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# European Reviews of Chemical Research

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Has been issued since 2014.  
E-ISSN 2413-7243  
2021. 8(1). Issued once a year

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Release date 15.12.21  
Format 21 × 29,7/4.

Website: <https://ercr.cherkasgu.press>  
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Founder and Editor: Cherkas Global University Press  
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**European Reviews of Chemical Research**

2021

Is. **1**

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Published in the USA  
 European Reviews of Chemical Research  
 Has been issued since 2014.  
 E-ISSN: 2413-7243  
 2021. 8(1): 3-16

DOI: 10.13187/ercr.2021.1.3  
<https://ercr.cherkasgu.press>



## Articles

### Biological Potential of Pyrazole, and Triazole Derivatives: A Mini Review

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#### Abstract

The main objectives of this review to study of synthetic routes and biological activities of substituted Pyrazole, and 1,2,4-Triazole derivatives. These Pyrazole, and 1,2,4-Triazole derivatives have various types of biological activities such as antibacterial, antifungal, analgesic, antimicrobial, antiinflammatory, anticancer, antidepressant, anticonvulsant, anti-hyperglycemic, antipyretic, fungicidal, anti-arthritis and other biological activities. Moreover, some of the currently available drugs have been shown to exhibit unfavourable side effects and toxicity. It is well established that small modifications in the structure of the targets are altering their biological character as well as their physicochemical properties.

**Keywords:** biological potential, heterocyclic, pyrazoles, triazole, drugs.

#### 1. Introduction

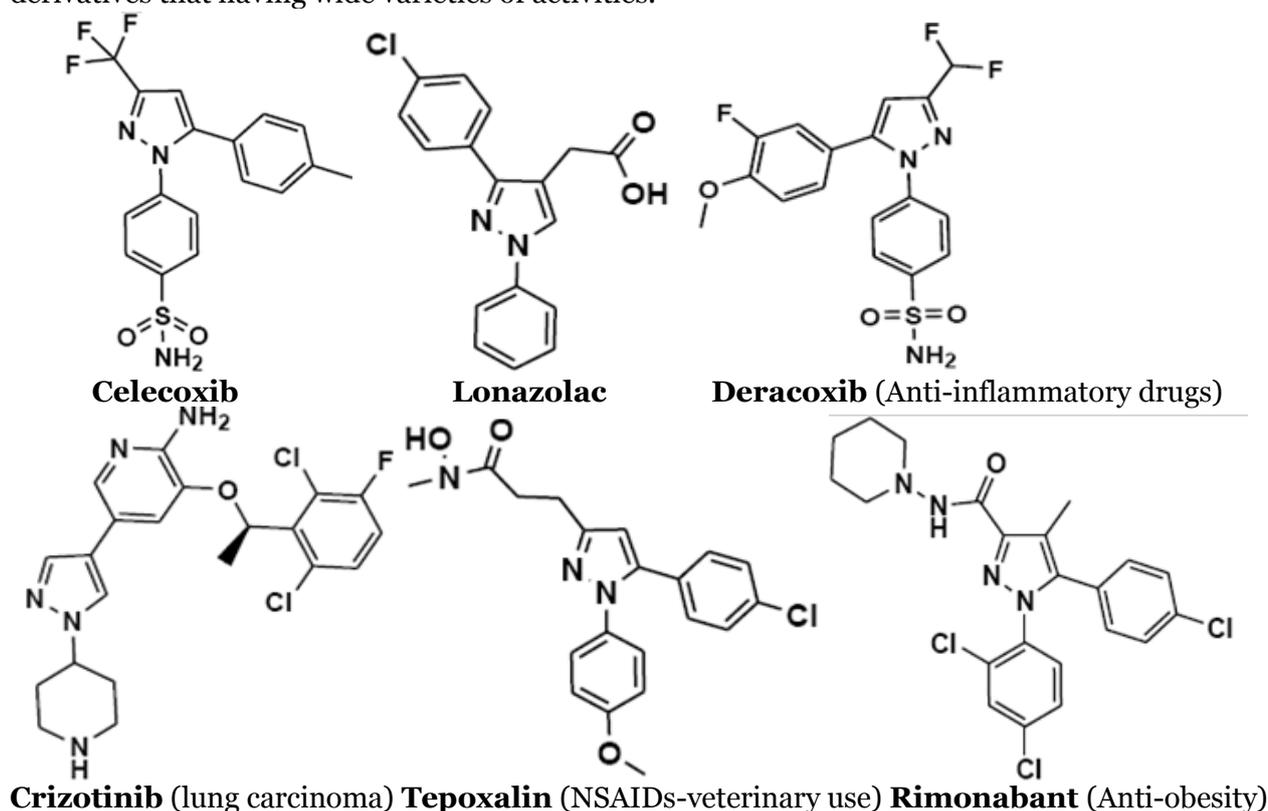
Heterocyclic compounds offer a high degree of structural diversity and have proven to be broadly and economically useful as therapeutic agents (Harikishan, Kapoor, 2008). It is well-known that heterocyclic compounds having azole nucleus are important pharmacophore that appear extensively in various types of pharmaceutical agents, widely implicated in biochemical processes and display diversity of pharmacological activities. These heterocyclic compounds form a major part of organic chemistry; they are widely distributed in nature and play a vital role (Agarwal, 2002; Kalpesh et al., 2009). Their practical applications range from extensive clinical use to fields as diverse as medicine, agriculture, photochemistry, biocidal activities. Many heterocyclic compounds synthesized have been successfully used as clinical agents. The chemistry of heterocycles has played a vital role in combating many deadly diseases. Various heterocyclic compounds are essential to our life and their functions are often of fundamental importance for living systems (Ravindra et al., 2006). Amongst the heterocyclic compounds pyrazoles and Triazoles have attracted a tremendous attention, in the biological and industrial applications (Chen et al., 1999; Moise et al., 2009). The biological activities are invariably associated with a large variety of heterocyclic systems such as Pyrazole and Triazole. Various new derivatives have been synthesized and extensively studied for various pharmacological properties.

#### 2. Results and discussion

**Biological importance of Pyrazoles.** The pyrazole ring system consists of a doubly unsaturated five member ring containing two adjacent nitrogen atoms. The procedures for its

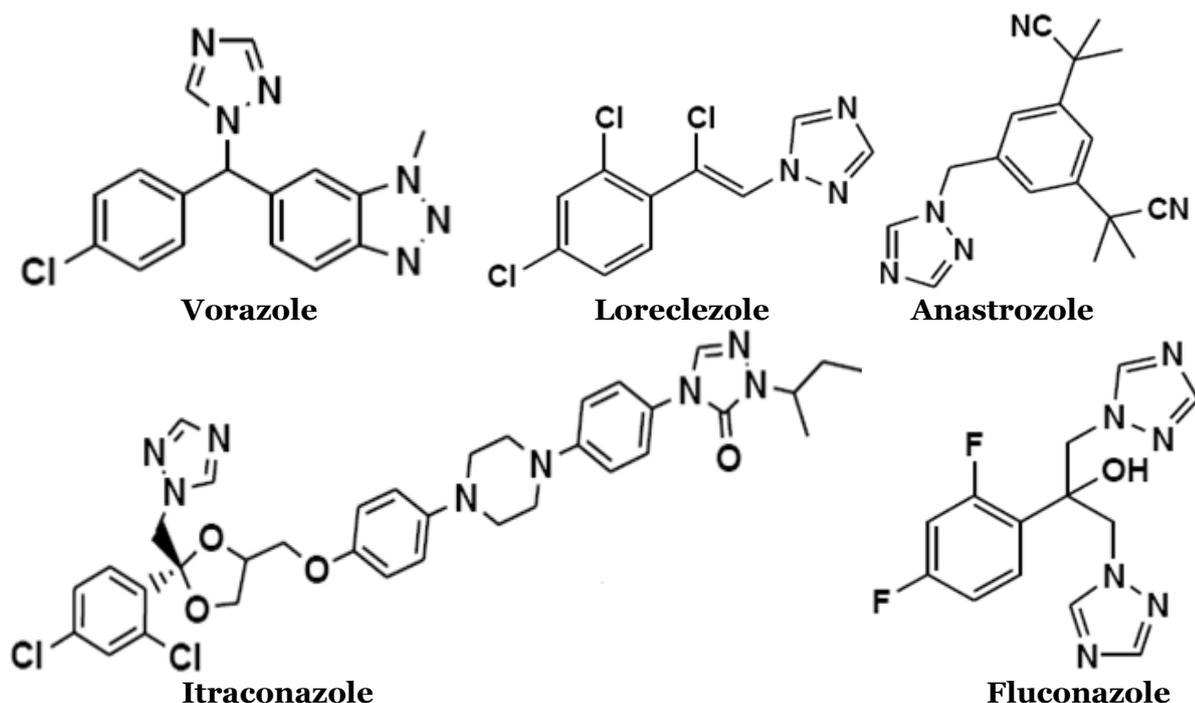
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synthesis have been extensively studied and such studies have been stimulated by various promising applications, especially in the case of highly substituted pyrazole derivatives. In fact, certain substituted pyrazoles are used as antimicrobial (Pimerova, Voronina, 2001), anticancer (Magedov et al., 2007), antiinflammatory (Rovnyak et al., 1982), antidepressant (Prasad et al., 2005), anticonvulsant (Ozdemir et al., 2007), antihyperglycemic (Ozdemir et al., 2007), antipyretic (Sener et al., 2002), antibacterial (Liu et al., 2008), antifungal (Akbas, Berber, 2005; Delany, 1991), anti-arthritic (Rangari et al., 1990) activities. The 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. A synthetic approach on medicinal properties of pyrazole derivatives that having wide varieties of activities.



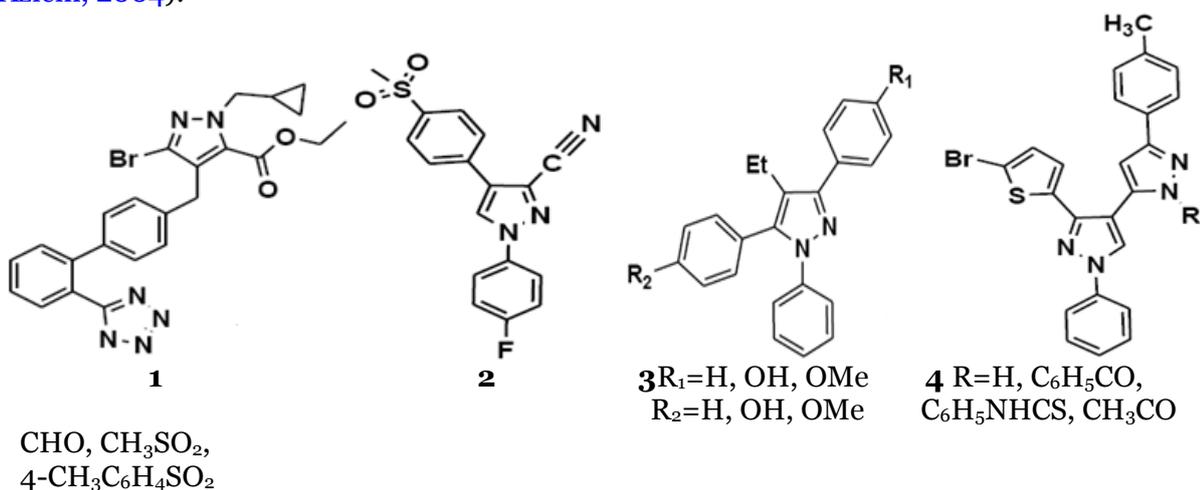
**Fig. 1.** Some Pyrazole containing drugs

**Biological importance of 1,2,4-Triazole:** The drug molecule is one of the most challenging tasks to the medicinal chemist. The synthesis of high nitrogen containing heterocyclic systems has been attracting increasing interest over the past decade because of their utility in various applications, such as propellants, explosives, pyrotechnics and especially chemotherapy. The chemistry of Triazoles and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance. The derivatization of Triazole is considered to be based on the phenomenon of bioisosterism in which replacement of oxygen of oxadiazole nucleus with nitrogen atom yields triazole analogue. 1,2,4-Triazole moiety is of great importance to chemists as well as biologist as it is chemically useful molecules having diverse biological activities. Triazole, a heterocyclic nucleus has attracted a wide attention of the medicinal chemist in search for the new therapeutic molecules. Out of its two possible isomers, 1,2,4-triazole is which posses almost all types of biological activities. Some of the drugs which are having Triazole as core molecule are given below (Figure 2), several 1,2,4-Triazole containing compounds are used as drugs for instance Fluconazole is used as an antimicrobial drug, while Vorozole, Letrozole and Anastrozole are used as non steroidal drugs used for the treatment of cancer. Loreclezole is used as an antifungal agent.

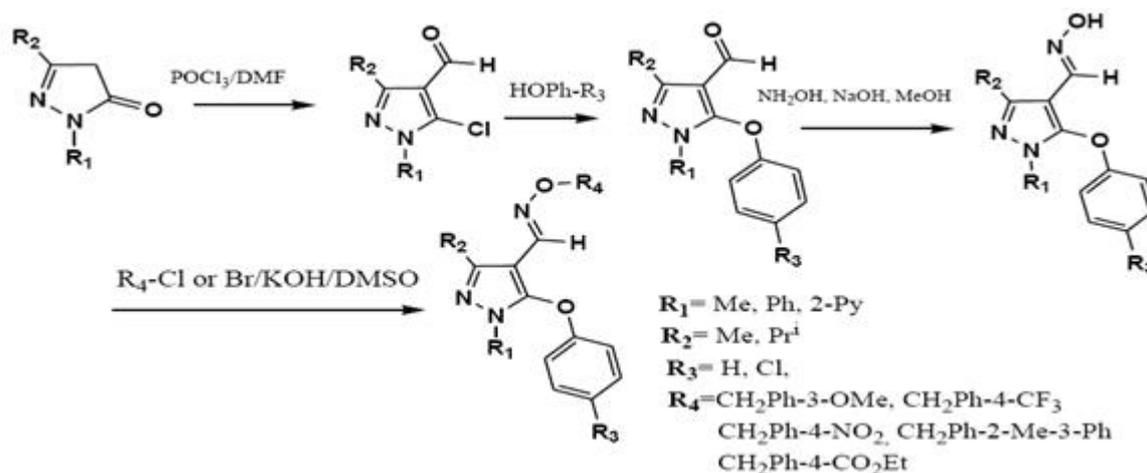


**Fig. 2.** 1,2,4-triazole containing drugs

The N-alkyl biaryl tetrazoles containing pyrazole nucleus, among the synthesized compound, (1) was found to be highly potent antagonists of angiotensin II (Watson et al., 1994). 1,4-Diaryl pyrazole derivative (2) was tested for anti-inflammatory and analgesic activities to develop anti-inflammatory agents with fewer side effects than existing non-steroidal anti-inflammatory drugs (Kiyoshi et al., 1997). The 4-alkyl-1,3,5-triarylpyrazoles (3) which are useful as estrogen receptor (Huang, Katzenellenbogen, 2000). The novel series of structurally related 1*H*-pyrazolyl derivatives (4) were tested for their anti-inflammatory and antimicrobial activities. COX-1 and COX-2 inhibitory activities, ulcerogenic effects and acute toxicity were also determined (Bekhit, Abdel-Aziem, 2004).

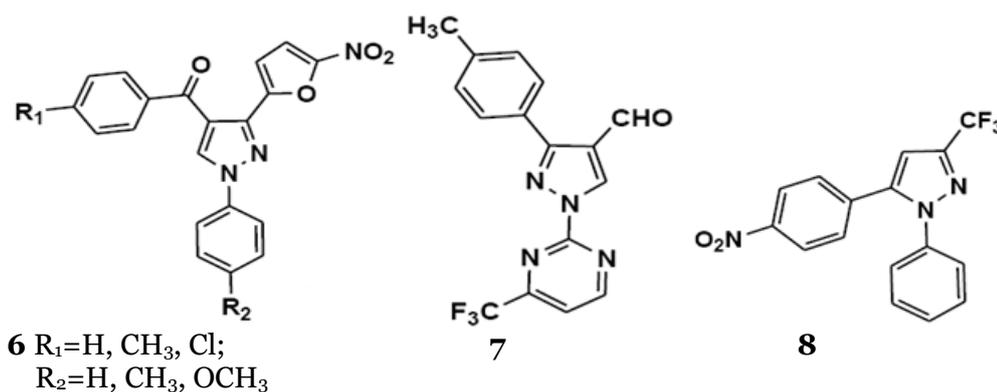


A series of Pyrazole oxime ether derivatives (5) were examined its cytotoxicity activities. Among those, 5-phenoxy pyrazole exhibited very potent cytotoxicity comparable to Doxorubicin (Scheme 1) (Park et al., 2005).

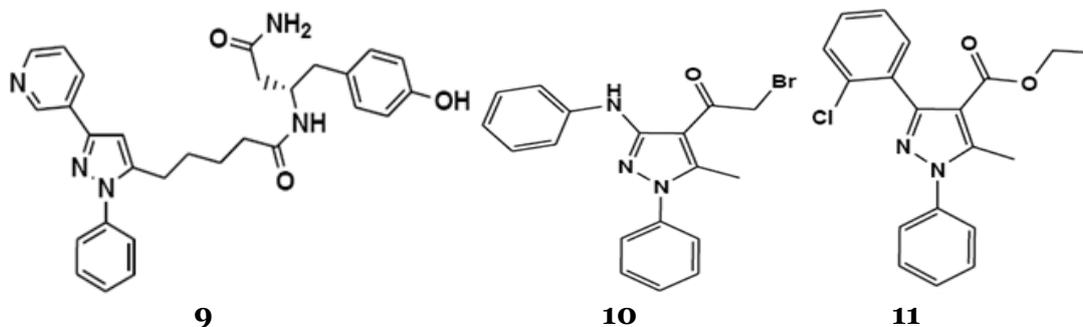


**Scheme 1.4:** pyrazole oxime ethers derivatives

A series of pyrazole derivatives (**6**) via 1,3-dipolar addition of sydnone and nitro furan acetylenic ketones were reported (Rai et al., 2006). A series of 1,5-diaryl and 1,3-diaryl substituted pyrazoles were evaluated for their ability to inhibit enoyl-ACP reductase of *Plasmodium falciparum*. The inhibitory activity of these compounds was evaluated in a continuous spectrophotometric assay. Of all the tested compounds (**7**) and (**8**) inhibited the enzyme with IC<sub>50</sub> values of 30  $\mu\text{M}$  and 50  $\mu\text{M}$ , respectively (Kumar et al., 2006).

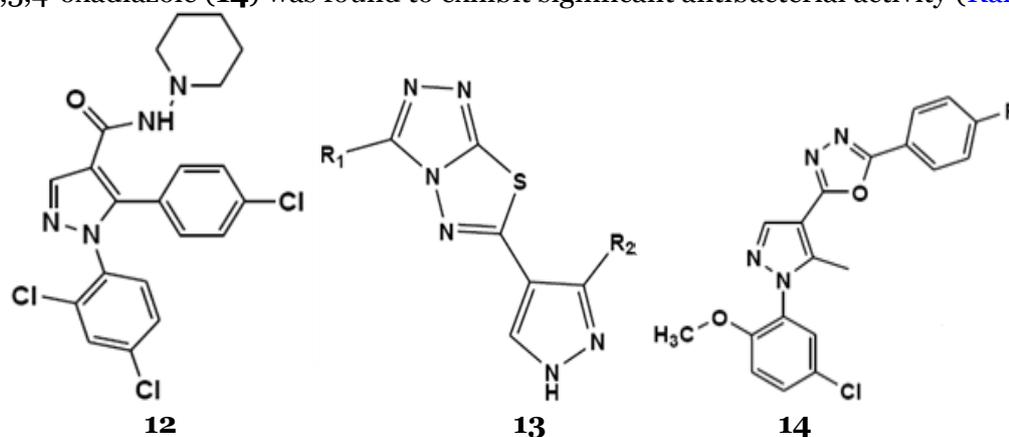


The pyrazole compounds (**9**) as low molecular weight luteinizing hormone receptor agonists (Jorand-Lebrun, 2007). The N-pyrazole derivatives were used as antimicrobial. Among the compounds tested for antimicrobial, compound (**10**) shown very good activity against pathogenic mould (*Aspergillus*) (Farag et al., 2008). Synthesized and discovered a novel pyrazole derivative (**11**) as an inhibitor of apoptosis through modulating integrin  $\beta_4$ , ROS, and p53 levels in vascular endothelial cells (Zhao et al., 2008).

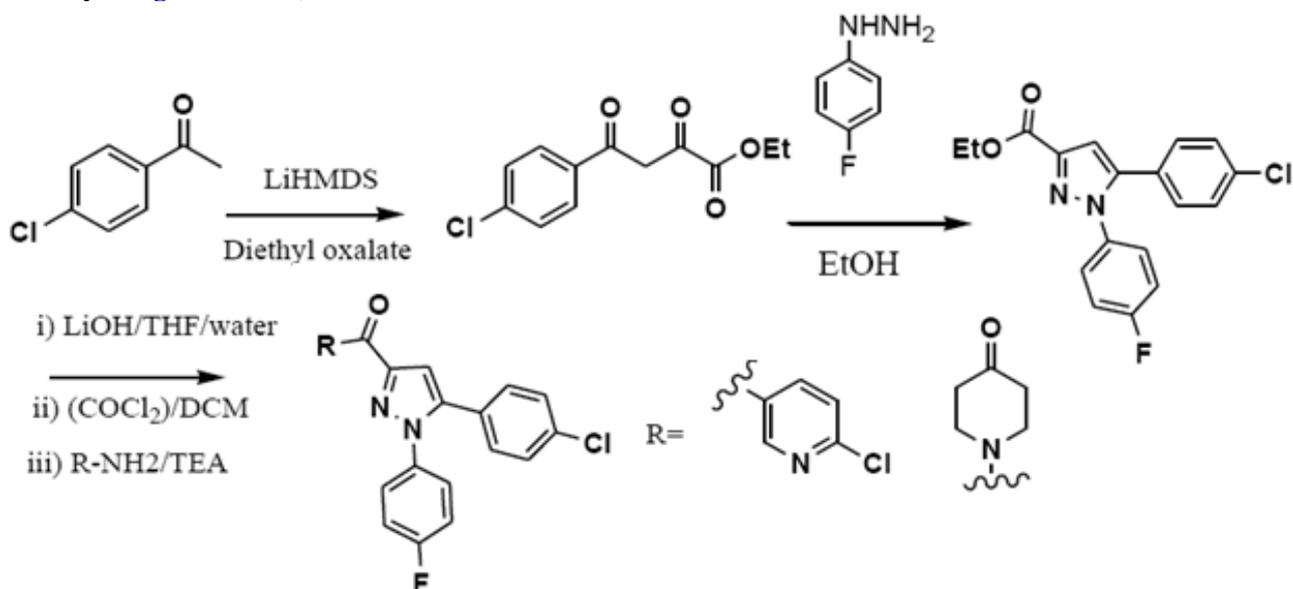


A set of 1-(2,4-dichlorophenyl)-5-arylpyrazoles were evaluated in vitro for their affinity on human CB1 and CB2 receptors. Among the compounds (**12**) was the closest rimonabant analogue

and showed competitive binding of 79 % and 37 % against CB1 and CB2 receptor respectively (Menozzi et al., 2008). The synthesis and anticancer activity of 3,6-disubstituted 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles containing pyrazole moiety (**13**) (Dhanya et al., 2009). A series of 1,3,4-oxadiazole containing pyrazole derivatives and studied its antibacterial activities. Among the compounds, 2-[1-(5-chloro-2-methoxyphenyl)-5-methyl-1H-pyrazol-4-yl]-5-(4-fluorophenyl)-1,3,4-oxadiazole (**14**) was found to exhibit significant antibacterial activity (Rai et al., 2009).

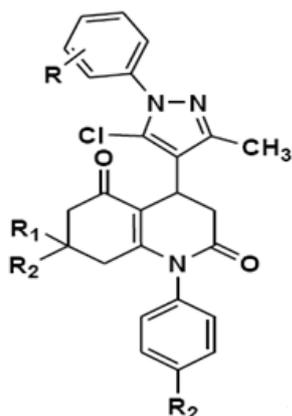


A series of 1,5-diaryl pyrazole derivatives (**Scheme-2**) (**15**) and screened for their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. Similarly all these compounds were screened for their antifungal activity against *Aspergillus flavus*, *Aspergillus fumigates*, *Penicillium marneffe* and *Trichophyton mentagrophytes*. Some of the synthesized compounds exhibited good antibacterial and antifungal activity (Ragavan et al., 2010).



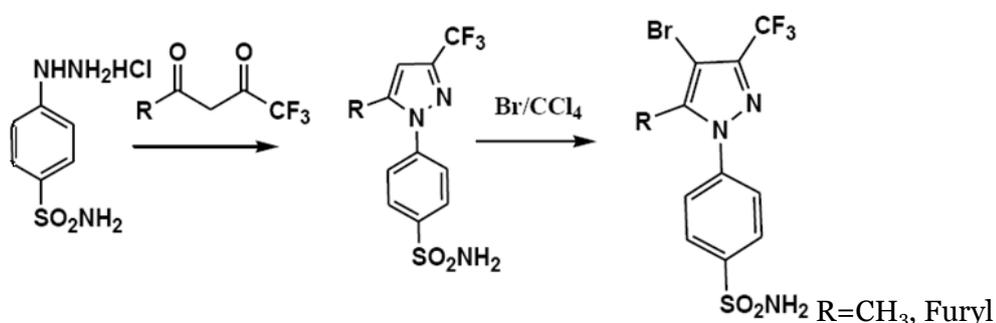
**Scheme 2.** Synthesis of novel 1,5-diaryl pyrazoles

A series of 4-pyrazolyl-*N*-arylquinoline-2,5-dione derivatives (**16**) and are screened, against some of the bacterial pathogens. Some of the compounds were found to be equipotent or more potent than commercial drugs (Thumar, Patel, 2011).



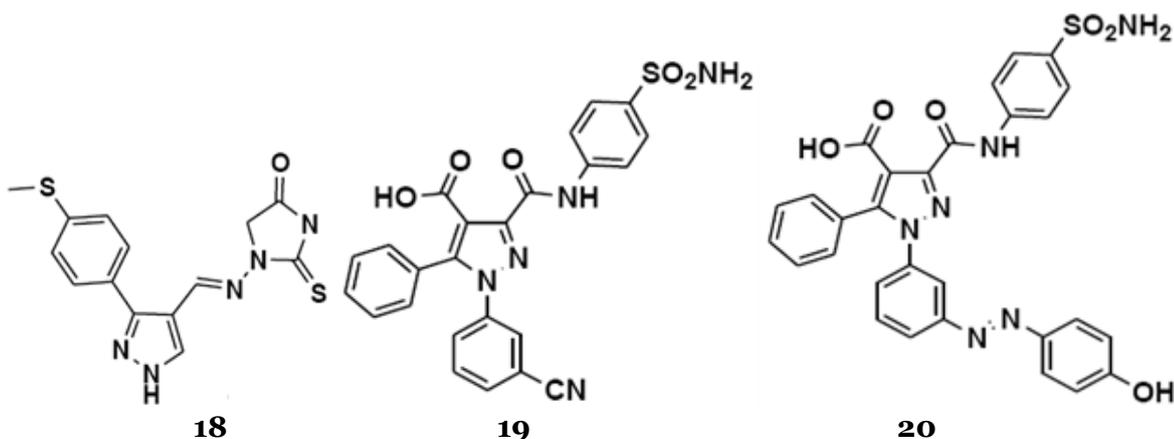
**16** R=3-Cl, 4-Me R<sub>1</sub>=H, Me R<sub>2</sub>=F, OMe

The fluorinated pyrazoles (**17**) (**Scheme-3**) has been observed that Preliminary biological screening of the compounds revealed significant antidiabetic and antibacterial activities ([Faidallah et al., 2011](#)).

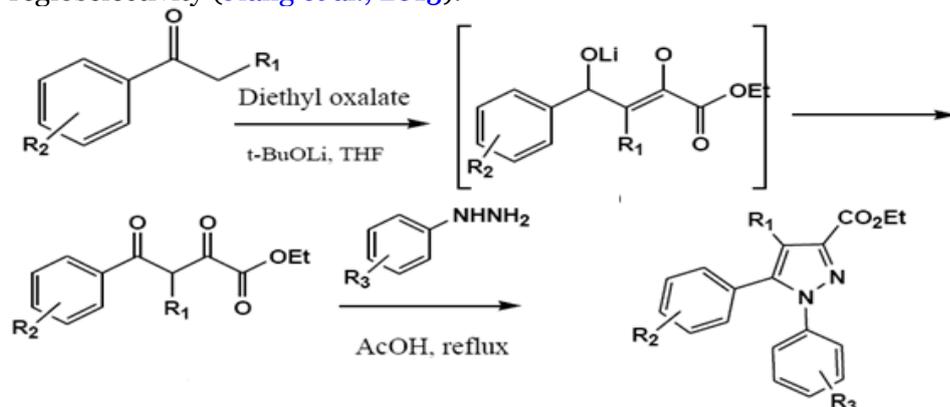


**Scheme 3.** 3-Trifluoromethylpyrazolesulfonyl-urea and thiourea derivatives

A series of novel imidazole derivatives containing substituted pyrazole moiety. Among the compounds, compound (**18**) was found to be potent antimicrobial agent. The acute oral toxicity study for the compound was carried out and the experimental studies revealed that compound is safe up to 3000 mg/kg and no death of animals were recorded ([Vijesh et al., 2011](#)). A series of pyrazole-sulfonamide derivatives (**19**) and (**20**) were synthesized and the inhibition effects of the derivatives on human carbonic anhydrases (hCA I and hCA II) were investigated as in vitro. Almost all the compounds have good inhibition effects on the CA I and CA II isoenzymes ([Balseven et al., 2013](#)).

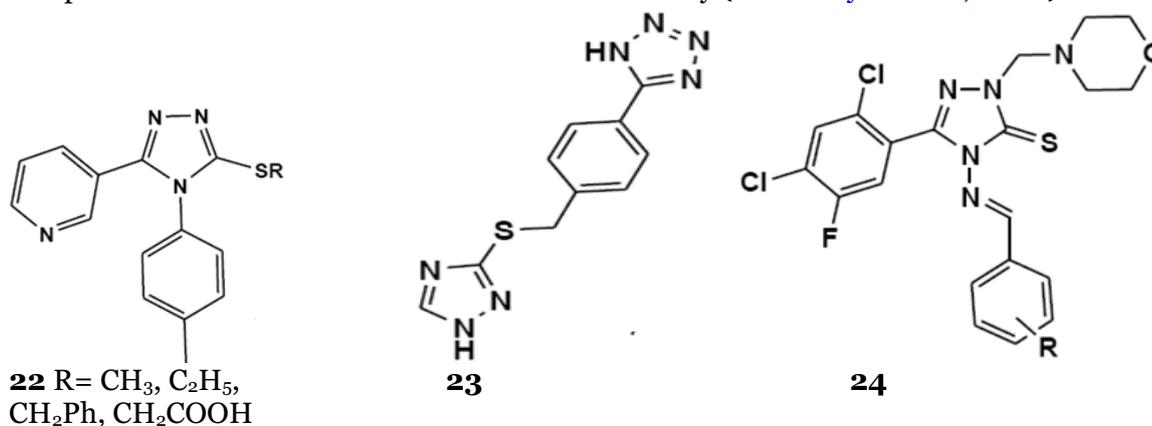


A concise 'one-pot' synthesis of a variety of 4-substituted 1,5-diaryl-1*H*-pyrazole-3-carboxylates (**21**) has been developed in moderate to good yields (**Scheme 4**) with excellent regioselectivity (Jiang et al., 2013).

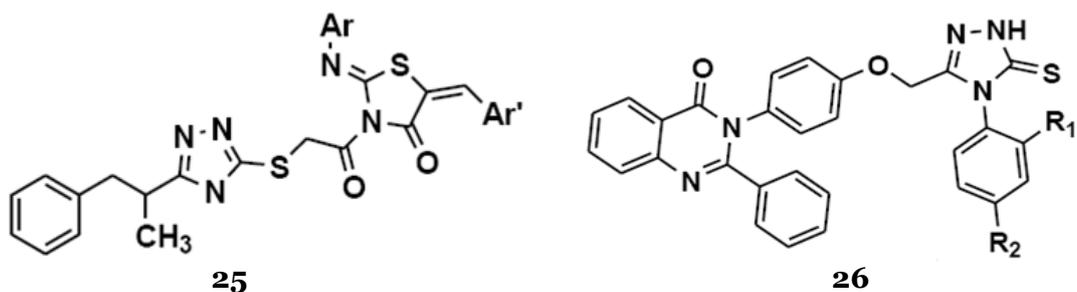


**Scheme-4.** Synthesis of 4-substituted 1,5-diaryl-1*H*-pyrazole-3-carboxylates via lithium *tert*-butoxide medium

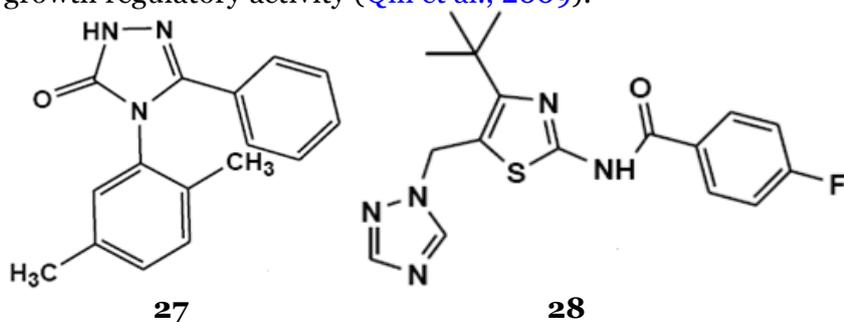
A series of 1,5-(isomeric pyridyl)-4-aryl-1,2,4-triazole-3-thiol, -thioethyl, thiomethyl, thiobenzyl derivatives from pyridine carboxylic acid hydrazide (**22**) (Zamani et al., 2003). Series of 3-benzylsulfanyl derivatives of 1,2,4-triazole were synthesized by alkylation of starting triazole-3-thiol with appropriately substituted benzyl halide (**23**). All members of the set were evaluated for *in vitro* anti-TB activity. The compounds exhibited only a moderate or slight anti-TB activity. MICs fall into a range of 32->1000  $\mu\text{mol/l}$ . The most active substances bear two nitro groups or a thioamide group on the benzyl moiety (Klimesova et al., 2004). Some Schiff bases bearing 2,4-dichloro-5-fluorophenyl moiety (**24**) by condensing triazole with aromatic aldehydes. Synthesized compounds were tested for their antimicrobial activity (Karthikeyan et al., 2006).



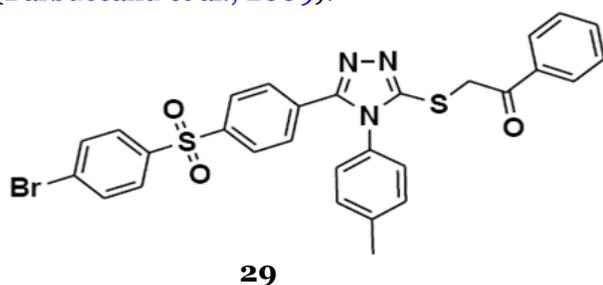
Anticonvulsant activity of Thiazolidinone-triazole derivatives (**25**), By the treatment of (2-chloroacetyl)-2-arylimino-5-[(*Z*)-arylmethylidene]-1,3-thiazolan-4-ones with 5-(1-phenoxyethyl)-4*H*-1,2,4-triazole-3-thiol in identical conditions provided a set of bulkier derivatives which have also shown the anticonvulsant potential (Shiradhar, Nikalje, 2007). The 3-[4-(4-substituted phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-ylmethoxy)-phenyl]-2-phenyl-3*H*-quinazolin-4-one (**26**). The synthesized compounds were evaluated *in vitro* for their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* by the ditch-plate technique using concentrations of 50  $\mu\text{g/mL}$ . The compounds synthesized were screened for their antifungal activity against *Aspergillus niger*, *Candida albicans* and *Cryptococcus neoformans* at concentrations of 50  $\mu\text{g/mL}$  (Havaldar, Patil, 2008).



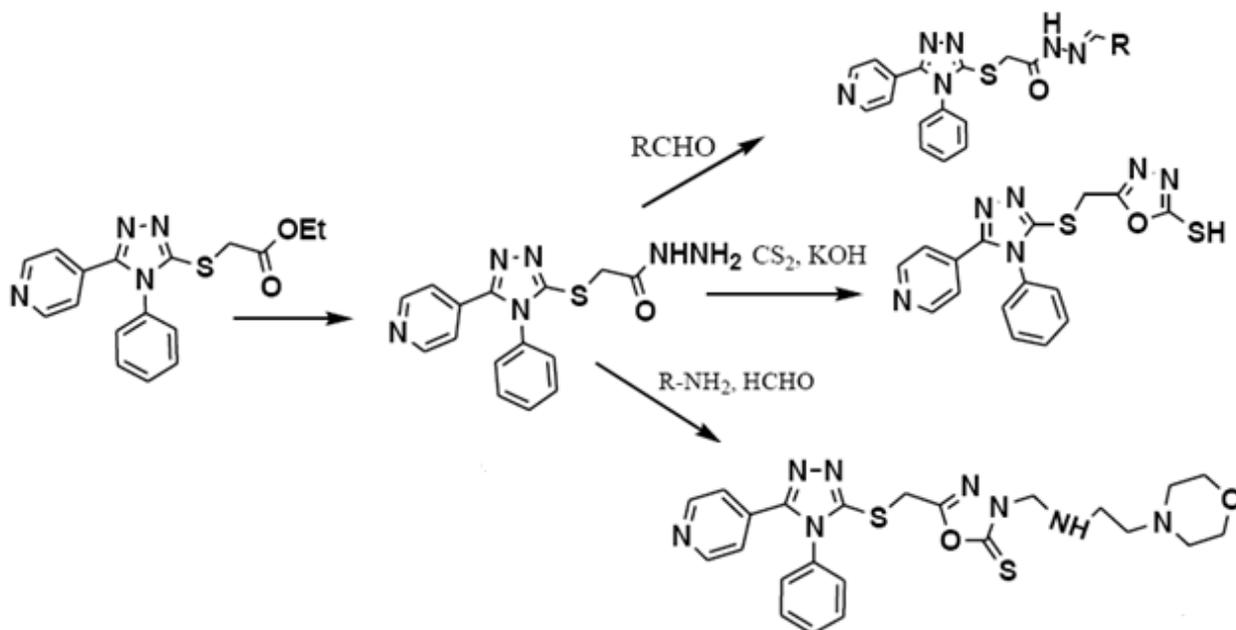
Some substituted diphenyl-1,2,4-triazole-3-ones by the condensation of substituted benzoyl chlorides and substituted phenyl semicarbazides. The anticonvulsant activities of these compounds were screened by using different animal models. Results show that compound (**27**) exhibited anticonvulsant activity in all the four animal models of seizure. A series of N-(5-((1H-1,2,4-triazol-1-yl)methyl)-4-tertbutylthiazol-2-yl)-4-carboxamide derivatives from 3, 3-dimethyl butan-2-one. The presence of fluorine atom at position 2,3,4 of phenyl ring are crucial for exhibited plant-growth regulatory activities and the substitution with chlorine atom at both 2nd position and 4th position of benzene ring caused a decrease of the activity while the presence of a strong electron-withdrawing group such as nitrogroup led to decrease in activity. Compound (**28**) having fluorine atom at 4th position connected to the phenyl ring produced excellent plant-growth regulatory activity ([Qin et al., 2009](#)).



The 1,2,4-triazoles incorporated diphenyl sulfone was synthesized. The compounds were tested for its antibacterial activity. The compounds were tested for their *in vitro* growth inhibitory activity against the following Gram-negative bacteria and Gram-positive bacteria using the paper disk diffusion method. Among these compound (**29**) showed more active against the tested strains ([Barbuceanu et al., 2009](#)).

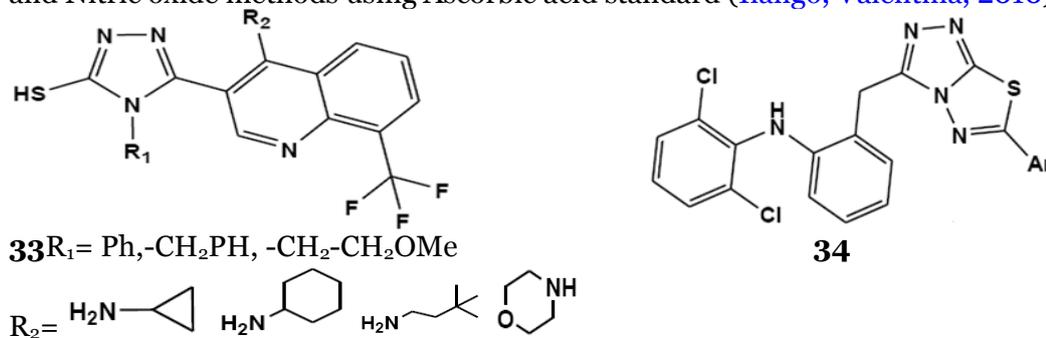


Some new 1,2,4-triazoles and their Schiff and Mannich bases (**Scheme 5**) (**30-32**) and screened for their antimicrobial activities. Some of the screened compounds showed good activity ([Bayrak et al., 2009](#)).

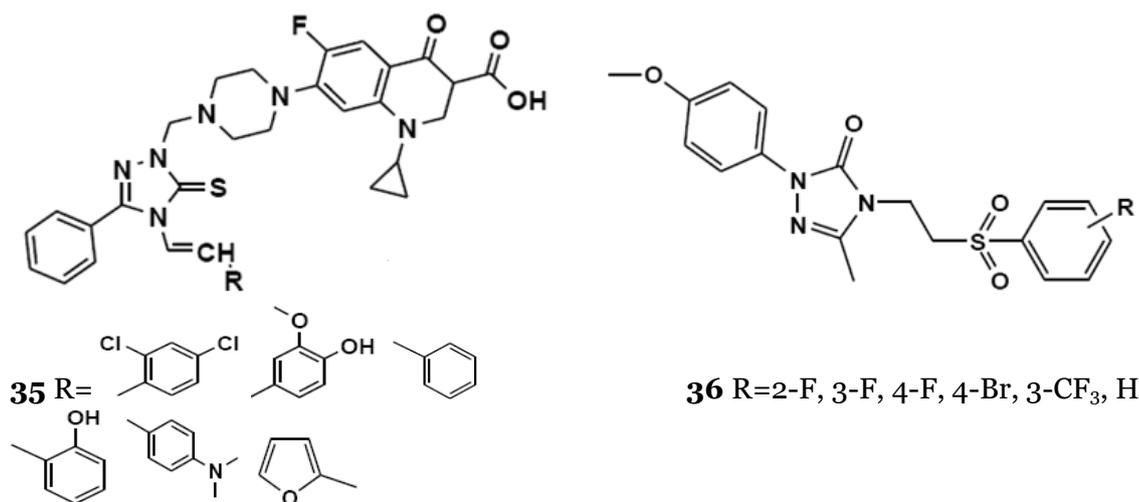


**Scheme 5.** 1,2,4-Triazoles, their Schiff and Mannich bases

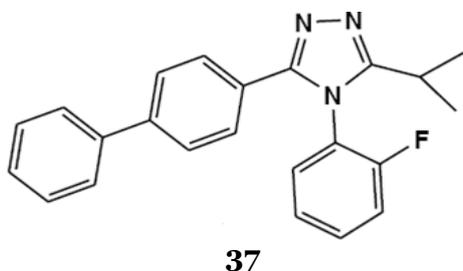
A class of quinoline derivatives containing 1,2,4-triazole moiety (**33**). The compounds were tested for their in vitro antibacterial and antifungal activities against four strains each. Preliminary results indicated that most of the compounds demonstrated very good antimicrobial activity, comparable to the first line standard drugs. The most effective compounds have exhibited activity at MIC of 6.25  $\mu\text{g}/\text{mL}$  (Eswaran et al., 2009). A series of 3, 6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles. The compounds (**34**) were screened for antifungal activity against *Candida albicans* and *Aspergillus Niger* using Ketoconazole as standard and antioxidant activity by DPPH and Nitric oxide methods using Ascorbic acid standard (Ilango, Valentina, 2010).



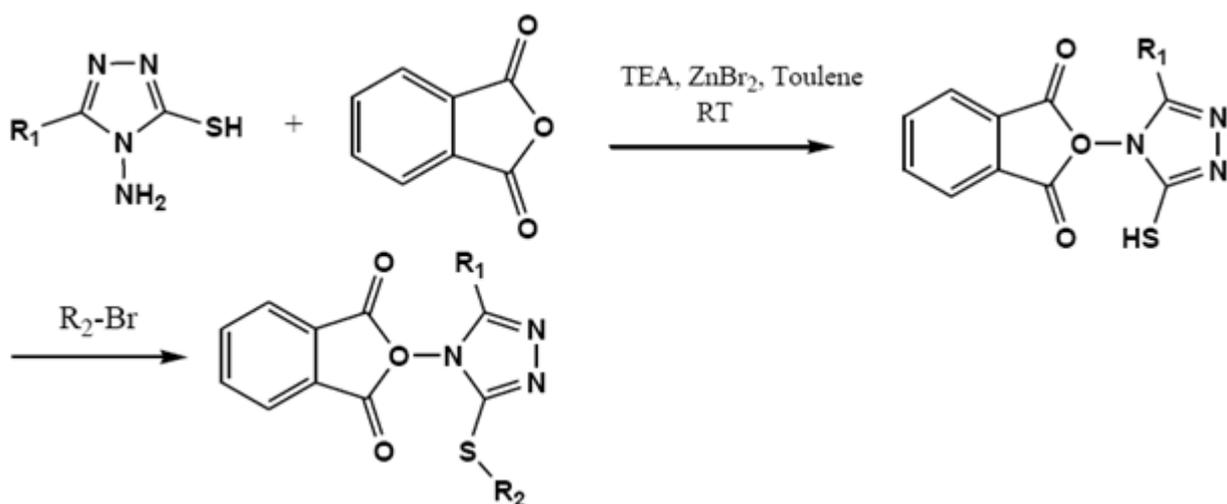
Some novel Ciprofloxacin analogues (**35**) as antimicrobial agents, Ciprofloxacin have been incorporated to the new series of Schiff and Mannich reaction. The compounds have been evaluated in vitro for their antimicrobial activity against *B. subtilis*, *K. pneumoniae*, and *P. aeruginosa* at 10  $\mu\text{g}/\text{mL}$  concentration. All the compounds showed in vitro gram positive and gram negative activity generally comparable or superior to that of reference ciprofloxacin (Jubie et al., 2010). A series of sulfone containing 1,2,4-triazole derivatives. The compounds were screened for their antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The antifungal activity was tested against *Rhizopus oryzae*, *Aspergillus Niger*, *Aspergillus flavus*, *Candida albicans* and *Saccharomyces cerevisiae*. Among all the compounds synthesized, compound (**36**) exhibited significant antibacterial activity (Patil et al., 2010).



A series of glycine transporter 1 inhibitors derived from a high-throughput screening hit. A pharmacokinetic study was showed that compound (**37**) showed very good oral bioavailability and ameliorated learning impairment in passive avoidance tasks in mice (Sugane et al., 2011).



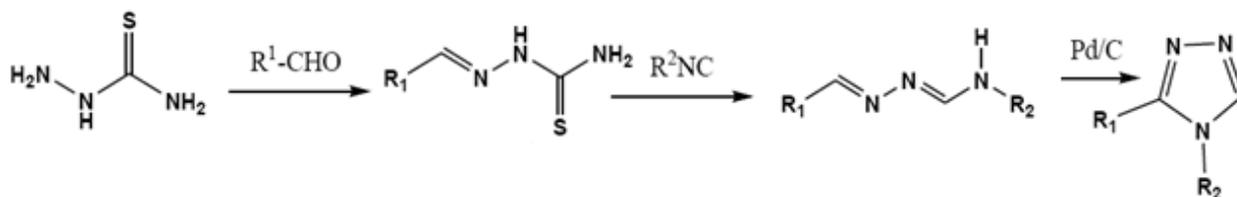
A series of novel isoindoline-1,3-diones containing 1,2,4-triazole moiety were synthesized *via* a one-pot reaction (Scheme 6). Antifungal and cytotoxic activities of these compounds were evaluated. Antifungal studies of the novel compounds showed promising activity (**38**). Some compounds displayed much stronger antitumor activity than Fluorouracil (Zhao et al., 2012).



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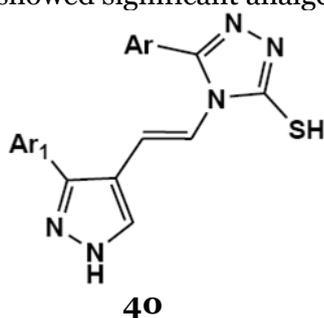
**heme 6.** Novel isoindoline-1,3-dione derivatives bearing 1,2,4-triazole derivatives

A two-step synthesis of medicinally important 1,2,4-triazoles from isocyanides and thiosemicarbazones (Scheme 7). The method is based on the discovered TMSCl-promoted reaction of isocyanides that yields rare N1, N3-disubstituted formamidrazones (**39**) (Sarnpitak, Krasavin, 2013).



**Scheme 7.** Synthesis of novel 1,2,4-triazoles from isocyanides and thiosemicarbazones

A series of new 1,2,4-triazole derivatives. All the synthesized compounds were screened for their analgesic activity by the tail flick method. The antimicrobial activity of the new derivatives was also performed by MIC by the serial dilution method. The results revealed that the compound having 2,5-dichlorothiophene substituent on pyrazole moiety and a triazole ring (**40**) showed significant analgesic and antimicrobial activity (Vijesh et al., 2013).



### 3. Conclusion

The present work, involving design, synthesis and characterization Pyrazole and Triazole derivatives and evaluation of their preliminary antibacterial, antifungal activities, has been aimed at development of new active compounds, which may have future commercial applications. To modify the structure of already existing drugs by improving the binding affinity to the receptor. Also, the correlation between structure activities of the new compounds would impart valuable information to assist the development of new types of drugs in new millennium. Furthermore, the results of research may be useful in understanding the mechanism of drug action. The research study is expected to add some more data to the chemistry of new heterocyclic compounds. The utility of above new heterocyclic compounds may be explored in other area of applications also.

### 4. Acknowledgements

We are thankful to the Department of Pharmaceutical Chemistry, Glocal University, Saharanpur, Uttar Pradesh, India for providing the necessary facilities.

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Published in the USA  
 European Reviews of Chemical Research  
 Has been issued since 2014.  
 E-ISSN: 2413-7243  
 2021. 8(1): 17-21

DOI: 10.13187/ercr.2021.1.17  
<https://ercr.cherkasgu.press>



## Isolation and Identification of Bacteria from Used Face Masks in Bauchi State University, Gadau

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### Abstract

The most traditional and realistic technique to avert the blowout of respiratory contaminations is to wear face masks; quite a few research clusters have established its efficacy against the spread of respiratory viruses before the COVID-19 pandemic. The current study aimed to isolate and identify bacteria from disposable face masks after use in Bauchi state university, Gadau. A total of 100 used face masks were collected from students of the university and subjected to microbiological and biochemical tests in order to isolate and identify the organisms from both the inner and outer surfaces of the used face masks. Most isolated bacterial contamination on both the outside area of the used face masks were *Staphylococcus aureus* 41.6 %, *Escherichia coli* 45.8 %, *Streptococcus pneumoniae* 8.3 % and *Streptococcus mutans* with 4.1 % but the number of organisms found on the outside were much lower. This study shows that the most abundant bacteria found in used face masks was *Escherichia coli* with the highest percentage of occurrence. The findings from this study show that bacteria were isolated from both the inner and outer surfaces of all the face masks used for this study, majority of which were Gram positive cocci. Only one-gram negative rod was identified and isolated.

**Keywords:** COVID-19, pandemic, contamination, microbiological, biochemical.

### 1. Introduction

The most traditional and reasonable method to prevent the spread of respiratory infections is to wear face masks; several research groups have demonstrated its effectiveness against the transmission of respiratory viruses before the COVID-19 pandemic (Chughtaita et al., 2020; Park et al., 2022). Non-woven, polyurethane, and gauze or cloth masks are the most popular types of face masks that are available commercially worldwide (Martinelli et al., 2021). Non-woven masks are commonly used worldwide to prevent droplet infections by most respiratory microbes, including SARS-CoV-2 (Furnaz et al., 2022). Among environmental pathogens, viruses cannot replicate without infecting host cells, however most bacteria and fungi can survive and grow on various materials depending on the environmental conditions (Park et al., 2022). Bacteria and fungi are widely present on the surface of the materials used in our daily lives such as currency notes and in public transportation systems (Jefferson et al., 2020; Zhao et al., 2022). Although a few studies reported bacterial or viral contamination on masks in experimental and clinical settings, there is a

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need to study the characteristics of the various microbes that are present on face masks used daily in our day-to-day activities (Park et al., 2022; Gonçalves et al., 2018). Since masks can be a direct source of infection to the respiratory tract, digestive tract, and skin, it is pertinent to maintain their hygiene to prevent life potentially threatening bacterial and fungal infections (CDC, 2020).

## 2. Materials and methods

### Sample collection

The partakers were males and females aged  $\geq 18$  years, and have voluntarily participated and signed the consent form. Furthermore, 100 disposable facemask samples were collected from school Gate, Faculty and Hostels in the same days during the 2 Weeks observations to investigate the microbial air quality (bacterial counts). The study was conducted with the ethical approval of the ethical committee of Bauchi State University Gadau. The 100 students were study from different departments such as: male and female school library, Male hostel, Female hostel, faculty of science, Faculty of medicine in the university.

A total of 100 disposable facemasks samples collected from the student study in university including: 60 samples from males and 40 samples from females.

Sampling was done during 12:00 to 15:00 p.m. in Monday, Wednesday and Friday for 1 week to assess bacterial counts. Sampling collection points includes school gate, male hostel, and female hostel, faculty of science faculty of medicine and school library.

## 2. Methods

A cross-sectional study was conducted to assess the bacterial contamination on 100 used disposable face masks from 100 students. The inside and outside area of the masks were analyzed.

The used disposable face masks, which were 100 in number, were collected to culture the bacterial counts. Inside and outside areas of the disposable face masks were and put in a sterile container consisting of distill water for 20 minutes. A spread plate method was used for determining total bacterial counts. General bacteria were cultivated in nutrient agar. The plates were incubated at  $37^{\circ}\text{C}$  for 48 hours to get the bacterial counts, and observation was done daily. After counting the isolated bacteria, they were preliminarily identified by Gram's stain and microscopic morphology (lacto-phenol cotton blue) following (Larone, 1995).

### Characterization and identification of the Isolates

Cultural characteristics, gram stain reaction and biochemical tests were used to characterize and identify the isolates.

### Gram Staining

A drop of water was placed on a sterile grease free slide, using a sterile wire loop, a small portion of inoculum were picked from 24 hours culture of isolate and emulsified on the drop of the distilled water until a thick homogeneous smear were made and it was heat fixed.

The smear was flooded with crystal violet for 60 seconds, then rinsed with water and afterwards a few drops of Lugol's iodine was added to the smear for 30 seconds and rinsed with water and 95 % alcohol was added drop wise until no drop of the crystal violet was seen dripping from the smear, it was then rinsed with water. Then the smear was flooded with safranin for 60 seconds then rinsed with water. It was air dried, then a drop of immersion oil placed on the smear and viewed under  $\times 100$  objective lens. The gram reaction of each of the isolate was noted and recorded.

### Biochemical Test

These include catalase test, coagulase test, oxidase test and indole test

### Catalase test

Two (2) drops of hydrogen peroxide solution was placed on a clean grease free slide, using sterile wire loop, a small portion of the isolates picked and emulsified on the drop of hydrogen peroxide and the presence and absence of effervescence was noted and recorded.

### Coagulase Test

A drop of distilled water was placed on a slide and the test colony was emulsified on the drop of the distilled water to make a thick suspension A loopful of plasma was added to the suspension and mixed gently and the presence or absence of agglutination was checked within 10 seconds

### Oxidase Test

A filter paper was placed in a clean Petri dish and two (2) drops of freshly prepared oxidase reagent added using a glass rod. A colony of the test organism was removed and smeared on the filter paper the presence or absence of blue-purple colour was taken note of and recorded.

### Indole test

The test organism was inoculated in a test tube containing 3ml of sterile tryptone water. It was incubated at 37°C for 48 hours. 0.5ml Kovac's reagent was added and shaken gently. The reaction was observed for the presence or absence of red layer on the surface and was taken note of.

## 3. Results

A total of one hundred (100) used face masks were collected from one hundred students to isolate and identify bacterial contamination. Results revealed that the bacterial contamination on the inside and outside of the used face masks was 22-25 cfu/ml/piece.

**Table 1.** Bacterial contamination (cfu/ml/piece.) observed on inner surface of the used face masks (N = 100)

BACTERIA	FREQUENCY (CFU/ml)	% OF OCCURRENCE (%)
S. aureus	5	20.8
E. coli	6	25.0
S. pneumoniae	7	29.1
S. mutans	6	25.0
TOTAL	24	100

Key: E. coli = Escherichia coli, S. pneumonia = Streptococcus pneumonia, S. mutans = Streptococcus mutans, S. aureus = Staphylococcus aureus.

**Table 2.** Bacterial contamination (cfu/ml/piece.) observed on the outer surface of the used face masks (N = 100)

BACTERIA	FREQUENCY (CFU/ml)	% OF OCCURRENCE (%)
S. aureus	10	41.6 %
E. coli	11	45.8 %
S. pneumoniae	2	8.3 %
S. mutans	1	4.1 %
TOTAL	24	100 %

Key: E. coli = Escherichia coli, S. pneumonia = streptococcus pneumoniae, S. mutans = streptococcus mutans, S. aureus=staphylococcus aureus

**Table 3.** Biochemical identification of bacteria & Gram staining

ORGANISM	CATALASE TEST	COAGULASE TEST	OXIDASE TEST	INDOLE TEST	GRAM REACTION	SHAPE
S. aureus	+	+	+	+	G+	Coccus
S. pneumoniae	-	-	-	-	G+	Coccus
E. coli	+	-	-	+	G-	Bacillus
S. mutans	-	-	-	-	G+	Coccus
Salmonella	+	-	-	-	G-	Bacillus

Key: E. coli=Escherichia coli. S. pneumoniae=streptococcus pneumoniae. S. mutans=streptococcus mutans. S. aureus=staphylococcus aureus.

#### 4. Discussion

This study was done to assess bacterial contamination on used face masks. The findings from this study show that bacteria were isolated from both the inner and outer surfaces of all the face masks used for this study, majority of which were Gram positive cocci. Only one gram negative rod was identified and isolated. This is in agreement with similar studies conducted by Chughtai et al. (2019) and those of Delanghe et al. (2021), there were more organisms on the outer surface than those detected on the inner surface, this maybe because of the difference in exposure to the outside between the two surfaces. Though the total number of organisms found were quite different, the findings were also in agreement with those of Gund et al. (2021), Pullangott et al. (2021) and those of Pullangott et al. (2021).

#### 5. Conclusion

The present study revealed high bacterial contamination on the outside and inside areas of the used disposable masks. Wearing a mask is an easy way to reduce the risk of unknowingly spreading the infection. To reduce the load of bacterial contamination on the used masks, the university environments, especially microbial air quality in the student hostels, lecture theaters and general school premises should be improved.

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