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Synthesis of Some New Binary and Spiro Heterocyclic Thiazolo[4,3-b][1,3,4]thiadiazole Ring Systems and Their Antimicrobial Evaluation

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ABSTRACT

Schiff's bases 2-5 were synthesized by reaction of 5-phenyl-5H-thiazolo[4,3-b][1,3,4]thiadiazol-2-amine (1) with some aldehydes and ketone. Cycloaddition reaction of ketimine 5 with thioglycolic acid or thiosemicarbazide *afforded spirothiazolidinone and spirotriazole derivatives 6, 7, respectively. Transformation of 1 with the appropriate α-halocarbonyl compounds produced thiazolo[4,3-b][1,3,4]thiadiazole derivatives 8 and 9. The newly synthesized compounds were screened for their* antimicrobial *activities. Furthermore, their geometrical optimization calculations through the Density Functional Theory (DFT) method for the new synthesized compounds were in a good agreement with the proposed structures.*

Keywords: Schiff's bases, Aldehydes, Isatin, α-halocarbonyl compounds, Thiazolidinone, Thioglycolic acid, Antimicrobial activity

INTRODUCTION

The development of new antimicrobial agents is becoming the major interest in many academic and industrial research laboratories all over the world with the aim to develop more potent molecules with higher specificity.

Heterocyclic compounds play an important role in biological processes, especially those heterocycles containing bridgehead-nitrogen are of interest because of their wide use in medicinal and agrochemistry as scaffolds for active agents [1,2] such as antiviral, antiulcer, antimalarial, antibacterial, antifungal, herbicidal, antileprotic and immune suppressive agents [3]. Schiff's bases bearing heterocyclic rings possess excellent biological activities, which has attracted many researcher's attention in recent years [4-6]. In particular, Schiff's bases of 1,3,4-thiadiazole derivatives have been studied because of their interesting pharmacological properties including analgesic [3], antimicrobial [4-9], anti-inflammatory [10], antitubercular [11], anticonvulsant [12], anticancer [13], anthelmintic [14], antiplatelet [15] and diuretic activity [16]. 2-Amino-1,3,4-thiadiazole derivatives are also used as antitumor and antituberculosis drugs [17,18]. Recently, *N*-(substituted)-2-amino-5-aryl-5*H*-thiazolo[4,3-b]-1,3,4-thiadiazoles have drawn the attention of researchers due to its antitubercular, antibacterial and antifungal activities. They have interesting activity against pathogenic fungi of agricultural crops [19-24]. Thus, in view of the above mentioned characteristics and in continuation of our research program as well as our interest in bridgehead-nitrogen heterocyclic systems, we synthesized new compounds containing thiazolo[4,3-b][1,3,4]thiadiazole moieties and their antimicrobial activity has been studied. The synthesis of the target compounds is outlined in Schemes 1-3.

EXPERIMENTAL

Instruments

All melting points are in degree centigrade (uncorrected) and were determined on Gallenkamp electric melting point apparatus. Elemental analyses were carried out at Micro analytical Center, Faculty of Science, Cairo University. IR spectra were recorded (KBr), (ύ cm^{-1}) on a Mattson 5000 FTIR Spectrophotometer at Microanalytically Center Faculty of Science, Mansoura University. ^{1}H NMR Spectra were measured on a Varian Spectrophotometer at 300 MHz, using TMS as an internal reference and DMSO-*d6* as solvent at Chemistry Department, Faculty of Science, Cairo University. ^{13}C NMR (100 MHz) was recorded in DMSO-d6 using a Bruker AV 400 spectrometer at Chemistry Department, Faculty of Science, Assiut University. Mass spectra were recorded on (Kratos, 70 eV) MS equipment and/or a Varian MAT 311 A Spectrometer, at Microanalytical Center, Faculty of Science, Cairo University. Reaction mixtures were monitored by thin layer chromatography (TLC) using EM science silica gel coated plates with visualization by irradiation with ultraviolet lamp. Biological Testing was carried out at Drug Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

Synthesis of Heterocyclic Schiff's bases 2-5

General procedure

To a stirred solution of compound 1 (1 mmol) in ethanol (50 ml) containing sulphuric acid (2 ml) was added appropriate aldehyde (2,4-dihydroxybenzaldehyde, 2-hydroxy naphthaldehyde and 1,3-diphenyl-1*H*-pyrazole-5-carbaldehyde) or isatin (1 mmol), the mixture was refluxed for 6 h. The separated solid was filtered off and recrystallized from ethanol to give the corresponding Schiff's bases 2-5 respectively.

(E)-4-(((5-phenyl-5H-thiazolo[4,3-b][1,3,4]thiadiazol-2-yl)imino) methyl)benzene-1,3-diol (2)

Yield 55% (Brown crystals); m. >300^{o}C; R_f = 0.88 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr):V_{max}, cm^{-1}: 3422 (OH), 2928 (C-H), 1616 (-N=CH), 701 (C-S-C); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.09-7.86 (m, 8H, Ar-CH), 7.88 (s, 1H, N=CH), 8.28 (s, 1H, thiazolyl 7-H), 8.34 (s, 1H, thiazolyl 5-H), 11.65 (s, 1H, OH-*p*), 12.03 (s, 1H, OH-*o*); MS (EI, 70 ev) m/z (%) = 355 (M$^+$, 3.34), 341 (14.5), 313 (38.6), 133 (13.1), 92 (10.6), 82 (100, base peak); Anal. Calcd. For $C_{17}H_{13}N_3O_2S_2$ (355.43): C, 57.45; H, 3.69; Found: C, 57.36; H, 3.62%.

(E)-1-((5-phenyl-5H-thiazolo[4,3-b][1,3,4]thiadiazol-2-ylimino)methyl)naphthalen-2-ol (3)

Yield 60% (Yellow crystals); m.p 295-297^{o}C; R_f = 0.76 [pet. ether(60-80)/ethyl acetate (4:1)]; IR(KBr):V_{max}, cm^{-1}: 3445 (OH), 1580 (-N=CH), 721 (C-S-C); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.27-8.05 (m, 11H, Ar-CH), 8.63 (s, 1H, N=CH), 8.66 (s, 1H, thiazolyl 7-H), 9.99 (s, 1H, thiazolyl 5-H), 12.86 (s, 1H, OH); ^{13}C NMR (DMSO-d_6) δ (ppm): 87.10 (thiazolyl 7-C), 108.48 (thiazolyl 5-C), 115.16, 118.91, 119.32, 121.79, 123.88, 127.63, 127.86, 128.04, 128.95, 128.99, 129.32, 130.00, 130.11 (2 C), 132.37, 133.11, 134.77, 160.08(CH=N), 161.29 (C-OH); MS (EI, 70 ev) m/z (%) = 391 (M$^+$+2, 0.74), 389 (M$^+$, 3.0), 218 (26.1), 148 (12.1), 142 (42.5), 129 (4.8), 115 (100, base peak); Anal. Calcd. For $C_{21}H_{15}N_3OS_2$ (389.49): C, 64.76; H, 3.88; Found: C, 64.69; H, 3.80%.

(Z)-1-(1,3-diphenyl-1H-pyrazol-5-yl)-N-(5-phenyl-5H-thiazolo[4,3-b][1,3,4]thiadiazol-2-yl)methanimine (4)

Yield 58 % (Yellow crystals); m.p 233-238^{o}C; R_f = 0.85 [pet. ether (60-80)/ethyl acetate (4:1)]; IR(KBr):V_{max}, cm^{-1}: 3140 (ArC-H), 2924 (C-H), 2854 (C-H thiazole), 1596 (N=CH), 712 (C-S-C); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.16-7.86 (m, 15H, Ar-CH and CH-pyrazolyl), 8.11 (s, 1H, N=CH), 8.23 (s, 1H, thiazolyl 7-H), 8.46 (s, 1H, thiazolyl 5-H); MS (EI, 70 ev) m/z (%) = 466 (M$^+$+1, 0.4), 465 (M$^+$, 2.3), 463 (14.4), 346 (14.4), 246 (100, base peak), 231 (38.2), 217 (15.4), 116 (23.4),77 (91.6), 65 (3.4); Anal. Calcd. For $C_{26}H_{19}N_5S_2$ (465.59): C, 67.07; H, 4.11; Found: C, 67.20; H, 4.17%.

(Z)-3-(5-phenyl-5H-thiazolo[4,3-b][1,3,4]thiadiazol-2-ylimino)indolin-2-one (5)

Yield 65% (purple crystals); m.p 285-290^{o}C; R_f = 0.12 [pet. ether(60-80)/ethyl acetate (4:1)]; IR (KBr):V_{max} cm^{-1}: 3444 (NH), 2797 (ArC-H), 1683 (C=O), 1557 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.55-7.96 (m, 9H, Ar-CH), 8.17 (s, 1H, thiazolyl 7-H), 9.80 (s, 1H, thiazolyl 5-H), 10.11 (s, 1H, NH isatin); MS (EI, 70 ev) m/z (%) = 366 (M$^+$+2, 1.1), 364 (M$^+$, 21.6), 233 (0.5), 128 (16.6), 102 (77.9), 95 (35.8), 76 (100, base peak), 55 (56.1); Anal. Calcd. For $C_{18}H_{12}N_4OS_2$ (364.44): C, 59.32; H, 3.32; Found: C, 59.44; H, 3.39%.

Synthesis of spiroindolines 6 and 7

A mixture of (3.75 g, 1 mmol) and thioglycolic acid and/ or thiosemicarbazide (1 mmol) in DMF (10 ml) was refluxed for 10 h. The reaction mixture was then poured into 25 ml of ice-cold and the resulted solid was filtered off, dried and recrystallized from ethanol afforded 6 and /or **7**.

3'-(5-Phenyl-5H-thiazolo[4,3-b][1,3,4]thiadiazol-2-yl)spiro[indoline-3,2'-thiazolidine]-2,4'-dione (6)

Yield 66% (black crystals); m.p 298-300^{o}C, R_f = 0.73 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr) $_{max}$ cm^{-1}: 3443 (NH), 1664, 1631 (2 CO), 1587 (C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.30 (s, 2H, CH_2 thiazole), 6.94-8.03 (m, 9 H, ArC-H), 8.12 (s, 1H, thiazolyl 7-H), 9.25 (s, 1H, thiazolyl 5-H), 9.91 (s, 1H, NH isatin); MS (EI, 70 ev) m/z % = 438 (M$^+$, 5.5), 408 (0.2), 364 (2.2), 243 (0.3), 121 (17.1), 105 (100, base peak), 95 (1.9), 65 (30.6), 51 (62.1); Anal. Calcd. For $C_{20}H_{14}N_4O_2S_3$ (438.54): C, 54.78; H, 3.22; Found: C, 54.86; H, 3.28%.

5'-Amino-4'-(5-phenyl-5H-thiazolo[4,3-b][1,3,4]thiadiazol-2-yl)-2',4'-dihydrospiro [indoline-3,3'-[1,2,4]triazol]-2-one (7)

Yield 67% (red crystal); m.p 300-305^{o}C; R_f = 0.76 [pet. ether(60-80)/ethyl acetate (4:1)], IR (KBr):V_{max} cm^{-1}: 3442, 3310, 3295, 3108 (NH_2 and 2 NH), 2927 (C-H, str.), 1621 (C=O), 1592 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 6.19 (br. s, 2H, NH_2), 7.34-8.11 (m, 10H, Ar-CH and NHtriazole), 8.77 (s, 1H, thiazolyl 7-H), 9.25 (s, 1H, thiazolyl 5-H), 9.90 (s, 1H, NHindole); MS (EI, 70 ev) m/z (%) = 421 (M$^+$, 0.4), 445 (50.7), 412 (45.6), 330 (100, base peak), 225 (34.9), 206 (33.0), 131 (6.3), 83 (66.8); Anal. Calcd. For $C_{19}H_{15}N_7OS_2$ (421.50): C, 54.14; H, 3.59; Found: C, 54.26; H, 3.63%.

1-phenyl-2-((5-phenyl-5H-thiazolo[4,3-b][1,3,4]thiadiazol-2-yl)amino)ethan-1-one (8)

A mixture of 5-phenyl-5*H*-thiazolo[4,3-b][1,3,4]thiadiazol-2-amine (1) (2.35 g, 1 mmol) and phenacyl bromide (1.99 g, 1 mmol) in absolute ethanol was refluxed for 1 h. The reaction mixture was allowed to cool to room temperature, and formed precipitate was filtered off, washed with cold ethanol and recrystallized from ethanol furnished 8.

Yield 64% (white crystals); m.p 230-232^{o}C; R_f = 0.57 [pet. ether(60-80)/ethyl acetate(4:1)]; IR (KBr):V_{max} cm^{-1} 3421 (NH), 1674 (CO), 1621 (C=N), 1560 (C=C), ^{1}H NMR (DMSO-d_6) δ (ppm): 4.12 (s, 2H, CH_2), 7.13-7.85 (m, 10H, Ar-CH), 7.98 (s, 1H, CH thiazolyl 7-H), 9.00 (s, 1H, thiazolyl 5-H), 12.00 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 40.13 (CH_2CO), 85.70 (thiazolyl 7-C), 103.79 (thiazolyl 5-C), 125.57, 126.31, 126.46, 127.63, 127.88, 128.37, 128.67, 128.88, 129.36, 134.36, 134.51, 141.45, 142.42, 150.25(C=NH), 168.24 (C=O); MS (EI, 70 ev) m/z (%) = 354 (M$^+$+1, 0.8), 353 (M$^+$, 4.7), 277 (4.0), 148 (46.1), 134 (17.9), 106 (10.9), 82 (100, base peak); Anal. Calcd. For $C_{18}H_{15}N_3OS_2$ (353.46): C, 61.17; H, 4.28; Found: C, 61.21; H, 4.31%.

3-((5-phenyl-5H-thiazolo[4,3-b][1,3,4]thiadiazol-2-yl)glycyl)-2H-chromen-2-one (9)

A mixture of 1 (2.35 g, 1 mmol) and 3-(2-bromoacetyl)-2*H*-chromen-2-one (2.76 g, 1 mmol) in glacial acetic acid (10 ml) was refluxed for 3 h the formed precipitate was filtered off, washed with cold ethanol and recrystallized from ethanol furnished 9. Yield 70% (yellow crystals); m.p 218- 220^{o}C; R_f = 0.47 [pet. ether(60-80)/ethyl acetate (4:1)]; IR (KBr):V_{max}, cm^{-1}: 3413 (NH), 2717, 2923 (CH str.,), 1711 (OC=O), 1662 (C=O), 1596 (C=N), 1580 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.42 (s, 2H, CH_2), 7.16-7.87 (m, 9H, ArC-H), 8.31 (s, 1H, thiazolyl 7-H), 8.55 (s, 1H, CH-coumarin), 9.00 (s, 1H, thiazolyl 5-H), 12.19 (s, 1H, NH); MS (EI, 70 ev) m/z (%) = 422 (M^++1, 0.3), 421 (M^+, 10.4), 234 (9.3), 218 (6.2), 173 (94.8), 145 (32.9), 76 (100, base peak); Anal. Calcd. For $C_{21}H_{15}N_3O_3S_2$ (421.49): C, 59.84; H, 3.59; Found: C, 59.78; H, 3.54%.

Antimicrobial activity

Chemical compounds were individually tested against a panel of Gram positive such as *Bacillus Subtilis*, Gram Negative such as *Escherichia coli* and *Fungi such as Candida albicans* by the agar diffusion technique. Each of the screened compounds was dissolved in DMSO and solutions of the concentration 1 mg/ml were prepared separately. Paper discs of what man filter paper were prepared with standard size (5 cm) were cut and sterilized in an autoclave. The paper discs soaked in the desired concentration of the complex solution were placed aseptically in the petridishes containing nutrient agar media (agar 20g + beef extract 3g + peptone 5g) seeded with *Bacillus Subtilis, E. coli and Candida albicans*. The petri dishes were incubated at 36°C and the diameter of inhibition zone (millimeter) was measured, after 24 h of incubation. Each treatment was replicated three times whereby the average value is recorded. The antibacterial activity of a common standard antibiotic ampicillin and antifungal clotrimazole was also recorded using the same procedure as above at the same concentration and solvents [25-27].

RESULTS AND DISCUSSION

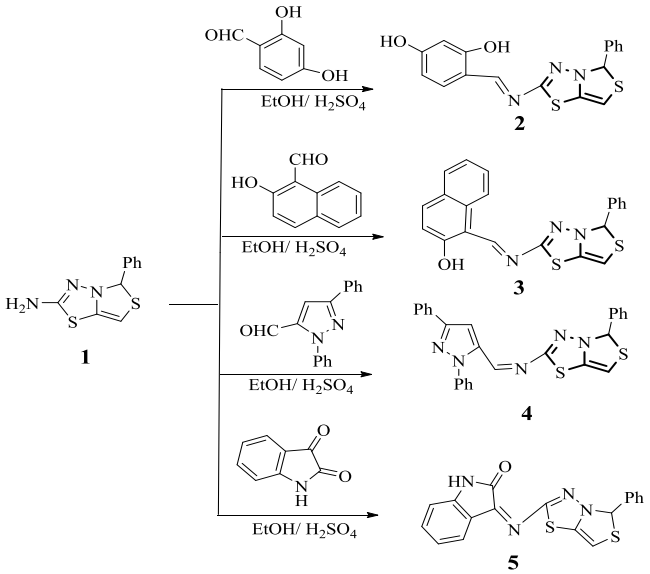
Chemistry

The starting material, 5-phenyl-5*H*-thiazolo[4,3-b][1,3,4]thiadiazol-2-amine (1) was synthesized according to published work [28,29]. The acid-catalyzed condensation of 1 with aromatic aldehydes namely 2,4-dihydroxybenzaldehyde and 2-hydroxy naphthalene-1-carbaldehyde or with heterocyclic aldehyde such as 1,3-diphenyl-1*H*-pyrazole-5-carbaldehyde [30] was carried out by heating under reflux in ethanol containing catalytic amount of conc. sulphuric acid yielded the corresponding Schiff's bases, *N*-(benzylidene)-5-phenyl-5*H*-thiazolo[4,3-b][1,3,4]thiadiazol-2-amine derivatives 2-4, respectively (Scheme 1).

The chemical structures of the newly synthesized Schiff's bases were confirmed by IR, ^{1}H-NMR spectral measurements and elemental analyses which indicate a good agreement with the proposed structures. The IR spectrum of 2 showed stretching absorption band at 3422, 1616 cm^{-1}, attributed to the OH and N=CH functional groups, respectively, while the absorption band due to NH_2 disappeared. Compound 3 displayed similar absorption bands at 3445 for OH, 1580 cm^{-1} for N=CH functional groups, respectively. Whereas, compound 4 showed a similar IR pattern. The ^{1}H NMR spectrum of 2 showed signals at δ 7.88 (CH=N), 7.09-7.86 (8 aromatic protons), 8.28, 8.34 for 7-H and 5-H of thiazole moiety, 11.65 (OH-*p*) and at 12.03 ppm (OH-*o*). In addition compound 2 gave a molecular ion peak at m/z 355 (M^+, 3.34) and a base peak at m/z 82.

However, the ^{1}H NMR spectrum of 3 is characterized by chemical shifts at δ 7.27-8.05, 8.63, 8.66 and 9.99 due to (11 aromatic protons), N=CH, 7-H and 5-H of thiazole moiety, respectively and 12.86 ppm for OH proton. Moreover, ^{13}C NMR of 3 displayed a full agreement with its structure, While the ^{1}H NMR spectrum of 4 showed signals at δ 7.16-7.86 corresponding to 15 aromatic protons and CH of pyrazolyl ring, 8.11 consequent to N=CH, 8.23 and 8.46 for 7-H and 5-H of thiazole moiety, respectively. Moreover, the elemental microanalyses of compounds 2-4 were all in good agreement with the assigned structures.

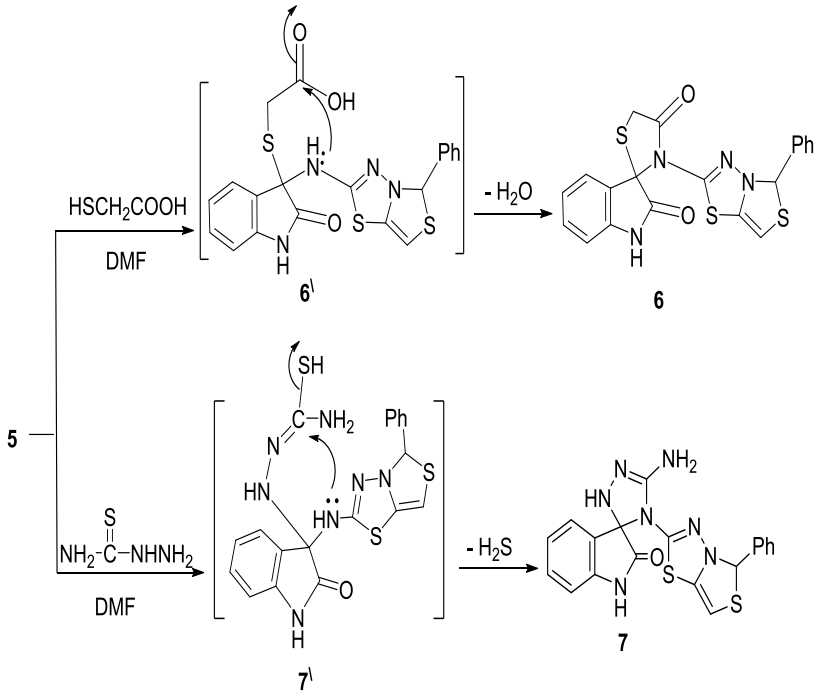
Also, the condensation of 1 with isatin in ethanol contain drops of conc. H_2SO_4 under reflux gave the corresponding Schiff's base 5. Its structure was characterized from the spectral analysis. Thus, its IR showed the presence of C=O and NH bands for indole at 1683 and 3444, respectively and C=N- functional group at 1557 cm^{-1}. Its ^{1}H NMR spectrum showed signals at δ 7.55-7.96 for (9 aromatic protons), 7-H and 5-H of thiazole moiety appeared at δ 8.17 and 9.80, respectively and a singlet signal at 10.11 ppm for indole NH. Its mass spectrum showed a molecular ion peak at *m*/*z* 364 (M^+, 21.6) and a base peak at *m*/*z* 76.



Scheme 1: Synthesis of aldimines 2-4 and ketimine 5

On the other hand, keeping in view the diverse biological activities associated with spiroindoles, thiazolo[1,3,4]thiadiazole and spiro indo linothiazolidine, and/or spiro indoline[1,2,4]triazol, it inspired us to construct a novel system which may combine these bioactive rings together in a single molecular framework to see the additive reinforcement towards their biological activities. Furthermore, a literature survey reveals no report on the synthesis of the target novel spiroindolinothiazolidine, and/or spiro indolino[1,2,4]triazol yet so far. As a part of our ongoing program to develop efficient methods for the synthesis of biologically relevant compounds [31] from readily available building blocks, via Michael addition of ketimine 5 with thioglycolic acid and thiosemicarbazide in DMF to afford the respective spiro[indoline-3,2'-thiazolidine]-2,4'-dione 6 and spiro[indoline-3,3'-[1,2,4]triazol]-2-one derivatives 7 (Scheme 2) were implemented. Thus, compounds 6 and 7 were formed via Michael addition followed by elimination of either water or hydrogen sulfide from non-isolable intermediates 6' or 7'.

The assigned structures of 6 and 7 were proved based on elemental and spectral analyses. The IR spectrum of the isolated product 6 showed absorption bands at 1664 for spiro indole -C=O and 1631 cm^{-1} for thiazolidinone C=O. While, the ^1H NMR spectrum of 6 revealed signals at δ 3.30 attributed to CH_2 thiazole ring protons, multiplet at δ 6.94-8.03 corresponds to (9 aromatic protons) and also broad peak at 9.91 ppm for NH (indole moiety). Regarding compound 7, both elemental and spectroscopic data are consistent with the assigned structure. Thus, its IR spectrum showed absorption bands at 3442, 3310, 3295 and 3108 cm^{-1} for NH_2 and 2NH functional groups. The ^1H NMR of 7 showed the NH_2 and NH of triazole moiety at δ 6.19 and 8.11 ppm, respectively. While the NH of indole moiety appeared at 9.90, besides a signals at 8.77 and 9.25 respective to 7-H and 5-H of thiazole moiety. The mass spectrum of 7 revealed a molecular ion peak at m/z 421 (M^+, 0.4) which corresponds to the molecular formula $C_{19}H_{15}N_7OS_2$ and a base peak at m/z 330.

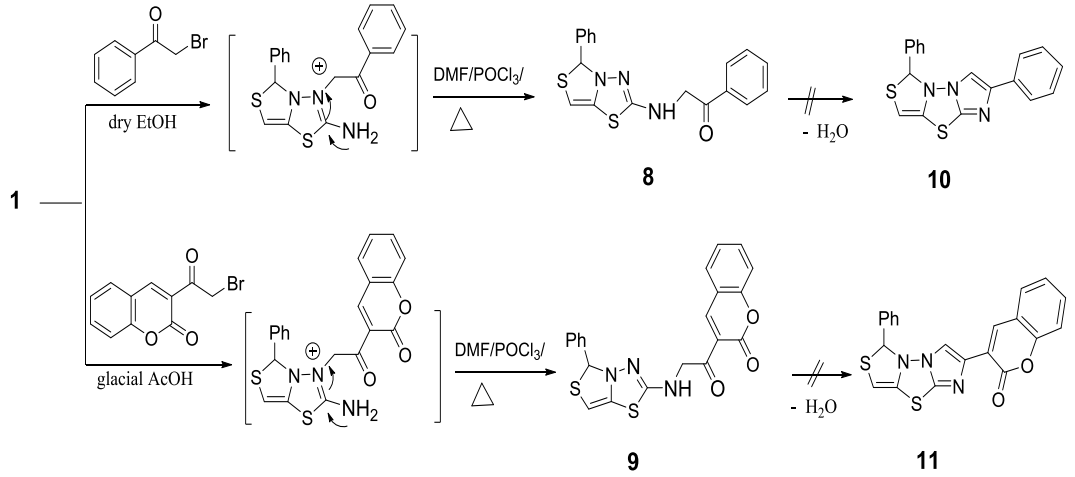


Scheme 2: Synthetic pathways of spirocompounds 6 and 7

Furthermore, the incorporation of imidazole ring into different aryl or heteroaryl ring systems was reported to exhibit significant biological activities [32-34]. Thus, the reaction of compound 1 with α-halocarbonyl compounds such as phenacyl bromide in dry ethanol or with 3-(2-bromoacetyl)-2*H*-chromen-2-one in refluxing glacial acetic acid furnished 1-phenyl-2-((5-phenyl-5H-thiazolo[4,3-b][1,3,4]thiadiazol-2-yl)amino)ethan-1-one (8) and 3-((5-phenyl-5H-thiazolo[4,3-b][1,3,4]thiadiazol-2-yl)glycyl)-2H-chromen-2-one (9), respectively, whereby the reaction proceeds *via* nucleophilic displacement of bromide derivatives to give *N*-alkylated intermediate. Cyclization of either compound 8 or 9 by nucleophilic addition of NH group to the carbonyl group in refluxing DMF/$POCl_3$ mixture in order to furnish the imidazo[2,1-b] thiazolo[3,4-d][1,3,4]thiadiazol derivatives 10 and 11 according to literature procedure [35] were unsuccessful (Scheme 3).

The structures of the new compounds 8 and 9 were firmly established on the basis of their elemental analyses and spectral data. The IR spectrum of 8 is characterized by the presence of absorption bands at 3421 and 1674 cm^{-1} for the NH and CO functional groups, The ^1H NMR spectrum of 8 is characterized by the presence of signals at δ 4.12, 7.13-7.85, 7.98, 9.00 and 12.00 ppm corresponding to CH_2, (10 aromatic proton), 7-H and 5-H of thiazole moiety and NH, respectively. The carbon skeleton of compound 8, taken as an example of the series prepared, was assigned using ^{13}C NMR spectrum (experimental section).

IR spectrum of 9 showed the disappearance of the NH_2 group present in compound 1, and revealed band at 1711 and 1662 cm^{-1} assignable to the two C=O groups. ^1H NMR spectrum of 9 showed characteristic signals at 4.42 and 8.55 for CH_2 and CH-coumarin, respectively. Also ^1H NMR spectrum of 9 showed 7-H and 5-H of thiazole moiety at 8.31 and 9.00, while NH proton appears at 12.19 ppm. Mass spectrum of 9 revealed a molecular ion peak at m/z 421 (M^+, 10.4) and a base peak at 76. The structures of the new produced compounds were elucidated by elemental analyses and the data were in agreement with the proposed structures (see experimental section).



Scheme 3: Behaviour of compound 1 with *α*-halocarbonyl compounds

Antimicrobial activity

All the newly synthesized compounds 1-9 were screened for *in vitro* antibacterial activity against the Gram-positive bacteria *Bacillus subtilis* (*B.* subtilis), Gram-negative bacteria Escherichia coli (*E.* coli) and the fungal *Candida albicans* (*C.* albicans) with the antibiotic ampicillin and clotrimazole as reference drugs [36]. The results were summarized in Table 1.

Table 1: Antimicrobial activity of the tested compounds

Compound no.	Diameter of inhibition z one(mm) bacteria				Fungi	
	Gram (+) bacteria		Gram (-) bacteria			
	Bacillus subtilis	**%Activity Index**	***Escherichia coli***	**% Activity Index**	***Candida albicans***	**%Activity Index**
1	4	16.9	NA	NA	4	16.6
2	15	60	22	84.64	5	17.85
3	4	16	NA	NA	6	21.42
4	NA	NA	NA	NA	6	21.42
5	NA	NA	NA	NA	4	14.28
6	9	40.9	7	31.8	6	24.0
7	12	48	5	19.23	6	21.42
8	NA	NA	6	23.07	NA	NA
9	NA	NA	NA	NA	NA	NA
Ampicillin	25	100	26	100	NA	NA
Clotrimazole NA		NA	NA	NA	28	100
NA: no activity						

In vitro antimicrobial screening of compounds 1-9 prepared in this study was carried out using cultures of two bacteria species, namely, Gram positive bacteria, *Bacillus subtilis* (RCMB 000107, *BS*), Gram negative bacteria, *Escherichia coli* (RCMB 000103, *EC*) as well as one fungal strain, *Candida albicans* (RCMB 005002, *CA*). The antibiotic ampicillin and Clotrimazole were used as references for antibacterial agent for Gram positive and Gram negative bacteria's and as antifungal agent, respectively to evaluate the potency of the tested compounds under the same conditions.

The results of antimicrobial activities for some of the newly synthesized compounds showed promising effects compared to control drugs (Table 1). Compounds 2, 6 and 7 have high activity as antibacterial agent against the Gram positive microorganisms *B.* subtilis with inhibition zones 15, 4 and 12 mm, respectively compared to 5-phenyl-5*H*-thiazolo[4,3-b][1,3,4]thiadiazol-2-amine (1), that exhibited inhibition zone 4 mm while they have moderate activity compared with 25 mm for Ampicillin, but compound 3 has the same activity as compound 1, while compounds 4, 5, 8 and 9 have no activity against *B.Subtilis* bacteria. On the other hand, compound 2 has the most potent activity against the Gram negative microorganisms *E. coli* with inhibition zone 22 mm, while compounds 6, 7 and 8 have moderate activity against the Gram negative bacterium *E. coli*, with inhibition zones 7, 5 and 6 mm, respectively. Compounds 1, 3, 4, 5 and 9 showed negative results against the Gram negative bacterium *E. coli*. In addition, the results depicted in Table 1 showed that compounds 2-7 have moderate efficiency against the fungus *C. albicans* with inhibition zones 5, 6, 6, 4, 6 and 6 mm compared with 28 mm for the reference Clotrimazole, while they have higher activity than compound 1. Lastly, compounds 8 and 9 have no activity against *C. albicans*.

Structure Activity Relationships (SAR's)

From the results of antimicrobial activity compound 2 showed the most prominent activity against Gram-positive and Gram negative bacteria and this result suggest that 2 phenolic OH an effective function for antimicrobial activity, the presence of spiro indolethiazolidin-4-one in compound 6 increases the antibacterial potency compared to 1. Compound 7 with a spiro indole 1,2,4-triazol-3-amine exhibits the highest activity than 1. Compounds 2-7 showed moderate antifungal activity than 1 and this mean that introducing the basic side chain in compound 1 increases the antifungal activity.

CONCLUSION

The Aim of the present search focusing on the synthesis and assess of some new Schiff's bases, spirothiazole, spirotriazole, chromene derivatives Bearing Thiazolo[4,3-b][1,3,4]thiadiazole moiety for detect new systems act as antimicrobial agent. The data display that some of these compounds displayed high, good to moderate *in vitro* antimicrobial activities.

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REFERENCES

[1] C. Enguehard-Gueiffier, A. Gueiffier, *Mini-Rev. Med. Chem*., **2007**, 7, 888.
[2] W.S. Hamama, H.H. Zoorob, *Tetrahedron.*, **2002**, 58, 6143.
[3] M. Abass, M.M. Ismail, W.R. Abdel-Monem, A.S. Mayas, *Chem. Papers.*, **2010**, 64, 72.
[4] A.S. Abu-Khadra, R.S. Farag, A.E.M. Abdel-Hady, *Am. J. Anal. Chem*., **2016**, 7, 233.
[5] B.S. Holla, B.S. Rao, K. Shridhara, P.M. Akberali, *Farmaco.*, **2000**, 55, 338.
[6] F.T. Esmadi, O.F. Khabour, K. Abbas, A. Mohammad, R.T. Obeidat, D. Mfady *Drug. Chem. Toxicol*., **2016**, 39, 41.
[7] K. Marina, M. Anastssia, M. Panagiotis, P. Nicole, P.G. Spyroula, P. Christophe, W. Myriam, D.C. Erik, *Farmaco.*, **2002**, 57, 253.
[8] M. Aslam, I. Anis, R. Mehmood, L. Iqbal, S. Iqbal, I. Khan, M.S. Chishti, S. Perveen, *Med. Chem. Res*., **2016**, 25, 109.
[9] V.K. Chaha, N.S. Ranwa, P.K. Dadheech, *J. Phytol. Res*., **1998**, 11, 201.
[10] M.S. Alexandre-Moreira, M.R. Piuvezam, C.C. Araújo, G.Thomas, *J. Ethnopharmacol*., **1999**, 67, 171.
[11] S.N. Sawhney, A. Gupta, P.K. Sharma, *İndian J. Heterocycl. Chem*., **1991**, 1, 8.
[12] A. Foroumadi, S. Mansouri, Z. Kirani, A. Rahman, *Eur. J. Med. Chem*., **2003**, 38, 851.
[13] R.K. Khare, H. Sing, A.K. Srivastava, *Indian J. Chem.*, **2007**, 46B, 875.
[14] M. Amir, K. Shikha, *Eur. J. Med. Chem*., **2004**, 39, 535.
[15] M.D. Mullican, M.W. Wilson, D.T. Connor, C.R. Konstlan, D.J. Schrier, R.D. Dyer, *J. Med. Chem*., **1993**, 36, 1090.
[16] K. Srivastava, S.N. Pandeya, *Bioorg. Med. Chem*., **1993**, 3, 547.
[17] J. Matysiak, *Eur. J. Med. Chem*., **2007**, 42, 940.
[18] B.H. Lee, F.E. Dutton, M.F. Clothier, J.W. Bowman, J.P. Davis, S.S. Johnson, E. M. Thomas, M.R. Zantello, E.W. Zinser, J.C. McGuire, D.P. Thompson, T.G. Geary, *Bioorg. Med. Chem. Lett*., **1999**, 9, 1727.
[19] H.M. Abo-Salem, M.S. Ebaid, E.R. El-Sawy, A.E. El-Gendy, A. H. Mandour, *Egypt Pharmaceut. J.*, **2013**, 12, 11.
[20] M. Bülbül, N. Saraçoğlu, O.I. Küfrevioğlu, M. Ciftçi, *Bioorg. Med. Chem*., **2002**, 10, 2561.
[21] H. Malipeddi, A.A. Karigar, V.R. Malipeddi, M. S. Sikarwar, *Trop. J. Pharm. Res*., **2012**, 11, 611.
[22] A.A. Karigar, M. Himaja, S.V. Mali, K.P. Jagadeesh, M.S. Sikarwar, *Int. Res. J. Pharm*., **2011**, 2, 153.
[23] E.E. Oruç, S. Rollas, F. Kandemirli, N. Shvets, A.S. Dimoglo, *J. Med. Chem*. 2004, 47, 6760.
[24] A. Nizamuddin, A. Singh, *Indian. J. Chem*., **2004**, 43B, 901.
[25] M. Himaja, Asif Karigar, M.V. Ramana, D. Munirajasekhar, S.S. Mukesh, *Lett. Drug. Des. Discov*., **2016**, 13, 611.
[26] M. Molnar, M. Cacic, S. Zec Zrinusic, *Lett. Org. Chem.*, **2012**, 9, 401
[27] A.A. Karigar, M. Himaja, M.V. Ramana, S. Mukesh, *IJRAP*., **2011**, 2, 170.
[28] S. Sh Shukurov, M. A. Kukaniev, A.M. Alibaeva, *Russ. Chem. Bull*., **1996**, 45, 724,
[29]W.S. Hamama, M.E. Ibrahim, H.H. Zoorob, *J. Heterocycl. Chem*., **2016**, 53, 2007.
[30] M.A. Kira, M.O. Abdel-Rahman, K.Z. Gadalla, *Tetrahedron Lett*., **1969**, 10, 109.
[31] A. Dandia, G. Sharma, R. Singh, A. Laxkar, *RKIVOC.*, **2009**, 100.
[32] R.S. Lamani, N.S. Shetty, R.R. Kamble, I.A.M. Khazi, *Eur. J. Med. Chem*., **2009**, 44, 2828.
[33] A.R. Ali, E.R. El-Bendary, M.A. Ghaly, I.A. Shehata, *Eur. J. Med. Chem*., **2014**, 75, 492.
[34] I.A.M. Khozi, A.K. Gadad, R.S. Lamoni, B.A. Bhongade, *Tetrahedron.*, **2011**, 67, 3289.
[35] W.S. Hamama, M.A. Gouda, M.H. Badr, H.H. Zoorob, *J. Heterocyclic. Chem*., **2013**, 50, 787.
[36] R. Cruickshank, J.P. Duguid, B.P. Marion, R.H.A. Swain, *Med. Microbiol.*, Churchill Livingstone, London, **1975**, 196.