Synthesis, Characterization and Antimicrobial Activity of Some Novel 2-(2-hydroxy 5-(substitutedphenyldiazy)l) -N-[(4-oxo -2-phenylquinazoline 3(4H)–yl)]-4-oxo 1,3 thiazolidine-1-carbothioamide

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ABSTRACT: The development of resistance to current antibacterial therapy continues to drive the search for effective agents. A new series of 2-(2 –hydroxyl -5 (substituted phenyldiazy)l) -N-[(4-oxo -2-phenylquinazoline 3(4H) – yl)]-4-oxo 1,3 thiazolidine-1-carbothioamide have been synthesized by the reaction of substituted thiosemicarbazones with thioglycolic acid in presence of zinc chloride in DMF. The structure of synthesized compounds is confirmed by IR, NMR and Mass spectral studies. The antimicrobial activities of the synthesized compounds were evaluated by screening on different human pathogens using the disc diffusion assay.

KEYWORDS: Quinazoline; thiosemicarbazones; thiazolidinones; antimicrobial activity.

Introduction

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and increasing number of multi drug resistant microbial pathogens with particular relevance for Gram-positive bacteria. Multidrug resistance (MDR) is a phenotype of cross resistance to multiple drugs with diverse chemical structure. The development of new potential drugs, which will be devoid of side effect profile of currently available drugs, will be one of the possible solutions to treat various infectious diseases with multidrug treatment over a long period of the time.

The chemistry and biological study of heterocyclic compounds has been interesting field for a long time due to medicinal reasons. The number of heterocyclic derivatives containing nitrogen and sulphur atom possess broad spectrum of biological activities. 4-thiazolidinones have received considerable attention due to their wide range of biological activities. 4-thiazolidinone derivatives are known to possess antimicrobial, anticancer, anti-inflammatory, anti-HIV activity, cytoxic, anticonvulsant, antiproliferative activity. 4-thiazolidinone core structure was found to selectively kill drug-resistant cancer cells and induce apoptosis. Quinazoline nucleus possessed the potent pharmacodynamic nucleus. In addition several quinazoline derivative posses diverse biological activities.

Biological Results

The antimicrobial activities of the synthesized compounds were screened in vitro using the Disc Diffusion technique against bacterial and fungal pathogens Escherichia coli, Salmonella typhimurium, Klebsiella pneumoniae and Aspergillus niger, Aspergillus fumigatus, Curvularia lunata strains at 600 µg /ml. All the compounds were active against human pathogens. Compounds T2 and T3 showed good activity against K. pneumoniae and C. lunata. All the compounds showed moderate activity against A. fumigatus and minimum to E.coli (Table 1).

Conclusion

Conclusively, a series of thiazolidinone derivatives successfully synthesized in appreciable yields and screened in vitro for their antimicrobial activities against different human pathogens. Such a SAR evaluation would open future perspectives to use these compounds as new lead compounds in clinical trials.

The major findings gathered from the antimicrobial study of the compounds are highlighted as follow:

(i) p-nitro substitution enhanced the antimicrobial profile.
(ii) p-methoxy substituent in aryl ring showed poor antimicrobial activity.
(iii) p-nitro group showed higher antimicrobial activity than p-chloro and p-methoxy derivatives.
Experimental Methods

The synthetic route to the required compounds is outlined in scheme 1. For the synthesis of the titled compounds, substituted 2- hydroxyl 5-(phenylazinyl) benzaldehyde (I) required as a starting material was prepared by the diazotization and coupling method and quinazoline derivative of thiosemicarbazide (II) was prepared by reacting benzyolated anthranilic acid and thiosemicarbazide in the presence of ethanol. The reaction of equimolar quantities of (I) with (II) in the presence of DMF resulted in the formation of compounds (III). The titled compounds (IV T1–T6) synthesized by reacting compounds (III) with thioglycolic acid and anhydrous ZnCl2 in the presence of DMF.

\[
\begin{align*}
\text{(I) } & \text{(II)} \\
\text{(I) + (II) } & \text{DMF/} \triangle 8h \\
\text{(III) } & \text{HSCH}_{2}\text{COOH} \text{ ZnCl}_2 / \text{DMF} 12 - 14h \\
\end{align*}
\]

R = -H, m -NO2, p - NO2, m - OCH3, p - OCH3, p -Cl

Scheme 1 Synthetic route of novel compounds.
Synthesis of substituted 2- hydroxyl 5-(phenyldiazenyl) benzaldehyde (I).

Substituted primary amine (0.01 M) were dissolved in aqueous hydrochloric acid (28 ml, 6 N) and mechanically stirred at 0°C-5°C. A cold solution of sodium nitrite (5 gm/10 ml water) was added drop wise to the constantly stirred reaction mixture. The diazotized solution was immediately added in small portion to salicylaldehyde (5 ml dissolved in 40 ml, 6 N NaOH), with constant stirring at 0°C-5°C. The stirring was continued for 4 hours. The solid obtained was filtered under suction washed with cold water and recrystallized from glacial acetic acid.

Synthesis of quinazoline derivative of thiosemicarbazide (II)

Thiosemicarbazide has been synthesized in two steps.

1. Synthesis of 2-phenyl-3,1-benzoxazine – 4(3H) – one

To a stirred solution of anthranilic acid (0.01 M) in pyridine (0.01 M), benzoyl chloride (0.01 M) were added drop wise maintaining the temperature near 8°C for 1 hour. Reaction mixture was stirred for another 2 hours at room temperature while stirring a solid product separates out and then whole reaction mixture was neutralized with NaHCO3 solution. A pale yellow solid deposited which was filtered, washed with water and recrystallized from ethanol.

2. Synthesis of N-[2-phenyl-4 (3H)-oxo-quinazoline-3-yl] thiourea

2-phenyl 3,1-benzoxazine – 4 (3H) – one (0.01 M) were dissolved in ethanol and thiosemicarbazide (0.01 M) in ethanol were added to it with a catalytic amount of pyridine. Reaction mixture was refluxed for 4 hours and after cooling at room temperature a crystalline product was obtained. It was filtered and recrystallized from ethanol to yield needle shaped shiny white crystals.

Synthesis of 1-[2-hydroxy-5-(Substituted phenyl)diazylbenzylidene -3- (4-oxo-2- phenyl quinazolin-3(4H) yl) thiourea (III)

A mixture of the appropriate, 2-hydroxy-5- (phenyldiazenyl) benzaldehyde (I) (0.01 M) and N-[2-phenyl-4 (3H)- oxo-quinazoline-3-yl] thiourea (II) (0.01 M) were refluxed for 8 hours in DMF (30 ml). The mixture was then cooled in an ice bath and the product separated was repeatedly washed with water followed by ethanol and recrystallized from diethyl ether.

Synthesis of 2-(2-hydroxy-5 (substitutedphenyldiazy)-N-[(4-oxo -2-phenylquinazoline 3(4H)- yl)-4-oxo 1,3 thiazolidine-1-carbothioamide (IV T1–T6)

In a round bottom flask, were taken a solution of 1-[2-hydroxy-5-(4-Substituted phenyl) diazyl benzylidene -3- (4-oxo-2-phenyl quinazolin-3(4H)-yl) thiourea (0.001 M), dissolved in minimum amount of DMF to which were added a solution of thiglycolic acid (0.001 M, in DMF ) with continuous shaking. A pinch of anhydrous ZnCl2 was then added and the solution was refluxed for 12 hours. The reaction mixture was then allowed to cooled and finally poured into crushed ice. The solid thus obtain was filtered, washed and recrystallized from ethanol.

2-(2-hydroxy-5(phenyldiazy)-N-[(4-oxo -2-phenylquinazoline 3(4H)- yl)-4-oxo 1,3 thiazolidine-1-carbothioamide (T1)

Melting point 225°C; yield 55%; recrystallization solvent: Ethanol ; IR (KBr) ν cm1 : 3333 (-OH and -NH), 1720 -1600 (-C=O), 1541 (-N=N=), 1450 (S-CH2 ), 1377 (-C=S). 1H NMR (DMSO)δ in ppm: 12.23 (s, 1H, NH), 8.73 (s,1H -CHN), 7.57-7.96 (4H, Ar-H), 7.1 (s,1H ,-OH), 3.76 (s,2H, CH2 thiazolidinones), 2.5- 3.0 (s, C6H5 ). Analysis Calculated for C30H22O3N6S2: C, 62.72; H, 3.83; N, 14.52; Found C, 62.55; H, 3.65; N, 13.45. MS m/z 505.

2-(2-hydroxy-5(4-nitrophenyldiazy)-N-[(4-oxo -2-phenylquinazoline 3(4H)- yl)-4-oxo 1,3 thiazolidine-1-carbothioamide (T2)

Melting point 225°C; yield 55%; recrystallization solvent: Ethanol ; IR (KBr) ν cm1 : 3333 (-OH and -NH), 1720 -1600 (-C=O), 1541 (-N=N=), 1450 (S-CH2 ), 1377 (-C=S). 1H NMR (DMSO)δ in ppm: 12.23 (s, 1H, NH), 8.73 (s,1H -CHN), 7.57-7.96 (4H, Ar-H), 7.1 (s,1H ,-OH), 3.76 (s,2H, CH2 thiazolidinones), 2.5- 3.0 (s, C6H5 ). Analysis Calculated for C30H22O3N6S2: C, 62.55; H, 3.65; N, 13.45. MS m/z 505.
2-(2-hydroxy-5(4-methoxyphenyldiazy)-N-(4-oxo-2-phenylquinazoline 3(4H)-yl)-4-oxo 1,3 thiazolidine-1-carbothioamide (T4)

Melting point 198°C; yield 50%; recrystallization solvent: Ethanol; IR (KBr) cm⁻¹: 3333 (-OH and -NH), 1715 -1600 (-C=O), 1377 (-C=S).

\[ ^1 \text{H} \text{NMR (DMSO)} \delta \text{ in ppm: 12.55 (s, 1H, -NH), 8.73(s,1H-CN), 7.57-7.96 (4H, Ar-H), 6.5 (s,1H, -OH), 3.71 (s,2H, CH₂ thiazolidinones), 3.4(s.3H, OCH₃), 2.5- 3.0 (s, C₆H₅).} \]

Analysis Calculated for C₃₁H₂₄O₄N₆S₂: C, 61.17; H, 3.97; N, 13.81; Found C, 60.15; H, 3.05; N, 11.81. MS m/z 535.

2-(2-hydroxy-5(3-methoxyphenyldiazy)-N-(4-oxo-2-phenylquinazoline 3(4H)-yl)-4-oxo 1,3 thiazolidine-1-carbothioamide (T5)

Melting point 166°C; yield 52%; recrystallization solvent: Ethanol; IR (KBr) cm⁻¹: 3333 (-OH and -NH), 1715-1600 (-C=O), 1565 (-N=O), 1440 (S-CH₂), 157-7.96 (4H, Ar-H), 6.5 (s,1H, -OH), 3.71 (s,2H, CH₂ thiazolidinones), 3.5(s.3H, OCH₃), 2.5- 3.0 (s, C₆H₅).

Analysis Calculated for C₃₁H₂₄O₄N₆S₂: C, 61.17; H, 3.97; N, 13.81; Found C, 60.15; H, 3.05; N, 11.81. MS m/z 535.

2-(2-hydroxy-5(4-Chlorophenyldiazy)-N-(4-oxo-2-phenylquinazoline 3(4H)-yl)-4-oxo 1,3 thiazolidine-1-carbothioamide (T6)

Melting point 188°C; yield 48%; recrystallization solvent: Ethanol; IR (KBr) cm⁻¹: 3333 (-OH and -NH), 1720 - 1600 (-C=O), 1541 (-N=O), 1450 (S-CH₂), 1377 (-C=S ) 754 (C-Cl.) \[ ^1 \text{H} \text{NMR (DMSO)} \delta \text{ in ppm: 12.22 (s, 1H, -NH), 8.73(s,1H-CN), 7.57-7.96 (4H, Ar-H), 6.5 (s,1H ,-OH), 3.71 (s,2H, CH₂ thiazolidinones), 3.5(s.3H, OCH₃), 2.5- 3.0 (s, C₆H₅).} \]

Analysis Calculated for C₃₀H₂₁O₃N₆S₂Cl: C, 61.72; H, 3.53; N, 14.52; Found C, 60.55; H, 3.25; N, 13.45. MS m/z 540.

All melting points were determined in open capillaries and are uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel 60G (Merck) with petroleum ether: ethyl acetate (5:1). Spectroscopic data were recorded using following instruments-IR: Shimadzu (FTIR) spectrophotometer, NMR : Bruker DRX 300 (300 MHz, FT NMR), MS – FAB: Jeol –SX 102 Mass spectrometer and elemental (C, H, N) analysis.

Antimicrobial activity

All the synthesized compounds were screened for their in vitro antimicrobial activity against 24 hours old cultures of bacterial and fungal pathogens. Antimicrobial activity was determined against Escherichia coli, Salmonella typhimurium, Klebsiella pneumoniae and Aspergillus niger, Aspergillus fumigatus, Curvularia lunata strains using the disc diffusion assay. For this, a sterile filter paper disc (5 mm) impregnated with fixed does (600 µg/ml) of the synthesized compounds under investigation were placed upon the seeded petridishes. Similar disc were prepared for the standard drugs, chloramphenicol, fluconazole and the solvent control, dimethyl formamide. The plates were incubated for 24 hours at 37°C for the bacterial and fungal strains. The zone of inhibition, observed around the disc after incubation was measured. The results are represented in Table 1.

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<th>Comp</th>
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<th>S.typhimurium</th>
<th>K.pneumoniae</th>
<th>A. niger</th>
<th>A. fumigatus</th>
<th>C. lunata</th>
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600 µg/ml

Zone of inhibition in (mm)
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References