

SYNTHESIS AND IN-VITRO STUDIES OF SOME NEW QUINOLINE 1,3,4-THIADIAZOLO PYRIMIDIN DERIVATIVES

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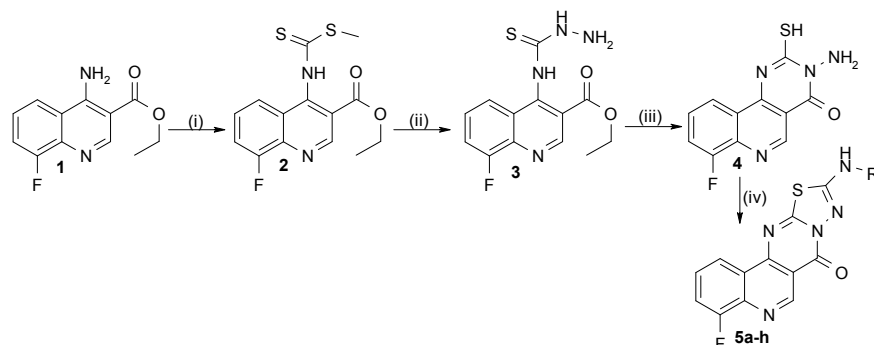
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ABSTRACT. A series of eight new quinoline associated 1,3,4-thiadiazolo pyrimidin derivatives (**5a-h**) have been developed using 4-amino-8-fluoro-quinoline-3-carboxylic acid ethyl ester (**1**) as raw material and by involving 8-fluoro-4-methylsulfanyltiocarbonylamino-quinoline-3-carboxylic acid ethyl ester (**2**), 8-fluoro-4-hydrazine thiocarbonylamino-quinoline-3-carboxylic acid ethyl ester (**3**) and 3-amino-7-fluoro-2-mercapto-3H-pyrimido-[5,4-c]quinolin-4-one (**4**) as intermediates. The title compounds after structure elucidation were used in vitro to find their antibacterial ability towards different micro-organisms.

KEY WORDS: Synthesis, Quinoline, 1,3,4-thiadiazolo pyrimidin, Spectral data, Potential activity

INTRODUCTION

The quinoline skeleton is often used for the design of many synthetic compounds with diverse pharmacological properties such as anti-inflammatory [1], antimicrobial [2], cytotoxic [3], antitumor [4], antimalarial [5], anti-cancer [6], HIV protease inhibitor [7] and anti-viral [8] activities. It is known that many 1,3,4-thiadiazoles have biological activities like antibacterial [9], antimycobacterial [10], antimycotic [11], antifungal [12], antidepressant [13], analgesic [14] and anti-inflammatory [15]. Nitrogen containing heterocyclic ring such as pyrimidine and its derivatives have been reported as anti-microbial [16], analgesic, anti-viral, anti-inflammatory [17], anti-HIV [18], anti-tubercular [19], anti-tumour [20], anti-neoplastic [21], anti-malarial [22], diuretic [23], cardiovascular [24] agents.



Scheme 1. (i) CS₂, NaOH, DMSO, RT, 2 h; (ii) NH₂-NH₂, EtOH, reflux, 6 h; (iii) EtOH, reflux, 4 h; (iv) ArNCS, K₂CO₃, DMF, reflux, 20-22 h **5 (a-h)**: R = a) C₆H₅; b) 4-CH₃-C₆H₄; c) 3-OCH₃-C₆H₄; d) 4-OCH₃-C₆H₄; e) 3-Cl-C₆H₄; f) 4-Cl-C₆H₄; g) 3NO₂-C₆H₄; h) 4-NO₂-C₆H₄.

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Here in, we report a series of eight new quinoline associated 1,3,4-thiadiazolo pyrimidin derivatives. Compound **5e** displayed activity more than standard benzyl penicillin against *B. subtilis* and considerable activity against streptomycin, and compounds **5b** and **5f** are significant active towards *M. luteus* compared to standard drug. The general synthetic scheme is presented in Scheme 1.

EXPERIMENTAL

All the reagents and solvents were used as purchased without further purification. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer BX serried FTIR 5000 spectrometer using KBr pellet. NMR spectra were recorded on a Varian 300 MHz spectrometer for ¹H-NMR and 100 MHz for ¹³C-NMR. The chemical shifts were reported as ppm down field using TMS as an internal standard. Mass spectra were recorded on a VG-Micromass 7070H spectrometer operating at 70 eV.

The antimicrobial studies were conducted by broth method. The broth culture is incubated in non-CO₂ incubator at 35 °C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours). By obtaining isolated colonies of bacterial strains to test and combine the colonies and culture in rich media, incubate overnight and count colonies. The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard and verified cfu/mL count of visible media and the collective results were noted.

Synthesis of 8-fluoro-4-methylsulfanylthiocarbonylamino-quinoline-3-carboxylic acid ethyl ester (2)

To a mixture of 4-amino-8-fluoro-quinoline-3-carboxylic acid ethyl ester (**1**) (0.01 mol) in dimethyl sulphoxide (10 mL) at room temperature was added carbon disulfide (1.4 mL) and aqueous sodium hydroxide (1.0 mL) solution in drop wise. After 30 min dimethyl sulfate (2.3 g) was added under cold conditions by keeping in an ice bath. The reaction mixture then constantly stirred at room temperature for 1.5 h. After completion of the reaction (monitored by TLC), the resultant solution was poured in ice water. The solid that separated out was filtered, dried and recrystallized from ethanol to get pure 8-fluoro-4-methylsulfanylthiocarbonylamino-quinoline-3-carboxylic acid ethyl ester (**2**).

Synthesis of 8-fluoro-4-hydrazine thiocarbonylamino-quinoline-3-carboxylic acid ethyl ester (3)

To the mixture of 8-fluoro-4-methylsulfanylthiocarbonylamino-quinoline-3-carboxylic acid ethyl ester (**2**) (0.01 mol) in ethanol (10 mL) was added hydrazine hydrate (15 mL) and the resulted solution was refluxed for 6 h on uniform stirring. After completion of the reaction (examined by TLC), the mixture was cooled to room temperature, poured in ice-cold water (15 mL) and filtered. The obtained crude solid was recrystallized from ethanol to give 8-fluoro-4-hydrazine thiocarbonylamino-quinoline-3-carboxylic acid ethyl ester (**3**).

Synthesis of 3-amino-7-fluoro-2-mercapto-3H-pyrimido-[5,4-c]quinolin-4-one (4)

A mixture of 8-fluoro-4-hydrazine thiocarbonylamino-quinoline-3-carboxylic acid ethyl ester (**3**) (0.01 mol) in ethanol (10 mL) was heated under reflux with steady stirring for 4 h. After completion of the reaction (watched by TLC), the residual mass was poured over crushed ice and the precipitated crude product was filtered, washed with water, dried and recrystallized from ethanol to get the pure 3-amino-7-fluoro-2-mercapto-3H-pyrimido-[5,4-c]quinolin-4-one (**4**).

Synthesis of 2-arylamino-8-fluoro-[1,3,4]thiadiazolo-[3,2-a]pyrimidin-[5,4-c]quinolin-5-ones (5a-h)

A mixture of 3-amino-7-fluoro-2-mercapto-3*H*-pyrimido-[5,4-*c*]quinolin-4-one (**4**) (0.01 mol), small amount of anhydrous potassium carbonate and suitable aryl isothiocyanates in DMF (15 mL) was refluxed with consistent stirring for 20-22 h. After completion of the reaction (monitored by TLC), the reaction mixture was cooled to room temperature and poured in crushed ice. The resulted solid was filtered off. The crude product thus obtained was purified by column chromatography on silica gel with hexane-ethyl acetate as eluent to afford corresponding 2-arylamino-8-fluoro-[1,3,4]thiadiazolo-[3,2-*a*]pyrimidin-[5,4-*c*]quinolin-5-ones (**5a-h**) in pure form.

Physical and spectral data

8-Fluoro-4-methylsulfanylthiocarbonylamino-quinoline-3-carboxylic acid ethyl ester (2). Yellow solid, yield: 70%, mp: 184-186 °C, IR (KBr): 3347 (N-H), 3052 (C-H, Ar), 2960 (C-H, CH₃), 1740 (C=O), 1640 (C=C, Ar), 1576 (C=N), 1240 (C=S), 1085 (C-O) cm⁻¹; ¹H NMR (300 MHz, DMSO-*d*₆): δ ppm 12.21 (s, 1H, NH), 7.84 (s, 1H, Ar-H), 7.68-7.42 (m, 3H, Ar-H), 3.68 (q, 2H, J = 5.4 Hz, OCH₂), 2.89 (s, 3H, SCH₃), 1.82 (t, 3H, J = 5.4 Hz, CH₃). ¹³C NMR (100 MHz, DMSO-*d*₆): δ ppm 178.9, 165.2, 158.7, 155.4, 152.3, 139.5, 124.2, 118.7, 116.3, 113.8, 106.9, 54.7, 14.8, 12.8. MS: 324 m/z (M⁺). Elemental analysis: calculated for C₁₄H₁₃FN₂O₂S₂: C-51.84, H-4.04, F-5.86, N-8.64, O-9.86, S-19.77. Found: C-49.98, H-3.87, F-5.12, N-8.28, O-9.02, S-18.84.

8-Fluoro-4-hydrazine thiocarbonylamino-quinoline-3-carboxylic acid ethyl ester (3). Brown solid, yield: 69%, mp: 169-171 °C, IR (KBr): 3358 (N-H), 3245 (N-H), 3048 (C-H, Ar), 2954 (C-H, CH₃), 1738 (C=O), 1648 (C=C, Ar), 1580 (C=N), 1236 (C=S), 1092 (C-O) cm⁻¹; ¹H NMR (300 MHz, DMSO-*d*₆): δ ppm 12.36 (s, 1H, NH), 7.89 (s, 1H, Ar-H), 7.68 (s, 1H, NH), 7.60-7.47 (m, 3H, Ar-H), 4.42 (s, 2H, NH₂), 3.61 (q, 2H, J = 5.0 Hz, OCH₂), 1.79 (t, 3H, J = 5.0 Hz, CH₃). ¹³C NMR (100 MHz, DMSO-*d*₆): δ ppm 174.3, 165.3, 156.7, 150.2, 147.6, 143.2, 124.8, 120.3, 118.7, 113.5, 105.8, 57.4, 14.2. MS: 308 m/z (M⁺). Elemental analysis: calculated for C₁₃H₁₃FN₄O₂S: C-50.64, H-4.25, F-6.16, N-17.17, O-10.38, S-10.40. Found: C-49.16, H-4.12, F-6.10, N-17.00, O-9.84, S-9.85.

3-Amino-7-fluoro-2-mercapto-3H-pyrimido-[5,4-c]quinolin-4-one (4). Pale yellow solid, yield: 71%, mp: 154-156 °C, IR (KBr): 3214 (N-H), 3056 (C-H, Ar), 2966 (C-H, CH₃), 2562 (S-H), 1684 (C=O), 1656 (C=C, Ar), 1588 (C=N) cm⁻¹; ¹H NMR (300 MHz, DMSO-*d*₆): δ ppm 7.84 (s, 1H, Ar-H), 7.63-7.58 (m, 3H Ar-H), 5.79 (s, 2H, NH₂), 3.21 (s, 1H, S-H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ ppm 165.7, 163.2, 158.6, 157.4, 147.8, 136.5, 127.8, 123.5, 122.8, 122.0, 113.5. MS: 262 m/z (M⁺). Elemental analysis: calculated for C₁₁H₇FN₄OS: C-50.38, H-2.69, F-7.24, N-21.36, O-6.10, S-12.23. Found: C-49.68, H-2.45, F-7.12, N-20.87, O-5.89, S-11.98.

2-Phentlamino-8-fluoro-[1,3,4]thiadiazolo-[3,2-a]pyrimidin-[5,4-c]quinolin-5-one (5a). Yellow solid, yield: 70%, mp: 144-146 °C, IR (KBr): 3164 (N-H, NH₂), 3062 (C-H, Ar), 1683 (C=O), 1615 (C=C, Ar), 1577 (C=N) cm⁻¹; ¹H NMR (300 MHz, DMSO-*d*₆): δ ppm 9.69 (s, 1H, NH), 7.89 (s, 1H, Ar-H), 7.74-7.48 (m, 8H, Ar-H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ ppm 168.9, 162.3, 160.2, 156.7, 152.3, 147.2, 144.2, 136.4, 127.6, 125.7, 123.6, 122.7, 121.4, 119.5, 116.8, 114.2. MS: 363 m/z (M⁺). Elemental analysis: calculated for C₁₈H₁₀FN₅OS: C-59.50, H-2.77, F-5.23, N-19.27, O-4.40, S-8.82. Found: C-58.02, H-2.61, F-5.02, N-18.47, O-4.21, S-8.26.

2-(4-Methyl phenylamino)-8-fluoro-[1,3,4]thiadiazolo-[3,2-a]pyrimidin-[5,4-c]quinolin-5-one (5b). Pale brown solid, yield: 68%, mp: 135-137 °C, IR (KBr): 3178 (N-H, NH₂), 3068 (C-H, Ar), 1679 (C=O), 1628 (C=C, Ar), 1584 (C=N) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ ppm 9.62 (s, 1H, NH), 7.69 (s, 1H, Ar-H), 7.65-7.51 (m, 3H, Ar-H), 7.64 (d, 2H, J = 7.4 Hz, Ar-H), 7.42 (d, 2H, J = 7.4 Hz, Ar-H), 2.15 (s, 3H, CH₃). ¹³C NMR (100 MHz, DMSO-d₆): δ ppm 166.7, 161.8, 158.7, 155.9, 154.2, 148.6, 143.2, 137.4, 129.6, 124.7, 122.0, 121.8, 120.3, 118.4, 115.6, 113.8, 36.3. MS: 377 m/z (M⁺). Elemental analysis: calculated for C₁₉H₁₂FN₅O₂S: C-60.47, H-3.20, F-5.03, N-18.56, O-4.24, S-8.50. Found: C-58.98, H-3.12, F-4.87, N-17.98, O-4.12, S-8.14.

2-(3-Methoxy phenylamino)-8-fluoro-[1,3,4]thiadiazolo-[3,2-a]pyrimidin-[5,4-c]quinolin-5-one (5c). White solid, yield: 69%, mp: 128-130 °C, IR (KBr): 3162 (N-H, NH₂), 3073 (C-H, Ar), 1684 (C=O), 1644 (C=C, Ar), 1578 (C=N), 1158 (C-O) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ ppm 9.55 (s, 1H, NH), 7.65 (s, 1H, Ar-H), 7.72-7.55 (m, 6H, Ar-H), 7.64 (s, 1H, Ar-H), 3.21 (s, 3H, OCH₃). ¹³C NMR (100 MHz, DMSO-d₆): δ ppm 166.7, 164.0, 163.6, 155.8, 154.7, 149.5, 146.1, 137.4, 129.6, 128.4, 127.5, 126.3, 125.3, 124.7, 122.8, 121.7, 119.5, 116.3, 44.8. MS: 393 m/z (M⁺). Elemental analysis: calculated for C₁₉H₁₂FN₅O₂S: C-58.01, H-3.07, F-4.83, N-17.80, O-8.13, S-5.15. Found: C-56.23, H-2.98, F-4.54, N-17.02, O-7.84, S-5.02.

2-(4-Methoxy phenylamino)-8-fluoro-[1,3,4]thiadiazolo-[3,2-a]pyrimidin-[5,4-c]quinolin-5-one (5d). Yellow solid, yield: 72%, mp: 147-149 °C, IR (KBr): 3166 (N-H, NH₂), 3078 (C-H, Ar), 1688 (C=O), 1636 (C=C, Ar), 1580 (C=N), 1164 (C-O) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ ppm 9.71 (s, 1H, NH), 7.65 (s, 1H, Ar-H), 7.62-7.44 (m, 3H, Ar-H), 7.58 (d, 2H, J = 7.2 Hz, Ar-H), 7.40 (d, 2H, J = 7.2 Hz, Ar-H), 3.12 (s, 3H, OCH₃). ¹³C NMR (100 MHz, DMSO-d₆): δ ppm 169.1, 166.3, 162.4, 155.8, 154.3, 149.2, 146.4, 138.1, 129.2, 127.1, 125.2, 123.0, 120.8, 119.5, 118.1, 112.3, 46.4. MS: 393 m/z (M⁺). Elemental analysis: calculated for C₁₉H₁₂FN₅O₂S: C-58.01, H-3.07, F-4.83, N-17.80, O-8.13, S-5.15. Found: C-56.23, H-2.98, F-4.54, N-17.02, O-7.84, S-5.02.

2-(3-Chloro phenylamino)-8-fluoro-[1,3,4]thiadiazolo-[3,2-a]pyrimidin-[5,4-c]quinolin-5-one (5e). Pale yellow solid, yield: 66%, mp: 174-176 °C, IR (KBr): 3184 (N-H, NH₂), 3066 (C-H, Ar), 1669 (C=O), 1640 (C=C, Ar), 1585 (C=N) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ ppm 9.65 (s, 1H, NH), 7.71 (s, 1H, Ar-H), 7.69-7.44 (m, 6H, Ar-H), 7.58 (s, 1H, Ar-H). ¹³C NMR (100 MHz, DMSO-d₆): δ ppm 167.0, 163.0, 162.4, 158.6, 150.2, 144.7, 142.0, 136.7, 129.4, 126.3, 125.3, 123.4, 122.0, 121.7, 120.2, 116.3, 114.8, 113.1. MS: 397 m/z (M⁺). Elemental analysis: calculated for C₁₈H₉ClFN₅O₂S: C-54.35, H-2.28, Cl-8.91, F-4.78, N-17.60, O-4.02, S-8.06. Found: C-52.69, H-2.20, Cl-8.12, F-4.68, N-16.98, O-3.87, S-7.85.

2-(4-Chloro phenylamino)-8-fluoro-[1,3,4]thiadiazolo-[3,2-a]pyrimidin-[5,4-c]quinolin-5-one (5f). Brown solid, yield: 68%, mp: 123-125 °C, IR (KBr): 3192 (N-H, NH₂), 3062 (C-H, Ar), 1674 (C=O), 1635 (C=C, Ar), 1592 (C=N) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ ppm 9.66 (s, 1H, NH), 7.69 (s, 1H, Ar-H), 7.65-7.42 (m, 3H, Ar-H), 7.55 (d, 2H, J = 7.0 Hz, Ar-H), 7.48 (d, 2H, J = 7.0 Hz, Ar-H). ¹³C NMR (100 MHz, DMSO-d₆): δ ppm 170.5, 166.3, 164.2, 154.3, 150.7, 145.3, 143.7, 134.6, 129.8, 126.3, 125.7, 121.8, 119.8, 117.4, 113.2, 112.0. MS: 397 m/z (M⁺). Elemental analysis: calculated for C₁₈H₉ClFN₅O₂S: C-54.35, H-2.28, Cl-8.91, F-4.78, N-17.60, O-4.02, S-8.06. Found: C-52.69, H-2.20, Cl-8.12, F-4.68, N-16.98, O-3.87, S-7.85.

2-(3-Nitro phenylamino)-8-fluoro-[1,3,4]thiadiazolo-[3,2-a]pyrimidin-[5,4-c]quinolin-5-one (5g). Greenish yellow solid, yield: 67%, mp: 130-132 °C, IR (KBr): 3168 (N-H, NH₂), 3058 (C-H, Ar), 1670 (C=O), 1633 (C=C, Ar), 1625 (N=O), 1577 (C=N) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ ppm 9.78 (s, 1H, NH), 7.76 (s, 1H, Ar-H), 7.71-7.54 (m, 6H, Ar-H), 7.62 (s, 1H,

Ar-H). ^{13}C NMR (100 MHz, DMSO- d_6): δ ppm 167.2, 160.2, 159.8, 155.2, 151.4, 144.8, 141.0, 135.3, 129.8, 126.3, 125.7, 124.7, 122.3, 120.7, 119.7, 118.7, 115.6, 111.3. MS: 408 m/z (M^+). Elemental analysis: calculated for $\text{C}_{18}\text{H}_9\text{FN}_6\text{O}_3\text{S}$: C-52.94, H-2.22, F-4.65, N-20.58, O-11.75, S-7.85. Found: C-51.58, H-2.15, F-4.26, N-19.87, O-11.12, S-7.57.

2-(4-Nitro phenylamino)-8-fluoro-[1,3,4]thiadiazolo-[3,2-*a*]pyrimidin-[5,4-*c*]quinolin-5-one (**5h**). Brown solid, yield: 73%, mp: 140-142 °C, IR (KBr): 3170 (N-H, NH_2), 3054 (C-H, Ar), 1668 (C=O), 1630 (C=C, Ar), 1632 (N=O), 1587 (C=N) cm^{-1} ; ^1H NMR (300 MHz, DMSO- d_6): δ ppm 9.62 (s, 1H, NH), 7.66 (s, 1H, Ar-H), 7.60-7.45 (m, 3H, Ar-H), 7.51 (d, 2H, J = 7.3 Hz, Ar-H), 7.45 (d, 2H, J = 7.3 Hz, Ar-H). ^{13}C NMR (100 MHz, DMSO- d_6): δ ppm 165.6, 161.3, 157.4, 153.5, 150.2, 144.7, 143.4, 138.9, 125.7, 124.2, 121.4, 120.8, 119.8, 116.4, 114.9, 112.8. MS: 408 m/z (M^+). Elemental analysis: calculated for $\text{C}_{18}\text{H}_9\text{FN}_6\text{O}_3\text{S}$: C-52.94, H-2.22, F-4.65, N-20.58, O-11.75, S-7.85. Found: C-51.58, H-2.15, F-4.26, N-19.87, O-11.12, S-7.57.

RESULTS AND DISCUSSION

These initial reports stimulated us to integrate 1,3,4-thiadiazole and pyrimidine moieties into quinoline frame work, since these systems possess well documented biological activity. The target compounds, 2-arylamino-8-fluoro-[1,3,4]thiadiazolo-[3,2-*a*]pyrimidin-[5,4-*c*]quinolin-5-ones (**5a-h**) have been prepared by using commercially available 4-amino-8-fluoro-quinoline-3-carboxylic acid ethyl ester (**1**) as raw material (Scheme 1). The initial intermediate, 8-fluoro-4-methylsulfanylthiocarbonylamino-quinoline-3-carboxylic acid ethyl ester (**2**) has been prepared on constant stirring of a mixture of compound **1**, carbon disulfide and dimethyl sulfoxide in aq. NaOH for 2 h at room temperature. Then compound **2** was reacted with hydrazine hydrate in ethanol solvent on uniform stirring under reflux for 6 h to get the next intermediate, 8-fluoro-4-hydrazine thiocarbonylamino-quinoline-3-carboxylic acid ethyl ester (**3**). Further, the final intermediate, 3-amino-7-fluoro-2-mercapto-3*H*-pyrimido-[5,4-*c*]quinolin-4-one (**4**) for the synthesis of title compounds was prepared by the cyclization of compound **3** in ethanol solvent at reflux for 4 h with steady stirring. Finally, the compound **4** has been cyclized successively with a variety of aryl isothiocyanates and K_2CO_3 in dimethyl formamide under reflux for 20-22 h to get the title compounds, 2-arylamino-8-fluoro-[1,3,4]thiadiazolo-[3,2-*a*]pyrimidin-[5,4-*c*]quinolin-5-ones (**5a-h**). The chemical structures of all newly discovered compounds were established by IR, ^1H & ^{13}C -NMR, mass spectral data and elemental analysis. Further, the target compounds were used to find their antimicrobial ability against different microorganisms.

Antibacterial activity

The *in vitro* antibacterial activity of all newly prepared compounds 2-arylamino-8-fluoro-[1,3,4]thiadiazolo-[3,2-*a*]pyrimidin-[5,4-*c*]quinolin-5-ones (**5a-h**) were evaluated against three gram-positive organisms like *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus* and five gram-negative bacteria such as *Proteus vulgaris*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salomonella paratyphi A* by broth dilution method [25] by using benzyl penicillin and streptomycin as standard drugs for comparison with title compounds. The results of the screening compounds are summarized in Table 1.

The *in-vitro* antibacterial activity of compounds **5a-h** towards different tested bacterial organisms disclosed significant activity with a degree of variation (Table1). It is found that compound **5e** displayed considerable activity against *B. subtilis*. Compounds **5b** and **5f** are significant active towards *M. luteus* compared to standard drug. Notable activity is also achieved for compound **5d** against *S. aureus*. All the quinoline based 1,3,4-thiadiazolo pyrimidin derivatives (**5a-h**) have performed significant to moderate activity against gram-negative bacteria. Derivative **5g** has displayed marked activity against *S. typhimurium*. Remaining

derivatives such as, compounds **5a** and **5h** displayed least activity against all the tested microorganisms. Compounds **5c** and **5d** were showing significant activity against *E. coli*. The outstanding properties of this new class of antibacterial substances deserve further investigation in order to clarify the mode of action at molecular level, responsible for the activity observed.

Table 1. The *in-vitro* antibacterial activity of compounds 5a-h (zone of inhibition in mm).

Entry	<i>B.s.</i>	<i>M.l.</i>	<i>S.a.</i>	<i>P.v.</i>	<i>S.t.</i>	<i>P.a.</i>	<i>E.c.</i>	<i>S.p.</i>
5a	10	14	13	12	14	13	15	17
5b	13	21	16	17	15	18	17	21
5c	18	19	18	16	18	17	19	17
5d	16	21	20	14	16	17	19	16
5e	25	19	15	13	15	15	21	21
5f	22	23	14	17	16	17	20	22
5g	15	15	15	18	24	18	18	19
5h	13	14	15	14	13	15	16	15
Benzyl penicillin	24	26	28	27	28	29	28	29
Streptomycin	29	28	31	30	31	28	32	28

B.s. - *B. subtilis*; *M.l.* - *M. luteus*; *S.a.* - *S. aureus*; *P.v.* - *P. vulgaris*; *S.t.* - *S. typhimurium*; *P.a.* - *P. aeruginosa*; *E.c.* - *E. coli*; *S.p.* - *S. paratyphi*.

CONCLUSIONS

From the current investigation of anti-microbial and antibacterial screening of all the synthesized compounds, it was proved that all the compounds exhibiting better activity against positive organisms and five gram-negative bacteria. From these results, it was observed that **5e** was exhibiting activity more than the slandered drug Benzyl Penicillin against *B. subtilis* and also considerable active against the second slandered drug Streptomycin. Similarly **5b** and **5f** are active against *M. luteus*, makeable activity of compound **5d** against *S. aureus*. Compounds **5c** and **5d** were showing similar activity against *E. coli*. Also among **5a-h**, it was a common observation that **5a** was showed lowest activity against all the bacteria. This was may be because of no substitution on phenyl ring. **5a-h** exhibited the maximum activity by inhibiting the growth of all the bacteria to a greater extent in comparison with the standard drug Streptomycin. From the structure-activity relationship and to optimize the effectiveness of this series of molecules extensive study is also warranted to determine additional physicochemical and biological parameters.

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