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SYNTHESIS, ANALGESIC, ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITIES OF NOVEL PYRAZOLINE DERIVATIVES.

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ABSTRACT

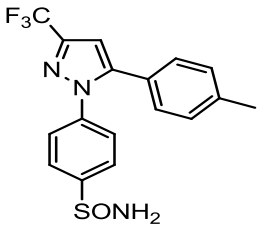
A series of novel pyrazoline derivatives **3a-p** were synthesized *via* the reaction of aryl hydrazine derivatives **2a-d** with chalcones **1a-d.** The new compounds were screened for their analgesic, anti- inflammatory and antimicrobial activities. Compounds **3a** and **3l** showed a greater analgesic activity than Celecoxib. Moreover compounds **3a** and **3n** showed good anti-inflammatory compared to Celecoxib.However their efficacies were associated with lower gastric ulcerogenecity compared with Indomethacin.Compounds **3e** and **3f** showed high activities against both gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria *(Pseudomonas aruginosa*) while compounds**3e, 3k** and **3 m** have high activity against *Candida albicans*.

KEY WORDS: Analgesic, anti-inflammatory, antimicrobial activity, synthesis, pyrazoline derivatives.

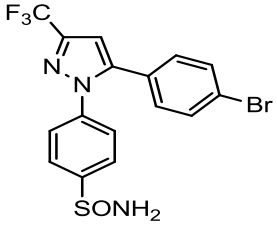
INTRODUCTION

Inflammation is a tissue response from immune system against injury and plays a significant role in the pathogenesis of various diseases. Prostaglandin has important role and its biosynthesis is increased during inflammatory responces.[1] Acute inflammation is rapid and

quick process and leads to healing of wound. Chronic inflammation is a long lasting pain mainly occurs in cancer, rheumatoid arthritis and other sever disorders.[2] Synthesis of prostaglandin is dependent on cyclooxygenase enzyme. There are two isoforms of cyclooxgenase COX-1 and COX-2.[1] Inhibition of cyclooxgenase enzyme is considered as one of the important target in the anti-inflammatory drug discovery. Various drugs are well known as COX inhibitors as Rofecoxib, Meloxicam, Diclofenac, Indomethacin and Ibuprofen.[3] However pyrazole is an interesting class of heterocyclic compounds possessing synthetic versatility and effective biological activities. Some pyrazole derivatives have anti inflammatory properties as Celecoxib and SC-558[4] representing (non steroidal anti-inflammatory drugs).



Celecoxib

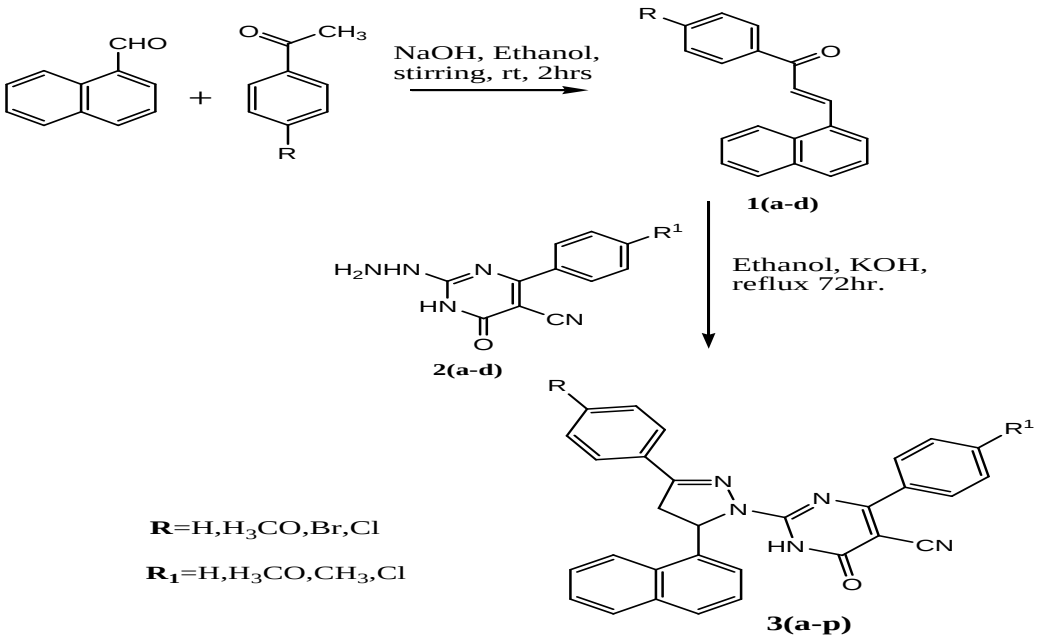


SC-558

Pyrazolines have also been reported to possess antimicrobial[5-8], anti-inflammatory[9-11], analgesic[12-14], antipyretic[15], antiviral[16], anticonvulsant[17], antihypertensive[18], antidepressant[19] and anticancer[20.21] ,anthelminitic activity[22], antioxidant[23], anti tubercular[24] activity. So in this work we direct our attention for synthesis of some pyrazoline derivatives **3a-p** and investigate their analgesic, anti-inflammatory and antimicrobial activities.

RESULTS AND DISCUSSION

Chemistry: 4-(4-Substituted phenyl)-2-(5-(naphthalen-1-yl)-3-(4-substituted phenyl) -4, 5-dihydro-1H-pyrazol-1-yl)-6-oxo-1, 6-dihydro pyrimidine-5-carbonitrile **3(a-p)** were prepared *via* refluxing chalcone derivatives **1a-d** and aryl hydrazine derivatives **2a-d** in ethanol containing potassium hydroxide (scheme1). The structures of all new compounds **3a-p** were identified by IR, ^{1}HNMR and Mass Spectrometry. The purity of these compounds was checked by elemental analysis.



Scheme 1

IR spectra showed peaks at 3113-3352 corresponding to (NH) group, 3050-3060 (CH, aromatic), 2967-2920 (CH, aliphatic). Moreover appearance of strong absorption band at υ = 2220-2213 cm^{-1} corresponding to (CN) group.

Measurement of chemical shift prove pyrazoline structure where, H_4 (trans) appeared as double doublets at δ = 3.21-3.23 ppm. The peak of H_4 (cis) of pyrazoline appeared in the range of δ = 4.64-4.68 ppm. The H_5 of pyrazoline appeared at δ = 5.56-5.58 ppm. Appearance of multiplet signals at δ = 7.10-9.08 ppm, singlet at δ = 2.40, 3.80 and 12.64 ppm due to (ArH), CH_3, OCH_3 and (NH, D_2O exchangeable) respectively.^{13}CNMR confirmed the proposed structures due to appearance of characteristic peaks at δ ppm: 162.48 (C═O), 114.27 (C≡N), 55.60(C_5 of pyrazole) and 28.60 (C_4 of pyrazole).

Biological Activity

In vivo anti-inflammatory studies: Compounds **3a, 3f, 3h, 3i, 3l, 3m, 3n** and **3o** were evaluated for their anti-inflammatory activity using carrageenan-induced edema bioassay method in rats.[25]

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 1**).

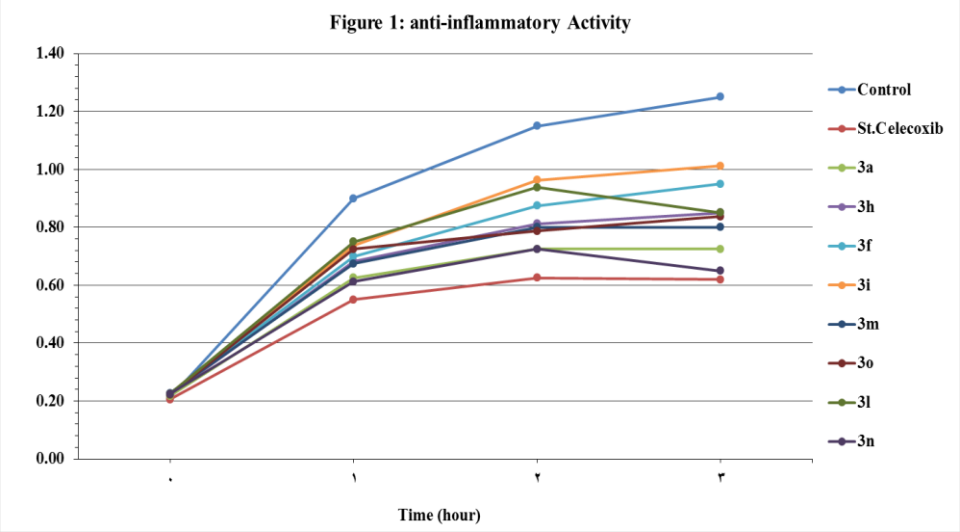
Table 1: Anti-inflammatory evaluation of the tested compounds and Celecoxib at a dose of 0.9mg/100mg body weight of rats on inflamed rat paw (n=4 rats).

Comp. no.	Thickness of paw skin in mm after			
	Zero time	1st hr.	2nd hr.	3rd hr.
Control	0.21 ± 0.01^{a}	0.90 ± 0.02^{a}	1.15 ± 0.03^{a}	1.25 ± 0.02^{a}
Celecoxib	0.21 ± 0.01^{a}	0.55 ± 0.03^{b}	0.63 ± 0.01^{b}	0.62 ± 0.02^{b}
3a	0.22 ± 0.01^{a}	0.63 ± 0.05^{bc}	0.73 ± 0.05^{bc}	0.73 ± 0.05^{bc}
3f	0.23 ± 0.01^{a}	0.70 ± 0.04^{bc}	0.88 ± 0.01^{cdegi}	0.95 ± 0.03^{df}
3h	0.22 ± 0.01^{a}	0.68 ± 0.02^{bc}	0.81 ± 0.04^{cegi}	0.85 ± 0.04^{cdf}
3i	0.23 ± 0.01^{a}	0.74 ± 0.02^{c}	0.96 ± 0.05^{e}	1.01 ± 0.08^{de}
3l	0.23 ± 0.01^{a}	0.75 ± 0.03^{ace}	0.94 ± 0.02^{eh}	0.85 ± 0.02^{cdh}
3m	0.23 ± 0.01^{a}	0.68 ± 0.03^{bc}	0.80 ± 0.04^{cefgi}	0.80 ± 0.02^{bfgh}
3n	0.23 ± 0.02^{a}	0.61 ± 0.01^{bc}	0.73 ± 0.03^{bi}	0.65 ± 0.02^{bi}
3o	0.22 ± 0.01^{a}	0.73 ± 0.03^{cd}	0.79 ± 0.02^{bgh}	0.84 ± 0.02^{cdgi}

Means are expressed as Mean ± S.E

Means within the same column carrying different superscript letters are significantly different at ($P< 0.05$) using the Bonferroni correction.

Compounds **3a** and **3n** exhibited good anti-inflammatory activity compared to **Celecoxib**. Compounds **3f, 3h, 3m** and **3o** showed moderate anti-inflammatory activity, while compounds **3i** and **3l** exhibited the least activity among the tested compounds as shown in **figure (1)**.



The presences of different substituents at p- position of both phenyl rings which are attached to the newly synthesized scaffold of pyrazoline incorporating pyrimidine and naphthalene moieties is very important and directly affect on the lipophillic nature of the tested compounds, hence affect on the rate of absorption and transportation in vivo.

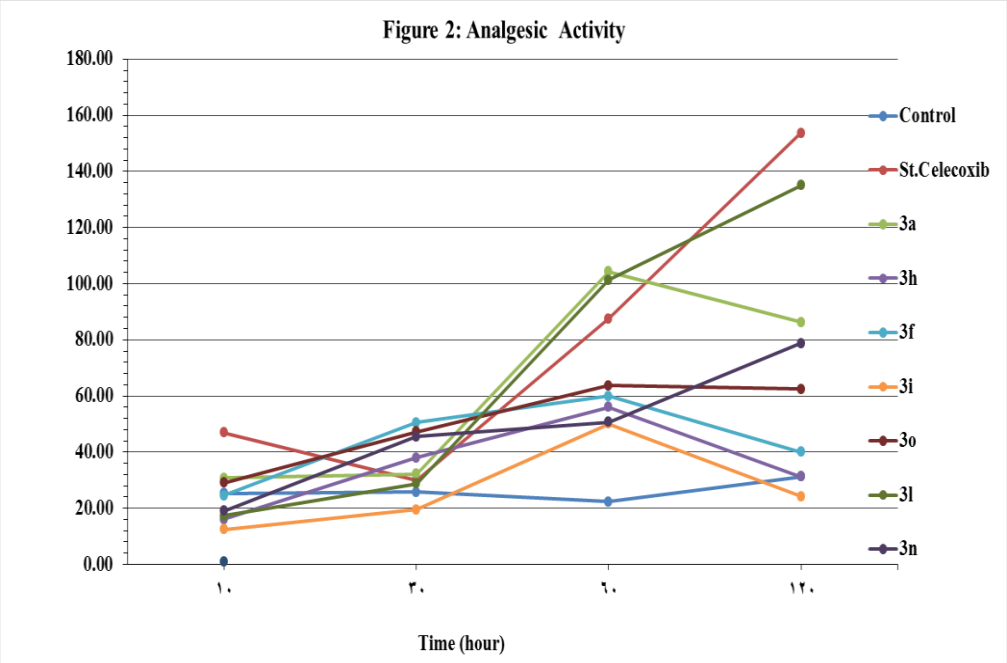
Analgesic activity: Compounds **3a, 3f, 3h 3i, 3l, 3n, 3o** drug were screened for their analgesic activity Celecoxib (COX-2 inhibitor) as reference drug using the hot plate method of Jacob and Bsovski.[26] **Figure (2)** showed that the compounds **3a** and **3l** show a significant increase in the reaction time slightly better than the reference drug **Celecoxib**. The results presented in **(table 2)** indicate that all the tested compounds elicited a significant change in the reaction time when compared with the control group.

Table 2: Analgesic evaluation of the tested compounds and Celecoxib at a dose of 1.3mg/100mg body weight of mice (n=4 mice).

Comp. no.	Reaction time in seconds after			
	10 min	30 min	60 min	120 min
Control	25.25 ± 2.06^{adf}	25.75 ± 2.10^{ae}	22.25 ± 1.03^{a}	31.25 ± 3.33^{ac}
Celecoxib	47.00 ± 3.63^{b}	30.00 ± 4.08^{ace}	87.50 ± 8.54^{ac}	153.75 ± 12.81^{b}
3a	30.75 ± 4.64^{ac}	32.25 ± 3.54^{ab}	104.25 ± 8.13^{b}	82.34 ± 6.99^{ac}
3f	24.50 ± 2.99^{adf}	50.50 ± 3.97^{b}	60.00 ± 5.77^{ac}	40.00 ± 4.08^{ac}
3h	16.00 ± 3.19^{df}	38.00 ± 5.07^{ab}	56.00 ± 4.95^{ac}	31.25 ± 3.15^{ac}
3i	12.50 ± 1.26^{de}	19.50 ± 0.50^{a}	50.00 ± 6.47^{ac}	24.25 ± 1.49^{a}
3l	17.25 ± 1.03^{adf}	28.75 ± 3.20^{ade}	101.25 ± 8.26^{c}	135.00 ± 17.08^{b}
3n	19.00 ± 1.29^{adf}	45.50 ± 5.11^{be}	50.75 ± 4.35^{ac}	78.75 ± 16.63^{c}
3o	29.00 ± 3.32^{cf}	47.25 ± 5.41^{bcd}	63.75 ± 7.18^{ac}	62.50 ± 9.24^{ac}

Means are expressed as Mean ± S.E

Means within the same column carrying different superscript letters are significantly different at ($P< 0.05$) using the Bonferroni correction.



Ulcerogenic activity

The target compounds **3a, 3f, 3h, 3i, 3l, 3m, 3n** and **3o** 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.[27] The ulcer index was calculated according to the method of Pauls et al.[28] All drug doses were calculated according to Paget and Barnes.[29] As illustrated in **(table 3)**, all the tested compounds are having less ulcerogenic activity than the 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	0.5±0.29	50	25
3a	2.5±0.29	100	250
3f	3±0.00	100	300
3h	2.25±0.25	100	225
3i	0.5±0.29	50	25
3l	0.25±0.25	25	6.5
3m	2±0.41	100	200
3n	1.75±0.25	100	175
3o	0.25±0.25	25	6.5
Indomethacin	4.5±0.29	100	450

Means are expressed as Mean ± S.E.

Compound **3o** which have moderate anti-inflammatory activity as shown in **(table 1)** is less ulcerogenic than **Celecoxib** reference drug.

Compounds **3i** and **3l** which unfortunately showed the least anti-inflammatory activity within all the tested compounds are having the same ulcerogenic activity or slightly better than **Celecoxib** reference drug. Moreover, compounds **3a** and **3l** which are the most active analgesic compounds according to the previous data shown in **(table 2)**, having low gastric ulcerogenicity compared with **Indomethacin** reference drug.

Antimicrobial Activity

Some of the new compounds have been investigated against Gram+ve bacteria *(Staphylococcus aureus* ATCC 6538), Gram -ve bacteria (*Pseudomonas aeruginosa* ATCC 9027) and *(Escherichia coli* ATCC 10536) and Fungi *(Candida albicans* ATCC 10231) using cup plate diffusion method.[30-33] The results were reported as zone of inhibition compared to **Cefotaxime** and **Nystatin** as standard for bacterial and fungal strains respectively.

The results obtained in (table 4) showed that compounds **3e**, **3f** are the most active against Gram +ve bacteria *(Staphylococcus aureus*) and Gran –ve bacteria (*Pseudomonas aeruginosa*). Moreover, compounds **3g, 3k** and **3n** showed moderate activity against Gram +ve bacteria *(Staphylococcus aureus*) and Gran –ve bacteria (*Pseudomonas aeruginosa*). Compounds **3e, 3f, 3g, 3h**, **3k, 3o** and **3p** showed moderate activity against Gram –ve bacteria*(Escherichia coli*). Furthermore, compounds **3e, 3k & 3m** have high activity against fungi(*Candida albicans*) ,while compounds **3g, 3h, 3j, 3n, 3o** and **3p** have moderate activity against *Candida albicans*.

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.			
	Gm+ve bacteria	Gm-ve bacteria		Fungi
	Staphylococcus aureus **ATCC 6538**	***Pseudomonas aeruginosa*** **ATCC 9027**	***Escherichia coli*** **ATCC 10536**	***Candida albicans*** **ATCC 10231**
3e	40	38	25	26
3f	30	26	22	18
3g	25	20	21	25
3h	21	20	20	20
3i	20	18	11	-
3j	18	19	15	24
3k	25	20	20	30
3m	18	20	-	30
3n	25	26	19	25
3o	20	20	20	20
3p	22	20	20	20
Cefotaxime (5mg/ml) (control)	30	30	35	-
Nystatin (5mg/ml) (control)	-	-	-	24
DMF (control)	-	-	-	-

EXPERIMENTAL

Chemistry

Melting points were determined with Gallenkamp melting point apparatus and are uncorrected. IR spectra (KBr, cm^{-1}) were recorded on Bruker or Testscan shimadzu FT8000 spectrometer. ^{1}HNMR (300 MHz) spectra were recorded on a Bruker AC 300 MHz spectrometer in DMSO-d_6 as a solvent and tetra methyl silane (TMS) as an internal standard (chemical shift in ppm).

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).

Compounds 1a-d& 2a-d were prepared according to the reported procedure.[34-36]

General procedure for synthesis of: 4-(4-Substituted phenyl)-2-[5-(naphthalen-1-yl)-3-(4-substituted phenyl)-4, 5-dihydro-1H-pyrazol-1-yl]-6-oxo-1, 6-dihydro pyrimidine-5-carbonitrile. 3(a-p)

An equimolecular mixture of chalcones **1a-d**, aryl hydrazine derivatives **2a-d and** KOH (0.007 mol) in ethanol (50ml) was heated under reflux for 72hrs. After cooling, the reaction mixture was poured into ice-cold water and neutralized with glacial acetic acid, then filtered, dried and crystallized from an appropriate solvent to give compounds **3a-p** as shown in **(table 5)**.

Table 5: 4-(4-Substituted phenyl)-2-(5-(naphthalen-1-yl)-3-(4-substituted phenyl)-4, 5-dihydro-1H-pyrazol-1-yl)-6-oxo-1, 6-dihydro pyrimidine-5-carbonitrile. 3(a-p)

Comp. No.	R	R^1	M.F. (M.W.)	M.P. (°C)	Yield (%) Crys.solvent	% analysis of C, H, N (Calcd./found)		
						C	H	N
3a	H	H	$C_{30}H_{21}N_5O$ (467)	290-293	62 a	77.07 77.23	4.53 4.61	14.98 15.09
3b	H_3CO	H	$C_{31}H_{23}N_5O_2$ (497)	287-290	46 a	74.83 74.98	4.66 4.72	14.08 14.23
3c	Br	H	$C_{30}H_{20}BrN_5O$ (546)	232-235	42 a	65.94 66.17	3.69 3.74	12.82 12.97
3d	Cl	H	$C_{30}H_{20}ClN_5O$ (501.5)	228-230	49 a	71.78 71.89	4.02 3.95	13.95 14.12
3e	H	CH_3	$C_{31}H_{23}N_5O$ (481)	114-116	94 a	77.32 77.49	4.81 4.89	14.54 14.69
3f	H_3CO	CH_3	$C_{32}H_{25}N_5O_2$ (511)	123-125	89 a	75.13 75.21	4.93 4.97	13.69 13.88
3g	Br	CH_3	$C_{31}H_{22}BrN_5O$ (560)	124-126	89 b	66.44 66.58	3.96 3.98	12.50 12.59

3h	Cl	CH_3	$C_{31}H_{22}ClN_5O$ (515.5)	153-156	84 b	72.16 72.31	4.30 4.37	13.57 13.71
3i	H	H_3CO	$C_{31}H_{23}N_5O_2$ (497)	119-121	74 a	74.83 74.97	4.66 4.73	14.08 14.24
3j	H_3CO	H_3CO	$C_{32}H_{25}N_5O_3$ (527)	192-195	62 b	72.85 73.04	4.78 4.85	13.27 13.39
3k	Br	H_3CO	$C_{31}H_{22}BrN_5O_2$ (576)	127-130	92 b	64.59 64.63	3.85 3.81	12.15 12.51
3l	Cl	H_3CO	$C_{31}H_{22}ClN_5O_2$ (531.5)	134-136	48 b	69.99 69.87	4.17 4.24	13.16 13.42
3m	H	Cl	$C_{30}H_{20}ClN_5O$ (501.5)	120-122	88 a	71.78 71.89	4.02 4.11	13.95 14.08
3n	H_3CO	Cl	$C_{31}H_{22}ClN_5O_2$ (531.5)	218-221	79 b	69.99 70.17	4.17 4.28	13.16 13.39
3o	Br	Cl	$C_{30}H_{19}BrClN_5O$ (580.5)	132-135	84 b	62.03 62.18	3.30 3.27	12.06 12.18
3p	Cl	Cl	$C_{30}H_{19}Cl_2N_5O$ (536)	124-127	58 b	67.17 67.24	3.57 3.64	13.06 13.21

$EtOH/H_2O$ = a*

$HOAc/H_2O$ = b*

Compound **3a**; IR (KBr, cm^{-1}): 3113 (NH), 3046 (CH, aromatic), 2936 (CH, aliphatic), 2213 (C≡N), 1660 (C=O), 1568 (C=N), 1501 (C=C); ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 3.43-3.45 (d, 1H, C_4-H of pyrazole), 4.18-4.28 (dd, 1H, C_4-H of pyrazole), 6.60-6.61 (d, 1H, C_5-H of pyrazole), 7.14-8.19 (m, 17H, ArH), 12.64 (s, 1H, NH, D_2O exchangeable); MS: m/z (%) = 467 (50.70) M^+, 364 (69.08), 363 (100), 152 (51.72).

Compound **3b**; IR (KBr, cm^{-1}): 3435 (NH), 3050 (CH, aromatic), 2927 (CH, aliphatic), 2210 (C≡N), 1660 (C=O), 1575 (C=N), 1505 (C=C); ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 3.31-3.32 (dd, 1H, C_4-H of pyrazole), 3.83 (s, 3H, H_3CO), 4.19-4.20 (dd, 1H, C_4-H of pyrazole), 6.56-6.57 (d, 1H, C_5-H of pyrazole), 7.05-8.18 (m, 16H, ArH), 12.51 (s, 1H, NH, D_2O exchangeable).

Compound **3c**; IR (KBr, cm^{-1}): 3429 (NH), 3050 (CH, aromatic), 2920 (CH, aliphatic), 2211 (C≡N), 1661 (C=O), 1575 (C=N), 1495 (C=C); ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 3.21-3.23 (dd, 1H, C_4-H of pyrazole), 4.18-4.19 (dd, 1H, C_4-H of pyrazole), 6.58-6.59 (d, 1H, C_5-H of pyrazole), 7.00-8.02 (m, 16H, ArH), 12.45 (s, 1H, NH, D_2O exchangeable).

Compound **3d**; IR (KBr, cm^{-1}): 3424 (NH), 3054 (CH, aromatic), 2967, 2928 (CH, aliphatic), 2214 (C≡N), 1659 (C=O), 1577 (C=N), 1494 (C=C); ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm:

2.96-2.98 (dd, 1H, C_4-H of pyrazole), 4.11-4.23 (dd, 1H, C_4-H of pyrazole), 6.56-6.58 (d, 1H, C_5-H of pyrazole), 6.89-8.01 (m, 16H, ArH), 13.31 (s, 1H, NH, D_2O exchangeable).

Compound **3e**; IR (KBr, cm^{-1}): 3352 (NH), 3056 (CH, aromatic), 2924 (CH, aliphatic), 2221 (C≡N), 1679 (C=O), 1615 (C=N), 1483 (C=C); ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 2.40 (s, 3H, CH_3), 3.43-3.51 (d, 1H, C_4-H of pyrazole), 4.64-4.68 (d, 1H, C_4-H of pyrazole), 5.89-5.91 (d, 1H, C_5-H of pyrazole), 7.14-9.08 (m, 16H, ArH), 11.47 (s, 1H, NH, D_2O exchangeable); MS: m/z (%) = 481 (0.63) M^+, 393 (61.62), 140 (100), 105 (72.17), 83 (70.63).

Compound **3f**; IR (KBr, cm^{-1}): 3447 (NH), 3057 (CH, aromatic), 2925(CH, aliphatic), 2219 (C≡N), 1672(C=O), 1613 (C=N), 1492 (C=C); ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 2.41 (s, 3H, CH_3), 3.39-3.51 (d, 1H, C_4-H of pyrazole), 3.79 (s, 3H, H_3CO), 3.82-3.85 (dd, 1H, C_4-H of pyrazole), 4.84-4.86 (d, 1H, C_5-H of pyrazole), 6.98-9.37 (m, 15H, ArH), 11.43 (s, 1H, NH, D_2O exchangeable); MS: m/z (%) = 511 (0.49) M^+, 391 (45.67), 140 (77.99), 135 (86.16), 83 (100).

Compound **3g**; IR (KBr, cm^{-1}): 3319 (NH), 3050 (CH, aromatic), 2919 (CH, aliphatic), 2220 (C≡N), 1676 (C=O), 1614 (C=N), 1481 (C=C); ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 2.40 (s, 3H, CH_3), 3.20-3.22 (d, 1H, C_4-H of pyrazole), 4.18-4.22 (d, 1H, C_4-H of pyrazole), 5.76-5.78 (d, 1H, C_5-H of pyrazole), 7.43-9.35 (m, 15H, ArH), 11.47 (s, 1H, NH, D_2O exchangeable); ^{13}CNMR (75 MHz, DMSO-d6) d ppm: 161.20, 158.99, 158.62, 133.63, 133.44, 131.72, 131.23, 131.06, 130.94, 130.68, 130.18, 129.80, 129.26, 128.68, 128.30, 127.76, 126.39, 125.49, 123.05, 117.41, 115.63, 71.26, 28.52, 21.08; MS: m/z (%) = 562 (5.57) M^++2, 561 (527)M^++1, 560 (9.32)M^+, 248 (21.38), 124 (100), 59 (39.01).

Compound **3h**; IR (KBr, cm^{-1}): 3447 (NH), 3055 (CH, aromatic), 2969-2922 (CH, aliphatic), 2221 (C≡N), 1677 (C=O), 1614 (C=N), 1538 (C=C); ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 2.41 (s, 3H, CH_3), 3.19-3.21 (d, 1H, C_4-H of pyrazole), 4.16-4.18 (d, 1H, C_4-H of pyrazole), 5.80-5.82 (d, 1H, C_5-H of pyrazole), 7.41-9.39 (m, 15H, ArH), 11.50 (s, 1H, NH, D_2O exchangeable); ^{13}CNMR (75 MHz, DMSO-d_6) δ ppm: 160.00, 158.90, 158.56, 133.64, 133.32, 131.23, 130.67, 129.97, 129.32, 128.79, 128.61, 128.49, 128.27, 128.14, 127.43, 126.90, 126.39, 125.88, 125.49, 123.04, 118.00, 55.60, 28.54, 21.07.

Compound **3i**; IR (KBr, cm^{-1}): 3322 (NH), 3054 (CH, aromatic), 2937, 2839 (CH, aliphatic), 2219 (C≡N), 1675 (C=O), 1610 (C=N), 1481 (C=C); ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 3.30-3.43 (d, 1H, C_4-H of pyrazole), 3.85 (s, 3H, H_3CO), 3.88-3.89 (d, 1H, C_4-H of pyrazole), 5.88-5.90 (d, 1H, C_5-H of pyrazole), 7.14-9.08 (m, 16H, ArH), 11.25 (s, 1H, NH, D_2O exchangeable).

Compound **3j**; IR (KBr, cm^{-1}): 3440 (NH), 3055 (CH, aromatic), 2935, 2837 (CH, aliphatic), 2213 (C≡N), 1673 (C=O), 1605 (C=N), 1483 (C=C); ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 3.30-3.41 (d, 1H, C_4-H of pyrazole), 3.85 (s, 6H, $2H_3CO$), 3.89-3.90 (d, 1H, C_4-H of pyrazole), 5.78-5.80 (d, 1H, C_5-H of pyrazole), 7.09-9.08 (m, 15H, ArH), 11.31 (s, 1H, NH, D_2O exchangeable).

Compound **3k**; IR (KBr, cm^{-1}): 3341 (NH), 3055 (CH, aromatic), 2933, 2838 (CH, aliphatic), 2220 (C≡N), 1677 (C=O), 1611 (C=N), 1539 (C=C); ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 3.28-3.29 (d, 1H, C_4-H of pyrazole), 3.86 (s, 3H, H_3CO), 3.87-3.89 (dd, 1H, C_4-H of pyrazole), 5.89-5.91 (d, 1H, C_5-H of pyrazole), 7.18-9.38 (m, 15H, ArH), 11.44 (s, 1H, NH, D_2O exchangeable); ^{13}CNMR (75 MHz, DMSO-d_6) δ ppm: 162.48, 149.87, 133.65, 131.72, 131.33, 131.31, 130.95, 130.42, 129.89, 129.08, 128.78, 127.55, 126.89, 126.46, 126.20, 125.88, 125.49, 123.57, 123.05, 114.27, 113.62, 55.60, 55.55, 28.52.

Compound **3l**; IR (KBr, cm^{-1}): 3421 (NH), 3058 (CH, aromatic), 2930 (CH, aliphatic), 2221 (C≡N), 1677 (C=O), 1612 (C=N), 1516 (C=C); ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 3.15-3.16 (d, 1H, C_4-H of pyrazole), 3.87 (s, 3H, H_3CO), 3.88-3.89 (d, 1H, C_4-H of pyrazole), 5.81-5.84 (d, 1H, C_5-H of pyrazole), 7.18-9.40 (m, 15H, ArH), 11.47 (s, 1H, NH, D_2O exchangeable); MS: m/z (%) = 533 (1.07) M^++2, 531 (2.57) M^+, 407 (82.80), 393 (39.36), 152 (45.17), 83 (100).

Compound **3m**; IR (KBr, cm^{-1}): 3447 (NH), 3057 (CH, aromatic), 2930 (CH, aliphatic), 2219 (C≡N), 1675 (C=O), 1616 (C=N), 1537 (C=C); ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 3.11-3.12 (dd, 1H, C_4-H of pyrazole), 4.10-4.12 (dd, 1H, C_4-H of pyrazole), 5.89-5.90 (dd, 1H, C_5-H of pyrazole), 7.16-9.10 (m, 16H, ArH), 11.25 (s, 1H, NH, D_2O exchangeable); MS: m/z (%) = 503 (0.13)M^++2, 501 (0.33)M^+, 413 (46.37), 154 (41.08), 140 (100), 139 (61.83).

Compound **3n**; IR (KBr, cm^{-1}): 3316 (NH), 3055 (CH, aromatic), 2938 (CH, aliphatic), 2214 (C≡N), 1674 (C=O), 1599 (C=N), 1493 (C=C); ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 3.30-

3.43 (dd, 1H, C_4-H of pyrazole), 3.86 (s, 3H, H_3CO), 4.10-4.12 (dd, 1H, C_4-H of pyrazole), 5.88-5.90 (dd, 1H, C_5-H of pyrazole), 7.12-9.29 (m, 15H, ArH), 11.56 (s, 1H, NH, D_2O exchangeable).

Compound **3o**; IR (KBr, cm^{-1}): 3309 (NH), 3051 (CH, aromatic), 2923 (CH, aliphatic), 2217 (C≡N), 1674 (C=O), 1613 (C=N), 1536 (C=C); ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 3.31-3.32 (dd, 1H, C_4-H of pyrazole), 4.08-4.10 (d, 1H, C_4-H of pyrazole), 5.85-5.86 (dd, 1H, C_5-H of pyrazole), 7.16-9.28 (m, 15H, ArH), 11.52 (s, 1H, NH, D_2O exchangeable); ^{13}CNMR (75 MHz, DMSO-d_6) δ ppm: 162.00, 136.37, 133.62, 131.71, 131.48, 131.41, 131.22, 130.86, 130.31, 129.97, 129.27, 128.79, 128.64, 128.53, 127.56, 126.43, 125.47, 123.56, 123.02, 114.00, 113.50, 55.60, 28.99.

Compound **3p**; IR (KBr, cm^{-1}): 3448 (NH), 3062 (CH, aromatic), 2930 (CH, aliphatic), 2222 (C≡N), 1672 (C=O), 1614 (C=N), 1540 (C=C); ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 3.32-3.33 (d, 1H, C_4-H of pyrazole), 4.09-4.11 (d, 1H, C_4-H of pyrazole), 5.76-5.78 (dd, 1H, C_5-H of pyrazole), 7.32-8.06 (m, 15H, ArH), 11.55 (s, 1H, NH, D_2O exchangeable).

Pharmacology

In vivo Anti-inflammatory activity: The rat hind paw edema method [25] 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 10 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 1)**.

Analgesic activity

The hot plate method of Jacob and Bsovski[26] was used to evaluate the analgesic activity. Mature albino male mice weighing 20-25gm were classified into 9 groups (each of 4). The first group was left as control and injected (i.p.) with the solvent (DMSO), whereas, the second group was injected (i.p.) with **Celecoxib** at a dose of 1.3mg/100gm. Each of the remaining groups was injected (i.p.) with the test compound in the same dose 1.3mg/100gm. Ten minutes later, each mouse was placed in a two liter–beaker immersed in a water bath

thermostatically controlled at 56^0C. The time elapsed till the mouse clicks its paw or jumps was considered as the reaction time and was taken as a measure of the analgesic effect. Readings were taken at 10, 30, 60 and 120 minutes post treatment **(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 hours prior to drug administration. The animals were divided into 11 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[27] as follows

1) 1 or 2 minute sporadic punctuate 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.[28]

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.

Antimicrobial screening

Media and chemicals

Müller-Hinton broth was obtained in dehydrated form from Oxoid, Hampshire, England. Agar was supplied by Biolife, Milano, Italy. Dimethyl formamide (DMF) used as a negative control.

Microorganisms

A total of four standard microbial strains were used in this study. They were obtained from the Egyptian Pharmaceutical Industries Company (EPICO), Egypt which were *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 10536, and *Candida albicans* ATCC 10231.

Antimicrobial activity

The antibacterial activities of the samples were determined by the agar well diffusion method as modified from NCCLS.[33] 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.

CONCLUSION

In the present work, we submitted very efficient method for the synthesis of some new 4-(4-Substituted phenyl)-2-[5-(naphthalen-1-yl)-3-(4-substituted phenyl)-4, 5-dihydro-1H-pyrazol-1-yl]-6-oxo-1, 6-dihydro pyrimidine-5-carbonitriles. 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. Some of newly synthesized compounds were investigated for anti-inflammatory and analgesic activities using Celecoxib as a reference standard. Ulcerogenicity was done using Indomethacin as standard.The result showed that compounds **3a & 3n** have good anti-inflammatory compared to Celecoxib.Moreover compounds **3a & 3l** were the most active analgesic greater than Celecoxib with low ulcerogenecity. Furthermore, most of the synthesized compounds were screened in vitro for antimicrobial activity compared with Cefotaxime as antibacterial drug and Nystatin as

antifungal drug using cup-plate diffusion method and the results showed that the tested compounds have good antimicrobial activity.

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