Synthesis of 2-Amino-*s*-triazino[1,2-*a*]benzimidazoles as Potential Antifolates from 2-Guanidino- and 2-Guanidino-5-methylbenzimidazoles

Anton V. Dolzhenko and Wai-Keung Chui*

Department of Pharmacy, Faculty of Science, National University of Singapore, 18 Science Drive 4, Singapore 117543, Singapore E-mail: phacwk@nus.edu.sg

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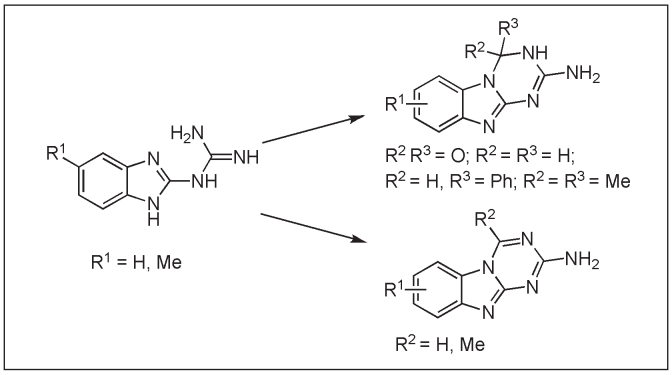

The syntheses of 2-amino-*s*-triazino[1,2-*a*]benzimidazoles from 2-guanidinobenzimidazoles were successfully carried out by a ring annelation reaction. The regiochemistry of the ring closure of 5-methyl-2-guanidinobenzimidazole with diethyl azodicarboxylate, aldehydes, acetone, diethyl ethoxymethylenemalonate and orthoesters, leading to the formation of *s*-triazine ring was studied. High regioselectivity was not observed in any of these reactions. However, the synthesis of *s*-triazino[1,2-*a*]benzimidazole system was found to be more regioselective than its 3,4-dihydro analogue. NOESY experiment indicated that the compound, 2-amino-4,4-dimethyl-3,4-dihydro-*s*-triazino[1,2-*a*]benzimidazole existed predominantly as the 3,4-dihydro tautomer in dimethyl sulfoxide. It was found to inhibit bovine dihydrofolate reductase with IC_{50} 10.9 μM.

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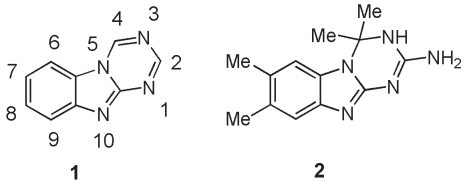
Our laboratory has been working on the *s*-triazine class of dihydrofolate reductase (DHFR) inhibitors in the search for potential antibacterial and anticancer agents [1]. It is therefore of interest to us to explore the synthesis of fused *s*-triazines in an attempt to discover new heterocyclic structures that may inhibit DHFR. It has been noted from some recent reports [2,3] that compounds having the core structure of *s*-triazino[1,2-*a*]benzimidazole (**1**), with particular reference to 2-amino-4,4,7,8-tetramethyl-3,4-dihydro-*s*-triazino[1,2-*a*]benzimidazole (**2**), have demonstrated inhibitory activity against the plasmodial DHFR.



1 **2**

Figure 1

The synthesis of the 2-amino-*s*-triazino[1,2-*a*]benzimidazoles has been achieved through the ring closure of 2-guanidinobenzimidazoles [4-13]. To date, only a limited number of reports [8,9,12] have discussed the ring closure of unsymmetrical 2-guanidinobenzimidazoles, with respect to 5-methyl-2-guanidinobenzimidazole (**5b**). However, the regiochemistry of the reactions has not yet been investigated thoroughly and the existing reports appear to be incomplete and controversial. For example, 2-amino-7(8)-methyl-*s*-triazino[1,2-*a*]benzimidazoles and 2-amino-7-methyl-4-oxo-4,10-dihydro-*s*-triazino[1,2-*a*]benzimidazole have been shown to be the products when **5b** was allowed to react with triethyl orthoformate [8,9] or diethyl azodicarboxylate [12], respectively. In the first case [8,9] the analysis of the mixture of isomers has not been described fully; while in the second case [12], no evidence to support the participation of N-3 instead of N-1 atom of **5b** in the annulation step has been demonstrated.

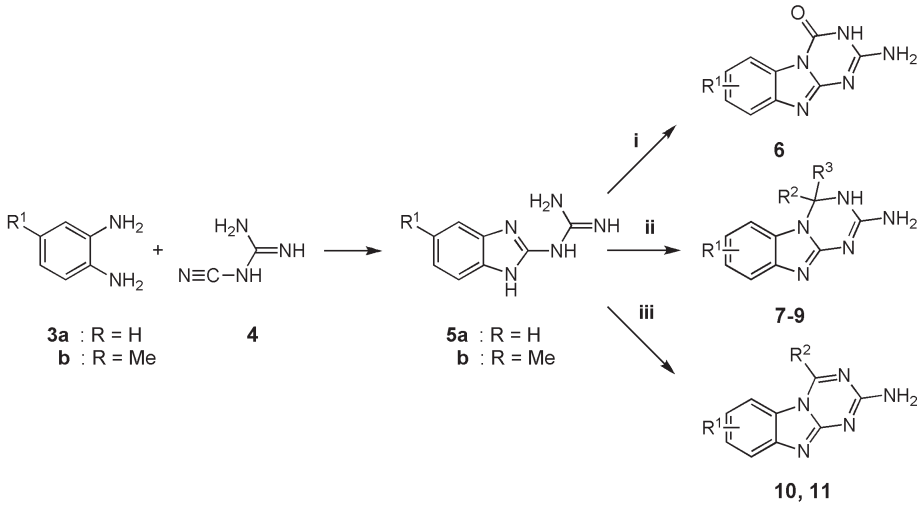
Therefore, this study is designed with the objective to synthesize and investigate the nature of the ring closure step during the formation of a series of 2-amino-*s*-triazino[1,2-*a*]benzimidazoles. In addition, the inhibitory activity of the series of analogues against the bovine DHFR will be evaluated. This study will report for the first time, a simple and effective method for the preparation of compounds related to fused structure **2** and having two geminal methyl groups at position 4. At the same time the regiochemistry of the ring closure of **5b**, with the formation of the fused s-triazine ring will also be discussed.

Guanidinobenzimidazoles (**5a**,**b**) were prepared from a reaction between an appropriate *o*-phenylenediamine (**3**) with cyanoguanidine (**4**) as described in previous general method [14]. The synthesized 2-guanidinobenzimidazoles (**5**) were then cyclized using different reagents to give 2-amino-*s*-triazino[1,2-*a*]benzimidazoles with different substitutions at position 4 (Scheme 1). The structures of the synthesized 2-amino-[1,3,5]triazino[1,2-*a*]benzimidazoles (**6**-**11**) were elucidated with the help of ir and nmr spectral and elemental analyses. The ratios of the isomeric products (**6**-**11b**,**c**) prepared from **5b** were determined, without separation of mixtures, using nmr spectroscopic techniques. The assignment of ^{1}H and ^{13}C nmr signals for the 7(8)-methyl substituted compounds (**6**-**11b**,**c**) in the mixtures was made based on the data for analogous compounds (**6**-**11a**) prepared from the unsubstituted 2-guanidinobenzimidazole (**5a**) with the help of COSY, DEPT90 and DEPT135 experiments.

Table 1
2-Amino-*s*-triazino[1,2-*a*]benzimidazoles

Compound	R^1	R^2	R^3	Ratio of regioisomers (**b** / **c**), %
6a	H	-	-	-
6b,c	7(8)-Me	-	-	55 / 45
7a	H	H	H	-
7b,c	7(8)-Me	H	H	50 / 50
8a	H	H	Ph	-
8b,c	7(8)-Me	H	Ph	55 / 45 (method A), 50 / 50 (methods B and C)
9a	H	Me	Me	-
9b,c	7(8)-Me	Me	Me	54 / 46
10a	H	H	-	-
10b,c	7(8)-Me	H	-	20 / 80 (method A), 17 / 83 (method B)
11a	H	Me	-	-
11b,c	7(8)-Me	Me	-	65 / 35

Scheme 1



Reagents and reaction conditions: R^1 = H, Me (**i**) diethyl azodicarboxylate, EtOH, reflux, 3 h; (**ii**) $R^2 = R^3$ = H, 37% HCHO, dioxane, reflux, 2 h; R^2 = H, R^3 = Ph, PhCHO, KOH (method A), piperidine (method B) or *N*-methylpiperazine (method C), reflux, 3-5 h; $R^2 = R^3$ = Me, acetone, piperidine, reflux, 7 h; (**iii**) R^2 = H, $EtOCH{=}C(COOEt)_2$, MeCN, reflux, 5 h (method A) or $HC(OMe)_3$, DMF, reflux, 3 h (method B); R^2 = Me, $MeC(OEt)_3$, DMF, reflux, 18 h.

Contrary to the previously reported information [12], it was found that the ring closure of **5b** with diethyl azodicarboxylate did not proceed regioselectively and afforded a mixture of 2-amino-7(8)-methyl-4-oxo-3,4-dihydro-*s*-triazino[1,2-*a*]benzimidazoles (**6b**,**c**) that could be clearly observed from the ^{1}H nmr spectrum. Regioisomers (**6b**,**c**) were formed in almost equal proportion (Table 1).

The presence of the carbonyl group at C4 in the structure was supported with a significant stretching absorption signal at 1717 cm^{-1} ($\nu_{C=O}$) in the ir spectra of compound (**6a**).The anisotropic effect of the oxygen atom of this carbonyl group led to the downfield shift of the signal of H-6 to 7.92-8.09 ppm in the ^{1}H nmr spectra of the compounds (**6**). These findings confirmed the *s*-triazine ring formation and are in agreement with literature data [15] for related fused heterocyclic systems with 4-oxo-*s*-triazine ring.

Reactions of **5** with aldehydes afforded 2-amino-3,4-dihydro-*s*-triazino[1,2-*a*]benzimidazoles (**7**-**8**). Refluxing of 2-guanidinobenzimidazoles (**5**) in acetone under piperidine catalysis led to the formation of 2-amino-3,4-dihydro-*s*-triazino[1,2-*a*]benzimidazoles (**9**) with two geminal methyl groups. Cycloadditions of these carbonyl reagents to **5b** did not show regioselectivity and gave the isomeric compounds **7**-**9b** and **7**-**9c** in equal or almost equal proportion (Table 1). The methyl group of **5b** did not have an influence on the ring closure taking place at N-1 and N-3.

The nature of catalyst used did not have a significant impact on the ratio of isomeric forms. Thus, it was observed that with the use of potassium hydroxide as a catalyst, the reaction resulted in the formation of 55% of **8b** and 45% of **8c**; while reactions catalyzed separately by piperidine and *N*-methylpiperazine afforded a mixture of isomers (**8b**,**c**) of 50% each.

The formation of the dihydro-*s*-triazine ring with a sp^3 hybridized C-4 was confirmed by the signals of C-4 in separate ^{13}C nmr spectra, namely the signals at 53.0, 65.7-65.8 and 69.3-69.4 ppm for secondary, tertiary and quaternary carbon atom of compounds (**7**-**9**), respectively. The signals of the other two sp^2 carbon atoms of the formed *s*-triazine ring (C-2 and C-10a), surrounded by three nitrogen atoms each, were located at 155.2-156.5 and 153.0-153.7 ppm, respectively. The ^{13}C nmr spectral data were in good agreement with the work carried out on the formation of the spiro- analogue, as described in another report (**7**, $R^2R^3 = C_5H_{10}$ [8]).

Evidence indicating that the dihydro-*s*-triazino[1,2-*a*]-benzimidazoles (**6**-**9**) could exist in 3,4-dihydro-, 1,4-dihydro- and 4,10-dihydro- tautomeric forms was observed (Figure 2). The prototropic interconversion between these tautomeric forms led to the broadening of the signals of C-2, C-4, C-10a atoms in the ^{13}C nmr spectra that was observed for the compounds **6-9**. In a NOESY experiment conducted on compound **9**, strong cross-peaks were observed for the signal at 1.82 ppm and the signals of 6-H as well as the N-H protons. The close spatial relationship of the geminal methyl groups and proton at the annular nitrogen atom corresponded to the 3,4-dihydrotautomeric form. In the condition of the experiment, no cross-peak was found for N-H and 9-H that indicated the predominance of 3,4-dihydro- tautomeric form in the dimethyl sulfoxide solution.

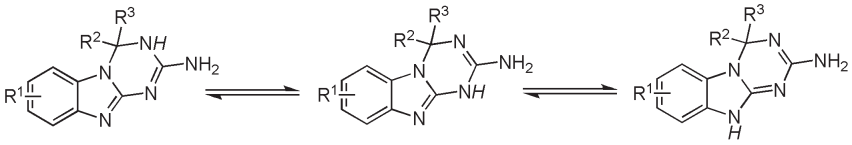


Figure 2. Annular tautomerism in 2-amino-dihydro-*s*-triazino[1,2-*a*]benzimidazoles.

The completely conjugated 2-amino-*s*-triazino[1,2-*a*]-benzimidazoles (**10-11**) was prepared from the reaction of **5** with diethyl ethoxymethylenemalonate or orthoesters. These types of annulations when applied to unsymmetrical **5b** seemed to be more regioselective than the other reactions described above. Thus, reaction of **5b** with diethyl ethoxymethylenemalonate or trimethyl orthoformate led to the formation of a mixture of 7(8)-methyl-2-amino-*s*-triazino[1,2-*a*]benzimidazoles (**10b**,**c**). The 8-methyl substituted isomeric form **10c** was predominant (80-83%) in the mixture with 17-20% of the minor compound **10b**. Interestingly, almost an inverse ratio (65% and 35%) of 4-methyl substituted analogues (**11b**,**c**) was observed in the result of the reaction when triethyl orthoacetate was used for cycloaddition instead.

It should be noted that several signals in the ^{13}C nmr spectrum of **10a** have not been correctly assigned in a previous report [13]. For example, the signal of methine carbone C(4)H could not be located at 163 ppm as was indicated in [13]. A signal was not found in this region in DEPT90 experiment, at the same time the C-4 signal was observed at 148.8 ppm. The corrected assignments for **10a** are presented below in experimental section.

DHFR inhibitory activity of the individual compounds **6-10a** was evaluated using bovine DHFR (Fluka Chemie) according to a previously described method [1]. The compounds for the DHFR inhibition bioassays were dissolved in dimethyl sulfoxide. In order to ensure that the solvent *per se* did not have an effect on the enzymatic activity, negative control test was performed using dimethyl sulfoxide at the same concentration. 2-Amino-4-methyl-*s*-triazino[1,2-*a*]benzimidazole (**11a**) was not tested because of its very poor solubility in the bioassay medium. The most active compound in the series was 2-amino-4,4-dimethyl-3,4-dihydro-*s*-triazino[1,2-*a*]benzimidazole (**9a**). This compound was found to inhibit DHFR with IC_{50} value 10.9 μM. All the other compounds in the series possessed IC_{50} values more than 100 μM and were therefore considered as inactive.

EXPERIMENTAL

Melting points (uncorrected) were determined on a Gallenkamp melting point apparatus. The infrared spectra were performed on a Jasco FT-IR-430 spectrophotometer in potassium bromide pellets. ^1H and ^{13}C nmr spectra were recorded on a Bruker DPX-300 spectrometer in the dimethyl sulfoxide-d_6 solution using TMS as an internal reference. The samples prepared in the reaction of 2-guanidino-5-methylbenzimidazole (**5b**) described below were analyzed by nmr spectroscopy without recrystallization in order to avoid changes in the isomers ratio. The satisfied purity of the samples was achieved by careful washing with ethanol (acetone) that confirmed by nmr spectral data and elemental analyses.

General Procedure for Preparation of 2-Benzimidazolylguanidines (**5**).

The mixture of the appropriate *o*-phenylenediamine **3** (0.1 mol), cyanoguanidine **4** (8.4 g, 0.1 mol) and conc. hydrochloric acid (20 mL, 0.2 mol) in 200 mL of water was heated under reflux for 1 h, cooled to 0° and 50 mL of 10% solution of potassium hydroxide was added slowly. Precipitated 2-guanidinobenzimidazole **5** was filtered, washed with water, dried and used in subsequent steps of the reactions without further purification.

2-Guanidinobenzimidazole (**5a**).

Compound **5a** was obtained in 78% yield; mp 243-244 °, (Lit. [14] mp 245 °).

2-Guanidino-5-methylbenzimidazole (**5b**).

Compound **5b** was obtained in 82% yield; mp 220-221 °; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 2.32 (s, 3H, Me), 6.72 (dd, 1H, 6-H, J = 7.9, 0.8 Hz), 6.7 (br s, 4H, NH-C(=NH)NH_2, deuterium oxide-exchangeable), 6.96 (s, 1H, 4-H), 7.03 (d, 1H, 7-H, J = 7.9 Hz), 10.84 (br s, 1H, N1(3)-H, deuterium oxide-exchangeable).

General Procedure for Preparation of 2-Amino-4-oxo-3,4-dihydro-*s*-triazino[1,2-*a*]benzimidazoles (**6**).

To a solution of the appropriate 2-guanidinobenzimidazole **5** (0.01 mol) in ethanol (15 mL), diethyl azodicarboxylate (1.74 g, 0.01 mol) was added dropwise with stirring and the mixture was heated under reflux for 3 h. After cooling, the product was filtered, washed with ethanol and dried.

2-Amino-4-oxo-3,4-dihydro-*s*-triazino[1,2-*a*]benzimidazole (**6a**).

Compound **6a** was obtained in 76% yield; mp >360° (DMF) (Lit. mp >360° [5,8,10], mp > 300° [11]); ir (potassium bromide): NH 3415, NH 3219, NH 3123, 2997, 2886, 2800, C=O 1717, NH_2 1616, 1601, 1526, 1457, 1405, 774, 736 cm^{-1}; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 7.19 (br s, 1H, NH_2, deuterium oxide-exchangeable), 7.25 (td, 1H, 7-H, J = 7.7, 1.1 Hz), 7.34 (td, 1H, 8-H, J = 7.7, 1.5 Hz), 7.42 (d, 1H, 9-H, J = 7.2 Hz), 8.09 (d, 1H, 6-H, J = 7.5 Hz), 12.20 (br s, 1H, NH, deuterium oxide-exchangeable); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 112.6 (C-6), 113.9 (C-9), 121.8 (C-7), 124.7 (C-8), 126.6 (C-5a), 133.1 (C-9a), 150.3 (C-10a), 153.7 (CO), 162.9 (C-2).

Anal. Calcd. for $C_9H_7N_5O$: C, 53.73; H, 3.51; N, 34.81. Found: C, 53.68; H, 3.60; N, 34.72.

2-Amino-7(8)-methyl-4-oxo-3,4-dihydro-*s*-triazino[1,2-*a*]benzimidazoles (**6b**,**c**)

The mixture of the regioisomers **6b**,**c** was obtained in 58% yield; mp >360 °; ^{1}H nmr (dimethyl sulfoxide-d_6): δ **6b**: 2.42 (s, 3H, C(7)Me), 7.08 (br s, 2H, NH_2, deuterium oxide-exchangeable), 7.15 (d, 1H, 8-H, J = 8.3 Hz), 7.28 (d, 1H, 9-H, J = 8.3 Hz), 7.92 (s, 1H, 6-H); **6c**: 2.41 (s, 3H, C(8)Me), 7.07 (d, 1H, 7-H, J = 8.3 Hz), 7.08 (br s, 2H, NH_2, deuterium oxide-exchangeable), 7.20 (s, 1H, 9-H), 7.93 (d, 1H, 6-H, J = 8.3 Hz).

Anal. Calcd. for $C_{10}H_9N_5O$: C, 55.81; H, 4.22; N, 32.54. Found: C, 55.84; H, 4.27; N, 32.15.

General Procedure for Preparation of 2-Amino-3,4-dihydro-*s*-triazino[1,2-*a*]benzimidazoles (**7a**-**c**).

A solution of the appropriate 2-guanidinobenzimidazole **5** (0.01 mol) and 1.00 mL (0.01 mol) 37% formaldehyde in dioxane (20 mL) was heated under reflux for 2 h. After cooling, the product was filtered, washed with ethanol and dried.

2-Amino-3,4-dihydro-*s*-triazino[1,2-*a*]benzimidazole (**7a**).

Compound **7a** was obtained in 68% yield; mp 284-285° (DMF), (Lit. mp 304-305° [8], mp 307-308° [9]); ir (potassium bromide): NH 3323, NH 3144, CH (aromatic) 3058, CH (aliphatic) 2898, C=N 1649, NH_2 1620, 1588, 1526, 1467, 1439, 1375, 1279, 1244, 758, 740 cm^{-1}; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 5.40 (s, 2H, CH_2), 6.83 (s, 2H, NH_2, deuterium oxide-exchangeable), 6.97 (td, 1H, 7-H, J = 7.3, 1.3 Hz), 7.02 (td, 1H, 8-H, J = 7.3, 1.3 Hz), 7.17 (dd, 1H, 9-H, J = 7.3, 1.1 Hz), 7.29 (dd, 1H, 6-H, J = 7.3, 1.1 Hz), 7.89 (br s, 1H, NH, deuterium oxide-exchangeable); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 53.0 (C-4), 107.3 (C-6), 115.5 (C-9), 119.0 (C-8), 120.7 (C-7), 131.5 (C-5a), 142.7 (C-9a), 153.7 (C-10a), 156.5 (C-2).

Anal. Calcd. for $C_9H_9N_5$: C, 57.74; H, 4.85; N, 37.41. Found: C, 57.62; H, 4.92; N, 37.24.

2-Amino-7(8)-methyl-3,4-dihydro-*s*-triazino[1,2-*a*]benzimidazoles (**7b**,**c**).

The mixture of the regioisomers **7b**,**c** was obtained in 70% yield; mp 253-254°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ **7b**: 2.35 (s, 3H, Me), 5.30 (s, 2H, CH_2), 6.40 (s, 2H, NH_2, deuterium oxide-exchangeable), 6.81 (d, 1H, 8-H, J = 7.9 Hz), 7.00 (d, 1H, 9-H, J = 7.9 Hz), 7.06 (s, 1H, 6-H), 7.36 (br s, 1H, NH, deuterium oxide-exchangeable); **7c**: 2.33 (s, 3H, Me), 5.30 (s, 2H, CH_2), 6.38 (s, 2H, NH_2, deuterium oxide-exchangeable), 6.77 (d, 1H, 7-H, J = 7.9 Hz), 6.94 (s, 1H, 9-H), 7.13 (d, 1H, 6-H, J = 7.9 Hz), 7.36 (br s, 1H, NH, deuterium oxide-exchangeable).

Anal. Calcd. for $C_{10}H_{11}N_5$: C, 59.69; H, 5.51; N, 34.80. Found: C, 59.34; H, 5.29; N, 34.45.

General Procedure for Preparation of 2-Amino-4-phenyl-3,4-dihydro-*s*-triazino[1,2-*a*]benzimidazoles (**8**).

A solution of the appropriate 2-guanidinobenzimidazole **5** (0.01 mol), 1.00 mL (0.01 mol) benzaldehyde and 0.4 g KOH (*method A*) or 0.50 mL piperidine (*method B*) or 0.50 ml N-methylpiperazine (*method C*) in ethanol (20 mL) was heated under reflux for 5 h (*A*) or 3 h (*B and C*). After cooling, the product was filtered, washed with ethanol and dried.

2-Amino-4-phenyl-3,4-dihydro-*s*-triazino[1,2-*a*]benzimidazole (**8a**).

Compound **8a** was obtained in 85% yield (*method B*); mp 292-293° (DMF/ethanol), (Lit. [4,8] mp 294-295°); ir (potassium bromide): NH 3407, NH 3321, NH 3224, CH (aromatic) 3052, C=N 1660, NH_2 1613, 1590, 1571, 1459, 1423, 1400, 1279, 1246, 740, 699 cm^{-1};^{1}H nmr (dimethyl sulfoxide-d_6): δ 6.58 (s, 2H, NH_2, deuterium oxide-exchangeable), 6.75 (dd, 1H, 9-H, J = 7.2, 1.5 Hz), 6.77 (s, 1H, H-4), 6.79 (td, 1H, 8-H, J = 7.3, 1.1 Hz), 6.93 (td, 1H, 7-H, J = 7.3, 1.5 Hz), 7.22 (d, 1H, 6-H, J = 7.5 Hz), 7.33-7.42 (m, 5H, phenyl protons), 8.20 (s, 1H, NH, deuterium oxide-exchangeable); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 65.8(C-4), 108.1 (C-6), 115.8 (C-9), 118.8 (C-8), 120.7 (C-7), 126.2 (C-2' and -6'), 128.8 (C-3' and -5'), 129.1 (C-4'), 131.2 (C-5a), 140.4 (C-1'), 143.2 (C-9a), 153.4 (C-10a), 155.2 (C-2).

Anal. Calcd. for $C_{15}H_{13}N_5$: C, 68.42; H, 4.98; N, 26.60. Found: C, 68.26; H, 5.14; N, 26.42.

2-Amino-7(8)-methyl-4-phenyl-3,4-dihydro-*s*-triazino[1,2-*a*]-benzimidazoles (**8b**,**c**).

The mixture of the regioisomers **8b**,**c** was obtained in 76% (*method A*), 85% (*method B*) and 81% (*method C*) yields; mp 284-286°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ **8b**: 2.26 (s, 3H, Me), 6.36 (s, 2H, NH_2, deuterium oxide-exchangeable), 6.56-6.62 (m, 2H, 8- and 9-H), 7.02 (s, 1H, 6-H), 7.30-7.42 (m, 5H, phenyl protons), 7.98 (s, 1H, NH, deuterium oxide-exchangeable); **8c**: 2.20 (s, 3H, Me), 6.35 (s, 2H, NH_2, deuterium oxide-exchangeable), 6.60 (s, 1H, 9-H), 6.72 (s, 1H, 4-H), 6.75 (d, 1H, J = 7.9 Hz, 7-H), 7.11 (d, 1H, J = 7.9 Hz, 6-H), 7.30-7.42 (m, 5H, phenyl protons), 7.95 (s, 1H, NH, deuterium oxide-exchangeable); (**8b**+**8c**) ^{13}C nmr (dimethyl sulfoxide-d_6): δ 21.1 (Me), 21.2 (Me), 65.7 (C-4), 65.8 (C-4), 107.8 (C-6 **8c**), 108.4 (C-6 **8b**), 115.4 (C-9 **8b**), 116.0 (C-9 **8c**), 119.9 (C-8 **8b**), 121.9 (C-7 **8c**), 126.1 (C-2'), 126.2 (C-2' and -6'), 127.8 (C-8 **8c**), 128.8 (C-3' and -5'), 129.1 (C-7 **8b**), 129.5 (C-5a **8c**), 131.3 (C-5a **8b**), 140.4 (C-1'), 140.6 (C-1'), 140.9 (C-9a **8b**), 143.3 (C-9a **8c**), 153.0 (C-10a **8c**), 153.5 (C-10a **8b**), 155.2 (C-2), 155.3 (C-2).

Anal. Calcd for $C_{16}H_{15}N_5$: C, 69.29; H, 5.45; N, 25.25. Found: C, 68.93; H, 5.39; N, 24.90.

General Procedure for Preparation of 2-Amino-4,4-dimethyl-3,4-dihydro-*s*-triazino[1,2-*a*]benzimidazoles (**9**).

A solution of the appropriate 2-guanidinobenzimidazole **5** (0.01 mol) and 0.50 mL piperidine in acetone (20 mL) was heated under reflux for 7 h. After cooling, the product was filtered, washed with acetone and dried.

2-Amino-4,4-dimethyl-3,4-dihydro-*s*-triazino[1,2-*a*]benzimidazole (**9a**).

Compound **9a** was obtained in 84% yield; mp 295-296°; ir (potassium bromide): NH 3285, NH 3132, CH (aliphatic) 2980, CH (aliphatic) 2929, C=N 1659, NH_2 1616, 1584, 1539, 1456, 1406, 1387, 1284, 1250, 762, 747, 549 cm^{-1}; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 1.82 (s, 6H, Me_2), 6.93 (s, 2H, NH_2, deuterium oxide-exchangeable), 6.95 (t, 1H, 7-H, J = 7.9 Hz), 7.02 (t, 1H, 8-H, J = 7.5 Hz), 7.29 (d, 1H, 9-H, J = 7.5Hz), 7.39 (d, 1H, 6-H, J = 7.9 Hz), 8.17 (br s, 1H, NH, deuterium oxide-exchangeable); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 28.5 (Me_2), 69.4 (C-4), 109.7 (C-6), 115.8 (C-9), 119.0 (C-8), 120.9 (C-7), 130.5 (C-5a), 143.3 (C-9a), 153.5 (C-10a), 155.3 (C-2).

Anal. Calcd. for $C_{11}H_{13}N_5$: C, 61.38; H, 6.09; N, 32.54. Found: C, 61.34; H, 6.36; N, 32.20.

2-Amino-4,4,7(8)-trimethyl-3,4-dihydro-*s*-triazino[1,2-*a*]benzimidazoles (**9b**,**c**).

The mixture of the regioisomers **9b**,**c** was obtained in 72% yield; mp 284-286°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ **9b** 1.84 (s, 6H, C(4)Me_2), 2.39 (s, 3H, C(7)Me), 6.86 (d, 1H, 8-H, J = 7.9 Hz), 7.13 (s, 2H, NH_2, deuterium oxide-exchangeable), 7.18 (d, 1H, 9-H, J = 7.9 Hz), 7.24 (s, 1H, 6-H), 8.32 (br s, 1H, NH, deuterium oxide-exchangeable); **9c** 1.82 (s, 6H, C(4)Me_2), 2.34 (s, 3H, C(8)Me), 6.78 (d, 1H, 7-H, J = 7.9 Hz), 7.10 (s, 1H, 9-H), 7.13 (s, 2H, NH_2, deuterium oxide-exchangeable), 7.26 (d, 1H, 6-H, J = 8.3 Hz), 8.32 (br s, 1H, NH, deuterium oxide-exchangeable); ^{13}C nmr (dimethyl sulfoxide-d_6): δ **9b** 21.1 (Me), 28.5 (Me_2), 69.3 (C-4), 110.1 (C-6), 115.4 (C-9), 120.1 (C-8), 128.5 (C-7), 130.7 (C-5a), 141.1 (C-9a), 153.2 (C-10a), 155.2* (C-2); **9c** 21.3 (Me), 28.6 (Me_2), 69.3 (C-4), 109.3 (C-6), 116.0 (C-9), 121.7 (C-7), 128.1 (C-8), 129.5 (C-5a), 143.5 (C-9a), 153.7 (C-10a), 155.3* (C-2). * - assignments may be reversed.

Anal. Calcd. for $C_{12}H_{15}N_5$: C, 62.86; H, 6.59; N, 30.54. Found: C, 62.73; H, 6.80; N, 30.24.

General Procedures for Preparation of 2-Amino-*s*-triazino[1,2-*a*]benzimidazoles (**10**).

Method A. A solution of the appropriate 2-guanidinobenzimidazole **5** (0.005 mol) and 1.00 ml (0.005 mol) diethyl ethoxymethylenemalonate in acetonitrile (20 mL) was heated under reflux for 5 h. After cooling, the product was filtered, washed with ethanol and dried.

Method B. A solution of the appropriate 2-guanidinobenzimidazole **5** (0.005 mol) and 0.55 ml (0.005 mol) trimethyl orthoformate in DMF (15 mL) was heated under reflux for 3 h. After cooling, the product was filtered, washed with ethanol and dried.

2-Amino-*s*-triazino[1,2-*a*]benzimidazole (**10a**).

Compound **10a** was obtained in 95% yield (*method A*); mp 301° (DMF) (Lit. mp 305-306° [6], mp 323-324° [8,9], mp 300-301° [13]); ir (potassium bromide): NH 3304, NH 3157, CH (aromatic) 3046, CH (aromatic) 3015, C=N 1685, NH_2 1632, 1603, 1480, 1450, 1343, 1306, 1275, 1243, 1184, 1091, 779, 758, 741 cm^{-1}; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 7.21 (td, 1H, 7-H, J = 7.4, 1.1 Hz), 7.36 (td, 1H, 8-H, J = 7.7, 1.1 Hz), 7.53 (d, 1H, 9-H, J = 7.9 Hz), 7.69 (br s, 2H, NH_2, deuterium oxide-exchangeable), 8.01 (d, 1H, 6-H, J = 7.9 Hz), 9.60 (s, 1H, 4-H); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 110.9 (C-6), 117.4 (C-9), 120.0 (C-7), 125.5 (C-8), 126.4 (C-5a), 144.1 (C-9a), 148.8 (C-4), 152.0 (C-10a), 161.1 (C-2).

Anal. Calcd. for $C_9H_7N_5$: C, 58.37; H, 3.81; N, 37.82. Found: C, 58.56; H, 3.72; N, 37.90.

2-Amino-7(8)-methyl-*s*-triazino[1,2-*a*]benzimidazoles (**10b**,**c**)

The mixture of the regioisomers **10b**,**c** was obtained in 95% (*method A*) and 86% (*method B*) yields; mp 306-308 °; ^{1}H nmr (dimethyl sulfoxide-d_6): δ **10b**: 2.44 (s, 3H, Me), 7.18 (d, 1H, 8-H, J = 8.3 Hz), 7.40 (d, 1H, 9-H, J = 8.3 Hz), 7.62 (s, 2H, NH_2, deuterium oxide-exchangeable), 7.82 (s, 1H, 6-H), 9.53 (s, 1H, 4-H); **10c**: 2.42 (s, 3H, Me), 7.02 (d, 1H, 7-H, J = 7.5 Hz), 7.32 (s, 1H, 9-H), 7.62 (s, 2H, NH_2, deuterium oxide-exchangeable), 7.87 (d, 1H, 6-H, J = 8.3 Hz), 9.52 (s, 1H, 4-H);^{13}C nmr (dimethyl sulfoxide-d_6): δ **10b**: 21.1 (Me), 110.8 (C-6), 117.0 (C-9), 126.4 (C-5a), 126.6 (C-8), 129.3 (C-7), 142.0 (C-9a), 148.5 (C-4), 151.6 (C-10a), 160.9 (C-2); **10c**: 21.4 (Me), 110.4 (C-6), 117.4 (C-9), 121.1 (C-7), 124.3 (C-5a), 134.9 (C-8), 144.4 (C-9a), 148.6 (C-4), 152.1 (C-10a), 160.9 (C-2).

Anal. Calcd. for $C_{10}H_9N_5$: C, 60.29; H, 4.55; N, 35.16. Found: C, 60.25; H, 4.49; N, 35.02.

General Procedures for Preparation of 2-Amino-4-methyl-*s*-triazino[1,2-*a*]benzimidazoles (**11**).

A solution of the appropriate 2-guanidinobenzimidazole **5** (0.005 mol) and 0.90 ml (0.005 mol) triethyl orthoacetate in DMF (15 mL) was heated under reflux for 18 h. After cooling, the product was collected by filration, washed with ethanol and dried.

2-Amino-4-methyl-*s*-triazino[1,2-*a*]benzimidazole (**11a**).

Compound **11a** was obtained in 76% yield; mp 324-325° (Lit. [8] mp 314-315°); ir (potassium bromide): NH 3329, NH 3166, CH (aliphatic) 2966, CH (aliphatic) 2923, C=N 1667, NH_2 1635, 1598, 1512, 1449, 1357, 1307, 1284, 1251, 1220, 1147, 779, 745,

726, 567 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 2.92 (s, 3H, Me), 7.20 (t, 1H, 7-H, J = 7.7 Hz), 7.37 (t, 1H, 8-H, J = 7.8 Hz), 7.51 (s, 1H, NH_2, deuterium oxide-exchangeable), 7.54 (d, 1H, 9-H, J = 7.9 Hz), 7.86 (d, 1H, 6-H, J = 7.9 Hz).

Anal. Calcd. for $C_{10}H_9N_5$: C, 60.29; H, 4.55; N, 35.16. Found: C, 60.34; H, 4.60; N, 35.04.

2-Amino-4,7(8)-dimethyl-*s*-triazino[1,2-*a*]benzimidazoles (**11b**,**c**).

The mixture of regioisomers **11b**,**c** was obtained in 65% yield; mp 319°; ^1H nmr (dimethyl sulfoxide-d_6): δ **11b**: 2.46 (s, 3H, C(7)Me), 2.90 (s, 3H, C(4)Me), 7.19 (d, 1H, 8-H, J = 8.3 Hz), 7.41 (d, 1H, 9-H, J = 8.3 Hz), 7.46 (s, 2H, NH_2, deuterium oxide-exchangeable), 7.67 (s, 1H, 6-H); **11c**: 2.43 (s, 3H, C(8)Me), 2.88 (s, 3H, C(4)Me), 7.01 (d, 1H, 7-H, J = 8.3 Hz), 7.33 (s, 1H, 9-H), 7.46 (s, 2H, NH_2, deuterium oxide-exchangeable), 7.71 (d, 1H, 6-H, J = 8.3 Hz).

Anal. Calcd. for $C_{11}H_{11}N_5$: C, 61.96; H, 5.20; N, 32.84. Found: C, 62.05; H, 5.25; N, 32.87.

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