂O exchangable.

[Insert Scheme 1 about here]

Indole-3-carbaldehyde derivatives (6a-c) were reacted with 4-substituted phenylhydrazine HCl to give
hydrazone derivatives \((7a-k)\) in good yield. The structure elucidation of hydrazone derivatives \((7a-k)\) was based on IR, \(^1\)H NMR, and \(^{13}\)C NMR spectral data. IR spectra showed bands at 1593-1597 cm\(^{-1}\) for C=N and disappearance of carbonyl absorption band at 1657-1670 cm\(^{-1}\) which confirm hydrazone formation. \(^1\)H NMR spectra showed a signal at \(\delta\) 8.24-8.36 ppm of hydrazone proton (H-C=N), 10.03-10.73 ppm of hydrazone NH which is D\(_2\)O exchangeable, 12.00 ppm for NH indole which is D\(_2\)O exchangeable and disappearance of aldehydic proton at \(\delta\) 10.00 – 10.04 ppm which confirm hydrazone formation. \(^{13}\)C NMR spectra showed a peak at 143-149 ppm of hydrazone carbon (C=N) which confirm hydrazone formation.

On the other hand, benzimidazole derivatives \((8a-c)\) are synthesized from the reaction of Indole-3-carbaldehyde derivatives \((6a-c)\) with 4-chloro-o-phenylenediamine in presence of sodium metabisulphite. IR spectra showed bands at 3272-3382 cm\(^{-1}\) (indole NH, benzimidazole NH) and disappearance of carbonyl absorption band at 1657-1670 cm\(^{-1}\). \(^1\)H NMR spectra showed the disappearance of aldehydic proton at \(\delta\) 10.00-10.04 ppm and presence of a signal at \(\delta\) (12.37-12.45) ppm of benzimidazole NH (D\(_2\)O exchangeable) in addition to a signal at \(\delta\) 12.04-12.18 ppm of indole NH (D\(_2\)O exchangeable).

Oxime derivatives \((9a-c)\) resulted from reflux of reaction of Indole-3-carbaldehyde derivatives \((6a-c)\) with hydroxylamine HCl. IR spectra lacked the carbonyl absorption band at 1657-1670 cm\(^{-1}\) and showed absorption bands at 3272-3382 cm\(^{-1}\) (NH, OH) and 1597 cm\(^{-1}\) (C=N). \(^1\)H NMR spectra showed singlet signal at \(\delta\) 8.32 ppm of azomethine proton H-C=N, 10.89 ppm of OH (D\(_2\)O exchangeable) in beside to signal at \(\delta\) 11.79-12.04 ppm of indole NH (D\(_2\)O exchangeable) and disappearance of aldehydic proton at \(\delta\) 10.00-10.04 ppm which confirm oxime formation.

[Insert Scheme 2 about here]

**Biological evaluation**

**Antimicrobial screening**

The antimicrobial study was performed by CO-ADD (The Community for Antimicrobial Drug Discovery), funded by the Wellcome Trust (UK) and The University of Queensland (Australia).
Evaluation of all synthesized compounds for their antimicrobial activity against five pathogenic bacteria, *methicillin-resistant Staphylococcus aureus* (ATCC 43300) as Gram-positive bacteria, *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 700603), *Acinetobacter baumannii* (ATCC 19606) and *Pseudomonas aeruginosa* (ATCC 27853) as Gram-negative bacteria and antifungal activity against two pathogenic fungal strains *Candida albicans* (ATCC 90028) and *Cryptococcus neoformans var. grubii* (H99; ATCC 208821) (Table 1).

[Insert Table 1 about here]

Results revealed that hydrazone derivatives 7c, 7e, 7f, 7h, and 7j have moderate antibacterial activity against Gram-negative *A. baumannii* with growth inhibition 43.29, 43.64, 66.69, 51.82 and 46.23 %, respectively while the hydrazone derivatives 7a, 7g, and 7i have high antibacterial activity against MRSA bacteria and *E. coli, K. pneumoniae, P. aeruginosa,* and *A. baumannii* with growth inhibition ranged from 85.76 to 97.76%.

Additionally the oxime derivatives 9a showed moderate antibacterial activity against Gram-negative *A. baumannii* with growth inhibition 42.1% while benzimidazole derivatives (8a-c) showed weak antibacterial activity.

On the other hand, all compounds have weak antifungal activity against *C. albicans* and *C. neoformans var. grubii*.

The Minimal inhibitory concentrations (MIC µg/mL) measurements were performed for compounds with significant microbial growth inhibition (7a, 7g and 7i) using ceftriaxone and amphotericin B as a reference drugs for antibacterial and antifungal activity, respectively.

As shown in Table 2, compounds 7a, 7g and 7i have best antibacterial activity comparable to that of ceftriaxone against MRSA, *E. coli, K. pneumoniae, P. aeruginosa* and *A. baumannii,* respectively.

The safety margin for the active compounds toward human cells was determined through cytotoxicity against human embryonic kidney cell line and hemolysis of human red blood cells. The tested compounds 7a, 7g and 7i were tolerated and non-toxic for human cells as the cytotoxic and hemolytic dose was higher than the therapeutic dose (Table 2).

Compound 7a showed a high therapeutic index as the largest therapeutic dose (8 µg/mL against *A.
baumannii) was highly lower than cytotoxic and hemolytic dose (>32, >32 µg/mL respectively). Also, compound 7g showed a good therapeutic index for all therapeutic doses against all tested microbes except for A. baumannii (4 µg/mL) which is near to cytotoxic concentration (4.2 µg/mL). Otherwise, compound 7i showed good therapeutic index for all therapeutic doses against all tested microbes except for A. baumannii (4 µg/mL) which is higher than cytotoxic concentration (2.987 µg/mL).

[Insert Table 2 about here]

In vitro cyclooxygenase (COX) inhibition assay
The in vitro assay evaluated the ability of compounds 7a-k, 8a-c, and 9a-c to inhibit Ovine COX-1 and human recombinant COX-2. All tested compounds have weak COX-1 inhibition activity (IC$_{50}$ = 9.14 - 13.2 µM) in comparison with indomethacin (IC$_{50}$ = 0.039 µM). They also exerted potent COX-2 inhibitory activity (IC$_{50}$ = 0.1 - 0.31 µM) with high COX-2 selectivity (SI = 132 - 31.29) in comparison with reference drugs, indomethacin and celecoxib.

Hydrazone derivatives 7a-k showed potent COX-2 inhibitory activity (IC$_{50}$ = 0.10 - 0.31 µM) with high selectivity (SI = 132 - 31.29) more than another compounds. Likewise, benzimidazole 8a-c and oxime derivatives 9a-c showed good COX-2 inhibitory activity (IC$_{50}$ = 0.13 - 0.35 µM) in comparison with reference drugs.

Generally, all tested compounds were more selective toward the COX-2 enzyme (SI = 31.29 - 132) than indomethacin (SI = 0.079) (Table 3) due to the size of synthesized compounds was too large to fit into the small COX-1 active site in addition to the presence of diaryl structure bearing SO$_2$CH$_3$ or SO$_2$NH$_2$ group.

[Insert Table 3 about here]

In vivo anti-inflammatory activity
The results listed in (Table 4) showed that compounds 7a-k, 8a-c, and 9a-c offered good anti-inflammatory activity (56.4 - 93.5% reduction of inflammation) after 6 h. in comparison with celecoxib and indomethacin (94.7, 96.6 % reduction of inflammation, respectively) after 6 h.

Hydrazone derivatives (7a-k) showed good anti-inflammatory activity (66.3 - 93.5% reduction of inflammation) after 6 h., Compounds that contained two SO$_2$CH$_3$ group or one SO$_2$CH$_3$ and one
SO$_2$NH$_2$ group (7b, 7c, 7d, 7e, 7h, and 7i) showed a reduction of inflammation by 93.5, 82.5, 78.6, 79.9, 92.7 and 90.1% after 6 h, respectively more than other derivatives. Also, benzimidazole and oxime derivatives (8a-c, 9a-c) showed good inhibition of inflammation ranged from 56.4 - 76.2 % % after 6 h. Compounds 7b, 7c, 7h and 7i that showed the highest COX-2 inhibitory activity (IC$_{50}$=0.1, 0.11, 0.11 and 0.1 respectively) with high selectivity (S.I. = 124.2, 103.7, 112.7 and 132 respectively) were found to have excellent anti-inflammatory activity (edema inhibition= 93.5, 82.5, 92.7 and 90.1%, respectively) after 6 h.

[Insert Table 4 about here]

**In vitro nitric oxide release**
The NO-releasing properties of compounds 9a-c were assessed in phosphate buffer of pH 7.4 with Griess reagent [23]. As shown in Table 5, compounds 9a-c were found to release moderate amounts of NO compared to the sodium nitrite standard solution. Therefore, insertion of nitric oxide releasing group (oxime) can offer a method to decrease the cardiovascular side effects of selective COX-2 inhibitors.

[Insert Table 5 about here]

**Structure-activity relationship**
Interpretation of the antimicrobial results revealed that introduction of arylhydrazone derivatives 7a-k at position 3 of indole, can possess antimicrobial activity against strains of Gram-positive MRSA bacteria and Gram-negative *E. coli, K. pneumoniae, P. aeruginosa,* and *A. baumannii* in addition to their COX-2 inhibitory activity.

Concerning the anti-inflammatory activity, replacement of methyl group in position 2 in indomethacin by *p*-methylsulfonyl phenyl moiety in all synthesized compounds, increased the anti-inflammatory activity and selectivity toward COX-2 receptor through increasing the interaction with hydrophobic residue of COX-2 active site as the resulted compounds was large to bind to COX-1 active site [24]. Also, presence of two SO$_2$CH$_3$ group or one SO$_2$CH$_3$ and one SO$_2$NH$_2$ group (7b, 7c, 7d, 7e, 7h, and 7i) have selective COX-2 inhibitory activity more than other derivatives.
Omission of acidic center (CH$_2$COOH) moiety in position 3 in indomethacin and replaced by benzimidazole moiety 8a-c, as a rigid isostere of p-chlorobenzoyl moiety of indomethacin, showed selective COX-2 inhibitory activity. The introduction of oxime group 9a-c at position 3 of indole, showed release of nitric oxide and have COX-2 inhibitory activity.

**Molecular modeling**

To understand the nature of the interaction of the most active synthesized compounds and COX-2 active site, a molecular docking study was performed using crystal structure data for COX-2 (PDB: ID 3LN1) active site obtained from protein data bank [25]. Molecular modeling of compounds 7h, 7i, 7b and co-crystalized ligand, celecoxib using MOE 2018.0101 modeling software. The docking results of compounds 7h, 7i, 7b, and celecoxib were presented in [Table 6](#). Hydrazine derivatives 7b, 7h and 7i were fully fitted within COX-2 active site with high affinity (-17.19, -16.71 and -16.42 Kcal/mol, respectively) in comparison with celecoxib (-14.12 kcal/mol). Compounds 7b, 7h and 7i contained one SO$_2$CH$_3$ and one SO$_2$NH$_2$ group or two SO$_2$CH$_3$ groups that formed hydrogen bonds with different amino acids (Leu338, Arg499, Ser339, Val335, Arg106 and His75). Also, the indole ring of compound 7h and 7i offered hydrophobic interaction with Val509 ([Fig. 3, 4](#)). Thus, the molecular docking results ensure that compounds 7b, 7h and 7i bind to COX-2 active site with the same manner of celecoxib.

[Insert Table 6 about here]

[Insert Figures 3, 4 about here]

3. Conclusion

A three series of 2-(4-methylsulfonylphenyl) indole derivatives 7a-k, 8a-c and 9a-c were evaluated for their antimicrobial and anti-inflammatory activities.

Compounds 7a, 7g and 7i show antimicrobial activity against strains of MRSA bacteria and many species of Gram-negative with growth inhibition ranged from 85.76% to 97.76%.

Furthermore, compounds 7a-k, 8a-c and 9a-c showed potent and selective COX–2 inhibitory activity (IC$_{50}$ = 0.1—0.31 µM, SI = 132—31.29) more than indomethacin in comparison with reference drugs; Hydrazine derivatives 7a, 7g and 7i exerted dual antimicrobial/anti-inflammatory activity. Oxime
derivatives 9a-c showed selective COX-2 inhibitory activity and moderate *in vitro* nitric oxide release which can offer valuable drug design to decrease the cardiovascular problems.

The molecular modeling study ensured *in vitro* COX-2 inhibition assay results. Compounds 7b, 7h, and 7i fitted to a COX-2 enzyme with three to five hydrogen bonding interactions in a similar manner to celecoxib.

4. Experimental Chemistry

A Thomas-Hoover capillary apparatus used to determine melting points. Infrared (IR) spectra were recorded as films on KBr plates using FT-IR spectrometer.

Thin-layer chromatography (Merck, Darmstadt, Germany) was used for monitoring the reaction mixture, purity, and homogeneity of the synthesized compounds. UV was used as the visualizing agent.

$^1$H NMR and $^{13}$C NMR spectra were measured on a Bruker Avance III 400 MHz for $^1$H NMR and 100 MHz for $^{13}$C NMR (Bruker AG, Switzerland) with BBFO Smart Probe and Bruker 400 AEON Nitrogen-Free Magnet, Faculty of Pharmacy, Beni-Suef University, Egypt in DMSO-$d_6$ with TMS as the internal standard, where $J$ (coupling constant) values are estimated in Hertz (Hz) and chemical shifts were recorded in ppm on $\delta$ scale.

Microanalyses for C, H, and N were carried out on Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA) at the Microanalytical unit of Al Azhar University, Egypt and all compounds were within $\pm$ 0.5% of the theoretical values.

$p$-Methylthioacetophenone (2) and $p$-methylsulfonyl acetophenone (3) and 5-Un/substituted-2-(4-(methylsulfonyl)phenyl)-1$H$-indole (5a-c) were prepared according to a previous procedure [13]. The compounds were confirmed by matching their physical properties with the reported ones.

**4.1.1. General procedure for synthesis of 5-substituted-2-(4-(methylsulfonyl)phenyl)-1$H$-indole-3-carbaldehyde 6a-c**

A mixture of phosphorous oxychloride POCI$_3$ (1.53 g, 10 mmol) and DMF (0.73 g, 10 mmol) was stirred for 30 minutes at room temperature, the solution of respective indole (1 mmol) in DMF (5 mL) was
added slowly to the mixture which allowed to stir overnight. The reaction mixture was poured into ice-cold water and neutralized with 40% NaOH. The separated solid was filtered, dried and recrystallized from ethyl alcohol (yield: 70-80%).

4.1.1.1. 2-(4-(Methylsulfonyl)phenyl)-1H-indole-3-carbaldehyde (6a)

Yellow solid; Yield 70%; mp 232-235 °C; IR (KBr) 3205 (NH), 3065-3042 (CH aromatic), 2929-2871 (CH aliphatic), 1657 (C=O), 1305, 1150 (SO₂) cm⁻¹; ¹H NMR (DMSO-d₆) δ (ppm): 3.21 (s, 3H, SO₂CH₃), 7.27-7.36 (m, 2H, indole H-5, H-6), 7.57 (d, 1H, J = 8 Hz, indole H-7), 8.08 (d, 2H, J = 8.4 Hz, phenyl H-2, H-6), 8.15 (d, 2H, J = 8.4 Hz, phenyl H-3, H-5), 8.26 (d, 1H, J = 7.6 Hz, indole H-4), 10.04 (s, 1H, aldehydic H), 12.64 (s, 1H, indole NH, D₂O exchangeable). Anal.Calcd for C₁₆H₁₃NO₃S: C, 64.20; H, 4.38; N, 4.68. Found: C, 64.48; H, 4.40; N, 4.84.

4.1.1.2. 5-Methyl-2-(4-(methylsulfonyl)phenyl)-1H-indole-3-carbaldehyde (6b)

Brown solid; Yield 80%; mp 244-246 °C; IR (KBr) 3279 (NH), 3059-3029 (CH aromatic), 2927-2856 (CH aliphatic), 1670 (C=O), 1301, 1148 (SO₂) cm⁻¹; ¹H NMR (DMSO-d₆) δ (ppm): 2.45 (s, 3H, CH₃), 3.17 (s, 3H, SO₂CH₃), 7.17 (d, 1H, J = 8 Hz, indole H-6), 7.46 (d, 1H, J = 8 Hz, indole H-7), 8.06-8.14 (m, 5H, indole H-4, phenyl H-2, H-3, H-5, H-6), 10.00 (s, 1H, aldehydic H), 12.62 (s, 1H, indole NH, D₂O exchangeable). Anal.Calcd for C₁₇H₁₅NO₃S: C, 65.16; H, 4.82; N, 4.47. Found: C, 65.27; H, 4.68; N, 4.52.

4.1.1.3. 5-Fluoro-2-(4-(methylsulfonyl)phenyl)-1H-indole-3-carbaldehyde (6c)

Yellow solid; Yield 72%; mp 195-197 °C; IR (KBr) 3320 (NH), 3064-3027 (CH aromatic), 2928-2853 (CH aliphatic), 1661 (C=O), 1302, 1146 (SO₂) cm⁻¹; ¹H NMR (DMSO-d₆) δ (ppm): 3.18 (s, 3H, SO₂CH₃), 7.2 (d, 1H, J = 8Hz, indole H-6), 7.58 (s, 1H, indole H-4), 7.91 (d, 1H, J = 9.6 Hz, indole H-7), 8.09 (d, 2H, J = 8.4 Hz, phenyl H-2, H-6), 8.14 (d, 2H, J = 8.4 Hz, phenyl H-3, H-5), 10.00 (s, 1H, aldehydic H), 12.92 (s, 1H, indole NH, D₂O exchangeable). Anal.Calcd for C₁₆H₁₂FNO₃S: C, 60.56; H, 3.81; N, 4.41. Found: C, 60.73; H, 3.72; N, 4.62.

4.1.2. General procedure for synthesis of 5-substituted-3-((2-(4- substituted-
phenyl)hydrazono) methyl)-2-(4-(methylsulfonyl)phenyl)-1H-indole 7a-k

A mixture of an ethanolic solution of respective indole-3-carbaldehyde derivative (6a-c) (1 mmol) and 4-substituted phenylhydrazine HCl (1 mmol) was heated under reflux for 4-6 hours in presence of few drops of glacial acetic acid. After cooling, the reaction mixture was poured into ice-cold water and the separated solid was filtered, dried and recrystallized from methanol (yield: 73-92%).

4.1.2.1. 3-((2-(4-Fluorophenyl)hydrazono)methyl)-2-(4-(methylsulfonyl)phenyl)-1H-indole (7a)

Brown solid; Yield 73%; mp 204-206°C; IR (KBr) 3282-3317 (indole NH, hydrazone NH), 3063 (CH aromatic), 2927-2843 (CH aliphatic), 1597 (C=N), 1302, 1148 (SO2) cm−1; 1H NMR (DMSO-d6) δ (ppm):
3.26 (s, 3H, SO2CH3), 7.04-7.18 (m, 4H, phenyl hydrazone H-3, H-5, indole H-5, H-6), 7.44 (d, 1H, J = 8 Hz, indole H-4), 7.59 (d, 2H, J = 8.4 Hz, phenyl hydrazone H-2, H-6), 7.99 (d, 2H, J = 8.4 Hz, phenyl H-2, H-6), 8.12 (d, 2H, J = 8.4 Hz, phenyl H-3, H-5), 8.27 (s, 1H, CH), 8.4 (d, 1H, J = 8 Hz, indole H-7), 10.01 (s, 1H, hydrazone NH, D2O exchangeable), 11.79 (s, 1H, indole NH, D2O exchangeable).

Anal. Calcd for C22H18FN3O2S: C, 64.85; H, 4.45; N, 10.31. Found: C, 65.08; H, 4.33; N, 9.95.

4.1.2.2. 2-(4-(Methylsulfonyl)phenyl)-3-((2-(4-(methylsulfonyl)phenyl)hydrazono)methyl)-1H-indole (7b)

Yellow solid; Yield 85%; mp 228-230°C; IR (KBr) 3262-3309 (indole NH, hydrazone NH), 3017 (CH aromatic), 2934-2863 (CH aliphatic), 1593 (C=N), 1299, 1150 (SO2) cm−1; 1H NMR (DMSO-d6) δ (ppm):
3.11 (s, 3H, SO2CH3), 3.33 (s, 3H, SO2CH3), 7.17 (d, 2H, J = 8 Hz, phenyl hydrazone H-3, H-5), 7.24-7.33 (m, 2H, indole H-5, H-6), 7.51 (d, 1H, J = 8 Hz, indole H-4), 7.75 (d, 2H, J = 8 Hz, phenyl hydrazone H-2, H-6), 7.95 (d, 2H, J = 8 Hz, phenyl H-2, H-6), 8.13 (d, 2H, J = 8 Hz, phenyl H-3, H-5), 8.3 (s, 1H, CH), 8.4 (d, 1H, J = 8 Hz, indole H-7), 10.72 (s, 1H, hydrazone NH, D2O exchangeable), 11.98 (s, 1H, indole NH, D2O exchangeable); 13C NMR (DMSO-d6) δ (ppm): 43.96 (SO2CH3), 44.85 (SO2CH3), 110.54, 111.29, 112.26, 121.53, 122.76, 124.10, 125.72, 127.94, 128.76, 129.52, 130.23, 136.81, 137.35, 137.66, 137.89, 140.66, 149.82 (CH=N). Anal. Calcd for C23H21N3O4S2: C, 59.08; H,
4.53; N, 8.99. Found: C, 59.27; H, 4.68; N, 9.12.

4.1.2.3. 4-(2-((2-(4-(Methylsulfonyl)phenyl)-1H-indol-3-yl)methylene)hydrazinyl)benzene sulfonamide (7c)

Yellow solid; Yield 83%; mp 203-204°C; IR (KBr) 3298-3325 (NH, indole NH, hydrazone NH), 3014 (CH aromatic), 2924-2853 (CH aliphatic), 1593 (C=N), 1276, 1089 (SO₂) cm⁻¹; ¹H NMR (DMSO-d₆) δ (ppm): 3.11 (s, 3H, SO₂CH₃), 7.17 (d, 2H, J = 8 Hz, phenyl hydrazone H-3, H-5), 7.24-7.33 (m, 2H, indole H-5, H-6), 7.5 (d, 1H, J = 8 Hz, indole H-4), 7.75 (d, 2H, J = 8 Hz, phenyl hydrazone H-2, H-6), 7.95 (d, 2H, J = 8 Hz, phenyl H-2, H-6), 8.13 (d, 2H, J = 8 Hz, phenyl H-3, H-5), 8.36 (s, 1H, CH), 8.39 (d, 1H, J = 8 Hz, indole H-7), 10.71 (s, 1H, hydrazone NH, D₂O exchangeable), 11.97 (s, 1H, indole NH, D₂O exchangeable), NH₂ not distinguished; ¹³C NMR (DMSO-d₆) δ (ppm): 43.95 (SO₂CH₃), 110.53, 111.28, 112.25, 121.53, 122.75, 124.10, 125.72, 127.93, 128.76, 129.52, 130.22, 136.80, 137.34, 137.65, 137.89, 140.67, 149.81 (CH=N). Anal.Calcd for C₂₂H₂₉N₄O₄S₂: C, 56.39; H, 4.30; N, 11.96. Found: C, 56.45; H, 4.17; N, 12.28.

4.1.2.4. 5-Methyl-2-(4-(methylsulfonyl)phenyl)-3-((2-(4-(methylsulfonyl)phenyl)hydrazono)methyl)-1H-indole (7d)

Brown solid; Yield 85%; mp 262-264°C; IR (KBr) 3319-3340 (indole NH, hydrazone NH), 3023 (CH aromatic), 2932-2856 (CH aliphatic), 1595 (C=N), 1300, 1140 (SO₂) cm⁻¹; ¹H NMR (DMSO-d₆) δ (ppm): 2.55 (s, 3H, CH₃), 3.11 (s, 3H, SO₂CH₃), 3.31 (s, 3H, SO₂CH₃), 7.12-7.18 (m, 3H, indole H-6, phenyl hydrazone H-3, H-5), 7.4 (d, 1H, J = 8.4 Hz, indole H-7), 7.76 (d, 2H, J = 8.4 Hz, phenyl hydrazone H-2, H-6), 7.92 (d, 2H, J = 8 Hz, phenyl H-2, H-6), 8.11 (d, 2H, J = 8 Hz, phenyl H-3, H-5), 8.35 (s, 1H, CH), 8.18 (s, 1H, indole H-4), 10.71 (s, 1H, hydrazone NH, D₂O exchangeable), 11.88 (s, 1H, indole NH, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ (ppm): 22.06 (CH₃), 44.00 (SO₂CH₃), 44.87 (SO₂CH₃), 110.16, 111.26, 111.92, 122.24, 125.62, 126.02, 126.84, 127.86, 128.79, 129.51, 129.88, 130.12, 135.73, 136.92, 137.81, 140.61, 149.88(CH=N). Anal.Calcd for C₂₄H₂₃N₃O₄S₂: C, 59.86; H, 4.81; N, 8.73. Found: C, 59.67; H, 4.82; N, 8.97.
4.1.2.5. 4-(2-((5-Methyl-2-(4-(methylsulfonyl)phenyl)-1H-indol-3-yl)methylene) hydrazine-yl) benzenesulfonamide (7e)

Yellow solid; Yield 87%; mp 186-188°C; IR (KBr) 3300-3341 (NH$_2$, indole NH, hydrazone NH), 3023 (CH aromatic), 2927-2854 (CH aliphatic), 1595 (C=N), 1300, 1130 (SO$_2$) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ (ppm): 3.1 (s, 3H, CH$_3$), 3.33 (s, 3H, SO$_2$CH$_3$), 7.12-7.18 (m, 4H, phenyl hydrazone H-3, H-5, indole H-4, H-6), 7.39 (d, 1H, $J = 8$ Hz, indole H-7), 7.76 (d, 2H, $J = 8$ Hz, phenyl hydrazone H-2, H-6), 7.93 (d, 2H, $J = 8$ Hz, phenyl H-2, H-6), 8.12 (d, 2H, $J = 8$ Hz, phenyl H-3, H-5), 8.18 (s, 2H, NH$_2$, D$_2$O exchangeable), 8.35 (s, 1H, CH), 10.7 (s, 1H, hydrazone NH, D$_2$O exchangeable), 11.88 (s, 1H, indole NH, D$_2$O exchangeable); $^{13}$C NMR (DMSO-$d_6$) $\delta$ (ppm): 22.09 (CH$_3$), 43.96 (SO$_2$CH$_3$), 110.11, 111.23, 111.96, 122.36, 125.64, 125.99, 127.91, 128.66, 129.56, 129.88, 130.12, 135.71, 136.91, 137.80, 137.88, 140.53, 149.86 (CH=N). Anal. Calcd for C$_{23}$H$_{22}$N$_4$O$_4$S$_2$: C, 57.24; H, 4.60; N, 11.61. Found: C, 57.56; H, 4.53; N, 11.89.

4.1.2.6. 3-((2-(4-Fluorophenyl)hydrazono)methyl)-5-methyl-2-(4-(methylsulfonyl)phenyl)-1H-indole (7f)

Yellow solid; Yield 80%; mp 159-161°C; IR (KBr) 3250-3307 (indole NH, hydrazone NH), 3065 (CH aromatic), 2928-2859 (CH aliphatic), 1597 (C=N), 1300, 1146 (SO$_2$) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ (ppm): 2.49 (s, 3H, CH$_3$), 3.4 (s, 3H, SO$_2$CH$_3$), 7.02 (d, 2H, $J = 8.4$ Hz, phenyl hydrazone H-3, H-5), 7.04-7.1 (m, 3H, phenyl hydrazone H-2, H-6, indole H-7), 7.37 (d, 1H, $J = 8$ Hz, indole H-6), 7.92 (d, 2H, $J = 8$ Hz, phenyl H-2, H-6), 8.1 (d, 2H, $J = 8$ Hz, phenyl H-3, H-5), 8.18 (s, 1H, indole H-4), 8.25 (s, 1H, CH), 10.03 (s, 1H, hydrazone NH, D$_2$O exchangeable), 11.75 (s, 1H, indole NH, D$_2$O exchangeable); $^{13}$C NMR (DMSO-$d_6$) $\delta$ (ppm): 22.66 (CH$_3$), 43.66 (SO$_2$CH$_3$), 110.41, 111.51, 112.15, 116.21, 122.36, 125.41, 126.17, 127.82, 129.89, 134.67, 135.79, 136.49, 137.28, 140.28, 143.16 (CH=N), 154.75, 157.07. Anal. Calcd for C$_{23}$H$_{22}$FN$_3$O$_2$S: C, 65.54; H, 4.78; N, 9.97. Found: C, 65.6; H, 4.6; N, 9.94.

4.1.2.7. 5-Methyl-2-(4-(methylsulfonyl)phenyl)-3-((2-(p-tolyl)hydrazono)methyl)-1H-indole
Brown solid; Yield 84%; mp 166-168°C; IR (KBr) 3214-3306 (indole NH, hydrazone NH), 3023 (CH aromatic), 2926-28658 (CH aliphatic), 1598 (C=N), 1302, 1149 (SO₂) cm⁻¹; ¹H NMR (DMSO-d₆) δ (ppm): 2.32 (s, 3H, CH₃), 2.48 (s, 3H, CH₃), 3.25 (s, 3H, SO₂CH₃), 6.95-7.07 (m, 3H, indole H-6, phenyl hydrazone H-3, H-5), 7.39 (d, 1H, J = 8.4 Hz, indole H-7), 7.62 (s, 2H, NH₂, D₂O exchangeable), 7.80 (d, 2H, J = 8.4 Hz, phenyl hydrazone H-2, H-6), 7.92 (d, 2H, J = 8.4 Hz, phenyl H-2, H-6), 8.10 (d, 2H, J = 8.4 Hz, phenyl H-3, H-5), 8.2 (s, 1H, CH), 8.25 (s, 1H, indole H-4), 9.91 (s, 1H, hydrazone NH, D₂O exchangeable), 11.71 (s, 1H, indole NH, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ (ppm): 20.55 (CH₃), 22.12 (CH₃), 44.08 (SO₂CH₃), 111.00, 111.94, 120.53, 122.50, 125.64, 126.16, 126.87, 127.21, 127.87, 128.13, 129.60, 130.08, 135.27, 136.13, 137.77, 140.75, 144.17(CH=N). Anal.Calcd for C₂₄H₂₀N₃O₁₂S₂: C, 69.04; H, 5.55; N, 10.06. Found: C, 68.82; H, 5.68; N, 10.32.

4.1.2.8. 5-Fluoro-2-(4-(methylsulfonyl)phenyl)-3-((2-(4-(methylsulfonyl)phenyl)hydrazono)methyl)-1H-indole (7h)

Bale yellow solid; Yield 92%; mp 187-188°C; IR (KBr) 3265-3337 (indole NH, hydrazone NH),3025 (CH aromatic), 2925-2854 (CH aliphatic), 1593 (C=N), 1321, 1140 (SO₂) cm⁻¹; ¹H NMR (DMSO-d₆) δ (ppm): 3.12 (s, 3H, SO₂CH₃), 3.34 (s, 3H, SO₂CH₃), 7.15-7.20 (m, 3H, phenyl hydrazone H-3, H-5, indole H-6), 7.51 (s, 1H, indole H-4), 7.77 (d, 2H, J = 8 Hz, phenyl hydrazone H-2, H-6), 7.95 (d, 2H, J = 8 Hz, phenyl H-2, H-6), 8.04 (d, 1H, J = 8 Hz, indole H-7), 8.14 (d, 2H, J = 8 Hz, phenyl H-3, H-5), 8.34 (s, 1H, CH), 10.73 (s, 1H, hydrazone NH, D₂O exchangeable), 12.11 (s, 1H, indole NH, D₂O exchangeable); ¹³C NMR (DMSO-d₆) δ (ppm): 44.00 (SO₂CH₃), 44.84 (SO₂CH₃), 107.07, 111.3, 112.16, 113.45, 125.9, 127.93, 129.05, 129.50, 130.23, 134.03, 136.5, 137.42, 139.48, 140.98, 149.79 (CH=N), 157.26, 159.59. Anal.Calcd for C₂₃H₂₀FN₃O₄S₂: C, 56.89; H, 4.15; N, 8.65. Found: C, 57.17; H, 4.23; N, 8.58.

4.1.2.9. 4-(2-((5-Fluoro-2-(4-(methylsulfonyl)phenyl)1H-indol-3yl)methylene)hydrazinyl)benzene sulfonamide (7i)
Yellow solid; Yield 82%; mp 212-214°C; IR (KBr) 3260-3315 (NH$_2$, indole NH, hydrazone NH), 3026 (CH aromatic), 2927 (CH aliphatic), 1594 (C=N), 1295, 1140 (SO$_2$) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ (ppm): 3.24 (s, 3H, SO$_2$CH$_3$), 7.14 (d, 2H, $J = 8$ Hz, phenyl hydrazone H-3, H-5), 7.51 (s, 1H, indole H-4), 7.67 (d, 1H, $J = 8$ Hz, indole H-6), 7.76 (d, 2H, $J = 8$ Hz, phenyl hydrazone H-2, H-6), 7.91 (s, 2H, NH$_2$, D$_2$O exchangeable), 7.95 (d, 2H, $J = 8$ Hz, phenyl H-2, H-6), 8.03 (d, 1H, $J = 8$ Hz, indole H-7), 8.13 (d, 2H, $J = 8$ Hz, phenyl H-3, H-5), 8.34 (s, 1H, CH), 10.62 (s, 1H, hydrazone NH, D$_2$O exchangeable), 11.99 (s, 1H, indole NH, D$_2$O exchangeable); $^{13}$C NMR (DMSO-$d_6$) $\delta$ (ppm): 44.00 (SO$_2$CH$_3$), 107.04, 110.60, 112.41, 113.55, 125.99, 127.53, 129.06, 129.26, 130.23, 134.02, 135.55, 136.47, 139.48, 140.84, 141.00, 149.77 (CH=N), 157.24. Anal. Calcd for C$_{22}$H$_{19}$FN$_4$O$_4$S$_2$: C, 54.31; H, 3.94; N, 11.52. Found: C, 54.67; H, 3.82; N, 11.73.

4.1.2.10. 5-Fluoro-3-((2-(4-fluorophenyl)hydrazono)methyl)-2-(4-(methylsulfonyl)phenyl)-1H-indole (7j)

Yellow solid; Yield 82%; mp 200-202°C; IR (KBr) 3217-3250 (indole NH, hydrazone NH), 3065 (CH aromatic), 2928-2863 (CH aliphatic), 1597 (C=N), 1302, 1145 (SO$_2$) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ (ppm): 3.33 (s, 3H, SO$_2$CH$_3$), 7.01 (d, 2H, $J = 8$ Hz, phenyl hydrazone H-3, H-5), 7.09-7.17 (m, 3H, phenylhydrazone H-2, H-6, indole H-6), 7.5 (s, 1H, indole H-4), 7.94 (d, 2H, $J = 8$ Hz, phenyl H-2, H-6), 8.03 (d, 1H, $J = 8$ Hz, indole H-7), 8.12 (d, 2H, $J = 8$ Hz, phenyl H-3, H-5), 8.25 (s, 1H, CH), 10.09 (s, 1H, hydrazone NH, D$_2$O exchangeable), 11.99 (s, 1H, indole NH, D$_2$O exchangeable); $^{13}$C NMR (DMSO-$d_6$) $\delta$ (ppm): 44.03 (SO$_2$CH$_3$), 107.30, 111.15, 112.01, 112.70, 113.09, 116.32, 125.97, 127.94, 130.19, 134.08, 136.73, 137.95, 140.63, 142.94 (CH=N), 154.76, 157.07, 159.36. Anal. Calcd for C$_{22}$H$_{17}$F$_2$N$_3$O$_2$S: C, 62.11; H, 4.03; N, 9.88. Found: C, 62.32; H, 4.11; N, 10.16.

4.1.2.11. 5-Fluoro-2-(4-(methylsulfonyl)phenyl)-3-((2-(p-tolyl)hydrazono)methyl)-1H-indole (7k)

Brown solid; Yield 75%; mp 151-153°C; IR (KBr) 3220-3270 (indole NH, hydrazone NH), 3034 (CH
aromatic), 2927, 2860 (CH aliphatic), 1597 (C=N), 1303, 1146 (SO$_2$) cm$^{-1}$; $^1$H NMR (DMSO-d$_6$) $\delta$ (ppm): 2.23 (s, 3H, CH$_3$), 3.33 (s, 3H, SO$_2$CH$_3$), 6.94 (d, 2H, $J = 12$ Hz, phenyl hydrazone H-3, H-5), 7.07 (d, 2H, $J = 12$ Hz, phenyl hydrazone H-2, H-6), 7.15 (d, 1H, $J = 8$ Hz, indole H-6), 7.48 (s, 1H, indole H-4), 7.94 (d, 2H, $J = 8$ Hz, phenyl H-2, H-6), 8.05 (d, 1H, $J = 12$ Hz, indole H-7), 8.12 (d, 2H, $J = 8$ Hz, phenyl H-3, H-5), 8.24 (s, 1H, CH), 10.01 (s, 1H, hydrazone NH, D$_2$O exchangeable), 11.96 (s, 1H, indole NH, D$_2$O exchangeable; $^{13}$C NMR (DMSO-d$_6$) $\delta$ (ppm): 20.71 (CH$_3$), 43.96 (SO$_2$CH$_3$), 105.31, 111.45, 112.23, 113.36, 125.92, 126.95, 127.37, 128.20, 129.97, 134.03, 134.80, 137.76, 137.95, 140.54, 144.00 (CH=N), 157.00, 159.32. Anal.Calcd for C$_{23}$H$_{20}$FN$_3$O$_2$S: C, 65.54; H, 4.78; N, 9.97

4.1.3. General procedure for synthesis of 2-(5-substituted-2-(4-(methylsulfonyl)phenyl)-1H-indol-3-yl)-6-chloro-1H-benzo[d]imidazole 8a-c

A mixture of 4-chloro phenylene diamine (0.142 g, 1 mmol), sodium metabisulfite (0.19 g, 1 mmol) and respective indole-3-carbaldehyde derivative (6a-c) (1 mmol) in DMF was heated under reflux for 6 hours. After cooling, the reaction mixture was poured into ice cold water and the separated solid was filtered, dried and recrystallized from ethanol (yield: 60-70%).

4.1.3.1. 5-Chloro-2-(2-(4-(methylsulfonyl)phenyl)-1H-indol-3-yl)-1H-benzo[d]imidazole (8a)

Yellow solid; Yield 60%; mp 210-212 °C; IR (KBr) 3285-3382 (indole NH, benzimidazole NH), 3065-3021 (CH aromatic), 2926-2853 (CH aliphatic), 1660 (benzimidazole C=N), 1301, 1149 (SO$_2$) cm$^{-1}$; $^1$H NMR (DMSO-d$_6$) $\delta$ (ppm): 3.27 (s, 3H, SO$_2$CH$_3$), 7.19-7.22 (m, 2H, indole H-5, benzimidazole H-6), 7.3 (t, 1H, $J = 7.4$ Hz, indole H-6), 7.47 (s, 1H, benzimidazole H-4), 7.55 (d, 1H, $J = 8$ Hz, benzimidazole H-7), 7.69 (s, 1H, indole H-7), 7.89-7.92 (m, 3H, phenyl H-2, H-6, indole H-4), 7.99 (d, 2H, $J = 8.4$ Hz, phenyl H-3, H-5), 12.18 (s, 1H, indole NH, D$_2$O exchangeable), 12.45 (s, 1H, benzimidazole NH, D$_2$O exchangeable); $^{13}$C NMR (DMSO-d$_6$) $\delta$ (ppm): 43.88 (SO$_2$CH$_3$), 105.14, 112.40, 116.49, 116.80, 117.57, 120.79, 121.25, 122.52, 123.83, 127.72, 128.08, 129.57, 131.54, 135.34, 136.24, 136.85, 136.98, 140.55, 145.85. Anal.Calcd for C$_{22}$H$_{16}$ClN$_3$O$_2$S: C, 62.63; H, 3.82; N, 9.96. Found: C, 62.89; H,
4.1.3.2. 5-Chloro-2-(5-fluoro-2-(4-(methylsulfonyl)phenyl)-1H-indol-3-yl)-1H-benzo[d]imidazole (8b)

Pale yellow; Yield 67%; mp 202-204°C; IR (KBr) 3348-3360 (indole NH, benzimidazole NH), 3008-3063 (CH aromatic), 2854-2928 (CH aliphatic), 1659 (benzimidazole C=N), 3348-3360 (indole NH, benzimidazole NH), 3008-3063 (CH aromatic), 2854-2928 (CH aliphatic), 1659 (benzimidazole C=N), 1300, 1148 (SO$_2$) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ (ppm): 3.29 (s, 3H, SO$_2$CH$_3$), 7.13-7.22 (m, 2H, indole H-6, benzimidazole H-6), 7.46 (d, 1H, $J = 8$ Hz, benzimidazole H-7), 7.55 (s, 1H, indole H-4), 7.66-7.72 (m, 2H, benzimidazole H-4, indole H-7), 7.91 (d, 2H, $J = 8$ Hz, phenyl H-2, H-6), 8.02 (d, 2H, $J = 8$ Hz, phenyl H-3, H-5), 12.25 (s, 1H, indole NH, D$_2$O exchangeable), 12.37 (s, 1H, benzimidazole NH, D$_2$O exchangeable); $^{13}$C NMR (DMSO-$d_6$) $\delta$ (ppm): 43.93 (SO$_2$CH$_3$), 105.42, 106.30, 112.15, 113.64, 117.37, 118.34, 122.00, 123.87, 125.26, 127.72, 129.72, 133.57, 136.68, 138.18, 140.41, 140.95, 141.98, 146.36, 149.03. Anal. Calcd for C$_{22}$H$_{15}$ClFN$_3$O$_2$S: C, 60.07; H, 3.44; N, 9.55. Found: C, 60.31; H, 3.20; N, 9.79.

4.1.3.3. 5-Chloro-2-(5-methyl-2-(4-(methylsulfonyl)phenyl)-1H-indol-3-yl)-1H-benzo[d]imidazole (8c)

Yellow solid; Yield 70%; mp 217-219 °C; IR (KBr) 3272-3322 (indole NH, benzimidazole NH), 3192, 3072 (CH aromatic), 2927, 2857 (CH aliphatic), 1620 (benzimidazole C=N), 1301, 1149 (SO$_2$) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ (ppm): 2.43 (s, 3H, CH$_3$), 3.27 (s, 3H, SO$_2$CH$_3$), 7.12 (d, 1H, $J = 8.4$ Hz, indole H-6), 7.2 (d, 1H, $J = 8.4$ Hz, benzimidazole H-6), 7.43-7.47 (m, 2H, indole H-7, benzimidazole H-7), 6.88-7.71 (m, 2H, indole H-4, benzimidazole H-4), 7.88 (d, 2H, $J = 8.4$ Hz, phenyl H-2, H-6), 7.98 (d, 2H, $J = 8.4$ Hz, phenyl H-3, H-5), 12.04 (s, 1H, indole NH, D$_2$O exchangeable), 12.45 (s, 1H, benzimidazole NH, D$_2$O exchangeable); $^{13}$C NMR (DMSO-$d_6$) $\delta$ (ppm): 21.78 (CH$_3$), 43.88 (SO$_2$CH$_3$), 104.72, 112.12, 113.23, 118.42, 118.68, 120.12, 122.76, 125.51, 126.26, 127.68, 128.37, 129.43, 130.00, 134.33, 135.24, 136.18, 137.09, 140.40, 145.79. Anal. Calcd for C$_{23}$H$_{18}$ClN$_3$O$_2$S: C, 63.37; H, 4.16; N, 9.64. Found: C, 63.24; H, 4.25; N, 9.88.

4.1.4. General procedure for synthesis of 5-un/substituted-2-(4-
A mixture of an ethanolic solution of respective indole-3-carbaldehyde derivative (6a-c) (1 mmol) and hydroxylamine HCl (0.08 g, 1 mmol) was heated under reflux for 4-6 hours in presence of few drops of pyridine. After cooling, the reaction mixture was poured into ice-cold water and the separated solid was filtered, dried and recrystallized from ethanol (yield: 55-70%).

4.1.4.1. 2-(4-(Methylsulfonyl)phenyl)-1H-indole-3-carbaldehyde oxime (9a)

Yellow solid; Yield 62%; mp 199-201°C; IR (KBr) 3282-3385 (indole NH, OH), 3010-3028 (CH aromatic), 2928-2951 (CH aliphatic), 1596 (C=N), 1302, 1146 (SO\textsubscript{2}) cm\textsuperscript{-1}; \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}) \(\delta\) (ppm): 3.31 (s, 3H, SO\textsubscript{2}CH\textsubscript{3}), 7.16 - 7.26 (m, 2H, indole H-5, H-6), 7.48 (d, 1H, \(J = 8\) Hz, indole H-7), 7.89 (d, 2H, \(J = 8\) Hz, phenyl H-2, H-6), 8.10-8.12 (m, 3H, phenyl H-3, H-5, indole H-4), 8.32 (s, 1H, CH), 10.89 (s, 1H, OH, D\textsubscript{2}O exchangeable), 11.96 (s, 1H, indole NH, D\textsubscript{2}O exchangeable). Anal.Calcd for C\textsubscript{16}H\textsubscript{14}N\textsubscript{2}O\textsubscript{3}S: C, 61.13; H, 4.49; N, 8.91. Found: C, 61.48; H, 4.61; N, 8.62.

4.1.4.2. 5-Fluoro-2-(4-(methylsulfonyl)phenyl)-1H-indole-3-carbaldehyde oxime (9b)

Yellow solid; Yield 55%; mp 226-228°C; IR (KBr) 3366-3463 (indole NH, OH), 3013-3029 (CH aromatic), 2918-2997 (CH aliphatic), 1598 (C=N), 1298, 1143 (SO\textsubscript{2}) cm\textsuperscript{-1}; \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}) \(\delta\) (ppm): 3.3 (s, 3H, SO\textsubscript{2}CH\textsubscript{3}), 7.12 (d, 1H, \(J = 8\) Hz, indole H-6), 7.48 (s, 1H, indole H-4), 7.8 (d, 1H, \(J = 8\)Hz, indole H-7), 7.89 (d, 2H, \(J = 8\)Hz, phenyl H-2, H-6), 8.11 (d, 2H, \(J = 8\) Hz, phenyl H-3, H-5), 8.31 (s, 1H, CH), 10.89 (s, 1H, OH, D\textsubscript{2}O exchangeable), 12.04 (s, 1H, indole NH, D\textsubscript{2}O exchangeable); \textsuperscript{13}C NMR (DMSO-d\textsubscript{6}) \(\delta\) (ppm): 43.98 (SO\textsubscript{2}CH\textsubscript{3}), 107.27, 112.28, 113.39, 126.22, 128.03, 129.53, 133.81, 136.31, 139.31, 140.52, 144.02 (CH=N), 157.12, 159.44. Anal.Calcd for C\textsubscript{16}H\textsubscript{13}FN\textsubscript{2}O\textsubscript{3}S: C, 57.82; H, 3.94; N, 8.43. Found: C, 57.58; H, 4.06; N, 8.75.

4.1.4.3. 5-Methyl-2-(4-(methylsulfonyl)phenyl)-1H-indole-3-carbaldehyde oxime (9c)

Yellow solid; Yield 70%; mp 212-214°C °C; IR (KBr) 3362 (indole NH, OH), 3025-3060 (CH aromatic), 2857-2928 (CH aliphatic), 1597 (C=N), 1300, 1145 (SO\textsubscript{2}) cm\textsuperscript{-1}; \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}) \(\delta\) (ppm): 2.42 (s, 3H, CH\textsubscript{3}), 3.29 (s, 3H, SO\textsubscript{2}CH\textsubscript{3}), 7.09 (d, 1H, \(J = 8\) Hz, indole H-7), 7.36 (d, 1H, \(J = 8\) Hz, indole H-6),
7.86 (d, 2H, J = 8 Hz, phenyl H-2, H-6), 7.94 (s, 1H, indole H-4), 8.09 (d, 2H, J = 8 Hz, phenyl H-3, H-5), 8.31 (s, 1H, CH), 10.8 (s, 1H, OH, D$_2$O exchangeable), 11.79 (s, 1H, indole NH, D$_2$O exchangeable);

$^{13}$C NMR (DMSO-$d_6$) $\delta$ (ppm): 21.79 (CH$_3$), 44.02 (SO$_2$CH$_3$), 107.42, 111.87, 122.24, 125.47, 126.22, 127.72, 129.07, 129.91, 135.55, 136.78, 137.70, 140.57, 144.36 (CH=N). Anal. Calcd for C$_{17}$H$_{16}$N$_2$O$_3$S: C, 62.18; H, 4.91; N, 8.53. Found: C, 62.42; H, 4.83; N, 8.79.

**Biological evaluation**

**Antimicrobial and antifungal activities**

The antimicrobial and antifungal screening was performed according to CO-ADD (The Community for Antimicrobial Drug Discovery) procedures [26].

**COX-1/COX-2 inhibition colorimetric assay**

Measurement of the ability of the synthesized compounds to inhibit COX isozymes by using colorimetric COX (ovine) Inhibitor Screening Assay Kit (Kit catalog number 760111, Cayman Chemical, Ann Arbor, MI, USA) following the manufacturer’s instructions and as mentioned before [27].

**Carrageenan-induced rat edema assay**

Pretreatment of rats with compounds 7a-k, 8a-c, and 9a-c before injection with carrageenan in rat paw which induce inflammation and then the percentage of paw edema reduction was measured after certain hours according to previously reported procedures [28].

**In vitro nitric oxide release assay**

Different solutions of the tested compounds 9a-c in DMF were diluted using phosphate buffer (pH 7.4) till a final concentration of 100 µM (test solutions). To 100 µl of different test solutions, 100 µl of N-acetyl cysteine solution was added and the obtained solution was kept in an incubator at 37 °C (treated solutions). The solutions were treated similarly as for nitrite standard solution with Griess reagent components, 100 µl of sulphanilamide solution was added to each tube of the treated solution, the mixture was left at 25 °C for 5-10 minutes, protected from light. To this mixture 100 µl of the NED solution was added, the mixture was again left for 5-10 minutes at 25 °C, protected from light.

The absorbance of the formed purple color, if any, was measured within 30 minutes at $\lambda$ 546 nm, a blank experiment was performed under the same conditions, the procedure was repeated three times.
for each tested compound and the average absorbance values were calculated. The corresponding concentration of nitrite was determined by comparison to the nitrite standard calibration curve and the amount of NO released (revealed by the corresponding nitrite concentration) was calculated as percentage of moles of NO released from 1 mole of the tested compounds.

**Molecular modeling and docking**
Molecular modeling studies were performed by using Molecular Operating Environment MOE version 2018.0101. Structures of 7b, 7h, and 7i were built in MOE. The X-ray crystal structure of celecoxib bound to the COX-2 (PDB: ID 3LN1) active site was obtained from protein data bank at research collaboration for Structural Bioinformatics (RSCB) protein database [PDB]. Preparation of the enzyme for docking by removing the Co-crystallized ligand and water molecules then the enzyme was 3D protonated, in which hydrogen atoms were added to their standard geometry. The conformers generated were docked into the COX-2 receptor with MOE-dock using the triangle matcher placement method and the GBVI/WSA dG scoring function. A molecular mechanics force field refinement was carried out on the top 30 poses generated. Celecoxib was redocked into the active site of 3LN1 to validate the docking protocol. Amino acid interactions and the hydrogen bond lengths were summarized in (Table 6).

**Declarations**

**Availability of data and materials**
The datasets and samples of the compounds used during the current study are available from the corresponding author on reasonable request.

**Authors’ contributions**
All authors wrote the final version of the manuscript. All authors read and approved the final manuscript.

**Competing interests**
The authors declare that they have no competing interests.

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Tables

Table 1: The antibacterial and antifungal activities (growth inhibition %) for compounds 7a-k, 8a-c and 9a-c at 32 µg/mL concentration.

| Compound No. | Sa<sup>a</sup> | Ec<sup>b</sup> | Kp<sup>c</sup> | Pa<sup>d</sup> | Ab<sup>e</sup> | Ca<sup>f</sup> | Cn<sup>g</sup> |
|--------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| 7a           | 95.76         | 96.48         | 97.64         | 97.76         | 96.66         | 6.28          | -64.35        |
| 7b           | 25.62         | -8.09         | -5.34         | 3.8           | 35.54         | 9.55          | -280.7        |
| 7c           | 21.88         | 3.17          | 12.8          | 11.3          | 43.29         | 4.13          | -110.9        |
| 7d           | 15.6          | -5.77         | 4.86          | -8.11         | 34.95         | 2.54          | -177.2        |
| 7e           | 7.58          | -11.49        | -14.6         | -22.55        | 43.64         | 28.66         | -59.93        |
| 7f           | 8.33          | -9.43         | 8.05          | -7.9          | 66.69         | 3.5           | -118.8        |
| 7g           | 96.15         | 86.42         | 87.53         | 94.63         | 85.76         | 4.88          | -57.42        |
| 7h           | 30.26         | -13.86        | 23.59         | 7.97          | 51.82         | 25.34         | -99           |
| 7i           | 95.22         | 96.45         | 94.4          | 96.93         | 94.34         | 15.32         | -104.5        |
| 7j           | 30.59         | -2.24         | 12.27         | -0.85         | 46.23         | 1.91          | -80.19        |
| 7k           | 28.25         | -0.72         | 8.77          | 2.23          | 31.42         | 1.79          | -55.44        |
| 8a           | 13.6          | -45.65        | -22.34        | -28.34        | -15.51        | 7.71          | -292.1        |
| 8b           | 11.28         | -8.78         | 6.56          | 14.46         | 22.42         | 13.22         | -114.4        |
| 8c           | 4.62          | -25.19        | -8.38         | -10           | -13.94        | 1.65          | -288.1        |

<sup>a</sup> MRSA; <sup>b</sup> E. coli; <sup>c</sup> K. pneumoniae; <sup>d</sup> P. aeruginosa; <sup>e</sup> A. baumannii; <sup>f</sup> C. albicans; <sup>g</sup> C. neoformans var. grubii
Table 2: Minimum inhibitory concentrations (MIC µg/mL) of most active compounds 7a, 7g, 7i and reference drugs, ceftriaxone and amphotericin B.

| Compound No. | Sa<sup>a</sup> | Ec<sup>b</sup> | Kp<sup>c</sup> | Pa<sup>d</sup> | Ab<sup>e</sup> | Ca<sup>f</sup> | Cn<sup>g</sup> | CC<sub>50</sub><sup>h</sup> | HC<sub>10</sub><sup>i</sup> |
|--------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|----------------|----------------|
| 7a           | 8             | ≤0.25         | 8             | 4             | 16            | >32           | >32           | >32            | >32            |
| 7g           | 1             | ≤0.25         | 1             | 1             | 4             | >32           | >32           | 4.2            | >32            |
| 7i           | 2             | ≤0.25         | 2             | 2             | 4             | >32           | >32           | 2.987          | >32            |
| Ceftriaxone  | 32            | 0.125         | 16            | 32            | 32            | NT            | NT            | NT             | NT             |
| Amphotericin B | NT         | NT            | NT            | NT            | NT            | 1.56          | 1.56          | NT             | NT             |

<sup>a</sup> MRSA, <sup>b</sup> E. coli, <sup>c</sup> K. pneumoniae, <sup>d</sup> P. aeruginosa, <sup>e</sup> A. baumannii, <sup>f</sup> C. albicans, <sup>g</sup> C. neoformans var. grubii, <sup>h</sup> CC<sub>50</sub> is the concentration at 50% cytotoxicity, <sup>i</sup> HC<sub>10</sub> is the concentration at 10% hemolysis.

Table 3: In vitro COX-1 and COX-2 inhibition for compounds 7a-k, 8a-c, 9a-c and reference drugs.
| Compounds | COX Inhibition (IC$_{50}$ µM) | Selectivity index$^a$ (SI) |
|-----------|-------------------------------|--------------------------|
|           | COX-1                        | COX-2                    |
| Celecoxib | 14.8                         | 0.05                     | 296                     |
| Indomethacin | 0.039                       | 0.49                     | 0.079                   |
| 7a        | 10.32                        | 0.11                     | 93.81                   |
| 7b        | 12.41                        | 0.10                     | 124.1                   |
| 7c        | 11.41                        | 0.11                     | 103.72                  |
| 7d        | 10.4                         | 0.15                     | 69.33                   |
| 7e        | 9.7                          | 0.31                     | 31.29                   |
| 7f        | 9.73                         | 0.17                     | 57.23                   |
| 7g        | 7.9                          | 0.2                      | 39.5                    |
| 7h        | 12.4                         | 0.11                     | 112.72                  |
| 7i        | 13.2                         | 0.10                     | 132                     |
| 7j        | 10.8                         | 0.11                     | 98.18                   |
| 7k        | 8.24                         | 0.21                     | 39.2                    |
| 8a        | 10.64                        | 0.13                     | 81.84                   |
| 8b        | 9.41                         | 0.15                     | 62.73                   |
| 8c        | 11.23                        | 0.12                     | 93.58                   |
| 9a        | 10.64                        | 0.13                     | 81.84                   |
| 9b        | 9.42                         | 0.21                     | 44.85                   |
| 9c        | 8.24                         | 0.24                     | 34.33                   |

$^a$ Selectivity index (COX-1 IC$_{50}$/COX-2 IC$_{50}$)

Table 4: Anti-inflammatory activities for compounds 7a-k, 8a-c, 9a-c and reference drug in
Table 5: The amount of NO released from tested compounds **9a-c** in phosphate buffer pH = 7.4 (% mol/mol)
| Compound No. | Amount of NO released (% mol/mol)±standardization error (in phosphate buffer PH 7.4) |
|-------------|----------------------------------------------------------------------------------|
|             | 1 h                                | 2 h                                | 3 h                                | 4 h                                | 5 h                                |
| 9a          | 0.027±0.002                        | 0.065±0.002                        | 0.194±0.007                        | 0.165±0.002                        | 0.138±0.002                        |
| 9b          | 0.086±0.001                        | 0.147±0.003                        | 0.210±0.002                        | 0.198±0.003                        | 0.218±0.003                        |
| 9c          | 0.061±0.001                        | 0.130±0.002                        | 0.187±0.001                        | 0.198±0.003                        | 0.225±0.003                        |

Table 6: Molecular docking data for compounds 7b, 7h, 7i and celecoxib in COX-2 active site (PDB ID: 3LN1).

| Compound No. | Affinity (kcal/mol) | Affinity kcal/mol | Distance (in Å) from main residue | Functional group | Interaction |
|--------------|---------------------|-------------------|-----------------------------------|------------------|-------------|
| Celecoxib    | -14.12              | -2.7              | 3.07                              | Leu338           | -NH₂        | H-donor     |
|              | -1.6                | 2.99              | -NH₂                              |                 |             |             |
|              | -0.8                | 3.54              | -SO₂                              |                 |             |             |
| 7b           | -17.198             | -1.5              | 3.18                              | Leu338           | -SO₂CH₃     | H-donor     |
|              | -0.7                | 2.70              | -SO₂                              |                 |             |             |
|              | -2.3                | 2.84              | -SO₂                              |                 |             |             |
| 7h           | -16.71              | -1.4              | 3.23                              | Leu338           | -SO₂CH₃     | H-donor     |
|              | -2.7                | 2.83              | -SO₂                              |                 |             |             |
|              | -0.6                | 4.71              | -Ph-ring                          |                 |             | H-pi        |
| 7i           | -16.42              | -0.9              | 3.36                              | Val335           | -NH         | H-donor     |
|              | -0.6                | 3.47              | -SO₂CH₃                           |                 |             |             |
|              | -4.5                | 2.86              | -SO₂                              |                 |             |             |
|              | -1.5                | 2.94              | -SO₂                              |                 |             |             |
|              | -0.9                | 3.77              | -Ph-ring                          |                 |             | H-pi        |

Figures
Figure 1

Chemical structures of selective cyclooxygenase-2 (COX-2) inhibitor drugs (I, II, III).
Figure 2

Hybridization of chemical structures of indomethacin V, celecoxib II, nitrofurantoin IV to design indole derivatives 7a-k, 8a-c, and 9a-c.
Figure 3

Binding of celecoxib inside COX-2 active site. a) 2D interaction, the most important amino acids are shown together with their respective numbers. b) The 3D proposed binding mode inside the active site of COX-2 resulted from docking.

Figure 4

Binding of compound 7b inside COX-2 active site. a) 2D interaction, the most important amino acids are shown together with their respective numbers. b) The 3D proposed binding mode inside the active site of COX-2 resulted from docking.

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