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SYNTHESIS, ANTI-INFLAMMATORY AND ANTIMICROBIAL EVALUATION OF NICOTINAMIDES BEARING THIAZOLE AND PYRAZOLINE PHARMACOPHORES.

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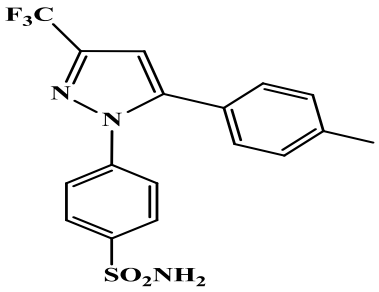
ABSTRACT

Searching novel, safe and effective anti-inflammatory and antimicrobial agents has remained an evolving research enquiry in the mainstream of inflammatory disorders and microbial infections. In the present investigation series of pyrazoles bearing thiazole and pyridyl moiety as a possible pharmacophore and nicotinamides bearing thiazole moiety were synthesized and assessed for their *in vitro* anti-inflammatory and antimicrobial activity. The structure of newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}CNMR, and mass spectral analysis. Cyclooxygenase I and II (COX-I and COX-II) inhibition assays were carried out using Celecoxib ,Diclofenac and Indomethacin as standards.The results obtained clearly focus the significance of compounds 2a,2b,2e,2j,2k,2l and 6 as selective COX-II inhibitors. However,their efficacies were associated with lower gastric ulcerogenicity compared to Indomethacin. Compounds 4a,4b,4c,5a, 5b,5c and 5d showed high antimicrobial activity against Gram positive bacteria (*Staphylococcus aureus and Micrococcus luteus*), moderate activity against Gram negative bacteria (*Salmonella typhimurium and Escherchia coli*) and high antifungal activity against *Candida albicans.*

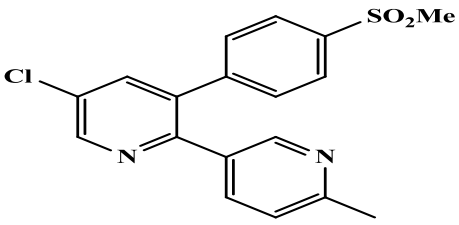
KEYWORDS: Anti-inflammatory, antimicrobial, pyrazoline, pyridine, thiazole, nicotinamide, COX-I, COX-II, ulcerogenicity.

INTRODUCTION

Despite the continuous efforts to improve the pharmacological index of NSAIDs, ulcerogenicity remains the most restraining obstructions in their clinical use.Cyclooxygenase (COX), the rate limiting enzyme of the prostanoid biosynthetic pathway, catalyzes the conversion of arachidonic acid to important anti-inflammatory mediators such as prostaglandins (PGs), prostacyclin (PGI) and thromboxane (TXA) These enzymes catalyze the formation of key mediators in recruiting inflammatory response. There are two isoforms of COXs (COX-I and COX-II) The COX-I is constitutively expressed in most tissues and organs and catalyzes the synthesis of PGs involved in the regulation of physiological, cellular activities, whereas COX-II is mainly stimulated by various stimuli such as cytokines, mitogens, and endotoxins in inflammatory sites . Inhibition of cyclooxgenase enzyme is considered as one of the important targets in the anti-inflammatory drug discovery. Traditional NSAIDs as aspirin, ibuprofen and indomethacin interact with both forms (COX-I and COX-II) and therefore their long administration even at low prophylactic doses resulting in gastro intestinal side effects ranging from ulcers to perforation and bleeding.For this reason ,synthesis of selective COX-II inhibitor drugs (coxibs) takes much consideration in recent years that achieve the same anti-inflammatory activity as traditional NSAIDs but with minimal risk of undesirable gastrointestinal side effects.Various drugs are well known as COX inhibitors as Rofecoxib, Meloxicam, Diclofenac, Indomethacin and Ibuprofen.However pyrazole is an interesting class of heterocyclic compounds possessing diverse and effective biological activities. Some pyrazole derivatives have anti-inflammatory properties as Celecoxib representing non steroidal anti-inflammatory drugs.



celecoxib



etoricoxib

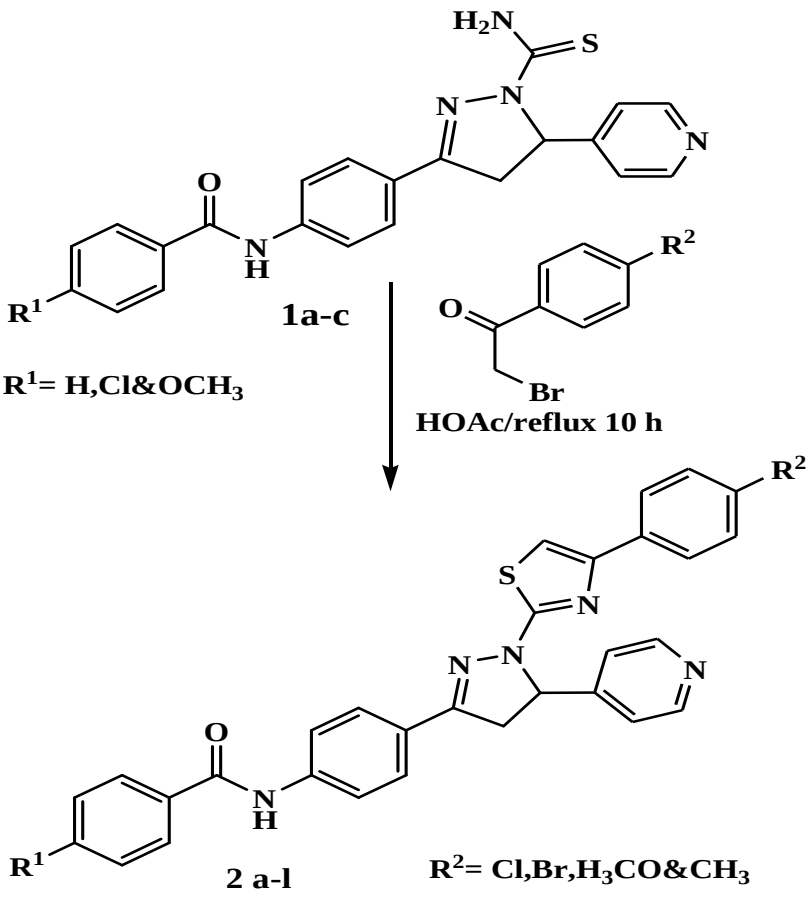
Pyrazolines have also been reported to possess antimicrobial[1–3],anti-inflammatory[4–6], antidepressant[7] and anticancer activity.[8–10] Numerous reports have appeared in the literature describing a broad spectrum of biological properties of pyridine as anticancer[11–14],antiinflammatory[15–17] and antimicrobial agents.[18–20] Similarly thiazole analogs exhibit various biological activities such as anti-inflammatory[21,22], and antimicrobial agents.[23]

RESULTS AND DISCUSSION

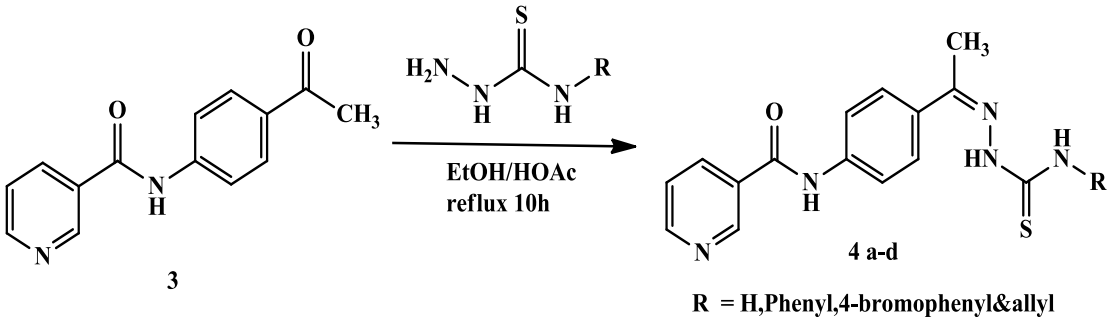
Chemistry

The general methods for the synthesis of the target pyrazole, pyridine and thiazole derivatives are depicted in schemes 1 and 2. Thiocarbamyol pyrazole derivatives 1a-c were cyclized to pyrazolothiazole derivatives 2a-l through their reaction with phenacyl bromide derivatives (scheme1). Moreover, the new nicotinamide derivatives containing thiosemicarbazone moiety 4a-d were obtained by refluxing equimolar amounts of N-(4-acetylphenyl) nicotinamide 3 and the corresponding thiosemicarbazide derivatives in ethanol containing catalytic amount of acetic acid (Scheme 2). Furthermore the nicotinamide derivative with thiosemicarbazone moiety 4a was cyclized to either thiazolyl nicotinamide derivatives 5a-d , thiazolyl-4-(5H)-one-2-yl nicotinamide 6 and nicotinamidothiazole carboxylate 7 through their reaction with phenacyl bromide derivatives , ethyl bromoacetate or ethyl-2-chloro acetoacetate respectively (Scheme 3). All the target compounds were characterized using thin layer chromatography and m.p. Analytical and spectral data of all the compounds are in full agreement with the proposed structures.

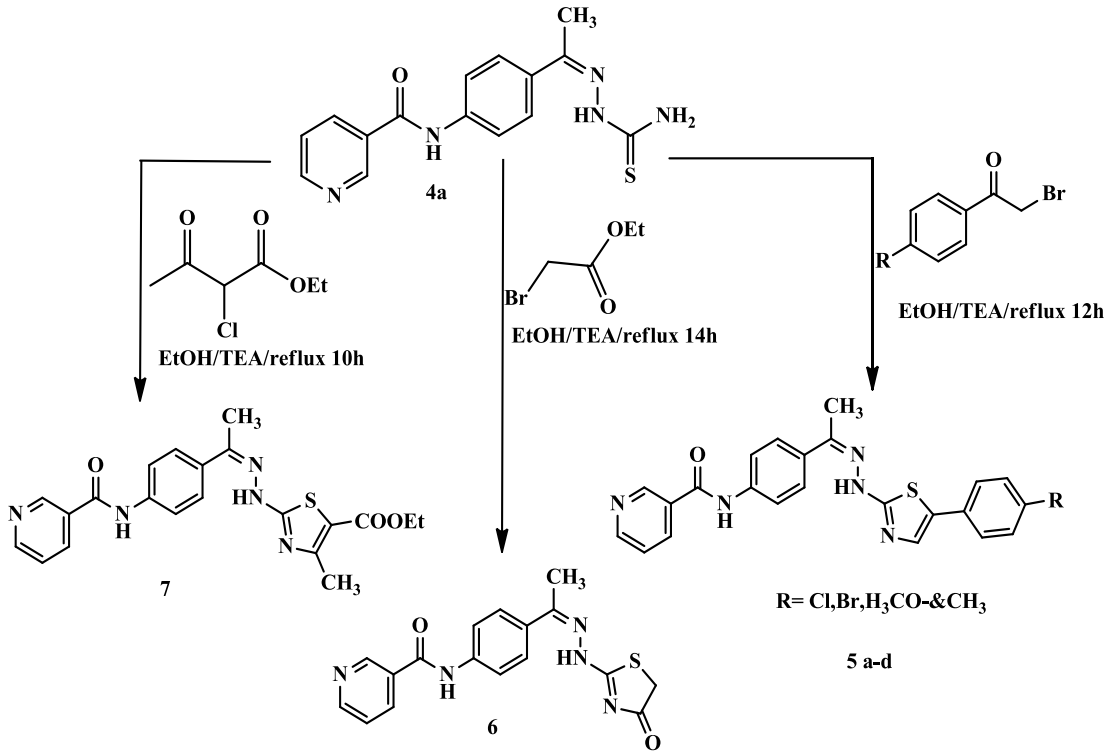
Scheme 1



Scheme 2



Scheme 3



IR spectra showed peaks at 3433-3271 corresponding to (NH) group, 3050-3025 (CH, aromatic), 2967-2924 (CH, aliphatic).

Measurement of chemical shift prove pyrazoline structure where, H4 of pyrazoline appeared as double doublets at δ = 3.14-3.78 ppm. The peak of H4 of pyrazoline appeared in the range of δ = 3.36-3.96 ppm. The H5 of pyrazoline appeared at δ = 5.69-5.95 ppm. Appearance of multiplet signals at δ = 6.55-8.56 ppm, singlet at δ = 1.90-2.31, 3.83-3.84 and 10.30-10.60 ppm due to (ArH), CH_3, OCH_3 and (NH, D_2O exchangeable) respectively.[13] CNMR spectra confirmed the proposed strutures, where, the signal due to carbonyl carbon of compounds 2a-l appeared at 164–166ppm and the signal due to C=N of thiazole ring at 174-175. The ^{13}CNMR chemical shift values of the carbon atoms at 40.95–44.50 ppm due to (C4) of

pyrazole, 55-62 ppm due to (C5) of pyrazole. The ^{13}CNMR of compounds **4a-d** showed signal in the region of 164-165 ppm due to (C=O) and 174-175 ppm due to (C=S). Compounds **5a-d** showed signal at 177-178 ppm due to (C=N) of thiazole ring and signal at 163-164 ppm due to(C=O). The aromatic carbon atoms appeared in the region of 113.6-147.4 ppm. The mass spectrum and elemental analysis supported the structure of various synthesized compounds.

Biological Activity

In vitro anti-inflammatory studies: Compounds 2a,2b,2e,2f,2j,2k,2l,4c,5a,5b,6 and7 were screened for their in vitro anti-inflammatory activity by evaluation of IC50 of COX-I and COX-II.

Celecoxib was used as a reference standard representing selective COX-2 inhibitor, Indomethacin and Diclofenac as non-steroidal anti-inflammatory agents. The results in the current investigation revealed that all the tested compounds exhibited a promising anti-COX-II inhibitory activity especially compounds 2a,2b,2e,2j,2k,2l and 6 as shown in (table 1).

Table 1: IC50 of the synthesized compounds against COX-I and COX-II.

Compound	COX-I µm IC50	COX-II µm IC50
Celecoxib	14.8	0.05
Diclofenac sodium	3.9	0.8
Indomethacin	0.039	0.49
2a	9.4	0.11
2b	13.6	0.11
2e	11.6	0.12
2f	10.9	0.19
2j	10.9	0.10
2k	11.4	0.10
2l	13.4	0.10
4c	8.9	0.23
5a	7.9	0.34
5b	9.6	0.17
6	12.1	0.10
7	8.6	0.22

In vivo anti-inflammatory studies: Compounds 2a, 2b, 2e, 2j, 2k, 2l, and 6 were evaluated for their anti-inflammatory activity using carrageenan-induced edema bioassay method in rats.[24] Celecoxib was used as a reference standard representing selective COX-2 inhibitor non-

steroidal anti-inflammatory agents. The results in the current investigation revealed that all the tested compounds exhibited a promising anti-inflammatory activity as shown in (table 2).

Table 2: Anti-inflammatory evaluation of the tested compounds and Celecoxib at a dose of 0.9mg/100mg body weight of rats on inflamed rat paw (n=4 rats).

Comp. no.	Thickness of paw skin in mm after			
	Zero time	1st hr.	2nd hr.	3rd hr.
Control	0.21±0.00	0.90±0.00	1.15±0.00	1.25±0.00
Celecoxib	0.21±0.00	0.55±0.00	0.63±0.00	0.62±0.00
2a	0.24±0.01	0.65±0.06	0.77±0.05	0.79±0.05
2b	0.21±0.01	0.62±0.05	0.73±0.05	0.73±0.05
2e	0.21±0.01	0.90±0.02	1.15±0.03	1.25±0.02
2j	0.22±0.01	0.68±0.04	0.79±0.04	0.88±0.03
2k	0.22±0.01	0.68±0.01	0.79±0.02	0.85±0.04
2l	0.22±0.01	0.70±0.04	0.87±0.01	0.86±0.02
6	0.22±0.01	0.73±0.03	0.79±0.02	0.85±0.02

Means are expressed as Mean ± S.E

Compounds 2a and 2b exhibited good anti-inflammatory activity compared to Celecoxib. Compounds 2j,2k, 2l and 6 showed moderate anti-inflammatory activity, moreover compound 2e is completely inactive as you see in table(2).

Ulcerogenic activity

The target compounds 2a, 2b, 2e, 2j, 2k, 2l and 6 were evaluated for their ulcerogenic potential in rats using Indomethacin as a reference drug. The incidence of ulcer score was calculated according to 1 to 5 scoring system of Wilhelmi and Menasse-Gdynia.[25] The ulcer index was calculated according to the method of Pauls et al.[26] All drug doses were calculated according to Paget and Barnes.[27] As illustrated in table (3), all the tested compounds exhibited low ulcerogenic activity compared to reference drug Indomethacin.

Table 3: Ulcerogenic activity of the tested compounds and Indomethacin (n=4 rats).

Group	Ulcer score	Ulcer incidence (%)	Ulcer index
Control	0±0.00	0.0	0.0
Celecoxib	0.40±0.25	40	16
2a	1.40±0.40	60	84
2b	1.40±0.60	60	84
2e	0.40±0.25	40	16
2j	0.40±0.25	40	16
2k	0.40±0.25	40	16
2l	0.80±0.37	60	48
6	0.40±0.25	40	16

Indomethacin	2.60±0.25	100	260

Means are expressed as Mean ± S.E.

Compounds 2a and 2b which have good anti-inflammatory activity compared to celecoxib as shown in (table 2), exhibited low ulcerogenicity compared to indomethacin .Moreover, compound 2j, 2k and 6 which have moderate anti-inflammatory activity exhibited the same ulcerogenic activity of Celecoxib reference drug .2l is slightly more ulcerogenic than Celecoxib.

Antimicrobial activity

All the new compounds have been investigated against Gram+ve bacteria *(Staphylococcus aureus, Micrococcus luteus)* Gram -ve bacteria (*salmonella typhimurium and Escherchia coli*) and Fungi *(Candida albicans*) using cup plate diffusion method.[28] The results were reported as zone of inhibition compared to Cefotaxime and Nystatin as standard for bacterial and fungal strains respectively. The results of antimicrobial evaluation revealed that compounds 4a,4b,4c,5a,5b,5c and 5d showed high antibacterial activity against Gram positive bacteria (*Staphylococcus aureus and Micrococcus luteus*) ,moderate activity against Gram negative bacteria (*salmonella typhimurium and Escherchia coli*) and showed high antifungal activity against *Candida albicans*.Compounds 2a,2b,2c,2d,2e,2f,4d and 6 showed moderate antibacterial activity against Gram positive and Gram negative bacteria.All the investigated compounds exhibited good antifungal activity against*(Candida albicans*)as shown in table 4.

Table 4: Antimicrobial activity evaluation of the newly synthesized compounds.

Tested samples	Diameter (mm) of inhibition zones against the corresponding standard strains of different microorganisms				
	G+ve bacteria		G-ve bacteria		Fungi
	Staphylococcus aureus ATCC 6538	***Micrococcus luteus ATCC 10240***	***Salmonella typhimurium ATCC 40028***	***Escherichia coli ATCC 10536***	***Candida albicans ATCC 10231***
2a	22	25	19	20	28
2b	20	24	19	20	24
2c	25	30	22	22	26
2d	23	27	17	18	32
2e	24	26	21	23	27
2f	23	26	19	20	34
2g	22	26	20	21	26
2h	21	25	20	20	28
2i	25	27	23	21	27

2j	22	25	17	19	25
2k	23	26	19	20	26
2l	25	29	20	20	26
4a	26	30	23	24	30
4b	28	31	26	25	31
4c	28	30	27	26	30
4d	23	26	18	18	31
5a	28	32	24	24	24
5b	26	30	22	20	22
5c	29	32	23	23	23
5d	27	31	20	20	24
6	22	26	25	25	30
7	22	25	19	20	26
Cefotaxime (control)	31	28	32	34	-
Nystatin (control)	-	-	-	-	25
DMF (control)	-	-	-		-

MATERIAL AND METHODS

Melting points (°C) were determined with Gallenkamp melting point apparatus and are uncorrected. IR spectra (KBr, cm^{-1}) were recorded on Bruker or Testscanshimadzu FT8000 spectrometer.^{1}HNMR (400 MHz) spectra were recorded on a Bruker AC 400 MHz spectrometer in DMSO-d_6 as a solvent and tetramethylsilane (TMS) as an internal standard (chemical shift in δ ppm).^{13}CNMR (100 MHz) spectra were recorded on a Bruker AC 400 MHz spectrometer in DMSO-d_6 as a solvent, the chemical shifts are expressed in δ units.

Mass spectra were determined using a GC/MS Mat 112 S at 70ev spectrometer. Elemental analysis was carried out at the Regional center of Mycology and Biotechnology, Al-Azhar University, Nasr City, Egypt. All the results of elemental analysis corresponded to the calculated values within experimental error.

TLC was performed on silica gel (Merck 60 F254) and spots were visualized by iodine vapours or irradiation with UV light (254 nm).

Experimental

Chemistry

Compounds 1a-c were prepared according to reported procedure[29]

General procedure for the synthesis of N-(4-(1-(4-Substitutedphenylthiazol-2-yl)-5-(pyridin-4-yl)-4, 5-dihydro-1H-pyrazol-3-yl) phenyl) 4-substituted benzamides (2 a-l).

To a suspension of compounds 1 a-c (0.01mmol) in acetic acid (20ml), phenacyl bromide derivatives (0.01mmol) and sodium acetate (0.015mmol) were added and heated under reflux for 10 h. After cooling the obtained product was collected by filteration and crystallized from ethanol to give compounds 2 a-l (table 5).TLC: (ethyl acetate: pet. ether, 2: 3)

Table 5: **Physical and analytical data for N-(4-(1-(4-Substituted phenylthiazol-2-yl)-5-(pyridin-4-yl)-4, 5-dihydro-1H-pyrazol-3-yl) phenyl) 4-substituted benzamides (2 a-l).**

Comp. No.	R^1	R^2	M.F. (M.W.)	M.P. (°C)	Yield (%)	% Analysis of C,H,N (Calcd./Found)		
						C	H	N
2a	H_3CO	Cl	$C_{31}H_{24}ClN_5O_2S$ 566.07	240-242	85%	56.77 56.65	4.27 4.14	12.37 12.25
2b	H_3CO	Br	$C_{31}H_{24}BrN_5O_2S$ 610.52	230-232	89%	60.99 60.88	3.96 3.85	11.47 11.38
2c	H_3CO	CH_3	$C_{32}H_{27}N_5O_2S$ 545.65	210-212	88%	70.44 70.29	4.99 4.86	12.83 12.72
2d	H_3CO	H_3CO	$C_{32}H_{27}N_5O_3S$ 561.65	215-217	85%	68.43 68.32	4.85 4.72	12.47 12.38
2e	H	Cl	$C_{30}H_{22}ClN_5OS$ 536.05	203-205	90%	67.22 67.05	4.14 4.03	13.06 12.96
2f	H	Br	$C_{30}H_{22}BrN_5OS$ 580.50	224-226	92%	62.07 61.99	3.82 3.71	12.06 12.01
2g	H	CH_3	$C_{31}H_{25}N_5OS$ 515.63	218-220	88%	72.21 72.10	4.89 4.69	13.58 13.39
2h	H	H_3CO	$C_{31}H_{25}N_5O_2S$ 531.63	206-208	86%	70.04 70.00	4.74 4.62	13.17 13.06
2i	Cl	Cl	$C_{30}H_{21}Cl_2N_5OS$ 570.49	194-196	87%	63.16 63.05	3.71 3.60	12.28 12.19
2j	Cl	Br	$C_{30}H_{21}BrClN_5OS$ 614.94	200-202	92%	58.59 58.47	3.44 3.32	11.39 11.26
2k	Cl	CH_3	$C_{31}H_{24}ClN_5OS$ 550.07	191-193	91%	67.69 67.55	4.40 4.28	12.73 12.61
2l	Cl	H_3CO	$C_{31}H_{24}ClN_5O_2S$ 566.07	197-199	86%	65.77 65.69	4.27 4.19	12.37 12.25

Compound 2a :IR (KBr, cm^{-1}): 3419(NH), 3025 (CH, aromatic),2925 (CH,aliphatic), 1668 (C=O), 1599 (C=N),1462(C=C);^{1}HNMR (400MHz ,DMSO-d6) δ ppm:3.14-3.18(dd,1H,C_4-H of pyrazole), 3.36(dd, 1H,C_4-H of pyrazole), 3.84(s,3H,H_3CO-),5.92-5.94(dd,1H,C_5-H of

pyrazole),7.07-8.51(m,17H,ArH+1H of thiazole ring), 10.30 (s,1H,NH,D_2O exchangeable); MS: m/z(%) = 566.38(2.67) M^+, 138.95 (100).

Compound 2b: IR (KBr, cm^{-1}): 3413(NH), 3030 (CH, aromatic),2927 (CH,aliphatic), 1664 (C=O), 1596 (C=N),1460 (C=C);^{1}HNMR (400MHz ,DMSO-d6) δ ppm: 3.14-3.18(dd,1H,C_4-H of pyrazole), 3.44(dd, 1H,C_4-H of pyrazole), 3.84(s,3H,H_3CO-),5.92-5.94(dd,1H,C_5-H of pyrazole),7.05-8.51(m,17H,ArH+1H of thiazole ring), 10.32 (s,1H,NH,D_2O exchangeable); ^{13}C NMR(100MHz, DMSO-d6) δ ppm:175.92, 165.06, 162.07, 154.69, 151.42, 149.85, 140.64, 131.99, 129.71, 127.80, 126.60, 125.42, 120.56, 119.79, 113.64, 66.33, 62.44, 41.80.

Compound 2c :IR (KBr, cm^{-1}): 3407(NH), 3035 (CH, aromatic),2928 (CH,aliphatic), 1667 (C=O), 1598 (C=N),1459(C=C); ^{1}HNMR (400MHz, DMSO-d6) δ ppm: 2.31 (s,3H,CH_3), 3.14-3.18(dd,1H,C_4-H of pyrazole), 3.41(dd, 1H,C_4-H of pyrazole), 3.84(s,3H,H_3CO-), 5.92-5.94(dd,1H,C_5-H of pyrazole),7.06-8.51(m,17H,ArH+1H of thiazole ring), 10.30 (s,1H,NH,D_2O exchangeable);^{13} C NMR(100MHz, DMSO-d6) δ ppm:175.91, 172.00, 165.05, 162.07, 154.68, 151.41, 149.84, 141.62, 129.70, 127.80, 126.58, 125.43, 120.56, 119.78, 113.64, 62.01, 55.44, 41.79; MS: m/z(%) = 545.27(4.62) M^+,135.06(100).

Compound 2d: IR (KBr, cm^{-1}): 3409(NH), 3028 (CH, aromatic),2933 (CH,aliphatic), 1667 (C=O), 1596 (C=N),1461(C=C); ^{1}HNMR (400MHz, DMSO-d6) δ ppm: 3.13-3.18(dd,1H,C_4-H of pyrazole), 3.41(dd, 1H,C_4-H of pyrazole), 3.84(s,6H,H_3CO-), 5.92-5.94(dd,1H,C_5-H of pyrazole),7.07-8.51(m,17H,ArH+1H of thiazole ring), 10.33 (s,1H,NH,D_2O exchangeable);^{13}C NMR(100MHz, DMSO-d6) δ ppm:175.92, 173.36, 169.83, 165.06, 162.07, 154.69, 151.42, 149.85, 141.65, 132.50, 129.72, 127.80, 126.60, 125.42, 121.80, 120.56, 119.79, 113.64, 112.50, 66.13, 62.01, 56.81, 41.79; MS: m/z(%) = 561.72(2.19) M^+,135.13 (100).

Compound 2e: IR (KBr, cm^{-1}): 3430(NH), 3050 (CH, aromatic), 2925 (CH,aliphatic), 1656 (C=O), 1592 (C=N), 1477(C=C); ^{1}HNMR (400MHz ,DMSO-d6) δ ppm: 3.14-3.18(dd,1H,C_4-H of pyrazole), 3.48-3.96 (dd,1H,C_4-H of pyrazole),5.70-5.94 (dd,1H,C_5-H of pyrazole), 6.55-8.51 (m,18H,ArH+1H of thiazole ring), 10.49 (s,1H,NH,D_2O exchangeable); MS: m/z(%) =537.58(1.36), M^++1, 536.25(10.99) M^+, 105.06 (100)

Compound 2f: IR (KBr, cm^{-1}): 3432(NH), 3032 (CH, aromatic), 2930 (CH,aliphatic), 1658 (C=O), 1591 (C=N),1480 (C=C);^{1}HNMR (400MHz, DMSO-d6) δ ppm: 3.14-3.18(dd,1H,C_4-

H of pyrazole), 3.89-3.93(dd, 1H,C_4-H of pyrazole),5.92-5.95(dd,1H,C_5-H of pyrazole),7.15-8.51(m,18H,ArH+1H of thiazole ring), 10.54 (s,1H,NH,D_2O exchangeable);MS: m/z(%) = 580.00(2.26) M^+,77.10 (100).

Compound 2g: IR (KBr, cm^{-1}): 3429(NH), 3040 (CH, aromatic),2924 (CH,aliphatic), 1660 (C=O), 1596 (C=N),1482 (C=C);^{1}HNMR (400MHz ,DMSO-d6) δ ppm: 3.14-3.18(dd,1H,C_4-H of pyrazole), 3.89-3.96 (dd,1H,C_4-H of pyrazole), 5.92-5.94 (dd,1H,C_5-H of pyrazole), 6.61-8.51 (m,18H,ArH+1H of thiazole ring), 10.57 (s,1H,NH,D_2O exchangeable);^{13}C NMR(100MHz, DMSO-d6) δ ppm:175.95, 174.22, 165.77, 154.66, 151.43, 149.86, 141.47, 134.63, 131.77, 129.06, 128.41, 127.75, 125.68, 125.39, 121.74, 120.57, 119.90, 113.43, 113.20, 63.35, 41.81, 24.19, 20.78; MS: m/z(%) = 515.39(3.68) M^+,119.12 (100).

Compound 2h: IR (KBr, cm^{-1}): 3428(NH), 3045 (CH, aromatic),2932 (CH,aliphatic), 1665 (C=O), 1595 (C=N),1482 (C=C);^{1}HNMR (400MHz ,DMSO-d6) δ ppm: 3.57-3.78 (dd,1H,C_4-H of pyrazole), 3.83 (s,3H,H_3CO-), 3.87-3.95 (dd, 1H,C_4-H of pyrazole),5.69-5.94 (dd,1H,C_5-H of pyrazole),6.89-8.56 (m,18H,ArH+1H of thiazole ring), 10.60 (s,1H,NH,D_2O exchangeable); MS: m/z(%) = 531.80(1.66) M^+,43.23 (100).

Compound 2i: IR (KBr, cm^{-1}): 3432(NH), 3038 (CH, aromatic), 2964 (CH,aliphatic), 1652 (C=O), 1513 (C=N),1433 (C=C);^{1}HNMR (400MHz ,DMSO-d6) δ ppm: 3.15(dd,1H,C_4-H of pyrazole), 3.78(dd, 1H,C_4-H of pyrazole),5.71(dd,1H,C_5-H of pyrazole),6.55-8.51(m,17H, ArH+1H of thiazole ring), 10.51 (s,1H,NH,D_2O exchangeable);MS: m/z(%) = 570.90(12.54) M^+, 176.12 (100).

Compound 2j :IR (KBr, cm^{-1}): 3431(NH), 3043 (CH, aromatic),2926 (CH,aliphatic), 1653 (C=O), 1516 (C=N),1409(C=C);^{1}HNMR (400MHz ,DMSO-d6) δ ppm: 3.15(dd,1H,C_4-H of pyrazole), 3.91(dd, 1H,C_4-H of pyrazole), 5.75(dd,1H,C_5-H of pyrazole), 6.57-8.55(m,17H, ArH+1H of thiazole ring), 10.51 (s,1H,NH,D_2O exchangeable);MS: m/z(%) = 614(0.84) M^+,106.15 (100).

Compound 2k: IR (KBr, cm^{-1}): 3433(NH), 3041 (CH, aromatic),2924 (CH,aliphatic), 1663 (C=O), 1515 (C=N),1411 (C=C);^{1}HNMR (400MHz ,DMSO-d6) δ ppm: 2.30 (s,3H,CH_3), 3.15(dd,1H,C_4-H of pyrazole), 3.93 (dd, 1H,C_4-H of pyrazole), 5.69(dd,1H,C_5-H of pyrazole), 6.57-8.55(m,17H,ArH+1H of thiazole ring), 10.53 (s,1H,NH,D_2O exchangeable); MS: m/z(%) = 550.11(2.76) M^+,105.03 (100).

Compound 2l :IR (KBr, cm^{-1}): 3434(NH), 3039 (CH, aromatic),2967 (CH,aliphatic), 1668 (C=O), 1516 (C=N),1410 (C=C);^{1}HNMR (400MHz, DMSO-d6) δ ppm: 3.42-3.78(dd,1H,C_4-H of pyrazole), 3.83 (s,3H,H_3CO-), 3.93-3.95 (dd, 1H,C_4-H of pyrazole),5.42-5.69 (dd,1H,C_5-H of pyrazole),6.60-8.56(m,17H,ArH+1H of thiazole ring), 10.55 (s,1H,NH,D_2O exchangeable);MS: m/z(%) = 566.10(3.46) M^+,135.06 (100).

General procedure for the synthesis of N-(4-(1-(2-(Substituted thiocarbamoyl) hydrazono) ethyl) phenyl) nicotinamides (4a-d)

A mixture of compound 3 (0.001 mmol) and thiosemicarbazide (0.001 mmol) in ethanol (50 ml) and acetic acid (5 ml) was refluxed with vigorous stirring for 10 h.then allowed to cool. The resulting precipitate was filtered off, dried and crystallized from ethanol to give compounds 4 a-d (table 6).

Table 6: Physical and analytical data for N-(4-(1-(2-(Substituted thiocarbamoyl) hydrazono) ethyl) phenyl) nicotinamides (4a-d).

Comp. No.	R	M.F. (M.W.)	M.P. (°C)	Yield (%)	% Analysis of C,H,N (Calcd./Found)		
					C	H	N
4a	H	$C_{15}H_{15}N_5OS$ 313.38	125-127	95%	57.49 57.35	4.82 4.71	22.35 22.19
4b	Phenyl	$C_{21}H_{19}N_5OS$ 389.47	112-114	92%	64.76 64.65	4.92 4.81	17.98 17.87
4c	4-Br-phenyl	$C_{21}H_{18}BrN_5OS$ 468.37	152-156	89%	53.85 53.72	3.87 3.77	14.95 14.86
4d	Allyl	$C_{18}H_{19}N_5OS$ 353.44	122-124	91%	61.17 61.05	5.42 5.31	19.81 19.70

Compound 4a: IR (KBr, cm^{-1}): 3349,3263(NH_2),3172 (NH), 3030 (CH, aromatic),2950 (CH,aliphatic), 1656 (C=O), 1597 (C=N), 1499 (C=C),1272 (C=S);^{1}HNMR (400MHz, DMSO-d6) δ ppm: 2.29 (s,3H,CH_3), 7.56-9.11(m,10H,ArH+NH_2, D_2O exchangeable), 10.18(s,1H,NH,D_2O exchangeable), 10.54(s,1H,NH,D_2Oexchangeable); ^{13}CNMR(100MHz,DMSOd6)δppm: 176.75,164. 11, 152.16, 148.69, 147.40, 147.40, 139.81, 135.48, 133.00, 130.46, 127.13, 123.48, 119.64, 13.73.

Compound 4b: IR (KBr, cm^{-1}): 3433, 3307 & 3233 (NH), 3038 (CH, aromatic),2966 (CH,aliphatic), 1656 (C=O), 1597 (C=N),1484 (C=C),1253(C=S);^{1}HNMR (400MHz, DMSO-d6) δ ppm: 2.32 (s,3H,CH_3), 7.19-9.12 (m,13H,ArH), 10.03 (s,1H,NH,D_2O exchangeable), 10.61 (s,1H,NH,D_2Oexchangeable), 10.93(s,1H,NH,D_2

Oexchangeable),;^{13}CNMR(100MHz,DMSOd6)δppm:176.87,164.17,157.47,152.18,148.70,140.29,139.19,135.50,132.77,130.46,129.33,127.43,125.93,121.00,119.85,26.48,14.53.

Compound 4c: IR (KBr, cm^{-1}): 3378,3279(NH), 3030 (CH, aromatic),2960 (CH,aliphatic), 1676 (C=O), 1594 (C=N),1482 (C=C),1267 (C=S);^{1}HNMR (400MHz ,DMSO-d6) δ ppm: 2.38 (s,3H,CH_3), 7.54-9.12(m,12H,ArH),10.05 (s,1H,NH,D_2O exchangeable),10.65 (s,1H,NH,D_2O exchangeable), 10.93 (s,1H,NH,D_2O exchangeable);^{13} C NMR(100MHz, DMSO-d6) δ ppm:176.80, 164.17, 152.16, 149.02, 148.93, 140.09, 138.62, 135.53, 132.75, 130.83, 129.33, 127.92, 127.48, 123.50, 119.84, 117.54, 14.23;MS: m/z(%) =470.11(2.44)M^++2, 468.69(2.98) M^+,279.15 (100).

Compound 4d: IR (KBr, cm-1): 3413,3297,3237 (NH), 3032 (CH, aromatic), 2950 (CH,aliphatic), 1663 (C=O), 1592 (C=N),1408 (C=C), 1271(C=S);1HNMR (400MHz, DMSO-d6) δ ppm: 2.30(s,3H,CH_3), 4.09 (s,2H, N-CH_2-), 5.03-5.05(d,1H,=CH_2), 5.08-5.16 (d,1H,CH=CH_2), 5.18-5.85(m,1H,CH=),7.57-9.34(m,8H,ArH),9.34(s,1H,NH, D_2Oexchangeable),10.65(s,1H,NH,D_2O exchangeable), 10.73 (s,1H,NH,D_2O exchangeable); ^{13}C NMR(100MHz, DMSO-d6) δ ppm:178.06, 164.52, 152.35, 148.77, 147.52, 143.22, 139.85, 135.61, 134.72, 132.99, 130.48, 129.34, 127.14, 115.36, 45.95, 13.84; MS: m/z(%) = 353.07(0.94) M+, 134.96 (100).

General procedure for the synthesis of N-(4-(1-(2-(5-(4-Substitutedphenyl) thiazol-2-yl) hydrazono) ethyl) phenyl) nicotinamides(5a-d)

To a solution of 4a (0.001 mmol) in absolute ethanol (20 ml), triethyl amine (0.5 ml) and phenacylbromides (0.001 mmol) were added, the reaction mixture was refluxed for 8 h. The progress of the reaction was monitored by TLC. The reaction mixture was allowed to cool, poured into ice water (50 ml) and neutralized with diluted HCl to give a precipitate that was filtered, washed with water (30 ml), air dried and then crystallized from the ethanol to give compounds 5a-d (table 7).

Table 7:- Physical and analytical data for N-(4-(1-(2-(5-(4-Substituted phenyl)thiazol-2-yl)hydrazono)ethyl)phenyl)nicotinamides(5a-d).

Comp. No.	R	M.F. (M.W.)	M.P. (°C)	Yield(%)	% Analysis of C,H,N (Calcd./Found)		
					C	H	N
5a	Cl	$C_{23}H_{18}ClN_5OS$ 447.94	170-172	87%	61.67 61.56	4.05 3.95	15.63 15.51

5b	Br	$C_{23}H_{18}BrN_5OS$ 492.39	185-187	85%	56.10 56.00	3.68 3.57	14.22 14.13
5c	H_3CO-	$C_{24}H_{21}N_5O_2S$ 443.52	168-170	82%	64.99 64.88	4.77 4.64	15.79 15.64
5d	CH_3	$C_{24}H_{21}N_5OS$ 427.52	173-175	79%	67.43 67.31	4.95 4.86	16.38 16.28

Compound 5a: IR (KBr, cm^{-1}): 3267,3173(NH), 3022 (CH, aromatic),2960 (CH,aliphatic), 1656 (C=O), 1591 (C=N),1406 (C=C);^{1}HNMR (400MHz ,DMSO-d6) δ ppm: 2.29 (s,3H,CH_3), 7.56-9.11(m,13H,ArH+1H of thiazole ring), 10.17 (s,1H,NH,D_2O exchangeable), 10.54 (s,1H,NH,D_2O exchangeable);^{13}CNMR(100MHz,DMSO-d6)δ ppm:178.75,164.11, 152.16, 148.69, 147.38, 139.81, 135.48, 132.99, 130.46, 127.12, 123.48, 119.63, 13.71.

Compound 5b: IR (KBr, cm^{-1}): 3265,3172(NH), 3020 (CH, aromatic),2960 (CH,aliphatic), 1656 (C=O), 1590 (C=N),1402 (C=C);^{1}HNMR (400MHz ,DMSO-d6) δ ppm: 2.29 (s,3H,CH_3), 7.56-9.11(m,13H,ArH+1H of thiazole ring), 10.17 (s,1H,NH,D_2O exchangeable), 10.54 (s,1H,NH,D_2O exchangeable).

Compound 5c: IR (KBr, cm^{-1}): 3264,3172(NH), 3036 (CH, aromatic),2965 (CH,aliphatic), 1657 (C=O), 1595 (C=N), 1497 (C=C);^{1}HNMR (400MHz ,DMSO-d6) δ ppm: 2.29 (s,3H,CH_3),3.84(s,3H,H_3CO-), 7.58-9.13(m,13H,ArH+1H of thiazole ring), 10.18 (s,1H,NH,D_2O exchangeable), 10.55 (s,1H,NH,D_2O exchangeable);^{13}C NMR(100MHz, DMSO-d6) δ ppm: 178.74, 163.91, 164.02, 151.67, 148.28, 147.38, 139.77, 136.07, 133.03, 130.64, 129.34, 127.13, 123.70, 119.64, 119.51, 114.77, 114.11, 113.78, 56.01, 13.73; MS: m/z(%) = 443.14(1.21) M^+,78.03 (100).

Compound 5d: IR (KBr, cm^{-1}): 3268,3173(NH), 3044 (CH, aromatic),2968 (CH,aliphatic), 1656 (C=O), 1597 (C=N),1411 (C=C);^{1}HNMR (400MHz ,DMSO-d6) δ ppm: 2.29 (s,3H,CH_3),2.32(s,3H,CH_3), 7.56-9.11(m,13H,ArH+1H of thiazole ring), 10.17 (s,1H,NH,D_2O exchangeable), 10.54 (s,1H,NH,D_2O exchangeable); MS: m/z(%)= 427.13(3.28) M^+,78.06 (100)

Procedure for the synthesis of N-(4-(1-(2-(4-oxo-4, 5-dihydrothiazol-2-yl) hydrazono) ethyl) phenyl)nicotinamide (6).

To a solution of compounds 4a (0.01 mmol) in ethanol (20 ml), ethyl bromoacetate (0.01 mmol) and triethyl amine (0.5 ml) were added and heated under reflux for 14 h. The reaction

mixture was poured into ice-cold water. The obtained precipitated was filtered, dried and crystallized from ethanol/H_2O to give compound 6.

Yield(%)82%, M.P.(°C) 166-168 ^{0}C;IR (KBr, cm^{-1}): 3334,3181(NH), 3065 (CH, aromatic),2931 (CH,aliphatic), 1680 (C=O), 1599 (C=N),1402(C=C);^{1}HNMR (400MHz, DMSO-d6) δ ppm: 2.29 (s,3H,CH_3),4.09(s,2H,CH_2 of thiazolone), 7.56-9.12(m,8H,ArH), 10.57 (s,1H,NH,D_2O exchangeable), 10.58 (s,1H,NH,D_2O exchangeable); ^{13}C NMR(100MHz, DMSO-d6) δ ppm:178.75, 171.65, 169.13, 164.13, 157.12, 140.59,139.85, 132.85, 129.34, 126.62, 61.23, 56.02, 43.74, 33.81, 14.26**;**MS: m/z(%) = 354.16(0.80) M^++1,353.14(5.41)M^+, 78.09(100)**;** Microanalysis for $C_{17}H_{15}N_5O_2S$(353.40) Calcd.%C, 57.78, H.4.28, N, 19.82, found %C.57.69, H.4.18, N, 19.73**.**

Procedure for synthesis of ethyl 4-methyl-2-(2-(1-(4-(nicotinamido) phenyl)ethylidene) hydrazinyl)thiazole-5-carboxylate 7.

To mixture of 4a (0.001 mmol) in absolute ethanol (20 ml), triethyl amine (0.5 ml),ethyl-2-chloroacetoacetate (0.001 mmol) was added and the reaction mixture was refluxed for 10 h. The progress of the reaction was monitored by TLC. The reaction mixture was allowed to cool, poured into ice water (50 ml) and neutralized with diluted HCl to afford a precipitate that was filtered, washed with water (30 ml), air dried and then crystallized from ethanol to give compound 7. Yield(%)86%, M.P.(°C) 158-160 ^{0}C;IR (KBr, cm^{-1}): 3299,3161(NH), 3051 (CH, aromatic), 2981, 2916 (CH,aliphatic), 1682,1654 (C=O), 1590 (C=N), 1446(C=C); ^{1}HNMR (400MHz, DMSO-d6) δ ppm: 1.25-1.28 (t,3H,CH_3-), 2.32 (s,3H,CH_3), 2.48(s,3H,CH_3), 4.18-4.23(q,2H, -CH_2-), 7.56-9.12(m,8H,ArH), 10.59 (s,1H,NH,D_2O exchangeable), 11.73 (s,1H,NH,D_2O exchangeable); Microanalysis for$C_{21}H_{21}N_5O_3S$(423.49)Calcd.%C,59.56,H.5.00,N,16.54,found%C,59.46,H,4.95,N,16.43.

Biological activity

Antimicrobial activity

The antibacterial activities of the samples were determined by the agar well diffusion method[28,30,31] as modified from NCCLS.[32] Mueller-Hinton agar plates were surface-inoculated with the tested strains suspensions adjusted to match 0.5 McFarland standard and the inocula were spread over the surfaces of plates using sterile cotton swabs. After drying of the plates, cups (10 mm diameter) were punched in the agar and 100 µl of the samples in DMF or the antimicrobial agents were added into the wells. The plates were incubated at 37 ºC for 24 hours. The antibacterial activity was determined by measuring the diameter of the

zone of inhibition. The test was repeated three times and the mean inhibition zones were calculated.

In vivo Anti-inflammatory activity: The rat hind paw edema method was applied to determine the anti-inflammatory activity of the test compounds using Celecoxib as a standard. Mature albino male rats weighing 200-250gm were used. The animals were divided into 9 equal groups (each of 4). The first group was left as control, while the second group was injected (i.p.) with Celecoxib at a dose of 0.9mg/100gm. The test compounds were injected (i.p.) to the remaining groups at a dose of 0.9mg/100gm. One hour later, edema in the right hind paw was induced by injection of 0.1ml of 10% carragenan. The thickness of the paw was measured at 60, 120 and 180 minutes after carragenan injection to determine the anti-inflammatory activity of the test compounds (table 2).

Ulcerogenic activity

The anti-inflammatory tested compounds and Celecoxib were tested for their ulcerogenic activity using Indomethacin as a reference drug. Male albino rat's weighing180-200gm were fasted for 12 h prior to drug administration. The animals were divided into 10 equal groups (each of 4). The first received 1% gum acacia (suspending vehicle) orally once a day and was left as a control, whereas, the second group received Indomethacin at a dose of 0.9mg/100gm/day orally. The third group received Celecoxib at a dose of 0.9mg/100gm/day orally. The remaining groups received the test compounds at a dose of 0.9mg/100gm/day orally. The drugs were administered once a day for three successive days. The animals were killed by overdose of ether 6 hours after the last dose. The stomach was removed, opened along the great curvature and examined for ulceration. The number and severity of discrete areas of damage in the glandular mucosa were scored (table 3). The ulcer score was calculated according to the 1 to 5 scoring system of Wilhemi and Menasse-Gdynia as follows

1) 1 or 2 minute sporadic punctuates lesions.
2) Several small lesions.
3) One extensive lesion or multiple moderate-sized lesions.
4) Several large lesions.
5) Several large lesions with stomach perforation.

Stomach ulceration was expressed in terms of ulcer index (U.I. = mean ulcer score of a group of animals similarly tested multiplied by the percentage of ulcerated animals of this group.

Statistical analysis.

Data were analyzed using one-way analysis of variance (***ANOVA***) through the general linear models (GLM) procedure of the Statistical Package for Social Sciences version 21.0 (SPSS for Windows 21.0, Inc., Chicago, IL, USA).

The comparison of means was carried out with *Bonferroni multiple comparison* procedure.

Results were presented as mean ± standard errors (SE). The value of $P < 0.05$ was used to indicate statistical significance.

CONCLUSION

In the present work, we submitted very efficient method for the synthesis of some new nicotinamides bearing thiazole and pyrazoline pharmacophores. All the synthesized compounds were obtained in good to moderate yield. The synthesized compounds were characterized by IR, ^{1}HNMR, Mass spectroscopy and elemental analysis. The obtained results are showing good agreement with the proposed structures. Cyclooxygenase I and II (COX-I and COX-II) inhibition assays were carried out using Celecoxib, Diclofenac and Indomethacin as standards. Compounds which showed high inhibition of COX enzyme were further investigated for *in vivo* anti-inflammatory activity using Celecoxib as standard. Ulcerogenicity was done using Indomethacin as standard.The result showed Compounds 2a and 2b which have good antiinflammatroy activity compared to celecoxib as shown in (table 2), exhibited low ulcerogenicity compared to indomethacin .Moreover, compound 2j, 2k and 6 which have moderate anti-inflammatory activity exhibited the same ulcerogenic activity of Celecoxib reference drug . Furthermore, most of the synthesized compounds were screened *in vitro* antimicrobial activity compared with Cefotaxime as antibacterial drug and Nystatin as antifungal drug using cup-plate diffusion method , the results revealed that compounds 4a,4b,4c,5a,5b,5c and 5d showed high antibacterial activity against Gram positive bacteria (*Staphylococcus aureus and Micrococcus luteus*) ,moderate activity against Gram negative bacteria (*salmonella typhimurium and Escherchia coli*) and showed high antifungal activity against *Candida albicans* as shown in table (4).

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REFERENCES

1. Mert S., Kasımoğulları R., İça T., Çolak F., Altun A.and Ok S. Synthesis, structure–activity relationships, and in vitro antibacterial and antifungal activity evaluations of novel pyrazole carboxylic and dicarboxylic acid derivatives. *Eur J Med Chem*., 2014; 78: 86-96.
2. Rajendra Prasad Y., Kumar G.V.S and Chandrashekar S.M. Synthesis and biological evaluation of novel 4,5-dihydropyrazole derivatives as potent anticancer and antimicrobial agents. *Med Chem Res*, 2013; 22(5): 2061-2078.
3. Basha S.S., Ramachandra Reddy P., Padmaja A., Padmavathi V., Mouli K.C.and Vijaya T. Synthesis and antimicrobial activity of 3-aroyl-4-heteroaryl pyrroles and pyrazoles. *Med Chem Res*, 2015; 24(3): 954-964.
4. 4. Bandgar B.P., Adsul L.K.and Chavan H.V. et al. Synthesis, biological evaluation, and docking studies of 3-(substituted)-aryl-5-(9-methyl-3-carbazole)-1H-2-pyrazolines as potent anti-inflammatory and antioxidant agents. *Bioorg Med Chem Lett*, 2012; 22(18): 5839-5844.
5. Viveka S., Dinesha Shama P., Nagaraja G.K., Ballav S. and Kerkar S. Design and synthesis of some new pyrazolyl-pyrazolines as potential anti-inflammatory, analgesic and antibacterial agents. *Eur J Med Chem*, 2015; 101: 442-451.
6. Abdulla M.M., Amr A.E-G.E., Al-Omar M.A., Hussain A.A. and Shalaby A.F.A. Synthesis and pharmacological activities of some novel 5-chloro-N-(4-(1,5-(disubstituted)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-2-methoxybenzamide derivatives. *Med Chem Res*, 2014; 23(4): 2113-2121.
7. Özdemir A., Altıntop M.D., Kaplancıklı Z.A., Can Ö.D., Demir Özkay Ü.and Turan-Zitouni G. Synthesis and Evaluation of New 1,5-Diaryl-3-[4-(methyl-sulfonyl)phenyl]-4,5-dihydro-1H-pyrazole Derivatives as Potential Antidepressant Agents. *Molecules*, 2015; 20(2): 2668.

8. Qin Y-J., Li Y.and Jiang A-Q., et al. Design, synthesis and biological evaluation of novel pyrazoline-containing derivatives as potential tubulin assembling inhibitors. *Eur J Med Chem*, 2015; 94: 447-457.
9. Yang Y-S., Yang B., Zou Y., Li G.and Zhu H-L. Design, biological evaluation and 3D {QSAR} studies of novel dioxin-containing triaryl pyrazoline derivatives as potential B-Raf inhibitors. *Bioorg Med Chem*, 2016; 24(13): 3052-3061.
10. Karabacak M., Altıntop M.D. and İbrahim Çiftçi H., et al. Synthesis and Evaluation of New Pyrazoline Derivatives as Potential Anticancer Agents. *Molecules*, 2015; 20(10): 19066.
11. Burger M.T., Nishiguchi G.and Han W., et al. Identification of N-(4-((1R,3S,5S)-3-Amino-5-methylcyclohexyl)pyridin-3-yl)-6-(2,6-difluorophenyl)-5-fluoropicolinamide (PIM447), a Potent and Selective Proviral Insertion Site of Moloney Murine Leukemia (PIM) 1, 2, and 3 Kinase Inhibitor in Clinical Tri. *J Med Chem*, 2015; 58(21): 8373-8386.
12. Westaway S.M., Preston A.G.S.and Barker M.D., et al. Cell Penetrant Inhibitors of the KDM4 and KDM5 Families of Histone Lysine Demethylases. 1. 3-Amino-4-pyridine Carboxylate Derivatives. *J Med Chem*, 2016; 59(4): 1357-1369.
13. Jain R., Mathur M.and Lan J., et al. Discovery of Potent and Selective RSK Inhibitors as Biological Probes. *J Med chr*, 2015; 58(17): 6766-6783.
14. Singh R.K., Prasad D.N.and Bhardwaj T.R. Hybrid pharmacophore-based drug design, synthesis, and antiproliferative activity of 1,4-dihydropyridines-linked alkylating anticancer agents. *Med Chem Res*, 2015; 24(4): 1534-1545.
15. Brun P., Dean A. and Marco V. Di., et al. Peroxisome proliferator-activated receptor-γ mediates the anti-inflammatory effect of 3-hydroxy-4-pyridinecarboxylic acid derivatives: Synthesis and biological evaluation. *Eur J Med Chem*, 2013; 62: 486-497.
16. 1Ulloora S., Shabaraya R., Ranganathan R. and Adhikari A.V. Synthesis, anticonvulsant and anti-inflammatory studies of new 1,4-dihydropyridin-4-yl-phenoxyacetohydrazones. *Eur J Med Chem*, 2013; 70: 341-349.
17. Lu X., Zhang H. and Li X., et al. Design, synthesis and biological evaluation of pyridine acyl sulfonamide derivatives as novel COX-2 inhibitors. *Bioorg Med Chem*, 2011; 19(22): 6827-6832.
18. Lone I.H., Khan K.Z. and Fozdar BI. Synthesis, physicochemical properties, antimicrobial and antioxidant studies of pyrazoline derivatives bearing a pyridyl moiety. *Med Chem Res*, 2014; 23(1): 363-369.
19. Patel N.B., Patel H.R., Shaikh F.M. and Rajani D. New 4-thiazolidinones from 5-ethyl

pyridine-2-ethanol: their antibacterial, antifungal, and antitubercular activity. *Med Chem Res*, 2014; 23(3): 1360-1370.

20. Pitucha M., Woś M. and Miazga-Karska M., et al. Synthesis, antibacterial and antiproliferative potential of some new 1-pyridinecarbonyl-4-substituted thiosemicarbazide derivatives. *Med Chem Res*, 2016; 1-12.
21. Kamble R.D., Meshram R.J. and Hese S.V., et al. Synthesis and in silico investigation of thiazoles bearing pyrazoles derivatives as anti-inflammatory agents. *Comput Biol Chem*, 2016; 61: 86-96. doi:http://dx.
22. Abdelall E.K.A and Kamel G.M. Synthesis of new thiazolo-celecoxib analogues as dual cyclooxygenase-2/15-lipoxygenase inhibitors: Determination of regio-specific different pyrazole cyclization by 2D {NMR}. *Eur J Med Chem*, 2016; 118: 250-258.
23. Pawar C.D., Sarkate A.P. and Karnik K.S., et al. Synthesis and antimicrobial evaluation of novel ethyl 2-(2-(4-substituted)acetamido)-4-subtituted-thiazole-5-carboxylate derivatives. *Bioorg Med Chem Lett*, 2016; 26(15): 3525-3528.
24. Winter C., Risley E. and Nuss G.W, Proc. Soc. Exp. Biol. Med., 1962; 111: 544.
25. Wilhelmi G., Menasse-Gdynia R., Pharmacology, 1972; 8(321).
26. Pauls F., Wick A.N. and Mackay E.M., Gastroenterology, 1997; 8(774).
27. S. P, Barnes R. Evaluation of drug activities",. *pharmacometrics*, 1964; I.
28. Febiger I. The antimicrobial susceptibility test: Principle and practices. *Biol Abstr*, 1976; 65: 25183.
29. Zakaria K. A., Hanan A. A., Amany M. E. and Elsayed M.M.Docking,synthesis and biolobical evaluation of novel pyrazoline derivatives as potential antimicrobial and antitumor agents.World J.Pharm. Res, 2016; 5(11): 1523-1551.
30. William H. Microbiological Assay, An introduction to quantitative principles and evaluation. *Acad Press york*, 1977; 1-68.
31. Bauer R., Kirby M.D., Sherris J.C and Turck M. Antibiotic susceptibility testing by standard singl diffusion method. *Amer J Clin Pathol*, 1966; 45: 493.
32. (NCCLS). committee for clinical laboratory standards. Methods for dilution antimicrobial susceptibility tests of bacteria that grow aerobically. *Approv Stand M100-512, Wayne P.A,NCCLS*, 2002.