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Synthesis and biological evaluation of new cytotoxic azanaphthoquinone pyrrolo-annelated derivatives

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ABSTRACT

A series of azanaphthoquinone pyrrolo-annelated derivatives attached to basic side chains have been synthesized. The antiproliferative activities of all compounds were evaluated on at least four different cell lines. The effects on cell cycle and intercalation were investigated.

Mitoxantrone is a widely used anticancer agent.[1,2] The most distinctive disadvantage of this intercalating drug molecule is cardiotoxicity.[3] It seems that a solution of this problem has been found by the development of BBR2778 (Pixantrone) which is currently in phase III clinical trials in patients with Non-Hodgkin's Lymphoma.[4] This compound exhibits less cardiotoxicity[5] and it is evident that this advantage is based on the substitution of the 5,8-dihydroxy phenyl moiety in Mitoxantrone by a pyridine ring.[6] On the basis of Pixantrone-type compounds the authors[7] previously reported the synthesis of a series of azanaphthoquinone pyrrolo-annelated compounds. The phenyl ring in Pixantrone connected to two amino groups with distinct electron donating effects is substituted by a pyrrole ring. Compound **2a** of this pyrrolo-annelated azanaphthoquinone series exhibited significant antiproliferative activity, which does not arise from intercalation but rather induction of caspase 3/7 activity. Thus, the rationale for the reported synthesis is the question whether different annelation patterns of the pyridine ring to the quinone nucleus as well as the substitution by equally electron withdrawing heteroaromatic ring systems like pyrazine or pyrimidine would lead to enhanced cytotoxic activities. All target cores are attached to a dimethylaminoethyl or dimethylaminopropyl side chain, respectively (**1a**,**b**–**6a**,**b**; Fig. 1). In addition to cytotoxicity the effects on cell cycle, caspase activity, and intercalation were investigated.

Our continuous efforts of designing new cytotoxic compounds[7,8] led to the study presented in this Letter. Regarding the previously characterized compound **2**,[9] which serves as one of the key compounds in our investigations, a series of novel azanaphthoquinone pyrrolo-annelated derivatives was synthesized. With the exception of compound **1**, which was prepared as previously described by the authors,[9] the syntheses of all compounds **2**–**6** were realized according to the methodology as depicted in Scheme 1. This circumstance is due to the regioselectivity of the reaction shown in Scheme 1 which results only in the synthesis of compound **2** but not **1**. Compound **7** was straightforwardly obtained by oxidation of readily available 5-hydroxyisochinoline. Compounds **8** and **11** were synthesized in a four-step reaction, whereas compound **9** was prepared in five steps including a final oxidation of 5,8-dimethoxychinoline, referring to Kitahara

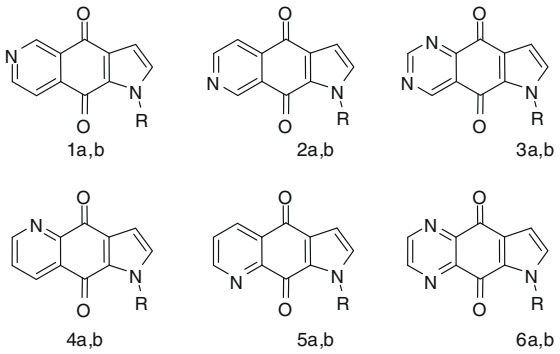

Figure 1.

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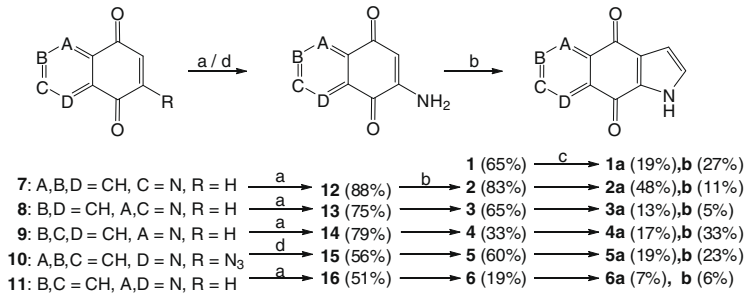

Scheme 1. Reagents and conditions: (a) NaN_3/H_2O, AcOH, THF, 40 °C, 2 h; (b) $MeCH(OEt)_2$, $Mn(OAc)_3$, AcOH, 60 °C, 18 h; (c) $ClCH_2CH_2NMe_2{\cdot}HCl$ or $ClCH_2CH_2CH_2NMe_2{\cdot}HCl$, NaH, DMF, 67 °C, 4 h; (d) Ph_3P/CH_2Cl_2, AcOH, rt, 1 h.

et al.[10] The following amination of **9** gave stereoisomere **14** with high selectivity, thus a literature approach[11] to compound **15** was adapted which suggested the reduction of 7-azidochinoline-5,8-dione (**10**) (Scheme 1, reaction d). Amino quinones **12** and **14** were prepared according to Shanab et al.[7] with sodium azide in acetic acid/THF. The new amino quinones **13** and **16** were made accessible analogously. In accordance to Wu et al.,[12] the azidation reaction of **8** leading to amino derivative **13** occurred regioselectively, which was confirmed by INEPT and HSQC NMR experiments.

The amino quinones **12**–**16** underwent cyclization to pyrrolo quinones **2**–**6** by exploiting Wu's method[12] of a free radical reaction in the presence of $Mn(OAc)_3$/acetaldehyde diethylacetal. Subsequently, **1**–**6** were N-alkylated with *N,N*-dimethylaminoethyl chloride or *N,N*-dimethylaminopropyl chloride, respectively, to furnish target compounds **1a**,**b**–**6a**,**b**.

Pyrrole annelated isoquinoline diones **1a**,**b**, **2a**,**b**, quinazoline diones **3a**,**b**, quinoline diones **4a**,**b**, **5a**,**b**, and quinoxaline diones **6a**,**b** were screened for antiproliferative activity against different cancer cell lines KB/HeLa (cervical carcinoma), SKOV-3 (ovarian carcinoma), SF-268 (CNS, glioma), NCI-H460 (non-small cell lung carcinoma (NSCLC)), and of RKOp27 (colon adenocarcinoma).[13] The concentration of the compound that inhibits 50% (EC_{50}) of cell proliferation after 48 h was calculated by nonlinear regression (GraphPad Prism™) using the data of at least two independent XTT cytotoxicity assays.[14] Results of the cytotoxicity assays are shown in detail in Table 1. Quinazolines **3** exhibited highest inhibition across all cell lines. In particular, the quinazoline core with the dimethylaminoethyl chain **3a** displayed the highest inhibition with a mean EC_{50} of 0.08 μg/mL against NCI-H460 and 0.11 μg/mL against SF-268 cells. Quinazoline derivative **3b** bearing a dimethylaminopropyl chain is slightly less active, showing mean EC_{50} of 0.18 μg/mL in NCI-H460 and 0.2 μg/mL in SF-268 cell lines. The next active compound within this series is quinoxaline **6a**. Activity has dropped by a factor of nearly 10. Compound **6b** incorporating a dimethylaminopropyl chain is regarded as inactive in this experimental setting, which parallels the activity drop from **3a** to **3b**. Furthermore, isoquinolines **1a**,**b** and **2a**,**b** showed a measurable antiproliferative activity, being less potent than **6a** by a factor of at least two. In contrast, the quinolines **4a**,**b** and **5a**,**b** are regarded as inactive.

As a general observation, activity clearly falls into groups of positional isomers. Ring systems containing two nitrogens exhibit the best antiproliferative activity (quinazolines and quinoxalines),

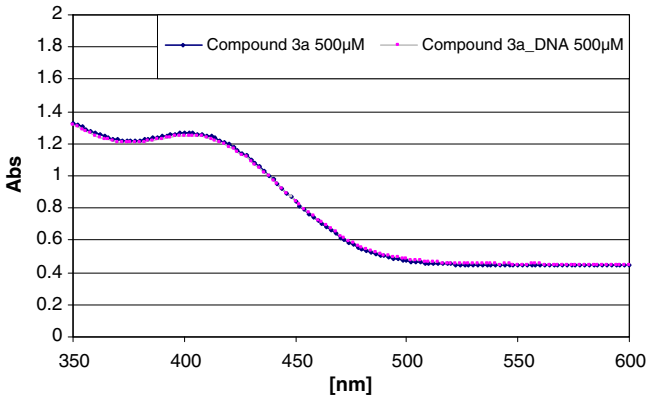

Figure 2. DNA intercalating UV experiment with compound **3a**.

Table 1
In vitro cytotoxicity of compounds **2**–**6** towards different cell lines

Cells (origin)/compound	EC_{50}[a] (μg/mL)					
	KB/HeLa (cervix)	SKOV-3 (ovarian)	SF-268 (CNS)	NCI-H460 (NSCLC)	RKOp27 (colon)	RKOp27IND (colon)
1a	[3–10]	[3–10]	[3–10]	[3–10]	[3–10]	[3–10]
1b	[3–10]	[3–10]	[3–10]	[3–10]	[3–10]	[3–10]
2a[7]	[3–10]	[3–10]	[3–10]	[3–10]	[3–10]	[3–10]
2b[7]	[3–10]	[3–10]	[3–10]	[3–10]	[3–10]	[3–10]
3a	0.173 ± 0.044 (*n* = 3)	0.239 ± 0.015 (*n* = 3)	0.107 ± 0.020 (*n* = 3)	0.082 ± 0.006 (*n* = 3)	0.173 ± 0.021 (*n* = 3)	0.377 ± 0.018 (*n* = 3)
3b	0.280 ± 0.039 (*n* = 3)	0.452 ± 0.054 (*n* = 3)	0.198 ± 0.025 (*n* = 3)	0.177 ± 0.007 (*n* = 3)	0.246 ± 0.052 (*n* = 3)	0.440 ± 0.056 (*n* = 3)
4a	No inhib.[b]	No inhib.	No inhib.	No inhib.	No inhib.	No inhib.
4b	No inhib.	No inhib.	No inhib.	No inhib.	No inhib.	No inhib.
5a	No inhib.	No inhib.	No inhib.	No inhib.	No inhib.	No inhib.
5b	No inhib.	No inhib.	No inhib.	No inhib.	No inhib.	No inhib.
6a	1.368 ± 0.058 (*n* = 2)	[3–10]	2.699 (*n* = 1)	1.121 ± 0.820 (*n* = 3)	1.194 ± 0.304 (*n* = 3)	2.973 (*n* = 1)
6b	No inhib.	No inhib.	No inhib.	No inhib.	No inhib.	No inhib.

[a] EC_{50} values <3 μg/ml are given as mean values ± standard deviation with number of replicates given in round brackets. EC_{50} values reported in the range of 3–10 μg/ml reveal activity in the highest concentrations during EC_{50} determination, but do not give full sigmoidal curves. Therefore, activities were only estimated within a range, marked by square brackets.

[b] No inhib. = no inhibition, that is, <50% inhibition in the highest concentration (3.16 μg/ml) during EC_{50} determination.

while ring systems containing one nitrogen (with the exception of **2**) exhibit low antiproliferative activity.

Surprisingly, it was demonstrated by standard DNA intercalating UV experiments that the most active compound **3a** does not intercalate into DNA (as exemplified in Fig. 2).[15]

As demonstrated earlier by the authors,[7] oxime derivatives of compound **2a** with excellent antiproliferative activities were also identified as not DNA binding. Comparing the activities for **3a** against RKOp27 with or without p27 induced cell cycle arrest suggest, that a G2/M-phase cell cycle arrest (as displayed by e.g., tubilin inhibitors) is not involved. However, the mode of action remains to be elucidated.

In conclusion, pyrrole annelated isoquinolines, quinazolines, quinolines and quinoxalines were synthesized and characterized.[16] These compounds were screened for cytotoxic activity against different cell lines. Compounds containing a quinazoline core exhibited superior EC_{50} values compared with compounds containing different positional isomers on nitrogen containing ring systems. Quinazoline **3a** does 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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15. The UV absorption curve of compound **3a** shows a complete overlay to an UV absorption curve of compound **3a** added to DNA. A second experiment using a compound concentration of 125 μmol and determining UV absorptions between 230 nm and 350 nm shows again no shift in absorption maxima. Using Acridinorange as reference compound, a shift in absorption maxima for about 25 nm was observed. Set-up of DNA intercalation experiment: stock solution of reference and test compounds up to 5 mM concentration in 5% (v:v) DMSO or methanol were prepared. The compound solution was diluted stepwise in reaction buffer (5 mM NaH_2PO_4, 5 mM Na_2HPO_4, 70 mM NaCl, pH 7) and a compound concentration resulting in absorbance [abs max] of approx. 1–2 was determined in 96 well UV plates (Costar, Acton, MA) between 350 nm and 600 nm using a Spectramax 190 plus reader (Molecular Devices, Sunnyvale, CA). Finally, curves of the determined compound test concentration using reaction buffer with or without 2 mg/ml (w:v) calf thymus DNA (Sigma–Aldrich Corp., St. Louis, MO) were compared.
16. All of the final structures were confirmed by ^{1}H NMR, ^{13}C NMR, IR, and MS as the following. Compound **1a**: Mp = 99 °C. ^{1}H NMR ($CDCl_3$): δ (ppm): 2.27 (s, 6H), 2.69 (dt, J = 5.2 Hz und 1.1 Hz, 2H), 4.52 (t, J = 6.4 Hz, 2H), 6.73 (d, J = 2.5 Hz, 1H), 7.11 (dd, J = 2.6 Hz und 1.3 Hz, 1H), 7.89 (d, J = 4.9 Hz, 1H), 8.96 (dd, J = 4.9 Hz und 1.9 Hz, 1H), 9.33 (s, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm): 45.5, 47.3, 59.4, 108.2, 118.7, 126.2, 129.0, 129.7, 132.6, 139.6, 148.4, 154.9, 174.4, 180.2. IR (KBr): ν_{max} 3102, 2771, 1661, 1586, 1499, 1399, 1247, 1025, 934, 734 cm^{-1}. MS: m/z (% relative intensity) 269 (M^+, 1), 225 (1), 211 (1), 128 (1), 101 (7), 71 (15), 58 (100), 42 (9).
Compound **1b**: ^{1}H NMR ($CDCl_3$): δ (ppm): 1.89–2.03 (m, 2H), 2.23 (t, J = 7.1 Hz, 2H), 2.91 (s, 6H), 4.47 (t, J = 5.6 Hz, 2H), 6.72 (d, J = 2.6 Hz, 1H), 7.07 (d, J = 2.8 Hz, 1H), 7.88 (dd, J = 5.1 Hz und 0.8 Hz, 1H), 8.95 (d, J = 4.9 Hz, 1H), 9.32 (s, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm): 28.3, 45.2, 47.4, 55.6, 107.9, 118.7, 126.2, 129.0, 129.8, 132.6, 139.6, 148.4, 154.9, 174.2, 180.2. IR (KBr): ν_{max} 1668, 1652, 1584, 1496, 1392, 1368, 1253, 1238, 1183, 1036, 931, 731 cm^{-1}. MS: m/z (% relative intensity) 283 (M^+, 2), 211 (4), 141 (2), 101 (3), 84 (8), 77 (10), 58 (100), 42 (12).
Compound **2a**: 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 = 4.9 Hz, 1H), 8.98 (d, J = 5.0 Hz, 1H), 9.37 (s, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm): 45.6, 47.3, 59.5, 108.2, 118.8, 126.8, 128.7, 129.8, 132.1, 139.2, 148.4, 154.9, 175.4, 179.7. IR (KBr): ν_{max} 1668, 1646, 1583, 1502, 1378, 1247 cm^{-1}. MS: m/z (% relative intensity) 269 (M^+, 1), 211 (1), 155 (1), 128 (3), 101 (1), 71 (6), 58 (100).
Compound **2b**: ^{1}H NMR ($CDCl_3$): δ (ppm): 1.88–2.06 (m, 2H), 2.21 (s, 6H), 2.26 (t, J = 6.8 Hz, 2H), 4.50 (t, J = 7.0 Hz, 2H), 6.75 (d, J = 2.4 Hz, 1H), 7.06 (d, J = 2.7 Hz, 1H), 7.93 (d, J = 4.9 Hz, 1H), 8.97 (d, J = 4.9 Hz, 1H), 9.35 (s, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm): 28.4, 45.2, 47.5, 55.7, 108.0, 118.7, 126.8, 128.8, 129.8, 132.1, 139.2, 148.4, 154.9, 175.2, 179.6. IR (KBr): ν_{max} 3430, 2815, 1667, 1652, 1583, 1497, 1389, 1251 cm^{-1}. MS: m/z (% relative intensity) 283 (M^+, 1), 211 (13), 98 (26), 84 (12), 72 (16), 58 (100).
Compound **3a**: Mp = 177–178 °C. ^{1}H NMR ($CDCl_3$): δ (ppm): 2.23 (s, 6H), 2.67 (t, J = 6.37 Hz, 2H), 4.50 (t, J = 6.44 Hz, 2H), 6.79 (d, J = 2.64 Hz, 1H), 7.13 (d, J = 2.78 Hz, 1H), 9.45 (s, 1H), 9.51 (s, 1H). ^{13}C NMR. ($CDCl_3$): δ (ppm): 45.4, 47.2, 59.2, 109.0, 125.7, 129.0, 129.2, 132.8, 154.7, 156.8, 173.2, 177.9. IR (KBr): ν_{max} 3092, 2761, 1670, 1566, 1498, 1372, 1249, 1040, 955 cm^{-1}. MS: m/z (% relative intensity) 270 (M^+, 1), 226 (1), 120 (1), 106 (1), 79 (1), 65 (1), 58(100).
Compound **3b**: Mp = 88 °C. ^{1}H NMR ($CDCl_3$): δ (ppm): 1.97 (m, 2H), 2.23–2.31 (m, 8H), 4.52 (t, J = 6.94 Hz, 2H), 6.88 (d, J = 2.64 Hz, 2H), 7.15 (d, J = 2.66 Hz, 1H), 9.53 (s, 1H), 9.60 (s, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm): 28.3, 45.2, 47.6, 55.6, 109.0, 125.9, 129.2, 129.5, 133.0 154.9, 157.0, 173.3, 178.1. IR (KBr): ν_{max} 3116, 2805, 1656, 1548, 1498, 1404, 1371, 1254, 1192, 935 cm^{-1}. MS: m/z (% relative intensity) 284 (M^+, 5), 213 (4), 212 (4), 149 (2), 84 (12), 72 (12), 59 (5), 58(100).
Compound **4a**: Mp = 148–149 °C. ^{1}H NMR ($CDCl_3$): δ (ppm): 2.29 (s, 6H), 2.72 (t, J = 6.50 Hz, 2H), 4.55 (t, J = 6.50 Hz, 2H), 6.85 (d, J = 2.78 Hz, 1H), 7.10 (d, J = 2.78 Hz, 1H), 7.60 (dd, J = 4.67/7.83 Hz, 1H), 7.47 (dd, J = 1.70/7.90 Hz, 1H), 8.95 (dd, J = 1.70/4.62 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm): 45.6, 47.2, 59.5, 108.8, 126.7, 129.4, 129.6, 130.8, 132.2, 134.6, 149.5, 153.4, 174.7, 179.0. IR (KBr): ν_{max} 3415, 1677, 1655, 1578, 1388, 1250, 940 cm^{-1}. MS: m/z (% relative intensity) 269 (M^+, 1), 86 (2), 78 (2), 71 (12), 70 (2), 69 (2), 58 (100).
Compound **4b**: Mp = 99 °C. ^{1}H NMR ($CDCl_3$): δ (ppm): 1.94 (m, 2H), 2.13–2.24 (m, 8H), 4.44 (t, J = 6.75 Hz, 2H), 6.68 (d, J = 2.66 Hz, 1H), 7.02 (d, J = 2.26 Hz, 1H), 7.56 (dd, J = 4.66/6.94 Hz, 1H), 8.41 (d, J = 7.84 Hz, 1H), 8.89 (d, J = 4.56 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm): 28.3, 45.1, 47.3, 55.6, 108.5, 126.6, 129.3, 129.5, 130.6, 132.2, 134.4, 149.4, 153.2, 174.3, 174.3. IR (KBr): ν_{max} 3413, 2945, 1651, 1500, 1394, 1243, 936, 706 cm^{-1}. MS: m/z (% relative intensity) 283 (M^+, 6), 212 (5), 211 (7), 156 (2), 155 (2), 113 (2), 58(100).
Compound **5a**: Mp = 269 °C. ^{1}H NMR ($CDCl_3$): δ (ppm): 2.25 (s, 6H), 2.69 (t, J = 6.63 Hz, 2H), 4.53 (t, J = 6.62 Hz, 2H), 6.68 (d, J = 2.78 Hz, 1H), 7.04 (d, J = 2.64 Hz, 1H), 7.54 (dd, J = 4.74/7.88 Hz, 1H), 8.40 (dd, J = 1.64/7.82 Hz, 1H), 8.88 (dd, J = 1.70/4.74 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm): 45.4, 47.2, 59.3, 107.8, 126.6, 128.4, 130.2, 130.3, 131.9, 134.5, 149.5, 153.3, 173.7, 179.0. IR (KBr): ν_{max} 3092, 2945, 2771, 1661, 1573, 1500, 1394, 1243 cm^{-1}. MS: m/z (% relative intensity) 269 (M^+, 1), 78 (2), 71 (15), 70 (13), 59 (4), 58 (100).
Compound **5b**: ^{1}H NMR ($CDCl_3$): δ (ppm): 1.97 (m, 2H), 2.17–2.26 (m, 8H), 4.48 (t, J = 6.94 Hz, 2H), 6.69 (d, J = 2.66 Hz, 1H), 7.02 (d, J = 2.64 Hz, 1H), 7.55 (dd, J = 4.72/7.76 Hz, 1H), 8.41 (dd, J = 1.58/7.76 Hz, 1H), 8.89 (dd, J = 1.70/4.60 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm): 28.2, 45.1, 47.6, 55.7, 107.6, 126.6, 128.5, 130.2, 130.4, 132.0, 134.5, 149.6, 153.4, 173.6, 179.3. IR (KBr): ν_{max} 2943, 2818, 2767, 1663, 1578, 1501, 1399, 1240, 1064, 939 cm^{-1}. MS: m/z (% relative intensity) 283 (M^+, 2), 212 (6), 211 (10), 95 (4), 85 (8), 84 (98), 81 (11), 71 (4), 69 (19), 59 (5), 58(100).
Compound **6a**: Mp = 189–190 °C. ^{1}H NMR ($CDCl_3$): δ (ppm): 2.29 (s, 6H), 2.74 (t, J = 6.57 Hz, 2H), 4.59 (t, J = 6.63 Hz, 2H), 6.86 (d, J = 2.66 Hz, 1H), 7.17 (d, J = 2.66 Hz, 1H), 8.91 (s, 2H). ^{13}C NMR ($CDCl_3$): δ (ppm): 45.4, 47.3, 59.2, 109.0, 129.3, 130.2, 133.0, 145.5, 145.9, 147.4, 147.5, 172.5, 177.7. IR (KBr): ν_{max} 1664, 1500, 1396, 1254, 1191, 1084, 994 cm^{-1}. MS: m/z (% relative intensity) 270 (M^+, 1), 78 (2), 71 (29), 70 (4), 64 (2), 59 (5), 58 (100).
Compound **6b**: ^{1}H NMR ($CDCl_3$): δ (ppm): 2.06 (m, 2H), 2.28 (s, 6H), 2.38 (t, J = 6.82 Hz, 2H), 4.55 (t, J = 7.01 Hz, 1H), 6.88 (d, J = 2.52 Hz, 1H), 7.15 (d, J = 2.76, 1H), 8.94 (s, 2H). ^{13}C NMR ($CDCl_3$): δ (ppm): 28.0, 44.8, 47.7, 55.5, 108.9, 129.4, 130.3, 133.0, 145.6, 145.9, 147.4, 147.5, 172.4, 177.7. IR (KBr): ν_{max} 3420, 2963, 2930, 1667, 1502, 1397, 1254, 1085 cm^{-1}. MS: m/z (% relative intensity) 284 (M^+, 2), 215 (3), 212 (5), 120 (3), 85 (8), 84 (81), 72 (6), 59 (5), 58(100).