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Synthesis and biological activity of benzamide DNA minor groove binders

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ABSTRACT

A range of di- and triaryl benzamides were synthesised to investigate the effect of the presence and nature of a polar sidechain, bonding and substitution patterns and functionalisation of benzylic substituents. These compounds were tested for their antiproliferative activity as well as their DNA binding activity. The most active compounds in all assays were unsymmetrical triaryl benzamides with a bulky or alkylating benzylic substituent and a polar amino sidechain.

DNA minor groove binders (MGB's) are a diverse group of compounds, of both natural (e.g., distamycin **1a**)[1] and synthetic (e.g., tallimustine **1b**)[2] origins (Fig. 1).[3] Although wide-ranging in nature, they all have a concave-shaped aromatic framework that can fit in to the minor groove of DNA in addition to containing groups that are capable of hydrogen bonding.[4]

Distamycin **1a** is a naturally occurring MGB isolated from *Streptomyces distallicus*, and despite having little antitumor activity and low cytotoxicity owing to their reversible binding to DNA, **1a** has been a lead compound in the synthesis of DNA MGB's,[1] with a large number of polypyrrolic analogues synthesised, a notable of example of which is **1b**.[2,5] Unfortunately, there are significant problems associated with polypyrrole MGB's; due to their electron rich nature there are limited reaction conditions that they can withstand and that can be used to functionalise them. Also, added substituents on the nitrogen have been known to undergo unwanted intramolecular cyclisation, negating the benefits these substituents could provide to activity.[3,6] Additionally, to construct MGB's with appropriate curvature, only substitution at the 2- and 4-positions would provide compounds with the desired curvature. A strategy to overcome these problems is to synthesise benzamide derived MGB's. The synthesis of benzamide derivatives (e.g., **2**) is known,[7] however these benzamide derivatives are either simple symmetrical diaryl derivatives or triaryl amides, none with alkylating functional groups; there is little literature precedent for the synthesis of non-symmetrical and more complicated symmetrical oligoamides with various additional functionalities. Herein we report the synthesis and biological evaluation of complex diaryl and symmetrical and unsymmetrical triaryl benzamide MGB's. These structures have been chosen to explore various structural features that can be incorporated in to benzamide MGB's, including the effect of the presence and nature of a polar sidechain, bonding and substitution patterns and functionalisation of benzylic substituents.

We envisioned that complex symmetric triaryl benzamide MGB's could be formed through the coupling of a symmetric diacid chloride **3**. To incorporate benzylic substitution into the resultant triaryl oligoamide that could be functionalised or allow for conjugation to other bioactive molecules, we wished to synthesise an appropriate aniline for the coupling; aniline **4**, with an easily removable TBDMS (*tert*-butyldimethylsilyl) group was thought to be suitable for this purpose. To provide **4**, dinitrobenzyl alcohol **5** was mono-reduced using Zinin reduction procedures,[8] and then both the resultant amine and alcohol groups were acetylated to give diacetate **6** (Scheme 1). Diacetate **6** was then selectively hydrolysed with sodium hydroxide in ethanol to provide an alcohol which was then protected to give aniline **4** in a very high yield of 97% over two steps.[9] With required aniline **4** in hand, we next sought to achieve dicoupling of **3** and **4**. After screening various reaction conditions, we found that using K_2CO_3 (5.4 equiv) in THF at rt efficiently produced the desired triaryl benzamide in an excellent 96% yield, which was then deprotected with TBAF (tetra-*n*-butylammonium fluoride) to provide diol **7** in a high yield.

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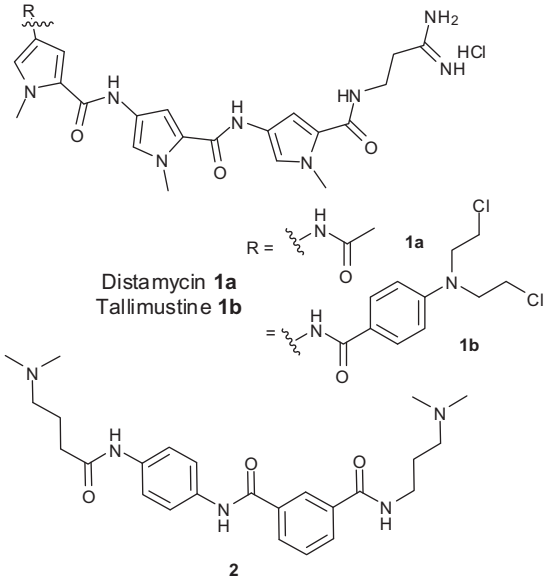
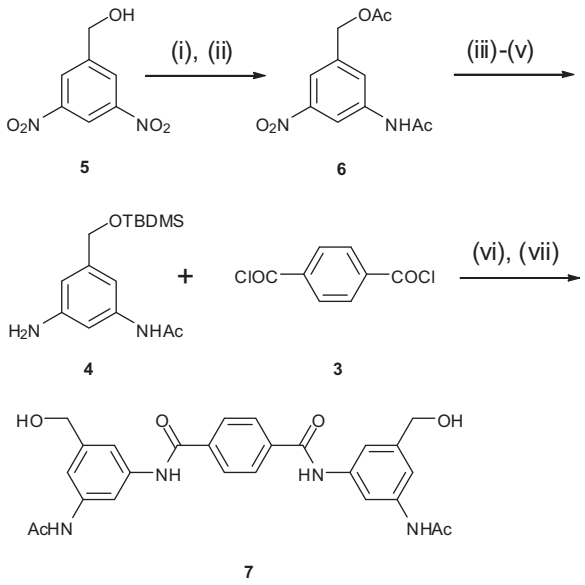
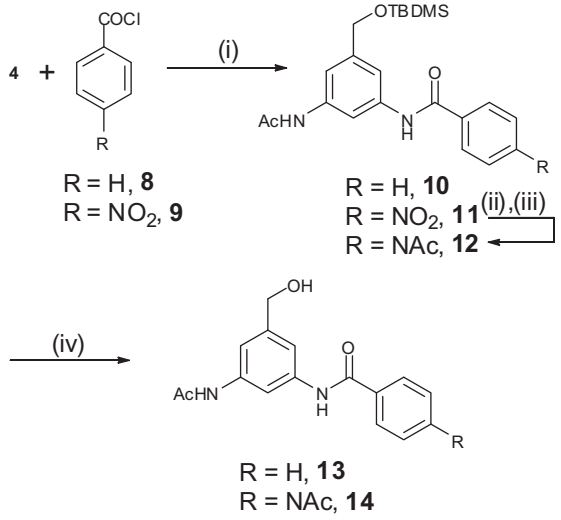


Figure 1. Distamycin **1a**, tallimustine **1b** and benzamide **2**.



Scheme 1. Reagents, conditions and yields: (i) $(NH_4)_2S$, MeOH, reflux then rt, 6 h then 20 h, 81%; (ii) Ac_2O, Et_3N, DMF, rt, 24 h, 99%; (iii) NaOH, EtOH, rt, 3 h, 98%; (iv) TBDMSCl, imidazole, DMF, rt, 5 h, 99%; (v) 10% Pd/C, H_2, MeOH, rt, 3 h, quant; (vi) K_2CO_3, THF, rt, 30 min, 96%; (vii) TBAF, THF, rt, 24 h, 97%.

In order to study the effect of an increased number of aryl groups on the binding of these compounds, we decided to synthesise a range of diaryl benzamides so they could be compared to triaryl benzamides. We first sought to produce simple benzamides with no additional sidechains (Scheme 2) and then follow this with exploration into the addition of polar sidechains to the MGB compounds, which should increase the solubility and binding capabilities of these compounds. Synthesising diaryl benzamides that lack these functionalities would allow the determination of their effect on the biological activity. Aniline **4** was reacted with benzoyl chlorides **8** and **9** to give benzamides **10** and **11** (Scheme 2). The nitro group in **11** was then reduced in quantitative yield to provide benzamide **12**. Finally, deprotection of the TBDMS groups in **10** and **12** gave diaryl benzamides **13** and **14** in high yields.



Scheme 2. Reagents, conditions and yields: (i) pyridine, rt, 16 h, 84% **10**, 77% **11**; (ii) 10% Pd/C, H_2, MeOH, rt, 3 h, quant; (iii) Ac_2O, Et_3N, DMF, rt, 24 h, 89%; (iv) TBAF, AcOH, THF, rt, 24 h, 84% **13**, 93% **14**.

After successfully synthesising simplified benzamides **13** and **14**, we wished to adapt our methods to produce diaryl benzamides with additional sidechains, which as stated above, should aid in solubility and binding of the resultant MGB's. Aniline **4** was coupled[11] with *meta* and *para* regioisomers of various previously prepared[10] benzamides **15**–**18** to provide benzamides **19**–**22** which were then deprotected to provide alcohols **23**–**26**. Synthesising both *meta* and *para* substituted benzamides would allow us to investigate the effect of curvature on the activity.

Additionally, we sought to investigate the effect of altering the pattern of amide bonding (from ArNHCOR to ArCONHR); we anticipated that this alteration would have a strong effect on the electronic character in the molecule which could also play an important role in the solubility and activity of the compounds. Once again aniline **4** was used; it was coupled[11] to benzoic acids **27** and **28**[10] to provide benzamides **29**[12] and **30** in very good yields of 96% (**29**) and 80% (**30**). Silyl group deprotection with TBAF gave alcohols **31** and **32**. Alcohol **31** was further functionalised to the mesylate which was immediately converted[13] to the corresponding chloride **33**,[14] an alkylating agent, in 96% over two steps (see Scheme 3).

In addition to the symmetrical triaryl benzamide synthesised earlier, we also sought to construct various unsymmetrical triaryl compounds to further explore the activity of these larger compounds. The acid coupling partners **34** and **35** were synthesised through the coupling of amides **36**[15] and **37**[7b] which contain a polar amino sidechain, with acid chloride **38** followed by hydrogenolysis to give **34** and **35**. Benzoic acids **34** and **35** were then coupled[11] with aniline **4** to provide triaryl benzamides **39** and **40**[16] which were then deprotected to provide alcohols **41** and **42**. Chloride derivatives **43** and **44**[17] were also synthesised from alcohols **41** and **42** in very high yields of 91% and 97%, respectively, over two steps (see Scheme 4).

With various classes of benzamide MGB's synthesised, we next sought to investigate their biological activities and the effect of various structural changes on their activity. As part of the National Cancer Institute's Developmental Therapeutics Program, 15 of the synthesised compounds were selected to be tested for their antiproliferative activity against 60 human tumour cell lines.[18] The compounds were not toxic to all cell lines and instead generally showed very high selectivity for certain cell lines, particularly leukaemia lines K-562, CCRF-CEM, MOLT-4 and SR lines (see Support-