Pergamon

*Bioorganic & Medicinal Chemistry Letters*, Vol. 5, No. 16, pp. 1869-1874, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00310-X

# The *in vitro* Cytotoxicity and DNA Alkylating Ability of the Simplest Functional Analogues of the *seco* CC-1065 Alkylating Subunit

Rodney H. White,[†] Peter G. Parsons,*[‡] Arungundrum S. Prakash,*[§] and David J. Young*[†]

[†]Faculty of Science and Technology, Griffith University, Nathan, 4111, Australia; [‡]Queensland Institute of Medical Research, Herston, 4029; [§]National Research Centre for Environmental Toxicology, Kessels Rd, Coopers Plains, 4108.

**ABSTRACT:** The *p*-hydroxyphenethyl halides **1(b-d)** possess the minimum structural requirements for alkylating DNA with Ar-3' participation and exhibit *in vitro* cytotoxicity and DNA alkylating ability/sequence selectivity which is diminished relative to more functionalised *seco* analogues of the antitumor agent CC-1065 alkylating subunit.

The antitumor agent CC-1065[1,2] (Figure 1) and recently developed synthetic analogues exhibit exceptional potency against a variety of tumour cell lines via intense and sequence selective DNA alkylation.[3] This intensity and selectivity is a function of non covalent binding within AT-rich regions of the minor groove of double stranded B DNA[4,5] and the unique reactivity of the cyclopropane containing (CPI) subunit which primarily alkylates N3 of susceptible adenine residues.[6] Uncyclised (*seco* ) CPI containing analogues such as carzelesin and the bifunctional alkylating agent bizelesin (Figure 1) also exhibit these remarkable DNA-binding properties as well as improved antitumor efficacy *in vitro* and are prodrugs.[7-11]

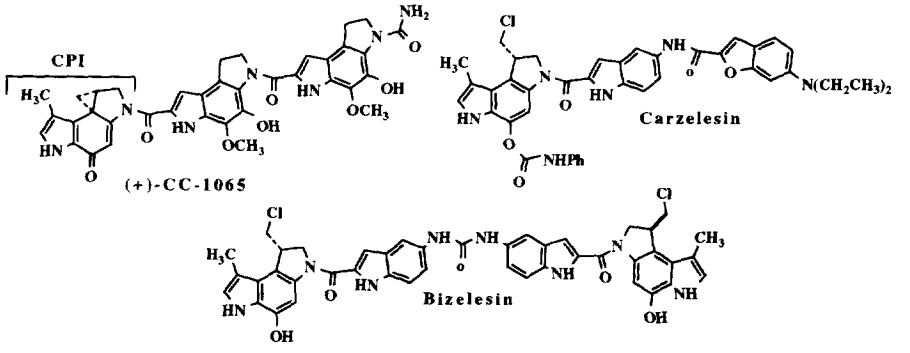
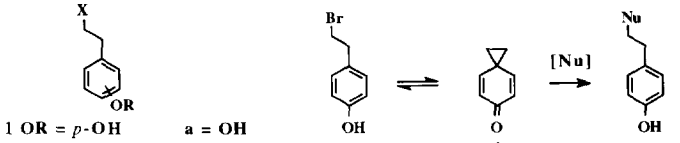



**Figure 1**: Structures of CC-1065, carzelesin and bizelesin

In the quest for improved antitumour agents of this type, investigations involving the isolated alkylating and non-alkylating moieties have provided valuable information with regard to the origins of the DNA sequence selectivity[4,12] and the effect of altered chemical reactivity on cytotoxic potency.[13,14,15] In this vein,

we have sought to determine the minimal structural features of the alkylating subunit necessary for a "CC-1065 mode" of biological activity and to compare the biological properties of this minimal functional agent with those of previously reported, more highly functionalised alkylating subunits. Spiro[2.5]octa-1,4-dien-3-one (**4**) lacks the fused aromatic and pyrrolidine functionality of the CPI and other known alkylating subunits, but is too hydrolytically unstable[16] for study in biological systems. Baird and Winstein have, however, unambiguously established the intermediacy of **4** in the reaction of bromide **1c** with simple nucleophiles (Scheme 1). Therefore, we decided to investigate the cytotoxicity and DNA alkylating ability of the simplest *seco* CPI functional analogues, the 2-*p*-hydroxyphenethyl halides (**1b - d**) and now report our preliminary findings.




**Scheme 1**

We first determined the *in vitro* cytotoxicity of bromide **1c** towards a variety of tumour cells lines relative to the standard DNA alkylating agent methylmethanesulfonate (Table 1). Cells were exposed to the compounds (diluted in RPMI 1640 with 5% fetal calf serum) and incubated for 3 days at 37 °C. Survival curves were obtained by graphing the log of the percent survival compared to the control against the drug concentration, using 5 concentrations (in quadruplicate) which spanned the 1 - 100% survival range.

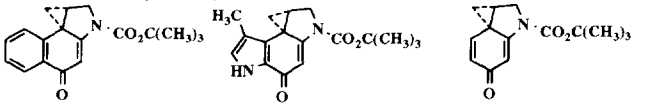
**Table 1**: *In vitro* cytotoxicity of **1c** towards several cell lines

| cell line | He La[b] | MM96L[c] | CI80-13S[d] | MM418cl5a[e] | B16[f] |
|---|---|---|---|---|---|
| **1c** $D_{37}$ μM[a] | 18 | 16 | 27 | 25 | 44 (32) |
| MMS $D_{37}$ μM[a] | 35 | 56 | 59 | 58 | -- |

[a]The dose required to reduce cell survival to a factor of 0.37 relative to untreated controls. [b]Mer- human cervical carcinoma. [c]Human melanoma. [d]DNA cross-linking resistant human ovarian carcinoma. [e]Human melanoma with high melanin content. [f]Murine melanoma. $IC_{50}$ (μM) in parenthesis.

Cell death appeared to be turnover dependent in each case with no obvious membrane disruption. Bromide **1c** was consistently *ca* 2-fold more cytotoxic to all cell lines than the simple $S_N2$ guanine alkylating agent MMS. The almost identical cytotoxicity towards the human melanoma cell lines MM96L and the darker MM418cl5a indicated no significant melanin binding; and the lack of sensitivity of the $O^6$- alkylguanine repair - deficient line HeLa confirmed the expectation that $O^6$ - alkylation was not significant. The $IC_{50}$ for **1c**

towards murine melanoma B16 is considerably larger (*ca* 2 x $10^6$ fold) than that reported for (+) CC-1065.[17] Interestingly, however, this value may not be dissimilar to the corresponding value for (±) $N^2$-BOC-CPI (reported to be >3.3 μM) and is *ca* 34 times that for the benzannelated analogue (±) $N^2$-BOC-CBI (Figure 3).[17] The decreased activity of **1c** relative to these other alkylating subunits is consistent with the inverse relationship between chemical stability and cytotoxic potency determined by Boger *et al*. [13]




(+) $N^2$-BOC-CBI (+) $N^2$-BOC-CPI (+) $N^2$-BOC-CI

**Figure 2**

We then compared the *in vitro* cytotoxicity of the *p*- hydroxyphenethyl halides (**1**) with that for the corresponding *m*-hydroxy- (**2**) and *p*-methoxy- (**3**) derivatives against the cell lines He La and MM96L (Table 2).

**Table 2:** *In vitro* cytotoxicity and DNA alkylation intensity of *p*-hydroxy-, *m*-hydroxy- and *p*-methoxyphenethyl compounds (**1** - **3**).

| Comp'd | **1a** | **1b** | **1c** | **1d** | **2a** | **2b** | **2c** | **2d** | **3a** | **3b** | **3c** | **3d** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| He La $D_{37}$, μM | 1420 | 32 | 18 | 33 | 7530 | 450 | 75 | 19 | 4420 | 670 | 220 | 22 |
| MM96L $D_{37}$, μM | 905 | 42 | 16 | 43 | 2530 | 450 | 60 | 20 | 3330 | 1970 | 520 | 25 |
| DNA alkylation | | + | +++ | ++ | | | | | | | + | |

This study revealed the following relationships which held for both cell lines: (i) the alcohols were much less cytotoxic than the corresponding halides, suggesting activity for the latter by virtue of biomolecule alkylation; (ii) the relative cytotoxicities of the halides for compounds **2** and **3** correlated with leaving group ability, ie I > Br > Cl which is consistent with $S_N2$ alkylation, while for compound **1** the corresponding order was Br > I ≈ Cl (although the differences were much less pronounced than for compounds **2** and **3**); (iii) for X = Cl and Br, the order of cytotoxicity was **1** > **2** > **3** while the cytotoxicity of the three iodides was approximately equivalent. The greater potency of *p*-hydroxyphenethyl chloride (**1b**) and bromide (**1c**), relative to the corresponding *m*- hydroxy (**2**) and *p*-methoxy (**3**) derivatives, correlates with the greater electrophilicity of these substrates by virtue of Ar-3' participation.[16]

The alkylation of DNA by compounds **1** - **3** was studied using a 375 base pair EcoRI to BamHI fragment of pBR322 DNA which was 3'-end labelled at the EcoRI site using Klenow fragment and α-$^{32}$P-

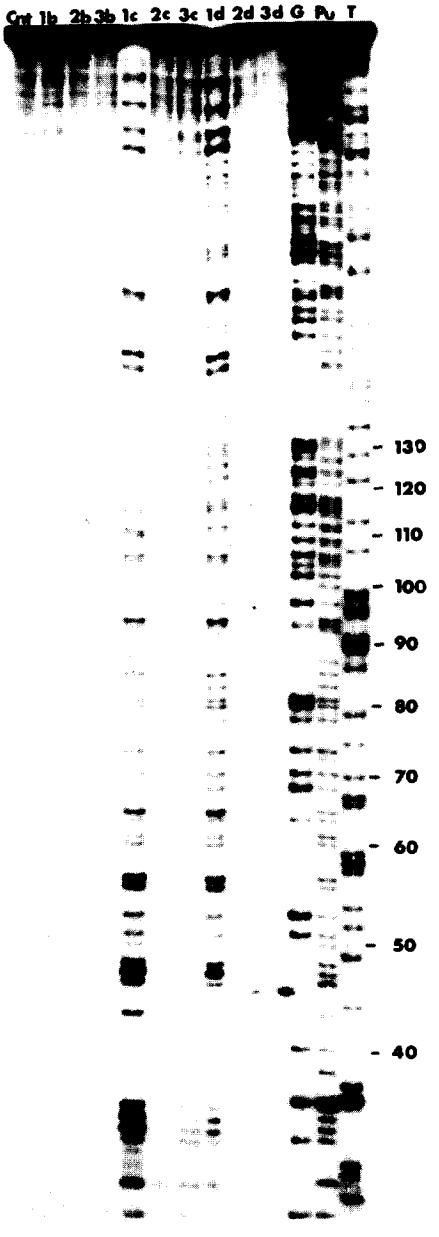



**Figure 3.** Autoradiogram of piperidine - induced strand breaking pattern following treatment of a 375-bp fragment of pBR322 plasmid DNA with 500 μM of compounds **1** - **3** (1 hr, 37 °C, pH 6.7).

dATP according to published procedures.[18] The autoradiogram (Figure 3) revealed that bromide **1c**, iodide **1d** and, to a lesser extent, chloride **1b** exhibited higher levels of DNA alkylation than the corresponding *m*-hydroxy- and *p*- methoxy- derivatives under these conditions (Table 2). This is also reflected in reduced intensities of the bands corresponding to unmodified labelled DNA at the top of the gel in these lanes by comparison to that of the control lane. Considerably higher concentrations of **1c** and **1d** were required to observe DNA alkylation than is required for CC-1065, but these conditions (500μM of agent, 37 $^{o}$C, 1 hr) are, however, comparable to that required to observe alkylation by CPI and CI derivatives.[4] The intensity of alkylation for compounds of type **1** followed the order Br > I > Cl. This increased intensity of DNA alkylation by bromide **1c** correlates with the higher *in vitro* cytotoxicity of this compound noted above. Likewise, the decreased intensity of DNA alkylation by iodide **1d** (relative to **1c**) also correlates with the order of *in vitro* cytotoxicity and may indicate prior non-productive hydrolysis. The lower intensity of alkylation and *in vitro* cytotoxicity of chloride **1b** (relative to **1c**) correlates with the decreased leaving group ability of Cl suggesting that, for this compound at least, ring closure to the spirocyclopropane intermediate (**4**) in the proposed alkylation mechanism (Scheme 1) is rate determining under these conditions.

All compounds exhibited a comparable alkylation pattern, differing in band intensities. Compounds **1c**, in particular, demonstrated a strong preference for adenines and especially for adenines with a T on the 5' side. Runs of adenines were also alkylated in preference to isolated adenines. Within runs of adenines, the middle adenine was alkylated preferentially. The order of A alkylation preference observed with this fragment was 5' - TTAAA (bases 37 - 33) = 5' - TAAA (bases 49 - 46) > 5' - TTTAA (bases 60 - 56) > 5' - TTA (bases 67 - 65 and 96 - 94) > 5' - TA (bases 86 - 85) > 5' - AATTT (bases 62 - 58) > 5' - AT (bases 38, 50, 87 and 100) = 5' - NAN (bases 73 and 83). All guanines were also alkylated under these conditions but with a much reduced intensity relative to adenines and with no obvious sequence selectivity.

While this preference of **1c** for runs of A and particularly those with a T on the 5' side broadly mirrors that of CC1065,[3] this compound was dramatically less sequence selective indicating the important directing influence of the non covalent binding subunits. Even compared to the isolated CPI and CI subunits[7,19] and their *seco* precursors, **1c** appeared to be less discriminating indicating a directing influence for the additional functionality, presumably by virtue of moderating reactivity.[14,20]

The authors gratefully acknowledge the financial support of the Australian Research Council and the Queensland Cancer Fund.

## REFERENCES

(1) Martin, D. G.; Chidester, C. G.; Duchamp, D. J.; Mizsak, S. A. *J. Antibiot.* **1980**, *33*, 902.

(2) Hanka, L. J.; Dietz, A.; Gerpheide, S. A.; Kuentzel, S. L.; Martin, D. G. *J. Antibiot.* **1978**, *31*, 1211.

(3) Hurley, L. H.; Lee, C.-S.; McGovren, P.; Warpehoski, M. A.; Mitchell, M. A.; Kelly, R. C.; Aristoff, P. A. *Biochemistry* **1988**, *27*, 3886.
(4) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H. *J. Am. Chem. Soc.* **1991**, *113*, 3980.
(5) Boger, D. L.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 1431.
(6) Hurley, L. H.; Reynolds, V. L.; Swenson, D. H.; Petzold, G. L.; Scahill, T. A. *Science* **1984**, *226*, 843.
(7) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Tshizaki, T.; Haught, J.; Bina, M. *Tetrahedron* **1991**, *47*, 2661.
(8) Li, L. H.; DeKoning, T. F.; Kelly, R. C.; Kreuger, W. C.; McGovren, J. P.; Padbury, G. E.; Petzold, G. L.; Wallace, T. L.; Ouding, R. J..; Prairie, M. D.; Gebhard, I. *Cancer Res.* **1992**, *52*, 4904.
(9) Lee, C.-S.; Gibson, N. W. *Cancer Res.* **1991**, *51*, 6586.
(10) Lee, C.-S.; Gibson, N. W. *Biochemistry* **1993**, *32*, 2592.
(11) Sun, D.; Hurley, L. H. *J. Am. Chem. Soc.* **1993**, *115*, 5925.
(12) Boger, D. L.; Johnson, D. S. *J. Am. Chem. Soc.* **1995**, *117*, 1443.
(13) Boger, D. L.; Mesini, P.; Tarby, C. M. *J. Am. Chem. Soc.* **1994**, *116*, 6461.
(14) Boger, D. L.; Yun, W. *J. Am. Chem. Soc.* **1994**, *116*, 5523-.
(15) Boger, D. L.; Nishi, T.; Teegarden, B. R. *J. Org. Chem.* **1994**, *59*, 4943.
(16) Baird, R.; Winstein, S. *J. Am. Chem. Soc.* **1963**, *85*, 567.
(17) Boger, D. L.; Ishizaki, T.; Wysocki, R. J. Jr.; Munk, S. A. *J. Am. Chem. Soc.* **1989**, *111*, 6461.
(18) Maxim, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499.
(19) Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Kitos, P. A.; Suntornwat, O. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 55.
(20) Boger, D. L.; Munk, S. A.; Ishizaki, T. *J. Am. Chem. Soc.* **1991**, *113*, 2779.

(*Received in USA* 25 *May* 1995; *accepted* 14 *July* 1995)