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Thiazolidinone As An Antimicrobial Agent: Synthesis And Biological Evaluation Of Their Derivative.

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ABSTRACT

Numerous studies have contributed to the development of natural and synthetic antimicrobial peptides as a prospective source of antibiotic agents. Based on the concept that cationic charge, bulk, and lipophilicity are major factors determining antibacterial activity in these peptides, we designed and screened several combinatorial libraries based on 1,3,5-triazine as a template. A set of compounds were identified to show potent antimicrobial activity together with low hemolytic activity.

Keywords: 1, 3, 5-S-triazine, Schiff base, Thiazolidinone, IR/NMR Spectroscopy, Antibacterial and Antifungal activity.

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INTRODUCTION

Thiazolidinone which are part of antibiotics structure are known to exhibit interesting biological activities. A large number of 3-chloro monocyclic β-lactam possesses powerful antibacterial, antimicrobial (1-3), anti-inflammatory (4), anticonvulsant & antitubercular (4-5) activities. They also function as enzyme inhibitors & are effective on the central nervous system. (6-8) they are the carbonyl derivatives of azetidines containing carbonyl group at the position-2. These are also known as 2-azetidinones or more commonly β-lactam (4). Azetidinones or β-lactam chemistry is of great importance because of the use of β-lactam derivatives as antibacterial agents. (9)

Cycloaddition of monochloroacetylchloride with imine (schiff base) result in formation of 2-azetidinone (β-lactam). The reaction involves direct acylation of imine with monochloroacetylchloride. The reaction is carried out with base as triethylamine gives β-lactam.(5) Although variety of drugs have been developed for treating bacterial and fungal diseases, the basic difficulty experienced with these infections are the rapid development of drug résistance to the infectious strains. Review of literature reveals that 2-azetidinones are reported to possess significant antitubercular, antibacterial & antifungal activities. In view of these facts, synthesis of certain Azetidinone containing ciprofloxacin and isoniazid moiety has been undertaken in the hope of getting better bioactive agents. The constitution of all compounds synthesized was established by elemental analysis, IR and H1 NMR spectral study. Compounds were also evaluated for anti bacterial and anti fungal activites.

MATERIAL AND METHODS

All the chemicals used were of pure grade (Merck and B.D.H). The melting points of all complexes were determined by open capillary method and were uncorrected.

EXPERIMENTAL

Preparation of 1-Cyclopropyl-7-[4-(4, 6-dichloro-[1, 3, 5] triazin-2-yl)-piperazin-1-yl]-6-fluoro-4-oxo-1, 4-dihydro-quinoline-3-carboxylic acid

In 250ml F.B.F, the Cyanuric chloride (1.84gm, 0.01mole) in acetone and K_2CO_3 (1.12gm, 0.01mole) were taken. Ciprofloxacin (3.31gm, 0.01mole) in acetone was added drop wise with stirring and temperature was maintaining between0- 5^0C by using external cooling. After the completion of the addition, the clear reaction mixture was stirred at room temperature

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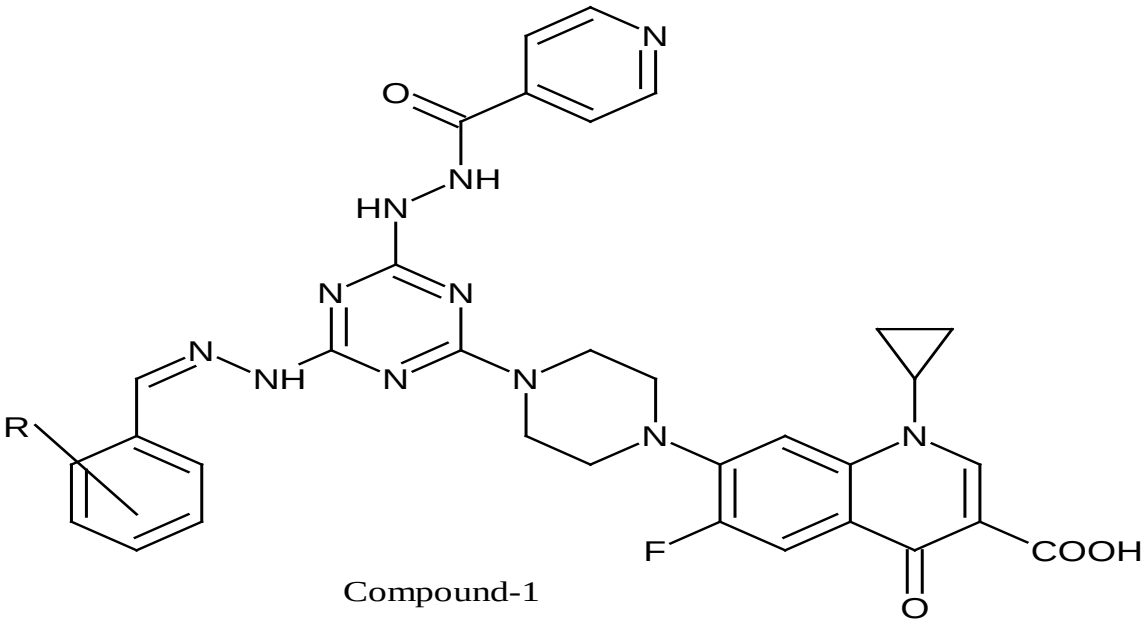
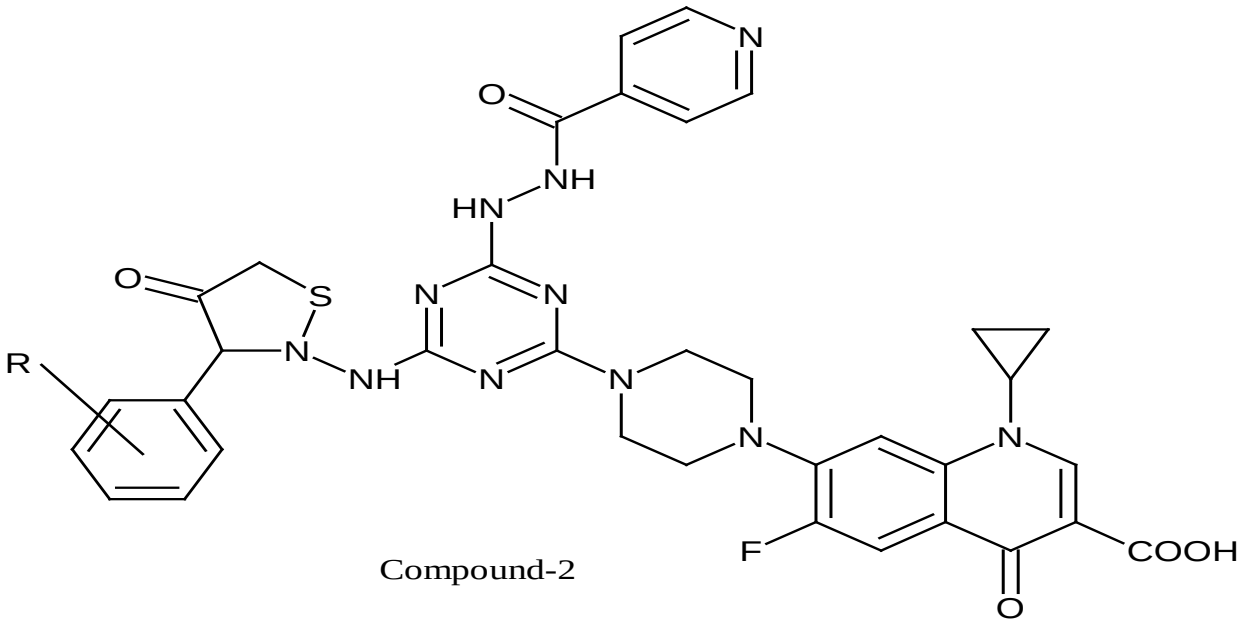
REACTION SCHEME



Compound-1

DMF
Thio Glycolic Acid **7-9hrs**



Compound-2

Where R = 4-CH_3,3-CH_3-4-OH, 4-Cl, 2-NO_2, 2:4-Cl_2, 2-OCH_3, 4-NO_2, 4-H, 4-N $(CH_3)_2$, 2-OH

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for 2-4 hrs. The off white precipitate was isolated on filter paper and washed with 1:1 Acetone: Water. The precipitate was dried and recrystallized using ethanol.

Preparation of 7-(4-{4-chloro-[N'-(pyridine-4-carbonyl)-hydrazino]-[1, 3, 5] triazin-2-yl}-piprazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1, 4-dihydro-quinoline-3-carboxylic acid

In 250ml F.B.F, the 1-Cyclopropyl-7-[4-(4,6-dichloro-[1, 3, 5]triazin-2-yl)-piperazin-1-yl]-6-fluoro-4-oxo-1, 4-dihydro-quinoline-3-carboxylic acic (0.01mole) in acetone and K_2CO_3 (1.12gm, 0.01mole) were taken. isoniazid (1.37gm, 0.01mole) in acetone was added drop wise with stirring and temperature was maintaining between30- 35^0C. After the completion of the addition, the clear reaction mixture was stirred for 2-4 hrs. The white precipitate was isolated on filter paper and washed with 1:1 Acetone: Water. The precipitate was dried and recrystallized using ethanol.

Preparation of 1-cyclopropyl-6-fluoro-7-(4-(4-hydrazinyl-6-(2-isonicotinoylhydrazinyl)-1,3,5-triazine-2-yl)piprazin-1-yl)-4-oxo-1,4dihydroquinolinec-3-carboxylic acid

7-(4-{4-chloro-[N'-(pyridine-4-carbonyl)-hydrazino]-[1, 3, 5]triazin-2-yl}-piprazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1, 4-dihydro-quinoline-3-carboxylic acid(0.01 mole) in THF (25 ml) were taken in a 250 ml R.B.F. Hydrazine (0.052ml, 0.01 mole) was added and the reaction mixture was refluxed at80^0-90^0C for 6-8 hr. Excess solvent was removed by distillation under reduced pressure and the residue was poured into ice-cold water. The resulting solid was filtered, washed, dried and recrystallised by using methanol.

Preparation of (E)-1-cyclopropyl-6-fluoro- 7-(4-(4-(2-isonicotinoylhydrazinyl)-6-(2-(4-substituted-benzylidene) hydrazinyl-1, 3, 5,-triazin-2-yl) piperazin-1-yl)-4-oxo-1, 4-dihydroquinoline -3-carboxylic acid

In a 250 ml R.B.F1-cyclopropyl-6-fluoro-7-(4-(4-hydrazinyl-6-(2-isonicotino ylhydrazinyl)-1,3,5-triazine-2-yl)piprazin-1-yl)-4-oxo-1,4dihydroquinolinec-3-carboxylic acid (0.01 mole) and substituted aldehyde (0.01 mole) were taken in THF (30 ml) as a solvent. The reaction mixture was refluxed for 8-9 hr in presence of acid catalyst (H_2SO_4) (2 ml to 4 ml). The resulting solution was cooled and poured into crushed ice. The product obtained was filtered, washed, dried and recrystallized by ethanol.

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Preparation of 1-cyclopropyl-6-fluoro-7-(4-(4-(2-isonicotinoylhydrazinyl -6-(substitutedbenzaldehyde-4-oxo-isothiazolidin-2-ylamino)-1,3,5-tri azin-2-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

In a 250 ml R.B.F, (E)-1-cyclopropyl-6-fluoro-7-(4-(4-(4-(2-isonicotinoylhydrazinyl)-6-(2-(4-substituted benzylidene) hydrazinyl)-1, 3, 5 -triazin-2-yl) piperazine-1-yl) -4-oxo-1,4dihydro quinoline-3-carboxylic acid (0.01 mole) and thiogylcolic acid (0.03 mole) were taken in DMF (25 ml) as a solvent. The reaction mixture was refluxed for 7-9 hr. After the completion of the reaction (monitored by TLC using xylene: ethyl acetate, 60:40) the excess of solvent was removed by distillation and cooled. The solid thus separated filetered, washed, dried and recrystallised from glacial acetic acid.

RESULTS AND DISCUSSION

All the synthesized final compounds were first purified by successive recrystallization using appropriate solvents. The purity of the synthesized compounds was checked by performing thin layer chromatography and determining melting points. Then the synthesized compounds were subjected to spectral analysis such as IR and NMR to confirm the structures. All the analytical details show satisfactory results. Our titled compounds are known to possess antimicrobial activity; the compounds were screened for their antibacterial and antifungal activity by cup-plate method. Two gram positive bacteria such as S.aureus and S.pyogenus two gram negative bacteria such as E.coli and P.aeruginosa and three fungal species such as A.Niger, A.clavatus and C.albicans are tested for the activities. The concentration of 250, 500 and 750 µg/ml of our titled compounds has been used. Ampicillin and chloramphenicol have been used as standards for anti-bacterial activity and nystatin and greseofulvin have been used as standards for anti-fungal activity. All the compounds have shown mild to moderate activities.

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TABLE- 1

Characterization Table of 1-cyclopropyl-6-fluoro-7-(4-(4-(2-isonicotinoylhydrazinyl)-6-(substituted benzaldehyde-4-oxo-isothiazolidin-2-ylamino)-1,3,5-triazin-2-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

No	R	Molecular formula (M. wt.)	Yield (%) (per./ hrs.)	M.P. ^{0}C.
2a	4-CH_3	$C_{36}H_{34}FN_{11}O_5S$ (751.80)	71 (7)	281-82
2b	3-$0CH_3$,4-OH	$C_{36}H_{34}FN_{11}O_7S$ (783.80)	83 (9)	310-11
2c	2-NO_2	$C_{35}H_{31}FN_{12}O_7S$ (782.77)	80 (8.5)	299-00
2d	4-Cl	$C_{35}H_{31}ClFN_{11}O_5S$ (772.21)	73 (7.5)	272-73
2e	2,4-$(Cl)_2$	$C_{35}H_{30}Cl_2FN_{11}O_5S$ (806.66)	64 (9)	324-325
2f	2-OCH_3	$C_{36}H_{34}FN_{11}O_6S$ (767.80)	77 (8)	330-31
2g	4-NO_2	$C_{35}H_{31}FN_{12}O_7S$ (782.77)	75 (8.5)	292-93
2h	4-H	$C_{35}H_{32}FN_{11}O_5S$ (737.77)	87 (9)	280-81
2i	4-$N(CH_3)_2$	$C_{37}H_{37}FN_{12}O_7S$ (780.84)	61 (8.5)	335-36
2j	2-OH	$C_{35}H_{32}FN_{11}O_6S$ (753.77)	70 (8)	307-08

Infrared spectra

The systematic interpretation of the infra - red spectrum is based upon the empirical data obtained by assigning infra-red absorption values to the structural units a characteristic of different structural units.Infra - red spectra were recorded in KBr on a Shimadzu FTIR spectrophotometer.The data of the structure is summarized in table-2 as below.

TABLE-2

Adsorption	2b	2d
N-H (st)	3424.62	3334.10
O-H (st)	3329.63	3166.19
-CH_3	2956.95	-----
-OCH_3	-----	1250.66
-CO	1672.53	1683.44
-C-F	1032.73	1036.59
-C-S-C	765.63	768.04
-N-C=O	1639.75	1655.17
Ar (C=C)	1602.14	1604.55
Ar (C-N) (st)	1560.67	1559.35
(C-N) (st)	1369.27	1370.71
In plane Ar-H	1140.25	1139.28
In plane -CH_2-	976.32	973.91
Ar-H (b) Vib.	840.85	865.91
Out plane ST	793.59	789.74
Out plane -CH_2-	719.83	713.08
Out plane Ar-H	681.26	694.75

^{1}H NMR Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is one of the latest physical methods of investigating organic compounds. The scale of the spectrum is usually marked in parts per million (ppm) of the applied field or in frequency units (Hz). ^{1}H-NMR spectra were recorded on Bruker WM 400FT MHz NMR instrument using $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal reference.The data of compound (2a) is summarized in table -3.

TABLE-3

PROTONS	VALUE
–CH2 protons cyclopropen	0.71
–CH proton of cyclopropen	2.15
-CH2 Proton of Piperazine	2.66 & 3.01
-CH3 Group	2.33
-NH (a)	8.30
-NH(b)	9.50

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-NH(c)	6.01

Antimicrobial activity

The examination of antimicrobial activity of organic compound and its all substitution reveals that the compound is moderately more or less active against various organisms. the synthesized compounds were screened for their antibacterial activity using S. aureous, P.aeruginosa, E. coli and.S.pyogenus. Control experiment was carried out under similar condition by using ampicillin and chloramphenicol as standard. And were screened for their antifungal activity using *C.albicans,A.niger and A.clavatus.* Control experiment was carried out under similar condition by using nystatin and greseofulvin.

Anti-bacterial activity

Anti-bacterial activity of Compound 2a—j was investigated via the broth dilution method [10-12]. Bacteria strains *S.aureus, E. coli, P.aeruginosa and S. pyogenus* were used. Microorganisms were cultured on water aminopeptide solution (pH 7.2).The amount of bacteria in 1 ml of solvent was 2.5105 colony forming unit (c.f.u.) after 18 h of treatment. Antimicrobial activity was estimated by minimum inhibitory concentration (MIC) the lowest concentration to completely inhibit bacterial growth of the compound shown in mg/ml. Compounds with MIC500mg/ml were considered to be in-active. Every experiment was repeated three times (Table-4).

Table-4 Anti-bacterial activity of compound

Code No.	*S. aureus*	*P.aeruginosa*	*E. coli*	*S. pyogenus*
2a	250	200	125	500
2b	125	250	200	200
2c	200	100	100	200
2d	125	500	250	125
2e	100	250	200	250
2f	1000	250	250	200
2g	250	200	200	125
2h	125	250	250	200
2i	200	200	125	125
2j	62.5	250	125	100
Ampicillin	250	100	100	100

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Chloramphenicol	50	50	50	50

Anti-fungal activity

The investigation of antifungal activity of Compound4a-j was carried out with the stiff plate agar diffusion method [13] against *C.albicans,A.niger and A.clavatus.* The amount of microbial cells was 109c.f.u. /ml. Incubation period was 24 h at 35 °C for bacteria. Antibiotics nystatin and greseofulvin were used as standards. The bacterial cultures, standards, and obtained substances in 5 mg/ml concentration were streaked across grooves and then allowed to dif-fuse in the agar nutrient plate (Table-5).

Table-4 Anti-fungal activity of compound

Code No.	*C.albicans*	*A.niger*	*A.clavatus*
2a	100	250	250
2b	1000	1000	250
2c	250	125	100
2d	125	250	100
2e	500	100	500
2f	100	100	1000
2g	>1000	500	1000
2h	500	200	1000
2i	250	125	100
2j	125	250	125
nystatin	100	100	100
greseofulvin	500	100	100

CONCLUSION

The work has approached towards the synthetic and biological approach of these wonder molecules. Anti-bacterial property of the synthesized compounds has exhibited very good inhibition; all compounds have exhibited outstanding activity towards gram positive becteria S. aureous, B.Substilis, when compound 2a shows outstanding activity againstgram negative becteria E. coli and P.aeruginosa as compare to both standared. Compound **2d**, **2e**, **2g**, **2h** and **2j** shows mild to moderate activity against different gram negative becteria as compare to standared. But the systematic substitution at various position and other derived compounds have shown remarkable antifungal properties. The compounds **2e** and **2i** have exhibited outstanding activity towards *A.niger,A.clavatus* and C. *albicans.*Compound 2f and 2g shows good activity against C. *albicans.* The remaining compounds have shown poor antifungal activity indicating less biological importance for a synthetic chemist.

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Efforts are under progress in evaluation of these synthesized compounds for *in vivo* studies especially the Anti tubercular and anti-malarial agents and the results will be published in later communications. This class of compounds has a great scope compared to other organic moieties because of their mesoionic nature, solubility and high sensitiveness towards the biological behaviors.

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