Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 1, pp. 93-96, 1995

0960-894X/95 $9.50+0.00

0960-894X(94)00465-X

Tricarbonyl η6 Arene Chromium (0) Based Antitumor Agents

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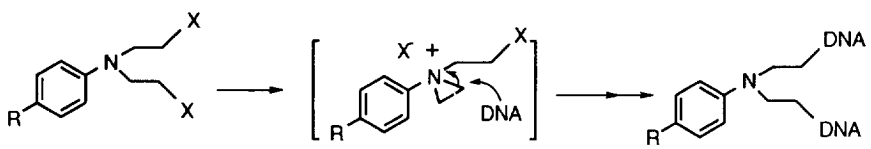
Abstract: Novel arene chromium carbonyl complexed mustard agents have been prepared. The metal carbonyl group modulates mustard alkylative ability as evidenced by 4-NBP assay. Antitumor evaluation against the human colon tumor line HCT-116 however showed no clear correlation between alkylative ability and antitumor activity of the mustard agents, in part a consequence of the inherent cytotoxicity of the metal carbonyl group in this screen.

Nitrogen mustard agents which have the capacity to cross link DNA have received widespread attention due to their potential as antineoplastic agents.[1] In an effort to achieve selective cytotoxicity, a number of studies targeted at the hypoxic tumor cell environment indicate the utility of aniline mustards which bear electron withdrawing functions.[2,3] Such mustards, e.g.**1** (R=NO_2 or $SOCH_3$), when subjected to a bioreductive environment, undergo chemical activation since in the reduced form (**1**, R=NH_2 or SCH_3, X=Cl), the nucleophilicity of the aniline nitrogen atom is enhanced. The net effect is an increase in the alkylative capacity of the β chloroethyl group, due to ease of formation of the requisite aziridinium ion for DNA alkylation viz. **2**, Scheme 1, giving the potential to form bis DNA adducts **3** and possibly cross links.

Scheme 1. DNA crosslinking *via* aniline mustard agents



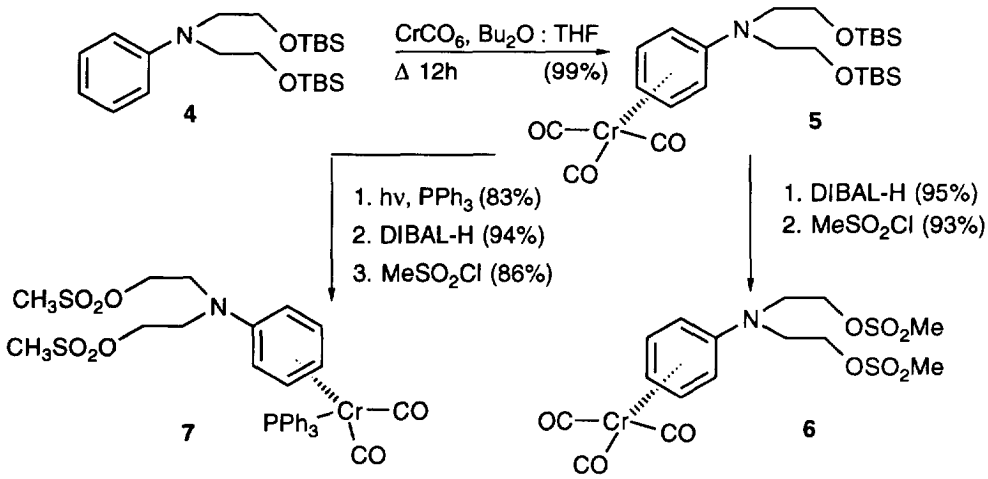
1 R =H, SOMe, NO_2
X= Cl, OSO_2Me **2** **3**

In connection with our studies on antitumor agents,[4] we wished to investigate the utility of the η6 arene metal carbonyl group as an aniline mustard triggering device. A variety of agents effect decomplexation, and this may potentially serve as an activation protocol.[5] It is well known that the relative (aryl) electron density of tricarbonyl chromium (0) complexes can be influenced by ligand replacement, such that complexes $ArCr(CO)_3$ are electron deficient, wheras those of type $ArCr(CO)_2P(R)_3$ are electron rich relative to the parent arene.[6] Thus, preparation of chromium carbonyl complexed aniline mustard agents would potentially allow relative control of aziridinium ion formation by variation of the attached ligands.

The common precursor **4** was formed by addition of 2-chloroethanol to aniline, followed by subsequent silylation with tertbutyldimethylsilyl triflate (Scheme 2).[7] The silyl ether was subjected to typical complexation conditions using hexacarbonyl chromium, giving the desired chromium complex **5** in nearly quantitative yield.[8] Liberation of the hydroxyl groups was eventually accomplished using DIBAL-H,[9] since deprotection using hydrogen fluoride-pyridine resulted in partial decomplexation. Finally, the methanesulfonyl (mesyl) mustard agent

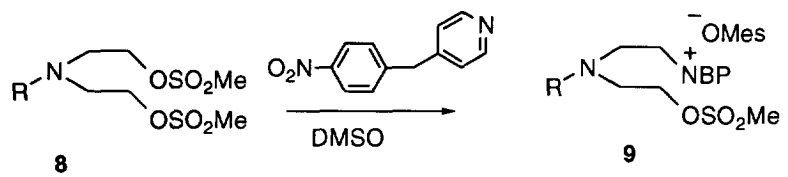
6 was prepared, using methanesulfonyl chloride with triethylamine. The mesyl group was chosen in favor of the chloro group due to both its hydrophilicity (p=-0.88 versus 0.71 Cl),[2] and leaving group ability (L=1.57 versus -1.61 Cl),[10] important considerations for the desired *in vitro* testing. With variation of the electron withdrawing

Scheme 2. Preparation of metal carbonyl based mustard agents



ability of the metal carbonyl group a critical feature of our approach, we prepared the mixed ligand system **7**, via irradiation (Hg lamp) of complex **5** with triphenylphosphine in benzene, deprotection and mesylation giving the desired product in high yield (Scheme 2). With complexes **6** and **7** available, the ability of the metal carbonyl group to influence aziridinium ion formation was probed, using the established 4-(4'-nitrobenzyl)pyridine (NBP) assay.[11] Nucleophilic addition of 4-NBP to agents **6**, **7** and the uncomplexed mustard **8**, R=Ph (NSC 71035) were carried out, and the relative rate of formation of adducts **9** (Scheme 3) compared. The results, shown in Figure 1., demonstrate clearly the retardative effect on the addition to the complex **6** (relative to uncomplexed mustard NSC 71035) which may be a consequence of sluggish formation of the derived aziridinium ion, in turn a consequence of reduced nucleophilicity of the aniline nitrogen atom. The mixed ligand complex **7**, on the other hand, forms the adduct more readily, in agreement with a trend based on the electron donor capacity of mixed arene chromium carbonyl complexes.[6]

Scheme 3. 4-(4'-nitrobenzyl)pyridine (NBP) aniline (N) nucleophilicity assay



Biological evauation of the mustards was then conducted using the human colon tumor line HCT-116.[12] The computed IC_{50} values are presented in Table 1. In agreement with the trend observed in the NBP assay (Figure 1), the mixed ligand chromium complexed mustard **7** was an order of magnitude more active than control mustard

NSC 71035 (entries 1, 2). The tricarbonyl complex **6** however also showed high cytotoxocity (entry 3) leading to the conclusion that observed cytotoxicity may be due in part to the metal carbonyl group. Evidence for this theory was supported by comparison of cytotoxicities of compounds **4** and **5**, neither of which bears a potent electrophilic center (entries 4, 5), yet **5** is highly cytotoxic to the HCT-116 cell line.

Figure 1. Plot of NBP adduct formation vs. time for agents 6, 7, 8 (R=Ph)

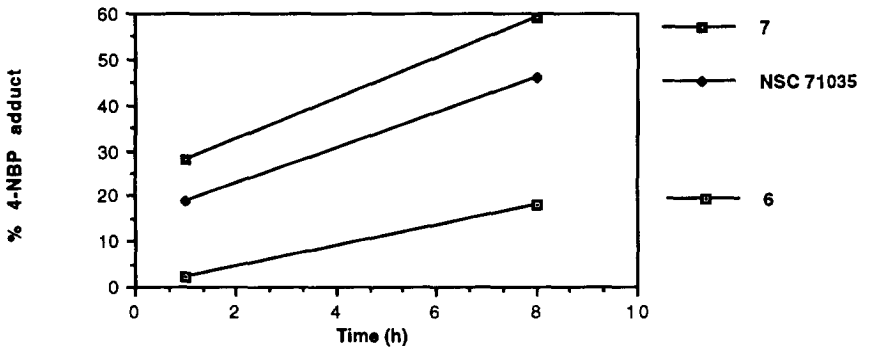
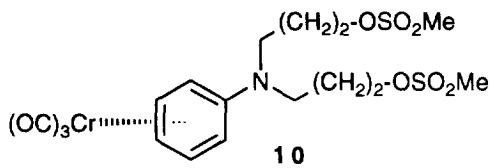


Table 1: Activity against HCT-116 Tumor line

entry	compound	IC_{50} (M)
1	**7**	1.4×10^{-9}
2	NSC 71035	2.5×10^{-8}
3	**6**	1.9×10^{-9}
4	**4**	1.9×10^{-6}
5	**5**	1.7×10^{-8}
6	**10**	1.5×10^{-8}
7	**11**	9.9×10^{-8}

In order to separate out the relative cytotoxic effects into contributions from the metal carbonyl group and those derived from aziridinium ion formation, chain extended mustard agents **10** and **11** were prepared using similar methods.[7] The IC_{50} values for these compounds (entries 6, 7) when compared to their more chemically reactive homologs (**6** and NSC 71035 respectively) indicates that the metal carbonyl group contributes to cytotoxicity observed in this assay, partially masking the subtle differences in alkylative ability based on potential for aziridinium ion formation.



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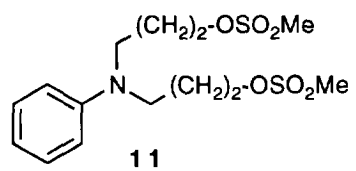

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Development of this family of tricarbonyl arene chromium (0) complexes as potential antitumor agents was based on the premise that under certain environments, the chromium tricarbonyl group could be decomplexed, yielding an activated aniline mustard agent. Since a variety of biological agents may, in principle, initiate such decomplexation, we investigated the conversion of **6** to mustard NSC 71035 using a variety of mimics, and the

results are shown in Table 2. As can be seen, commonly encountered agents such as peroxides and quinols result in rapid decomplexation (entries 1-2), making it entirely likely that the decomplexed species was formed to some extent during bioassay. The derived chromium based decomplexation products very likely contribute to overall cytotoxicity, and may explain the unexpected high cytotoxicity observed for **5** and **6**.

Table 2. Decomplexation of **6** to mustard NSC 71035 using external agents[a]

entry	agent	% conversion	
		1h	12 hr
1	H_2O_2	55%	95%[b]
2	hydroquinone	21%	59%
3	hv[c]	40 %	99%
4	AIBN	35%	75%
5	2-mercaptoethanol	17%	64%[b]
6	control	0%	<5%[b]

a: all incubations carried out in the dark at 37°C in degassed DMF

b: accompanied by mesylate hydrolysis; c: 450W Hg lamp.

In summary, a new family of tunable alkylating agents has been investigated. The arene chromium carbonyl complex is able to control aziridinium ion formation in aniline mustards as evidenced by nucleophilic addition assays. Initial *in vitro* antitumor evaluation shows the mustard derivatives to be highly cytotoxic, in part a consequence of the metal carbonyl group itself. Ongoing studies are designed to ratify the origin of non alkylative cytotoxicity, as a function of both assay conditions, and cell lines.

References and Notes

1. For discussion see Rink, S. M.; Solomon, M. S.; Taylor, M. J.; Rajur, S. B.; McGlaughlin, L. W.; Hopkins, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 2551.
2. Palmer, B. D.; Wilson, W. R.; Pullen, S. M.; Denny, W. A. *J. Med. Chem.* **1990**, *33*, 112.
3. Kwon, C. H.; Blanco, D. R.; Baturay, N. *J. Med. Chem.* **1992**, *35*, 2137.
4. Jones, G. B.; Huber, R. S. *Tetrahedron Lett* **1994**, *35*, 2655; Jones, G. B.; Mathews, J. E.; Banner, W. K. *Heterocycles* **1994**, *38*, 1221; Jones, G. B.; Huber, R. S.; Chau, S. *Tetrahedron* **1993**, *49*, 369.
5. Davies, S. G. *Organotransition Metal Chemistry: Applications to Synthesis*; Pergamon Press: Oxford, (**1982**).
6. Benzoic acid pKa = 5.68; tricarbonyl chromium (0) benzoic acid complex pKa = 4.77; dicarbonyl (triphenylphosphine) chromium (0) benzoic acid complex pKa = 6.15. For discussion see: Solladie-Cavallo, A. *Polyhedron* **1985**, *4*, 901.
7. Reaction of aniline with 2-chloroethanol gave the bis β hydroxy alcohol in 88%, and with 3-chloro propanol the γ hydroxy alcohol in 85% yield. Subsequent silylation is achieved in quantitative yield (TBSOTf) in both cases. Mustard **10** was prepared by complexation of the C3-TBS ether (90%), deprotection (DIBAL-H, 95%) and mesylation ($MeSO_2Cl$, Et_3N, 91%). Mustards NSC 71035 and **11** were prepared by direct mesylation of the alcohols.
8. For general complexation conditions employed see: Jones, G. B.; Heaton, S. B. *Tetrahedron: Asymmetry* **1993**, *4*, 267.
9. Corey, E. J.; Jones, G. B. *J. Org. Chem.* **1992**, *57*, 1028.
10. Price, C. C. in *Antineoplastic and Immunosuppressive Agents*, Vol. 2, pp. 1-5, Sartorelli, A. C.; Johns, D. G. Eds; Springer-Verlag: New York, **1975**.
11. Spears, C. P. *Molecular Pharmacology* **1981**, *19*, 496.
12. All *in vitro* analysis was performed by Dr Louis R. Barrows at the Center For Drug Discovery, University of Utah. HCT-116 assays were performed at drug concentrations ranging from 0.005mg/mL through 50 mg/mL using the MTT protocol (*Int. J. Radiat. Oncol. Biol. Phys.* **1989**, *16*, 973). Drug exposure lasted 18h. All IC_{50} determinations reported are +/- 0.15.

(*Received in USA* 4 *August* 1994; *accepted* 5 *December* 1994)