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Full Paper

Synthesis and biological activity of some new fused thieno[2,3-*b*] pyridine derivatives; pyridothienopyrimidinones and pyridothienotriazinones

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Abstract
3-Amino-4-aryl-5-ethoxycarbonyl-6-methylthieno[2,3-b]pyrindine-2-carboxamides 3a,b were prepared and reacted with triethyl orthoformate to give pyridothienopyrimidinone derivatives 4a,b. The reaction of compound 3b with potassium ethyl xanthate and/or phenyl isothiocyanate produced thioxopyridothieno-pyrimidinones 5 and 6 respectively. Diazotisation of compounds 3a,b led to the formation of 9-aryl-8-ethoxycarbonyl-7-methylpyrido[3',2':4,5]thieno[3,2-d] [1,2,3]triazine-4(3H)-ones (7a,b). Compounds 5, 6 and 7a,b underwent some reactions to furnish other new pyridothienopyrimidinones 8, 9a,b and pyridothienotriazinones 10-13a,b. The structural formulas of all newly synthesized compounds were confirmed by elemental and spectral analyses. Also, the biological activity of fifteen compounds as antibacterial and antifungal agents were studied.

Keywords: Thienopyridines, pyridothienopyridines, pyridothienotriazines, Mannich reaction.

1. Introduction

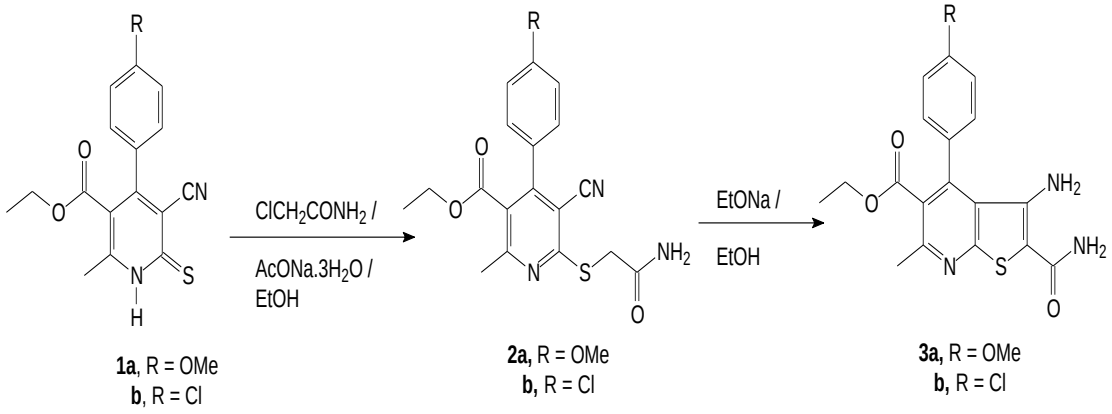
Many thieno[2,3-b]pyridines have been synthesized and investigated in relation with their biological and pharmacological importance [1,2]. Some of them proved to possess antiviral [3-6], antidiabetic [7], antimicrobial [8-10], anti-inflammatory [11], antitumor [12], antiparasitic [13] and neurotropic activities [14]. Also, some pyrido[3',2':4,5]thieno[3,2d]pyrimidines are reported to exhibit antiallergic [15], antiprotozoal [16], antianaphylactic [17,18] and antimicrobial activities [8-10]. Furthermore, a number of pyridothieno triazines have been used as antiprotozoal [16], antitumor [19], antiangiogenic [20] and antimicrobial agents [8]. In view of the above observations, we report herein the synthesis and biological activity of the title compounds which are expected to be biologically active ones owing to incorporation of different pharmacophores.

2. Results and discussion

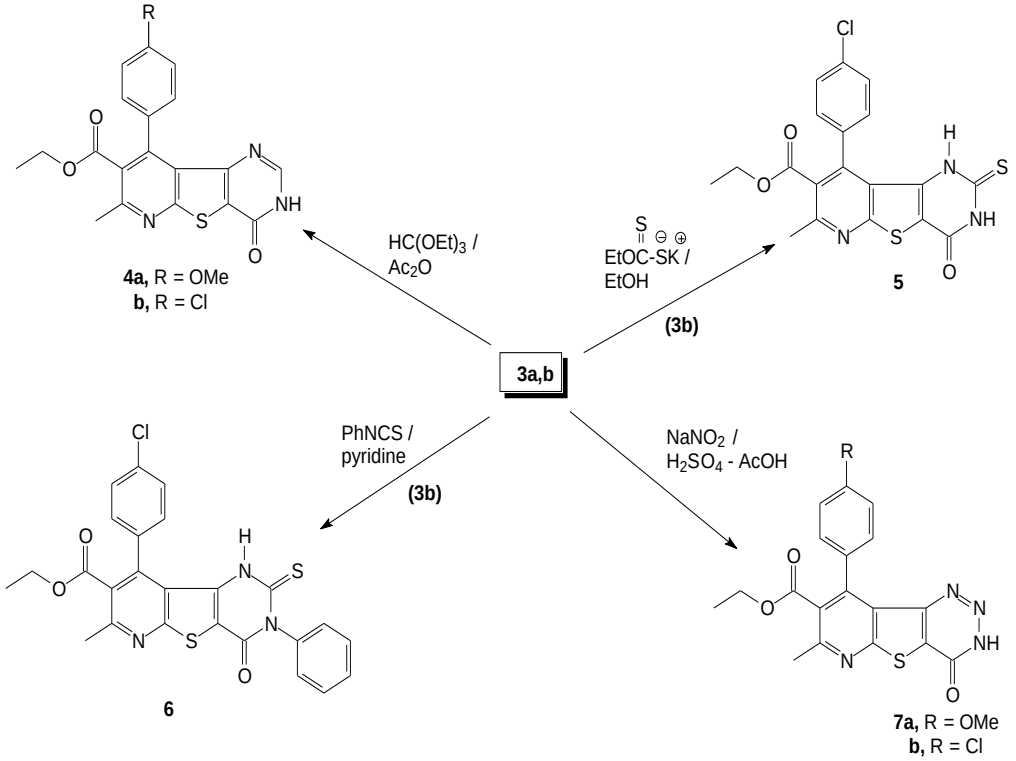
Our approach to the synthesis of the target compounds started from 3-amino-4-aryl-5-ethoxycarbonyl-6-methylthieno [2,3-b]pyrindine-2-carboxamides **3a,b** which were prepared from the reaction of ethyl 4-aryl-3-cyano-6-methyl-2-thioxo-

1,2-dihydropyridine-5-carboxylates**(1a,b)** with chloroa-cetamide according to the reported method [21] (Scheme 1).

Condensation of compounds **3a,b** with triethyl orthoformate by refluxing in acetic anhydride produced the pyrimidinone derivatives **4a,b**. Upon refluxing of **3b** with potassium ethyl xanthate in ethanol, ethyl 9-(*p*-chloro-phenyl)-7-methyl-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrido[3',2':4,5] thieno[3,2-d]pyrimidine-8-carboxylate (**5**) was obtained. The 3-phenyl analogue **6** was synthesized by reacting **3b** with phenyl isothiocyanate in hot pyridine. Treatment of compounds **3a,b** in H_2SO_4-AcOH mixture with sodium nitrite solution at low temperature resulted in diazotization followed by self coupling to furnish the 1,2,3-triazinones **7a,b** (Scheme 2).

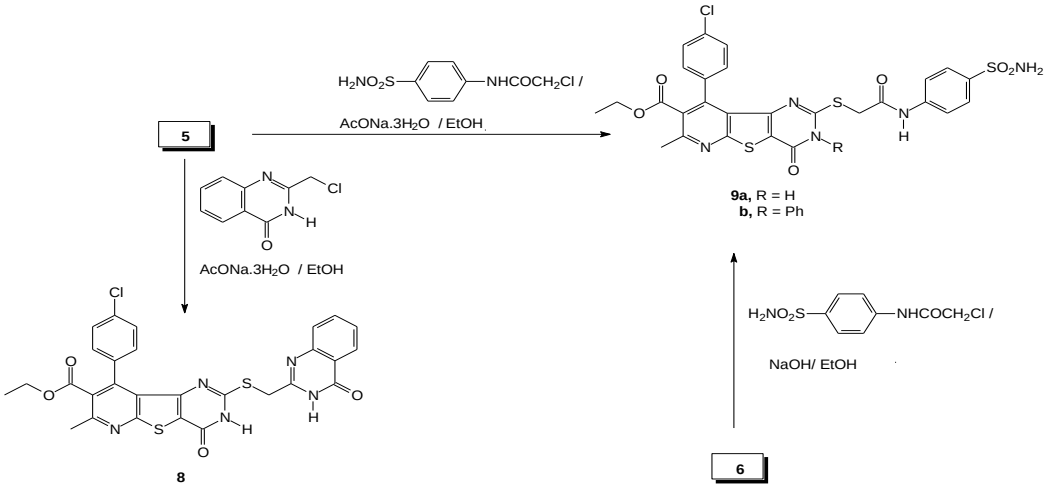


Scheme 1



Scheme 2

The compounds **5**, **6** and **7a,b** were utilized as precursors for synthesizing other new pyridothienopyrimidinones and pyrido-thienotriazinones. Thus, reaction of thioxopyrimidinone **5** with 2-chloro-methylquinazoline-4(3*H*)-one and/ or N^4-chloroacetylsulphanilamide, by refluxing in ethanol containing sodium acetate, gave ethyl 9-(*p*-chlorophenyl)-7-methyl-4-oxo-2-(4-oxo-3,4-dihydro-quinazolin-2-yl) methyl-thio-3,4-dihydropyrido[3',2':4,5] thieno-[3,2-d]pyrimidine-8-carboxylate **(8)** and ethyl 2-[(*p*-aminosulfonylphenyl)-carbamoyl]methylthio-9-(*p*-chloro-phenyl)-7-methyl-4-oxo-3,4-dihydro pyrido[3',2':4,5]thieno[3,2-d]pyrimi-dine-8-carboxylate (**9a)** respectively. Whereas, the reaction of thioxopyrimi-dinone **6** with N^4-chloro-acetylsulpha-nilamide in an ethanolic sodium hydroxide solution produced ethyl 2-[(*p*-aminosulfonylphenyl)carbamoyl] methylthio-9-(*p*-chlorophenyl)-7-methyl-4-oxo-3-phenyl-3,4-dihydro-pyrido[3',2':4,5]thieno[3,2-d]pyrim-idine-8-carboxylate (**9b)** in a high yield (Scheme 3). Reaction of compound **7a** or **7b** with ethyl chloroacetate or chloroacetamide in DMF containing anhyd. K_2CO_3 at room temperature gave the *N*-alkylated triazinones **10** and **11** respectively. When compound **7a** was allowed to react with aromatic or heterocyclic amines in the presence of of formaldehyde, the corresponding Mannich bases **12a,b** and **13a,b** were obtained in high yields (Scheme 4). The structural formulas of all newly synthesized compounds were confirmed by elemental and spectral analyses (*cf.* experimental part).Among the synthesized compounds, fifteen samples were selected and evaluated for their antimicrobial activities against six strains of bacteria and six strains of fungi. The bioassay results, which are given in tables 1 and 2, revealed that: (i) most of the tested compounds possess mild to strong activity against, at least, one bacterial or fungal strain, (ii) only three compounds (**2a**, **9a** and **9b**) showed no activity against all used strains of bacteria and fungi, (iii) Most of the tested compounds possess moderate to very strong inhibition activity against *Staphylococcus aureus*, (iv) each of triazinone derivatives **7a**, **7b** and **13b** showed mild to moderate activity against all tested strains of bacteria, (v) compounds **1a, 1b** and **12b** exhibit no activity against all fungal strains used and (vi) Compounds **4b**, **5**, **6**, **7a**, **7b** and **8** showed partial inhibition zones of 18, 14, 14, 18 and 18 mm., respectively, towards *G. candidum*



Scheme 3

3. Experimental

All melting points were measured on a Gallen-Kamp melting point apparatus and are uncorrected. IR Spectra were obtained on a Pye-Unicam SP3-100 spectrophotometer using KBr disc technique. ^{1}H NMR Spectra were recorded on a Bruker 400 MHz NMR spectrometer (Sohag University) or a Varian EM-390, 90 MHz spectrometer (Assiut University). Elemental analyses were performed on an Elemental Analyser system GmbH VARIO EL $V_{2.3}$1998 CHNS Mode. Compounds **1a,b**, **2a,b** and **3a,b** were prepared according to our reported methods **[**21**]**.

3.1. Reaction of compounds 3a,b with triethylorthoformate; formation of pyridothienopyrimidinones 4a,b; general procedure

A mixture of compound **3a,b** (0.005 mol) and triethyl orthoformate (3 ml) in redistilled acetic anhydride (10 ml) was heated under reflux for 4 h. The precipitate that formed while hot was collected by filtration, washed with ethanol and crystallized from DMF to give compounds **4a,b** in the form of white needles.

3.1.1. Ethyl 9-(p-methoxyphenyl)-7-methyl-4-oxo-3,4-dihydropyrido[3',2':4,5] thieno[3,2-d]pyrimidine-8-carboxylate (4a)

Yield: 80 %, m.p.: 298-299 °C. IR: 3220 (NH), 1731 (C=O, ester), 1659 (C=O, pyrimidinone) cm.$^{-1}$ ^{1}H NMR (400 MHz, DMSO-d_6)): δ = 12.875 (br. s, 1H, NH), 8.03 (s, 1H, CH pyrinidinone), 7.26-7.28 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.98-7.00 (d, *J* = 8.0 Hz, 2H, Ar-H), 4.06-4.08 (q, 2H, OCH_2), 3.83 (s, 3H, OCH_3), 2.64 (s, 3H, CH_3 at C-7), 0.95-0.97 (t, 3H, CH_3 of ester). Anal. Calcd. for $C_{20}H_{17}N_3O_4S$ (395.43): C, 60.75; H, 4.33; N, 10.63; S, 8.11 %. Found: C, 60.66; H, 4.21; N, 10.41; S, 8.25 %.

3.1.2. Ethyl 9-(p-chlorophenyl)-7-methyl-4-oxo-3,4-dihydropyrido[3',2':4,5]thieno [3,2-d]pyrimidine-8-carboxylate (4b)

Yield: 81 %, m.p.: 334-336 °C. IR: 3200 (NH), 1728 (C=O, ester), 1655 (C=O, pyrimidinone) cm.$^{-1}$ ^{1}H NMR (90 MHz, DMSO-d_6)): δ = 12.8 (br. s, 1H, NH), 8.1 (s, 1H, CH pyrimidinone), 7.5-7.9 (dd, 4H, Ar-H), 4.0-4.3 (q, 2H, OCH_2), 2.6 (s, 3H, CH_3 at C-7), 0.9-1.1 (t, 3H, CH_3 of ester). Anal. Calcd. for $C_{19}H_{14}ClN_3O_3S$ (399.85): C, 57.07; H, 3.53; N, 10.51; S, 8.02 %. Found: C, 57.00; H, 3.61; N, 10.38; S, 7.84 %.

3.2. Ethyl 9-(p-chlorophenyl)-7-methyl-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrido [3',2':4,5]thieno[3,2-d]pyrimidine-8-carboxylate (5)

A mixture of compound **3b** (1.95 g, 0.005 mol) and ethyl potassium xanthate (1.12 g, 0.007 mol) in absolute ethanol (15 ml) was heated on a water bath under reflux for 6 h. The reaction mixture was cooled, diluted with water (20 ml) and then acidified with dil. HCl. The precipitated solid was collected by filtration, air-dried and crystallized from DMSO to give compound **5**. Yield: 87 %, m.p.: 282-283 °C (Lit. m.p.: 281 °C) [22]. IR: 3388 (NH), 3368 (NH), 1727 (C=O, ester), 1694 (C=O, pyrimidinone) cm.$^{-1}$ Anal. Calcd. for $C_{19}H_{14}ClN_3O_3S_2$ (431.91): C, 52.84; H, 3.27; N, 9.73; S, 14.85 %. Found: C, 52.93; H, 3.25; N, 9.64; S, 15.00 %.

3.4. Ethyl 9-(p-chlorophenyl)-7-methyl-4-oxo-3-phenyl-2-thioxo-1,2,3,4-tetrahydro- pyrido[3',2':4,5]thieno[3,2-d]pyrimidine-8-carboxylate (6)

A mixture of compound **3b** (1.95 g, 0.005 mol) and phenyl isothiocyanate (0.7 ml, 0.005 mol) in redistilled pyridine (15 ml) was heated on a water bath for 3 h. The precipitate that formed while hot was collected by filtration, washed with ethanol and recrystallized from DMF. Yield: 87 %, m.p.: 268-270 °C (Lit. m.p.: 267-268 °C) [22]. IR: 3398 (NH), 1733 (C=O, ester), 1697 (C=O, pyrimidinone) cm.$^{-1}$ Anal. Calcd. for $C_{25}H_{18}ClN_3O_3S_2$ (508.01): C, 59.11; H, 3.57; N, 8.27; S, 12.62 %. Found: C, 59.24; H, 3.50; N, 8.59; S, 12.72 %.

3.5. Diazotisation of compounds 3a,b; formation of triazinone derivatives 7a,b; general procedure

To a chilled mixture of compound **3a,b** (0.005 mol) and conc H_2SO_4 (5 ml) in glacial acetic acid (15 ml), 5 ml of sodium nitrite solution (10 %) was added dropwise with stirring during about 10 min. After the completion of addition, stirring was continued for 2 h. The precipitate that formed after dilution with water (10 ml) was collected by filtration and crystallized from DMSO in the form of white crystals.

3.6.1. Ethyl 9-(p-methoxyphenyl)-7-methyl-4-oxo3,4dihydropyrido[3',2':4,5] thieno[3,2-d][1,2,3]triazine-8-carboxylate (7a)

Yield: 80 **%**, m.p.: 184-185 °C. IR: 3205 (NH), 1724 (C=O, ester), 1671 (C=O, triazinone) cm.$^{-1}$ ^{1}H NMR (400 MHz, $CDCl_3$,): δ = 12.55 (br. s, 1H, NH), 7.25-7.27 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.91-6.93 (d, *J* = 8.0 Hz, 2H, Ar-H), 4.03-4.06 (q, *J* = 4.0 Hz, 2H, OCH_2), 3.81 (s, 3H, OCH_3), 2.71 (s, 3H, CH_3 at C-7), 0.96 - 0.98 (t, *J* = 4.0 Hz, 3H, CH_3 of ester). Anal. Calcd. for $C_{19}H_{16}N_4O_4S$ (396.42): C, 57.57; H, 4.07; N, 14.13; S, 8.09 %. Found: C, 57.44; H, 4.08; N, 14.33; S, 7.92 %**.**

3.6.2. Ethyl 9-(p-chlorophenyl)-7-methyl-4-oxo-3,4-dihydropyrido[3',2':4,5]thieno [3,2-d][1,2,3]triazine -8-carboxylate (7b)

Yield: 86 %, m.p.: 201-203 °C; IR: 3203 (NH), 1739 (C=O, ester), 1671 (C=O, triazinone) cm.$^{-1}$ ^{1}H NMR (90 MHz, DMSO-d_6): δ = 12.5 (br. s, 1H, NH), 7.4-7.8 (dd, 4H, Ar-H), 4.0-4.3 (q, 2H, OCH_2), 2.70 (s, 3H, CH_3 at C-7), 0.9 -1.1 (t, 3H, CH_3 of ester). Anal. Calcd. for $C_{18}H_{13}ClN_4O_3S$ (400.84): C, 53.94; H, 3.27; N, 13.98; S 8.00 %. Found: C, 54.09; H, 3.21; N, 13.78; S, 8.11 %**.**

3.7. Reaction of phenylpyrimidinethione 5 with 2-chloromethylquinazoline-4(3H)-one and/ or N^4-chloroacetylsulph-anilamide; formation of compounds 8 and 9a; general procedure

To a mixture of compound **5** (2.16 g, 0.005 mol) and sodium acetate trihydrate (1.0 g, 0.007 mol) in ethanol (25 ml), 2-chloromethylquinazoline-4(3*H*)-one or N^4-chloroacetylsulphanilamide (0.005 mol) was added. The resulting mixture was heated under reflux for 2 h. The precipitate that formed while hot was collected and recrystallized from ethanol to give compounds **8** or **9a** respectively**.**

3.7.1 9-(4-chlorophenyl)-7-methyl-4-oxo-2-(4-oxo-3,4-dihydroquinazolin-2-yl) methylthio-3,4-dihydropyrido[3',2':4,5] thieno[3,2-d]pyrimidine-8-carboxylate (8)

Yield: 87 %, m.p.: 340-341 °C. IR: 3250 (2 NH), 1717 (C=O, ester), 1647 (C=O, pyrimidinone and quinazolinone), 1621 (C=N) cm.$^{-1}$ ^{1}H NMR (90 MHz, $CDCl_3$): δ = 11.6 (br. s, 2H, 2 NH of pyrimidinone and quinazolinone), 7.1- 8.2 (m, 8H, Ar-H), 4.3 (s, 2H, SCH_2), 3.8-4.2 (q, 2H, OCH_2), 2.7 (s, 3H, CH_3 at C-7), 0.8 – 1.1 (t, *J* = 6.0 Hz, 3H, CH_3 of ester). Anal. Calcd. for $C_{28}H_{20}ClN_5O_4S_2$ (590.07): C, 56.99; H, 3.42; N, 11.87; S, 10.87%. Found: C, 56.73; H, 3.44; N, 11.80; S, 10.66 %.

3.7.2. Ethyl 9-(4-chlorophenyl)-7-methyl-4-oxo-2-[(p-aminosulfonylphenyl)carbamoyl]methylthio3,4dihydropyrido[3',2':4,5]thieno[3,2-d]pyrimidine-8-carboxylate (9a)

Yield:; mp. 310-311 °C. IR: 3323 (NH), 3231 (NH), 1721 (C=O, ester), 1659 (C=O, pyrimidinone and anilide) cm.$^{-1}$ ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.24 (s, 1H, NH of pyrimidinone), 10.24 (s, 1H, NH of sulphanilamide moiety), 7.79-7.81 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.71-7.73 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.44-7.46 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.35-7.37 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.20 (s, 2H, SO_2NH_2), 4.01-4.06 (q, *J* = 6.0 Hz, 2H, OCH_2), 3.41 (s, 2H, SCH_2), 2.67 (s, 3H, CH_3 at C-7), 0.91-0.94 (t, *J* = 6.0 Hz, 3H, CH_3 of ester). Anal. Calcd. for $C_{27}H_{22}ClN_5O_6S_3$ (644.13): C, 50.35; H, 3.44; N, 10.87; S, 14.93 %.

Found: C, 50.18; H, 3.40; N, 11.01; S, 15.12 %.

3.8. Ethyl 9-(4-chlorophenyl)-7-methyl-4-oxo-3-phenyl-2-[(p-aminosulfonylphenyl) carbamoyl]methylthio-3,4-dihydropyrido [3',2':4,5]thieno[3,2-d]pyrimidine-8-carboxylate (9b)

To a solution of compound **6** (1.01 g, 0.002 mol) in an ethanolic sodium hydroxide 5 % (10 ml), N^4-chloroacetylsulphanilamide (0.5 g, 0.002 mol) was added. The reaction mixture was heated under reflux for one hour and then allowed to cool. The precipitate that formed was collected and recrystallized from isopropanol. Yield: 87 %, m.p.: 221-222 °C. IR: 3300 (NH), 1716 (C=O, ester), 1660 (2 C=O, anilide) $cm.^{-1}$

^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.22 (s, 1H, NH of sulphanilamide moiety), 7.77-7.79 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.67-7.69 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.58 (s, 3H, Ar-H), 7.49-7.51 (d, *J* = 8.0 Hz, 4H, Ar-H), 7.40-7.42 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.19 (s, 2H, SO_2NH_2), 4.03-4.08 (q, *J* = 6.0 Hz, 2H, OCH_2), 3.24 (s, 2H, SCH_2, overlapped with DMSO signal), 2.70 (s, 3H, CH_3 at C-7), 0.93-0.96 (t, *J* = 6.0 Hz, 3H, CH_3 of ester). Anal. Calcd. for $C_{33}H_{26}ClN_5O_6S_3$ (720.23): C, 55.03; H, 3.64; N, 9.72; S, 13.35 %. Found: C, 55.18; H, 3.34; N, 9.50; S, 13.37 %.

3.9. Alkylation of triazinones 7a,b, formation of compounds 10 and 11; general procedure

To a mixture of compound **7a,b** (0.005 mol) and ethyl chloroacetate or chloroacetamide (0.005 mol) in DMF (20 ml), anhyd. K_2CO_3 (1.0 g) was added. The reaction mixture was stirred at room temperature for 5 h and then diluted with water. The solid that precipitated was collected and recrystallized from ethanol**.**

3.9.*1.* ***8-Ethoxycarbonyl-3-ethoxyca-bonylmethyl-9-(p-methoxyphenyl)-7-methyl-pyrido[3',2':4,5]thieno[3,2-d][1,2,3] triazine-4(3H)-one (10)***

7a with ethyl chloroacetate following the above general procedure. Yield: 76 %; m.p.: 143-144 °C. IR: 1751 (C=O, non conjugated ester), 1710 (C=O, conjugated ester), 1693 (C=O, triazinone). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.92-7.28 (dd, 4H, Ar-H), 5.09 (s, 2H, NCH_2), 4.19 (q, 2H, OCH_2), 4.05 (q, 2H, OCH_2), 3.79 (s, 3H, OCH_3), 2.70 (s, 3H, CH_3 at C-7), 1.19-1.22 (t, 3H, CH_3 of ester group), 0.97-0.99 (t, 3H, CH_3 of ester group). Anal. Calcd. for $C_{23}H_{22}N_4O_6S$ (482.51): C, 57.25; H, 4.60; N, 11.61; S, 6.64 %. Found: C, 57.00; H, 4.53; N, 11.50; S, 7.01 %.

3.9.*2.* ***3-Carbamoylmethyl-9-(p-chlorophenyl)-8-ethoxycarbonyl-7-methylpyrido [3',2':4,5]thieno[3,2-d][1,2,3]triazine-4(3H)-one (11)***

It was prepared by reaction of compound **7b** with chloroacetamide following the above general procedure. Yield: 81 %; m.p.: 262-264 °C. IR: 3419, 3212 (NH_2), 1696 (C=O, ester), 1680 (2 C=O, triazinone and amide). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.70 (s, 1H, OH), 7.57-7.59 (d, *J* = 8 Hz, 2H, Ar-H), 7.45-7.47 (d, *J* = 8 Hz, 2H, Ar-H), 7.30 (s, 1H, NH), 5.02 (s, 2H, NCH_2), 4.09-4.14 (q, *J* = 8 Hz, 2H, OCH_2), 2.73 (s, 3H, CH_3 at C-7), 0.97-1.01(t, *J* = 8 Hz, 3H, CH_3 of ester group). Anal. Calcd. for $C_{20}H_{16}ClN_5O_4S$ (457.89): C, 52.46; H, 3.52; N, 15.29; S, 7.00 %. Found: C, 52.69; H, 3.38; N, 15.27; S, 7.11 %.

3.10. Reaction of triazinone 7a with aromatic or heterocyclic amines and formaldehyde, formation of Mannich bases 12a,b and 13a,b; general procedure

To a stirred mixture of compound **7a** (1.98 g, 0.005 mol), aromatic or heterocyclic amine (0.005 mol) in ethanol (30 ml), formalin solution 37 % (10 ml) was added. The stirring was continued for 4 h. The reaction mixture was then allowed to stand at room temperature overnight. The product that precipitated was collected and recrystallized from proper solvent.

3.10.1. 8-Ethoxycarbonyl-9-(p-methoxyphenyl)-7-methyl-3-(p-tolylaminomethyl) pyrido[3',2':4,5]thieno[3,2-d][1,2,3]triazine-4(3H)-one (12a)

It was prepared by using *p*-toludine in the above general procedure. Yield: 80 %; m.p.: 153-155°C (EtOH). IR: 3446 (NH), 1730 (C=O, ester), 1689 (C=O, triazinone). Anal. Calcd. for $C_{27}H_{25}N_5O_4S$ (515.59): C, 62.90; H, 4.89; N, 13.58; S, 6.22 %. Found: C, 62.68; H, 4.94; N, 13.44; S, 6.00 %.

3.10.2. 3-(p-Chlorophenylaminomethyl)-8-ethoxycarbonyl-9-(p-methoxyphenyl)-7-methylpyrido[3',2':4,5]thieno[3,2-d][1,2,3]triazine-4(3H)-one (12b)

It was prepared by using *p*-chloroaniline in the above general procedure. Yield: 83%; m.p.: 167-168 °C (DMSO). IR: 3388 (NH), 1725 (C=O, ester), 1681 (C=O, triazinone). ^{1}H NMR (90 MHz, $CDCl_3$): δ = 6.7-7.6 (m, 8H, Ar-H), 5.7-5.9 (d, 2H, NCH_2N, appears as a singlet after deutration), 5.3-5.5 (t, 1H, NH, exchangeable with D_2O), 4.0-4.3 (q, 2H, OCH_2), 3.9 (s, 3H, OCH_3), 2.8 (s, 3H, CH_3 at C-7), 0.9-1.2 (t, 3H, CH_3 of ester group). Anal. Calcd. for $C_{26}H_{22}ClN_5O_4S$ (536.00): C, 58.26; H, 4.14; N, 13.07; S, 5.98 %. Found: C, 58.18; H, 4.34; N, 13.13; S, 6.10 %.

3.10.3. 8-Ethoxycarbonyl-9-(p-methoxyphenyl)-7-methyl-3-piperdinomethylpyrido [3',2':4,5]thieno[3,2-d][1,2,3]triazine-4(3H)-one (13a)

It was prepared by using piperidine in the above general procedure. Yield: 78 %; m.p.:174-175 °C. IR: 2936 (C-H, aliphatic), 1717 (C=O, ester), 1675 (C=O, triazinone). ^{1}H NMR (90 MHz, $CDCl_3$): δ = 7.0-7.5 (dd, 4H, ArH), 5.4 (s, 2H, NCH_2), 4.0-4.3 (q, 2H, OCH_2), 3.9 (s, 3H, OCH_3), 2.7-2.9 (m, 7H, CH_2NCH_2 and CH_3 at C-7), 1.3-1.7 (m, 6H, three methylene groups of piperidine ring), 0.9-1.1 (t, 3H, CH_3 of ester group). Anal. Calcd. for $C_{27}H_{29}N_5O_4S$ (519.62): C, 60.48; H, 5.51; N, 14.19; S, 6.50 %. Found: C, 60.55; H, 5.47; N, 14.27; S, 6.34 %.

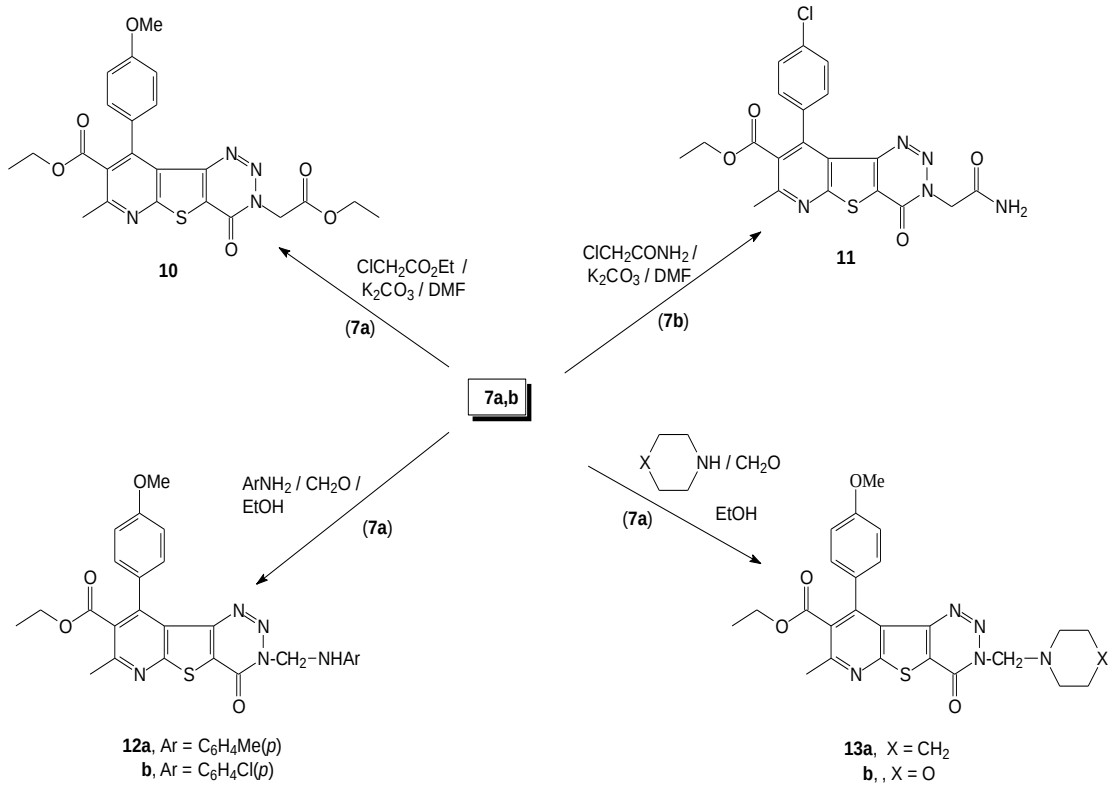
3.10.4. 8-Ethoxycarbonyl--9-(p-methoxy-phenyl)-7-methyl-3-morpholinomethyl pyrido[3',2':4,5]thieno[3,2-d][1,2,3] triazine-4(3H)-one (13b)

It was prepared by using morpholine in the above general procedure. Yield: 82 %; m.p.:181-182 °C. IR: 2941 (C-H, aliphatic), 1710 (C=O, ester), 1671 (C=O, triazinone).. ^{1}H NMR (90 MHz, $CDCl_3$): δ = 7.0-7.5 (dd, 4H, ArH), 5.4 (s, 2H, NCH_2), 4.0-4.3 (q, 2H, OCH_2), 3.9 (s, 3H, OCH_3), 3.6-3.8 (t, 4H, CH_2OCH_2), 2.7-2.9 (m, 7H, CH_2NCH_2 and CH_3 at C-7), 1.9-1.1 (t, 3H, CH_3 of ester group). Anal. Calcd. for $C_{24}H_{25}N_5O_5S$ (495.55): C, 58.17; H, 5.08; N, 14.13; S, 6.47 %. Found: C, 58.00; H, 4.81; N, 14.00; S, 6.76 %.

3.11. Biological activity

The antimicrobial activity of fifteen compounds was tested against six bacterial and six fungal strains provided by the Assiut University Mycological Centre (AUMC). These strains were isolated from different sources in Egypt and some of them were involved in human diseases *(Staphylococcus aureus, Aspergillus flavus, Candida albicans, Geotrichum candidum, Scopulariopsis brevicaulis and Trichophyton rubrum)*, plant diseases *(Fusarium oxysporum)* or frequently reported from contaminated soil, water and food substances *(Bacillus cereus, Micrococcus luteus, Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens).* 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 conical containing 30 ml Sabouraud's dextrose broth. Bioassay was performed 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 [23]. After solidification of media, 5 mm diameter cavities were cut in the solidified agar (4 cavities/ plate) using sterile cork borer. Organic compounds dissolved in dimethyl sulfoxide (DMSO) at 2 % w/ v (=20 mg / ml) were pipette in the cavities (20 ul/ cavity). Cultures were then incubated at 28 C for 48 h in case of bacteria and up to 7 days in case of fungi. Results were read as the diameter of inhibition zone around cavities [24].



Scheme 4

Table 1: Antibacterial activities of some representative compounds (inhibition zones in mm).

Compd. No.	*B. cereus*	*M. luteus*	*S. aureus*	*E. Coli*	*P. aeruginosa*	*S. marcescens*
1a	0	0	6	0	0	0
1b	0	0	7	0	0	0
2a	0	0	0	0	0	0
3a	0	6	6	9	0	0
4b	0	6	12	0	8	0
5	6	10	11	0	10	12
6	0	0	0	0	0	0
7a	7	12	11	7	11	12
7b	11	9	11	6	9	11
8	9	8	8	0	9	9
9a	0	0	0	0	0	0
9b	0	0	0	0	0	0
11	6	0	6	0	0	0
12b	0	0	6	0	0	6
13b	9	13	12	12	12	13
Control	11	20	13	16	13	20

Control = Chloramphenicol

Table 2: Antifungal activities of some representative compounds (inhibition zones in mm).

Compd. No.	*A. Flavus*	*C. albicans*	*F. oxysporum*	*G. candidum*	*S. brevicaulis*	*T. rubrum*
1a	0	0	0	0	0	0
1b	0	0	0	0	0	0
2a	0	0	0	0	0	0
3a	7	10	0	0	0	0
4b	0	0	0	18 p.i.	0	0
5	0	0	0	14 p.i.	0	13
6	0	0	0	14 p.i.	0	0
7a	0	0	0	16 p.i.	0	13
7b	0	7	0	18 p.i.	0	12
8	0	7	0	12	0	10
9a	0	0	0	0	0	0
9b	0	0	0	0	0	0
11	0	9	0	0	0	8
12b	0	0	0	0	0	0
13b	0	9	8	16 p.i.	0	13
Control	40	12	12	14	28	40

Control = Clotrimazole
p.i. = partial inhibition

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