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Synthesis of some pyrazolyl benzenesulfonamide derivatives as dual anti-inflammatory antimicrobial agents

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Abstract

Four series of pyrazolylbenzenesulfonamide derivatives were synthesized and evaluated for their anti-inflammatory activity using cotton pellet induced granuloma and carrageenan-induced rat paw edema bioassays. Moreover, COX-1 and COX-2 inhibitory activity, ulcerogenic effect and acute toxicity were also determined. Furthermore, the target compounds were screened for their *in-vitro* antimicrobial activity against *Eischerichia coli*, *Staphylococcus aureus* and *Candida albicans*. Compounds 4-(3-Phenyl-4-cyano-1H-pyrazol-1-yl)benzenesulfonamide **9a** and 4-(3-Tolyl-4-cyano-1H-pyrazol-1-yl)benzenesulfonamide **9b** were not only found to be the most active dual anti-inflammatory antimicrobial agents in the present study with good safety margin and minimal ulcerogenic effect but also exhibited good selective inhibitory activity towards COX-2. A docking pose for **9a** and **9b** separately in the active site of the human COX-2 enzyme was also obtained. Therefore, these compounds would represent a fruitful matrix for the development of dual anti-inflammatory antimicrobial candidates with remarkable COX-2 selectivity.

Keywords: *Pyrazole, anti-inflammatory, antimicrobial, ulcerogenic effect, docking, acute toxicity, cox-2, inhibitors*

Introduction

Inflammatory diseases continue to be treated primarily by non steroidal anti-inflammatory drugs (NSAIDs). However, long term use of NSAIDs has been associated with gastrointestinal mucosal damage, bleeding [1,2], intolerance [3], renal toxicity [4] and contributing to an estimated 16500 deaths each year among rheumatoid arthritis and osteoarthritis patients in the USA alone [5]. Consequently, extensive work has been made to improve the pharmacological profile of NSAIDs, which resulted in a major breakthrough in anti-inflammatory research with the discovery that cyclooxygenase (COX) exists in three isoforms COX-1, COX-2 and COX-3 that are regulated differently [6,7]. The discovery of the inducible enzyme COX-2 has stimulated the search for anti-inflammatory agents that specifically target COX-2 and avoid the undesirable side-effects associated with classical non selective NSAIDs. Much effort has been addressed to the diaryl-heterocycle class. Among this class of COX-2 inhibitors that comprise the pyrazole nucleus; celecoxib occupies a unique position as a potent and gastrointestinal (GI) safe anti-inflammatory and analgesic agent. Celecoxib is considered as a typical model of diaryl-heterocycles template that is known to inhibit selectively COX-2 enzyme [8].

From pharmacoeconomic point of view, and to better patient compliance, an anti-inflammatory

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antimicrobial agent with minimum adverse effects and high safety margin is highly desirable. Drugs that serve dual anti-inflammatory antimicrobial functions will have greater potential than multi drugs regimen to treat inflammatory conditions that might be associated with microbial infections especially in patients with impaired liver or kidney functions. Compounds containing pyrazole functionality have been reported to exhibit promising anti-inflammatory activity [9–13]. Moreover, several pyrazole derivatives exhibited antimicrobial activity [14–16]. Taken together, the above reasons stimulated the research in our lab for agents that would have a dual anti-inflammatory antimicrobial effect. Several lead compounds containing a pyrazole moiety attached to various heterocyclic rings have been reported from our lab [17–23]. Some of these reported compounds **A** [19], **B** [20], **C** [18,21] **D** [18], and **E** [23] showed pronounced dual anti-inflammatory and antimicrobial activities (Figure 1).

In extension to our efforts in the discovery of novel anti-inflammatory antimicrobial agents; we explored a new approach to, design new compounds by incorporating some pharmacophoric assemblies in celecoxib represented by an aminosulfonylphenyl group attached to a pyrazole ring bearing a lipophilic moiety at position 3, in addition to hydroxyiminomethyl or cyano group at position 4. In order to acquire new insights on the structure-activity relationship of this class of compounds, the dimethylaminomethyleneaminosulfonyl derivatives were pharmacologically evaluated to investigate the influence of this moiety on the anti-inflammatory activity compared to the free aminosulfonyl derivatives. The structural changes in such moiety might be expected to improve the pharmacokinetic parameters of these compounds. In addition to the targeted anti-inflammatory and antimicrobial activities, the ulcerogenic and acute toxicity profiles of the active compounds were determined.

Material and methods

Melting points were determined in open glass capillaries using a Gallenkamp melting point apparatus and are uncorrected. Infra-red (IR) spectra was recorded on Perkin-Elmer 1430 infrared spectrophotometer using the KBr disc technique. ^{1}H NMR spectra was recorded on JEOL 300 MHz, 400 MHz or 500 MHz NMR spectrometer (DMSO-d_6), and the chemical shifts are given in δ (ppm) downfield from tetramethylsilane (TMS) which served as an internal standard. Splitting patterns were designated as follows: s: singlet; d: doublet; m: multiplet. Elemental analyses were performed on Vario El Fab-Nr elemental analyzer 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 GF254, Merck) and the spots were detected by exposure to UV-lamp at $\lambda = 254$ nm for few seconds.

Synthesis

*4-(2-Arylethylidenehydrazono) benzenesulfonamide **3a–e***. A mixture of the appropriate acetophenone derivative (**1a–e**) (16.6 mmol), 4-hydrazinobenzenesulfonamide hydrochloride (**2**) (16.6 mmol) and anhydrous sodium

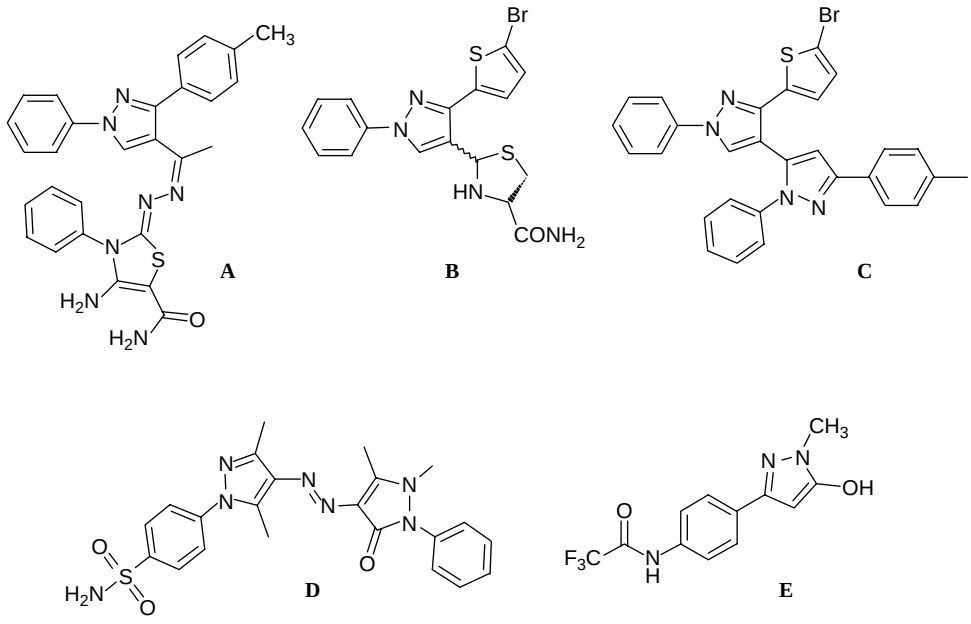


Figure 1. Structure of reported active pyrazole derivatives **A**, **B**, **C**, **D** and **E**.

Table I. Physical data of compounds **3–9**.

Comp. No.	R	Yield %	M.P (°C) Cryst. Solvent	Mol. Formula (Mol. wt)	Comp. No.	R	Yield %	M.P (°C) Cryst. Solvent	Mol. Formula (Mol. wt)
3a	H	89	247–248 (DMF/H_2O)(4:1)	$C_{14}H_{15}N_3O_2S$ (289.36)	**6d**	Cl	74	240–241 (EtOH)	$C_{19}H_{18}ClN_5O_3S$ (431.90)
3b	CH_3	85	224–226 (Ethanol)	$C_{15}H_{17}N_3O_2S$ (303.39)	**6e**	NO_2	75	243–244 (EtOH)	$C_{19}H_{18}N_6O_5S$ (442.46)
3c	Br	83	210–212 (Ethanol)	$C_{14}H_{14}BrN_3O_2S$ (368.25)	**7a**	H	90	227–228 (EtOH)	$C_{16}H_{14}N_4O_3S$ (342.38)
3d	Cl	87	221–222 (DMF/H_2O) (4:1)	$C_{14}H_{14}ClN_3O_2S$ (323.80)	**7b**	CH_3	89	220–221 (MeOH)	$C_{17}H_{16}N_4O_3S$ (356.41)
3e	NO_2	89	254–255 (DMF/H_2O) (4:1)	$C_{14}H_{14}N_4O_4S$ (334.36)	**7c**	Br	89	240–242 (EtOH/H_2O) (5:1)	$C_{16}H_{13}BrN_4O_3S$ (421.27)
4a	H	87	162–163 (DMF/H_2O) (4:1)	$C_{19}H_{18}N_4O_3S$ (382.44)	**7d**	Cl	88	230–232 (EtOH/H_2O) (5:1)	$C_{16}H_{13}ClN_4O_3S$ (376.82)
4b	CH_3	86	129–130 (EtOH)	$C_{20}H_{20}N_4O_3S$ (396.47)	**7e**	NO_2	89	237–238 (EtOH)	$C_{16}H_{13}N_5O_5S$ (387.38)
4c	Br	84	214–215 (EtOH)	$C_{19}H_{17}BrN_4O_3S$ (461.34)	**8a**	H	85	190–191 (MeOH)	$C_{19}H_{17}N_5O_2S$ (379.44)
4d	Cl	85	210–212 (EtOH)	$C_{19}H_{17}ClN_4O_3S$ (416.89)	**8b**	CH_3	84	236–237 (MeOH)	$C_{20}H_{19}N_5O_2S$ (393.47)
4e	NO_2	81	190–191°C (DMF/H_2O) (4:1)	$C_{19}H_{17}N_5O_5S$ (427.44)	**8c**	Br	83	257–258 (EtOH)	$C_{19}H_{16}BrN_5O_2S$ (458.34)
5a	H	75	182–183 (EtOH)	$C_{16}H_{13}N_3O_3S$ (327.36)	**8d**	Cl	82	270–272 (EtOH)	$C_{19}H_{16}ClN_5O_2S$ (413.89)
5b	CH_3	73	170–171 (EtOH)	$C_{17}H_{15}N_3O_3S$ (341.39)	**8e**	NO_2	86	235–236 (DMF/H_2O) (8:1)	$C_{19}H_{16}N_6O_4S$ (424.44)
5c	Br	71	166–167 (EtOH)	$C_{16}H_{12}BrN_3O_3S$ (406.26)	**9a**	H	89	219–220 (EtOH)	$C_{16}H_{12}N_4O_2S$ (324.36)
5d	Cl	70	162–163 (EtOH)	$C_{16}H_{12}ClN_3O_3S$ (361.81)	**9b**	CH_3	88	200–202 (EtOH)	$C_{17}H_{14}N_4O_2S$ (338.39)
5e	NO_2	65	210–212 (EtOH)	$C_{16}H_{12}N_4O_5S$ (372.36)	**9c**	Br	90	252–253 (EtOH)	$C_{16}H_{11}BrN_4O_2S$ (403.26)
6a	H	77	220–221°C (EtOH)	$C_{19}H_{19}N_5O_3S$ (397.46)	**9d**	Cl	89	244–245 (EtOH)	$C_{16}H_{11}ClN_4O_2S$ (358.81)
6b	CH_3	75	218–219 (EtOH/H_2O) (5:1)	$C_{20}H_{21}N_5O_3S$ (411.48)	**9e**	NO_2	90	260–261 (DMF/H_2O) (8:1)	$C_{16}H_{11}N_5O_4S$ (369.36)
6c	Br	75	212–13°C (EtOH)	$C_{19}H_{18}BrN_5O_3S$ (476.36)					

acetate (1.36 g, 16.6 mmol) in ethanol (15 mL) was heated under reflux for 2–3 h. The reaction mixture was allowed to attain room temperature. The separated solid product was filtered, washed with ethanol, dried and crystallized from the proper solvent (Tables I & II).

Z-N,N-Dimethylaminomethylene-4-(3-aryl-4-formyl-1H-pyrazol-1-yl) benzenesulfonamide ***4a-e***. To an ice cold dimethylformamide (6.54 g, 6.93 ml, 89.5 mmol), a phosphorous oxychloride (2.42 g, 1.47 mL, 15.8 mmol) was added drop-wise with stirring over a period of 30 min and stirring was continued for further 45 min with the reaction mixture kept at 0°C. The appropriate hydrazone (**3a-e**) (6.88 mmol) was then added and the reaction mixture was allowed to attain room temperature. The mixture was heated at 70°C for 2–3 h, allowed to cool to room temperature and then poured onto crushed ice and water. The mixture was boiled and the copious white precipitate obtained after cooling was filtered, dried and crystallized from the proper solvent (Tables I & II).

4-(3-Aryl-4-formyl-1H-pyrazol-1-yl) benzenesulfonamide ***5a-e***. A solution of the proper aldehyde (**4a-e**) (5.24 mmol) in concentrated HCl (15 mL) was heated under reflux for 3 h. The reaction mixture was left to attain room temperature. The precipitated solid was filtered, washed with water, dried and crystallized from the proper solvent (Tables I & II).

(Z,E)-N,N-Dimethylaminomethylene-4-(3-aryl-4-hydroxyliminomethyl-1H-pyrazol-1-yl) benzenesulfonamide ***6a-e*** *and E-4-(3-Aryl-4-hydroxyliminomethyl-1H-pyrazol-1-yl) benzenesulfonamide* ***7a-e***. A solution of the selected aldehyde **4a-e** or **5a-e** (6.1 mmol), hydroxylamine hydrochloride (0.42 g, 6.1 mmol) and anhydrous sodium acetate (0.5 g, 6.1 mmol) in ethanol (15 mL) was heated under reflux for 4 h. The reaction mixture was concentrated and left to attain room temperature. The separated solid product was filtered, washed with water, dried and crystallized from the proper solvent (Tables I & II).

Z-N,N-Dimethylaminomethylene-4-(3-aryl-4-cyano-1H-pyrazol-1-yl) benzenesulfonamide ***8a-e***. A solution of the appropriate oxime **6a-e** (5.85 mmol) in acetic anhydride (10 mL) was heated under reflux for 2–3 h. The reaction mixture was left overnight at room temperature. The separated solid product was filtered, washed with water, dried and crystallized from the proper solvent (Tables I & II).

Table II. **2:** IR and ^{1}H-NMR spectra of compounds **3–9**.

Comp. No.	IR (KBr, cm^{-1})	^{1}H-NMR (DMSO-d_6)
3a	3362, 3335, 3266(NH_2, NH), 1595 (C = N), 1342, 1147 (SO_2)	2.29 (s, 3H, CH_3), 7.08, (s, 2H, NH_2, D_2O exchangeable), 7.30–7.43 (m, 5H, phenyl-H), 7.66, 7.82 (2d, J = 8.7 Hz, 4H, benzenesulfonamide $C_{3,5}$ H & $C_{2,6}$H), 9.73 (s, 1H, NH, D_2O exchangeable).
3b	3360, 3333, 3264 (NH_2, NH), 1596, (C = N), 1340, 1146 (SO_2)	2.27 (s, 3H, phenyl CH_3), 2.33 (s, 3H, CH_3), 7.07 (s, 2H, NH_2, D_2O exchangeable), 7.21, 7.31 (2d, J = 8.0 Hz, 4H, phenyl $C_{3,5}$H & $C_{2,6}$H), 7.66, 7.71 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$H & $C_{2,6}$H), 9.67 (s, 1H, NH, D_2O exchangeable).
3c	3333, 3305, 3262 (NH_2, NH), 1599 (C = N), 1339, 1151 (SO_2)	2.28 (s, 3H, CH_3), 7.10 (s, 2H, NH_2, D_2O exchangeable), 7.32, 7.58 (2d, J = 8.0 Hz, 4H, phenyl $C_{2,6}$H & $C_{3,5}$H), 7.68, 7.76 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$H & $C_{2,6}$H), 9.80 (s, 1H, NH, D_2O exchangeable).
3d	3335, 3305, 3193, (NH_2, NH), 1598 (C = N), 1339, 1151 (SO_2)	2.28 (s, 3H, CH_3), 7.09 (s, 2H, NH_2, D_2O exchangeable), 7.33, 7.45 (2d, J = 8.0, Hz, 4H, phenyl $C_{2,6}$H & $C_{3,5}$H), 7.68, 7.83 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$H & $C_{2,6}$H), 9.81 (s, 1H, NH, D_2O exchangeable).
3e	3303, 3246, 3111 (NH_2, NH), 1594 (C = N), 1344, 1148 (SO_2)	2.35 (s, 3H, CH_3), 7.13 (s, 2H, NH_2, D_2O exchangeable), 7.40 (d, J = 8.8 Hz, 2H, phenyl $C_{2,6}$H), 7.71 (d, J = 9.1 Hz, 2H, benzenesulfonamide $C_{3,5}$H), 8.07 (d, J = 8.8 Hz, 2H, phenyl $C_{3,5}$H), 8.23 (d, J = 9.1 Hz, 2H, benzenesulfonamide $C_{2,6}$H), 10.08 (s, 1H, NH, D_2O exchangeable).
4a	1679 (C = O), 1627, 1596 (C = N), 1338, 1147 (SO_2).	2.92, 3.15 (2s, 6H, -N(CH_3)$_2$), 7.48–7.53 (m, 3H, phenyl $C_{3,4,5}$H), 7.91 (d, J = 7.6 Hz, 2H, phenyl $C_{2,6}$-H), 7.94, 8.14 (2d, J = 8.7 Hz, 4H, benzenesulfonamide $C_{3,5}$H & $C_{2,6}$H), 8.25 (s, 1H, N = CH), 9.41 (s, 1H, pyrazole C_5H), 9.99 (s, 1H, CHO).
4b	1678 (C = O), 1627, 1595 (C = N), 1341, 1147 (SO_2).	2.37 (s, 3H, phenyl CH_3), 2.92, 3.15 (2s, 6H, N(CH_3)$_2$, 7.31, 7.80 (2d, J = 8.0 Hz, 4H, phenyl $C_{3,5}$H & $C_{2,6}$H), 7.94, 8.12 (2d, J = 8.7 Hz, 4H, benzenesulfonamide $C_{3,5}$H & $C_{2,6}$H), 8.25 (s, 1H, N = CH), 9.38 (s, 1H, pyrazole C_5H), 9.97 (s, 1H, CHO).
4c	1690 (C = O); 1630, 1593 (C = N); 1339, 1150 (SO_2).	2.92, 3.16 (2s, 6H, -N(CH_3)$_2$), 7.71, 7.92 (2d, J = 8.4 Hz, 4H, phenyl $C_{2,6}$H & $C_{3,5}$H), 7.95, 8.13 (2d, J = 9 Hz, 4H, benzenesulfonamide $C_{3,5}$H & $C_{2,6}$H), 8.25 (s, 1H, N = CH), 9.45 (s, 1H, pyrazole C_5H), 9.98 (s, 1H, CHO).
4d	1690 (C = O); 1636, 1593 (C = N); 1337, 1150 (SO_2).	2.92, 3.16 (2s, 6H, N(CH_3)$_2$), 7.58, 7.94 (2d, J = 8.4 Hz, 4H, phenyl $C_{2,6}$H & $C_{3,5}$H), 8.00, 8.14 (2d, J = 8.7 Hz, 4H, benzenesulfonamide $C_{3,5}$H & $C_{2,6}$H), 8.26 (s, 1H, N = CH), 9.44 (s, 1H, pyrazole C_5 H), 9.98 (s, 1H, CHO).
4e	1688 (C = O); 1627, 1595 (C = N); 1344, 1149 (SO_2).	2.93, 3.16 (2s, 6H, N(CH_3)$_2$), 7.96 (d, J = 8.7 Hz, 2H, phenyl $C_{2,6}$H), 8.16 (d, J = 8.8 Hz, 2H, benezenesulfonamide $C_{3,5}$H), 8.25 (d, J = 8.7 Hz, 2H, phenyl $C_{3,5}$H), 8.28 (S, 1 h, CH = N), 8.36 (d, J = 8.8 Hz, 2H, benzenesulfonamide $C_{2,6}$H)), 9.52 (s, 1H, pyrazole C_5H), 10.02 (s, 1H, CHO).
5a	3347, 3236 (NH_2); 1665 (C = O); 1596 (C = N); 1339, 1158 (SO_2).	7.47 (s, 2H, NH_2, D_2O exchangeable), 7.48–7.54 (m, 3H, phenyl $C_{3,4,5}$H), 7.92 (d, J = 7.8 Hz, phenyl $C_{2,6}$H), 8.00, 8.21 (2d, J = 8.7 Hz, 4H, benzenesulfonamide $C_{3,5}$H & $C_{2,6}$H), 9.43 (s, 1H, pyrazole C_5H), 10.00 (s, 1H, CHO).
5b	3351, 3234 (NH_2); 1661 (C = O); 1595 (C = N); 1346, 1163 (SO_2).	2.38 (s, 3H, CH_3), 7.33 (d, J = 8.0 Hz, 2H, phenyl $C_{3,5}$H), 7.47 (s, 2H, NH_2, D_2O exchangeable), 7.83 (d, J = 8.0 Hz, 2H, phenyl $C_{2,6}$H), 8.00, 8.19 (2d, J = 8.6 Hz, 2H, benzenesulfonamide $C_{3,5}$H & $C_{2,6}$H), 9.40 (s, 1H, pyrazole C_5H), 9.99 (s, 1H, CHO).
5c	3344, 3254 (NH_2); 1681 (C = O); 1596 (C = N); 1336, 1165 (SO_2).	7.48 (s, 2H, NH_2, D_2O exchangeable), 7.73 (d, J = 8.4 Hz, 2H, phenyl $C_{2,6}$H), 7.93 (d, J = 8.7 Hz, 2H, benzenesulfonamide $C_{3,5}$H), 8.00 (d, J = 8.4 Hz, 2H, phenyl $C_{3,5}$H), 8.20 (d, J = 8.7 Hz, 2H, benzenesulfonamide $C_{2,6}$H), 9.46 (s, 1H, pyrazole C_5H), 9.99 (s, 1H, CHO).
5d	3347, 3260 (NH_2); 1680 (C = O); 1596 (C = N); 1335, 1161 (SO_2).	7.47 (s, 2H, NH_2, D_2O exchangeable), 7.72 (d, J = 8.2 Hz, 2H, phenyl $C_{2,6}$H), 7.92 (d, J = 8.7 Hz, 2H, benzenesulfonamide $C_{3,5}$H), 7.99 (d, J = 8.2 Hz, 2H, phenyl $C_{3,5}$H), 8.18 (d, J = 8.7 Hz, 2H, benzenesulfonamide $C_{2,6}$H), 9.45 (s, 1H, pyrazole C_5H), 9.97 (s, 1H, CHO).
5e	3350, 3263 (NH_2); 1681 (C = O); 1596 (C = N); 1344, 1166 (SO_2).	7.50 (s, 2H, NH_2, D_2O exchangeable), 8.01 (d, J = 8.7 Hz, 2H, phenyl $C_{2,6}$H), 8.20 (d, J = 9.0 Hz, 2H, benzenesulfonamide $C_{3,5}$H), 8.26 (d, J = 8.7 Hz, 2H, phenyl $C_{3,5}$H), 8.36 (d, J = 9.0 Hz, 2H, benzenesulfonamide $C_{2,6}$H), 9.52 (s, 1H, pyrazole C_5H), 10.03 (s, 1H, CHO).
6a	3373 (OH); 1643, 1595 (C = N); 1334, 1161 (SO_2).	2.91, 3.15 (2s, 6H, -N(CH_3)$_2$), 7.43 (s, 1H, CH = N), 7.49–7.54 (m, 3H, phenyl $C_{3,4,5}$H), 7.66 (d, J = 7.6 Hz, 2H, phenyl $C_{2,6}$H), 7.89, 8.10 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$H $C_{2,6}$H), 8.23 (s, 1H, N = CH), 9.22 (s, 1H, pyrazole C_5H), 11.86 (s, 1H, OH, D_2O exchangeable).

Table II – *continued*

Comp. No.	IR (KBr, cm^{-1})	^{1}H-NMR (DMSO-d_6)
6b	3244 (OH); 1628, 1595 (C = N); 1340, 1161 (SO_2).	2.35 (s, 3H, phenyl CH_3), 2.90, 3.13 (2s, 6H, $N(CH_3)_2$), 7.31 (d, J = 8.0 Hz, 2H, phenyl $C_{3,5}H$), 7.40 (s, 1H, CH = N), 7.53 (d, J = 8.0 Hz, 2H, phenyl $C_{2,6}H$), 7.87, 8.07 (2d, J = 9 Hz, 4H, benzenesulfonamide $C_{3,5}H$ $C_{2,6}H$), 8.23 (s, 1H, N = CH), 9.20 (s, 1H, pyrazole C_5H), 11.84 (s, 1H, OH, D_2O exchangeable).
6c	3391 (OH); 1631, 1595 (C = N); 1344, 1152 (SO_2).	2.92, 3.15 (2s, 6H, $N(CH_3)_2$), 7.44 (s, 1H, CH = N), 7.58 (d, J = 8.4 Hz, 2H, phenyl $C_{2,6}H$), 7.71 (d, J = 8.7 Hz, 2H, benzenesulfonamide $C_{3,5}H$), 7.96 (d, J = 8.4 Hz, 2H, phenyl $C_{3,5}H$), 8.16 (d, J = 8.7 Hz, 2H, benzenesulfonamide $C_{2,6}H$), 8.23 (s, 1H, N = CH), 9.26 (s, 1H, pyrazole C_5H), 11.91 (s, 1H, OH, D_2O exchangeable).
6d	3238 (OH); 1627, 1596 (C = N); 1340, 1161 (SO_2).	2.89, 3.13 (2s, 6H, $N(CH_3)_2$), 7.41 (s, 1H, CH = N), 7.56 (d, J = 8.4 Hz, 2H, phenyl $C_{2,6}H$), 7.67 (d, J = 8.6 Hz, 2H, benzenesulfonamide $C_{3,5}H$), 7.86 (d, J = 8.4 Hz, 2H, phenyl $C_{3,5}H$), 8.07 (d, J = 8.6 Hz, 2H, benzenesulfonamide $C_{2,6}H$), 8.22 (s, 1H, N = CH), 9.21 (s, 1H, pyrazole C_5H), 11.94 (s, 1H, OH, D_2O exchangeable).
6e	3248 (OH); 1629, 1596 (C = N); 1343, 1149 (SO_2).	2.92, 3.15 (2s, 6H, $N(CH_3)_2$), 7.53 (s, 1H, CH = N), 7.93 (d, J = 8.7 Hz, 2H, phenyl $C_{2,6}H$), 8.03 (d, J = 9 Hz, 2H, benzenesulfonamide $C_{3,5}H$), 8.13 (d, J = 8.7 Hz, 2H, phenyl $C_{3,5}H$), 8.25 (s, 1H, N = CH), 8.35 (d, J = 9 Hz, 2H, benzenesulfonamide $C_{2,6}H$), 9.0 (s, 1H, pyrazole C_5H), 11.25 (s, 1H, OH, D_2O exchangeable).
7a	3350, 3255, 3147 (NH_2, OH), 1595 (C = N), 1334, 1161 (SO_2).	7.42 (s, 3H, CH = N & NH_2, D_2O exchangeable), 7.46–7.57 (m, 3H, phenyl $C_{3,4,5}H$), 7.69 (d, J = 8.1 Hz, 2H, phenyl $C_{2,6}H$), 7.95, 8.16 (2d, J = 8.7 Hz, 4H, benzenesulfonamide $C_{3,5}H$ & $C_{2,6}H$), 9.26 (s, 1H, pyrazole C_5H), 11.88 (s, 1H, OH, D_2O exchangeable).
7b	3335, 3256, 3115 (NH_2, OH), 1595 (C = N), 1336, 1160 (SO_2).	2.36 (s, 3H, CH_3), 7.34 (d, J = 7.8 Hz, 2H, phenyl $C_{3,5}H$), 7.43 (s, 3H, CH = N & NH_2), 7.56 (d, J = 7.8 Hz, phenyl $C_{2,6}H$), 7.95, 8.16 (2d, J = 9.0 Hz, 4H, benzenesulfonamide $C_{3,5}H$ & $C_{2,6}H$), 9.24 (s, 1H, pyrazole C_5H), 11.86 (s, 1H, OH, D_2O exchangeable).
7c	3335, 3250, 3186 (NH_2, OH), 1596 (C = N), 1340; 1162 (SO_2).	7.46 (s, 2H, NH_2, D_2O exchangeable), 7.48 (s, 1H, CH = N), 7.63, 7.71, 7.94, 8.15 (4d, J = 8.4 Hz, each 2H, phenyl $C_{2,6}H$ & $C_{3,5}H$, benzenesulfonamide $C_{3,5}H$ & $C_{2,6}H$), 9.25 (s, 1H, pyrazole C_5H), 11.95 (s, 1H, OH, D_2O exchangeable).
7d	3353, 3254, 3188 (NH_2, OH), 1595 (C = N), 1342, 1158 (SO_2).	7.43 (s, 1H, CH = N), 7.44 (s, 2H, NH_2, D_2O exchangeable), 7.57, 7.69, 7.94, 8.14 (4d, J = 8.4 Hz, each 2H, phenyl $C_{2,6}H$ & $C_{3,5}H$, benzenesulfonamide $C_{3,5}H$ & $C_{2,6}H$), 9.25 (s, 1H, pyrazole C_5H), 11.91 (s, 1H, OH, D_2O exchangeable).
7e	3330, 3250, 3150 (NH_2, OH), 1596 (C = N), 1340, 1162 (SO_2).	7.48 (s, 2H, NH_2, D_2O exchangeable), 7.55 (s, 1H, CH = N), 7.98, 8.02, 8.20, 8.40 (4d, J = 8.8 Hz, each 2H, phenyl $C_{2,6}H$, benzenesulfonamide $C_{3,5}H$, phenyl $C_{3,5}H$ & benzenesulfonamide $C_{2,6}H$), 9.33 (s, 1H, pyrazole C_5H), 12.01 (s, 1H, OH, D_2O exchangeable).
8a	2230 (C ≡ N), 1631, 1592 (C = N), 1338, 1152 (SO_2).	2.93, 3.16 (2s, 6H, $N(CH_3)_2$), 7.54–7.62 (m, 3H, phenyl $C_{3,4,5}H$), 7.97 (d, J = 8.8 Hz, 2H, phenyl $C_{2,6}H$), 7.99, 8.10 (d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}H$ & $C_{2,6}H$), 8.27 (s, 1H, N = CH), 9.56 (s, 1H, pyrazole C_5 H).
8b	2232 (C ≡ N), 1635, 1592 (C = N), 1340, 1152 (SO_2).	2.39 (s, 3H, phenyl CH_3), 2.93, 3.20 (2s, 6H, $N(CH_3)_2$), 7.39, 7.87, 7.97, 8.09 (4d, J = 8 Hz, each 2H, phenyl $C_{3,5}H$ &$C_{2,6}H$, benzenesulfonamide $C_{3,5}H$ & $C_{2,6}H$), 8.27 (s, 1H, N = CH), 9.53 (s, 1H, pyrazole C_5 H).
8c	2233 (C ≡ N), 1636, 1594 (C = N), 1339, 1153 (SO_2).	2.93, 3.16 (2s, 6H, $N(CH_3)_2$), 7.82, 7.94 (2d, J = 8 Hz, 4H, phenyl $C_{2,6}H$ & $C_{3,5}H$), 7.97, 8.10 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}H$ & $C_{2,6}H$), 8.27 (s, 1H, N = CH), 9.57 (s, 1H, pyrazole C_5 H).
8d	2233 (C ≡ N), 1637, 1593 (C = N), 1340, 1152 (SO_2).	2.93, 3.16 (2s, 6H, $N(CH_3)_2$), 7.67, 7.96, 8.00, 8.10 (4d, J = 8.8 Hz, each 2H, phenyl $C_{2,6}H$ & $C_{3,5}H$, benzenesulfonamide $C_{3,5}H$ & $C_{2,6}H$), 8.27 (s, 1H, N = CH), 9.57 (s, 1H, pyrazole C_5 H).
8e	2233 (C ≡ N), 1631, 1595 (C = N), 1341, 1146 (SO_2).	2.90, 3.14 (2s, 6H, $N(CH_3)_2$), 7.96 (d, J = 9.2 Hz, 2H, phenyl $C_{2,6}H$), 8.08 (d, J = 8.4 Hz, 2H, benzenesulfonamide $C_{3,5}H$), 8.21 (d, J = 9.2 Hz, 2H, phenyl $C_{3,5}H$), 8.24 (s, 1H, N = CH), 8.41 (d, J = 8.4 Hz, 2H, benzenesulfonamide $C_{2,6}H$), 9.59 (s, 1H, pyrazole C_5H).

9a	3372, 3279 (NH_2), 2225 (C ≡ N), 1594 (C = N), 1338, 1157 (SO_2).	7.51 (s, 2H, NH_2, D_2O exchangeable), 7.54–7.62 (m, 3H, phenyl $C_{3,4,5}H$), 7.99 (d, $\mathcal{J}$ = 7.8 Hz, 2H, phenyl $C_{2,6}H$), 8.02, 8.16 (2d, $\mathcal{J}$ = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}H$ & $C_{2,6}H$), 9.57 (s, 1H, pyrazole C_5 H).
9b	3347, 3258 (NH_2), 2227 (C ≡ N), 1596 (C = N), 1330, 1164 (SO_2).	2.39 (s, 3H, CH_3), 7.40 (d, $\mathcal{J}$ = 8 Hz, 2H, phenyl $C_{3,5}H$), 7.51 (s, 2H, NH_2, D_2O exchangeable), 7.89 (d, $\mathcal{J}$ = 8 Hz, 2H, phenyl $C_{2,6}$ H), 8.01, 815 (2d, $\mathcal{J}$ = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$ H & $C_{2,6}$H), 9.54 (s, 1H, pyrazole C_5 H).
9c	3325, 3231 (NH_2), 2228 (C ≡ N), 1595 (C = N), 1337, 1157 (SO_2).	7.50 (s, 2H, NH_2, D_2O exchangeable), 7.77, 7.91 (2d, $\mathcal{J}$ = 8.4 Hz, 4H, phenyl $C_{2,6}H$ & $C_{3,5}H$), 8.00, 8.08 (2d, $\mathcal{J}$ = 9 Hz, 4H, benzenesulfonamide $C_{3,5}H$ & $C_{2,6}H$), 9.53 (s, 1H, pyrazole C_5H).
9d	3271, 3209 (NH_2); 2234 (C ≡ N), 1595 (C = N), 1343, 1166 (SO_2).	7.52 (s, 2H, NH_2, D_2O exchangeable), 7.68, 8.00, 8.03, 8.15 (4d, $\mathcal{J}$ = 8.8 Hz, 8H, phenyl $C_{2,6}H$ & $C_{3,5}H$, benzenesulfonamide$C_{3,5}H$ & $C_{2,6}H$), 9.58 (s, 1H, pyrazole C_5H).
9e	3262, 3227 (NH_2); 2232 (C ≡ N), 1596 (C = N), 1345, 1163 (SO_2).	7.47 (s, 2H, NH_2, D_2O exchangeable), 8.12, 8.20, 8.25, 8.45 (4d, $\mathcal{J}$ = 8.8 Hz, 8H, phenyl $C_{2,6}H$, benzenesulfonamide $C_{3,5}H$, phenyl $C_{3,5}H$, benzenesulfonamide $C_{2,6}H$), 9.67 (s, 1H, pyrazole C_5H).

4-(3-Aryl-4-cyano-1H-pyrazol-1-yl)benzenesulfonamide ***9a–e***. A solution of the selected oxime (**7a-e**) (2.92 mmol) in phosphorous oxychloride (4.48 g, 2.72ml, 29.2 mmol) and triethylamine (0.44 g, 0.61 ml, 4.35 mmol) was heated at 80°C for 2 h. The reaction mixture was left to attain room temperature, then poured onto ice water. The separated solid was filtered, dried and crystallized from the proper solvent (Tables I & II).

Anti-inflammatory testing

Cotton pellet induced granuloma bioassay [24]. Adult male Sprague-Dawley rats (120–140 g; sourced from Medical Research Institute, Alexandria University) were used to investigate the anti-inflammatory efficacy of the synthesized compounds. The rats were acclimated one week prior to use and were given *ad libitum* 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 0.01 mmol) of a solution of the test compound in chloroform or acetone 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 anaesthesia. Two groups of animals were treated as above with the substitution of tested compounds with standard reference (indomethacin or celecoxib) and the antibiotics at the same level. Pellets containing only the antibiotics were similarly implanted in the control rats. After seven days, 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. All experiments were conducted within Institute guidelines laid down for animal experimentation.

Carrageenan-induced rat paw edema bioassay [25]. Male albino rats weighing 120–150 g (Medical Research Institute, Alexandria University) were used to investigate the carrageenan-induced rat paw edema. The rats were kept in 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, celecoxib and test compounds were dissolved in dimethylsulfoxide (DMSO) and injected subcutaneously in a dose of 10 μmol/ kg body weight, one hour prior to carrageenan injection. DMSO alone was injected to the control group. The volume of paw edema (mL) was determined by means of water plethysmometer immediately after injection of carrageenan and 4 h later. The increase in paw volume between time 0 to 4 h was measured [25]. The percentage protection against inflammation was calculated as follows:

$$\frac{V_c - V_d}{V_c} \times 100$$

where V_c is the increase in paw volume in absence of test compound (control) and V_d is the increase of paw volume after injection of the test compound. Data were expressed as the mean ± SEM. Significant difference between the control and treated groups was performed using Student's *t*-test and *P* values were 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 the literature protocol [30]. COX-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 [27]. COX-2 assay was performed utilising human recombinant COX-2 (hrCOX-2) purchased from Sigma-Aldrich. 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 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 (COX-1) or 5 min (COX-2) at 24 °C. The reaction was stopped by addition of 400 μL of a stop solution composed of Et_2O/MeOH/1M 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 (TLC) plate. TLC was performed at 4 °C with a 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 a radiometric photographic system. The concentration of the compound causing 50% inhibition (IC_{50}) was calculated.

Ulcerogenic effects

All synthesized compounds were evaluated for their ulcerogenic potential in rats [28]. Indomethacin was used as a 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 μM/kg 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 (Normal stomach: Score 0.0, Petechial hemorrhage or hyperemia: Score 1, Definite hemorrhagic erosions: Score 2, Very small ulcers (< 1 mm long): Score 3, Small ulcers (from 1 to < 2 mm long): Score 4, Medium ulcers (from 2 to < 3 mm long): Score 5, Large ulcers (from 3 to < 4 mm long): Score 6, Very large ulcers (> 4 mm long: Score 7) was used to calculate the ulcer index which indicates the severity of stomach lesions [28]. The percentage ulceration for each group was calculated as follows:

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

Acute toxicity

The biologically significant compounds (**7a**, **7b**, **9a** and **9b**) were further investigated for their oral acute toxicity in male mice (each 20 g, supplied by Medical Research Institute, Alexandria University) as reported in previous reports [26,29]. Groups of mice each of six animals were used for the assay. The compounds were given orally suspended in 1% gum acacia, in doses of 1, 10, 100, 200, 250, 300 mg/kg. After 24 h, the % mortality in each group/compound was recorded. Moreover, these compounds were further tested for their parenteral acute toxicity using groups of mice each of six animals. The compounds or their vehicle, propylene glycol (control), were given by intraperitoneal injection in doses of 10, 25, 50, 75, 100 mg / kg. Survival of the mice was followed up to 7 days [20].

Modeling 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 [31].

Docking Protocol. The coordinates from the X-ray crystal structure of human COX-2 used in this simulation were obtained from the Protein Data Bank (PDB ID 1CX2), where the active site is bound to the selective COX-2 inhibitor SC-588. 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.

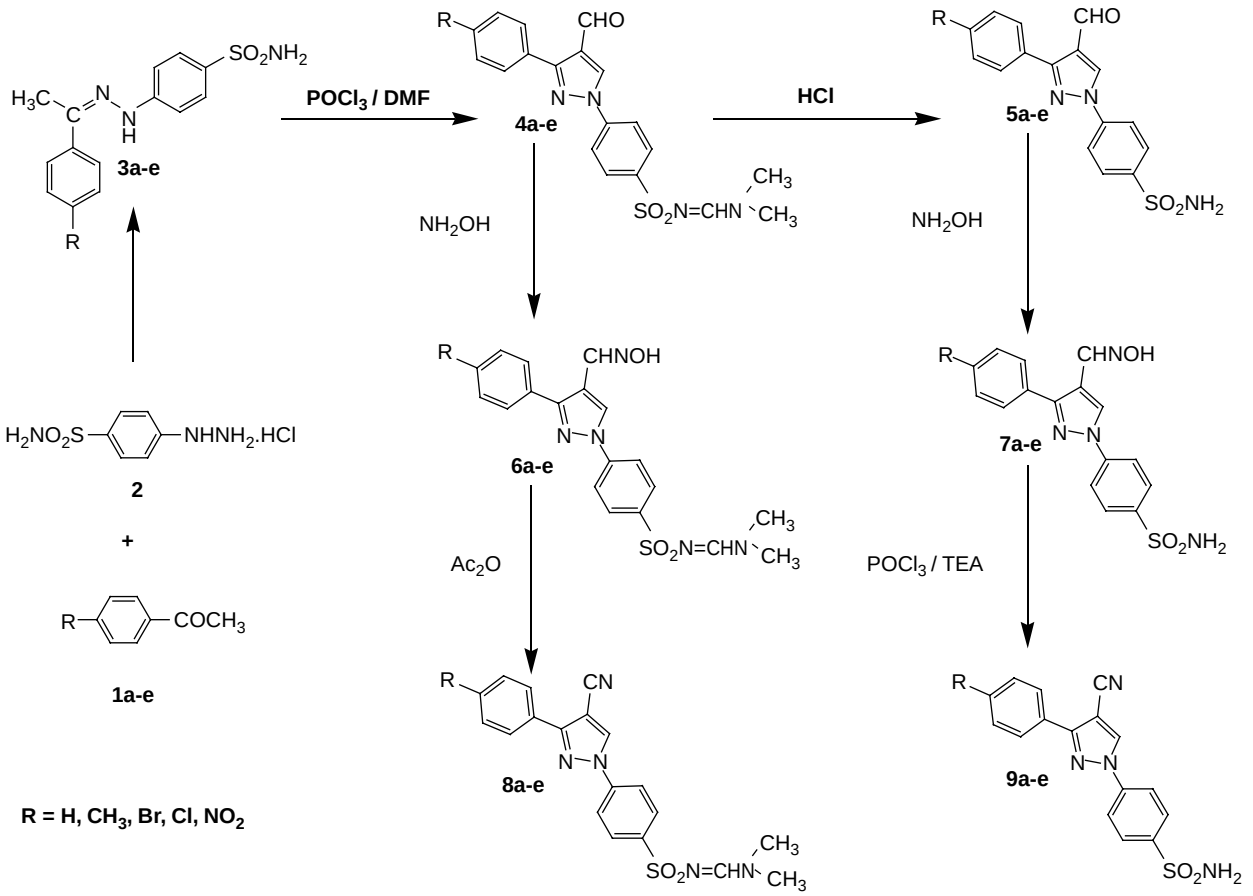
In vitro antimicrobial activity

The microdilution susceptibility test in Müller-Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) were used for determination of antibacterial and antifungal activity [32]. Test organisms were *Escherichia coli* ATCC 25922 as Gram-negative bacteria, *Staphylococcus aureus* ATCC 19433 as Gram-positive bacteria and *Candida albicans* as a yeast-like fungi. 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, serial dilutions of the compounds (800, 400, . . . , 6.25 μg/mL) were prepared. The microorganism suspensions at concentration of 10^6 CFU/ml (colony forming unit/ml) were inoculated to the corresponding wells. Plates were incubated at 36 °C for 24–48 h. The incubation chamber was kept sufficiently humid during the incubation period and 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 same conditions.

Results and discussion

Chemistry

The target compounds were synthesized according to the steps outlined in Scheme 1. Condensation of the appropriate acetophenone derivatives **1a–e** with 4-hydrazinobenzenesulfonamide hydrochloride **2** and



Scheme 1. Synthesis of test compounds **3-9a–e**

Table III. The anti-inflammatory activity (ED_{50}, μmol / cotton pellet)[a] and ulcerogenic activity[a].

Test Compound	ED_{50} (μmol)	% Ulceration
Indomethacin	9.68 ± 0.27	100
Celecoxib	16.74 ± 0.22	0.0
6a	12.36 ± 0.28	0.0
6b	10.64 ± 0.32	10
6c	10.72 ± 0.18	10
6d	11.96 ± 0.27	0.0
6e	11.76 ± 043	0.0
7a	9.46 ± 0.28	0.0
7b	9.38 ± 0.36	0.0
7c	11.06 ± 0.45	10
7d	10.98 ± 26	10
7e	10.34 ± 34	0.0
8a	10.96 ± 0.52	0.0
8b	11.36 ± 26	0.0
8c	11.58 ± 33	10
8d	13.28 ± 0.44	10
8e	10.34 ± 0.42	0.0
9a	8.58 ± 0.16	0.0
9b	8.94 ± 0.25	0.0
9c	10.88 ± 0.24	0.0
9d	11.06 ± 0.27	20
9e	13.82 ± 0.35	10

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

anhydrous sodium acetate in ethanol afforded the corresponding hydrazones **3a-e.** The hydrazones **3a-e** were subjected to Vilsmeyer Haack reaction [33], to yield the dimethylaminomethyleneamino aldehydes **4a-e**. The structures of these compounds were confirmed by IR spectra that showed lack of the absorption bands characteristic for NH_2 group. Moreover, ^{1}H-NMR spectra for these compounds revealed two singlets at δ 2.9 and 3.15 ppm characteristic for two methyl groups, in addition to a singlet at δ8.23–8.27 ppm characteristic for methine proton. The latter signal appeared at high chemical shift indicating presence of compounds **4a-e** as Z-isomers [34]. These results are in accordance to the findings of Sen and Mukhopadhyay [35] who reported the synthesis of dimethylaminomethyleneaminosulfonyl derivative by reaction of sulfonamido derivative with Vilsmeyer reagent. Compounds **4a-e** were hydrolyzed to the free aminosulfonyl derivatives **5a-e** by heating under reflux with concentrated HCl for 3 h. Reaction of the selected aldehyde **4a-e** and **5a-e** with hydroxylamine hydrochloride in ethanol containing anhydrous sodium acetate yielded the corresponding oximes **6a-e** and **7a-e** respectively. Inspection of ^{1}H-NMR spectra for compounds **6a-e** and **7a-e** revealed iminomethyl proton at δ = 7.40–7.55 ppm confirming existence of these compounds as E-isomers [36]. Dehydration of the oximes **6a-e** with acetic anhydride [21] afforded the cyano derivatives **8a-e**. ^{1}H-NMR spectra of the latter compounds showed methine proton at δ = 8.24–8.27 ppm indicating their existence as Z-isomers similar to their precursors **4a-e**. Dehydration of the oximes **7a-e** was accomplished using $POCl_3$ in presence of triethylamine [37] rather than acetic anhydride to avoid acetylation of the sulfonamido group. This method resulted in the corresponding cyano derivatives **9a-e** in high yield.

Anti-inflammatory activity

Cotton pellet-induced granuloma bioassay. The anti-inflammatory activity of the synthesized compounds **6**, **7**, **8**, **9** was evaluated by applying the cotton-pellet granuloma bioassay in rats [24] using celecoxib and indomethacin as reference standards. The ED_{50} values were determined for each compound and expressed as the mean ± SEM. Significant differences between the control and the treated groups was obtained using student's t-test and P values. The difference in results was considered significant at $P < 0.001$ (Table III). All test compounds showed anti-inflammatory activity higher than celecoxib (ED_{50} = 16.74 μmol), whereas, compounds **7a**, **7b**, **9a** and **9b** (ED_{50} = 9.46, 9.38, 8.58 and 8.94 μmol respectively) possessed anti-inflammatory activity comparable to that of indomethacin (ED_{50} = 9.68 μmol). In general, the free aminosulfonyl pyrazole derivatives were more active than their dimethylaminomethyleneamino analogues. The replacement of hydroxyliminomethyl group at position 4 of the pyrazole ring with a cyano group (compounds **8a**, **8e** and **9a-c**) enhanced the anti-inflammatory activity. Within the active series **7**

Table IV. Effect of compounds **7a**, **7b**, **9a** and **9b** on carrageenan-induced rat paw edema[a] (mL) at a single dose (10 μmol/kg), % protection and activity relative to indomethacin.

Test compound	Increase in paw edema (ml) ± SEM[b,c]	% 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
7a	0.30 ± 0.022	69.3	93.14
7b	0.32 ± 0.018	67.3	90.45
9a	0.23 ± 0.016	76.5	102.82
9b	0.26 ± 0.026	73.4	98.65

[a] Paw edema was Induced by subplantar injection of 50 μL of 2% carrageenan soultion in saline (0.9%); [b] SEM denotes the standard error of the mean; [c] All data are significantly different from control ($P < 0.001$).

Table V. In vitro human COX-2[a] and COX-1[b] enzymes inhibitory activities of compounds **7a**, **7b**, **9a** and **9b**.

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
7a	1.35 ± 0.02	83.62 ± 0.29	61.94
7b	1.26 ± 0.06	76.41 ± 0.22	60.64
9a	0.78 ± 0.08	>100	128.20
9b	0.92 ± .0.02	94.22 ± 0.18	102.41

[a] Human recombinant COX-2 enzyme; [b] Human COX-1 enzyme from human platelets; [c] Values are means of at least four experiments.

and **9**, the introduction of a methyl group at position 4 of the phenyl moiety didn't alter the activity. However the bromo, chloro and nitro groups exhibited reduced anti-inflammatory activity. Compounds **9a** and **9b** were found to be the most active anti-inflammatory agents in the present study (ED_{50} = 8.58 and 8.94 μmol, respectively).

Carrageenan-induced rat paw edema bioassay. Compounds **7a**, **7b**, **9a** and **9b** that showed anti-inflammatory activity comparable to that of indomethacin and higher than celecoxib in the cotton pellet–induced granuloma bioassay, were further evaluated for their *in-vivo* systemic effect using the carrageenan–induced paw edema bioassay in rats [25]. The results (Table IV) revealed that compounds **9a** and **9b** exhibited systemic anti-inflammatory activity (% protection = 76.5 and 73.4, respectively) comparable to that of indomethacin and celecoxib (% protection = 74.4 and 77.5, respectively) whereas compounds **7a** and **7b** (% protection = 69.3 and 67.3) were relatively less active.

Human COX-1 and COX-2 enzymatic assay

Compounds **7a**, **7b**, **9a** and **9b** that exhibited potent anti-inflammatory profiles in the pre-mentioned animal models were further tested for their ability to inhibit human COX-1 and COX-2 enzymes *in-vitro* applying the methodology of Wakitani *et al* [30]. The concentration of the compound causing 50% enzyme inhibition (IC_{50} μmol) is shown in Table V. The test compounds exhibited much weaker inhibitory activity against COX-1 enzyme (IC_{50} values range 76.41– > 100 μmol) compared with indomethacin (IC_{50} = 0.26 μmol). In addition, the test compounds showed higher inhibitory profile against COX-2 (IC_{50} values were between 0.78 and 1.35 μmol) compared with indomethacin (IC_{50} = 2.63 μmol). This led to a very high approximate selectivity ratio (COX-1/COX-2) for the test compounds (ranged between 60.6–128.2) compared with indomethacin (selectivity ratio is 0.098). However, all test compounds showed approximate selectivity ratio (COX-1/COX-2) lower than that of celecoxib. In general, oxime derivatives (compounds **7a**, **7b**) showed lower selectivity towards COX-2 enzyme than cyano derivatives (compounds **9a**, **9b**). Compound **9a** was the most selective COX-2 inhibitor (approximate selectivity ratio = 128.20) in the present study, but the proximate selectivity was >40% of that found with celecoxib (approximate selectivity ratio >333).

Ulcerogenic effects

The synthesized compounds were evaluated for their ulcerogenic potential in rats [28]. All test compounds proved to have superior gastrointestinal safety profiles (0–20% ulceration) in the population of the tested animals at oral doses of 30 μmol/kg per day, compared with indomethacin which was found to cause 100% ulceration under the same experimental conditions (Table III). Compounds having bromo, chloro or nitro group **6c**, **7c**, **7d**, **8c**, **8d**, **9d**, **9e** showed a slight ulcerogenic effect (10–20%). Gross observation of the isolated rat stomachs showed a normal stomach texture for the other compounds.

Acute Toxicity

Compounds **7a**, **7b**, **9a** and **9b** were further evaluated for their oral acute toxicity in male mice according to established methods [26,29]. The results indicated that most of the tested 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 parenteral route [20]. The results revealed that all compounds were non-toxic up to 65 mg /kg.

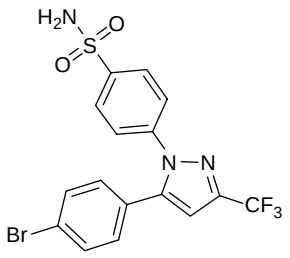


Figure 2. Structure of SC-558.

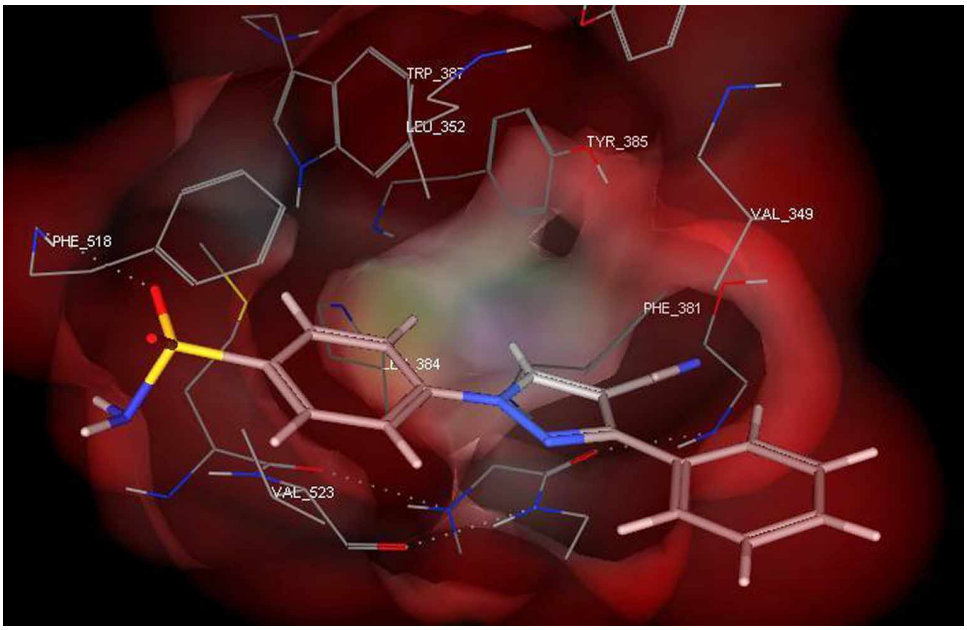


Figure 3. 3D View from a molecular modeling study, of the minimum-energy structure of the complex of **9a** docked in COX-2 (PDB ID: 1CX2). Viewed using Molecular Operating Environment (MOE) module.

Docking Studies

Molecular docking as well as conformational alignment studies of compounds **(9a, 9b)** were performed using Molecular Operating Environment's (MOE-Dock 2005) module [31] in order to rationalize the obtained biological results. Molecular docking studies further helps in understanding the various interactions between the ligands and enzyme active sites in detail.

The determination of the three-dimensional co-crystal structure of COX-2 complexed with a selective inhibitor, SC-558 (Figure 2; PDB ID: 1CX2) has led to the development of a model for the topography of the NSAIDs binding site in human COX-2 [38]. Therefore herein, we performed the docking studies using this COX-2 co-crystal with SC-558 as a template.

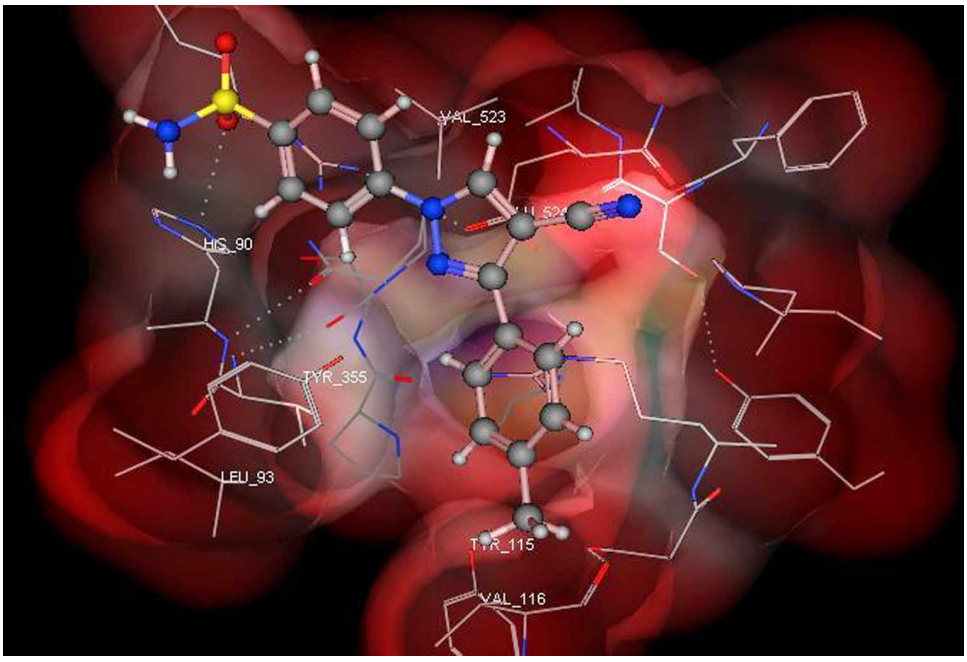


Figure 4. 3D View from a molecular modeling study, of the minimum-energy structure of the complex of **9b** docked in COX-2 (PDB ID: 1CX2). Viewed using Molecular Operating Environment (MOE) module.

Figures 3 and 4 shows the binding interactions of both compounds **9a**, **9b** to the active site of COX-2, respectively where both of them exhibited some similar interactions as SC-558. In general, compounds **9a**, **9b** showed a hydrogen bonding interactions with His90, Ile517, Phe518, Ser146, Ser38, Glu671 and a hydrophobic interaction with: Met113, Val116, Val349, Leu352, Leu359, Phe518, Val523, Leu531.

In a similar pattern to SC-588, one of the O-atoms of the SO_2NH_2 substituent of both **9a** and **9b** forms a hydrogen bond with the NH hydrogen of His90 (Figure 4) and the other oxygen forms hydrogen bonds with backbone amide hydrogens of Phe518 (Figure 3). Furthermore, the phenyl ring bearing the sulfonamide is surrounded by a hydrophobic pocket of Val349, Leu352, Phe518, and the more important Val523 (found only in COX-2). On the other hand, the conformational alignment of SC-558 and **9b** showed an overlapping between the phenylsulfonamide moiety of both SC-558 and compound **9b** while the 3-tolyl ring was inserted into the trifluoromethyl zone.

From the abovementioned data, the docking results of the examined compounds **(9a, 9b)** provide a good explanation for the potent inhibitory activity of both compounds and give an insight about the COX-2 binding of the pyrazole nucleus with a 1,3-diaryl substituted pattern as a suitable scaffold for the development of selective COX-2 inhibitors.

Table VI. Minimal inhibitory concentrations (MIC μg/mL) of test compounds.

Test compound	*E. coli*	*S. aureus*	C. albicans
6a	100	>200	>200
6b	100	100	>200
6c	200	50	>200
6d	25	>200	>200
6e	100	50	>200
7a	>200	100	>200
7b	25	100	>200
7c	100	25	>200
7d	25	50	>200
7e	50	100	>200
8a	50	100	>200
8b	25	>200	>200
8c	100	100	>200
8d	100	100	>200
8e	50	>200	>200
9a	50	50	>200
9b	25	25	>200
9c	50	50	>200
9d	50	100	>200
9e	50	100	>200
Ampicillin	25	12.5	–
Clotrimazole	–	–	12.5

Antimicrobial activity

The designed compounds **6a-e**, **7a-e**, **8a-e** and **9a-e** were evaluated for their 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 [32]. The minimal inhibitory concentration (MIC) revealed that all test compounds have no antifungal activity (Table VI). The antibacterial activity of the test compounds **6d**, **7b**, **7d**, **8b** and **9b** against *E. coli* was similar to the reference antimicrobial compound (ampicillin). Compounds **7e**, **8a**, **8e**, **9a** and **9c–e** showed half the antimicrobial activity of ampicillin against *E. coli*. In general cyano derivatives **8a-c**, **8e**, **9a** and **9c** possessed better antimicrobial activity than their oxime precursors **6a-c**, **6e**, **7a** and **7c** against *E. coli*. Moreover, aminosulfonyl derivatives **7b**, **7c** and **7e** had higher activity than their dimethylaminomethyleneamino analogs **6b**, **6c** and **6e** may be attributed to the benzenesulfonamido moiety. Furthermore, the antibacterial activity against *E. coli* was enhanced when dimethylaminomethyleneamino group of **8c** and **8d** was hydrolyzed to the corresponding aminosulfonyl analogs **9c** and **9d**. Regarding the antibacterial activity against *S. aureus*, all test compounds showed weak or no antibacterial activity except for compounds **7c** and **9b** which showed half the activity of ampicillin.

Conclusion

The investigated compounds **7a**, **7b**, **9a** and **9b** were found to possess anti-inflammatory activity comparable to that of indomethacin and celecoxib. Meanwhile, they displayed higher selective inhibitory activity to COX-2 when compared to indomethacin. Moreover, compounds **7b** and **9b** exhibited similar antimicrobial activity to that of ampicillin against *E. coli*. Compounds **9a** and **9b** showed anti-inflammatory activity profile lower than previously reported compound **A** [19] and comparable to that of compounds **B** [20], **C** [18,21], **D** [18] and **E** [23]. In addition, compounds **9a** and **9b** exhibited COX-1/COX-2 selectivity ratio higher than compound **A** and lower than compound **C**. Compound **9a** displayed antimicrobial activity against *E. coli*. comparable to compounds **A**, **B**, **C** and **E**, whereas, the antimicrobial activity of **7b** and **9b** was comparable to compound **D**. The antimicrobial activity of compounds **7a**, **7b** and **9a** against *S. aureus* was lower than compounds **A**, **B**, **C** and **D**, while the activity of **9b** was comparable to compound **D**. Overall, it can be concluded that compounds **9a** and **9b** proved to be the most active anti-inflammatory agents ($ED_{50} = 8.58$ and 8.94 μmol) in the present study with no ulcerogenic effect and good safety margin. However, the antibacterial activity of compound **9b** was twice that found with compound **9a** against *E. coli* and *S. aureus*.

The antibacterial activity of compound **9b** against *E. coli* was equivalent to that of ampicillin, whereas, the antibacterial activity against *S. aureus* is about 50% that of ampicillin.

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