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Bioorganic & Medicinal Chemistry Letters 18 (2008) 2594–2598
Bioorganic & Medicinal Chemistry Letters

Synthesis of new benzimidazole linked pyrrolo[2,1-*c*][1,4]benzodiazepine conjugates with efficient DNA-binding affinity and potent cytotoxicity

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Received 25 August 2007; revised 16 February 2008; accepted 14 March 2008
Available online 16 March 2008

Abstract—The synthesis of new benzimidazole linked pyrrolobenzodiazepine conjugates is described. Some of these conjugates show significant DNA-binding affinity and, a representative compound **4c** shows promising in vitro cytotoxicity against a number of human cancer cell lines.

Benzimidazole moiety is structurally related to purine bases and is found in a variety of naturally occurring compounds such as vitamin B_{12}. The benzimidazoles are potent antitumour,[1] antifungal[2] and antiparasitic agents,[3] whose mode of action is thought to result from their inhibition of microtubule formations.[4] Substituted benzimidazoles have proven as drug leads, which have exhibited pharmacological interest.[5] A series of 2-substituted benzimidazole-4-carboxamides (**1**) have been synthesized and evaluated for in vitro and in vivo antitumour activity and DNA-binding affinity.[1] Moreover, the architecture of benzimidazole moiety as a new platform for the DNA-minor groove recognition elements[6] for, selective base pair recognition can be achieved by introduction of heteroatoms and substituents in this ring system.[7] This ring system can also be considered as a new tool for the target specific transcription factor at the binding sites relevant to biological systems. Recently, Dervan and co-workers reported the down-regulation of the angiogenetic vascular endothelial growth factor (VEGF) by a DNA-binding fluorescein–polyamide conjugate in cell culture.[8]

On the other side, there is considerable interest in the development of low molecular weight DNA-binding agents towards their application on various biological responses particularly anticancer activity. In this context pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a group of potent DNA interactive antitumour antibiotics derived from *Streptomyces* species,[9] well-known members include DC-81 (**2**), anthramycin, chicamycin and tomaymycin. Their interaction with DNA has been extensively investigated and it is considered unique since they bind within the minor groove of duplex DNA forming a covalent aminal bond with N2-amino group of guanine base,[10] giving rise to preference for Pu–G–Pu sequences.[11] PBDs containing an N10–C11 lactam moiety instead of an electrophilic N10–C11 imine or carbinolamine, and thus unable to interact covalently with DNA, are also known. For example, Kaneko and co-workers first reported[12] that the PBD dilactam **3a** has significant in vivo antitumour activity in a P388 lymphocytic leukaemia mouse model but did not propose a mechanism of action. Jones and co-workers followed up this observation by demonstrating, through DNA thermal denaturation studies, that dilactam **3a** and a number of related analogues such as **3b** can still bind to DNA but through a non-covalent mechanism which was suggested to account for the biological activity[13,14] (Fig. 1).

In the literature, some PBD conjugates have been synthesized and evaluated for their biological activity, particularly for their antitumour potential.[15–17] Wang and co-workers have synthesized indole linked PBD conjugates as potential antitumour agents.[18] We have also been engaged for the last few years towards the structural modifications[19–21] and the development of new synthetic strategies[22–24] for this ring system. Recently, we have designed and synthesized a number of PBD hy-

Keywords: Pyrrolobenzodiazepine; Benzimidazoles; In vitro cytotoxicity; DNA-binding affinity.
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doi:10.1016/j.bmcl.2008.03.039

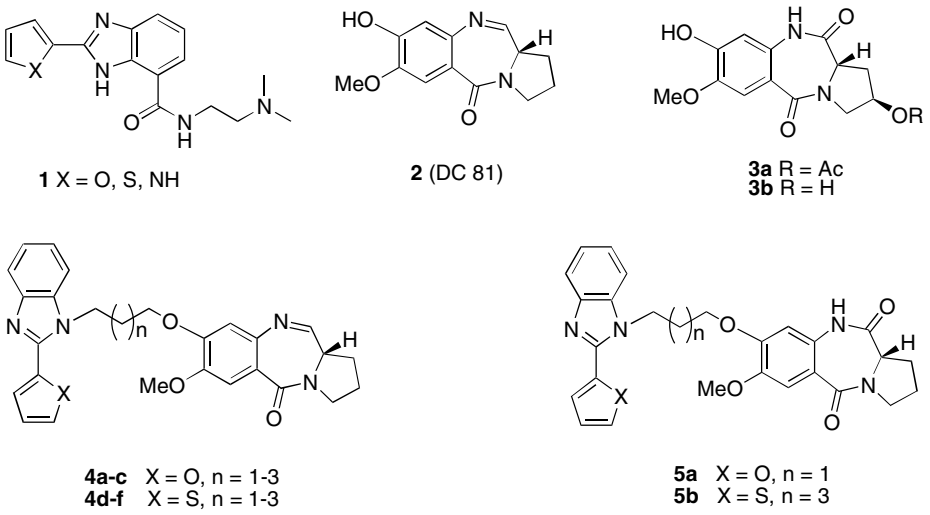
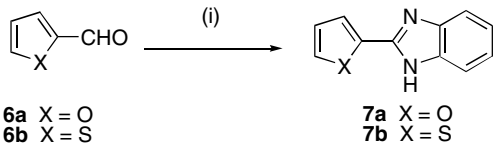
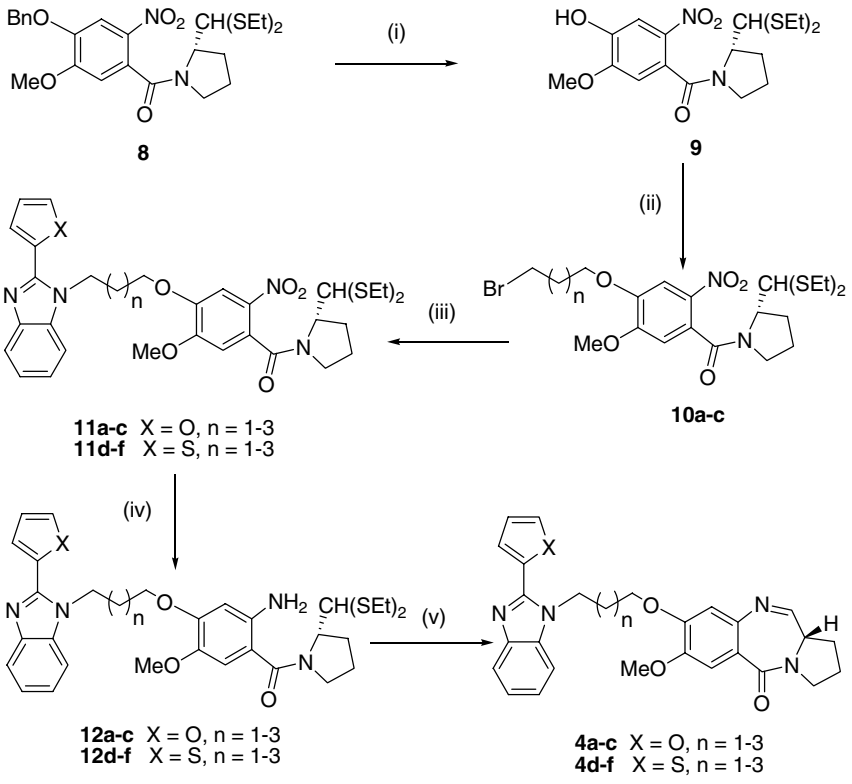


Figure 1. Chemical structures of 2-substituted benzimidazole-4-carboxamide (**1**), DC-81 (**2**), PBD dilactoms (**3**), new benzimidazole–PBD conjugates (**4a**–**f**) and benzimidazole–PBD dilactom conjugates (**5a**–**b**).

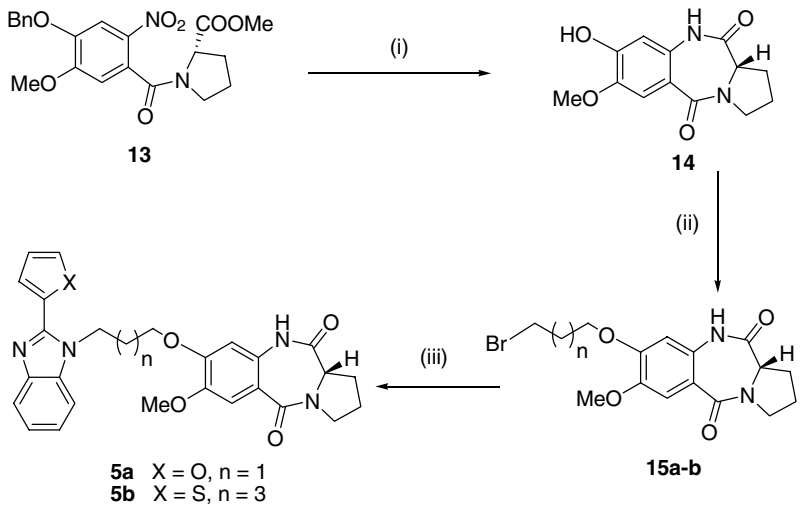
brids and amongst them C8-linked PBD–benzimidazole conjugates exhibited remarkable DNA-binding affinity.[25] In continuation of these efforts, we herein report the synthesis of new benzimidazoles linked to pyrrolo-[2,1-*c*][1,4]benzodiazepine conjugates as a significant and important aspect in the development of novel PBD conjugates with potential anticancer activity and DNA-binding ability.[26]



Scheme 1. Reagents and condition: (i) *O*-phenelenediamine, $Na_2S_2O_5$, EtOH, reflux, 4 h, 75%.



Scheme 2. Reagents and conditions: (i) EtSH–BF_3OEt_2, CH_2Cl_2, 12 h, rt, 75%; (ii) dibromoalkanes, K_2CO_3, DMF, rt, 28 h, 70%; (iii) **7a**–**b**, K_2CO_3, acetone, reflux, 24 h, 70%; (iv) $SnCl_2 2H_2O$, MeOH, reflux, 60 min, 70%; (v) $HgCl_2$–$CaCO_3$, CH_3CN/H_2O (4:1), 8–12 h, 52%.



Scheme 3. Reagents and conditions: (i) 10% Pd/C, H_2, EtOH, 8 h, rt, 65%; (ii) dibromoalkanes, K_2CO_3, DMF, rt, 28 h, 75%; (iii) **7a**–**b**, K_2CO_3, acetone, reflux, 24 h, 70%.

Synthesis of benzimidazole–PBD conjugates **4a**–**f** has been carried out by employing the benzyloxy nitrothioacetal compound **8** as the starting material, which has been prepared by the literature method.[27] This upon debenzylation gives hydroxy nitrothioacetal compound **9** and further etherification by dibromoalkanes provide monoalkylated compounds **10a**–**c**. The oxidative cyclization of *O*-phenylenediamine and aldehyde compounds **6a**–**b** with $Na_2S_2O_5$ in ethanol provides the benzimidazole compounds **7a**–**b** (Scheme 1).[28] Further, N-alkylation of these benzimidazole compounds with monoalkylated compounds **10a**–**c** give the benzimidazole linked nitrothioacetal precursors **11a**–**c**, which upon reduction followed by deprotection of thioacetal group afford the target imine compounds **4a**–**f** (Scheme 2).[29]

Synthesis of dilactam compounds **5a**–**b** has been carried out by employing the benzylated ester compound **13** as the starting material, which was prepared by the literature method[30] and further debenzylation followed by reductive cyclization with 10% Pd/C–H_2 provides hydroxydilactom compound **14**. This on monoalkylation by dibromoalkanes provides monoalkylated dilactom compounds **15a**–**b**. The benzimidazole compounds **7a**–**b** coupled with monoalkylated dilactom compounds **15a**–**b** afford the desired final dilactom compounds **5a**–**b** (Scheme 3).[31]

The DNA-binding ability of the novel PBD conjugates (**4a**–**f** and **5a**–**b**) has been investigated by thermal denaturation studies using calf thymus (CT) DNA at pH 7.0, incubated at 37 °C, where PBD/DNA molar ratio is 1:5.[32] In this assay, it is interesting to observe that compounds **4a**, **4c**, **4d** and **4f** elevates the helix melting temperature of CT-DNA by 6.1, 7.0, 5.1 and 6.1 °C, respectively, after incubation for 18 h at 37 °C. The enhancement of DNA-binding ability of these conjugates can be correlated to other interactions produced by the benzimidazole component in addition to covalent linkage of the imine component. Moreover, for the compounds having odd number of carbon chain spacer there is a substantial increase in the DNA-binding affinity. Surprisingly, for compounds **4b** and **4e** having even number of carbon chain spacer possessing the ΔT_m values are almost negligible and particularly, compound **4b** has not exhibited any DNA-binding affinity. A similar phenomenon has been recently observed by us with other related PBD conjugates.[33] On the other hand, the dilactam PBD conjugates (**5a**–**b**) also show lower ΔT_m values due to the absence of imine functionality, that is, absence of covalent binding in these molecules. Moreover the naturally occurring compound DC-81 (**1**) exhibits a ΔT_m of 0.7 °C, under similar experimental conditions as illustrated in Table 1.

The restriction endonuclease inhibition studies carried out on these molecules also confirm the relative binding affinity of these PBD hybrids. The experimental proto-

Table 1. Thermal denaturation data for new benzimidazole–PBD conjugates with calf thymus DNA

PBD conjugates	[PBD]:[DNA] molar ratio[a]	ΔT_m[b] (°C) after incubation at 37 °C for	
		0 h	18 h
4a	1:5	5.1	6.1
4b	1:5	0.0	0.0
4c	1:5	5.1	7.0
4d	1:5	3.9	5.1
4e	1:5	0.1	1.0
4f	1:5	6.1	6.1
5a	1:5	0.3	0.7
5b	1:5	0.4	0.8
DC-81 (2)	1:5	0.3	0.7

[a] 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].

[b] For CT-DNA alone at pH 7.00 ± 0.01, T_m = 69.2 °C ± 0.01 (mean value from 10 separate determinations), all ΔT_m values are ±0.1–0.2 °C.

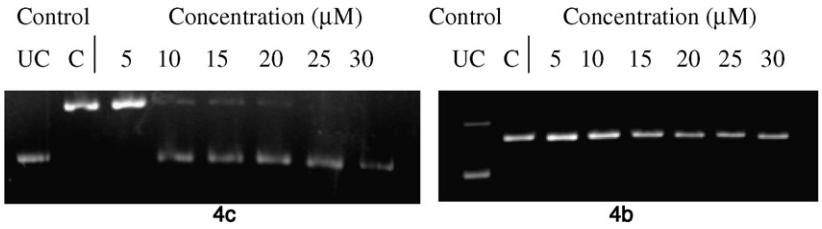


Figure 2. RED_{100}-restriction endonuclease digestion assay for benzimidazole–PBD conjugates with CT-DNA inhibitory activity of **4b** and **4c** on the cleavage of plasmid pBR322 by restriction endonuclease BamH1 (20 U in 2 μL) for 1 h at 37 °C. The cut (C) and uncut (UC) products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining under UV illumination. Lane 1, control pBR322; lane 2, complete digest of pBR322 by BamH1.

col described in the previous study has been employed.[32,34] The result of this experiment for a representative compound **4c** suggested that there is inhibition of BamH1 by this PBD hybrid. However, there is no inhibition by compound **4b** as shown in Figure 2. Similarly, compounds **5a** and **5b** have not shown noticeable inhibition of BamH1.

Compounds **4c** and **4e** have been evaluated in the 60 cell line cancer screen of NCI. Especially compound **4c** shows significant in vitro cytotoxic potency in a wide spectrum of cell lines in nine panels, with GI_{50} values less than 10 nM. Compound **4e** also possess cytotoxic potency against many cell lines with GI_{50} values ranging from 0.24 to 15.50 μM (Table 2). The GI_{50} values of compound **4e** against leukaemia cancer CCRF-CEM and RPMI-8226 cell lines are 0.24 and 0.31 μM, respectively. The in vitro cytotoxicity (IC_{50}) for the naturally occurring DC-81 is 0.38 and 0.33 μM in L1210 and PC6 cell lines, respectively.[35]

Table 2. In vitro cytotoxicity data for benzimidazole–PBD conjugates (**4c** and **4e**)

Panel/cell line	GI_{50} (μM)		Panel/cell line	GI_{50} (μM)	
	4c	**4e**		**4c**	**4e**
Leukemia			*Melanoma*		
CCRF-CEM	<0.01	0.24	LOX IMVI	<0.01	0.78
HL-60(TB)	<0.01	5.18	MALME-3M	0.01	1.74
K-562	<0.01	15.50	M14	<0.01	1.35
MOLT-4	<0.01	3.79	SK-MEL-2	0.02	1.47
RPMI-8226	<0.01	0.31	SK-MEL-28	<0.01	1.53
SR	<0.01	0.45	SK-MEL-5	<0.01	1.09
Lung cancer			UACC-257	<0.01	2.54
A549/ATCC	<0.01	3.22	UACC-62	<0.01	1.57
EKVX	<0.01	3.36	*Ovarian Cancer*		
HOP-62	<0.01	—	OVCAR-1	0.01	0.75
HOP-92	0.01	1.33	OVCAR-3	<0.01	1.72
NCI-H226	<0.01	2.13	OVCAR-4	<0.01	1.61
NCI-H23	<0.01	2.05	OVCAR-5	<0.01	3.27
NCI-H322M	0.03	2.70	OVCAR-8	<0.01	2.58
NCI-H460	<0.01	1.55	SK-OV-3	<0.01	3.32
NCI-H522	<0.01	1.04	*Renal cancer*		
Colon cancer			786-0	<0.01	1.71
COLO 205	<0.01	1.07	A498	<0.01	1.68
HCC-2998	—	2.08	ACHN	<0.01	2.81
HCT-116	<0.01	1.75	CAKI-1	<0.01	0.94
HCT-15	0.02	3.19	RXF 393	<0.01	1.34
HT29	<0.01	1.97	SN 12C	<0.01	2.50
KM12	<0.01	1.92	TK-10	0.01	2.09
SW-620	<0.01	1.84	UO-31	0.10	1.23
Breast cancer			*Prostate cancer*		
MCF7	<0.01	0.66	DU-145	0.02	1.32
NCI/ADR-RES	0.23	3.24	*CNS cancer*		
MDA-MB-231	0.01	1.39	SF-268	<0.01	1.80
HS 578T	<0.01	1.70	SF-539	<0.01	1.72
MDA-MB-435	<0.01	1.39	SNB-19	<0.01	1.53
BT-549	<0.01	0.41	SNB-75	<0.01	1.22
T-47D	<0.01	0.50	U251	<0.01	1.77
MDA-MB-468	<0.01	0.33			

In conclusion, new benzimidazole linked–PBD conjugates have been synthesized that exhibit significant DNA-binding ability. More importantly, compound **4c** exhibits potential in vitro cytotoxicity in a number of cancer cell lines. This investigation further reveals the significance of combining a non-covalent DNA-binding component (benzimidazole core) to the covalent binding PBD moiety. The detailed anticancer activity and molecular modelling studies will be published in due course.

Acknowledgments

We thank the National Cancer Institute (NCI), Maryland, for the primary anticancer assay in human cancer cell lines. We are also grateful to CSIR, New Delhi, for the award of research fellowships to P.P.K., K.S., B.N.S. and P.R.

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31. *Spectral data for compound (**4a**):* ^{1}H NMR ($CDCl_3$): δ 7.82 (d, J = 7.8 Hz, 1H), 7.67 (s, 2H), 7.55 (s, 1H), 7.42–7.52 (m, 3H), 7.07–7.19 (m, 2H), 6.72 (s, 1H), 5.06–5.18 (m, 1H), 4.58–4.76 (m, 2H), 4.05–4.15 (m, 1H), 4.00 (s, 3H), 3.78–3.90 (m, 1H), 3.65–3.75 (m, 1H), 3.45–3.65 (m, 1H), 2.38–2.49 (m, 1H), 2.25–2.36 (m, 1H), 1.95–2.15 (m, 4H); ESIMS: *m*/*z* 471 (M^++1).
*Compound (**4b**):* ^{1}H NMR ($CDCl_3$): δ 7.77 (d, J = 7.5 Hz, 1H), 7.62–7.69 (d, J = 3.7 Hz, 1H), 7.57 (s, 1H), 7.44–7.55 (m, 2H), 7.10–7.33 (m, 4H), 6.50 (s, 1H), 5.00–5.52 (m, 1H), 4.76 (t, J = 5.0 Hz, 1H), 4.65 (t, J = 6.7 Hz, 1H), 4.10–4.19 (m, 2H), 3.92 (s, 3H), 3.45–3.87 (m, 2H), 2.30–2.39 (m, 2H), 1.85–2.20 (m, 6H); ESIMS: *m*/*z* 485 (M^+).
*Compound (**4c**):* ^{1}H NMR ($CDCl_3$): δ 7.79 (d, J = 7.5 Hz, 1H), 7.63–7.68 (d, J = 3.7 Hz, 1H), 7.57 (s, 1H), 7.45–7.55 (m, 2H), 7.10–7.35 (m, 4H), 6.5 (s, 1H), 5.00–5.52 (m, 1H), 4.78 (t, J = 5.0 Hz), 4.67 (t, J = 6.7 Hz, 2H), 4.10–4.20 (m, 2H), 3.90 (s, 3H), 3.50–3.70 (m, 2H), 2.40–2.55 (m, 2H), 2.20–2.35 (m, 2H), 1.56–2.40 (m, 6H); ESIMS: *m*/*z* 499 (M^+).
*Compound (**4d**):* ^{1}H NMR ($CDCl_3$): δ 7.77–7.84 (m, 1H), 7.67 (d, J = 4.5 Hz, 1H), 7.56 (s, 1H), 7.48 (d, J = 4.53 Hz, 1H), 7.45 (d, J = 2.26 Hz,1H), 7.23–7.36 (m, 1H), 7.11–7.16 (m, 2H), 6.71 (s, 1H), 5.08–5.12 (m, 1H), 4.69 (t, J = 7.45 Hz, 2H), 4.04–4.18 (m, 1H), 3.99 (s, 3H), 3.78–3.92 (m, 1H), 3.50–3.75 (m, 2H), 2.25–2.50 (m, 4H), 2.00–2.15 (m, 2H); ESIMS: *m*/*z* 487 (M^++1).
*Compound (**4e**):* ^{1}H NMR ($CDCl_3$): δ 7.75–7.85 (m, 1H), 7.69 (d, J = 4.5 Hz, 1H), 7.54 (s, 1H), 7.50 (d, J = 4.5 Hz, 1H), 7.40–7.47 (m, 1H), 7.26–7.34 (m, 3H), 7.07–7.16 (dd, J = 3.7, J = 5.2 Hz, 1H), 6.80 (s, 1H), 4.94–5.04 (m, 1H), 4.53 (t, J = 7.5 Hz, 2H), 4.00–4.25 (m, 2H), 3.92 (s, 3H), 3.45–3.89 (m, 2H), 2.30–2.40 (m, 2H), 1.85–2.19 (m, 6H); ESIMS: *m*/*z* 501 (M^+).
*Compound (**4f**):* ^{1}H NMR ($CDCl_3$): δ 7.72–7.80 (dd, J = 1.7, J = 6.0 Hz, 1H), 7.62–7.65 (m, 1H), 7.52 (s, 1H), 7.45 (d, J = 4.3 Hz, 1H), 7.32–7.41 (m, 1H), 7.20–7.30 (m, 2H), 7.08–7.18 (m, 1H), 6.6 (s, 1H), 4.84 (d, J = 4.3 Hz, 1H), 4.64–4.76 (m, 3H), 4.05–4.15 (t, J = 4.3 Hz, 2H), 4.00 (s, 3H), 3.50–3.70 (m, 2H), 2.40–2.55 (m, 2H), 2.20–2.35 (m, 2H), 1.56–2.40 (m, 6H); ESIMS: *m*/*z* 516 (M^++1).
*Compound (**5a**):* ^{1}H NMR($CDCl_3$) δ 8.9 (s, 1H), 7.74 (d, J = 6.79 Hz, 1H), 7.55–7.40 (m, 2H), 7.37 (s, 1H), 7.36–7.16 (m, 3H), 7.15–7.08 (q, 1H), 6.32 (s, 1H), 4.40 (t, J = 7.5 Hz, 2H), 4.00 (d, J = 5.2 Hz, 1H), 3.7 (s, 3H), 3.6–3.48 (m, 2H), 2.7–2.67 (m, 2H), 2.20–2.00 (m, 4H), 1.84–1.64 (m, 2H), 1.60–1.48 (m, 2H), 1.20–1.36 (m, 2H); MS (ESI) 487 $[M]^+$.
*Compound (**5b**):* ^{1}H NMR ($CDCl_3$) δ 8.25 (s, 1H), 7.77 (d, J = 8.08 Hz, 1H), 7.40–7.56 (m, 3H), 7.17–7.30 (m, 3H), 6.57 (d, J = 2.20 Hz, 1H), 6.22 (s, 1H), 4.75 (t, J = 6.5 Hz, 2H), 3.81–4.11 (m, 6H), 3.52–3.83 (m, 2H), 2.42 (m, 2H), 1.96–2.12 (m, 4H); MS (ESI) 531 $[M]^+$.
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