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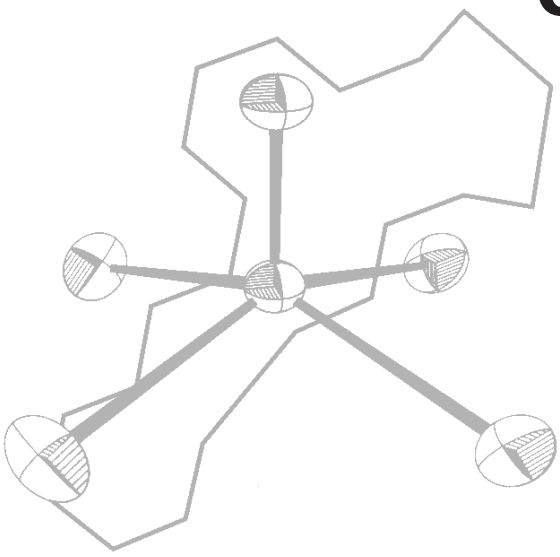

Volume 51, 1998

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Aust. J. Chem., 1998, **51**, 243–247

Synthesis and Binding Studies of a New DNA Minor Groove Binder

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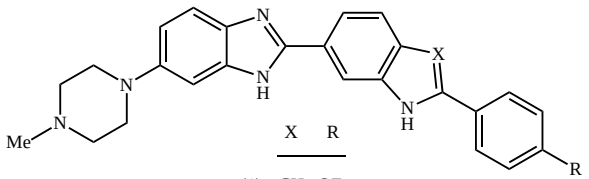
2-[2′-(4′′-Ethoxyphenyl)-1*H*-indol-6′-yl]-5-(4′′′-methylpiperazin-1′′′-yl)-1*H*-benzimidazole (1) has been synthesized from 2-(4′-ethoxyphenyl)-1*H*-indole-6-carbaldehyde (4b) and 4-(4′-methylpiperazin-1′-yl)benzene-1,2-diamine (3b). The aldehyde (4b) was prepared in five steps by using a modified Leimgruber–Batcho indole synthesis. Evaluation of (1) as a DNA ligand is described.

Introduction

Minor-groove-binding DNA ligands include a variety of structural types. Classic examples[1] are naturally occurring amide-linked bis- and tris-pyrroles, namely netropsin and distamycin A, and synthetic ligands, first the bibenzimidazole Hoechst 33258 (2)[2] and then the diamidino phenyl indole DAPI.[3] An early interest in these compounds was in the mode of binding to DNA, being distinct from intercalation, and their potential pharmacological properties, which led to the synthesis of a number of analogues.[4] However, it is their AT selectivity that has attracted sustained attention. DNA sequencing techniques enable discrete ligand-binding sites to be identified by footprinting[5] or by affinity cleavage with ligands modified to include DNA cleavage ability by chemical,[6] photolytic[7,8] or radiolytic[9] mechanisms, even in DNA in intact cells.[10]



	X	R
(1)	CH	OEt
(2)	N	OH

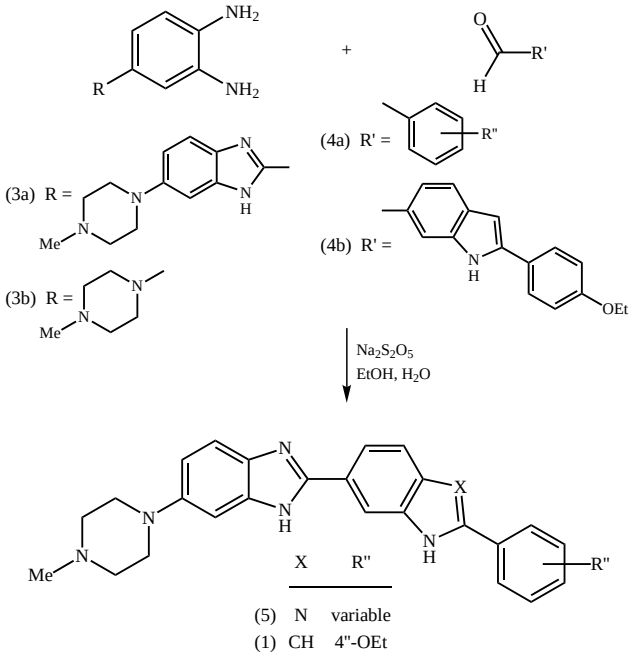
The availability of synthetic oligodeoxynucleotides has enabled ligand binding to particular sites, such as $d(AATT)_2$, to be examined by X-ray crystallography[11–13] and solution n.m.r.[14,15] To a large extent this activity is aimed at the development of the capability to design ligands with defined sequence selectivity.[16] The AT-selectivity of many of these ligands can be attributed to hydrogen bond donors on the ligands, making contacts in the minor groove with hydrogen bond acceptors on A and T sites.[17] Thus, there have been a number of attempts to modify bibenzimidazole ligands, for example by introducing heterocyclic groups that will act as hydrogen bond acceptors, so as to confer GC-selectivity.[16,18–20]

We have also undertaken changes to the bibenzimidazole structure, but for quite different reasons. Our interest in these ligands is in their potential use as radiomodifiers in cancer radiotherapy. This potential includes the use of iodinated analogues as radiosensitizers,[7] whereas the non-iodinated bibenzimidazoles have radioprotective activity.[21–23] There is some evidence to suggest that this radioprotective activity may be mediated by reduction by the ligand of radiation-induced transient oxidizing species on the DNA.[24] Accordingly, we have sought to modify the electronic properties of the ligands, and we report here the synthesis of a new ligand in which one of the benzimidazoles is replaced by an indole, in order to increase the π-electron density in the molecule. Aside from the classic case of DAPI[3] there are a number of examples of minor-groove-binding ligands including an indole moiety,[25–28] but to our knowledge this is the first report of a 2-(indol-6-yl)benzimidazole DNA binding ligand.

Synthesis of the 2-(Indol-6-yl)benzimidazole (1)

We have previously detailed the synthesis of a series of bibenzimidazoles related to Hoechst 33258 (2).[29,30] These derivatives were prepared by coupling aromatic *o*-diamines with either imino ether hydrochlorides or benzaldehyde derivatives through linear and convergent pathways. Scheme 1 details a linear synthesis of bibenzimidazole (5) involving the coupling of diamine (3a) to

Manuscript received 4 July 1997 0004-9425/98/030243$05.00

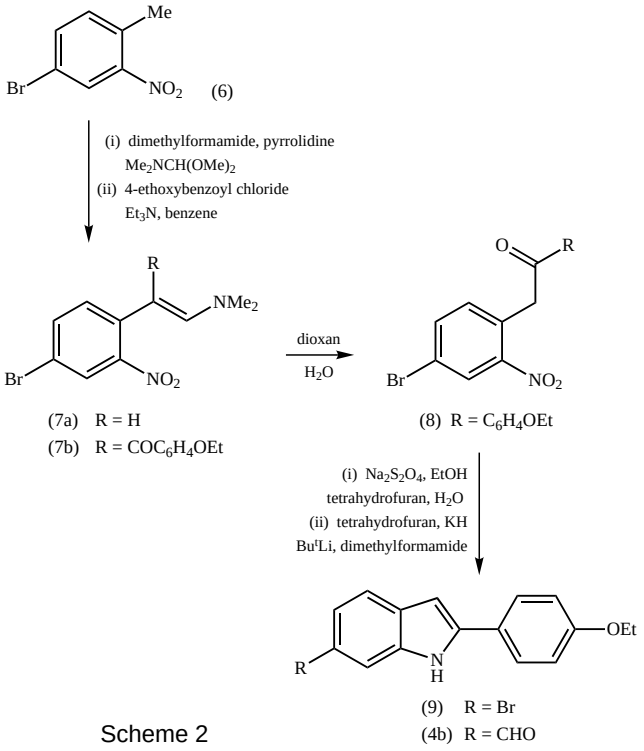


Scheme 1

benzaldehyde (4a). In the course of seeking a pathway to the 2-(indol-6-yl)benzimidazole (1) we entertained the idea that it could be produced by a convergent synthesis between diamine (3b) and indole-substituted aldehyde (4b).

We initially proposed to synthesize 2-(4′-ethoxyphenyl)-1*H*-indole-6-carbaldehyde (4b) via formylation of an appropriately substituted indole. Given that Vilsmeier formylation of indole and C 2 substituted indoles has been shown to occur at C 3,[31] not C 6 as required for (4b), we decided to prepare the aldehyde via an organometallic route analogous to that reported for indole-6-carbaldehyde by Rapoport *et al.*[32] This involved treating bromoindole (9) with potassium hydride, t-butyllithium and dimethylformamide. We chose to synthesize (9) via a modified Leimgruber–Batcho[33] indole synthesis. This involves a *de novo* construction of the indole nucleus with introduction of a C 2 aryl substituent,[34] and is shown in Scheme 2.

Under standard conditions[33] the nitrotoluene (6) was treated with *N,N*-dimethylformamide dimethyl acetal and pyrrolidine in dimethylformamide to afford enamine (7a). The enamine was treated with 4-ethoxybenzoyl chloride and triethylamine in benzene until t.l.c. indicated most of the starting material had been consumed. Enamine (7b) was isolated and, without purification, hydrolysed and deformylated in dioxan/water over 24 h to give ketone (8) in 50% overall yield from (6). Intramolecular cyclization of (8) was effected by chemical reduction with sodium dithionite in ethanol/tetrahydrofuran/water to give 6-bromo-2-(4′-ethoxyphenyl)-1*H*-indole (9) in 63% yield. Bromoindole (9) was formylated under standard conditions[32] in tetrahydrofuran[35] to afford aldehyde (4b) in 36% yield. This was later increased to a moderate 42%. In the final step the coupling[36] of aromatic diamine (3b) and formylindole (4b) proceeded smoothly to afford (1) in 65% yield.



Scheme 2

Estimation of Dissociation Constants

The binding of the ligand (L) to DNA was studied with the synthetic oligoDNA d$(CGCGCGAATTCGCGCG)_2$, which is expected to contain a single ligand-binding site. The expression for the dissociation equilibrium

$$\text{DNA–L}_n \overset{K_\text{d}}{\rightleftharpoons} n\text{L} + \text{DNA} \qquad (1)$$

can be written

$$K_\text{d} = L(1-\alpha)(nx - L\alpha)L\alpha \qquad (2)$$

where α is the fraction of bound ligand, L is the total ligand concentration (mol l^{-1}), n is the number of ligand binding sites per DNA base pair (bp), and x is the total DNA concentration (mol bp l^{-1}).

The spectrophotometric data from titration with DNA was processed by regression analysis. Each intermediate spectrum during the titration process was represented as a combination of the spectra of free $[y_\text{f}(\lambda)]$ and bound $[y_\text{b}(\lambda)]$ ligand in following form:

$$y(\lambda) = (1-\alpha)y_\text{f}(\lambda) + \alpha y_\text{b}(\lambda) \qquad (3)$$

The regression analysis was then applied to expression *(3)*, to yield values for α. These values were used to

build a binding curve (fraction of bound ligand against DNA concentration) and Scatchard plot. The values for K_d and n were calculated from the Scatchard plot.

Results

The DNA binding experiments yielded a K_d value of $2{\cdot}8(\pm0{\cdot}7)\times10^{-8}$ mol l^{-1} with $n = 0{\cdot}042(\pm0{\cdot}001)$. Similar experiments with Hoechst 33258 (2) gave values of $K_d = 1{\cdot}1(\pm0{\cdot}2)\times10^{-8}$ and $n = 0{\cdot}023(\pm0{\cdot}001)$. These values are averages and standard deviations of the results of our experiments. The data from a typical experiment are shown in Fig. 1 for (1) and Fig. 2 for Hoechst 33258 (2). As indicated in Table 1, the apparent binding is around 5–10-fold lower than values reported in the Hoechst 33258 in ethanol-free aqueous buffer.[37] The ethanol was included in our experiments to improve ligand solubility and thus allow comparisons between a variety of new ligands, and to minimize non-specific binding to DNA and glassware. The extent of the effect of ethanol on K_d is similar to that reported previously for poly[d(A–T)],[37] as shown in Table 1. Given the limited precision, the K_d value for the 2-(indol-6-yl)benzimidazole ligand (1) is comparable to that for Hoechst 33258 (2). However, the value for the number of binding sites of the new ligand per oligoDNA molecule is about twice that for Hoechst 33258 (2). This could reflect the binding of two molecules of the ligand, as reported for DAPI[38,39] and distamycin.[40]

While the relative values of n for the 2-(indol-6-yl)benzimidazole (1) and Hoechst 33258 (2) are close to 2 : 1, we cannot explain why the absolute values are less than expected.

In conclusion, (1) is the first compound in a new class of DNA binders, the 2-(indol-6-yl)benzimidazoles. Work is in progress to synthesize more derivatives of (1) and other types of compounds with increased π-electron density over (1).

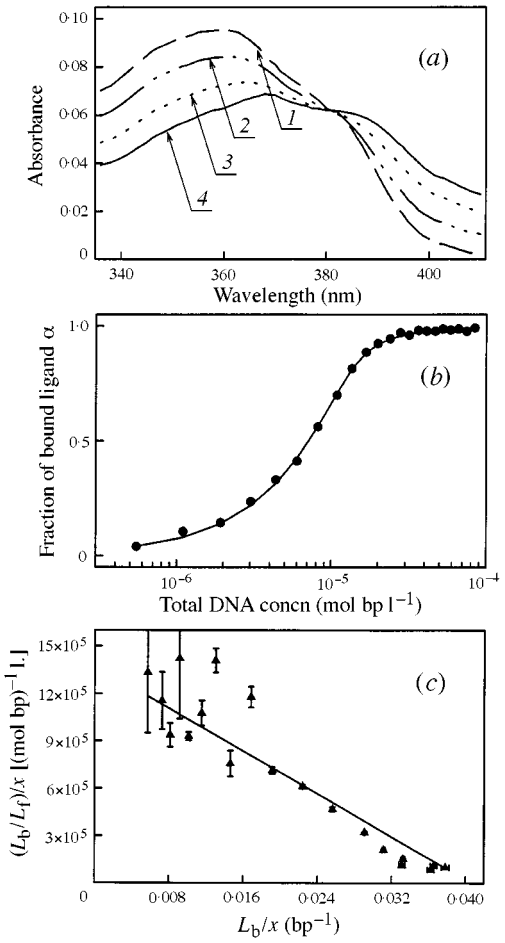


Fig. 1. Results for ligand (1). (*a*) Effect of DNA on ligand absorption. Spectra: *1*, free ligand; *2,3* examples of intermediate spectra; *4*, bound ligand. Only two intermediate spectra are shown to avoid complication of the figure. (*b*) Binding curve. (*c*) Scatchard plot. L_b and L_f are the concentrations of bound and free ligand respectively; x is the DNA concentration. The closed symbols on panels (*b*) and (*c*) represent experimental data; the solid lines are the result of regression analysis.

Experimental

The buffer used for the DNA/ligand titration was 20 mM phosphate buffer pH 7·4, with 100 mM NaCl, containing 20% ethanol to minimize problems due to solubility, adsorption and non-specific binding of the ligand. The DNA concentration was determined by using the extinction coefficient of the

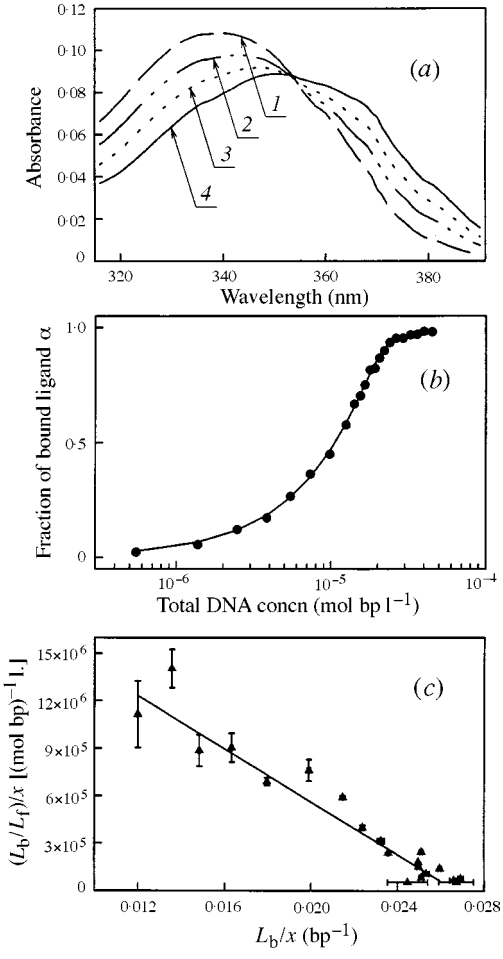


Fig. 2. Results for ligand (2): see caption to Fig. 1.

Table 1. Binding of ligands (1) and (2) to synthetic DNA

DNA	Ligand	Ethanol (%)	K_d (mol l^{-1})	Ref.
$d(CGCGCGAATTCGCGCG)_2$	(2)	20	$(1{\cdot}1\pm0{\cdot}2)\times10^{-8}$	this study
$d(CGCGCGAATTCGCGCG)_2$	(1)	20	$(2{\cdot}8\pm0{\cdot}7)\times10^{-8}$	this study
$d(CGCGAATTCGCG)_2$	(2)	—	$0{\cdot}25\times10^{-8}$	37
poly[d(A–T)]	(2)	—	$0{\cdot}26\times10^{-8}$	37
poly[d(A–T)]	(2)	25	$6{\cdot}8\times10^{-8}$	37

duplex oligoDNA (286000 M^{-1} cm^{-1}), determined from that for single-stranded DNA (161600 M^{-1} cm^{-1}) by measuring the relative extinction of native and heat denatured DNA. The concentration of ligand used in titration experiments was in the range 0·4–0·2 μM. The absorption spectra were measured in 50 mm cuvettes. The ultraviolet spectroscopic data obtained for (1) (>90% pure by h.p.l.c.), in 0·1% CF_3CO_2H in 45% MeOH in water (v/v/v), were characterized by maxima at 385 nm (ϵ_{385} $3{\cdot}98\times10^4$ mol^{-1} l. cm^{-1}) and 249 nm (ϵ_{249} $2{\cdot}94\times10^4$ mol^{-1} l. cm^{-1}).

Melting points are uncorrected. ^1H, ^{13}C and two-dimensional spectra (HMBC, HMQC) were recorded on a Varian Unity 400 spectrometer operating at 399·5 MHz for ^1H and 100 MHz for ^{13}C. *J* values are given in Hz. The addition of a few drops of CF_3CO_2D to the CD_3OD solution of (1) was found to reduce peak broadening and enhance peak definition in the aromatic region of the ^1H n.m.r. spectra. For the acquisition of the carbon spectrum of (1) in CD_3OD the addition of a few drops of CH_3SO_3H was used to increase solubility. Positive-ion electron ionization mass spectra were recorded on a Micromass VG7070F instrument at an ionization potential of 70 eV. Elemental analyses were performed by Chemical and Micro Analytical Services Pty Ltd Melbourne, Victoria.

2-(4′′-Bromo-2′′-nitrophenyl)-1-(4′-ethoxyphenyl)ethanone (8)

To (*E*)-2-(4-bromo-2-nitrophenyl)-*N,N*-dimethylethenamine (7a) (3·1 g, 11·57 mmol),[32] prepared from 4-bromo-1-methyl-2-nitrobenzene (6),[41] was added 4-ethoxybenzoyl chloride (2·14 g, 11·57 mmol), triethylamine (1·6 ml, 11·57 mmol) and dry benzene (15 ml). The mixture was refluxed for 7 days at which time most of the enamine had been consumed. Upon washing with water the organic layer was refluxed with dioxan (15 ml) and water (5 ml) for 24 h. The solvents were removed and the crude material in dichloromethane was 'flashed' through silica to yield (8) (2·12 g, 50·4%) as a tan crystalline *solid*, m.p. 117–118°C (from dichloromethane/light petroleum (b.p. 40–60°C)) (Found: C, 52·8; H, 3·8; Br, 21·8; N, 3·8%; M^+ 363·0124. $C_{16}H_{14}BrNO_4$ requires C, 52·7; H, 3·9; Br, 22·0; N, 3·9%; M^+ 363·0106). δ_H ($CDCl_3$) 8·265. 1H, d, *J* 2, H3′′; 7·97, 2H, d, *J* 9·1, H2′,6′; 7·71, 1H, dd, *J* 8·1, 2, H5′′; 7·205, 1H, d, *J* 8·1, H6′′; 6·945, 2H, d, *J* 9, H3′,5′, 4·63, 2H, s, H2; 4·11, 2H, q, *J* 7, OC**H**$_2$CH$_3$; 1·45, 3H, t, *J* 7·1, OCH_2C**H**$_3$. δ_C ($CDCl_3$) 193·1. C1; 163·29, C4′; 149·47, C2′′; 136·26, C5′′; 134·82, C6′′; 130·51, C2′,6′; 129·87, C1′′; 128·92, C1′; 128·10, C3′′; 121·25, C4′′; 114·28, C3′,5′; 63·80, O**C**H_2CH$_3$; 43·21, C2; 14·63, OCH_2**C**H$_3$; *m/z* 363 (M^+, 0·31%), 149 (100), 121 (37·73), 65 (16·62), 57 (86·95).

*6-Bromo-2-(4′-ethoxyphenyl)-1*H*-indole (9)*

The ketone (8) (284 mg, 0·780 mmol) and sodium dithionite (313 mg, 1·80 mmol) in tetrahydrofuran (2 ml), ethanol (2 ml) and water (1·3 ml) were refluxed for 20 min. The reaction was monitored by silica t.l.c. (benzene; R_F 0·4 for (8), R_F 0·64 for (9)). Additional sodium dithionite and tetrahydrofuran/ethanol/water were added and the solution was reheated if required. Upon completion the organic solvents were removed and (9) was isolated by filtration. The filtrate was acidified with dilute HCl and heated and further product was obtained. Upon drying the title compound was obtained as a beige *solid* (158 mg, 63%), m.p. 225–226°C (repeated trituration with ethyl acetate/light petroleum) (Found: C, 60·9; H, 4·5; Br, 25·3; N, 4·4%; M^+ 315·0133. $C_{16}H_{14}BrNO$ requires C, 60·8; H, 4·5; Br, 25·3; N, 4·4%; M^+ 315·0259. δ_H ($CDCl_3$) 8·25. 1H, br s, NH; 7·56, 2H, d, *J* 8·7, H2′,6′; 7·52, 1H, br s, H7; 7·45, 1H, d, *J* 8·4, H4; 7·2, 1H, dd, *J* 8·3, 1·4, H5; 6·97, 2H, d, *J* 8·7, H3′,5′; 6·665, 1H, d, *J* 1·4, H3; 4·08, 2H, q, *J* 7, OC**H**$_2$CH$_3$; 1·45, 3H, t, *J* 6·95, OCH_2C**H**$_3$. δ_C ($(CD_3)_2SO$) 158·27, C4; 138·84, C2; 137·77, C7; 127·85, C3a; 126·45, C2′,6′; 124·16, C1′; 122·02, C5; 121·25, C4; 114·78, C3′,5′; 113·44, C6 or C7; 113·40, C6 or C7; 97·36, C3; 63·08, O**C**H_2CH$_3$; 14·54, OCH_2**C**H$_3$). *m/z* 315 (M^+, 98·92%), 286 (59·75), 236 (4·64), 207 (12·03).

*2-(4′-Ethoxyphenyl)-1*H*-indole-6-carbaldehyde (4b)*

To potassium hydride (24 mg, 0·6 mmol) in tetrahydrofuran (0·5 ml) at 0°C was added dropwise a solution of the indole (9) (189 mg, 0·598 mmol) in tetrahydrofuran (3 ml).[32,35] When the hydride had been consumed the solution was cooled to −100°C and t-butyllithium (0·71 ml, 1·694 M, 1·196 mmol), at −100°C, was added via cannula. Upon warming to −80°C, dimethylformamide (46 μl, 0·598 mmol) in tetrahydrofuran (1 ml) was added and the solution was stirred at −80°C for 15 min before being warmed to room temperature. The reaction mixture was poured into ice-cold 1 M phosphorous acid and extracted with ethyl acetate. Washing with saturated sodium bicarbonate, drying with sodium sulfate and removal of the solvent gave a residue which was subject to column chromatography on silica gel (ethyl acetate/light petroleum (b.p. 40–60°C) (32:68)). The title compound (4b) (R_F 0·32) was obtained as a crystalline *solid* (57 mg, 36%), m.p. 199–201°C (Found: C, 77·2; H, 5·8; N, 5·2%; M^+ 265·1254. $C_{17}H_{15}NO_2$ requires C, 77·0; H, 5·7; N, 5·3%; M^+ 265·1103). δ_H ($(CD_3)_2CO$) 11·15, 1H, br s, NH; 9·99, 1H, s, CHO; 7·94, 1H, br s, H7; 7·845, 2H, d, *J* 8·8, H2′,6′; 7·675, 1H, d, *J* 8·3, H4; 7·575, 1H, dd, *J* 8·2, 1·4, H5; 7·035, 2H, d, *J* 8·8, H3′,5′; 6·915, 1H, d, *J* 1·7, H3; 4·11, 2H, q, *J* 7, OC**H**$_2$CH$_3$; 1·38, 3H, t, *J* 6·95, OCH_2C**H**$_3$. δ_C ($(CD_3)_2SO$) 192·47, CHO; 158·89, C4′; 142·72, C2; 136·32, C7a; 133·93, C3a; 130·02, C6; 127·09, C2′,6′; 123·72, C1′; 119·89, C4,5; 114·92, C3′,5′; 114·44, C7; 98·30, C3; 63·22, OCH_2CH$_3$; 14·65, OCH_2**C**H$_3$. *m/z* 265 (M^+, 89·36%), 236 (68·08), 208 (20·92), 149 (32·35), 71 (57·59), 57 (100).

*2-[2′-(4′′-Ethoxyphenyl)-1*H*-indol-6′-yl)]-5-(4′′′-methylpiperazin-1′′′-yl)-1*H*-benzimidazole (1)*

To freshly prepared 4-(4′-methylpiperazin-1′-yl)benzene-1,2-diamine (3b)[29] (44 mg, 0·21 mmol) in dry ethanol (2 ml) was added a solution of thiosulfate aldehyde complex (prepared by adding sodium metabisulfite (39·5 mg, 0·208 mmol) in ethanol/water (0·5:0·5 ml) to a refluxing solution of (4b) (55 mg, 0·208 mmol) in ethanol (5 ml)). The resultant solution was heated at reflux for 12 h. The solution was cooled, basified with ammonium hydroxide and put in a freezer for several hours. The solid obtained via filtration was washed with water, diethyl ether and dried in vacuum to give (1) as a yellow *solid* (65 mg, 69%), m.p. 294°C (dec.) (Found: C, 74·0; H, 6·7; N, 15·6; O, 3·3%; M^+ 451·2343. $C_{28}H_{29}N_5O$ requires C, 74·5; H, 6·5; N, 15·5; O, 3·5%; M^+ 451·2372). δ_H

(CD_3OD+CF_3CO_2D) 8·055, 1H, d, *J* 1·5, H 7′; 7·745, 2H, d, *J* 8·7, H 2″,6″; 7·73, 1H, d, *J* 8·8, H 4′; 7·620, 1H, d, *J* 8·7, H 7; 7·619, 1H, dd, *J* 8·7, 1·8, H 5′; 7·305, 1H, dd, *J* 9, 2·2, H 6; 7·21, 1H, d, *J* 2·1, H 4; 6·98, 2H, d, *J* 8·8, H 3″,5″; 6·78, 1H, br s, H 3′, exchangeable; 4·065, 2H, q, *J* 7, OC**H**$_2$$CH_3$; 3·885 2H, br d, *J* 13·4, piperazinyl H; 3·655, 2H, br d, *J* 12·1, piperazinyl H; 3·29, 2H, br t, *J* 9·7, piperazinyl H; 3·15, 2H, br t, *J* 11·9, piperazinyl H; 2·98, 3H, s, NCH_3; 1·41, 3H, t, *J* 7·05, OCH_2C**H**$_3$. δ_C (CD_3OD+CH_3SO_3H) 160·49, C 4″; 150·42, C 2 or C 3a′; 149·51, C 3a or C 5; 143·98, C 2′; 137·41, C 6′ or C 7a′; 134·15, C 2 or C 3a′; 133·31, C 3a or C 5; 127·88, C 2,6″; 126·75, C 7a; 124·68, C 1″, 121·55, C 4′; 118·66, C 5′; 117·86, C 6; 115·66, C 3,5″; 114·74, C 7; 113·99, C 6′ or C 7a′; 111·10, C 7′; 100·26, C 4; 99·3, C 3′; 64·47, O**C**H_2CH_3; 54·41, piperazinyl C; 47·99, piperazinyl C; 43·57, (N**C**H_3; 15·19, OCH_2**C**H_3. *m/z* 451 (M^+, 100%), 381 (12·32), 253 (14·59), 157 (13·27), 71 (17·21).

Acknowledgments

We are grateful for the financial support of a grant from the Australian Research Council.

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