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Research Article

Synthesis And Antimirobial Screening Of Some Triazol Schiff Bases

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ABSTRACT: Schiff bases of Fluroquinolones with 1,2,4-triazol were prepared by the reaction of intermediate (4-amino-3-(4-pyridyl)-5-mercapto-1,2,4-triazol) in dimethyl formamide with the various fluroquinolones in acidic medium. The chemical structures of prepared compounds were confirmed by ^{1}H NMR, ^{13}C NMR, IR, Mass and elemental analysis. Antimicrobial activity studies were performed, in order to provide insights into the mechanism of action of potential antimicrobial drugs for resistant microorganisms. Antimicrobial activity of compounds was investigated *in vitro* under aseptic conditions, using the disk diffusion method, against various gram positive and gram negative pathogenic microorganisms such as *Pseudomonas aeruginosa (P.A.), Staphylococcus aureus (S. aureus), Helicobacter pylori (H. pylori), Escherichia coli (E. coli), Methicillin-resistant Staphylococcus aureus (MRSA)* and some fungal strains such as, *Aspergillus fumigatus*, *Pneumocystis carinii* and *Aspergillus niger*. A series of these compounds were prepared and have been shown to inhibit pathogenic growth, judging from the area of the zone of inhibition.

Keywords: *Antimicrobial, Schiff base, Triazoles, Zone of Inhibition, Fluoroquinolone*

Introduction:

Among pharmacologically active compounds the arrangement of atoms >N-C-C-C-N<, >N-O-C-C-C-N< and >N-C-C-O-NH- & >N-C-C-C-NH-, are of importance, as far as inhibitors of histamine. Our interest in 1- benzhydrylpiperazine-4-substituted piperazine as biological active compounds prompted us to prepare their compounds having above arrangement and to undertake biological evaluation for antihistaminic activity.

The benzhydryl moeity is a fundamental component present in drugs which are anti-histaminic, anti-hypertensive, antimigraine, and anti-allergenic agents. The piperazine nucleus is capable of binding

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to multiple receptors with high affinity and therefore it has been classified as a privileged structure. Piperazines are found in various biologically active compounds across a number of different therapeutic areas which include anti-fungal, anti-bacterial, antimalarial, anti-psychotic, and anti-depressant agents. They are reported to possess good anti-tumor activity against colon, prostate, breast, lung, and leukemia tumors. The piperazine ring and its derivatives are important cyclic components in the field of industry since there used as raw materials for hardening of the epoxy resins, corrosion inhibitors, insecticides, accelerators for rubber, urethane catalysts, and anti oxidants.1-Benzylpiperazine was originally synthesized as a potential anti-helminthic. These derivatives of piperazine are found to possess excellent pharmacological activities such as vasodilator, hypotensive, anti-viral, and cerebral blood flow increasing actions. They are found to have broad pharmacological action on central nervous system (CNS), especially on dopaminergic neurotransmission.

Experimental:-

Melting points were determined by using melting point apparatus of Scientech Company. Reactions were monitored by thin layer chromatography (TLC) on silica gel G plates using Ethyl acetate:n-Hexane (5:5), Cyclohexane:Ethyl acetate (1:1), and iodine vapours and UV chamber as visualizing agent. Infrared (IR) spectra were recorded using a Perkin Elmer FTIR model. Nuclear magnetic resonance (1H NMR) spectra were recorded on a Bruker DRX-300 and chemical shifts (ppm, for d) relative to TMS as an internal standard. Spin multiplets are given as s (singlet), d (doublet), t (triplet), m (multiplet). Mass spectra were recorded on the DART-MS was recorded on a JEOL-Accu TOF JMS-T100 LC mass spectrometer. Dry helium was used with 4L PM flow rate for ionization at 350^0 C. Silica gel column. Chromatography was performed using Merck silica gel (60–120 mesh) and Merck made TLC plates.

Synthesis of 4-amino-3–(4-pyridyl)-5-mercapto-4*H*-1,2,4-triazol (2)

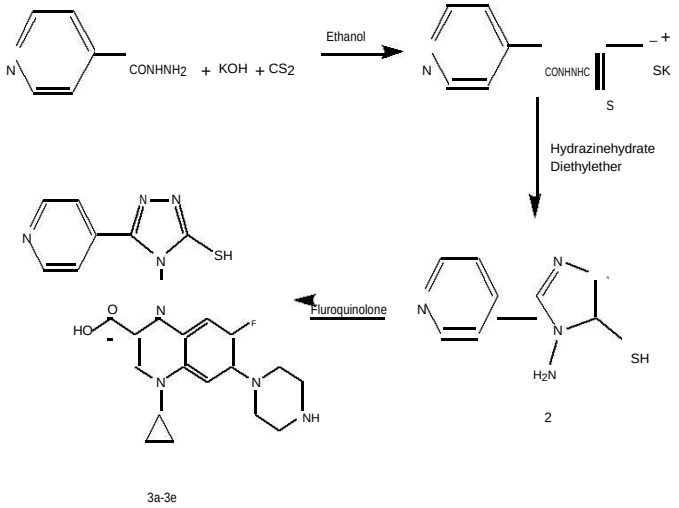
Isonicotinic acid hydrazide 13.7 g (0.1 mol) was dissolved in 200 mL absolute alcohol containing potassium hydroxide 11.2 g (0.1 mol) at room temperature 12.5 mL carbondisulfide was added in parts and was stirred for 16 hours at room temperature. 100 mL of diethyl ether was added and stirred for further 3 h. 10.3 g (0.1 mol, 99%) hydrazine hydrate was added gradually to the potassium dithiocarbazinate salt dissolved in 100 mL water with stirring and was refluxed for 8 h during which hydrogen sulphide gas evolved and the colour of the reaction mixture changed to deep green. It was then cooled and acidified with hydrochloric acid to pH 1. The yellow colored solid was isolated by filtration and recrystallised from ethanol to give compound (2)

Synthesis of 1-cyclopropyl-6-fluoro-4-(3-mercapto-5-(pyridin-4-yl)-4H-1,2,4-triazol -4-ylimino)-7-(piperazin-1-yl)-1,4-dihydroquinoline -3-carboxylic acid (3a-3e)

Few drops of glacial acetic acid were added to a solution of 0.01 mol of compound 2 (4-amino-3-(4-pyridyl)-5-mercapto-4*H*-1,2,4-triazol) in dimethyl formamide (20 mL) and 0.01 mol of various fluroquinolones derivatives was added and refluxed

for 9 hours. The reaction mixture was cooled and the precipitate precipitate obtained was filtered, dried in vacuum and recrystallised from ethanol to give 1-cyclopropyl-6-fluoro-4-(3-mercapto-5-(pyridin-4-yl)-4H-1,2,4-triazol-4-ylimino)-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**3a-3e)**.

Reaction Scheme:-



Spectral data of compounds:-

1-cyclopropyl-6-fluoro-4-(3-mercapto-5-(pyridin-4-yl)-4H-1,2,4-triazol-4-ylimino)-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (3a):-

H^1 NMR:- (300 MHz, $CDCl_3$) : δ ,7.24-8.34 (6H , m , Ar-H) , 13.2-13.6 (s, aromatic C-SH), 2.50 (t , 4H , piperazine) , 3.44 (t, 4 H, piperazine) , 4.22 (1H, S, -CH of benzhydryl moiety MS: (ESI+) m/z =506 (M^+) , 507 (M^++1), 508 (M^++2).

Chemical Formula: $C_{24}H_{23}FN_8O_2S$

Molecular Weight: 506.56

Elemental Analysis: C, 56.91; H, 4.58; F, 3.75; N, 22.12; O, 6.32; S, 6.33%

1-ethyl-6-fluoro-4-(3-mercapto-5-(pyridin-4-yl)-1,2,4-triazol-4-ylimino)-7-(piperazin-1-yl)-1,2,3,4-tetrahydroquinoline-3-carboxylic acid (3b):-

H^1 NMR:- (300 MHz, $CDCl_3$) : δ ,1.15 (t-methyl), 2.0 (NH- amine), 7.24-8.34 (6H , m , Ar-H), 13.2-13.6 (s, aromatic C-SH), 2.50 (t , 4H , piperazine), 12.38 (s,OH, carboxylic acid) ,3.48(m, methylene), 7.24 (t, methylene), 7.44 (m,CH, benzylidenimin) 3.44 (t, 4 H, piperazine) , 4.22 (1H, S, -CH of benzhydryl moiety MS: (ESI+) m/z =496 (M^+) , 497 (M^++1), 498 (M^++2).

Chemical Formula: $C_{23}H_{25}FN_8O_2S$

Molecular Weight: 496.56

Elemental Analysis: C, 55.63; H, 5.07; F, 3.83; N, 22.57; O, 6.

9-fluoro-7-(3-mercapto-5-(pyridin-4-yl)-4H-1,2,4-triazol-4-ylimino)-3-methyl-10-(4-methylpiperazin-1-yl)-3,7-dihydro-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (3c):-

H^1 NMR:- (300 MHz, $CDCl_3$) : δ ,1.15 (t-methyl), 7.24-8.34 (6H , m , Ar-H), 13.6 (s, aromatic C-SH), 8.7 (t , CH , 4-pyridine), 15.38 (s, OH, carboxylic acid) ,3.48(m, methylene), 7.24 (t, methylene), 7.44 (m,CH, benzylidenimin), 4.22 (1H, S, -CH of benzhydryl moiety MS: (ESI+) m/z =536 (M^+) , 537 (M^++1), 538 (M^++2).

Chemical Formula: $C_{25}H_{25}FN_8O_3S$

Molecular Weight: 536.58

Elemental Analysis: C, 55.96; H, 4.70; F, 3.54; N, 20.88; O, 8.95; S, 5.98 1-cyclopropyl-6-fluoro-4-(3-mercapto-5-(pyridin-4-yl)-4H-1,2,4-triazol-4-ylimino)-8-methoxy-7-(3-

methylpiperazin-1-yl)-1,2,3,4-tetrahydroquinoline-3-carboxylic acid (3d):-

H^1 NMR:- (300 MHz, $CDCl_3$) : δ ,1.15 (d-methyl), 2.0 (NH- amine), CH_2, cyclopropane)7.24-8.34 (6H , m , Ar-H), 13.5 (s, aromatic C-SH), 8.7 (t , CH , 4-pyridine), 15.38 (s, OH, carboxylic acid) ,3.48(m, methine), 1.24 (t, methylene), 7.44 (m,CH, benzylidenimin), 7.22 (1H, S, -CH of benzhydryl moiety MS: (ESI+) m/z =552 (M^+) , 553 (M^++1), 554 (M^++2).

Chemical Formula: $C_{26}H_{29}FN_8O_3S$

Molecular Weight: 552.62

Elemental Analysis: C, 56.51; H, 5.29; F, 3.44; N, 20.28; O, 8.69; S, 5.80% 5-fluoro-7-(3-mercapto-5-(pyridin-4-yl)-4H-1,2,4-triazol-4-ylimino)-3-methyl-4-(piperazin-1-yl)-3,7-dihydro-2H-[1,2]oxazino[4,3,2-ij]quinoline-8-carboxylic acid (3e)

H^1 NMR:- (300 MHz, $CDCl_3$) : δ ,1.15 (t-methyl), 7.24-8.34 (6H , m , Ar-H), 13.6 (s, aromatic C-SH), 8.7 (t , CH , 4-pyridine), 15.38 (s, OH, carboxylic acid) ,3.48(m, methylene), 7.24 (t, methylene), 7.44 (m, CH, benzylidenimin), 4.22 (1H, S, -CH of benzhydryl moiety MS: (ESI+) m/z =522 (M^+) , 523 (M^++1), 524 (M^++2).

Chemical Formula: $C_{24}H_{23}FN_8O_3S$

Molecular Weight: 522

Elemental Analysis: C, 55.16; H, 4.44; F, 3.64; N, 21.44; O, 9.19; S, 6.14%

Pharmacological screening:-

In the effort of establish the effect of the synthesized compound, the antimicrobial activity was carried out.

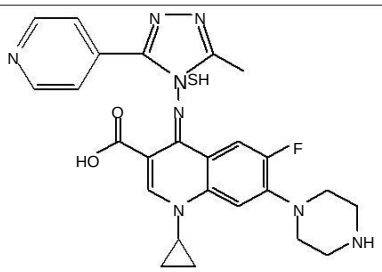
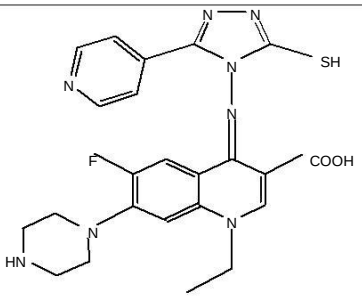
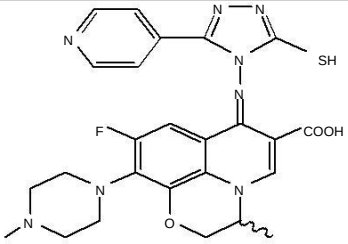
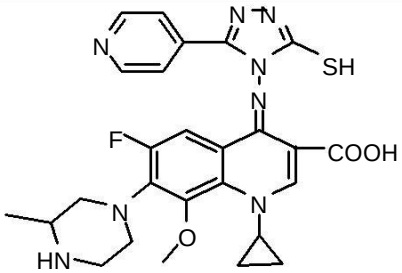
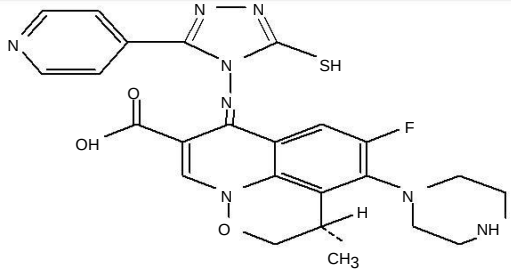
For the evaluation of antimicrobial activity of the synthesized compounds broth micro-dilution assay and agar diffusion method were employed.

Determination of minimum inhibitory concentration (MIC)

MIC was defined as the lowest concentration of a compound that inhibited visual growth, as indicated by TTC staining (dead cells are not stained by TTC). MICs were determined as the concentration with no visible growth. The MIC values (µg/ml) were determined by the checkerboard technique using nutrient agar medium. Five sets of dilution

(100 µg/ml) of the test compounds, ciprofloxacin and other fluoroquinolones (Solvent: DMSO) were prepared in a sterile McCarteney bottles. Sterile nutrient agar plates were prepared and incubated at 37 ºC for 24 hrs (for bacteria) and at 30 ºC for 72 hrs (for fungi) to check for any sort of contamination. Sterile filter paper discs (Whatman no. 1) of 6 mm diameter were soaked in the same dilution of the compounds and placed in appropriate positions on the surface of the flooded plates, marked as quadrant at the back of the petri dishes. The petri dishes were incubated at 37 ºC for 24 hrs (for bacteria) and at 30 ºC for 72 hrs (for fungi) and these plates were examined for the highest dilution which was inhibiting bacterial and fungal growth, i.e. the minimum inhibitory concentration (MIC). Similar procedure was adopted for the pure ciprofloxacin, norfloxacin, ofloxacin, gatifloxacin and sparfloxacin. All the experiments were performed in triplicates.

Table-1:-Physical data of compounds:-

S. No.	CODE NO.	STRUCTURE	MOLECULAR FORMULA	MOLECULAR WEIGHT	YIELD	Rf VALUE*
1.	3a		$C_{24}H_{23}FN_8O_2S$	506	71%	0.65
2.	3b		$C_{23}H_{25}FN_8O_2S$	496	54%	0.73
3.	3c		$C_{25}H_{25}FN_8O_3S$	536	59%	0.71
4.	3d		$C_{26}H_{29}FN_8O_3S$	552	60%	0.70
5.	3e		$C_{24}H_{23}FN_8O_3S$	522	45	0.640

Result and Discussion:-

Compounds 3a-3e were screened for their antibacterial activity against Gram-negative *Helicobacter. pylori* (ATCC 26695), *Klebsiella pneumoniae* (ATCC 15380), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27893), *Salmonella typhi* (MTCC 3216) and Gram-positive *Bacillus subtilis* (ATCC 6633), *Bacillus thuringiensis* (MTCC 4714), *Staphyloc-occus aureus* (ATCC 25323), methicillin resistant *Staphylococcus aureus* (ATCC 33591) (MRSA) bacterial strains by the agar dilution method Twofold serial dilutions of the compounds and reference drug 1 were prepared in Mueller-Hinton agar (Hi-media, Mumbai). Drugs (10.0 mg) were dissolved in DMSO (1 ml) and the solution was diluted with water (9 ml). Further progressive double dilution with melted Mueller-Hinton agar was performed to obtain the required concentrations of 100, 50, 25, 12.5 and 6.25 $\mu g.mL^{-1}$. The bacteria inocula were prepared by suspending overnight colonies from Mueller- Hinton agar media in 0.85% saline. The inocula were adjusted photometrically at 600 nm to a cell density equivalent to approximately 0.5 McFarland standards (1.5 ×108 CFU/ml). The suspensions were then diluted in 0.85% saline to give 107 CFU/ml. Petri dishes were spot inoculated with 1 µl of each prepared bacterial suspension (104 CFU/spot) and incubated at 35-37ºC for 18 hrs. The minimum inhibitory concentration (MIC) was the lowest concentration of the test compound, which resulted in no visible growth on the plate. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment.

Generally, the MICs of the test compounds indicate that compounds 3a-3e exhibit good activity against Gram-negative and Gram-positive bacteria. Result indicates that compounds 3a, 3b and 3e were active against *H. pylori* (ATCC 26695), *P. aeruginosa* (ATCC 27893), *S. typhi* (MTCC 3216) and against *P. aeruginosa* (ATCC 27893) the same compounds showed about four times better activity (MIC, 0.39 µg.mL-1) than the standard drugs ciprofloxacin (MIC, 1.56 µg.mL-1).

Conclusion

In Summary, a series Schiff bases have been synthesized in appreciable yields and screened for their *in vitro* antimicrobial activity against Gram-negative and Gram-positive bacterial strains. The role of the synthesized derivatives indicated the importance of fluroquinolone moieties. Finally it can be concluded from the presented research, that this work shows substantial promise for the development of novel antimicrobial agents.

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