Synthesis and Biological Evaluation of Novel Pyrazole Derivatives as Anti-Inflammatory Antimicrobial Agents

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Abstract: The synthesis of novel series of structurally related 4-pyrazolyl benzenesulfonamide derivatives is described. All the newly synthesized compounds were examined for their anti-inflammatory activity using cotton pellet induced granuloma and carrageenan induced rat paw edema bioassays. In addition the inhibitory activities of cyclooxygenase-1 and cyclooxygenase-2 (COX-1 and COX-2), ulcerogenic effect and acute toxicity were determined. Furthermore, all the compounds were evaluated for their *in vitro* antimicrobial activity against *Eischerichia coli, Staphylococcus aureus* and *Candida albicans*. Docking poses for compounds **6b** and **7b** separately in the active site of the human COX-2 enzyme and DNA-gyrase B were also obtained. The results revealed that compounds **3c, 4b, 4c, 5c, 6b** and **7b** exhibited comparable or better anti-inflammatory activity compared to indomethacin and celecoxib with no or minimal ulcerogenic effect and high safety margin. Compounds **3b, 3c, 4b, 4c, 5a-c, 6a, 6b** and **7a-c** displayed appreciable antibacterial activity against both *E. coli* and *S. aureus* compared with ampicillin. Compounds **5a-c** and **7a** had antibacterial activity against *E. coli* similar to ampicillin whereas compounds **3b, 3c, 4b, 4c, 6a** and **7b** displayed considerable activity against the microorganism. Compounds **3a, 3c, 4c, 5a-c, 6b** and **7a-c** had appreciable activity against *S. aureus*. Overall, compounds **4c, 6b** and **7b** are the most distinctive derivatives in the present study because of their remarkable anti-inflammatory potency and significant antibacterial activity. Furthermore, compounds **6b** and **7b** exhibited good selective inhibitory activity against COX-2 enzyme. Therefore, such compounds would represent a suitable template for the design of anti-inflammatory antimicrobial candidates with reasonable COX-2 selectivity.

Key Words: Pyrazoles, anti-inflammatory activity, COX inhibitory activity, acute toxicity, ulcerogenic effect, antimicrobial activity.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) have been recognized as important therapeutic agents for the treatment of rheumatoid arthritis and its variants. However, large doses of NSAIDs or long term use usually results in gastrointestinal mucosal damage, intolerance and renal toxicity [1-4]. Despite the efforts that had been made to improve the pharmacological profile of NSAIDs, ulcerogenicity remains the most limiting problem in their clinical use. A major breakthrough in anti-inflammatory research occurred when it was discovered that COX exists in three isoforms COX-1, COX-2 and COX-3 which are regulated differently [5, 6]. The discovery of the inducible isoform COX-2 spurred the search for novel anti-inflammatory agents devoid of the undesirable effects associated with classical non selective NSAIDs. Consequently, a new generation of COX-2 selective inhibitors has been clinically used with the hope that they would exhibit a reduced risk in gastrointestinal events. Among this class, celecoxib; 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide, Fig. (**1**); was shown to be a potent and gastrointestinal safe anti-inflammatory and analgesic agent. It is considered a typical model of pyrazole containing diaryl heterocyclic template that is known to inhibit COX-2 selectively [7]. Several other compounds containing pyrazole functionality were also reported to exhibit anti-inflammatory activity [8-12]. Furthermore, much attention has been focused towards pyrazoles as antimicrobial agents after the discovery of the natural pyrazole C-glycoside pyrazofurin; 4-hydroxy-3-β-D-ribo-furanosyl-1H-pyrazole-5-carboxamide, Fig. (**1**); which demonstrated a broad spectrum of antimicrobial activities [13, 14]. This led to the synthesis of several pyrazole derivatives that exhibited antimicrobial activity by Tanitame and coworkers [15-17].

Co-administration of multiple drugs for treatment of inflammatory conditions associated with microbial infection is a major risk especially in case of patients with impaired liver or kidney functions. A mono therapy of a drug with dual anti-inflammatory antimicrobial activity would be preferred from the pharmacoeconomic and patient compliance point of view. This premise was one of the goals of our research program aimed at the discovery of new pyrazolyl compounds that would possess dual anti-inflammatory antimicrobial activities [18-25]. Some of our reported pyrazolyl compounds [19-22] showed pronounced dual activities. Encouraged by these results it was decided to synthesize novel pyrazolylbenzenesulfonamide derivatives [26] in which the

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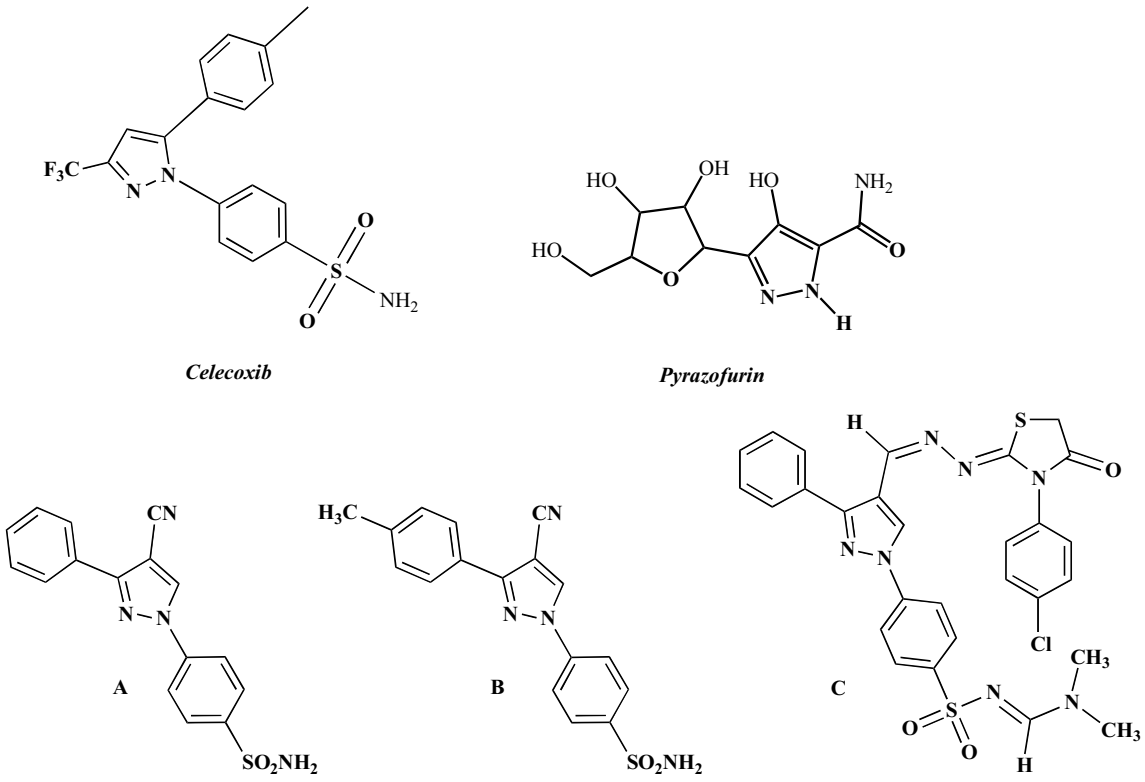
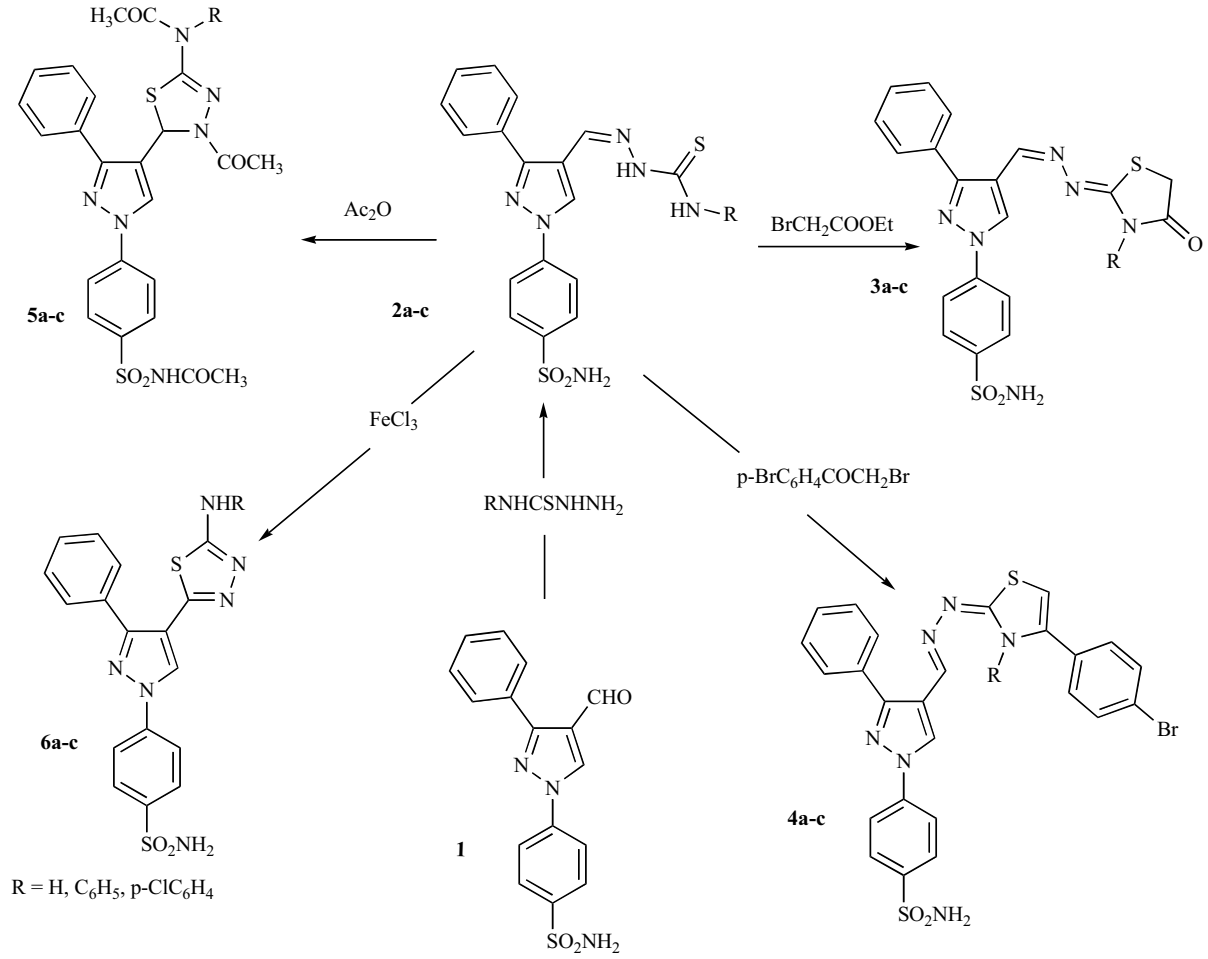


Fig. (1). Structures of celecoxib and reported active pyrazole derivatives A, B and C.

substitution pattern of the pyrazole ring was rationalized so as to be closely correlated to celecoxib. In addition, pyrazolyl N,N-dimethylaminomethylenebenzenesulfonamide derivatives [27] in which the pyrazole moiety is attached to different heterocyclic ring systems known to possess anti-inflammatory and/or antimicrobial activity [28, 29] were also prepared. Three of these lead compounds **A** and **B** [26] and **C** [27] displayed reasonable anti-inflammatory and selective COX-2 inhibitory activity, Fig. (**1**). Therefore, we extended the same pharmacological screening to new pyrazolylbenzenesulfonamide derivatives incorporating an aminosulfonyl moiety instead of N,N-dimethylaminomethyleneaminosulfonyl group. Furthermore, the *N*-acetyl benzenesulfonamide derivatives were pharmacologically evaluated with the aim of clarifying whether the introduction of the acetamidosulfonyl moiety instead of aminosulfonyl group was able to affect the anti-inflammatory and antimicrobial activities of this class of compounds. Moreover, the inhibitory activities of COX-1 and COX-2 enzymes were investigated. A docking pose of the most active compounds (**6b** and **7b**) separately in the active site of the human COX-2 enzyme and DNA-gyrase B were also obtained. Additionally, the ulcerogenic and acute toxicity profiles of the active compounds were determined.

CHEMISTRY

Synthesis of the intermediate and target compounds was performed according to the reactions illustrated in Scheme **1** and Scheme **2**. The key intermediates 4-[3-phenyl-4-(thiocarbamoyl or substituted thiocarbamoylhydrazonomethyl)-1H-pyrazol-1-yl]benzenesulfonamides **2a-c** were obtained in a good yield by condensation of 4-(4-formyl-3-phenyl-1H-pyrazol-1-yl) benzenesulfonamide [26] with thiosemicarbazide or N^4-substituted thiosemicarbazides. ^{1}H-NMR spectra for these compounds revealed a singlet characteristic for methine proton at 8.19 – 8.35 ppm. The appearance of signals for methine proton at a high chemical shift indicates that these compounds exist as E-isomers [30]. Heating the key intermediates **2a-c** with ethyl bromoacetate in boiling ethanol resulted in the respective thiazolidinonyl derivatives **3a-c**. Similarly, reaction of **2a-c** with 4-bromophenacyl bromide yielded the proposed thiazolinyl derivatives **4a-c**. The target compounds, 1,3,4-thiadiazolines **5a-c** were obtained by reflux of the intermediates **2a-c** in acetic anhydride for 2 h. Furthermore, heating **2a-c** with 2M $FeCl_3$ solution, gave the expected 1,3,4-thiadiazole derivatives **6a-c** in a poor yield (Method I). Therefore it was decided to prepare the latter compounds by applying a different pathway involving hydrazinolysis of compounds **5a-c** followed by oxidation using 2M $FeCl_3$ solution according to a previously reported procedure [31]. Thus, stirring of compounds **5a-c** with hydrazine hydrate 85% at room temperature for 3 h afforded the corresponding thiadiazolinyl benzenesulfonamide derivatives **7a-c**. IR spectra of the latter compounds showed absorption bands characteristic for SO_2NH_2, NH and C=O functions whereas, their ^{1}H-NMR spectra revealed signals for one methyl group and thiadiazole C_2-H. Heating compounds **7a-c** with 2M $FeCl_3$ solution in Dioxane/ethanol mixture (2:1), in attempts to get the proposed 1,3,4-thiadiazole derivatives **6a-c** were unsuccessful. However, we were able to obtain the target products **6a-c** through oxidation of compounds **5a-c** using $KMnO_4$ followed by hydrazinolysis of the obtained



Scheme 1.

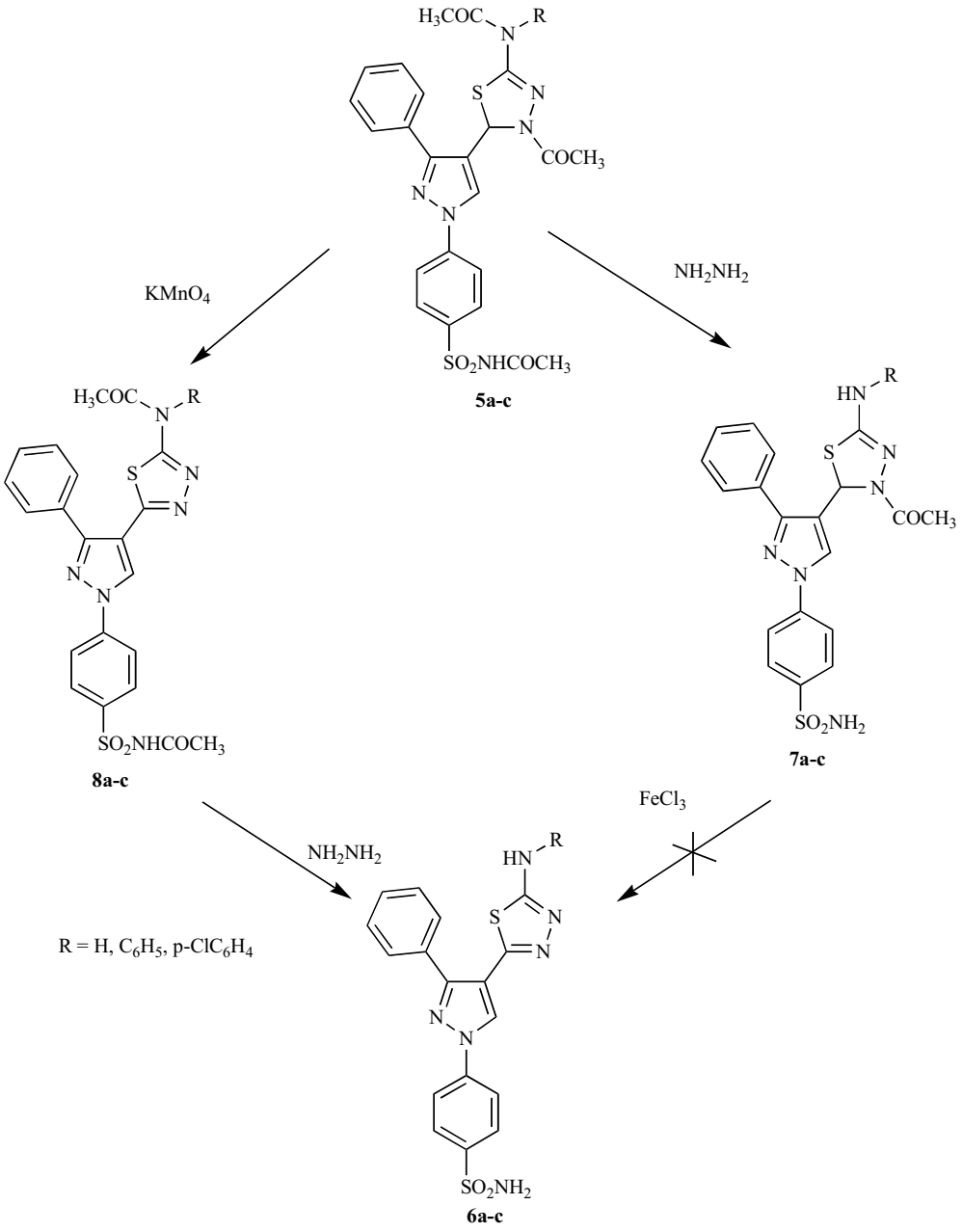
products [31]. Hence, stirring the selected N-acetyl thiadiazolinyl benzenesulfonamide derivatives **5a-c** with $KMnO_4$ in acetic acid at 10-15°C furnished the corresponding N-acetyl thiadiazolyl benzenesulfonamide derivatives **8a-c** in an excellent yield. IR spectra of the latter compounds revealed absorption bands characteristic for NH and two C=O functions while ^{1}H-NMR spectra lacked signal characteristic for thiadiazoline C_2-H. Hydrazinolysis of the latter compounds using hydrazine hydrate 85% at room temperature resulted in the expected thiadiazolyl benzenesulfonamide derivatives **6a-c** in a high yield (Method II). IR spectra of compounds **6a-c** lacked C=O absorption bands, while their ^{1}H-NMR spectra lacked signals characteristic for $COCH_3$ protons. Products from both methods (I & II) gave identical R_f value in TLC, melting point and IR spectra.

RESULTS AND DISCUSSION

Anti-Inflammatory Activity

Cotton Pellet Induced Granuloma Bioassay

The anti-inflammatory activity of the target compounds **3, 4, 5, 6, 7** and **8** was evaluated by applying the cotton pellet-induced granuloma bioassay in rats as described earlier [27] using indomethacin and celecoxib as reference standards. The ED_{50} value for each of the test compounds was expressed as the mean ± SEM. Significant difference between the control and treated groups was estimated using student's t-test and was considered significant at $P < 0.001$ (Table **1**). Most of the test compounds showed significant anti-inflammatory activity comparable to indomethacin and celecoxib, and four of them **4c, 5c, 6b** and **7b** possessed anti-inflammatory activity (ED_{50} = 8.16, 9.08, 8.06 and 8.32 µmol respectively) surpassing that of indomethacin and celecoxib (ED_{50} = 9.68 and 16.74 µmol respectively). The results revealed that in general, pyrazolyl benzenesulfonamide derivatives having thiazolidinonyl and thiazolinyl moieties separated from the pyrazole ring by a hydrazonomethyl link (compounds **3a-c** & **4a-c)** as well as N-acetylthiadiazolinyl benzenesulfonamides (compounds **5a-c)** appeared to possess higher anti-inflammatory activity than pyrazolyl benzenesulfonamides having thiadiazolyl and thiadiazolinyl moieties directly linked to the pyrazole ring (compounds **6** and **7)** except for compounds **6b** and **7b** which were nearly equipotent with **4c**. Within the three series **3, 4** and **5**, a direct association between the lipophilic character of the substituents (from hydrogen to phenyl to p-chlorophenyl) and the anti-inflammatory activity may exist. Moreover, the anti-inflammatory activity of the N-acetyl thiadiazolinyl benzenesulfonamide derivatives **5a-c** was found to be greater than the activity of their oxidized N-acetyl thiadiazolyl benzenesulfonamide analogs **8a-c**. Furthermore, a marked increase in the anti-inflammatory activity was observed when



Scheme 2.

the N-acetyl thiadiazolyl benzenesulfonamide derivatives **8a-c** were hydrolysed to the corresponding thiadiazolyl benzenesulfonamides **6a-c**. Compounds **4c, 6b** and **7b** were proved to be the most potent anti-inflammatory agents (ED_{50} = 8.16, 8.06 and 8.32 μmol respectively) whereas compounds **8a-c** (ED_{50} values range 14.52 – 16.98 μmol) were found to be the least active as anti-inflammatory agents (Table **1**).

Carrageenan-Induced Rat Paw Edema Bioassay

Compounds showing promising anti-inflammatory activity in the cotton pellet-induced granuloma bioassay (**3c, 4b, 4c, 5c, 6b** and **7b**) were further evaluated for their *in vivo* systemic effect using carrageenan-induced paw edema bioassay in rats as described earlier [27]. The results showed that compounds **6b** and **7b** displayed systemic anti-inflammatory activity (% protection = 75.5 and 76.5, respectively) slightly less than celecoxib (% protection = 77.5) but slightly higher than indomethacin (% protection = 74.4). However, the other test compounds **3c, 4b, 4c,** and **5c** (% protection = 72.4, 72.4, 71.4 and 73.4 respectively) were slightly less active than both celecoxib and indomethacin (Table **2**).

Human COX-1 and COX-2 Enzymatic Activity

Compounds **3c, 4b, 4c, 5c, 6b** and **7b** that showed potent anti-inflammatory profiles in animal models were further tested for their ability to inhibit human COX-1 and COX-2 enzymes *in vitro* as described by Wakitani *et al*. [32]. COX-

Table 1. The Anti-Inflammatory Activity for Compound 3-8 (ED_{50}, μmol/Cotton Pellet)[a] and Ulcerogenic Activity Compounds 3-8[a]

Test Compound	ED_{50} (μmol)	% Ulceration
Indomethacin	9.68 ± 0.27	100
Celecoxib	16.74 ± 0.22	0.0
3a	10.22 ± 0.18	0.0
3b	10.12 ± 0.27	0.0
3c	9.62 ± 0.12	0.0
4a	10.84 ± 0.14	10
4b	9.75 ± 0.14	0.0
4c	8.16 ± 0.32	0.0
5a	10.28 ± 0.26	0.0
5b	10.02 ± 0.14	0.0
5c	9.08 ± 0.16	20
6a	10.22 ± 0.17	0.0
6b	8.06 ± 0.32	0.0
6c	13.28 ± 0.44	10
7a	11.58 ± 0.33	20
7b	8.32 ± 0.15	10
7c	10.34 ± 0.42	20
8a	14.52 ± 0.22	0.0
8b	16.68 ± 0.34	0.0
8c	16.98 ± 0.18	0.0

[a] All data are significantly different from control ($P < 0.001$)

1 assay was carried out using platelets microsome fraction. Human platelets were prepared from NSAID-free normal human volunteers according to the method of Hammarström and Falardeau [33]. COX-2 assay was performed utilizing human recombinant COX-2 purchased from Sigma-Aldrich. The inhibitory activity was expressed as the concentration of the compound causing 50% enzyme inhibition (IC_{50} μmol) (Table **3**). The results revealed that the test compounds exhibited weak inhibitory activity against COX-1 enzyme (IC_{50} values between 79.52 and > 100 μmol) compared with indomethacin (IC_{50} = 0.26 μmol), however compounds **3c, 4b** and **5c** were more potent than celecoxib (IC_{50} = > 100 μmol). The test compounds showed higher inhibitory profile against COX-2 (IC_{50} values between 0.43 and 1.85 μmol) compared with indomethacin (IC_{50} = 2.63 μmol). All test compounds showed approximate selectivity ratio (COX-1 / COX-2) lower than that of celecoxib. In general, the results revealed that compounds having thiadiazolyl and thiadiazolinyl moieties directly attached to the pyrazole ring (compounds **6b** and **7b)** possessed higher selectivity towards COX-2 enzyme than the N-acetyl thiadiazolinyl derivative **5c** and those derivatives having thiazolidinonyl and thiazolinyl moieties separated from the pyrazole ring by a hydrazonomethyl link (compounds **3c, 4b** and **4c)**. However, within this latter class of compounds, the selective COX-2 inhibition of thiazolinyl derivatives **4b** and **4c** was greater than the thiazolidinonyl derivative **3c**. Compounds **6b** and **7b**, the most selective COX-2 inhibitors (approximate selectivity ratio = 232.55 & 196.07) in the present study, had lower selectivity as compared with celecoxib (approximate selectivity ratio > 333).

Ulcerogenic Effect

The target compounds were evaluated for their ulcerogenic potential in rats [34]. All the test compounds proved to have superior gastrointestinal safety profiles (0-20% ulceration) in the population of test animals at oral doses of 30 μmol / kg per day, compared with indomethacin, the reference drug, which was found to cause 100% ulceration under the same experimental conditions (Table **1**). Compounds **4a, 5c, 6c** and **7a-c** showed a slight ulcerogenic effect (10-20%). Gross observation of the isolated rat stomachs showed a normal stomach texture for the other compounds.

Table 2. Effect of Compounds 3c, 4b, 4c, 5c and 6b on Carrageenan-Induced Rat Paw Edema (ml), % Protection and Activity Relative to Indomethacin

Test Compound	Increase in Paw Edema (ml) ± SEM[a,b]	% Protection	Activity Relative to Indomethacin
Control	0.98 ± 0.027	0.0	0.0
Indomethacin	0.25 ± 0.024	74.4	100
Celecoxib	0.22 ± 0.016	77.5	104.23
3c	0.27 ± 0.021	72.4	97.31
4b	0.27 ± 0.016	72.4	97.31
4c	0.28 ± 0.026	71.4	95.96
5c	0.26 ± 0.022	73.4	98.65
6b	0.24 ± 0.018	75.5	101.47
7b	0.23 ± 0.018	76.5	102.82

[a] SEM denotes the standard error of the mean.

[b] All data are significantly different from control ($P < 0.001$)

Acute Toxicity

Compounds **3c, 4b, 4c, 5c, 6b** and **7b** were further evaluated for their oral acute toxicity in male mice using a previously reported method [27, 35]. The results indicated that most of the test compounds proved to be non-toxic and well tolerated by experimental animals up to 140 mg/kg. Moreover, these compounds were tested for their toxicity through the parenteral route [20]. The results revealed that all test compounds were non toxic up to 75 mg/kg.

Docking Studies

Molecular docking studies of compounds **- 6b** and **7b** were performed using Molecular Operating Environment (MOE-Dock 2006) module [36] in order to rationalize the obtained biological results. Molecular docking studies further helps in understanding the various interactions between the ligand and enzyme active site in detail.

The determination of the three-dimensional co-crystal structure of COX-2 complex with a selective inhibitor, **SC-558** [Fig. (**2**); PDB ID: 1CX2] has led to the development of a model for the topography of the NSAIDs binding site in human COX-2 [37]. Therefore herein, we performed the docking studies using this COX-2 co-crystal structure with **SC-558** as a template

The binding interactions of compounds **6b**, **7b** to the active site of COX-2, respectively, are shown in Figs. (**3**, **4**); where they exhibited some similar interactions as **SC-558**. Compound **6b** showed hydrogen bond interactions with Arg

Table 3. In Vitro Human COX-2[a] and COX-1[b] Enzymes Inhibitory Activities of Compounds 3c, 4b, 4c, 5c, 6b and 7b

Test compound	COX-2 IC_{50} (μM)[c]	COX-1 IC_{50} (μM)[c]	Approximate Selectivity Ratio COX-1/COX-2
Indomethacin	2.63 ± 0.02	0.26 ± 0.32	0.098
Celecoxib	< 0.3	> 100	333
3c	1.85 ± 0.04	86.62 ± 0.26	46.82
4b	1.42 ± 0.06	79.52 ± 0.24	56
4c	0.61 ± 0.06	> 100	163.93
5c	0.98 ± 0.02	96.32 ± 0.16	98.28
6b	0.43 ± 0.04	> 100	232.55
7b	0.51 ± 0.06	> 100	196.92

[a] Human recombinant COX-2 enzyme

[b] Human COX-1 enzyme from human platelets

[c] Values are means of at least four experiments

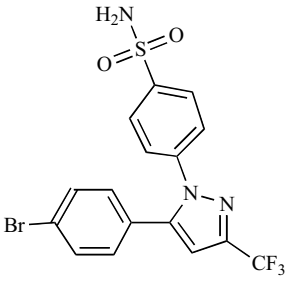


Fig. (2). Structure of SC-558.

120, Gly 354 and Tyr 355 and hydrophobic interactions with His 90, Arg 120, Val 349, Leu 352, Ser 353, Tyr 355, Leu 359, Ala 516, Val 523 and Leu 531. Compound **7b** displayed hydrogen bond interactions with Arg 513 and Asp 515 and hydrophobic interactions with His 90, Arg 120, Gln 192, Leu 352, Ser 353, Tyr 355, Arg 513, Asp 515, Ala 516 and Val 523. Similar interactions have been found with Nimesulide [38] and diaryloxazolone derivatives [39] suggesting similar steric interactions to those reported for celecoxib, rofecoxib and oxazolones [39]. In addition, the *in vitro* study showed weak inhibitory activity against COX-1 for these compounds.

Antimicrobial Activity

The designed compounds **3, 4, 5, 6, 7** and **8** were evaluated for their *in vitro* antimicrobial activity against *Escherichia coli* (*E. coli* ATCC 25922), as an example of Gram negative bacteria, *Staphylococcus aureus* (*S. aureus* ATCC 19433) as an example of Gram positive bacteria, and Candida albicans (*C. albicans*) as a representative of fungi. The micro dilution susceptibility test in Müller-Hinton Broth (Oxoid) and Sabouraud liquid Medium (Oxoid) were used for determination of antibacterial and antifungal activities [40]. The minimal inhibitory concentration (MIC; μg/ml) of the test compounds are shown in Table **4**. The results revealed that most of the newly synthesized compounds exhibited appreciable antibacterial activity. Acetylthiadiazoline derivatives **5a, 5b, 5c** and **7a** exhibited antibacterial activity against *E. coli* comparable to ampicillin, whereas thiazolidinones **3b, 3c,** thiazolines **4b, 4c,** thiadiazoles **6a, 6b** and acetylthiadiazoline **7b** derivatives showed half the antibacterial activity of ampicilin. On the other hand thiadiazole **6b** showed antibacterial activity against *S. aureus*. comparable to ampicillin. In addition, compounds **3c, 4c, 5b, 7b** and **7c** displayed half the activity of ampicillin against *S. aureus*. The rest of the test compounds were weakly active against both organisms. However, none of the screened compounds showed significant activity against *Candida albicans* (MIC values > 200) compared with the reference antifungal agent clotrimazole (MIC 12.5 μg/ml).

In a study to investigate the mechanism for the antimicrobial activity of the most active thiadiazole **6b** and acetylthiadiazoline **7b** derivatives, we examined the compounds for their inhibition of DNA gyrase [41] (full data will be reported elsewhere). Compounds **6b** and **7b** showed promising DNA gyrase inhibitory activity, the IC_{50} is 2.5 μg / ml and 5 μg / ml, respectively. Furthermore, we performed the docking studies of **6b** and **7b** with DNA-gyrase B (PDB ID: 1EI1). The binding of compounds **6b** and **7b** to the active site of DNA-gyrase are shown in Fig. (**5** and **6**). Compound **6b** showed hydrogen bond interactions with Asp 173 and Tyr 109 and hydrophobic interactions with Val 43, Val 44, Asn 46, Ala 47, Val 71, Gln 72, Ile 78, Ala 100, Gly 101, Tyr 109, Thr 165 and Val 167. Compound **7b** displayed hydrogen bond interactions with Gly 102 and hydrophobic interactions with Val 43, Asn 46, Ala 47, Val 71, Asp 73, Ile

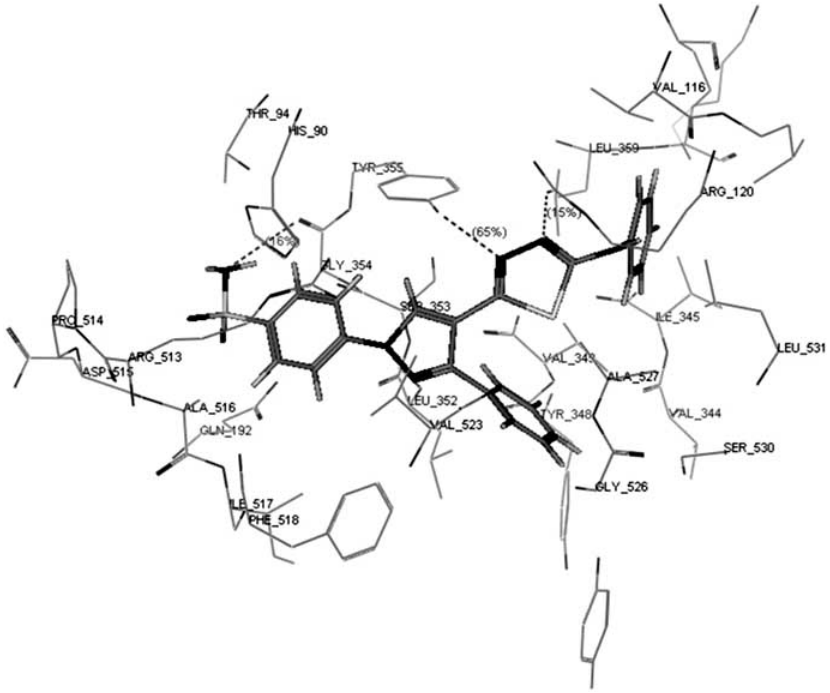


Fig. (3). 3D View from a molecular modeling study, of the minimum-energy structure of the complex of **6b** docked in COX-2 (PDB ID: 1CX2). Viewed using Molecular Operating Environment (MOE) module.

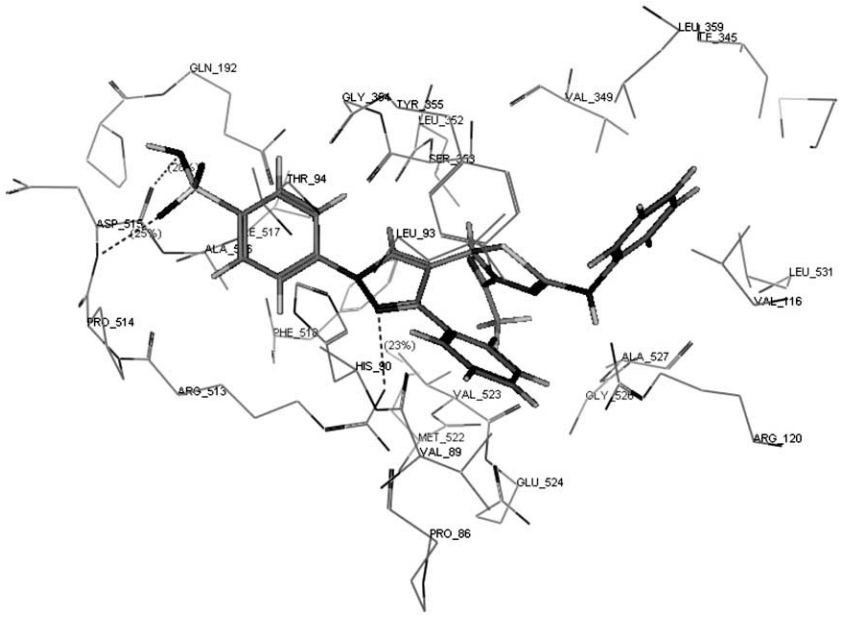


Fig. (4). 3D View from a molecular modeling study, of the minimum-energy structure of the complex of **7b** docked in COX-2 (PDB ID: 1CX2). Viewed using Molecular Operating Environment (MOE) module.

94, Gly 101, Lys 103, Ser 108, Thr 165, Met 166 and Val 167.

CONCLUSION

Most of the synthesized compounds exhibited moderate to good anti-inflammatory and antibacterial activity with no or minimal ulcerogenic effect and good safety margin. Compounds **6b** and **7b** were found to be the most potent anti-inflammatory agents in the present study. These compounds (**6b** and **7b)** displayed higher selective inhibitory activity towards COX-2 compared with indomethacin and exhibited promising antibacterial activity against both *E. coli* and *S. aureus*. Docking studies for both **6b** and **7b** with COX-2 (PDB ID: 1CX2) and DNA-gyrase B (PDB ID: 1EI1) showed good binding profile. Therefore, compounds **6b** and **7b** would represent a fruitful matrix for the development of a new class of dual anti-inflammatory antimicrobial agents that would deserve further investigation and derivatization.

It is worth mentioning that compounds **6b** and **7b** displayed anti-inflammatory profile comparable to our previously reported pyrazolyl benzenesulfonamide compounds **A**, **B** [26] and **C** [27]. However, the selective COX-2 inhibition of compounds **6b** and **7b** was higher than compounds **A, B** and **C**.

EXPERIMENTAL SECTION

Chemistry

Melting points were determined in open glass capillaries using Thomas Capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on Perkin-Elmer 1430 infrared spectrophotometer. ^{1}H-NMR spectra were scanned on Jeol-400 MHz or 500 MHz Spectrometer, and chemical shifts are given in δ (ppm) downfield from tetramethylsilane (TMS) as internal standard. Splitting patterns were designated as follows; s: singlet; d: doublet; t: triplet; m: multiplet. Elemental analyses were performed on Vario El Fab.-Nr. elemental analyzer, Faculty of Science, Assiut University, Assiut, Egypt and were found within ± 0.4% of the theoretical values. Follow up of the reactions and checking the purity of the compounds was made by thin layer chromatography (TLC) on silica gel-precoated aluminium sheets (Type 60 GF_{254} Merck) and the spots were detected by exposure to UV lamp at λ_{254} nm for few seconds.

4-[3-Phenyl-4-(thiocarbamoyl; or substituted thiocarbamoylhydrazonomethyl)-1H-pyrazol-1-yl]benzenesulfonamides (2a-c)

To a suspension of 4-(4-formyl-3-phenyl-1H-pyrazol-1-yl)benzenesulfonamide **1** (3 g, 9.17 mmol) in ethanol (30 ml), an equivalent amount of thiosemicarbazide or N^4-substituted thiosemicarbazide was added. The reaction mixture was heated under reflux for 2 h and allowed to cool to room temperature. The separated solid product was filtered, washed with ethanol and crystallized from the proper solvent (Table **5**). IR (cm^{-1}), **2a-c**: 3358-3313, 3301-3198 (NH_2), 3131-3113 (NH), 1595-1591 (C=N), 1339-1336 & 1160-1158 (SO_2), 1548-1525, 1328-1308, 1199-1184, 995-952 (NCS amide I, II, III and IV bands respectively). ^{1}H-NMR (DMSO-d_6), **2a**: δ 7.45 (s, 2H, SO_2NH_2, D_2O exchangeable), 7.48-7.54 (m, 3H, phenyl $C_{3,4,5}$-H), 7.67 (d, J = 7.65 Hz, 2H, phenyl $C_{2,6}$-H), 7.77 (s, 2H, NH_2, D_2O exchangeable), 7.96, 8.07 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$-H & $C_{2,6}$-H), 8.19 (s, 1H, CH=N), 9.30 (s, 1H, pyrazole C_5-H), 11.36 (s, 1H, NH, D_2O exchangeable). ^{1}H-NMR (DMSO-d_6), **2b**: δ 7.24 (t, J = 7.4 Hz, 1H, phenylthiocarbamoyl C_4-H), 7.41 (t, J = 7.7 Hz, 1H, phenyl C_4-H), 7.48 (s, 2H, NH_2, D_2O exchangeable), 7.50-7.61 (m, 6H, phenylthiocarbamoyl $C_{2,3,5,6}$-H and phenyl $C_{3,5}$-H), 7.74 (d, J = 7.7 Hz, 2H, phenyl $C_{2,6}$-H), 8.00, 8.11 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$-H & $C_{2,6}$-H), 8.35 (s, 1H, CH = N), 9.35 (s, 1H, pyrazole C_5-H), 9.84, 11.80 (2s, 2H, 2NH, D_2O exchangeable). ^{1}H-NMR

Table 4. Minimal Inhibitory Concentrations (MIC μg/ml) of Test Compounds

Test Compound	*E. coli*	*S. aureus*	*C. albicans*
3a	100	50	> 200
3b	50	> 200	> 200
3c	50	25	> 200
4a	> 200	100	> 200
4b	50	> 200	> 200
4c	50	25	> 200
5a	25	50	> 200
5b	25	25	> 200
5c	25	50	> 200
6a	50	100	> 200
6b	50	12.5	> 200
6c	> 200	100	> 200
7a	25	50	> 200
7b	50	25	> 200
7c	100	25	> 200
8a	> 200	100	> 200
8b	100	100	> 200
8c	> 200	100	> 200
Ampicillin	25	12.5	--
Clotrimazole	--	--	12.5

(DMSO-d_6), **2c**: δ 7.44-7.65 (m, 9H, phenyl-$C_{3,4,5}$-H, chlorophenyl-H and NH_2), 7.73 (d, J = 8.0 Hz, phenyl $C_{2,6}$-H), 8.01, 8.11 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$-H & $C_{2,6}$-H), 8.35 (s, 1H, CH=N), 9.34 (s, 1H, pyrazole C_5-H), 9.89, 11.88 (2s, 2H, 2NH, D_2O exchangeable).

4-[4-(4-Oxothiazolidin-2-ylidenehydrazonomethyl)-3-phenyl-1H-pyrazol-1-yl]benzenesulfonamide (3a), 4-[4-(4-Oxo-3-phenyl; or substituted phenylthiazolidin-2-ylidenehydrazonomethyl)-3-phenyl-1H-pyrazol-1-yl]benzenesulfonamides (3b and 3c)

To a suspension of the appropriate thiosemicarbazone **2a-c** (2.1 mmol) in absolute ethanol (15 ml), an equivalent amount of ethyl bromoacetate (0.26 g, 0.18 ml) was added. The reaction mixture was heated under reflux for 2 h and allowed to cool to room temperature. The separated solid product was filtered, washed with ethanol, dried and crystallized from the proper solvent (Table **5**). IR (cm^{-1}) for compounds **3a-c**: 3383-3351 & 3289-3276 (NH_2), 3105 (NH), 1715-1699 (C=O), 1643-1638 & 1608-1593 (C=N), 1341-1338 & 1162-1153 (SO_2). ^{1}H-NMR (DMSO-d_6), **3a**: δ 3.91 (s, 2H, thiazole C_5-H_2), 7.46 (s, 2H, NH_2, D_2O exchangeable), 7.48-7.56 (m, 3H, phenyl $C_{3,4,5}$-H), 7.86 (d, J = 7.3 Hz, 2H, phenyl $C_{2,6}$-H), 7.98, 8.21 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$-H & $C_{2,6}$-H), 8.42 (s, 1H, CH=N), 9.08 (s, 1H, pyrazole C_5-H), 11.91 (s, 1H, NH, D_2O exchangeable). ^{1}H-NMR (DMSO-d_6), **3b**: δ 4.11 (s, 2H, thiazole C_5-H_2), 7.38-7.68 (m, 10H, thiazolidinephenyl -H, phenyl $C_{3,4,5}$-H and NH_2), 7.83 (d, J = 7.3 Hz, 2H, phenyl $C_{2,6}$-H), 7.97, 8.17 (2d, J = 8.8 Hz, 4H benzenesulfonamide $C_{3,5}$-H and $C_{2,6}$-H), 8.27 (s, 1H, CH=N), 9.04 (s, 1H, pyrazole C_5-H). ^{1}H-NMR (DMSO-d_6), **3c**: δ 4.05 (s, 2H, thiazole C_5-H_2), 7.28 (d, J = 8.4 Hz, 2H, chlorophenyl $C_{2,6}$-H), 7.32-7.41 (m, 3H, phenyl $C_{3,4,5}$-H), 7.48 (d, J = 8.4 Hz, 2H, chlorophenyl $C_{3,5}$-H), 7.60 (s, 2H, NH_2, D_2O exchangeable), 7.74 (d, J = 7.0 Hz, 2H, phenyl $C_{2,6}$-H), 7.88, 8.04 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$-H & $C_{2,6}$-H), 8.14 (s, 1H, CH=N), 9.16 (s, 1H, pyrazole C_5-H).

4-[4-(4-Bromophenyl-2,3-dihydrothiazol-2-ylidenehydrazonomethyl)-3-phenyl-1H-pyrazol-1-yl]benzenesulfonamide (4a), 4-[4-(4-Bromophenyl-3-phenyl; or substituted phenyl-2,3-dihydrothiazol-2-ylidenehydrazonomethyl)-3-phenyl-1H-pyrazol-1-yl]benzenesulfonamides (4b and 4c)

A mixture of the selected thiosemicarbazone **2a-c** (2.1 mmol) and p-bromophenacyl bromide (0.58 g, 2.1 mmol) in ethanol (15 ml) was heated under reflux for 2 h and left to attain room temperature. The separated solid product was

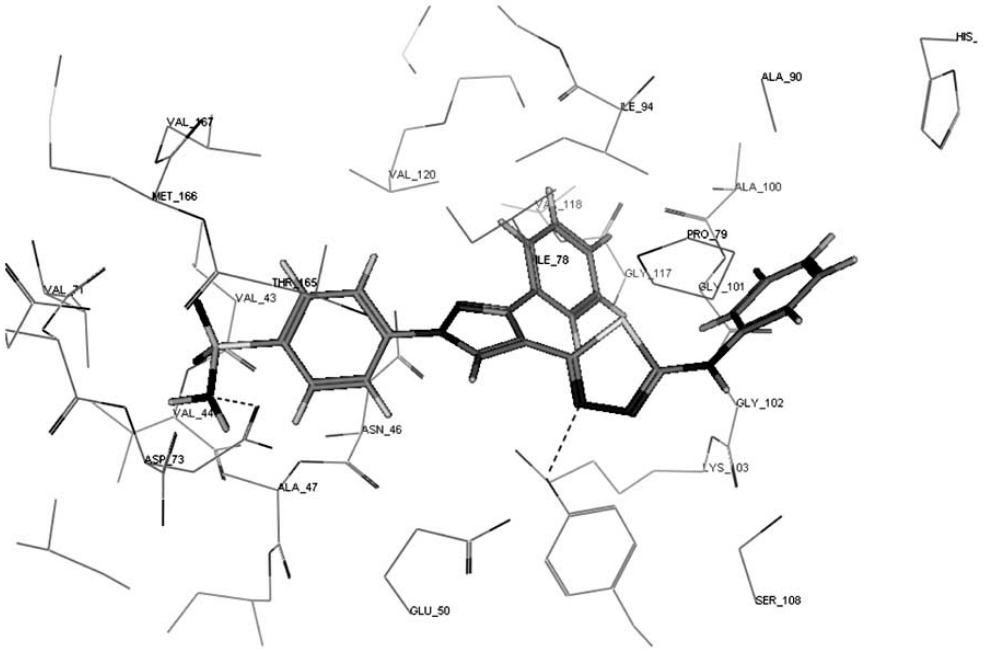


Fig. (5). 3D View from a molecular modeling study, of the minimum-energy structure of the complex of **6b** docked in DNA-gyrase B (PDB ID: 1EI1). Viewed using Molecular Operating Environment (MOE) module.

filtered, washed with ethanol, dried and crystallized from the proper solvent (Table **5**). IR (cm^{-1}), **4a-c**: 3380-3352 & 3300-3282 (NH_2), 3209 (NH), 1616-1592 (C=N), 1335-1334 & 1164-1163 (SO_2). ^{1}H-NMR (DMSO-d_6), **4a**: δ 7.40 (s, 1H, thiazole C_5-H), 7.47 (s, 2H, NH_2, D_2O exchangeable), 7.49-7.58 (m, 5H, phenyl $C_{3,4,5}$-H & bromophenyl $C_{2,6}$-H), 7.60, 7.79, 7.97, 8.21 (4d, J = 8.8 Hz, 8H, phenyl $C_{2,6}$-H, bromophenyl $C_{3,5}$-H, benzenesulfonamide $C_{3,5}$-H and $C_{2,6}$-H), 8.17 (s, 1H CH=N), 9.02 (s, 1H, pyrazole C_5-H), 12.04 (s, 1H, NH, D_2O exchangeable). ^{1}H-NMR (DMSO, d_6), **4b**: δ 6.76 (s, 1H, thiazole C_5-H), 7.13 (d, J = 8.6 Hz, 2H, thiazolephenyl $C_{2,6}$-H), 7.43-7.56 (m, 14H, thiazolephenyl $C_{3,4,5}$-H, phenyl-H, bromophenyl-H and NH_2), 7.63, 7.97 (2d, J = 8.0 Hz, 4H, benzenesulfonamide $C_{3,5}$-H, and $C_{2,6}$-H), 7.92 (s, 1H, CH=N), 8.57 (s, 1H, pyrazole C_5-H). ^{1}H-NMR (DMSO, d_6), **4c**: δ 6.82 (s, 1H, thiazole C_5-H), 7.17 (d, J = 8.0 Hz, 2H, chlorophenyl $C_{2,6}$-H), 7.52 (s, 2H, NH_2, D_2O exchangeable), 7.53-7.69 (m, 14H, phenyl-H, chlorophenyl $C_{3,5}$-H, bromophenyl-H, benzenesulfonamide $C_{3,5}$-H & CH=N), 8.04 (d, J = 8.8 Hz, 2H, benzenesulfonamide $C_{2,6}$-H), 8.60 (s, 1H, pyrazole C_5-H).

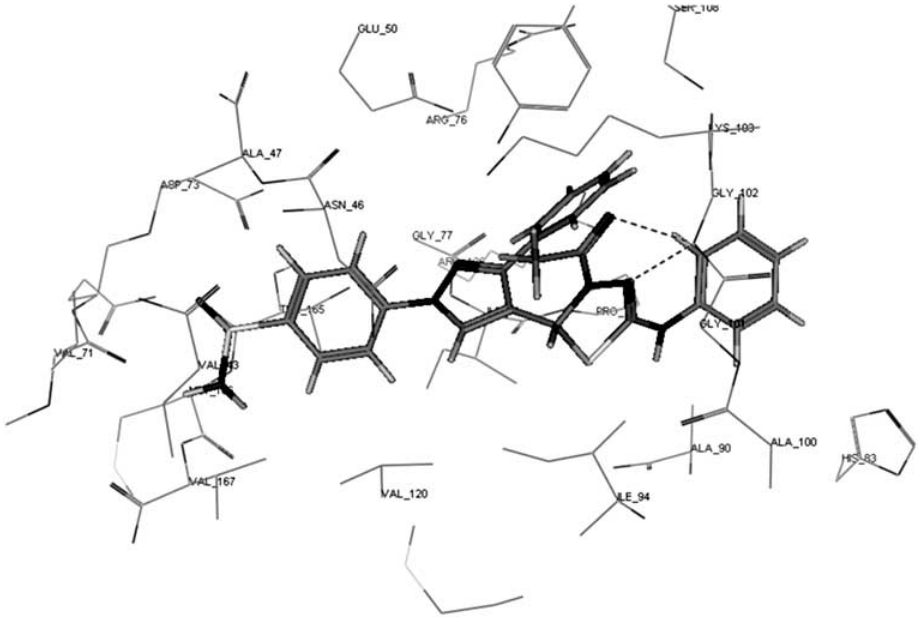


Fig. (6). 3D View from a molecular modeling study, of the minimum-energy structure of the complex of **7b** docked in DNA-gyrase B (PDB ID: 1EI1). Viewed using Molecular Operating Environment (MOE) module.

Table 5. Physical and Analytical Data of Compounds 2-8

Comp. No.	R	Yield %	M.P.(°C) Cryst. Solvent	Mol. Formula (Mol. wt)
2a	H	82	240-241 (Dioxane/H_2O)(8:1)	$C_{17}H_{16}N_6O_2S_2$ (400.48)
2b	C_6H_5	84	208-210 (Dioxane/H_2O)(8:1)	$C_{23}H_{20}N_6O_2S_2$ (476.58)
2c	4-ClC_6H_4	85	262-264 (Dioxane/H_2O)(8:1)	$C_{23}H_{19}ClN_6O_2S_2$ (511.02)
3a	H	79	292-294 (DMF/H_2O)(6:1)	$C_{19}H_{16}N_6O_3S_2$ (440.50)
3b	C_6H_5	82	296-298 (DMF/H_2O)(6:1)	$C_{25}H_{20}N_6O_3S_2$ (516.60)
3c	4-ClC_6H_4	82	309-310 (DMF/H_2O)(6:1)	$C_{25}H_{19}ClN_6O_3S_2.½\ H_2O$ (560.05)
4a	H	72	239-240 (DMF/H_2O)(6:1)	$C_{25}H_{19}BrN_6O_2S_2$ (579.50)
4b	C_6H_5	74	228-229 (DMF/H_2O)(6:1)	$C_{31}H_{23}BrN_6O_2S_2.\ 1\ H_2O$ (673.61)
4c	4-ClC_6H_4	74	179-181 (DMF/H_2O)(6:1)	$C_{31}H_{22}BrClN_6O_2S_2.1\ H_2O$ (708.06)
5a	H	84	301-303 (EtOH)	$C_{23}H_{22}N_6O_5S_2.\ ½\ H_2O$ (535.60)
5b	C_6H_5	85	157-158 (EtOH/H_2O)(6:1)	$C_{29}H_{26}N_6O_5S_2.2\ H_2O$ (638.72)
5c	4-ClC_6H_4	85	164-165 (EtOH/H_2O)(6:1)	$C_{29}H_{25}ClN_6O_5S_2.\ 2\ H_2O$ (673.17)
6a	H	73 (Method II)	230-232 (DMF/H_2O)(5:1)	$C_{17}H_{14}N_6O_2S_2.\ ½\ H_2O$ (407.47)
6b	C_6H_5	71 (Method II)	258-259 (DMF/H_2O)(5:1)	$C_{23}H_{18}N_6O_2S_2.\ ½\ H_2O$ (483.57)
6c	4-ClC_6H_4	71 (Method II)	295-296 (DMF/H_2O)(5:1)	$C_{23}H_{17}ClN_6O_2S_2$ (509.01)
7a	H	67	290 (EtOH/H_2O)(5:1)	$C_{19}H_{18}N_6O_3S_2$ (442.52)
7b	C_6H_5	65	227-228 (Dioxane/H_2O)(8:1)	$C_{25}H_{22}N_6O_3S_2.\ 1\ H_2O$ (536.63)
7c	4-ClC_6H_4	66	196-198 (Dioxane/H_2O)(8:1)	$C_{25}H_{21}ClN_6O_3S_2.\ 1\ H_2O$ (571.08)
8a	H	71	310-312 (DMF/H_2O)(6:1)	$C_{21}H_{18}N_6O_4S_2.\ ½\ H_2O$ (491.55)
8b	C_6H_5	73	265-266 (DMF/H_2O)(6:1)	$C_{27}H_{22}N_6O_4S_2$ (558.64)
8c	4-ClC_6H_4	73	262-263 (DMF/H_2O)(6:1)	$C_{27}H_{21}ClN_6O_4S_2.\ ½\ H_2O$ (602.08)

N-Acetyl-4-[4-(5-acetamido; or N-substituted acetamido-3-acetyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)-3-phenyl-1H-pyrazol-1-yl]benzenesulfonamides (5a-c)

A solution of the selected thiosemicarbazone **2a-c** (2 mmol) in acetic anhydride (10 ml) was heated under reflux for 2 h. The reaction mixture was left to attain room temperature, cold water (10 ml) was then added and the mixture was stirred for 30 min to decompose excess acetic anhydride. The separated solid product was filtered, washed with water, dried and crystallized from the proper solvent (Table **5**). IR (cm^{-1}) **5a-c**: 3454-3305 (NH), 1717-1640 (C=O), 1597-1593 (C=N), 1366-1343, 1163-1161 (SO_2). ^{1}H-NMR (DMSO-d_6), **5a**: δ 1.85, 1.93, 2.07 (3s, each 3H, 3 $COCH_3$), 6.90 (s, 1H, thiadiazole C_2-H), 7.32-7.42 (m, 3H, phenyl $C_{3,4,5}$-H), 7.55 (d, J = 6.9 Hz, phenyl $C_{2,6}$-H), 7.86, 8.05 (2d, J = 8.7 Hz, 4H, benzenesulfonamide $C_{3,5}$-H and $C_{2,6}$-H), 8.43 (s, 1H, pyrazole C_5-H), 11.94 (s, 1H, NH, D_2O exchangeable), 12.17 (s, 1H, SO_2NH, D_2O exchangeable). ^{1}H-NMR (DMSO-d_6), **5b**: δ 1.86, 1.87, 1.94 (3s, each 3H, 3 $COCH_3$), 7.11 (s, 1H, thiadiazole C_2-H), 7.46-7.58 (m, 8H, acetamidophenyl-H and phenyl $C_{3,4,5}$-H), 7.76 (d, J = 7.3 Hz, 2H, phenyl $C_{2,6}$-H), 8.02, 8.16 (2d, J = 9.0 Hz, 4H, benzenesulfonamide $C_{3,5}$-H and $C_{2,6}$-H), 8.47 (s, 1H, pyrazole C_5-H), 12.15 (s, 1H, NH, D_2O exchangeable). ^{1}H-NMR (DMSO-d_6), **5c**: δ 1.87, 1.89, 1.94 (3s, each 3H, 3 $COCH_3$), 7.12 (s, 1H, thiadiazole C_2-H), 7.47-7.66 (m, 7H, phenyl $C_{3,4,5}$-H and chlorophenyl-H), 7.77 (d, J = 7.3 Hz, 2H, phenyl $C_{2,6}$-H), 8.03, 8.15 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$-H and $C_{2,6}$-H), 8.46 (s, 1H, pyrazole C_5-H), 12.15 (s, 1H, NH, D_2O exchangeable).

4-[4-(5-Amino; or substituted amino-1,3,4-thiadiazol-2-yl)-3-phenyl-1H-pyrazol-1-yl]benzenesulfonamides (6a-c)

Method I

To a boiling well stirred solution of the proper thiosemicarbazone **2a-c** (3 mmol) in a mixture of dioxane/ethanol (15 ml) (2:1), an aqueous solution of 2M $FeCl_3$ (0.6 ml) was added dropwise. The reaction mixture was heated under reflux for 30 min and then concentrated under vacuum. The concentrated reaction mixture was allowed to attain room temperature and treated with cold water. The separated solid product was filtered, dried and crystallized from the proper solvent (Table **5**).

Method II

A mixture of the appropriate N-acetyl thiadiazolyl benzenesulfonamide derivatives **8a-c** (1.8 mmol) and hydrazine hydrate 85% (6 ml) was stirred at room temperature for 3 h. The reaction mixture was diluted with cold water and left to stand overnight. The separated solid product was filtered, dried and crystallized from the proper solvent (Table **5**). IR (cm^{-1}) **6a-c** : 3399-3171 (NH_2), 3125-3119 (NH), 1597-1594 (C=N), 1343-1339, 1162-1159 (SO_2). ^{1}H-NMR (DMSO-d_6), **6a**: δ 7.28 (s, 2H, SO_2NH_2, D_2O exchangeable), 7.44-7.50 (m, 5H, phenyl-H), 7.72 (s, 2H, NH_2, D_2O exchangeable), 7.97, 8.18 (2d, J = 9.1 Hz, 4H, benzenesulfonamide $C_{3,5}$-H & $C_{2,6}$-H), 9.14 (s, 1H, pyrazole C_5-H). ^{1}H-NMR (DMSO-d_6), **6b**: δ 7.1 (t, J = 7.3 Hz, 1H, phenylamino C_4-H), 7.35 (t, J = 7.9 Hz, 1H, phenyl C_4-H), 7.45 (s, 2H, NH_2, D_2O exchangeable), 7.48-7.54 (m, 4H, phenylamino $C_{2,3,5,6}$-H), 7.63 (d, J = 7.9 Hz, 2H, phenyl $C_{3,5}$-H), 7.75-7.78 (m, 2H, phenyl $C_{2,6}$-H), 7.99, 8.20 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$-H & $C_{2,6}$-H), 9.26 (s, 1H, pyrazole C_5-H), 10.41 (s, 1H, NH, D_2O exchangeable). ^{1}H-NMR (DMSO-d_6), **6c**: δ 7.40 (d, J = 8.8 Hz, 2H, chlorophenyl $C_{2,6}$-H), 7.48 (s, 2H, NH_2, D_2O exchangeable), 7.49-7.53 (m, 3H, phenyl $C_{3,4,5}$-H), 7.68 (d, J = 8.8 Hz, 2H, chlorophenyl $C_{3,5}$-H), 7.74-7.78 (m, 2H, phenyl $C_{2,6}$-H), 7.99, 8.21 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$-H & $C_{2,6}$-H), 9.26 (s, 1H, pyrazole C_5-H), 10.55 (s, 1H, NH, D_2O exchangeable).

4-[4-(3-Acetyl-5-amino; or substituted amino-2,3-dihydro-1,3,4-thiadiazol-2-yl)-3-phenyl-1H-pyrazol-1-yl]benzenesulfonamides (7a-c)

A mixture of the proper N-acetyl thiadiazolinyl benzenesulfonamide derivatives **5a-c** (2 mmol) and hydrazine hydrate 85% (8 ml) was stirred at room temperature for 3 h. The reaction mixture was diluted with cold water and left to stand overnight. The separated solid product was filtered, dried and crystallized from the proper solvent (Table **5**).

IR (cm^{-1}), **7a-c**: 3322-3141 (NH_2, NH), 1619-1617 (C=O), 1597-1595 (C=N), 1356-1338 & 1162-1155 (SO_2). ^{1}H-NMR (DMSO-d_6), **7a**: δ 2.15 (s, 3H, $COCH_3$), 6.69 (s, 2H, NH_2, D_2O exchangeable), 7.23 (s, 1H, thiadiazole C_2-H), 7.40-7.53 (m, 5H, phenyl $C_{3,4,5}$-H and SO_2NH_2), 7.73 (d, J = 8.4 Hz, 2H, phenyl $C_{2,6}$-H), 7.98, 8.11 (2d, J = 8.4 Hz, 4H, benzenesulfonamide $C_{3,5}$-H & $C_{2,6}$-H), 8.40 (s, 1H, pyrazole C_5-H). ^{1}H-NMR (DMSO-d_6), **7b**: δ 2.33 (s, 3H, $COCH_3$), 6.98 (t, J = 7.3 Hz, 1H, phenylamino C_4-H), 7.32 (t, J = 7.7 Hz, 1H, phenyl C_4-H), 7.35 (s, 2H, NH_2, D_2O exchangeable), 7.42 (s, 1H, thiadiazole C_2-H), 7.45-7.54 (m, 6H, phenylamino $C_{2,3,5,6}$-H & phenyl $C_{3,5}$-H), 7.78 (d, J = 7.7 Hz, 2H, phenyl $C_{2,6}$-H), 7.92, 8.14 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$-H & $C_{2,6}$-H), 8.53 (s, 1H, pyrazole C_5-H), 9.58 (s, 1H, NH, D_2O exchangeable). ^{1}H-NMR (DMSO-d_6), **7c**: δ 2.32 (s, 3H, $COCH_3$), 7.36 (s, 2H, NH_2, D_2O exchangeable), 7.38 (s, 1H, thiadiazole C_2-H), 7.42-7.55 (m, 7H, chlorophenyl $C_{2,3,5,6}$-H and phenyl $C_{3,4,5}$-H), 7.78 (d, J = 7.4 Hz, 2H, phenyl $C_{2,6}$-H), 7.92, 8.14 (2d, J = 8.8 Hz, 4-H, benzenesulfonamide $C_{3,5}$-H & $C_{2,6}$-H), 8.54 (s, 1H, pyrazole C_5-H), 9.73 (s, 1H, NH, D_2O exchangeable).

N-Acetyl-4-[4-(5-acetamido; or N-substituted acetamido-1,3,4-thiadiazol-2-yl)-3-phenyl-1H-pyrazol-1-yl]benzenesulfonamides (8a-c)

Potassium permanganate (1.5 g, 10 mmol) was added portion-wise at 10-15^{o}C to a stirred suspension of the selected N-acetylthiadiazolinyl benzenesulfonamide derivatives **5a-c** (4 mmol) in acetic acid (25 ml). After being stirred for 2 h at room temperature, the mixture was diluted with water, treated with 30% H_2O_2 in an ice-water bath, and left to stand overnight. The separated solid product was filtered, washed with water, dried and crystallized from the proper solvent (Table **5**). IR (cm^{-1}), **8a-c**: 3246-3184 (NH), 1725-1686 (C=O), 1593 (C=N), 1349-1347 & 1161-1160 (SO_2). ^{1}H-NMR (DMSO-d_6), **8a**: δ 1.96, 2.19 (2s, each 3H, 2 $COCH_3$), 7.45-7.55 (m, 3H, phenyl $C_{3,4,5}$-H), 7.73 (m, 2H, phenyl $C_{2,6}$-H), 8.07, 8.25 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$-H and $C_{2,6}$-H), 9.35 (s, 1H, pyrazole C_5-H), 12.19 (s, 1H, NHCO, D_2O exchangeable), 12.60 (s, 1H, SO_2NH, D_2O exchangeable). ^{1}H-NMR (DMSO-d_6), **8b**: δ 1.95, 1.99 (2s, each 3H, 2 $COCH_3$), 7.45-7.62 (m, 8H, phenyl $C_{3,4,5}$-H and

acetamidophenyl-H), 7.74-7.78 (m, 2H, phenyl $C_{2,6}$-H), 8.07, 8.26 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$-H and $C_{2,6}$-H), 9.35 (s, 1H, pyrazole C_5-H), 12.19 (s, 1H, NH, D_2O exchangeable). ^{1}H-NMR (DMSO-d_6), **8c**: δ 1.95, 2.01 (2s, each 3H, 2 $COCH_3$), 7.45-7.50 (m, 3H, phenyl $C_{3,4,5}$-H), 7.63, 7.67 (2d, J = 8.8 Hz, 4H, chlorophenyl-H), 7.74-7.79 (m, 2H, phenyl $C_{2,6}$-H), 8.08, 8.27 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$-H and $C_{2,6}$-H), 9.35 (s, 1H, pyrazole C_5-H), 12.19 (s, 1H, NH, D_2O exchangeable).

ANTI-INFLAMMATORY ACTIVITY

Cotton Pellet-Induced Granuloma Bioassay

Adult male Sprague-Dawley rats (120-140 g) obtained from Medical Research Institute, Alexandria University, were used. The rats were acclimated one week prior to use and allowed unlimited access to standard rat chow and water. Prior to the start of experiment, the animals were randomly divided into groups of six rats each. Cotton pellet (35 ± 1 mg) cut from dental rolls were impregnated with 0.2 ml (containing 10 µmol) of a solution of the test compound in chloroform and the solvent was allowed to evaporate. Each cotton pellet was subsequently injected with 0.2 ml of an aqueous solution of antibiotics (1 mg penicillin G and 1.3 mg dihydrostreptomycin/ml). Two pellets were implanted subcutaneously, one in each axilla of the rat, under mild general anesthesia. One group of animals received the standard reference indomethacin and the antibiotics at the same level. Pellets containing only the antibiotics were similarly implanted in the control rats. Seven days later, the animals were sacrificed and the two cotton pellets, with adhering granulomas, were removed, dried for 48 h at 60°C and weighed. The increment in dry weight (difference between the initial and final weights) was taken as a measure of granuloma ± SEM. This was calculated for each group and the percentage reduction in dry weight of granuloma from control value was also calculated. The ED_{50} values were determined through dose response curves using doses of 4, 7, 10 and 15 µmol for each compound.

Carrageenan-Induced Rat Paw Edema

Male albino rats weighing 120-150 g (Medical Research Institute, Alexandria University) were used throughout the work. They were kept in the animal house under standard conditions of light and temperature with free access to food and water. The animals were randomly divided into groups of six rats each. The paw edema was induced by subplantar injection of 50 µl of 2% carrageenan solution in saline (0.9%). Indomethacin and the test compounds were dissolved in DMSO and were injected subcutaneously in a dose of 10 µmol/kg body weight, 1 h prior to carrageenan injection. Control group was injected with DMSO only. The volume of paw edema (ml) was determined by means of plethysmometer immediately after injection of carrageenan and 4 h later. The increase in paw volume between time 0 and 4 h was measured as described earlier [27]. The percentage protection against inflammation was calculated as follows:

$$\frac{V_c - V_d}{V_c} \text{ X } 100$$

Where V_c is the increase in paw volume in the absence of test compound (control) and V_d is the increase in paw volume after injection of the test compound. Data were expressed as the mean ± SEM. Significant difference between the control and the treated groups was performed using Student's t-test and P values. The difference in the means was considered significant at $P < 0.001$. The anti-inflammatory activity of the test compounds relative to that of indomethacin was also calculated.

Human COX-1 and COX-2 Enzymatic Assay

Human COX-1 and COX-2 activities were determined according to Wakitani *et al*. [32]. Human COX-1 (0.3 mg protein/assay) or COX-2 (1 mg protein/assay) was suspended in 0.2 ml of 100 mmol Tris/HCl buffer (pH 8) containing hematin (2 mmol) and tryptophan (5 mmol) as cofactors. The reaction mixture was pre-incubated with each test compound individually for 5 min at 24°C. [^{14}C]-Arachidonic acid (100.00 dpm, 30 mmol) was added to the mixture and then incubated for 2 min (for COX-1) or 45 min (for COX-2) at 24°C. The reaction was stopped by addition of 400 µl of a solution composed of Et_2O/MeOH/1 M Citric acid (30:4:1, v/v/v). After centrifugation of the mixture at 1700 ×g for 5 min at 4°C, 50 µl of the upper phase was applied to a thin layer chromatography plate. Thin layer chromatography was performed at 4°C with solvent system consisting of Et_2O/MeOH/AcOH (90:2:0.1, v/v/v). Enzyme activity was calculated from the percent conversion of arachidonic acid to PGH2 and its decomposition products, using radiometric photographic system. The concentration of the compound causing 50% inhibition (IC_{50}) was calculated.

Ulcerogenic Effects

All target compounds were evaluated for their ulcerogenic potential in rats [34]. Indomethacin was used as reference standard. Male albino rats (100-120 g) were fasted for 12 h prior to administration of the compounds. Water was given *ad libitum*. The animals were divided into groups of six rats each. Control group received 1% gum acacia orally. Other groups received indomethacin or test compounds orally in two equal doses at 0 and 12 h for three successive days at a dose of 30 µmol/kg body weight per day. Animals were sacrificed by diethyl ether 6 h after the last dose and the stomach was removed. An opening at the greater curvature was made and the stomach was cleaned by washing with cold saline and inspected with a 3X magnifying lens for any evidence of hyperemia, hemorrhage, definite hemorrhagic erosion or ulcer. An arbitrary scale was used to calculate the ulcer index which indicates the severity of stomach lesions [34]. The percentage ulceration for each group was calculated as follows:

$$\% \text{ Ulceration} = \frac{\text{Number of animals bearing ulcer in a group}}{\text{Total number of animals in the same group}} \text{ X } 100$$

Acute Toxicity

The oral acute toxicity of compounds **3c, 4b, 4c, 5c, 6b** and **7b** was investigated using male mice (20 g each, Medical Research Institute, Alexandria University) according to previously reported methods [27, 35]. The animals were di-

vided into groups of six mice each. The compounds were given orally, suspended in 1% gum acacia, in doses of 1, 10, 100, 200, 250, 300 mg/kg. The mortality percentage in each group was recorded after 24 h. Additionally the test compounds were investigated for their parenteral acute toxicity in groups of mice of six animals each. The compounds or their vehicle, propylene glycol (control), were given by intraperitoneal injection in doses of 10, 25, 50, 75, 100 mg/kg. The percentage survival was followed up to 7 days [21].

Docking Studies

Computer assisted simulated docking experiments were carried out under an MMFF94X force field in (PDB ID: 1CX2) using chemical computing group's Molecular Operating Environment (MOE-Dock 2005) software, Montréal, Canada [36].

Docking of Human COX-2

The coordinate from the X-ray crystal structure of human COX-2 used in this simulation was obtained from the Protein Data Bank (PDB ID: 1CX2), where the selective COX-2 inhibitor **SC-588** is bound to the active site. The ligand molecules were constructed using the builder module and were energy minimized. The active site of COX-2 was generated using the MOE-Alpha Site Finder, and then ligands were docked within this active site using the MOE-Dock. The lowest energy conformation was selected and the ligand interactions (hydrogen bonding and hydrophobic interaction) with COX-2 were determined.

Docking of DNA-Gyrase B

The coordinate from the X-ray crystal structure of DNA-gyrase B used in this simulation was obtained from the Protein Data Bank (PDB ID: 1EI1). The ligand molecules were constructed using the builder module and were energy minimized. The active site of DNA-gyrase B was generated using the MOE-Alpha Site Finder, and then ligands were docked within this active site using the MOE-Dock. The lowest energy conformation was selected and the ligand interactions (hydrogen bonding and hydrophobic interaction) with DNA-gyrase B were determined.

Antimicrobial Activity

The microdilution susceptibility test in Müller-Hinton Broth (oxoid) and Sabouraud Liquid Medium (oxoid) were used for determination of antibacterial and antifungal activities [40]. Test organisms were *Escherichia coli* (*E. coli*) ATCC 25922 as an example of Gram-negative bacteria, *Staphylococcus aureus* (*S. aureus*) ATCC 19433 as an example of Gram-positive bacteria and *Candida albicans* (*C. albicans*) as yeast like fungus. Ampicillin trihydrate and clotrimazole were used as standard antibacterial and antifungal agents, respectively. Solutions of the test compounds, ampicillin trihydrate and clotrimazole were prepared in DMSO at concentration of 1600 µg/ml. From this stock solution, two-fold dilutions of the compounds (800, 400, … 6.25 µg/ml) were inoculated to the corresponding wells. Plates were incubated at 36°C for 24-48 h, with the incubation chamber kept sufficiently humid. At the end of the incubation period, the minimal inhibitory concentrations (MIC) were determined. Controls with DMSO and uninoculated media were run parallel to the test compounds under the same conditions.

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