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Synthesis and evaluation of new nifurtimox-adamantane adducts with trypanocidal activity

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In memory of Professor George B. Foscolos, Department of Pharmacy, National and Kapodistrian University of Athens

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Abstract: The synthesis and pharmacological evaluation of the C-1 substituted adamantane hydrazones **1a-d**, their C-2 substituted isomers **2a-d** and the C-1 substituted adamantane furanico carboxamides **3a-c** is described. The new adamantane derivatives exhibited an interesting pharmacological profile, in terms of trypanocidal activity and selectivity. Of the compounds tested, the phenylacetoxo hydrazone **1b** showed the most promising profile against African trypanosomes ($EC_{50}=11 \pm 0.9$ nM; $SI_{Tb}=770$).

Vector-borne kinetoplastid diseases, such as Chagas disease (CD),^[1] leishmaniasis and human African trypanosomiasis (HAT)^[2] threaten almost a billion people worldwide.^[3] The available drugs against these neglected tropical diseases (NTDs) are characterized by toxicity, limited efficacy and increasing resistance.^[4] For example, nifurtimox and benznidazole which are used against CD, can cause severe side-effects and treatment is often unsuccessful.^[5] This has led the World Health Organization (WHO) to coordinate public sector and private partnerships as part of a global effort to develop new and safer drugs.^[3] Fexinidazole, which was recommended by the European Medicines Agency in November 2018, is a successful example of the collaboration between the Drugs for Neglected Diseases initiative (DNDi) and pharmaceutical chemistry sector.^[6]

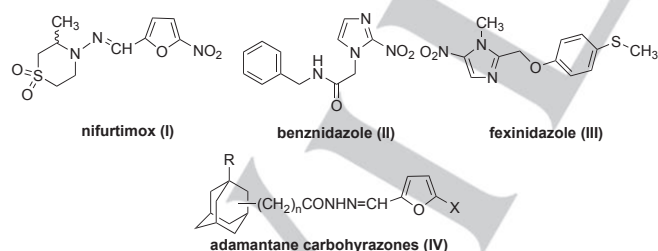


Figure 1. Nifurtimox, benznidazole, fexinidazole and adamantane carbohydrazone derivatives.

We have been interested in adamantane chemistry^[7-21] and have prepared a large number of analogues in an attempt to exploit adamantane's role in bioactivity. We prepared a series of adamantane carbohydrazones,^[22] and showed that these derivatives are very potent trypanocidals. Following this work, we now describe the preparation of a series of phenylhydrazone analogues, **1a-d** and **2a-d**, which in general have very promising antitrypanosomal activity. The new derivatives share common structural features with nifurtimox and also contain a phenyl

substituted adamantane ring. The adamantane core of the novel adducts accommodates aromatic substitutions at the C-1 and C-2 positions. In addition, the 5-nitro-2-furanyl pharmacophore group is attached to the phenyladamantane by a hydrazone bond with a linker, consisting of one or two methylene groups and an oxygen atom. This bond is altered to carboxamide in compounds **3a-c**, with compound **3c** also bearing a benzyl group on the amide nitrogen, to mimic the benznidazole skeletal arrangement (**Figure 2**).

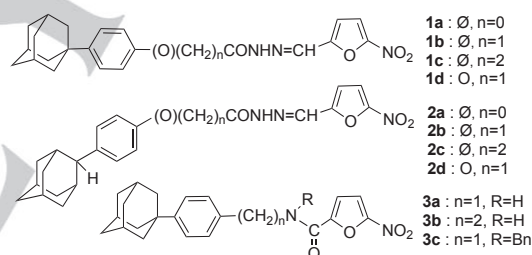
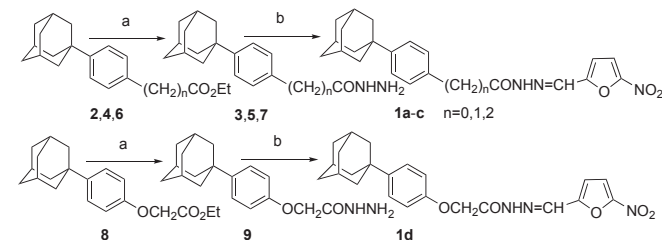


Figure 2. New nifurtimox-adamantane adducts with trypanocidal activity.

The 4-(adamant-1-yl)phenylhydrazides, **1a-d**, were prepared by previously described methods,^[22] using as starting materials ethyl 4-(adamant-1-yl)benzoate (**2**),^[18] ethyl 4-(adamant-1-yl)phenyl acetate (**4**),^[18] ethyl 4-(adamant-1-yl)phenylpropionate (**6**)^[18] and ethyl 4-(adamant-1-yl)phenoxyacetate (**8**)^[23] as shown in **Scheme 1**.



Scheme 1. Reagents and conditions: (a) hydrazine hydrate, EtOH, **3** : 160 °C, autoclave, 6 d, 81 %; **5** : 130 °C, autoclave, 5 d, 85 %; **7** : reflux, 2 d, 80 %, **9** : reflux, 1d, 98 %; (b) 5-nitro-2-furaldehyde, EtOH, r.t., 12 h, 55-80 %.

The preparation of analogues **2a-c** was realised by the reaction sequence illustrated in **Scheme 2**.

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(I) and 3–9 fold more effective against *T. brucei* than *T. cruzi*. The most active adduct, with the best selectivity, was the phenylacetoxo hydrazone **1b** ($EC_{50}=11\pm 0.9$ nM and $SI_{Tb}=770$). The spacer between the phenyl ring and the carbonyl group seems to have a significant impact on activity and cytotoxicity. It seems that the present structural modification comprising of a phenyl ring insertion between the adamantane core and the hydrazone side chain has improved the pharmacological characteristics of the new molecules, in terms of activity and toxicity, compared to the adamantane carbohydrazones (IV), we previously reported.^[22] The direct attachment of the hydrazone linker to the phenyl ring decreased the potency, and a one or two methylene spacer was associated with enhanced activity. Adducts **1b**, **1c** and **2b**, **2c** exhibited higher trypanocidal activity than analogues **1a** and **2a**, respectively. Conversely, the replacement of one methylene by an oxygen atom had a detrimental impact on activity. The position of substitution on the adamantane core (C-1, C-2), influenced cytotoxicity and the C-1 substituted hydrazones **1b** ($SI_{Tb}=770$) and **1c** ($SI_{Tb}=520$) were found to be more selective than the corresponding C-2 substituted adducts **2b** ($SI_{Tb}=235$) and **2c** ($SI_{Tb}=215$). The same pattern was also observed in the *T. cruzi* results. The third series of derivatives, the 5-nitro-furonic carboxamides **3a-c**, exhibited reduced activity in comparison to the hydrazones, which implies that these analogues may follow the same mechanistic pathway as nifurtimox.^[30–32] This observation is in agreement with our previous test results on adamantane carbohydrazones (IV).^[22]

In conclusion, the new nifurtimox-adamantane hydrazone adducts show higher trypanocidal potency than both the parent drug (I) and the adamantane carbohydrazones (IV). The optimum potency arose from the combination of 4-(adamant-1-yl)phenyl substitution and a two methylene spacer, whilst their 4-(adamant-2-yl)phenyl substituted isomers were almost equipotent but less selective.

Experimental Section

General procedure for the preparation of hydrazones 1a-d and 2a-d.

A solution of the respective hydrazide (0.96 mmol) in ethanol (5 mL) was added to a solution of 5-nitro-2-furaldehyde (200 mg, 1.42 mmol) in ethanol (5 mL). The mixture was stirred under Argon in darkness for 12 h. The resulting precipitate was filtered, washed with a small amount of ethanol and dried to give the corresponding product as a yellow solid, which was recrystallized from chloroform.

General procedure for the preparation of carboxamides 3a-c.

5-Nitro-2-furoic acid (240 mg, 1.52 mmol) was treated with $SOCl_2$ (10 mL) at 60–65 °C for 60 min. Excess $SOCl_2$ was removed under reduced pressure and subsequently by azeotropic distillation with benzene (5 mL). The residue obtained was dissolved in anhydrous acetone (10–15 mL) and a solution of the respective amine (1.44 mmol) in anhydrous pyridine (2 mL) was added dropwise under stirring. The reaction mixture was stirred at room temperature for 1 d, the solvent removed *in vacuo* and the residue was treated with water. The resulting mixture was extracted with CH_2Cl_2 and the organic extracts washed with water, 10% sol. HCl and water. The combined organic phases were dried over Na_2SO_4 and the solvent evaporated. The crude product was purified by gradient flash column chromatography, using as eluent a mixture of $CH_2Cl_2/MeOH$, to give the corresponding carboxamide as an orange viscous semi-solid, which was recrystallized from chloroform.

Conflicts of interest

The authors have no conflicts to declare.

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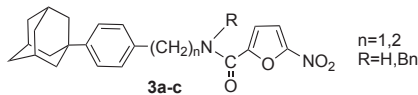
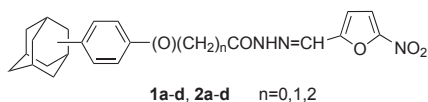
Keywords: 4-(adamant-1-yl)phenyl substitution • 4-(adamant-2-yl)phenyl substitution • hydrazone • nifurtimox • trypanocidal activity

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The insertion of a phenyl ring between the adamantane core and the hydrazone side chain of the new nifurtimox-adamantane adducts led to a higher trypanocidal activity and lower toxicity than the parent drug. The most active adduct with the best selectivity is the phenylacetoxo hydrazone **1b** ($EC_{50}=11 \pm 0.9$ nM and $SI_{Tb}=770$).