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Reinvestigation of the regiospecific outcome of heterocyclization of 3-hydrazino-1,2,4-triazino[5,6-*b*]indoles: angular versus linear annelated 1,2,4-triazolo-1,2,4-triazino[5,6-*b*]indole structures

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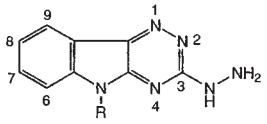
Divergences of the structural assignments reported in the literature for the products of cyclization of 3-hydrazino-1,2,4-triazino[5,6-*b*]indoles **1** with one-carbon cyclizing reagents have been reinvestigated. The linear annelated 1,2,4-triazolo[4,3-*b*]1,2,4-triazino[5,6-*b*]indoles **3** were found to be the products regiospecifically formed, rather than the angular annelated 1,2,4-triazolo[3,4-*c*]1,2,4-triazino[5,6-*b*]indoles **2**. Our findings were based on comparison of the UV spectral data of the cyclization products with those of unequivocally as well as inevitably linear annelated compounds. Calculations of optimized geometries and electronic structures accord with the results obtained. Divergences reported in the literature were, therefore, attributed to erroneous structural assignments rather than to cyclization by different regiochemical pathways.

1. Introduction

Reactions of 3-hydrazino-1,2,4-triazino[5,6-*b*]indoles **1** with one-carbon cyclizing reagents may involve the triazine ring N-4 or N-2. The products may, therefore, be assigned the angular annelated 1,2,4-triazolo[3,4-*c*]1,2,4-triazino-[5,6-*b*]indole (**2**) [1–10] or the linear annelated 1,2,4-triazolo[4,3-*b*]1,2,4-triazino[5,6-*b*]indole (**3**) [9, 10, 11–23] structures respectively. Formation of a mixture of both regioisomers (**2** and **3**) is hypothetically feasible, but in practice only one isomer is obtained [1–23]. In many cases no coherent proof has been given in support of the structure assignment [1–8, 14, 17, 19, 20]. Publications from our laboratory [22–24] rejected the angular annelated 1-(alditol-1-yl)-10-alkyl-1,2,4-triazolo[3,4-*c*]1,2,4-triazino[5,6-*b*]indole structure (**2**, R^1 = polyhydroxyalkyl chains) previously assigned [10] to 1,2,4-triazolo-1,2,4-triazino[5,6-*b*]indole acyclo *C*-nucleosides. The linear annelated 3-(alditol-1-yl)-10-alkyl-1,2,4-triazolo[4,3-*b*]1,2,4-triazino[5,6-*b*]indole structure (**3**, R^1 = polyhydroxyalkyl chains) was established on theoretical grounds as well as on the basis of experimental findings including X-ray crystallographic analysis [22–24]. Surveying the literature, we encountered many contradicting structural assignments for similarly obtained products. Thus, products resulting from the same cyclization reactions using the same reaction conditions were ascribed divergent structures (compare for example, Refs [2] with [14] and [16]; [2] with [18]; [4] with [20]; [9] and [10] with [22–24]). We undertook the work described in this article in an attempt to resolve such discrepancies. Settling structure divergences is important not only from an academic point of view but also from a pharmacological perspective. Subtle differences in structure such as those existing between isomers may fundamentally alter the type or magnitude of biological activities. The case of thalidomide is a well known example [25]. Resolving the differences in structural assignment of 1,2,4-triazolo-1,2,4-triazino[5,6-*b*]indoles acquires importance in view of their antibacterial [5] and antiviral [21–23, 26–29] activities.

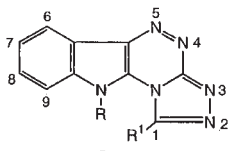
2. Investigations, results and discussion

Cyclization of hydrazine **1a** with formic acid has been described, giving a product which has been assigned the angular structure **2c** by one group [2] and the linear structure **3c** (Scheme 1) by two other independent authors [14, 16, 30]; no justification has been given in support of either of these assignments. In our work, this cyclization also gave a single product which melted with decomposition at 360–362 °C (Ref [14]: m.p. >300 °C). It showed four UV absorption maxima at 317, 268, 261.4 (sh), and 224.2 nm; the same absorptions as those reported [21] for 10-methyl-1,2,4-triazolo[4,3-*b*]1,2,4-triazino[5,6-*b*]indole **3e**, unequivocally prepared [11, 21] by cyclocondensation of 1-methyl isatin (**4**) [11] with 3,4-diamino-1,2,4-triazole (**5**) [31] (Scheme 1). It is worth mentioning that regioisomeric 1,2,4-triazolo-1,2,4-triazines have been reported to show different UV absorption patterns [32–34]. Thus, whereas 6,7-diphenyl-1,2,4-triazolo[4,3-*b*]1,2,4-triazine showed three absorptin maxima at 333, 243 (infl.), and 217 nm [32], the 6,7-diphenyl-1,2,4-triazolo[5,1-*c*]1,2,4-triazine regioisomer showed only two absorption maxima at 349 and 248 nm [33]. 3-Amino-6,7-diphenyl-1,2,4-triazolo[4,3-*b*]1,2,4-triazine also showed four maxima at 420, 335 (infl.), 284, and 243 nm [33] as distinct from its 3-amino-6,7-diphenyl-1,2,4-triazolo[5,1-*c*]1,2,4-triazine regioisomer which showed only two maxima at 349 and 248 nm [33]. It may be concluded, therefore, that the cyclization product obtained possesses the linear structure **3c**



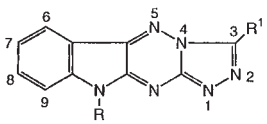
1

a, R = H
b, R = Me



2

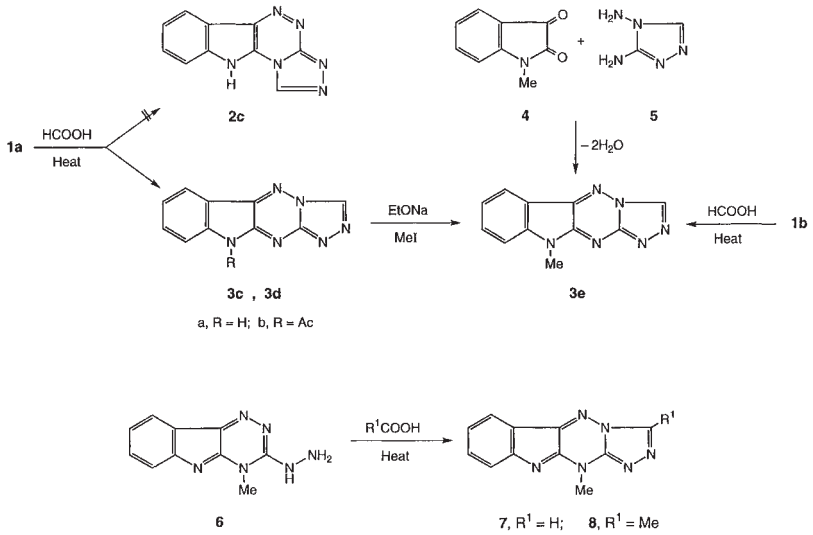
c, R = R^1 = H
f, R = H; R^1 = Me



3

d, R = Ac; R^1 = H
e, R = Me; R^1 = H
g, R = H; R^1 = Ac
h, R = R^1 = Me

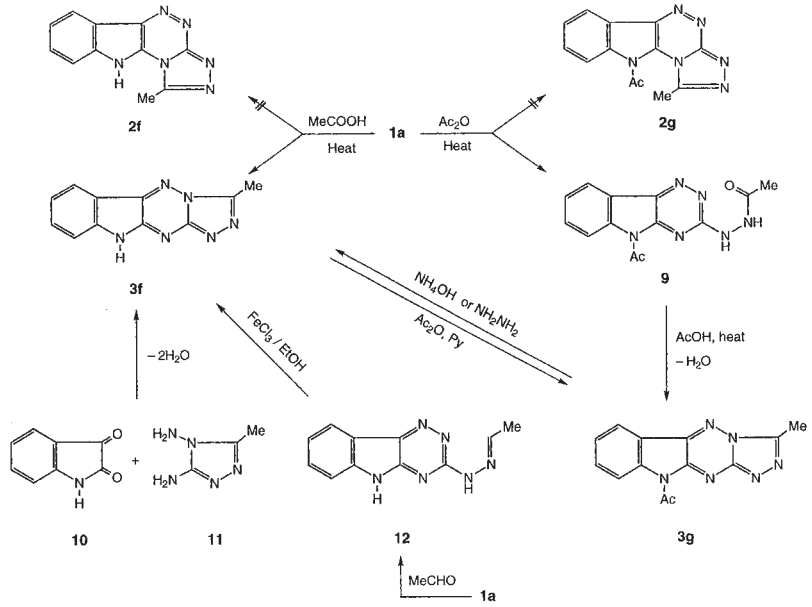
Scheme 1



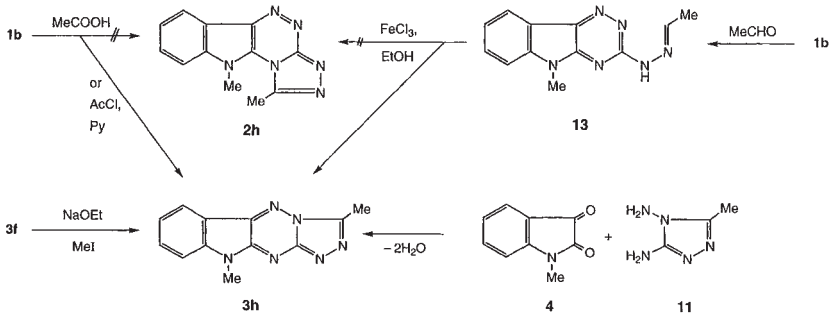
as described by Ram [14, 30] and Tomchin [16] rather than the angular structure **2c** as reported by Monge et al. [2]. Further confirmation of this result was obtained by: (a) methylation of the sodium salt of the cyclization product we obtained **(3c)** with methyl iodide gave a methylation product that is fully identical to **3e** (Scheme 1) and (b) the UV spectral pattern of the methylated product obtained **(3e)** is identical to that of the inevitably-linear 11-methyl-1,2,4-triazolo[4,3-*b*]1,2,4-triazino[5,6-*b*]indole (**7**). Compound **7** was obtained by cyclization of the hitherto unknown N-4-blocked 3-hydrazino-4-methyl-1,2,4-triazino[5,6-*b*]indole (**6**) [35] with formic acid (Scheme 1). Acetylation of **3c** with acetic anhydride/pyridine gave 10-acetyl-1,2,4-triazolo[4,3-*b*]1,2,4-triazino[5,6-*b*]indole **(3d)** (Scheme 1).

Ram [14, 30] and El Ashry et al. [18] reported that cyclization of the unsubstituted hydrazine **1a** by boiling with acetic acid yielded the linear annelated 3-methyl-1,2,4-triazolo[4,3-*b*]1,2,4-triazino[5,b-*b*]indole **(3f)** (Scheme 2). On the other hand, Abdel-Latif et al. [5] described cyclization of the same hydrazine by boiling with acetic ahydride which took place with concomittant acetylation of the pyrrole ring nitrogen to produce the angular annelated product 10-acetyl-1-methyl-1,2,4-triazolo[3,4-*c*]1,2,4-triazino[5,6-*b*]indole **(2g)** (Scheme 2). No rationalization has been given to the divergent regiochemical outcomes of these reactions. Heating **1a** with acetic acid, we obtained a product that melted with decomposition at 400–402 °C (Refs [14] and [18], m.p. >300 °C). The same product was also obtained by cyclodehydrogenation of the hydrazone **12**, derived from **1a** and acetaldehyde, with $FeCl_3$ (Scheme 2). The latter product showed UV absorptions at 315, 266.2, 262 (sh), and 216.2 nm similar to those which appeared in the spectrum of the linear regioisomer **3f** unequivocally prepared [11] by cyclocondensation of isatin **(10)** and 3,4-diamino-5-methyl-1,2,4-triazole **(11)** [31]

Scheme 2



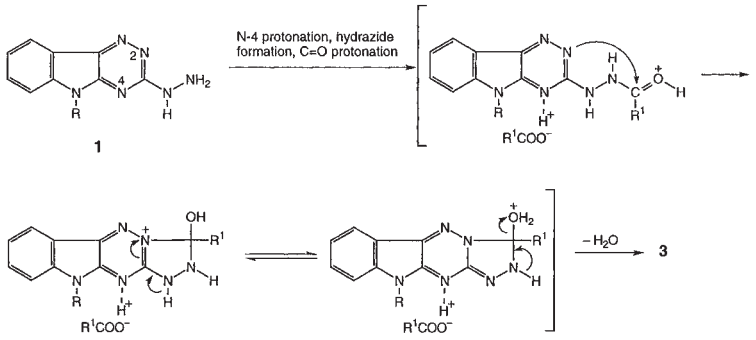
Scheme 3



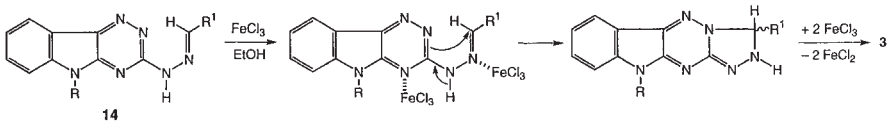
(Scheme 2). Attempted cyclization of **1a** with acetic anhydride according to Abdel-Latif et al. [5] to obtain **2g**, led only to the formation of 5-acetyl-3-acetylhydrazino-1,2,4-triazino [5,6-*b*]indole **(9)** which showed an NH and two CON absorptions at 3449, 1773, and 1710 cm^{-1} respectively (Scheme 2). Several other attempts comprising various modifications of the conditions of the latter reaction also gave **9**. Heating **9** with acetic acid caused its cyclization to a product that melted with decomposition at 360 °C (Ref [5]: m.p. of **2g** >300 °C). The latter product was also obtained by acetylation of the linearly annelated **3f** with acetic anhydride in pyridine and was, therefore, assigned the linear structure 10-acetyl-3-methyl-1,2,4-triazolo[4,3-*b*]1,2,4-triazino[5,6-*b*]indole (**3g**) (Scheme 2). The UV spectrum of **3g** prepared by the two routes showed the same UV absorptions at 317, 269.4, 263, 220 (sh), and 216.8 nm. N-deacetylation of **3g** with hydrazine hydrate or with methanolic ammonium hydroxide at ambient temperature gave **3f** (Scheme 2). Obviously, these results are in harmony with the linear structures **3f** and **3g** assigned by Ram [14, 30] and El Ashry et al. [18], but in contradiction with the angular structures **2f** and **2g** assigned by Abdel-Latif et al. [5].

The above-mentioned results and those described in the literature [14, 18] show that cyclocondensation of unsubstituted hydrazine **1a** with acetic acid or cyclodehydrogenation of aldehyde hydrazones **12** derived from it with $FeCl_3$ resulted in the same regiochemical outcome: the linear 1,2,4-triazolo-1,2,4-triazino[5,6-*b*]indoles **3**. Surprisingly, however, similar cyclizations performed with the N-5-substituted hydrazine **1b** and aldehyde hydrazones derived from it have been described as yielding triazolotriazino-indoles with divergent regiochemical outcomes [10]. Thus, **1b** was reported to cyclize with acetic acid to give the linear product **3h** [10] (Scheme 3). On the other hand, cyclization with $FeCl_3$ of the hydrazone **13,** derived from **1b**, and acetaldehyde was reported to yield the angular regioisomer **2h** [10] (Scheme 3). No plausible justification has been offered to explain such regiochemical divergence. Reinvestigating the latter reactions, we found: (a) reaction of **1b** with boiling acetic acid or with acetyl chloride/pyridine gave a product which melted with decomposition at 338 °C (Ref. [21], m.p. 338 °C; Ref. [10], m.p. 330 °C) (Scheme 3); (b) cyclization of hydrazone **13**, derived from **1b** and acetaldehyde, with ethanolic $FeCl_3$ gave a product that melted with decomposition at 338 °C (Ref. [10], m.p. 234–235 °C); (c) compounds prepared according to (a) and (b) were indistinguishable from those obtained by methylation of **3f** with methyl iodide/sodium ethoxide or from the linear regioisomer **3h** unequivocally prepared [10, 11, 21, 22] by cyclocondensation of 1-methylisatin (**4**) [11] and 3,4-diamino-5-methyl-1,2,4-triazole **(11)** [31] (Scheme 3). The UV spectra of **3h** prepared by the four methods were identical; they showed absorptions at 318, 273, 262 (sh), and 216 nm, and (d) cyclization of the hithertho unknown N-4-blocked 3-hydrazino-4-methyl-1,2,4-triazino[5,6-*b*]indole (**6**) [35] with boiling acetic acid gave the inevitably-linear annelated 3,11-dimethyl-1,2,4-triazolo[4,3-*b*]1,2,4-triazino[5,6-*b*]indole **(8)** (Scheme 1). Compound **8** showed UV absorptions at 320, 269.6, 262 (sh), and 213.2 nm; the same absorption pattern as that of **3h**. These results show that cyclization of 5-methylhydrazine **(1b)** with acetic acid or of the acetaldehyde hydrazone **13** derived from it led to products having the same regiochemical outcomes namely the linear annelated triazolotriazinoindoles (**3**), in contradiction to previous results [10]. It follows that the divergences described in the literature regarding the regiochemical outcome of the cyclization reactions of hydrazines **1** and their aldehyde hydrazones are due to erroneous structural assignments rather than to cyclization by different regiochemical pathways.

Scheme 4



Scheme 5



We have shown [21–23] that molecular models and computer optimized geometries predict that the cyclization of N-5-substituted 3-hydrazino-1,2,4-triazino[5,6-*b*]indoles (e.g. **1b**) and their aldehyde hydrazones is sterically controlled to produce preferentially the linearly annelated cyclic products **3**; the corresponding angular products **2** suffer crowding of the C-1 and N-10 substituents. N-5-unsubstituted hydrazine **(1a)** and its hydrazones are predicted to cyclize to the angular (**2**) and/or linear (**3**) regioisomers, both of which are free of adverse steric interactions. The present study shows, however, that **1a** also cyclizes regiospecifically to the linear triazolotriazinoindoles **3c**, and **3f** (Schemes 1 and 2). A reasonable explanation should, therefore, be given to justify this finding. Asaad et al. [36] studied computationally the optimized geometries and electronic structures of 1,2,4-triazine and condensed heterocyclo[*e*]1,2,4-triazines and found N-4 of the triazine ring to possess the highest electronic charge. Accordingly, we postulate that during cyclization of **1a** with carboxylic acids (Scheme 4) or the corresponding hydrazones **(14)** with $FeCl_3$ (Scheme 5), the electron-deficient entity (H^+ or $FeCl_3$) of the cyclizing agent will coordinate preferentially to the hard nucleophilic center N-4. As a consequence, N-2 will be the only available nucleophilic cyclization center leading to the regiospecific formation of the linear products **3**. This justification may also be extended to include the cyclization of N-5-substituted hydrazines (e.g **1b**) and their hydrazones, operating synergistically with the steric control factor to ensure the regiospecific production of **3**.

3. Experimental

Melting points were determined on a MEL-TEMP II melting point apparatus in open glass capillaries and are uncorrected. The UV spectra were recorded on a Shimadzu UV-160A UV/VIS spectrophotometer. The IR spectra were recorded for KBr discs on a Pye-Unicam SP1025 spectrophotometer. ^{1}H NMR spectra were carried out at ambient temperature (~25 °C) with a Varian EM-390 or a Brucker AC-250 spectrometer using TMS as an internal standard. Homogeneity of the products and follow up of the reactions were checked by TLC on precoated silica gel G plates (E. Merck; layer thickness 0.25 mm). Solvent systems: V/V; the distance of solvent travel was 5 cm and the spots were visualized by iodine vapour. Elemental microanalyses: Microanalytical Unit, Cairo University, Cairo, Egypt. The prepared compounds gave satisfactory elemental analyses to (+/−) 0.3%.

3.1. 10H-1,2,4-Triazolo[4,3-b]1,2,4-triazino[5,6-b]indole (3c)

A mixture of hydrazine **1a** (5 mmol) and formic acid (20 ml) was heated under reflux for 3 h. The product which separated upon cooling, was filtered, washed with water, and crystallized from DMF to give **3c** as yellow crystals (78%); m.p.: 360–362 °C/dec., lit. [14], m.p.: >300 °C; TLC in 9: $CHCl_3$/MeOH, R_f: 0.51; IR: 3146 (NH) and 1611 cm^{-1} (C=N); λ_{max}^{EtOH} nm (log ε): 317 (3.53), 268 (4.24), 261.4 (sh), and 224.2 (3.80).

3.2. 10-Acetyl-1,2,4-triazolo[4,3-b]1,2,4-triazino[5,6-b]indole (3d)

Compound **3c** (5 mmol), pyridine (5 ml) and acetic anhydride (20 ml) were stirred at room temperature for 18 h. The product was filtered, washed with water, and crystallized from DMF to give **3d** as yellow crystals (81%); m.p.: 293–295 °C/dec.; TLC in 9 : 1 $CHCl_3$/MeOH, R_f: 0.49; IR: 1725 (CON) and 1603 cm^{-1} (C=N); λ_{max}^{EtOH} nm log ε): 316 (4.74), 268.8 (6.23), 261.8 (sh), and 225.6 (6.3); ^{1}H NMR ($CDCl_3$): δ 9.16 (s, 1 H, triazolyl H), 8.69 (d, H-6, $J_{6,7}$ 8.7 Hz), 8.20 (d, H-9, $J_{9,8}$ 7.8 Hz), 7.79 (t, H-8, $J_{8,9}$ 8.1 Hz), 7.54 (t, H-7, $J_{7,8}$ 7.5 Hz), and 3.15 (s, 3 H, CH_3).
$C_{12}H_8N_6O$ (252).

3.3. 10-Methyl-1,2,4-triazolo[4,3-b]1,2,4-triazino[5,6-b]indole (3e)

3.3.1. Method A

A mixture of 1-methylisatin (**10**, 6 mmol), 3,4-diamino-1,2,4-triazole (**11**, 6 mmol), and sodium acetate (6 mmol) in 25% aqueous ethanol (25 ml) was heated under reflux for 1 h. Acetic acid (0.2 ml) was added and heating was continued for 2 h. The product which separated after attaining room temperature was crystallized from $CHCl_3$-EtOH (1 : 1) to give **3e** as orange crystals (58%); m.p.: 321 °C, lit. [11], m.p.: 309 °C; TLC in 9 : 1 $CHCl_3$/MeOH, R_f: 0.42; IR: 1608 cm^{-1} (C=N); λ_{max}^{EtOH} nm (log ε): 315 (3.95), 266.2 (4.65), 262 (sh), and 216.2 (4.33); ^{1}H NMR [$(CD_3)_2SO$]: δ 9.55 (s, 1 H, triazolyl H), 7.75 (m, 4 H, ArH), and 3.75 (s, 3 H, CH3).
$C_{11}H_8N_6$ (224)

3.3.2. Method B

Compound **3c** (5 mmol) was treated with a solution of sodium (4 mmol) in absolute ethanol (20 ml) and the mixture was stirred at room temperature for 30 min. The product was filtered and washed with ether to give the sodium salt of compound **3c** (4 mmol). A mixture of the sodium salt (4 mmol) and methyl iodide (0.4 ml) was heated at 100 °C in a closed tube for 20 min. The mass obtained was crystallized from $CHCl_3$-EtOH to give **3e** as orange crystals (62%); m.p. and mixed m.p.: 321 °C/dec. lit. [21], m.p.: 321 °C. TLC, IR, and UV are identical to those of **3e** prepared by method A.

3.3.3. Method C

A mixture of hydrazine **1b** (5 mmol) and formic acid (20 ml) was heated under reflux for 10 h and then evaporated to dryness. The residue obtained was crystallized from $CHCl_3$-EtOH to give **3e** (82%), m.p. and mixed m.p.: 321 °C/dec. TLC, IR and UV of **3e** prepared by methods A and B are identical.

3.4. 3-Methyl-10H-1,2,4-triazolo[4,3-b]1,2,4-triazino[5,6-b]indole (3f)

3.4.1. Method A

A mixture of hydrazine **1a** (5 mmol) and acetic acid (20 ml) was heated under reflux for 4 h. The product which separated after attaining room temperature was washed with water and crystallized from DMF to give **3f** as dark yellow crystals (84%); m.p.: 400–402 °C/dec., lit. [18], m.p.: >300 °C; TLC in 9 : 1 $CHCl_3$/MeOH, Rf: 0.41; IR: 3443 (NH) and 1614 cm^{-1} (C=N); λ_{max}^{EtOH} nm (log ε): 315 (4.61), 268.6 (4.30), 262 (sh), and 215.8 (4.20).
$C_{11}H_8N_6$ (224)

3.4.2. Method B

A suspension of hydrazone **12** (5 mmol) in ethanol (50 ml) was treated with 10% ethanolic iron (III) chloride solution (20 ml) and heated under reflux for 30 min. The mixture was evaporated to dryness and the residue obtained was triturated with water, filtered, washed with water, and crystallized from DMF to give **3f** (62%); m.p. and mixed m.p.: 400–402 °C/dec., lit. [18], m.p.: >300 °C. TLC, IR, and UV are identical to those of **3f** prepared by method A.

3.4.3. Method C

A mixture of isatin (**10**, 6 mmol), 3,4-diamino-5-methyl-1,2,4-triazole (**11**, 6 mmol), and sodium acetate (6 mmol) in 25% aqueous ethanol (25 ml), was heated under reflux for 1 h. Acetic acid (1 ml) was added to the mixture and heating was continued for 2 h. The product which separated after attaining room temperature was washed with ethanol, and crystallized from DMF to give **3f** (65%); m.p.: 400–402 °C/dec., lit. [18], m.p.: >300 °C. TLC, IR, and UV are identical to those of **3f** prepared by methods A and B.

3.4.4. Method D

A solution of **3g** (5 mmol) in hydrazine hydrate (10 ml) was stirred at room temperature for 3 h. The product which separated after the addition of ethanol (30 ml), was crystallized from DMF to give **3f** (62%); m.p.: 400–402 °C/dec. TLC, IR, and UV are identical to those of **3f** prepared by methods A–C.

3.4.5. Method E

A mixture of **3g** (5 mmol), methanol (20 ml), and ammonium hydroxide (30%, 20 ml), was kept at room temperature for 48 h and then evaporated to dryness. The resulting residue was crystallized from DMF to give **3f** (59%); m.p. and mixed m.p.: 400–402 °C/dec. TLC, IR, and UV spectra of **3f** prepared by methods A–E are identical.

3.5. 10-Acetyl-3-methyl-1,2,4-triazolo[4,3-b]1,2,4-triazino[5,6-b]indole (**3g**)

3.5.1. Method A

A mixture of compound **9** (5 mmol) and acetic acid (20 ml) was heated under reflux for 3 h and then evaporated under reduced pressure. The residue obtained was crystallized from $CHCl_3$-EtOH to give **3g** as yellow crystals (78%); m.p.: 360 °C/dec., lit [5], m.p.: >300 °C, [18], m.p.: >335 °C; TLC in 9 : 1 $CHCl_3$/MeOH, Rf: 0.45; IR: 1716 (CON) and 1604 cm^{-1} (C=N); λ_{max}^{EtOH} nm (log ε): 317 (4.87), 269.4 (5.33), 263 (sh), and 216.8 (5.48); ^1H NMR (CDCl3): δ 8.69 (d, H-6, $J_{6,7}$ 8.1 Hz), 8.21, 7.77, 7.54 (3 m, 1 H each, ArH), 3.15 (s, 3 H, NCH_3), and 2.91 (s, 3 H, CH_3).
$C_{13}H_{10}N_6O$ (266)

3.5.2. Method B

A mixture of compound **3f** (5 mmol), pyridine (5 ml), and acetic anhydride (20 ml) was stirred at room temperature for 18 h. The product was filtered, washed with water and crystallized from $CHCl_3$-EtOH to give **3g** (85%); m.p. and mixed m.p.: 360 °C/dec. TLC, IR, and UV of **3g** prepared by methods A and B are identical.

3.6. 3,10-Dimethyl-1,2,4-triazolo[4,3-b]1,2,4-triazino[5,6-b]indole (**3h**)

3.6.1. Method A

A mixture of hydrazine **1b** (5 mmol) and acetic acid (20 ml) was heated under reflux for 3 h. The mixture was poured into water and the product was filtered and crystallized from $CHCl_3$-EtOH to give **3h** as yellow crystals (65%); m.p.: 338 °C/dec, lit. [10], m.p.: 330 °C, [21], m.p.: 338 °C; TLC in 9 : 1 $CHCl_3$/MeOH, Rf: 0.43; IR: 1604 cm^{-1} (C=N); λ_{max}^{EtOH} nm (log ε): 317 (3.55), 272.8 (4.32), 263 (sh), and 216 (4.08); ^1H NMR ($CDCl_3$): δ 8.28 (d, H-6, $J_{6,7}$ 8.7 Hz), 7.80 (t, H-8, $J_{8,9}$ 8.5 Hz), 7.42 (m, 2 H, ArH), and 3.86, 2.93 (2s, 3 H each, CH_3).
$C_{12}H_{10}N_6$ (238)

3.6.2. Method B

A mixture of hydrazine **1b** (5 mmol) and pyridine (5 ml), was treated with acetyl chloride (5 ml) and heated at 100 °C for 3 h. The mixture was cooled to room temperature, poured into a cold saturated solution of sodium bicarbonate and the product which separated was washed with water and crystallized from $CHCl_3$-EtOH to give **3h** (61%); m.p.: 338 °C/dec. TLC, IR, and UV are identical to those of **3h** prepared by method A.

3.6.3. Method C

The title compound was prepared from a solution of hydrazone **13** (5 mmol) in ethanol (50 ml) and 10% ethanolic $FeCl_3$ solution (20 ml) as described for the preparation of **3f**. It crystallized from $CHCl_3$-EtOH to give **3h** (65%); m.p.: 338 °C/dec., lit. [10], m.p.: 234–235 °C. TLC, IR, and UV are identical to those of **3h** prepared by method A and B.

3.6.4. Method D

Compound **3f** (5 mmol) was treated with a solution of sodium (4 mmol) in absolute ethanol (20 ml), and the mixture was stirred at room temperature for 30 min. The product which separated was filtered and washed with ether to give the sodium salt of compound **3f** (4 mmol). A mixture of this sodium salt (4 mmol) and methyl iodide (0.4 ml) in a closed tube was heated at 100 °C for 20 min. The mass obtained was crystallized from $CHCl_3$-EtOH to give **3h** (82); m.p. and mixed m.p.: 338 °C/dec. TLC, IR, and UV are identical to those of **3h** prepared according to methods A–C.

3.6.5. Method E

A mixture of 1-methylisatin (**10**, 6 mmol), 3,4-diamino-1,2,4-triazole (**11**, 6 mmol) and sodium acetate (6 mmol) in 25% aqueous ethanol (25 ml) was heated under reflux for 1 h. Acetic acid (0.2 ml) was added and heating was continued for 2 h. The product which separated after attaining ambient temperature was crystallized from $CHCl_3$-EtOH to give **3h** (63%); m.p.: 338 °C/dec., lit. [10], m.p.: 330 °C, [11], m.p.: 332 °C, [21, 22], m.p.: 338 °C. TLC, IR, and UV are identical to those of **3h** prepared by methods A–D.

3.7. 11-Methyl-1,2,4-triazolo[4,3-b]1,2,4-triazino[5,6-b]indole (**7**)

A mixture of 3-hydrazino-4-methyl-1,2,4-triazino[5,6-*b*]indole (**6**, 5 mmol) [35] and formic acid (20 ml) was heated under reflux for 10 h and then evaporated to dryness. The residue was crystallized from EtOH to give **7** as yellowish brown crystals (61%); m.p.: 320–325 °C/dec; TLC in 9 : 1 $CHCl_3$/MeOH, Rf: 0.44; IR: 1612 cm^{-1} (C=N); λ_{max}^{EtOH} nm (log ε): 320 (3.69), 268.4 (4.29), 261.2 (sh), and 224 (3.93).
$C_{11}H_8N_6$ (224)

3.8. 3,11-Dimethyl-1,2,4-triazolo[4,3-b]1,2,4-triazino[5,6-b]indole (**8**)

A mixture of hydrazine **6** (5 mmol) and acetic acid (20 ml) was heated under reflux for 4 h and then evaporated to dryness. The residue was crystallized from EtOH to give **8** as yellowish brown crystals (65%); m.p.: 420–424 °C/dec; TLC in 9 : 1 $CHCl_3$/MeOH, Rf: 0.47; IR: 1614 cm^{-1} (C=N); λ_{max}^{EtOH} nm (log ε): 313 (3.76), 269.5 (4.51), 262 (sh), and 213.2 (4.30).
$C_{12}H_{10}N_6$ (238)

3.9. 5-Acetyl-3-acetylhydrazino-1,2,4-triazino[5,6-b]indole (**9**)

3.9.1. Method A

A mixture of hydrazine **1a** (5 mmol), pyridine (5 ml), and acetic anhydride (10 ml) was stirred at room temperature for 18 h, poured into ice water, and the product which separated was crystallized from $CHCl_3$-EtOH to give **9** as pale yellow crystals (80%); m.p.: 175 °C; TLC in 9 : 1 $CHCl_3$/MeOH, Rf: 0.42; IR: 3249 (NH), 1733, 1710 (CON), 1607 cm^{-1} (C=N); λ_{max}^{EtOH} nm (log ε): 326 (3.97), 268.4 (4.35), and 225.8 (4.37); ^1H NMR ($CDCl_3$): δ 8.68 (d, H-6, $J_{6,7}$ 8.4 Hz), 8.42 (d, H-9, $J_{9,8}$ 7.8 Hz), 7.76 (t, H-8, $J_{8,9}$ 7.2 Hz), 7.63 (t, H-7, $J_{7,8}$ 7.8 Hz)), and 2.96, 2.80 (2s, 3 H each, CH_3).
$C_{13}H_{12}N_6O_2$ (284)

3.9.2. Method B

A mixture of hydrazine **1a** (5 mmol) and acetic anhydride (20 ml) was heated under reflux for 3 h. The mixture was poured into ice water and the product was filtered and crystallized from $CHCl_3$-EtOH to give **9** (82%); m.p. and mixed m.p.: 175 °C. TLC, IR and UV spectra of **9** prepared by methods A and B were identical.

3.10. 3-Ethylidenehydrazino-5H-1,2,4-triazino[5,6-b]indole (**12**)

A solution of acetaldehyde (0.3 ml) in absolute ethanol (5 ml), was treated with 2 drops of acetic acid. This mixture was added to a solution of hydrazine **1a** (5 mmol) in DMF (30 ml) in a closed tube and the mixture was heated at 100 °C for 2 h. The product obtained after attaining ambient temperature was crystallized from DMF to give **12** as orange crystals (81%); m.p.: 268–270 °C/dec., lit. [18], m.p.: 260–262 °C; TLC in 9 : 1 $CHCl_3$/MeOH, Rf: 0.47; IR: 3214 cm^{-1} (NH), and 1613 (C=N); λ_{max}^{EtOH} nm (log ε): 365.5 (4.75), 277 (5.29), and 224 (4.92).
$C_{11}H_{10}N_6$ (226)

3.11. 3-Ethylidenahydrazino-5-methyl-1,2,4-triazino[5,6-b]indole (**13**)

The title compound was prepared from a solution of **1b** (5 mmol) in absolute ethanol (30 ml) by treatment with acetaldehyde (0.3 ml) as described for the preparation of **12**. It crystallized from EtOH to give **13** as yellow crystals (78%); m.p.: 245 °C, lit. [10], m.p.: 304–305 °C; TLC in 19 : 1 $CHCl_3$/MeOH, Rf: 0.56; IR: 3216 (NH) and 1604 cm^{-1} (C=N); λ_{max}^{EtOH} nm (log ε): 364 (4.75), 276.6 (5.28), and 223.4 (5.04).
$C_{12}H_{12}N_6$ (240)

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