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SYNTHESIS OF (HET)ARYLAMIDOGUANIDINES AND THEIR ANTICOAGULANT ACTIVITY EVALUATION

Anton V. Dolzhenko,[1] Anna A. Krasnova,[2] Svetlana A. Ustinova,[2] Dmitry V. Kalinin,[2] Olga V. Sherbakova,[2] Boris Ya. Syropyatov,[2] Anna V. Dolzhenko,[1] and Wai-Keung Chui[1]

[1] *Department of Pharmacy, Faculty of Science, National University of Singapore, Singapore, phada@nus.edu.sg*

[2] *Department of Physiology, Perm State Pharmaceutical Academy, Perm, Russian Federation, syropyatov@mail.ru*

Abstract. (Het)arylamidoguanidines were synthesized from corresponding hydrazides and *S*-methyl isothiourea. They were screened *in vitro* for anticoagulant activity using whole canine blood. The most active compounds, namely 4-methylbenzamidoguanidine and nicotinamidoguanidine were also tested on platelet free plasma. No effect was observed for these compounds on the clotting time of platelet free plasma. Therefore, platelets were suspected as a possible target for the anticoagulant activity of the (het)arylamidoguanidines.

Introduction.

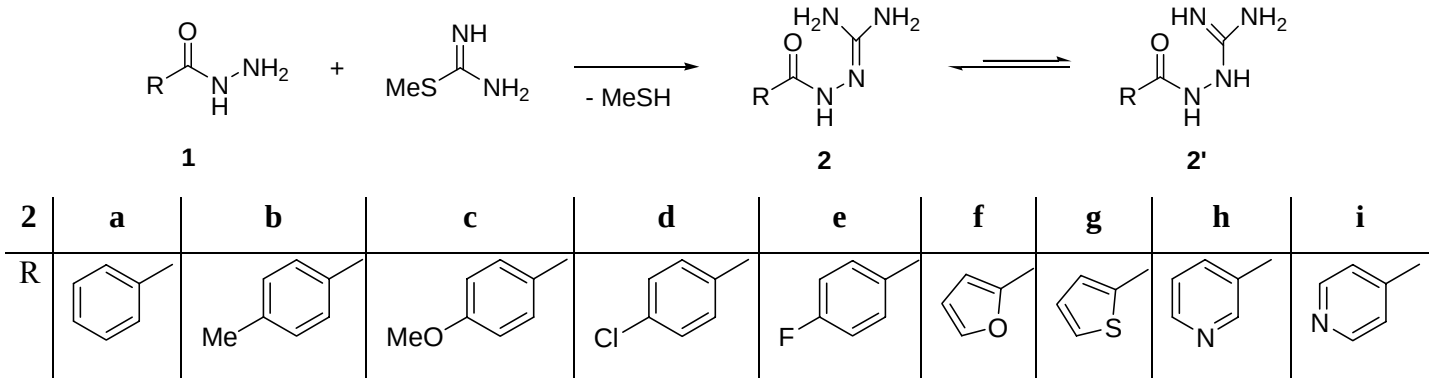
Thromboembolic diseases are well recognized as one of the major causes of morbidity and mortality in the world. The known drugs against these diseases are limited in use by narrow therapeutic windows. Therefore, need to search for new anticoagulant agents is obvious.

Recently, some substituted amidoguanidines were reported [1] to inhibit thrombin in enzyme assay. In this report we present the synthesis of several (het)arylamidoguanidines and their anticoagulant profile evaluation.

Results and discussion.

The (het)arylamidoguanidines (**2**) were synthesized *via* reaction of appropriate (het)arylhydrazides (**1**) with *S*-methyl isothiourea (Scheme 1). The representative set of 4-substituted benzamidoguanidines (**2b-e**) as well as heterylamidoguanidines (**2f,g**) was successfully prepared (Table 1). The synthesis of compounds **2a,h,i** we

reported previously [2]. The structures of the compounds obtained were established using ^{1}H and ^{13}C NMR spectral data (Table 2 and 3).

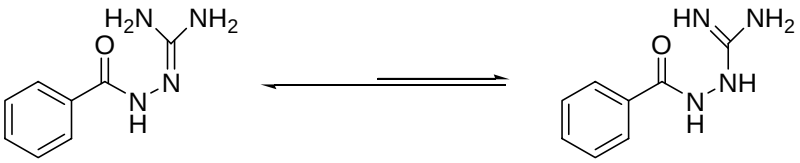


Scheme 1

Table 1. (Het)arylamidoguanidines (**2b-g**)

Compound	mp, °C (solvent)	mp, °C [lit]
2b	189-190 (water)	191 [3]
2c	222-223 (water)	218-220 [4]
2d	225-227 (40% aqueous EtOH)	194-196 [4]
2e	185 (10% aqueous EtOH)	-
2f	208 (water)	199-200 [5]
2g	212 (water)	215-216 [6]

Theoretically, two possible tautomeric forms **2** and **2'** for the (het)aryamidoguanidines were obtainable. The two signals of NH_2 groups in ^{1}H NMR spectra of compounds **2a-c,f-i** indicated that tautomeric form **2**, rather than form **2'**, was preferred in DMSO solution. The activation energy ($\Delta G^{\ddagger}$) of the tautomeric exchange at the coalescence temperature was estimated for the model compound **2a** using dynamic ^{1}H NMR experiments (Scheme 2).



2a: $\Delta G^{\ddagger}_{316}$ = 67.6 kJ

Scheme 2

Table 2. ^{1}H NMR spectral data for compounds **2b-g**, 300 MHz, DMSO-d_6 (δ, ppm)

Compound	-N=C(N**H**$_2$)$_2$	N**H**	R
2b	6.89 (2H, br. s, NH_2), 7.06 (2H, br. s, NH_2)	10.91 (1H, br. s)	2.30 (3H, s, Me), 7.10 (2H, d, *J* = 7.9 Hz, H-3 and H-5), 7.82 (2H, d, *J* = 7.9 Hz, H-2 and H-6)
2c	6.88 (2H, br. s, NH_2), 7.03 (2H, br. s, NH_2)	10.86 (1H, br. s)	3.76 (3H, s, OMe), 6.84 (2H, d, *J* = 8.7 Hz, H-3 and H-5), 7.87 (2H, d, *J* = 8.7 Hz, H-2 and H-6)
2d	7.01 (4H, br. s)	10.78 (1H, br. s)	7.33 (2H, d, *J* = 8.3 Hz, H-3 and H-5), 7.96 (2H, d, *J* = 8.3 Hz, H-2 and H-6)
2e	6.98 (4H, s)	10.74 (1H, br. s)	7.09 (2H, dd, *J* = 8.7, 8.9 Hz, H-3 and H-5), 7.98 (2H, dd, *J* = 8.7, 6.0 Hz, H-2 and H-6)
2f	6.78 (2H, s, NH_2), 6.95 (2H, s, NH_2)	10.87 (1H, s)	6.44 (1H, dd, *J* = 3.0, 1.9 Hz, H-4), 6.63 (1H, d, *J* = 3.0 Hz, H-3), 7.55 (1H, s, H-5)
2g	6.80 (2H, s, NH_2), 6.95 (2H, s, NH_2)	10.77 (1H, s)	6.98 (1H, dd, *J* = 4.9, 3.4 Hz, H-4), 7.34 (1H, d, *J* = 3.4 Hz, H-3), 7.36 (1H, d, *J* = 4.9 Hz, H-5)

Table 3. ^{13}C NMR spectral data for compounds **2b-g**, 75 MHz, DMSO-d_6 (δ, ppm)

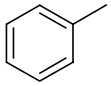
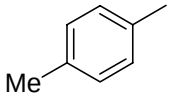
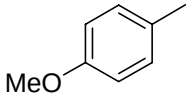
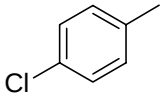
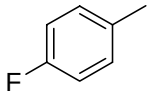
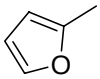
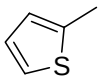
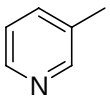
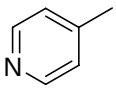
Compound	-N=**C**(NH$_2$)$_2$	**C**=O	R
2b	152.7	160.7	20.8 (Me), 126.5 (2C), 127.8 (2C), 135.8, 137.1
2c	152.7	160.5	54.9 (OMe), 112.5 (2C), 127.9 (2C), 131.1, 159.3
2d	152.8	159.5	127.2 (2C), 128.4 (2C), 132.5, 137.6
2e	152.7	159.7	113.8 (d, *J* = 21.2 Hz, C-3 and C-5), 128.6 (d, *J* = 8.2 Hz, C-2 and C-6), 135.1 (d, *J* = 2.4 Hz, C-1), 162.2 (d, *J* = 244.0 Hz, C-4)
2f	152.8*	155.0	108.1, 110.7, 141.8 (C-5), 152.7 (C-2)*
2g	152.5	157.5	124.9, 125.7, 126.7, 144.3 (C-2)

* - assignments may be reversed

Among the benzamidoguanidines (**2a-e**) screened for anticoagulant activity (Table), 4-methyl and 4-methoxy substituted compounds **2b,c** were found to possess some anticoagulant properties. The pyridyl substituted amidoguanidines (**2h,i**) also increase the clotting time in our experiments. The highest level of the anticoagulant

activity was observed for nicotinamidoguanidine (**2h**). The anticoagulant effect of this compound on whole blood at the screening concentration was found to be higher than that of heparin.

Table 4. Anticoagulant activity of (het)aryamidoguanidines (**2a-i**)

Compound	R	Clotting time, sec		Anticoagulant effect, %
		Control	Experiment	
2a		41.9±3.44	41.1±2.31	na
2b		49.5±3.27	60.2±2.78	21.1*
2c		37.1±1.62	41.0±5.12	10.5
2d		50.7±3.25	51.3±3.98	na
2e		36.7±2.72	36.3±2.28	na
2f		36.9±2.32	36.5±2.16	na
2g		48.4±3.96	48.6±3.81	na
2h		39.4±2.63	50.9±3.24	29.2*
2i		36.3±1.80	38.1±2.33	5.0
Heparin	-	29.9±0.48	36.6±1.82	22.4*

na – not active; * - $p < 0.05$

Interestingly, the effect of the most active anticoagulant compounds from this study, *viz.* 4-methylbenzamidoguanidine (**2b**) and nicotinamidoguanidine (**2h**), on the blood coagulation was observed only

when whole blood was used and no significant changes in the platelet free plasma clotting time were observed in our experiments (Figure 1). We were unable to find a correlation between the anticoagulant activity and thrombin inhibition. These findings clearly indicated that factors other than thrombin, most probably platelets are targets for the (het)aryamidoguanidines and particularly nicotinamidoguanidine (**2h**).

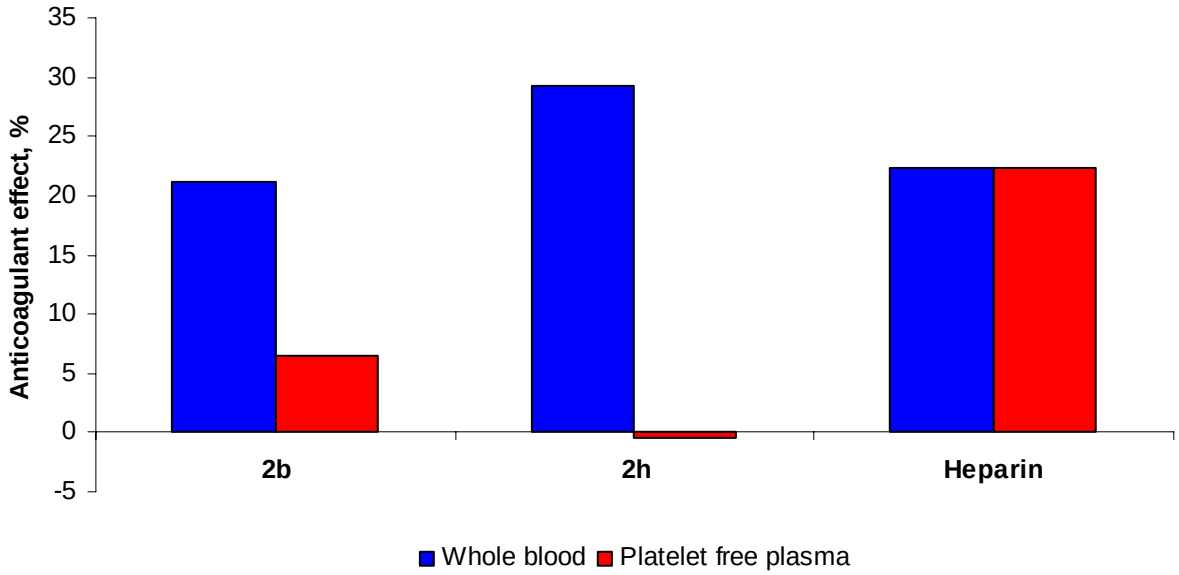


Figure 1

Conclusions.

(Het)arylamidoguanidines were found to be an attractive class of compounds for the search of new potent anticoagulants. Further investigations of the anticoagulant activity mechanism of nicotinamidoguanidine (**2h**) are in progress.

Experimental.

General.

Melting points (uncorrected) were determined on a Gallenkamp melting point apparatus. ^{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. The energy of activation ($\Delta G^{\ddagger}$) for the equilibrium between **2a** and **2a'** was estimated in DMSO-d_6 solution at the temperature of coalescence using dynamic ^{1}H NMR experiment.

Synthesis of (het)arylamidoguanidines.

The mixture of hydrazide (**1**, 10.0 mmol) and *S*-methyl isothiouronium sulfate (1.39 g, 5.0 mmol) in 1% aqueous sodium hydroxide solution (40 ml) was stirred at rt for 72 h and then heated to 50 °C for another 3 h. After cooling, the precipitated product **2** was filtered, washed with ice-cold water and dried. In case of **2d** 20% ethanol was used as a solvent.

Anticoagulant activity.

The effect of the compounds on the clotting time of whole canine blood and platelet free plasma was estimated *in vitro* using coagulometer "Minilab 701". The compounds were tested at the concentration of 1 mg/ml. The heparin solution (1 ED/ml) was used in the experiments as a positive control.

Acknowledgement.

This work is supported by the Academic Research Fund (WBS R-148-000-069-112) from the National University of Singapore.

References.

[1] De Simone, G.; Menchise, V.; Omaggio, S.; Pedone, C.; Scozzafava, A.; Supuran, C. T. *Biochemistry* **2003,** 42(30), 9013-9021.

[2] Dolzhenko, A. V.; Chia, H. S.; Chui, W. K. A026, *ECSOC-9*, **2005,** 1-30 November 2005, http://www.usc.es/congresos/ecsoc/9/ECSOC9.HTM

[3] Hegarty, A. F.; O'Mahony, T. A. F.; Quain, P.; Scott, F. L. *J. Chem. Soc., Perkin Trans. 2* **1973,** (15), 2047-2054.

[4] Hoggarth, E. *J. Chem. Soc.* **1950,** 612-614.

[5] Grinstein, V.; Chipen, G. I. *Zh. Obshch. Khim.* **1961,** 31, 886-890.

[6] Yale, H. L.; Losee, K. A.; Perry, F. M.; Bernstein, J. *J. Am. Chem. Soc.* **1954,** 76, 2208-2211.