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Bioorganic & Medicinal Chemistry Letters 13 (2003) 3577–3581

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis of Novel C2 and C2–C8 Linked Pyrrolo[2,1-*c*][1,4]benzodiazepine-naphthalimide Hybrids as DNA-Binding Agents

Ahmed Kamal,* O. Srinivas, P. Ramulu, G. Ramesh and P. Praveen Kumar

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

Received 2 May 2003; accepted 24 June 2003

Abstract—Synthesis of C2 and C2-C8 linked pyrrolobenzodiazepine-naphthalimide hybrids have been prepared that exhibit significant DNA-binding affinity and cytotoxicity.

The pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) antitumour antibiotics have generated interest as potential anticancer and gene-targeting agents.[1] These compounds show their biological activity by forming a covalent bond between the C11 position of the PBD and the exocyclic N2 group of guanine, giving rise to preference for Pu-G-Pu sequences.[2] Typical examples of PBD natural products such as tomaymycin and DC-81, possess the same aromatic substitution pattern. Moreover, tomaymycin possesses C2-*exo* unsaturation and its cytotoxic activity more than DC-81.[3,4] Thurston and coworkers have synthesized a series of novel C ring modified C2-*exo* and C2-C3-*endo* unsaturated pyrrolo[2,1-*c*][1,4]-benzodiazepines, that have shown interesting DNA binding ability and in vitro cytotoxicity.[5] Recently, C2-aryl substituted pyrrolobenzodiazepines have also been prepared with potent antitumour activity.[6] We have also designed and synthesized non-cross-linking mixed imine–amide PBD dimers that have significant DNA-binding ability and promising anticancer activity.[7]

Naphthalimides are DNA interclating agents with potential anti-cancer activity and two members of this class amonafide and mitonafide are in clinical trails.[8] These mono interclating chromophores have been linked by a flexible alkylamine chain to bis-naphthalimides (DMP-840,[9] LU 79553[10]), which are more potent than the corresponding monomers. Recently, we have reported the design, synthesis and biological evaluation of C8 linked PBD-naphthalimide hybrids as new anticancer agents.[11] Therefore, it has been considered of interest to design and synthesize C2 linked PBD-naphthalimide hybrids that could improve the DNA binding ability and biological activity. In this endeavor we have also synthesized novel C2–C8 linked PBD-naphthalimide hybrid to explore its DNA binding potential. This is in continuation to our efforts in the structural modification of PBD ring system[12] and also the development of new synthetic strategies[13] for this ring system. We herein report the synthesis, DNA-binding ability and antitumour activity of novel C2 and C2–C8 linked PBD–naphthalimide hybrids with different alkylamide spacers.

Synthesis of C2 linked PBD-naphthalimide hybrids has been carried out by employing 4-benzyloxy-5-methoxy-2-nitrobenzoic acid **7** as the starting material, which has been obtained by the procedure described in the literature.[14] *trans*-4-Hydroxy L-proline methylester hydrochloride has been coupled to compound **7** to give the nitro ester **8**. The hydroxy group is protected with TBDMS-Cl followed by reduction with DIBAL-H to produce the corresponding aldehyde, which is protected with EtSH/TMS-Cl. Surprisngly, in this reaction protection of aldehyde to diethyl thioacetal and deprotection of TBDMS takes place in the same step to afford the compound **11**. Then C2 hydroxy group is oxidized with TPAP/NMO to give compound **12**, which upon Horner-Emmons olefination with methyl (diethyl phosphono) acetate affords compound **13**. In this reaction,

*Corresponding author. Tel.: +91-40-2719-3159; fax: +91-40-2719-3189; e-mail: ahmedkamal@iict.ap.nic.in

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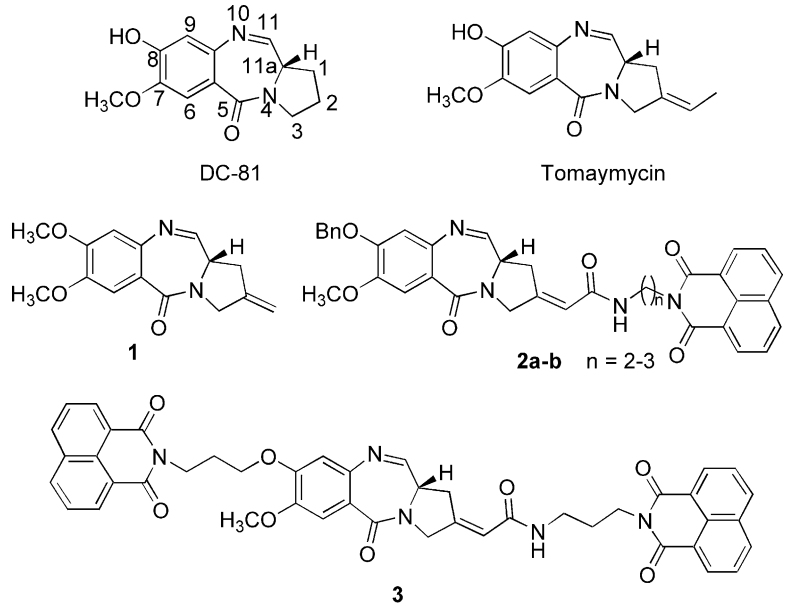
doi:10.1016/S0960-894X(03)00718-2



(E) ester[15] (**13**) has been obtained exclusively which upon hydrolysis affords the corresponding acid **14**.

The key intermediates **15a–b** have been prepared by amidation of compound **14** with **6a–b**, that have been obtained from commercially available 1,8-naphthalimide as shown in Scheme 1. The compounds **15a–b** are reduced with $SnCl_2{\cdot}2H_2O$ to afford the corresponding amino diethyl thioacetal (**16a–b**). Deprotection of amino diethyl thioacetal with $HgCl_2/CaCO_3$ provides the target molecules **2a–b**[16] (Scheme 2).

Compound **17** has been prepared by the methodology described in our previous report.[7] This upon protection with TBDMS-Cl, reduction followed by ethanethiol protection affords compound **20**. The coupling of this compound with 1,8-naphthalimide affords the intermediate **21**. This upon oxidation, Horner–Emmons olefination and ester hydrolysis gives compound **24**.

Table 1. In vitro one-dose primary anticancer assay[a] of C2 and C2–C8 linked PBD-naphthalimide hybrids

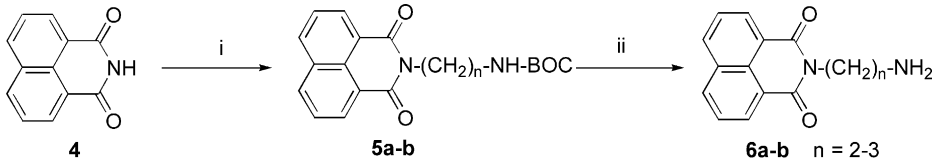
PBD hybrids	Growth percentages		
	(Lung) NCI-H460	(Breast) MCF7	(CNS) SF-268
2a	101	89	95
2b	0	0	1
3	102	86	97

[a]One dose of **2a–b** and **3** at 10^{-4} molar concentration.

Further amidation of the compound **24** with **6b** provides compound **25**. Then subsequent reduction followed by deprotection of diethyl thioacetal group affords the desired compound **3**[17] (Scheme 3).

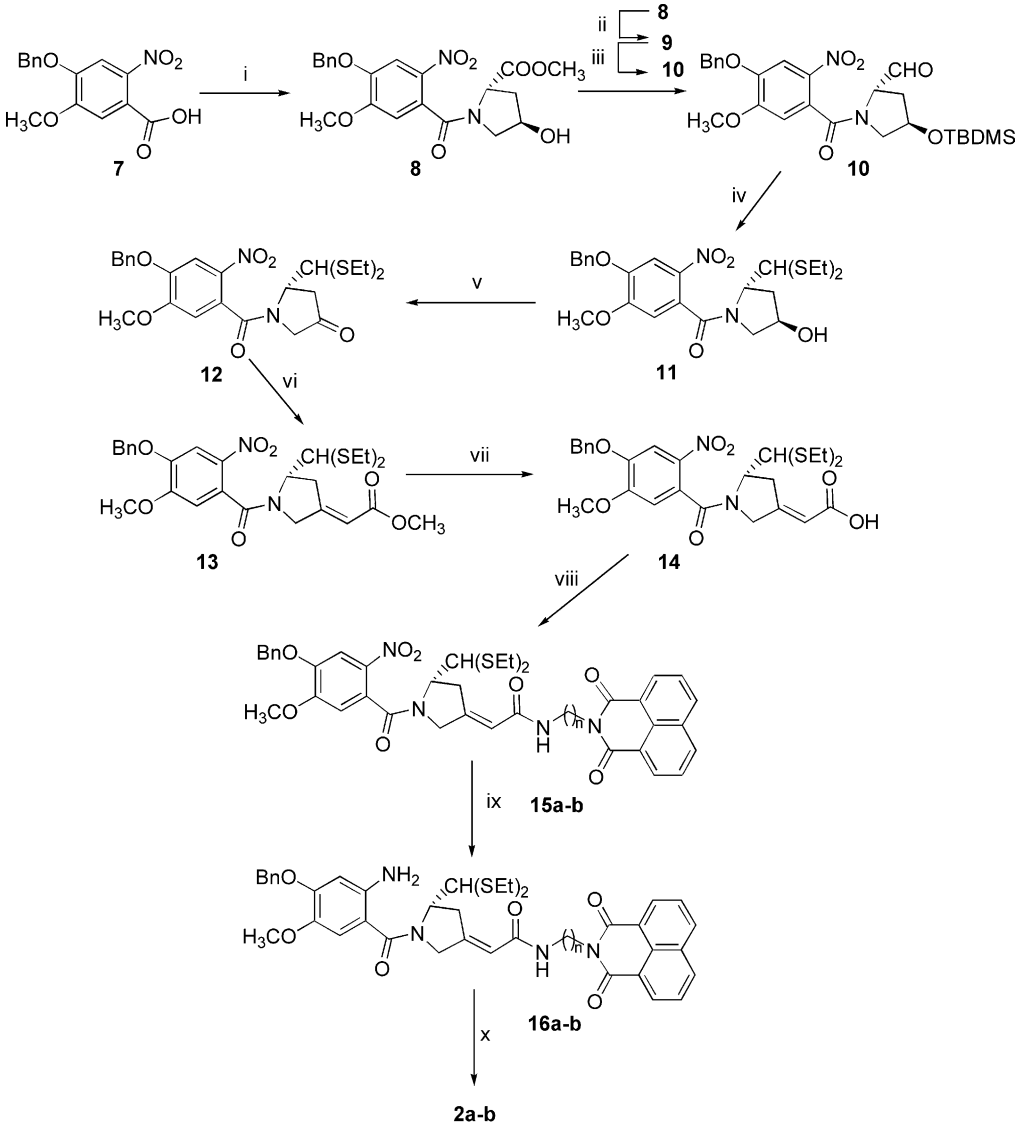
Compounds **2a–b** and **3** (Table 1) have been evaluated for the primary anticancer activity in the standard three-cell line panel consisting of the MCF7 (breast), NCI-H460 (lung) and SF-268 (CNS). Amongst these hybrids **2b** has promising anticancer activity in a 60-cell line panel. The GI_{50} value of compound **2b** (Table 2) against leukemia cancer CCRF-CEM, HL-60 (TB), MOLT-4 and SR cell lines are 0.062, 0.091, 0.055 and <0.01 μM respectively. Compound **2b** exhibits a cytotoxic potency in CNS cancer panel, in which SNB-19 cell line affected, with GI_{50} value of 0.080 μM. Compound **2b** exhibited cytotoxic potency against breast cancer cell line T-47D with GI_{50} value of 0.034 μM.

The DNA binding ability of these novel C2 and C2–C8 linked PBD-naphthalide hybrids has been investigated by thermal denaturation studies using calf thymus (CT) DNA at pH 7.0, incubated at 37 °C. It is interesting to observe that these hybrid molecules elevate the helix melting temperature of the CT-DNA significantly (Table 3). One of these hybrids (**2b**) elevates the helix melting temperature of CT-DNA by 13.5 °C after incubation for 18 h. In the same experiment the naturally occurring DC-81 having one imine group exhibits a ΔT_m of 0.7 °C. Further, synthetically designed C2-*exo* unsaturated PBDs have been reported[5] to exhibit lower



Scheme 1. (i) Br–$(CH_2)_n$–NHBOC, K_2CO_3, DMF, 12 h, rt, 94%; (ii) TFA, DCM, 0 °C, 8 h, 70%.

ΔT_m values (1.33–2.38 °C) and, for example, ΔT_m of compound **1** is 2.38 °C. Therefore, the enhancement of DNA binding ability of these hybrids can be correlated to other interactions produced by the naphthalimide component in addition to covalent linkage of the imine. Moreover, as the carbon chain increases from two to three as in the case of **2b**, there is a substantial increase in the DNA binding affinity, whereas, **3**, that is C2–C8 linked PBD to two naphthalimide moieties, also shows substantial DNA binding ability.



Scheme 2. (i) $SOCl_2$, C_6H_6, *trans*-4-hydroxy L-proline methylester hydrochloride, Et_3N, H_2O, THF, 0 °C, 1 h, 75%; (ii) TBDMS-Cl, imidazole, DCM, 12 h, rt, 92%; (iii) DIBAL-H, DCM, −78 °C, 45 min, 64%; (iv) EtSH-TMS-Cl, $CHCl_3$, 18 h, rt, 72%; (v) TPAP–NMO, DCM–MeCN, 2.5 h, rt, 78%; (vi) methyl (diethyl phosphono) acetate, NaH, THF, 0 °C, 2 h, 85%; (vii) 1 N LiOH, THF–H_2O–MeOH, 2 h, rt, 83%; (viii) EDCI-HOBt, compound **6a–b**, DCM–H_2O, 24 h, rt, 60–62%; (ix) $SnCl_2{\cdot}2H_2O$, MeOH, reflux, 2 h, 75–78%; (x) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O, 12 h, rt, 56–58%.

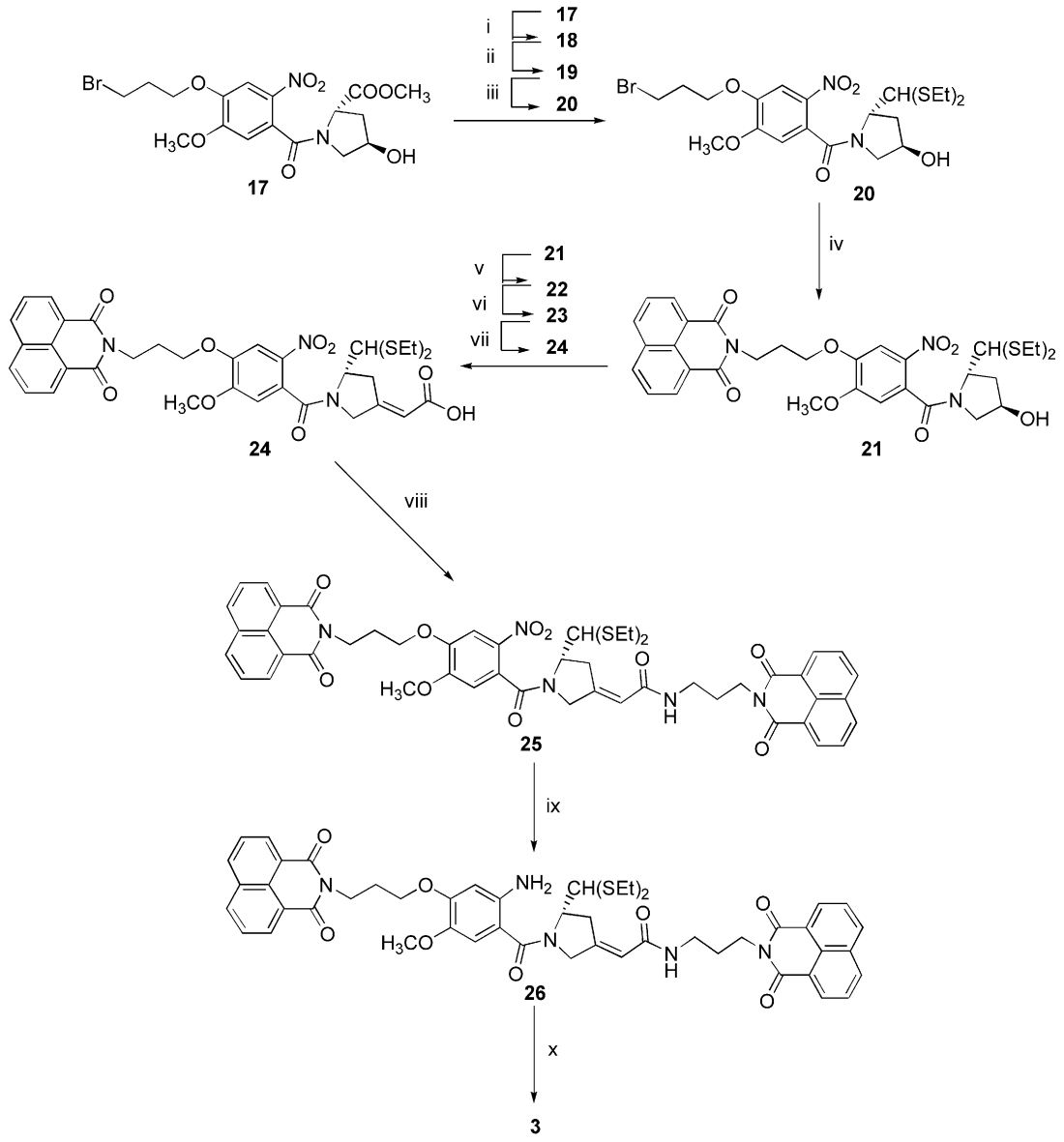
Table 2. In vitro cytotoxicity of compound **2b** in selected cancer cell lines

Cancer panel/cell line	GI_{50} (μM)
Leukemia	
CCRF-CEM	0.062
HL60 (TB)	0.091
MOLT-4	0.055
SR	<0.010
CNS	
SNB-19	0.08
Breast	
T-47D	0.034

Table 3. Thermal denaturation data for C2 and C2–C8 linked PBD–nathphalimide hybrids with CT-DNA

PBD hybrids	[PBD]/[DNA] molar ratio[b]	ΔT_m (°C)[a] after 18 h incubation at 37 °C for
2a	1:5	8.7
2b	1:5	13.5
3	1.5	10.5
DC-81	1:5	0.7

[a]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.
[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].



Scheme 3. (i) TBDMS-Cl, imidazole, DCM, 12 h, rt, 88%; (ii) DIBAL-H, DCM, −78 °C, 60 min, 68%; (iii) EtSH–TMS–Cl, $CHCl_3$, 18 h, rt, 85%; (iv) compound **4**, K_2CO_3, DMF, 12 h, rt, 90%; (v) TPAP–NMO, DCM–MeCN, 2.5 h, rt, 72%; (vi) methyl (diethyl phosphono) acetate, NaH, THF, 0 °C, 2 h, 71%; (vii) 1 N LiOH, THF–H_2O–MeOH, 2 h, rt, 78%; (viii) EDCI–HOBt, compound **6b**, DCM–H_2O, 24 h, rt, 62%; (ix) $SnCl_2{\cdot}2H_2O$, MeOH, reflux, 2 h, 72%; (x) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O, 12 h, rt, 52%.

In conclusion, novel C2 and C2–C8 linked PBD-naphthalimide hybrids have been synthesized that exhibit significant DNA-binding ability. Further, these compounds exhibit promising anticancer activity in certain cancer cell lines. The detailed mechanistic and molecular modeling studies for these PBD hybrids are in progress.

Acknowledgements

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

References and Notes

1. Thurston, D. E. *Br. J. Cancer* **1999**, *80* (Suppl 1), 65.
2. Thurston, D. E. In *Molecular Aspects of Anticancer Drug-DNA Interactions*; Neidle, S.; Waring, M. J., Eds.; Macmillan: UK, 1993; Vol. 1, p 54.
3. Puvvada, M. S.; Hartley, J. A.; Jenkins, T. C.; Thurston, D. E. *Nucleic Acids Res.* **1993**, *21*, 3671.
4. Thurston, D. E.; Bose, D. S.; Howard, P. W.; Jenkins, T. C.; Leoni, A.; Baraldi, P. G.; Guiotto, A.; Cacciari, B.; Kelland, L. R.; Foloppe, M. P.; Rault, S. *J. Med. Chem.* **1999**, *42*, 1951.
5. (a) Gregson, S. J.; Howard, P. W.; Corcoran, K. E.; Barcella, S.; Yasin, M. M.; Hurst, A. A.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. *Bioorg. Med. Chem. Lett.* **2000**, *10*,

1845. (b) Gregson, S. J.; Howard, P. W.; Barcella, S.; Nakamya, A.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 1849.
6. Cooper, N.; Hagan, D. R.; Tiberghien, A.; Ademefun, T.; Matthews, C. S.; Howard, P. W.; Thurston, D. E. *Chem. Commun*. **2002**, 1764.
7. Kamal, A.; Ramesh, G.; Laxman, N.; Ramulu, P.; Srinivas, O.; Neelima, K.; Kondapi, A. K.; Srinu, V. B.; Nagarajaram, H. M. *J. Med. Chem*. **2002**, *45*, 4679.
8. Brana, M. F.; Ramos, A. *Curr. Med. Chem. Anti-Cancer Agents* **2001**, *1*, 237.
9. O'Reilly, S.; Baker, S. D.; Sartorius, S.; Rowinsky, E. K.; Finizio, M.; Lubiniecki, G. M.; Grochow, L. B.; Gray, J. E.; Pieniaszek, H. J.; Donehower, R. C. *Ann. Oncol*. **1998**, *9*, 101.
10. Bousquet, P. F.; Brana, M. F.; Conlon, D.; Fitzgerald, K. M.; Perron, D.; Cocchiaro, C.; Miller, R.; Moran, M.; George, J.; Qian, X. D. *Cancer Res*. **1995**, *55*, 1176.
11. Kamal, A.; Reddy, B. S. N.; Reddy, G. S. K.; Ramesh, G. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 1933.
12. Kamal, A.; Laxman, N.; Ramesh, G.; Srinivas, O.; Ramulu, P. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 1917.
13. (a) Kamal, A.; Rao, M. V.; Laxman, N.; Ramesh, G.; Reddy, G. S. K. *Curr. Med. Chem. Anti-Cancer Agents* **2002**, *2*, 215. (b) Kamal, A.; Reddy, G. S. K.; Reddy, K. L.; Raghavan, S. *Tetrahedron Lett*. **2002**, *43*, 2103. (c) Kamal, A.; Reddy, P. S. M. M.; Reddy, R. *Tetrahedron Lett*. **2002**, *43*, 6629.
14. Thurston, D. E.; Murty, V. S.; Langley, D. R.; Jones, G. B. *Synthesis* **1990**, 81.
15. Reddy, B. S. P.; Damayanthi, Y.; Lown, J. W. *Synlett* **1999**, *7*, 1112.
16. Selected data for compound **2b**. ^{1}H NMR ($CDCl_3$) δ 1.80–2.2 (m, 2H), 3.2–3.35 (m, 4H), 3.98 (s, 3H), 4.20–4.30 (m, 2H), 4.45–4.65 (m, 3H), 5.20 (s, 2H), 6.0 (s, 1H), 6.85 (s, 1H), 6.92 (m, 1H), 7.25–7.45 (m, 5H), 7.55 (s, 1H), 7.75 (m, 2H), 7.84 (d, $J=3.8$ Hz, 1H), 8.23 (d, $J=7.8$ Hz, 2H), 8.56 (d, $J=7.0$ Hz, 2H); MS (FAB) 629 $[M+H]^{+\cdot}$
17. Selected data for compound **3**. ^{1}H NMR ($CDCl_3$) δ 1.94–2.40 (m, 4H), 3.20–3.40 (m, 4H), 3.45 (s, 3H), 4.20–4.60 (m, 9H), 6.05 (s, 1H), 6.80 (s, 1H), 6.93 (m, 1H), 7.32 (s, 1H), 7.70–7.85 (m, 4H), 7.88 (d, $J=4.08$ Hz, 1H), 8.18–8.30 (m, 4H), 8.56 (d, $J=7.2$ Hz, 4H); MS (FAB) 776 $[M+H]^{+\cdot}$.