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Synthesis and dihydrofolate reductase inhibitory activity of 2-amino-[1,3,5]triazino[1,2-*a*]benzimidazoles

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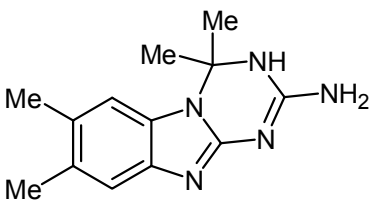
Abstract

This report describes the syntheses of 2-amino-[1,3,5]triazino[1,2-*a*]benzimidazoles (**2-6**) *via* reactions berween 2-guanidinobenzimidazole (**1**) and selected aldehydes, ketones and diethyl ethoxymethylenemalonate were reported. Data of NMR analysis including NOESY experiment indicated that 2-amino-dihydro[1,3,5]triazino[1,2-*a*]benzimidazoles (**2-5**) existed predominantly as the 3,4-dihydro tautomeric forms in DMSO solution. The compounds (**2-6**) were evaluated for potential dihydrofolate reductase inhibitory activity. 2-Amino-4,4-dimethyl-3,4-dihydro[1,3,5]triazino[1,2-*a*]benzimidazole (**4**) was found to be the most active compound (IC_{50} 10.9 μM).

Key words: fused 1,3,5-triazines, 1,3,5-triazino[1,2-*a*]benzimidazoles, tautomerism, antifolate, dihydrofolate reductase inhibitors.

Introduction

Inhibitors of enzyme dihydrofolate reductase (DHFR) are known to be effective antibacterial, antiparasitic and antitumor agents [1-4]. The investigations in this area have identified several chemical classes, including dihydro-*s*-triazines, to be promising in the development of new DHFR inhibitors [5]. However *s*-triazines fused with another heterocyclic ring have not been studied thoroughly. Only one compound, namely, 2-amino-4,4,7,8-tetramethyl-3,4-dihydro-[1,3,5]triazino[1,2-*a*]benzimidazole (Figure 1) has been shown to possess inhibitory activity towards plasmodial DHFR [6,7]. Using this compound as a prototype, we designed and synthesized several analogues without methyl groups at positions 7 and 8. It was investigated whether the substituents at position 4 as well as the conjugation in heterocyclic nucleus have an influence on DHFR inhibitory activity. At the same time we explored tautomeric preferences in the prepared compounds.



IC_{50} = 1.4 μM

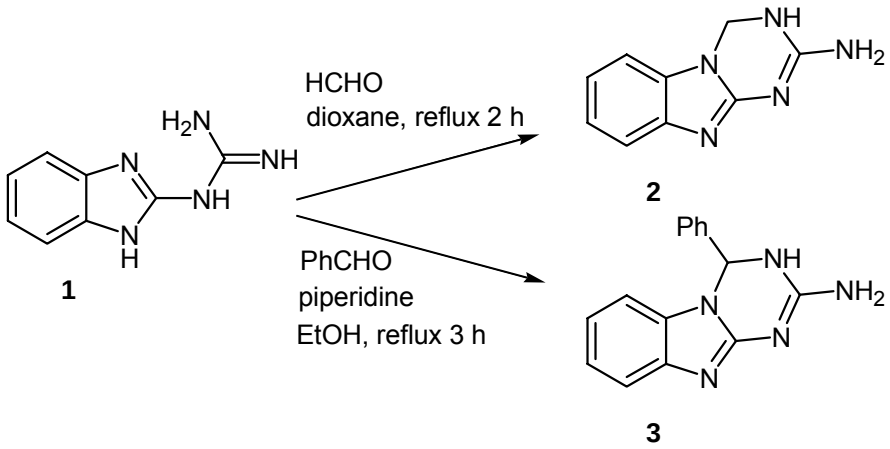
Figure 1.

Results and discussion

The target 2-amino-[1,3,5]triazino[1,2-*a*]benzimidazoles (**2-6**) were synthesized *via* 5+1 heterocyclization of 2-guanidinobenzimidazole (**1**) using aldehydes, ketones or diethyl ethoxymethylenemalonate as the one-carbon cyclizing agents.

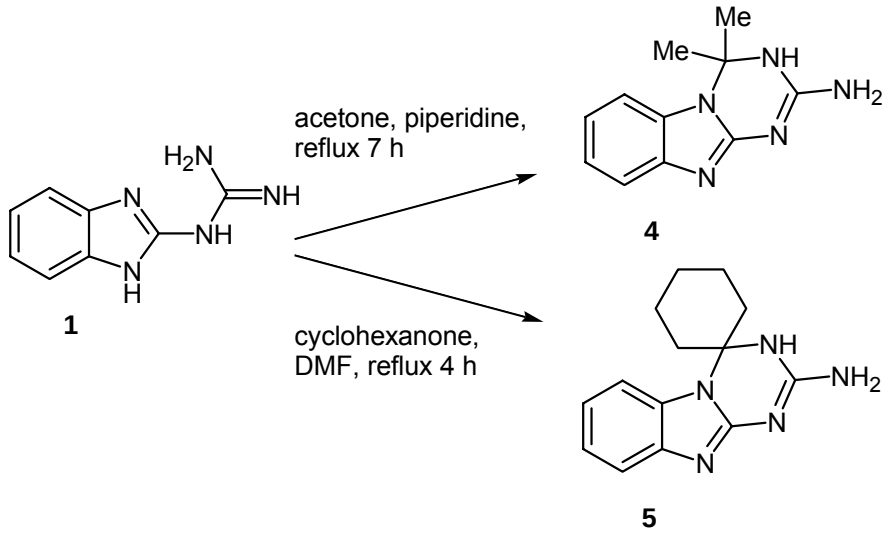
Reactions of 2-guanidinobenzimidazole (**1**) with formaldehyde and benzaldehyde afforded 2-amino-3,4-dihydro-[1,3,5]triazino[1,2-*a*]benzimidazoles (**2**,**3**) (Scheme 1).

Scheme 1



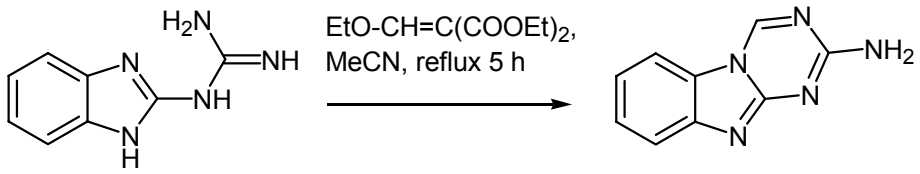
Refluxing of 2-guanidinobenzimidazole (**1**) in acetone under piperidine catalysis led to the formation of 2-amino-3,4-dihydro[1,3,5]triazino[1,2-*a*]benzimidazole (**4**) with two geminal methyl groups (Scheme 2). The spiro cyclohexyl analogue was formed when **1** reacted with cyclohexanone in refluxing DMF (Scheme 2).

Scheme 2



The completely conjugated 2-amino-[1,3,5]triazino[1,2-*a*]benzimidazole (**6**) was prepared from the reaction of **1** with diethyl ethoxymethylenemalonate in refluxing acetonitrile (Scheme 3).

Scheme 3



1 **6**

The prepared compounds (**2-6**) were fully characterized according to the analytical and spectroscopic properties (Tables 1-3).

Table 1

Physicochemical characteristics of 2-amino-[1,3,5]triazino[1,2-*a*]benzimidazoles (**2-6**)

Compound	Molecular Formula*	Yield (%)	Mp ,°C (solvent)	IR (KBr), ν (cm^{-1})
2	$C_9H_9N_5$ (187.2)	68	284-285 (DMF)	3323 (NH), 3144 (NH), 3058 (CH), 2898 (CH), 1649 (C=N), 1620 (NH_2), 1588, 1526, 1467, 1439, 1375, 1279, 1244, 758, 740
3	$C_{15}H_{13}N_5$ (263.3)	85	292-293 (DMF-ethanol)	3407 (NH), 3321 (NH), 3224 (NH), 3052 (CH), 1660 (C=N), 1613 (NH_2), 1590, 1571, 1459, 1423, 1400, 1279, 1246, 740, 699
4	$C_{11}H_{13}N_5$ (215.3)	84	295-296 (DMF-ethanol)	3285 (NH), 3132 (NH), 2980 (CH), 2929 (CH), 1659 (C=N), 1616 (NH_2), 1584, 1539, 1456, 1406, 1387, 1284, 1250, 762, 747, 549
5	$C_{14}H_{17}N_5$ (255.3)	56	375 (DMF)	3433 (NH), 3317 (NH), 3135 (NH), 2978 (CH), 2928 (CH), 2856 (CH), 1667 (C=N), 1615 (NH_2), 1528, 1456, 1381, 1285, 1256, 761, 745
6	$C_9H_7N_5$ (185.2)	95	301 (DMF)	3304 (NH), 3157 (NH), 3046(CH), 3015 (CH), 1685 (C=N), 1632 (NH_2), 1603, 1480, 1450, 1343, 1306, 1275, 1243, 1184, 1091, 779, 758, 741

* Satisfactory elemental analyses were obtained.

According to ^{1}H NMR data (Table 2) the prepared compounds (**2-6**) existed in amino forms. However the dihydro[1,3,5]triazino[1,2-a]benzimidazoles (**2-5**) could be involved in annular prototropic tautomerism with concurrent presence of 3,4-dihydro (**A**), 1,4-dihydro (**B**) and 4,10-dihydro (**C**) tautomeric forms (Scheme 4). 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 **2-5**. The comparison of ^{13}C NMR spectral data for compounds **2** and **6** was useful for the analysis of tautomeric equilibrium. The significant downfield shift of C-2 signal (Δδ = 4.6 ppm) in the spectrum of **6** and less evident shift of C-10a (Δδ = 1.7 ppm) indicated that 3,4-dihydro tautomeric form (**A**) is predominant in the equilibrium. In a NOESY experiment conducted on compound **4**, 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 form **A**. 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 (**A**) in the DMSO solution.

Scheme 4

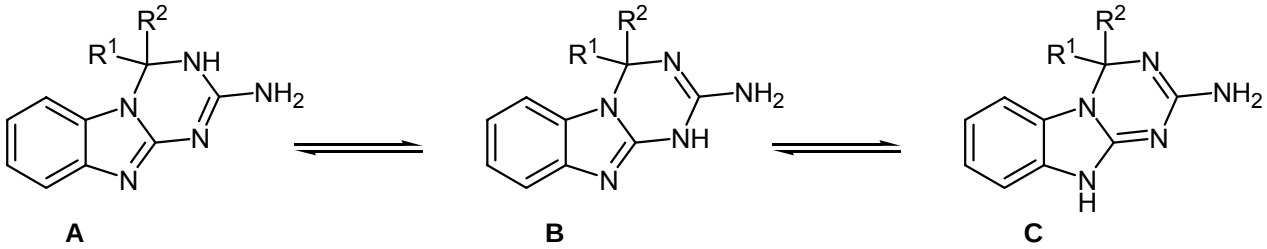


Table 2

^{1}H NMR spectral data for 2-amino-[1,3,5]triazino[1,2-*a*]benzimidazoles (**2-6**)

(300 MHz, DMSO-d_6/TMS, δ, ppm; *J*, Hz)

Compd.	NH_2	NH	H-6	H-7	H-8	H-9	H-4 (group at C-4)
2	6.83 s	7.89 br. s	7.29 dd, *J* = 7.3; 1.1	6.97 td, *J* = 7.3; 1.3	7.02 td, *J* = 7.3; 1.3	7.17 dd, *J* = 7.3; 1.1	5.40 s, 2H, CH_2
3	6.58 s	8.20 s	7.22 d, *J* = 7.5	6.93 td, *J* = 7.3, 1.5	6.79 td, *J* = 7.3, 1.1	6.75 dd, *J* = 7.2, 1.5	6.77 s, 1H, H-4; 7.33-7.42 m, 5H, Ph
4	6.93 s	8.17 br. s	7.39 d, *J* = 7.9	6.95 t, *J* = 7.9	7.02 t, *J* = 7.5	7.29 d, *J* = 7.5	1.82 s, 6H, 2Me
5	6.36 s	7.48 br. s	7.43 d, *J* = 7.9	6.90 t, *J* = 7.2	6.98 t, *J* = 7.3	7.24 d, *J* = 7.5	1.31-1.90 m, 8H, 2'-H, 3'-H, 5'-H and 6'-H; 2,41 td, 2H, *J* = 12.8; 3.4, 4'-H
6	7.69 br. s	-	8.01 d, *J* = 7.9	7.21 td, *J* = 7.4; 1.1	7.36 td, *J* = 7.7; 1.1	7.53 d, *J* = 7.9	9.60 s, 1H, CH

Table 3

^{13}C NMR spectral data for 2-amino-[1,3,5]triazino[1,2-*a*]benzimidazoles (**2-6**) (75 MHz, DMSO-d_6/TMS, δ, ppm)

Compd.	C-2	C-4	C-5a	C-6	C-7	C-8	C-9	C-9a	C-10	Group at C-4
2	156.5	53.0	131.5	107.3	120.7	119.0	115.5	142.7	153.7	-
3	155.2	65.8	131.2	108.1	120.7	118.8	115.8	143.2	153.4	126.2 (C-2' and C-6'), 128.8 (C-3' and C-5'), 129.1 (C-4'), 140.4 (C-1')
4	155.3	69.4	130.5	109.7	120.9	119.0	115.8	143.3	153.5	28.5 (2Me)
5	154.6	71.1	130.6	110.1	120.4	118.8	116.1	143.6	153.6	20.8 (C-3' and C-5'), 23.6 (C-4'), 35.3 (C-2' and C-6'),
6	161.1	148.8	126.4	110.9	120.0	125.5	117.4	144.1	152.0	-

DHFR inhibitory activity of the synthesized compounds **2-6** was evaluated using bovine DHFR (Fluka Chemie) according to a previously described method [8]. The compounds (**2-6**) for the DHFR inhibition bioassays were dissolved in DMSO. In order to ensure that the solvent *per se* did not have an effect on the enzymatic activity, negative control test was performed using DMSO at the same concentration. IC_{50} was calculated for the active compounds.

The results of the biological assay are presented in Table 4. Compound **4** with *gem*-dimethyl group at position 4 and structurally related to the prototype molecule was found to be most active. Compound **2** which does not have substituents at position 4 was more than 50 times less active than **4**. Similar inhibition of DHFR was observed for the spiro compound **5**. Compound **3** with phenyl ring at position 4 was not active.

Interestingly, compound **6**, the fully conjugated analogue of **2**, was twice more active. This is an indirect evidence that other forms either than 3,4-dihydro tautomeric form contributed to the inhibition of DHFR.

Table 4

DHFR inhibitory activity of 2-amino-[1,3,5]triazino[1,2-*a*]benzimidazoles (**2-6**)

Compound	IC_{50}, μM
2	570
3	> 1000
4	10.9 (7.2 – 16.4)*
5	470
6	280

* - interval for p = 0.05

In conclusion, the 2-amino-[1,3,5]triazino[1,2-*a*]benzimidazoles were found to be a group of compounds with potential DHFR inhibitory activity, considering 2-amino-4,4-dimethyl-3,4-dihydro[1,3,5]triazino[1,2-*a*]benzimidazole (**4**) as a lead compound.

Experimental

Melting points (uncorrected) were determined on a Gallenkamp melting point apparatus. IR spectra were performed on a Jasco FT-IR-430 spectrophotometer in KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-300 spectrometer, using DMSO-d_6 as a solvent and TMS as an internal reference.

*2-Amino-3,4-dihydro[1,3,5]triazino[1,2-a]benzimidazole (**2**).*

A solution of 1.75 g (10 mmol) 2-guanidinobenzimidazole (**1**) and 1.00 ml (10 mmol) 37% formaldehyde in dioxane (20 ml) was heated under reflux for 2 h. After cooling, the product was filtered, washed with ethanol, dried and recrystallized from DMF.

*2-Amino-4-phenyl-3,4-dihydro[1,3,5]triazino[1,2-a]benzimidazole (**3**).*

A solution of 1.75 g (10 mmol) 2-guanidinobenzimidazole (**1**), 1.00 ml (10 mmol) benzaldehyde and 0.50 ml piperidine in ethanol (20 ml) was heated under reflux for 3 h. After cooling, the product was filtered, washed with ethanol, dried and recrystallized from DMF-ethanol.

*2-Amino-4,4-dimethyl-3,4-dihydro[1,3,5]triazino[1,2-a]benzimidazole (**4**).*

A solution of 1.75 g (10 mmol) 2-guanidinobenzimidazole (**1**) 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, dried and recrystallized from DMF-ethanol.

*2'-Amino-3'H-spiro[cyclohexane-1,4'-[1,3,5]triazino[1,2-a]benzimidazole] (**5**).*

A solution of 1.75 g (10 mmol) 2-guanidinobenzimidazole (**1**) and 1.55 ml (15 mmol) cyclohexanone in DMF (15 ml) was heated under reflux for 4 h. The reaction mixture was concentrated under vacuum. After cooling, the product was filtered, washed with ethanol, dried and recrystallized from DMF.

*2-Amino-[1,3,5]triazino[1,2-a]benzimidazole (**6**).*

A solution of 0.88 (5 mmol) 2-guanidinobenzimidazole (**1**) and 1.00 ml (5 mmol) diethyl ethoxymethylenemalonate in acetonitrile (20 ml) was heated under reflux for 5 h. After cooling, the product was filtered, washed with ethanol, dried and recrystallized from DMF.

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