2-(3-ARYLACRYLOYL)-3-METHYLQUINOXALINE 1,4-DIOXIDES AS POTENTIAL HYPOXIC SELECTIVE CYTOTOXINS

Kristin Dittenhafer[1], Umashankar Das[2], Brent L. Younglove[1], Hilary Mackay[1], Toni Brown[1], Jonathan R. Dimmock,[2] Moses Lee[1*] and Hari Pati[2]

[1]Department of Chemistry, Hope College, Holland, MI 49423, USA
[2]College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 5C9, Canada.
e-mail: lee@hope.edu

Abstract: The synthesis of a series of 2-(3-arylacryloyl)-3-methylquinoxaline 1,4-dioxides is reported. *In-vitro* cytotoxic activity of these compounds was evaluated via the MTT assay in B16 murine melanoma and L1210 murine leukemia cell lines. A dichloro analogue was found to be the most cytotoxic and was > 30-fold more active than the quinoxaline control (IC_{50} = 2 vs. 67 μM in L1210, respectively). These results suggest that compounds containing two bioactive moieties could be developed as hypoxic selective cytotoxins.

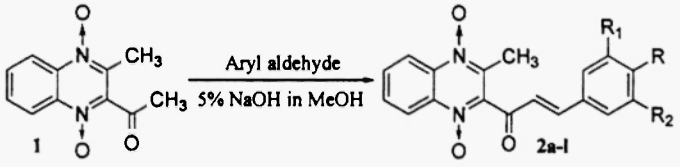
Introduction

Tumor hypoxia is a condition caused by deficient delivery of oxygen to tumor cells due to inefficient vasculature.[1] Hypoxia appears to be a common and distinct property of cells in solid tumors and has thus become a target for therapeutic intervention. Bioreductive prodrugs, which are activated under hypoxic conditions, have been developed to selectively deliver cytotoxic moieties to the tumor mass.[2] Examples of these include *N*-oxides such as the benzotriazine-di-*N*-oxide tirapazamine, which has shown significant activity in phase III clinical trials in combination with cisplatin.[3] The proposed mechanism of cytotoxicity involves one electron reductive activation to produce •OH radicals. These radicals subsequently attack DNA, generating base damage and single and double strand breaks. However, the byproducts of tirapazamine reduction are non-toxic to either hypoxic or aerobic cells. Several groups have investigated hybrid molecules, containing two bioactive moieties; an *N*-oxide and a π-stacking moiety.[4-6] Such molecules have the potential to damage hypoxic cells via free radical generation, as with tirapazamine, and additionally by direct interaction with DNA or DNA-related biomolecules.

We report herein the synthesis and cytotoxicity of a series of bioactive hybrid compounds **2a-l** containing a quinoxaline 1,4-dioxide motif and an acryloyl group. These compounds should readily diffuse into hypoxic tumor tissue and, via a bioreductive mechanism, promote oxidative DNA cleavage. Subsequently, the acryloyl group is expected to bind with free cysteine sulfhydryl groups, including glutathione (GSH), to form Michael adducts. GSH levels increase rapidly prior to and during mitosis and are, hence, more susceptible to alkylation. Hybrid compounds **2a**, **2b**, **2f**, **2g**, **2i**, and **2k** are known in the literature, and are reported to possess antibacterial and growth promoting activities in animals.[7] However, cytotoxic data on these compounds have not been published, thus they were included in this study. Compound **1**, which lacks the acryloyl group, serves as a control.[8]

Results and Discussion

According to equation given below, compounds **2a-l** were prepared via Aldol chemistry by combining 2-methyl-3-acetylqunioxaline-1,4-oxide, **1** and the appropriate aryl aldehyde in methanolic sodium hydroxide solution for five minutes at 5-10°C.[9] The product was then recrystallized in chloroform/ethanol.



a: R = R_1 = R_2 = H
b: R = OCH_3, R_1 = R_2 = H
c: R= R_1= OCH_3, R_2 = H
d: R = R_1= R_2= OCH_3
e: R = R_1 = OCH_2O; R_2 = H
f: R = CH_3; R_1 = R_2 = H
g: R = Cl, R_1 = R_2 = H
h: R = R_1 = Cl, R_2 = H
i: R = Br, R_1 = R_2 = H
j: R = F, R_1= R_2 = H
k: R = NO_2, R_1 = R_2 = H
l: R = R_1 = H, R_2 = NO_2

In-vitro cytotoxicity in B16 (murine melanoma) and L1210 (murine leukemia) cell lines was evaluated using a 72-hour, continuous exposure MTT assay.[10] Concentrations of compounds that inhibited the growth of tumor cells by 50% relative to an untreated control, or IC_{50} values, are shown in table I. Data could not be obtained for **2k** due to its insolubility in DMSO.

All eleven hybrid compounds tested **2a-j, 2l** were more cytotoxic in the B16 cell line than quinoxaline species **1**, whoch does not have the unsaturated keto group. Compound **1** was not active against B16 cells, IC_{50} >100 □M. This indicates that both bioactive motifs are possibly contributing to the observed cytotoxicity. This trend is less distinct in the L1210 cell line, however, as monomethoxy derivative **2b** and dioxolane derivative **2e** have comparably low IC_{50} values to **1** (59, 65 and 67 μM, respectively).

Table 1. Cytotoxicities of compounds **2a-l**.

	IC_{50} (μM)			IC_{50} (μM)	
Compound (m.p.)	**B16**	**L1210**	Compound (m.p.)	**B16**	**L1210**
2a (172-173 °C)	16	5	**2g** (196-197 °C)	41	5
2b (169-170 °C)	70	59	**2h** (211-212 °C)	11	2
2c (179-180 °C)	40	12	**2i** (209-210 °C)	50	7
2d (180-182 °C)	5.1	4	**2j** (195-197 °C)	11	4
2e (185-186 °C)	31	65	**2k** (222-224 °C)	Insoluble	Insoluble
2f (189-191 °C)	30	13	**2l** (206-208 °C)	19	3

IC_{50} values (μM) of compounds **2a-l** in B16 and L1210 cell line as determined by MTT assay. The IC_{50} value of control compound **1** were determined to be > 100 and 67 μM in B16 and L1210, respectively.

With the exception of compound **2e**, all molecules were more cytotoxic in the L1210 cell line. However, general trends in activity are comparable within each cell line. Considering compounds **2a-d** in both cell lines it can be observed that introduction of a *p*-methoxy-group **2b** reduces the activity drastically. Introduction of a second methoxy- **2c** brings the activity back slightly, and trimethoxy- derivative **2d** yields the most cytotoxic analogue of the series in this cell line (IC_{50} = 5 μM). This observation

is reminiscent with the disposition of methoxy groups found in combretastatin A4, a well-known tubulin inhibitor and potent anti-cancer compound.[11]

Compounds **2f**, **2g**, **2i** and **2j** investigate the effects of mono-substitution at the *p*-position. In the B16 cell line, *p*-substitution with methyl- **2f**, chloro- **2g**, or bromo- **2i**, yields derivatives with reduced activity as compared to unsubstituted **2a** (IC_{50} = 30, 41, and 50 μM vs. 16 μM). Fluoro- derivative **2j** however, has a slightly improved activity over **2a** (IC_{50} = 11 μM). This trend is identical in the L1210 cell line with the exception of chloro- **2g** which, instead of reducing the activity, has the same IC_{50} value as **2a** (5 μM).

Dichloro derivative **2h** has increased activity over its monochloro- counterpart **2g** in both cell lines, the same trend as was observed with mono- and dimethoxy-derivatives **2b** and **2c**. Dichloro- **2h** is also the most potent analogue in the L1210 cell line (IC_{50} = 2 μM), although compounds **2a**, **2d**, **2e**, **2i**, **2j** and **2l** all have similar IC_{50} values (5, 4, 5, 7, 4 and 3 μM, respectively). In conclusion, these results suggest that hypoxic selective toxins containing two bioactive structural motifs have the potential to be developed as useful pharmaceuticals. Compound **2d** is worthy of further biological testing especially under hypoxic conditions.

Experimental

Each of the synthesized compounds[9] was subjected to *in-vitro* cytotoxicty screening using a 72-hour continuous exposure MTT assay as previously described.[10] Concentrations of compounds that inhibited tumor cell growth by 50% relative to an untreated control, or IC_{50} (μM) values, for both B16 and L1210 cell lines (murine melanoma and leukemia, respectively) are shown in Table I.

All compounds were characterized by melting point, IR, elemental analysis and ^{1}H NMR. Melting points were recorded on a Gallen Kamp apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker 500 MHz spectrometer using TMS as internal standard (δ in ppm). CHN analyses were carried out using Elemental CHN analyzer. Compounds possessing water of crystallization were confirmed by CHN analyses.

Acknowledgement The authors thank Hope College and Canada's CIHR for support of this work.

References and notes

1. M. Jaffar, N. Abou-Zeid, L. Bai, I. Mrema, I. Robinson, R. Tanner and I. J. Stratford, Curr.Drug Delivery **1**, 345 (2004)
2. W.A. Denny, Oncology **1**, 25 (2000)
3. M.S. Kovacs, D.J. Hocking, J.W. Evans, B.G. Siim, B.G. Wouters and J.M. Brown, Br. J. Cancer **90**, 1245 (1999)
4. L.H. Patterson, Cancer Met. Rev. **12**, 119 (1993)
5. H.H. Lee, W.R. Wilson, D.M. Ferry, P. van Zijl, S.M. Pullen and W.A. Denny, J. Med. Chem. **39**, 2508 (1996)
6. (a) A. Monge, F.J. Martinez-Crespo, A. Lopez de Cerain, J.A. Palop, S. Narro, V. Senador, A. Marin, Y. Sainz, M. Gonzalez, E. Hamilton and A.J. Barker, J. Med. Chem. **38**, 1786 (1995); (b) M. Boiani, H. Cerecetto, M. Gonzalez, M.

Risso, C. Olea-Azar, O.E. Piro, E.E. Castellano, A. Lopez de Cerain, O. Ezpeleta and A. Monge-Vega, Eur. J. Med. Chem. **36**, 771 (2001); (c) M. Boiani, H. Cerecetto, M. Gonzalez, Farmaco, **59**, 405 (2004)

7. (a) R. Zhao, Y. Wang, F. Xue, Z. Xu, J. Li, Ji. Li, X. Yan, X. Du, X. Miao, Faming Zhuanli Shenqing Gongkai Shuomingshu (2006), 6 pp. CODEN: CNXXEV CN 1785979 A 20060614 Patent written in Chinese. Application: CN 1007-3376 20041207. Priority: CAN 145:62928 AN 2006:584980; (b) P. Benko, D. Bozsing, J. Gundel, K. Magyar, (E. Gy. T. Gyogyszervegyeszeti Gyar, Hung.).Fr. Demande (1981), 18 pp. CODEN: FRXXBL FR 2483415 A1 19811204 Patent written in French. Application: FR 81-10867 19810602. Priority: HU 80-1385 19800603. CAN 96:217878 AN 1982:217878; (c) R. Lu, H. Wang and L. Tong, Biomimetic and Supramolecular Systems **C10**, 7 (1999); (d) M.S. El-Halim, A.S. El-Ahl, H.A. Etman, M.M. Ali, A. Fouda and A.A. Fadda, Monatshefte fuer Chemie **126**, 1217 (1995); (e) K. Matoba, T. Terada, M. Sugiura and T. Yamazaki, Heterocycles **26**, 55 (1987); (f) A. Monge, M.J. Gil, M.A. Gastelurrutia and M. Pascual, Anales de la Real Academia de Farmacia **48**, 533 (1982)

8. (a) H.U. Gali-Muhtasib, M. Diab-Assaf, and M.J. Haddadin, Cancer Chemotherapy and Pharmacology **55**, 369 (2005); (b) M. Diab-Assef, M.J. Haddadin, P. Yared, C. Assaad and H.U. Gali-Muhtasib, Molecular Carcinogenesis **33**, 198 (2002); (c) H.U. Gali-Muhtasib, M.J. Haddadin, D.N. Rahhal and I.H. Younes, Oncology Reports 8, 679 (2001)

9. General synthetic procedure: A mixture of 2-methyl-3-acetylquinoxaline 1, 4-oxide, **1** (0.003 mol) and appropriate aryl aldehyde (0.0035 mol) in methanolic sodium hydroxide solution (10 ml, 5% w/v) was stirred for 5-10 min at 5-10 °C. The precipitate obtained was filtered, washed with water and recrystallised from chloroform/ethanol to provide the title compounds **2a-l.** Compound **2a**: Yield: 62%: m.p. 172-173 °C: IR (neat) 3102, 3009, 2919, 1657, 1597, 1334, 1096, 766 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 2.65 (s, 3H, CH_3), 7.16 (d, 1H, =CH, J=16.15 Hz), 7.45 (m, 3H, Ar-H), 7.59 (m, 3H, =CH and Ar-H), 7.90 (m, 2H, Ar-H), 8.60 (d, 1H, Ar-H, J=8.6 Hz), 8.70 (d, 1H, Ar-H, J=8.40 Hz); Anal. Calcd for $C_{18}H_{14}N_2O_3$. 0.5 H_2O: C 68.55; H 4.47; N 8.88. Found C 68.56; H 4.58; N 9.10

10. R. LeBlanc, J. Dickson, T. Brown, M. Stewart, H. Pati, D. VanDerveer, H. Arman, J. Harris, W. Pennington, H. Holt and M. Lee, Bioorg. Med. Chem **13**, 6025 (2005)

11. G. R. Pettit, S. Bux, B. Singh, M.L. Niven, E. Hamel and J.M. Schimdt, J. Nat. Prod. **50**, 119 (1987)

Received on 12 June, 2008