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Synthesis and biological evaluation of novel benzodioxinocarbazoles (BDCZs) as potential anticancer agents

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ABSTRACT

We report the efficient synthesis and biological evaluation of new benzodioxinoindolocarbazoles heterocycles (BDCZs) designed as potential anticancer agents. Indolic substitution and maleimide variations were performed to design a new library of BDCZs and their cytotoxicity were evaluated on two representative cancer cell lines. Several derivatives have shown a marked cytotoxicity with IC_{50} values in the nanomolar range. Results are reported in this Letter.

The indolocarbazole alkaloids are a family of natural products isolated from marine invertebrates and cultures of diverse microorganisms.[1] Rebeccamycin (Fig. 1), an important member of this family, is potent inhibitor of topoisomerase I. Its biological activities have made it a high profile lead compound for the development of anticancer drugs,[2] and several analogues (i.e., NB-506 and J-107088 or edotecarin) have entered clinical trials.[3]

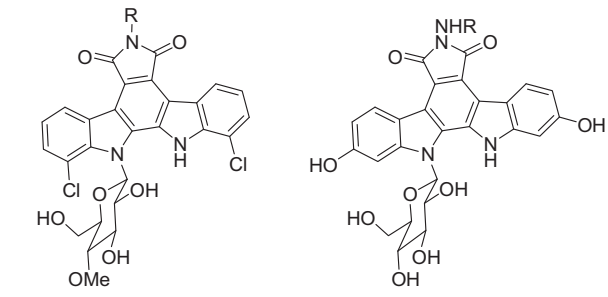
A number of research groups, including ours, have sought to synthesize various simplified derivatives (Fig. 2), lacking the sugar moiety to obtain strong protein kinase inhibitors and/or in vitro cytotoxic agents.[4] In order to develop such selective series, bioisosteric replacement of one indole ring by another heteroaryl moiety appears indeed to be a valid alternative compared to the restrictive synthesis of glycosylated compounds.[5]

On the background of these findings, we describe herein the preparation of new potential anticancer lead compounds bearing an original benzodioxinocarbazole moiety.[4f] For the establishment of primary structure–activity relationships of these new functionalized carbazoles, cell studies were performed.

We endeavored to prepare original benzodioxino-pyrrolocarbazoles (BDCZs) **V** (Fig. 3) using a general synthetic route that relied on a photochemically induced 6π-electrocyclization as a key step.[4a,d,6] The synthetic strategy is also based on a Stille Pd(0)-catalyzed cross-coupling reaction starting from intermediate **VII** and 2-trialkylstannylbenzodioxine.

Structural variations were performed on the indolic part, for fine-tuning developments and for establishing structure–activity relationships. Electron rich and acceptor/donor hydrogen bond groups were used. In addition, to induce a selective cytotoxic activity and to increase water solubility of our new heterocyclic scaffold, we though to introduce a dimethylaminoalkyl chain onto the maleimide moiety.[4e–i]

The synthesis began with the construction of the upper side of the molecule via a Stille palladium cross-coupling reaction[7]



Rebeccamycin R = H
NSC 655 649 R = $(CH_2)_2N(C_2H_5)_2$

NB-506 R = CHO
J-107088 R = $CH(CH_2OH)_2$

Figure 1.

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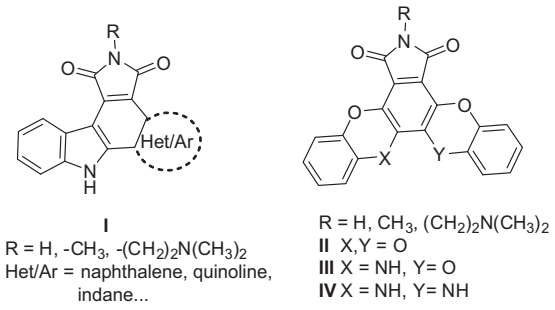


Figure 2. Examples of indolocarbazoles bioisosteres **I**–**IV**.

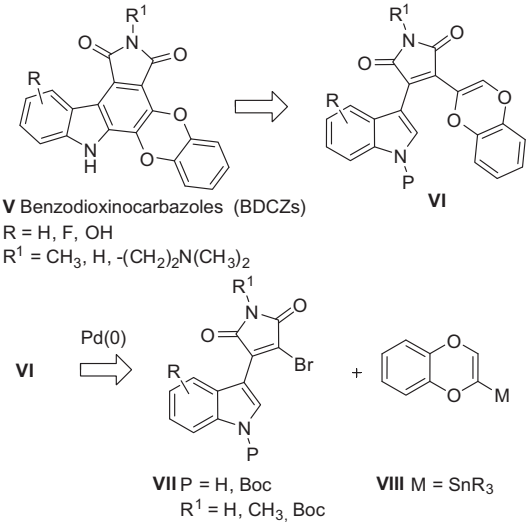


Figure 3. General synthetic scheme.

(Table 1) to afford in fair to good yields desired compounds **16–27**. Reactions were performed starting from 2-trimethylstannyl-benzodioxine and indolo-derivatives **1–15**[8] in the presence of $Pd(PPh_3)_4$ and CuI in dioxane at 65 °C or 100 °C. The palladium cross-coupling reaction could be conducted with *N*-Me, *N*-Boc or *N*-H maleimide and Boc protected or unprotected indolyl compounds. Indole could be functionalized by electro-donor or -withdrawing groups. For benzyloxy derivatives (entries 5–8, 12–15) best yields were obtained after protection of the indolyl nitrogen with a Boc group.[8]

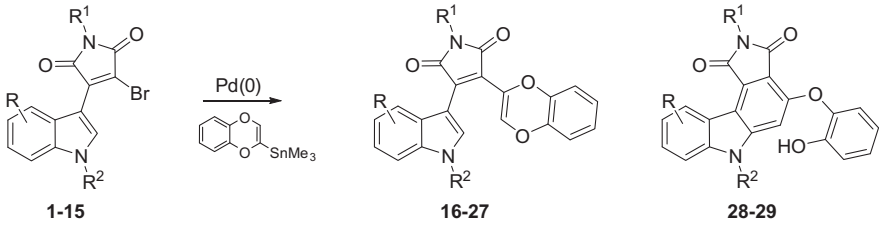
It is worth noting that ring-opened phenols **28** or **29** were isolated as separable by-products starting from **7** or **8**, respectively. As previously demonstrated, these phenolic derivatives result from the ring opening of the benzodioxane moiety obtained via a spontaneous [4+2] cycloaddition/ring opening sequence.[9]

Next, our attention turned to study the key cyclization step. Good results were obtained by performing a photochemical induced 6π-electrocyclization (Table 2). This key step was accomplished by submitting coupled derivatives **16–27** to irradiation (UV 500 W DEMA-lamp) and led to the attempted BDCZs **30–40**.[10] The reaction was conducted in the presence of an excess of diiodine in order to induce a further spontaneous aromatization.

Starting from unprotected derivatives **17–19** or **23–25**, the reaction led respectively to carbazoles **31–33** or **36–38** isolated as the sole products in moderate yields (entries 2–4, 7–9). With Boc derivatives (entries 1, 5, 6, 10, 11), both processes of β-elimination and of aromatization concomitantly occurred which afforded a separable mixture of attempted BDCZS and phenolic derivatives **41–45**. It is noteworthy that the Boc protective group of the indolyl nitrogen survives to the experimental conditions whereas the maleimide Boc protection is cleaved (entries 10 and 11).

To fully prepare our derivatives for biological assays and to enhance their solubility, additional reactions were next performed (Table 3). Maleimide substitution was carried out by heating the starting carbazoles **31–35** in the presence of *N*,*N*-dimethylethylenediamine (entries 1–5).[11] The transimidification process led to

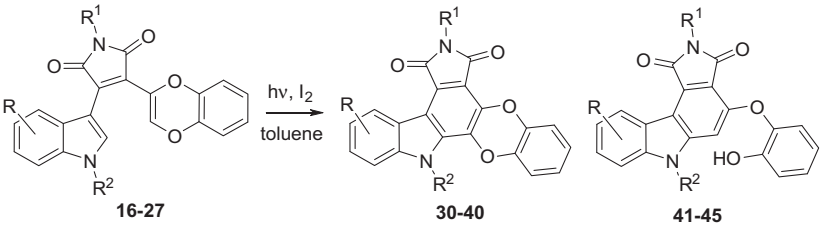
Table 1
Stille palladium cross-coupling procedure[a]



Entry	Compound	R	R^1	R^2	Time	CuI (mol %)	Products[b] (yields %)
1	**1**	H	CH_3	Boc	1 h	20	**16**[c] (82%)
2	**2**	H	CH_3	H	1 h 45 min	20	**17** (79%)
3	**3**	5-F	CH_3	H	1 h 40 min	10	**18** (81%)
4	**4**	6-F	CH_3	H	45 min	10	**19** (82%)
5	**5**	5-OBn	CH_3	H	1 h	20	Degradation
6	**6**	6-OBn	CH_3	H	50 min	10	**20** (64%)
7	**7**	5-OBn	CH_3	Boc	50 min	20	**21** (76%), **28** (20%)
8	**8**	6-OBn	CH_3	Boc	30 min	10	**22** (84%), **29** (16%)
9	**9**	H	H	H	2 h	10	**23** (76%)
10	**10**	5-F	H	H	2 h40	10	**24** (82%)
11	**11**	6-F	H	H	1 h50	10	**25** (85%)
12	**12**	5-OBn	H	H	1 h	10	Degradation
13	**13**	6-OBn	H	H	1 h	10	Degradation
14	**14**	5-OBn	Boc	Boc	1 h10	10	**26** (69%)
15	**15**	6-OBn	Boc	Boc	2 h	10	**27** (65%)

[a] Reagents and conditions: (a) $PdCl_2(PPh_3)_2$ 10 mol %, CuI 10 or 20 mol %, dioxane 65 °C.
[b] Yields are indicated as isolated products.
[c] The reaction was conducted at 100 °C.

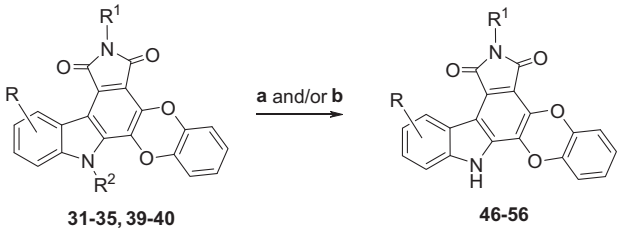
Table 2
Photochemical step[a]



Entry	Compound	R	R^1	R^2	Time	Products[b] (yields %)
1	**16**	H	CH_3	Boc	30 min	**30** (23%)[c]
2	**17**	H	CH_3	H	1 h 10 min	**31**(51%)
3	**18**	5-F	CH_3	H	30 min	**32** (55%)
4	**19**	6-F	CH_3	H	30 min	**33** (38%)
5	**21**	5-OBn	CH_3	Boc	40 min	**34** (38%)[c]
6	**22**	6-OBn	CH_3	Boc	30 min	**35** (21%)[c]
7	**23**	H	H	H	45 min	**36** (52%)
8	**24**	5-F	H	H	40 min	**37** (25%)
9	**25**	6-F	H	H	1 h 30 min	**38** (24%)
10	**26**	5-OBn	Boc	Boc	30 min	**39** (27%)[c,d]
11	**27**	6-OBn	Boc	Boc	30 min	**40** (27%)[c,d]

[a] Conditions: h*ν*, 500 W DEMA-lamp, I_2 (14.0 equiv), toluene.
[b] Yields are indicated as isolated product.
[c] Starting from **16**, **21**, **22**, **26** and **27**, the corresponding opened compounds **41** (12%), **42** (13%), **43** (22%), **44** (non determined) and **45** (27%, R^1 = H) were, respectively, isolated as by-products.
[d] R^1 = H.

Table 3
Deprotections of BDCZs **30**–**40** and maleimide reactions



Entry	Starting materials	R	R^1	R^2	Products[d] (yields %)	R	R^1
1[a]	**31**	H	CH_3	H	**46** (75%)	H	
2[a]	**32**	5-F	CH_3	H	**47** (75%)	5-F	
3[a]	**33**	6-F	CH_3	H	**48** (76%)	6-F	
4[a]	**34**	5-OBn	CH_3	Boc	**49** (89%)	5-OBn	
5[a]	**35**	6-OBn	CH_3	Boc	**50** (75%)	6-OBn	
6[b]	**34**	5-OBn	CH_3	Boc	**51** (79%)	5-OH	CH_3
7[b]	**35**	6-OBn	CH_3	Boc	**52** (94%)	6-OH	CH_3
8[b]	**39**	5-OBn	H	Boc	**53** (80%)	5-OH	H
9[b]	**40**	6-OBn	H	Boc	**54** (74%)	6-OH	H
10[b,c]	**49**	5-OBn		H	**55**[12] (98%)	5-OH	
11[b,c]	**50**	6-OBn		H	**56** (71%)	6-OH	

[a] Reagents and conditions: $NH_2(CH_2)_2N(CH_3)_2$, rflx, 22 h.
[b] Reagents and conditions: BBr_3 (2.0 equiv), CH_2Cl_2, 0 °C (15 min) then rt (1 h15 min).
[c] Additional neutralization step with aqueous Na_2CO_3 solution.
[d] Yields are indicated as isolated products.

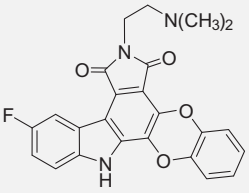
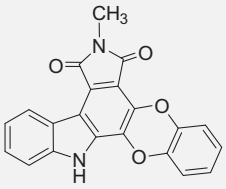
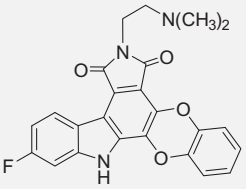
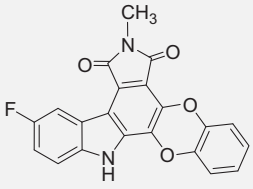
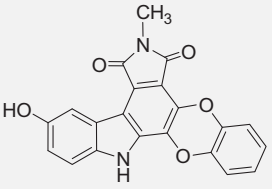
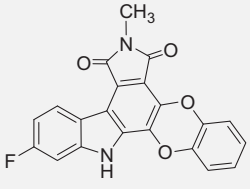
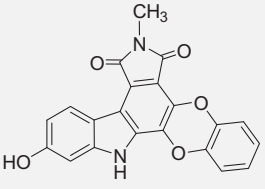
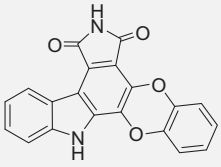
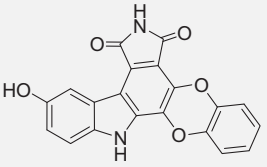
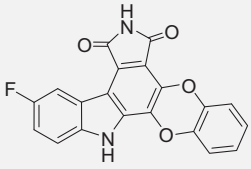
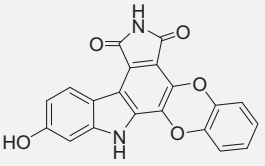
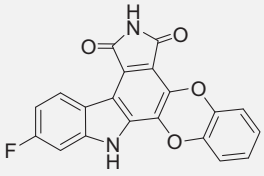
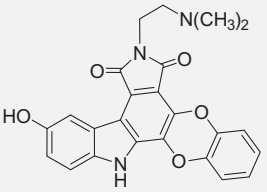
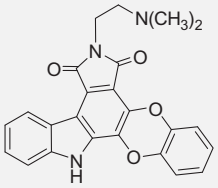
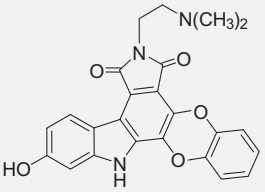
the desired products **46–50** in good yields. To achieve the BDCZs, removal of the benzyl group was performed from **34**, **35, 39**, **40** and from newly formed **49** and **50** compounds using BBr_3 in dichloromethane (entries 6–11). As attempted, spontaneous removal of the Boc protective groups was observed under acid or basic conditions.

Antiproliferative activities of the newly synthesized compounds **31–33**, **36–38**, **46–48** and **51–56** were evaluated toward two tumor

cell lines: murine leukemia L1210 and human prostate carcinoma DU145 and compared with those of rebeccamycin (Table 4). The cytotoxicity of compounds was measured using a conventional microculture tetrazolium assay. As expected, significant variations of the cytotoxicity were observed according to the nature and the position of substituents.

The unprotected NH maleimide derivatives **36**, **37**, **38** are quite inactive against L1210 cell line. Some SAR in indolocarbazole

Table 4
In vitro antiproliferative activities against a murine leukemia (L1210) and a human prostate cancer (DU145)

Entry	Compound		IC_{50} L1210 (μM)	IC_{50} DU145 (μM)	Entry	Compound		IC_{50} L1210 (μM)	IC_{50} DU145 (μM)
1	Rebeccamycin	—	0.14	ND	8		**47**	0.63	0.96
2		**31**	0.64	ND	9		48	0.23	1.2
3		**32**	0.64	0.29	10		**51**	28.1	13.0
4		**33**	2.2[a]	0.32	11		**52**	21.3	2.0
5		**36**	24.8	1.6	12		**53**	0.33	0.82
6		**37**	12.5	8.4	13		**54**	0.58	0.89
7		**38**	13.0	3.3	14		**55**	0.074	0.179
8		**46**	0.2	0.65			**56**	0.18	0.45

[a] Precipitate. IC_{50} values are the average of at least four determinations in triplicate obtained in independent experiments. Variation between replicates was less than 5%.

analogues series confirms this result. Despite the presence of a lipophilic methyl group on the maleimide moiety, *N*-methyl maleimide compounds **31** and **32** exhibit a surprising and significant cytotoxicity. This cell activity decreased by changing the position of the fluorine atom (**33**) or by introducing hydroxyl function (**51** or **52**). In this sub-class, sub micromolar cell effects seem to be dependent from either lipophilicity, minimal steric hindrance and position of the substituents (*C*-5 vs *C*-6) on the indolic part.

Substitution of the maleimide with a *N,N*-dimethylaminoethyl chain resulted in more soluble derivatives that retained better cytotoxicity. The *N,N*-dimethylaminoethyl substitution induced a clear improvement of the cytotoxicity compared to their *NH* or *N*-methyl substituted counterparts. Compounds **46**, **47**, **48**, **55**, and **56** inhibited the cellular proliferation in the nanomolar range. Without any indolyl substituent or in the presence of fluorine atom at *C*-5 or *C*-6 position of the indole ring, IC_{50} were limited to some hundred nanomolar. For compounds **55** and **56**, the presence of hydrophilic function on the indolyl part enhanced considerably the cytotoxicity. Indolic *C*-5 position was privileged and compound **55** was the most active of this subset with IC_{50}'s (L1210) of 74 nM.

Without exception, all compounds described were significantly less cytotoxic on the HT29 than on the L1210 cell lines. As seen with tests on L1210, compound **55** was the most potent with an IC_{50} (HT29) of 179 nM.

In order to find the biological target of our new lead compounds, DNA flow cytometric analyses were next performed on compounds **33**, **47**, **48**, **55**, **56** using propidium iodide staining. Compound **33** was the sole derivative inducing an accumulation of cells in G1 phase (53% at 50 μM). Compounds **47**, **55** and **56** showed a G2 + M phase accumulation of cells (**47**, 62% at 5 μM; **55**, 77% at 0.5 μM; **56**, 48% at 1 μM). The most cytotoxic compound **55** was also the most active on the cell cycle. This original derivative is two times more active than rebeccamycin. Further investigations onto DNA showed a strong intercalation but topoisomerase I or II inhibitions were quite insufficient to explain the observed cytotoxicity (data not shown).

In conclusion, new indolocarbazole analogues based on original benzodioxinoindolocarbazoles heterocycles (BDCZs) has been prepared. The efficient synthetic strategy employed a Stille palladium cross-coupling approach combined with a photochemical induced 6π-electrocyclisation. The synthesis was generalized to several substituted indoles, and maleimide substitutions led to the series of representative original BDCZs. Compound **55** was the most active derivative with a strong effect on cell cycle. Efforts are being made to find the biological target and to optimize the structure of our lead.

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7. *Representative procedure for the preparation of* **16**–**27**: To a solution of compound **2** (176 mg, 0.59 mmol) in 1,4-dioxane (10 mL) were successively added under argon 2-trimethylstannylbenzodioxine (200 mg, 1.49 mmol),[9] 20 mol % of copper iodide and 10 mol of $PdCl_2(PPh_3)_2$. The reaction mixture was poured under stirring in a pre-heated oil bath (100 °C) for 1 h 45 min. After cooling, the solution was filtered over Celite and the precipitate was washed wit EtOAc (20 mL). The combined organic layers were removed under reduced pressure. The crude was purified without any further treatment by flash chromatography on silica gel (petroleum ether/EtOAc 6:4) to afford compound **17** as a red solid (79% yield). Mp: 219–221 °C; R_f: 0.41 (petroleum ether/EtOAc 7:3); ^{1}H NMR (acetone-d_6, 250 MHz): δ 3.04 (s, 3H), 5.67 (dd, 1H, *J* = 1.6 Hz and *J* = 7.8 Hz), 6.67 (dt, 1H, *J* = 7.8 Hz and *J* = 1.6 Hz,), 6.74–6.88 (m, 2H), 6.96 (dt, 1H, H_{Ar}, *J* = 7.8 Hz and *J* = 1.0 Hz), 7.15 (dt, 1H, *J* = 7.8 Hz and *J* = 1.0 Hz), 7.25 (s, 1H), 7.53 (d, 1H, *J* = 7.8 Hz), 7.72 (d, 1H, *J* = 7.8 Hz), 7.91 (d, 1H, *J* = 2.8 Hz), 10.99 (br s, 1H, NH, exchangeable D_2O); ^{13}C NMR (DMSO-d_6, 62.9 MHz): δ 24.0 (CH_3), 105.1 (Cq), 112.1 (CH), 116.1 (CH), 116.2 (CH), 119.3 (Cq), 120.3 (CH + Cq), 121.0 (CH), 122.0 (CH), 124.4 (CH), 124.7 (CH), 126.5 (Cq), 130.3 (Cq), 130.8 (CH), 131.1 (CH), 136.0 (Cq), 140.9 (Cq), 141.1 (Cq), 169.4 (C=O), 170.3 (C=O); HRMS (TOF ES+) *m*/*z* $[M+Na]^+$ calcd for $C_{21}H_{14}N_2O_4{}^{23}Na$: 381.0851; found 381.0848.
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10. *Representative procedure for the preparation of* **30**–**40**. A solution of compound **17** (150 mg, 0.418 mmol) and diiodine (1.50 g, 5.91 mmol) in toluene (500 mL) was irradiated for 1 h 10 min in a quartz vessel with a 'DEMA UV-lamp TQ-718 at 500 W. The reaction mixture was diluted with EtOAc (150 mL) and washed with an aqueous solution of sodium thiosulfite 20% (80 mL) The organic layers were evaporated under reduced pressure and the residue was washed successively with Et_2O (2 × 50 mL), THF (50 mL). The filtrate was removed under reduced pressure and the crude material was purified by flash chromatography (petroleum ether/EtOAc 6:4) to afford compound **31** as a yellow solid (76 mg, 51%). Mp: 258 °C (dec.); R_f: 0.59 (petroleum ether/EtOAc 6:4); ^{1}H NMR (DMSO-d_6, 250 MHz): δ 3.02 (s, 3H), 7.10 (m, 4H), 7.27 (dt, 1H, *J* = 7.4 Hz and *J* = 2.2 Hz,), 7.48–7.55 (m, 2H), 8.71 (d, 1H, *J* = 7.4 Hz), 12.18 (br s, 1H, NH, exchangeable D_2O); ^{13}C NMR (DMSO-d_6, 62.9 MHz): δ 23.7 (CH_3), 111.8 (CH), 114.0 (Cq), 114.9 (Cq), 116.7 (CH), 117.1 (CH), 120.6 (CH), 124.3 (CH), 125.3 (2 × CH), 128.1 (CH), 129.7 (Cq), 130.5 (Cq), 132.1 (Cq), 135.3 (Cq), 140.5 (Cq), 140.6 (Cq), 142.3 (Cq), 157.7 (Cq), 166.0 (C=O), 167.7 (C=O); HRMS (TOF ES+) *m*/*z* $[M+Na]^+$ calcd for $C_{21}H_{12}N_2O_4{}^{23}Na$: 379.0689; found 379.0690.
11. *Representative procedure for the preparation of* **46**–**50**. A solution of compound **35** (45 mg, 0.8 mmol) in *N,N*-dimethylethylenediamine (4 mL) was refluxed for 22 h. After evaporation, the residue was triturated in methanol and filtered to afford **50** as a yellow solid (75% yield). Mp: 262 °C; R_f: 0.21 (acetone); ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.57 (s, 6H), 2.94 (t, 2H, *J* = 5.1 Hz), 3.88 (t, 2H, *J* = 5.1 Hz), 5.25 (s, 2H), 6.55 (dd, 1H, *J* = 2 Hz and *J* = 8.8 Hz), 6.65 (dd, 1H, *J* = 1.7 Hz and *J* = 7.7 Hz), 6.78 (d, 1H, *J* = 2 Hz), 6.86–7.03 (m, 3H), 7.37–7.49 (m, 3H), 7.56–7.59 (m, 2H), 8.13 (d, 1H, *J* = 8.8 Hz), 11.07 (br s, 1H, NH, exchangeable D_2O). NMR DEPT 135 ($CDCl_3$, 62.9 MHz): δ 34.9 (CH_2), 45.7 (2 × CH_3), 58.1 (CH_2), 70.5 (CH_2), 96.9 (CH), 110.9 (CH), 116.2 (CH), 117.8 (CH), 124.3 (2 × CH), 125.4 (CH), 127.5 (2 × CH), 128.0 (CH), 128.7 (2 × CH). HRMS (TOF ES+) *m*/*z* $[M+Na]^+$ calcd for $C_{31}H_{25}N_3O_5{}^{23}Na$: 542.1691; found 542.1688.
12. Removal of the benzyl group give compound **55** as an orange–yellow solid (98%). Mp >360 °C; R_f: 0.07 (acetone); ^{1}H NMR (DMSO-d_6, 250 MHz): δ 2.18 (s, 6H), 3.68 (t, 2H, *J* = 6 Hz), 7.01 (dd, 1H, *J* = 2.4 Hz and *J* = 8.9 Hz), 7.11 (m, 4H), 7.37 (d, 1H, *J* = 8.9 Hz), 8.18 (d, 1H, *J* = 2.4 Hz). NMR DEPT 135 (DMSO-d_6, 62.9 MHz): δ 36.2 (CH_2), 46.0 (2 × CH_3), 57.6 (CH_2), 109.7 (CH), 113.3 (CH), 117.6 (CH), 117.9 (CH), 118.6 (CH), 126.0 (CH), 126.1 (CH). HRMS (TOF ES+) *m*/*z* $[M+Na]^+$ calcd for $C_{24}H_{19}N_3O_5{}^{23}Na$: 452.1222; found 452.1218.