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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL FLUOROQUINOLONE CLUBBED THIADIAZOLE ANALOGS

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ABSTRACT

An efficient and facile procedure for synthesis of novel series of fluoroquinolone analogs clubbed with thiadiazoles is described herein. Antibacterial screening of synthesized compounds was carried out against a range of Gram-positive and Gram-negative bacterial strains. Some of synthesized compounds 5i (MIC: 0.625 µg/mL) and 5k (MIC: 0.3125 µg/mL) exhibited promising activities against the *E. coli* and *S. aureus* respectively. The analogs 5c, 5d, 5f and 5j displayed good anthelmintic activity against *Eisemia foetida* when compared with standard drug.

KEYWORDS: Fluoroquinolones; thiadiazole; infection; antibacterial; anthelmintic.

INTRODUCTION

Heterocyclic moieties have been found in a large number of compounds exhibiting a great biological potential.[1] Thiadiazole is a heterocyclic moiety commonly known to exhibit diverse antiviral, antibacterial, antifungal and antitubercular potential.[2-5] In recent years, scientific researchers across the world are taking considerable interest in this important biological scaffold due to its wide spectrum of applications and accordingly have been actively involved in the progression of thiadiazole chemistry.[6]

Bacterial infections had been the chief cause of death across the world since the time immemorial.[7,8] Antimicrobial agents provide an effective way for the management and treatment of infectious diseases caused by bacteria as well as other microbes.[9] Fluoroquinolones, a series of synthetic antibacterial agents are being utilized as a great weapon against infection by the clinicians and has been proved one of the most regularly prescribed categories of antibacterial drugs.[10] The mechanism of action by which they act, slightly differs from other antibacterial drugs.[11,12] They act on two enzymes primarily involved in DNA synthesis: DNA gyrase and topoisomerase IV and thus obstruct DNA replication, transcription as well as cell repair, leading to cell death.[13] Some other fluoroquinolones derivatives with anti-fungal, anti-tubercular and anti-viral activities are well recognized.[14-22] The promising role of fluoroquinolones in carcinogenesis and mutagenesis, an immense research endeavors are being carried out.[23,24] Despite of availability of various accepted fluoroquinolones for curing a number of infections, continuous efforts have been carried out for the invention of fluoroquinolones having desired pharmacokinetic profile and therapeutic index for solving the problems of rising bacterial resistance.[25,26]

Microbial resistance is the serious concern in the medical community to antimicrobial agents. This global crisis has caught the attention of medicinal chemists to overcome the pitfalls of presently accessible antimicrobial therapy against microbial infections.[27] Therefore, development of novel derivatives/analogs of antimicrobial agents having significant potency and broad spectrum of activity is highly desired.[28]

Keeping in view, the biological significance of thiadizole moiety as well as fluoroquinolones, it is the task of curiosity to keep these both moieties in a singular framework which would results in compounds of interesting biological profile. As a part of our ongoing research in developing new bioactive molecule, we tried to study the influence of fluoroquinolone and thiadiazole scaffold combination on the antimicrobial activity. In this present study, we have attempted to incorporate both these biologically active moieties together to give a confined structure for evaluating their anti-bacterial and anthelmintic activities.

EXPERIMENTAL

General

Laboratory chemicals and solvents used were procured from Merck AG (Mumbai, India), Qualigens (Navi Mumbai, India), Sigma Aldrich (Bangalore, India) and SD Fines (Mumbai, India). Melting points (uncorrected) were recorded on a Labindia MR-VIS visual melting

range apparatus (Mumbai, India). The infrared (IR) spectra were obtained on a Perkin Elmer (Waltham, MA), IR spectrophotometer (KBr disk). ^{1}H NMR spectra were run on Bruker 400 (Fallanden, Switzerland) spectrometer using tetramethylsilane as an internal standard in DMSO.

Chemistry

Synthesis of 2-amino-5-aryl-1, 3, 4-thiadiazole (2a-e)

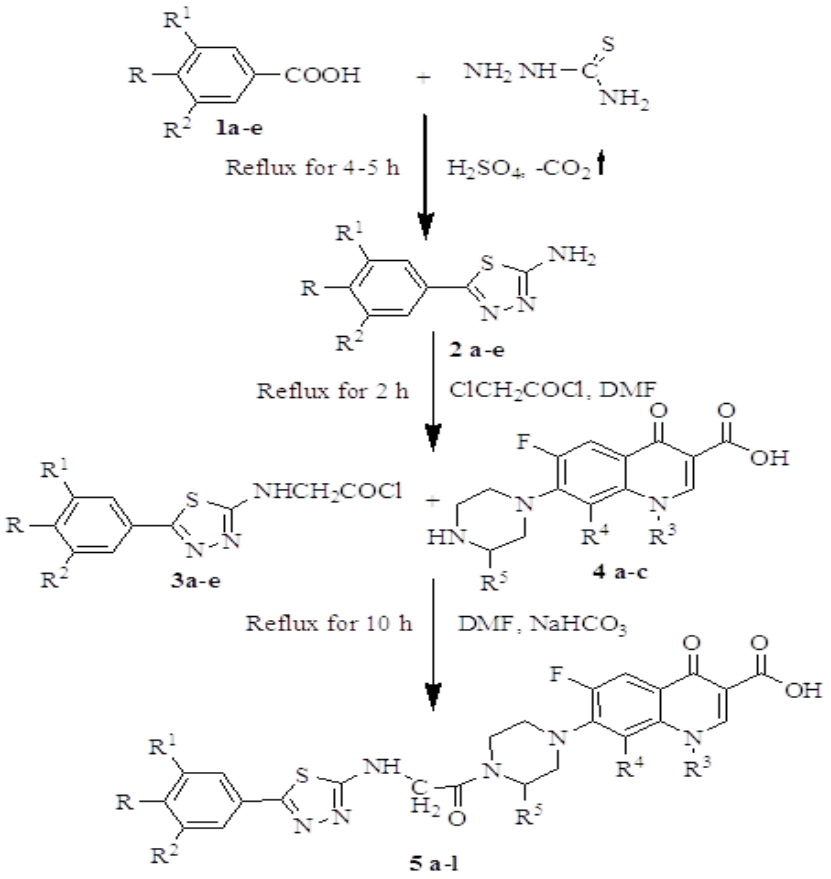
Thiosemicarbazide (9.1 g, 0.1 mol) and aryl carboxylic acid (0.1 mol) was taken in round bottom flask and allowed to reflux for 4-5 h in presence of concentrated sulphuric acid (10 drops). The mixture was poured over crushed ice. The solid thus obtained was filtered and given washing with water. The product was purified by recrystallization process from ethanol.[3]

Synthesis of substituted N-(5-aryl-1, 3,4-thiadiazole-2-yl)-2-chloroacetamide (3a-e)

To a solution of 2-amino-5-aryl-1, 3, 4-thiadiazoles (2a-e) (0.1mol) in 25 mL of *N*,*N* dimethyl formamide (DMF). Chloroacetyl chloride was added by drop wise manner (7.9 mL, 0.1 mol) to avoid any vigorous reaction to take place. The reaction mixture was refluxed for 2 h and poured into crushed ice. The precipitates separated out were filtered and washed with cold water. It was purified by recrystallization from absolute alcoh.[4]

Synthesis of fluoroquinolone derivatives bearing acetamide linkage (5a-l)

Equimolar quantity of thiadiazole **3a-e** (0.03 mmol), fluoroquinolone **4a-e** (0.03 mmol) and $NaHCO_3$ (0.03 mmol) in DMF (10 mL), was taken in round bottom flask and heated at temperature 85-90˚C. After completion of reaction as observed by TLC, 15 mL of water was added in reaction mixture. The precipitates were isolated and washed with water. The product was purified by passing through silica gel column (solvent: chloroform-ethanol; 19:1) and recrystallized from DMF-H_2O to yield compounds **5a-l**.[5] The new analogs were synthesized following the versatile synthetic method, given in scheme **1** (Figure **1**).



R= -H, -Cl, $-NO_2$,- OCH_3

R^1= $-OCH_3$, $-NO_2$

R^2= $-NO_2$

Ciprofloxacin R^3= cyclopropyl, R^4= -H, R^5= -H

Norfloxacin R^3= $-CH_2CH_3$, R^4= -H, R^5= -H

Gatifloxacin R^3= cyclopropyl, R^4= $-OCH_3$, R^5= $-CH_3$

Scheme 1

Compd	R	R^1	R^2	R^3	R^4	R^5	Molecular Formula
5a	-Cl	-H	-H	—◁	-H	-H	$C_{27}H_{24}ClFN_6O_4S$
5b	$-NO_2$	-H	-H	—◁	-H	-H	$C_{27}H_{24}FN_7O_6S$
5c	$-OCH_3$	$-OCH_3$	-H	—◁	-H	-H	$C_{29}H_{29}FN_6O_6S$
5d	$-OCH_3$	-H	-H	—◁	-H	-H	$C_{28}H_{27}FN_6O_5S$
5e	-H	$-NO_2$	$-NO_2$	—◁	-H	-H	$C_{27}H_{23}FN_8O_8S$
5f	$-NO_2$	-H	-H	$-CH_2CH_3$	-H	-H	$C_{26}H_{26}FN_7O_5S$
5g	-Cl	-H	-H	$-CH_2CH_3$	-H	-H	$C_{26}H_{24}ClFN_6O_4S$
5h	$-OCH_3$	-H	-H	$-CH_2CH_3$	-H	-H	$C_{27}H_{27}FN_6O_5S$
5i	-H	$-NO_2$	$-NO_2$	$-CH_2CH_3$	-H	-H	$C_{26}H_{23}FN_8O_8S$
5j	-Cl	-H	-H	—◁	$-OCH_3$	$-CH_3$	$C_{29}H_{28}ClFN_6O_5S$
5k	$-NO_2$	-H	-H	—◁	$-OCH_3$	$-CH_3$	$C_{29}H_{28}FN_7O_7S$
5l	-H	$-NO_2$	$-NO_2$	—◁	-H	-H	$C_{29}H_{27}FN_8O_9S$

Figure 1. Synthetic scheme for preparation of fluoroquinolone compounds 5a-l

1-Cyclopropyl-6-fluoro-7-(4-{2-(5-(4-chloro-phenyl-1,3,4-thiadiazol-2-yl-)amino)-acetyl}-piperazin-1-yl)- 4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ***(5a)***. Yield 62%, m.p. 298-301°C, IR (KBr) (cm^{-1}): 3374 (NH str), 3063 (C-H str), 1744 (C=O str), 1682 (CONH str), 1636 (C=O str), 1535 (C=C str), 1311 (C-O str), 1111 (C-N str); **^{1}H NMR (DMSO-d_6)** δ ppm: 2.34 (m, 4H, -CH_2CH_2- cyclopropyl), 2.62-2.73 (m, 9H, piperazine-H and cyclopropyl-H), 3.30 (s, 2H, -CH_2 methylene bridge), 7.28-8.14 {m, 6H, aromatic (H_5, H_8- quinolone and $H_{2'}$, $H_{3'}$, $H_{5'}$ and $H_{6'}$- phenyl thiadiazole)}, 8.06 (s, 1H, H_2-quinolone).

1-Cyclopropyl-6-fluoro-7-(4-{2-(5-(4-nitro-phenyl-1,3,4-thiadiazoyl-)amino)-acetyl}-4-oxoethyl) piperazin-1-yl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ***(5b)***. Yield 52%, m.p. 280-282°C. IR (KBr) (cm^{-1}): 3342 (NH str), 1744 (C=O str), 1682 (CONH str), 1628 (C=O str), 1535 (C=C str), 1227 (C-O str), 1196 (C-N str); **^{1}H NMR (DMSO-d_6) δ ppm:** 1.45-1.60 (m, 4H, -CH_2CH_2- cyclopropyl), 2.62 (m, 9H, piperazine-H and cyclopropyl-H), 4.09 (s, 2H, -CH_2 methylene bridge), 7.97-8.02 {m, 2H, aromatic (H_5, H_8- quinolone) 8.806 (s, 1H, H_2-quinolone), 9.13-9.20{m, 4H, aromatic (and $H_{2'}$, $H_{3'}$, $H_{5'}$ and $H_{6'}$- phenyl thiadiazole)}.

1-Cyclopropyl-6-fluoro-7-(4-{2-(5-(3,4-dimethoxy-phenyl-1,3,4-thiadiazol-2-yl)amino)-acetyl}-piperazin-1-yl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ***(5c)***. Yield 57%, m.p. 272-274°C. IR (KBr) (cm^{-1}): 3370 (NH str), 3145-2850 (C-H str), 1715 (C=O str), 1672 (CONH str), 1633 (C=O str), 1550 (C=C str), 1262 (C-O str), 1160 (C-N str); **^{1}H NMR (DMSO-d_6) δ ppm:** 0.98 (m, 4H, -CH_2CH_2- cyclopropyl), 3.43-3.70 (m, 9H, piperazine-H and cyclopropyl-H), 3.73 (s, 6H, -OCH_3 of phenyl ring of thiadiazole), 3.95 (s, 2H, -CH_2 methylene bridge), 6.92-7.78 {m, 5H, aromatic (H_5, H_8- quinolone and $H_{2'}$, $H_{5'}$ and $H_{6'}$- phenyl-thiadiazole)}, 7.96 (s, 1H, H_2-quinolone).

1-Cyclopropyl-6-fluoro-7-(4-{2-(5-(4-methoxy-phenyl-1,3,4-thiadiazol-2-yl)amino)-acetyl}-piperazin-1-yl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ***(5d)***. Yield 65%, m.p. 287-288°C. IR (KBr) (cm^{-1}): 3380 (NH str), 3160-2850 (C-H str), 1750 (C=O str), 1665 (CONH str), 1628 (C=O str), 1551 (C=C str), 1227 (C-O str), 1183 (C-N str); **^{1}H NMR (DMSO-d_6) δ ppm:** 0.87 (m, 4H, -CH_2CH_2- cyclopropyl), 3.30-3.60 (m, 9H, piperazine-H and cyclopropyl-H), 3.65 (s, 3H, -OCH_3 of phenyl ring of thiadiazole), 4.11 (s, 2H, -CH_2 methylene bridge), 7.11-7.78 {m, 6H, aromatic (H_5, H_8- quinolone and $H_{2'}$,$H_{3'}$,$H_{5'}$ and $H_{6'}$- phenyl-thiadiazole)}, 8.26 (s, 1H, H_2-quinolone).

1-Cyclopropyl-6-fluoro-7-(4-{2-(5-(3,5-dinitro-phenyl-[1,3,4]-thiadiazol-2-yl)amino)-acetyl}-piperazin-1-yl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ***(5e)***. Yield 58%, m.p. 283-285°C. IR (KBr) (cm^{-1}): 3360 (NH str), 3056-2875 (C-H str), 1720 (C=O str), 1674 (CONH str), 1630 (C=O str), 1535 (C=C str), 1227 (C-O str), 1145 (C-N str); **^{1}H NMR (DMSO-d_6) δ ppm:** 0.9 (m, 4H, -CH_2CH_2- cyclopropyl), 3.45-3.87 (m, 9H, piperazine-H and cyclopropyl-H), 3.95 (s, 2H, -CH_2 methylene bridge), 7.56-8.56 {m, 5H, aromatic (H_5, H_8-quinolone and $H_{2'}$,$H_{4'}$and $H_{6'}$- phenyl-thiadiazole)}, 8.26 (s, 1H, H_2-quinolone).

1-Ethyl-6-fluoro-7-(4-{2-(5-(4-nitro-phenyl-[1,3,4]-thiadiazol-2-yl)amino)-acetyl}-piperazin-1-yl)- 4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ***(5f)***. Yield 69%, m.p. 256-258°C. IR (KBr) (cm^{-1}): 3618 (NH str), 1744 (C=O str), 1682 (CONH str), 1628 (C=O str), 1535 (C=C str), 1227 (C-O str), 1196 (C-N str); **^{1}H NMR (DMSO-d_6) δ ppm:** 1.06 (s, 3H, -CH_3 ethyl), 1.04 (s, 2H, -CH_2 ethyl), 2.50-2.90 (m, 9H, piperazine-H and cyclopropyl-H), 3.46 (s, 2H, -CH_2 methylene bridge), 8.048-8.067 {m, 6H, aromatic (H_5, H_8-quinolone and $H_{2'}$,$H_{3'}$,$H_{5'}$ and $H_{6'}$- phenyl-thiadiazole)}, 8.508 (s, 1H, H_2-quinolone).

1-Ethyl-6-fluoro-7-(4-{2-(5-(4-chloro-phenyl-[1,3,4]-thiadiazol-2-yl)amino)-acetyl}-piperazin-1-yl)- 4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ***(5g).*** Yield 59%, m.p. 269-272°C. IR (KBr) (cm^{-1}): 3350 (NH str), 3068-2876 (C-H str), 1713 (C=O str), 1668 (CONH str), 1625 (C=O str), 1550 (C=C str), 1257 (C-O str), 1157 (C-N str); **^{1}H NMR (DMSO-d_6) δ ppm:** 1.27 (s, 3H, -CH_3 ethyl), 2.24 (s, 2H, -CH_2 ethyl), 3.16-3.67 (m, 9H, piperazine-H and cyclopropyl-H), 3.57 (s, 2H, -CH_2 methylene bridge), 7.35-8.09 {m, 6H, aromatic (H_5, H_8-quinolone and $H_{2'}$,$H_{3'}$,$H_{5'}$ and $H_{6'}$- phenyl-thiadiazole)}, 8.52 (s, 1H, H_2-quinolone).

1-Ethyl-6-fluoro-7-(4-{2-(5-(4-methoxy-phenyl-[1,3,4]-thiadiazol-2-yl)amino)-acetyl}-piperazin-1-yl)- 4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ***(5h).*** Yield 61%, m.p. 263-265°C. IR (KBr) (cm^{-1}): 3340 (NH str), 3055-2876 (C-H str), 1721 (C=O str), 1678 (CONH str), 1649 (C=O str), 1557 (C=C str), 1254 (C-O str), 1165 (C-N str); **^{1}H NMR (DMSO-d_6) δ ppm:** 1.35 (s, 3H, -CH_3 ethyl), 2.26 (s, 2H, -CH_2 ethyl), 3.10-3.61 (m, 9H, piperazine-H and cyclopropyl-H), 3.65 (s, 3H, -OCH_3), 4.05 (s, 2H, -CH_2 methylene bridge), 6.83-7.37 {m, 6H, aromatic (H_5, H_8-quinolone and $H_{2'}$,$H_{3'}$,$H_{5'}$ and $H_{6'}$- phenyl-thiadiazole)}, 7.96 (s, 1H, H_2-quinolone).

1-Ethyl-6-fluoro-7-(4-{2-(5-(3,5-dinitro-phenyl-[1,3,4]-thiadiazol-2-yl)amino)-acetyl}-piperazin-1-yl)- 4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ***(5i).*** Yield 63%, m.p. 263-265°C. IR (KBr) (cm^{-1}): 3350 (NH str), 3085-2834 (C-H str), 1712 (C=O str), 1666 (CONH str), 1628 (C=O str), 1535 (C=C str), 1255 (C-O str), 1160 (C-N str); **^{1}H NMR (DMSO-d_6) δ ppm:** 1.31 (s, 3H, -CH_3 ethyl), 2.20 (s, 2H, -CH_2 ethyl), 3.31-3.76 (m, 9H, piperazine-H and cyclopropyl-H), 4.08 (s, 2H, -CH_2 methylene bridge), 7.35-8.21 {m, 6H, aromatic (H_5, H_8-quinolone and $H_{2'}$,$H_{4'}$ and $H_{6'}$- phenyl-thiadiazole)}, 8.36 (s, 1H, H_2-quinolone).

1-Cyclopropyl-6-fluoro-7-(4-{2-(5-(4-chloro-phenyl-[1,3,4]-thiadiazol-2-yl)amino)-acetyl}-3-methyl-piperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ***(5j).*** Yield 49%, m.p. 164-166°C. IR (KBr) (cm^{-1}): 3350 (NH str), 3049-2864 (C-H str), 1713 (C=O str), 1669 (CONH str), 1627 (C=O str), 1549 (C=C str), 1255 (C-O str), 1160 (C-N str); **^{1}H NMR (DMSO-d_6) δ ppm:** 1.27 (m, 4H, -CH_2CH_2- cyclopropyl), 2.34 (m, 3H, -CH_3), 3.21-367 (m, 9H, piperazine-H and cyclopropyl-H), 3.77 (s, 3H, -OCH_3), 4.14 (s, 2H, -CH_2 methylene bridge), 6.96-7.85 {m, 6H, aromatic (H_5, H_8-quinolone and $H_{2'}$,$H_{3'}$ $H_{5'}$ and $H_{6'}$-phenyl-thiadiazole)}, 8.12 (s, 1H, H_2-quinolone).

1-Cyclopropyl-6-fluoro-7-(4-{2-(5-(4-nitro-phenyl-[1,3,4]-thiadiazol-2-yl)amino)-acetyl}-3-methyl-piperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ***(5k).*** Yield 43%, m.p. 175-179°C. IR (KBr) (cm^{-1}): 3350 (NH str), 3020-2765(C-H str), 1711(C=O str), 1665(CONH str), 1630 (C=O str), 1545(C=C str), 1245(C-O str), 1165 (C-N str); **^{1}H NMR (DMSO-d_6) δ ppm:** 1.08 (m, 4H,-CH_2CH_2- cyclopropyl), 2.28 (m, 3H, -CH_3), 3.45-3.82 (m, 9H, piperazine-H and cyclopropyl-H), 3.75 (s, 3H,-OCH_3), 4.12 (s, 2H,-CH_2 methylene bridge), 7.96-8.85 {m, 6H, aromatic (H_5, H_8-quinolone and $H_{2'}$,$H_{3'}$,$H_{5'}$ and $H_{6'}$- phenyl-thiadiazole)},8.12 (s, 1H, H_2-quinolone).

1-Cyclopropyl-6-fluoro-7-(4-{2-(5-(4-nitro-phenyl-[1,3,4]-thiadiazol-2-yl)amino)-acetyl}-3-methyl-piperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ***(5l).*** Yield 56%, m.p. 171-173°C. IR (KBr) (cm^{-1}): 3320 (NH str), 3056-2841 (C-H str), 1718 (C=O str), 1665 (CONH str), 1635 (C=O str), 1555(C=C str), 1215(C-O str),1160 (C-N str); **^{1}H NMR (DMSO-d_6) δ ppm:** 1.0 (m, 4H, -CH_2CH_2- cyclopropyl), 2.28 (m, 3H, -CH_3), 3.34-3.65 (m, 9H, piperazine-H and cyclopropyl-H), 3.89 (s, 3H, -OCH_3), 4.18 (s, 2H, -CH_2 methylene bridge), 7.10-7.96 {m, 5H, aromatic (H_5, H_8-quinolone and $H_{2'}$,$H_{4'}$ and $H_{6'}$- phenyl-thiadiazole)}, 8.22 (s, 1H, H_2-quinolone).

Biological evaluation

Anti-bacterial activity

Antibacterial screening for all the synthesized compounds **5a-5l**, against two Gram-negative bacteria *i.e. E. coli* (MTCC 119), *P. aeruginosa* (MTCC 7453) and two Gram-positive strains *i.e. B. pumilus* (MTCC 7411), *S. aureus* (MTCC 96) was carried out. The antibacterial evaluation of synthesized compounds was carried out by minimum inhibitory concentration (MIC) method. Microorganisms were inoculated and incubated according to procedure provided by NDRI, Karnal. The bacterial cultures were grown on nutrient agar plate and kept at 4°C. The experimental MIC values of the target compounds against selected bacterial strains have been shown in table **1**.

Table 1: Minimum inhibitory concentration (MIC) of target compounds 5a-l in µg/mL against *B. pumilus, S. aureus, E. coli and P. aeruginosa* bacterial strains.

Compounds	Gram (+)		Gram (-)	
	B. pumilus	***S. aureus***	***E. coli***	***P. aeruginosa***
5a	0.3125	0.3125	0.625	1.25
5b	0.625	0.625	0.625	2.50
5c	0.3125	0.3125	0.3125	0.3125
5d	0.625	0.3125	0.625	0.625
5e	0.625	0.3125	0.625	1.25
5f	0.625	0.3125	1.25	1.25
5g	2.50	2.50	2.50	2.50
5h	10	10	10	10
5i	0.625	0.3125	0.625	0.3125
5j	0.625	0.625	0.3125	0.3125
5k	0.3125	0.3125	0.3125	0.3125
5l	10	2.50	2.50	10
Ciprofloxacin	<0.3125	<0.3125	0.3125	0.3125
Norfloxacin	0.625	<0.3125	1.25	0.625
Gatifloxacin	0.625	0.625	<0.3125	0.3125

Anthelmintic evaluation

The anthelmintic activity is a test to determine the capability of a chemical agent to eliminate worms. The newly synthesized compounds were screened for anthelmintic activity *in vitro* using adult Indian earth worm *E. foetida* due to its analogous anatomical and physiological features with the intestinal roundworm, parasites of human beings.[29,30] Earthworms (*E. foetida*) were purchased from Department of Agriculture, Gurukul, Kurukshetra. The suspensions of the synthesized compounds were prepared by using 0.5% tween 80 as a suspending agent and distilled water. The mechanical stirrer was utilized for stirring the

resulting suspension for half an hour. Anthelmintic activities of these suspensions were determined. Piperazine citrate in the suspension form was also used as reference standard and taken in the equal concentration as the vehicle control.

Petri dishes having 50 mL suspension of the standard drug (piperazine citrate) for comparison, 50 mL suspension of distilled water and 0.5% tween 80 as control and 50 mL suspension of each test samples were taken at room temperature. Five earthworms having comparable sizes and varieties were taken in each dish and examined for paralysis and death. The death time was recorded after ascertaining that earthworm didn't move on dropping the paralysed worm in water having temperature up to 50°C.

The mean paralysing time and mean death time was calculated. All the outcomes of anthelmintic activity have been summarized in Table 2.

Table 2. Anthelmintic activity of synthesized derivatives 5a-l.

Compounds	Concentration of compounds (mg/100mL)	Mean paralysing time (min)+S.E.	Mean death time (min)+S.E.
5a	200	26.00±2.0*	38.40±0.9**
5b	200	32.60±1.1	43.20±1.2
5c	200	22.80±1.9**	38.20±0.6**
5d	200	23.20±1.3**	44.40±1.2
5e	200	27.80±0.7	44.40±1.3
5f	200	23.60±0.7**	39.20±1.2**
5g	200	26.80±1.5*	43.20±2.1
5h	200	27.00±1.4	40.40±1.0*
5i	200	28.40±1.2	47.80±1.5
5j	200	23.60±1.8**	42.00±1.0
5k	200	24.60±1.6	43.60±1.9
5l	200	29.00±2.7	44.80±1.4
Control	-	-	-
Standard	200	24.40±1.0	34.80±1.0

Statistical analysis: The results were calculated as mean ± (SEM) of five worms in every group. Dunnett's 't' test have been performed after one way ANOVA method for statistical analysis.

RESULTS AND DISCUSSION

Chemistry

Detailed synthetic pathways of various fluoroquinolone derivatives **5a-l** are depicted in scheme **1**. Compounds **2a-e** were synthesized by refluxing substituted aryl carboxylic acid

1a-g and thiosemicarbazide in acidic environment for 4-5 hrs. The intermediate compounds **2a-e** after reaction with chloroacetyl chloride in DMF gave substituted N-(5-aryl-1, 3,4-thiadiazole-2-yl)-2-chloroacetamide **3a-e**. Finally the compounds **3a-e** were allowed to reflux with fluoroquinolones **4a-e** in DMF as solvent and basic environment provided by sodium bicarbonate at 85-90°C. Product obtained was collected, dried and recrystallized from DMF and water to give **5a-l**.

The structures, molecular formula of newly synthesized compounds have been supported and confirmed by spectral analysis (IR and ^{1}H NMR). Detailed study of IR spectra of the target compounds **5a-l** revealed the characteristic absorption band at 1720-1705 cm^{-1} and 1674-1666 cm^{-1}, corresponds to C=O and CONH stretching vibration respectively. The ^{1}H NMR spectra displayed proton of methylene bridge at about 3.35-4.12 (singlet 2H) ppm, evidently demonstrating the occurrence of acetamide linkage in fluoroquinolone derivatives **5a-l**.

Pharmacological screening

Antibacterial and anthelmintic screening of synthesized compounds was performed to find out their pharmacological prospective.

Anti-bacterial activity

The synthesized compounds **5a-l** were investigated for anti-bacterial activity by minimum inhibitory concentration method. The activity was carried out against selected Gram-negative bacteria *i.e. E. coli* (MTCC 119), *P. aeruginosa* (MTCC 7453) and Gram-positive strains *i.e. B. pumilus* (MTCC 7411), *S. aureus* (MTCC 96). These novel derivatives demonstrated varying antibacterial activities against different strains. Compounds **5i** and **5k** (MIC= 0.3125-0.625 µg/mL) having nitro functional group at aromatic ring attached to thiadiazole moiety showed significantly potent antibacterial activities against selected bacterial strains using norfloxacin and gatifloxacin (MIC= 0.625-1.25 µg/mL) as standard antibiotics. These compounds with nitro substituted derivatives were observed to be more potent against bacterial strains compared to methoxy, chloro and dimethoxy substituted derivatives. Ciprofloxacin derivative **5c** (MIC= 0.3125 µg/mL) was found to exhibit equivalent MIC on comparing with its standard drug (MIC= 0.3125 µg/ml) against Gram-negative bacteria like *E. coli* and *P. aeruginosa*. Similarly when nofloxacin derivative **5f** (MIC= 0.625-1.25 µg/mL) against *E. coli* along with *B. pumilus* and gatifloxacin derivative **5j** (MIC= 0.3125-0.625 µg/mL) against *P. aeruginosa*, *B. pumilus* and *S. aureus* were tested, they were found to exhibit equivalent MIC against antibiotics norfloxacin and gatifloxacin used as standards.

The results of antibacterial activity reveal that norfloxacin and gatifloxacin analogs were proved to be more efficacious as compared to ciprofloxacin derivatives.

Anthelmintic activity

The synthesized compounds were evaluated for anthelmintic activity against *E. foetida*. All the compounds were found to exhibit significant anthelmintic activity. Compound **5c, 5d, 5f** and **5j** possessed good activity having mean paralysis time within the range 22.80±1.9** to 23.60±1.8* min whereas majority of the compounds expressed death time varies from 38.20 ±0.9** min to 39.20±1.2** min. Piperazine citrate used as standard drug at concentration of 200 mg/mL exhibited mean paralysis time 24.40± 1.0 min and mean death time 34.80 ± 1.0 min. Anthelmintic activity of synthesized analogs revealed that fluoroquinolone derivatives bearing dimethoxy, methoxy, nitro and chloro group showed good activity compared to other substitution at thiazdiazole ring. It has been proved from the results that activity of the compounds can be improved by substitution of more electro negativity group.

CONCLUSION

In current investigation, a facile novel synthesis of fluoroquinolone clubbed thiadiazole analogs with acetamide linkage is reported. The *in vitro* screening of newly synthesized compounds showed improved therapeutic efficiency as compared to parent drugs. Some of the synthesized compounds demonstrated more potent or equipotent antibacterial activities against the selected bacterial strains. A few analogs have also possesses promising antihelmintic activity. The significant findings of the present research work may be utilized by the researchers for development of better antibacterial and antihelmintic agents in future.

Conflict of interest

The authors have declared no conflict of interest.

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