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Sterically controlled regiospecific heterocyclization of 3-hydrazino-5-methyl-1,2,4-triazino[5,6-*b*]indole to 10-methyl-1,2,4-triazolo[4′,3′:2,3]1,2,4-triazino[5,6-*b*]indoles

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Abstract

3-Hydrazino-5-methyl-1,2,4-triazino[5,6-*b*]indole underwent sterically controlled regiospecific heterocyclizations with a variety of one-carbon cyclizing agents to give the sterically more favored linearly annulated 10-methyl-1,2,4-triazolo[4′,3′:2,3]1,2,4-triazino[5,6-*b*]indoles rather than the sterically less favored angularly annulated 10-methyl-1,2,4-triazolo[3′,4′:3,4]1,2,4-triazino[5,6-*b*]indoles. The assigned structures were corroborated by comparison with unequivocally synthesized authentics, chemical and spectral data. The antimicrobial activity of some of the prepared compounds was investigated.

Keywords: Hydrazones; Hydrazides; Sterically controlled heterocyclization

1. Introduction

The synthesis of 1,2,4-triazolo-1,2,4-triazino[5,6-*b*]indoles has been surveyed in two recent reviews [1,2]. 3-Hydrazino- and 3-thiosemicarbazido- derivatives of 1,2,4-triazino[5,6-*b*]indoles showed antimicrobial [3–6] and antitumor activities against P388 lymphocytic leukemia in mice [7]. Furthermore, 1,2,4-triazolo-1,2,4-triazino[5,6-*b*]indoles revealed antimicrobial [6], antiviral [8,9] and anti-inflammatory activities [10]. We became interested in the synthesis of 1,2,4-triazolo-1,2,4-triazino[5,6-*b*]indoles by cyclocondensation of various 3-hydrazono- and 3-thiosemicarbazido- derivatives of 1,2,4-triazino[5,6-*b*]indoles in order to investigate the antimicrobial activity, as well as to resolve the contradictions [1,2] regarding the assignment of their correct structures. Thus, linearly or angularly annulated structures were assigned to the products of cyclization of 3-hydrazino-1,2,4-triazino[5,6-*b*]indoles with one-carbon cyclizing agents. In continuation of our interest in the synthesis of various condensed 1,2,4-triazolo-heterocycles [11–14] and in an attempt to settle such a structural controversy, we report here the results obtained on the synthesis and structure elucidation of the title compounds.

2. Chemistry

Products of heterocyclization of 5-substituted-3-hydrazino-1,2,4-triazino[5,6-*b*]indoles such as **1** (R ≠ H) with one-carbon cyclizing agents were assigned either the angularly annulated 1,2,4-triazolo[3′,4′:3,4]1,2,4-triazino[5,6-*b*]indole (**2**) structure [15–17] or its linearly annulated regioisomeric 1,2,4-triazolo[4′,3′:2,3]1,2,4-triazino[5,6-*b*]indole (**3**) structure [8,9,18–20] (Scheme 1); in no case has the formation of a mixture of both regioisomers (**2** and **3**) been reported. Assignment of either of these two structures was based only on electronic factors that enhance the nucleophilicites of N2 and N4 in the 1,2,4-triazine ring. Unexpectedly, steric factors that may dominate electronic influences in determining the regiospecific (or regioselective) outcome of this reaction have been overlooked. Inspection of molecular models indicated that cyclization of the N5-unsubstituted hydrazine **1** (R = H) with one-carbon cyclizing reagents would only be electronically controlled and might, accordingly, lead to **2** (R = H) and/

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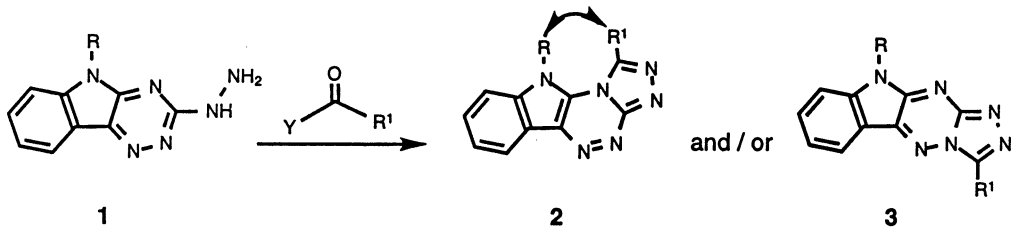


Scheme 1.

or **3** (R = H) depending on factors affecting the comparative nucleophilicities of N2 and N4 of the 1,2,4-triazine ring. Molecular models and computer optimized geometries (Figs. 1–4) predicted, on the other hand, that introduction of even the least bulky methyl group at N5 (**1**, R = Me) would impose a steric control upon the regiochemical outcome of this cyclization. Proximity of the C1 and N10 substituents in the angular regioisomers (**2**, R = Me, R^1 = Me, Ph) (Figs. 1 and 2) would generate enough steric interaction to force the direction of cyclization towards the formation of the linear regioisomers (**3**, R = Me, R^1 = Me, Ph) (Figs. 3 and 4), which are free of unfavorable steric interactions.

In order to validate or refute the aforementioned observations, some relevant heterocyclization reactions of 3-hydrazino-5-methyl-1,2,4-triazino[5,6-*b*]indole (**4**) [21] have been studied and are described here. Thus, heating the latter hydrazine with acetic acid gave a single product which showed IR and ^{1}H NMR spectral data consistent with either of the angular or the linear tetracyclic structures **6a** or **10a** (Scheme 2). Formation of **6a** or **10a** must have taken place through the dehydrative cyclization of the unisolable hydrazide **5a**. The UV spectrum of the product was found to be identical with the spectrum of the authentic sample of **10a** unequivocally prepared by cyclocondensation of 1-methylisatin **8** with 3,4-diamino-5-methyl-1,2,4-triazole (**9a**) [18]; both showed two absorption maxima at 317, 268 nm, and a shoulder at 263 nm. Further corroboration of the assigned linear structure **10a** has been pinpointed by its failure to undergo Dimroth-like rearrangement upon heating with acetic acid or piperidine. This result agrees with a 1,2,4-triazolo[4,3-*b*]1,2,4-triazine type of fusion (e.g. **10a**), which is known to be unable to undergo Dimroth rearrangement [22,23] and not with the 1,2,4-triazolo[3,4-*c*]1,2,4-triazine type of fusion (e.g. **6a**) which undergoes [22,23] facile rearrangement to the thermodynamically more stable 1,2,4-triazolo[5,1-*c*]1,2,4-triazine (e.g. **7a**). It is evident that the enhanced nucleophilicity of triazine ring N2 together with the steric control of the N5 methyl group in **4** operated synergistically toward the regiospecific formation of the sterically preferable linearly annulated regioisomer **10a** (Fig. 3). X-ray crystal structure analysis would be the most adequate technique to differentiate between the two regioisomeric structures **6** and **10** [24], yet attempts to obtain well developed crystals suitable for this type of analysis were unsuccessful.

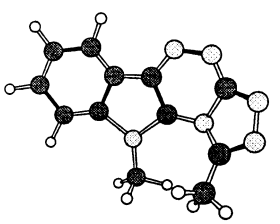


Fig. 1. Computer optimized geometry of **2** (R = R^1 = Me).

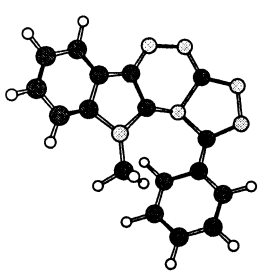


Fig. 2. Computer optimized geometry of **2** (R = Me, R^1 = Ph).

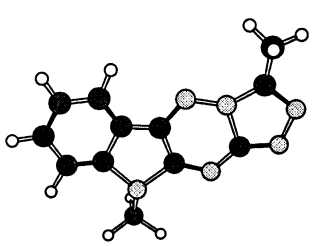


Fig. 3. Computer optimized geometry of **3** (R = R^1 = Me).

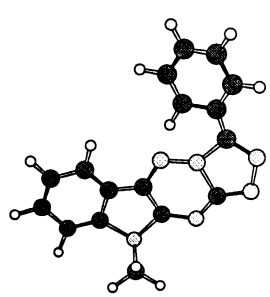
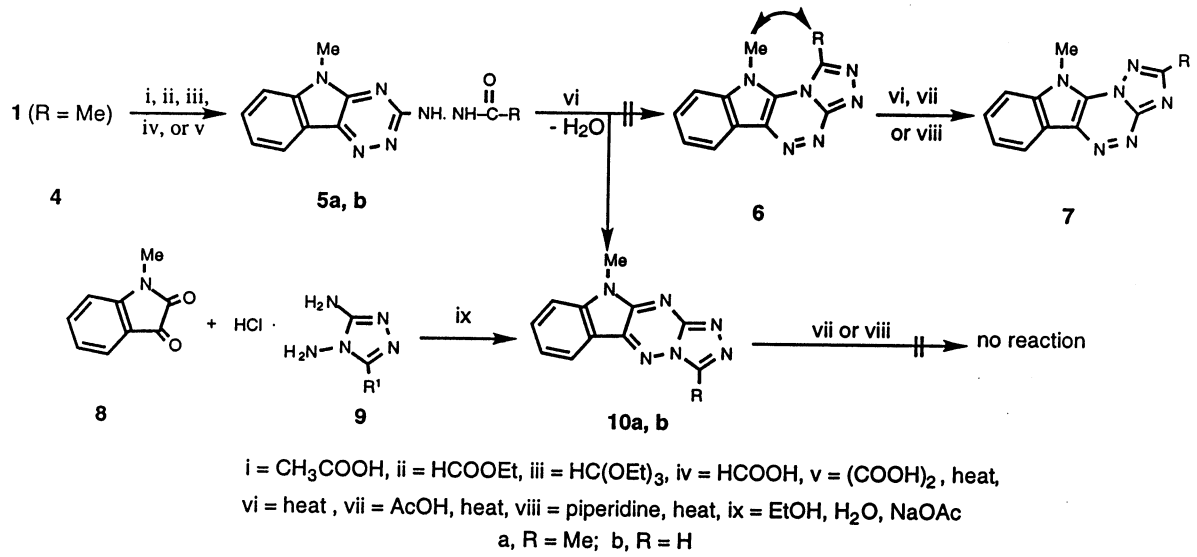


Fig. 4. Computer optimized geometry of **3** (R = Me, R^1 = Ph).



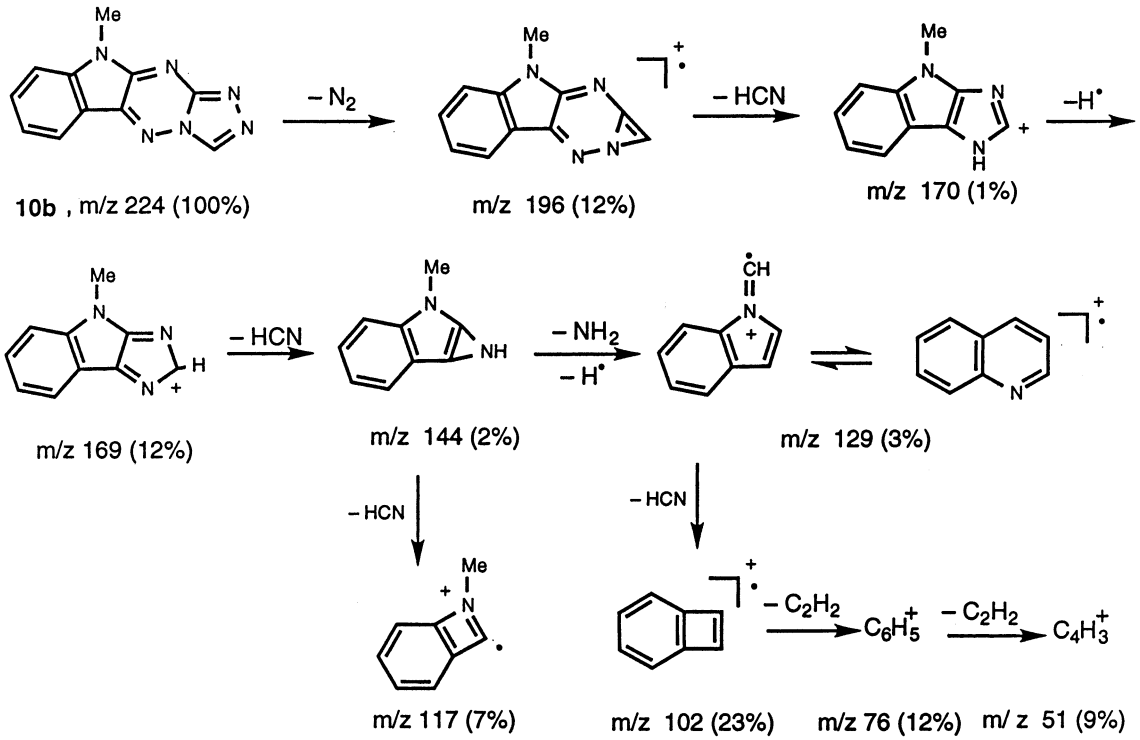
Scheme 2.

Cyclization of **4** with a methine moiety has been made under neutral conditions by heating with ethyl formate or triethyl orthoformate as well as under acidic conditions by heating with formic or oxalic acids. With ethyl formate, an intermediate was obtained which showed NH and CONH IR absorptions and a formyl, a hydrazino, and a hydrazido ^{1}H NMR proton signals. These data, together with elemental analysis, indicated that the intermediate is the formylhydrazino derivative **5b** (Scheme 2). Fusion of **5b** caused its dehydrocyclization to a single product which was assigned the linearly annulated tetracyclic structure **10b** rather than the angularly annulated structure **6b** on the following bases: (a) direct comparison with an unequivocally prepared sample obtained [18] by cyclocondensation of 1-methylisatin (**8**) with 3,4-diamino-1,2,4-triazole (**9b**) (Scheme 2); both compounds showed two UV absorption maxima at 315 and 267 nm, and a shoulder at 262 nm, (b) resistance of the obtained cyclization product to undergo acid-, base-, or thermally-induced Dimroth rearrangement to **7b**, and (c) ^{1}H NMR absorption of the 1,2,4-triazole ring proton of the product which appeared at δ 9.55, a value that is closer to those (δ 9.15–9.30) for the more deshielded H3 of 1,2,4-triazolo[4,3-*b*]1,2,4-triazines (e.g. **10b**) than to the values (δ 8.50–8.80) for H1 of 1,2,4-triazolo[3,4-*c*]1,2,4-triazines (e.g. **6b**) [22,25]. MS of **10b** showed its $M+1$ (12%) and M^+ (100%) ions at m/z 225 and 224, respectively, and underwent the characteristic fragmentation shown in Scheme 3. Triethyl orthoformate, formic acid and oxalic acid also reacted with **4** to give the same product **10b**. It is worth mentioning that oxalic acid behaved in this reaction as a one-carbon cyclizing agent similarly to its reaction with 2-hydrazinopyridine [12] and 2-hydrazinoquinoline [12], but differed from its reaction as a two-carbon cyclizing agent with 2-hydrazinobenzothiazole [12].

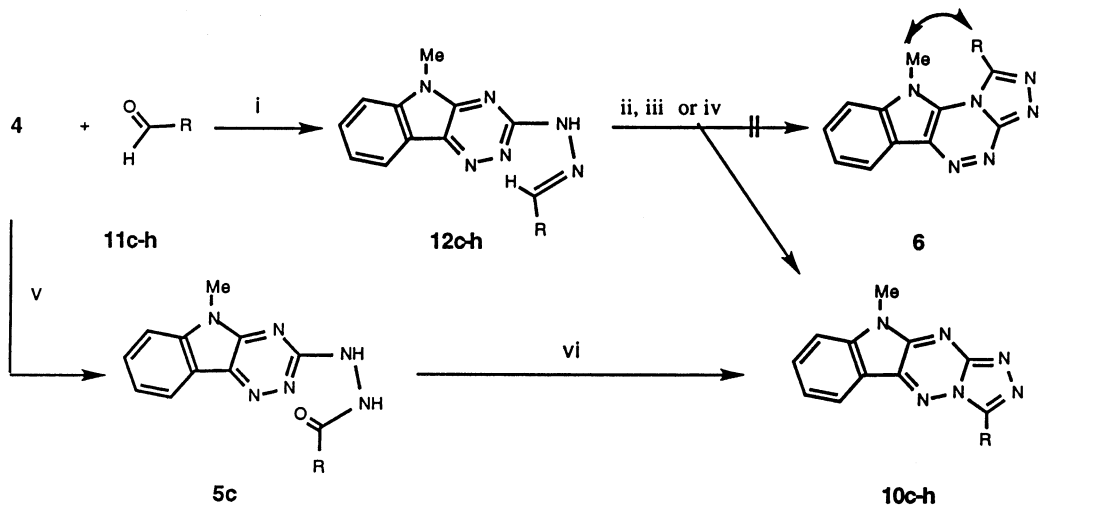
Condensation of **4** with aromatic aldehydes (**11c–h**) gave the corresponding 3-arylidenehydrazino-1,2,4-triazino[5,6-*b*]indoles **12c–h** (Scheme 4) which showed C=N absorptions at 1635–1557 cm^{-1}; their azomethine proton (–CH=N–) is included in the downfield ^{1}H NMR signals for the aromatic protons that appear between δ 8.20–7.50 ppm. The MS of one of these hydrazones (**12c**) showed its M^+ ion at m/z 302 (21%).

Oxidative cyclization of **12c–h** (Scheme 4) was accomplished by heating with three different reagents: (a) 10% ethanolic iron (III) chloride, (b) thionyl chloride or (c) 30% aqueous sodium hypochlorite solution in dioxane; the three reagents produced the same cyclic product from a particular hydrazone. Taking the purity and yield of the products as criteria to evaluate the efficacy of the three cyclizing reagents, it is possible to conclude that sodium hypochlorite is the most useful reagent. The cyclic products were also assigned the linear structure **10** and not the angular regioisomeric structure **6** on the bases of the aforementioned steric and electronic factors and further corroborated by their failure to undergo acid- or base-induced Dimroth rearrangement. Compound **10c** was also prepared through the benzoylation of **4** with benzoyl chloride followed by the acid-catalyzed dehydrocyclization of the obtained benzoylhydrazide **5c** (Scheme 4).

Reaction of **4** with phenyl isocyanate at 100°C afforded the phenylsemicarbazide **13a** (Scheme 5) which showed NH, CON and C=N absorptions as well as three exchangeable ^{1}H NMR proton signals (three NH). Heating **13a** above its melting point gave only one product to which the 10-methyl-3-oxo-1,2,4-triazolo[4′,3′:2,3]1,2,4-triazino[5,6-*b*]indole (**15a**) structure



Scheme 3.

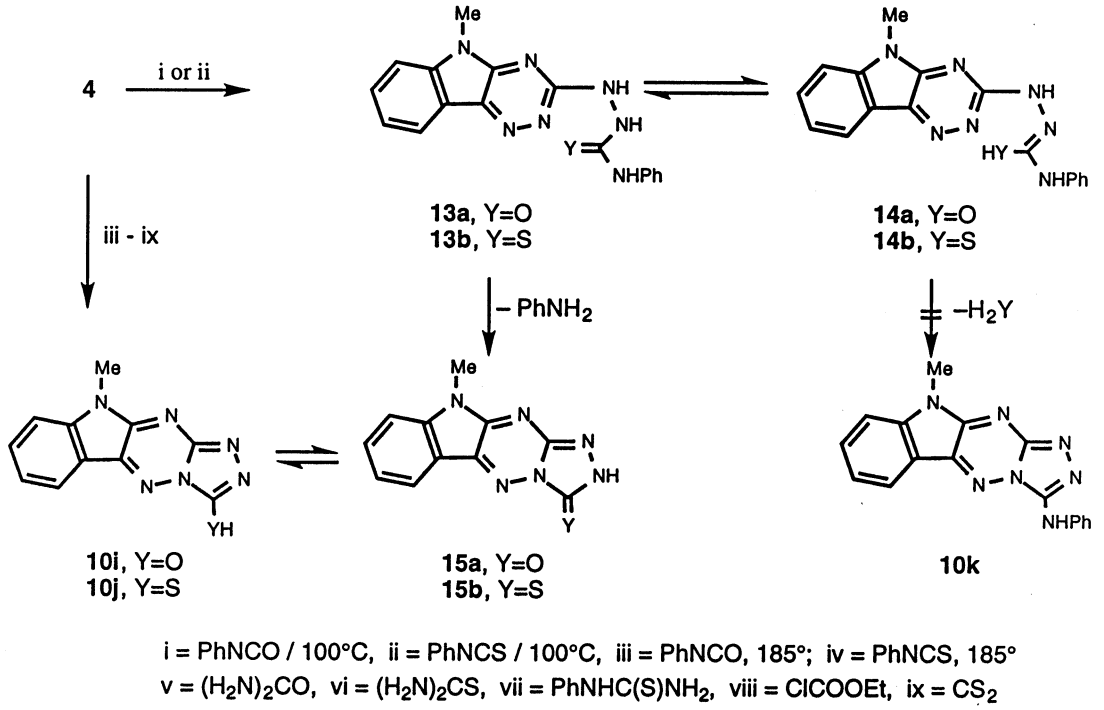


i = EtOH, heat , ii = 10% $FeCl_3$ / EtOH, iii = $SOCl_2$, iv = 30% NaOCl / H_2O, dioxane,
v = RCOCl, pyr. RT, vi = AcOH, heat

c, Ar = C_6H_5; d, Ar = 4–MeOC $_6H_4$; e, Ar = 4–ClC $_6H_4$; f, Ar = 4–BrC $_6H_4$, g, Ar= 4–Me $_2$NC$_6H_4$; h, Ar = 4–O $_2$NC$_6H_4$

Scheme 4.

was assigned. Formation of **15a** from **13a** took place throughout the elimination of an aniline molecule. Under this thermal cyclization condition, aniline appears to be a better leaving entity than water since the 3-phenylamino derivative **10k** has not been formed. Molecular models showed that, whereas the C3 carbonyl and N10 methyl groups in the linear structure **15a** are far apart, the C1 carbonyl and N10 methyl groups in the corresponding angular structure are in close proximity to each other and should suffer mild steric crowding. ^{1}H NMR spectra of **15a** showed an exchangeable one-proton broad signal at δ 5.30 ppm indicating that, in solution, this compound exhibits amide–imidic acid equilibrium (**15** $\rightleftharpoons$ **10**) [26]. Com-



Scheme 5.

pound **15a** was also obtained directly by heating **4** with phenyl isocyanate, urea or ethyl chloroformate (Scheme 5).

Reaction of **4** with phenyl isothiocyanate gave the phenylthiosemicarbazide **13b** which was thermally cyclized to the 10-methyl-3-thioxo-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-*b*]indole (**15b**). Compound **15b** was also obtained by reacting **4** with phenyl isothiocyanate, thiourea, phenylthiourea or carbon disulfide.

3. Experimental

3.1. Chemistry

Melting points were determined on MEL-TEMP II melting point apparatus in open glass capillaries and are uncorrected. The ultraviolet spectra (UV) were recorded on a Perkin–Elmer Lambda 4B UV–Vis spectrophotometer. The infrared spectra (IR) were recorded for potassium bromide discs on a Pye-Unicam SP1025 spectrophotometer. Magnetic resonance (^{1}H NMR) spectra were carried out at ambient temperature ($\sim$25°C) with a Varian EM-390 spectrometer using tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were performed at 70 eV on an analytical system consisting of a Du Pont 21-419 mass spectrometer interfaced with a Du Pont 492-094 data acquisition station or on a Hewlett–Packard 5995 gas chromatography–mass spectrometer system. Structure geometries were optimized using molecular mechanics (program CHEM 3D PLUS). Homogeneity of the products and follow up of the reactions were checked by ascending thin-layer chromatography (TLC) on plates precoated with silica gel G (E. Merck; layer thickness 0.25 mm), used without pretreatment. All ratios of the used solvent systems were volume to volume (v/v); the eluent path was 5 cm and the spots were visualized by exposure to iodine vapor for a few minutes. Elemental microanalyses were performed at the Microanalytical Unit, Cairo University, Cairo, Egypt, and the results for the indicated elements were within $\pm$0.4% of the theoretical values.

3.1.1. 3,10-Dimethyl-1,2,4-triazolo[4′,3′:2,3]1,2,4-triazino[5,6-b]indole (**10a**)

3.1.1.1. Method (A). A mixture of hydrazine **4** (1 g) and glacial acetic acid (20 ml) was heated at reflux for 3 h. The mixture was poured into water and the product which separated was filtered and crystallized from 1:1 chloroform–ethanol to give **10a** as yellow needles (65%); m.p. 338°C, Ref. [18] 332°C; TLC in 9:1 chloroform–methanol, R_f: 0.43; IR: 1606 cm^{-1} (C=N); λ_{max}^{MeOH} nm (log ε) 317 (4.02), 268 (4.77), 263 (sh); ^{1}H NMR ($CDCl_3$): δ 2.93, 3.86 (2s, 3H each, CH_3), 7.42 (m, 2H, ArH), 7.80 (t, 1H, ArH), 8.28 (d, 1H, ArH). *Anal.* Calc. for $C_{12}H_{10}N_6$ (238).

3.1.1.2. Method (B). A mixture of 1-methylisatin (**8**, 1 g), 3,4-diamino-5-methyl-1,2,4-triazole hydrochloride (**9a**, 0.9 g) and sodium acetate (0.19 g) in 25% aqueous ethanol (25 ml) was heated at reflux for 1 h. Acetic acid (0.2 ml) was added and heating was continued for an additional 2 h. The product which separated after attaining ambient temperature was filtered and crystallized from 1:1 ethanol–chloroform to give yellow needles of **9a** (62%); m.p. and mixed m.p.: 338°C. TLC, IR, UV and ^{1}H NMR of **10a** prepared by methods A and B were identical.

*3.1.2. 3-Formylhydrazino-5-methyl-1,2,4-triazino-[5,6-b]indole (**5b**)*

A mixture of **4** (1 g) and ethyl formate (20 ml) was heated at reflux for 3 h. The product which separated upon cooling, was filtered, washed with ether and crystallized from ethanol to give **5b** as pale yellow needles (65%); m.p.: 282°C; TLC in 9:1 chloroform–ethanol, R_f: 0.51; IR: 1585, 1608 (C=N), 1670 (CON), 3270 cm^{-1} (NH); ^{1}H NMR [$(CD_3)_2SO$]: δ 3.68 (s, 3H, CH_3), 7.40 (m, 3H, ArH), 8.15 (d, 1H, ArH), 8.25 (s, 1H, CHO), 9.70, 10.15 (2s, 1H each, exchangeable, 2NH). *Anal.* Calc. for $C_{11}H_{10}N_6O$ (242).

*3.1.3. 10-Methyl-1,2,4-triazolo[4′,3′:2,3]1,2,4-triazino-[5,6-b]indole (**10b**)*

3.1.3.1. Method (A). Compound **5b** (1 g) was heated at 285°C for 30 min and then cooled to ambient temperature. The obtained mass was crystallized from 1:1 ethanol–chloroform to give **10b** as yellow crystals (75%); m.p.: 321°C, Ref. [18] 309°C; TLC in 9:1 chloroform–methanol, R_f: 0.42; IR: 1590, 1620 cm^{-1} (C=N); λ_{max}^{MeOH} nm (log ε) 315 (4.28), 267 (5.03), 262 (sh); ^{1}H NMR [$(CD_3)_2SO$]: δ 3.75 (s, 3H, CH_3), 7.75 (m, 4H, ArH), 9.55 (s, 1H, triazole ring H). *Anal.* Calc. for $C_{11}H_8N_6$ (224).

3.1.3.2. Method (B). A mixture of 1-methylisatin (**8**, 1 g), 3,4-diamino-1,2,4-triazole hydrochloride (**9b**, 0.8 g) and sodium acetate (0.1 g) in 25% aqueous ethanol (25 ml) was heated at reflux for 1 h. Acetic acid (0.2 ml) was added and heating was continued for an additional 2 h. The product which separated after attaining ambient temperature was filtered and crystallized from 1:1 ethanol–chloroform to give **10b** (58%); m.p. and mixed m.p.: 321°C. TLC, IR, UV, and ^{1}H NMR of **10b** prepared by methods A and B were identical.

3.1.3.3. Method (C). A mixture of **4** (1 g) and triethyl orthoformate (20 ml) was heated at reflux for 10 h and then evaporated under reduced pressure. The obtained residue was triturated with water, filtered, washed with ether and crystallized from 1:1 ethanol–chloroform to give **10b** (85%).

3.1.3.4. Method (D). A mixture of **4** (1 g) and formic acid (20 ml, 99%) was heated at reflux for 10 h and then evaporated under reduced pressure. The obtained residue was crystallized from 1:1 ethanol–chloroform to give **10b** (82%).

3.1.3.5. Method (E). A mixture of **4** (1 g) and oxalic acid (0.6 g) was heated at 185°C for 30 min. The obtained mass was crystallized from 1:1 ethanol–chloroform to give **10b** (69%).

*3.1.4. 3-Benzoylhydrazino-5-methyl-1,2,4-triazino-[5,6-b]indole (**5c**)*

A mixture of **4** (1 g), pyridine (5 ml) and benzoyl chloride (0.68 g) was heated at reflux at 100°C for 1 h. To the reaction mixture, water (50 ml) was added and the product which separated was filtered, washed with water and crystallized from ethanol to give **5c** as pale yellow needles (44%), m.p.: 300°C; TLC in 9:1 chloroform–methanol, R_f: 0.52; IR:1601, 1631 (C=N), 1659 (CON), 3456 cm^{-1} (NH); ^{1}H NMR [$(CD_3)_2SO$]: δ 3.66, (s, 3H, CH_3), 8.15 (m, 9H, ArH), 11.10, 10.50 (2s, 1H each, exchangeable, 2NH). *Anal.* Calc. for $C_{17}H_{14}N_6O$ (318).

*3.1.5. General procedure for the preparation of 3-arylidenehydrazino-5-methyl-1,2,4-triazino[5,6-b]indoles (**12c–h**)*

A solution of **4** (2 mmol) in ethanol (30 ml) was treated with a solution of the appropriate aromatic aldehyde **11c–f** (2 mmol) in ethanol (20 ml) and the mixture was heated at 100°C for 10 min. The reaction mixture was kept at room temperature for 24 h and the product which separated was filtered, washed with ether and crystallized from ethanol. The following compounds were prepared.

*3.1.5.1. 3-Benzylidenehydrazino-5-methyl-1,2,4-triazino-[5,6-b] indole (**12c**).* Yield: 82%, yellow needles; m.p.: 304°C, Ref. [7] 286°C; TLC in 9:1 chloroform–methanol, R_f: 0.63, IR: 1605 cm^{-1} (C=N); ^{1}H NMR (CF_3COOD): δ 3.85 (s, 3H, CH_3), 7.80 (m, 10 H, ArH + CH=N). *Anal.* Calc. for $C_{17}H_{14}N_6$ (302).

*3.1.5.2. 3-(4-Methoxybenzylidenehydrazino)-5-methyl-1,2,4-triazino[5,6-b]indole (**12d**).* Yield: 63%; yellow needles, m.p.: 294°C, Ref. [7] 284°C; TLC in 9:1 chloroform–methanol, R_f: 0.62; IR: 1595, 1615 cm^{-1} (C=N); ^{1}H NMR (CF_3COOD): δ 3.85, 3.95 (2s, 3H each, 2 CH_3), 7.60 (m, 9H, ArH + CH=N). *Anal.* Calc. for $C_{18}H_{16}N_6O$ (332).

*3.1.5.3. 3-(4-Chlorobenzylidenehydrazino)-5-methyl-1,2,4-triazino[5,6-b]indole (**12e**).* Yield: 75%; yellow needles; m.p.: 316°C; TLC in 9:1 chloroform–methanol, R_f: 0.71; IR: 1595, 1610 cm^{-1} (C=N); ^1H NMR (CF_3COOD): δ 3.80 (s, 3H, CH_3), 7.80 (m, 9H, ArH + CH=N). *Anal.* Calc. for $C_{17}H_{13}N_6Cl$ (336.5).

*3.1.5.4. 3-(4-Bromobenzylidenehydrazino)-5-methyl-1,2,4-triazino[5,6-b]indole (**12f**).* Yield: 80%; yellow needles, m.p.: 318°C; TLC in 9:1 chloroform–methanol, R_f: 0.60; IR: 1600, 1625 cm^{-1} (C=N); ^1H NMR (CF_3COOD): δ 3.85 (2, 3H, CH_3), 7.90 (m, 9H, ArH + CH=N). *Anal.* Calc. for $C_{17}H_{13}N_6Br$ (381).

*3.1.5.5. 3-(4-N,N-Dimethylaminobenzylidenehydrazino)-5-methyl-1,2,4-triazino[5,6-b]indole (**12g**).* Yield: 71%; orange needles; m.p.: 243°C, Ref. [7] 296°C; TLC in 9:1 chloroform–methanol; R_f: 0.62; IR: 1557, 1606 cm^{-1} (C=N); ^1H NMR [$(CD_3)_2SO$]: δ 3.00 (s, 6H, $N(CH_3)_2$), 3.80 (s, 3H, CH_3), 7.50 (m, 9H, aromatic H + CH=N). *Anal.* Calc. for $C_{19}H_{19}N_7$ (345).

*3.1.5.6. 3-(4-Nitrobenzylidenehydrazino)-5-methyl-1,2,4-triazino[5,6-b]indole (**12h**).* Yield: 84%; orange needles; m.p.: 376°C, Ref. [7] 300°C; TLC in 9:1 chloroform–methanol, R_f: 0.58; IR: 1605, 1635 cm^{-1} (C=N); ^1H NMR (CF_3COOD): δ 3.95 (s, 3H, CH_3), 8.20 (m, 9H, ArH + CH=N). *Anal.* Calc. for $C_{17}H_{13}N_7O_2$ (347).

*3.1.6. General procedure for the preparation of 3-aryl-10-methyl-1,2,4-triazolo[4′,3′: 2,3]1,2,4-triazino-[5,6-b]indoles (**10c–h**)*

3.1.6.1. Method (A). A suspension of the particular 3-arylidenehydrazino-5-methyl-1,2,4-triazino[5,6-*b*]-indole (**12c–h**, 2 mmol) in dioxane (5 ml) was treated with 30% aqueous sodium hypochlorite solution (15 ml) and the mixture was heated at 100°C for 10 min. The product, obtained after attaining ambient temperature, was filtered, washed with water, dried and crystallized from ethanol.

3.1.6.2. Method (B). A suspension of the particular hydrazone (**12c–h**, 2 mmol) in toluene (30 ml) was treated with 10% ethanolic iron (III) chloride solution (20 ml) and heated at reflux for 3 h. The mixture was evaporated to dryness and the obtained residue was triturated with water, filtered, washed with water and crystallized from ethanol.

3.1.6.3. Method (C). A mixture of the appropriate hydrazone (**12c–h**, 2 mmol) and thionyl chloride (25 ml) was heated at reflux for 5 h. The mixture was evaporated and the resulting residue was crystallized from ethanol.

3.1.6.4. Method (D). A mixture of 3-benzoylhydrazino-5-methyl-1,2,4-triazino[5,6-*b*]indole (**5c**, 1 g) in glacial acetic acid (20 ml) was heated at 100°C for 3 h. The reaction mixture was evaporated and the resulting residue was crystallized from ethanol to give **10c** (52%) as orange needles, m.p.: 330°C, identical in every respect to **10c** prepared according to methods A, B and C. The following compounds were prepared according to methods A–C.

*3.1.6.5. 10-Methyl-3-phenyl-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indole (**10c**).* Yield: method (A), 65%; method (B); 43%; method (C), 50%; orange needles, m.p: 330°C, Ref. [18] 336; TLC in 9:1 chloroform-methanol, R_f: 0.22; IR: 1600 cm^{-1} (C=N); ^1H NMR (CF_3COOD): δ 4.05 (s, 3H, CH_3), 8.35 (m, 9H, ArH). *Anal.* Calc. for $C_{17}H_{12}N_6$ (300).

*3.1.6.6. 3-(4-Methoxyphenyl)-10-methyl-1,2,4-triazolo-[4′,3′:2,3]1,2,4-triazino[5,6-b]indole (**10d**).* Yield: method (A), 52%; method (B), 39%; method (C), 45%; orange needles, m.p.: 312°C; TLC in 9:1 chloroform–mehonol, R_f: 0.36; IR: 1607 cm^{-1} (C=N); ^1H NMR (CF_3COOD): δ 3.95, 4.05 (2s, 3H each, CH_3), 8.00 (m, 8H, ArH). *Anal.* Calc. for $C_{18}H_{14}N_6O$ (300).

*3.1.6.7. 3-(4-Chlorophenyl)-10-methyl-1,2,4-triazolo-[4′,3′:2,2]1,2,4-triazino[5,6-b]indole (**10e**).* Yield: method (A), 57%; method (B), 48%; method (C), 46%; orange needles, m.p.: 360°C Ref. [18] 348°C; TLC in 19:1 chloroform–methanol, R_f: 0.41; IR: 1601 cm^{-1} (C=N); ^1H NMR (CF_3COOD): δ 4.05 (s, 3H, CH_3), 8.10 (m 8H, ArH). *Anal.* Calc. for $C_{17}H_{11}N_6Cl$ (334.5).

*3.1.6.8. 3-(4-Bromophenyl)-10-methyl-1,2,4-triazolo-[4′,3′:2,3]1,2,4-triazino[5,6-b]indole (**10f**).* Yield: method (A), 62% method (B), 54%; method (C), 56%; orange needles, m.p.: 370°C; TLC in 19:1 chloroform, R_f: 0.40; IR: 1601 cm^{-1} (C=N); ^1H NMR (CF_3COOD): δ 3.80 (s, 3H, CH_3), 8.00 (m, 8H, ArH). *Anal.* Calc. for $C_{17}H_{11}N_6Br$ (379).

*3.1.6.9. 3-(4-N,N-Dimethylaminophenyl)-10-methyl-1,2,4-triazolo[4′,3′:2,3]1,2,4-triazino[5,6-b]indole (**10g**).* Yield: method (A), 55%; method (B), 41%; method (C), 42%; orange needles, m.p.: 315°C; TLC in 9:1 chloroform–methanol, R_f: 0.69; IR: 1606 cm^{-1} (C=N); ^1H NMR (CF_3COOD): δ 3.50 (s, 6H, $N(CH_3)_2$), 3.90 (s, 3H, CH_3), 8.00 (m, 8H, ArH). *Anal.* Calc. for $C_{19}H_{17}N_7$ (343).

*3.1.6.10. 10-Methyl-3-(4-nitrophenyl)-1,2,4-triazolo-[4′,3′:2,3]1,2,4-triazino[5,6-b]indole (**10h**).* Yield: method (A), 67%; method (B), 63%; method (C), 57%, orange needles; m.p.: 407°C; TLC in 19:1 chloroform–methanol, R_f: 0.35; IR: 1601 cm^{-1} (C=N); ^1H NMR

(CF_3COOD): δ 3.90 (s, 3H, CH_3), 8.10 (m, 8H, ArH). *Anal.* Calc. for $C_{17}H_{11}N_7O_2$ (345).

*3.1.7. 1-{5-Methyl-1,2,4-triazino[5,6-b]indol-3-yl}-4-phenylsemicarbzide (**13a**)*

A solution of **4** (1 g) in ethanol (25 ml) was treated with phenyl isocyanate (0.6 g) and the mixture was heated at reflux for 15 min. The reaction mixture was allowed to attain ambient temperature and the product which separated was filtered, washed with ether and crystallized from ethanol to give **13a** as pale yellow needles (52%); m.p.: 230°C; TLC in 19:1 chloroform–methanol, R_f: 0.53, IR: 1595 (C=N), 1670 (CON), 3222, 3293 cm^{-1} (NH); ^1H NMR [$(CD_3)_2SO$]: δ 3.66 (s, 3H, CH_3), 7.80 (m, 9H, ArH), 9.25 (broad, s, 3H, exchangeable NH). *Anal.* Calc. for $C_{17}H_{15}N_7O$ (333).

*3.1.8. 10-Methyl-3-oxo-1,2,4-triazolo[4′,3′:2,3]1,2,4-triazino[5,6-b]indole (**15a**)*

3.1.8.1. Method (A). Compound **13a** (1 g) was heated at 240°C for 1 h. The obtained mass was crystallized from ethanol–chloroform to give **15a** as red needles (57%); m.p.: 388°C; TLC in 9:1 chloroform–methanol, R_f: 0.44; IR: 1580, 1630 (C=N), 1750 (CON), 3400 cm^{-1} (NH); ^1H NMR [$(CD_3)_2SO$]: δ 3.50 (s, 3H, CH_3), 5.30 (broad s, 1H, OH), 7.60 (m, 4H, ArH). *Anal.* Calc. for $C_{11}H_8N_6O$ (240).

3.1.8.2. Method (B). A mixture of **4** (1 g) and phenyl isocyanate (0.6 g) was heated at 185°C for 1 h. The obtained mass was crystallized from ethanol–chloroform to give **15a** (48%).

3.1.8.3. Method (C). A mixture of **4** (1 g) and urea (0.4g) was heated at 185°C for 1 h. The resulting mass was crystallized from ethanol–chlorform to give **15a** (55%).

3.1.8.4. Method (D). A solution of **4** (1 g) in pyridine (3 ml) was treated with ethyl chloroformate (20 ml) and the mixture was heated at reflux for 3 h. The reaction mixture was poured onto crushed ice and the product which separated was filtered, washed with water and crystallized from ethanol–chloroform to give **15a** (63%).

*3.1.9. 1-{5-Methyl-1,2,4-triazino[5,6-b]indol-3-yl}-4-phenylthiosemicarbazide (**13b**)*

The title compound was prepared from **4** (1 g) and phenyl isothiocyanate (0.8 g) as described for the preparation of **13a**. It crystallized from ethanol as pale yellow needles (44%); m.p.: 185°C, Ref. [4] 199°C); TLC in 19:1 chloroform–methanol, R_f: 0.44; IR: 1247 (C=S), 1594 (C=N), 3209, 3367 cm^{-1} (NH); ^1H NMR [$(CD_3)_2SO$]: δ 3.73 (s, 3H, CH_3), 7.33 (m, 7H, ArH), 8.16 (d, 2H, ArH), 9.80 (broad s, 3H, exchangeable, NH). *Anal.* Calc. for $C_{17}H_{15}N_7S$ (349).

*3.1.10. 10-Methyl-3-thioxo-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indole (**15b**)*

3.1.10.1. Method (A). Compound **13b** (1 g) was heated at 185°C for 1 h. The obtained mass was crystallized from ethanol to give **15b** as red needles (47%); m.p.: 348°C; TLC in 9:1 chloroform–methanol, R_f: 0.54; IR: 1230 (C=S), 1600 (C=N), 3420 cm^{-1} (NH); ^1H NMR (CF_3COOD): δ 3.95 (s, 3H, CH_3), 7.66 (m, 2H, ArH), 7.95 (t, 1H, ArH), 8.35 (d, 1H, ArH). *Anal.* Calc. for $C_{11}H_8N_6S$ (256).

3.1.10.2. Method (B). A mixture of **4** (1 g) and phenyl isothiocyanate (0.8 g) was heated at 185°C for 1.5 h. The obtained mass was crystallized from ethanol to give **15b** (48%).

3.1.10.3. Method (C). Hydrazine **4** (1 g) and thiourea (0.4 g) were heated at 185°C for 1 h. The obtained mass was crystallized from ethanol to give **15b** (45%).

3.1.10.4. Method (D). Hydrazine **4** (1 g) and phenylthiourea (0.8g) were heated at 185°C for 1 h. The obtained mass was crystallized from ethanol to give **15b** (47%).

3.1.10.5. Method (E). A solution of **4** (1 g) in pyridine (3 ml) and carbon disulfide (20 ml) was heated at reflux for 3 h. The mixture was evaporated and the obtained residue was crystallized from ethanol to give **15b** (51%).

3.1.11. Attempted acid- or base-catalyzed Dimroth rearrangement

Solutions of compounds **10a**–**e** (1 g) in acetic acid or piperidine (20 ml) were heated at reflux for 2 h. The reaction mixture, in each case, was evaporated and the obtained residue was crystallized from chloroform–methanol. TLC, m.p., and mixed m.p. of each product were identical with those of the respective starting compounds.

3.2. Antimicrobial screening

Sterile nutrient agar plates (100 ml) were separately inoculated with a 24 h broth culture (1 ml) of *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. Solutions (60 ml) of the tested compounds (0.24 mg) in DMF (1 ml) were placed in wells (6 mm diameter) cut in the agar media and the plates were incubated at 37°C in the case of bacteria and at 25°C in the case of yeast. The diameters of the resulting inhibition zones were measured after 28 h for bacteria and after 96 h for yeast [27].

4. Results of antimicrobial screening

The 3-formylhydrazino- and 3-benzoylhydrazino-5-methyl-1,2,4-triazino[5,6-*b*]indoles (**5b** and **5c**), respectively, were found inactive against *E. coli* and *S. aureus* but were slightly active against *C. albicans* (Table 1).

We have previously screened the antibacterial and antifungal activity of some 3-(alditol-1-ylidene)hydrazino-5-methyl-1,2,4-triazolo[5,6-*b*]indoles (the monosaccharide hydrazone analogs of **12**) and 3-(alditol-1-yl)- 10 - methyl - 1,2,4 - triazolo[4′,3′:2,3]1,2,4 - triazino - [5,6,-*b*]-indoles (the acyclo *C*-nucleoside analogs of **10**) against *E. coli*, *S. aureus*, and *C. albicans* [11], and found these compounds inactive or marginally active [11]. Screening the two 3-arylidenehydrazino-5-methyl-1,2,4-triazino[5,6-*b*]indoles (**12g** and **12h**) showed that both were inactive against *E. coli* and *S. aureus* and weakly active against *C. albicans*. Whereas the 3,10-dimethyl - 1,2,4 - triazolo[4′,3′:2,3]1,2,4 - triazino[5,6 - *b*] - indole **10a** showed fair activity against *E. coli* and lacked activity against *S. aureus* and *C. albicans*, its 3-(4-chlorophenyl) congener (**10e**) was found active against *C. albicans* only (Table 1).

Omar et al. [4] reported that the 5-unsubstituted thiosemicarbazide congener of **13b** possessed potent antibacterial activity against *E. coli* and *S. aureus*, as well as potent antifungal activity against *C. albicans*. The 5-methyl semicarbazide analog **13a** lacked antibacterial activity against these microorganisms and only showed weak antifungal activity against *C. albicans* (Table 1).

10-Methyl-3-oxo-1,2,4-triazolo[4′,3′:2,3]1,2,4-triazino-[5,6-*b*]indole (**15a**) was devoid of activity toward both bacterial strains and weakly active toward *C. albicans*. The 3-thioxo congener (**15b**) showed activity against *S. aureus* only (Table 1).

Table 1
Results of antimicrobial activity

Comp.	Inhibition zones [a]			
	Concentration (mg/ml)	*E. coli*	*S. aureus*	*C. albicans*
5b	3/1			12.0
5c	3/1			13.0
10a	3/1	19.0		
10e	1/3			11.5
12g	3/2			10.0
12h	1/3			8.0
13a	3/1			9.0
15a	3/1			9.0
15b	3/1		11.0	
Ampicillin	3/1	21	17	
Streptomycin	3/1	30	22	

[a] Inhibition zones of less than 10 mm in diameter were considered to indicate weak activity.

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