MedChemComm

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CONCISE ARTICLE

View Article Online
View Journal | View Issue

Cite this: *Med. Chem. Commun.*, 2013, **4**, 856

Received 13th March 2013
Accepted 31st March 2013

DOI: 10.1039/c3md00081h

www.rsc.org/medchemcomm

Synthesis and trypanocidal action of new adamantane substituted imidazolines†

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Introduction of the hydrophobic substituents cyclopentyl, cyclohexyl and phenyl into adamantane imidazolines was accomplished with the synthesis of compounds **4**. Members of this series were found to display a range of activities against bloodstream forms of *Trypanosoma brucei*.

Introduction

Human African trypanosomiasis (HAT) is caused by tsetse fly transmitted protozoan parasites of the *Trypanosoma brucei* species complex. HAT is endemic in several areas of Sub-Saharan Africa and subject to epidemic outbreaks. Drugs used to treat[1] HAT are thought to target a range of biological molecules. Elfornithine[2,3] is a specific irreversible inhibitor of the first step in polyamine biosynthesis, which is the formation of putrescine from ornithine by ornithine decarboxylase. Eflornithine is relatively expensive and requires administration over 14 days, under medical supervision. Suramin[4,5] is a large polyanion which exerts inhibitory activity on a variety of enzymes involved in several protozoan metabolic pathways. Melarsoprol[6,7] and its metabolite melarsen oxide inhibit many enzymes and substrates that contain vicinal thiol groups, *e.g.* trypanothione, and disrupt the intracellular thiol-redox balance in *T. brucei*. Melarsoprol is extremely toxic, with reactive encephalopathy killing up to 5% of patients. Diamidines[8–10] are nucleic acid binding drugs that target the kinetoplast. The trypanocidal activity of pentamidine is the result of selective accumulation leading to multiple lethal effects, rather than a single effect on a specific "diamidine target". More recently, nifurtimox–eflornithine combination therapy (NECT) has been introduced and has led to some improvements in treatment.[1] Other promising drug targets are *N*-myristoyltransferase,[11] protozoan caspases[12] and kinases.[13]

It has been reported that various anti-*Infuenza A* virus aminoadamantane derivatives,[14] *e.g.* rimantadine and to a lesser extent amantadine, are effective against the bloodstream forms of *T. brucei.* These adamantane amino derivatives readily cross the BBB and are well absorbed by the gastrointestinal tract.[15] Furthermore, other adamantane derivatives with hydrophobic substituents on the adamantane ring show increased trypanocidal activity.[16]

We have previously reported[17] the synthesis of compounds **1** and **2** (Fig. 1). The non-spiro analogues **2** were found to be potent against bloodstream form *T. brucei*.

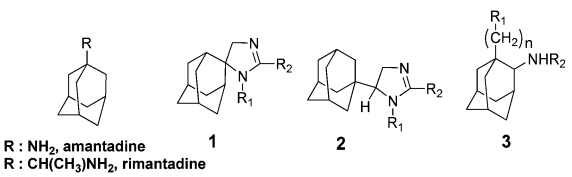
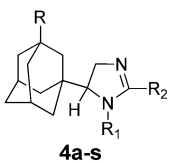


Fig. 1 Amantadine, rimantadine, adamantane analogues **1**, **2** and **3**.



Cmpd	R	R_1	R_2	Cmpd	R	R_1	R_2
4a	cyclopentyl	H	H	**4j**	Phenyl	H	H
4b	cyclopentyl	CH_3	H	**4k**	Phenyl	CH_3	H
4c	cyclopentyl	H	CH_3	**4l**	Phenyl	H	CH_3
4d	cyclopentyl	CH_3	CH_3	**4m**	Phenyl	CH_3	CH_3
4e	cyclopentyl	H	NH_2	**4n**	Phenyl	H	NH_2
4f	cyclopentyl	CH_3	NH_2	**4o**	Phenyl	CH_3	NH_2
4g	cyclohexyl	H	H	**4p**	cyclopentyl	cyclohexyl	H
4h	cyclohexyl	H	NH_2	**4q**	cyclopentyl	cyclohexyl	NH_2
4i	cyclohexyl	CH_3	NH_2	**4r**	cyclopentyl	phenyl	H
				4s	cyclopentyl	phenyl	NH_2

Fig. 2 5-(3-Substituted-1-adamantyl)-2-imidazolines **4a–s**.

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† Electronic supplementary information (ESI) available: Experimental data for the compounds, materials and methods for pharmacological assay. See DOI: 10.1039/c3md00081h

In this communication, we extend our previous work to 5-(3-substituted-1-adamantyl)-2-imidazolines of the general type **4** (Fig. 2), which, besides the imidazoline ring, also bear hydrophobic substituents at C3 of the adamantane skeleton. This increase in lipophilicity is expected to lead to more active trypanocidals, as in the case of previously reported analogues **3**.[16]

Results and discussion

The synthesis of 2-imidazolines **4a–s** was accomplished by the reaction shown in Scheme 1.

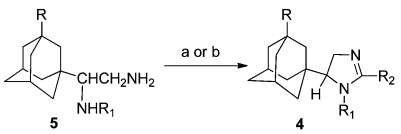
The imidazolines **4a**, **4b**, **4g**, **4j**, **4k**, **4p** and **4r** and the 2-methyl-imidazolines **4c**, **4d**, **4l** and **4m** were prepared by condensing 1-(1-adamantyl)ethylenediamines **5a–c** with either formamidine acetate or acetamidine hydrochloride in ethanol. The free bases that were obtained were then transformed to either the corresponding hydrochloride or fumarate.

Treatment of diamines **5a–h** with cyanogen bromide led to 2-amino-2-imidazolines ($R_2 = NH_2$) (**4e**, **4f**, **4h**, **4i**, **4n**, **4o**, **4q** and **3s**) in the form of hydrobromides. The preparation of ethylenediamines **5** was realized by reduction of the corresponding α-aminoacetonitriles **6a–f**, under the appropriate conditions, as illustrated in Scheme 2. Catalytic hydrogenation of α-aminonitriles **6a** and **6b** under heat and pressure in the presence of PtO_2 in ethanol gave the diamines **5a** and **5b**, respectively. Conversely, prolonged hydrogenation of the phenyl-substituted nitriles **6c** and **6d**, under the same conditions, led to cyclohexyldiamines **5c** and **4d**. Under less harsh conditions, however, the catalytic hydrogenation of nitriles **6c** and **6d** led to the formation of the phenyl-substituted diamines **5e** and **5f**. Last, reduction of the anilinonitrile **6e**, depending on the conditions used, gave three different products (**5g**, **5h** and **7**). Thus, *N*-cyclohexyldiamine **5g** was formed from **6e** upon complete hydrogenation of its benzene ring and the concomitant reduction of the CN group, when the reaction was performed at room temperature and under pressure. The synthesis of the phenyl-substituted diamine **5h** was effected by reducing aminonitrile **6e** with $BH_3 \cdot THF$ complex. Previous attempts to prepare diamine **5h** by reducing aminonitrile **5c** with $LiAlH_4$ in THF, led to the removal of the cyano group and the formation of aniline **7**.

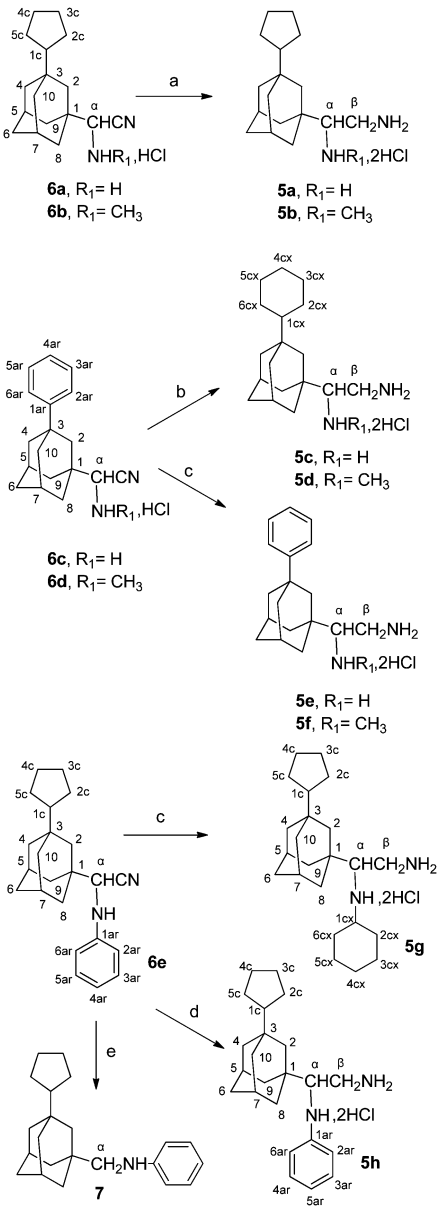
The preparation of α-aminoacetonitriles **6** involved the Strecker reaction[18] on the 3-substituted 1-adamantanecarboxaldehydes **8a** and **8b** (Scheme 3).

3-Cyclopentyl-1-adamantanecarboxaldehyde (**8a**) was prepared from 3-cyclopentyl-1-adamantanecarboxylic acid (**9**), as shown in Scheme 4.

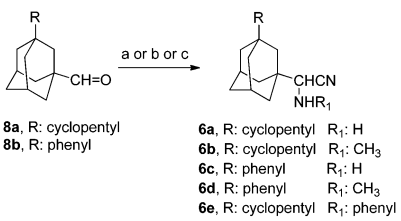
The synthesis of 3-phenyl-1-adamantanecarboxaldehyde (**8b**) (Scheme 5) was accomplished by the same reaction sequence followed for the preparation of aldehyde **8a**.



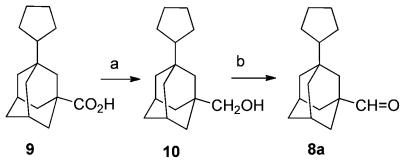
Scheme 1 *Reagents and conditions*: (a) formamidine acetate ($R_2 = H$) or acetamidine hydrochloride ($R_2 = CH_3$) in EtOH; (b) cyanogen bromide ($R_2 = NH_2$).



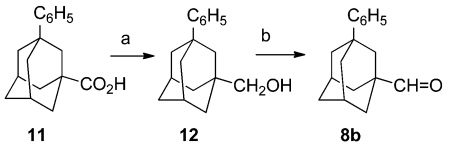
Scheme 2 *Reagents and conditions*: (a) H_2/PtO_2, gas HCl in EtOH/MeOH, 50–60 psi, 60 °C, 4 h; (b) H_2/PtO_2, gas HCl in EtOH/MeOH, 50–60 psi, 60 °C, 8 h; (c) H_2/PtO_2, gas HCl in EtOH, 40–50 psi, rt, 2–3 h; (d) (i) BH_3 in THF, 0 °C, (ii) reflux, 2 h, (iii) H_2O, 0 °C, (iv) HCl 18%, reflux, 4 h; (e) (i) $LiAlH_4$, THF, 0 °C, 1 h, (ii) H_2O/NaOH, 0 °C.



Scheme 3 *Reagents and conditions*: (a) (i) Me_3SiCN, $CHCl_3$, ZnI_2, (ii) NH_3/MeOH, 70–75 °C, autoclave, 20 h (**6a**, **6c**); (b) NaCN, $MeNH_2 \cdot HCl$, DMSO/H_2O, rt for 48 h and then 60 °C for 7 h (**6b**, **6d**); (c) Me_3SiCN, aniline, ACN, rt (**6e**).



Scheme 4 *Reagents and conditions*: (a) (i) $LiAlH_4$, THF, rt, 3 h, (ii) H_2O/NaOH, 0 °C; (b) PCC, DCM, 2 h.



Scheme 5 *Reagents and conditions*: (a) (i) $LiAlH_4$, THF, rt, 3 h, (ii) H_2O/NaOH, 0 °C; (b) PCC, DCM, 2 h.

The results of anti-*T. brucei* screening are summarized in Table 1. In general, the introduction of a cyclopentyl or cyclohexyl group in C3 of adamantane seems to increase the trypanocidal activity in comparison to the previously reported unsubstituted adamantane analogues **2**. The introduction of phenyl substitution either in C3 of adamantane or in *N*1 of imidazoline causes, in all cases, a drastic reduction of trypanocidal action. Cyclopentyl or cyclohexyl substituted derivatives present similar activity with the exception of compound **4q**, the most active of this series, which bears a cyclopentyl group at C3 adamantane and a cyclohexyl substituent at *N*1 imidazoline. In previous studies with other classes of adamantane derivatives, we have found that anti-parasite activity is enhanced by these chemical features.[14,16] Interestingly, there was no general correlation between trypanocidal activity and cytotoxicity. For example, the addition of the amino group at the C2 position of the imidazoline ring of the 3-cyclopentyl or 3-cyclohexyl derivatives **4e**, **4f** and **4h** significantly reduced cytotoxicity, while having little effect on trypanocidal activity. In contrast, with derivative **4q**, the *N*1 cyclohexyl substitution was associated with enhancement of both trypanocidal activity and cytotoxicity. These insights should provide the basis for the design of compounds with greater selectivity indices. Calculated log *P* and PSA values (Table 2 in ESI†), imply that compounds **4** should be able to cross the BBB, as various other trypanocidals, with analogous physicochemical properties, do.[19]

Table 1 Activity[a] and cytotoxicity[b] of 5-(3-substituted-1-adamantyl)-2-imidazolines **4a–s**

Cmpd	*T. brucei* IC_{50} (μM)	*T. brucei* IC_{90} (μM)	L6 cells IC_{50} (μM)	Selectivity index
4a[c]	1.57 ± 0.18	2.42 ± 0.03	3.70 ± 0.15	2.4
4b[c]	1.50 ± 0.03	1.83 ± 0.03	5.77 ± 0.72	3.9
4c[d]	2.16 ± 0.05	2.77 ± 0.12	3.56 ± 0.56	1.6
4d[d]	2.97 ± 0.24	4.51 ± 0.15	3.77 ± 0.33	1.3
4e[e]	2.17 ± 0.19	2.63 ± 0.03	17.22 ± 1.63	7.9
4f[e]	1.65 ± 0.10	2.23 ± 0.29	11.74 ± 1.96	7.1
4g[d]	2.89 ± 0.09	4.20 ± 0.09	7.22 ± 1.74	2.5
4h[e]	1.86 ± 0.08	2.52 ± 0.03	19.00 ± 2.57	10.2
4i[d]	1.72 ± 0.03	2.35 ± 0.03	7.39 ± 0.33	4.3
4j[d]	7.09 ± 0.32	9.02 ± 0.10	nd	—
4k[d]	7.91 ± 0.24	13.05 ± 0.18	nd	—
4l[d]	4.88 ± 0.11	8.11 ± 0.06	nd	—
4m[d]	7.16 ± 0.12	10.32 ± 0.23	nd	—
4n[e]	7.39 ± 0.19	11.81 ± 0.19	nd	—
4o[e]	4.53 ± 0.12	5.58 ± 0.49	nd	—
4p[d]	1.82 ± 0.03	2.41 ± 0.06	3.46 ± 0.18	1.9
4q[e]	0.65 ± 0.01	0.84 ± 0.01	3.22 ± 0.13	5.0
4r[d]	16.4 ± 3.8	24.2 ± 2.5	nd	—
4s[e]	11.1 ± 1.7	15.5 ± 0.7	nd	—
Rimantadine	7.04 ± 0.27	13.97 ± 1.67		
Amantadine	>132	>132		

[a] Derivatives **4a–s** were tested against *in vitro* bloodstream form *T. brucei* (pH 7.4) (ESI). IC_{50} and IC_{90} data are the mean of triplicate experiments ± SEM. [b] Cytotoxicity tests were carried out using rat L6 cells (ESI). Selectivity indices were calculated as the ratio of the IC_{50} values for L6 cells and *T. brucei*. [c] Fumarate. [d] Hydrochloride. [e] Hydrobromide.

Conclusion

It is clear from our results that introduction of the cyclopentyl or cyclohexyl lipophilic substituents into the scaffold of 5-(1-adamantyl)-2-imidazolines **2** increases efficacy against cultured bloodstream forms of the African trypanosome. Both the antiviral and the CNS activity of aminoadamantane derivatives is explainable in terms of their channel blocking properties. In *T. brucei* however, the precise target(s) of these compounds remain to be determined.

Experimental section

General method of preparation of compound 4a–d, 4g, 4j, 4k–m, 4p and 4r: 5-(3-substituted-1-tricyclo[3.3.1.1^{3,7}]decyl)-4,5-dihydro-1*H*-imidazoles

The requisite diamine **5** (1.3 mmol) was dissolved in absolute ethanol (10 mL) and formamidine acetate or acetamidine hydrochloride (1.7 mmol) was added. The mixture was stirred under an argon atmosphere at ambient temperature for 24 h and at 50 °C for another 24 h. The solvent was then evaporated under vacuum and the residue obtained was treated with HCl 4% (30 mL). The resulting mixture was washed with ethyl acetate, the aqueous phase was made alkaline with NaOH 4% and extracted with ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give the respective imidazoline, which was then converted to its salt, on treatment with the appropriate acid.

General method of preparation of compound 4e, 4f, 4h, 4i, 4n, 4o, 4q and 4s: 5-(3-substituted-1-tricyclo[3.3.1.1^{3,7}]decyl)-4,5-dihydro-1*H*-2-imidazolamines

To a stirred solution of the respective diamine **5** (2.9 mmol) in dry dichloromethane (10 mL) a solution of cyanogen bromide (375 mg, 3.52 mmol) in dry dichloromethane (5 mL) was added, under an argon atmosphere, dropwise and under cooling. Stirring was continued at ambient temperature for 48 h and the solvent evaporated under vacuum to leave a residue, which crystallized as hydrobromide salt on treatment with anhydrous ether.

Acknowledgements

JMK was funded by the Wellcome Trust (grant number 092573).

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