INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

SYNTHESIS, *INVIVO* ANALGESIC AND *INVITRO* ANTI-MICROBIAL ACTIVITY OF3-AMINO-4-[2-(SUBSTITUTED PHENYL) HYDRAZIN-1-YLIDENE]-1-[(2-OXO-2H-CHROMEN-3-YL) CARBONYL]-4, 5-DIHYDRO-1H-PYRAZOL-5-ONE AND ITS SCHIFF BASES

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ABSTRACT

A series of 3-amino-4-[2-(4-chlorophenyl)hydrazin-1-ylidene]-1-[(2-oxo-2H-chromen-3-yl)carbonyl]-4,5-dihydro-1H-pyrazol-5-one (**4a-j**) and its Schiff bases (**5a-i**) have been synthesized. The structures of the newly synthesized compounds were confirmed by IR, NMR and Mass spectral analysis. The titled compounds were screened for their i*nvivo*analgesic activity by acetic acid-induced writhing model in mice. All compounds were evaluated for their *invitro* anti-bacterial and anti-fungal activity. Among the synthesized compounds, compounds **4f**, **4g** demonstrated 1.06 times more potent anti bacterial activity aganist*E coil* and *K. pneumoniae*and corresponding Schiff base compounds **5d** and **5e**demonstrated 1.00 and 1.06 times more potent anti bacterial activity aganist*E coil.* Compounds 4**b** and **4c** displayequipotent anti-fungal activity aganist***C. albicans*** and *A. niger*. Compound**5i**showing 1.04 times more potent than standard Indomethacin and also the compounds 4f**, 4g, 5b,** and **5j** exhibited significant analgesic activity compared with the standard drug (Indomethacin0.03 mM/kg) on oral administration.

Keywords: Antimicrobial, Analgesic, Coumarin, Pyrazolone.

INTRODUCTION

Since resistance of pathogenic bacteria towards available antibiotics is rapidly becoming a major world-wide problem, the design of new compounds to deal with resistant bacteria has become one of the most important areas of antibacterial research today. In addition, primary and opportunistic fungal infections continue to increase rapidly because of the increased number of immune compromised patients. As known, not only biochemical similarity of the human cell and fungi forms a handicap for selective activity, but also the easily gained resistance is the main problem encountered in developing safe and efficient antifungal. Antipyrine[1] (2, 3-dimethyl-1-phenyl-3-pyrazolin-5-one) was the first pyrazolone derivative introduced in the year 1884 for the management of pain, inflammation and fever. Many of this pyrazole derivatives[2-4] such as Phenyl butazone, Febrazone, Feclobuzone, Metobutazoneand Ramitenazone has found their clinical application as NSAID's. Pyrazolone and their derivatives have attracted the attention of several research groups due to their wide range of pharmacological activities[5-9] which exhibited anti-inflammatory,analgesic,antipyretic,anti-tubercular, anti-bacterial,anticonvulsant, and antidepressant, anticancer. On the other hand coumarins and their derivatives have engrossed substantial attention from organic and medicinal chemists for many years as

they belong to a class of compounds with proven utility in medicinal chemistry. Coumarin is an important scaffold since several coumarin derivatives have wide range of biological activities[10-14] such as antimicrobial, antitubercular, anticancer agent etc. As a part of our continued program on the chemistry of pyrazolone ring systems, we recently developed a simple and efficient approach to a wide range of such derivatives. These results prompted us to synthesize a series of new pyrazolone derivatives containing coumarin ring that would act as potent analgesic and agent anti-microbial.

EXPERIMENTAL

MATERIALS AND METHODS

Starting materials were obtained from commercial sources and were used without further purification. Reaction progress was observed by thin layer chromatography making the use of commercial silica gel plates (Merck Ltd., Mumbai). Melting points were determined in open capillary tubes on a Sonar melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were determined by Bruker 300 MHz FT- NMR spectrometer in appropriate deuterated solvents and are expressed in parts per million (δ, ppm) downfield from tetramethylsilane (internal standard). NMR data are given as multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet) and number of protons. Elemental analysis (C, H, and N) was undertaken with Elemental vario EL III Carlo Erba 1108 analyzer. The infrared (IR) spectra were run as KBr disk on Jasco FTIR 4100 spectrophotometer. Mass spectra of the synthesized compounds were recorded in MS (EI) JEOL GC mate spectrometer.

Synthesis of Ethyl-2-oxo-2H-chromene-3-carboxylate[15] (1a)

To a cold mixture of salicylaldehyde (0.2M) and diethyl malonate (0.2M), 2 ml of Piperidine was added by rapid stirring. After 20 min the yellow solid separated was filtered off subsequently washed with ethanol and was recrystallized from water: ethanol (2:8), M.p.128°C and yield was 85 **%.**

Synthesis of 2-oxo-2H-chromene-3-carbohydrazide[12] (1b)

A mixture of Ethyl-2-oxo-2H-chromene-3-carboxylate (0.01M) and hydrazine hydrate 98 % (0.5M) was refluxed for an appropriate time. The reaction mixture was poured into water and the solid which separate filtered off. It was the recrystallized from ethanol to give the title compound, M.p.145°C and yield was 72 %.

Diazotization of substituted aniline [16] (2a-j)

A mixture of different substituted aniline (0.01M) in concentrated HCl (3ml) was cooled to 0-50°C under ice, and cooled sodium nitrite solution (1.5 g in 10ml of water) added to it drop wise during 10 minutes. The reaction mixture was then stirred for 30 minutes.

Synthesis of Ethyl-2(substituted phenyl) hydrazono-3-oxobutyrate[17] (3a-j)

To an ice-cold mixture of the active methylene compound (Ethyl cyano acetate) (0.01M) and sodium acetate (4.10 g ; 0.05M) in ethanol (50 ml), was added drop wise with stirring a solution of diazonium salt compound **(2a-j)**(0.01M) over 15 minutes. The stirring was continued for 30 minutes and the reaction mixture then left for 2 hours at room temperature. The product was collected and recrystallized from ethanol to give the corresponding hydrazone derivatives (**3a-j**).

Synthesis of 3-amino-4-[2-(substituted phenyl) hydrazin-1-ylidene]-1-[(2-oxo-2H-chromen-3-yl) carbonyl]-4,5-dihydro-1H-pyrazol-5-one [18] (4a – j)

Ethyl-2(substituted phenyl) hydrazono-3-oxobutyrate (**3a-j**) (0.003 M) dissolved in acetic acid (10-15 ml) was added a solution of 2-oxo-2H-chromene-3-carbohydrazide (**1b**) (0.003 M) in acetic acid (15-20 ml) and the mixture was refluxed for 6 h. On cooling, the resulting sticky product was obtained. Sticky mass was allowed to stand overnight and extracted ether (55 %) to get title compounds (**4a-j**).

Synthesis of schiff base of 3-amino-4-[2-(substituted phenyl)hydrazin-1-ylidene]-1-[(2-oxo-2H-chromen-3-yl)carbonyl]-4,5-dihydro-1H-pyrazol-5-one(5a-j)

The mixture of 3-amino-4-[2-(substituted phenyl)hydrazin-1-ylidene]-1-[(2-oxo-2H-chromen-3-yl)carbonyl]-4,5-dihydro-1H-pyrazol-5-one **(4a-j)**(0.005 mol) and corresponding aldehydes (0.005 mol) in 20 ml of ethanol was heated under reflux for 2-3 h in the presence of glacial acetic acid. After completion of reaction, the resulting mixture was poured into crushed ice and the precipitated title compound (**5a-j)** was filtered, dried and recrystallized from ethanol.

BIOLOGICAL EVALUATION

Animals

Albino mice of either sex weighing 25-30 g were used for acute toxicity studies and

analgesics activity. Animals were kept in KMCH College of Pharmacy, Coimbatore. Animal are housed individually in polypropylene cages, maintained under standard conditions of alternating 12 h light and dark cycles at a constant temperature (25±2°C and 35-60 % RH). Animals were fed with standard rat pellet and water *ad libitum.*

Acute toxicity

The acute toxicity test was carried out according to an organization for Economic co-operation and development (OECD) guidelines [19] to establish the effective dose of test compounds after obtaining ethical clearance from animal ethics committee of KMCH College of pharmacy, Coimbatore. Albino mice of either sex weighing between 25-30 g were grouped into 12 groups of six animals each, starved for 24 h with water *ad libitum* prior to test. On the drug of experiment animals were administered with different compounds to different groups in an increasing dose of 10, 20, 100, 200, 1000, 1500, 2000, 2500 mg/kg orally. The animals were then observed continuously for 3 h for general behavioral neurological, autonomic profiles and then every 30 min for next 3hr and finally for 24 h or till death.

Analgesic activity by acetic acid-induced writhing model in mice[20]

Twenty-four hours prior to actual testing a large number of mice received intraperitoneally 10 ml/kg of 0.6 % glacial acetic acid. Animals were observed for writhing movements. Only these showing one or other type of writhing movements (positive responders) were chosen for the test on the next day. On the test day the responders received synthesized compounds (**4a-j** and **5a-j**) half an hour prior to glacial acetic acid challenge. Synthesized compounds were orally administrated at a dose of 0.03 mM/kg as a suspension in 0.5 Sodium Carboxyl Methyl Cellulose (CMC). Each mouse was then observed for the total number of stretching episodes or writhing for 15minutes following glacial acetic acid injection. Percentage of writhing was calculated using the relation.

Inhibition of Writhing (%) =100 X (1-b⁄a).

where; a=Mean writhing number of control mice, b=Mean writhing number of treated mice. The results of the analgesic studies are listed in Table **III**, respectively.

Antimicrobial activity

Determination of zone of inhibition

All the previously reported our synthesized compounds were screened for their preliminary antibacterial activity against two Gram-positive strains: *Micrococcus luteus, Staphylococcus aureus, two* Gram-negative strains: *Escherichia coli, Klebsiellapneumoniae*and two fungal strains: *Candida albicans, Aspergillusniger,* by disk diffusion method[21]. Ciprofloxacin (5 µg/disk) and Clotrimazole (5 µg/disk) were used as standard drugs for antibacterial and antifungal activity, respectively. Activity was determined by measuring the diameter of the zone showing complete inhibition. The results of the antibacterial and antifungal studies are listed in Tables **IV**and **V**, respectively.

Determination ofminimum inhibitory concentration

The minimum inhibitory concentrations (MIC) of µg/ml values of the titled compounds were carried out by two-fold serial dilution method [22]. The MICs are recorded through visual observations after 24 h (for bacteria) and 72–96 h (for fungi) of incubation. Ciprofloxacin was used as standard for bacterial studies and Clotrimazole was used as standard for fungal studies. The lowest concentration at which there was no visible growth was taken as MIC. The results of the MIC study were listed in Tables **IV** and **V**, respectively.

RESULTS AND DISCUSSION

Chemistry

The synthetic strategies adopted for the synthesis of the intermediate and target compounds are depicted in the Scheme I. Ethyl-2-oxo-2H-chromene-3-carboxylate (**1a**) and 2-oxo-2H-chromene-3-carbohydrazide (**2a**) were synthesized according to the literature procedures. The aryl diazonium salts (**3a-i**) were synthesized by diazotization of different derivatives of anilines using a mixture of sodium nitrite and HCl at 0-5°C. The diazonium salts thus obtained were treated in ethanol in the presence of sodium acetate with calculated amounts of ethyl acetoacetate to afford the corresponding key intermediate ethyl-2(substituted phenyl) hydrazono-3-oxobutyrate **(4a-i)**.Compound 2-oxo-2H-chromene-3-carbohydrazide (**2a**) and ethyl-2(substituted phenyl)hydrazono-3-oxobutyrate (**4a-i**) were dissolved in glacial acetic acid and reflux to form 3-methyl-1-[(2-oxo-2*H*-chromen-4-yl)carbonyl]-1*H*-pyrazole-4,5-dione 4-[(4-substitutedphenyl)hydrazone] (**5a–i**). Melting point was uncorrected determined by open capillary tube. Synthesized compounds were

characterized by their elemental analysis, IR, ^{1}H-NMR which is listed in Table I. From the structural investigation, IR spectra of the compound **1a**showed stretching frequency at 1798 cm^{-1}, which evinced the presence of carboxylic esterand theappearance ofCONH peak for the synthesized compound **2a** showed stretching frequency at 1650 cm^{-1}. IR spectrums of compounds (**3a-j**) were showed appearance of the characteristic band of the cyano group and carboxylic acid ester at 2262 -2270 and 1744-1756 cm^{-1} respectively.Dependant substitution of double-bonded nitrogen group of imine C=N could be the reason for the characteristic absorption close to the carbonyl C=O of amide (1630-1680 cm^{-1}) or C=C of alkenes (1600-1680 cm^{-1}) double bond stretching region[23]. Compounds **4a-j** the appearance of a band at 3312-3328 and 1635-1663 cm^{-1} attributed to the stretching vibration of the NH_2 group, pyrazolone ring C=O groups and also disappearance of the characteristic band of the cyano group and carboxylic acid ester.The IR spectra of the Schiff base (5a-j) confirmed the presence of -NH-N=C, N-C= O and C=O lactone present in the synthesized compounds by presence of IR stretching bands at 3426-3456, 1715-1728, 1635-1663, and 1602-1623 cm^{-1} respectively. Disappearance of -NH_2 primary amine signal at 3312-3328 cm^{-1}. ^{1}H-NMR spectrum showed singlet signals derived from -NH-N=C protons at 6.92-7.11 δ ppm indicate the hydrazone formation. Compounds **4a-j** containing NH_2 protons signal appeared as singlet range from 1.75-2.12 δ ppm. The aromatic protons appeared as multiplets ranging from 6.75-7.32(coumarin ring) and 6.14-6.68 (substituted phenyl) δ ppm. In case of Schiff bases (**5a-j**) singlet signals appeared within the range 6.14-6.68 δ ppm due to –N=CH- proton which confirmed the formation of Schiff bases. Further, the formations of title compounds were confirmed by recording their Mass spectrums which were in full agreement with their molecular weights and results of elemental analysis were ±0.4 % of the theoretical values.^{1}H-NMR spectra give a characteristic proton resonance shifts for all the synthesized compound derivatives, which ensured the existence of aromatic, hydrazones, and imines' protons.

Acute toxicity

The compound showed a high safety margin when screened at graded doses 0.01-2.5g/kgfor their acute lethal doses (ALD_{50}). The (LD_{50}) values were found to be more than 2.5 g/kg.

Analgesic activity

Table-II reveals the analgesic activity of serious titled compounds **(4a-j and 5a – j)** at a dose of 0.03mM/kg body weight by acetic acid induced writhing method. Abdominal constriction response induced by acetic acid is a sensitive procedure to establish efficacy of peripherally acting analgesic. The synthesized compounds **4a, 4b, 4c, 4f, 4g**and **4h** show significant analgesic activity by acetic acid induced writhing model at 0.03 mM/kg when orally administrated in mice compared to standard drug Indomethacin 0.03 mM/kg dose level. Analgesic activity of synthesized compounds (4a-j) demonstrated that compounds having para substituted electron donating group (**4a, 4b, 4c, 4f** and **4g**) in the phenyl ring at 4th position in the pyrazoline nucleus attached throughazomethine (–NHN=CH) linkagepossess percentage of protection range between 75.90-97.57% compared to standard drug Indomethacin. On the other hand, compound **4h** illustrated that disubstituted electron withdrawing group (NO_2) bearing 3 & 4th at position in the phenyl ring showed significant analgesic activity with 80.23 % of protection. However, disubstitutedcompound **4i** and **4j** illustrated that electron donating group (Cl&Br) bearing at 3 & 4th in the phenyl ring showed 4.00 and 2.53 fold timesdecreaseanalgesic activity compared with corresponding compounds bearing paramonosubstituted (Cl& Br) electron donating group compounds (**4a** and **4b**) with 78.02 and 82.42 % of protection, respectively. Compound 3-(benzylamino)-4-[2-(3,4-dichlorophenyl)hydrazin-1-ylidene]-1-[(2-oxo-2H-chromen-3-yl)carbonyl]-4,5-dihydro-1H-pyrazol-5-one (**5i**) demonstrate 1.04times more potent analgesic activity than Indomethacin and 5.33 times more analgesic activity than corresponding pyrazolone derivatives (**4i**), Compound 3-(benzylamino)-4-[2-(3,4-dibromophenyl)hydrazin-1-ylidene]-1-[(2-oxo-2H-chromen-3-yl)carbonyl]-4,5-dihydro-1H-pyrazol-5-one (**5j**) display nearly equipotent analgesic activity compared to standard and 2.93 times more analgesic activity than corresponding pyrazolone derivatives(**4j**), indicated that disubstituted electron donating group (Cl&Br) bearing 3 & 4th at position in the phenyl ring improved analgesic activity.In general, compounds (**5a**, **5b**, **5c**, **5i** and **5j**) bearingdisubstitutedhalogen atom at 3 & 4th position in the phenyl ring improved analgesic activity compared to compounds (**4a**, **4b**, **4c**, **4i** and **4j**) bearing mono substituted halogen atom at 4th position in the phenyl ring. On the other hand, compounds (**5d**,**5e, 5f &5g**) bearing

disubstituted electron donating group (-CH_3& -OCH_3) and electron withdrawing group (NO_2& COOH) bearing 3 & 4th at position in the phenyl ring decrease analgesic activity compared to compounds (**4d**, **4e**, **4f**&**4g**) bearing monosubstituted electron donating group (-CH_3& -OCH_3) and electron withdrawing group(NO_2& COOH).

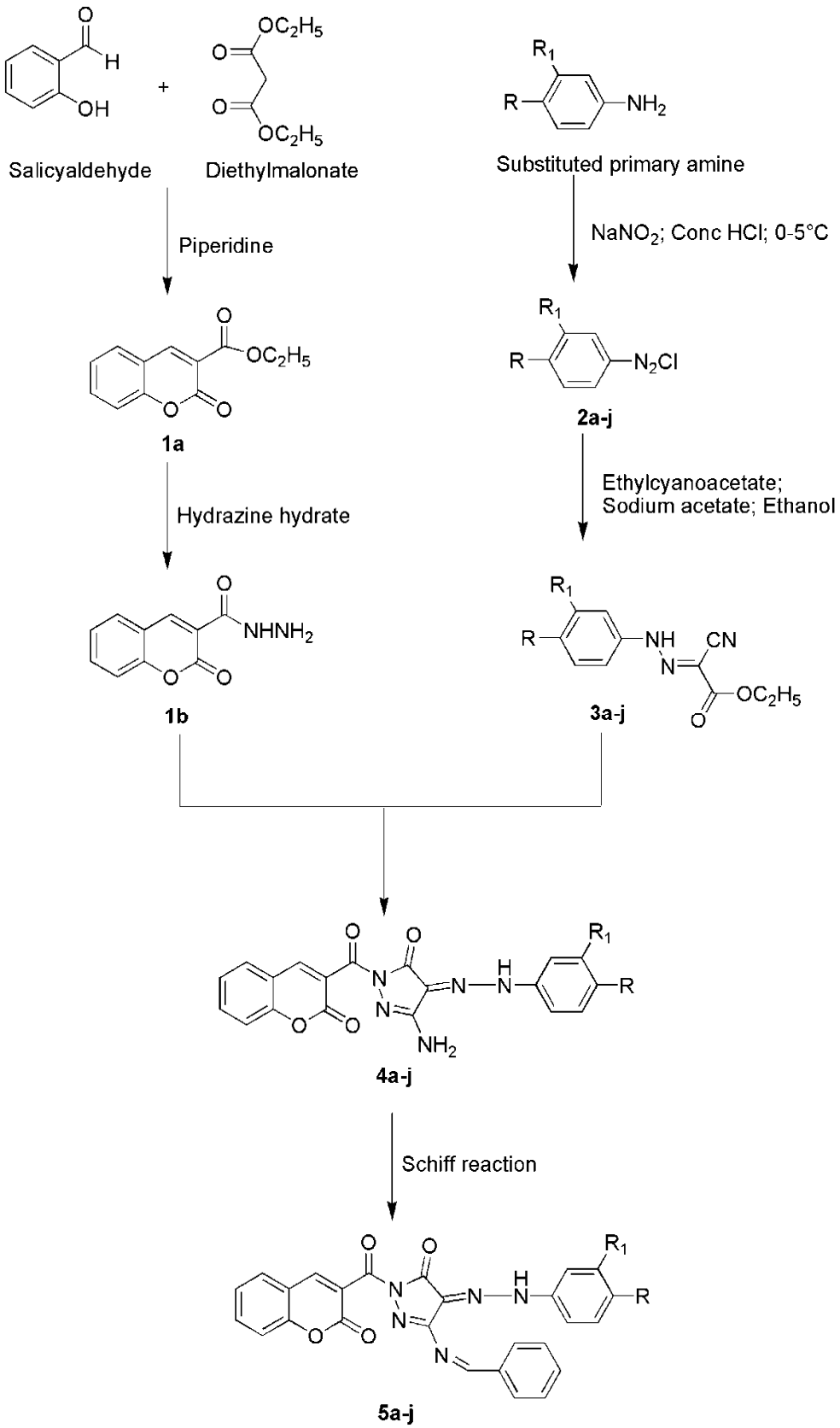
Among o-substituted phenyl derivatives and o,p-disubstituted phenyl derivatives of pyrazolones, highest analgesic activity was observed with substituent possessing highest lipophilicity (i.e., dichloro compound **5i**).

Antimicrobial activity

All the synthesized compounds (**4a-j** and **5a-j**) were screened for their antimicrobial activity against two gram-positive, two gram negative bacterial strains and two fungal strains by disc diffusion method and the results are presented in Tables **IV** and **V**.*In vitro* antibacterial activity results revealed that compounds **4f** and **4g** demonstrated 1.06 times more potent anti bacterial activity aganist*E coil* and *K. pneumoniae*and corresponding Schiff base compounds **5d** and **5e**demonstrated 1.00 and 1.06 times more potent anti bacterial activity aganist*E coil.* Compounds **4a**, 4**b** and **4c** display significant anti-fungal activity aganist*C. albicans*and *A. niger*.Monosubstituted Schiff base compounds (**5a**, **5b**, **5c**, **5d**, **5e**, **5f** and **5g**) showed significant antibacterial activity aganist*S.aureus*with percentage of inhibition range between 63-83%. Compounds (5h, 5i and 5j) display antifungal activity aganist*C. albicans*with zone of inhibition 71, 95 and 95 %, respectively. However all other synthesized compounds demonstrated mild antifungal activity aganist*C. albicans*with zone of inhibition range between 48-57 %. Compounds **4f** and **4g** showed better Minimum Inhibitory Concentration among the synthesized compound in the series **4a-j**aganist the tested bacterial strains. MIC against *C. albicans*and *A.niger*compounds **4a. 4b** and **4c** showed enhanced MIC antifungal activity with 14.95, 13.75 and 15.89µM/mLx10^{-3}. All the synthesized in the Schiff base pyrazolone derivatives (5a-j) devoid antifungal activity against *A.niger* except compound 5e which showing 61% zone of inhibition compared to Clotrimazole.

CONCLUSION

In the present paper, we report the synthesis, spectral studies, evaluation of antimicrobial and analgesic activity of 3-amino-4-[2-(substituted phenyl) hydrazin-1-ylidene]-1-[(2-oxo-2H-chromen-3-yl) carbonyl]-4, 5-dihydro-1H-pyrazol-5-one (4a-j) and its schiff bases (**5a-j).***In vitro* antibacterial activity results revealed that compounds **4f** and **4g** demonstrated 1.06 times more potent anti bacterial activity aganist*E coil* and *K. pneumoniae and* showed better Minimum Inhibitory Concentration among the synthesized compound in the series **4a-j**aganist the tested bacterial strains. Compound3-(benzylamino)-4-[2-(3,4-dichlorophenyl)hydrazin-1-ylidene]-1-[(2-oxo-2H-chromen-3-yl)carbonyl]-4,5-di hydro-1H-pyrazol-5-one (**5i**) demonstrate 1.04 times more potent analgesic activity than Indomethacin. Thus these compounds constitute an interesting template for the evaluation of new antimicrobial and analgesic inhibitors. In conclusion, we reported herein a simple and convenient route for the synthesis of some new heterocyclic based on pyrazolinone for antimicrobial evaluation and analgesic activity.



Scheme. 1: Synthesis of Schiff base pyrazolone derivatives

Table 1: Physicochemical data of synthesized compounds (4a-j and 5a-j)

Cpd Code	R	R1	Molecular formula	Molecular weight	R_f value	Melting point	% yield	Log P	Polarizability	Refractivity
4a	Cl	H	$C_{19}H_{12}Cl\ N_5O_4$	409.78	0.865	195	65	3.22	38.91	105.10
4b	Br	H	$C_{19}H_{12}BrN_5O_4$	454.23	0.765	232	68	3.39	39.88	107.91
4c	F	H	$C_{19}H_{12}FN_5O_4$	393.33	0.812	215	62	2.76	36.74	100.51
4d	NO_2	H	$C_{19}H_{12}N_6O_6$	419.34	0.723	240	54	2.56	38.98	107.62
3e	COOH	H	$C_{20}H_{13}N_5O_6$	417.36	0.890	210	60	2.28	39.42	107.55
4f	CH_3	H	$C_{20}H_{15}N_5O_4$	387.37	0.912	270	70	3.13	38.75	105.33
4g	OCH_3	H	$C_{20}H_{15}N_5O_5$	403.37	0.832	215	71	2.46	39.54	106.75
4h	NO_2	NO_2	$C_{19}H_{11}N_7O_8$	463.34	0.687	224	55	2.50	41.02	114.94
4i	Cl	Cl	$C_{19}H_{11}Cl_2N_5O_4$	442.24	0.824	232	60	3.83	40.88	109.90
4j	Br	Br	$C_{19}H_{11}Br_2N_5O_4$	531.13	0.824	230	62	4.16	42.88	155.54
5a	Cl	H	$C_{26}H_{16}Cl\ N_5O_4$	497.89	0.784	245	55	5.50	49.85	134.77
5b	Br	H	$C_{26}H_{16}Br\ N_5O_4$	542.34	0.635	232	60	5.66	50.76	137.59
5c	F	H	$C_{26}H_{16}F\ N_5O_4$	481.43	0.854	221	58	5.04	47.66	130.18
5d	NO_2	H	$C_{26}H_{16}\ N_6O_6$	508.44	0.752	185	62	4.83	49.89	137.29
5e	COOH	H	$C_{27}H_{17}\ N_5O_6$	507.45	0.812	275	65	4.55	50.34	140.37
5f	CH_3	H	$C_{27}H_{19}\ N_5O_4$	477.47	0.755	245	58	5.41	49.73	135.01
5g	OCH_3	H	$C_{27}H_{19}\ N_5O_5$	493.47	0.881	210	65	4.74	50.49	136.43
5h	NO_2	NO_2	$C_{26}H_{15}\ N_7O_8$	553.44	0.843	187	61	4.77	51.88	144.62
5i	Cl	Cl	$C_{19}H_{11}Cl_2N_5O_4$	532.33	0.755	165	62	6.10	51.79	139.58
5j	Br	Br	$C_{19}H_{11}Br_2N_5O_4$	621.24	0.874	214	64	6.43	53.67	145.21

Table II: Characterization of the synthesized compounds (4a-j)

Cpd Code	Elemental analysis % calculated/ found			IR (cm^{-1})	^{1}H-NMR
	C	H	N		
4a	58.72/ 58.20	3.20/ 3.40	13.70/ 13.60	3434 (NH), 3312 (-NH_2),1720 (C=O, lactone), 1655 (C=O in Pyrazolone ring), 1640 (C=N), 826 (CH=CH str., Aromatic), 807 (C-Cl)	δ 8.51(1H, Ar CH of CH=C in coumarin ring), 6.89-7.29(4H, Ar-CH of coumarin), 6.22 -6.53(4H, Ar-CH of substituted phenyl),7.03 (1H, NH), 1.89 (2H, NH_2).
4b	52.95/ 53.02	2.88/ 2.60	12.36/ 12.40	3426 (NH),), 3318 (-NH_2),1717 (C=O, lactone), 1663 (C=O in Pyrazolone ring), 1634 (C=N), 917 (-NH_2),815 (CH=CH str., Aromatic), 612 (C-Br)	δ 8.49(1H, Ar CH of CH=C in coumarin ring), 6.91-7.31(4H, Ar-CH of coumarin), 6.20 -6.49(4H, Ar-CH of substituted phenyl),7.11 (1H, NH), 2.04 (2H, NH_2).
4c	61.22/ 60.02	3.33/ 3.15	14.28/ 13.98	3432 (NH),), 3313 (-NH_2), 1722 (C=O, lactone), 1659 (C=O in Pyrazolone ring), 1636 (C=N), 1056 (C-F), 910 (-NH_2),	δ 8.51(1H, Ar CH of CH=C in coumarin ring), 6.89-7.29(4H, Ar-CH of coumarin), 6.22 -6.53(4H, Ar-CH of substituted phenyl),7.05 (1H, NH), 1.89 (2H, NH_2).
4d	64.88/ 62.80	4.15/ 4.02	14.42/ 14.02	3449 (NH),), 3319 (-NH_2), 1715 (C=O, lactone), 1661 (C=O in Pyrazolone ring), 1640 (C=N), 1510 (C-NO_2 str., aromatic), 814 (CH=CH str., Aromatic), 909 (-NH_2)	δ 8.54(1H, Ar CH of CH=C in coumarin ring), 6.85-7.25(4H, Ar-CH of coumarin), 6.19 -6.43(4H, Ar-CH of substituted phenyl),6.93 (1H, NH), 1.94 (2H, NH_2).
4e	62.37/ 58.26	3.98/ 3.60	13.85/ 13.60	3457 (NH),), 3322 (-NH_2), 3032(COOH), 1719 (C=O, lactone), 1649(C=O in Pyrazolone ring), 1629 (C=N), 915 (-NH_2), 819 (CH=CH str., Aromatic)	δ 11.86 1H, OH of carboxylic acid), 8.45(1H, Ar CH of CH=C in coumarin ring), 6.95-7.32(4H, Ar-CH of coumarin), 6. 17 -6.51(4H, Ar-CH of substituted phenyl),7.09 (1H, NH), 2.11 (2H, NH_2).
4f	57.28/ 58.02	3.12/ 3.06	16.70/ 16.75	3439 (NH),), 3322 (-NH_2),1725 (C=O, lactone), 1651 (C=O in Pyrazolone ring), 1640 (C=N), 914 (-NH_2), 831 (CH=CH str., Aromatic),	δ 8.36(1H, Ar CH of CH=C in coumarin ring), 6.79-7.19(4H, Ar-CH of coumarin), 6.21 -6.43(4H, Ar-CH of substituted phenyl),6.98 (1H, NH), 2.67 (3H, CH_3), 1.75(H, NH_2).
4g	59.26/ 58.45	3.73/ 3.23	17.28/ 17.10	3445 (NH),), 3320 (-NH_2), 1728 (C=O, lactone), 1659 (C=O), 1635 (C=N), 914 (-NH_2), 816 (CH=CH str., Aromatic),	δ 8.55(1H, Ar CH of CH=C in coumarin ring), 6.75-7.21(4H, Ar-CH of coumarin), 6.21 -6.52(4H, Ar-CH of substituted phenyl),7.10 (1H, NH), 3.65 (3H, OCH_3), 2.12 (2H, NH_2).
4g	51.73/ 50.92	2.79/ 2.08	18.09/ 17.90	3452 (NH),), 3317 (-NH_2),1726 (C=O, lactone), 1660 (C=O in Pyrazolone ring), 1632 (C=N), 919 (-NH_2), 819 (CH=CH str., Aromatic),	δ 8.43(1H, Ar CH of CH=C in coumarin ring), 6.88-7.14(4H, Ar-CH of coumarin), 6.12 -6.43(3H, Ar-CH of substituted phenyl),7.11 (1H, NH), 1.95(2H, NH_2).
4h	54.19/ 53.90	2.72/ 2.60	12.64/ 12.06	3432 (NH),), 3322 (-NH_2),1724 (C=O, lactone), 1645 (C=O in Pyrazolone ring), 1641 (C=N), 1516 (C-NO_2 str., aromatic), 926 (-NH_2), 812(CH=CH str., Aromatic)	δ 8.59(1H, Ar CH of CH=C in coumarin ring), 6.92-7.19(4H, Ar-CH of coumarin), 6.20 -6.55(3H, Ar-CH of substituted phenyl),6.92 (1H, NH), 1.99 (2H, NH_2).
4i	45.14/ 45.06	2.27/ 2.30	10.52/ 10.20	3439 (NH),), 3318 (-NH_2),1710 (C=O, lactone), 1652 (C=O in Pyrazolone ring), 1637 (C=N), 914 (-NH_2), 821 (C-Cl)	δ 8.43(1H, Ar CH of CH=C), 6.99-7.36(4H, Ar-CH of coumarin), 6.30 -6.63(3H, Ar-CH of substituted phenyl),7.13 (1H, NH), 2.08 (2H, NH_2).

4j	42.80/ 42.61	2.08/ 1.98	13.14/ 12.89	3447 (NH),), 3317 (-NH_2),1722 (C=O, lactone), 1639 (C=O in Pyrazolone ring), 1640 (C=N), 915 (-NH_2), 816 (CH=CH str., Aromatic),626 (C-Br)	δ 8.50(1H, Ar CH of CH=C in coumarin ring), 6.84-7.21(4H, Ar-CH of coumarin), 6.20 -6.55(3H, Ar-CH of substituted phenyl),7.01 (1H, NH), 1.92 (2H, NH_2).

Table III: Characterization of the synthesized compounds (5a-j)

Cpd Code	Elemental analysis % calculated/Found			IR (cm^{-1})	^{1}H-NMR
	C	H	N		
5a	62.72/ 62.54	3.24/ 3.10	14.07/ 13.98	3444 (NH), 1717 (C=O, lactone), 1657(C=O in Pyrazolone ring), 1639 (C=N), 26 (CH=CH str., Aromatic), 807 (C-Cl).	δ 8.65(1H, Ar CH of CH=C in coumarin ring), 8.14(1H, HC=N- of Schiff base), 7.25-7.70 (5H, Ar-CH of Schiff base phenyl), 6.96-7.14 (4H, Ar-CH of coumarin), 6.35-6.60 (4H, Ar-CH of substituted phenyl).
5b	57.58/ 57.42	2.97/ 2.89	12.91/ 12.85	3428 (NH), 1715 (C=O, lactone), 1663 (C=O in Pyrazolone ring), 1634 (C=N), 815 (CH=CH str., Aromatic), 547 (C-Br)	δ 8.49(1H, Ar CH of CH=C in coumarin ring), 8.09(1H, HC=N- of Schiff base), 7.28-7.68 (5H, Ar-CH of Schiff base phenyl), 6.82-7.22(4H, Ar-CH of coumarin), 6.21 -6.49(4H, Ar-CH of substituted phenyl).
5c	64.86/ 64.72	3.35/ 3.24	14.55/ 14.63	3432 (NH), 1722 (C=O, lactone), 1659 (C=O in Pyrazolone ring), 1636 (C=N), 1007 (C-F).	δ 8.48(1H, Ar CH of CH=C in coumarin ring), 8.17(1H, HC=N- of Schiff base), 7.17-7.67 (5H, Ar-CH of Schiff base phenyl), 6.79-7.21(4H, Ar-CH of coumarin), 6.19 -6.43(4H, Ar-CH of substituted phenyl).
5d	61.42/ 61.34	3.17/ 3.09	16.53/ 16.12	3449 (NH), 1725 (C=O, lactone), 1646 (C=O in Pyrazolone ring), 1639 (C=N), 1512 (C-NO_2 str., aromatic), 811 (CH=CH str., Aromatic).	δ 8.54(1H, Ar CH of CH=C), 8.12(1H, HC=N- of Schiff base), 7.22-7.69 (5H, Ar-CH of Schiff base phenyl), 6.75-7.25(4H, Ar-CH of coumarin), 6.19 -6.43(4H, Ar-CH of substituted phenyl).
5e	63.91/ 63.82	3.38/ 3.28	13.80/ 13.69	3456 (NH), 3021 (COOH), 1728(C=O, lactone), 1658(C=O in Pyrazolone ring), 1619 (C=N), 821 (CH=CH str., Aromatic)	δ 12.36 1H, OH of carboxylic acid), 8.59(1H, Ar CH of CH=C in coumarin ring), 8.19(1H, HC=N- of Schiff base), 7.26-7.68 (5H, Ar-CH of Schiff base phenyl), 6.55-7.08(4H, Ar-CH of coumarin), 6.14 -6.38(4H, Ar-CH of substituted phenyl).
5f	67.92/ 67.71	4.01/ 3.89	14.67/ 14.52	3435 (NH), 1722 (C=O, lactone), 1658 (C=O in Pyrazolone ring), 1637 (C=N), 911 (-NH_2), 833 (CH=CH str., Aromatic).	δ 8.26(1H, Ar CH of CH=C), 8.15(1H, HC=N- of Schiff base), 7.18-7.70 (5H, Ar-CH of Schiff base phenyl), 6.79-7.09(4H, Ar-CH of coumarin), 6.19 -6.45(4H, Ar-CH of substituted phenyl),2.67 (3H, CH_3).
5g	65.72/ 65.50	3.88/ 3.62	14.19/ 14.02	3441 (NH), 1721 (C=O, lactone), 1652(C=O in Pyrazolone ring), 1636 (C=N), 815 (CH=CH str., Aromatic).	δ 8.34(1H, Ar CH of CH=C in coumarin ring), 8.15(1H, HC=N- of Schiff base), 7.15-7.65 (5H, Ar-CH of Schiff base phenyl), 6.85-7.09(4H, Ar-CH of coumarin), 6.22 -6.62(4H, Ar-CH of substituted phenyl),3.45 (3H, OCH_3).
5h	56.43/ 56.31	2.73/ 2.56	17.72/ 17.56	3432 (NH), 1724 (C=O, lactone), 1645 (C=O in Pyrazolone ring), 1641 (C=N), 1516 (C-NO_2 str., aromatic),812(CH=CH str., Aromatic).	δ 8.41(1H, Ar CH of CH=C in coumarin ring), 8.15(1H, HC=N- of Schiff base), 7.24-7.68 (5H, Ar-CH of Schiff base phenyl), 6.76-7.12(4H, Ar-CH of coumarin), 6.18 -6.46(3H, Ar-CH of substituted phenyl).
5i	58.66/ 5.42	2.84/ 2.62	13.16/ 13.00	3436 (NH), 1710 (C=O, lactone), 1656 (C=O in Pyrazolone ring), 1622(C=N), 819 (C-Cl).	δ 8.55(1H, Ar CH of CH=C in coumarin ring), 8.08(1H, HC=N- of Schiff base), 7.15-7.72 (5H, Ar-CH of Schiff base phenyl), 6.82-7.09(4H, Ar-CH of coumarin), 6.22 -6.59(3H, Ar-CH of substituted phenyl).
5j	50.27 / 49.84	2.43/ 2.22	11.27/ 11.02	3452 (NH), 1724(C=O, lactone), 1653 (C=O in Pyrazolone ring), 1635 (C=N), 816 (CH=CH str., Aromatic), 622 (C-Br).	δ 8.33(1H, Ar CH of CH=C in coumarin ring), 8.22(1H, HC=N- of Schiff base), 7.15-7.75 (5H, Ar-CH of Schiff base phenyl), 6.82-7.16(4H, Ar-CH of coumarin), 6.28 -6.66(3H, Ar-CH of substituted phenyl).

Table IV: *In-vivo* analgesic activity of synthesized compounds by acetic acid induced writhing method in mice

Cpd code	Dose (0.03 mM/Kg) qty in mg/kg	Writhing reflex (mean ± SEM)	Inhibition %	Potency %
4a	12.26	31±41*	53.73	78.06
4b	13.60	29±19**	56.72	82.41
4c	11.77	32±22*	52.24	75.90
4d	12.58	44±23**	34.32	49.86
3e	12.55	46±31***	31.34	45.53
4f	11.65	22±37*	67.16	97.57
4g	12.13	24±14*	64.18	93.24
4h	13.93	30±33**	55.22	80.23
4i	13.30	58±21**	13.43	19.51
4j	15.96	52±35*	22.38	32.51
5a	14.94	27±11	59.70	86.74
5b	16.27	25±32	62.68	91.06
5c	14.44	28±24	58.21	84.46
5d	1525	48±31	28.36	41.20
5e	15.22	50±14	25.37	36.86
5f	14.32	35±12	47.76	69.39
5g	14.80	32±08	52.24	75.90
5h	16.60	34±16	49.25	71.55
5i	15.97	19±21	71.64	104.08
5j	18.64	23±16	65.67	95.40
Control	-	67±33*	-	-
Indomethacin	10.34	20±51**	68.83	100

The result are expressed as mean ± SEM (n=5). Significance was calculated by using one-way ANOVA with Dunnet`s t-test. The different in result was considered significant When $p < 0.05$, *$p < 0.05$ vs control at .03mmol/kg, **$p < 0.01$ vs control at .03mmol/kg; ***$p < 0.001$ vs control at 0.03mmol/kg.

Table V: *In-vitro* antimicrobial activity of synthesized compounds (4a-j)

Cpd code	Zone of inhibition in mm (ZI), Percentage of inhibition (%) & Minimum Inhibition Concentration (MIC=µM/mLx10^{-3})																	
	M.luteus			*S.aureus*			*E. coli*			*K. pneumoniae*			*C. albicans*			*A.niger*		
	ZI in mm	(%)	MIC	ZI in mm	(%)	MIC	ZI in mm	(%)	MIC	ZI in mm	(%)	MIC	ZI in mm	(%)	MIC	ZI in mm	(%)	MIC
4a	-	-	NT	-	-	NT	16	94	29.89	-	-	NT	17	81	14.95	15	83	14.95
4b	-	-	NT	-	-	NT	18	106	13.75	-	-	NT	20	95	13.75	18	100	13.75
4c	-	-	NT	-	-	NT	12	71	31.77	-	-	NT	22	105	15.89	19	106	15.89
4d	12	48	59.61	11	49	29.81	10	59	29.81	12	71	29.81	10	48	14.91	-	-	NT
3e	14	56	29.95	14	58	29.51	13	76	29.51	14	82	14.76	12	57	14.76	-	-	NT
4f	20	80	16.13	18	75	16.13	18	106	8.07	18	106	8.07	-	-	NT	-	-	NT
4g	24	96	15.49	20	83	15.49	18	106	15.49	18	106	7.75	-	-	NT	-	-	NT
4h	-	-	NT	-	-	NT	10	59	53.96	12	71	53.96	-	-	NT	10	56	107.91
4i	-	-	NT	-	-	NT	10	59	28.26	12	71	28.26	14	67	28.26	-	-	NT
4j	-	-	NT	-	-	NT	09	53	23.53	13	76	23.53	-	-	NT	-	-	NT
CPN	25	100	0.59	24	100	9.43	17	100	04.72	17	100	0.59	-	-	-	-	-	-
CTZ	-	-	-	-	-	-	-	-	-	-	-	-	21	100	12.5	18	100	25.5
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

MIC -Minimum Inhibitory Concentration; - Indicate No inhibition; NT- Not Test; CFN-Ciprofloxacin; CTZ-Clo-trimazole; DMSO-Dimethyl sulphoxide

Table VI: *In vitro* antimicrobial activity of synthesized compounds (5a-j)

Cpd code	Zone of inhibition in mm (ZI), Percentage of inhibition (%) & Minimum Inhibition Concentration (MIC=µM/mLx10^{-2})																	
	M.luteus			*S.aureus*			*E. coli*			*K. pneumoniae*			*C. albicans*			*A.niger*		
	ZI in mm	(%)	MIC	ZI in mm	(%)	MIC	ZI in mm	(%)	MIC	ZI in mm	(%)	MIC	ZI in mm	(%)	MIC	ZI in mm	(%)	MIC
5a	10	40	50.21	22	92	25.11	13	76	50.21	-	-	NT	12	57	50.21	-	-	NT
5b	11	44	46.09	20	83	23.05	15	88	23.05	-	-	NT	11	52	46.09	-	-	NT
5c	10	40	51.92	18	75	25.96	10	59	25.96	-	-	NT	11	52	25.96	-	-	NT
5d	16	64	24.58	18	75	12.29	17	100	12.29	10	59	12.29	11	52	12.29	-	-	NT
5e	14	56	24.63	15	63	24.63	18	106	24.63	12	71	24.63	10	48	12.31	11	61	12.31
5f	-	-	NT	20	83	52.35	10	59	52.35	10	59	52.35	16	76	26.18	-	-	NT
5g	-	-	NT	20	83	50.66	10	59	25.33	10	59	25.33	12	57	25.33	-	-	NT
5h	-	-	NT	10	42	90.34	-	-	NT	-	-	NT	15	71	90.34	-	-	NT
5i	-	-	NT	10	42	93.93	-	-	NT	-	-	NT	20	95	93.93	-	-	NT
5j	-	-	NT	12	50	80.48	-	-	NT	-	-	NT	20	95	80.48	-	-	NT
CFN	25	100	0.59	24	100	9.43	17	100	04.72	17	100	0.59	-	-	-	-	-	-
CTZ	-	-	-	-	-	-	-	-	-	-	-	-	21	100	12.5	18	100	25.5
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

MIC -Minimum Inhibitory Concentration; - Indicate No inhibition; NT- Not Test; CFN-Ciprofloxacin; CTZ-Clo-trimazole; DMSO- Dimethyl sulphoxide

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