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# Synthesis and Evaluation of Some Novel Pyrazole Derivatives for Anti-Inflammatory and Antimicrobial Activity

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**ABSTRACT:**

Pyrazole and their derivatives are found to have profound biological activity. In the present work some novel substituted Pyrazole derivatives were synthesized. Pyrazole are synthesized by treating ethyl bis [methyl thio] -2- cyanoacrylate with hydrazide derivatives. The Derivatives of Pyrazole were prepared by Schiff base reaction. All the synthesized compounds were characterized by IR, $^{1}$H-NMR and Elemental Analysis. All the compounds were evaluated for antimicrobial activity of newly synthesized derivatives was carried out on different micro-organisms (*E.coli, S. aureus, A.niger, C. albicans*) at the concentration of 200µcg/mL by using cup-plate agar diffusion method. The activity was measured in terms of zone of inhibition and compared with standard drug ciprofloxacin for antibacterial and griseofulvin for antifungal activity. All the compounds were screened for *in-vitro* anti-inflammatory activity at different concentration like200 µg/ml, and 300 µg/ml, by inhibition of protein denaturation method. Ibuprofen was used as standard drug. Some of them Compounds were screened for the *in vivo* anti-inflammatory activity at 200 µg/ml concentration. Compound 6b shown the most promising anti-inflammatory activity

**KEY WORDS:** Pyrazole; Pyrazole derivatives; Antibacterial and Anti-inflammatory

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## INTRODUCTION:

Pyrazoles[1]represent one of the most active classes of compounds possessing a wide spectrum of biological activities. Many of the therapeutically useful compounds such as phenylbutazone, oxyphenbutazone, antipyrine and aminopyrine are having analgesic and muscles relaxant action[2]. Pyrazoles exhibit anti-inflammatory, antipyretic activity is associated with several compounds possessing pyrazole and benzothiazole ring[3]. A dramatic increase in the anti-inflammatory activity of cortisone and other steroids, in corporating pyrazole nucleus in the meolecule[4]. Antipyrine is the one of the earliest synthetic drugs and is named after its antipyretic properties. Butazolidine, another pyrazolone is a powerful anti-inflammatory[5] drug used in rheumatic conditions, but it has dangerous side effects. Many pyrazole derivatives are associated with anti-fungal, anti-diabetic and anti-inflammatory properties.[6]

Pyrazoles and their derivatives have been investigated extensively by the organic chemists due to their close association with biological activities.[7,8]Pyrazole derivatives have been reported to show a broad spectrum of biological activity including antimicrobial[9-15], anti-inflammatory[16-20], antituberculosis[21,22], antiviral[23,24], hypoglycemic[25,26], anti tumor[27,28],antihypertensive[29]. Due to its wide range of biological activity, pyrazoles have received a considerable interest in the field of drug discovery and therefore pyrazolering constitutes a relevant synthetic target in

pharmaceutical industry.

**Chemistry**

The synthesis of the Pyrazole derivatives **6a-l**was carried out following *Scheme 1*.Theethyl bis(methylthio)- 2 cynoacrylate**1** was reflux with hydrazides derivative in methanoland Produce Pyrazoles**3**. Then compounds **3** react with hydrazine hyadrate and produced hydrazide derivative **4** by replacing ethoxy group. Then compounds **5** react with ethylacetoacetate and produce another Pyrazolone ring. The final derivatives **6a-l**was prepared by Schiff base reaction by react with aldehyde compounds under microwave.

**SCHEME 1**

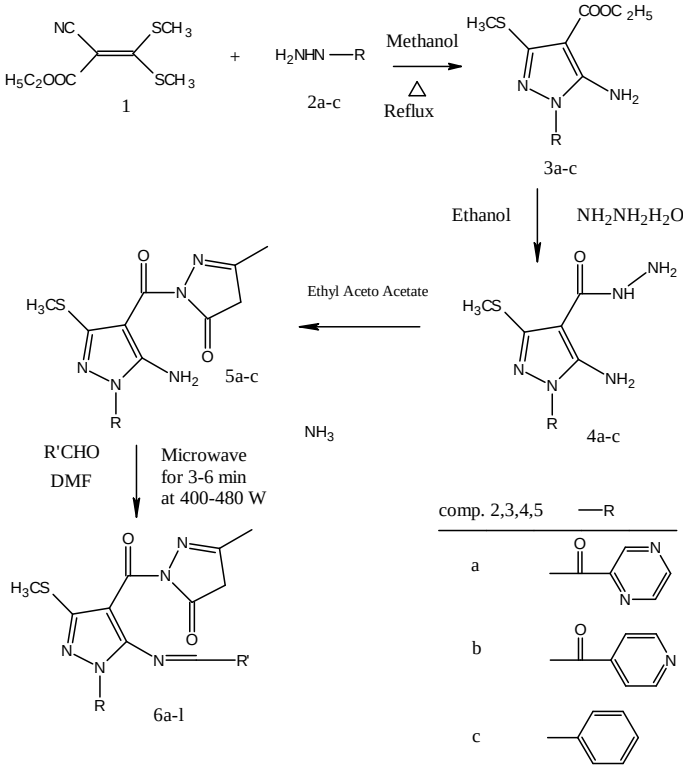



Table1: Substitution of Synthesized compounds

| Comp.(6) | R- | R'- |
|---|---|---|
| **6a** | Pyrazine-2CO- | Ph- |
| **6b** | Pyrazine-2CO- | 4-$OCH_3$-Ph- |
| **6c** | Pyrazine-2CO- | 2-OH-Ph- |
| **6d** | Pyrazine-2CO- | Ph-CH=CH- |
| **6e** | Pyridine-4CO- | Ph- |
| **6f** | Pyridine-4CO- | 4-$OCH_3$-Ph- |
| **6g** | Pyridine-4CO- | 2-OH-Ph- |
| **6h** | Pyridine-4CO- | Ph-CH=CH- |
| **6i** | Ph- | Ph- |
| **6j** | Ph- | 4-$OCH_3$-Ph- |
| **6k** | Ph- | 2-OH-Ph- |
| **6l** | Ph- | Ph-CH=CH- |

**Results and Discussion**

Formation of pyrazole(6) was confirmed on the basis of elemental analysis, NMR and IR spectral data. In the IR spectrum of 6c, 6g and 6k a broad absorption band around 3394-3400$cm^{-1}$ (O-H str.) indicates the presence of hydrogen bonded hydroxyl group in the compound. In the IR spectrum of 6b, 6c, 6f, 6g, 6j and 6k a broad absorption band around 1012-1104$cm^{-1}$ (C-O), indicates the presence of hydroxy and methoxy group in the compound. The shift in the frequency to lower values could be explained on the basis of the mesomeric shift and intramolecular hydrogen bonding. The other prominent absorption bands observed in the IR spectrum are 1698-1732$cm^{-1}$ for (C=O),1630-1659$cm^{-1}$ (C=N),1015-1286$cm^{-1}$ (C-N),1012-1104$cm^{-1}$ (C-O), 662-694$cm^{-1}$ (C-S). $^1$H NMR spectrum of 6a showed a singlet at $\delta$9.38(*s*, 1H of 2- pyrazine) 8.76(*d*, 2H of 2- pyrazine), 8.36(*s*, 1H of CH), 7.80(*d*, 2H of benzylidenimine), 7.55(*t*, 3H of benzylidenimine), 2.58(*s*, 3H of $SCH_3$), 2.24(*s*, 1H of $CH_2$), 1.90(*s*, 1H of $CH_3$);. Compound 6c containing proton of hydroxyl group resonated as a singlet at $\delta$10.98. Singlet at $\delta$3.80 in 6b due to 3H of methoxy group.

All the compounds were screened for antibacterial activity at 200 μg/ml concentration. However the compounds **6b**, **6c**, **6f**, **6j** and **6k** have shown maximum antibacterial activity, while the remaining compounds have also shown moderate antibacterial activity, when compared with standard drug **Ciprofloxacin** against *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative).

All the compounds were screened for antifungal activity. However Compound **6b, 6f, 6g**, **6j**, and **6k** have showed maximum activity, while the remaining compounds have also shown moderate Antifungal activity, when compared with standard **Griseofulvin** against*Aspergillus niger* & *Candida albicans*.

All the compounds were screened for *in-vitro* anti-inflammatory activity at different concentration like200 μg/ml, and 300 μg/ml, by inhibition of protein denaturation method. Compounds **6b, 6c, 6f, 6g**, **6k** and **6l** have shown promising anti-inflammatory activity. **Ibuprofen** was used as standard drug. The Compounds**6a, 6b, 6c, 6d**, **6b** and **6c** were screened for the *in vivo* anti-inflammatory activity at 200 μg/ml concentrations. Compound**6b, 6c, 6d**, **6b** and **6c** shows good anti-inflammatory activity.

Regarding the above result, it is suggested that compounds substituted with electron-releasing groups (-$OCH_3$, -OH) increase the antimicrobial activity and anti-inflammatory activity

**Pharmacological and Microbiological Screening**

Anti-inflammatory activity:

In-vitro anti-inflammatory activity- *Inhibition of protein denaturation*:[30,31]

The standard drug and synthesized compounds (**6**) were dissolved in minimumquantity of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M,pH 7.4). Final concentration of DMF in all solution was less than2.5%. Testsolution (1ml) containing different concentrations of drug was mixed with 1 ml of1mM albumin solution in phosphate buffer and incubated at 27° + 1° C in BODincubator for 15 min. Denaturation was induced by keeping the reaction mixtureat 60° + 1° C in water bath for 10 min. After cooling, the turbidity was measured denaturation was calculated from control where no drug was added.Each experiment was done in triplicate and average is taken. The Ibuprofen was use as standard drug.

**Table 2: *In-vitro* Anti-inflammatory activity of the synthesized compounds**

| Compound | Absorbance Value (Mean + SE) | Inhibition of Denaturation (in %) |
|---|---|---|
| | 200 µg/mL | 200 µg/mL |
| **Control** | 0.095 | - |
| **Ibuprofen** | 0.180 | 89.4% |
| **6a** | 0.142 | 49.47% |
| **6b** | 0.162 | 70.52% |
| **6c** | 0.156 | 64.21% |
| **6d** | 0.130 | 36.84% |
| **6e** | 0.134 | 41.05% |
| **6f** | 0.146 | 53.68% |
| **6g** | 0.155 | 63.15% |
| **6h** | 0.135 | 42.10% |
| **6i** | 0.130 | 36.84% |
| **6j** | 0.141 | 48.42% |
| **6k** | 0.160 | 68.42% |
| **6l** | 0.142 | 49.47% |

The percentage inhibition of denaturation was calculated byusing following formula.

% of Inhibition = 100 X [Vt / Vc – 1]
Where,
Vt = Mean absorbence of test sample.
Vc = Mean absorbence of control

**In –Vivo Anti-Inflammatory Activity:**

Oedema was produced by using type IV lambada carrageenan from sigma laboratories. Foot volumes were measured in Plethysmometer by water displacement.

The instrument was calibrated before performing the experiment using standard calibrated probe number and standard drug used Diclofenac sodium.

**Method:**

**Acute oral toxicity – Acute toxic class method:[32]**

The acute oral toxicity was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423, received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

The method enables judgment with respect to classifying the test substance to one of the series of toxicity classics defined by fixed $LD_{50}$ cut off values. Healthy young Swiss Albino mice of either sex weighing 20-25 gms were grouped into 4 groups of six animals each, starved for 24 h with water ad libitum prior to test. On the day of the experiment animals were administered with different compounds to different groups in an increasing dose of 10, 20, 100, 200, 1000 and 2000 mg/kg body weight orally. The animals were then observed continuously for 3 h for general behavioral, neurological, autonomic profiles and then every 30 min for next 3 h and finally for next 24 h or till death. The $LD_{50}$ of the compounds was found to be 2000mg/kg body wt. one tenth of the dose was selected as a therapeutic dose for evaluation (i.e. 200mg/kg).

**Acute anti-inflammatory method:**

***Carrageenan Induced Rat hind Paw Edema:*** [33, 34]

Anti-inflammatory activity was determined by Carrageenan Induced Rat hind Paw method of winter et al. wistar rats (170-220 g) was used for the experiment. The conventional laboratory diet was fed with adequate supply of

drinking water. The animals were randomly selected, marked to permit individual identification and kept in polypropylene cages for one week prior to dosing to allow acclimatization of them to laboratory conditions. The drugs were prepared as a suspension by triturating with water and 0.5% sodium CMC. The standard group received 50mg/kg body weight of Diclofenac sodium, test group received 200mg/kg body weight of synthesized compounds and the control group received 1% w/v of CMC.

All the treatment compounds were administered 30min. prior to carrageenan. Acute inflammation was induced in each group by injecting 0.1ml of freshly prepared carrageenan (0.1 ml of 1% suspension in 0.9% saline) into the sub-plantar region of right hind paw. A mark was put on the leg at the mallaleous region to facilitate the dipping of the leg to the same level at the second and subsequent times. The initial reading was taken at 0hr., i.e., immediately after injecting carrageenan and the procedure was repeated at 1, 2, and 3 hours after carrageenan injection with the help of Plethysmometer.

**Table 3: In-vivo Anti-inflammatory activity of the synthesized compounds**

| Compound | Increase paw volume | | | % Decrease paw volume after 3 hour |
|---|---|---|---|---|
| | **1 hour** | **2 hour** | **3 hour** | |
| Control | 0.37(±0.02) | 0.39(±0.008) | 0.45(±0.03) | |
| Diclofenac sodium | 0.10(±0.03) *** | 0.10(±0.05) *** | 0.12(±0.011) *** | **73.33** |
| **6a** | 0.12(±0.007) *** | 0.23(±0.03) ** | 0.26(±0.02) ** | **42.22** |
| **6b** | 0.08(±0.02) *** | 0.09(±0.05) *** | 0.13(±0.009) *** | **71.22** |
| **6g** | 0.08(±0.009) *** | 0.12(±0.05) *** | 0.17(±0.011) *** | **62.23** |
| **6h** | 0.10(±0.06) *** | 0.13(±0.009) *** | 0.16(±0.02) *** | **64.45** |
| **6j** | 0.07(±0.04) *** | 0.09(±0.05) *** | 0.14(±0.009) *** | **68.89** |
| **6k** | 0.11(±0.03) *** | 0.14(±0.07) *** | 0.16(±0.012)*** | **64.45** |

The difference between 0 hour reading and one of the subsequent readings provides the actual edema volume at that time. The mean paw volume at different times was calculated and compared with the control. The percentage inhibition of inflammation after 3 hour was then calculated by using the formula.

% of Inhibition = 100 X [1– Vt / Vc]

Where,

Vt = Edema volume in the rat treated with test drugs
Vc = Edema volume in the rat treated with control Antibacterial studies

The newly synthesized compounds were screened for theirantibacterial activity against Escherichia coli (ATCC-25922) and Staphylococcus aureus (ATCC-25923) bacterial strains by disc diffusionmethod.[35,36] Discs measuring 6.25 mm in diameter werepunched from Whatman no.1 filter paper. Batches of 100 discswere dispensed to each screw capped bottles and sterilized bydry heat at 140 $^{o}$C for an hour. The test compounds were preparedwith different concentrations using dimethylformamide.One milliliter containing 100 times the amount of chemical ineach disc was added to each bottle, which contains 100 discs.The discs of each concentration were placed in triplicate in nutrientagar medium seeded with fresh bacteria separately. Theincubation was carried out at 37 $^{o}$C for 24 h. Ciprofloxacinwas used as a standard drug. Solvent and growth controlswere prepared and kept. Zones of inhibition and minimum inhibitoryconcentrations (MICs) were noted. The results of antibacterialstudies are given in Table 4.

The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. Compounds**6b**,**6c**,**6f**, **6j** and **6k** showed very good activity almost equivalentto that of standard against all the bacterial strains.

**Antifungal studies**

Newly prepared compounds were screened for their antifungal activity against *A. niger* NCIM 596 and*C. albicans*(NCIM 3102) in DMSO by serial plate dilution method.[37,38] Sabourands agar media were prepared by dissolvingpeptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water(100 ml) and adjusting pH to 5.7. Normal saline was used tomake a suspension of spore of fungal strain for lawning. Aloopful of particular fungal strain was transferred to 3 ml salineto get a suspension of corresponding species. Agar media(20 ml) were poured into each Petri dish. Excess of suspensionwas decanted and the plates were dried by placing in an incubatorat 37 $^{o}$C for 1 h. Using an agar punch, wells were madeand each wells were labeled. A control was also prepared in triplicate and maintained at 37 $^{o}$C for 3-4 days. Zone of inhibitionand minimum inhibitory concentration (MIC) were noted. The activity of each compound was compared with Griseofulvin as the standard drug. The results of antifungal studiesare given in Table 4.

Table 4: Anti-bacterial and Anti-fungal activity of synthesized compounds

| Compd. | Zone of inhibition at 200µcg/mL (in mm.) | | | |
|---|---|---|---|---|
| | ***E. coli*** | ***S. aureus*** | ***A. niger*** | ***C. albicans*** |
| | **ATCC 25922** | **ATCC 25923** | **NCIM 596** | **NCIM 3102** |
| **6a** | 16 | 14 | 12 | 16 |
| **6b** | 18 | **23** | **21** | 23 |
| **6c** | **21** | **24** | **21** | 18 |
| **6d** | 15 | 16 | 14 | 18 |
| **6e** | 12 | 14 | 16 | 14 |
| **6f** | 18 | **22** | **24** | **25** |
| **6g** | 16 | 19 | **23** | **24** |
| **6h** | 18 | 17 | 14 | **22** |
| **6i** | 13 | 14 | 12 | 20 |
| **6j** | 18 | **21** | **22** | **24** |
| **6k** | **22** | **24** | 20 | **26** |
| **6l** | 18 | 12 | 17 | 13 |
| **Ciprofloxacin** | **28** | **26** | - | **-** |
| **Griseofulvin** | - | - | **28** | 25 |

The antifungal screening data showed moderate to good activitybut compounds particularly **6b, 6f,6g**, **6j**, and**6k**emerged as very active against both the fungal strains.

**Conclusion**

Around 12 new Pyrazole derivatives were synthesized, with the standard chemicals and well established procedures. The synthesized compounds were tested for their Preliminary Tests, Physical Constants, TLC etc.The structures of the final compounds were confirmed by IR, $^{1}$H-NMR Spectra and CHN analysis.The proposed compounds were screened for their Antibacterial, Antifungal and and Anti-inflammatory activities with the standard drugs in the well-equipped microbiology and pharmacology lab by using standard methods.Compounds**6b**, **6c**, **6f**, **6j and 6k**were found to posses maximum activity against both*escherichia coli*, *staphylococcus aureus*.Compounds **6b, 6f, 6g**, **6j**, **and6k**showed maximal activity againstboth*aspergillus niger* and *candida albicans*.Compounds **6b, 6c, 6f, 6g**, **6kand6l**have shown promising *in-vitro* anti-inflammatory activity.Compound**6b, 6c, 6d**, **6band6c**shows good anti-inflammatory activity.Compounds substituted with electron-releasing groups (-OCH3, -OH) increase the antimicrobial activity and anti-inflammatory activity.

The proposed work has given out many active Antibacterial, Antifungal, Anti-tubercular and Anti-inflammatory agents. Some of the compounds have showed excellent activities. These compounds with suitable modification can be explored better for their therapeutic activities in the future.

**Experimental protocols**

Introduction:

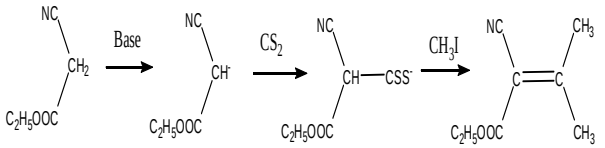
The identification and characterization of the prepared compounds were carried out by the following procedure to ascertain that all prepared compounds were of different chemical nature than the respective parent compound. Physical constant, Thin Layer Chromatography (TLC),FT-Infrared Spectroscopy (FT-IR),Nuclear Magnetic Resonance Spectroscopy ($^{1}$H-NMR),Elemental Analysis (C,H,N).

The melting points of the organic compounds were determined by open capillary in a heavy liquid paraffin bath. Melting point is a valuable criterion of purity for an organic compound, as a pure crystal is having definite and sharp melting point.Chromatography is an important technique to identify the formulation of new compounds and also to determine the purity of the compounds. The $R_f$ value is the characteristic for each compound and it was measured by using TLC solnent Methanol:Benzene(1:9).FTIR can be

routinely used to identify the functional groups and for quality control of raw materials/finished products. Jasco FT/IR 4100 offers fast throughput and rapid access to reliable and dependable IR results.$H^1$NMR spectra were recorded on sophisticated multinuclear NMR Spectrometer model Advance-II (Bruker), DMSO-*d6* as internal standards. The instrument is equipped with a Gyromagnet of field strength 9.4 T. Its $^1$H frequency is 400 MHz.

Synthesis of ethyl bis(methylthio)- 2 cynoacrylate(1):[39]

Pulvirised potassium hydroxide(0.2mol) was suspended in dioxane(100mL) and a solution of ethylcynoacetate(0.1mol) and carbon disulphide(0.1mol) in dioxane(50mL) was added with stirring and cooling to maintaine temp. of 15-20$^o$c. After stirred for 20 min. dilute with 250 mL ether. The yellow precipitate filtetered, wash with dioxane-ether (1:1) dried in vacuo over NaOH and $P_2O_5$. A solution of dithiolates(2mM) and methyl iodide(4mM) in abs. ethanol was kept at 0$^o$c for 2 days. The ethanol was removed by evaporation in vacuo and water added to the residue. The insoluble solid was filtered and dried on recrystallised form ether it yield colorless crystal.




Step 1: General procedure for synthesis of ethyl 5-amino-3-(methylthio)-1-substituted-1H-pyrazole-4-carboxylate (3a-c):[40]

A hydrazide derivative (100 mM) and ketene dithioacetal derivative (150 mM) in methanol (70 mL) was heated under reflux till completion of reaction. The reaction was monitored by thin layer chromatography using mixture of chloroform and methanol (8:2) as eluent. The reaction mass was cooled to 0-5° C to crystallize the product. On filtration and washing with chilled methanol it afforded pyrazole derivatives.

Step 3: General procedure for synthesis of 1-substituted-5-amino-4- [(hydrazinooxy)carbonyl]-1H-pyrazole(4a-c):[41]

A mixture of 0.01 mole of com.(3a-c) and 0.2 mole (10mL) of Hydrazine hydrate were taken in 250 mL round bottom flask attached to a refluxed condenser and refluxed with 50 ml of 95% ethanol for 15 hrs. The resultant mixture was concentrated in 250 ml beaker. It was cooled at room temperature and kept in refrigerator for 2 hrs. The solid mass thus separated out was filtered, dried and purified by recrystallization from ethanol.

Step 4: General Procedure for synthesis of 2-{[5-amino-1-substituted-3(methylthio)-1H-pyrazole-4-yl]carbonyl}-5-methyl-2,4-dihydro-3H-pyrazole-3-one.(5a-c):[42]

A mixture of the hydrazide (4a-c) (10 mM) and ethyl acetoacetate (10 mM) in absolute ethanol was heated at reflux for 3 h. The reaction mixture was cooled and the formed precipitate was filtered off, dried and recrystallized from acetic acid.

Step 5: General procedure for Microwave Assisted synthesis of derivative of pyrazole(6a-l):[43]

A mixture of 2 mM of aldehyde and 2 mM of different aryl or alkyl amines (5a-c) was taken and triturated in a mortar pestle. Then above mixture was transferred to a vessel which was then kept in microwave for synthesis. 4 to 5 mL of DMF was also added to mixture before putting it in microwave. Microwave was run at 400-480 W for 3 to 6 min for depending on reaction mixtures. Reaction completion was monitored continuously after each run by TLC. Then product was washed with ethanol, solvent was evaporated, dried and recrystalized with ethanol.

Spectral and Analytical data of synthesized compound

1-(5-(benzylideneamino)-3-(methylthio)-1-(pyrazine-2-carbonyl)-1*H*–pyrazole-4-yl)-3-methyl-1*H*–pyrazole-5(4*H*)-one**6a**:Yield: 71%, m.p.:140-142, Rf value: 0.74,IR ($cm^{-1}$):2997,1712,1630,1602,1112,668; $^1$HNMR:$\delta$9.38(*s*, 1H of 2-pyrazine) 8.76(*d*, 2H of 2- pyrazine), 8.36(*s*, 1H of CH), 7.80(*d*, 2H of benzylidenimine), 7.55(*t*, 3H of benzylidenimine),, 2.58(*s*, 3H of $SCH_3$), 2.24(*s*, 1H of $CH_2$), 1.90(*s*, 1H of $CH_3$); CHN Analysis for $C_{20}H_{17}N_7O_2S$, calcd: C,57.27; H,4.09; N,23.37 Found: C,57.22; H,4.26; N,21.81

1-(5-(4-methoxybenzylideneamino)-3-(methylthio)-1-(pyrazine-2-carbonyl)-1*H*–pyrazole-4-yl)-3-methyl-1*H*–pyrazole-5(4*H*)-one**6b**:Yield: 67%, m.p.:143-145,Rf value: 0.70, IR ($cm^{-1}$): 2992,1710,1645,1610,1142,1086,686; $^1$HNMR:$\delta$9.44(*s*, 1H of 2- pyrazine) 8.66(*d*, 2H of 2- pyrazine), 8.32(*s*, 1H of CH), 7.73(*d*, 2H of benzylidenimine), 7.02(*d*, 2H of benzylidenimine),3.80(*s*, 3H of $OCH_3$), 2.50(*s*, 3H of $SCH_3$), 2.19(*s*, 1H of $CH_2$), 1.94(*s*, 1H of $CH_3$); CHN Analysis for $C_{21}H_{19}N_7O_3S$, calcd: C,56.11; H,4.26; N,21.81 Found: C,56.42; H,4.43; N,21.48

1-(5-(2-hydroxybenzylideneamino)-3-(methylthio)-1-(pyrazine-2-carbonyl)-1*H*–pyrazole-4-yl)-3-methyl-1*H*–pyrazole-5(4*H*)-one**6c**:Yield: 63%, m.p.:152-154,Rf value: 0.67, IR ($cm^{-1}$):3398,2988,1698,1634,1592,1286,1046,694; $^1$HNMR:$\delta$10.98(*s*, 1H of OH), 8.79(*s*, 1H of 2- pyrazine),8.66(*d*, 2H of 2- pyrazine), 8.07(*s*, 1H of CH),7.65-7.03(*m*, 4H of benzylidenimine), 2.86(*s*, 3H of $SCH_3$), 2.51(*s*, 1H of $CH_2$), 1.80(*s*, 1H of $CH_3$); CHN Analysis for $C_{20}H_{17}N_7O_3S$, calcd: C,55.16; H,3.93; N,22.52 Found: C,55.47; H,3.83; N,22.26

3-methyl-1-(3-(methylthio)-5-(3-phenylallylideneamino)-1-(pyrazine-2-carbonyl)-1*H*–pyrazole-4-yl)-1*H*–pyrazole-5(4*H*)-one**6d**:Yield: 79%, m.p.:194-196,Rf value: 0.72,IR ($cm^{-1}$): 2998,1702,1653,1612,1574,1086,674; $^1$HNMR:$\delta$9.21(*s*, 1H of 2- pyrazine) 8.76(*d*, 2H of 2- pyrazine), 7.50 (*d*, 1H of CH), 6.86,7.19 (*dd*, 2H of CH=CH),7.35-7.62(*m*, 5H of benzene), 2.64(*s*, 3H of $SCH_3$), 2.14(*s*, 1H of $CH_2$), 1.96(*s*, 1H of $CH_3$); CHN Analysis for $C_{22}H_{19}N_7O_2S$, calcd: C,59.31; H,4.30; N,22.01 Found: C,57.42; H,4.02; N,22.10

1-(5-(benzylideneamino)-1-isonicotinoyl-3-(methylthio)-1*H*–pyrazole-4-yl)-3-methyl-1*H*–pyrazole-5(4*H*)-one**6e**:Yield: 58%, m.p.:178-180,Rf value: 0.76,IR ($cm^{-1}$):3008,1699,1634,1597,1080,683;$^1$HNMR: $\delta$ 8.46(*d*, 2H of 4-pyridine),7.93(*s*, 1H of CH),7.72(*d*, 2H of 4- pyridine), 7.63(*d*, 2H of benzylidenimine), 7.26(*t*, 3H of benzylidenimine), 3.29(*s*, 3H of $SCH_3$), 2.56(*s*, 1H of $CH_2$), 2.13(*s*, 1H of $CH_3$); CHN Analysis for $C_{21}H_1N_6O_2S$, calcd: C,60.27; H,4.34; N,20.08 Found: C,60.12; H,4.26; N,20.81

1-(1-isonicotinoyl-5-(4-methoxybenzylideneamino)-3-3-(methylthio)-1*H*–pyrazole-4-yl)-3-methyl-1*H*–pyrazole-5(4*H*)-one**6f**:Yield: 56%, m.p.:145-147,Rf value: 0.75,IR ($cm^{-1}$): 3010,1710,1632,1590,1243,1012,662;$^1$HNMR: $\delta$ 8.93(*d*, 2H of 4- pyridine),8.56(*s*, 1H of CH), 7.94(*d*, 2H of 4- pyridine), 7.83(*d*, 2H of benzylidenimine), 7.51(*d*, 2H of benzylidenimine),3.86(*s*, 3H of $OCH_3$), 2.63(*s*, 3H of $SCH_3$), 2.36(*s*, 1H of $CH_2$), 2.03(*s*, 1H of $CH_3$); CHN Analysis for $C_{22}H_{20}N_6O_3S$, calcd: C,58.92; H,4.49; N,18.74; Found: C,57.79; H,4.26; N19.38

1-(5-(2-hydroxybenzylideneamino)-1-isonicotinoyl-3-(methylthio)-1*H*–pyrazole-4-yl)-3-methyl-1*H*–pyrazole-5(4*H*)-one**6g**:Yield: 65%, m.p.:146-148,Rf value: 0.71, IR ($cm^{-1}$):3400,2994,1689,1622,1586,1124,1078,697$^1$HNMR: $\delta$ 11.12(*s*, 1H of OH),8.83(*d*, 2H of 4- pyridine),8.59(*s*, 1H of CH), 7.91(*d*, 2H of 4- pyridine, 7.01-7.61(*m*, 4H of benzylidenimine),, 2.72(*s*, 3H of $SCH_3$), 2.15(*s*, 1H of $CH_2$), 1.93(*s*, 1H of $CH_3$); CHN Analysis for $C_{21}H_{18}N_6O_3S$, calcd:C,58.05; H,4.18; N,19.34; Found: C,58.03; H,4.24; N,19.33

1-(1-isonicotinoyl-3-(methylthio)-5-(3-phenylallylideneamino)-1*H*–pyrazole-4-yl)-3-methyl-1*H*–pyrazole-5(4*H*)-one**6h**:Yield: 70%, m.p.:186-188,Rf value: 0.68, IR ($cm^{-1}$): 2997,1732,1659,1621,1015,681;$^1$HNMR: $\delta$ 8.83(*d*, 2H of 4-pyridine),, 7.89(*d*, 2H of 4- pyridine),7.66(*d*, 1H of CH)7.31-7.60(*m*, 5H of benzene),6.61,7.69 (*dd*, 2H of CH=CH), 2.65(*s*, 3H of $SCH_3$), 2.43(*s*, 1H of $CH_2$), 1.848(*s*, 1H of $CH_3$); CHN Analysis for $C_{23}H_{20}N_6O_2S$, calcd:C62.15; H,4.54; N,18.91; Found: C,62.43; H,4.24; N,18.73

1-(5-(benzylideneamino)-3-(methylthio)-1-phenyl-1*H*–pyrazole-4-yl)-3-methyl-1*H*–pyrazole-5(4*H*)-one**6i**:Yield: 63%, m.p.:126-128,Rf value: 0.76, IR ($cm^{-1}$): 3016,1709,1642,1602,1087,690; $^1$HNMR:$\delta$8.34(*s*, 1H of CH), 8.04-7.44(*m*, 10H of 2benzene), 2.73(*s*, 3H of $SCH_3$), 2.33(*s*, 1H of $CH_2$), 1.90(*s*, 1H of $CH_3$); CHN Analysis for $C_{20}H_{17}N_7O_2S$, calcd: C,57.27; H,4.09; N,23.37 Found: C,57.22; H,4.26; N,21.81;CHN Analysis for $C_{21}H_{19}N_5OS$, calcd: C,64.47; H,4.92; N,17.98; Found: C,64.65; H,4.96; N,17.81

1-(5-(4-methoxybenzylideneamino)-3-(methylthio)-1-phenyl-1*H*–pyrazole-4-yl)-3-methyl-1*H*–pyrazole-5(4*H*)-one**6j**:Yield: 64%, m.p.:139-141,Rf value: 0.68, IR ($cm^{-1}$): 3021,1702,1641,1578,1210,1043,673;$^1$HNMR: $\delta$ 8.27(*s*, 1H of CH), 8.06-7.06(*m*, 9H of 2benzene),3.79(*s*, 3H of $OCH_3$) 2.64(*s*, 3H of $SCH_3$), 2.29(*s*, 1H of $CH_2$), 2.04(*s*, 1H of $CH_3$); CHN Analysis for $C_{22}H_{21}N_5O_2S$, calcd: C,62.99; H,5.05; N,16.69 Found: C,62.78; H,5.31; N,16.58

1-(5-(2-hydroxybenzylideneamino)-3-(methylthio)-1-phenyl-1*H*–pyrazole-4-yl)-3-methyl-1*H*–pyrazole-5(4*H*)-one**6k**:Yield: 68%, m.p.:146-148,Rf value: 0.65, IR ($cm^{-1}$): 3394,3024,1731,1651,1597,1253,1104,686; $^1$HNMR:$\delta$11.24(*s*, 1H of OH),8.59(*s*, 1H of CH), 8.04-7.02(*m*, 9H of 2benzene), 2.54(*s*, 3H of $SCH_3$), 2.31(*s*, 1H of $CH_2$), 1.94(*s*, 1H of $CH_3$); CHN Analysis for $C_{21}H_{19}N_5O_2S$, calcd: C,62.21; H,4.72; N,17.21 Found: C,62.42; H,4.76; N,16.91

3-methyl-1-(3-(methylthio)-5-(3-phenylallylideneamino)-1-phenyl-1*H*–pyrazole-4-yl)-1*H*–pyrazole-5(4*H*)-one**6l**:Yield: 72%, m.p.:165-167;Rf value: 0.72, IR ($cm^{-1}$): 3026,1714,1640,1600,1131,692;$^1$HNMR: $\delta$ 7.86(*d*, 1H of CH), 7.30-7.69(*m*, 10H of 2benzene),5.42,6.02 (*dd*, 2H of CH=CH), 3.05(*s*, 3H of $SCH_3$), 2.39(*s*, 1H of $CH_2$), 1.84(*s*, 1H of $CH_3$); CHN Analysis for $C_{23}H_{21}N_5OS$, calcd: C,66.48; H,5.09; N,16.85 Found: C,66.29; H,5.02; N,22.22

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