Synthesis and antimicrobial activity of thiazolidinone norfloxacin hybrids

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A new series of 1-ethyl-6-fluoro-7-piperazinyl-4-oxo-3-(substitutedarylidinylcarboxy-hydrazido)quinolines 3-7 and 1-ethyl-6-fluoro-7-piperazinyl-4-oxo-3-(2'-substitutedaryl-4'-oxo-1,3'-thiazolidin-3'-yl)aminocarboxyquinolines 8-12 have been synthesized in order to determine their antimicrobial activities and feasible structure-activity relationships. The synthesized compounds and reference drugs have been tested in vitro against various strains of bacteria: E. coli ATCC 25922, B. subtilis ATCC 1633 and S. aureus ATCC 25923 and the fungi: C. albicans ATCC 2091, A. niger ATCC 9029 and C. krusei ATCC 6258. Microbiological results showed that the synthesized compounds possessed a broad spectrum of antimicrobial activity against the microorganisms tested. 1-Ethyl-6-fluoro-7-piperazinyl-4-oxo-3-[2'-o-methoxyphenyl]-4'-oxo-1',3'-thiazolidin-3'-yl)aminocarboxyquinoline 10 has displayed more potent antibacterial activity as compared to standard drug, chloroamphenicol and rest of the compounds of this series. This compound has also exhibited significant antifungal activities, which is not more than that of fluconazole. The structural assignments of newly synthesized compounds are based on IR, 1H NMR, mass spectral studies and elemental analysis.

Keywords: Quinoline, Schiff base, thiazolidinone, antibacterial activity, antifungal activity

Nalidixic acid is the oldest member of the quinolone class of synthetic antimicrobial agents, and has been used for the treatment of urinary tract infections for many years. This drug is of relatively minor significance because of its limited therapeutic utility and the rapid development of bacterial resistance. Against this backdrop, fluorinated 4-quinolones like norfloxacin, ciprofloxacin, ofloxacin, etc. have been developed. Since these agents have broad antimicrobial profile, they are effective after oral administration for the treatment of a wide variety of infectious diseases. However, serious side effects are associated with these drugs along with development of antimicrobial resistance. Both the discovery and development of new antimicrobial agents are required to overcome these drawbacks. For this purpose extensive literature survey has been carried out, which has indicated that derivatives of quinoline, Schiff bases and thiazolidinone ring exhibit good antimicrobial profile.

The present study contemplates the synthesis of some new norfloxacin (1-ethyl-6-fluoro-7-piperazinyl-4-oxo-3-carboethoxy-quinoline) hybrid molecules through the combination of different pharmacophores like thiazolidinone at its 3rd-position in one frame, and may lead to compounds with interesting biological profiles.

The starting compound was norfloxacin: 1-ethyl-6-fluoro-7-piperazinyl-4-oxo-3-carboxylic-quinoline, which on reaction with concentrated sulphuric acid gave 1-ethyl-6-fluoro-7-piperazinyl-4-oxo-3-(carboxyethoxy)quinoline 1. This compound was reacted with hydrazine hydrate to furnish hydrazido congener: 1-ethyl-6-fluoro-7-piperazinyl-4-oxo-3-(carboxyhydrazido)quinoline 2, which on treatment with different aromatic aldehydes in presence of glacial acetic acid yielded the Schiff bases: 1-ethyl-6-fluoro-7-piperazinyl-4-oxo-3-(substitutedarylidinylcarboxyhydrazido)quinolines 3-7. Finally, substituted thiazolidinone congeners: 1-ethyl-6-fluoro-7-piperazinyl-4-oxo-3-(2'-substitutedaryl-4'-oxo-1',3'-thiazolidin-3'-yl)aminocarboxyquinolines 8-12 have been prepared from the reaction of compounds 3-7 with thioglycolic acid and zinc chloride. The synthetic route for the above mentioned compounds is given in Scheme I.
Antibacterial and antifungal screening of compounds 3-12 and reference drugs have been carried out, and the results of inhibition zone and MIC noted and displayed in Table I.

**Antibacterial activity**

Compounds 3-12 and standard drug chloramphenicol were screened for their antibacterial activity against *E. coli ATCC 25922*, *B. subtilis ATCC 1633* and *S. aureus ATCC 25923*. These compounds displayed varying degrees of antibacterial activity (Table I). Only compound 10 revealed maximal inhibition with MIC 1.592-6.25 µg/mL against bacterial strains used when compared to the standard drug. Compounds 11 and 12 exhibited prominent antibacterial activity. The rest of the compounds investigated in the present study have not shown significant activity.

**Antifungal activity**

Antifungal activity of the compounds 3-12 along with reference drug fluconazole was carried out against *C. albicans ATCC 2091*, *A. niger ATCC 9029* and *C. krusei ATCC 6258* at a concentration of 250 µg/mL and the results of inhibition of growth of these strains are shown in Table I. Compound 10 has displayed potential antifungal activity against the above strains with MIC 3.25, 12.5 and 1.592 µg/mL, respectively, as compared to fluconazole with MIC 6.25, 12.5 and 3.125 µg/mL respectively. Compounds 3, 4 and 11, 12 have shown not affected the growth of fungal strains. Compounds 5, 6, 7, 11 and 12 have shown mild to significant antifungal activity (Table I).

**Discussion**

All the newly synthesized compounds 3-12 of the present study were evaluated *in vitro* to determine their antibacterial and antifungal activities against numerous pathogens. The results of screening are given in Table I. It is significant to note from the results that the conversion of Schiff base congeners 3-7 into thiazolidinone 8-12 congeners increased the inhibition action against various microorganisms.

Furthermore, the effects of various substitutions on antibacterial and antifungal activities were examined and after reviewing the results for the compounds 3-12, a few conclusions could be drawn, such as:

- It has been observed that compounds 5 and 10 bearing o-methoxypheynyl group reflected most potent antibacterial and antifungal activities.
- Compounds having furfuryl group as in compounds 7 and 12 yielded remarkable inhibitory action against bacteria and fungi.
- Compounds 6 and 11 bearing p-hydroxyphenyl group furnished adequate antibacterial and antifungal activity.
It is also interesting to point out that substitution of \( o \)-chlorophenyl and \( p \)-chlorophenyl as in compounds 3, 8 and 4, 9, respectively, yielded less antibacterial activity. And these compounds have not exhibited antifungal activity.

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### Experimental Section

All the reagents and solvents were received from commercial suppliers. Reactions were carried out in dried glassware. Melting points were obtained in open capillaries by thermonic melting point apparatus.
(Campbell Electronic Mumbai, India) and are uncorrected. The homogeneity of the newly synthesized compounds was checked by thin layer chromatography (TLC) on silica gel-G coated plates by using different solvent systems. Infrared (IR) spectra were determined on Bruker IFS-66 FTIR instrument (Bruker Bioscience, USA) using KBr pallets and wave number (ν) was reported in cm⁻¹. The ¹H NMR spectra were recorded on Jeol GSX-300 FT NMR (Jeol, Tokyo, Japan) in CDCl₃ or DMSO-d₆, and chemical shifts are reported in δ (ppm). Tetramethylsilane (TMS) was used as internal reference standard. Mass spectra were recorded on Spec Finnigan Mat 8230 MS. The carbon, hydrogen and nitrogen analysis were performed on Carlo Erba-1108 (Carlo Erba, Milan, Italy), and the results were found within ±0.4% of the theoretical values.

1-Ethyl-6-fluoro-7-piperazinyl-4-oxo-3-(carboethyloxy)quinoline 1. A mixture of 1-ethyl-6-fluoro-7-piperazinyl-4-oxo-3-carboxylic-quinoline (0.01 mol) and concentrated sulphuric acid (0.01 mol) in methanol (110 mL) was refluxed for 5 hr. After refluxing, the excess of solvent was distilled off and the residue was allowed to cool. The resultant mixture was poured over ice-cold water. The product thus found within +0.4% of the theoretical values.

To the solution of compound 1 (0.01 mol) in methanol (80 mL), hydrazine hydrate (0.02 mol) was added drop-wise with constant stirring. Further, this reaction mixture was refluxed by using different solvent systems. Infrared (IR) spectra were determined on Bruker IFS-66 FTIR instrument (Bruker Bioscience, USA) using KBr pellets and wave number (ν) was reported in cm⁻¹. The ¹H NMR spectra were recorded on Jeol GSX-300 FT NMR (Jeol, Tokyo, Japan) in CDCl₃ or DMSO-d₆, and chemical shifts are reported in δ (ppm). Tetramethylsilane (TMS) was used as internal reference standard. Mass spectra were recorded on Spec Finnigan Mat 8230 MS. The carbon, hydrogen and nitrogen analysis were performed on Carlo Erba-1108 (Carlo Erba, Milan, Italy), and the results were found within ±0.4% of the theoretical values.

1-Ethyl-6-fluoro-7-piperazinyl-4-oxo-3-(carboxyhydrazido)quinoline 2. To the solution of compound 1 (0.01 mol) in methanol (80 mL), hydrazine hydrate (0.02 mol) was added drop-wise with constant stirring. Further, this reaction mixture was refluxed for 8.0 hr. Then, this mixture was concentrated and then cooled to RT. The mixture was poured onto crushed ice and then filtered. Solid thus separated out was dried and purified by recrystallization from ethanol to give compound 2: m.p. 234°C, yield 62%. Anal. Found: C, 60.44; H, 6.42; N, 12.10%. IR (KBr): 3245 (NH), 3045 (C-H aromatic), 2963 (C-H aliphatic), 1675, 1710 (C=O), 1563 (C----C of aromatic ring), 1145 (C-N), 1035 (N-N), 886 cm⁻¹ (C-F); ¹H NMR (DMSO-d₆): δ 9.65 (bs, 1H, CONHNH₂, exchangeable with D₂O), 7.79 (s, 1H, H₃ of quinoline), 7.72 (s, 1H, H₄ of quinoline), 5.48 (s, 1H, NH of piperazine, exchangeable with D₂O), 4.58 (s, 2H, CONHNH₂, exchangeable with D₂O), 3.73 (m, 2H, NCH₂CH₃), 2.39 (m, 8H, CH₂ × 4 of piperazine), 1.09 (t, 3H, N-CH₂CH₃, J = 6.0 Hz); MS: m/z 333 [M⁺].

1-Ethyl-6-fluoro-7-piperazinyl-4-oxo-3-(substitutedarylidinylcarboxyhydrazido) quinolines 3-7. A mixture of compound 2 (0.01 mol) and proper substituted aromatic aldehyde (0.01 mol) in ethanol (70 mL) along with a few drops of glacial acetic acid was heated under reflux for 6-8 hr. Progress and completion of the reaction was checked on TLC. Then, the solvent was removed by distillation, and the residue was poured on ice-water, filtered and purified by recrystallization from appropriate solvent to yield the compounds 3-7. By this procedure, compounds 3, 4, 5, 6 and 7 were obtained starting form o-chlorobenzaldehyde, p-chlorobenzaldehyde, o-methoxybenzaldehyde, p-hydroxybenzaldehyde and furfuraldehyde. Their physical, analytical and spectral data are given below:

1-Ethyl-6-fluoro-7-piperazinyl-4-oxo-3-(o-chloroarylidinylcarboxyhydrazido)quinoline 3. Reagents: compound 2 (0.01 mol), o-chlorobenzaldehyde (0.01 mol), ethanol (70 mL), few drops of glacial acetic acid. m.p. 222°C, yield 62%, recrystallized from methanol. Anal. Found: C, 60.44; H, 4.88; N, 15.40. Calculated for C₁₆H₂₀N₂O₅FCl: C, 60.59; H, 5.05; N, 15.37%. IR (KBr): 3265 (NH), 3056 (C-H aromatic), 2952 (C-H aliphatic), 1666, 1710 (C=O), 1604 (C=O), 1565 (C----C of aromatic ring), 1185 (C-N), 1040 (N-N), 868 (C-F) 720 cm⁻¹ (C-Cl); ¹H NMR (DMSO-d₆): δ 9.85 (bs, 1H, CONHNH₂, exchangeable with D₂O), 8.17 (s, 1H, CH-Ar), 7.74 (s, 1H, H₂ of quinoline), 7.69 (s, 1H, H₃ of quinoline), 7.52 (s, 1H, H₄ of quinoline), 7.23-7.44 (m, 4H, Ar), 5.41 (s, 1H, NH of piperazine, exchangeable with D₂O), 4.21 (t, 3H, COOCH₂CH₃, J = 7.12 Hz), 3.70 (m, 2H, N-CH₂CH₃), 2.37 (m, 8H, CH₂ × 4 of piperazine), 1.25-1.35 (m, 2H, COOCH₂CH₃), 1.05 (t, 3H, N-CH₂CH₃, J = 6.0 Hz); MS: m/z 455.5 [M⁺], 457.5 [M+2].

1-Ethyl-6-fluoro-7-piperazinyl-4-oxo-3-(p-chloroarylidinylcarboxyhydrazido)quinoline 4. Reagents: compound 2 (0.01 mol), p-chlorobenzaldehyde (0.01 mol), ethanol (70 mL), few drops of glacial acetic acid. m.p. 251°C, yield 65%, recrystallized from ethanol. Anal. Found: C, 60.75; H, 5.27; N,
15.32. Calcd for C$_{23}$H$_{22}$N$_{5}$O$_{3}$FCl: C, 60.59; H, 5.05; N, 15.37%. IR (KBr): 3275 (NH), 3056 (C-H aromatic), 2965 (C-H aliphatic), 1666, 1710 (C=O), 1640 (C=N), 855 cm$^{-1}$ (C-Cl); $^1$H NMR (CDCl$_3$): $\delta$ 8.94 (bs, 1H, CONH, exchangeable with D$_2$O), 8.15 (s, 1H, CH-Ar), 7.71 (s, 1H, H$_5$ of quinoline), 7.67 (s, 1H, H$_6$ of quinoline), 7.51 (s, 1H, H$_2$ of quinoline), 7.21-7.41 (m, 4H, Ar-H), 5.43 (s, 1H, NH of piperazine, exchangeable with D$_2$O), 3.72 (m, 2H, NCH$_2$CH$_3$), 2.39 (m, 8H, CH$_2 \times 4$ of piperazine), 1.07 (t, 3H, N-CH$_3$CH$_3$, $J = 6.0$ Hz); MS: $m/z$ 455.5 [M$^+$], 457.5[M+2].

1-Ethyl-6-fluoro-7-piperazinyl-4-oxo-3-((o-methoxyarylidinylcarboxyhydrazido)quinoline 5. Reagents: compound 2 (0.01 mol), o-methoxybenzaldehyde (0.01 mol), ethanol (70 mL), few drops of glacial acetic acid, m.p. 228°C, yield 60%, recrystallized from ethyl acetate. Anal. Found: C, 63.77; H, 5.81; N, 15.75. Calcd for C$_{23}$H$_{22}$N$_{5}$O$_{3}$F: C, 63.86; H, 5.76; N, 15.52%. IR (KBr): 3265 (NH), 3056 (C-H aromatic), 2952 (C-H aliphatic), 1666, 1710 (C=O), 1610 (C=N), 1565 (C-C of aromatic ring), 1185 (C-N), 1075 (C-O-C), 1040 (N-N), 868 cm$^{-1}$ (C-F); $^1$H NMR (DMSO-d$_6$): $\delta$ 9.85 (bs, 1H, CONH, exchangeable with D$_2$O), 8.17 (s, 1H, CH-Ar), 7.74 (s, 1H, H$_5$ of quinoline), 7.69 (s, 1H, H$_6$ of quinoline), 7.52 (s, 1H, H$_2$ of quinoline), 7.22-7.42 (m, 4H, Ar-H), 5.41 (s, 1H, NH of piperazine, exchangeable with D$_2$O), 3.73 (m, 2H, NCH$_2$CH$_3$), 2.37 (m, 8H, CH$_2 \times 4$ of piperazine), 1.09 (t, 3H, N-CH$_3$CH$_3$, $J = 6.0$ Hz); MS: $m/z$ 451 [M$^+$].

1-Ethyl-6-fluoro-7-piperazinyl-4-oxo-3-((p-hydroxyarylidinylcarboxyhydrazido)quinoline 6. Reagents: compound 2 (0.01 mol), p-hydroxybenzaldehyde (0.01 mol), ethanol (70 mL), few drops of glacial acetic acid. m.p. 209°C, yield 55%, recrystallized from DMF. Anal. Found: C, 63.35; H, 5.65; N, 16.25. Calcd for C$_{23}$H$_{22}$N$_{5}$O$_{3}$F: C, 63.16; H, 5.49; N, 16.02%. IR (KBr): 3515 (OH), 3275 (NH), 3065 (C-H aromatic), 2975 (C-H aliphatic), 1666, 1715 (C=O), 1640 (C=N), 1565 (C-C of aromatic ring), 1190 (C-N), 1055 (N-N), 855 cm$^{-1}$ (C-F); $^1$H NMR (CDCl$_3$): $\delta$ 10.14 (s, 1H, OH, exchangeable with D$_2$O), 9.83 (bs, 1H, CONH), exchangeable with D$_2$O), 8.19 (s, 1H, CH-Ar), 7.74 (s, 1H, H$_5$ of quinoline), 7.68 (s, 1H, H$_6$ of quinoline), 7.52 (s, 1H, H$_2$ of quinoline), 7.23-7.42 (m, 4H, Ar-H), 5.41 (s, 1H, NH of piperazine, exchangeable with D$_2$O), 3.71 (m, 2H, NCH$_2$CH$_3$), 2.37 (m, 8H, CH$_2 \times 4$ of piperazine), 1.07 (t, 3H, N-CH$_3$CH$_3$, $J = 6.0$ Hz); MS: $m/z$ 437 [M$^+$].

1-Ethyl-6-fluoro-7-piperazinyl-4-oxo-3-((fururyldenylcarboxyhydrazido)quinoline 7. Reagents: compound 2 (0.01 mol), furfuraldehyde (0.01 mol), ethanol (70 mL), few drops of glacial acetic acid. m.p. 193°C, yield 62%, recrystallized from benzene. Anal. Found: C, 61.45; H, 5.19; N, 16.85. Calcd for C$_{23}$H$_{22}$N$_{5}$O$_{3}$F: C, 61.31; H, 5.35; N, 17.03%. IR (KBr): 3265 (NH), 3056 (C-H aromatic), 2952 (C-H aliphatic), 1666, 1710 (C=O), 1637 (C=N), 1565 (C-C of aromatic ring), 1185 (C-N), 1075 (C-O-C), 1040 (N-N), 868 cm$^{-1}$ (C-F); $^1$H NMR (DMSO-d$_6$): $\delta$ 9.85 (bs, 1H, CONH, exchangeable with D$_2$O), 8.17 (s, 1H, CH-Ar), 7.74 (s, 1H, H$_5$ of quinoline), 7.69 (s, 1H, H$_6$ of quinoline), 7.52 (s, 1H, H$_2$ of quinoline), 7.13-7.32 (m, 3H, Ar-H), 5.41 (s, 1H, NH of piperazine, exchangeable with D$_2$O), 3.73 (m, 2H, NCH$_2$CH$_3$), 2.39 (m, 8H, CH$_2 \times 4$ of piperazine), 1.09 (t, 3H, N-CH$_3$CH$_3$, $J = 6.0$ Hz); MS: $m/z$ 411 [M$^+$].

1-Ethyl-6-fluoro-7-piperazinyl-4-oxo-3-((2′-substitutedarylylidinyl-4-oxo-1′,3′-thiazolidin-3′-yl)-aminocarboxyquinoilies 8-12. To a solution of compound (3-7. 0.02 mol) in dioxane (50 mL), thioglycolic acid (0.02 mol) and anhydrous ZnCl$_2$ (2.0 g) were added. This reaction mixture was refluxed for 7-10 hr. Reaction was followed at regular intervals by TLC. After completion of the reaction, the excess of solvent was distilled off, and the solid thus obtained was cooled and poured onto crushed ice, then filtered and dried. The product thus separated out was purified by recrystallization from appropriate solvent to yield the compounds 8-12. Their physical, analytical and spectral data are given below:

1-Ethyl-6-fluoro-7-piperazinyl-4-oxo-3-[(2′-o-chlorophenyl)-4′-oxo-1′,3′-thiazolidin-3′-yl)-aminocarboxyquinoline 8. Reagents: compound 3 (0.02 mol), thioglycolic acid (0.02 mol), dioxane (50 mL), ZnCl$_2$ (2.0 g), m.p. 198°C, yield 55%, recrystallized from DMF. Anal. Found: C, 56.80; H, 4.62; N, 13.30. Calcd for C$_{25}$H$_{24}$N$_{5}$O$_{3}$F: C, 56.66; H, 4.72; N, 13.22%. IR (KBr): 3215 (NH), 3056 (C-H aromatic), 2953 (C-H aliphatic), 1668, 1705, 1725 (C=O), 1565 (C-C of aromatic ring), 1135 (C-N), 1075 (C-O-C), 1065 (N-N), 865 (C-F), 725 (C-Cl), 665 cm$^{-1}$ (C-S-C); $^1$H NMR (CDCl$_3$): $\delta$ 9.82 (bs, 1H, CONH), exchangeable with D$_2$O), 8.15 (s, 1H, CH-Ar), 7.73 (s, 1H, H$_5$ of quinoline), 7.69 (s, 1H, H$_6$ of quinoline), 7.51 (s, 1H, H$_2$ of quinoline), 7.24-7.45 (m, 4H, Ar-H), 5.42 (s, 1H, NH of piperazine, exchangeable with D$_2$O), 3.71 (m, 2H, NCH$_2$CH$_3$), 3.63 (s, 2H, CH$_2$ of thiazolidinone), 2.37 (m, 8H, CH$_2 \times 4$ of piperazine),
1.10 (t, 3H, N-CH₂CH₃, J = 6.0 Hz); MS: m/z 529.5 [M]⁺, 531.5 [M+2].

1-Ethyl-6-fluoro-7-piperazinyl-4-oxo-3-[2′-(p-chlorophenyl)-4′-oxo-1′,3′-thiazolidin-3′-yl]-aminocarboxyquinoline 9. Reagents: compound 4 (0.02 mol), thiglycolic acid (0.02 mol), dioxane (50 mL), ZnCl₂ (2.0 g), m.p. 208°C, yield 60%, recrystallized from acetone. Anal. Found: C, 56.85; H, 4.55; N, 13.42.

Calcd for C₃₈H₃₅N₅O₇S₂: C, 56.92; H, 4.63; N, 13.85. IR (KBr): 3235 (NH), 3056 (C-H aromatic), 2954 (C-H aliphatic), 1668, 1705, 1735 (C=O), 1585 (C———C of aromatic ring), 1145 (C-N), 1055 (N-N), 868 (C-F) 725 (C-Cl), 660 cm⁻¹ (C-S-C); ¹H NMR (DMSO-d₆): δ 9.83 (bs, 1H, CONH, exchangeable with D₂O), 8.17 (s, 1H, CH-Ar), 7.73 (s, 1H, H₇ of quinoline), 7.71 (s, 1H, H₈ of quinoline), 7.24-7.45 (m, 4H, Ar-H), 5.41 (s, 1H, NH of piperazine, exchangeable with D₂O), 3.73 (m, 2H, NCH₂CH₃), 3.61 (s, 2H, CH₂ of thiazolidinone), 2.35 (m, 8H, CH₂ × 4 of piperazine), 1.09 (t, 3H, N-CH₂CH₃, J = 6.0 Hz); MS: m/z 529.5 [M]⁺, 531.5 [M+2].

1-Ethyl-6-fluoro-7-piperazinyl-4-oxo-3-[2′-(o-methoxyphenyl)-4′-oxo-1′,3′-thiazolidin-3′-yl]-aminocarboxyquinoline 10. Reagents: compound 5 (0.02 mol), thiglycolic acid (0.02 mol), dioxane (50 mL), ZnCl₂ (2.0 g). m.p. 181°C, yield 58%, recrystallized from acetic acid. Anal. Found: C, 57.10; H, 5.05; N, 14.55.

Calcd for C₃₈H₃₅N₅O₇S₂: C, 56.91; H, 4.95; N, 14.43%. IR (KBr): 3215 (NH), 3055 (C-H aromatic), 2954 (C-H aliphatic), 1668, 1705, 1725 (C=O), 1585 (C———C of aromatic ring), 1140 (C-N), 1055 (N-N), 865 (C-F) 725 (C-Cl), 660 cm⁻¹ (C-S-C); ¹H NMR (CDCl₃): δ 9.87 (bs, 1H, CONH, exchangeable with D₂O), 8.16 (s, 1H, CH-Ar), 7.79 (s, 1H, H₇ of quinoline), 7.64 (s, 1H, H₈ of quinoline), 7.56 (s, 1H, H₂ of quinoline), 7.25-7.45 (m, 4H, Ar-H), 5.39 (s, 1H, NH of piperazine, exchangeable with D₂O), 3.75 (m, 2H, NCH₂CH₃), 3.65 (s, 2H, CH₂ of thiazolidinone), 3.47 (s, 3H, OCH₃), 2.36 (m, 8H, CH₂ × 4 of piperazine), 1.06 (t, 3H, NCH₂CH₃, J = 6.0 Hz); MS: m/z 525 [M]⁺.

Antimicrobial screening

All the compounds 3-12 prepared herein were screened for antibacterial and antifungal activities against different strains of bacteria and fungi.

Minimal inhibitory concentration (MIC)

The antimicrobial activity was assayed in vitro by two-fold broth dilution²⁻ against bacteria: Escherichia coli ATCC 25922, Bacillus subtilis ATCC 1637 and Staphylococcus aureus ATCC 25923 and fungi: Candida albicans ATCC 2091, Aspergillus niger ATCC 9029 and Candida krusei ATCC 6258. The minimal inhibitory concentrations (MIC in µg/mL) were defined as the lowest concentrations of compound that completely inhibited the growth of each strain. All compounds dissolved in dimethylsulfoxide (DMSO) were added to culture media. Mueller-Hinton Broth for bacteria and Sabouraud...
Liquid Medium for fungi were used to obtain the final concentrations ranging from 125 µg/mL to 1.592 µg/mL. The amount of DMSO never exceeded 1% v/v. Inocula consisted of 5.0 × 10^4 bacteria/mL and 1.0 × 10^3 fungi/mL. The MICs were noted after incubation at 37°C for 24 hr (bacteria) and at 30°C for 48 hr (fungi). Media with 1% v/v DMSO were employed as growth controls. Chloroamphenicol and fluconazole were used as reference antibacterial and anti-fungal drugs, respectively.

**Antibacterial activity**

The newly synthesized compounds 3-12 and standard drug, chloroamphenicol were screened for antibacterial activity against bacterial strains namely *Eschericia coli* ATCC 25922, *Bacillus subtilis* ATCC 1633 and *Staphylococcus aureus* ATCC 25923 at a concentration of 250 µg/mL by using filter paper disc method. DMSO served as control. The discs of Whatman filter paper were prepared with standard size (7.0 mm) and kept into 1.0 Oz screw-capped wide-mouthed containers for sterilization. These bottles were kept in a hot-air oven at a temperature of 150°C. Then, the prepared solution of test compounds and standard drug (dissolved in DMSO) of desired concentration were poured into their respective bottles. Further, the discs were transferred to the inoculated plates with a pair of fine pointed tweezers. To prevent contamination, tweezers may be kept with their tips in 70% alcohol and flamed off before used. Before using the test organisms, which were grown on nutrient agar, they were sub-cultured in nutrient broth at a temperature of 37°C for 18-20 hr. Each disc was applied carefully to the surface of agar without lateral movement once the surface had been touched. Thereafter, the plates were incubated for 24 hr at a temperature of 37°C. Care was taken not to stockpile the plates.

**Antifungal activity**

The newly synthesized compounds 3-12 and standard drug, fluconazole, were evaluated for their antifungal activity by employing the standard agar disc diffusion method. The following strains of fungi have been used in this study: *Candida albicans* ATCC 2091, *Aspergillus niger* ATCC 9029 and *Candida krusei* ATCC 6258. All cultures were maintained on Sabouraud Dextrose Agar (SDA) and incubated at 30°C. To prepare homogeneous suspensions of the above-mentioned fungi for the disc assays, they were grown in Sabouraud broth, centrifuged to collect the pellet, and buffered with saline. The fungal pellet was homogenized in a sterile hand held homogenizer. This suspension was then plated onto SDA using a fungal spreader to obtain an even growth field. The discs of Whatman filter paper were prepared with standard size (6.0 mm) and kept into 1.0 Oz screw capped wide mouthed containers for sterilization. These sterilized discs were impregnated with 250 µg/mL concentrations of the various test compounds and standard drug, fluconazole. These discs were then placed in the centre of each quadrant of an SDA plate. Each plate had one control disc impregnated with DMSO. The plates were incubated at 30°C. After 48 hr, the plates were removed and the radius of the zone of inhibition (in mm) was measured. Care was taken not to stockpile the plates.

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**Reference**