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Design, Synthesis And Biologicalevaluation Of Novel Series Of*n*-Pyrazole Derivatives As Potential Anti-Inflammatory And Antibacterial Agents.

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ABSTRACT

A novel series of N-pyrazole-5-carboxylate bearing aryl α,γ-diketobutanoic acid moiety **4**was synthesized in a series of synthetic steps and was used as key intermediate for the synthesis of 4-(3-hydroxy-4-isopropoxy-4-oxobut-2-enoyl)benzyl-1H-pyrazole-5-carboxylate **(5)** and 4-(3-hydroxy-4-methylene-1-(4-substituted phenyl)pyrrolidin-2-enoyl)benzyl) -1H-pyrazole-5-carboxylate derivatives**(7a,b**). Compounds 4-{3-hydroxy-4-[(1-hydroxypropan-2-yl) amino]-4-oxobut-2-enoyl}benzyl-1H-pyrazole-5-carboxylate (**9**) and 4-[3-hydroxy-(4-benzyl)-4-oxobut-2-enoyl]amino}propyl-4-substituted phenyl-carboxamido}-1H-pyrazole-5-carboxylate derivatives(**10a, b**) were also synthesized.On the other hand, compound **1** was used as key intermediate for the synthesis of novel series of N-pyrazolylderivatives with different substitution on terminal phenyl(**12**-**15**),**17a, b**,**19a, b**and**21a, b.**respectivelyvia treatment with various reagents.The structural interpretations of newly synthesized compounds were based on IR, ^{1}H, ^{13}C NMR, mass spectral data and elemental analysis. All the synthesized compounds were screened for their in vitroanti-inflammatory and antibacterial activities. Compounds **7a**, **10b**, **19a** and **21a** showed the greatest anti-inflammatory activity which were more potent than the reference drug celecoxib. Furthermore, compounds **19a**, **21a** exhibited maximum reduction in ulcerogenic activity. Most of the newly synthesized compounds showed highantibacterial activity. In addition, ethyl 4-amino-3-(4-chlorophenyl)-1-{4-[(isoquinolin-5-sulfonyl)oxy]-benzyl}-1*H*-pyrazole-5-carboxy-late **13** exhibited antibacterial activity higher than cefatoxime as reference drug.

Keywords: *N*-pyrazolylderivatives, anti-inflammatory, antibacterial and ulcerogenic activities.

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) plays a fundamental role for the relief of pain and inflammation through inhibition of cyclooxygenase (COX) enzyme which performs the first step in prostaglandins creation via catalyzing the double dioxygenation of arachidonic acid to prostaglandin endoperoxides and thromboxane [1]. NSAIDs lead to increased secretion of stomach acid which causes ulceration and bleeding of the gastrointestinal tract [2], the discovery of new compounds with potent anti-inflammatory activities and selective COX-2 inhibitiondevoid of the undesirable effects associated with classical NSAIDs is still the objective of this research. Celecoxib is one of the most safely anti-inflammatory agents [3] which has pyrazole moiety, there are more than NSAIDs containing pyrazole moiety among this Lonazolac [4] andRimonabant [5](Fig.1).

Besides the anti-inflammatory drugs, there is an imperious need to develop new antibacterial agents due to increasing bacterial resistance. Pyrazole derivatives displayed a multitude of biological activities, including antitumor [6, 7], antimicrobial [8, 9],anti-tobacco mosaic virus (TMV) activity [10],sodium channel blocker[11],antitubercular[12] antipyreticand anti-inflammatory [13,14]

Furthermore, pyrazole and its synthetic analogs have also been reported to possess antidiabetic [15], analgesic [16], antihypertensive [17], anticonvulsant [18], and also used as human acyl-cholesterol acyltransferase inhibitors [19].

According to the aforementioned information and in continuation of our previous work on pyrazole, we describe the synthesis of anti-inflammatory activity, ulcerogenic liability and antibacterial for aseries of novel N-pyrazole derivatives incorporated with other biologically active heterocycles.

MATERIALS AND METHODS

Experimental

Microanalytical data were gathered with a Vario Elementar apparatus (Shimadzu). Elemental analyses of all compounds were within ± 0.4% of the theoretical values. The IR spectra (KBr) were recorded on a Perkin Elmer 1650 spectrometer (USA).^{1}H and^{13}C NMRspectra(500MHz for ^{1}Hand 125 MHzfor ^{13}C NMR) were recorded on JEOL ECA-500 (Shimadzu) instruments. Chemical shifts were expressed in ppm relative to $SiMe_4$ as internal standard in DMSO-d_6 as a solvent. Mass spectra were recorded on a 70 eV Finnigan SSQ 7000 spectrometer (Thermo-Instrument System Incorporation, USA).All melting points were measured on an Electrothermal 9100 series digital melting point apparatus (Shimadzu, Japan). The purity of the compounds was checked on aluminum plates coated with silica gel (Merck, Germany).Chemicals and solvents (Analar ≥99%) were purchased from Sigma-Aldrich (USA).Cefatoxime was supplied by Hoechst-Roussel Pharmaceuticals Inc., Somerville, New Jersey, USA.

Syntheses

Ethyl 4-amino-3-(4-chlorophenyl)-1H-pyrazol-5-carboxylate (**1**) was achieved by a reported method [20].

Ethyl 1-(4-acetylbenzyl)-4-amino-3-(4-chlorophenyl)-1H-pyrazole-5-carboxylate(**2**).To a solution of compound **1** (2.65 g, 10 mmol) in DMF (30 mL) was added 4-(bromo-methyl) acetophenone (2.13 g, 10 mmol) and K_2CO_3 (2.76, 20 mmol). The mixture was stirred at room temperature during 36 h and evaporated under reduced pressure. The residue was purified over silica gel column chromatography with Petroleum ether/ EtOAc to afford compound **2**as a pale yellow powder.

Yield 72%;m.p. 260-262°C; IR (KBr)3390(brs, NH_2), 3030(CH aryl), 2978(CH alkyl) ,1725, 1680 (2C=O), 1610(C=N) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6 ,ppm): δ 1.35 (t, 3H, CH_3), 2.65 (s, 3H, $COCH_3$), 3.40 (s, 2H, N-CH_2), 4.25 (q, 2H, OCH_2), 7.25 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.50 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.82 (d, 2H, *J* = 8.2 Hz, Ar-H), 8.10 (d, 2H, *J* = 8.2 Hz, Ar-H), 9.08 (br, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 13.6 (CH_3), 26.6 ($COCH_3$), 44.7 (CH_2), 48.5 (OCH_2) 120.4, 127.9, 128.2, 130.8, 136.7, 137.6, 141.8, 157.2, 159.4, 160.1 (11 sp^2 carbons) 170.9, 186.8 (2CO); its MS (m/z): 397 (M^+, 73%), 399 (M^++2, 21%); $C_{21}H_{20}ClN_3O_3$ (397.8); calcd. %C:63.40, %H: 5.07, %N: 10.56. found: %C:63.37, %H: 5.05, %N: 10.54.

Ethyl-4-amino-3-(4-chlorophenyl)-1-({4-[(4-ethoxy-3-hydroxy-4-oxobut-2-enoyl]benzyl})-1H-pyrazole-5-carboxylate(**3**).A solution of **2** (0.1 mol) in dry THF (0.1 M, 50 mL) under nitrogen pressure was treated with LiHDMS (0.15mol) at -80°C. After stirring 40 min at -80°C, diethyloxalate (0.15 mol) was added dropwise and let the mixture to reach room temperature. Stirring was continued for 3 h and the solution was concentrated under reduced pressure. The residue was taken with 1 M HCl and extracted with CH_2Cl_2. Combined organic layers were washed with water and dried over $MgSO_4$then evaporated, collect the solid and crystallized from dioxane as a white powder.

Yield 67%;m.p. 230-232°C; IR (KBr)3443(brs, NH_2), 3020(CH aryl), 2980(CH alkyl), 1728, 1678 (2C=O), 1620(C=N), 1595 (C=C)cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6 ,ppm): δ1.34-1.40 (m, 6H, $2CH_3$), 3.45 (s, 2H, N-CH_2), 3.85-4.35 (m, 4H, $2OCH_2$), 5.40 (s, 1H, OH, D_2O exchangeable), 6.09 (s, 1H, CH), 7.20 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.46 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.80 (d, 2H, *J* = 8.2 Hz, Ar-H), 8.05 (d, 2H, *J* = 8.2 Hz, Ar-H), 9.15 (br, 2H, NH_2, D_2O exchangeable);its MS (m/z):497(M^+, 66%),499(M^++2, 19%); $C_{25}H_{24}ClN_3O_6$ (497.9);calcd. %C:63.30, %H: 4.86, %N: 8.44. found: %C:60.27, %H: 4.82, %N: 8.40.

4-[4-(4-amino-3-(4-chlorophenyl)-5-ethoxycarbonyl-pyrazol-1-yl)methyl]-phenyl]-3-hydroxy-4-oxo-but-2-enoic acid (**4**).Compound **3** (4.98 g, 10 mmol) was dissolved in THF (0.1 M, 50mL) and 1 M LiOH (10 mL) was added. At room temperaturethe mixture was stirred for 3 h, acidified with 1M HCl (5 mL) and extracted with dichloromethane. Combined organic layers were washed with water and dried over $MgSO_4$then evaporated. The residue was purified over short reverse phase silica gel column with H_2O/ACN to afford **4** as a gray powder. Yield 75%;m.p. 273-275°C; IR (KBr)3425(brs, NH_2), 3025(CH aryl), 2899(CH alkyl), 1729, 1688(2C=O), 1618(C=N), 1590(C=C);^{1}H NMR (500 MHz, DMSO-d_6 ,ppm): δ1.32 (t, 3H, CH_3), 3.44 (s, 2H, N-CH_2), 4.35 (q, 2H, OCH_2), 5.77 (s, 1H, OH, D_2O exchangeable), 6.00 (s, 1H, CH), 7.25 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.60 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.85 (d, 2H, *J* = 8.0 Hz, Ar-H), 8.15 (d, 2H, *J* = 8.0 Hz, Ar-H), 9.23 (br, 2H, NH_2, D_2O exchangeable), 13.90 (s, 1H, COOH); ^{13}C NMR (125MHz, DMSO-d_6, *δ*, ppm); 13.8 (CH_3) , 44.3 (CH_2), 47.9 (OCH_2), 115.64, 118.2, 120.4, 128.05, 128.1, 130.41, 132.8, 143.0, 149.0, 158.2, 159.3, 159.9, 160.3, 186.1, 174.4, 173.1(3CO);its MS (m/z):469 (M^+, 85%), 471 (M^++2, 25%); $C_{23}H_{20}ClN_3O_6$(469.8); calcd. %C:58.79, %H: 4.29, %N: 8.94. found: %C:58.75, %H: 4.26, %N: 8.94.

Ethyl-4-amino-3-(4-chlorophenyl)-1-(4-(3-hydroxy-4-isopropoxy-4-oxobut-2-enoyl)benzyl)-1*H*-pyrazole-5-carboxylate (5).A solution of **4** (2.35 g, 5 mmol) in 30 mL of isopropyl alcohol, conc. sulfuric acid (3.5 mL)was added with stirring then the mixture was heated under reflux for 4 h. After completion of the reaction, the solvent was evaporated under reduced pressure. The solid product was collected and crystallized from ethanol as white powder.

Yield 75%;m.p. 273-275°C; IR (KBr)3430(brs, NH_2), 3020(CH aryl), 2970(CH alkyl), 1730, 1690(C=O), 1610(C=N), 1590(C=C); ^{1}H NMR (500 MHz, DMSO-d_6 ,ppm): δ1.30 (t, 3H, CH_3), 1.41 (s, 6H, $2CH_3$), 3.45 (s, 2H, N-CH_2), 3.70 (sept, 1H, OCH isopropyl), 4.30 (q, 2H, OCH_2), 5.60 (s, 1H, OH, D_2O exchangeable), 6.05 (s, 1H, CH), 7.15 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.49 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.75 (d, 2H, *J* = 8.2 Hz, Ar-H), 8.10 (d, 2H, *J* = 8.2 Hz, Ar-H), 9.15 (br, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (125MHz, DMSO-d_6, *δ*, ppm); 14.5, 21.7, 23.2 ($3CH_3$), 43.1 (CH_2),43.9 (OCH), 48.3 (OCH_2), 128.2, 128.4, 129.2, 134.4, 137.3, 142.5, 145.3, 148.7, 158.5, 159.3, 160.3, 161.5, 162.1 (13 sp^2),185.1, 173.9, 170.4 (3CO);its MS (m/z):511 (M^+, 69 %), 513(M^++2, 22%); $C_{26}H_{26}ClN_3O_6$ (511.9); calcd. %C:61.00, %H: 5.12, %N: 8.21. found: %C:61.00, %H: 5.10, %N: 8.18.

Ethyl 4-amino-3-(4-chlorophenyl)-1-[4-(3-hydroxy-4-methylene-1-(4-substituted phenyl)pyrrolidin-2-enoyl)benzyl)]-1*H*-pyrazole-5-carboxylate derivatives(7a,b).*General procedure*.Compound **4** (4.7 g, 10 mmol) and 1-(4-trifluoromethoxy-phenyl)-4-hydroxymethyl-pyrrolid-2-one **(6a**)(2.73 g, 10 mmol) or1-(4-methoxyphenyl)-4-hydroxymethyl-pyrrolid-2-one(**6b**)(2.19 g, 10 mmol), Ph_3P (2.62g, 10mmol) and triethyl amine (1.01ml, 10mmol) in dry toluene (50ml) at 0 °C, diisopropyl azodicarboxylate (DEAD) (2mL, 10mmol) was added drop-wise. The mixture was cooled to room temperature and heated at 90 °C for 24h. 5% HCl (30 ml) was added to the mixture and the compound was extracted with dichloromethane. The organic layer was dried and concentrated to afford the crude product which was purified by flash column chromatography to obtain **7a,b**.

Ethyl 4-amino-3-(4-chlorophenyl)-1-[4-(3-hydroxy-4-methylene-1-(4-triflouromethoxy-phenyl) pyrrolid-2-onyl) benzyl)]-1*H*-pyrazole-5-carboxylate(7a)

Yield 68%;m.p. 190-192°C; IR (KBr)3425 (brs, NH_2), 3310 (brs, OH), 3025 (CH aryl), 2980 (CH alkyl), 1730, 1688 (C=O), 1612 (C=N), 1600 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6 ,ppm): δ1.34 (t, 3H, CH_3), 2.31-2.41 (m, 1H, CH of pyrolidine), 2.60-2.66 (m, 2H, CH_2); 2.96-2.98 (m, 2H, CH_2), 3.46 (s, 2H, N-CH_2), 4.05 (s, 2H, OCH_2), 4.25 (q, 2H, OCH_2CH_3), 5.45 (s, 1H, OH, D_2O exchangeable), 6.10 (s, 1H, CH), 7.35-7.40 (m, 6H, Ar-H), 7.50-7.98 (m, 6H, Ar-H), 9.20 (br, 2H, NH_2, D_2O exchangeable);its MS (m/z):727 (M^+, 64%),729 (M^++2, 22%)$C_{35}H_{30}ClF_3N_4O_8$ (727.0); calcd. %C:57.82, %H: 4.16, %N: 7.71. found: %C:57.80, %H: 4.13, %N: 7.68.

Ethyl 4-amino-3-(4-chlorophenyl)-1-[4-(3-hydroxy-4-methylene-1-(4-methoxy-phenyl) pyrrolid-2-onyl)benzyl)]-1*H*-pyrazole-5-carboxylate (7b).

Yield 72%;m.p. 220-222°C; IR (KBr)3450 (brs, NH_2), 3300 (brs, OH), 3025 (CH aryl), 2988 (CH alkyl), 1732, 1688 (C=O), 1610 (C=N), 1590 (C=C);^{1}H NMR (500 MHz, DMSO-d_6 ,ppm): δ1.30 (t, 3H, CH_3), 2.30-2.39 (m, 1H, CH of pyrolidine), 2.59-2.70 (m, 2H, CH_2); 2.94-2.98 (m, 2H, CH_2), 3.40 (s, 2H, N-CH_2), 3.75 (s, 3H, OCH_3), 4.10 (s, 2H, OCH_2), 4.29 (q, 2H, OCH_2CH_3), 5.40 (s, 1H, OH, D_2O exchangeable), 6.12 (s, 1H, CH), 7.30-7.38 (m, 6H, Ar-H), 7.49-7.95 (m, 6H, Ar-H), 9.16 (br, 2H, NH_2, D_2O exchangeable);its MS (m/z):673 (M^+, 54%), 675 (M^++2, 18%)$C_{35}H_{33}ClN_4O_8$ (673.1);calcd. %C:62.45, %H: 4.94, %N: 8.32. found: %C:62.41, %H: 4.92, %N: 8.30.

Ethyl 4-amino-3-(4-chlorophenyl)-1-(4-[4-chloro-3-hydroxy-4-oxobut-2-enoyl] benzyl)-1*H*-pyrazole-5-carboxylate(**8**).To a solution of $SOCl_2$ (5.35 g, 30 mmol), the compound **4** (4.7 g, 10 mmol) was added dropwisely. The reaction mixture was heated under reflux for 5 h. After completion the reaction, the excess of $SOCl_2$ was evaporated to give **8** as a yellow liquid that was used without further purification.

IR (KBr)3435 (brs, NH_2), 3320 (brs, OH), 3020 (CH aryl), 2980 (CH alkyl), 1730, 1689 (C=O), 1620 (C=N), 1595 (C=C);^{1}H NMR (500 MHz, DMSO-d_6 ,ppm):δ1.34 (t, 3H, CH_3), 3.41 (s, 2H, N-CH_2), 4.30 (q, 2H, OCH_2), 5.73 (s, 1H, OH, D_2O exchangeable), 6.09 (s, 1H, CH), 7.19 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.52 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.80 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.98 (d, 2H, *J* = 8.0 Hz, Ar-H), 9.28 (br, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6, *δ*, ppm); 13.8 (CH_3), 42.7 (CH_2), 49.5 (OCH_2), 115.6, 119.2, 121.4, 127.1, 128.0, 131.41, 135.8, 137.6, 144.3, 147.5, 158.2, 158.9, 159.4 (13 sp^2),171.5, 173.2, 184.1(3CO);$C_{23}H_{19}Cl_2N_3O_5$ (488.3).

Ethyl 4-amino-3-(4-chlorophenyl)-4-{3-hydroxy-4-[(1-hydroxypropan-2-yl) amino]-4-oxobut-2-enoyl}benzyl-1*H*-pyrazole-5-carboxylate(**9**).To a solution of compound **8** (4.88 g, 10 mmol) in CH_2Cl_2 (30 mL), 2-amino-propanol(1.5 mL, 20 mmol) and Et_3N (3.03 mL, 30 mmol) in CH_2Cl_2 (50 mL) was added dropwisely under 0-5 °C for 1 h. The mixture was vigorously stirred at ambient temperature for 5 h, then evaporated and the residue was purified by column chromatography eluting with a mixture of hexane/ethyl acetate (5:5) to obtain white solid.

Yield 85%;m.p. 254-256°C; IR (KBr)3443 (brs, NH_2), 3300 (brs, OH), 3016 (CH aryl), 2989 (CH alkyl), 1730, 1670 (C=O), 1615 (C=N), 1585 (C=C);δ^{1}H NMR (500 MHz, DMSO-d_6 ,ppm):

1.16 (d, 3H, CH_3), 1.30 (t, 3H, CH_3), 3.50 (s, 2H, N-CH_2), 4.10-4.30 (m, 4H, 2OCH_2), 4.39-4.50 (m, 1H, NCH), 5.66 (s, 1H, OH, D_2O exchangeable), 6.05 (s, 1H, CH), 6.73 (s, 1H, NH), 7.05 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.37 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.50 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.88 (d, 2H, *J* = 8.4 Hz, Ar-H), 9. 20 (br, 2H, NH_2, D_2O exchangeable), 9.50 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR (500 MHz, DMSO-d_6, *δ*, ppm); 13.8, 18.28 (2CH_3), 43.1(CH), 43.6, 45.6 (2CH_2), 48.67(OCH_2), 115.6, 117.3, 119.2, 120.8,121.4, 127.1, 131.41, 134.6, 137.4, 145.3, 147.8, 158.4, 159.1(13 sp^2), 172.3, 174.1, 180.2 (3CO) ;its MS (m/z):526 (M^+, 66%), 528 (M^++2, 24%)$C_{26}H_{27}ClN_4O_6$ (526.9)calcd. %C:59.26, %H: 5.16, %N: 10.63. found: %C:59.22, %H: 5.31, %N: 10.60.

Ethyl 4-amino-3-(4-chlorophenyl)-1-{4-[3-hydroxy-(4-benzyl)-4-oxobut-2-enoyl]amino}propyl-4-substituted phenyl-carboxamido}-1*H*-pyrazole-5-carboxylate derivatives(**10a, b**).*General procedure.* Compound **9** (5.27g, 10 mmol) with phenylisocyanate (1.19 g, 10 mmol) or p-fluoro- phenylisocyanate (1.37 g, 10 mmol) in THF (60 mL). The mixture was stirred at room temperature for overnight. The solid wasfiltered washed with ethanol, dried and crystallized from dioxane.

Ethyl 4-amino-3-(4-chlorophenyl)-1-{4-[3-hydroxy-(4-benzyl)-4-oxobut-2-enoyl]amino} propyl-phenyl-carboxamido}-1*H*-pyrazole-5-carboxylate (**10a**)

Yield 62%;m.p. 210-212°C; IR (KBr); 3440 (brs, NH_2), 3330 (brs, OH), 3020 (CH aryl), 2989 (CH alkyl), 1732, 1669 (C=O), 1620 (C=N), 1590 (C=C);δ^1H NMR (500 MHz, DMSO-d_6 ,ppm):1.14 (d, 3H, CH_3), 1.39 (t, 3H, CH_3), 3.44 (s, 2H, N-CH_2), 4.07- 4.21 (m, 4H, 2OCH_2), 4.29 (m, 1H, NCH), 5. 54(s, 1H, OH), 6.05 (s, 1H, CH), 6.98 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.25 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.45-7.65 (m, 4H, Ar-H), 7.80- 8.04 (m, 5H, Ar-H), 8.58 (s, 1H, NH), 9. 33 (br, 2H, NH_2, D_2O exchangeable), 9.69 (s, 1H, NH),1.15 (d, 3H, CH_3), 1.40 (t, 3H, CH_3), 3.39 (s, 2H, N-CH_2), 4.03- 4.19 (m, 4H, 2OCH_2), 4.24 (m, 1H, NCH), 5.58 (s, 1H, OH, D_2O exchangeable), 6.12 (s, 1H, CH), 7.25-7.35 (m, 6H, Ar-H), 7.59-7.97 (m, 6H, Ar-H), 8.60 (s, 1H, NH), 9. 24 (br, 2H, NH_2, D_2O exchangeable), 9.60 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm); 14.3, 19.20 (2CH_3), 42.2 (CH), 42.9 (CH_2), 48.2, 48.6 (2OCH_2), 115.4, 117.2, 120.8, 122.3, 124.4, 125.8, 129.9, 130.8, 131.6, 134.5, 137.4, 145.3, 147.8, 151.4, 153.1, 157.4, 158.9 (17 sp^2) 171.5, 174.2, 178.3, 181.2 (4CO); its MS (m/z):646 (M^+, 71%), 648 (M^++2, 22%) $C_{33}H_{32}ClN_5O_7$ (646.1),calcd. %C:61.35, %H: 4.99, %N: 10.84. found: %C:61.32, %H: 4.95, %N: 10.80.

Ethyl 4-amino-3-(4-chlorophenyl)-1-{4-[3-hydroxy-(4-benzyl)-4-oxobut-2-enoyl]amino} propyl-4-fluorophenyl-carboxamido}-1*H*-pyrazole-5-carboxylate (**10b**).

Yield 65%;m.p. 220-222°C; IR (KBr);3441(brs, NH_2), 3335 (brs, OH), 3020 (CH aryl), 2989 (CH alkyl),1733, 1669 (C=O), 1618 (C=N), 1595 (C=C); δ^1H NMR (500 MHz, DMSO-d_6 ,ppm):

δ1.15 (d, 3H, CH_3), 1.40 (t, 3H, CH_3), 3.39 (s, 2H, N-CH_2), 4.03- 4.19 (m, 4H, 2OCH_2), 4.24 (m,1H, NCH), 5.58 (s, 1H, OH, D_2O exchangeable), 6.12 (s, 1H, CH), 7.25-7.35 (m, 6H, Ar-H), 7.59-7.97 (m, 6H, Ar-H), 8.60 (s, 1H, NH), 9. 24 (br, 2H, NH_2, D_2O exchangeable), 9.60 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm); 14.3, 19.20 (2CH_3), 42.2 (CH), 42.9 (CH_2), 48.2, 48.6 (2OCH_2), 115.4, 117.2, 120.8, 122.3, 124.4, 125.8, 129.9, 130.8, 131.6, 134.5, 137.4, 145.3, 147.8, 151.4, 153.1, 157.4, 158.9 (17 sp^2) 171.5, 174.2, 178.3, 181.2 (4CO);its MS (m/z):

664 (M^+, 59 %),666 (M^++2, 18%), $C_{33}H_{31}ClFN_5O_7$ (664.1); calcd. %C:59.68, %H: 4.71, %N: 10.55. found: %C:59.65, %H: 4.70,%N: 10.53.

Ethyl 4-amino-3-(4-chlorophenyl)-1-(4-hydroxybenzyl)-1*H*-pyrazole-5-carboxylate (12).

A mixture of compound **1** (2.65 g, 10 mmol) and potassium carbonate (1.65 g, 12 mmol) was stirred in 4-bromomethyl-phenol (10 mL) under reflux for 5 h. After completion the reaction, the mixture was cooled to room temperature, the excess of 4-bromomethylphenol was evaporated and water was added. The residue was extracted with chloroform, the combined organic phase was dried over anhydrous Na_2SO_4, and then the residue was purified by column chromatography (eluent, chloroform/methanol 50:1, v/v) to give compound 1**2** as a pale yellow solid.

Yield 77%;m.p. 240-242°C; IR (KBr);3443(brs, NH_2), 3333 (brs, OH), 3028 (CH aryl), 2980 (CH alkyl), 1725 (C=O),1620 (C=N), 1590(C=C);δ^1H NMR (500 MHz, DMSO-d_6 ,ppm):1.34 (t, 3H, CH_3), 3.43 (s, 2H, N-CH_2), 4.22 (q, 2H, OCH_2), 5.20 (s, 1H, OH, D_2O exchangeable), 7.10 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.32 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.54 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.90 (d, 2H, *J* = 8.2 Hz, Ar-H), 9.20 (br, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm); 16.5 (CH_3), 42.7 (CH_2), 47.9 (OCH_2), 119.4, 121.6, 127.6, 128.2, 129.2, 131.8, 136.8, 137.5, 143.8, 157.7, 159.1 (11sp^2), 170.4 (CO);its MS (m/z): 371 (M^+, 85 %), 373 (M^++2, 23%); $C_{19}H_{18}ClN_3O_3$ (371.8);calcd. %C:61.38, %H: 4.88, %N: 11.30. found: %C:61.34, %H: 4.86, %N: 11.28.

Ethyl 4-amino-3-(4-chlorophenyl)-1-{4-[(isoquinolin--5-sulfonyl) oxy] benzyl}-1*H*-pyrazole-5-carboxylate(**13**).

A solution of **12** (3.72 g, 10mmol), 5-isoquinoline -sulfonyl chloride (2.28g, 10 mmol), HCl (5mL) and triethylamine (TEA) (3mL), was stirred in dried dichloromethane for 3 h at room temperature. Then the mixture was treated with saturated aq. NH_4Cl (25 mL). The organic phase was extracted with dichloromethane (120 mL),and the combined organic phases were dried over sodium sulfate, filtered and concentrated under vacuum. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH 45:1, v/v) to afford **13** as a pale brown powder.

Yield 80%;m.p. 278-280°C; IR (KBr);3420 (brs, NH_2), 3023 (CH aryl), 2978 (CH alkyl), 1730 (C=O), 1622 (C=N), 1595 (C=C), 1358, 1152 (SO_2); ^{1}H NMR (500 MHz, DMSO-d_6 ,ppm):δ1.33 (t, 3H, CH_3), 3.40 (s, 2H, N-CH_2), 4.20 (q, 2H, OCH_2), 6.80 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.05 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.35 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.70 (d, 2H, *J* = 8.2 Hz, Ar-H), 8.20-8.32 (m, 3H, Ar-H), 8.39- 8.55 (m, 3H, isoqunoline), 9.35 (br, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6, *δ*, ppm); 16.5 (CH_3), 42.7 (CH_2), 47.9 (O*CH*$_2$), 117.4, 122.2, 125.8, 128.4, 128.7, 130.2, 131.6, 134.9, 135.2, 136.0, 137.5, 143.8, 145.8, 148.6, 152.0, 153.3, 156.2, 159.3 (18 sp^2), 171.4 (CO); its MS (m/z): 563 (M^+, 73 %),565 (M^++2, 20%), $C_{28}H_{23}ClN_4O_5S$ (563.0); calcd. %C:59.73, %H: 4.12, %N: 9.95. found: %C:59.70, %H: 4.10,%N: 9.93.

Ethyl 4-amino-3-(4-chlorophenyl)-1-(methoxymethyl)-1*H*-pyrazole-5-carboxylate(**14**).

To a cooled solution (−2 °C) of compound **1**(1.32 g, 5 mmol) and K_2CO_3 (1.65 g, 12 mmol) in anhydrous DMF (25 mL) chloromethyl methyl ether (MOMCl) (1.25 mL, 14 mmol) was added. Reaction mixture was stirred at room temperature for 24 h and solvent was evaporated to dryness. Column chromatography purification (CH_2Cl_2 : CH_3OH = 40 : 1) gave a pale yellow powder.

Yield 65%;m.p. 188-190°C; IR (KBr);3395(brs, NH_2), 3020 (CH aryl), 2984 (CH alkyl), 1725 (C=O), 1615 (C=N), 1590 (C=C); ^{1}H NMR (500MHz, DMSO-d_6 ,ppm):δ1.30 (t, 3H, CH_3), 3.22 (s, 3H, OCH_3), 4.22 (q, 2H, OCH_2), 4.90 (s, 2H, N-CH_2), 7.25 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.57 (d, 2H, *J* = 8.2 Hz, Ar-H), 8.98 (br, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6, *δ*, ppm); 13.6 (CH_3) , 45.2 (OCH_3), 48.5 (OCH_2 ester), 52.24 (OCH_2 ether), 120.4, 127.9, 128.2, 138.2, 140.8, 157.2, 159.1(7 sp^2), 173.9 (CO);its MS (m/z):309 (M^+, 88 %), 311 (M^++2, 26%), $C_{14}H_{16}ClN_3O_3$ (309.7);calcd. %C:54.29, %H: 5.21, %N: 13.57. found: %C:54.25, %H: 5.19,%N: 13.54.

Ethyl4-amino-3-(4-chlorophenyl)-1-{[2-(dimethylsilyl)ethoxy]methyl}-1*H*-pyrazole-5-carboxylate (15).

A mixture of compound **1**(2.65 g, 10 mmol) and ammonium sulfate (0.65 mg, 5 mmol) in hexamethyldisilazane (HMDS) (30 mL) was heated under reflux for 4 h. Obtained colorless solution was cooled to (−3°C) and 2-(trimethylsilyl)ethoxymethyl chloride (SEMCl) (1.2 mL) was added. The mixture was stirring at room temperature for 20 h and solvent was evaporated to dryness. The product was purified by column chromatography (CH_2Cl_2: CH_3OH = 20:1) to give **15** as a yellow crystal.

Yield 70%;m.p. 263-265°C; IR (KBr);3397 (brs, NH_2), 3022 (CH aryl), 2984 (CH alkyl), 1728 (C=O), 1620 (C=N), 1593 (C=C);^{1}H NMR (500MHz, DMSO-d_6 ,ppm):δ0.02 (9H, s, $SiCH_3$), 0.86 (2H, m, CH_2Si), 1.29 (t, 3H, CH_3), 3.42 (2H, m, OCH_2), 4.25 (q, 2H, OCH_2), 4.95 (s, 2H, N-CH_2), 7.18 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.50 (d, 2H, *J* = 8.2 Hz, Ar-H), 9.00 (br, 2H, NH_2, D_2O exchangeable); its MS (m/z): 395 (M^+, 68 %), 397 (M^++2, 14%); $C_{18}H_{26}ClN_3O_3Si$ (395.9);calcd. %C:54.60, %H: 6.62, %N: 10.61. found: %C:54.58, %H: 6.62,%N: 10.60.

Ethyl 4-amino-3-(4-chlorophenyl)-1-[2-(1-(4substituted phenyl)-5-oxopyrrolidin-3-yl)ethyl]-1*H*-pyrazole-5-carboxylate derivatives (17a,b).

*General procedure.*A mixture ofcompound **1** (2.65 g, 10 mmol) withmethanesulfonic acid 1-[5-oxo-1-(4-trifluoromethyl-phenyl)-pyrrolidin-3-yl]-ethyl ester(**16a)** (3.51 g, 10 mmol) ormethanesulfonic acid 1-[5-oxo-1-(4-methyl-phenyl)-pyrrolidin-3-yl]-ethyl ester(**16b)** (2.91 g, 10 mmol) andpotassium carbonate (1.38 g, 10 mmol) was stirred under reflux in DMF at 80 °C for 16h.After the reaction was completed the mixture was evaporated and the soild product crystallized from an appropriate solvent to produce **17a**,**b** in high yields.

Ethyl 4-amino-3-(4-chlorophenyl)-1-[2-(1-(4-trifluoromethyl benzene)-5-oxopyrrolidin-3-yl) ethyl]-1*H*-pyrazole-5-carboxylate (17a).

Yield 80%;m.p. 267-269°C (from ethanol); IR (KBr);3425 (brs, NH_2), 3020 (CH aryl), 2980 (CH alkyl), 1725, 1680 (C=O), 1615 (C=N), 1600 (C=C); ^{1}H NMR (500MHz, DMSO-d_6 ,ppm):δ1.32 (t, 3H, CH_3), 2.19-2.24 (m, 1H, CH of pyrolidine), 2.27-2.34 (m, 2H, CH_2); 2.40-2.44 (m, 2H, CH_2), 2.48- 2.53 (m, 2H, CH_2), 3.54 (m, 2H, N-CH_2), 4.20 (q, 2H, OCH_2CH_3), 6.90 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.20 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.42 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.78 (d, 2H, *J* = 8.2 Hz, Ar-H), 9.11 (br, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6, *δ*, ppm); 18.3 (CH_3), 24.3, 25.4, 39.4, 40.5, 44.7(5 CH_2), 48.6 (OCH_2), 76.5 (CF_3), 119.4, 121.6, 123.4, 127.5, 129.2, 130.2,

131.9, 136.2, 141.7, 157.3, 159.5, 171.3, 173.5 (2CO); its MS (m/z):520 (M^+, 65 %), 522(M^++2, 21%), $C_{25}H_{24}ClF_3N_4O_3$ (520.9), calcd. %C:57.64, %H: 4.64, %N: 10.76. found: %C:57.61, %H: 4.62,%N: 10.72.

Ethyl-4-amino-3-(4-chlorophenyl)-1-[2-(1-(4-tollyl)-5-oxopyrrolidin-3-yl)ethyl]-1*H*-pyrazole-5-carboxylate (17b).

Yield 77%;m.p. 258-260°C(from ethanol); IR (KBr);3426 (brs, NH_2), 3025 (CH aryl), 2984 (CH alkyl), 1727, 1679 (C=O), 1620 (C=N), 1600 (C=C); ^{1}H NMR (500MHz, DMSO-d_6 ,ppm):δ1.18 (s, 3H, CH_3), 1.35 (t, 3H, CH_3), 2.20-2.25 (m, 1H, CH of pyrolidine), 2.29-2.32 (m, 2H, CH_2); 2.40-2.46 (m, 2H, CH_2), 2.52- 2.60 (m, 2H, CH_2), 3.46 (m, 2H, N-CH_2), 4.21 (q, 2H, OCH_2CH_3), 7.05 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.35 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.49 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.89 (d, 2H, *J* = 8.4 Hz, Ar-H), 9.20 (br, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6,*δ*, ppm); 16.2, 18.5 ($2CH_3$), 23.3, 24.5, 39.0, 41.6, 45.7 (5 CH_2), 48.2 (OCH_2), 118.5, 125.5, 128.2, 130.0, 131.4, 132.6, 135.2, 137.3, 144.7, 156.5, 158.4 (11sp^2), 171.6 , 173.8 (2CO);its MS (m/z):466 (M^+, 70 %), 468 (M^++2, 22%),$C_{25}H_{27}ClN_4O_3$ (466.9), calcd. %C:64.30, %H: 5.83, %N: 12.00. found: %C:64.27, %H: 5.82,%N: 11.98.

Ethyl 4-amino-3-(4-chlorophenyl)-1-[2-(1-{substitutedphenyl)-pyrrolidin-3-yl)ethyl]-1*H*-pyrazole-5-carboxylate (19a,b).

General procedure. A mixture ofcompound **1** (2.65 g, 10 mmol) with methanesulfonic acid 1-(4-fluorophenyl)-pyrrolidin-3-yl-methyl ester **18a** (2.87 g, 10 mmol) ormethanesulfonic acid 1-(3-fluorophenyl)-pyrrolidin-3-yl-methyl ester **18b** (2.87 g, 10 mmol) andpotassium carbonate (1.38 g, 10 mmol) was stirred under reflux in DMF at 80 °C for 12 h, after the reaction was completed the reaction mixture was evaporated under reduced pressure. The soild product crystallized from an appropriate solvent to produce **19a**,**b** in moderate yields.

Ethyl-4-amino-3-(4-chlorophenyl)-1-[2-(1-{4-fluorophenyl)-pyrrolidin-3-yl)ethyl]-1*H* pyrazole-5-carboxylate (19a).

Yield 60%;m.p. 254-256 °C (from DMF); IR (KBr);3410 (brs, NH_2), 3023 (CH aryl), 2989 (CH alkyl), 1728 (C=O), 1618 (C=N), 1590 (C=C); ^{1}H NMR (500MHz, DMSO-d_6 ,ppm):δ1.33 (t, 3H, CH_3), 1.93-1.99 (m, 1H, CH of pyrolidine), 2.05-2.12 (m, 2H, CH_2); 2.16-2.23 (m, 2H, CH_2), 2.35- 2.41 (m, 2H, CH_2), 2.50- 2.54 (m, 2H, CH_2), 3.42 (m, 2H, N-CH_2), 4.23 (q, 2H, OCH_2CH_3), 6.97 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.25 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.40 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.69 (d, 2H, *J* = 8.0 Hz, Ar-H), 9.18 (br, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6, *δ*, ppm); 18.3(CH_3), 24.3, 26.4, 39.0, 41.8, 44.6, 46.6 (6 CH_2), 48.9 (OCH_2), 119.4, 121.6, 123.4, 127.5, 129.2, 130.2, 131.9, 136.2, 149.4, 156.9, 159.2 (11sp^2), 172.4 (CO);its MS (m/z):457 (M^+, 69 %),459 (M^++2, 25%), $C_{24}H_{26}ClFN_4O_2$ (456.9), calcd. %C:64.08, %H: 5.74, %N: 12.26. found: %C:63.04, %H: 5.73,%N: 12.22.

Ethyl-4-amino-3-(4-chlorophenyl)-1-[2-{1-(3-fluorophenyl)-pyrrolidin-3-yl)ethyl]-1*H* pyrazole-5-carboxylate (19b).

Yield 60%;m.p. 254-256 °C(from DMF); IR (KBr);3400 (brs, NH_2), 3023 (CH aryl), 2988 (CH alkyl), 1728 (C=O), 1620 (C=N), 1593 (C=C);^{1}H NMR (500MHz, DMSO-d_6 ,ppm):δ1.34 (t, 3H, CH_3), 1.93-1.98 (m, 1H, CH of pyrolidine), 2.07-2.15 (m, 2H, CH_2); 2.19-2.25 (m, 2H, CH_2), 2.30- 2.41 (m, 2H, CH_2), 2.49- 2.55 (m, 2H, CH_2), 3.40 (m, 2H, N-CH_2), 4.21 (q, 2H, OCH_2CH_3), 7.04 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.20 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.35 (s, 1H, Ar-H), 7.65-7.75 (m, 3H, Ar-H), 9.20 (br, 2H, NH_2, D_2O exchangeable); its MS (m/z):456 (M^+, 71 %), 458 (M^++2, 21%), $C_{24}H_{26}ClFN_4O_2$ (456.9), calcd. %C:63.08, %H: 5.74, %N: 12.26. found: %C:63.06, %H: 5.74, %N: 12.24.

Ethyl 4-amino-3-(4-chlorophenyl)-1-[2-oxo-2-(4-{substituted phenyl}-piperazin-1-yl)ethyl]-1H-pyrazole-5-carboxylate(21a,b).

*General procedure.*A mixture ofcompound **1** (2.65 g, 10 mmol) with compound 2-chloro-1-[4-(4-fluorophenyl)-piprazine-1-yl]-ethanone(**20a**) (2.57g, 10 mmol) or 2-chloro-1-[4-(3-fluorophenyl)-piprazine-1-yl]-ethanone(**20b**) (2.57 g, 10 mmol)andpotassium carbonate (1.38 g, 10 mmol) was stirred under reflux in DMF at 80 °C for 8 h, after the reaction was completed the reaction mixture was evaporated under reduced pressure. The soild product was crystallized from an appropriate solvent to produce **21a**,**b** in moderate yields.

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Ethyl 4-amino-3-(4-chlorophenyl)-1-[2-oxo-2-(4-{4-fluoro phenyl}-piperazin-1-yl)ethyl]-1H-pyrazole-5-arboxylate (**21a**).

Yield 60%;m.p. 254-256 ^{o}C (from hexane); IR (KBr); 3425 (brs, NH_2), 3015 (CH aryl), 2995 (CH alkyl), 1730, 1678 (C=O), 1615 (C=N), 1600 (C=C); ^{1}H NMR (500MHz, DMSO-d_6 ,ppm):δ1.32 (t, 3H, CH_3),2.68 (brs, 4H, piperazinyl), 3.36 (m, 2H, N-CH_2), 3.43 (brs, 4H, piperazinyl), 4.15 (q, 2H, OCH_2CH_3), 7.01 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.35 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.45 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.82 (d, 2H, *J* = 8.0 Hz, Ar-H), 9.23 (br, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6,*δ*, ppm); 16.7 (CH_3), 29.5, 44.6, 46.3, 46.9, 48.1 (5 CH_2), 48.6 (OCH_2), 119.0, 122.4, 127.2, 129. 5, 131.8, 137.4, 141.8,145.4,150.3, 156.1, 158.3, 171.8, 173.5 (2CO);its MS (m/z):486 (M^+, 79 %), 488 (M^++2, 23%),$C_{24}H_{25}ClFN_5O_3$ (485.9)calcd. %C:59.32, %H: 5.19, %N: 14.41. found: %C:59.32, %H: 5.15, %N: 14.39.

Ethyl 4-amino-3-(4-chloro-phenyl)-1-[2-oxo-2-(4-{3-fluoro-phenyl}-piperazin-1-yl)ethyl]-1H-pyrazole-5-carboxylate(21b).

Yield 60%;m.p. 254-256 ^{o}C(from hexane); IR (KBr); 3420 (brs, NH_2), 3015(CH aryl), 2997 (CH alkyl), 1729, 1680 (C=O), 1615 (C=N), 1958 (C=C); ^{1}H NMR (500MHz, DMSO-d_6 ,ppm):δ1.33 (t, 3H, CH_3),2.65 (brs, 4H, piperazinyl), 3.34 (m, 2H, N-CH_2), 3.45 (brs, 4H, piperazinyl) 4.15 (q, 2H, OCH_2CH_3), 6.90 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.20 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.45 (s, 1H, Ar-H), 7.70-7.82 (m, 3H, Ar-H), 9.20 (br, 2H, NH_2, D_2O exchangeable); its MS (m/z):485 (M^+, 71 %) , 486 (M^++1, 18%), $C_{24}H_{25}ClFN_5O_3$ (485.9)calcd. %C:59.32, %H: 5.19, %N: 14.41. found: %C:59.29, %H: 5.17, %N: 14.38.

Biological screening

Anti-inflammatory assay:

Animals:

Albino mice of both sexes weighing (20–25) gm (were obtained from the animal house colony of the National Research Center, Cairo, Egypt) were used. The animals left for two days for acclimatization to animal room conditions and were maintained on standard pellet diet and water ad libitum [21]. The food was withdrawn on the day before the experiment, but allowed free access of water. All procedures involving animals were carried out in accordance with the guide for the care and use of laboratory animals (National Academy of Science of Egypt) and were approved by the Animals Studies Committee at Washington University.

Carrageenan induced rat paw edema

The anti-inflammatory activity was evaluated using carrageenan-induced rat paw edema assay [22]. The animals were divided into 21equal groups. Mice were divided into twenty-one groups. The first group was administered vehicle, the second group was administrated celecoxib (50 mg/kg) while the other remaining groups were administrated orally (50 mg/kg) immediately prior to induction of inflammation by carrageenan subcutaneous injection. The animals received 100 µl of vehicle or carrageenan (1% in saline) S.C. on the plantar surface of the left hind paw. The development of paw edema was assessed by measuring paw-volume changes at 1, 2, 3, 4 and 5 h after carrageenan injection using a pair of dial thickness gauge calipers accurate to 0.001 cm. The right hind paw served as a reference of non-inflamed paw for comparison. Results are expressed as paw volume change (mL). Edema values, expressed as mean standard deviation, were compared statistically using one-way-ANOVA followed by Dunnet's test Table 1.

Table 1: Anti-inflammatory activity of N-pyrazole derivatives

Comp.[a]	Volume of edema (mL) [b]				
	1h	2h	3h	4h	5h
2	0.67 ± 0.01 (17%)	0.64 ± 0.01 (22%)	0.62 ± 0.01 (29%)	0.54 ± 0.01 (43%)	0.46 ± 0.0152 (60%)
4	0.66 ± 0.01	0.59 ± 0.01	0.58 ± 0.0115*	0.38 ± 0.0264	0.35 ± 0.0152

	(21%)	(36%)	(50%)	(75%)	(86%)
5	0.67 ± 0.0152 (15%)	0.65 ± 0.0057 (25%)	0.62 ± 0.01 (40%)	0.44 ± 0.01 (58%)	0.37 ± 0.0057 (66%)
7a	0.66 ± 0.005 (17%)	0.65 ± 0.0055 (30%)	0.48 ± 0.0054 (60%)	0.34 ± 0.0051 (90%)	0.34 ± 0.0173 (93%)
7b	0.67 ± 0.005** (16%)	0.65 ± 0.005** (20%)	0.60 ± 0.005** (30%)	0.54 ± 0.023** (45%)	0.48 ± 0.005** (65%)
8	0.66 ± 0.01 (18%)	0.62 ± 0.0054 (21%)	0.58 ± 0.0115 (38%)	0.40 ± 0.01 (42%)	0.38 ± 0.0115 (58%)
9	0.65 ± 0.005 (16%)	0.65 ± 0.01 (22%)	0.56 ± 0.0115* (35%)	0.43 ± 0.01 (50%)	0.48 ± 0.011* (65%)
10a	0.65 ± 0.01 (19%)	0.66 ± 0.0054 (21%)	0.56 ± 0.0115 (35%)	0.40 ± 0.01 (70%)	0.38 ± 0.0052 (72%)
10b	0.63 ± 0.0055 (22%)	0.55 ± 0.0057 (40%)	0.40 ± 0.0115 (80%)	0.34 ± 0.0057 (90%)	0.35 ± 0.0057 (90%)
12	0.66 ± 0.01 (16%)	0.62 ± 0.0115 (23%)	0.63 ± 0.02 (30%)	0.48 ± 0.0057* (60%)	0.40 ± 0.0152 (75%)
13	0.68 ± 0.01 (17%)	0.64 ± 0.0054 (22%)	0.53 ± 0.023 (30%)	0.48 ± 0.0057(50%)	0.46 ± 0.0152 (60%)
14	0.65 ± 0.01 (19%)	0.66 ± 0.0054 (22%)	0.58 ± 0.0115 (40%)	0.41 ± 0.01 (60%)	0.40 ± 0.0115 (71%)
15	0.64 ± 0.01 (16%)	0.60 ± 0.0052 (21%)	0.56 ± 0.0115 (35%)	0.46 ± 0.0057 (50%)	0.48 ± 0.0152 (60%)
17a	0.58 ± 0.01(20%)	0.56 ± 0.0115 (35%)	0.64 ± 0.0057 (40%)	0.37 ± 0.0264 (68%)	0.36 ± 0.0152 (81%)
17b	0.66 ± 0.01 (23%)	0.59 ± 0.0054 (40%)	0.57 ± 0.0054 (42%)	0.47 ± 0.0173 (60%)	0.46 ± 0.0152 (60%)
19a	0.66 ± 0.01 (18%)	0.60 ± 0.01 (42%)	0.48 ± 0.01 (60%)	0.39 ± 0.0054 (80%)	0.35 ± 0.011 (90%)
19b	0.64 ± 0.0075 (21%)	0.59 ± 0.0052 (40%)	0.60 ± 0.0173 (40%)	0.48 ± 0.0153 (60%)	0.46 ± 0.0115 (61%)
21a	0.66 ± 0.01 (19%)	0.60 ± 0.01 (42%)	0.51 ± 0.01 (60%)	0.39 ± 0.0054 (80%)	0.35 ± 0.011 (90%)
21b	0.68 ± 0.01 (17%)	0.63 ± 0.0115 (22%)	0.64 ± 0.02 (30%)	0.49 ± 0.0057 (60%)	0.40 ± 0.011 (71%)
Control	0.82 ± 0.02	0.88 ± 0.002	0.93 ± 0.001	0.97 ± 0.02	0.97 ± 0.02
celecoxib	0.65 ± 0.01 (23%)	0.59 ± 0.005 (40%)	0.56 ± 0.01 (49%)	0.38 ± 0.153 (79%)	0.34 ± 0.01 (89%)

[a] Dose levels: test compounds (50 mg/kg b.w), **celecoxib** (50 mg/kg b.w).
[b] n = 6, values are expressed as mean ± SEM and analysed by ANOVA.
[c] Values in parentheses (percentage anti-inflammatory activity, AI%).
* Means significant difference with celecoxib at $p < 0.05$.
** Means significant difference with celecoxib at $p < 0.01$.

Ulcerogenic liability study:

Ulcerogenic liability for the most biologically active synthesized compounds (**4**, **7a**, **10b**, **17a**, **19a** and **21a**) and celecoxib was evaluated [23]. Adult male albino rats weigh between 120 and 150 g were used in this study and divided into 8 groups. The animals were fasted for 20 h before drug administration. The first group control rats received p.o. the vehicle (suspension of 1% carboxy methylcellulose). The second group received celecoxib used as a reference drug in a dose 200 mg/kg. The remaining groups received the synthesized compounds (**4**, **7a**, **10b**, **17a**, **19a** and **21a**) in a dose 200 mg/ kg suspended. Treatment was continued once daily for 3 successive days in all groups. Two hours after the last dose, rats were sacrificed under general anesthesia and the stomach was removed, opened along the greater curvature and rinsed with saline. The gastric mucosa was examined with a magnifying lens for the presence of lesions in the form of hemorrhages or

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linear breaks and erosions. The expression of the degree of ulcerogenic effect was in term of dividing the percentage incidence of ulcers in each group of animal by ten, the average number of ulcers per stomach and the visual observation of the ulcer score (average severity of ulcer)Table 2.

Table 2: Ulcerogenic activity of the most biologically active synthesized compounds

Compound No.	Dose (mg kg^{-1}, 1% CMC)	Ulcer index[a]
Control		0.0± 0.0[b]
4	200	0.8± 0.2[b]
7a	200	0.5± 0.1[c]
10b	200	0.6± 0.2[c]
17a	200	0.8± 0.2[d]
19a	200	0.4± 0.0[b]
21a	200	0.4± 0.2[b]
celecoxib	200	0.9 ± 0.2

Each value represents the mean ± SD [a] n= 6, [b] P< 0.0001, [c] P< 0.001 and [d] P< 0.05.

Antibacterial activity. - Antibacterial activity of the newly synthesized compounds was tested *in vitro* against the Gram-negative bacteria: *Klebsiella pneumonia* ATCC 26845, *Escherichia coli* ATCC and Gram-positive bacteria: *Streptococcus pneumonia* ATCC 19681, *Staphylococcus aureus* ATCC 15923. All microorganisms were purchased from the American Type Culture Collection (Manassas, USA). Agar disk diffusion technique [24] has been used for determining the antibacterial activity of the synthesized compounds. One mg of each of the newly synthesized compounds was dissolved in DMSO (1 mL) then made up to 10 mL with sterile water to give a concentration of 100 µg mL^{-1}.A microplate-wells, 1 cm in diameter have been used. A solution of the tested compounds was placed separately on an agar plate containing a standard suspension of microorganisms. Cefatoxime (109.8 µmol L^{-1}) was used as a standard drug. Diametersof the inhibition zones were measured after 24 h incubation, were measured with calipers or automated scannersand were compared with those of the standards.

Minimum inhibitory concentration (*MIC*) [25], were carried out for all synthesized compounds using serial plate dilution technique. The MIC is defined as the lowest concentration of the test compound that showed no bacterial growth. DMSO was used as a solvent control to ensure that the solvent had no effect on bacterial growth. The stock solution of the tested compounds have been prepared by dissolving 5 mg of each test compounds in 1 mL of dimethylsulfoxide. The plates were incubated at 37 °C for 24h. The inhibition zone diameters and MIC (µmol L^{-1}) values are revealed in Table 3.

Table 3: *Antibaerial activity of newly synthesized compounds*

Compound	Microorganisms			
	Gram -ve bacteria		Gram + ve bacteria	
	Klebsiella pneumonia/ MIC	***Escherichia coli/ MIC***	***Streptococcus pneumonia/ MIC***	***Staphylococcus aureus/ MIC***
2	18/16	19/14	20/16	20/18
4	21/14	18/12	18/16	18/14
5	18/16	16/14	20/18	16/12
7a	22/10	20/12	18/16	19/13
7b	20/16	22/18	20/18	16/18
8	27/12	26/10	18/16	16/18
9	21/16	18/14	18/18	20/18
10a	21/18	19/16	17/18	18/20
10b	20/12	20/12	21/13	22/16
12	18/16	19/14	16/18	18/16
13	28/10	27/10	22/13	29/10
14	16/20	16/18	18/20	16/19

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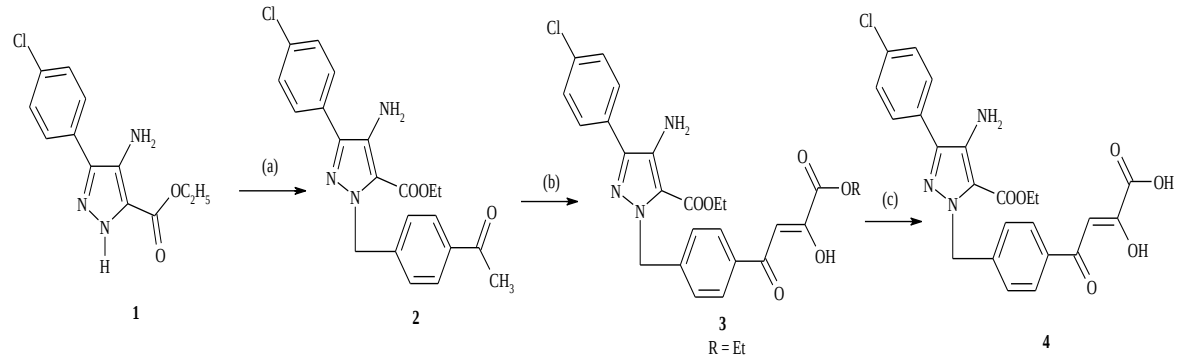
15	20/14	18/12	18/16	20/16
17a	24/12	20/14	22/13	20/13
17b	21/18	18/20	20/19	20/21
19a	18/14	16/12	16/15	18/14
19b	16\18	16/18	20/18	16/18
21a	20/12	22/12	19/16	24/16
21b	16/18	18/18	20/18	16/18
Cefatoxime	27/12	25/10	22/13	26/13

Results expressed as inhibition zone diameter in mm. / Minimal inhibitory concentration (MIC, $\mu molL^{-1}$) of the newly synthesized compounds.

RESULTS AND DISCUSSION

Chemistry

Recently, we have designed and synthesized a variety of pyrazole derivatives with the high potency of anticancer and antimicrobial activities [6-8]. Ethyl 4-amino-3-(4-chlorophenyl)-1*H*-pyrazol-5-carboxylate **1** was synthesized according to the literature [20]. Compound **1** is a precursor to synthesize *N*-pyrazole derivatives which was alkylated with 4-(bromomethyl)-acetophenone.The alkylated derivative ethyl 1-(4-acetyl-benzyl)-4-amino-3-(4-chlorophenyl)-1*H*-pyrazole-5-carboxylate**2** was isolated in good yield (72%). The methyl ketone intermediate was treated with LiHMDS and reacted with diethyl oxalate to produce the α, γ-diketoester derivative **3**,whichacidified with 1M HCl to afford the key intermediate of α, γ-diketobutanoic acids **4** in good yield scheme 1.



Scheme 1: Reagent and conditions a) 4-(bromomethyl)acetophenone, K_2CO_3, DMF,70C, 3h; b) LiHMDS, THF, -80 °C, 40 min, then diethyl oxalate; c) LiOH (1 M), THF, rt,3 h

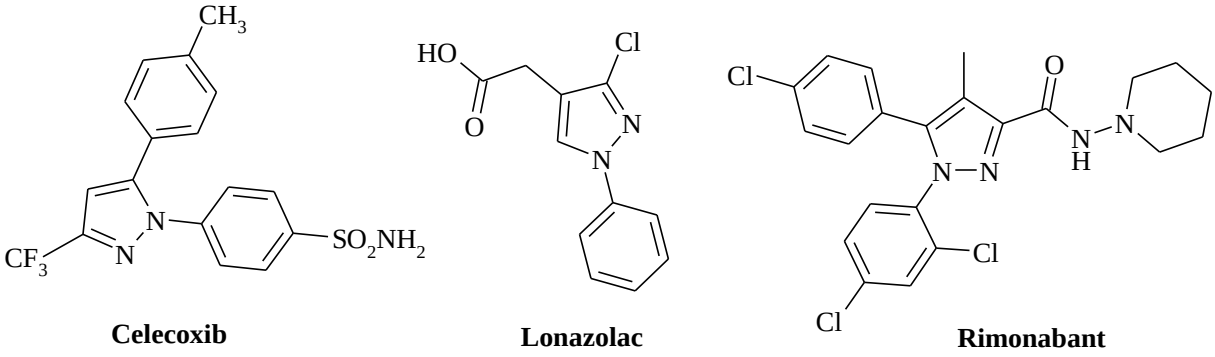


Fig 1: Chemical structures of the selective cyclooxygenase-2 inhibitors containing pyrazole moiety celecoxib, Lonazolac and Rimonabant.

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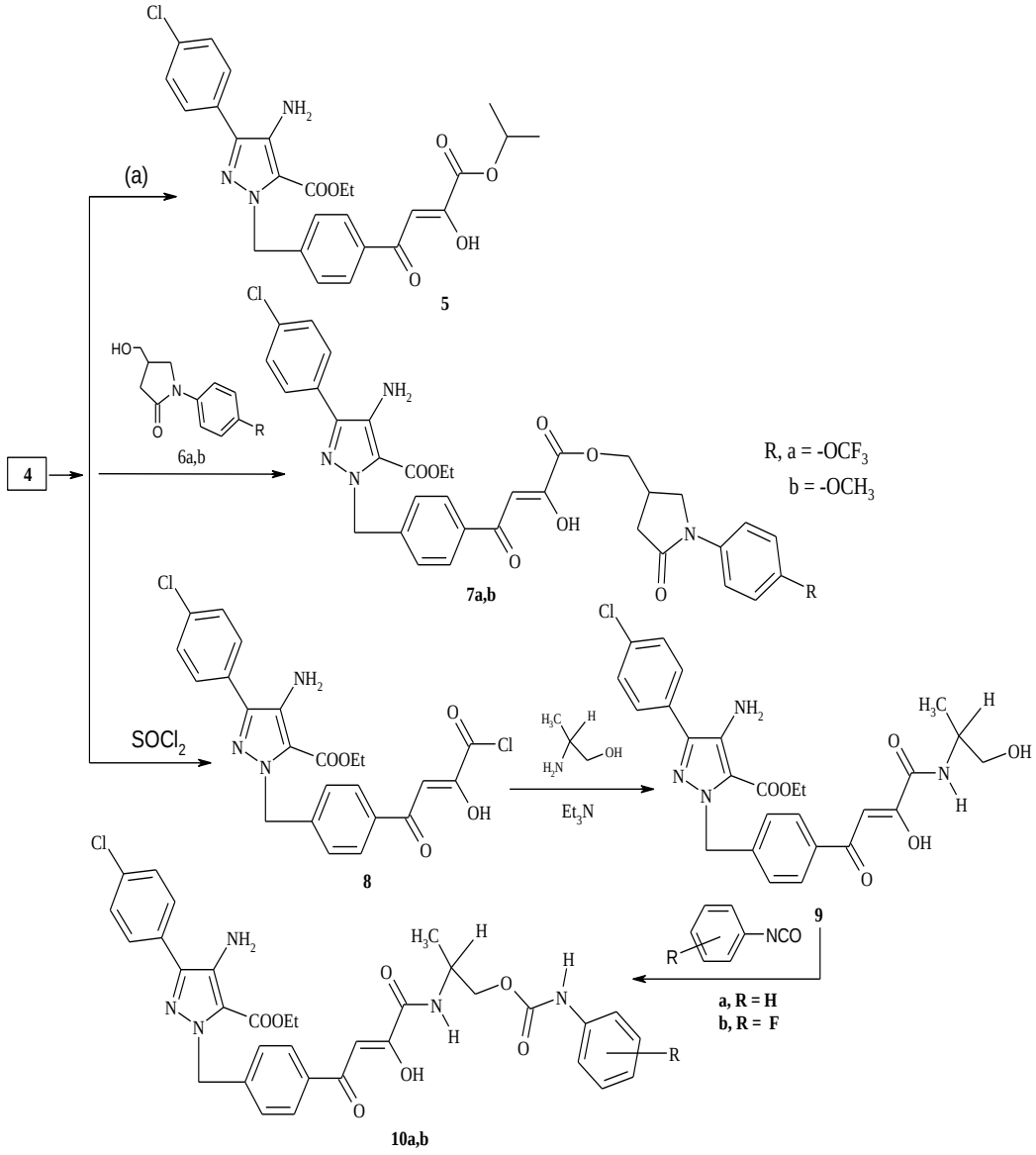
4-[4-(4-Amino-3-(4-chlorophenyl)-5-ethoxycarbonyl- pyrazol-1-yl) methyl] phenyl]-3-hydroxy-4-oxo-but-2-enoic acid **4**was esterifiedwith isopropyl alcohol to give isopropyl ester **5**. Treatment of **4**with4-hydroxymethyl-1-(4-substituted-phenyl)-pyrrolid-2-one**6a**,**b** under Mitsunobu conditions afforded ethyl 4-amino-3-(4-chlorophenyl)-1-[4-(3-hydroxy-4-methylene-1-(4-substituted phenyl)pyrrolid-2-enoyl)benzyl)]-1*H*-pyrazole-5-carboxylate derivatives**7a**,**b**. Also, compound **4** was refluxed in $SOCl_2$ to give acid chloride derivatives **8**, the structure of compound **8** was confirmed by ^{1}H NMR (DMSO-d_6) which revealed the disappearance of hydroxyl group of carboxylic acid. Following, ethyl 4-amino-3-(4-chlorophenyl)-4-{3-hydroxy-4-[(1-hydroxypropan-2-yl) amino]-4-oxobut-2-enoyl} benzyl-1*H*-pyrazole-5-carboxylate**9** were obtained by reacting acid chloride derivatives **8** with 2-amino-propanol under Et_3N condition in CH_2Cl_2. The treatment of **9**with substituted phenylisocyanate, the mixture was stirred for 10 h at 60 °C the final products are given in moderate yield**10a**,**b**scheme 2.



Scheme 2: Reagent and conditions a) Isopropyl alcohol, conc. H_2SO_4, Δ, 4h.

The alkylation of compound **1** with 4-(bromo-methyl) phenol**11** generated ethyl 4-amino-3-(4-chlorophenyl)-1-(4-hydroxybenzyl)-1*H*-pyrazole-5-carboxylate **12**. On the other hand, the sulfonylation of *N*-hydroxybenzyl compound **12** with 5-isoquinoline sulfonyl chloride resulted in compound **13**,the structure of compound **13** was confirmed by ^{1}H NMR (DMSO-d_6) which revealed the disappearance of hydroxyl group and the appearance of isoquinoline moiety as multiplet from the range δ:8.39 to 8.55 ppm.

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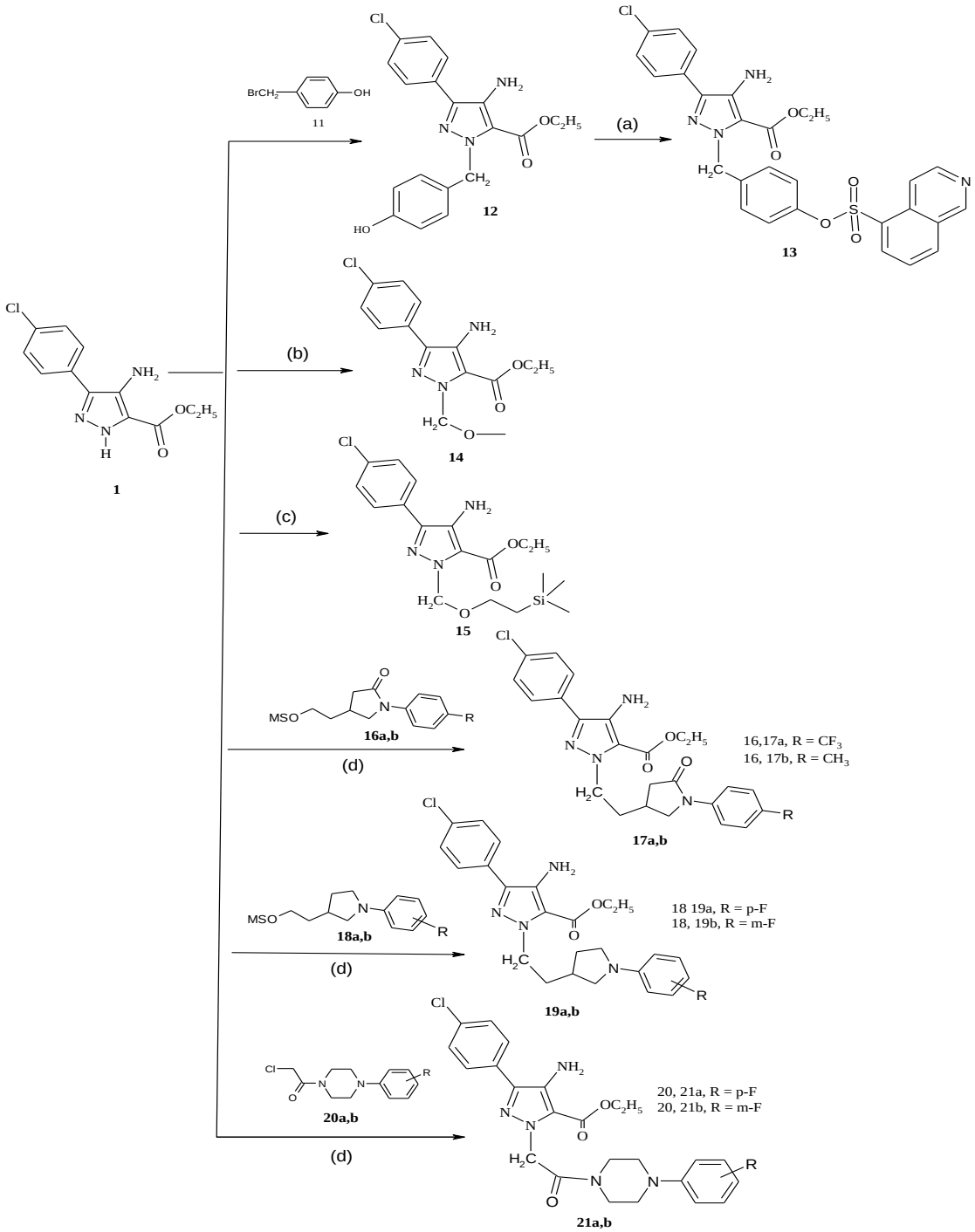
Methoxymethylation reaction of **1** with MOMCl (methoxymethylchloride) gave *N*-1-methoxymethylene pyrazol derivative **14**,alsothe treatment of**1** with trimethylsilylethoxy-methyl chloride (SEMCl) in 1,1,1,3,3,3-hexamethyldisylazane (HMDS) gave *N*-1-trimethylsilyl-ethoxymethyl pyrazole derivative **15**scheme 3**.**



Scheme 3: Reagent and conditions a) 5-isoquinolinesulfoyl chloride, TEA, DCM, rt.; b) K_2CO_3, DMF, MOMCl, rt; c) HMDS, SEMCl, reflux, 14 h; d) K_2CO_3, DMF, 90°C.

Compounds **17a,b**, **19a**,**b** were prepared by alkylation of compound**1** with different mesylates derived from N-phenyl substituted 5-oxopyrrolidines **16a**, **b** and N-phenyl substituted pyrrolidines **18a**,**b** which were synthesized according to literature procedure [26, 27], by heating under reflux with potassium carbonate in DMF. Finally, the treatment of **1** withpiperazinyl derivatives2-chloro-1-[4-(p- or m-fluoro-phenyl)-piprazine-1-

yl]ethanone**20a**,**b** using potassium carbonate in DMF by heating under reflux afforded ethyl 4-amino-3-(4-chlorophenyl)-1-[2-oxo-2-(4-{substituted phenyl}-piperazin-1-yl)ethyl]-1*H*-pyrazole-5-carboxylate**21a**,**b**scheme 3.

Anti-inflammatory activity:

All compounds were tested for anti-inflammatory activity and the results are shown in Table 1. The data revealed that most of the test compounds showed significant anti-inflammatory effects with inhibition ranging from 58% to 93%.

Compounds **7a**, **10b**, **19a** and **21a** exhibited the strongest inflammatory inhibition higher thancelecoxib (89%). It was clear that compounds bearing pyrrolidone moiety withsubstituent electron withdrawing group (-OCF_3) **7a**displayed the best activity(93%).Also for compound **10b** the presence of (p-fluoro) substituent on phenyl carboxamide increases the anti-inflammatory activity (90%), compound **19a** with pyrrolidine moiety attached to *p*-fluorophenyl showed higher activity than the standard drug celecoxib (90%). Furthermore, compound **21a** with piperazine moiety attached to *p*-fluorophenyl exhibited potent anti-inflammatory activity higher than celecoxib (90%). In addition to compound **4** and **17a** showed high anti-inflammatory activity (86%), (81%) but still less than the standard drug celecoxib (89%). However, the other remaining compounds exhibited good to moderate activityTable 1.

Ulcerogenic liability:

Compounds which exhibited high anti-inflammatory **4**, **7a**, **10b**, **17a**, **19a** and **21a** were screened for their ulcerogenic activity Table 2. The maximum reduction in ulcerogenic activity was 0.4 ±0.0, found in ethyl 4-amino-3-(4-chlorophenyl)-1-[2-(1-{4-fluorophenyl)-pyrrolidin-3-yl) ethyl]-1*H*-pyrazole-5-carboxylate(**19a**). Maximum reduction (0.4± 0.2) was found in ethyl 4-amino-3-(4-chlorophenyl)-1-[2-oxo-2-(4-{4-fluorophenyl}-piperazin-1-yl) ethyl]-1H-pyrazole-5-carbox-ylate**21a**. Also 4-[4-(4-amino-3-(4-chlorophenyl)-5-ethoxycarbonyl-pyrazol-1-yl) methyl]phenyl]-3-hydroxy-4-oxo-but-2-enoic acid **4** (0.8 ± 0.2) exhibitedmaximum reduction . The other three compounds **7a**, **10b** and **17a** showed moderate severity indexes.

Antibacterial activity and structure activity relationship

All the newly synthesized compounds were alsoscreened for antibacterial activity. The obtained results as depicted in Table 3 revealed that compound **13** with phenyl isoquinoline-5-sulfonate derivative attached to the pyrazole moiety exhibited stronger antibacterial efficacy and broader bioactive spectrum against Klebsiella pneumonia and*Staphylococcus aureus* with MIC (10, 10 µmol L^{-1}) than cefatoxime with MIC (12, 13 µmol L^{-1}) respectively, and equipotent with MIC (10, 13 µmol L^{-1}) as cefatoxime against *Escherichia coli* and *Streptococcus pneumonia* .

Compounds **4**, **7a**, **10b**, **15**, **17a**, **19a** and **21a** could effectively inhibit the growth of tested bacteria. Compound **4** with increasing number of hydroxyl groups exhibited high activity with MIC nearly as active as cefatoxime. Compound **7a** nearly as active as cefatoxime due to the presence ofpyrrolidone moiety with substituent electron withdrawing group (-OCF_3), compound **10b** exhibited high activity against all bacteria which has the same MIC value (12 µmol L^{-1}) against *Klebsiella pneumonia*, *Streptococcus pneumonia* and MIC value (12, 16 µmol L^{-1}) against *Escherichia coli* and*Staphylococcus aureus.* This activity may be attributedto the presence of (p-fluoro) substituent on phenyl carboxamide. Furthermore, compound **15** with ether linkage has high antibacterial activity, compound **17a** with electron withdrawing group (p-CF_3) substituent on phenyl pyrazolidinone derivatives has equipotent activity against Klebsiella pneumonia, Streptococcus pneumonia and Staphylococcus aureus while good activity with MIC value (14 µmol L^{-1}) against *Escherichia coli*. Compounds **19a** and **21a** have high activities against all bacteria this was attributed to the presence of (p-fluoro) substituent on phenyl pyrrolidine which attached to the pyrazole **19a** and(p-fluoro) substituent on phenyl piprazine **21a** while compounds **2**, **5**, **9** exhibited good activities against all the tested bacterial strains due to the presence of *N*-methyl acetophenone **2**, oxyisopropyl **5**, hydroxy-propane carboxamide **9.**In addition, compound**8** wasequipotent against*Klebsiella pneumonia* and*Escherichia coli* due to the presence of electron withdrawing group (p-chloro), while has good activity against *Streptococcus pneumonia* and *Staphylococcus aureus*, while the other compounds **5**, **7b**, **9**, **10a**, **12**, **14**, **17b**, **19b**and **21b** exhibited moderate activities toward all bacterial strains.

CONCLUSION

We have synthesized novel compounds **2**, **4**, **5**, (**7a**, **b**), **8**, **9**, (**10a**, **b**), **12**, **13**, **14**, **15**, (**17a**, **b**), (**19a**, **b**), (**21a**, **b**) and these compounds were investigated for their in vitroantiinflammatory andantibacterial activities. Among the synthesized compounds, compounds **7a**, **10b**, **19a** and **21a** exhibited higher anti-inflammatory activity than the celecoxib. Interestingly compounds **4**, **19a** and **21a** exhibitedmaximum reduction in ulcerogenic activity. In addition to,compound **13** exhibited antibacterial activity higher than the reference drug,compounds **4**, **7a**, **10b**, **15**, **17a**, **19a** and **21a** showed excellent antibacterial activity in comparison with cefatoxime as a standard drug.

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