Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 2669–2672
Bioorganic & Medicinal Chemistry Letters

The effect of C2-fluoro group on the biological activity of DC-81 and its dimers

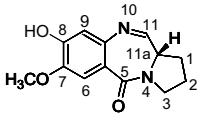
Ahmed Kamal,* P. S. M. M. Reddy and D. Rajasekhar Reddy

Biotransformation Laboratory, Division of Organic Chemistry, Indian Institute of Chemical Technology, Hyderabad 500007, India

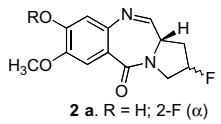
Received 27 October 2003; accepted 10 February 2004

Abstract—C2-Fluoro substituted pyrrolobenzodiazepines (PBDs) have been synthesized that exhibit potential anticancer activity in a number of human tumour cell lines. These C2-fluoro substituted PBDs also exhibit significant DNA-binding ability.

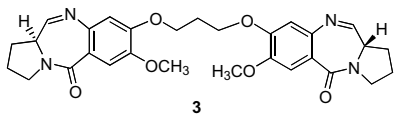
A number of pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) possessing antibiotic and antitumour activities have been isolated from *Streptomyces* species.[1] Later extensive studies have been devoted towards the structural modifications of the ring system particularly by linking this ring system to other DNA-interactive moieties[2] apart from the synthesis of different type of dimers of this class of compounds.[3] Several naturally occurring PBDs that have been isolated exhibit remarkable cytotoxicity particularly with substitutions in the C-ring.[4] In order to understand the structure–activity relationship studies many synthetic analogues have been prepared mainly with modifications and substitutions in the aromatic A-ring and not much attention has been given to the C-ring modifications. Recently, C2-aryl substituted PBDs have been prepared that have exhibited selective cytotoxicity at the sub-nanomolar level towards melanoma and ovarian cancer cell lines.[5] Furthermore, the discovery of the antimetabolic properties of fluoroacetic acid[6] and fluorouracil[7] initiated interest incorporating fluorine into other structures for exploring the effect on the anticancer properties such as in case of combretastatin A-4.[8] There are also some recent reports in the literature wherein fluorinated analogues have improved the biological activity profile of some pharmacologically important compounds.[9,10]



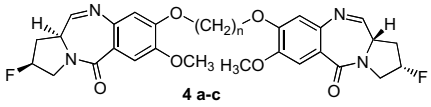
1



2 a. R = H; 2-F (α)
b. R = benzyl; 2-F (α)
c. R= H; 2-F (β)



3



4 a-c

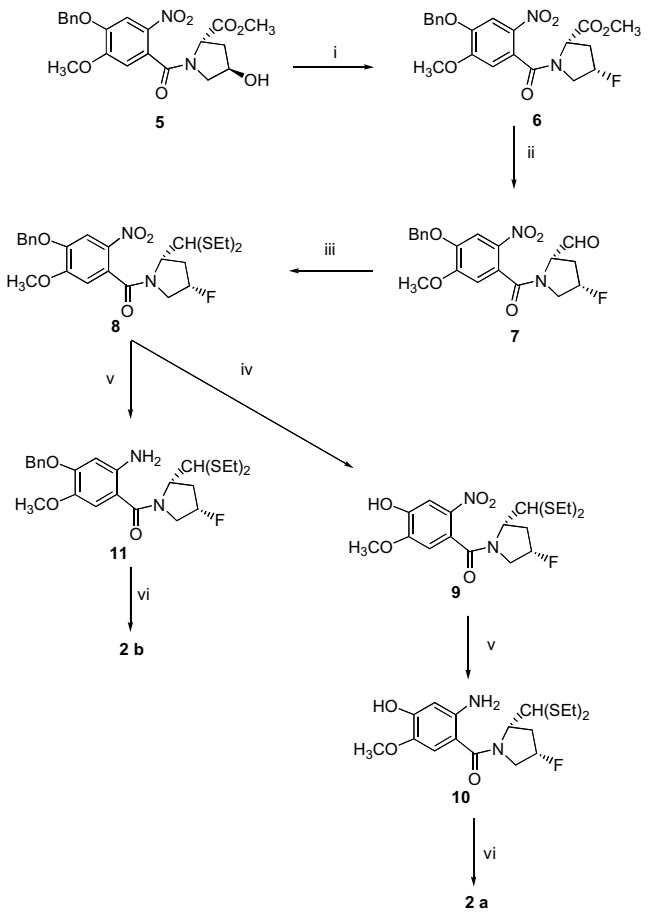
where n = 3, 4, 5

Our interest in the design and synthesis of PBDs has led to the development of some new PBD hybrids with potential antitumour activity. In continuation of these efforts, it has been considered of interest to prepare C2-fluoro substituted DC-81 and its dimers. During the course of this work a report has appeared on the synthesis of C2-fluorinated A-ring unsubstituted PBDs with some enhancement in cytotoxicity and marginal increase of ΔT_m values in comparison to DC-81.[11]

The preparation of these C2-fluoro substituted PBDs has been carried out by employing (2*S*)-*N*-[4-benzyloxy-5-methoxy-2-nitrobenzoyl]-4-fluoropyrrolidine-2-carboxylate **6** as one of the starting material and this has

Keywords: C2-Fluoropyrrolobenzodiazepines; Cytotoxicity and DNA-binding affinity.
* Corresponding author. Tel.: +91-40-27193157; fax: +91-40-271931-89; e-mail: ahmedkamal@iict.ap.nic.in

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.02.063



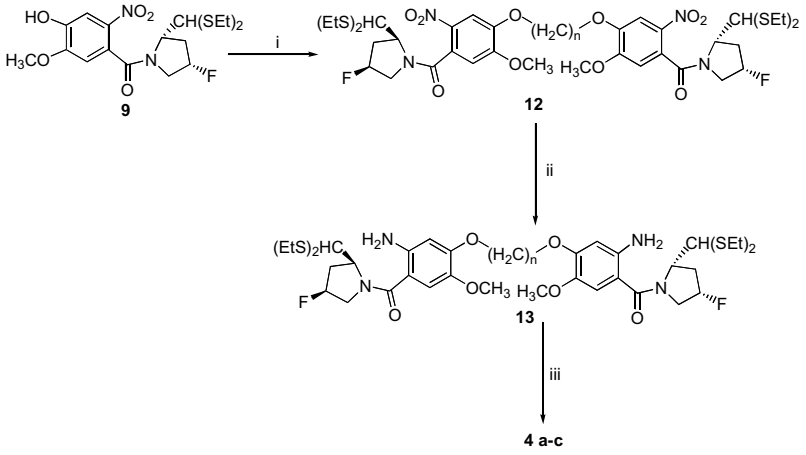
Scheme 1. Reagents and conditions: (i) DAST, CH_2Cl_2, −78 °C, 12 h, rt, 85%; (ii) DIBAL-H, CH_2Cl_2, −78 °C, 45 min, 80–85%; (iii) EtSH–TMSCl, $CHCl_3$, 24 h, rt, 90%; (iv) EtSH–BF_3OEt_2, CH_2Cl_2, 12 h, rt, 75%; (v) $SnCl_2{\cdot}2H_2O$, CH_3OH, reflux, 2 h, 80–85%; (vi) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O, 12 h, rt, 68–86%.

been obtained by the treatment of the (2*S*)-*N*-(4-benzyloxy-5-methoxy-2-nitrobenzoyl)-4-hydroxypyrrolidine-2-carboxylate **5** by DAST in CH_2Cl_2 (Scheme 1). Whereas, (2*S*)-*N*-(4-benzyloxy-5-methoxy-2-nitrobenzoyl)-4-fluoropyrrolidine-2-carboxaldehyde diethyl thioacetal **8** has been prepared by literature method,[12] which upon debenzylation gives **9**. Further, these upon reduction and followed by deprotection of aminothioacetal precursors (**10** and **11**) afford the target C2-fluoro substituted PBDs **2a**–**b** as shown in Scheme 1.[13]

The synthesis of the dimers has been carried out by the etherification of (2*S*)-*N*-(4-hydroxy-5-methoxy-2-nitrobenzoyl)-4-fluoropyrrolidine-2-carboxaldehyde diethyl thioacetal **9** with dibromoalkanes to provide **12a**–**c**. Further, these upon reduction and followed by deprotection of aminothioacetal precursors (**13a**–**c**) afford the desired C2-fluorinated PBD dimers **4a**–**c** in good yields (Scheme 2).[13]

Representative compounds **2a**, **2b**, **4a** and **4c** (Table 1) have been evaluated for the primary anticancer activity in the standard three-cell line panel consisting of the NCI-H460 (lung), MCF7 (breast), SF-268 (CNS). These fluorinated PBDs were also evaluated for in vitro anticancer activity in human 60-cell line panel. Amongst these **2b** and **4c** have exhibited promising anticancer activity. The GI_{50} value of compounds **2b** and **4c** (Table 2) against leukemia cancer (CCRF-CEM and SR) and renal (UO-31) cell lines is <0.01 μM whereas in CNS cancer panel in which SF-268, SF-295 cell lines affected, GI_{50} values are 0.01, <0.01, 0.03 and <0.01 μM, respectively. Compounds **2a**, **2b** and **4c** exhibited cytotoxic potency against breast cancer cell line HS-578T with GI_{50} values of 0.01 and <0.01 μM. The in vitro cytotoxicity (IC_{50}) for the naturally occurring DC-81[14] is 0.38, 0.33 and 0.1 μM whereas for DC-81 dimer (DSB-120)[14] is 0.01, 0.0005 and 0.003 μM in L1210, PC6 and CH1 cell lines, respectively. The in vitro cytotoxicity (IC_{50}) reported[11] for A-ring unsubstituted 2α-fluoro-PBD is 1.1, 0.22 and 1.9 μM in A2780, CH1 and L1210 cell lines, respectively, whereas for 2β-fluoro-PBD it is 0.43, 0.076 and 0.64 μM in A2780, CH1 and L1210 cell lines.

The DNA-binding ability of these new C2-fluorinated PBDs and their dimers have been investigated by thermal denaturation studies using calf thymus (CT) DNA



Scheme 2. Reagents and conditions: (i) dibromoalkanes, K_2CO_3, acetone, 36–48 h, reflux, 89–91%; (ii) $SnCl_2{\cdot}2H_2O$, CH_3OH, reflux, 2 h, 70–72%; (iii) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O, 12 h, rt, 65–70%.

Table 1. In vitro one dose primary anticancer assay[a] of C2-fluorinated PBDs

PBD	Growth percentages		
	(Lung) NCI-H460	(Breast) MCF7	(CNS) SF-268
2a	0	0	0
2b	1	0	19
4a	0	0	0
4c	0	0	0

[a] One dose of **2a**, **2b**, **4a** and **4c** at 10^{-4} M concentration.

Table 2. In vitro cytotoxicity of compounds **2a**, **2b**, **4a** and **4c** in selected human cancer cell lines

Cancer panel/cell line	GI_{50} (μM)			
	2a	**2b**	**4a**	**4c**
Leukemia				
CCRF-CEM	0.30	<0.01	—	<0.01
SR	0.20	<0.01	1.54	<0.01
Colon				
SW-620	1.19	<0.01	4.2	0.02
Non-small cell lung				
NCI-H522	0.41	<0.01	1.88	<0.01
CNS				
SF-268	0.35	0.01	2.2	<0.01
SF-295	0.32	0.03	1.96	<0.01
Renal				
UO-31	0.17	<0.01	2.40	<0.01
Breast				
HS-578T	0.01	<0.01	—	<0.01

at pH 7.0, incubated at 37 °C. In case of C2α-fluorinated DC-81 (**2a**) the helix melting temperature has marginally increased after 18 h of incubation in comparison to naturally occurring DC-81. The C8-benzylated C2-fluorinated DC-81 (**2b**) exhibited further enhancement in the melting temperatures. Similarly, C2β-fluorinated DC-81 (**2c**) exhibited slightly higher DNA melting temperatures compared to the α-isomer. On the other hand in case of C2-fluorinated dimers it is interesting to observe that C2-fluorinated dimer (**4a**) shows lower DNA melting temperatures compared to DC-81 dimer (**3**), while as the linker length increases from 3 to 5 the helix melting temperature of CT-DNA increases to 16 °C after incubation of 18 h for compound **4c** as shown in Table 3. It is well known in case of pyrrolobenzodiazepine compounds that substitution in A-ring have often exhibited higher DNA melting and in vitro antitumour activity, and this is true in case of present study when compared to the report on C2-fluorinated A-ring unsubstituted PBDs.[11]

In summary new C2-fluorinated DC-81 and its dimers have been synthesized that exhibits significant DNA-binding ability. Moreover, these new fluorinated compounds possess in vitro anticancer activity in a number of human cancer cell lines. The detailed mechanistic and molecular modeling studies for these fluorinated PBDs are in progress and further the effect of fluorination for the C2/C2′-*exo* unsaturated PBDs is also under investigation.

Table 3. Thermal denaturation data for fluorinated PBDs with CT-DNA

Compounds	[PBD]:[DNA] molar ratio[b]	ΔT_m (°C)[a] after incubation at 37 °C for		
		0 h	18 h	36 h
2a	1:5	0.3	1.0	2.2
2b	1:5	1.1	2.0	2.5
2c	1:5	0.6	1.5	2.25
4a	1:5	3.1	4.9	6.16
4b	1:5	4.6	12.3	13.8
4c	1:5	14.2	16.0	17.4
3	1:5	10.2	15.4	
1	1:5	0.3	0.7	

[a] For CT-DNA alone at pH 7.00 ± 0.01, $T_m = 69.8$ °C ± 0.01 (mean value from 10 separate determinations), all ΔT_m values are ±0.1–0.2 °C.

[b] For a 1:5 molar ratio of [PBD]/[DNA], where CT-DNA concentration = 100 μM and ligand concentration = 20 μM in aqueous sodium phosphate buffer (10 mM sodium phosphate+1 mM EDTA, pH 7.00 ± 0.01).

Acknowledgements

We thank the National Cancer Institute, Maryland, USA for the in vitro anticancer assay in human cancer cell lines. One of the authors P.S.M.M.R. is grateful to CSIR, New Delhi for the award of research fellowship.

References and notes

1. Kamal, A.; Rao, M. V.; Laxman, N.; Ramesh, G.; Reddy, G. S. K. *Curr. Med. Chem.—Anti-Cancer Agents* **2002**, *2*, 215.
2. (a) Kamal, A.; Ramesh, G.; Ramulu, P.; Srinivas, O.; Rehana, T.; Sheelu, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3451; (b) Kamal, A.; Ramulu, P.; Srinivas, O.; Ramesh, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3517; (c) Kamal, A.; Srinivas, O.; Ramulu, P.; Ramesh, G.; Kumar, P. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3577; (d) Zhou, Q.; Duan, W.; Simmons, D.; Shayo, Y.; Raymond, M. A.; Dorr, R. T.; Hurley, L. H. *J. Am. Chem. Soc.* **2001**, *123*, 4865; (e) Tercel, M.; Stribbling, S. M.; Sheppard, H.; Siim, B. G.; Wu, K.; Pullen, S. M.; Botting, K. J.; Wilson, W. R.; Denny, W. A. *J. Med. Chem.* **2003**, *46*, 2132; (f) Reddy, B. S. P.; Damayanthi, Y.; Reddy, B. S. N.; Lown, W. J. *Anti-Cancer Drug Des.* **2000**, *15*, 225; (g) Kamal, A.; Laxman, N.; Ramesh, G.; Srinivas, O.; Ramulu, P. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1917; (h) Kamal, A.; Reddy, B. S. N.; Reddy, G. S. K.; Ramesh, G. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1933.
3. (a) Kamal, A.; Ramesh, G.; Laxman, N.; Ramulu, P.; Srinivas, O.; Neelima, K.; Kondapi, A. K.; Sreenu, V. B.; Nagarajaram, H. A. *J. Med. Chem.* **2002**, *45*, 4679; (b) Gregson, S. J.; Howard, P. W.; Hartley, J. A.; Brooks, N. A.; Adams, L. J.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. *J. Med. Chem.* **2001**, *44*, 737; (c) Kamal, A.; Ramulu, P.; Srinivas, O.; Ramesh, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3955.

4. (a) Tozuka, Z.; Takasugi, H.; Takaya, T. *J. Antibiot.* **1983**, *36*, 276; (b) Tozuka, Z.; Yazawa, H.; Murata, M.; Takaya, T. *J. Antibiot.* **1983**, *36*, 1699.
5. (a) Cooper, N.; Hagan, D. R.; Tiberghein, A.; Ademefun, T.; Matthews, C. S.; Howard, P. W.; Thurston, D. E. *Chem. Commun.* **2002**, 1764; (b) Kang, G.-D.; Howard, P. W.; Thurston, D. E. *Chem. Commun.* **2003**, 1688.
6. Peters, R. *Endeavour* **1954**, *13*, 147.
7. Duschinsky, R.; Pleven, E.; Heidelberger, C. *J. Am. Chem. Soc.* **1957**, *79*, 4559.
8. Lawrence, N. J.; Hepworth, L. A.; Rennison, D.; McGown, A. T.; Hadfield, J. A. *J. Fluorine Chem.* **2003**, *123*, 101.
9. Ming Pu, Y.; Torok, D. S.; Ziffer, H. *J. Med. Chem.* **1995**, *38*, 4120.
10. Wu, Y.-J.; Davis, C. D.; Dworetzky, S.; Fitzpatrick, W. C.; Harden, D.; He, H.; Knox, R. J.; Newton, A. E.; Philip, T.; Polson, C.; Sivarao, D. V.; Sun, L.-Q.; Tertyshnikova, S.; Weaver, D.; Yeola, S.; Zoeckler, M.; Sinz, M. W. *J. Med. Chem.* **2003**, *46*, 3778.
11. O'Neil, I. A.; Thompson, S.; Kalindjian, S. B.; Jenkins, T. C. *Tetrahedron Lett.* **2003**, *44*, 7809.
12. Thurston, D. E.; Murty, V. S.; Langley, D. R.; Jones, G. B. *Synthesis* **1990**, 81.
13. Spectral data for compound **2a**: ^{1}H NMR ($CDCl_3$) δ 2.58–2.76 (m, 2H), 3.56 (m, 3H), 3.98 (s, 3H), 5.35 (dt, 1H, $J = 53.0$, 5.2 Hz), 6.95 (s, 1H), 7.56 (s, 1H), 7.82 (d, 1H, $J = 4.2$ Hz); MS 264 $(M+H)^{+\cdot}$. Compound **4c**: ^{1}H NMR ($CDCl_3$) δ 1.58–1.81 (m, 4H), 1.90–2.01 (m, 2H), 2.38–2.50 (m, 4H), 3.08–3.24 (m, 6H), 3.95 (s, 6H), 4.01–4.20 (m, 4H), 5.39 (dt, 2H, $J = 52.5$, 5.3 Hz), 6.81 (s, 2H), 7.49 (s, 2H), 7.83 (d, 2H, $J = 4.4$ Hz); FABMS 596 $(M+H)^+$.
14. Bose, D. S.; Thompson, A. S.; Ching, J.; Hartley, J. A.; Berardini, M. D.; Jenkins, T. C.; Neidle, S.; Hurley, L. H.; Thurston, D. E. *J. Am. Chem. Soc.* **1992**, *114*, 4939.