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Synthesis of Some New Fused 1,2,4-Triazines and Their Antimicrobial Activity[1]

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Abstract—The starting material 4-amino-4*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol **1** was synthesized by refluxing a mixture of isatin and thiocarbohydrazide in glacial acetic acid. Compound **1** reacted with various types of reagents including ammonia, hydrazine hydrate, semicarbazide HCl in various media to give triazino-[5,6-*b*]indole-3,4-diamine **2**, 3-hydrazino-4*H*-[1,2,4]triazino[5,6-*b*]indol-4-amine **3** and 3,4-dihydro[1,2,4,5]-tetrazino[6',1':3,4][1,2,4]triazino[5,6-*b*]indole-2(1*H*)-one **5,** respectively. Reactions of compounds **2** and **3** with ethyl chloroformate in boiling DMF led to 1*H*-[1,2,4]triazolo[5',1':3,4][1,2,4]triazino[5,6-*b*]indol-3(4*H*)-one **4** and compound **5,** respectively. Microwave irradiation of compound **1** with maleic anhydride yielded 1-(3-mercapto-4*H*-[1,2,4]triazino[5,6-*b*]indol-4-yl)-1*H*-pyrrole-2,5-dione **6**. Reactions of **1** with different aldehydes in EtOH-HCl gave the corresponding Schiff bases. Treatment of substrate **1** with NH_4SCN in glacial AcOH yielded *N*-(3-mercapto-4*H*-[1,2,4]triazino[5,6-*b*]indol-4-yl)thiourea **9** and 1*H*-[1,2,4]triazolo[5',1':3,4][1,2,4]-triazino[5,6-*b*]indole-3(2*H,*4*H*)-thione **10**, respectively. Reaction of substrate **1** with phenyl isothiocyanate in refluxing dioxan gave *N*-(3-mercapto-4*H*-[1,2,4]triazino[5,6-*b*]indol-4-yl)-*N*'-phenylthiourea **11**. Its reaction with ethyl cyanoacetate upon refluxing in EtONa/EtOH afforded ethyl (4-amino-4*H*-[1,2,4]triazino[5,6-*b*]-indol-3-yl)(cyano)acetate **12**, which upon boiling gave ethyl 2-amino-1*H*-pyrazolo[5',1':3,4][1,2,4]triazino-[5,6-*b*]indole-3-carboxylate **13**. Structures of new compounds were confirmed by elemental analysis and spectroscopic data (IR, ^{1}H and ^{13}C NMR). The newly synthesized compounds were subjected to the biological screening, which demonstrated promising results.

Keyword: triazinoindole, tetrazinotriazinoindole, triazolotriazinoindole, biological activity

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INTRODUCTION

Isatin has been used in synthesis of fused indole derivatives, such as indolothiazoles [1], thiadiazinoindole [2], pyrazinoindole [3], tris-indolobenzene [4], and 1,2,4-triazinoindole derivatives [5].

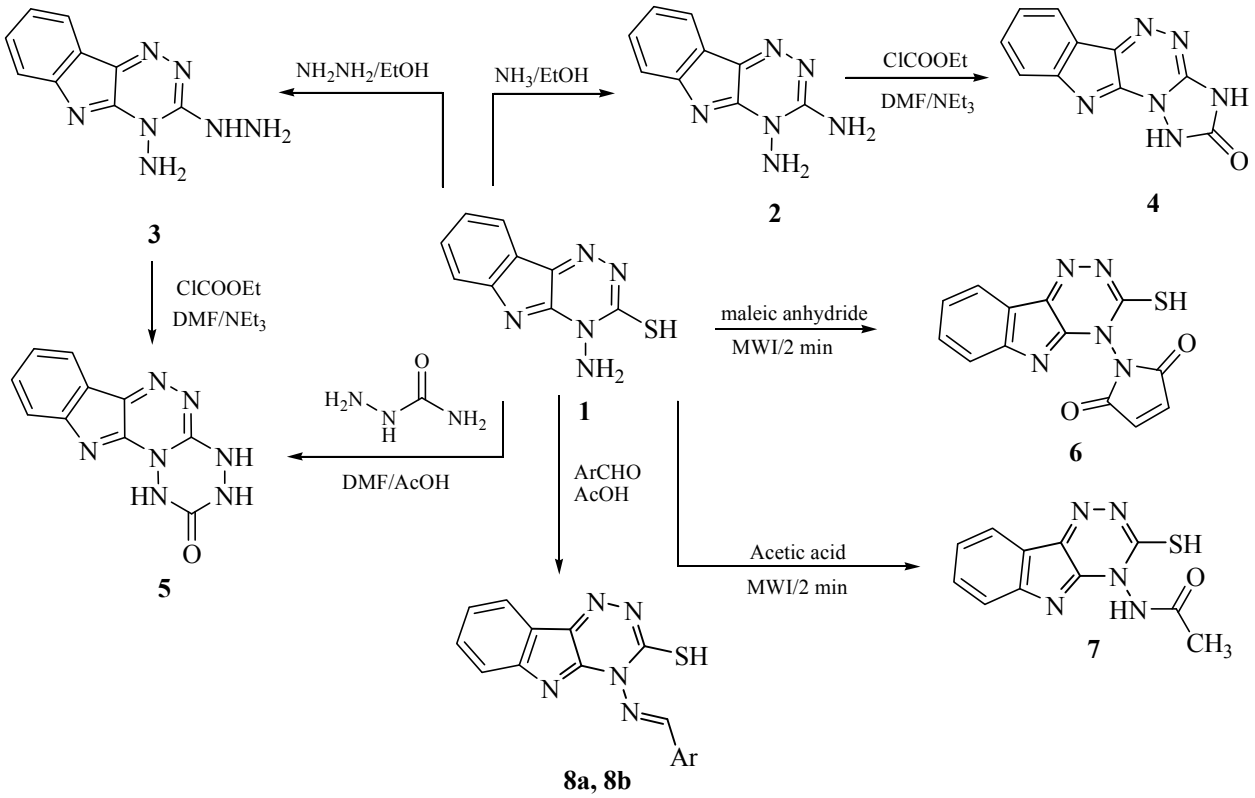
Isatin derivatives can undergo nucleophilic attack at positions C-2 and/or C-3. Chemoselectivity of such reactions depends on the nature of nucleophiles, nature of substituents attached to the isatin nucleus and particularly the nitrogen atom, as well as, upon solvents and the process temperature. The primary products reacted with a second nucleophilic group to give cyclization products [6]. In the current study we pursued synthesis of fused heterocyclic systems that contained an indole moiety. 4-Amino-4*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol was synthesized and used as the starting material in further transformations by traditional or microwave methods that led to new derivatives. Biological activity of the synthesized compounds was tested.

RESULTS AND DISCUSSION

Results of earlier studies of antimicrobial activity of 4-amino-4*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol **1** [7] initiated further functionalization of that key intermediate targeting its derivatives with higher antimicrobial activities. The compound **1** was subjected to a series of reactions that led to various azoles and/or azines fused with the starting [1,2,4]triazolo[5,6-*b*]-indoles. It reacted with ammonia and/or hydrazine hydrate in boiling ethanol to give 4*H*-[1,2,4]triazino-

[1] The text was submitted by the authors in English.

Scheme 1.



Ar = Ph (**a**), thinyl (**b**).

[5,6-*b*]indole-3,4-diamine **2** and/or 3-hydrazinyl-4*H*-[1,2,4]-triazino[5,6-*b*]indol-4-amine **3**, respectively (Scheme 1).

Structures of compounds **2** and **3** were elucidated from their IR, ^{1}H and ^{13}C NMR data. The IR spectrum of **2** exhibited $2NH_2$ bands in the range 3349–3328 cm^{-1} and disappearance of the signal at 2615 cm^{-1} (C=S). Presence of the $2NH_2$ groups was supported by signals at 10.44 and 11.11 ppm with disappearance of the signal at 12.67 ppm (SH). The IR spectrum of **3** demonstrated a band at 3350–3319 cm^{-1} attributed to one NH and two NH_2 groups. The ^{1}H NMR spectrum of the product **3** exhibited signals of the NH proton at 8.50 ppm and two signals at 10.43 and 11.39 ppm. No signal of the SH group was recorded.

Compounds **2** and **3** (Scheme 1) reacted with ethyl chloroformate in boiling DMF under the action of catalytic amount of triethylamine to give 1*H*-[1,2,4]-triazolo[5',1':3,4][1,2,4]triazino[5,6-*b*]indol-2(3*H*)-one **4** and 3,4-dihydro[1,2,4,5]tetrazino[6',1':3,4][1,2,4]-triazino[5,6-*b*]indole-2(1*H*)-one **5**, respectively. IR spectrum of compound **4** demonstrated the new band at 1642 cm^{-1} (urea derivative C=O). In ^{1}H NMR spectrum two NH signals appeared at 7.23 and 11.30 ppm while the NH_2 signals were not recorded. ^{1}H NMR spectrum of compound **5** exhibited three NH signals at 11.30, 12.99 and 13.25 ppm. Signal of the C=O group was recorded in ^{13}C NMR spectra at 160.5 ppm.

The compound **1** reacted with semicarbazide in boiling DMF with small amount of glacial acetic acid. Formation of compound **5** (80%) in the process confirmed the structure of **1**.

Use of microwave irradiation is an established tool in organic synthesis for achieving higher selectivity, rate enhancement and reduction of thermal degradation byproducts formation [8, 9]. However such procedures are carried out under high pressure developed by a solvent upon heating in an oven. One of the ways to overcome the problem is the use of organic reagents on solid inorganic supports [10, 11]. It also provides an opportunity to work with open vessels and allows to scale up reactions [12, 13].

Reaction of **1** with maleic anhydride under microwave irradiation gave 1-(3-mercapto-4*H*-[1,2,4]-triazino[5,6-*b*]indol-4-yl)-1*H*-pyrrole-2,5-dione **6**

Table 1. Antibacterial screening data for positively responding compounds according to Kirby–Bauer disc diffusion metho[a,b]

Sample		Inhibition zone diameter, mm/mg					
		Bacillus subtilis (G^+ bacteria)	*Escherichia coli* (G^- bacteria)	*Pseudomonas. Aeruginosa* (G^- bacteria)	*Staphylococcus aureus* (G^+ bacteria)	*Aspergillus flavus*	*Candida albicans*
Control: DMSO		0.0	0.0	0.0	0.0	0.0	0.0
Standard	Ampicillin antibacterial agent	20	18	17	18	0.0	0.0
Standard	Amphotericin B antifungal agent	0.0	0.0	0.0	0.0	17	19
1		12	14R[c]	14R	14	0.0	0.0
2		0.0	0.0	7	0.0	0.0	0.0
3		0.0	0.0	6	0.0	0.0	0.0
5		15	13	11	13	0.0	8
6		10	8	9	9	0.0	9
7		7	5	8	10	0.0	9
8		0.0	0.0	0.0	0.0	0.0	0.0
9		9	7	7	5	0.0	0.0
10		9	12	17	14	0.0	10
11		9	7	7	6	0.0	0.0
12		0.0	0.0	0.0	9	0.0	0.0
13		0.0	0.0	0.0	9	0.0	0.0

[a] (G) Gram reaction. [b] Solvent DMSO. [c] (R) Repellent action (not complete inhibition).

(Scheme 1). Its IR spectrum demonstrated a broad band at 1675–1667 cm^{-1} due to two C=O groups and ^1H NMR spectrum exhibited a signal at 7.58 ppm of pyrrole protons. The ^{13}C NMR showed one signal attributed to two symmetrical C=O groups at 160.7 ppm.

Microwave irradiation of compound **1** in the presence of few drops of glacial acetic acid yielded *N*-(3-mercapto-4*H*-[1,2,4]triazino[5,6-*b*]indol-4-yl)-acetamide **7** (Scheme 1).

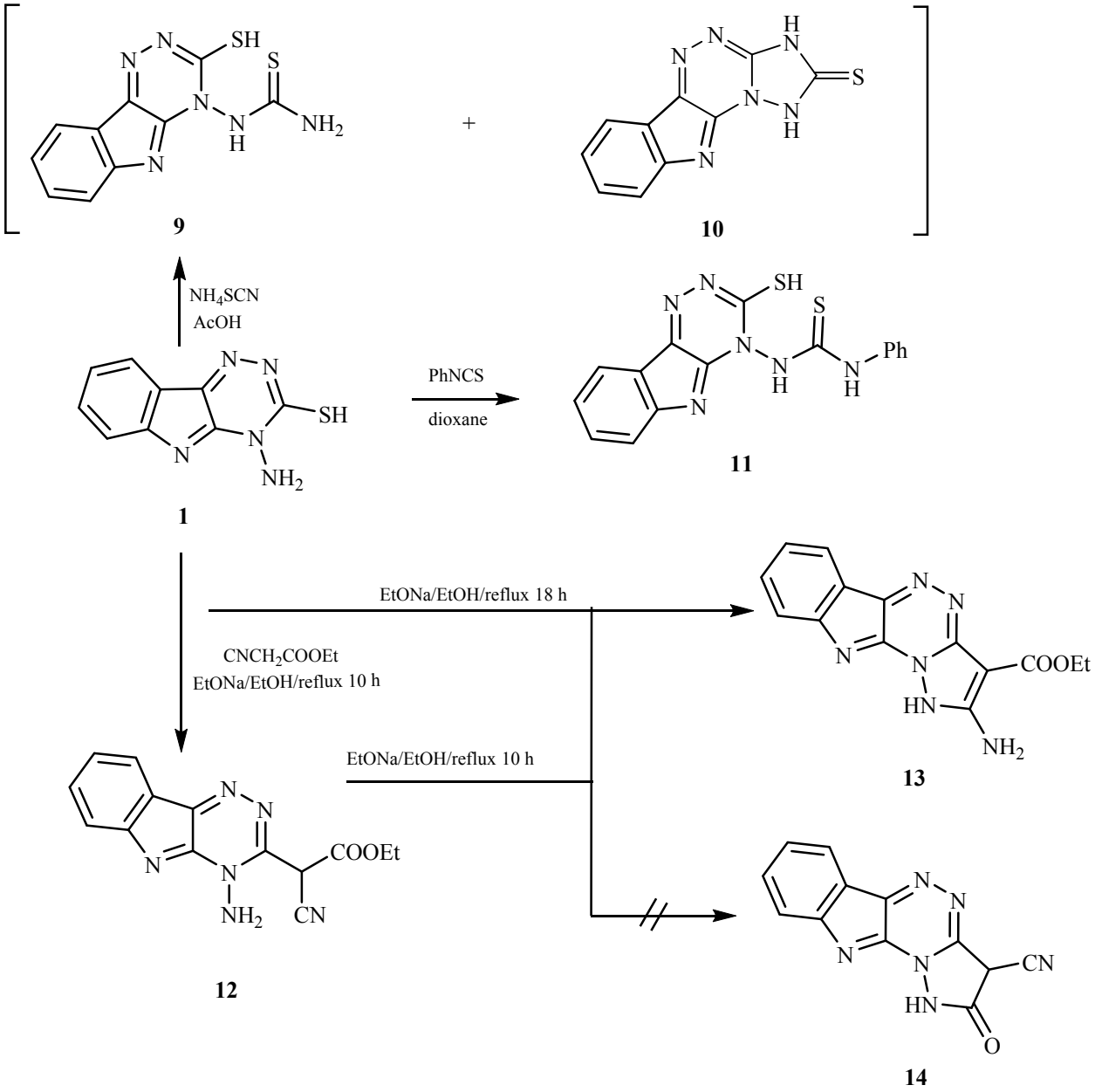
In pursue of expanding biological activity of the new derivatives, compound **1** was condensed with aromatic aldehydes such as benzaldehyde or 2-thiophenaldehyde in EtOH–HCl with formation Schiff bases (**8a** and **8b**), respectively (Scheme 1).

Addition of ammonium thiocyanate to compound **1** in glacial acetic acid led to *N*-substituted thiourea derivatives *N*-(3-mercapto-4*H*-[1,2,4]triazino[5,6-*b*]indol-4-yl)thiourea **9** and 1*H*-[1,2,4]triazolo-[5',1':3,4][1,2,4]-triazino[5,6-*b*]indole-2(3*H*)-thione **10**, respectively (Scheme 2), that were separated upon crystallization. The reaction of **1** with phenyl isothiocyanate under reflux in dioxane yielded *N*-(3-mercapto-4*H*-[1,2,4]-triazino[5,6-*b*]indol-4-yl)-*N*'-phenylthiourea **11** (Scheme 2).

Compound **1** was refluxed with ethyl cyanoacetate in ethanolic sodium ethoxide to afford ethyl (4-amino-4*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)(cyano)acetate **12.** More prolonged boiling of the mixture led to ethyl 2-amino-1*H*-pyrazolo[5',1':3,4][1,2,4]triazino[5,6-*b*]-indole-3-carboxylate **13** with no evidence of formation of 2-oxo-2,3-dihydro-1*H*-pyrazolo[5',1':3,4][1,2,4]-triazino[5,6-*b*]indole-3-carbonitrile **14** (Scheme 2).

IR spectra of **13** revealed disappearance of the band of the CN group, hence, supporting addition of the

Scheme 2. Syntes is of some fused triazin oindoles.



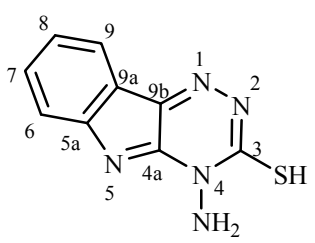
amino group to it. Such result excluded formation of 2-oxo-2,3-dihydro-1*H*-pyrazolo[5',1':3,4][1,2,4]triazino-[5,6-*b*]indole-3-carbonitrile **14**. ^{13}C NMR spectrum of the product **13** also demonstrated no signals characteristic to the *sp* carbon atom.

Antimicrobial activity. The compound **1** had no effect on fungal strain and poor effect on all types of bacteria strains (Table 1). Fusion of the heterocyclic nuclei, azoles or azines, enhanced pharmacological activities of its corresponding derivatives [14]. All products demonstrated a moderate activity toward the studied bacterial strains. Presence of the C=O group in the tetrazine ring enhanced its activity. Whereas hiadiazole ring was inactivated by the same group. The C=S group supported some biological activity of the compounds.

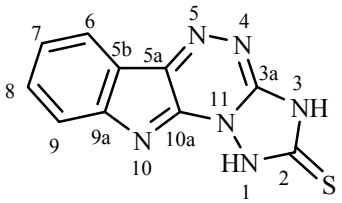
EXPERIMENTAL

All chemicals were purchased from Sigma (NY, USA). Melting points were measured by a digital

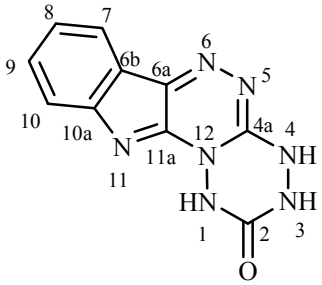
Scheme 3. Illustrates the numbering of different compounds.



1– 3, 6, 7, 8a, 8b, 9, 11, 12



4, 10, 13, 14



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Electrothermal IA 9100. IR spectra were recorded on an ATR-Alpha FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AC-600 Hz spectrometer in DMSO-d_6 solutions using TMS as the internal standard. Mass spectra were measured on a Shimadzu GC-MS-QP 1000 EX spectrometer. Microwave irradiation was performed in a domestic microwave oven (2450 MHz, 800 W).

4*H*-[1,2,4]Triazino[5,6-*b*]indole-3,4-diamine (2). A mixture of compound **1** (0.44 g, 2 mmol) with ammonia solution (3 mL, 50%) in ethanol (15 mL) was refluxed for 4 h. The reaction mixture was cooled down and the dark brown precipitate was purified with ethanol/charcoal. The product was recrystallized from ethanol as brown crystals, yield 53%, mp 282–284°C. IR spectrum, ν, cm^{-1}: 3328–3349 (NH_2), 1624 (C=N). ^{1}H NMR spectrum, δ, ppm: 6.90 d.d [1H, Ar-H (C^7)], 7.33 d [1H, Ar-H (C^6)], 7.51 d.d [1H, Ar-H (C^8)], 8.16 d [1H, Ar-H (C^9)], 10.44 s [2H, NH_2 (C^3)], 11.11 s (2H, N-N$\underline{H}_2$). ^{13}C NMR spectrum, δ, ppm: 111.2 (C_{9a}), 121.4 (C_9), 122.0 (C_8), 126.4 (C_6), 131.7 (C_7), 138.6 (C_{4a}), 140.3 (C_3), 152.1 (C_{9b}) and 155.1 (C_{5a}). Found, %: C 54.01; H 4.00; N 41.93. $C_9H_8N_6$. Calculated, %: C 53.99; H 4.03; N 41.98.

3-Hydrazinyl-4*H*-[1,2,4]triazino[5,6-*b*]indol-4-amine (3). A mixture of compound **1** (0.44 g, 2 mmol) with hydrazine hydrate (3 mL, 80%, excess) in ethanol (15 mL) was refluxed for 4 h. The dark brown precipitate was purified with ethanol/charcoal, the product was crystallized from ethanol to yield brown crystals, 53%, mp 289–291°C. IR spectrum, ν, cm^{-1}: 3319–3350 (NH and 2NH_2) and 1614 (C=N). ^{1}H NMR spectrum, δ, ppm: 6.90 d.d [1H, Ar-H (C^7)], 7.21 d [1H, Ar-H (C^6)], 7.31 d.d [1H, Ar-H (C^8)], 7.64 d [1H, Ar-H (C^9)], 8.50 s [1H, N$\underline{H}$NH_2 (C^3)], 10.43 s [2H, NHN$\underline{H}_2$ (C_3)], 11.39 s (2H, N^4-N$\underline{H}_2$). ^{13}C NMR spectrum, δ, ppm: 113.1 (C_{9a}), 122.4 (C_9), 124.0 (C_8), 125.6 (C_6), 133.0 (C_7), 137.7 (C_{4a}), 141.3 (C_3), 151.3 (C_{9b}) and 157.2 (C_{5a}). Found, %: C 50.29; H 4.20; N 45.54. $C_9H_8N_6$. Calculated, %: C 50.23; H 4.22; N 45.56.

1*H*-[1,2,4]triazolo[5',1':3,4][1,2,4]triazino[5,6-*b*]-indol-2(3*H*)-one (4). A mixture of compound **2** (0.2 g, 0.01 mol) with ethyl chloroformate (0.1 mL, 0.01 mol) in DMF (15 mL) and catalytic amount of triethylamine was refluxed for 6 h, cooled down and poured onto ice/cold water. The precipitate formed was filtered off and recrystallized from DMF/ethanol as brownish powder. Yield 79%, mp 321–323°C. IR spectrum, ν, cm^{-1}: 3218–3129 (2NH), 1642 (C=O) and 1620 (C=N). ^{1}H NMR spectrum, δ, ppm: 7.23 d.d (1H, Ar-H (C^7)], 7.63 d [1H, Ar-H (C^8)], 7.78 s [1H, N$\underline{H}$ (N^1)], 8.24 d [1H, Ar-H (C^6)], 8.27 d [1H, Ar-H (C^9)], 11.30 s [1H, N$\underline{H}$ (N^3)]. ^{13}C NMR spectrum, δ, ppm: 115.8 (C_{5b}), 119.6 (C_6), 121.9 (C_7), 125.6 (C_9), 133.5 (C_8), 134.7 (C_{10a}), 156.0 (C_{3a}), 157.2 (C_{9a}), 158.9 (C=O), and 160.5 (C_{5a}). Found, %: C 53.15; H 2.66; N 37.11. $C_{10}H_6N_6O$. Calculated, %: C 53.10; H 2.67; N 37.15.

3,4-Dihydro[1,2,4,5]tetrazino[6',1':3,4][1,2,4]triazino[5,6-b]indole-2(1*H*)-one (5). *Method a.* A mixture of compound **3** (0.21 g, 0.01 mol) with ethyl chloroformate (0.1 mL, 0.01 mol) in DMF (15 mL) and catalytic amount of triethylamine was refluxed for 6 h, cooled down and poured onto ice. The precipitate was filtered off and recrystallized from DMF/ethanol as orange brown powder. Yield 71%, mp 324–326°C.

Method b. A mixture of compound **1** (0.44 g, 2 mmol) with semicarbazide (0.15 g, 2 mmol) in DMF (15 mL) was refluxed for 6 h, cooled down and the precipitate was crystallized from DMF/ethanol as orange brown powder. Yield 80%, mp 325–327°C. IR spectrum, ν, cm^{-1}: 3221–3321 (3NH), 1648 (C=O) and 1617 (C=N). ^{1}H NMR spectrum, δ, ppm: 6.82 d.d [1H, Ar-H (C^9)], 6.94 d [1H, Ar-H (C^{10})], 7.24 d.d [1H, Ar-H (C^8)], 8.18 d [1H, Ar-H (C^7)], 11.30 s [1H, N$\underline{H}$ (N^4)], 12.99 s [1H, N$\underline{H}$ (N^1)], 13.25 s [1H, N$\underline{H}$ (N^3)].

^{13}C NMR spectrum, δ, ppm: 113.5 (C_{6b}), 120.8 (C_7), 122.9 (C_8), 124.5 (C_{10}), 133.2 (C_{11a}), 137.5 (C_9), 145.2 (C_{4a}), 153.9 (C=O), 157.1 (C_{10a}), and 160.5 (C_{5a}). Found, %: C 49.72; H 2.90; N 40.61. $C_{10}H_7N_7O$. Calculated, %: C 49.79; H 2.93; N 40.60.

1-(3-Mercapto-4*H*-[1,2,4]triazino[5,6-*b*]indol-4-yl)-1*H*-pyrrole-2,5-dione (6). A mixture of **1** (0.44 g, 2 mmol) with maleic anhydride (0.2 g, 2 mmol) was dissolved in a mixture of methylene chloride–methanol (4 : 1, 15 mL) followed by addition of silica gel (1.0 g, 200–400 mesh). The solvent was removed by evaporation. The dry residue was transferred into a glass beaker and irradiated for 1.5–2.0 min. The product was extracted from silica gel by DCM. Upon evaporation of the solvent under vacuum the product was purified on a silica gel column (eluent DCM) as yellow crystals. Yield 66%, mp 330–333°C. IR spectrum, ν, cm^{-1}: 2615 (SH) and 1675–1667 br (2C=O amide). ^{1}H NMR spectrum, δ, ppm: 6.90 d.d [1H, Ar-H (C^7)], 7.31 d [1H, Ar-H (C^6)], 7.48 d.d [1H, Ar-H (C^8)], 7.58 s (2H, Hz, pyrrole-H), 8.20 d [1H, Ar-H (C^9)], 12.70 s (1H, SH). ^{13}C NMR spectrum, δ, ppm: 116.2 (C_{9a}), 119.5 (C_9), 121.8 (C_8), 122.0 (C_{4a}), 125.6 (C_6), 132.4 (C_7), 133.6 (pyrrole C=C), 145.1 (C_3), 157.7 (C_{5a}), 160.7 (pyrrole 2C=O) and 168.8 (C_{9b}). Found, %: C 52.47; H 2.35; N 23.53. $C_{13}H_7N_5O_2S$. Calculated, %: C 52.52; H 2.37; N 23.56.

***N*-(3-Mercapto-4*H*-[1,2,4]triazino[5,6-*b*]indol-4-yl)acetamide (7).** To a solution of compound **1** (0.44 g, 2 mmol) in a mixture of methylene chloride–methanol (4 : 1, 15 mL), silica gel (1.0 g, 200–400 mesh) was added. The solvent was removed by evaporation, the dried residue was transferred into a glass beaker and drops of glacial acetic acid were added. The mixture was irradiated for 1.5–2.0 min. The product was extracted from silica gel by DCM. Upon evaporation of the solvent under vacuum the product was purified on a silica gel column, using DCM as an eluent to afford yellow crystals. Yield 85%, mp 265–267°C. IR spectrum, ν, cm^{-1}: 3202 (NH) and 1665 (C=O amide). ^{1}H NMR spectrum, δ, ppm: 7.50 d.d [1H, Ar-H (C^8)], 8.15 d [1H, Ar-H (C^9)], 12.66 s (1H, SH). ^{13}C NMR spectrum, δ, ppm: 21.58 (CH_3); 116.4 (C_{9a}), 118.4 (C_9), 120.0 (C_8), 124.4 (C_6), 138.6 (C_7), 143.2 (C_{4a}), 152.1 (C_{9b}), 156.0 (C_{5a}), 157.8 (C_3) and 166.3 (C=O). Found, %: C 50.88; H 3.47; N 27.00. $C_{11}H_9N_5OS$. Calculated, %: C 50.95; H 3.50; N 27.01.

Synthesis of compounds 8a and 8b. To a solution of **1** (0.22 g, 0.01 mol) in ethanol (20 mL), the appropriate aldehyde (benzaldehyde, 0.10 mL, 0.01 mol) or (2-thiophenecarboxaldehyde, 0.09 mL, 0.01 mol) was added followed by addition of HCl (1 mL). The reaction mixture was refluxed for 2 h, cooled down, poured onto crushed ice and neutralized with dil. ammonium hydroxide. The precipitate was recrystallized from ethanol (20 mL).

4-(Benzylideneamino)-4*H*-[1,2,4]triazino[5,6-*b*]-indole-3-thiol (8a). Yield 70% (orange crystals), mp 327–329°C. IR spectrum, ν, cm^{-1}: 2611 (SH). ^{1}H NMR spectrum, δ, ppm: 6.93 d.d [1H, Ar-H (C^7)], 7.33 d [1H, Ar-H (C^6)], 7.50 d.d [1H, Ar-H (C^8)], 7.58–8.00 m (5H, Ar-H), 8.15 d [1H, Ar-H (C^9)], 8.70 s (1H, N=C<u>H</u>Ph), 12.72 s (1H, SH). ^{13}C NMR spectrum, δ, ppm: 118.4 (C_9), 120.6 (C_{9a}), 123.1 (C_8), 124.5 (C_6), 129.5 (phenyl *meta* carbons), 130.7 (phenyl *para* carbon), 132.4 (phenyl *ortho* carbons), 133.6 (C_7), 135.2 (phenyl C_1 carbon), 137.9 (C_{4a}), 156.5 (C_{9b}), 160.8 (C_3), 162.3 (C_{5a}) and 164.1 (<u>H</u>C=N). Found, %: C 62.88; H 3.54; N 22.90; S 10.49. $C_{16}H_{11}N_5S$. Calculated, %: C 62.93; H 3.63; N 22.93; S 10.50.

4-[(2-Thienylmethylene)amino]-4*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (8b). Yield 70%, orange crystals, mp 327–329°C. IR spectrum, ν, cm^{-1}: 2620 (SH). ^{1}H NMR spectrum, δ, ppm: 6.77 d.d (1H, J = 3.90, 4.80 Hz, thiophene-H_b), 6.90 d.d [1H, Ar-H (C^7)], 7.29 d (1H, J = 3.6 Hz, thiophene-H_c), 7.35 d [1H, Ar-H (C^6)], 7.52 d.d [1H, Ar-H (C^8)], 7.62 d (1H, J = 5.40 Hz, thiophene-H_a), 8.14 d [1H, Ar-H (C^9)], 8.83 s (1H, N=C<u>H</u>-thienyl), 12.70 s (1H, SH). ^{13}C NMR spectrum, δ, ppm: 117.2 (C_{9a}), 120.7 (C_9), 122.4 (C_8), 126.8 (C_6), 127.1 (thienyl C_4), 129.5 (thienyl C_5), 133.0 (C_7), 135.7 (C_{4a}), 138.1 (thienyl C_2), 139.7 (thienyl C_3), 148.7 (<u>H</u>C=N), 157.4 (C_3), 158.2 (C_{5a}), and 161.1 (C_{9b}). Found, %: C 53.86; H 2.87; N 22.40; S 20.54. $C_{14}H_9N_5S_2$. Calculated, %: C 54.00; H 2.91; N 22.49; S 20.60.

Synthesis of compounds 9 and 10. A mixture of **1** (0.22 g, 0.01 mol) with dry ammonium thiocyanate (0.08 g, 0.01 mol) in glacial acetic acid (20 mL) was refluxed for 3 h. The solid residue was filtered off and cooled down. The precipitate was recrystallized from ethanol (20 mL).

***N*-(3-Mercapto-4*H*-[1,2,4]triazino[5,6-*b*]indol-4-yl)thiourea (9).** Yield 34%, mp 314–317°C. IR spectrum, ν, cm^{-1}: 3420–3280 (NH_2 and NH), 2613 (SH), 1335 (C=S). ^{1}H NMR spectrum, δ, ppm: 6.28 br (2H, NH_2), 6.90 d.d [1H, Ar-H (C^7)], 7.31 d [1H, Ar-H (C^6)], 7.52 d.d [1H, Ar-H (C^8)], 8.15 d [1H, Ar-H

(C^9)], 12.32 s (1H, NH), and 13.99 s (1H, SH). ^{13}C NMR spectrum, δ, ppm: 118.5 (C_{9a}), 119.3 (C_9), 121.1 (C_8), 125.3 (C_6), 131.6 (C_7), 133.5 (C_{4a}), 156.5 (C_3), 157.9 (C_{9b}), 159.9 (C_{5a}) and 166.9 (C=S). Found, %: C 43.45; H 2.90; N 30.35; S 23.18. $C_{10}H_8N_6S_2$. Calculated, %: C 43.46; H 2.92; N 30.41; S 23.21.

1*H*-[1,2,4]Triazolo[5',1':3,4][1,2,4]triazino[5,6-*b*]-indole-2(3*H*)-thione (10). Yield 42%, mp 310–312°C. IR spectrum, ν, cm^{-1}: 3243–3228 (2NH), 1328 (C=S). ^1H NMR spectrum, δ, ppm: 6.91 d.d [1H, Ar-H (C^7)], 7.31 d [1H, Ar-H (C^6)], 7.51 d.d [1H, Ar-H (C^8)], 8.14 d [1H, Ar-H (C^9)], 10.27 s (1H, NNH), 11.96 s (1H, NHCS). ^{13}C NMR spectrum, δ, ppm: 118.4 (C_{5b}), 119.0 (C_6), 121.1 (C_7), 125.0 (C_9), 131.7 (C_8), 136.3 (C_{10a}), 150.7 (C_{3a}), 153.1 (C_{5a}), 155.8 (C_{9a}) and 169.7 (C=S). Found, %: C 49.50; H 2.53; N 34.65; S 13.20. $C_{10}H_6N_6S$. Calculated, %: C 49.58; H 2.50; N 34.69; S 13.24.

***N*-(3-Mercapto-4*H*-[1,2,4]triazino[5,6-*b*]indol-4-yl)-*N*'-phenylthiourea (11).** To a solution of **1** (0.22 g, 0.01 mol) in dry dioxane (20 mL) phenyl isothiocyanate was added (0.1 mL, 0.01 mol) and the reaction mixture was refluxed for 2 h. Upon cooling the mixture down the precipitate was crystallized from ethanol (20 mL) as a yellow solid. Yield 62%, mp 326–328°C. IR spectrum, ν, cm^{-1}: 3280–3237 (NH), 2605 (SH) and 1328 (C=S). ^1H NMR spectrum, δ, ppm: 6.81 d (1H, phenyl group-H of C_4), 6.95 d.d [1H, Ar-H (C^7)], 7.20 d.d (2H, phenyl group-H of C_3,C_5), 7.27 d [1H, Ar-H (C^6)], 7.52 d.d [1H, Ar-H (C^8)], 7.20 d (2H, phenyl group-H of C_2,C_6), 8.09 d [1H, Ar-H (C^9)], 9.76 s (1H, N-NH), 12.32 s (1H, NHPh) and 13.92 s (1H, SH). ^{13}C NMR spectrum, δ, ppm: 118.1 (C_9), 119.3 (C_{9a}), 121.1 (C_8), 125.3 (C_6), 125.7 (phenyl *para* carbon), 125.8 (phenyl *ortho* carbons), 128.7 (phenyl *meta* carbons), 131.7 (C_7), 136.4 (C_{4a}), 144.2 (phenyl C_1 carbon), 157.2 (C_{9b}), 159.4 (C_3), 159.5 (C_{5a}) and 165.9 (C=S). Found, %: C 54.45; H 3.41; N 23.79; S 18.10. $C_{16}H_{12}N_6S_2$. Calculated, %: C 54.53; H 3.43; N 23.85; S 18.20.

Synthesis of compounds 12 and 13. Method A: A mixture of **1** (0.44 g, 0.02 mol), ethyl cyanoacetate (0.23 g, 0.02 mol) and a catalytic amount of sodium ethoxide in ethanol (15 mL) was refluxed for 10–18 h until the reaction was complete. The solvent was evaporated and the solid residue was recrystallized from dioxane to give compound **12** as a reddish powder and/or **13** as a brownish powder. Yield 70 %, mp 340–342°C.

Ethyl (4-amino-4*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)(cyano)acetate (12). Yield 82 %, mp 310–312°C. IR spectrum, ν, cm^{-1}: 3424 (NH_2), 2199 (CN), 1663 (C=O amide), and 1618 (C=N). ^1H NMR spectrum, δ, ppm: 1.29 t (3H, CH_3), 4.20 q (2H, CH_2), 4.93 s (1H, CH), 5.70 s (2H, NH_2), 6.89 d.d [1H, Ar-H (C^7)], 7.27 d [1H, Ar-H (C^6)], 7.51 d.d [1H, Ar-H (C^8)], 8.09 d [1H, Ar-H (C^9)]. ^{13}C NMR spectrum, δ, ppm: 14.25 (CH_3), 61.18 (CH_2), 63.39 (CH), 118.2 (C_9), 119.1 (C_{9a}), 121.8 (C_8), 124.5 (CN), 125.2 (C_6), 132.5 (C_7), 133.2 (C_{4a}), 154.0 (C_{9b}), 154.6 (C_3), 159.7 (C_{5a}) and 159.9 (C=O). Found, %: C 56.70; H 4.14; N 28.29. $C_{14}H_{12}N_6O_2$. Calculated, %: C 56.75; H 4.08; N 28.36.

Ethyl 2-amino-1*H*-pyrazolo[5',1':3,4][1,2,4]triazino[5,6-*b*]indole-3-carboxylate (13). *Method b*. Compound **12** (0.3 g, 0.01 mol) was refluxed in ethanolic sodium ethoxide (15 mL) for 10 h until the reaction was complete (TLC), the solvent was evaporated and the solid residue was recrystallized from dioxane to give compound **13** as a brownish powder**.** Yield 60 %, mp 337–339°C. IR spectrum, ν, cm^{-1}: 3402 (NH_2), 1649 (C=O amide) and 1623 (C=N). ^1H NMR spectrum, δ, ppm: 1.26 t (3H, CH_3), 4.21 q (2H, CH_2), 6.59 s (2H, NH_2), 6.89 d.d [1H, Ar-H (C^8)], 7.27 d [1H, Ar-H (C^9)], 7.51 d.d [1H, Ar-H (C^7)], 8.09 d [1H, Ar-H (C^6)], 11.01 s (1H, NH). ^{13}C NMR spectrum, δ, ppm: 13.93 (CH_3), 59.36 (CH_2), 82.0 (C_3), 118.4 (C_{5b}), 119.0 (C_6), 121.6 (C_7), 125.0 (C_9), 131.2 (C_8), 132.0 (C_{10a}), 150.2 (C_{3a}), 152.3 (C_2), 152.6 (C_{5a}), 158.6 (C_{9a}) and 159.8 (C=O). Found, %: C 56.68; H 4.12; N 28.31. $C_{14}H_{12}N_6O_2$. Calculated, %: C 56.75; H 4.08; N 28.36.

Antimictobial activity. The *in vitro* antimicrobial activity of the synthesized compounds was determined using a modified Kirby-Bauer disc diffusion method [15]. Ampicillin and Amphotericin B were used as bacterial and fungal positive controls, respectively, and DMSO was used as the solvent and negative control as well. Six microbial species were studied, namely, *E. coli* (G^- bacteria), *P. aeruginosa* (G^- bacteria), *S. aureus* (G^+ bacteria), *B. subtilis* (G^+ bacteria), *A. flavus* (filamentous fungi), and *C. albicans* (yeast).

Briefly, 100 mL of the test organism were grown in 10 mL of fresh media until they reached a count of 108 cells/mL for bacteria and 105 cells/mL for fungi. Approximately 100 ml of the microbial suspension was spread onto Muller-Hinton agar plates. Paper discs (Scleicher & Schull, Spain) with a diameter of 8.0 mm were impregnated by 10 mL of the test compound (4.0 mM), and the controls were treated similarly. The plates were incubated for 48 h at 35–37°C for bacterial

strains, 25°C for *A. flavus* and 30°C for *C. albicans*. Inhibition zone diameters were measured with slipping calipers.

CONCLUSIONS

New series of fused 1,2,4-triazino[5,6-*b*]indole derivatives were synthesized and tested for their antimicrobial activity. Compounds **5–7, 9, 10**, and **11** demonstrated some activity toward bacterial strain.

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