SYNTHESIS AND CHARACTERIZATION OF NOVEL POLY SUBSTITUTED PYRAZoles USING VARIOUS KETENE DITHIOACETALS.

Where $R_1$ = Aryl, $R_2$ = Benzyl, $p$-nitro benzyl.
2.1 INTRODUCTION

Pyrazoles are well known five member heterocyclic compounds and several procedures for its synthesis have been extensively studied (Figure-1). Such studies have been stimulated by various promising applications, especially in the case of highly substituted pyrazole derivatives. In fact, certain substituted pyrazoles are used as analgesic, anti-inflammatory, antipyretic, agrochemicals where as some others are being studied for their medicinal interest. The pyrazole ring system consists of a doubly unsaturated five member ring containing two adjacent nitrogen atoms. The knowledge of such applications has pointed out that trisubstituted pyrazole are important target to be prepared to our interest on synthesis and molecular structure determination of some types of pyrazole.

![Figure-1](image)

The discovery of pyrazole derivatives as antipyretic agents dates back to 1884, when the German chemist Ludwig Knorr\(^1\) attempted to synthesize quinoline derivatives with antipyretic activity and accidentally obtained antipyrine (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one), which has analgesic, antipyretic and antirheumatic activity. Pyrazoles is widely found as the core structure in a large variety of compounds that possess important agrochemical and pharmaceutical activities.\(^2,3\) Classical methods for the synthesis of substituted pyrazoles involve approaches based either on the condensations of hydrazines with 1,3-dicarbonyl compounds and their 1,3-dielectrophile equivalents, or on intermolecular [3+2] cycloadditions of 1,3-dipoles to alkynes.\(^4,5\)

2.2 Synthesis of functionalized pyrazole derivatives using various synthetic approaches.

S. Cacchi, et al.\(^6\) development of strategy based on the concept of palladium-catalyzed coupling/annulation was applied to the one-pot synthesis of a large number of 3-(5)aryl/vinyl-1H-pyrazole derivatives 3 from the readily available N-tosyl-N-propargylhydrazine 1 and aryl iodides or vinyl triflates 2.\(^5\) This process allows the
introduction of a variety of aryl groups bearing electron-donating and electron-withdrawing substituents (Figure-2).

![Figure-2](image)

**Figure-2**

A [1+4] approach was developed by Santagostino et al.\(^7\) to prepare 3-(5)-substituted pyrazoles 7 from diethoxyphosphorylacetaldehyde tosylhydrazone 4 and aldehydes 5 via \(\alpha,\beta\)-unsaturated tosylhydrazones 6. This method is quite general in that the reaction conditions leave a number of functional groups unscathed; in addition, it can be applied to enolizable as well as unsaturated or aromatic aldehydes (Figure-3).

![Figure-3](image)

**Figure-3**

The oxidation of pyrazolines is a suitable method for obtaining pyrazoles. One of the most common synthetic procedures for the preparation of pyrazolines is based on the cycloaddition of diazoalkanes to \(\alpha,\beta\)-unsaturated ketones. Silva et al.\(^8\) applied this approach to the synthesis of 3-benzoyl-4-styryl-2-pyrazolines 12 by treatment of \((E,E)\)-cinnamylideneacetophenones 11 with diazomethane. Subsequent oxidation with chloranil afforded 3-(5)benzoyl-4-styrylpyrazoles 13 in good yields Figure-4.

![Figure-4](image)

**Figure-4**

V. K. Aggarwal et al.\(^9\) The 1,3-dipolar cycloaddition of diazo compounds to triple bonds is an important [3+2] method for the preparation of pyrazoles. Thus,
diazo compounds 9 generated in situ from tosylhydrazones of aldehydes 8 react with $N$-vinylimidazole–an acetylene equivalent bearing a leaving group–to afford pyrazoles 10 in moderate yields **Figure-5**

![Figure-5](image-url)

Junjappa H. et al.\textsuperscript{10} have demonstrated that 1-bis(methoxy)-4-bis(methylthio)-3-buten-2-one has been to a useful three carbon synthon for efficient regiospecific synthesis of a variety pyrazoles with mask or unmask aldehyde functionality by cyclocondensation with hydrazine hydrate in alcohol (**Figure-6**).

![Figure-6](image-url)

In 2005, Sakya S. M. et al.\textsuperscript{11} have offered fluoride-mediated nucleophilic substitution reactions of 1-(4-methylsulfonyl (or sulfonamido)-2-pyridyl)-5-chloro-4-cyano pyrazoles (**Figure-7**) with various amines and alcohols under mild conditions. The further reaction of novel pyrazoles provides the 5-alkyl amino and ether pyrazoles in moderate to high yields.

![Figure-7](image-url)

Junjappa H. et al.\textsuperscript{12} have developed highly efficient and regioselective synthesis of 1-aryl-3,4-substituted/annulated-5-(methylthio)-pyrazoles and 1-aryl-3-
(methylthio)-4,5-substituted/annulated pyrazoles via cyclocondensation of arylhydrazines with either $\alpha$-oxoketene dithioacetals or $\alpha$-oxodithioesters (Figure-8).

A novel approach to the synthesis of pyrazole derivatives from tosylhydrazones of $\alpha,\beta$-unsaturated carbonyl compounds possessing a $\beta$-hydrogen was proposed by Rosa R. and coworkers (Figure-9),\textsuperscript{13} exploiting microwave (MW) activation coupled with solvent free reaction conditions. The cycloaddition was studied on three ketones ($\text{trans}$-4-phenyl-3-buten-2-one, $\beta$-ionone and $\text{trans}$-chalcone). The corresponding 3,5-disubstituted-1$H$-pyrazoles were obtained in high yields and short reaction times.

Tang L. et al.\textsuperscript{14} have synthesized the pyrazole analogs (Figure-10) from a common aryl isocyanide intermediate. The cyclization of isocyanide with the oxime or BOC-protected hydrazones of ethyl bromopyruvate furnished the pyrazole carboxy esters.

Kim J. N. et al.\textsuperscript{15} have reported the regio-selective synthesis of 1,3,4,5-tetrasubstituted pyrazole derivatives from the reaction of Baylis-Hillman adducts of
alkyl vinyl ketone and hydrazine derivatives (Figure-11). During the continuous studies on the chemical transformations of Baylis-Hillman adducts including the synthesis of pyrazole.

![Chemical structure](image)

**Figure-11**

Elgemeie G. H. et al.\(^{16}\) were readily prepared novel ketene \(N,S\)-acetals by the reaction of cyanoacetamide or cyanothioacetamide with phenylisothiocyanate in the presence of potassium hydroxide, followed by alkylation of the produced salts with methyl iodide. Further, the reaction of ketene \(N,S\)-acetals with hydrazine afforded different substituted pyrazoles in excellent yields (Figure-12).

![Chemical structure](image)

**Figure-12**

Kuettel S. et al.\(^{17}\) have synthesized 4-(3-phenylisoxazol-5-yl)morpholine derivatives (Figure-13) using ketene dithioacetals. The reaction of substituted acetophenones with carbon disulfide in the presence of base and followed by alkylation with methyl iodide afforded 4-phenoxyphenyl-2,2-bis(methylthio) ketones vinyl, which were further reacts with hydrazine hydrate to give substituted pyrazoles through *in situ* cyclization of the resulting \(N,S\)-acetals.

![Chemical structure](image)

**Figure-13**

Recently, Dong D. and coworkers\(^{18}\) have developed an efficient and divergent synthesis of fully substituted 1\(H\)-pyrazoles using cyclopropyl oximes (Figure-14).
Under Vilsmeier conditions (POCl₃/DMF), substituted 1H-pyrazoles were synthesized from 1-carbamoyl, 1-oximyl cyclopropanes via sequential ring-opening, chlorovinylation, and intramolecular aza-cyclization.

![Figure-14](Image)

**Figure-14**

- **Reported synthetic approaches pyrazoles**

  Kurz T. et al.¹⁹ have synthesized novel fluorinated ketene $N,S$-acetals by the reaction of fluoro substituted cyanoacetamide derivatives with aryl isothiocyanate in the presence of potassium hydroxide, followed by the alkylation with methyl iodide. The reaction of fluorinated ketene $N,S$-acetals with hydrazine afforded different fluoro substituted pyrazole derivatives in good yield (Figure-15).

![Figure-15](Image)

**Figure-15**

Elgemeie G. H. et al.²⁰ synthesized a variety of novel $\alpha$-cyanoketene $S,S$-acetals, readily prepared by the reaction of cyanoacetanilide or cyanothioacetamide with carbon disulfide, followed by alkylation, react smoothly with nucleophile to afford variously substituted methylthio derivatives of pyrazole (Figure-16).

![Figure-16](Image)

**Figure-16**

- **Synthesis of functionalized pyrazoles using combinatorial chemistry approach.**
In recent decades, combinatorial chemistry tools have enabled the rapid synthesis of a large number of heterocyclic small molecule libraries and it is recognized now as a key element of early drug discovery\textsuperscript{21}. The main advantage of the combinatorial technique is the speed at which diverse types of organic compounds can be synthesized, formulated, and tested for a particular application. Moreover, in combinatorial study the quantity of required material is less in comparison to conventional methods, which makes it more suitable when the materials are expensive\textsuperscript{22}.

Organ M. G. et al.\textsuperscript{23} have developed a library of 4-(5-Iodo-3-Methyl pyrazolyl) Phenyl- sulfonamide derivatives (Figure-17) via solution-phase Suzuki coupling using Pd/C as a solid-supported catalyst.

![Figure-17](image)

Ivachtchenko A. V. et al.\textsuperscript{24} have reported the parallel solution-phase approach of more than 2200 7-trifluoromethyl-substituted pyrazole[1,5-a]pyrimidine (Figure-18) and 4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine carboxamides on a 50-100-mg scale. The reactions were include assembly of the pyrazole[1,5-a]pyrimidine ring by condensation of 5-aminopyrazole derivatives with the corresponding trifluoromethyl-\(\alpha\)-diketones. Libraries were then obtained in good yields and purities using solution-phase acylation and reduction methodologies. Simple manual techniques for parallel reactions using special CombiSyn synthesizers were coupled with easy purification procedures (crystallization from the reaction mixtures) to give high-purity final products.

![Figure-18](image)
Yang H. Z. et al.\textsuperscript{25} have developed a small combinatorial library containing pyrazolyl- pyrazoles and pyrazole[1,5-\textalpha]pyrimidines (\textbf{Figure-19}) by traditional organic synthesis and parallel-liquid-phase combinatorial synthesis using \textalpha\texttext{-S,S}-acetal of ethyl cyanoacetate as key synthon and hydrazine hydrate.

![Figure-19](image)

Recently, Laufer, S. et al.\textsuperscript{26} have synthesized structurally diverse and medicinally interesting series of 1, 4-dihydropyrano[2,3-\textc]pyrazoles via a three-component reaction using solution phase synthesis in excellent yields (\textbf{Figure-20}).

![Figure-20](image)

In 2009, Laborde E. et al.\textsuperscript{27} have developed an efficient three-component, two-step “catch and release” solid-phase synthesis of 3,4,5-trisubstituted pyrazoles (\textbf{Figure-21}). The reaction involves a base-promoted condensation of a 2-sulfonyl acetonitrile derivative 1 with an isothiocyanate 2 and \textit{in situ} immobilization of the resulting thiolate anion 3 on Merrifield resin. Reaction of the resin-bound sulfonyl intermediate 4 with hydrazine, followed by release from the resin and intramolecular cyclization, afforded 3,5-diamino-4-(arylsulfonyl)-1\textit{H}-pyrazoles 5. However, this methodology has some drawback such as; long reaction time, isolation of product and high reaction temperature.
Taddei M. et al.\textsuperscript{28} have developed the libraries of substituted pyrazole through \textit{in situ} generation of polymer-bound enaminones (\textbf{Figure-22}). The synthetic protocol makes use of commercially available aniline cellulose, a low-cost and versatile biopolymer, under very mild conditions. This new support allowed carrying out reactions in polar solvents under both conventional heating and MW irradiation without degradation of the polymer. The reaction between cellulose-bound enaminones and hydrazine to afford the target heterocycles in high yields directly in solution is the key step. The support can be conveniently recycled.

![Figure-22]

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\textbf{Various N-substituted Pyrazoles synthetic approach}

Stephen R. Graham. et al.\textsuperscript{29} have described and scalable process for the preparation of 4-amino-1,3-dimethylpyrazole hydrochloride 1. Compound 1 was a useful starting material; its preparation is achieved via a three-step sequence from methyl hydrazine and technical grade acetaldehyde dimethylacetal. The target molecule is isolated in high chemical and isomeric purity (>99.0% with respect to 4-amino-1,5-dimethylpyrazole) (\textbf{Figure-23}).

![Figure-23]

Xiao-Ling Ding et al.\textsuperscript{30} have developed a series of novel 3-aryl-1-arylmethyl-1\textit{H}-pyrazole-5-carboxamide derivatives 3\textit{a}–\textit{i}, by the reaction of 3-aryl-1-arylmethyl-1\textit{H}-pyrazole-5-carbonyl chloride with substituted amine in excellent yields. The compounds 3\textit{e}–\textit{h} could suppress A549 lung cancer cell growth. More interestingly, compounds 3\textit{e} and 3\textit{f} might inhibit the A549 cell growth by inducing apoptosis; whereas compounds 3\textit{g} and 3\textit{h} with fluorine group might inhibit the A549 cell growth by inducing autophagy (\textbf{Figure-24}).
Anumula Raghupathi Reddy et al.\textsuperscript{31} have an improved, scalable and commercially viable process developed for an active pharmaceutical ingredient, celecoxib (Figure-25).

Christina Despotopoulou, Lydia Klier, and Paul Knochel.\textsuperscript{32} have reported full functionalization of the pyrazole ring achieved by successive regioselective metalations using MPMgCl-LiCl\textsuperscript{33} and TMP2Mg·2LiCl. Trapping with various electrophiles led to trisubstituted pyrazoles. An application to the synthesis of the acaricide Tebufenpyrad was reported (Figure-26).

Hee Jeong Seo et al.\textsuperscript{34} have been engaged in searching for novel CB1 receptor antagonists, since SR141716A (rimonabant), a CB1 receptor antagonist, proved to be efficacious in human for the treatment of obesity. In the present study, a series of 1,2,4-triazole-containing diarylpyrazolyl carboxamides based on the 1,5-diarylpyrazole template of rimonabant, was synthesized and tested for CB1 receptor binding affinity. The structure–activity relationship studies demonstrated that incorporation of 1,2,4-triazole ring onto the pyrazole scaffold \textit{via} a methylene linker led to a significant improvement for CB1 receptor binding affinity. Importantly, these
anallogues also exhibited excellent selectivity for CB1 receptor over CB2 receptor (Figure-27).

![Figure-27]

2.3 Biological activity of various substituted pyrazoles.

Pyrazole derivatives possessed diverse biological activities such as antihyperglycemic, analgesic, anti-inflammatory, antipyretic, antibacterial, and sedative-hypnotic activity, cyclooxygenase-2 (Cox-2) inhibitors, IL-1 synthesis inhibitors, protein kinase inhibitors, as well as useful activities in conditions like schizophrenia, hypertension, and Alzheimer’s disease.\textsuperscript{35} In addition, they also have agrochemical properties including herbicidal and soil fungicidal activity; thus, they have been used as pesticides and insecticides\textsuperscript{36} which are described briefly as follows.

Anti-inflammatory preparations are widely used in the modern clinic, as pathogenetic agents in the treatment of many illnesses and pathological processes, alone or more frequently in combination with other drugs. However many of the known anti-inflammatory agents cause a range of side phenomena and complications in addition to the main effect. Consequently the search for and study of new more active anti-inflammatory agents\textsuperscript{37} of low toxicity is one of the urgent problems of contemporary science (Figure-28).

![Figure-28]

Tanitame A. et al.\textsuperscript{38} have synthesized pyrazole derivatives possesses antibacterial activity and inhibitory activity against DNA gyrase and topoisomerase IV. They have synthesized new pyrazole derivatives and found that 5-[(E)-2-(5-chloroindol-3-yl)vinyl]pyrazole (Figure-29) possesses potent antibacterial activity
and selective inhibitory activity against bacterial topoisomerases. Many of the synthesized pyrazole derivatives were potent against clinically isolated quinolone coumarin-resistant Gram-positive strains and had minimal inhibitory concentration values against these strains equivalent to those against susceptible strains.

![Figure-29](image)

Figure-29

In 2004, Edwards P. J. et al.\textsuperscript{39} have synthesized numerous highly functionalized pyrazole derivatives (Figure-30) using various diketone and substituted hydrazine hydrate and screened for HIV mediated diseases. Among them such compounds were found to useful in the treatment of a variety of disorders including those in which the inhibition of reverse transcriptase is implicated. Disorders of interest include those caused by HIV and genetically related retroviruses, such as AIDS.

![Figure-30](image)

Figure-30

Bagley M. C. and co workers\textsuperscript{40} have synthesized substituted N-pyrazole urea under the microwave irradiation. The reaction of substituted hydrazines and β-ketoesters afforded 5-aminopyrazoles in excellent yield, which can be transformed to the corresponding N-carbonyl derivatives by treatment with an isocyanate or chloroformate. Derivatization of 4-nitronaphth-1-ol using predominantly microwave heating methods and reaction with an N-pyrazole carbamate provides a rapid route to the N-pyrazole urea BIRB 796 (Figure-31) in high purity, as a potent and selective inhibitor of p38a mitogen-activated protein kinase for the study of accelerated ageing in Werner syndrome cells.
Piscitelli et al.\textsuperscript{41} new 1-aryl-5-(1\textit{H}-pyrrol-1-yl)-1\textit{H}-pyrazole-3-carboxamides were synthesized as cannabinoid (CB) receptor ligands. Compound 11 (CB1 $K_i$ 2.3 nM, CB1 SI 163.6) showed CB1 receptor affinity and selectivity superior to Rimonabant and AM251. Acute administration of 2 mg/kg 11 reduced sucrose, but not regular food, intake in rats. On the other hand, compound 23 (CB2 $K_i$ 0.51 nM, CB2 SI 30.0) showed significant affinity and selectivity for the CB2 receptor. The results presented here show that the 1-aryl-5-(1\textit{H}-pyrrol-1-yl)-1\textit{H}-pyrazole-3-carboxamide may serve as an effective scaffold for the design of either CB1 or CB2 receptor ligands. This partial divergence between the in vitro data (suggesting that 11 has more affinity than 1 at the CB1 receptor binding) and the in vivo data (in which 1 appears to be more potent and effective than 11) may be due to presently unexplored differences in the pharmacokinetic profile (e.g., rate and extend of absorption, distribution, passage through the elucidation of the structural determinants for the CB1 versus CB2 selectivity and the pharmacological characterization of this class of CB receptor ligand are warranted (Figure-32).
Biologically active molecules containing alkyl and carboxamide functional groups

Pyrazoles bearing sulfones and carboxamide moieties demonstrated to have significant pharmacological applications which are discussed as under.

The role of the cyclooxygenase-2 isoform in inflammation and the attractiveness of COX-2 as a therapeutic target for the development of anti-inflammatory drugs are very well recognized. COX-2 selective inhibitors have proven to be effective anti-inflammatory and analgesic medicines with lower chronic gastrointestinal (GI) toxicity than traditional non-steroidal anti-inflammatory drugs (NSAIDs), which non-selectively inhibit COX-2 and COX-1. Prostaglandin (PG)-dependent and PG-independent factors are responsible for NSAID induced GI toxicity. Decreased PG production due to COX-1 inhibition may adversely affect mucus-bicarbonate secretion, acid secretion, and mucosal blood flow. COX inhibition may also elicit an increase in 5-lipoxygenase activity that would potentiate production of leukotriene-B4 and vasoconstrictor peptido-leukotrienes by the lipooxygenase pathway, and this may also contribute to the vascular and other mucosal damage induced by NSAIDs. Celecoxib (Figure-33) is one of the COX-2 selective inhibitors and are currently prescribed for the treatment of arthritis and inflammatory diseases. They show anti-inflammatory activity with reduced GI side effects.

![Figure-33](image)

The identification of several potent pyrazole-based inhibitors of bacterial dihydroorotate dehydrogenase (DHODase) via a directed parallel synthetic approach is described below (Figure-34). The initial pyrazole-containing lead compounds were optimized for potency against Helicobacter pylori DHODase. Using three successive focused libraries, inhibitors were rapidly identified with the following characteristics: $K_i < 10 \mu M$ against H. pylori DHODase, sub-$\mu g/mL$ H. pylori minimum inhibitory
Chapter 2

Synthesis of Novel Poly-substituted Pyrazoles

concentration activity, low molecular weight, and >10 000-fold selectivity over human DHODase. DHODase is an enzyme that catalyzes the fourth step in the de novo biosynthesis of pyrimidine. It converts dihydroorotate to orotate. The anti-inflammatory drug leflunomide has been shown to inhibit DHODH.

\[
\begin{align*}
\text{R}_1 &= \text{4-MeoPh, 4-FPh,} \\
\text{Cyclopentyl, pyridyl} \\
\text{R}_2 &= \text{4-PrPh, 4-CF}_3\text{Ph,} \\
\text{DiPh,}
\end{align*}
\]

Figure-34

P. K. Sasmal et al.\textsuperscript{46} describes the synthesis and biological evaluation of novel pyrazole-3-carboxamide derivatives as CB1 antagonists (Figure-35). As a part of eastern amide SAR, various chemically diverse motifs were introduced. In general, a range of modifications were well tolerated. Several molecules with high polar surface area were also identified as potent CB1 receptor antagonists. The in vivo proof of principle for weight loss is exemplified with a lead compound from this series.

\[
\begin{align*}
\text{X} &= \text{Cl, Br, CN, OSO}_2\text{Pr} \\
\text{Y} &= \text{H, Me} \\
\text{R}_3 &= -\text{Aryl/alkyl}
\end{align*}
\]

Figure-35

In addition, Bonacorso H. G. et al.\textsuperscript{47} have synthesized some novel \textit{N}-substituted pyrazoles containing sulfone and trifluoromethyl groups at \textit{N} and C5 position of pyrazole ring (Figure-36) and evaluated for antimicrobial activity. All the
synthesized compounds were shown promising antimicrobial activity. The best activity was obtained when the structure possessed a 4-fluorophenyl substituent linked at the carbon-3 of the pyrazoline ring.

\[ \text{Figure-36} \]

R.V. Ragavan et al.\(^{48}\) have synthesized novel 1,5-diaryl pyrazole derivatives viz. 5-(4-chlorophenyl)-1-(4-fluorophenyl)-1H-pyrazole-3-carboxamides, 2-(5-(4-chlorophenyl)-1-(4-fluorophenyl)-1H-pyrazole-3-yl)thiazoles by varying the active part (amide group) of pyrazole, characterized using IR, \(^1\)H NMR, mass spectral data and screened for their antibacterial activity against Escherichia coli (ATTC-25922), Staphylococcus aureus (ATTC-25923), Pseudomonas aeruginosa (ATTC-27853), Klebsiella pneumonia. Similarly all these compounds were screened for their antifungal activity against Aspergillus flavus (NCIM No. 524), Aspergillus fumigates (NCIM No. 902), Penicillium marneffei and Trichophyton mentagrophytes (Figure-37).

\[ \text{Figure-37} \]

Stephen Brand et al.\(^{49}\) have reported \(N\)-Myristoyltransferase (NMT) represents a promising drug target for human African trypanosomiasis (HAT), which was caused
by the parasitic protozoa *Trypanosoma brucei*. We report the optimization of a high throughput screening hit (1) to give a lead molecule DDD85646 (63), which has potent activity against the enzyme (IC$_{50}$ = 2 µM) and *T. brucei* (EC$_{50}$ = 2 µM) in culture. The compound has good oral pharmacokinetics and cures rodent models of peripheral HAT infection. This compound provides an excellent tool for validation of *T. brucei* NMT as a drug target for HAT as well as a valuable lead for further optimization (Figure-38).

![Chemical Structures](image)

**Figure-38**

*Helicobacter pylori* is a Gram-negative microaerophilic bacterium that infects up to 50% of the world’s human population.$^{50}$ *H. pylori* resides in the acidic surroundings of the stomach, utilizing a high urease enzyme activity to provide a locally alkaline environment. *H. pylori* has been implicated in numerous gastrointestinal disorders and is associated with gastric ulcers, gastritis, and gastric cancer.$^{51}$ The current treatment of *H. pylori* infections typically utilizes a multiple drug therapy involving at least one broad spectrum antibiotic (antimicrobial therapy) and a proton pump inhibitor (antisecretory therapy). However, a *H. pylori* specific antimicrobial would be very desirable; a specific agent should avoid many of the negative gastrointestinal side effects associated with a broad spectrum antibacterial resulting from eradication of the normal gastrointestinal flora.
2.4 Aim of Current research work

The pyrazole nucleus is present in a wide variety of biologically interesting compounds, which exhibit antihyperglycemic, analgesic, anti-inflammatory, antipyretic, antibacterial, hypoglycemic, sedative-hypnotic activities. As described above, the tremendous biological potential of the sulfone group and carboxamide group bearing pyrazole scaffolds have attracted many chemists to synthesize this class of molecules. Thus, continuous efforts have been devoted to the development of more general and versatile synthetic methodologies to this class of compounds. Many research groups have been synthesized pyrazole derivatives using various methods. However, the existing methods suffered with some drawbacks such as; long reaction time, product isolation, etc. Thus, the practical synthesis of structurally diverse pyrazole based small molecules is of great significance.

Nowadays, a great deal of effort has been focused on the field of green chemistry in adopting methods and processes. As a part of this “green” concept, toxic and/or flammable organic solvents are replaced by alternative non-toxic and nonflammable media. In this context, many efforts have been made to use aqueous media. Among alternative green solvents, water has been the solvent of choice for a variety of transformations. On the other hand; functionalized ketene dithioacetals are versatile intermediates in organic synthesis for the construction of substituted heterocycles. Given the importance of N-substituted and carboxamide group containing pyrazoles and our ongoing interest on the synthesis various bioactive heterocycles using novel ketene dithioacetals starting from starting from acetoacetylides encouraged us to utilized these ketene dithioacetals for the construction of small molecule library of 3-methyl-5-(methylthio)-N-benzyl-1H-pyrazole-4-carboxamidederivatives in aqueous medium. The newly synthesized compounds were characterized by IR, Mass, $^1$H NMR, $^{13}$C NMR spectroscopy and elemental analysis. The antiviral screening of the synthesized compounds are under process.
2.5 Results and discussion

Route of Synthesis for novel substituted Pyrazoles derivatives using versatile intermediate as ketene dithioacetals.

<table>
<thead>
<tr>
<th>Scheme-01</th>
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| \[
\begin{align*}
\text{NH}_2 & + \text{CO}_2\text{H} \quad \xrightarrow{\text{NaOH, Toluene, Reflux 16-20h}} \quad \text{3a-j} \\
1\text{a-k} & \quad 2 \\
\end{align*}
\] |

<table>
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<th>Scheme-02</th>
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| \[
\begin{align*}
R & \quad \text{H} \quad \text{N} \quad \text{O} \\
3\text{a-k} & \quad \xrightarrow{\text{K}_2\text{CO}_3, \text{CS}_2, \text{DMF}, 5-6 \text{ hrs}, \text{Stirring}} \quad \text{4a-k} \\
\end{align*}
\] |

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<th>Scheme-03</th>
</tr>
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</table>
| \[
\begin{align*}
\text{NH} & \quad \text{O} \\
4\text{a-k} & \quad \xrightarrow{\text{IPA, reflux 4-6 hrs}} \quad \text{6a-k} \\
\end{align*}
\] |

<table>
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<th>Scheme-04</th>
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</table>
| \[
\begin{align*}
\text{NH}_2 & \quad \text{O} \\
6\text{a-k} & \quad R & \quad \text{X} \\
\end{align*}
\] |

Where R = -CH\text{3, -OCH\text{3, -Cl, -Br, -F and R\text{1= -H, -NO\text{2}}}}

Preparation of the target compounds was initiated by the reaction of (1) with aryl amines (2) in toluene at reflux temperature to afford the acetoacetonilide (3) in 85-90\% yields (Scheme-1). This product undergoes reaction with carbon disulfide in the presence of base in DMF followed by methylation to afford corresponding 2-(bis(methylthio)methylene)-3-oxo-N-phenylbutanamide (4). Which on reaction with hydrazine hydrate undergoes cyclization to form 3-methyl-5-(methylthio)-N-aryl-1H-
pyrazole-4-carboxamide (6). Eleven different acetoacetanilide were synthesized bearing various electron donating and electron withdrawing groups such as 2,3-diCH₃; 3,4-diCH₃; 4-CH₃; H; 2,5-diCH₃; 2,4-diCH₃; 3-Cl-4-F; 4-F; 4-Cl; 2-F; 4-OCH₃; 2,5-diCl and 3-NO₂ on the phenyl ring. Thus, it has been found that reaction of substituted acetoacetanilide 3a-k derivatives with carbon disulfide in the presence of potassium carbonate followed by the Hydrazine hydrate (99%), (80-20%) with alkyl/aryl halide (7-9) (Scheme-2-5) gave the novel Pyrazoles 8a-k and 10a-k.

The structures of compound VBA-10a were established on the basis of their elemental analysis and spectral data (MS, IR, and ¹H NMR (400 MHz, CDCl₃)). The analytical data for 10a revealed a molecular formula C₁₉H₁₈N₄O₃S (m/z 382). The ¹H NMR spectrum revealed one singlet at δ_H= 2.25ppm assigned to 3 protons of (-CH₃) group of methyl, a singlet at δ_H= 2.59-2.61ppm assigned to 3 protons of (-SCH₃) group of methyl, a singlet at δ_H= 5.43ppm assigned to 1 protons of (-ArCH₂-) group of methylene, a singlet at δ_H= 5.62ppm assigned to 1 protons of (-ArCH₂) group of methylene, a triplet at δ_H= 7.13-7.30ppm assigned to 3 proton of (-Ar-H) group, one doublet at δ_H = 7.36-7.39ppm assigned to Ar-NO₂(2H),one doublet at δ_H= 7.64-7.66ppm assigned to Ar-H (2H),one doublet at δ_H= 8.21-8.23ppm assigned to ArHNO₂ (2H) one singlet at δ_H= 9.33-9.37ppm assigned to –ArCONH groups.

The structures of compound VBA-10i were established on the basis of their elemental analysis and spectral data (MS, IR, and ¹H NMR). The analytical data for 10i revealed a molecular formula C₂₀H₂₀N₄O₄S (m/z 412). The ¹H NMR spectrum revealed one singlet at δ_H= 2.25 ppm assigned to 3 protons of (-CH₃) one triplet at δ_H= 2.57-2.61 ppm assigned to 3 protons of (-SCH₃) group of methyl, a singlet at δ_H= 3.85ppm assigned to 3 protons of (-CH₃) group of methyl, a singlet at δ_H= 5.43ppm assigned to 1 protons of (-ArCH₂-) group of methylene, a singlet at δ_H= 6.89-6.93ppm assigned to 2 proton of (-Ar-H) group, one multiplet at δ_H = 7.37-7.40ppm assigned to Ar-HNO₂ (2H), one doublet at δ_H= 7.53-7.57ppm assigned to 2 proton of Ar-H (2H), one doublet at δ_H= 8.21-8.24ppm assigned to 2 proton of Ar-H (2H), one singlet at δ_H= 9.32-9.38 ppm assigned to –ArCONH groups.
<table>
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<tr>
<th>Entry</th>
<th>R</th>
<th>R¹</th>
<th>Yield %</th>
<th>Time h.</th>
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<td>C₆H₅</td>
<td>C₆H₂CH₂⁻</td>
<td>90</td>
<td>10-12</td>
</tr>
<tr>
<td>VBA-8b</td>
<td>4-ClC₆H₄</td>
<td>C₆H₂CH₂⁻</td>
<td>88</td>
<td>8-9</td>
</tr>
<tr>
<td>VBA-8c</td>
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<td>C₆H₂CH₂⁻</td>
<td>84</td>
<td>8-10</td>
</tr>
<tr>
<td>VBA-8d</td>
<td>4-BrC₆H₄</td>
<td>C₆H₂CH₂⁻</td>
<td>88</td>
<td>6-8</td>
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<td>86</td>
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<tr>
<td>VBA-8f</td>
<td>3,4Cl₂C₆H₃</td>
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<td>C₆H₂CH₂⁻</td>
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<td>6-8</td>
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<td>C₆H₂CH₂⁻</td>
<td>82</td>
<td>5-7</td>
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<td>VBA-8i</td>
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<td>C₆H₂CH₂⁻</td>
<td>82</td>
<td>5-7</td>
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<td>VBA-8j</td>
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<tr>
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<td>4-NO₂C₆H₄CH₂⁻</td>
<td>82</td>
<td>10-12</td>
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<td>VBA-10b</td>
<td>4-ClC₆H₄</td>
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<td>84</td>
<td>6-8</td>
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<td>4-NO₂C₆H₄CH₂⁻</td>
<td>80</td>
<td>6-8</td>
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<tr>
<td>VBA-10d</td>
<td>4-BrC₆H₄</td>
<td>4-NO₂C₆H₄CH₂⁻</td>
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<td>VBA-10e</td>
<td>4-FC₆H₄</td>
<td>4-NO₂C₆H₄CH₂⁻</td>
<td>80</td>
<td>6-8</td>
</tr>
<tr>
<td>VBA-10f</td>
<td>3,4Cl₂C₆H₃</td>
<td>4-NO₂C₆H₄CH₂⁻</td>
<td>78</td>
<td>4-6</td>
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<tr>
<td>VBA-10g</td>
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<td>4-NO₂C₆H₄CH₂⁻</td>
<td>80</td>
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<td>5-7</td>
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<td>5-7</td>
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<td>VBA-10j</td>
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<td>4-NO₂C₆H₄CH₂⁻</td>
<td>74</td>
<td>4-6</td>
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<td>4-NO₂C₆H₄CH₂⁻</td>
<td>72</td>
<td>5-7</td>
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</table>
Figure 39: Proposed mechanism for the formation of pyrazole.


2.6 Biological activity

EXPERIMENTAL

The solvent DMSO was also purified before use by standard method (37). All the synthesized compounds were recrystallized prior to use. For all the compounds, agar well diffusion method was used.

Test Microorganisms:

The synthesized compounds were tested for its antimicrobial susceptibility activity against two Gram positive bacteria *Micrococcus luteus* MTCC 106 and *Bacillus circulans* MTCC1197 and two Gram negative bacteria *Escherichia coli* MTCC729 and *pseudomonas aeruginosa* MTCC 4676. Microorganisms were obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh, India and were maintained at 37°C on nutrient agar slants.

Preparation of test compounds:

The solutions were prepared at a concentration of 1 mg/mL (Stock Solution) and further diluted to 0.1 mg/mL and 0.05 mg/mL for all the compounds.

Preparation of the plates and microbiological assay:

The antibacterial evaluation was done by agar well diffusion method (38, 39) using Mueller Hinton Agar No.2 as the nutrient medium. The agar well diffusion method was preferred to be used in this study because it was found to be better than the disc diffusion method (39). The bacterial strains were activated by inoculating a loop full of test strain in 25 mL of N-broth and the same was incubated for 24 h in an incubator at 37°C. 0.2 mL of the activated strain was inoculated in Mueller Hinton Agar. Mueller Hinton Agar kept at 45°C was then poured in the Petri dishes and allowed to solidify. After solidification of the media, 0.85 cm ditch was made in the plates using a sterile cork borer and these were completely filled with the test solution. The plates were incubated for 24 h at 37°C. The mean value obtained for the three wells was used to calculate the zone of growth inhibition of each sample. The controls were maintained for each bacterial strain and each solvent. The inhibition zone formed by these compounds against the particular test bacterial strain determined the antibacterial activities of these synthesized compounds.
Table 3: Biological Activity Result for Active compounds as following.

<table>
<thead>
<tr>
<th>Compound Code</th>
<th>Bacterial strain</th>
<th>50 µ/gm</th>
<th>100 µ/gm</th>
<th>1000 µ/gm</th>
<th>50 µ/gm</th>
<th>1000 µ/gm</th>
<th>50 µ/gm</th>
<th>1000 µ/gm</th>
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<th>1000 µ/gm</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>E. coli</td>
<td>P. aurogenosa</td>
<td>B. Circulance</td>
<td>M. Luteus</td>
<td></td>
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<td>-</td>
<td>-</td>
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<td>6</td>
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</tbody>
</table>
| VBA 10a       | -               | -        | -        | 2         | 4       | -        | 3       | 6        | -       | 2        | 4
2.7 CONCLUSION

In summary, we reported a facile synthesis of highly substituted pyrazoles VBA 8a-k and 10a-k having N-alkylated with benzyl chloride/p-nitro benzyl bromide was first investigated under a variety of reaction conditions. Best results were obtained when the reaction was conducted in the presence of K₂CO₃, KI, TBAB/TEAB in H₂O DMF and acetonitrile furnishing only one N-phenyl-1H-pyrazole VBA 8a-k and 10a-k in high yield (Table 1, entry 1). The structure of VBA 8a-k and 10a-k was established on the basis of its reported physical and spectral data. Out of 22 compounds screened for antimicrobial susceptibility assay 6 compounds shown positive activity against microbes and result shown in table 3. No compounds have shown activity against E-coli MTCC 729.
2.8 EXPERIMENTAL SECTION

Thin-layer chromatography was accomplished on 0.2-mm precoated plates of silica gel G60 F254 (Merck). Visualization was made with UV light (254 and 365nm) or with an iodine vapor. IR spectra were recorded on a SHIMADZU FTIR 8400 instrument by using DRS prob. $^1$H (400 MHz) and $^{13}$C(100 MHz) NMR spectra were recorded on a Bruker AVANCE II spectrometer in CDCl$_3$ and DMSO. $^{13}$C NMR were recorded on 100 MHz spectrometer, referred to the internal solvent signals (77.0 for CDCl$_3$ or 40.0 for DMSO). Chemical shifts are expressed in $\delta$ ppm downfield from TMS as an internal standard. Mass spectra were determined using direct inlet probe on a SHIMADZUGCMS-QP 2010 mass spectrometer. Solvents were evaporated with a BUCHI rotary evaporator. Melting points were measured in open capillaries and are uncorrected. The chemicals used in this work were purchased from Merck, Spectrochem and sisco Chemical Companies.

General synthesis of 3-oxo-N-arylbutanamide 2a-t.

A mixture containing the primary amine (10 mmol), Ethyl acetoacetate (10 mmol), and catalytic amount of sodium or potassium hydroxide (10 %) was refluxed at 110°C for the approximately 15-20 h. The reaction was monitored by TLC. After completion of reaction, the solvent was removed under vacuum when the reaction was completed. The solid or oil was crystallized from methanol to give pure product 2a-t.

General synthesis of ketene dithioacetals 3a-t.

A 100mL conical flask equipped with magnetic stirrer and septum was charged with a solution of 3-oxo-N-p-tolylbutanamide 2a-t, (10 mmol) in DMF (10 ml). Dried K$_2$CO$_3$ (10 mmol) was added and the mixture was stirred for 2 h at room temperature. Then CS$_2$ (30 mmol) was added and stirred for 2 h at room temperature. Then Methyl iodide (20 mmol) was added dropwise at 0-5°C during 30 min and the mixtures was stirred for 1 h at 0-5°C and allow to come at room temperature and stirred for 4 h. After completion of the reaction, the reaction mix was poured it in to ice cold water. The precipitated crude product was purified by filtration followed by crystallization from EtOH. In case of crude obtained as oil, the product was extracted with Et$_2$O. The combined organic extracts were washed with H$_2$O (2 × 10 mL), dried (MgSO$_4$), and concentrated in vacuum to afford ketene dithioacetals directly used for the next step.
General procedure for the synthesis of trisubstituted pyrazoles 6a-w.

To a suspension of various \( \alpha \)-acylketenedithioacetals 1a-w (10 mmol) in water (25 mL), hydrazine hydrate 80% (20 mmol) was added and the reaction mixture was refluxed for appropriate time (Table 2) with constant stirring. After completion of the reaction, the reaction mixtures were cooled to room temperature and add cold water (50 mL). The separated solid was filtered, washed with water (2 × 50 mL), dried and crystallized from methanol to afford analytically pure products which were used for next step without further purification.

General procedure for the synthesis of \( N \)-substituted pyrazoles VBA 8a-k and 10a-k

To a solution of (0.006 mol) trisubstituted pyrazoles 6 dissolved in 30 mL acetonitrile/DMF was added (0.012 mol) of potassium carbonate and (0.0006 mol) potassium iodide, (0.0006 mol) TBAB/TEAB and the mixture was stirred at room temperature for 30 min. To this mixture was then added (0.006 mol) of 4-nitro benzyl bromide dissolved in 20 mL acetonitrile or benzyl chloride dissolved in 20 mL DMF, in a dropwise manner over a period of 15 min and the mixture was stirred at room temperature for 12-14 h. The reaction was monitored by TLC. The reaction was completed than poured into crushed ice or solvent was evaporate. The product was obtained as white/yellow amorphous powder. The product was purified by Hexane/ether. The structures of compounds synthesized by this method are given in Table 2.

Spectral data of the synthesized compounds VBA 8a-j and VBA 10a-j

1-benzyl-3-methyl-5-(methylthio)-\(N\)-phenyl-1H-pyrazole-4-carboxamide (VBA-8a):

White solid; \( R_f \) 0.46 (7:3 H-E.A); mp 241-243°C; IR (KBr): 3315, 2982, 2854, 1651, 1645, 1589, 1545, 1532, 1510, 1477, 1385, 1253, 744, 651, cm \(^{-1} \); \( ^1 \)H NMR: (400 MHz, DMSO) \( \delta \) 2.27 (s, 3H, -CH\(_3\)), 2.47 (s, 3H, -SCH\(_3\)), 5.34 (s, 1H, -ArCH\(_2\)), 5.46 (s, 1H, -ArCH\(_2\)), 7.02-7.08 (dd, 1H \( J=24.0 \) Hz, Ar-H), 7.20-7.22 (t, 3H, Ar-H), 7.24-7.30 (m, 2H, Ar-H), 7.33-7.37 (m, 2H, Ar-H), 7.62-7.64 (d, 1H \( J=8.0 \) Hz, Ar-H), 7.67-7.69 (d, 1H \( J=8.0 \) Hz, Ar-H), 9.56 (s, 1H, -ArCONH); \( ^{13} \)C NMR: (100MHz, DMSO) \( \delta \) C 10.67, 13.20, 14.91, 19.47, 52.23, 52.33, 115.14, 119.40, 119.50, 119.99,
123.14, 123.28, 126.98, 127.32, 127.50, 128.40, 128.43, 128.50, 134.22, 136.42, 136.89, 138.93, 141.48, 144.73, 147.91, 161.45; MS m/z: 337.4(M⁺); Anal. Calcd. for C₁₉H₁₉N₃OS: C, 67.63; H, 5.68; N, 12.45%. Found: C, 66.58; H, 5.62; N, 12.47%.

1-benzyl-N-(4-chlorophenyl)-3-methyl-5-(methylthio)-1H-pyrazole-4-carboxamide (VBA-8b): White solid; Rf 0.52 (7:3 H-E.A); mp 220-222°C; IR (KBr): 3306, 2975, 2845, 1651, 1645, 1589, 1554, 1536, 1514, 1477, 1385, 1253, 815, 749, 665, cm⁻¹; MS m/z: 371.9(M⁺); Anal. Calcd. for C₁₉H₁₈ClN₃OS: C, 61.36; H, 4.88; N, 11.30%. Found: C, 61.29; H, 4.79; N, 11.25%.

1-benzyl-N-(3-chlorophenyl)-3-methyl-5-(methylthio)-1H-pyrazole-4-carboxamide (VBA-8c): White solid; Rf 0.50 (7:3 H-E.A); mp 255-257°C; IR (KBr): 3312, 2992, 2862, 1651, 1647, 1589, 1550, 1530, 1514, 1477, 1375, 1253, 754, 641, cm⁻¹; MS m/z: 371.9(M⁺); Anal. Calcd. for C₁₉H₁₈ClN₃OS: C, 61.36; H, 4.88; N, 11.30%. Found: C, 61.39; H, 4.79; N, 11.29%.

1-benzyl-N-(4-bromophenyl)-3-methyl-5-(methylthio)-1H-pyrazole-4-carboxamide (VBA-8d): White solid; Rf 0.53 (7:3 H-E.A); mp 268-270°C; IR (KBr): 3309, 2978, 2864, 1651, 1643, 1589, 1545, 1529, 1510, 1477, 1383, 1253, 813, 762, 644, cm⁻¹; MS m/z: 416.3(M⁺); Anal. Calcd. for C₁₉H₁₈BrN₃OS: C, 54.81; H, 4.36; N, 10.09%. Found: C, 54.77; H, 4.27; N, 10.05%.

1-benzyl-N-(4-fluorophenyl)-3-methyl-5-(methylthio)-1H-pyrazole-4-carboxamide (VBA-8e): White solid; Rf 0.54 (7:3 H-E.A); mp 200-202°C; IR (KBr): 3305, 2954, 2858, 1651, 1643, 1592, 1547, 1524, 1510, 1477, 1387, 1249, 815, 744, 667, cm⁻¹; ¹H NMR: (400 MHz, DMSO) δH 2.26 (s, 3H, -CH₃), 2.48 (s, 3H, -SCH₃), 5.33 (s, 1H, -ArCH₂-), 7.03-7.09 (dd, 2H, J=24.0Hz, Ar-H), 7.20-7.22 (t, 1H, Ar-H), 7.21-7.30 (m, 2H, Ar-H), 7.33-7.36 (m, 2H, Ar-H), 7.63-7.72 (m, 2H, Ar-H), 9.56 (s, 1H, -ArCONH); ¹³C NMR: (100MHz, DMSO) δC 10.66, 13.18, 14.93, 19.47, 52.25, 52.38, 114.78, 114.86, 115.00, 115.08, 119.87, 121.11, 121.19, 121.21, 121.29, 126.95, 127.31, 127.49, 128.37, 128.46, 134.24, 135.16, 135.19, 136.32, 136.81, 141.46, 144.74, 147.93, 156.90, 156.96, 159.29, 159.35, 161.38, 161.41; MS m/z: 355.4(M⁺); Anal. Calcd. for C₁₉H₁₈FN₃OS: C, 64.21; H, 5.10; N, 11.82%. Found: C, 64.25; H, 5.06; N, 11.83%.
Chapter 2

Synthesis of Novel Poly-substituted Pyrazoles

1-benzyl-N-(3,4-dichlorophenyl)-3-methyl-5-(methylthio)-1H-pyrazole-4-carboxamide (VBA-8f): White solid; $R_f$ 0.54 (7:3 H-E.A); mp 262-264°C; IR (KBr): 3299, 2962, 2863, 1653, 1648, 1588, 1544, 1521, 1508, 1487, 1379, 1253, 751, 655, cm$^{-1}$; MS $m/z$: 406.3(M$^+$); Anal. Calcd. for C$_{19}$H$_{17}$Cl$_2$N$_3$OS: C, 56.16; H, 4.22; N, 10.34%. Found: C, 55.56; H, 4.34; N, 10.27%.

1-benzyl-N-(3-chloro-4-fluorophenyl)-3-methyl-5-(methylthio)-1H-pyrazole-4-carboxamide (VBA-8g): White solid; $R_f$ 0.52 (7:3 H-E.A); mp 262-264°C; IR (KBr): 3309, 2973, 2842, 1656, 1649, 1588, 1544, 1534, 1521, 1474, 1368, 1249, 758, 642, cm$^{-1}$; MS $m/z$: 389.9(M$^+$); Anal. Calcd. for C$_{19}$H$_{17}$ClFN$_3$OS: C, 58.53; H, 4.40; N, 10.78%. Found: C, 58.64; H, 4.73; N, 10.36%.

1-benzyl-3-methyl-5-(methylthio)-N-p-tolyl-1H-pyrazole-4-carboxamide (VBA-8h): White solid; $R_f$ 0.48 (7:3 H-E.A); mp 214-216°C; IR (KBr): 3318, 2978, 2854, 1652, 1644, 1588, 1544, 1536, 1521, 1468, 1362, 1249, 809, 748, 638, cm$^{-1}$; MS $m/z$: 336.4(M$^+$); Anal. Calcd. for C$_{19}$H$_{18}$N$_3$OS: C, 67.83; H, 5.39; N, 12.49%. Found: C, 67.86; H, 5.28; N, 12.58%.

1-benzyl-N-(4-methoxyphenyl)-3-methyl-5-(methylthio)-1H-pyrazole-4-carboxamide (VBA-8i): White solid; $R_f$ 0.50 (7:3 H-E.A); mp 224-240°C; IR (KBr): 3315, 2963, 2856, 1662, 1642, 1588, 1544, 1532, 1521, 1478, 1366, 1252, 814, 745, 658, cm$^{-1}$; $^1$H NMR: (400 MHz, CDCl$_3$) $\delta$H 2.14 (s, 3H, -CH$_3$), 2.57 (s, 3H, -SCH$_3$), 3.82 (s, 3H, -OCH$_3$), 5.33 (s, 1H, -ArCH$_2$), 6.90-6.92 (d, 2H, $J=8.0$ Hz, Ar-H), 7.23-7.37 (m, 5H, Ar-H), 7.55-7.57 (d, 2H, $J=8.0$ Hz, Ar-H), 9.32-9.38 (d, 1H, -ArCONH); $^{13}$C NMR: (100 MHz, CDCl$_3$) $\delta$C 11.50, 14.68, 18.49, 19.95, 53.38, 55.53, 114.18, 114.21, 114.75, 121.63, 121.78, 126.86, 127.28, 128.04, 128.84, 128.91, 131.34, 135.62, 142.34, 145.15, 152.24, 156.27, 161.23; MS $m/z$: 367.5(M$^+$); Anal. Calcd. for C$_{20}$H$_{21}$N$_3$O$_2$S: C, 65.37; H, 5.76; N, 11.44%. Found: C, 65.32; H, 5.67; N, 11.57%.

1-benzyl-3-methyl-N-(3,4-dimethylphenyl)-5-(methylthio)-1H-pyrazole-4-carboxamide (VBA-8j): White solid; $R_f$ 0.54 (7:3 H-E.A); mp 250-252°C; IR (KBr): 3307, 2986, 2836, 1662, 1642, 1588, 1548, 1528, 1519, 1472, 1362, 1249, 759, 643,
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Synthesis of Novel Poly-substituted Pyrazoles

1-benzyl-3-methyl-5-(methylthio)-N-m-tolyl-1H-pyrazole-4-carboxamide (VBA-8k): Lemon white solid; \( R_f 0.51 \) (7:3 H-E.A); mp 278-280°C; IR (KBr): 3311, 2971, 2848, 1662, 1642, 1588, 1557, 1534, 1517, 1466, 1354, 1254, 749, 658, cm\(^{-1}\); MS \( m/z \): 351.5 (M\(^+\)); Anal. Calcd. for C\(_{20}\)H\(_{21}\)N\(_3\)OS: C, 68.35; H, 6.02; N, 11.96%. Found: C, 68.32; H, 6.08; N, 11.78%.

1-(4-nitrobenzyl)-3-methyl-5-(methylthio)-N-phenyl-1H-pyrazole-4-carboxamide (VBA-10a): Yellow solid; \( R_f 0.42 \) (7:3 H-E.A); mp 155-157°C; IR (KBr): 3292, 3064, 2988, 2845, 1664, 1645, 1608, 1555, 1526, 1372, 1262, 1214, 833, 812, 754, 717, 632, cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \)H 2.25 (s, 3H, -CH\(_3\)), 2.59-2.61 (s, 3H, -SCH\(_3\)), 5.43 (s, 1H, -ArCH\(_2\)), 5.62 (s, 1H, -ArCH\(_2\)), 7.13-7.30 (m, 3H, ArH), 7.36-7.39 (d, 2H \( J=12.0 \) Hz, ArHNO\(_2\)), 7.64-7.66 (d, 2H \( J=8.0 \) Hz, ArH), 8.21-8.23 (d, 2H \( J=8.0 \) Hz, ArHNO\(_2\)); \(^13\)C NMR (100MHz, CDCl\(_3\)): \( \delta \)C 11.44, 14.64, 18.13, 20.24, 52.37, 114.97, 119.89, 120.02, 124.14, 124.23, 124.33, 127.66, 128.08, 129.08, 129.13, 138.07, 143.45, 145.45, 147.72, 153.01, 160.64, 161.07; MS \( m/z \): 382.4(M\(^+\)); Anal. Calcd. for C\(_{19}\)H\(_{18}\)N\(_4\)O\(_3\)S: C, 59.67; H, 4.74; N, 14.65%. Found: C, 59.62; H, 4.68; N, 14.59%.

1-(4-nitrobenzyl)-N-(4-chlorophenyl)-3-methyl-5-(methylthio)-1H-pyrazole-4-carboxamide (VBA-10b): Yellow solid; \( R_f 0.44 \) (7:3 H-E.A); mp 176-178°C; IR (KBr): 3282, 3072, 2994, 2827, 1664, 1645, 1608, 1555, 1528, 1362, 1268, 1214, 833, 812, 758, 709, 632, cm\(^{-1}\); MS \( m/z \): 416.9(M\(^+\)); Anal. Calcd. for C\(_{19}\)H\(_{17}\)ClN\(_4\)O\(_3\)S: C, 54.74; H, 4.11; N, 13.44%. Found: C, 54.62; H, 4.01; N, 13.32%.

1-(4-nitrobenzyl)-N-(3-chlorophenyl)-3-methyl-5-(methylthio)-1H-pyrazole-4-carboxamide (VBA-10c): Yellow solid; \( R_f 0.44 \) (7:3 H-E.A); mp 162-164°C; IR (KBr): 3278, 3084, 2982, 2838, 1664, 1645, 1608, 1555, 1532, 1368, 1274, 1222, 833, 812, 762, 709, 632, cm\(^{-1}\); MS \( m/z \): 416.9(M\(^+\)); Anal. Calcd. for C\(_{19}\)H\(_{17}\)ClN\(_4\)O\(_3\)S: C, 54.74; H, 4.11; N, 13.44%. Found: C, 54.77; H, 4.08; N, 13.69%.

1-(4-nitrobenzyl)-N-(4-bromophenyl)-3-methyl-5-(methylthio)-1H-pyrazole-4-carboxamide (VBA-10d): Yellow solid; \( R_f 0.46 \) (7:3 H-E.A); mp 148-150°C; IR
1-(4-nitrobenzyl)-N-(4-fluorophenyl)-3-methyl-5-(methylthio)-1H-pyrazole-4-carboxamide (VBA-10e): Yellow solid; \( R_f \) 0.47 (7:3 H-E.A); mp 237-239°C; IR (KBr): 3437, 3286, 3064, 2999, 2838, 1637, 1599, 1521, 1438, 1350, 1238, 1034, 821, 758, 742, 707, 630, \( \text{cm}^{-1} \); MS \( m/z \): 400.4(M⁺); Anal. Calcd. for C\(_{19}\)H\(_{17}\)FN\(_4\)O\(_3\)S: C, 56.99; H, 4.28; N, 12.88%. Found: C, 56.83; H, 4.35; N, 12.87%.

1-(4-nitrobenzyl)-N-(3,4-dichlorophenyl)-3-methyl-5-(methylthio)-1H-pyrazole-4-carboxamide (VBA-10f): Lemon Yellow solid; \( R_f \) 0.46 (7:3 H-E.A); mp 281-284°C; IR (KBr): 3318, 3022, 2964, 2848, 1628, 1599, 1521, 1428, 1356, 1238, 1038, 821, 758, 742, 707, 630, \( \text{cm}^{-1} \); MS \( m/z \): 451.3(M⁺); Anal. Calcd. for C\(_{19}\)H\(_{16}\)Cl\(_2\)N\(_4\)O\(_3\)S: C, 50.56; H, 3.57; N, 12.41%. Found: C, 50.78; H, 3.65; N, 12.28%.

1-(4-nitrobenzyl)-N-(3-chloro-4-fluorophenyl)-3-methyl-5-(methylthio)-1H-pyrazole-4-carboxamide (VBA-10g): Lemon Yellow solid; \( R_f \) 0.46 (7:3 H-E.A); mp 256-258°C; IR (KBr): 3318, 3022, 2964, 2848, 1628, 1599, 1521, 1428, 1356, 1238, 1038, 821, 758, 742, 707, 630, \( \text{cm}^{-1} \); MS \( m/z \): 434.90(M⁺); Anal. Calcd. for C\(_{19}\)H\(_{16}\)ClFN\(_4\)O\(_3\)S: C, 52.48; H, 3.71; N, 12.88%. Found: C, 52.54; H, 3.85; N, 12.64%.

1-(4-nitrobenzyl)-3-methyl-5-(methylthio)-N-p-tolyl-1H-pyrazole-4-carboxamide (VBA-10h): Yellow solid; \( R_f \) 0.44 (7:3 H-E.A); mp 275-278°C; IR (KBr): 3349, 3015, 2978, 2858, 1628, 1599, 1521, 1428, 1358, 1232, 1024, 812, 748, 732, 709, 630 \( \text{cm}^{-1} \); 
\( ^1\)H NMR(400 MHz, CDCl\(_3\)): \( \delta_H \) 2.25-2.35 (s, 6H-CH\(_3\), -SCH\(_3\)), 2.58-2.60 (s, 3H, -CH\(_3\)), 5.43 (s, 1H, -ArCH\(_2\)), 5.62 (s, 1H, -ArCH\(_2\)), 7.17-7.19 (s, 2H, Ar-H), 7.28-7.39 (s, 2H, Ar-HNO\(_2\)), 7.52-7.60 (d, 2H \( J=32.0 \text{Hz} \), Ar-H), 8.22-7.23 (d, 2H \( J=4.0 \text{Hz} \), Ar-HNO\(_2\)), 9.30-9.36 (d, 1H, -ArCONH); \( ^{13}\)C NMR (100MHz, CDCl\(_3\)): \( \delta_C \) 11.46, 20.17, 20.93, 52.47, 119.88, 120.03, 124.15, 124.24, 127.64, 128.06, 129.62; MS \( m/z \): 396.5(M⁺); Anal. Calcd. for C\(_{20}\)H\(_{20}\)N\(_4\)O\(_3\)S: C, 60.59; H, 5.08; N, 14.13%. Found: C, 60.64; H, 5.27; N, 14.27%. 

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1-(4-nitrobenzyl)-N-(4-methoxyphenyl)-3-methyl-5-(methylthio)-1H-pyrazole-4-carboxamide (VBA-10i): Lemon Yellow solid; \( R_f \) 0.43 (7:3 H-E.A); mp 262-264°C; IR (KBr): 3406, 3271, 3001, 2933, 2835, 1741, 1637, 1599, 1521, 1460, 1438, 1350, 1228, 1107, 1030, 839, 817, 740, 707, cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 2.25 (s, 3H, -CH\(_3\)), 2.57-2.61 (t, 3H, -SCH\(_3\)), 3.85 (s, 3H, -OCH\(_3\)), 5.43 (s, 1H, ArCH\(_2\)-), 5.62 (s, 1H, ArCH\(_2\)-), 6.89-6.93 (d, 2H, \( J \)=16.0Hz, Ar-H), 7.37-7.40 (d, 2H, Ar-HNO\(_2\)), 7.53-7.57 (d, 2H, \( J \)=8.0Hz, Ar-H), 8.21-8.24 (d, 2H, \( J \)=12.0Hz, Ar-HNO\(_2\)), 9.20-9.24 (d, 1H, -ArCONH); \(^1\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \)C 11.42, 14.64, 18.11, 20.21, 52.35, 55.54, 114.20, 114.24, 121.70, 121.85, 124.13, 124.22, 127.64, 128.06, 133.08, 142.82, 143.67, 145.26, 147.68, 152.86, 156.39, 160.97; MS m/z: 412.5(M\(^+\)); Anal. Calcd. for C\(_{20}\)H\(_{20}\)N\(_4\)O\(_4\)S: C, 58.24; H, 4.89; N, 13.58%. Found: C, 58.33; H, 4.94; N, 13.18%.

1-(4-nitrobenzyl)-3-methyl-N-(3,4-dimethylphenyl)-5-(methylthio)-1H-pyrazole-4-carboxamide (VBA10j): Yellow solid; \( R_f \) 0.45 (7:3 H-E.A); mp 248-250°C; IR (KBr): 3358, 3009, 2964, 2828, 1634, 1599, 1521, 1428, 1352, 1236, 1028, 824, 764, 742, 709, 630, cm\(^{-1}\); MS m/z: 410.5(M\(^+\)); Anal. Calcd. for C\(_{21}\)H\(_{22}\)N\(_4\)O\(_3\)S: C, 61.44; H, 5.40; N, 13.65%. Found: C, 61.57; H, 5.37; N, 13.48%.

1-(4-nitrobenzyl)-3-methyl-5-(methylthio)-N-\( m \)-tolyl-1H-pyrazole-4-carboxamide (VBA-10k): Lemon yellow solid; \( R_f \) 0.44 (7:3 H-E.A); mp 258-260°C; IR (KBr): 3346, 3008, 2972, 2846, 1632, 1599, 1521, 1428, 1354, 1232, 1024, 812, 758, 742, 707, 630, cm\(^{-1}\); MS m/z: 396.5(M\(^+\)); Anal. Calcd. for C\(_{20}\)H\(_{20}\)N\(_4\)O\(_3\)S: C, 60.59; H, 5.08; N, 14.13%. Found: C, 60.64; H, 5.12; N, 14.27%.
2.9 Spectral representation of synthesized compounds:

$^1$H NMR spectrum of VBA 6 (intermediate)

$^1$H NMR spectrum of VBA- 8a
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$^1$H NMR Expanded spectrum of VBA 8a

$^{13}$C NMR spectrum of VBA 8a

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DEPT NMR spectrum of VBA 8a

13C NMR spectrum of VBA 8e
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$^1$H NMR spectrum of VBA- 8e

DEPT NMR spectrum of VBA 8e
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$^1$H NMR spectrum of VBA 8i

$^{13}$C NMR spectrum of VBA 8i
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$^1$H NMR spectrum of VBA 10a

$^{13}$C NMR spectrum of VBA 10a
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\[ \text{H NMR spectrum of VBA 10i} \]

\[ \text{\^{13}C NMR spectrum of VBA 10i} \]
Mass spectrum of VBA 8i

Mass spectrum of VBA 10a
Mass spectrum of VBA 10h

Mass spectrum of VBA 10i
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IR spectrum of VBA 10e

![Image of IR spectrum of VBA 10e]

IR spectrum of VBA 10i

![Image of IR spectrum of VBA 10i]
Chemical purity of VBA-6a

**Table 2.1**

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**Mobile phase:** H₂O: Acetonitrile (30:70) premix; Diluent: Methanol

Chemical purity of VBA-8i

**Table 2.2**

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Chemical purity of VBA-10i

Column: phenomenox (250X4.6), 5µ,
Injection volume: 20 µL,
Mobile phase: H₂O: Acetonitrile (30:70) premix;
Diluent: Methanol
Column Temp-30°C
Flow-1mL/min

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![Graph showing chemical purity of VBA-10i](image-url)
2.10 REFERENCES


44. Francesco Piscitelli, Alessia Ligresti b, Giuseppe La Regina, Valerio Gatti, Antonella Brizzi, Serena Pasquini, Marco Allarà, Mauro Antonio Maria Carai, Ettore Novellino, Giancarlo Colombo, Vincenzo Di Marzo, Federico Corelli, Romano Silvestri, European Journal of Medicinal Chemistry 2011, 46 5641.


