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Synthesis and biological evaluation of tetrazole derivatives as TNF-α, IL-6 and COX-2 inhibitors with antimicrobial activity: Computational analysis, molecular modeling study and region-specific cyclization using 2D NMR tools

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ABSTRACT

A group of tetrazole bearing compounds were synthesized and evaluated for their *in vitro* cyclooxygenase (COX) isozymes (COX-1/COX-2) inhibitory activity, *in vitro* anti-inflammatory activity through measuring levels of expression of IL-6 and TNF-α and antimicrobial activity. Cyclization of pyridine derivative **5b** was confirmed using 2D NMR such as NOESY and HMBC experiments. Within the synthesized compounds, compound **7c** was identified as effective and selective COX-2 inhibitors (COX-2 IC_{50} = 0.23 uM; COX-2 selectivity index = 16.91). Moreover **7c** was the most effective derivative on TNF-α (37.6 pg/ml). While, the most active compound on IL-6 was isoxazole derivative **6** (42.8 pg/ml). Dual inhibitory activity on both IL-6 and TNF-α was exhibited by compounds **2** and **3** (IL-6 = 47.5 and 82.7 pg/ml, respectively) and (TNF-α = 31.7 and 33.8 pg/ml, sequentially).

Additionally, compound **7a**, showed broad spectrum antimicrobial activity against Gram positive *cocci*, Gram positive rods and yeast fungus (inhibition zone = 20 and 19 mm). None of the test compounds exhibited activity against Gram negative rods. Compounds **3** and **7c** exhibited good antifungal activity at MIC equal to 64.5 μg/ml. While compound **6** showed antibacterial activities against *Micrococcus lysodicticus* and *Bacillus subtilis* at MIC = 32.25 and 64.5 μg/ml, respectively.

Computational analysis was used to predict molecular properties and bioactivity of the target compounds. To confirm the mode of action of the synthesized compounds as anti-inflammatory agents, molecular docking was done. Appreciable binding interactions were observed for compound **7c** containing COX-2 pharmacophore (SO_2NH_2), with binding energy −10.6652 Kcal/mol, forming two hydrogen bonding interactions with His90 and Tyr355 amino acids. It was fully fitted within COX-2 active site having the highest COX-2 selectivity index between all the test compounds (S.I. = 16.91).

1. Introduction

One of principals in modern drug discovery is the concept of "privileged medicinal scaffolds". It correlates molecular frameworks and biological activity through introducing different modified functional groups. These scaffolds can be considered as ligands for a number of biological receptors and can enhance physicochemical properties and biological activities for the prepared drugs [1–4].

On this base, we tried to design certain new compounds with tetrazole ring as a main scaffold merged with chalcones and different heterocyclic rings such as pyridine, pyrazoline and oxazole, decorated by certain pharmacophores - especially those with sulfonyl group – that reported to have many biological and pharmacological activities [5–9].

Tetrazole ring, the nitrogen-rich multi-electron, is often considered as a carboxylic acid bioisoster with close p*K*a, planarity and space requirements, in addition to provide maximum nitrogen content required from any heterocyclic compound [10,11].

Because of the unique structure and potential pharmacological activities such as antihypertensive, anti-allergic, antimicrobial, anticonvulsant and anticancer agents of tetrazole derivatives, they attracted the interest of scientists [12–20].

Although, anti-microbial activity of tetrazole derivatives has well

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been studied and established in many research papers, there are few reports on the anti-inflammatory activity of [1,2,3,4]-tetrazole ring against COX-2 enzyme or the pro-inflammatory cytokines (tumor necrosis factor-alpha, TNF-α and interleukin-6, IL-6) [21].

These cytokines are important for maintaining the normal cellular physiology. Thus, TNF-α can induce apoptosis and secretion of other cytokines such as IL-1, IL-6 and IL-10. Moreover, it helps in activation of *T*-cells and other inflammatory cells.

While, imbalance in regulation of TNF-α and IL-6 plays the major role in the pathogenesis of varies diseases containing autoimmune, inflammatory, cardiovascular, neurodegenerative, and cancer diseases promoting the inflammatory response and pathological processes [18,22].

Two effective strategies have been applied for the treatment of inflammation-related diseases, either by using small molecules that can inhibit the expression of inflammatory cytokines or through antagonism of their actions by antibodies [23].

The major challenge in the field of drug discovery is how to treat various disorders involving both inflammation and bacterial infections as infection is the primary cause of inflammation. Additionally, inflammation may also cause accumulation of fluid in the injured area which may lead to increase bacterial growth [24].

The main pharmacological action of non-steroidal anti-inflammatory drugs (NSAIDs) is through the enzymatic inhibition of cyclooxygenases (COX), production of pro-inflammatory mediators prostaglandins and thromboxane [11,25].

Due to frequently observed gastrointestinal ulceration and kidney damage side effects of non-steroidal anti-inflammatory drugs (NSAIDs) on long term use, their therapeutic utility has been limited. To overcome limitations of the non-selective NSAIDs, selective COX-2 inhibitors (coxibs) have been applied as safe anti-inflammatory agents. Moreover, there is also a need to develop an efficient class of antibacterial agents to overcome the antibiotic resistance developed by the pathogens. Celecoxib, is a good example for a selective COX-2 inhibitor beside its efficient antibacterial activity [26].

Guided by celecoxib, there is still a need to design and develop new compounds that can act as anti-inflammatory agents alternative to NSAIDs and at the same time have antimicrobial activity.

Encouraged by the results obtained from our previous work on tetrazole derivatives as anti-inflammatory agents [27], and to expand the scope of tetrazole as privileged medicinal scaffold, in this study, we focused on the synthesis, anti-inflammatory (COX-1, COX-2 and inhibition of the expression of cytokines IL-6 and TNF-α) and anti-microbial (antibacterial and antifungal) evaluation of novel tetrazole derivatives. Cheminformatics analyses were used to predict biochemical properties and bioactivity of the synthesized compounds. The mechanism of the tested compounds as anti-inflammatory agents was confirmed using molecular docking study.

2. Results and discussion

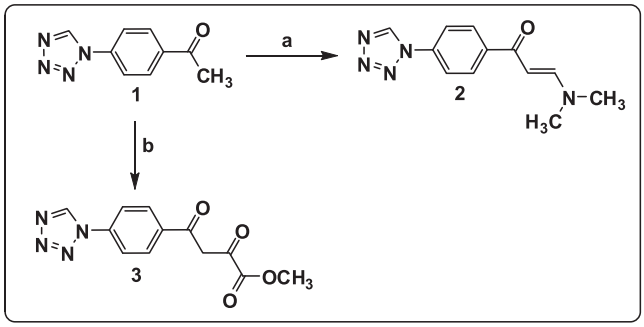
2.1. Chemistry

The synthetic procedures for preparation of N,N-dimethylaminoacryloyltetrazole derivative **2** and dioxomethylbutanoate derivative **3** from the reported tetrazole derivative [27] through condensation with dimethylformamidedimethylacetal (DMF-DMA) or diethyl oxalate, respectively, is depicted in Scheme 1.

The structure of the prepared compounds was confirmed by spectral data and elemental analyses. Thus, ^{1}H NMR spectrum of compound **2** revealed the presence of two doublet signals at δ 5.90 and 7.78 ppm with coupling constant $J = 12$ Hz due to olefinic protons (−COCH=CH−) in *E*-configuration.

While, IR of compound **3** showed three peaks for C=O groups at the range of 1688–1631 cm^{-1}.

Construction of pyridine ring in compounds **5a** and **b** was obtained



Scheme 1. Synthesis of the key intermediates **2** and **3**. ***Reagents and conditions:*** **(a)** DMF-DMA, toluene, refux 12 h, and **(b)** Diethyloxalate, methanol, NaOMe, r.t, stirring 24 h.

from reaction of enaminone derivative **2** with active methylene containing compounds such as acetyl acetone (2,4-pentadione) and ethyl acetoacetate (ethyl 3-oxo-butanoate) in glacial acetic acid in presence of ammonium acetate. The reaction proceeded *via* nucleophilic displacement of active methylene to dimethylamino group in enaminone derivative **2**, followed by elimination of one molecule of water from the non-separated intermediate (**I**), (Scheme 2 and Fig. 1).

The structure of compounds **5a** and **b** was confirmed by IR, ^{1}H NMR, ^{13}C NMR, 2D NMR (NOSY and HMBC) beside mass spectroscopy and elemental analysis.

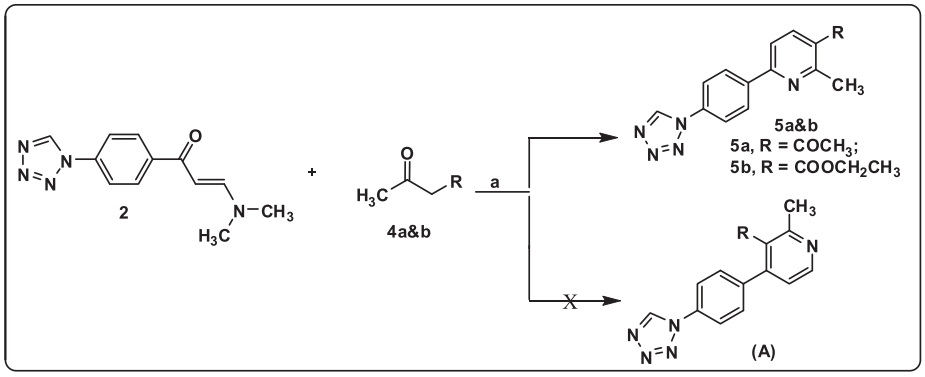
^{1}H NMR spectrum of **5a** showed two singlet signals at δ 2.55 and 2.71 ppm due to CH_3 and $COCH_3$ protons, respectively. Moreover, two additional signals was appeared in aromatic region due to pyridine H-4 and H-5.

To discard the other isomeric form (**A**), and confirm cyclization pathway of compounds **4a** and **b** to get compounds **5a** and **b**, 2D NMR experiments for compound **5b** was done. From NOESY experiment, there was a correlation between protons of ethyl group (δ 1.36 and 4.35 ppm) and pyridine H-4 (δ 8.31 ppm), H-5 (δ 8.07 ppm) and no observed relation between ester protons and phenyl ring protons.

By inspecting HMBC spectrum of compound **5b**, it was observed the presence of a correlation between ethyl CH_2 proton (δ 4.35 ppm) and pyridine H-4 (δ 8.31 ppm) with C=O carbon (δ 167.08 ppm). Other correlations were observed between pyridine H-4 (δ 8.31 ppm) and pyridine C-6 (δ 156.52 ppm), phenyl H-2, H-6 (δ 8.43 ppm) and phenyl C-1 (δ 138.93 ppm), phenyl H-2, H-6 (δ 8.43 ppm) and phenyl C-4 (δ 135.08 ppm), CH_3 (δ 2.83 ppm) and pyridine C-3 (δ 124.77 ppm), pyridine H-5 (δ 8.07 ppm) and phenyl C-2, C-6 (δ 121.82 ppm), finally, pyridine H-5 (δ 807 ppm) and its carbon (δ 118.44 ppm), (Fig. 2).

Two isomeric forms **6** or **B** might be obtained upon reaction of enaminone derivative **2** with hydroxyl amine hydrochloride in absolute ethanol containing potassium carbonate under reflux temperature for 8 h. Data obtained from ^{1}H NMR confirmed the formation of structure **6** rather than **B**. Thus, H-4 and H-5 of isoxazole ring appeared at δ 7.19 and 8.72 ppm, respectively. While, H-3 was expected to appear at higher field of ^{1}H NMR at δ 8.00 ppm [28]. The mechanism of the reaction assumed to be *via* condensation of amino group of hydroxylamine with carbonyl group of enaminone derivative **2** then elimination of dimethyl amine part, (Scheme 3).

Refluxing of enaminone derivative **2** with hydrazine hydrate, 4-methylsulfonylphenyl hydrazine hydrate hydrochloride or 4-benzene sulfonamide hydrazine hydrate hydrochloride in absolute ethanol for 8–12 h afforded pyrazole derivatives **7a–c**. Construction of pyrazole ring in the structure of the products was confirmed by spectral data and elemental analyses. ^{1}H NMR spectra of **7a–c** showed two characteristic doublet signals at δ 6.84–6.87 and 7.73–7.92 ppm attributed to pyrazole H-4 and H-5, sequentially. ^{13}C NMR spectra of **7a–c** showed pyrazole C-3 at δ 143.38–149.07 ppm. Mass spectrum of **7a** revealed molecular ion peak at *m/z* 212 (7.92%), (Scheme 3).



Scheme 2. Synthesis of pyridine containing compounds **5a** and **b**. ***Reagents and conditions:*** **(a)** acetyl acetone or ethyl acetoacetate, NH_4OAc, gl. AcOH, refux 8–10 h.

Another pathway used for the formation of pyrazole ring **8a** and **b** was obtained *via* reaction of oxobutanoate methyl ester **3** and 4-substitiutedphenyl hydrazine hydrochloride in absolute ethanol. ^{1}H NMR spectra of **8a** and **b** displayed a characteristic pyrazole H-4 singlet signal at δ 7.33 and 7.31, respectively. Moreover, ^{13}C NMR revealed the presence of peaks at δ (111.52 and 111.36), (141.03 and 141.66) and (144.66 and 144.72) ppm due to pyrazole C-4, C-5 and C-3, sequentially.

Mass spectrum of **8a** revealed molecular ion peak at *m/z* 424.68 (27.18%).

In an attempt to prepare furanone derivatives **C** through refluxing oxobutanoate methyl ester **3** with different aromatic aldehydes in absolute ethanol containing drops of piperidine for 6 h, chalcone derivatives **9a** and **b** were obtained instead. The expected mechanism for the non-formed product **C** was by Aldol condensation and intramolecularalcoholysis of compound **3** with aldehyde. While, the

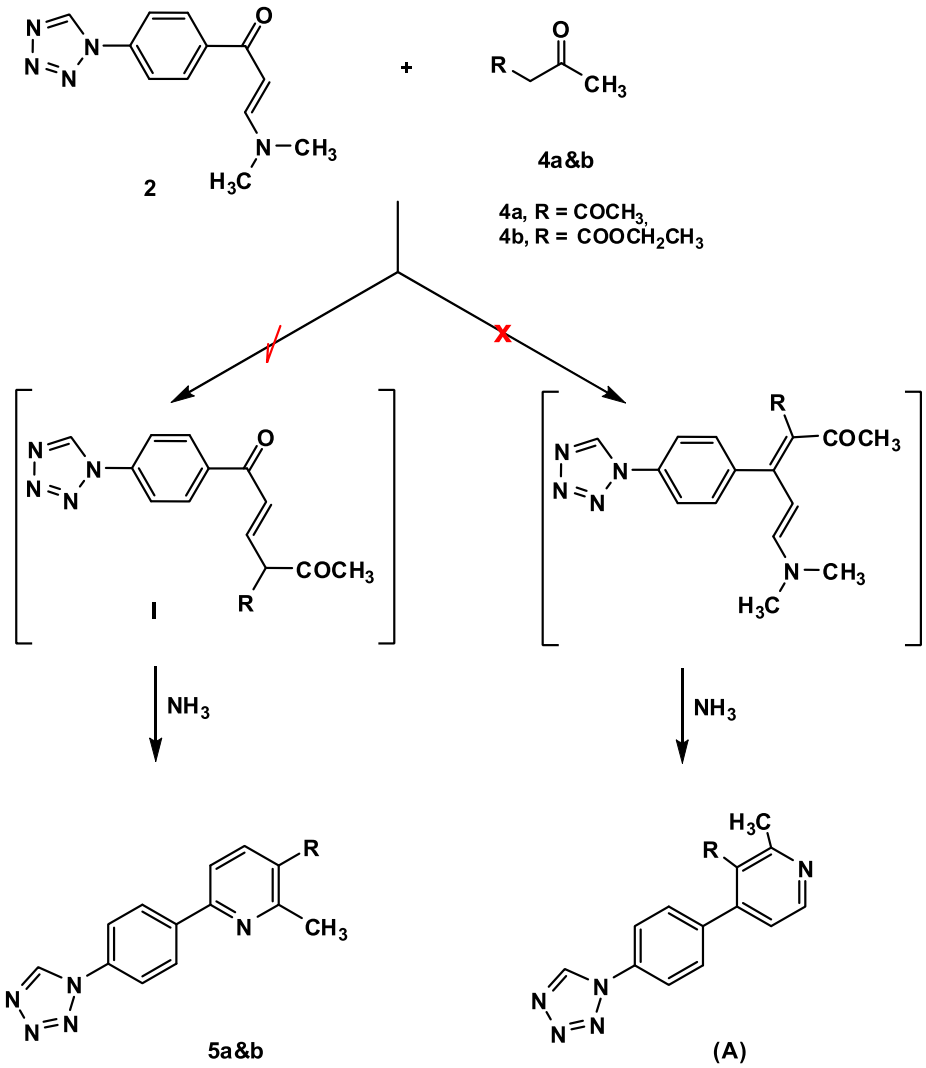
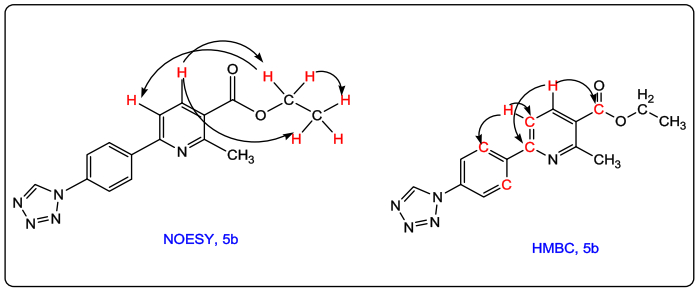


Fig. 1. Suggested mechanism for the formation of pyridine derivatives **5a** and **b**.



^{1}H NMR	^{1}H-^{1}H NOESY	Assignment
1.36 (t, *J* = 7.2 Hz, 3H)	8.07, 8.31	$COOCH_2\mathbf{CH_3}$
2.83 (s, 3H)		CH_3
4.35 (q, *J* = 7.2 Hz, 2H)	8.07, 8.31	$COO\mathbf{CH_2}CH_3$
8.07-8.10 (m, 3H)	1.36, 4.35	phenyl H-3, H-5, pyridine H-5
8.31 (d, *J* = 6.8 Hz, 1H)	1.36, 4.35	pyridine H-4
8.43 (d, *J* = 8.8 Hz, 2H)		phenyl H-2, H-6
10.21 (s, 1H)		tetrazole H
^{13}C NMR	**HMBC**	**Assignment**
14.52		$(CH_2\mathbf{CH_3})$
25.14		(CH_3)
61.61		(CH_2)
118.44	8.07	(pyridine C-5)
121.82	8.07	(phenyl C-2, C-6)
124.77	2.83	(pyridine C-3)
129.10		(phenyl C-3, C-5)
135.08	8.43	(phenyl C-4)
137.05		(pyridine C-4)
138.93	8.43	(phenyl C-1)
140.01		(tetrazole C)
156.52	8.31	(pyridine C-6)
165.21		(pyridine C-2)
167.08	4.35, 8.31	(C=O)

Fig. 2. NMR spectroscopic assignments and important correlations for compound **5b.**

reaction seemed to proceed *via* Claisen-Schmidt condensation between active methylene containing compound **3** and carbonyl of aromatic aldehyde in presence of piperidine as a base, then cleavage of the α, β-unsaturated carbonyl product by elimination of methyl-2-oxoacetate to afford the previously prepared chalcone derivatives **9a** and **b** [28]. Melting point and ^{1}H NMR of **9a** and **b** confirmed their structures (see supplementary data), (Scheme 4 and Fig. 3).

Two series of pyrazoline derivatives **10a** and **b** and **11a** and **b** were obtained from treatment compounds **9a** and **b** with hydrazine hydrate either in methanol or using glacial acetic acid as a solvent, respectively.

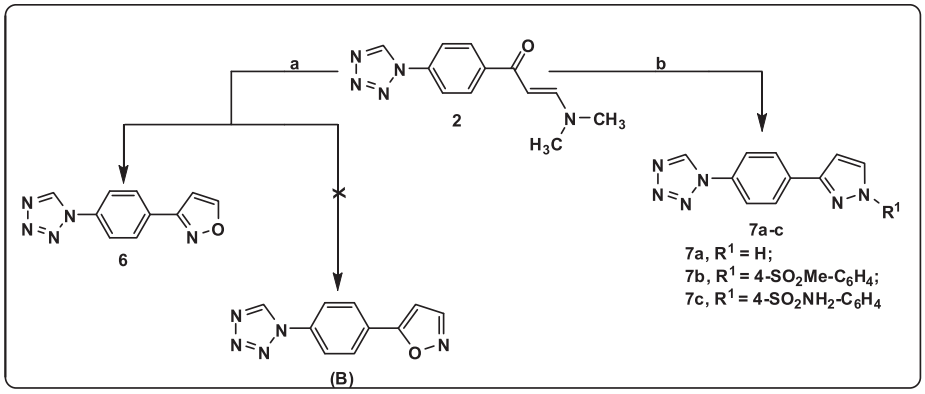
^{1}H NMR spectroscopy was used to differentiate between the two series. ^{1}H NMR spectra of **10a** and **b** showed singlet D_2O exchangeable signal at aromatic region attributed to NH proton. While, a singlet signal appeared at *δ* 2.34 and 2.37 ppm due to $COCH_3$ group in acetyl derivative **11a** and **b**.

^{13}C NMR spectra for **10a** and **b** and **11a** and **b** confirmed the formation of pyrazoline ring. Thus, pyrazoline C-4 appeared at *δ* 40.68–42.61 ppm. Besides, the appearance of pyrazoline C-5 which detected at *δ* 59.99–64.33 ppm.

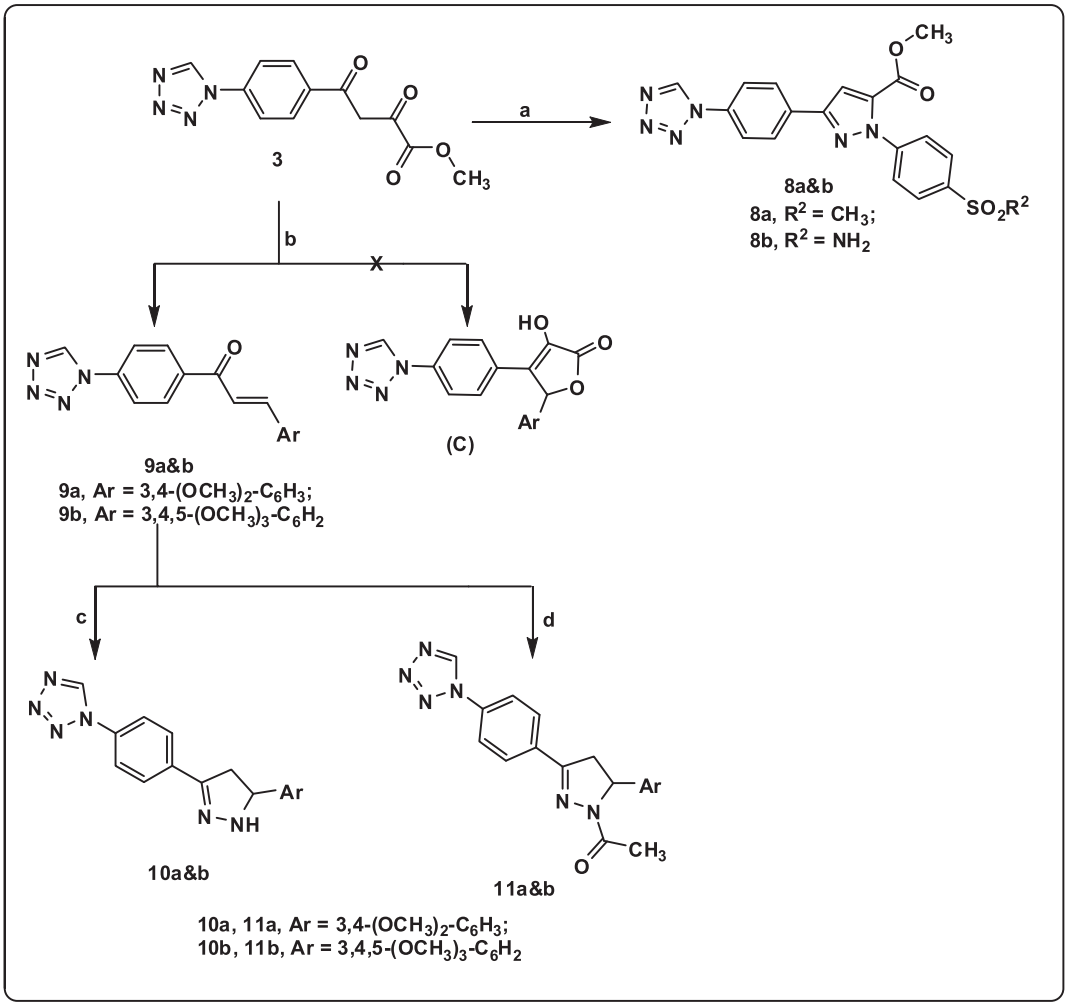
2.2. Biological activity

2.2.1. Anti-inflammatory activity

2.2.1.1. In vitro cyclooxygenases (COX-1 and COX-2) inhibition assay. A colorimetric enzyme immunoassay (EIA) kit assessed to test *in vitro* inhibition of the test compounds to both COX-1 and COX-2 enzymes.



Scheme 3. Synthesis of oxazole and pyrazole containing compounds **6** and **7a–c**. ***Reagents and conditions:*** **(a)** NH_2OH. HCl, K_2CO_3, EtOH, refux 8 h and **(b)** $NH_2NH_2{\cdot}H_2O$ or 4-methanesulfonylphenyl hydrazine hydrochloride or 4-aminosulfonylphenyl hydrazine hydrochloride, EtOH, reflux 8–12 h.



Scheme 4. Synthesis of pyrazole **8a** and **b** and pyrazoline derivatives **10a** and **b** and **11a** and **b**. ***Reagents and conditions:*** **(a)** 4-methanesulfonyl phenyl hydrazine hydrochloride or 4-aminosulfonylphenyl hydrazine hydrochloride, EtOH, reflux 8–12 h, **(b)** ArCHO, piperidine, EtOH, reflux 6 h, **(c)** $NH_2NH_2{\cdot}H_2O$, MeOH, reflux 6 h, and **(d)** $NH_2NH_2{\cdot}H_2O$, gl. acetic acid, reflux 5–8 h.

The oxidized form N,N,N',N'-tetramethyl-*p*-phenylenediamine (TMPD) was monitored at 590 nm. The potency of the test compounds was estimated as IC_{50} which is the *in vitro* test compound concentration required producing 50% inhibition of COX-1 or COX-2. The COX-2 selectivity index (S.I.) values were defined as [IC_{50} (COX-1)/IC_{50} (COX-2)] and calculated then compared to that of the standard drug celecoxib (as a selective COX-2 inhibitor) and indomethacin (selective COX-1 inhibitor). All the obtained data were listed in Table 1.

From the tabulated results, it was found that all the test compounds showed potent inhibitory activities toward COX-2 enzyme (IC_{50} = 0.23–0.66 μM) rather than COX-1 (IC_{50} = 3.89–9.34 μM) if compared to the reference drugs celecoxib (selective COX-2) (IC_{50} = 7.23 and 0.53 μM on COX-1 and COX-2, respectively) and indomethacin (selective COX-1) (IC_{50} = 0.63 and 11.47 μM on COX-1 and COX-2, respectively).

Pyrazole derivative **7c** bearing SO_2NH_2 group (COX-2

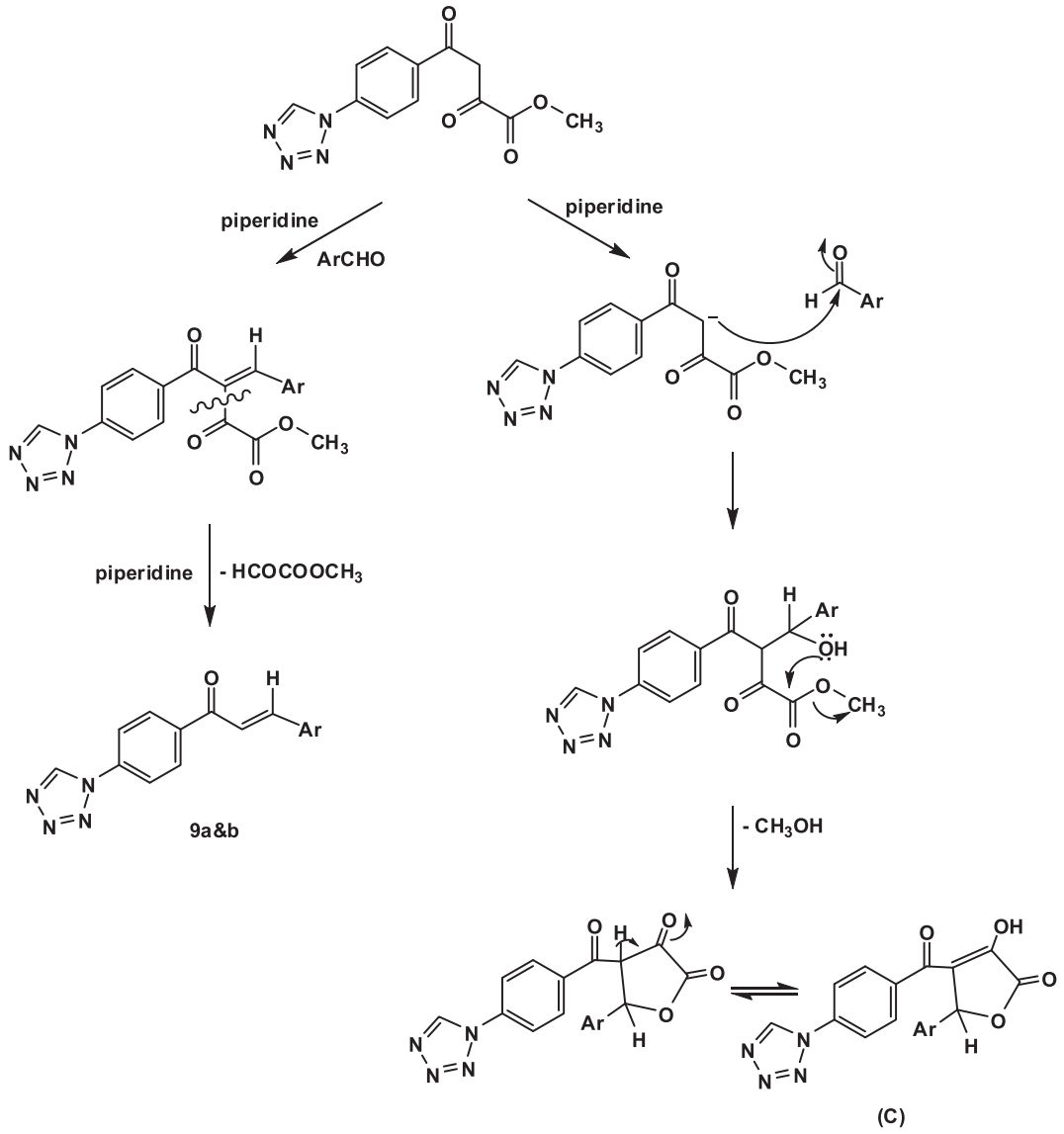


Fig. 3. Suggested mechanism for the formation of compound C and unexpected compounds **9a** and **b**.

pharmacophore) showed better COX-2 inhibitory activity (IC_{50} = 0.23 μM) and higher selectivity index value (S.I. = 16.91) than the reference drug celecoxib (IC_{50} = 0.53 μM and S.I. = 13.64). Moreover, compounds with dimethylamino, pyrazole or pyrazoline moiety (**2**, **7a**, **8a**, **10a** and **11a**) exhibited appreciable COX-2 inhibitory activity with IC_{50} values ranged from 0.36 to 0.51 μM comparing to celecoxib. Pyridine derivative **5a** was equal to celecoxib in its COX-2 inhibitory activity with IC_{50} = 0.53 μM. On the other hand, compounds with diketo-ester group, pyridine-ester, oxazole, pyrazole of methylsulfonyl, pyrazole ester of sulfamoyl, trimethoxyphenyl pyrazoline and *N*-acetyl trimethoxyphenyl pyrazoline scaffolds (**3**, **5b**, **6**, **7b**, **8b**, **10b** and **11b**) with IC_{50} ranged from 0.57 to 0.66 μM were slightly less potent than celecoxib (IC_{50} = 0.53 μM) toward COX-2 enzyme.

Additionally, the best selectivity index values in the second level after compound **7c** were observed in compounds (**2**, **5b** and **11b**) (S.I = 11.46–14.82) while that of celecoxib was equal to 13.64.

All of the other test compounds showed good selectivity index values ranged from 10.24 to 12.74 except pyridine derivative **5a** which had the lowest value (S.I. = 8.28).

From the above mentioned points, it was expected that most of the synthesized compounds might be safe with low ulcerogenicity on gastric mucosa.

2.2.1.2. Levels of TNF-α and IL-6. The expression of TNF-α and IL-6 levels in the supernatants of BEAS-2B cells were measured by ELISA kits, (Figs. 4 and 5). Excellent inhibitory activity was achieved by starting materials **2** and **3**, they could decrease the synthesis of both TNF-α and IL-6 significantly in BEAS-2B cells (47.5 and 82.7 pg/mL for TNF-α and 31.7 and 33.8 pg/mL for IL-6, respectively) if compared to dexamethasone (80 and 98 pg/mL for TNF-α and IL-6, respectively).

Regarding IL-6, Moreover, Compounds containing pyridine, isoxazole, pyrazole and pyrazoline ring system and methylsulfonyl, sulfamoyl or trimethoxyphenyl moieties, **5b**, **6**, **7b, 7c** and **10b** showed reduction in the level of IL-6 (83.2, 42.8, 94.2, 73 and 95.3, respectively).

N-Acetyl derivative of trimethoxyphenylpyrazoline scaffold **11b** exhibited reduction in the level of IL-6 (103.6 pg/ml) close to that of dexamethasone (98 pg/ml).

Concerning TNF-α, The most active derivative was 4-sulfamoylphenylpyrazole **7c** with a value 37.6 pg/ml. Also, isoxazole containing compound with a value of 66.5 pg/ml showed better action than dexamethasone (80 pg/ml).

The remaining prepared compounds might have anti-inflammatory activity by inhibiting other forms of cytokines.

Table 1
COX-1 and COX-2 inhibitory activities (IC_{50}, μM) and COX-2 selectivity index values for the synthesized target compounds and reference drugs, celecoxib and indomethacin.

Compound No.	IC_{50}[a] (μM)		COX-2 S.I.[b]
	COX-1	COX-2	
2	5.20	0.36	14.44
3	7.63	0.65	11.73
5a	4.39	0.53	8.28
5b	9.34	0.63	14.82
6	6.75	0.58	11.63
7a	5.15	0.46	11.19
7b	6.35	0.62	10.24
7c	3.89	0.23	16.91
8a	6.5	0.51	12.74
8b	6.16	0.57	10.80
10a	5.55	0.46	12.06
10b	8.2	0.65	12.61
11a	6.41	0.46	13.93
11b	7.57	0.66	11.46
Celebrex	7.23	0.53	13.64
Indomethacin	0.63	11.47	0.05

[a] The *in-vitro* test compound concentration required to produce 50% inhibition of COX-1 or COX-2. The result (IC50, μM) is the mean of two determinations acquired using an ovine COX-1/COX-2 assay Kit (Cayman Chemicals Inc, Ann Arbor, MI, USA) and the deviation from the mean is < 10% of the mean value.

[b] *In Vitro* COX-2 selectivity index (COX-1 IC50/COX-2 IC50).

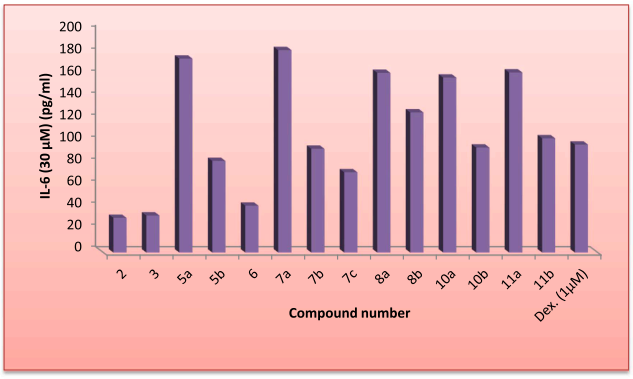


Fig. 4. Effects of the target compounds and dexamethason on IL-6 expression in supernatant of BEAS-2B cells.

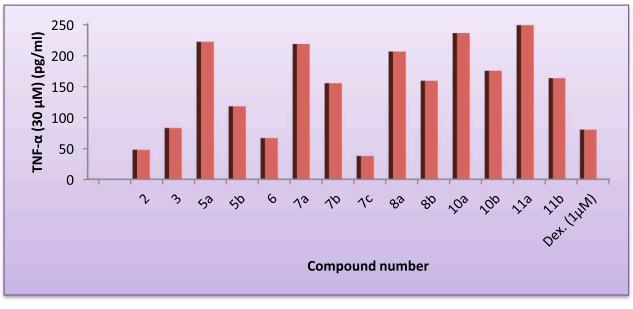


Fig. 5. Effects of the target compounds and dexamethason on TNF-α expression in supernatant of BEAS-2B cells.

2.2.2. Antimicrobial activity

Twelve compounds were selected and screened for their antimicrobial activity against six strains of Gram positive, Gram negative bacteria and fungi, namely, *E. coli*, *Staphylococcus aureus*, *Micrococcus lysodicticus*, *Bacillus subtilis*, *Mycobacterium* smegmatis, and *Candida albicans*.

The test compounds showed an excellent to moderate antibacterial activity against different types of tested microorganisms. Pyrazole derivative **7c** displayed broad spectrum activity against Gram positive *cocci*, Gram positive rods and Acid-fast *bacilli* (Fig. 6) while, compound **6**, bearing isoxazole ring, exhibited activity against both types of Gram positive bacteria (*rods* and *cocci*). Both diketoester derivative **3** and pyrazole derivative **7a** showed antibacterial and antifungal properties against *Micrococcus lysodicticus*, *Mycobacterium smegmatis* and *Candida albicans*, respectively. Other compounds like **11b** (trimethoxyphenyl derivative of *N*-acetylpyrazoline containing compound) demonstrated antibacterial activity against *Bacillus subtilis* and *Mycobacterium smegmatis*, (Table 2).

In conclusion, order of the synthetic compound activities against the Gram positive bacteria (*rods* and *cocci*) was as follows; **11b** > **7a** > **6** > **7c** > **2** = **5a** = **7b** = **8b** = **10a** = **11a** > **3** = **5b**.

For antifungal activity the order was, **3** > **7a**. No activity was observed against Gram negative bacteria.

Consequently, minimum inhibitory concentrations (MIC) were determined for compounds displayed the highest antimicrobial activity. The obtained results were summarized in Table 3. Between all test compounds, Isoxazole derivative **6** showed the highest potential MIC values ranged from 32.25 to 64.5 μg/ml against *Micrococcus lysodicticus* and *Bacillus subtilis* species. While compounds **3** and **7c** exhibited good antifungal activity at MIC values equal to 64.5 μg/ml.

2.3. Computational analysis

2.3.1. Biochemical properties and drug-likeness

The biochemical properties of the target compounds were predicted through Lipiniski's rule of five (RO5) analyses. To achieve the rule, molecular mass and Log*p* values should be less than 500 g/mol and 5, respectively. Moreover, HBA and HBD shouldn't exceed 10 and 5, sequentially. For the synthesize compounds, the molecular weight (g/mol) didn't exceed the acceptable value. Also, HBA and HBD were within the range (< 10 HBA and < 5 HBD) resulting in good permeation. Log*p* values for all the synthesized compounds justified the standard value (< 5).

To predict molecular flexibility and oral bioavailability, the number of rotatable bonds should be less than 10 and polar surface area (PSA) is less than 140 A^2. The obtained predictable results were comparable with standard values.

Drug-likeness is also considered as good descriptor for various molecular properties such as electronic distribution, molecule size, hydrophobicity and other various features. The obtained results showed acceptable to good drug score values. Compounds **10b** and **11b** were found to have positive values indicating good drug likeness behavior, (Table 4, Fig. 7).

2.3.2. Bioactivity prediction

The prediction of interaction of the synthesized compounds and drug targets such as G-protein-coupled receptor (GPCR), Ion channel modulator, Kinases, Nuclear receptor ligand and proteases was evaluated by Molinspiration (Table 5). To consider the synthesized compounds as good lead structures, they should possess > 0.00 bioactivity score. While, compounds having values in the range of −0.50 to 0.00 are classified as moderately active. Others with scores less than −0.50 are supposed to be inactive.

For our tested compounds, **7b** and **7c** were the most predictable active derivatives with positive values on protease receptor (0.05 and 0.04, respectively) and within intermediately active range on GPCR and kinases (for **7b** and **c**) and nuclear receptor (for **7b**). However, their activity predictions were not in the standard range against ion exchange receptor and nuclear receptor (in case of **7c**).

Additionally, compounds **8a** and **b** showed intermediate predicted activity against all drug targets except ion exchange receptor.

Whereas, the rest of the prepared derivatives, showed intermediate

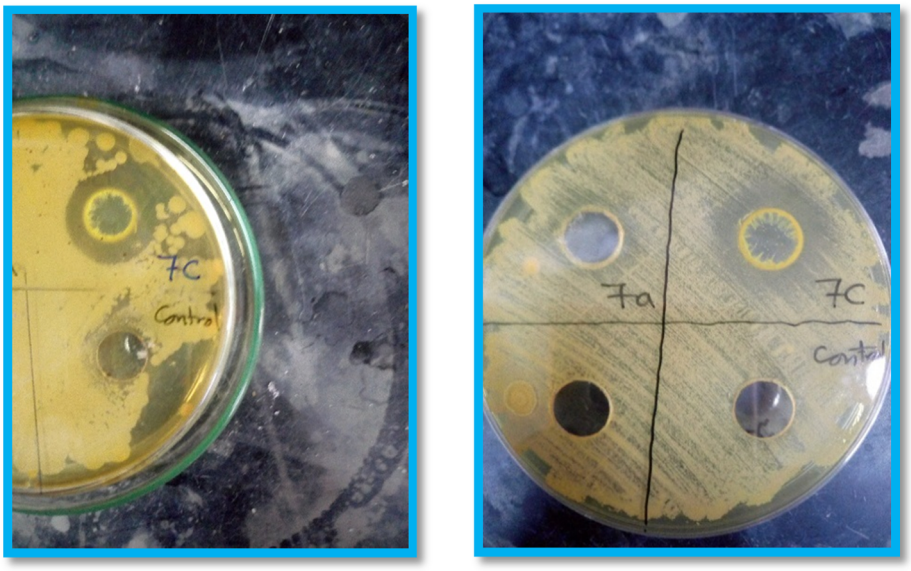


Fig. 6. Antibacterial activity of **7c** compound on: **(A)** *Bacillus subtilis* strain ATCC 6051 showing inhibition zone of 16 mm and **(B)** *Mycobacterium pheli* strain NCTC 10,266 showing inhibition zone of 16 mm.

Table 2
Inhibition zone diameter (mm) of the synthetic compounds against tested microorganisms.

Compound No.	Gram negative rods	Gram positive cocci		Gram positive rods		Yeast
	E. coli (ATCC 25922)	*Staphylococcus aureus* (ATCC 33592)	*Micrococcus lysodicticus* (ATCC 10240)	*Bacillus subtilis* (ATCC 6051)	*Mycobacterium smegmatis* (ATCC 19420).	*Candida albicans* (ATCC 90028)
2	N.D	12	12	13	19**	17
3	N.D	12	14	12	18**	21**
5a	N.D	12	12	14	19**	17
5b	N.D	12	12	12	18**	17
6	N.D	18**	19**	16**	12	17
7a	N.D	12	20**	12	20**	19**
7b	N.D	12	12	12	19**	17
7c	N.D	14	16**	16**	18**	14
8b	N.D	12	12	14	19**	17
10a	N.D	12	12	13	19**	17
11a	N.D	12	13	14	19**	17
11b	N.D	12	15	22**	22**	17
Control (DMSO)	N.D	12	12	11	14	14

N.D: Not determined.
** Indicates potential antimicrobial activity.

Table 3
MIC (μg/ml) of synthetic compounds with potential antimicrobial activity against tested microorganisms using standard antimicrobial compounds.

Compound No.	*Staphylococcus aureus* (ATCC 33592)	*Micrococcus lysodicticus* (ATCC 10240)	*Bacillus subtilis* (ATCC 6051)	*Mycobacterium smegmatis* (ATCC 19420).	*Candida albicans* (ATCC 90028)
3	–	–	–	> 500	64.5
6	> 500	32.25	64.5	–	–
7a	–	> 500	–	> 500	250
7c	–	> 500	125	500	64.5
11b	–	–	125	> 500	–
Ampicillin	> 50	25	7.25	N.D	N.D
Rifampicin	N.D	N.D	N.D	12.5	N.D
Fluconazole	N.D	N.D	N.D	N.D	> 50

to inactive predictable values on the tested drug targets, (Table 5).

2.4. Docking study

In docking study, ligand (SC-558)- obtained from the protein data bank (pdb: ID 1CX2) [29]- and all new prepared compounds were docked using Molecular Operating Environment (MOE, Version 2005.06, Chemical Computing Group Inc, Montreal, Quebec, Canada) into the COX-2 receptor. It was observed that two H-bonding interactions were achieved between selective COX-2 inhibitor (SC-558 ligand) and COX-2 receptor active site: (i) SO_2NH_2 with His90 (2.42 Å) and (ii) N-2 pyrazole with Tyr355 (2.77 Å).

Table 4
Molecular properties and drug-likeness of target compounds.

Compounds	Mol.weight (g/mol)	No. HBA	No. HBD	Rotatable Bonds	Mol. Log*p*	MolPSA (A^2)	Mol. Vol (A^3)	Drug likeness Score
2	243.11	4	0	4	0.88	56.75	243.03	−1.10
3	274.07	7	0	6	−0.27	87.67	248.53	−1.19
5a	279.11	5	0	3	1.75	62.75	260.48	−1.21
5b	309.12	6	0	5	2.54	69.68	287.66	−0.64
6	213.07	5	0	2	1.27	61.87	179.95	−1.78
7a	212.08	4	1	4	1.08	63.33	179.22	−1.68
7b	366.09	6	0	4	1.69	83.72	293.27	−0.82
7c	367.09	7	2	2	1.36	104.58	290.49	−0.83
8a	424.10	8	0	6	1.85	103.75	367.28	−0.76
8b	425.09	9	2	6	1.53	124.60	362.83	−0.67
10a	350.15	6	1	5	2.88	79.59	331.63	−0.16
10b	380.16	7	1	6	2.85	87.30	363.48	0.10
11a	392.16	7	0	5	2.16	82.88	380.44	−0.02
11b	422.17	8	0	6	2.13	90.60	412.28	0.26

The calculated binding energy was −10.0340 Kcal/mol, (Table 6 and Figs. 8 and 9).

These amino acids are important for new designed compounds, in addition to other amino acids such as His90, Tyr355, Tyr385, Ser530, and Gly354 amino acids, forming one to four H-bonds.

Appreciable binding interactions were observed for compound **7c** containing COX-2 pharmacophore (SO_2NH_2), with binding energy −10.6652 Kcal/mol and two hydrogen bonding interactions with His90 and Tyr355 amino acids. It was fully fitted within COX-2 active site having the highest COX-2 selectivity index between all the test compounds (S.I. = 16.91).

Compounds **2**, **5b** and **11b** which showed higher S.I. values (13.93–14.82) than the ligand drug (13.64), exhibited excellent binding interactions and their affinity range was from −18.7435 to −9.1501 Kcal/mol with different numbers of H-bonds from one to four with His90, Ser530, Tyr355 and Tyr385 amino acids.

Docking compounds **3**, **6**, **7a**, **7b**, **8a**, **8b**, **10a**, **10b** and **11a** (with COX-2 S.I. values = 10.24–12.74) in the COX-2 enzyme active site, forming one to three H-bonding interactions with His90, Tyr355, Tyr385, Arg513 and Ser530 amino acids exhibited good binding energy values between −18.5426 and −9.1501 Kcal/mol.

Compound **5a** with the lowest S.I. value of 8.28, showed the least binding energy (−8.3132 Kcal/mol) with only one H-bonding interaction with His90 amino acid.

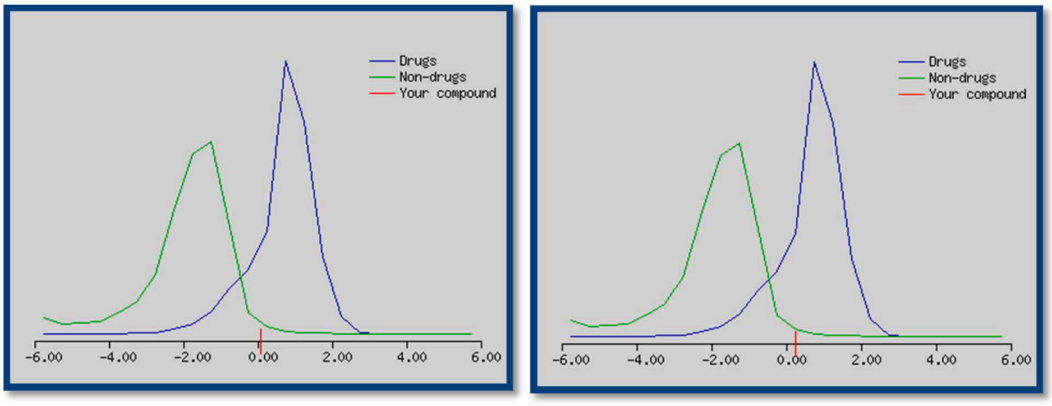
3. Conclusion

In conclusion, some novel tetrazole derivatives were prepared. Their stereogenic cyclization was confirmed by 2D NMR experiments. Their promising anti-inflammatory activity was evaluated against certain mediators (TNF-α and IL-6) and by measuring COX inhibitory activity (COX-1/2). All test compounds exhibited selective COX-2 inhibitory activity. The highest inhibitory activity was observed in pyrazole derivative **7c** – bearing COX-2 pharmacophoric group SO_2NH_2 – IC_{50} = 0.23 μM. Its selectivity index value was equal to 16.91 higher than that of the reference drug celecoxib (S.I. = 13.64). Molecular modeling studies showed that compound 7c was the most fitted one inside COX-2 active site by making two hydrogen bonds with His90 and Tyr355 amino acid residues and binding energy of −10.6652 Kcal/mol.

Excellent dual inhibitory activity was observed in starting materials **2** and **3**, they could decrease the synthesis of both TNF-α and IL-6

Table 5
Bioactivity prediction of target compounds.

Compounds	GPCR compound	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor
2	−0.54	−1.01	−0.85	−1.02	−0.54
3	−0.39	−0.75	−0.77	−0.51	−0.17
5a	−0.11	−0.68	−0.29	−0.50	−0.31
5b	−0.25	−0.65	−0.19	−0.38	−0.30
6	−0.44	−0.51	−0.60	−0.68	−0.52
7a	−0.45	−0.72	−0.24	−1.24	−0.58
7b	−0.06	−0.64	−0.15	−0.38	0.05
7c	−0.13	−0.56	−0.16	−0.68	0.04
8a	−0.22	−0.69	−0.28	−0.24	−0.09
8b	−0.28	−0.63	−0.29	−0.50	−0.10
10a	−0.48	−1.18	−0.63	−0.66	−0.56
10b	−0.46	−1.10	−0.58	−0.67	−0.53
11a	−0.46	−1.22	−0.72	−0.83	−0.49
11b	−0.44	−1.15	−0.67	−0.83	−0.47



10b **11b**

Fig 7. Drug likeness scores for compounds **10b** and **11b**.

Table 6
Results of binding free energies and hydrogen bonds for best poses of SC-558 and the prepared compounds.

Compound No.	E (kcal/mol)	No. of H-bonds	[Amino acids, bond length (Å)]
2	−10.9415	3	C═O (Tyr355, 2.60), N-3 tetrazole (Tyr385, 2.90), N-4 tetrazole (Tyr385, 2.78)
3	−7.0932	2	C═O (Tyr355, 2.57), N-2 tetrazole (His90, 2.74)
5a	−8.3132	1	N-2 tetrazole (His90, 2.65)
5b	−18.7435	2	N-2 tetrazole (His90, 2.93), O-oxazole (Tyr355, 2.94)
6	−7.8988	1	N-2 tetrazole (Tyr355, 2.81)
7a	−5.7998-	2	N-2 tetrazole (His90, 2.85), N-2 pyrazole (Tyr355, 3.08)
7b	−9.5584	2	N-2 tetrazole (Tyr355, 2.86), N-2 pyrazole (His90, 2.74)
7c	−10.6652	2	N-2 pyrazole (His90, 2.49), SO_2 (Tyr355, 2.61)
8a	−9.6577	2	SO_2 (Tyr385, 2.79) SO_2 (Ser530, 2.89)
8b	−16.3430	2	COOMe (Tyr355, 2.26), SO_2 (Tyr385, 2.81)
10a	−10.6625	3	N-2 tetrazole (His90, 2.50), 4-OMe (Tyr385, 2.44), 4-OMe (Ser530, 2.81)
10b	−18.5426	3	3-OMe (His90, 3.33), 4-OMe (Arg513, 2.87), N-2 tetrazole (Arg120, 3.11)
11a	−9.1501	3	N-2 tetrazole (His90, 2.51), 3-OMe (Ser530, 2.51), 3-OMe (Tyr385, 2.94)
11b	−13.1564	4	N-2 tetrazole (His90, 2.51), N-3 tetrazole (His90, 2.85), 3-OMe (Ser530, 2.85), 3-OMe (Tyr385, 2.49)
SC-558	−10.4592	2	SO_2 (His90, 2.42) N-2 pyrazole (Tyr385, 2.77)

significantly in BEAS-2B cells (47.5 and 82.7 pg/mL for TNF-α and 31.7 and 33.8 pg/mL for IL-6, respectively) if compared to dexamethasone (80 and 98 pg/mL for TNF-α and IL-6, respectively). Antimicrobial screening of the target compounds showed that compound **7a**, had broad spectrum antimicrobial activity against Gram positive *cocci*, Gram positive *rods* and yeast fungus. None of the test compounds exhibited activity against Gram negative rods. Compounds **3** and **7c** exhibited good antifungal activity at MIC equal to 64.5 µg/ml.

Prediction of molecular properties, drug likeness and bioactivity were achieved using computational analysis method.

4. Experimental

4.1. Chemistry

Griffin apparatus was used to determine melting points and was uncorrected. Shimadzu IR-435 spectrophotometer with KBr discs was used for IR spectra and values were represented in cm^{-1}. ^1H NMR and ^{13}C NMR (DEPT-Q) were carried out using the Bruker instrument at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR spectrophotometer, (Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt), in DMSO-d_6 (as a solvent), D_2O using TMS as an internal standard and chemical shifts were recorded in ppm on the δ scale using DMSO-d_6 (2.5) as a solvent. Coupling constant (J) values were estimated in Hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet, t, triplet; q, quartet; dd, doublet of doublet; m, multiplet. Hewlett Packard 5988 spectrometer (Palo Alto, CA) was used to record the electron impact (EI) mass spectra. Microanalysis was performed for C, H, N on Perkin-Elmer 2400 at the Microanalytical center, Cairo University, Egypt and was within ± 0.4% of theoretical values. Analytical thin layer chromatography (TLC), pre-coated plastic sheets, 0.2 mm silica gel with UV indicator (Macherey-Nagel) was employed routinely to follow the course of reactions and to check the purity of products. All other reagents, solvents and compound **1** were purchased from the Aldrich Chemical Company (Milwaukee, WI) and, were used without further purification.

4.1.1. General method for preparation of compound (2)

To a mixture of acetophenone derivative **1** (1.88 g, 0.01 mol) in toluene (20 mL), dimethylformamide-dimethylacetal (DMF-DMA) (2.38 g, 0.02 mol) was added. The mixture was heated under reflux temperature for 12 h. The resulting yellow precipitate was filtered out, dried and crystallized from ethanol 95%.

Yield 82%; yellow crystals; (ethanol 95%); mp 213–215 °C; IR (cm^{-1}): 3102 (CH aromatic), 2921 (CH aliphatic), 1639 (C═O), 1598 (C═N);^1H NMR (DMSO-d_6) δ 2.96 (s, 3H, NCH_3), 3.18 (s, 3H, NCH_3), 5.90 (d, J = 12 Hz, 1H, CO*CH*═CH), 7.78 (d, J = 12 Hz, 1H,

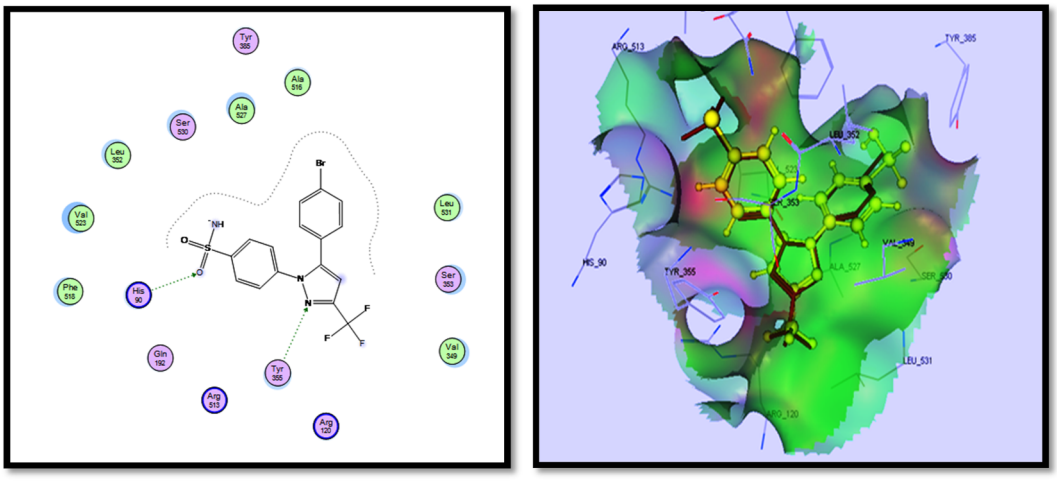


Fig. 8. (A) 2D image for compound SC-558 (ligand) inside COX-2 active site with its amino acids and hydrogen bonds, **(B)** 3D image for binding mode of the compound SC-558 into COX-2 pocket.

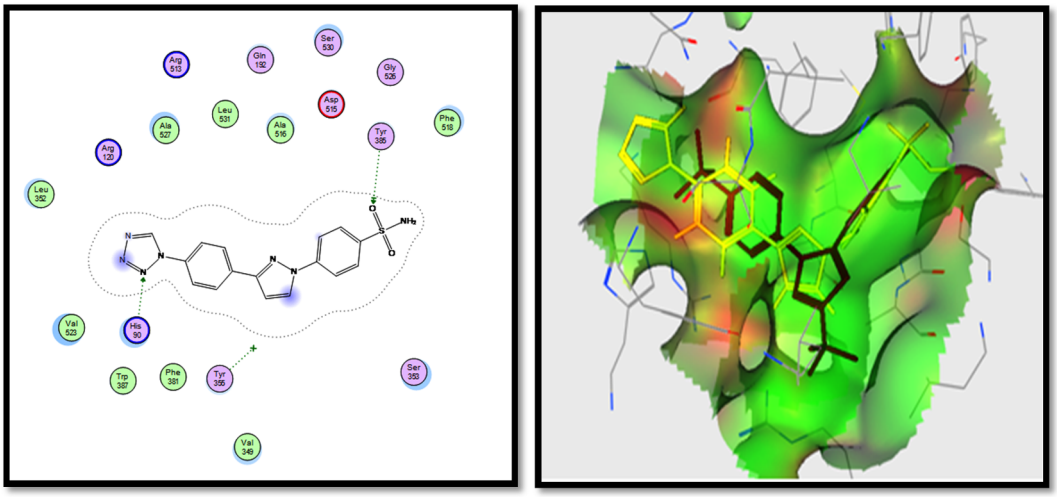


Fig. 9. (A) 2D image for compound **7c** inside COX-2 active site with its amino acids and hydrogen bonds, **(B)** 3D image for binding mode of the compound **7c (yellow)** above SC-558 (brown) into COX-2 pocket.

COCH = C*H*), 7.99 (d, J = 8.4 Hz, 2H, phenyl H-3, H-5), 8.15 (d, J = 8.4 Hz, 2H, phenyl H-2, H-6), 10.18 (s, 1H, tetrazole H); ^{13}C NMR (DMSO-d_6) δ 39.37 (NCH_3), 45.09 (NCH_3), 91.23 (CO*C*H=CH), 121.08 (phenyl C-3, C-5), 129.40 (phenyl C-2, C-6), 135.63 (phenyl C-1), 141.43 (phenyl C-4), 142.77 (tetrazole C-5), 155.25 (COCH=*C*H) 184.43 (C=O); EIMS (m/z): 244.00 (M + 1, 15.24%), 243.00 (M^+, 18.35%), 70.07 (100%); Anal. Calcd for $C_{12}H_{13}N_5O$ (243.26): C, 59.25; H, 5.39; N, 28.79. Found: C, 59.41; H, 5.07; N, 28.68.

*4.1.2. General method for preparation of compound (**3**)*

Asolution of acetophenone derivative **2** (2.43 g, 0.01 mol) and NaOMe (0.23 g Na in 10 mL MeOH) in MeOH (20 mL), was stirred for 30 min. then diethyloxalate (0.56 g, 0.01 mol) was added. The reaction mixture was further stirred at room temperature for 10 h. The yellow solid obtained was filtered, dried and crystalized from ethanol 95%: DMF mixture (4:1).

Yield 85%; yellow crystals; mp 213–215 °C; IR (cm^{-1}): 3129 (CH aromatic), 2918 (CH aliphatic), 1688–1631 (3C=O), 1600 (C=N); ^{1}H NMR (DMSO-d_6) δ 2.65 (s, 3H, OCH_3), 3. 80 (s, 2H, CH_2), 8.08 (d, J = 8.4 Hz, 2H, phenyl H-3, H-5), 8.19 (d, J = 8.4 Hz, 2H, phenyl H-2, H-6), 10.22 (s, 1H, tetrazole H); ^{13}C NMR (DMSO-d_6) δ 40.01 (CH_2), 49.90 (OCH_3), 128.18 (phenyl C-3, C-5), 129.08 (phenyl C-2, C-6), 133.25 (phenyl C-1), 139.36 (phenyl C-4), 142.31 (tetrazole C-5), 160.30 (*C*$OOCH_3$), 190.50 (*C*OCH_2CO), 193.28 (*C*OCH_2); EIMS (m/z): 274.05 (M^+, 22.08%), 205.13 (100%); Anal. Calcd for $C_{12}H_{10}N_4O_4$ (274.07): C, 52.56; H, 3.68; N, 20.43. Found: C, 52.48; H, 3.57; N, 20.28.

*4.1.3. General method for preparation of compounds (**5a** and **b**)*

To a solution of dimethylaminopropenone derivative **2** (2.43 g, 0.01 mol) in glacial acetic acid (20 mL), ammonium acetate (0.15 g, 0.02 mol) and acetyl acetone or ethylacetoacetate (0.01 mol) was added. The mixture was heated under reflux temperature for 8–10 h (monitored by TLC). After cooling, The resulting solution was poured onto ice. Theresulting precipitate was filtered out, dried and crystallized from ethanol/dioxane mixture (1:1).

*4.1.3.1. 1-{6-[4-(1H-Tetrazol-1-yl)phenyl]-2-methylpyridin-3-yl} ethanone (**5a**).* Yield 79%; yellow crystals; mp 171–173 °C; IR (cm^{-1}): 3127–3002 (CH aromatic), 2922 (CH aliphatic), 1687 (C=O), 1575 (C=N);^{1}H NMR (DMSO-d_6) δ 2.55 (s, 3H, $COCH_3$), 2.71 (s, 3H, CH_3), 8.05–8.07 (m, 3H, phenyl H-3, H-5, pyridine H-5), 8.35 (d, J = 8.4 Hz, 1H, pyridine H-4), 8.41 (d, J = 8.4 Hz, 2H, phenyl H-2, H-6), 10.19 (s, 1H, tetrazole H); ^{13}C NMR (DMSO-d_6) δ25.17 (CH_3), 29.94 (CO*C*H_3), 118.21 (pyridine C-5), 121.67 (phenyl C-2, C-6), 129.20(phenyl C-3, C-5), 132.11 (pyridine C-3), 134.97 (phenyl C-4), 138.97(phenyl C-1), 139.31 (pyridine C-4), 142.65 (tetrazole C), 155.69 (pyridine C-6), 157.61 (pyridine C-2), 200.86 (C=O); EIMS (m/z): 279.96 (M + 1, 22.99%), 279.04 (M^+, 20.69%), 251.21 (100.00%); Anal. Calcd for $C_{15}H_{13}N_5O$ (279.30): C, 64.51; H, 4.69; N, 25.07. Found: C, 64.91; H, 4.89; N, 25.37.

*4.1.3.2. Ethyl 6-[4-(1H-tetrazol-1-yl)phenyl]-2-methylnicotinate (**5b**).* Yield 85%; yellow crystals; mp 155–157 °C; IR (cm^{-1}): 3123 (CH aromatic), 2979–2934 (CH aliphatic), 1716 (C=O), 1580 (C=N); ^{1}H NMR (DMSO-d_6) δ 1.36 (t, J = 7.2 Hz, 3H, CH_2CH_3), 2.83 (s, 3H, CH_3), 4.35 (q, J = 7.2 Hz, 2H, CH_2CH_3), 8.07–8.10 (m, 3H, phenyl H-3, H-5, pyridine H-5), 8.31 (d, J = 8.4 Hz, 1H, pyridine H-4), 8.43 (d, J = 8.4 Hz, 2H, phenyl H-2, H-6), 10.21 (s, 1H, tetrazole H); ^{13}C NMR (DMSO-d_6) δ 14.52 (CH_2CH_3), 25.14 (CH_3), 61.61 (CH_2CH_3), 118.44 (pyridine C-5), 121.82 (phenyl C-2, C-6), 124.77 (pyridine C-3), 129.10 (phenyl C-3, C-5), 135.08 (phenyl C-4), 137.05 (pyridine C-4), 138.93 (phenyl C-1), 140.01 (tetrazole C), 156.52 (pyridine C-6), 165.21 (pyridine C-2), 167.08 (C=O); EIMS (m/z): 309.02 (M^+, 38.06%), 62.61 (100.00%); Anal. Calcd for $C_{16}H_{15}N_5O_2$ (309.32): C, 62.13; H, 4.89; N, 22.64. Found: C, 61.98; H, 5.01; N, 22.57.

*4.1.4. Method for preparation of 3-[4-(1H-Tetrazol-1-yl)phenyl]isoxazole (**6**)*

To a solution of dimethylaminopropenone derivative **2** (2.43 g, 0.01 mol) in absolute ethanol (20 mL), hydroxylamine hydrochloride (0.14 g, 0.02 mol) and anhydrous potassium carbonate (1.38 g, 0.01 mol) were added. The mixture was heated under reflux temperature for 8 h. After cooling, the resulting solution was poured onto ice cold water. The solid precipitate was filtered, dried and crystallized from ethanol 95%.

Yield 72%; yellow crystals; mp 155–157 °C; IR (cm^{-1}): 3129 (CH aromatic), 2927 (CH aliphatic), 1617 (C=N); ^{1}H NMR (DMSO-d_6) δ 7.19 (d, J = 8.2 Hz, 1H, isoxazole H-4), 8.10 (d, J = 8.4 Hz, 2H, phenyl H-3, H-5), 8.16 (d, J = 8.4 Hz, 2H, phenyl H-2, H-6), 8.72 (d, J = 8.2 Hz, 1H, isoxazole H-5),10.20 (s, 1H, tetrazole H); ^{13}C NMR (DMSO-d_6) δ101.56 (isoxazole C-4), 122.15 (phenyl C-2, C-6), 128.06 (phenyl C-3, C-5), 128.84 (phenyl C-1), 135.15(phenyl C-4), 142.74 (tetrazole C), 152.40(isoxazole C-5), 167.33(isoxazole C-3); EIMS (m/z): 213.77 (M + 1, 45.28%), 212.52 (M^+, 22.00%), 71.02 (100.00%); Anal. Calcd for $C_{10}H_7N_5O$ (213.20): C, 56.34; H, 3.31; N, 32.85. Found:

C, 56.26; H, 3.53; N, 32.67.

*4.1.5. General method for preparation of pyrazole derivatives (**7a–c**)*

To a solution of dimethylaminopropenone derivative **2** (2.43 g, 0.01 mol) in absolute ethanol (20 mL), the appropriate hydrazine or 4-substituted phenyl hydrazine derivative (0.02 mol) was added. The mixture was heated under reflux temperature for 8–12 h (monitored by TLC). After cooling, the resulting solution was poured onto ice cold water. Thesolid precipitate was filtered, dried and crystallized from ethanol 95%.

*4.1.5.1. 1-[4-(1H-Pyrazol-3-yl)phenyl]-1H-tetrazole (**7a**)*. Yield 65%; yellow crystals; mp 213–215 °C; IR (cm^{-1}): 3253 (NH), 3124 (CH aromatic), 2966 (CH aliphatic), 1601 (C=N); ^{1}H NMR (DMSO-d_6) δ 6.87 (d, J = 8.2 Hz, 1H, pyrazole H-4), 7.73 (d, J = 8.2 Hz, 1H, pyrazole H-5), 7.96 (d, J = 8 Hz, 2H, phenyl H-3, H-5), 8.08 (d, J = 8 Hz, 2H, phenyl H-2, H-6), 10.12 (s, 1H, tetrazole H), 13.07 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ102.94 (pyrazole C-4), 121.88 (phenyl C-2, C-6), 126.58 (phenyl C-3, C-5), 130.74 (pyrazole C-5), 133.06 (phenyl C-1), 135.68 (phenyl C-4), 142.57 (tetrazole C), 149.07 (pyrazole C-3); EIMS (*m/z*): 213.18 (M + 1, 3.37%), 212.09 (M$^+$, 7.92%), 63.00 (100.00%); Anal. Calcd for $C_{10}H_8N_6$ (212.21): C, 56.60; H, 3.80; N, 39.60. Found: C, 56.43; H, 3.67; N, 39.79.

*4.1.5.2. 1-{4-[1-(4-(Methylsulfonyl)phenyl)-1H-pyrazol-3-yl]phenyl}-1H-tetrazole (**7b**)*. Yield 68%; yellow crystals; mp 192–194 °C; IR (cm^{-1}): 3132–3008 (CH aromatic), 2922 (CH aliphatic), 1591 (C=N), 1213, 1146 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.27 (s, 3H, SO_2CH_3), 6.86 (d, J = 8.2 Hz, 1H, pyrazole H-4), 7.56–7.60 (m, 4H, phenyl H-2, H-3, H-5, H-6), 7.92 (d, J = 8.2 Hz, 1H, pyrazole H-5), 7.96–8.00 (m, 4H, 4-methylsulfonylphenylH-2, H-3, H-5, H-6), 10.14 (s, 1H, tetrazole H); ^{13}C NMR (DMSO-d_6) δ43.87 (SO_2CH_3), 110.34 (pyrazole C-4), 121.84 (4-methylsulfonylphenyl C-2, C-6), 125.90 (phenyl C-2, C-6), 128.74 (4-methylsulfonylphenyl C-3, C-5), 130.71 (phenyl C-3, C-5), 131.26 (phenyl C-1), 134.06 (phenyl C-4), 139.89 (4-methylsulfonylphenyl C-4), 141.99 (pyrazole C-5), 142.06 (4-methylsulfonylphenyl C-1), 142.69 (tetrazole C), 143.71 (pyrazole C-3); EIMS (*m/z*): 366.21 (M$^+$, 25.27%), 338.00 (100.00%); Anal. Calcd for $C_{17}H_{14}N_6O_2S$ (366.40): C, 55.73; H, 3.85; N, 22.94. Found: C, 55.60; H, 3.78; N, 23.05.

*4.1.5.3. 4-{3-[4-(1H-Tetrazol-1-yl)phenyl]-1H-pyrazol-1-yl} benzenesulfonamide (**7c**)*. Yield 63%; yellow crystals; mp 217–219 °C; IR (cm^{-1}): 3432–3347 (NH_2), 3134 (CH aromatic), 3067 (CH aliphatic), 1590 (C=N), 1296, 1208 (SO_2); ^{1}H NMR (DMSO-d_6) δ 6.84 (d, J = 8.2 Hz, 1H, pyrazole H-4), 7.47–7.55 (m, 4H, phenyl H-2, H-3, H-5, H-6),7.57 (s, 2H, SO_2NH_2, D_2O exchangeable), 7.87–7.89 (m, 3H, benzenesulfonamide H-3, H-5, pyrazole H-5), 7.97 (d, J = 8.4 Hz, 2H, benzenesulfonamide H-2, H-6), 10.13(s, 1H, tetrazole H); ^{13}C NMR (DMSO-d_6) δ110.03 (pyrazole C-4), 121.39 (benzenesulfonamide C-2, C-6), 125.77 (phenyl C-2, C-6), 127.31 (benzenesulfonamide C-3, C-5), 130.67 (phenyl C-3, C-5), 131.39 (phenyl C-1), 134.01 (phenyl C-4),140.03 (pyrazole C-5), 141.68 (benzenesulfonamide C-4), 142.33 (benzenesulfonamide C-1), 142.69 (tetrazole C), 143.38 (pyrazole C-3); EIMS (*m/z*): 368.42 (M + 1, 26.15%), 367.02 (M$^+$, 26.15%), 63.99 (100.00%); Anal. Calcd for $C_{16}H_{13}N_7O_2S$ (367.39): C, 52.31; H, 3.57; N, 26.69. Found: C, 52.11; H, 3.46; N, 26.73.

*4.1.6. General method for preparation of pyrazole methyl ester derivatives (**8a** and **b**)*

To a mixtureof methyl dioxobutanoate derivative **3** (2.74 g, 0.01 mol) in absolute ethanol (20 mL), the appropriate 4-substituted phenyl hydrazine derivative (0.01 mol) was added. The mixture was heated under reflux temperature for 8–12 h (monitored by TLC). The solid obtained on hot was filtered, dried and crystallized from methanol/chloroform mixture (5:2).

*4.1.6.1. Methyl3-[4-(1H-tetrazol-1-yl)phenyl]-1-[4-(methylsulfonyl) phenyl]-1H-pyrazole-5-carboxylate (**8a**)*. Yield 69%; yellow crystals; mp 236–238 °C; IR (cm^{-1}): 3130–3016 (CH aromatic), 2962 (CH aliphatic), 1709 (C=O), 1596 (C=N), 1205, 1152 (SO_2); ^{1}H NMR (DMSO-d_6) δ3.29 (s, 3H, SO_2CH_3), 3.89 (s, 3H, OCH_3), 7.33 (s, 1H, pyrazole H-4), 7.60 (d, J = 8.4 Hz, 2H, 4-methylsulfonylphenyl H-3, H-5), 7.67(d, J = 8.4 Hz, 2H, phenyl H-3, H-5),7.98 (d, J = 8.4 Hz, 2H, methylsulfonylphenyl H-2, H-6), 8.03 (d, J = 8.4 Hz, 2H, phenyl H-2, H-6), 10.16 (s, 1H, tetrazole H); ^{13}C NMR (DMSO-d_6) δ43.79 (SO_2CH_3), 52.52 (CH_3), 111.52 (pyrazole C-4), 121.80 (methylsulfonylphenylC-2, C-6), 126.66 (phenyl C-2, C-6), 128.84 (methylsulfonylphenyl C-3, C-5), 130.08 (phenyl C-1), 130.98 (phenyl C-3, C-5), 134.44 (phenyl C-4), 141.03 (pyrazole C-5), 142.74 (tetrazole C), 143.08 (methylsulfonylphenyl C-4), 143.85 (methylsulfonylphenyl C-1), 144.66 (pyrazole C-3), 162.19 (C=O); EIMS (*m/z*): 424.68 (M$^+$, 27.18%), 85.69 (100.00%); Anal. Calcd for $C_{19}H_{16}N_6O_4S$ (424.43): C, 53.77; H, 3.80; N, 19.80. Found: C, 53.62; H, 3.91; N, 19.77.

*4.1.6.2. Methyl 3-[4-(1H-tetrazol-1-yl)phenyl]-1-(4-(sulfamoylphenyl)-1H-pyrazole-5-carboxylate (**8b**)*. Yield 63%; yellow crystals; mp 264–266 °C; IR (cm^{-1}): 3352–3251 (NH_2), 3146–3081 (CH aromatic), 2923 (CH aliphatic), 1720 (C=O), 1591 (C=N), 1239, 1161 (SO_2); ^{1}H NMR (DMSO-d_6) δ3.89 (s, 3H, OCH_3), 7.31 (s, 1H, pyrazole H-4), 7.55 (s, 2H, NH_2, D_2O exchangeable), 7.59–7.62 (m, 4H, 4-sulfamoylphenyl H-3, H-5, phenyl H-3, H-5), 7.92 (d, J = 8.4 Hz, 2H, phenyl H-2, H-6), 7.97 (d, J = 8.4 Hz, 2H, 4-sulfamoylphenyl H-2, H-6), 10.13 (s, 1H, tetrazole H); ^{13}C NMR (DMSO-d_6) δ52.49(CH_3), 111.36 (pyrazole C-4), 121.77 (4-sulfamoylphenyl C-2, C-6), 126.45 (phenyl C-2, C-6), 127.39 (4-sulfamoylphenyl C-3, C-5), 130.22 (phenyl C-1), 130.96 (phenyl C-3, C-5), 134.40 (phenyl C-4), 141.66 (pyrazole C-5), 142.72 (tetrazole C), 143.74 (4-sulfamoylphenyl C-4), 144.45 (4-sulfamoylphenyl C-1), 144.72 (pyrazole C-3), 162.24 (C=O); EIMS (*m/z*): 425.20 (M$^+$, 51.76%), 369.25 (100.00%); Anal. Calcd for $C_{18}H_{15}N_7O_4S$ (425.42): C, 50.82; H, 3.55; N, 23.05. Found: C, 51.03; H, 3.37; N, 23.14.

*4.1.7. General method for preparation of chalcone derivatives (**9a** and **b**)*

Amixture of dioxomethylbutanoate derivative **3** (2.74 g, 0.01 mol) and the appropriate aromatic aldehyde (0.01 mol) in absolute ethanol (20 mL) containing piperidine (0.5 mL), was heated under reflux temperature for 6 h. The reaction mixture was evaporated to dryness. The obtained solid was crystallized from ethanol 95%.

(*E*)-1-[4-(1*H*-Tetrazol-1-yl)phenyl]-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (**9a**) and (*E*)-1-[4-(1*H*-Tetrazol-1-yl)phenyl]-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**9b**) [27].

*4.1.8. General method for preparation of pyrazoline derivatives (**10a** and **b**)*

Amixture of the appropriate chalcone derivative **9a** or **9b** (0.01 mol) and hydrazine hydrate 99.99% (1.5 g, 0.03 mol) in methanol (20 mL), was heated under reflux temperature for 6 h. The reaction mixture was evaporated to dryness. The obtained solid was crystallized from ethanol 95%.

*4.1.8.1. 1-{4-[5-(3,4-Dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl] phenyl}-1H-tetrazole (**10a**)*. Yield 54%; yellow crystals; mp 175–177 °C; IR (cm^{-1}): 3246 (NH), 3123 (CH aromatic), 2996–2914 (CH aliphatic), 1597 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.91–2.94 (m, 1H, pyrazoline H-4), 3.75–3.88 (m, 7H, 2(OCH_3) and pyrazoline H′-4), 4.865–4.87 (m, 1H, pyrazoline H-5), 6.91–7.01 (m, 4H, dimethoxyphenyl H-2, H-5, H-6 and NH, D_2O exchangeable), 7.86–8.10 (m, 4H, phenyl H-2, H-3, H-5, H-6), 10.11 (s, 1H, tetrazole H); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 40.68 (pyrazoline C-4), 56.24 (3,4-(OCH_3)$_2$), 64.28 (pyrazoline C-5), 109.40 (dimethoxyphenyl C-2), 118.18 (dimethoxyphenyl C-6), 121.57 (phenyl C-3, C-5), 121.89 (dimethoxyphenyl C-5), 127.12 (phenyl C-

2, C-6), 133.17 (phenyl C-4), 134.99 (phenyl C-1), 135.45 (dimethoxyphenyl C-1), 142.49 (tetrazole C-5), 148.50 (dimethoxyphenyl C-4), 149.44 (dimethoxyphenyl C-3), 153.83 (pyrazoline C-3); EIMS (*m/z*) 350.06 (M^+, 100%). Anal.Calcd for $C_{18}H_{18}N_6O_2$ (350.37): C, 61.70; H, 5.18; N, 23.99. Found: C, 61.56; H, 4.87; N, 23.75.

*4.1.8.2. 1-{4-[5-(3,4,5-Trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl] phenyl}-1H-tetrazole (**10b**)*. Yield 52%; yellow crystals; mp 172–174 °C; IR (cm^{-1}): 3433 (NH), 3127 (CH aromatic), 2936 (CH aliphatic), 1593 (C═N); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 3.49–3.61 (m, 1H, pyrazoline H-4), 3.66 (s, 3H, *p*-OCH_3), 3.85 (s, 6H, 2 *m*-OCH_3), 3.87–3.88 (m, 1H, pyrazoline H′-4), 4.92, 4.94 (dd, J = 12.4, 6.4 Hz, 1H, pyrazoline H-5), 6.77 (s, 2H, trimethoxyphenyl H-2, H-6), 7.90–8.13 (m, 5H, phenyl H-2, H-3, H-5, H-6 and NH, D_2O exchangeable), 10.25 (s, 1H, tetrazole H); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 40.87 (pyrazoline C-4), 56.32 (2 *m*-OCH_3), 60.47 (*p*-OCH_3), 64.33 (pyrazoline C-5), 100.87 (trimethoxyphenyl C-2, C-6), 121.63 (phenyl C-3, C-5), 127.55 (phenyl C-2, C-6), 133.24 (phenyl C-4), 134.37 (phenyl C-1), 137.17 (trimethoxyphenyl C-4), 137.87 (trimethoxyphenyl C-1), 142.55 (tetrazole C-5), 153.34 (pyrazole C-3), 153.74 (trimethoxyphenyl C-3, C-5); EIMS (*m/z*) 380.25 (M^+, 53.72%), 310.25 (100%). Anal.Calcd for $C_{19}H_{20}N_6O_3$ (380.40): C, 59.99; H, 5.30; N, 22.09. Found: C, 59.68; H, 5.09; N, 22.17.

*4.1.9. General method for preparation of pyrazoline derivatives (**11a** and **b**)*

Amixture of the appropriate chalcone derivative **9a** or **9b** (0.01 mol) and hydrazine hydrate 99.99% (1.5 g, 0.03 mol) in glacial acetic acid (20 mL), was heated under reflux temperature 5–8 h (monitored by TLC). The reaction mixture was evaporated to dryness. The obtained solid was crystallized from ethanol 95%.

*4.1.9.1. 1-{3-[4-(1H-Tetrazol-1-yl)phenyl]-5-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl}ethanone (**11a**)*. Yield 49%; yellow crystals; mp 216–218 °C; IR (cm^{-1}): 3121 (CH aromatic), 2968–2937 (CH aliphatic), 1662 (C═O), 1602 (C═N); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.34 (s, 3H, $COCH_3$), 3.21, 3.24 (dd, J = 18.4, 4.8 Hz, 1H, pyrazoline H-4), 3.72 (s, 3H, *p*-OCH_3), 3.73 (s, 3H, *m*-OCH_3), 3.86, 3.88 (dd, J = 18.4, 12 Hz, 1H, pyrazoline H′-4), 5.53, 5.54 (dd, J = 12, 4.8 Hz, 1H, pyrazoline H-5), 6.69 (d, J = 8.4 Hz, 1H, dimethoxyphenyl H-6), 6.83 (s, 1H, dimethoxyphenyl H-2), 6.88 (d, J = 8.4 Hz, 1H, dimethoxyphenyl H-5), 8.03–8.04 (m, 4H, phenyl H-2, H-3, H-5, H-6), 10.18 (s, 1H, tetrazole H); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 22.22 ($COCH_3$), 42.53 (pyrazoline C-4), 55.94 (3,5-OCH_3), 59.99 (pyrazoline C-5), 110.11 (dimethoxyphenyl C-2), 112.42 (dimethoxyphenyl C-6), 117.60 (dimethoxyphenyl C-5), 121.69 (phenyl C-3, C-5), 128.71 (phenyl C-2, C-6), 132.72 (dimethoxyphenyl C-1), 135.04 (phenyl C-4), 135.19 (phenyl C-1), 142.67 (tetrazole C-5), 148.45 (dimethoxyphenyl C-4), 149.26 (dimethoxyphenyl C-3), 153.56 (pyrazole C-3), 168.24 (C═O); EIMS (*m/z*) 392.00 (M^+, 100%). Anal.Calcd for $C_{20}H_{20}N_6O_3$ (392.41): C, 61.21; H, 5.14; N, 21.42. Found: C, 61.44; H, 5.37; N, 21.17.

*4.1.9.2. 1-{3-[4-(1H-Tetrazol-1-yl)phenyl]-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl}ethanone (**11b**)*. Yield 45%; yellow crystals; mp 226–228 °C; IR (cm^{-1}): 3119 (CH aromatic), 2930 (CH aliphatic), 1666 (C═O), 1595 (C═N); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.37 (s, 3H, $COCH_3$), 3.31–3.32 (m, 1H, pyrazoline H-4), 3.63 (s, 3H, *p*-OCH_3), 3.74 (s, 6H, 2 *m*-OCH_3), 3.98 (dd, J = 18, 12.4 Hz, 1H, pyrazoline H′-4), 5.52, 5.54 (dd, J = 12.4, 6.4 Hz, 1H, pyrazoline H-5), 6.49 (s, 2H, trimethoxyphenyl H-2, H-6), 7.63 (d, J = 8.8 Hz, 2H, phenyl H-3, H-5), 8.04 (d, J = 8.8 Hz, 2H, phenyl H-2, H-6), 10.19 (s, 1H, tetrazole H); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 22.24 ($COCH_3$), 42.61 (pyrazoline C-4), 56.34 (2 *m*-OCH_3), 60.39 (*p*-OCH_3), 60.44 (pyrazoline C-5), 102.99 (trimethoxyphenyl C-2, C-6), 121.68 (phenyl C-3, C-5), 128.76 (phenyl C-2, C-6), 132.69 (phenyl C-4), 135.08 (phenyl C-1), 137.02 (trimethoxyphenyl C-4), 138.55 (trimethoxyphenyl C-1), 142.71 (tetrazole C-5), 152.89 (pyrazole C-3), 153.54 (trimethoxyphenyl C-3, C-5), 168.36 (C═O); EIMS (*m/z*) 423.09 (M + 1, 26.72%), 422.23 (M^+, 51.87%), 249.29 (100%). Anal.Calcd for $C_{21}H_{22}N_6O_4$ (422.44): C, 59.71; H, 5.25; N, 19.89. Found: C, 59.56; H, 4.99; N, 19.73.

4.2. Biological evaluation

4.2.1. Anti-inflammatory assay

4.2.1.1. In vitro cyclooxygenases (COX-1 and COX-2) inhibition assay. Enzyme immune assay (EIA) kit (Cayman Chemical, Ann Arbor, MI) was used to test the ability of the prepared compounds to inhibit ovine COX-1 and COX-2 (IC_{50} value, lM) according to reported methods [30,31].

4.2.1.2. Measurements of TNF-α and IL-6 release. BEAS-2B cells- human bronchial epithelial cells- were obtained from American type culture collection (ATCC, Rockville, MD). The derived cells were cultured using DMEM/F12 medium supported with 10% fetal bovine serum and seeded in 96-well plates and placed in 5% CO_2 atmosphere at 37 °C.

Pro-inflammatory cytokine production of TNF-α and IL-6 in the supernatants of BEAS-2B cells was measured using commercial ELISA kits after the administration of dexamethasone (0.01 mM) or target compounds (0.3 mM). A Bio-Rad model 680 microplate reader was used to determine the absorbance of each sample at 450 nm. Levels of TNF-α and IL-6 were measured from standard curves and expressed as picograms per milliliter (pg/mL) according to the method described by Sudsai et al. [32] and Wang et al. [33].

4.2.2. Anti-microbial screening [34]

4.2.2.1. Bacterial strains, culture conditions and sensitivity testing. The target derivatives were screened *in vitro* against various types of Gram positive bacteria (*Staphylococcus aureus*, *Micrococcus lysodicticus, Bacillus subtilis*, *Mycobacterium smegmatis*) and Gram negative bacteria (*E. coli*). Their antifungal activities were tested against (*Candida albicans*).

Organisms were subcultured on Brain Heart infusion agar and Sabouraud's dextrose agar, incubated at 37 °C for 24 hr and 25 °C for 48 hr, respectively. Two to three colonies of bacterial isolates were emulsified in 5 mL saline to achieve 0.5 MacFarland turbidity.

All synthetic organic compounds were dissolved in dimethyl sulfoxide (DMSO) in a concentration of 10^4 μg/ml. Muller Hinton Agar and Sabouraud's dextrose agar were used for detection of antimicrobial activity using cup diffusion technique.

Half MacFarland suspension of each bacterial isolates were streaked 3 times over sensitivity medium with 45° angle in between, cups of 10 mm were made using a sterile corn borer. A hundred microliter that equal to 10^3 μg of organic compound were added, the plates were incubated at 37 °C for 24 hr for antibacterial activity and 25 °C for 48 hr for antifungal activity. The diameter of the inhibition zone was measured in millimeters.

4.2.2.2. Minimum inhibitory concentration (MIC). Minimum inhibitory concentration (MIC) were determined using microbroth dilution method according to CLSI, 2016. Serial dilutions of the test compounds were made in a concentration range from 25 to 1000 μg/ml. A sterile 96-well microplate were filled with 95 μl of double strength brain heart infusion broth or tryptone soya broth and 5 μl of the test microorganism. A hundred microliter of concentration 2000 μg/ml of test compounds were added in the first well to reach a final concentration of 1000 μg/ml. Serial dilutions were done from the first well to reach final concentration of 25 μg/ml. Ampicillin, Rifampicin and Fluconazole were used as a positive control with

concentration range from 100 μg/ml to 6.25 μg/ml while DMSO is used as a negative control.

4.3. Compuitional methodology

4.3.1. Molecular properties, drug likeness and bioactivity prediction

The synthesized compounds were drawn in ChemSketch in mol formate.

Molinspiration [35] and Molsoft [36] were used as online computational tools to predict the biochemical properties and Lipiniski's rule of five (RO5) for the synthesized derivatives, respectively. Moreover, the prediction of bioactivity scores for the target compounds was generated by Molinspiration.

4.4. Docking study

To predict the conformation and energy ranking between COX-2 receptor (PDB: 1CX2) and the designed compounds, docking was performed using Molecular Operating Environment (MOE, Version 2005.06, Chemical Computing Group Inc, Montreal, Quebec, Canada). To measure certain parameters such as energy, root mean standard deviation (RMSD) and amino acid interactions, the co-crystalized ligand (SC-558) was docked first into COX-2 active site. Docking was performed using London *dG* force. Refinement of the results was done using molecular mechanics force field energy (MMFF94x). RMSD, for COX-2 enzyme and the lead compound SC-558 was 3 Å.

3D structures of the prepared compounds were built using the MOE drawing tool bar. The conformer of lowest energy of the prepared compounds was docked into COX-2 active site using force field refinement and by applying the same docking protocol as with ligand. Binding energy was calculated and amino acid interactions and the hydrogen bond lengths were measured in Å.

4.5. Statistical analysis

One way ANOVA followed by Dunnett's test were used to analyze and compare the obtained results. Differences were considered significant at $^{*}P < 0.05$, and $^{**}P < 0.01$.

Declaration of Competing Interest

The authors state no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103301.

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