SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL PIPERAZINE DERIVATIVE

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Abstract:

Piperazine belongs to the family of medicines called anthelmintics. Anthelmintics are used in the treatment of worm infections.The synthesis and anti-bacterial activity of a series of 6 different derivatives of Piperazine moiety incorporatingdifferent aromatic halides moiety with increased antibiotic effect with the minimum side effect. The conventional methodology was adopted to synthesize the titled compound from starting material 4-(piperazine-1-yl) benzonitrile treated with anhydrous potassium carbonate which gives 4-(piperazine-1-yl)benzoic acid it was halogenated and reacted with hydrazine hydrate gives 4-(piperazine-1-yl) benzo hydrazide it was reacted with carbon disulfide followed by ammonia gives the title compound 5-[4piperazine-1-ylyl)phenyl]-4*H*-1,2,4-triazole-3-thiol (Q1-Q6). The synthesized compounds were characterized by TLC, melting point, IR, 1HNMR, and Mass spectral data. All synthesized Piperazine derivatives were screened for anti-bacterial activity using DMFas a solvent against the organisms, S. aureus and E.coli. The compound Q2,Q4, and Q5 have shown very good activity against S.aureus at 100μg/ml whencompared with the standard drug Ciprofloxacin. The compounds Q2, Q5, and Q6 haveshowngoodanti-bacterialactivity.

Keywords: Piperazine derivative, antibacterial activity, anthelmintic, S. aureus, E. Coli
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1. Introduction:

Piperazine were originally named because of their chemical similarity with piperidine, part of the structure of piperine in the black pepper plant (Piper nigrum). [1] The -az- infix added to "piperazine" refers to the extra nitrogen atom, compared to piperidine. It is important to note, however, that piperazine are not derived from plants in the Piper genus. Piperazine is freely soluble in water and ethylene glycol, but insoluble in diethyl ether. It is a weak base with two pKb of 5.35 and 9.73 at 25 °C.; the pH of a 10% aqueous solution of piperazine is 10.8–11.8. Piperazine readily absorbs water and carbon dioxide from the air. Although many piperazine derivatives occur naturally,

piperazine itself can be synthesized by reacting alcoholic ammonia with 1, 2-dichloroethane, by the action of sodium and ethylene glycol on ethylene diamine hydrochloride, or by reduction of pyrazine with sodium in ethanol. [2,3]

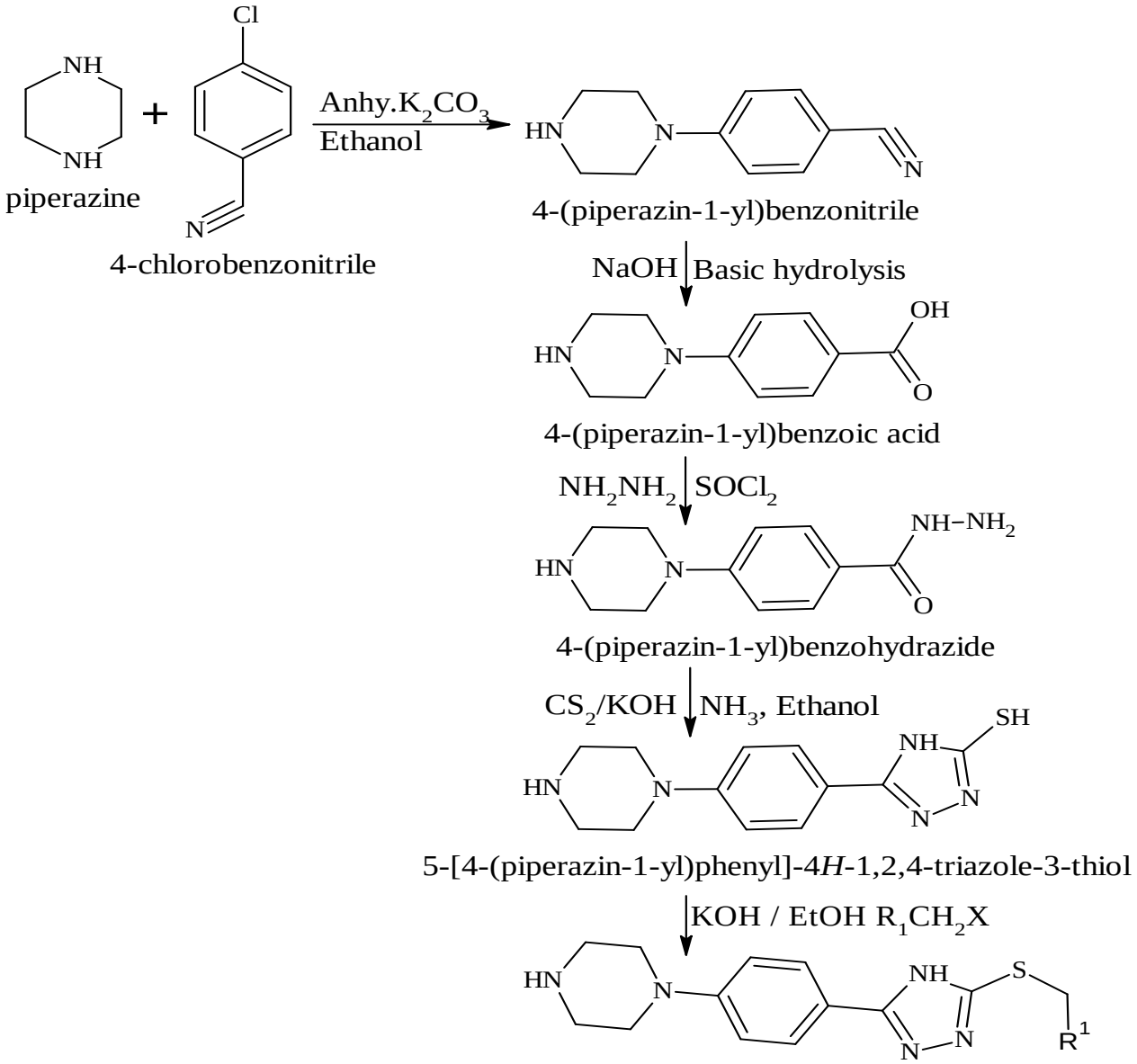
Piperazine is an anthelminthic especially useful in the treatment of partial intestinal obstruction caused by Ascaris worms, which is a condition primarily seen in children. Piperazine hydrate and piperazine citrate are the main anthelminthic piperazine. About 25% is metabolized in the liver. Piperazine is nitrosated to form N -mononitrosopiperazine (MNPz) in gastric juice, which is then metabolized to N-nitroso-3-hydroxypyrrolidine (NHPYR). LD50 = 5 g/kg (Human, oral). Symptoms of overdose include muscle fatigue, seizures, and difficulty breathing. [4]

Piperazine was marketed by Bayer as an anthelmintic in the early 20th century and was featured in print ads alongside other popular Bayer products at the time, including heroin. In fact, a large number of piperazine compounds have an anthelmintic action. Their mode of action is generally by paralyzing parasites, which allows the host body to easily expel the invasive organism. The neuromuscular effects are thought to be caused by blocking acetylcholine at the myoneural junction. This action is mediated by its agonist effects upon the inhibitory GABA (γ-aminobutyric acid) receptor. Its selectivity for helminths is because vertebrates use GABA only in the CNS, and the GABA receptor of helminths is of a different isoform from that of vertebrates. [3]

2. Materials and methods:

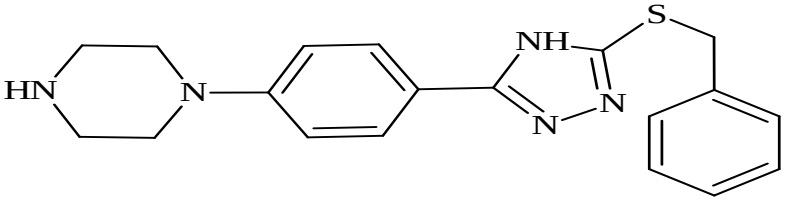
2.1 Experimental:

2.1.1 Scheme



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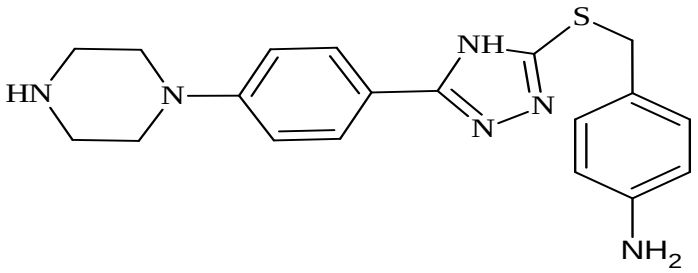
a) Preparationof1-{4-[5-(benzyl sulfanyl)-4H-1,2,4-triazol-3-yl]phenyl}piperazine (Q1):-[5,6]



1-{4-[5-(benzylsulfanyl)-4*H*-1,2,4-triazol-3-yl]phenyl}piperazine

$C_{19}H_{21}N_5S$, yield 78.70 %, Melting point 160-162˚C, $R_f$value 0.82, Recrystallization solvent Ethanol

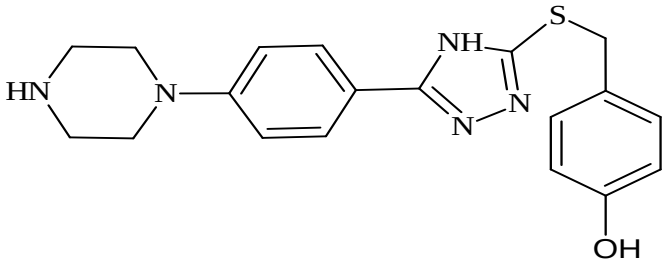
b) Preparation of 4-[({5-[4-(piperazin-1yl)phenyl]-4H-1,2,4-triazol-3yl}sulfanyl)methyl]aniline (Q2):-[7]



4-[({5-[4-(piperazin-1-yl)phenyl]-4*H*-1,2,4-triazol-3-yl}sulfanyl)methyl]aniline

$C_{19}H_{22}N_6S$, yield 90.74 %, Melting point 172-174˚C, $R_f$value 0.85, Recrystallization solvent Ethanol, -NHStretching 3138cm^{-1}, AromaticCHstretching 2950cm^{-1}, AliphaticCH 2890cm^{-1}, -C-CH2stretching 1150cm^{-1}, -C-S Stretching 1020 cm^{-1}, δ Value 0.00-3.00 -CH2, -CH2, δ Value 2.80-4.70 -NH, -NH2, δ Value 7.1-8.5 Ar-H multiplate.

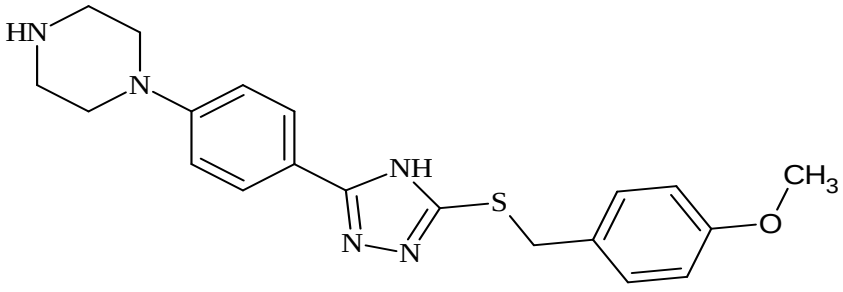
c) Preparation of 4-[({5-[4-(piperazin-1-yl)phenyl]-4H-1,2,4-triazol-3-yl}sulfanyl)methyl]phenol (Q3):-[8]



4-[({5-[4-(piperazin-1-yl)phenyl]-4*H*-1,2,4-triazol-3-yl}sulfanyl)methyl]phenol

$C_{19}H_{21}N_5SO$, yield 92.59 %, Melting point 186-188˚C, $R_f$value 0.94, Recrystallization solvent Ethanol.

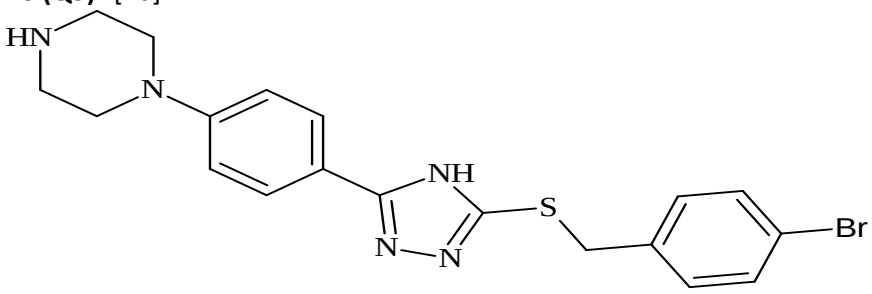
d) Preparation of 1-[4-(5-{[(4-methoxyphenyl)methyl]sulfanyl}-4H-1,2,4-triazol-3-yl)phenyl]piperazine (Q4):-[9]



1-[4-(5-{[(4-methoxyphenyl)methyl]sulfanyl}-4*H*-1,2,4-triazol-3-yl)phenyl]piperazine

$C_{20}H_{23}N_5SO$, yield 97.22 %, Melting point 180-182˚C, $R_f$value 0.88, Recrystallization solvent Ethanol.

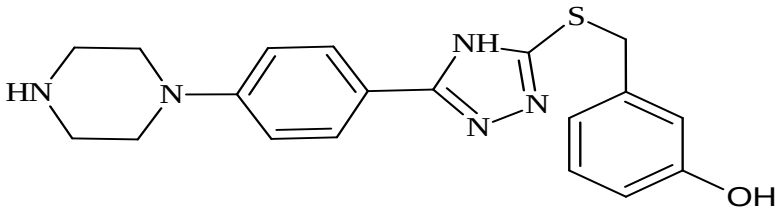
e) Preparation of 1-[4-(5-{[(4-bromophenyl)methyl]sulfanyl}-4H-1,2,4-triazol-3-yl)phenyl]piperazine (Q5):-[10]



1-[4-(5-{[(4-bromophenyl)methyl]sulfanyl}-4*H*-1,2,4-triazol-3-yl)phenyl]piperazine

$C_{19}H_{20}N_5SBr$, yield 69.44 %, Melting point 160-162°C, $R_f$value 1.02, Recrystallization solvent Ethanol, -NHStretching 3200 cm-1, AromaticCHstretching 3120cm-1, AliphaticCH 2890cm^{-1}, -C-CH2stretching 1200cm^{-1}, -C-S Stretching 950 cm^{-1}, -C-Br Stretching 800 cm^{-1}, δ Value 0.00-2.00 -CH2, -CH2, δ Value 3.50-4.60-NH, -NH2, δ Value 6.1-8.5 Ar-H multiplate.

f) Preparation of 3-[({5-[4-(piperazin-1yl)phenyl]-4H-1,2,4-triazol-3yl}sulfanyl)methyl]phenol (Q6):-[11]



3-[({5-[4-(piperazin-1-yl)phenyl]-4*H*-1,2,4-triazol-3-yl}sulfanyl)methyl]phenol

$C_{19}H_{21}N_5SO$, yield 82.40 %Melting point 158-160°C, $R_f$value 0.96, Recrystallization solvent Ethanol.

2.2 Biologicalevaluation:

Antimicrobial activity-

Turbidometric Method

We have used the cup plate method (well method) to evaluate the minimal inhibitory concentration (MIC) of synthesized compounds. It is one of the in-vitro bacterial susceptibility tests. This classic method yields accurate, precise, and quantitative results for the amount of antimicrobial agent that is required to inhibit the growth of specific microorganisms. [12]

Procedure

Weighed quantities of peptone and beef extract were dissolved in distilled water by gentle warming and then a specified amount of agar was dissolved by heating on water bath. Then the PH of the solution was adjusted to7.2 to7.4 by adding the sodium chloride and the volume of the final solution was made up to 1000 ml with distilled water. [13] Then it was transferred into a suitable container, plugged with non-adsorbent cotton and the media was sterilized by an autoclave at 121^0C for 20 minutes at 15 lbs pressure.

Preparation of Test Solution

10mg of the compound was dissolved in 10 ml of DMF. From this 1 ml of solution was taken and diluted up to 10 ml with DMF. Now the concentration of the test solution was 100µg/ml. From the stock solution 1ml of solution was taken and diluted with 1ml of DMF now the concentration is 50µg/ml.

Preparation of Standard Antibiotic Solution

Ciprofloxacin was used as standard antibiotics for comparison and solutions were prepared by using sterile water, as they were water-soluble. [14] The solutions are diluted by using sterile water so that the concentrations of the solutions were 100µg/ml and 50µg/ml.

Preparation of Disc

Discs of 6-7 mm in diameter were punched from NO: 1 Whattmann filter paper with a sterile cork borer of the same size. These discs were sterilized by keeping them in the oven at 140^0C for 60 minutes. [15] Then standard and test solutions were added to each disc and discs were air-dried.

Determination of Minimum Inhibitory Concentration(MIC) By Cup Plate Method

All the synthesized compounds were used for the antibacterial test procedure.

Methods Used For Screening

In screening, the test compounds were first dissolved in sterile Dimethylformamide (DMF) which has shown the inhibition of *E.coli,* so later sterile 1,4–Dioxane was used to dissolve the test compounds which was filter sterilized by using a membrane filter of 0.2µ, as its boiling point is below 121°C and thermo-instable. [16] Compounds were tested for their antibacterial activity at 25-200 µg/ml concentration. Ciprofloxacin was used as standard which was dissolved in sterile water.1, 4-Dioxane and water was also tested as a control. [17]

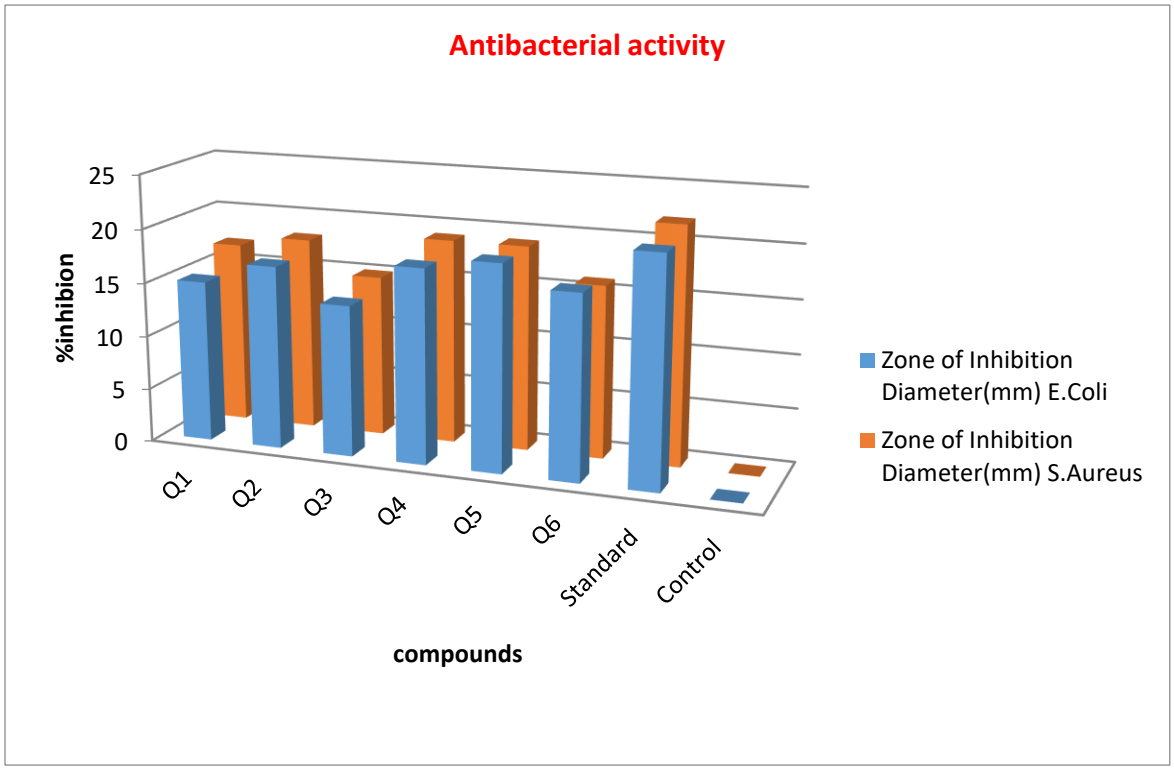
Table No 1:- The zone of inhibition antibacterial activity of Q1-Q6

Sr. No.	Product Code	Zone of Inhibition Diameter(mm)	
		E.Coli	***S.Aureus***
1	Q1	15	17
2	Q2	17	18
3	Q3	14	15
4	Q4	18	19
5	Q5	19	19
6	Q6	17	16
16	Standard	21	22
17	Control	-----	-----

(Against Bacteria)Note: -Standard(S) = CiprofloxacinControl (C) = DMF

Note: - 0-15 mm poor activity, 15-25 mm moderate activity, 25above good.

Figure no. 1:- Zone of inhibition of antibacterial activity of derivatives of 5-[4-(piperazin-1-yl) phenyl]-4*H*-1, 2, 4-triazole-3-thiol Q1-Q6



3. Results and Discussion:

All the reactions were monitored by TLC, structure, and purity of the anticipated compounds were characterized by physical constant and FTIR, 1HNMR, and Mass spectral studies. From the TLC we ensured to declare the completion of the reaction. The TLC plates were visualized either by iodine vapors or by viewing in a UV chamber. The reaction products of all the reactions were purified by different workup processes to remove unreacted starting material if any and recrystallization using suitable solvents. The literature survey reveals that piperazine has been reported for a number of pharmacological activities some molecules have shown significant activities and some show moderate and good activities.

The synthesized all piperazine derivatives were screened for anti-bacterial activity using DMF as a solvent against the organisms, S.aureus and E.coli. (Figure 1)The standard drug used was ciprofloxacin for antibacterial activity.The antimicrobial screening results presented in the above table reveal that compounds Q1,Q3, and Q6exhibited pooractivity at 50μg/ml, but at 100μg/mltheyhaveshown moderate activity against S. aureus, and moderate activity against E.coli. (Table 1)Q2,Q4, and Q5 have shown very good activity against S.aureus at 100μg/ml when compared with the standard drug Ciprofloxacin.

4. Conclusion:

From the results of antibacterial activity; it is clearly concluded that the synthesized compounds are promisingly

significant, good antimicrobial agents. The substituted Piperazine moieties are already known for different biological activities. Here we have synthesized some novel Piperazine analogs combined with different substituted a romatic and heterocyclic aldehydes ring systems with a view to getting a good antibacterial agent. From the above results, one can establish that the synthesized substituted Piperazine can berich source for the exploration. Therefore, in search ofa new generation of active compounds, itmay be worthwhile to explore the possibility in this area by making or introducing different functional groups to secondary amines or by cyclization as substitutions, which may result in better pharmacological agents.

5. Conflict of interest:

Author has no conflict of interest

6. Abbreviations:

S. aureus: Staphylococcus aureus

E. Coli: Escherichia coli

DMF: Dimethylformamide

FTIR: Fourier transform infrared

1HNMR: Proton nuclear magnetic resonance

TLC: Thin Layer Chromatography

$R_f$value: Retardation factor

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