ABSTRACT

In the present study, a series of 3-(8-hydroxyquinolin-5-yl)-5-oxo-5H-[1,3]thiazolo[3,2-a]pyrimidine-6-carbonitrile (3) and its derivatives (4a,b) were synthesized via the reaction of 7-methylthio-3-(8-hydroxyquinolin-5-yl)-5-oxo-5H-[1,3]thiazolo[3,2-a]pyrimidine-6-carbonitrile (3) with different N- and O-nucleophiles, such as phenyl, aryl amines, substituted phenols, and imidazoles in the presence of anhydrous potassium carbonate (K2CO3) and dimethyl formamide (DMF). Also, other fused tetracyclic thiazolo[2′,3′:1,2]pyrimido[5,4-d]thiazolo[3,2-a]pyrimidines (5, 6, 7) and (7a-c) were synthesized via the reaction of 7-methylthio-3-(8-hydroxyquinolin-5-yl)-5-oxo-5H-[1,3]thiazolo[3,2-a]pyrimidine-6-carbonitrile (3) with different N- and O-nucleophiles, such as ketenamides, ethyl acetate, and diethyl malonate to yield thiazolo[2′,3′:1,2]pyrimido[4,5-d]pyrazolo[2,3-alpyrimidine derivatives (12-14). On the other hand, treatment of 10 with appropriate aromatic aldehydes afforded the corresponding aryldiene derivatives (15a-c). Finally, reaction of 4-chlorobenzylidene derivative (15b) with thioglycolic acid and chloroacetyl chloride furnished the thiazolidine and azetidinone derivatives (16) and (17), respectively. All the new title compounds were characterized by elemental analysis and spectral data. The antimicrobial activity of some novel products was evaluated by agar well-diffusion.

Keywords: Thiazolo[3,2-a]pyrimidines, thiazolopyrimidinothiazolopyrimidines, pyrazolothiazolopyrimidine, thiazolidine, azetidone, antimicrobial activity.

INTRODUCTION

Heterocyclic compounds containing thiazole rings represent a very significant group of organic compounds [1-3], which are also found in certain natural products such as vitamin B1 (thiamine) and the penicillin thiazoles. Thiazolopyrimidines have become of interest due to their ability to inhibit 2-methylthreitol-2, 4-cyclodiphosphate synthase [4]. They have also been used as analgesic, antiparkinsonian agents [5], antitumor agents [6-8], phosphate inhibitors [9] and acetyl cholinesterase inhibitors [10].

Various condensed thiazolopyrimidines have been reported as antimicrobial substances [11, 12], anti-inflammatory [13], and antiviral activity and as inhibitors of HIV-1 reverse transcriptase [14]. With all the above facts in mind and as a part of our program directed towards the synthesis of polyfunctionally substituted 5-heterocyclic-8-quinolinoles of potential biological interest [15-20], we aimed to report herein the preparation of 3-(8-hydroxyquinolin-5-yl)-7-methylthio-5-oxo-5H-[1,3]thiazolo[3,2-a]pyrimidine-6-carbonitrile (3) as a conveniently accessible precursor for the synthesis of thiazolo[3,2-a]pyrimidines and other related heterocyclic systems.

MATERIALS AND METHODS

All chemicals used were of analytical grade (Qualigen, Merck). The melting points were determined on a KoFer melting point apparatus. IR spectra were recorded on a Pye Unicam SP-3100 spectrophotometer using the KBr wafer technique. The 1 H-NMR spectra were recorded on a Jeol LA-400 MHz at Assiut University. Elemental analyses were carried out using a Perkin-Elmer 240 C Micro analyzer at Assiut University and they were found to be within ± 0.4% of the theoretical values. The starting materials of 5-(2-aminothiazol-4-yl)-8-hydroquinoline 1 and ethyl 2-cyano-3-bis (methylthio) acrylate 2 were prepared according to reported literatures [19] and [21], respectively.

AN EFFICIENT SYNTHESIS AND REACTIONS OF 3-(8-HYDROXYQUINOLIN-5-YL)-7-METHYLTHIO-5-OXO-5H-[1,3] THIAZOLO[3,2-a] PYRIMIDINE-6-CARBONITRILE AS ANTIMICROBIAL AGENTS

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3-(8-Hydroxyquinolin-5-yl)-7-methylthio-5-oxo-5H-[1,3] thiazolo[3,2-a]pyrimidine-6-carbonitrile (3)

A mixture of 1 (2.43g, 0.01 mol) and ethyl 2-cyano-3,3-bis (methylthio) acrylate (2) (2.65 g, 0.01 mol) in 20 mL of N, N'-dimethylformamide and anhydrous potassium carbonate (10mg) was refluxed for 3 hours. The reaction mixture was cooled to room temperature and poured into ice cold water. The separated solid product was filtered, washed with water and recrystallized from n-hexane give pure 3 as orange powder, yield 2.96g (81%), mp 209–211°C; IR (KBr, cm⁻¹) υ 2225 (CN), 1660 (CO). 1 H NMR (DMSO-d⁶) δ = 2.75 (s, 3H, CH₃), 6.25 (s, 1H, thiazolyl), 7.20-8.80 (m, 6H, Ar- H). MS (70 eV) m/z = 366.29 (M+5). Anal. Calcd. for C₂₁H₁₇N₅O₆S₂: C, 62.85; H, 4.51; N, 17.63; S, 8.19%. Found: C, 62.63%; H, 4.56%; N, 17.60%; S, 8.10%.

General procedure for preparation of 4a, b, 5a-c, 7a-c, 8a, b and 9

A mixture of 3 (0.001 mol) was reacted independently with various heterocyclic compounds such as benzimidazole, substituted aromatic amines, substituted phenols, 4-substituted 2-aminothiazoles and 2-aminobenzothiazole (0.001 mol) in dimethyl formamide (20 mL) and anhydrous potassium carbonate (10mg) was refluxed for 4 to 6 hours. The reaction mixture was cooled to room temperature and poured into ice cold water. The separated solid product was filtered, washed with water and recrystallized from the proper solvent.

3-(8-Hydroxyquinolin-5-yl)-7-piperidino-5-oxo-5H-[1,3] thiazolo[3,2-a]pyrimidine-6-carbonitrile (4a)

Light brown crystals from ethanol, yield 0.30g (75%) mp 232–234°C; IR (KBr, cm⁻¹) υ 2218 (CN), 1660 (CO). 1 H NMR (DMSO-d₆) δ = 1.45 (s, 6H, 3OCH₃), 2.85 (t, J = 7.6 Hz, 4H, 2CH₂), 6.00 (s, 1H, thiazolyl), 7.15-8.80 (m, 6H, Ar-H). Anal. Calcd. for C₂₁H₁₇N₅O₆S₂: C, 62.52; H, 4.25; N, 17.36; S, 7.95%. Found: C, 62.68; H, 4.51; N, 17.63; S, 8.19%.

3-(8-Hydroxyquinolin-5-yl)-7-morpholino-5-oxo-5H-[1,3] thiazolo[3,2-a]pyrimidine-6-carbonitrile (4b)

Brown crystals from dioxane, yield 0.8g (69%) mp 214–216°C; IR (KBr, cm⁻¹) υ 2225 (CN), 1660 (CO). 1 H NMR (DMSO-d₆) δ = 2.85
Brown powder from dioxane, yield 0.35g (79%), mp 250–252°C; IR (KBr, cm⁻¹) v 3350 (OH), 1660 (CO). 1 H NMR (DMSO-d₆) δ = 4.85 (s, 2H, NH₂), 2.30 (s, 3H, CH₃). Anal. Calc'd. for C₃H₅N₂O₆S (555.56): C, 60.53; H, 3.81; N, 12.61; S, 5.77%. Found: C, 60.91; H, 4.12; N, 12.87; S, 6.13%.

3-Phenyl-8-(4-hydroxyquinolin-5-yl)-5-oxo-5-imino-thiazolo [3',2',1:2,1]pyrimido [4,5-d] thiazolo [3,2-a] pyrimidine (6b)

Brown crystals from dioxane, yield 0.39g (79%), mp >360°C; IR (KBr, cm⁻¹) v 3315 (NH), 1690 (CO). 1 H NMR (DMSO-d₆) δ = 6.35 (3H, 1H, thienyl), 6.48 (1H, 1H, thiazolyl), 7.65-8.85 (m, 10H, Ar-H), 9.15 (br.s, 1H, NH). Anal. Calc'd. for C₃H₅N₂O₆S (549.55): C, 60.72; H, 2.85; N, 16.99; S, 12.97%. Found: C, 60.98; H, 3.13; N, 17.27; S, 13.21.


Brown crystals from dioxane, yield 0.36g (77%), mp >360°C; IR (KBr, cm⁻¹) v 3255 (NH), 1677 (CO). 1 H NMR (DMSO-d₆) δ = 4.85 (s, 2H, NH₂), 6.00 (1H, 1H, thiazolyl), 7.10-8.80 (m, 6H, Ar-H), 13.10 (s, 1H, NH) MS (70eV) m/z = 532.12 (M⁺ 19). Anal. Calc'd. for C₁₉H₁₄N₄O₅S (532.12): C, 54.85; H, 2.88; N, 12.75; S, 9.15%. Found: C, 55.12; H, 3.01; N, 13.59; S, 9.33%.

A mixture of 3 (1.46g, 0.004 mol) and hydroxyl hydrate (15 mL) in absolute ethanol (40 mL) was refluxed for 5h. The reaction mixture was poured onto ice. The product was isolated and recrystallized from dioxane to yield 10 as white needles, yield 1.4g (74%), mp 208–282°C, IR (KBr, cm⁻¹) v 3440, 3333, 3215 (NH, NH), 1695 (CO). 1 H NMR (DMSO-d₆) δ = 4.85 (s, 2H, NH₂), 6.00 (1H, 1H, thiazolyl), 7.10-8.80 (m, 6H, Ar-H), 13.10 (s, 1H, NH) MS (70eV) m/z = 532.12 (M⁺ 19). Anal. Calc'd. for C₁₉H₁₄N₄O₅S (532.12): C, 54.85; H, 2.88; N, 12.75; S, 9.15%. Found: C, 55.12; H, 3.01; N, 13.59; S, 9.33%.

A mixture of 3 (1.46g, 0.004 mol) and hydroxyl hydrate (15 mL) in absolute ethanol (40 mL) was refluxed for 5h. The reaction mixture was poured onto ice. The product was isolated and recrystallized from dioxane to yield 10 as white needles, yield 1.4g (74%), mp 208–282°C, IR (KBr, cm⁻¹) v 3440, 3333, 3215 (NH, NH), 1695 (CO). 1 H NMR (DMSO-d₆) δ = 4.85 (s, 2H, NH₂), 6.00 (1H, 1H, thiazolyl), 7.10-8.80 (m, 6H, Ar-H), 13.10 (s, 1H, NH) MS (70eV) m/z = 532.12 (M⁺ 19). Anal. Calc'd. for C₁₉H₁₄N₄O₅S (532.12): C, 54.85; H, 2.88; N, 12.75; S, 9.15%. Found: C, 55.12; H, 3.01; N, 13.59; S, 9.33%.

3-Amino-6-(4-hydroxyquinolin-5-yl)pyrazolo [3,4-d] thiazolo [3,2-a] pyrimidin-4(1H)-one (11)

A mixture of 3 (1.46g, 0.004 mol) and hydroxyl hydrate (15 mL) in absolute ethanol (40 mL) was refluxed for 5h. The reaction mixture was poured onto ice. The product was isolated and recrystallized from dioxane to yield 10 as white needles, yield 1.4g (74%), mp 208–282°C, IR (KBr, cm⁻¹) v 3440, 3333, 3215 (NH, NH), 1695 (CO). 1 H NMR (DMSO-d₆) δ = 4.85 (s, 2H, NH₂), 6.00 (1H, 1H, thiazolyl), 7.10-8.80 (m, 6H, Ar-H), 13.10 (s, 1H, NH) MS (70eV) m/z = 532.12 (M⁺ 19). Anal. Calc'd. for C₁₉H₁₄N₄O₅S (532.12): C, 54.85; H, 2.88; N, 12.75; S, 9.15%. Found: C, 55.12; H, 3.01; N, 13.59; S, 9.33%.

To a mixture of compound 3 (0.35g, 0.001 mol) and diethyl malonate (0.17mL, 0.001 mol) was heated at 180 °C in an oil bath for 1h, the reaction mixture was then cooled and triturated with ethanol. The solid product was filtered off, dried and recrystallized from toluene as orange crystals, yield 0.30g (69%), mp 204–206°C; IR (KBr, cm⁻¹) 3630 (NH), 1715, 1670 (CO), 1H NMR (DMSO-d₆): δ = 3.40 (3H, CH₃), 6.50 (1H, thiazolyl), 7.05-8.80 (6H, Ar-H). Anal. Calcd. for C₂₃H₁₉ClN₅O₇S: C, 54.54; H, 2.41; N, 20.09; S, 7.66%. Found: C, 54.81; H, 2.55; N, 20.24; S, 7.91%.

General procedure for preparation of 15c
A mixture of compound 10 (0.001 mol) and the appropriate aldehydic aldehyde (0.001 mol) was stirred under reflux in ethanol (30 mL) in the presence of a few drops of glacial acetic acid for 5 h. The reaction mixture was allowed to cool to room temperature, poured into water, whereby a solid formed that was filtered off and crystallized from ethanol to produce 15c in good yields.

3-Benzylideneamino-6-(8-hydroxyquinolin-5-yl)pyrazolo[3,4-d]thiazolopyrimidine (15b)

Orange powder, yield 0.30g (70%), mp 204–206°C; IR (KBr, cm⁻¹) 3630 (NH), 1715, 1670 (CO), 1H NMR (DMSO-d₆): δ = 6.45 (3H, 1H, thiazolyl), 6.95-8.80 (8H, 1H, Ar-H), 9.25 (1H, N=CH), 13.15 (1H, NH). Anal. Calcd. for C₂₉H₁₉ClN₅O₇S: C, 54.16; H, 2.41; N, 20.28; S, 6.73%. Found: C, 53.87; H, 2.11; Cl, 7.87; N, 17.93; S, 8.06%.

3-(4-Chlorobenzylideneamino)-6-(8-hydroxyquinolin-5-yl)pyrazolo[3,4-d]thiazolo[3,2-a]pyrimidin-4(1H)-one (15b)

Orange powder, yield 0.30g (70%), mp 204–206°C; IR (KBr, cm⁻¹) 3330 (NH), 1680 (CO), 1H NMR (DMSO-d₆): δ = 6.30 (3H, 1H, thiazolyl), 6.90-8.90 (9H, 1H, Ar-H), 9.40 (1H, N=CH), 12.85 (1H, NH). Anal. Calcd. for C₂₉H₁₉ClN₅O₇S: C, 54.16; H, 2.41; N, 20.28; S, 6.63%. Found: C, 53.74; H, 2.30; Cl, 19.41; S, 8.06%.

3-(4-Nitrobenzylideneamino)-6-(8-hydroxyquinolin-5-yl)pyrazolo[3,4-d]thiazolo[3,2-a]pyrimidin-4(1H)-one (15c)

Orange powder, yield 0.31g (64%), mp 259-261°C; IR (KBr, cm⁻¹) 3330 (NH), 1680 (CO), 1H NMR (DMSO-d₆): δ = 6.20 (3H, 1H, thiazolyl), 6.80-8.90 (9H, 1H, Ar-H), 9.75 (1H, N=CH), 13.30 (1H, NH). Anal. Calcd. for C₂₉H₁₉ClN₅O₇S: C, 54.16; H, 2.41; N, 20.28; S, 6.63%. Found: C, 53.74; H, 2.30; Cl, 19.41; S, 8.06%.

3-(4-Chlorophenyl-4-oxo-1,3-thiazolin-3-yl)-6-(8-hydroxyquinolin-5-yl)pyrazolo[3,4-d][1,3]thiazolo[3,2-a]pyrimidine (15d)

A mixture of the Schiff base (15b) (0.47g, 0.001mol) and triethyl orthoformate (0.17mL, 0.001mol) was heated at 180 °C for 1h. The reaction mixture was then cooled and triturated with ethanol. The solid product was filtered off, dried and recrystallized from toluene as orange crystals, yield 0.76 g (70%), mp 180-182°C; IR (KBr, cm⁻¹) 3340 (NH), 1705, 1670 (CO), 1H NMR (DMSO-d₆): δ = 4.95 (3H, J = 6.0 Hz, 1H, CH=CH), 5.35 (1H, N=CH), 6.55 (1H, thiazolyl), 6.90-9.05 (9H, 1H, Ar-H), 13.25 (1H, NH). Anal. Calcd. for C₂₉H₂₅ClN₅O₇S: C, 54.65; H, 2.27; Cl, 13.14; N, 15.30; S, 6.84%. Found: C, 54.83; H, 2.77; Cl, 13.14; N, 15.49; S, 6.03%.

Antimicrobial activity

Using the agar well-diffusion method [22], the antimicrobial activity of the new chemical compounds was tested in vitro against six fungal species which are involved in human and animal diseases (Geotrichum candidum [AUMC No. 226], Candida albicans [AUMC No. 418], Aspergillus flavus [AUMC No. 321], Scopulariopsis brevicaulis [AUMC No. 729], Trichophyton rubrum [AUMC No. 1804] and plant diseases (Fusarium oysporum [AUMC No. 5119]). They were also tested against five bacterial species obtained from contaminated soil, water and food substances (Bacillus cereus [AUMC No. B-52], Staphylococcus aureus [AUMC No. B-54] as Gram positive bacteria [AUMC No:112] and Serratia marcescens [AUMC No. B-55], Escherichia coli [AUMC No. B-53], Pseudomonas aeruginosha [AUMC No. B-73] as Gram negative bacteria. These strains are common contaminants of the environment in Egypt and some of all microbial strains were kindly provided by the Assiut University Mycological Centre (AUMC). To prepare inocula for bioassay, bacterial strains were individually cultured for 48 h in 100 mL conical flasks containing 30 mL nutrient broth medium. Fungi were grown for 7 days in 100 mL conicals containing 30 mL Sabouraud’s dextrose broth. Bioassay was done in 10 cm sterile plastic Petri plates in which microbial suspension (1 mL plate) and 15 mL appropriate agar medium (15 mL plate) were poured. Nutrient agar and Sabouraud’s dextrose agar were respectively used for bacteria and fungi. After solidification of the media, 5 mm diameter cavities were made in the solidified agar (4 cavities/plate) using sterile cork borer. Chemical compounds dissolved in DMSO at 2%w/v (=20 mg/mL) were pipetted in the cavities. The screening tests were carried out in triplicate and the results were expressed as a mean of three determinations.

RESULTS AND DISCUSSION

According to the previously reported method [23], the parent compound 3-(8-hydroxyquinolin-5-yl)-7-methylothio-5-oxo-5H-[1,3]thiazolo[3,2-a]pyrimidine-6-carbonitrile 3 was obtained on refluxing 5-[2-Aminothiazol-4-yl]-8-hydroxyquinoline (1) and ethyl 2-cyano-3-bis(methylthio)acrylate (2) in the presence of dimethyl formamide and a catalytic amount of anhydrous potassium carbonate. The structure of 3 was confirmed according to its elemental analyses and spectral data. The IR spectra revealed the presence of characteristic absorption bands at ν2221 and at 1665 cm⁻¹ assignable to cyan and carbonyl groups, respectively. The H NMR spectra showed the presence of singlet signals at δ 2.75 and 6.25 ppm attributable to the methyl and thiazole proton H-2, respectively. Its mass spectrum showed a peak analogous to the molecular ion at m/z 366.29. Compound 3 possesses a replaceable active methylthio group at the 7-position that is activated by the ring 1-nitrogen atom and the electron withdrawing 6-cyano group. Literature [24] showed that thiazolopyrimidine type 3 is typically used as synthon for preparing of some novel substituted thiazolopyrimidines. In view of these, compound 3 was reacted with selected nitrogen and oxygen nucleophiles like heteroaryl amines, aryl amines and substituted phenols, respectively. These reactions resulted in the formation of 6,7-disubstituted derivatives of 5-oxo-3-(8-hydroxyquinolin-5-yl)-5H-[1,3]thiazolo[3,2-a]pyrimidine. According to this method, compound 3 was reacted with piperidine and morpholine in dimethyl formamide and a catalytic amount of anhydrous potassium carbonate afforded 7-piperidinomorpholin-3-(8-hydroxyquinolin-5-yl)-5H-[1,3]thiazolo[3,2-a]pyrimidine (4a,b). Under similar experimental conditions, compound 3 reacted independently with, 4-methoxyaniline, 4-chloroaniline and 4-
nitroaniline to yield 7-(p-methoxyanilino/ p-chloroanilino/ p-nitroanilino)-3-(8-hydroxyquinolin-5-yi)-5-oxo-5H-[1,3] thiazolo [3, 2-a] pyrimidine-6-carbonitrile (5a-c), respectively. 7-(phenoxy/p-methoxyphenoxyp/ p-nitrophenoxy)-3-(8-hydroxy quinolin -5-yi) -5-oxo-5H-[1,3] thiazolo [3,2-a] pyrimidine-6 carbonitrile (7a-c) derivatives were obtained by the condensation of compound 3 independently with phenol, 4-methoxyphenol and 4-nitrophenoil in N, N'-dimethyl formamide and a catalytic amount of anhydrous potassium carbonate (Scheme 1). The structures of the compounds 4a, b, 5a-c and 7a-c were elucidated on the basis of their spectral data and elemental analyses. For example, the IR spectra of 5a-c revealed absorption bands at ν 3330-3410 and at 2220 - 2245 cm⁻¹ assignable to NH and CN groups, respectively. The ¹H NMR spectrum of 7b exhibited two singlets at δ 3.75 and 6.15 ppm representing the protons of the methoxy (OCH₃) and thiazole H-2, respectively. Its mass spectrum showed a peak corresponding to the molecular ion at m/z 442.16 (M+15) reinforcing the structure of 7b. The 4-methoxyanilino derivative 5a was allowed to react with diethyl malonate in ethanolic sodium ethoxide solution to yield pyrido [2, 3-d] thiazolo [3, 2-a] pyrimidine derivative 6. The IR spectrum of compound 6 revealed the disappearance of an absorption band at 2245 cm⁻¹ due to the cyano group and the appearance of absorption bands at ν 3455, 3330 and 1735 cm⁻¹ attributed to the NH₂ and the CO ester groups, respectively.

The ¹H NMR spectrum of compound 6 revealed a triplet at δ 1.33 ppm (J = 7.8 Hz) assigned to the CH₃ protons and a quartet at δ 4.40 ppm (J = 7.7 Hz) for the CH₂ protons besides other signal at δ 4.15 ppm assigned to the NH₂ group, which ensure the possibility of closure of the pyridine ring via an intra-molecular cyclization, resulting in the formation of compound 6 (Scheme 1).

New tetracyclic thiazolo[2′,3′:1,2] pyrimido [5,4-d] thiazolo [3,2-a] pyrimidines 8a, b and 9 were obtained via the reaction of 3 with 4-methyl(phenyl)-2-aminothiazoles and 2-aminobenzothiazole independently in the presence of dimethyl formamide and a catalytic amount of anhydrous potassium carbonate, respectively.

It is clear that nucleophilic substitution with subsequent in situ cyclization took place (Scheme 2). The structures of compounds 8a, 8b and 9 were deduced on the basis of their spectral data and elemental analyses. The IR spectrum of compound 8a revealed the disappearance of an absorption band at ν 2220 cm⁻¹ due to CN function, and the appearance of an absorption band at ν 3320 for C=NH. ¹H NMR spectra exhibited singlet signal at δ 2.35 ppm for CH₃ protons. Two singlets at δ 5.95 and 6.15 ppm attributable to both thiazoles H-2 and H-9 protons. Its mass spectrum showed a peak analogous to the molecular ion at m/z 432.07 (M+48) (Scheme 2).
Scheme 2: Synthesis Of Compounds 8a,b and 9.

Scheme 3: Synthesis Of Compounds 10-15 a-c.
On the other hand, heating of compound 3 with hydrazine hydrate in ethanol afforded the corresponding 3-amino-6-(8-hydroxyquinolin-5-yl)pyrazolo[3,4-d]thiazolo[3,2-a]pyrimidin-4(1H)-one (10).

Reflexing of 10 with acetic anhydride afforded the expected 3-acetylamino derivative (11). The IR spectrum of compound 11 displayed absorption bands at ν 3340, 3220 and 1660 cm⁻¹ corresponding to the amide NH and amide carbonyl groups, respectively. In accord with the well known cyclodehydration of aminopyrazole with β-bifunctional reagents to yield pyrazolopyrimidines [25], compound (10) reacted with acetyl acetone, ethyl acetoacetate and diethyl malonate to yield pyrimido[3′,2′:1,5]pyrazolo[3,4-d]thiazolo[3,2-a] pyrimidine derivatives (12-14). The structure of compounds 12-14 was assigned on the basis of analytical and spectral data. 1H NMR spectra of compound 12 showed characteristic two singlets at δ 2.25 and 2.37 ppm attributable to both CH protons. The disappearance of the NH and NH₂ signals in the 1H NMR spectra of compounds 12 was in favor of an intramolecular cyclization. Condensation of 3 with aromatic aldehydes, such as benzaldehyde, 4-chlorobenzaldehyde and 4-nitrobenzaldehyde in refluxing ethanol and a few drops of acetic acid afforded the corresponding Schiff's bases (15a-c) (Scheme 3). The structure of compounds 15a-c was characterized by the analytical and spectral data. The 1H NMR spectra of these compounds are simple and consist of the two singlet signals related to the resonance of the C-H and N-H proton, which appeared at δ 9.25-9.75 and δ 12.85-13.30 ppm, respectively. The preparation of 4-thiazolidinone and 4-azetidinone (16) has been undertaken by the heterocyclisation of Schiff's bases with thiglycolic acid and chloroacetyl chloride, respectively. Thus, 4-chloro aryldine derivative 15b when heated with 2-marcapto acetic acid in the presence of anhydrous zinc chloride underwent cyclisation to give thiazolidin-4-one derivative (16). Also, compound 15b reacted with chloroacetyl chloride in the presence of triethylamine under reflux to give azetidin-2-one derivative (17). The structure 16 and 17 was supported by IR and 1H NMR spectral studies. The IR spectra of compound 16 exhibited an absorption band at ν 1715 cm⁻¹ this can be attributed to the cyclic C=O. The N-H is observed at ν 3320 cm⁻¹. The 1H NMR spectra displayed two significant singlets signals at δ 3.70 and 5.50 ppm attributed to the CH₂ (cyclic) and N-CH, respectively. Its mass spectrum showed a peak corresponding to the molecular ion at m/z 547.24 (M+ 31). Finally, the 1H NMR of the azetidinone derivative 17 exhibited CHC proton of β-lactam ring as doublet at δ 4.95 ppm (J = 6.9 Hz) and N-CH proton as multiplet at δ 5.35 ppm reinforcing the structure of 17. (Scheme 4).

Antimicrobial Evaluation

Fifteen selected derivatives (compounds 3, 4b, 5a, 5c, 6, 7b, 8a, 8b, 10, 12, 14, 15b, 15c, 16 and 17) were evaluated for their antifungal and antibacterial activities. Thus, these compounds were screened for their antifungal activities against six fungal strains: Geotrichum candidum, Candida albicans, Aspergillus flava, Scopularis brevis, Trichophyton rubrum and Fusarium oxysporum using clostimazole as control (Table 1). Also, for their antibacterial activity against Bacillus cereus, Staphylococcus aureus as a Gram positive bacteria and Serratia marcescens, Escherichia coli, Pseudomonas aeruginosa as Gram negative bacteria using chloramphenicol as control (Table 2). All experiments were carried out three times. The mean and standard deviation values were recorded using standard deviation calculator. The inhibition zones produced by the various synthesized compounds on the microbial growth were measured (diameter in mm).

Antifungal activity.

The results obtained from Table 1 revealed that the starting compound 3 has no effect against all tested fungi species except against C. albicans (72.6% inhibition) and Aspergillus flavus (98.6% inhibition). When the metlythio (SMe) group in compound 3 is substituted with a morpholino one (4b), it exhibit activity only against Trubrum (67.5% inhibition) and F. oxysporum (73.7% inhibition). Also, by the replacement of SMe group with the 4-methoxy and 4-nitrophenylamino compounds 5a and 5c, respectively we noticed that 5c is more potent against all of the tested fungi especially with A. flavus (99.4% inhibition) and S brevis (90.8% inhibition) than 5a. On heterocyclization of 5a to obtain pyridothiazolopyrimidine compound (6), the activity diminished to zero against most of the fungi species. A comparison of the antifungal activity of the thiazolopyrimidinotiazolopyrimidine derivatives 8a (R=CH₃) and 8b (R=Ph), indicated that the methyl derivative (8a) induces more fungitoxicity against all fungi tested except against Aflavus than the
other substituent 8b. It was noted that amongst pyrimidine derivatives 10, 12 and 14, only the pyrimidinedione (14) exhibit a moderate activity against Calicibicans (91.46% inhibition) and A. flavus (83.6% inhibition). A comparison of the antifungal activity data of compounds 15b (R = 4-Cl) and 15c (R = 4-NO2) clearly indicates that the 4-chloro derivative 15b is more active against all of the tested fungi except against Trubrum than the other substituent 15c. Also the thiazolidene derivative (16) is more active than the azetidineone (17) against all of the tested fungi species.

Table 1: Antifungal activity of some synthesized 3-(8-hydroxyquinolin-5-yl)-[1, 3] thiazolo [3, 2-a] pyrimidine derivatives

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>G. candidum</th>
<th>C. albicans</th>
<th>A. flavus</th>
<th>S. brevicaulis</th>
<th>T. rubrum</th>
<th>E. oxysporum</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>--</td>
<td>19.3±0.28</td>
<td>35.9±0.4</td>
<td>--</td>
<td>--</td>
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</tr>
<tr>
<td>4b</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>37.3±0.55</td>
<td>21.2±0.82</td>
<td>--</td>
</tr>
<tr>
<td>5a</td>
<td>12.4±0.42</td>
<td>8.06±0.40</td>
<td>38.4±0.31</td>
<td>26.5±1.13</td>
<td>15.47±0.45</td>
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</tr>
<tr>
<td>5c</td>
<td>22.6±0.55</td>
<td>25.8±0.61</td>
<td>38.6±0.31</td>
<td>29.9±0.82</td>
<td>24.3±0.50</td>
<td>--</td>
</tr>
<tr>
<td>6</td>
<td>--</td>
<td>8.5±0.50</td>
<td>12.7±0.80</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>7b</td>
<td>11.9±0.79</td>
<td>8.36±0.40</td>
<td>--</td>
<td>18.2±0.73</td>
<td>10.1±0.45</td>
<td>--</td>
</tr>
<tr>
<td>8a</td>
<td>23.9±0.90</td>
<td>25.8±0.46</td>
<td>40.1±1.01</td>
<td>43.5±0.40</td>
<td>26.1±0.70</td>
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<tr>
<td>8b</td>
<td>18.9±1.00</td>
<td>11.5±0.20</td>
<td>33.8±0.35</td>
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<td>6.43±0.45</td>
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</tr>
<tr>
<td>10</td>
<td>--</td>
<td>--</td>
<td>17.1±0.47</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>12</td>
<td>--</td>
<td>--</td>
<td>8.4±0.36</td>
<td>6.4±0.40</td>
<td>8.3±0.38</td>
<td>--</td>
</tr>
<tr>
<td>15b</td>
<td>18.7±0.71</td>
<td>22.1±0.21</td>
<td>34.7±0.66</td>
<td>30.2±0.25</td>
<td>--</td>
<td>19.9±0.81</td>
</tr>
<tr>
<td>15c</td>
<td>--</td>
<td>--</td>
<td>38.3±0.40</td>
<td>17.8±0.26</td>
<td>21.5±0.50</td>
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</tr>
<tr>
<td>16</td>
<td>23.5±0.60</td>
<td>21.1±0.15</td>
<td>36.2±0.31</td>
<td>41.0±0.31</td>
<td>20.8±0.92</td>
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<tr>
<td>17</td>
<td>--</td>
<td>--</td>
<td>20.1±0.17</td>
<td>11.2±0.37</td>
<td>8.2±0.31</td>
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</tr>
<tr>
<td>Clotrimazole</td>
<td>25.6±0.60</td>
<td>26.5±0.46</td>
<td>40.6±0.12</td>
<td>42.5±0.44</td>
<td>55.3±0.36</td>
<td>28.8±0.76</td>
</tr>
</tbody>
</table>

*All the test and the standard drug were tested at the concentration of 20 mg/ml Each result represents the average of triplicate readings.

- No inhibition observed.

Table 2: Antibacterial activity of some synthesized 3-(8-hydroxyquinolin-5-yl)-[1, 3] thiazolo [3, 2-a] pyrimidine derivatives

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>B. cereus (+ve)</th>
<th>S. aureus (+ve)</th>
<th>S. marcescens (-ve)</th>
<th>E. coli (-ve)</th>
<th>P. aeruginosa (-ve)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>16.4±0.40</td>
<td>28.7±0.80</td>
<td>18.7±0.68</td>
<td>34.5±0.47</td>
<td>21.6±0.53</td>
</tr>
<tr>
<td>4b</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>5a</td>
<td>19.9±0.90</td>
<td>20.6±0.81</td>
<td>23.0±0.12</td>
<td>18.3±0.30</td>
<td>23.2±1.08</td>
</tr>
<tr>
<td>5c</td>
<td>28.4±0.36</td>
<td>31.5±0.47</td>
<td>29.0±0.72</td>
<td>22.3±0.53</td>
<td>26.3±0.40</td>
</tr>
<tr>
<td>6</td>
<td>10.0±0.12</td>
<td>10.3±0.30</td>
<td>12.8±0.76</td>
<td>16.5±0.50</td>
<td>8.0±0.17</td>
</tr>
<tr>
<td>7b</td>
<td>16.1±0.29</td>
<td>12.3±0.35</td>
<td>10.6±0.65</td>
<td>8.1±0.23</td>
<td>14.3±0.35</td>
</tr>
<tr>
<td>8a</td>
<td>19.8±0.76</td>
<td>25.5±0.51</td>
<td>19.1±0.15</td>
<td>22.3±0.40</td>
<td>25.2±0.23</td>
</tr>
<tr>
<td>8b</td>
<td>15.2±0.32</td>
<td>20.1±0.29</td>
<td>15.7±0.67</td>
<td>17.5±0.50</td>
<td>14.7±0.45</td>
</tr>
<tr>
<td>10</td>
<td>12.1±0.23</td>
<td>17.1±0.38</td>
<td>13.3±0.26</td>
<td>20.6±0.53</td>
<td>22.1±0.17</td>
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<tr>
<td>12</td>
<td>16.4±0.35</td>
<td>20.3±0.58</td>
<td>16.6±0.53</td>
<td>23.7±0.35</td>
<td>19.0±1.10</td>
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<tr>
<td>14</td>
<td>15.4±0.74</td>
<td>24.7±0.64</td>
<td>10.2±0.20</td>
<td>19.1±0.17</td>
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<tr>
<td>15b</td>
<td>18.3±0.29</td>
<td>21.1±0.23</td>
<td>23.8±0.81</td>
<td>20.2±0.40</td>
<td>20.7±0.75</td>
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<td>8.3±0.29</td>
<td>15.6±0.56</td>
<td>20.6±0.53</td>
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<td>10.6±0.58</td>
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<td>16</td>
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<td>22.0±1.05</td>
<td>20.4±0.36</td>
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<td>17</td>
<td>21.2±0.40</td>
<td>11.5±0.50</td>
<td>17.1±0.15</td>
<td>18.1±0.29</td>
<td>20.1±0.23</td>
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<tr>
<td>Chloramphenicol</td>
<td>38.3±0.29</td>
<td>44.5±0.46</td>
<td>34.7±0.68</td>
<td>36.2±0.40</td>
<td>32.8±0.81</td>
</tr>
</tbody>
</table>

*All the test and the standard drug were tested at the concentration of 20 mg/ml Each result represents the average of triplicate readings.

- No inhibition observed.

Antibacterial activity.

The results of antibacterial activity of some synthesized compounds against different bacterial species were tabulated in Table 1. The study revealed that all compounds exerted moderate to significant antibacterial activity against the Gram positive as well as the Gram-negative bacteria, except 3-(8-Hydroxyquinolin-5-yl)-7-morpholinol-5-oxo-5H-[1,3] thiazolo [3, 2-a] pyrimidine-6-carbonitrile (4b) failed to exhibit inhibition against all the organisms. Compounds 3, 5a, 5c, 6a and 15b were found to possess appreciable antibacterial activity. The 3-(8-Hydroxyquinolin-5-yl)-7-methylthio-5-oxo-5H-[1,3] thiazolo [3, 2-a] pyrimidine-6-carbonitrile (3) showed an excellent inhibition zone against E. coli (95.30% inhibition). The 4-nitrophenylamino derivative (5c) showed good activity (83.63% inhibition against S. marcescens) comparable to the 4-methoxy one (5a) (66.32% inhibition against S. marcescens). Also, the methylthiazole derivative (8a) (76.87% inhibition against P. aeruginosa) is potent than the phenylthiazole derivative (8b). 4-Chlorobenzylideneamino derivative (15b) was found to be better than that of the 4-nitro derivative (15c). No definite trend was discernable that could lead to draw a correlation of the activities between these compounds.

CONCLUSION

It is concluded that the present work provides a convenient and efficient route for the preparation of new thiazolo [3, 2-a] pyrimidine derivatives and their fused thiazolo, pyrazolo, and pyrimidotropazarol ring systems. Fifteen of the newly synthesized compounds have been screened for their antimicrobial activities. Most of the tested compounds showed activities against the strains used. The results of the present study may serve as a ready reference for the researchers.
to take advantage of proficient procedure applied for the synthesis of novel series of derivatives and further plausible modifications which will augment the therapeutic potential of thiazolo [3,2-a] pyrimidine derivatives.

REFERENCES