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Synthesis of C-8 Alkylamino Substituted Pyrrolo[2,1-*c*][1,4]-benzodiazepines as Potential Anti-Cancer Agents

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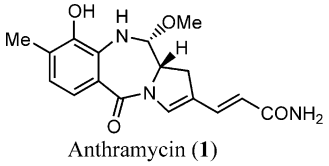
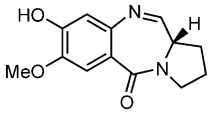
Abstract—The design and facile synthesis of C-8 alkylamino substituted pyrrolo[2,1-*c*][1,4]benzodiazepines is described. These have been prepared by linking the amines at C-8 position with propane spacer to improve solubility in water, and their in vitro cytotoxicity studies have been carried out.

Natural and synthetic pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are well known for their ability to bind covalently in the minor groove of DNA. Recently, there is much impetus for the PBD ring systems as they can recognize and bind to specific sequence of DNA.[1–5] Synthetic derivatives possessing PBD ring system are generating interest as potential anti-cancer and gene targeting agents due to their sequence specificity when binding to duplex DNA,[6–8] examples of naturally occurring PBDs include anthramycin, tomaymycin, sibiromycin and DC-81. The cytotoxicity and anti-tumour activity of these compounds are attributed to their property of covalent binding to the N-2 of guanine in the minor groove of duplex DNA via acid-labile aminal bond to the electrophilic imine at the N-10–C-11 position in a sequence selective manner. It has been well established that the imine functionality or its equivalent carbinolamine form is a primary requirement for the covalent binding.[9,10]

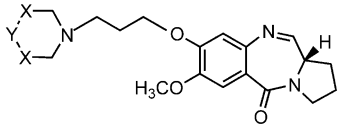
Recently a large number of structurally modified PBDs have been prepared and evaluated for their biological activity, particularly their anti-tumour potential.[11,12] A number of these compounds have been selected for pre-clinical studies but unfortunately most of them did not proceed beyond, due to problems related to water solubility. Therefore, it has been envisaged in the present work that incorporation of an amino functionality linked to the PBD ring system could improve its water solubility profile. We have been interested in the structural modifications of the PBD ring system and also towards the development of new synthetic strategies.[13–19] In continuation of these efforts, we report here the synthesis of PBD ring system linked to alkyl amines by a propane spacer at C-8 position with a view to enhance their lipophilicity.



Anthramycin (**1**)



DC-81 (**2**)



3a-c

a; X = CH_2, Y = O
b; X = CH_2, Y = N-CH_3
c; X = CH_3

The compounds (**3a–c**) were prepared from vanillic acid methyl ester **4** by its etherification with dibromo propane to afford **5**. The mono alkylation of **5** has been achieved by using 3 molar equivalents of the dibromo propane. Nitration of **5** followed by ester hydrolysis and coupling 2*S*-pyrrolidine methyl ester hydrochloride gives **8**, which is reduced with DIBAL-H to give corresponding aldehyde **9**. The aldehyde is protected with diethyl-thioacetal by using TMSCl–EtSH to afford **10**. The compound **10** is coupled with morpholine, *N*-methyl-piperzine and *N*,*N*-diethyl amine to give respectively the desired intermediates **11a–c**. The nitro thioacetal has

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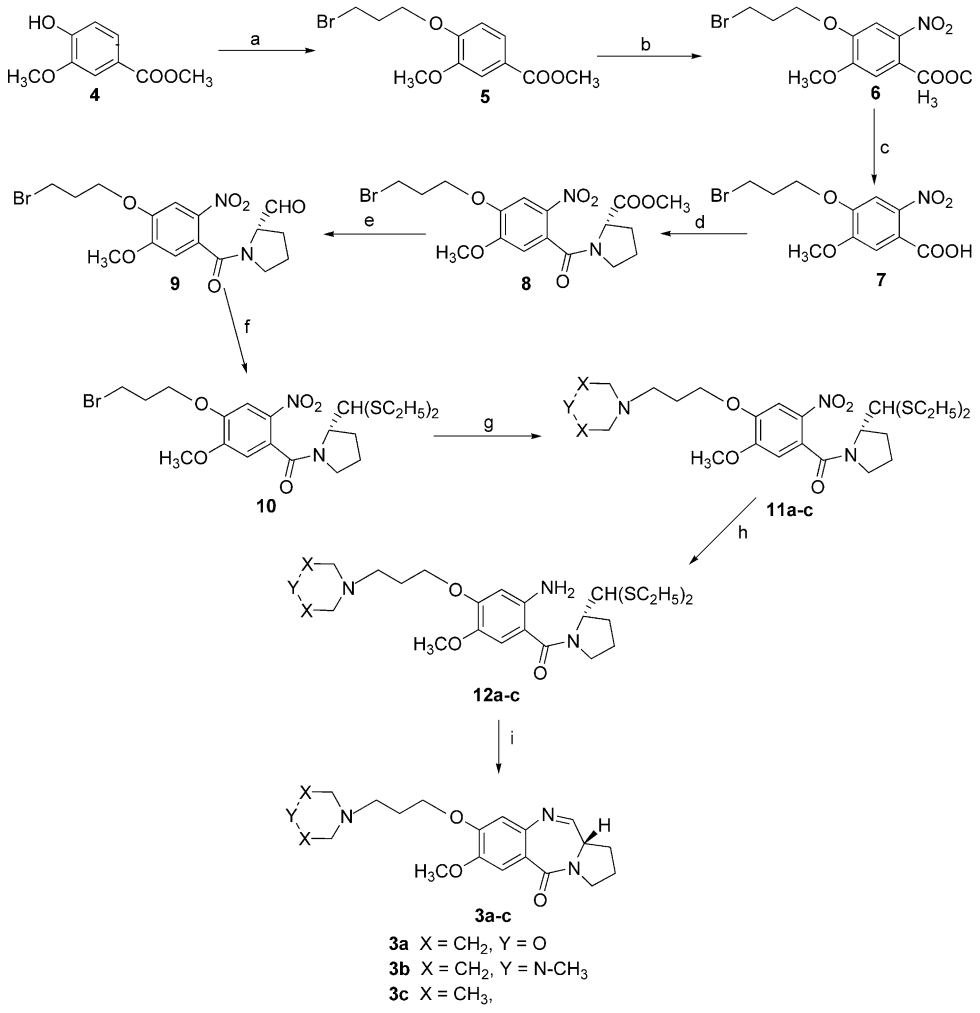

Scheme 1. (a) $Br(CH_2)_3Br$, K_2CO_3, CH_3COCH_3, reflux, 48 h, 82%; (b) $SnCl_4$–HNO_3, CH_2Cl_2, −25 °C, 5 min, 88%; (c) 1 M LiOH, THF–MeOH–H_2O (3:1:1), 12 h, rt, 90%; (d) $SOCl_2$, L-proline methyl ester hydrochloride, Et_3N, H_2O, 0 °C, 3 h, 80%; (e) DIBAL-H, CH_2Cl_2, −78 °C, 45 min, 65%; (f) EtSH–TMSCl, $CHCl_3$, rt 18 h, 82%; (g) morpholine or *N*-methyl piperazine or *N*,*N*-diethyl amine, K_2CO_3, CH_3CN, reflux, 48 h, 70–72%; (h) $SnCl_2{\cdot}2H_2O$, MeOH, reflux, 40 min, 70–75%; (i) $HgCl_2$, $CaCO_3$, CH_3CN–H_2O (4:1), 10 h, 50–52%.

been reduced with $SnCl_2{\cdot}2H_2O$ in methanol to give **12a–c**. Deprotection of the thioacetal group by using the method of Thurston and co-workers afforded the target molecules (**3a–c**) (Scheme 1).[20]

A series of PBD analogues (**3a–c**) were tested at the National Cancer Institute (Bethesda, MD, USA) using a Developmental Therapeutics Program involving tumour cell line cytotoxicity. From these screenings GI_{50} and LC_{50} values were obtained. The GI_{50} value is a response parameter and represents the concentration of the compound that induces 50% cell line growth inhibition. The LC_{50} value represents the compound concentration in mol/L causing 50% lethality (Table 1). Each cancer type represents the arithmetic average of six to eight different cancer cell lines. These compounds show moderate cytotoxic activity against some cancer cell lines. It is observed from the cytotoxicity data that the *N*-methylpiperizine linked to the PBD moiety (**3b**) has promising activity in comparison to the other alkyl amines like morpholine and *N*,*N*-diethyl amine and interestingly this compound is more potent for the melanoma cell lines.

Table 1. Log LC_{50} (concentration in mol/L causing 50% lethality) values for compounds **3a–c**[a] in different cancer cell lines

Cancer	LC_{50} values		
	3a	**3b**	**3c**
Leukaemia	−4.00	−4.28	−4.00
Non-small-cell lung	−4.02	−4.17	−4.00
Colon	−4.18	−4.26	−4.06
CNS	−4.02	−4.19	−4.00
Melanoma	−4.06	−4.51	−4.03
Ovarian	−4.00	−4.21	−4.00
Renal	−4.01	−4.25	−4.00
Prostate	−4.00	−4.00	−4.00
Breast	−4.01	−4.19	−4.00

[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.

In conclusion, new C-8 alkylamino linked PBDs have been synthesized which exhibit cytotoxic activity in some cancer cell lines. Moreover, these compounds with improved lyophilicity are promising for the develop-

ment of new cytotoxic agents and for which detailed investigations are in progress.

Acknowledgements

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20. Selected data for compound **3a**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.95–2.40 (m, 6H), 2.45–2.60 (m, 6H), 3.50–3.90 (m, 7H), 3.95 (s, 3H), 4.05–4.20 (m, 2H), 6.80 (s, 1H), 7.48 (s, 1H), 7.65 (d, 1H); MS (EI) *m*/*z* 373. Compound **3b**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.75–2.15 (m, 6H), 2.35 (s, 3H), 2.45–2.75 (m, 10H) 3.50–3.90 (m, 3H), 3.98 (s, 3H), 4.05–4.20 (m, 2H), 6.85 (s, 1H), 7.55 (s, 1H), 7.68 (d, 1H); MS (EI) *m*/*z* 386. Compound **3c**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.50–2.40 (m, 8H), 2.90–3.20 (m, 10H) 3.50–3.90 (m, 3H), 3.98 (s, 3H), 4.05–4.20 (m, 2H), 6.80 (s, 1H), 7.48 (s, 1H), 7.65 (d, 1H); MS (EI) *m*/*z* 359.