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Synthesis and antimicrobial activity of some new 1,3-thiazoles, 1,3,4-thiadiazoles, 1,2,4-triazoles and 1,3-thiazines incorporating acridine and 1,2,3,4-tetrahydroacridine moieties

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ABSTRACT

Some new sulfur-nitrogen heterocyclic systems such as 1,3-thiazoles, 1,3,4-thiadiazoles, 1,2,4-triazoles and 1,3-thiazines incorporating acridine and 1,2,3,4-tetrahydroacridine moieties were synthesized via heterocyclization of the key intermediate 4-(acridin-9-yl)-1-(1,2,3,4-tetrahydroacridin-9-ylcarbonyl)thiosemicarbazide. Structures of the new compounds were established by elemental analyses and spectral data. All the products were also screened in vitro for their antimicrobial activity.

1. Introduction

Acridine and 1,2,3,4-tetrahydroacridine derivatives, well known as DNA intercalates, have been widely studied from a variety of viewpoints such as synthesis [1,2], physicochemical properties [3,4], structural requirements [5] and biological activities [6,7]. Due to a polycyclic planar structure, acridine and its derivatives intercalate within DNA and RNA by forming hydrogen bonds and stacking between base pairs resulting in DNA cross links and strand breaks [8]. A variety of natural and synthetic acridine derivatives have also been tested for antimalarial [9], anti-inflammatory [10,11] and analgesic [12] activities and some of them have been approved for chemotherapy.

The 1,2,4-triazole nucleus has been incorporated into a wide variety of therapeutically important agents. Ribavirin (antiviral), Letrozole and Anastrozole (antitumor) are some examples of drugs containing the 1,2,4-triazole moiety [13–17]. 1,3,4-Thiadiazole derivatives are another important class of heterocycles due to their biological activities. The only commercially available 1,3,4-thiadiazole drugs are Desaglybuzole, Acetazolamide and Furidiazine [18-20]. Moreover, 1,3-thiazoles recently found application in drug development for the treatment of hypertension [21], schizophrenia [22], HIV infections [23] and as new inhibitors of bacterial DNA gyrase B [24]. Furthermore, 1,3-thiazine derivatives have antibacterial [25] and cannabinoid receptor agonist [26] activity.

In view of the above mentioned findings and in continuation of our efforts [27-29] to identify new candidates that may be of value in designing new and potent antimicrobial agents, we decided in the present work to synthesize new 1,3-thiazole, 1,3,4-thiadiazole, 1,2,4-triazole and 1,3-thiazine derivatives bearing acridine and 1,2,3,4-tetrahydroacridine moieties in order to investigate their antimicrobial activities.

2. Experimental

2.1. Instrumentation

Melting points were determined on a digital Stuart SMP-3 apparatus. Infrared spectra were measured on Perkin-Elmer 293 spectrophotometer (cm^{-1}), using KBr disks. ^1H NMR spectra were measured on Gemini-200 spectrometer (200 MHz), using DMSO-d_6 as a solvent and TMS (δ) as the internal standard. Elemental microanalyses were performed at Microanalysis Center in National Research Center, Giza. Mass spectra recorded on a Gas Chromatographic GCMSqp 1000 ex Shimadzu instrument at 70 eV. The purity of the synthesized compounds was checked by thin layer chromatography (TLC). Evaluation of antimicrobial activities was carried out by the Faculty of Agriculture for Girls, Al-Azhar University, Nasr City, Cairo, Egypt.

2.2. 4-(Acridin-9-yl)-1-(1,2,3,4-tetrahydroacridin-9-ylcarbonyl)thiosemicarbazide (4)

Equimolar amounts of 9-isothiocyanatoacridine (**2**) (0.005 mol, 1.18 g) and 1,2,3,4-tetrahydroacridine-9-carbohydrazide (**3**) (0.005 mol, 1.20 g) in ethanol (20 cm^3) was heated to reflux for 2 h, whereupon a solid product was separated out during heating (Scheme 1). The reaction mixture was cooled and the separated product was filtered off, washed with water, dried and recrystallized from dilute DMF. Yield 52%. M.p.: 218–220 °C. FT-IR, ν (cm^{-1}): 3275 (NH), 3238 (NH), 3181 (NH), 3098 (C–H_{arom}), 2942 (C–H_{aliph}), 1634 (C=O_{amide}), 1597 (C=N), 1538 (C=C), 1160 (C=S), 758 (NH bending, –NHCSNH–). ^1H NMR (200 MHz, DMSO-d_6), δ: 1.19–2.88 (m, 8H, 4 CH_2), 7.20–7.30

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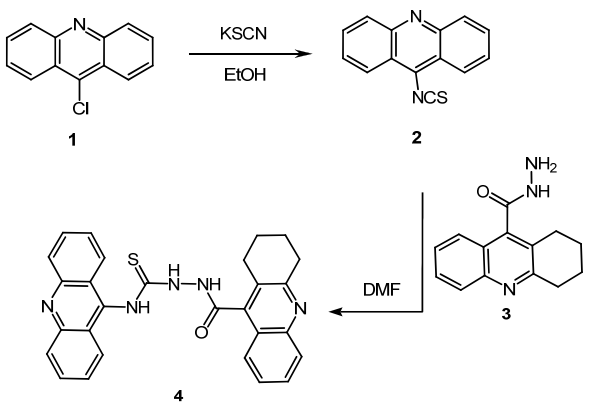
(m, 3H, Ar–H), 7.57 (d, 3H, *J*=7.8 Hz, Ar–H), 7.71 (d, 3H, *J*=6.8 Hz, Ar–H), 7.95 (d, 1H, *J*=7 Hz, Ar–H), 8.22 (d, 2H, *J*=7.6 Hz, Ar–H), 8.62 (br, 1H, NH exchangeable with D_2O), 8.87 (br, 1H, NH exchangeable with D_2O), 11.80 (s, 1H, NH exchangeable with D_2O). MS (*m/z*, I %): 478 (M+H, 64%), 473 (46), 407 (35), 310 (53), 252 (41), 189 (37), 174 (36), 123 (100), 114 (69), 83 (53). Anal. Calcd. for $C_{28}H_{23}N_5OS$ (477.59): C, 70.42; H, 4.85; N, 14.66; S, 6.71. Found: C, 70.01; H, 4.41; N, 14.23; S, 6.30%.



Scheme 1

2.3. General procedure for the synthesis of 1,3-thiazole derivatives 7, 8 and 9

A mixture of **4** (0.005 mol, 2.38 g) and chloroacetone, chloroacetyl chloride and/or chloroacetic acid (0.005 mol) was refluxed for 10 h, in dry DMF (20 cm^3) with the presence of few drops of piperidine. Cold water (10 cm^3) was added after cooling and the resulting precipitates were filtered off and crystallized from dilute DMF to afford compounds **7**, **8** and **9**, respectively (Scheme 2 and 3).

N-[2-(9-Acridinylimino)-4-methyl-1,3-thiazol-3(2H)-yl]-1,2,3,4-tetrahydroacridine-9-carboxamide (7): Yield: 64%. M.p.: 326–328 °C. FT-IR, *ν* (cm^{-1}): 3181 (NH), 3098 (C–H_{arom}), 2930 (C–H_{aliph}), 1635 (C=O_{amide}), 1592 (C=N), 1538 (C=C). ^1H NMR (200 MHz, DMSO-d_6), δ: 2.53 (s, 3H, CH_3), 1.22–2.88 (m, 8H, 4 CH_2), 7.28–7.98 (m, 10H, Ar–H and C$_5$–$H_{thiazole}$), 8.27 (d, 3H, *J*=8 Hz, Ar–H), 11.80 (br, 1H, NH exchangeable with D_2O). MS (*m/z*, I %): 338 (M–178, 1%), 325 (4), 195 (100), 178 (14), 167 (38), 152 (23), 139 (19), 84 (29), 63 (21). Anal. Calcd. for $C_{31}H_{25}N_5OS$ (515.64): C, 72.21; H, 4.89; N, 13.58; S, 6.22. Found: C, 71.75; H, 4.49; N, 13.18; S, 5.87%.

N-[2-(9-Acridinylimino)-5-oxo-1,3-thiazolidin-3-yl]-1,2,3,4-tetrahydroacridine-9-carboxamide (8): Yield: 56%. M.p.: > 350 °C. FT-IR, *ν* (cm^{-1}): 3425 (br, H-bonded, OH), 3240 (NH), 3048 (C–H_{arom}), 2985 (C–H_{aliph}), 1703 (C=$O_{thiazolidinone}$), 1636 (C=O_{amide}), 1601 (C=N). ^1H NMR (200 MHz, DMSO-d_6), δ: 1.90–2.85 (m, 8H, 4 CH_2), 3.93 (s, 2H, NCH_2), 7.16–7.26 (m, 3H, Ar–H), 7.57–7.72 (m, 6H, Ar–H), 8.22 (d, 3H, *J*=8 Hz, Ar–H), 11.73 (br, 1H, NH exchangeable with D_2O). MS (*m/z*, I %): 530 (M+2/3H_2O, 7%), 375 (7), 266 (7), 226 (17), 165 (13), 96 (29), 54 (100). Anal. Calcd. for $C_{30}H_{23}N_5O_2S$. 2/3H_2O (529.61): C, 67.97; H, 4.59; N, 13.21; S, 6.04. Found: C, 68.26; H, 4.21; N, 13.19; S, 5.84%.

N-[2-(9-Acridinylimino)-4-oxo-1,3-thiazolidin-3-yl]-1,2,3,4-tetrahydroacridine-9-carboxamide (9): Yield: 70%. M.p.: 346–348 °C. FT-IR, *ν* (cm^{-1}): 3404-3100 (br, H-bonded, OH, NH), 3083 (C–H_{arom}), 2924 (C–H_{aliph}), 1718 (C=$O_{thiazolidinone}$), 1627 (C=O_{amide}), 1589 (C=N). ^1H NMR (200 MHz, DMSO-d_6), δ: 1.20–2.88 (m, 8H, 4 CH_2), 3.83 (s, 2H, SCH_2), 7.16–7.26 (m, 3H, Ar–H), 7.56–7.96 (m, 6H, Ar–H), 8.24 (d, 3H, *J*=8 Hz, Ar–H), 11.74 (br, 1H, NH exchangeable with D_2O). MS (*m/z*, I %): 526 (M+1/2H_2O, 4%), 445 (3), 371 (3), 270 (3), 237 (3), 195 (100), 166 (39), 63 (34). Anal. Calcd. for $C_{30}H_{23}N_5O_2S$. 1/2H_2O (526.61): C, 68.36; H, 4.55; N, 13.29; S, 6.07. Found: C, 68.32; H, 4.13; N, 13.21; S, 5.82%.

2.4. 2-(1,2,3,4-Tetrahydroacridin-9-yl)-5-(9-acridinylimino)-1H-1,3-thiazolo[3,4-b] [1,2, 4]triazole (10)

A mixture of **9** (0.002 mol, 1.02 g) and anhydrous ammonium acetate (1.2 g) in absolute ethanol (20 cm^3) was refluxed for 10 h, whereupon a solid product was separated out during heating. The reaction mixture was cooled and the separated product was filtered off, washed with water, dried and recrystallized from dilute DMF. Yield: 40%. M.p.: > 350 °C. FT-IR, *ν* (cm^{-1}): 3427 (br, NH), 3083 (C–H_{arom}), 2924 (C–H_{aliph}), 1619 (C=N), 1589 (C=N), 1550 (C=C). ^1H NMR (200 MHz, DMSO-d_6), δ: 1.21–2.95 (m, 8H, 4 CH_2), 7.16–7.26 (m, 3H, Ar–H), 7.56–8.00 (m, 7H, Ar–H and C$_5$–$H_{thiazole}$), 8.24 (d, 3H, *J*=8 Hz, Ar–H), 11.74 (s, 1H, NH exchangeable with D_2O). MS (*m/z*, I %): 498 (M^+, 6%), 387 (11), 283 (10), 173 (12), 140 (17), 97 (19), 68 (76), 54 (100). Anal. Calcd. for $C_{30}H_{22}N_6S$ (498.61): C, 72.27; H, 4.45; N, 16.85; S, 6.43. Found: C, 71.89; H, 4.09; N, 16.39; S, 6.07%.

2.5. 2-(9-Acridinylamino)-5-(1,2,3,4-tetrahydroacridin-9-yl)-1,3,4-thiadiazole (11)

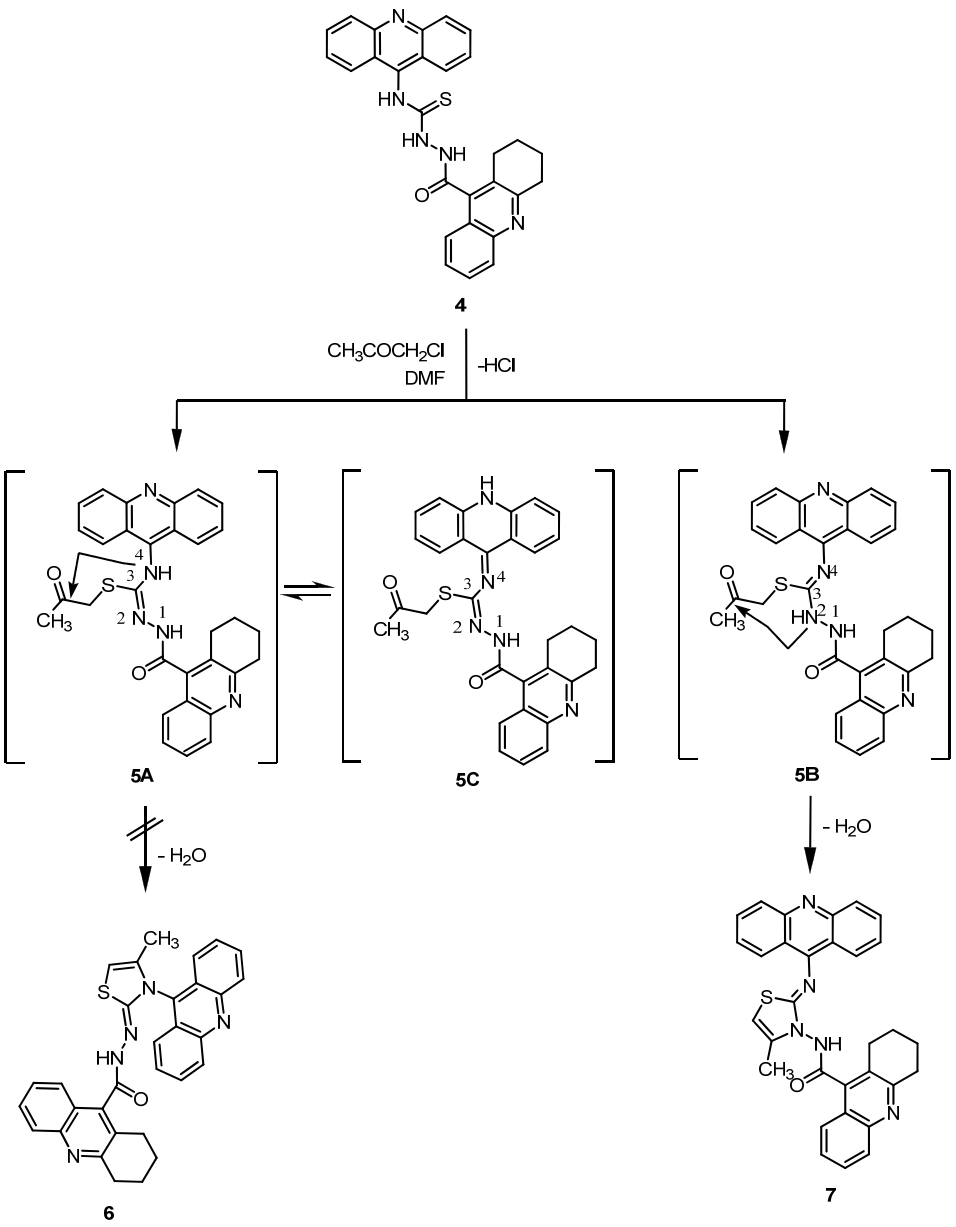
A mixture of **4** (0.005 mol, 2.38 g) and concentrated sulfuric acid (10 cm^3) was heated at 50 °C for 2 h. The reaction mixture was left overnight at room temperature. The resulting solution was poured into crushed ice and treated with dilute NH_4OH to pH~6. The formed precipitate was filtered off, washed thoroughly with water till neutral washings, dried and recrystallized from dilute DMF. Yield: 58%. M.p.: 196–198 °C. FT-IR, *ν* (cm^{-1}): 3394 (br, NH), 3085 (C–H_{arom}), 2920 (C–H_{aliph}), 1627 (C=N), 1600 (C=N), 1512 (C=C). ^1H NMR (200 MHz, DMSO-d_6), δ: 1.17–2.99 (m, 8H, 4 CH_2), 7.23–7.29 (m, 3H, Ar–H), 7.55 (d, 3H, *J*=7.8 Hz, Ar–H), 7.70 (d, 3H, *J*=7.6 Hz, Ar–H), 8.24 (d, 3H, *J*=7.8 Hz, Ar–H), 11.79 (s, 1H, NH exchangeable with D_2O). Anal. Calcd. for $C_{28}H_{21}N_5S$ (459.58): C, 73.18; H, 4.61; N, 15.24; S, 6.98. Found: C, 72.86; H, 4.23; N, 14.86; S, 6.62%.

2.6. 4-(9-Acridinyl)-5-(1,2,3,4-tetrahydroacridin-9-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (12)

A solution of **4** (0.005 mol, 2.38 g) in 5% sodium hydroxide (20 cm^3) was heated to reflux for 2 h. The reaction mixture was filtered off while hot, then cooled and acidified with dilute HCl to pH~6. The separated product was filtered off, washed well with water till neutral washings, dried and recrystallized from dilute DMF. Yield: 63%. M.p.: 253–256 °C. FT-IR, *ν* (cm^{-1}): 3280 (NH), 3090 (C–H_{arom}), 2925 (C–H_{aliph}), 1629 (C=N), 1589 (C=N), 1523 (C=C), 1161 (C=S). ^1H NMR (200 MHz, DMSO-d_6), δ: 1.21–2.91 (m, 8H, 4 CH_2), 7.22–7.29 (m, 3H, Ar–H), 7.64–7.72 (m, 6H, Ar–H), 8.25 (d, 3H, *J*=7.6 Hz, Ar–H), 12.22 (s, 1H, NH exchangeable with D_2O). MS (*m/z*, I %): 460 (M^+, 12%), 337 (18), 266 (21), 245 (100), 226 (23), 209 (21), 180 (20), 111 (24), 91 (12), 84 (9). Anal. Calcd. for $C_{28}H_{21}N_5S$ (459.58): C, 73.18; H, 4.61; N, 15.24; S, 6.98. Found: C, 72.85; H, 4.29; N, 14.70; S, 6.60%.

2.7. N-[2-(9-Acridinylimino)-5-cyano-4-imino-6-(methyl thio)-2H-1,3-thiazin-3(4H)-yl]-1,2,3,4-tetrahydroacridine-9-carboxamide (15)

A mixture of **4** (0.005 mol, 2.38 g) and [*bis*(methylthio)methylene]malononitrile (**13**) (0.005 mol, 0.85 g) in DMF (20 cm^3) was refluxed for 4 h. After cooling, cold water (10 cm^3) was added and the resulting precipitate was filtered off and crystallized from dilute DMF. Yield: 55 %. M.p.:



Scheme 2

270–272 °C. FT-IR, ν (cm^{-1}): 3275 (NH), 3178 (NH), 3098 (C–H$_{arom}$), 2924 (C–H$_{aliph}$), 2210 (C≡N), 1636 (C=O$_{amide}$), 1591 (C=N), 1539 (C=C). ^{1}H NMR (200 MHz, DMSO-d_6), δ: 1.19–2.80 (m, 8H, 4 CH$_2$), 3.34 (s, 3H, SCH$_3$), 7.22–7.29 (m, 3H, Ar–H), 7.50 (d, 3H, *J*=8 Hz, Ar–H), 7.73 (d, 3H, *J*=6.4 Hz, Ar–H), 8.24 (d, 3H, *J*=7.8 Hz, Ar–H), 9.94 (br, 1H, NH exchangeable with D$_2$O), 11.74 (s, 1H, NH exchangeable with D$_2$O). MS (*m/z*, I %): 386 (M–213, 8%), 194 (23), 140 (17), 105 (36), 60 (100). Anal. Calcd. for $C_{33}H_{25}N_7OS_2$ (599.74): C, 66.09; H, 4.20; N, 16.35; S, 10.69. Found: C, 65.69; H, 4.21; N, 16.78; S, 10.46%.

2.8. 5-(9-Acridinylimino)-7-(methylthio)-2-(1,2,3,4-tetra hydroacridin-9-yl)-[1,2,4] triazolo[1,5-c][1,3]thiazine-8-carbonitrile (16)

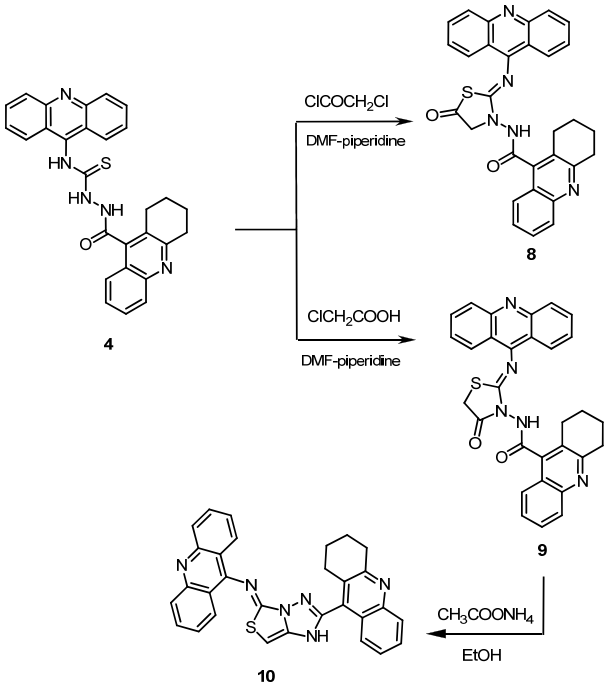
A mixture of **15** (0.002 mol, 1.19 g) and phosphorus oxychloride (7 cm^3) was heated at 80 °C for 2 h. The reaction mixture was left overnight at room temperature. The resulting solution was poured into crushed ice. The formed precipitate was filtered off, washed thoroughly with water, dried and recrystallized from dilute DMF. Yield: 69%. M.p.: > 350 °C. FT-IR, ν (cm^{-1}): 3100 (C–H$_{arom}$), 2923, 2852 (C–H$_{aliph}$), 2215 (C≡N), 1628 (C=N), 1591 (C=N), 1522 (C=C). ^{1}H NMR (200 MHz, DMSO-d_6), δ: 1.15–2.87 (m, 8H, 4 CH$_2$), 3.20 (s, 3H, SCH$_3$), 7.20–7.30 (m, 3H, Ar–H), 7.55 (d, 3H, *J*=8 Hz, Ar–H), 7.72 (d, 3H, *J*=6.5 Hz, Ar–H), 8.24 (d, 3H, *J*=7.8 Hz, Ar–H). MS (*m/z*, I %): 527 (M–54, 2%), 315 (3), 195 (100), 152 (20), 139 (19), 84 (30). Anal. Calcd. for $C_{33}H_{23}N_7S_2$ (581.73): C, 68.14; H, 3.99; N, 16.85; S, 11.02. Found: C, 68.59; H, 3.89; N, 16.49; S, 10.64%.

3. Results and Discussion

3.1. Synthesis

Treatment of 9-chloroacridine (**1**) [30] in absolute ethanol with potassium thiocyanate afforded the corresponding 9-isothiocyanatoacridine (**2**) [31] (Scheme 1). Compound **2** was refluxed for 2 h with 1,2,3,4-tetrahydroacridine-9-carbohydrazide (**3**) [32] in DMF, yielding 4-(acridin-9-yl)-1-(1,2,3,4-tetrahydroacridin-9-ylcarbonyl)thiosemicarbazide (**4**)

(Scheme 1). The IR spectrum of **4** showed three characteristic absorption bands at 3275, 3238 and 3181 cm^{-1} due to three NH functions in addition to the carbonyl absorption band at 1634 cm^{-1}. Its ^1H NMR spectrum revealed three characteristic signals (D_2O exchangeable) at δ 8.62, 8.87 and 11.80 ppm assigned to three NH protons. Also, its mass spectrum showed the molecular ion peak at *m/z* 478 [M+H] and the base peak at *m/z* 123.



Scheme 3

Thiosemicarbazide derivative **4** represents versatile synthons for several sulfur-nitrogen heterocycles. Thus, compound **4** reacts with chloroacetone in DMF containing piperidine as a base catalyst to give the product identified as *N*-[2-(9-acridinylimino)-4-methyl-1,3-thiazol-3(2*H*)-yl]-1,2,3,4-tetrahydroacridine-9-carboxamide (**7**) and not compound **6** (Scheme 2). The reaction proceeds *via* nucleophilic substitution attack of chloroacetone by the thiosemicarbazide sulfur atom (after tautomeric shift to the thiol form with the hydrogen emanating from either *N-2* or *N-4*) to remove hydrogen chloride. The first step may afford either of the two possible tautomeric intermediates, **5A** or **5B** (Scheme 2), which are then able to undergo differing reaction routes to form either of the cyclic products **6** or **7**. (A third tautomeric intermediate **5C**, in which the HN-10' tautomer is also plausible and indeed likely, but it must transpose into either **5A** or **5B** for the final step of the cyclization reaction to occur) [33,34]. Several recent articles reported [34-36] that when acridin-9-yl moiety was comprised of phenyl and pyridinyl moieties, the tautomer **5B** was thermodynamically more favorable while the tautomer **5A** was kinetically more disfavored, *i.e.* acridin-9-yl is better at conjugation and/or electron withdrawal in comparison to phenyl and pyridinyl positioned at *N-4* of thiosemicarbazide moiety, resulting in **7** to be produced exclusively. The structure of the latter product **7** was confirmed by its elemental and spectral data. Its IR spectrum showed absorption bands of the amide group at 3181 and 1635 cm^{-1} which is due to NH and $C=O_{amide}$ groups, respectively. Also, its mass spectrum did not show the molecular ion peak but showed a peak at *m/z* 338 (M−acridine moiety) and the base peak at *m/z* 195 which is due to 9-aminiumacridine cation.

It is known that the thiosemicarbazide reacts with halocarbonyl compounds (e.g. ethyl bromoacetate, chloroacetic acid) yielding 1,3-thiazolidinone derivatives [37,38]. Thus, two new isomers of 1,3-thiazolidinone derivatives **8** and **9** were obtained by cyclocondensation of **4** with chloroacetyl chloride and chloroacetic acid, respectively (Scheme 3). The structures of the latter products **8** and **9** were confirmed by the appearance of $C=O_{thiazolidinone}$, $C=O_{amide}$, and NH bands at 1703-1718, 1636-1627 and 3240-3100 cm^{-1}, respectively. The elemental analysis, IR and mass spectra of **8** and **9** confirmed the existence of water molecules in their crystallized states due to forming strong hydrogen bonds with water (Figure 1). Also, the ^1H NMR spectra of **8** and **9** revealed the methylene protons at δ 3.93 and 3.83 ppm, respectively.

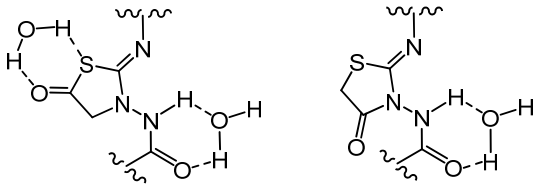
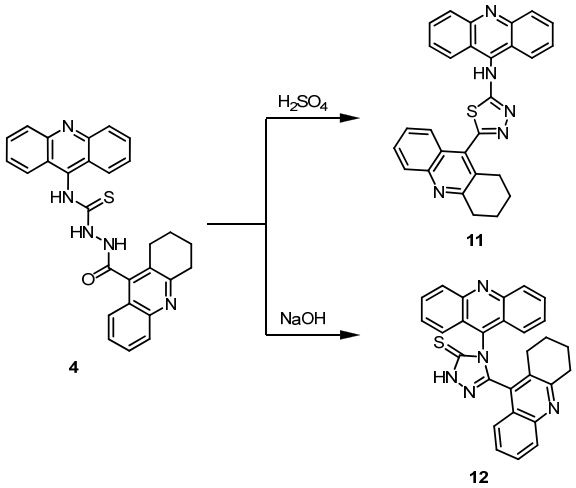


Figure 1. Hydrogen bonds of the compounds **8** and **9** with water.

Interestingly, interaction of compound **9** with ammonium acetate in refluxing ethanol furnished the corresponding 2-(1,2,3,4-tetrahydroacridin-9-yl)-5-(9-acridinylimino)-1*H*-1,3-thiazolo[3,4-*b*][1,2,4]triazole (**10**) (Scheme 3). The lack of $C=O_{thiazolidinone}$ and $C=O_{amide}$ functions in the IR spectrum of the isolated product supported the formation of compound **10**. Moreover, its mass spectrum showed the molecular ion peak at *m/z* 498 (6 %).

The vast pharmaceutical activities of the 1,3,4-thiadiazole and 1,2,4-triazole derivatives [14,19] encouraged us to synthesize *bis*(acridinyl)thiadiazole/triazole derivatives of potential biological interest. Thus, cyclodehydration of **4** with concentrated sulfuric acid afforded 2-(9-acridinylamino)-5-(1,2,3,4-tetrahydroacridin-9-yl)-1,3,4-thiadiazole (**11**), whereas its cyclization under reflux in sodium hydroxide solution afforded 4-(9-acridinyl)-5-(1,2,3,4-tetrahydroacridin-9-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**12**) (Scheme 4) [38]. The structures of the latter products **11** and **12** were established on the basis of their elemental analysis and spectral data (See experimental section).

N-[2-(9-Acridinylimino)-5-cyano-4-imino-6-(methylthio)-2*H*-1,3-thiazin-3(4*H*)-yl]-1,2,3,4-tetrahydroacridine-9-carboxamide (**15**) was achieved from refluxing compound **4** with [*bis*(methylthio)methylene]malononitrile (**13**) [39] through the nonisolable intermediate **14**, which underwent an



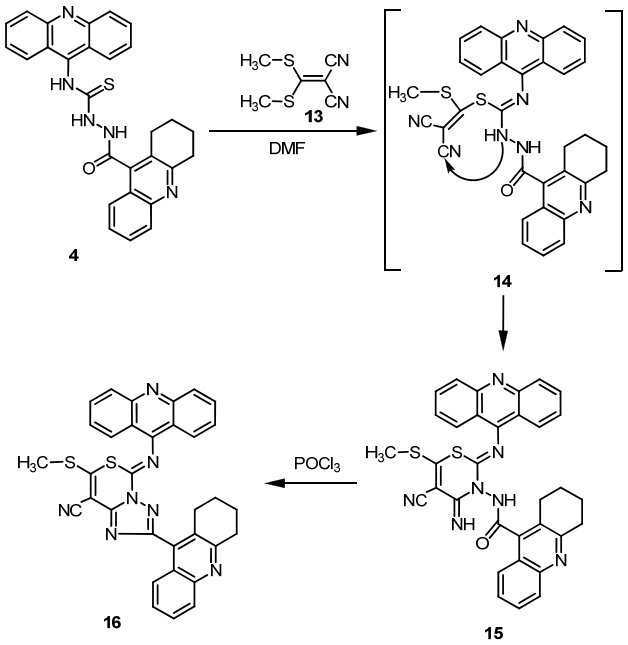
Scheme 4

Table 1. In *vitro* antibacterial activities of the prepared compounds 4-16*.

Compound No	Diameter of zone of inhibition in mm							
	Gram-positive bacteria				Gram-negative bacteria			
	S. aureus (ATCC 25923)		*S. pyogenes* (ATCC 19615)		*P. phaseolicola* (GSPB 2828)		*P. fluorescens* (S 97)	
	1 mg/mL	2 mg/mL	1 mg/mL	2 mg/mL	1 mg/mL	2 mg/mL	1 mg/mL	2 mg/mL
4	–	8	6	11	–	9	7	13
7	–	11	7	13	6	14	9	16
8	–	13	–	12	–	10	6	17
9	9	20	8	21	11	21	8	14
10	–	15	6	14	6	13	7	16
11	6	13	8	14	–	7	–	12
12	10	19	16	25	17	22	11	18
15	11	20	10	18	7	17	9	21
16	11	23	9	19	6	17	12	15
Cephalothin	28		30		NT		NT	
Chloramphenicol	NT		NT		25		30	

*Less active: 6–12 mm; moderately active: 13–19 mm; highly active: 20–30 mm; –: No inhibition or inhibition less than 5 mm; NT: not tested.

intramolecular cyclization *via* addition of NH functional group at C≡N group (Scheme 5). Both elemental analysis and spectral data of **15** were consistent with the assigned structure. Its IR spectrum revealed appearance of C=O$_{amide}$ and C≡N group at 1636 and 2210 cm^{-1}, respectively. Furthermore, its ^{1}H NMR spectrum exhibited signals of SCH$_3$ and two NH protons at δ 3.34, 9.94 and 11.74 ppm, respectively.



Scheme 5

Finally, cyclization of **15** *via* refluxing in phosphorus oxychloride for 3 h at 80 °C afforded 5-(9-acridinylimino)-7-(methylthio)-2-(1,2,3,4-tetrahydroacridin-9-yl)-[1,2,4]triazolo [1,5-*c*][1,3]thiazine-8-carbonitrile (**16**) (Scheme 5). The IR spectrum of **16** revealed disappearance of NH and C=O$_{amide}$ groups, while its ^{1}H NMR spectrum confirmed this structure by lacking two NH protons of **15**. However, the mass spectra of **15** and **16** did not show the molecular ion peaks indicating a fragile nature of these compounds which may loose the 9-hydroxymethyl-1,2,3,4-tetrahydroacridine (*m/z* 213) and triazete (*m/z* 54) moieties, yielding the fragments *m/z* 386 and 527 for **15** and **16**, respectively.

3.2. In vitro antimicrobial activity

All the synthesized compounds were evaluated *in vitro* for their antibacterial activities against *Staphylococcus aureus (ATCC 25923)* and *Streptococcus pyogenes (ATCC 19615)* as examples of Gram positive bacteria and *Pseudomonas fluorescens (S 97)* and *Pseudomonas phaseolicola (GSPB 2828)* as examples of Gram negative bacteria. They were also evaluated *in vitro* for their antifungal activities against the *Fusarium oxysporum* and *Aspergillus fumigatus* fungal strains.

Agar-diffusion technique was used for the determination of the preliminary antibacterial and antifungal activities [40,41]. Cephalothin, Chloramphenicol and Cycloheximide were used as reference drugs for Gram positive bacteria, Gram negative bacteria and fungi, respectively. The results were recorded for each tested compound as average diameter of inhibition zones of bacterial or fungal around the disks in mm at the concentrations of 1 and 2 mg/mL (Tables 1 and 2). The minimum inhibitory concentrations (MIC) for the compounds which showed growth inhibition zones >12 mm, were estimated by broth dilution assay [42].

Table 2. In *vitro* antifungal activity of the prepared compounds **4-16***.

Compound No	Diameter of zone of inhibition in mm			
	Fungi			
	F. oxysporum		*A. fumigatus*	
	1 mg/mL	2 mg/mL	1 mg/mL	2 mg/mL
4	6	11	6	12
7	8	18	–	12
8	-	16	–	12
9	12	21	8	19
10	–	9	–	13
11	–	10	6	10
12	12	20	9	22
15	14	23	13	21
16	8	19	14	22
Cycloheximide	28		31	

* Less active: 6–12 mm; moderately active: 13–19 mm; highly active: 20–30 mm; –: No inhibition or inhibition less than 5 mm.

1) The results depicted in Tables 1 and 2 revealed that most of the synthesized compounds were found to possess various antimicrobial activities towards all the microorganisms tested.
2) In general, most of the synthesized compounds have better activities against the microbial strains in comparison with the starting material **4** as a result of the construction of the bioactive heterocycles bearing two moieties of acridine derivatives.
3) Most of the synthesized compounds showed none or less inhibition at 1 mg/mL and moderate inhibition at 2 mg/mL concentrations.

4) Compounds **9**, **12, 15** and **16** were found to possess high inhibition activities at 2 mg/mL concentration against most microorganisms.
5) The results of the MIC values of the selected compounds in all cases were more than 500 µg/mL except for compounds **9**, **12**, **15** and **16**.
6) Compound **15** showed MIC values of 62.5 µg/mL against *S. aureus* (ATCC 25923) and *F. oxysporum* and 125 µg/mL against *S. pyogenes* (ATCC 19615), *P. phaseolicola* (GSPB 2828), *P. fluorescen* (S 97) and *A. fumigats*, whereas compound **16** showed MIC values of 125 µg/mL against all microbial strains. The bioactivities of the latter compounds may be due to the presence of methylthio and nitrile groups.
7) Compound **9** which contains the bioactive thiazolidinone moiety, showed MIC value of 125 µg/mL against all microbial strains.
8) Compound **12** showed MIC value of 62.5 µg/mL against all microbial strains and this noticeable activity may be due to the presence of the 1,2,4-triazolethione moiety.
9) In conclusion, compounds **9**, **12**, **15** and **16** are nearly as active as the reference drugs. However, none of them show superior activities compared to the reference drugs.

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