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"SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME PYRAZOLONE DERIVATIVES"

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Abstract: A series of twelve biologically active pyrazolone derivatives **(PA1-PA6 & PC1-PC6)** were synthesized by a condensation of 4-acyl-5-pyrazolones with aromatic amines, and the aromatic aldehydes added to the parent moiety. All the newly synthesized compounds were characterized by means of their FTIR, ^{1}H-NMR, MASS spectral data. All the compounds were tested for their antibacterial and antifungal activities by the agar well diffusion method. The Schiff base containing pyarazolones were produced significant activity in the antimicrobial properties against both gram positive and gram-negative bacterial strains at a 100 µg/ml concentration against standard Gentamycin (100 µg/ml).

Key Words: Pyrazolone, Schiff base, Benzylidene, Antibacterial activity, Antifungal activity.

INTRODUCTION

Among various heterocyclic frameworks, the science of five membered heterocyclic with in excess of one heteroatom has picked up significance the same number of the them display articulated bioactive nature. Pyrazolone and its derivatives are imperative class of heterocyclic compounds. Five-membered ring comprising of three carbon particles and to two nitrogen atoms neighboring each other is called pyrazole a carbonyl gathering at position C5 is known as pyrazole 5-one framework. They are very fascinating format for the scientists and medicinal chemistry due their simple preparation, wide biological activities which include antibacterial[1] antifungal, analgesic, antipyretic, anti-inflammatory,[2-10] antitubercular, antioxidant and antitumour activity[11] they have antiviral activity too[12]. Schiff bases containing the C=N group are known to have slight antitumour activities more of these compounds have been synthesized in order to find compounds with greater antitumour activity. Schiff bases and their complexes are versatile compounds synthesized from the condensation of an amino compound with carbonyl compounds and widely used for industrial purposes and also exhibit a broad range of biological activities including antifungal, antibacterial, antimalarial, an tiproliferative.[13]

In the view of above particulars and taking in to account a modification of the

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pyrazolone nucleus can bring significant changes in pharmacological activities and can afford new classes of therapeutically active moieties. In this current study the antimicrobial activity has been studied in detail. The aryl azopyrazolone derivative activities has been studied here. The aryl azo pyrazolones are prepared by treating ethyl aceto-derivatives with hydrazine derivatives. Along with this the benzylidene linked pyrazolone derivatives are also tried to synthesize. The newly synthesized pyrazolone derivatives (**PA1-PA6 & PC1-PC6**) are studied for its biological as well as insilico activity.

EXPERIMENTAL

Melting point were determined in open capillary tubes and were uncorrected. The IR spectra were recorded in KBr pellet on SHIMADZU FT-IR. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO with TMS as internal standard on a Bruker spectrometer at 400 MHz and 100 MHz, respectively. LC-MS. Purity of the compound was checked by TLC on silica gel plates and spots were visualized by iodine chamber using universal solvent system. Antimicrobial activity was evaluated using cup plate method.

Drugs and Chemicals: Phenyl hydrazine, Aceto acetic ester, Hydrochloric acid, Sodium hydroxide, Ethanol, Benzaldehyde, p-chloro benzaldehyde, o-nitro benzaldehyde, p-hydroxy benzaldehyde, p-methyl benzaldehyde, Dimethylamino benzaldehyde, Aniline, 2,4-dinitro aniline, Sulphanilic acid, Sulphaniliamide, Methanol, Benzoyl chloride. (Sigma Aldrich, Otto, CDH). All the prototypes were dissolved in DMSO and volume adjusted to 10ml for making concentration to 100 µg/ml.

Standard drug: Gentamycin and Ketoconazole were taken as reference standards and the concentration of standard drugs were prepared in DMSO to give 100 µg/ml.

CHEMICAL SYNTHESIS

General procedure for synthesis of 3-methyl-1-phenyl-5-pyrazolone

3-methyl-1-phenyl-5-pyrazolone (PMP) (**1**) was prepared according to a reported method. Pure ethyl acetoacetate (0.1 mol) was mixed with pure phenyl hydrazine (0.1 mol), followed by addition of 0.5ml of acetic acid and then heated on a boiling water bath for one hour with occasional stirring. The thick syrup was allowed to cool and 15-20 mL of ether was added and stirred the mixture vigorously to get crystalline pyrazolone. The product was filtered and the solid material washed thoroughly with ether and then recrystallized from a small quantity of equimolar ethanol and water mixture or ethanol. The methyl phenyl pyrazolone obtained was colourless, melting point 128˚C and yield was calculated as 85%[14].

General procedure for the synthesis of 4-benzoyl-5-pyrazolones

The 4-benzoyl-5-pyrazolones (**2**) were prepared by reported methods. Corresponding benzoyl chloride (0.1 mol) was added to well stirred mixture of 3-methyl-1-phenyl-5-pyrazolone (**1**) dissolved in 60-80 mL of dioxane, added with 12 g calcium hydroxide at room temperature. Temperature increases for the first 5 minutes due to the exothermic nature of the reaction and result in a thick paste texture. After the complete addition, the resultant mixture was heated to reflux for 30 min. Then the resultant mass obtained was then acidified by ice cold dilute hydrochloric acid (2N, 200ml), followed by continuous stirring to get cream colour crystals.[15]

The synthesis of Schiff bases **(PA1-PA6)**
A mixture of equimolar ethanolic hot solution (50 mL) of respective amine derivatives with corresponding benzoyl pyrazolones (0.1 mol) in the presence of a few drops of acetic acid. Refluxed for 3 hours, the reaction was monitored by TLC. The resultant mixture was cooled and poured into ice cold water and allowed to stand for overnight to separate out the coloured crystalline product (**PA1-PA6**). The product obtained with good yield with purity. It was washed with ethanol followed by drying.[16]

General procedure of preparation of 4-benzylidene-3-methyl-1-phenyl-5-pyrazolone derivatives (PC1-PC6)

Equimolar amounts of 3-methyl-1-phenyl-5-pyrazolone (**1**) and different aromatic aldehydes, in freshly prepared 20 % ethanolic NaOH solution for 8 hours and upon completion of the reaction was monitored by T.L.C. Later reaction mixtures were transferred into crushed ice and neutralized with dil. HCl to precipitate the product and has kept in freezer overnight (Scheme 1). It was then filtered and purified by recrystallization to get the pure products (**PC1-PC6**) [17]. the physiochemical and the spectral data were depicted **Table 2 & 3.**

BIOLOGICAL SCREENING

Antibacterial activities

Antibacterial activities of all compounds were studied against gram positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli* at a concentration of 100µg/ml. DMSO was used as control in the method. Under similar conditions using gentamycin as standard for comparison. A nutrient agar cup was prepared by dissolving a weighed ingredient in water and incubated at 35-37ºC for 24 h. Each sterile nutrient agar plate was then flooded with the corresponding peptone culture of the test organism, dried for 30 minutes and after drying of the flooded plate, wells were made using a cork borer (size-3) on the solidified medium. Prepared wells were filled with dilution of 100µg/ml of respective test and standard compounds. All the flooded plates were incubated at 35-37 ºC for 24 hours for bacterial strains and 72 hours old cultures for fungal strain. The diameters of the zones of inhibition were measured in mm and their means were compared accordingly [18]. Gentamycin and Ketoconazole were used as standard antibacterial and antifungal drugs, respectively. The Solvent was screened for the activity. The antibacterial screening results were presented in **Table 3.**

RESULTS AND DISCUSSION

The antimicrobial screening of test compounds (**PA1-PA6**) exhibited good activity compared to that of standard. All the compounds found to be active against both gram positive and gram-negative strains used in this *in vitro* bioassay at a concentration range of 100 µg/ml. From the activity in table 3, it shows that the tested compounds **PA1**, **PC2**, **PA4** & **PA6** shows significant activity against both strains *Bacillus subtilis* and *Escherichia coli.* In fact, these compounds exhibited equipotent activity at a 100 µg/ml concentration as that of standard gentamycin (100 µg/ml). However, the other set of compounds have shown moderate activity against the both strains at the same concentration. The compound having the pyrazolone as well as the electronegative group possess the significant activity against both strains of bacteria.

It was noticed that the Schiff base substituted pyrazolone nucleus (**PA1-**

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PA6) were found to possess comparatively equipotent activity than the benzylidene linked with pyrazolone (**PC1-PC6**) due to the presence of the N=C linkage with the parent moiety. While the antifungal activity results have shown that the compound PA1 and PA6 were having moderate activity against *Candida albicans* when compared to that of standard Ketoconazole at (100 µg/ml). Thus, from the structural examination, it concludes that the Schiff bases, particularly from the amine derived pyrazolone possess a good antibacterial and antifungal activities which may be due to the presence of electro negative groups likewise nitro and sulphamoyl moieties.

CONCLUSION

In this work all the synthesized compounds amine substituted pyrazolone's (**PA1-PA6**) and benzylidene substituted pyrazolone's (**PC1-PC6**) were found to be a significant action against both bacterial strains used in the *in vitro* bioassay at 100 µg/ml. In summary, Schiff base containing pyrazolone derivatives from amine derivatives have shown a promising antibacterial and antifungal activities. The presence of electro negative groups increases the effect as bacteriostatic or bactericidal action which may look up on further research of molecular pharmacology.

Table 1. Physio-chemical data of synthesized compounds (PA1-PA6 & PC1-PC6)

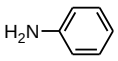
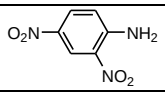
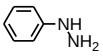
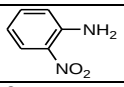
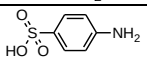
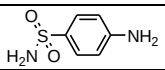
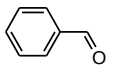
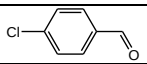
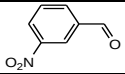
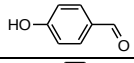
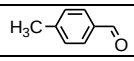
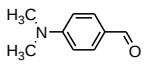
SL no:	Code	R (Substituent)	Molecular formula	Molecular weight	Melting point	Rf value	Colour
1	PA1		$C_{24}H_{21}N_3$	351.44	150-158	0.78	Pale yellow
2	PA2		$C_{24}H_{19}N_5O_4$	441.43	150-160	0.66	Pale yellow
3	PA3		$C_{24}H_{20}N_4O_2$	396.44	160-165	0.75	Pale yellow
4	PA4		$C_{24}H_{21}N_3O_3S$	431.50	80-100	0.51	Pale yellow
5	PA5		$C_{24}H_{22}N_4O_2S$	430.52	90-110	0.72	Yellow
6	PA6		$C_{24}H_{22}N_4$	366.45	190-196	0.70	Yellow
7	PC1		$C_{17}H_{14}N_2O$	262.312	180-196	0.68	Pale brown
8	PC2		$C_{17}H_{13}ClN_2O$	296.757	200-210	0.66	Brown
9	PC3		$C_{17}H_{13}N_3O_3$	307.309	196-200	0.58	Brown
10	PC4		$C_{17}H_{14}N_2O_2$	278.311	190-195	0.72	Brown
11	PC5		$C_{18}H_{16}N_2O$	276.339	120-126	0.71	Brown
12	PC6		$C_{19}H_{19}N_3O$	305.381	160-163	0.63	Reddish brown

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Table 1. Spectral data of synthesized compounds (PA1-PA6 & PC1-PC6)

Comp Code	FT-IR Infrared (KBr disc, cm-1)	^{1}H & ^{13}C NMR (400 MHz, CDCl3, δ ppm & 100 MHz, DMSO-d6, δ ppm)	ES-MASS*
PA1	1384 (Het. CH), 1485 (Aryl CH), 3283 (N-H Stretch), 3070(CH-Ar), 1428 (C-CH_3), 1735, 1676 (C=O), 1442 (C=N stretch).	^{1}H-NMR-δ ppm: 7.280-8.030 (m; 15H; Ar-H); 2.46 (s; 3H; CH_3) & 2.3-2.6 (s; 1 H; CH). ^{13}C-NMR-δ ppm: 132.30-153.48 (m; 18 C; Ar-C), 60.31 - 78.30 (s; 1 C; CH_3 & s; 1 C; CH), 190.0-195.2 (s; 1c; C=O), 40.0-65.2 (s; 1c; N=C), 160.0-165.2 (s; 1c; C=N).	351.44
PA2	1390 (Het. CH), 1475 (Aryl CH), 3273 (N-H Stretch), 3068(CH-Ar), 1418 (C-CH_3), 1670 (C=O), 1439 (C=N stretch).	^{1}H-NMR-δ ppm: 7.254-8.430 (m; 13H; Ar-H); 2.38 (s; 3H; CH_3) & 2.0-2.4 (s; 1H; CH) ^{13}C-NMR-δ ppm: 125.30-153.49 (m; 18 C; Ar-C), 71.31 - 79.30 (s; 1 C; CH_3 & s; 1 C; CH), 196.0-198.2 (s; 1c; C=O), 42.0-62.1 (s; 1c; N=C), 163.0-155.2 (s; 1c; C=N).	441.43
PA3	1392 (Het. CH), 1481 (Ar-CH), 3280 (N-H Stretch), 3072(CH-Ar), 1428 (C-CH_3), 1676 (C=O), 1602 (C=N stretch), 1080 C-N Stretch, 1386 (N=O)	^{1}H-NMR-δ ppm: 7.334-8.450 (m; 15H; Ar-H); 2.46 (s; 3H; CH_3), 2.3-2.6 (s; 1H; CH) & 4.3-4.6 (s; 1H; NH) ^{13}C-NMR-δ ppm: 128.30-153.50 (m; 18 C; Ar-C), 61.11 - 77.50 (s; 1 C; CH_3 & s; 1 C; CH), 191.0-194.2 (s; 1c; C=O), 40.0-65.2 (s; 1c; N=C), 162.1-165.4 (s; 1c; C=N).	396.44
PA4	1386 (Het. CH), 1479 (Ar-CH), 3279 (N-H Stretch), 3065(CH-Ar), 1438 (C-CH_3), 1679 (C=O), 1638 (C=N stretch), 1070 C-N Stretch, 1382 (N=O).	^{1}H-NMR-δ ppm: 6.584-8.530 (m; 14 H; Ar-H); 2.56 (s; 3H; CH_3) & 2.2-2.6 (s; 1H; CH) ^{13}C-NMR-δ ppm: 142.30-153.49 (m; 18 C; Ar-C), 60.31 - 78.30 (s; 1 C; CH_3 & s; 1 C; CH), 192.0-196.8 (s; 1c; C=O), 40.0-65.2 (s; 1c; N=C), 160.0-165.2 (s; 1c; C=N).	431.50
PA5	1395 (Het. CH), 1475 (Ar-CH), 3280 (N-H Stretch), 3075(CH-Ar), 1428 (C-CH_3), 1686 (C=O), 1642 (C=N stretch), 1082 C-N Stretch, 1396 (S=O).	^{1}H-NMR-δ ppm: 7.264-8.330 (m; 14 H; Ar-H); 2.40 (s; 3H; CH_3), 3.4-4.0 (s; 1H; OH) & 2.3-2.6 (s; 1H; CH.). ^{13}C-NMR-δ ppm: 132.30-153.48 (m; 18 C; Ar-C), 60.31 - 78.30 (s; 1 C; CH_3 & s; 1 C; CH), 190.0-195.2 (s; 1c; C=O), 40.0-65.2 (s; 1c; N=C), 160.0-165.2 (s; 1c; C=N).	430.52
PA6	1396 (Het. CH), 1485 (Ar-CH), 3278 (N-H Stretch), 3070 (CH-Ar), 1418 (C-CH_3), 1676 (C=O), 1638 (C=N stretch), 1080 C-N Stretch, 1406 (S=O).	^{1}H-NMR-δ ppm: 7.344-8.030 (m; 15 H; Ar-H); 2.39 (s; 3H; CH_3), 3.3-3.6 (s; 1H; OH)& 2.3-2.6 (s; 1H; CH.) ^{13}C-NMR-δ ppm: 141.50-152.48 (m; 18 C; Ar-C), 60.31 - 78.30 (s; 1 C; CH_3 & s; 1 C; CH), 190.0-195.2 (s; 1c; C=O), 42.0-63.2 (s; 1c; N=C), 159.0-163.5 (s; 1c; C=N).	366.45
PC1	1394 (Het. CH), 1585 (Ar-CH), 2150 (C=C Stretch), 3085(CH-Ar), 1425 (C-CH_3), 1656 (C=O), 1642	^{1}H-NMR-δ ppm: 7.264-8.027 (m; 10 H; Ar-H); 2.46 (s; 3H; CH_3), 2.3 – 3.3 (s; 1H; CH). ^{13}C-NMR-δ ppm: 132.30-153.48 (m; 15 C; Ar-C), 60.31 - 78.30 (s; 1 C; CH_3 & s; 1 C; CH).	262.312

	(C=N stretch), 1085 C-N Stretch.		
PC2	1385 (Het. CH), 1495 (Ar-CH), 2185 (C=C Stretch), 3076 (CH-Ar), 1458 (C-CH_3), 1666 (C=O), 1640 (C=N stretch), 1070 C-N Stretch, 796 (C-Cl stretch).	^{1}H-NMR-δ ppm: 7.668-8.330 (m; 9 H; Ar-H); 2.34 (s; 3H; CH_3), 2.3 – 5.3 (s; 1H; CH). ^{13}C-NMR-δ ppm: 122.30-153.48 (m; 15 C; Ar-C), 61.31 - 80.30 (s; 1 C; CH_3 & s; 1 C; CH).	296.757
PC3	1389 (Het. CH), 1485 (Ar-CH), 2135 (C=C Stretch), 3059(CH-Ar), 1418 (C-CH_3), 1686 (C=O), 1612 (C=N stretch), 1078 C-N Stretch.	^{1}H-NMR-δ ppm: 7.364-8.220 (m; 9 H; Ar-H); 2.38 (s; 3H; CH_3), 2.1 – 3.3 (s; 1H; CH) . ^{13}C-NMR-δ ppm: 142.30-153.58 (m; 15 C; Ar-C), 60.31 - 78.30 (s; 1 C; CH_3 & s; 1 C; CH).	307.309
PC4	3680 (OH stretch), 1392 (Het. CH), 1480 (Aryl CH), 2143 (C=C Stretch), 3060 (CH-Ar), 1410 (C-CH_3), 1710 (C=O), 1612 (C=N stretch), 1086 (C-N Stretch).	^{1}H-NMR-δ ppm: 7.384-8.210 (m; 9 H; Ar-H); 2.46 (s; 3H; CH_3), 3.0 – 3.3 (s; 1H; CH) & 2.3-2.6 (s; 1 H; OH). ^{13}C-NMR-δ ppm: 132.30-153.48 (m; 15 C; Ar-C), 61.21 - 76.30 (s; 1 C; CH_3 & s; 1 C; CH).	278.311
PC5	1380 (Het. CH), 1488 (Ar-CH), 2146 (C=C Stretch), 3072 (CH-Ar), 1420 (C-CH_3), 1686 (C=O), 1620 (C=N stretch), 1078 C-N Stretch.	^{1}H-NMR-δ ppm: 7.382-8.430 (m; 9 H; Ar-H); 2.46 (s; 6 H; CH_3), 3.1 – 3.3 (s; 1H; CH). ^{13}C-NMR-δ ppm: 138.80-163.48 (m; 15 C; Ar-C), 60.31 - 78.30 (s; 2 C; CH_3 & S; 1 C; CH).	276.339
PC6	1370 (Het. CH), 1481 (Ar-CH), 2145 (C=C Stretch), 3075 (CH-Ar), 1428 (C-CH_3), 1676 (C=O), 1602 (C=N stretch), 1068 (C-N Stretch).	^{1}H-NMR-δ ppm: 7.214-8.330 (m; 9 H; Ar-H); 2.46 (s; 9 H; CH_3), 2.3 – 3.3 (s; 1H; CH). ^{13}C-NMR-δ ppm: 135.36-163.48 (m; 15 C; Ar-C), 61.31 - 77.80 (s; 3 C; $(CH_3)_2$ & S; 1 C; CH).	305.381

***ES-MASS – Electro spray mass**

Table 3. Antibacterial and antifungal activities of compounds bold values indicate the highest value of the respective properties

Compound	Antibacterial activity		Antifungal activity
	***Bacillus subitilis* Gram +ve**	***Escherichia coli* Gram -ve**	***Candida albicans***
	Zone of inhibition (mm)	**Zone of inhibition (mm)**	**Zone of inhibition (mm)**
PA1	**21**	**22**	**10**
PA2	20	12	3
PA3	15	18	5

PA4	13	19	4
PA5	17	13	3
PA6	**23**	**26**	**11**
PC1	15	18	2
PC2	17	19	5
PC3	18	21	7
PC4	20	20	6
PC5	15	19	3
PC6	16	12	4
Gentamycin	23	25	-
Ketoconazole	-	-	13
Control	0	0	0

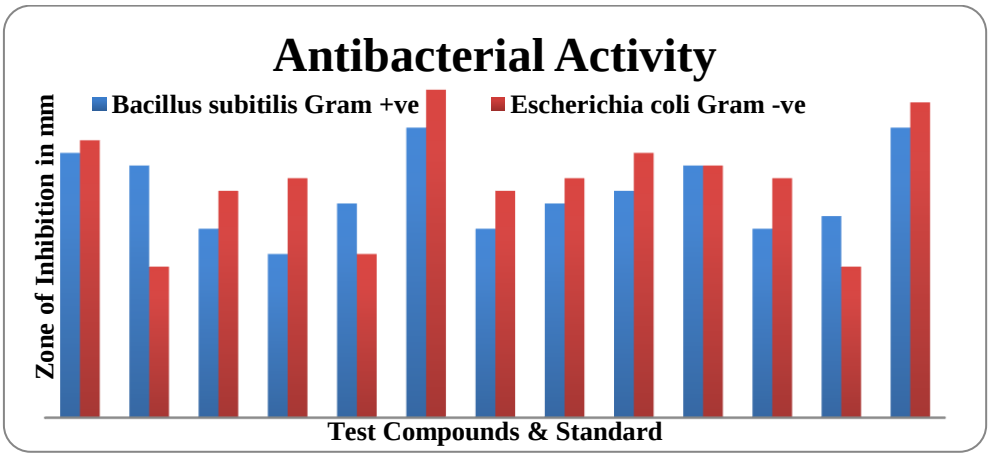

Figure: Anti-bacterial activities of the test compounds and standard against gram positive strains.

REFERENCES

1. Chaudhari, P. K. Preparaton and biological evaluation of 3-amino-4-aryl-4,5-dihydro -1-N-tolyl pyrazolo [3,4-d] pyrimidines derivative. 2012, Oriental Journal of Chemistry, 28, 507-512.
2. Gupta P, Gupta K J, Halve A K. Synthesis and biological significance of pyrazolones: a review. International Journal of Pharmaceutical Science and Research.2015; 6(6); 2291-2310
3. Usui Y, Matsumura C, Zasshi Ya.Synthesis and antifungal activity of halogen substituted phenyl pyrazolone derivatives. European PMC.1967; 87(1); 38-42.
4. Sathyanarayana K, Rao M N A. Synthesis of 4-[5-(substituted aryl)-4,5-dihydro-1*H*-pyrazol-3-yl]-3-phenyl-sydnones as antiinflammatory, antiarthritic and analgesic agents**.** European Journal of Medicinal Chemistry. 1995; 30(7-8); 641-645.

5. Antre V R, Cendilkumar A, Goli D, Andhale S G, Oswal R J.Microwave assisted synthesis of novel pyrazolone derivatives attached to a pyrimidine moiety and evaluation of their anti-inflammatory, analgesic and antipyretic activities**.**2011; 19(4); 233-243.
6. Mariappan G, Saha B P, Sutharson L, Singh A, Garg S, Pandey L, Deepak kumar. Analgesic, anti-inflammatory, antipyretic and toxicological evaluation of some newer 3-methyl pyrazolone derivatives. Saudi Pharmaceutical Journal. 2011; 19(2); 115-122.
7. Uramaru N, Shigematsu H, Toda A, Eyanagi R, Kitamura S, Ohta S.Design, Synthesis, and Pharmacological Activity of Nonallergenic Pyrazolone-Type Antipyretic Analgesics. Journal of medicinal chemistry 2010.53(24) 8727-8733.
8. Nassini R, Fusi C, Materazzi S, Coppi E, Preti D, Logu D F.*et,al.,*The TRPA1 channel mediates the analgesic action of dipyrone and pyrazolone derivatives. British Journal of Pharmacology.2015; 172; 3397-3411
9. Sivakumar K K, Rajasekaran A, Senthilkumar P, Wattamwar P P. Conventional and microwave assisted synthesis of pyrazolone mannich bases possessing anti-inflammatory, analgesic, ulcerogenic effect and antimicrobial properties. Bioorganic & Medicinal Chemistry Letters. 2014; 24(13); 2940-2944.
10. Khalil N A, Mohamed Ahmed E, Omar Mohamed K, Mohamed Nissan Y, Abo bakr Z S. Synthesis and biological evaluation of new pyrazolone-pyridiazine conjugates as anti-inflammatory and analgesic agents. Bioorganic & Medicinal Chemistry.2014; 22(7); 2080-2089.
11. Metwally M A, Suleiman A Y, Gouda M A, Harmal N A, Khalil A M. Synthesis, antitumour and antioxidant evaluation of some new antipyrine based azo dyes incorporating pyrazolone moiety. International Journal of Modern Organic Chemistry.2012; 1(3); 213-225.
12. Evstropov AN, Yavorovskaya VE, Vorob''ev ES, Khudonogova ZP, Medvedeva SG, Filimonov VD, Prishchep TP, Saratikov AS. ''Synthesis and antiviral activity of antipyrine derivatives'', Pharm. Chem. Jour. 1992, 26(5), 426-430.
13. M Ahmed, Abu-Dief, Ibrahim, Mohamed M A. A review on versatile applications of transition metal complexes incorporating Schiff bases.2015; 4(2); 119-133.
14. Mann F G, Saunders B C. Practical Organic Chemistry, J.Chem.Educ.,1937; 14(4); 200.
15. Jensen. The synthesis of 1-phenyl-3-methyl-4-acyl-pyrazolone-5. Acta Chemica Scandinavica. 1959; 13; 1668-1670.
16. Basaif AS thermal condensation of 1-aryl/hetaryl-3-methyl-2-pyrazolin-5-ones with aromatic aldehydes. Synthesis of 4-arylidene pyrazolones. JKAU;2008; 20(2); 93-100
17. Parmer N, Teraiya S, Patel R, Barad H, Jajda H, Thakkar V. Synthesis, antimicrobial and antioxidant activities of some 5-pyrazolone based Schiff bases. Journal of Saudi Chemical Society.2015; 19(1); 36-41.
18. Cruickshank R, Duguid J P, Marmion B P, Swain, R.H.A.Medical Microbiology, Churchill Livingstone, London, 1975; 2(12); 190.