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Synthesis and antiproliferative activity of new cytotoxic tri- and tetraazabenzo[3,2-*a*]fluorene-5,6-dione derivatives

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ABSTRACT

A new series of substituted tri-/tetraazabenzo[3,2-*a*]fluorene-5,6-diones and their corresponding oxime derivatives have been synthesized and spectroscopically characterized. The antiproliferative activities of all compounds were evaluated on at least three different cell lines.

The large and flat diazobenzo[*a*]fluorene moiety fulfills all requirements for DNA intercalation. Therefore, it is an astonishing fact that only one study focused on cytotoxic effects of diazobenzo[a]fluorene derivatives.[1] Instead of that much more examinations focused on properties of this type of compounds as photo sensitizers[2,3] and NOS-inhibitors for the treatment of hyptension, autoimmune diseases, venous insufficiency and/or inflammation edema.[4–6] Besides that two aza analogues of diazobenzo[a]fluorene were made accessible and their cytotoxic properties examined.[7,8] In continuation of our studies on novel cytotoxic compounds we report a new series of substituted tri-/tetraazabenzo[3,2-*a*]fluorene-5,6-diones. The rationale for the synthesis of compounds **1**–**9** is the reasonable assumption that the isoquinolinedione moiety in intercalating agents leads to anticancer compounds with favourable properties like reduced cardiotoxic effects.[9] Moreover, the isoquinolinedione nucleus is the crucial element of promising anticancer drug candidates like BBR 3422[10] or BBR 3438,[11] respectively, which finally led to the development and market release of BBR 2778 (Pix antrone, Pixuvri®) for the treatment of patients with multiply relapsed aggressive Non Hodgkin's Lymphoma.[12]

In consistent further development of our studies[13–17] we were interested in the antiproliferative effects triggered by an isoquinoline-1,2-dione moiety (instead of an isoquinoline-1,4-dione moiety) thus leading us to a series of compounds **1**–**9** as shown in Figure 1.

The synthesis of compounds **1**–**2** and **6** started from commercially available 5-hydroxyisoquinoline (**10**), which was converted into 6,7-dichloroisoquinoline-5,8-dione (**11**) with HCl/HNO_3 instead of the literature method[13] with sodium chlorate. The cyclization was accomplished by refluxing 6,7-dichloroisoquinoline-5,8-dione (**11**) with the appropriate aminopyridine **12** in ethanol in presence of potassium carbonate yielding two isomers **1**–**2** and **6** (Scheme 1).

Oximes **3**,**4** and **7**,**8** were prepared by reacting either **1** or **6** with 2-(aminooxy)-ethanol or 2-(aminooxy)-*N*,*N*-dimethylethanamine, respectively in presence of potassium hydroxide in methanol (Schemes 2 and 3).

When compound **2** was treated with N^1,N^1,N^2-trimethylethane-1,2-diamine substitution occurred on C-2 to afford compound **5**, leaving chlorine substituted C-9 unaffected. The structure of **5** was confirmed by 2D NMR technique, HMQC (Scheme 4).

Compound **9** was prepared in a 5-step reaction starting from commercially available 2,5-dimethoxybenz aldehyde (**13**), which upon treatment with silica gel impregnated with nitric acid gave **14**. Compound **14** was reacted with formamide under a HCl gas stream to yield the desired *N*,*N*′-diformamide (**15**) which was subjected to reductive cyclization with zinc in acetic acid to give **16**. After oxidation and chlorination of **16** to **17** with HCl/HNO_3, tetracyclic compound **9** was finally obtained as only isomer by refluxing **17** with 2-aminopyridine in ethanol in presence of potassium bicarbonate (Scheme 5).

In summary it can be said that in comparison to mitoxantron and doxorubicin the annelated derivatives **1** and **9**, respectively, exhibit satisfactory actitivities and therefore will serve as starting

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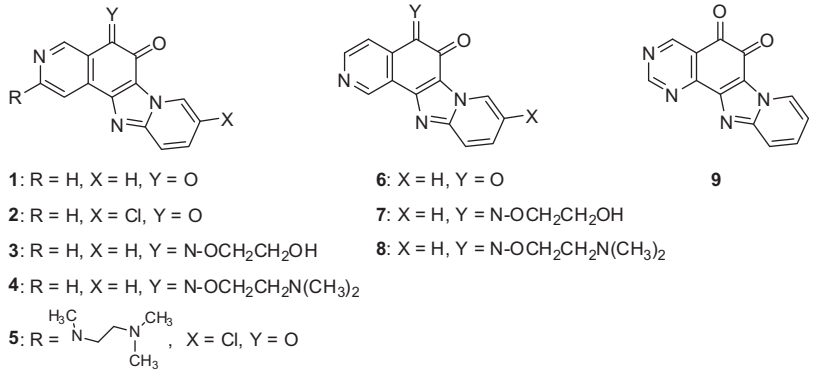
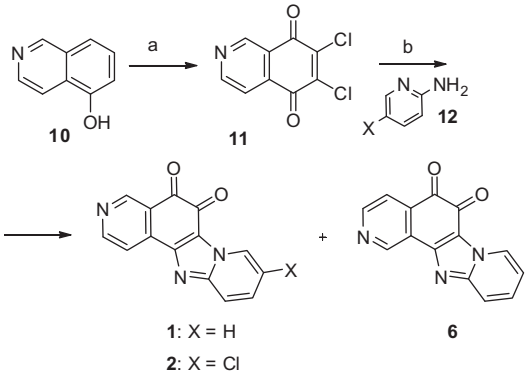
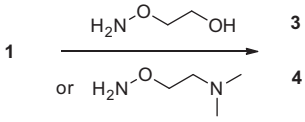


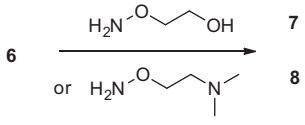
Figure 1.



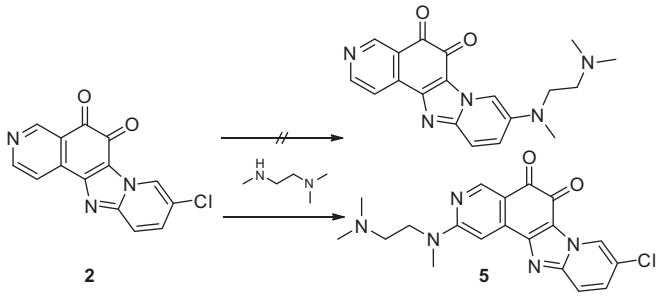
Scheme 1. Reagents and conditions: (a) HCl/HNO_3, 80–90 °C, 1 h; (b) K_2CO_3, EtOH, 85 °C, 5 h.



Scheme 2. Reagents: KOH, MeOH.

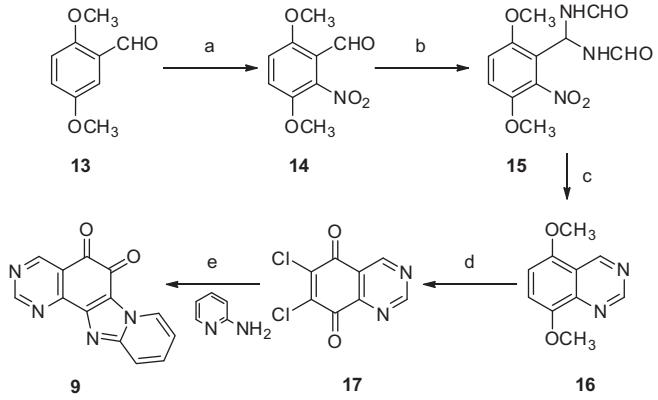


Scheme 3. Reagents: KOH, MeOH.



Scheme 4. Reagents and conditions: THF, EtOH, 85 °C.

points for a new series of cytotoxic compounds with a tri- and tetraazabenzo[3,2-*a*]fluorene-5,6-dione core (Table 1)[19].



Scheme 5. Reagents and conditions: (a) HNO_3–SiO_2, CH_2Cl_2, rt, 10 min, 69%; (b) formamide, HCl, 80 °C, 1 h, 75%; (c) Zn^0, CH_3COOH, 0 °C, 2.5 h, 54%; (d) HCl/HNO_3, 80–90 °C, 15 min, 5%; (e) K_2CO_3, EtOH, 85 °C, 5 h, 46%.

Table 1
In vitro cytotoxicity of compounds **1**–**9** against three human cancer cell lines

Cells (origin)/ compound	Compound activity (EC_{50} [μM][a])[18]		
	KB/HeLa (cervix)	SKOV-3 (ovarian)	NCI-H460 (NSCLC)
1	0.244 (1)	0.498 ± 0.001 (2)	0.242 ± 0.067 (3)
2	0.394 (1)	0.525 ± 0.048 (2)	0.342 ± 0.003 (3)
3	0.532 (1)	2.064 (1)	0.521 ± 0.221 (2)
4	0.406 (1)	0.931 ± 0.098 (2)	0.414 ± 0.097 (3)
5	2.802 (1)	No inhibition[b]	2.135 ± 1.166 (3)
6	0.917 (1)	1.222 ± 0.294 (2)	0.620 ± 0.176 (3)
7	4.349 (1)	14.732 (1)	3.875 ± 1.306 (2)
8	6.148 (1)	2.928 (1)	2.673 ± 0.457 (2)
9	0.145(1)	0.283 ± 0.043 (2)	0.337 ± 0.026 (3)
Mitoxantrone	0.420 ± 0.060 (2)	n.d.	0.030 ± 0.010 (2)
Doxorubicin	0.250 ± 0.140 (3)	0.290 ± 0.160 (10)	0.040 ± 0.010 (5)

[a] The data presented are EC_{50} values of cytotoxicity assessments with resazurin as detection reagent performed in quadruplicate measurements. EC_{50} values are depicted as mean values ± standard deviation with the number of replicates indicated in round brackets.

[b] No inhibition is defined as less than 30% inhibition in the highest final compound concentration analyzed (31.6 μM).

References and notes

1. Tapia, R. A.; Cantuarias, L.; Cuellar, M.; Villena, J. *Braz. Chem. Soc.* **2009**, *20*, 999.
2. Podemska, K.; Podsiadly, R.; Szymczak, A. M.; Chrzescijanska, E.; Sokolowska, J. *Dyes Pigm.* **2012**, *95*, 74.
3. Podemska, K.; Podsiadly, R.; Szymczak, A. M.; Chrzescijanska, E.; Sokolowska, J. *Dyes Pigm.* **2012**, *94*, 113.
4. Mijalli, A.M.M.; Sarshar, S.; Zhang, Ch. U.S. 1998, US5723451 A 19980303.
5. Boutherin-Falson, O.; Desquand-Billiald, St.; Favrou, A.; Finet, M.; Tembo, O.; Torregrosa, J.-L.; Yannic-Art PCT Int. Appl. 1997, WO 9721709 A 19970619.

6. Mijalli, A. M. M.; Sarshar, S.; Zhang, Ch. PCT Int. Appl. 1997, WO 9705871 A1 19970220.
7. Puetter, J. *Zeitschr. f. Phys. Chem.* **1963**, *332*, 1.
8. Kim, Y.-S.; Park, S.-Y.; Myung-Eun, L.; Schollmeyer, D. *Bioorg. Med. Chem.* **2003**, *1829*, 11.
9. Krapcho, A. P.; Menta, E. *Drugs Fut.* **1997**, *22*, 641.
10. Xu, L.-N.; Liang, G.-Z.; Li, Z.-L.; Wang, J.; Zhou, P. *J. Mol. Graph. Model* **2008**, *26*, 1252.
11. Hofheinz, R.-D.; Porta, C.; Hartung, G.; Santoro, A.; Hanauske, A.-R.; Kutz, K.; Stern, A.; Barbieri, P.; Verdi, E.; Hehlmann, R. *Invest. New Drugs* **2005**, *23*, 363.
12. EMA/CHMP/102366/2012.
13. Shahabi, M.; Schirmer, E.; Shanab, K.; Leepasert, T.; Ruzicka, J.; Holzer, W.; Spreitzer, H.; Aicher, B.; Schmidt, P.; Blumenstein, L.; Mueller, G.; Guenther, E. *Bioorg. Med. Chem. Lett.* **2013**, *1846*, 23.
14. Shanab, K.; Schirmer, E.; Wulz, E.; Weissenbacher, B.; Lassnig, S.; Slanz, R.; Foesleitner, G.; Holzer, W.; Spreitzer, H.; Schmidt, P.; Aicher, B.; Mueller, G.; Guenther, E. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3117.
15. Shanab, K.; Schirmer, E.; Knafl, H.; Wulz, E.; Holzer, W.; Spreitzer, H.; Schmidt, P.; Aicher, B.; Mueller, G.; Guenther, E. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3950.
16. Pongprom, N.; Mueller, G.; Schmidt, P.; Holzer, W.; Spreitzer, H. *Chem. Monthly* **2009**, *140*, 309.
17. Shanab, K.; Pongprom, N.; Wulz, E.; Holzer, W.; Spreitzer, H.; Schmidt, P.; Aicher, B.; Mueller, G.; Guenther, E. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6091.
18. Cytotoxic/antiproliferative activity.
Assessment of cytotoxic/antiproliferative activity was conducted with human cancer cell lines KB/HeLa (ATCC CCL17, cervix carcinoma), SKOV3 (ATCC HTB-77, ovarian carcinoma) and NCI-H460 (NCI 503473, large cell lung cancer). Measurement of the cellular cytotoxic/antiproliferative activity is based on the dye Resazurin (Sigma, cat. no. R7017), which exhibits fluorescence change in the appropriate oxidation–reduction range relating to cellular metabolic reduction [Nociari et al., *J. Immunol. Methods* **1998** *213*, 157] yielding a fluorescence signal at 590 nm. The increase of fluorescence at 590 nM is an indicator of cellular viability/cell number.
The cells were seeded in the respective growth medium recommended by the supplier (media and reagents purchased from Gibco-BRL) in 125 µl per 96 well and were grown for 24 h at 37 °C/5%CO_2. Cell numbers were adapted for each cell line to generate signals in the linear detection range under the experimental conditions applied. After 45 h of compound incubation at 37 °C/5%CO2 15 µl of the Resazurin detection reagent (0.2 mg/mL in DPBS (Gibco, 14190), steril filtered) was added for additional 3 h and after a total of 48 h of compound incubation cellular metabolic activity was quantified by measurement of fluorescence at 590 nm. Non-treated cells and blank controls w/o cells were set as reference values.
MS EXCEL was used for formating and analysis of data. All data were calculated as % efficacy compared to the mean of the respective negative (non-treated cells) and positive control wells (blank) on each assay plate. EC_{50} values were calculated by using non-linear regression software GraphPad Prism.
19. All of the final structures were confirmed by ^{1}H NMR, ^{13}C NMR, IR and MS as the following.
Compound **1** Mp = 300–302 °C (decomp.). ^{1}H NMR ($CDCl_3$): δ (ppm): 7.44 (m, 1H), 7.83 (m, 1H), 8.00 (d, J = 4.9 Hz, 1H), 8.02 (d, J = 4.9 Hz, 1H), 8.90 (d, J = 4.9 Hz, 1H), 9.03 (s, 1H), 9.23 (d, J = 6.6 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm): 117.6, 117.8, 118.4, 121.8, 125.3, 128.4, 132.7, 138.0, 149.2, 149.3, 149.6, 155.5, 166.9, 180.8. IR (KBr): ν_{max} 3121, 2950, 2919, 2847, 1690, 1659, 1599, 1488, 1405, 1253, 899 cm^{-1}. MS: m/z (% relative intensity) 249 (M^+, 17), 222 (15), 221 (100), 193 (39), 166 (23), 139 (19), 88 (39), 87 (19), 78 (41), 76 (22), 63 (26), 62 (27), 51 (56), 50 (26). HRMS: m/z calcd for $C_{14}H_7N_3NaO_2$: 272.0436 found: 272.0434.
Compound **2** Mp = 309–310 °C. ^{1}H NMR ($CDCl_3$): δ (ppm): 7.88 (dd, J = 2.1, 9.5 Hz, 1H), 8.02 (d, J = 5.0 Hz,1H), 8.05 (d, J = 9.5 Hz, 1H), 8.92 (d, J = 5.1 Hz, 1H), 9.07 (s, 1H), 9.24 (m, J = 2.1 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm): 117.5, 119.1, 121.8, 124.2, 125.0, 125.8, 132.9, 137.5, 147.4, 149.4, 149.7, 155.4, 167.0, 180.3. IR (KBr): ν_{max} 3101, 2923, 2852, 1700, 1651, 1595, 1487, 1408, 821 cm^{-1}. MS: m/z (% relative intensity) 283 (M^+, 23), 255 (100), 227 (16), 220 (8), 192 (23), 165 (17), 138 (8), 114 (24), 100 (16), 88 (27), 76 (45), 57 (28), 43 (25). HRMS: m/z calcd for $C_{14}H_6ClN_3NaO_2$: 306.0046 found: 306.0047.
Compound **3** Mp = 199–201 °C. ^{1}H NMR (DMSO-d_6): δ (ppm): 3.82 (m, 2H), 4.57 (m, 2H), 5.05 (s, 1H), 7.38 (m, 1H), 7.77 (m, 1H), 7.94 (m, 1H), 8.09 (d, J = 4.90 Hz, 1H), 8.76 (d, J = 4.90 Hz, 1H), 9.23, (d, J = 6.61 Hz, 1H), 9.91 (s, 1H). ^{13}C NMR (DMSO-d_6): δ (ppm): 59.4, 79.7, 116.8, 117.6, 117.9, 119.7, 121.9, 128.3, 132.0, 134.0, 144.0, 149.1, 149.3, 151.5, 151.6, 169. IR (KBr): ν_{max} 3250, 3038, 2919, 2847, 1651, 1630, 1604, 1501, 1423, 1400, 1253, 1077, 1041, 1005 cm^{-1}. MS: m/z (% relative intensity) 308 (M^+, 9), 260 (53), 248 (64), 247 (77), 220 (100), 219 (43), 208 (30), 192 (23), 165 (21), 100 (18), 88 (32), 78 (100), 63 (29), 51 (66), 45 (48), 43 (49). HRMS: m/z calcd for $C_{16}H_{12}N_4Na\ O_3$: 331.0807 found: 331.0800.
Compound **4** Mp = 140–142 °C. ^{1}H NMR ($CDCl_3$): δ (ppm): 2.34 (s, 6H), 2.80–2.86 (t, J = 5.81 Hz, 2H), 4.69–4.75 (t, J = 5.81 Hz, 2H), 7.13–7.20 (m, 1H), 7.55–7.63 (m, 1H), 7.77–7.82 (m, 1H), 8.12–8.14 (d, J = 4.92 Hz, 1H), 8.72–8.74 (d, J = 5.04 Hz, 1H), 9.33–9.36 (m,1H), 10.00 (s, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm): 45.8, 57.8, 76.4, 115.9, 117.7, 117.9, 120.0, 122.2, 128.7, 131.1, 134.2, 144.2, 149.8, 150.1, 151.3, 152.2, 170.9. IR (KBr): ν_{max} 3457, 3080, 3023, 2966, 2945, 2816, 2759, 1648, 1604, 1501, 1423, 1400, 1250, 1000 cm^{-1}. HRMS: m/z calcd for $C_{18}H_{18}N_5O_2$: 336.1460 found: 336.1469.
Compound **5** Mp = 230–231 °C. ^{1}H NMR ($CDCl_3$): δ (ppm): 2.34 (s, 6H), 2.62 (t, J = 6.9 Hz, 2H), 3.31 (s, 3H), 3.92 (s, 2H), 7.18 (s,1H), 7.58 (d, J = 9.5 Hz, 1H), 7.75 (d, J = 9.5 Hz, 1H), 8.90 (s, 1H), 9.37 (s, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm): 37.3, 45.6, 48.3, 56.7, 99.7, 114.7, 118.3, 121.7, 124.8, 126.6, 132.7, 138.1, 147.8, 151.3, 153.8, 160.9, 170.1, 178.4. IR (KBr): ν_{max} 2924, 2853, 1649, 1600, 1540, 730 cm^{-1}. HRMS: m/z calcd for $C_{19}H_{19}ClN_5O_2$ 383.1149 found [MH^+] 384.1222.
Compound **6** Mp = 283–285 °C. ^{1}H NMR ($CDCl_3$): δ (ppm): 7.42 (m, 1H), 7.82 (m, 1H), 8.01 (m, 1H), 8.87 (m, 1H), 9.23 (m, 1H), 9.31(s, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm): 117.5, 118.1, 120.7, 120.9, 124.7, 128.4, 132.7, 136.8, 145.3, 149.3, 150.6, 152.6, 166.8, 180.7. IR (KBr): ν_{max} 3121, 3028, 2919, 1700, 1646, 1622, 1566, 1493, 1478, 1382, 1250, 1137, 770 cm^{-1}. MS: m/z (% relative intensity) 249 (M^+, 11), 222 (11), 221 (45), 193 (15), 166 (8), 125 (13), 97 (25), 83 (35), 71 (33), 69 (33), 57 (68), 55 (49), 43 (100), 41 (36). HRMS: m/z calcd for $C_{14}H_7N_3NaO_2$: 272.0436 found: 272.0433.
Compound **7** Mp = 216–218 °C. ^{1}H NMR (DMSO-d_6): δ (ppm): 3.80–3.82 (m, 2H), 4.57–4.60 (m, 2H), 5.03 (s, 1H), 7.33–7.40 (m, 1H), 7.73–7.81 (m, 1H), 7.92–7.96 (m, 1H), 8.61–8.64 (m, 1H), 8.76–8.79 (m, 1H), 9.22–9.26 (m, 1H), 9.37 (s, 1H). ^{13}C NMR (DMSO-d_6): δ (ppm): 59.2, 80.1, 116.5, 117.6, 121.5, 124.2, 128.3, 131.8, 131.9, 144.4, 145.5, 149.5, 151.7, 169.4. IR (KBr): ν_{max} 3385, 2919, 2847, 1726, 1640, 1501, 1431, 1390, 1281, 1088, 1028, 889 cm^{-1}. MS: m/z (% relative intensity) 308 (M^+, 18), 264 (16), 260 (29), 248 (66), 247 (100), 220 (78), 219 (46), 206 (16), 192 (15), 165 (16), 88 (25), 78 (84), 51 (48), 45 (31). HRMS: m/z calcd for $C_{16}H_{12}N_4O_3Na$: 331.0807 found: 331.0803.
Compound **8** Mp = 149–151 °C. ^{1}H NMR ($CDCl_3$): δ (ppm): 2.3 (s, 6H), 2.78–2.84 (t, J = 5.81Hz, 2H), 4.68–4.74 (m, 2H), 7.10–7.16 (m, 1H), 7.57–7.61 (m, 1H), 7.75–7.61 (m, 1H), 8.56–8.59 (m, 1H), 8.70–8.72 (m, 1H), 9.29–9.32 (m, 1H), 9.50 (s, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm): 45.8, 57.9, 76.7, 115.6, 117.7, 119.1, 121.8, 124.2, 128.6, 131.1, 132.1, 144.6, 146.4, 150.0, 151.3, 151.7), 170.3. IR (KBr): ν_{max} 3405, 3131, 3033, 2940, 2852, 2816, 2764, 1651, 1633, 1498, 1429, 1256, 1026, 1000 cm^{-1}. HRMS: m/z calcd for $C_{18}H_{18}N_5O_2$: 336.1461 found: 336.1467.
Compound **9** Mp = 273 °C (decomp.). ^{1}H NMR ($CDCl_3$): δ (ppm): 7.48 (m, 1H), 7.85 (m, 1H), 8.07 (m, 1H), 9.20 (s, 1H), 9.29 (m, 1H), 9.46 (s, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm): 118.2, 118.9, 123.9, 124.7, 128.2, 132.6, 149.0, 149.1, 156.0, 156.2, 161.9, 166.7, 179.4. IR (KBr): ν_{max} 3123, 3029, 2923, 1709, 1669, 1653, 1646, 1363 cm^{-1}. MS: m/z (% relative intensity) 250 (M^+, 18), 222 (100), 194 (19), 167 (43), 84 (19), 78 (36), 51 (37). HRMS: m/z calcd for $C_{13}H_6N_4NaO_2$: 273.0388 found: 273.0387.