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Design and Synthesis of C-8 Linked Pyrrolobenzodiazepine–Naphthalimide Hybrids as Anti-Tumour Agents

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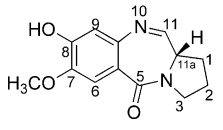
Abstract—The facile synthesis of C-8 linked pyrrolobenzodiazepine–naphthalimide hybrid analogues is described. The compounds are prepared with varying degrees of linker length in order to probe the structural requirements for optimal in vitro anti-tumour activity. Some of these new hybrid compounds showed higher cytotoxic activity than the existing natural and synthetic pyrrolo[2,1-*c*]-[1,4]benzodiazepines.

In the last few years, a growing interest has been shown in the development of DNA-minor groove binders acting as vehicles for the delivery of alkylating agents. It has been observed that small molecules may recognize an increasing range of DNA sequences,[1] which have DNA binding properties, including pyrrolo[2,1-*c*]-[1,4]benzodiazepines (PBDs) and lexitropsins. PBDs are a group of potent, naturally occurring anti-tumour antibiotics produced by various *Streptomyces* species and examples of which include anthramycin, tomaymycin, sibiromycin and the neothramycins A and B. A key feature of these molecules with respect to their mechanism of action is the N-10–C-11 carbinolamine (or imine equalent). Nucleophilic attack by 2-NH_2 of a guanine base at the C-11 position of PBD forms a covalent adduct in the minor groove of DNA.[2] Moreover, the PBDs bind to DNA sequence selectively and have potential not only as anti-tumour agents but also as gene regulators and probes of DNA structure. Further, a large number of methodologies have been developed for the synthesis of PBD ring system.[3]



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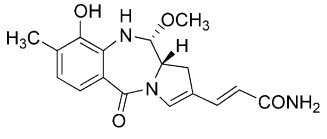
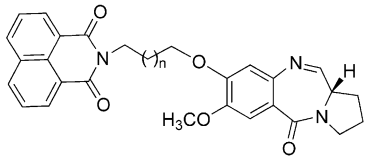


anthramycin



n = 1-3, 6, 8

1 a-e

Confalone and coworkers[4] have synthesized PBD analogues with an epoxide group attached at C-11a position with an objective of producing PBD system with DNA cross-linking activity. Recently, Thurston and coworkers, in order to increase cytotoxicity through the production of DNA cross-links, have designed and synthesized PBD dimers[5] comprising of two PBD units joined through their A-rings. Similarly, Baraldi and coworkers[6] as well as Lown and coworkers[7] have prepared new distamycin–PBD and lexitropin–PBD hybrids to explore their biological properties. We have also recently designed and synthesized non-cross-linking mixed imine–amide PBD dimers that have significant DNA-binding affinity and potent anti-tumour activity.[8] Naphthalimides are DNA intercalating agents with high anti-tumour activity[9] and two members of this class amonafide and mitonafide are in clinical trials. Therefore, it has been considered of interest to design and synthesize C-8 linked naphthalimide–PBD hybrids that could be attractive targets with a view to have a combination of both the DNA-binding and intercalating properties in the same molecule and may exhibit profound anti-tumour activity. We have been interested in the structural modifications of the PBD ring system and the development of new synthetic strategies.[10] In continuation of these efforts, we herein report the synthesis

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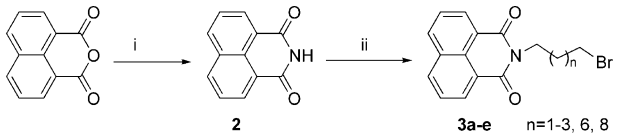
and in vitro anti-tumour activity of novel C-8 linked naphthalimide–PBD hybrids with different linker chain lengths.

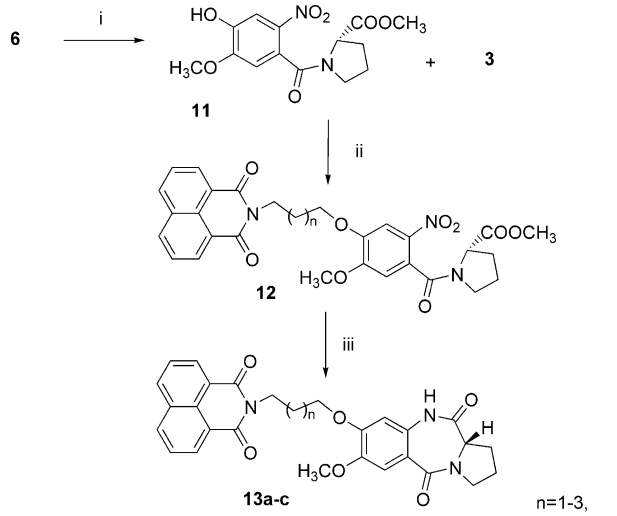
Synthesis of the naphthalimide–PBD hybrids[11] has been carried out employing the commercially available vanillin. Oxidation of vanillin followed by benzylation and nitration by literature methods provides the starting material (**5**). L-Proline methylester has been coupled to **5** to give the nitroester **6**. This nitroester **6** on treatment with DIBAL-H followed by protection of aldehyde with ethanethiol gives diethyl thioacetal (**7**). This upon debenzylation with $BF_3{\cdot}OEt_3$/EtSH provides the compound **8**. This nitro-diethyl thioacetal **8** has been coupled with appropriate bromo-*N*-alkyl-1,8-naphthalimide, which was prepared from commercially available naphthalic anhydride (Scheme 1) to give C-8 linked 1,8-naphthalimide nitro-diethyl thioacetal (**9**). The compound **9** is efficiently reduced employing $SnCl_2{\cdot}2H_2O$ to afford the corresponding amino thioacetal (**10**). Deprotection of amino diethyl thioacetal (**10**) with $HgCl_2$ and HgO affords the desired C-8 linked 1,8-naphthalimide-PBD imine[12] (**1**) as shown in Scheme 2.



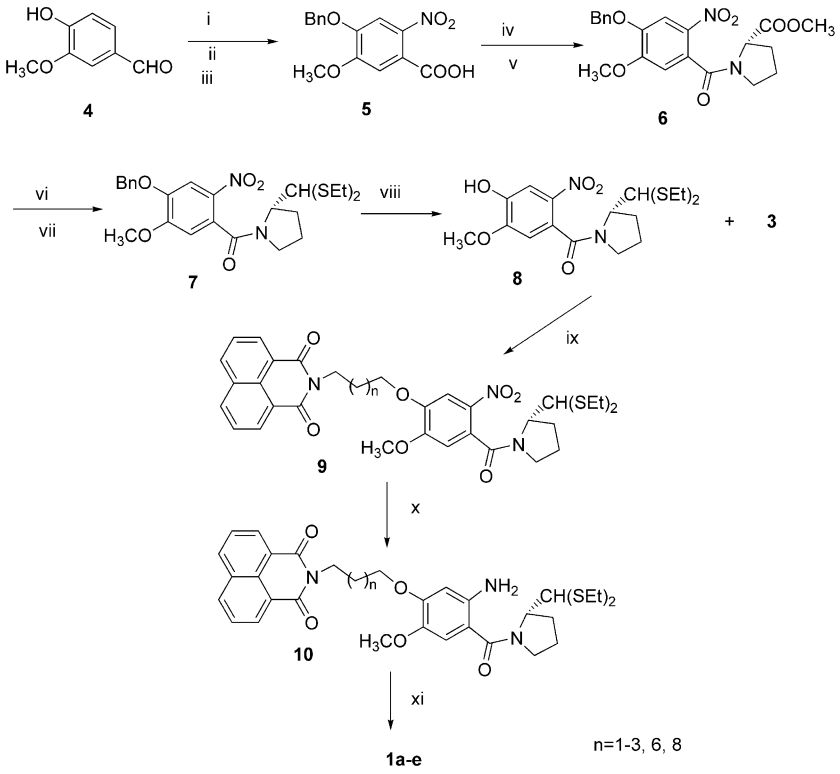
Scheme 1. (i) Liquid ammonia, reflux; (ii) dibromoalkane, K_2CO_3, acetone, reflux.

Further, compound **6** has been debenzylated with $BF_3{\cdot}OEt_3$/EtSH, and the resulting hydroxy compound is coupled with corresponding naphthalimide (**3**) to give the C-8 linked 1,8-naphthalimide nitroester (**12**). The nitroester (**12**) is reduced with $SnCl_2{\cdot}2H_2O$ to afford the desired naphthalimide linked PBD dilactam (**13**) as depicted in Scheme 3.

Interestingly, the data presented in Table 1 show that the size of the linker spacer plays an important role in this series of molecules for the anti-cancer activity.



Scheme 3. (i) $BF_3{\cdot}OEt_2$, EtSH, CH_2Cl_2; (ii) K_2CO_3, acetone, reflux; (iii) $SnCl_2{\cdot}2H_2O$, MeOH.



Scheme 2. (i) Sulphamic acid, $NaClO_2$; (ii) BnCl, NaOH; (iii) HNO_3/H_2SO_4, CH_2Cl_2, −25 °C; (iv) $SOCl_2$, C_6H_6, L-proline, TFA, THF; (v) H_2SO_4, MeOH, reflux; (vi) DIBAL-H, CH_2Cl_2, −78 °C; (vii) EtSH, TMSCl; (viii) $BF_3{\cdot}OEt_3$, EtSH, CH_2Cl_2; (ix) K_2CO_3, acetone, reflux; (x) $SnCl_2{\cdot}2H_2O$, MeOH; (xi) $HgCl_2$, HgO, CH_3CN/H_2O.

Table 1. Log LC_{50} (concentration in mol/L causing 50% lethality) values for compounds **1a**–**e**[a]

Cancer	1a	1b	1c	1d	1e
Leukaemia	−4.11	−4.14	−4.00	−4.11	−4.21
Non-small-cell lung	−4.05	−4.16	−4.34	−4.00	−4.08
Colon	−4.01	−4.34	−4.41	−4.08	−4.12
CNS	−4.00	−4.14	−4.00	−4.11	−4.29
Melanoma	−4.01	−4.23	−4.43	−4.09	−4.19
Ovarian	−4.01	−4.09	−4.06	−4.07	−4.11
Renal	−4.04	−4.57	−4.03	−4.02	−4.09
Prostate	−4.00	−4.21	−4.00	−4.03	−4.18
Breast	−4.00	−4.22	−4.36	−4.02	−4.07

[a]Each cancer type represents the average of six to eight different cancer cell lines. For each histolasic cancer type, the average −log LC_{50} value was determined from an NCI panel consisting of six to eight human cancer cell lines. The lower log LC_{50} values show the increase of cytotoxicity.

Compounds **1b** and **1c** have significant cytotoxic activity in the various types of cancer cell lines. It appears that cytotoxic activity is related to the length of the alkane chain spacer, thus allowing **1b** and **1c** with four- and five-carbon chain length for the proper snug fit in the minor groove of double helix DNA. Further, compound **1b** is more potent for colon and renal cancers, with compound **1c** likewise for colon and melanoma cancers. However, compounds **13a**–**c** did not exhibit any significant anti-cancer activity.

In summary, the new hybrid compounds synthesized by the combination of DNA-binding pyrrolobenzodiazepines and DNA-intercalating naphthalimides have exhibited promising in vitro anti-tumour activity and have the potential to be developed as novel anti-cancer agents. The detailed anti-cancer activity, molecular modelling studies and DNA binding affinity will be published in due course.

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12. Selected spectral data for **1c**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.2–2.4 (m, 10H), 3.5–3.8 (m, 4H), 3.9 (s, 3H), 4.0–4.3 (m, 3H), 6.8 (s, 1H), 7.5 (s, 1H), 7.65 (d, 1H, J = 4.6 Hz), 7.7 (t, 2H, J = 8.5 Hz), 8.2 (d, 2H, J = 8.2 Hz), 8.6 (d, 2H, J = 8 Hz). FABMS: m/z = 512 M + 1.