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Synthesis and biological evaluation of novel cytotoxic azanaphthoquinone annelated pyrrolo oximes

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Abstract—Two series of azanaphthoquinone annelated pyrrolo oximes have been synthesized. The antiproliferative activities of 10 compounds were evaluated on at least four different cell lines. One series of pyrrolo derivatives showed high cytotoxic activity. The effects on cell cycle and caspase activity were investigated. Compounds **9a** and **9b** showed an accumulation of cells in G2/M phase. Substantial and dose-dependent caspase activity was found after treatment of cells with **9a** and **9b**. This indicates an apoptosis inducing property of these compounds.

The anthracene-9,10-dione mitoxantrone (**1**) has probably become the most widely used synthetic DNA intercalating agent.[1] It is active against acute leukemia, lymphoma, breast cancer, and cervix carcinoma.[2] Mitoxantrone binds reversibly to DNA by intercalation, with the side chains lying in the major groove.[3] However, the cytotoxicity of mitoxantrone is caused largely by inhibition of topoisomerase II enzyme.[4] The use in clinics of topoisomerase II inhibitors is limited by their cardiotoxicity and drug resistance.[5] The search of new topoisomerase II inhibitors still constitutes an intensive field of research.

The development of aza-bioisosteric chemotypes related to mitoxantrone lacking the 5,8-dihydroxy substituted pattern led to the discovery of an aza-anthracene-9,10-dione BBR 2778 (**2**), which is currently in phase III clinical trials in patients with non-Hodgkin's lymphoma.[6] This compound showed a better therapeutic index and lower cardiotoxicity in comparison with mitoxantrone.[7] The 9-aza-anthrapyrazoles have also been developed as analogs of anthrapyrazoles. The position of the nitrogen has been found to exert a profound effect on the antitumor activity.[8] While 9-aza derivatives showed outstanding activity, the 8-aza congeners were essentially inactive. The studies on topoisomerase II poisoning effects showed an impaired interference of 8-aza compounds with the DNA processing enzyme, which explains the lack of cytotoxicity of these compounds.[9] A 9-aza-anthrapyrazole, BBR 3438 (**3**), is currently in phase II clinical trials in patients with gastric cancer.[10]

In our continuous effort to develop novel DNA interactive anticancer compounds based on the azanaphthoquinone annelated pyrrole structures,[11] we are reporting in this paper the synthesis of two series of azanaphthoquinone pyrrolo oximes of type **9** and **13** with the nitrogen atom in the pyrrole ring located at position 1 and 2, respectively. The cytotoxicity against at least four tumor cell lines is reported and compared between these two isomers. The active compounds have been further studied with regard to the effects on the cell cycle and caspase activity (Fig. 1).

Annelated pyrrolo oximes of type **9** were synthesized from 5-hydroxyisoquinoline (**4**) as shown in Scheme 1. Oxidation of **4** yielded isoquinoline-5,8-dione (**5**) in high yield.[12] Treatment of **5** with sodium azide gave 88% of 7-aminoisoquinoline-5,8-dione (**6**), which was reacted with $Mn(OAc)_3$/acetaldehyde diethylacetal[13] to furnish 1*H*-pyrrolo[3,2-*g*]isoquinoline-4,9-dione (**7**)[14] in 62% yield. N-Alkylation of **7** was carried out with NaH/*N*,*N*-dimethylaminoethyl chloride to obtain the alkylated product **8** in 48% yield. The aza-condensation of **8** with a series of hydroxylamine derivatives[15] occurred regiose-

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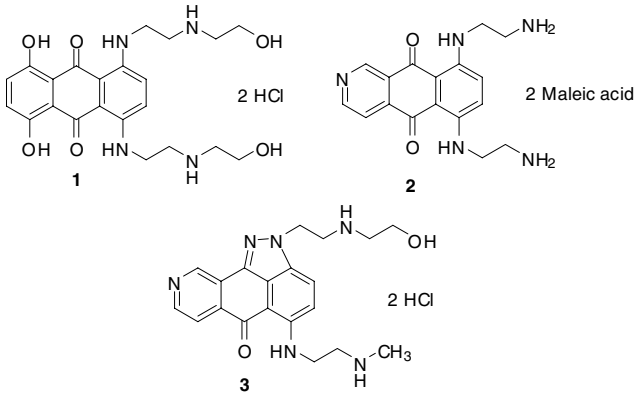
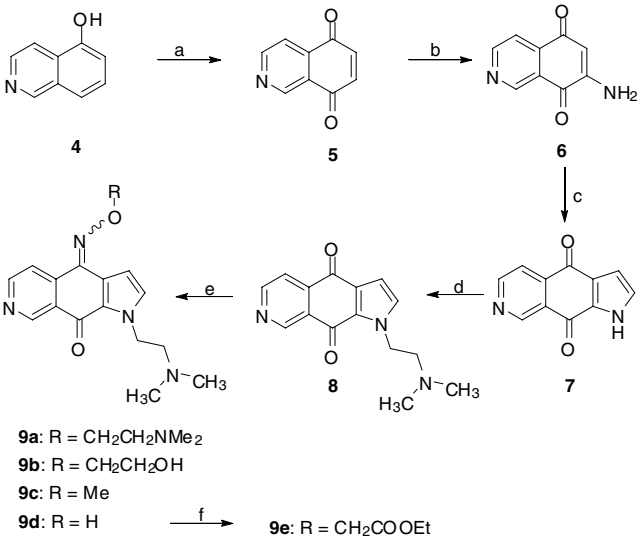


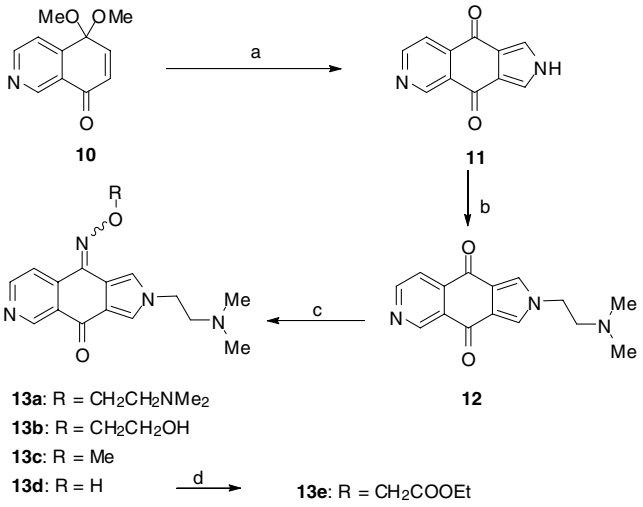
Figure 1.



Scheme 1. Reagents and conditions: (a) $PhI(OCOCF_3)_2$, $MeCN/H_2O$, 0 °C, 2 h; (b) NaN_3/H_2O, AcOH, THF, 40 °C, 2 h; (c) $MeCH(OEt)_2$, $Mn(OAc)_3$, AcOH, 60 °C, 18 h; (d) $ClCH_2CH_2NMe_2$·HCl, NaH, DMF, 67 °C, 4 h; (e) NH_2OR·HCl, 50% KOH, MeOH, rt; (f) $BrCH_2COOEt$, NaH, DMF, rt.

lectively at C-4 to furnish the corresponding oximes **9a**–**9d**. The structure elucidation of oxime **9c** was accomplished by 2D NMR and NOE experiments. Irradiation at OMe showed a significant nuclear Overhauser enhancement of C(3)-H and C(5)-H thus indicating the condensation at C-4 in the quinone system. The regioselectivity of the reaction could be explained by the stronger electrophilicity of the carbonyl group located in *para*-position to the nitrogen atom in the pyridine ring. Compound **9d** was converted into **9e** by O-alkylation with ethyl bromoacetate using NaH in DMF.

Pyrrolo oximes of type **13** were prepared as outlined in Scheme 2, starting from 5,5-dimethoxyisoquinolin-8(5*H*)-one (**10**).[14] The first step involves the Michael addition-cyclization of the unsaturated ketone **10** with tosylmethyl isocyanide (TosMIC) followed by acidic hydrolysis of the ketal to give pyrrole **11** in 79% overall yields.[14] N-Alkylation of **11** was carried out in the same way as mentioned above furnishing 59% yield of **12**,



Scheme 2. Reagents and conditions: (a) 1—TosMIC, KO*t*-Bu, THF, rt, 18 h; 2—PPTS/TsOH, acetone/H_2O, 35 °C, 24 h; (b) $ClCH_2CH_2NMe_2$·HCl, NaH, DMF, 67 °C, 4 h; (c) NH_2OR·HCl, 50% KOH, MeOH, rt; (d) $BrCH_2COOEt$, NaH, DMF, rt.

which was condensed regioselectively with hydroxyl amines at C-9 to the corresponding oximes **13a**–**13d** in good yields. The regioselectivity of the aza-condensation was determined by 2D NMR and NOE experiments. Irradiation at OMe showed a significant nuclear Overhauser enhancement of C(1)-H and C(8)-H, therefore indicating the condensation at C-9 in the quinone system. Oxime **13d** underwent reaction with ethyl bromo acetate to give **13e** in 26% yield.[22]

Compounds **9a**–**9e**, **13a**–**13e** as well as reference compounds paclitaxel and doxorubicin were screened for antiproliferative activity against different cancer cell lines KB/HeLa (cervical carcinoma), NCI-H460 (large-cell lung cancer), SKOV-3 (ovarian carcinoma), SF-268 (CNS, glioma), and RKOp27 (colon adenocarcinoma).[16] The quinones **8** and **12** were included in the screening in order to determine whether the oxime formation leads to improved cytotoxic activities. The concentration of the compound that inhibits 50% (IC_{50}) of cell proliferation after 48 h was calculated by nonlinear regression (GraphPad Prism™) using the data from at least two independent tetrazolium-based (XTT) cytotoxicity assays.[17] Results of the cytotoxicity assays are shown in detail in Table 1. Interestingly compounds **8** and **9a**–**9e** of the pyrrolo[2,3-*g*]isoquinoline series displayed significantly higher inhibition across all cell lines compared to compounds **12** and **13a**–**13e** of the pyrrolo[3,4-*g*]isoquinoline series (<50%). Cell lines NCI-H460 and RKOp27 were found to be most sensitive to compounds of the pyrrolo[2,3-*g*]isoquinoline series. In particular the *O*-(2-hydroxyethyl)oxime derivative **9b** exhibited the highest inhibition with a mean IC_{50} of 0.13 μM against NCI-H460 and 0.17 μM against RKOp27 cells. *O*-[2-(Dimethylamino) ethyl]oxime **9a** was found to be the second most active compound with an IC_{50} value of 0.23 μM, followed by derivative **9c** with 0.79 μM against NCI-H460. Compound **9d** showed moderate activity with IC_{50} values of >12 μM against all tested cell lines. It must be emphasized that oximation of **8** led to about

Table 1. In vitro cytotoxicity of compounds **8**, **9a**–**9e**, **12** and **13a**–**13e** across a panel of cancer cell lines

Cells (origin)	IC_{50}[a] (μmol)						
Compound	**8**	**9a**	**9b**	**9c**	**9d**	**9e**	**12**, **13a**–**13e**
KB/HeLa (cervix)	11.04	0.67 ± 0.06	0.66 ± 0.04	1.21 ± 0.05	>12	>12	n.a.[b]
NCI-H460 (lungs)	3.84	0.23 ± 0.01	0.13 ± 0.01	0.79 ± 0.05	>12	>12	n.a.
SF-268 (CNS)	4.71	1.23 ± 0.14	1.39 ± 0.01	2.43 ± 0.12	>12	>12	n.a.
SKOV-3 (ovaries)	13.87	0.94 ± 0.03	0.48 ± 0.02	2.24 ± 0.06	>12	>12	n.a.
RKOp27 (colon)	2.35	0.41 ± 0.07	0.17 ± 0.02	1.16 ± 0.01	>12	>12	n.a.

[a] Lower IC_{50} values are given as means ± standard deviation from at least two XTT assay experiments.
[b] n.a., not active, that is <50% inhibition at a compound concentration of 3.16 μg/ml.

Table 2. Cytotoxicity [μM] of selected compounds against drug-resistant cancer cell lines[19]

	LT12	LT12MDR	P388	P388ADR	L1210	L1210VCR
9a	0.03	0.07	0.02	0.40	0.05	0.11
9b	0.04	0.16	0.02	0.50	0.07	0.16
Pac	0.005	0.368	0.038	>3.4	0.052	>3.4
Dox	0.031	>3.4	0.52	>3.4	0.221	>3.4

Pac, paclitaxel; Dox, doxorubicin.

10-fold enhancement of cytotoxic activity. Compounds **9a** and **9b** were subjected to antiproliferative activity experiments against wild type and MDR cancer cell lines[18] for further characterization. As can be seen from the results summarized in Table 2, **9a** and **9b** show nearly the same activity against wild type/MDR cell line pairs as paclitaxel and doxorubicin, all compounds being tested in the same experimental setting.

Effects on the cell cycle were investigated by the treatment of KB/HeLa cells with different concentrations of selected compounds for 24 h, using untreated cells as reference. Propidiumiodide-stained cells were analyzed by flow cytometry analysis[20] and the resulting data of **9a** and **9b** confirmed the arrest of the cell cycle in the G2/M phase, Figure 2 (data for 9a).

Caspase 3/7 activity experiments were performed in U937 cells with DEVD rho-110 substrate after treatment with selected compounds for 24 h. Cells treated with **9a** and **9b** exhibited significant, dose-dependent caspase activity (⩽10 μM) which indicates apoptosis inducing properties, as shown in Figure 3.

Therefore we conclude that compounds **9a** and **9b** exert their antiproliferative/cytotoxic effect by arresting the cells in G2/M phase and activation of apoptosis pathways.

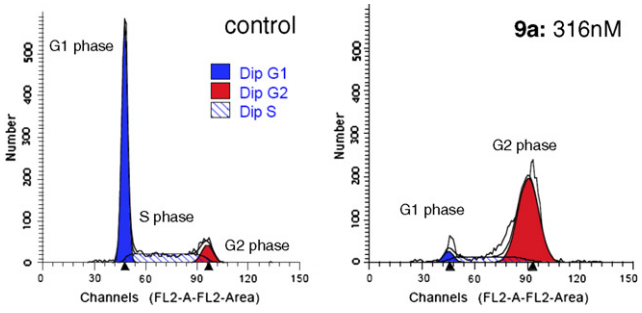


Figure 2. Representative FACS analysis diagrams for **9a** (right) showing the increase of cells arrested in G2/M phase compared to control (left).

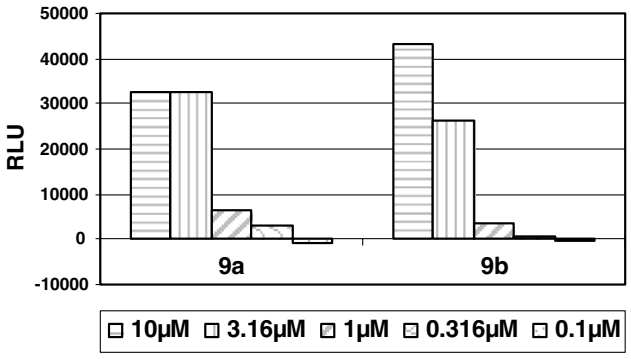


Figure 3. Results of caspase activity experiments for **9a** and **9b**.

Remarkably, it was demonstrated by standard DNA intercalating UV experiments that **9a** did not intercalate into DNA (data not shown).[21]

In conclusion, oximes of pyrrolo[3,2-*g*]isoquinoline-4,9-dione derivatives and pyrrolo[4,3-*g*]isoquinoline-4,9-dione derivatives have been synthesized and characterized. These compounds were screened for cytotoxic activity against different cell lines. All oximes of the pyrrolo[3,2-*g*]isoquinoline series exhibited superior IC_{50} values compared with the pyrrolo[4,3-*g*]isoquinoline series and the quinone precursors. Two lead compounds **9a** and **9b** were identified, that exhibited better antiproliferative effects than paclitaxel and doxorubicin on multidrug resistant cell lines. Caspase 3/7 induction indicated the arrest of the cell cycle in the G2/M phase of the cell cycle. These drugs do not intercalate into DNA, which suggests that the cytotoxic effects underlie a different mechanism than classical DNA intercalating agents. These results provide useful information for further studies and for the development of an extended compound library.

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18. From the readily available wild types LT12, P388, and L1210, cell lines were established, that were resistant against standard cancer reference compounds.

Wild type	Resistant type
L1210	L1210VCR (vincristine resist.)
P388	P388ADR (doxorubicin resist.)
LT12	LT12MDR (Ectopic expression of human MDR1 K. Nooter, University of Rotterdam)

19. It might attract attention that the activity of doxorubicin reported herein (see Table 2) differs from results published elsewhere (e.g., Furusawa, S.; Nakano, S.; Wu, J.; Sakaguchi, S.; Takayanagi, M.; Sasaki, K. I.; Satoh S.; *J. Pharm. Pharmacol.* **2001**, *53*, 1029). After carefully re-evaluating and confirming the results published herein, reasons for these differences might be found in different cell line development in the past or different experimental settings (e.g., use of the XTT vs MTT detection reagent, use of different cell counts, etc.). However, the difference in activity between wild type and resistant cell lines for test and reference compounds reported herein and the conclusions drawn from these results remain unaffected.
20. For FACS analysis, a FACSCalibur™ cytometer (Becton Dickinson, Heidelberg, GER) and Mod Fit LT VERITY (cell cycle analysis software) were used.
21. For DNA intercalation experiments following an in-house protocol, stock solution of reference and test compounds at 2–5 μM concentration in 5% DMSO (v/v) were prepared. After 1:10 (v/v) dilution of the compound stock in reaction buffer (5 mM NaH_2PO_4, 5 mM Na_2HPO_4, 70 mM NaCl, pH 7), the absorbance [Amax] ~1 was determined in 96-well UV plates (Costar, Acton, MA) between 230 and 570 nm using a Spectramax 190 plus reader (Molecular Devices, Sunnyvale, CA). The resulting curve was compared to a curve generated by using 2 mg/ml (w/v) calf thymus DNA (Sigma–Aldrich Corp., St. Louis, MO) solution in reaction buffer as compound diluent.
22. All of the final structures were confirmed by ^{1}H NMR, ^{13}C NMR, IR, and MS as the following.
Compound **6***:* Mp = 287 °C;^{1}H NMR (DMSO-d_6): δ (ppm) 5.89 (s, 1H), 7.48 (br s, 2H), 7.75 (d, J = 4.9 Hz, 1H), 8.99 (d, J = 4.9 Hz, 1H), 9.07 (s, 1H); MS: *m/z* 174 (M^+, 100), 147 (11), 130 (2), 118 (7), 106 (23).
Compound **8***:* Mp = 148 °C; ^{1}H NMR ($CDCl_3$): δ (ppm) 2.30 (s, 6H), 2.73 (t, J = 6.5 Hz, 2H), 4.57 (t, J = 6.5 Hz, 2H), 6.77 (d, J = 2.8 Hz, 1H), 7.10 (d, J = 2.8 Hz, 1H), 7.94 (d, J = 5.0 Hz, 1H), 8.98 (d, J = 5.0 Hz, 1H), 9.37 (s, 1H); MS: *m/z* 269 (M^+, 1), 211 (1), 155 (1), 58 (100).
Compound **9a***:* Mp = 70 °C; ^{1}H NMR ($CDCl_3$): δ (ppm) 2.28 (s, 6H), 2.33 (s, 6H), 2.71 (t, J = 6.5 Hz, 2H), 2.80 (t, J = 5.9 Hz, 2H), 4.57 (m, 4H), 7.12 (m, 2H), 8.10 (d, J = 5.4 Hz, 1H), 8.71 (d, J = 5.4 Hz, 1H), 9.42 (s, 1H); ^{13}C NMR ($CDCl_3$): δ (ppm) 45.6, 46.0, 47.3, 58.2, 59.8, 74.9, 117.0, 121.9, 126.0, 126.1, 131.6, 140.7, 140.9, 150.0, 151.6, 174.3; IR (KBr): ν_{max} 3427, 3100, 2947, 2762, 1639, 1576, 1460, 1427, 1379, 1270 cm^{-1}; MS: *m/z* 355 (M^+, 1), 284 (1), 282 (2), 269 (3), 267 (1), 266 (1), 222 (1), 167 (1), 149 (5), 125 (1).
Compound **9b***:* Mp = 154 °C; ^{1}H NMR ($CDCl_3$): δ (ppm) 2.25 (s, 6H), 2.62 (t, J = 6.7 Hz, 2H), 3.81 (s, 1H), 4.04 (t, J = 4.4 Hz, 2H), 4.43 (t, J = 6.8 Hz, 2H), 4.54 (t, J = 4.4 Hz, 2H), 6.97 (m, 2H), 7.86 (d, J = 5.3 Hz, 1H), 8.55 (d, J = 5.4 Hz, 1H), 9.20 (s, 1H); ^{13}C NMR ($CDCl_3$): δ (ppm) 45.5, 47.0, 59.5, 61.3, 59.8, 78.1, 111.7, 116.7, 121.5, 125.7, 125.8, 131.5, 140.3, 140.9, 148.6, 151.3, 173.7; IR (KBr): ν_{max} 3486, 3191, 3123, 2940, 2821, 2767, 1637, 1595, 1577, 1534, 1520, 1479, 1429 cm^{-1}; MS: *m/z* 328 (M^+, 1), 284 (1), 270 (1), 240 (1), 226 (1), 222 (1), 209 (1), 198 (2), 193 (1), 181 (1), 170 (1).
Compound **9c***:* Mp = 140 °C; ^{1}H NMR ($CDCl_3$): δ (ppm) 2.28 (s, 6H), 2.71 (t, J = 6.6 Hz, 2H), 4.25 (s, 3H), 4.59 (t, J = 6.6 Hz, 2H), 7.08 (m, 2H), 8.08 (d, J = 5.4 Hz, 1H), 8.70 (d, J = 5.3 Hz, 1H), 9.40 (s, 1H); ^{13}C NMR ($CDCl_3$): δ (ppm) 45.1, 47.3, 59.8, 64.0, 111.5, 117.0, 121.8, 126.0, 126.1, 131.5, 140.6, 140.7, 148.9, 151.6, 174.3; IR (KBr): ν_{max} 3422, 3118, 2972, 2934, 2818, 2767, 1639, 1576, 1481, 1425, 1381 cm^{-1}; MS: *m/z* 298 (M^+, 1), 208 (1), 195 (1), 180 (1), 155 (1), 154 (1), 140 (1), 127 (1).
Compound **9d***:* Mp (decomp.) = 173 °C; ^{1}H NMR ($CDCl_3$): δ (ppm) 2.51 (s, 6H), 3.10 (t, J = 5.5 Hz, 2H), 4.72 (t, J = 5.4 Hz, 2H), 7.03 (m, 2H), 7.57 (d, J = 5.4 Hz, 1H), 8.33 (d, J = 5.4 Hz, 1H), 9.20 (s, 1H); ^{13}C NMR ($CDCl_3$): δ (ppm) 45.1, 46.0, 59.7, 112.5, 116.1, 122.7, 125.7, 126.0, 130.9, 140.1, 141.2, 148.3, 150.8, 174.3; IR

(KBr): ν_{max} 3224, 2957, 2923, 1624, 1580, 1456, 1431, 1386, 1269 cm^{-1}; MS: *m/z* 284 (M^+, 1), 269 (1), 267 (1), 240 (1), 224 (1), 133 (1), 131 (1).

Compound **9e***:* Mp = 90 °C; ^1H NMR ($CDCl_3$): δ (ppm) 1.29 (t, J = 7.2 Hz, 3H), 2.29 (s, 6H), 2.71 (t, J = 6.6 Hz, 2H), 4.26 (q, J = 7.2 Hz, 2H), 4.59 (t, J = 6.6 Hz, 2H), 4.97 (s, 2H), 7.11 (d, J = 2.6 Hz, 1H), 7.19 (d, J = 2.6 Hz, 1H), 8.02 (d, J = 5.3 Hz, 1H), 8.70 (d, J = 5.3 Hz, 1H), 9.39 (s, 1H); ^{13}C NMR ($CDCl_3$): δ (ppm) 14.1, 45.5, 47.2, 59.7, 61.2, 72.4, 112.2, 117.1, 121.5, 126.0, 126.2, 131.7, 140.2, 140.9, 148.9, 151.8, 168.7, 174.2; IR (KBr): ν_{max} 3426, 3145, 3108, 2941, 2917, 2858, 2821, 2767, 1756, 1638, 1598, 1579, 1539, 1482 cm^{-1}; MS: *m/z* 370 (M^+, 1), 322 (1), 297 (1), 269 (1), 267 (1), 225 (1), 222 (1), 209 (1), 208 (1), 181 (1), 167 (1).

Compound **12***:* Mp = 180–181 °C; ^1H NMR ($CDCl_3$): δ (ppm) 2.28 (s, 6H), 2.71 (t, J = 6.1 Hz, 2H), 4.09 (t, J = 6.1 Hz, 2H), 7.53 (d, J = 1.8 Hz, 1H), 7.55 (d, J = 1.8 Hz, 1H), 8.01 (d, J = 5.0 Hz, 1H), 9.00 (d, J = 5.0 Hz, 1H), 9.47 (s, 1H); MS: *m/z* 270 (M^++1, 0.9), 225 (2.1), 211 (2.3), 183 (1.1), 155 (1.8), 58 (100).

Compound **13a***:* Mp = 83-85 °C; ^1H NMR ($CDCl_3$): δ (ppm) 2.29 (s, 6H), 2.35 (s, 6H), 2.72 (t, J = 6.5 Hz, 2H), 2.81 (t, J = 5.9 Hz, 2H), 4.10 (t, J = 6.5 Hz, 2H), 4.57 (t, J = 5.9 Hz, 2H), 7.60 (d, J = 2.0 Hz, 1H), 7.78 (d, J = 2.0 Hz, 1H), 8.13 (d, J = 5.4 Hz, 1H), 8.74 (d, J = 5.4 Hz, 1H), 9.47 (s, 1H); ^{13}C NMR ($CDCl_3$): δ (ppm) 45.6, 46.0, 48.8, 58.3, 59.7, 74.3, 114.1, 117.5, 120.2, 123.5, 126.2, 126.6, 140.8, 141.7, 149.7, 152.1, 179.7; IR (KBr): ν_{max} 2944, 2821, 1644, 1579, 1536, 1267 cm^{-1}; MS: *m/z* 355 (M^+, 0.2), 338 (0.6), 296 (0.3), 282 (2.5), 269 (3.2), 58 (100).

Compound **13b***:* Mp = 127–129 °C; ^1H NMR ($CDCl_3$): δ (ppm) 2.28 (s, 6H), 2.70 (t, J = 6.5 Hz, 2H), 4.06 (t, J = 6.5 Hz, 2H), 4.06 (t, J = 4.4 Hz, 2H), 4.56 (t, J = 4.4 Hz, 2H), 7.49 (d, J = 2.0 Hz, 1H), 7.68 (d, J = 2.0 Hz, 1H), 7.94 (d, J = 5.6 Hz, 1H), 8.64 (d, J = 5.6 Hz, 1H), 9.32 (s, 1H); ^{13}C NMR ($CDCl_3$): δ (ppm) 45.5, 48.7, 59.7, 61.7, 77.4, 113.8, 117.3, 120.0, 123.7, 126.1, 126.7, 141.0, 141.4, 149.5, 151.9, 179.4. IR (KBr): ν_{max} 3422, 2962, 2816, 1642, 1573, 1539, 1265, 1267 cm^{-1}; MS: *m/z* 329 (M^++1, 3.3), 265 (1.8), 208 (4.7), 180 (2.8), 58 (100).

Compound **13c***:* Mp = 121–123 °C; ^1H NMR ($CDCl_3$): δ (ppm) 2.28 (s, 6H), 2.73 (t, J = 6.5 Hz, 2H), 4.11 (t, J = 6.5 Hz, 2H), 4.27 (s, 3H), 7.61 (d, J = 2.0 Hz, 1H), 7.70 (d, J = 2.0 Hz, 1H), 8.13 (dd, J = 5.4, 0.6 Hz, 1H), 8.74 (d, J = 5.4 Hz, 1H), 9.46 (d, J = 0.6 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ (ppm): 45.6, 48.9, 59.8, 63.8, 114.1, 117.5, 120.3, 123.5, 126.3, 126.4, 140.6, 141.7, 149.7, 152.1, 179.8; IR (KBr): ν_{max} 3415, 2937, 2822, 1648, 1579, 1537, 1270 cm^{-1}; MS: *m/z* 299 (M^++1, 9.1), 265 (3.3), 240 (1.4), 208 (3.6), 180 (2.4), 58 (100).

Compound **13d***:* Mp >350 °C; ^1H NMR (DMSO-d_6): δ (ppm) 2.18 (s, 6H), 2.64 (t, J = 6.1 Hz, 2H), 4.22 (t, J = 6.1 Hz, 2H), 7.84 (d, J = 1.7 Hz, 1H), 8.01 (d, J = 1.7 Hz, 1H), 8.11 (d, J = 5.4 Hz, 1H), 8.75 (d, J = 5.4 Hz, 1H), 9.25 (s, 1H); ^{13}C NMR (DMSO-d_6): δ (ppm) 45.1, 47.4, 59.3, 113.1, 117.0, 118.8, 124.2, 125.6, 126.7, 140.2, 142.0, 148.7, 152.1, 178.6; IR (KBr): ν_{max} 3446, 2919, 2842, 1653, 1554, 1533, 1263 cm^{-1}; MS: *m/z* 284 (M^+, 0.4), 265 (0.5), 239 (0.5), 223 (0.3), 180 (0.5), 58 (100).

Compound **13e***:* Mp = 150–151 °C; ^1H NMR ($CDCl_3$): δ (ppm) 1.33 (t, J = 7.1 Hz, 3H), 2.29 (s, 6H), 2.73 (t, J = 6.5 Hz, 2H), 4.11 (t, J = 6.5 Hz, 2H), 4.30 (q, J = 7.1 Hz, 2H), 5.00 (s, 2H), 7.62 (d, J = 2.0 Hz, 1H), 7.86 (d, J = 2.0 Hz, 1H), 8.08 (dd, J = 5.4, 0.6 Hz, 1H), 8.74 (d, J = 5.4 Hz, 1H), 9.47 (d, J = 0.6 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ (ppm) 14.2, 45.5, 48.8, 59.7, 61.2, 72.3, 113.7, 117.6, 120.3, 123.7, 126.4, 127.4, 141.2, 142.2, 149.7, 152.3, 169.0, 179.6; IR (KBr): ν_{max} 3446, 2959, 1746, 1650, 1583, 1537, 1220 cm^{-1}; MS: *m/z* 370 (M^+, 0.6), 297 (0.7), 265 (1.2), 208 (1.9), 180 (1.3), 58 (100).