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ORIGINAL ARTICLE

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Thiolo-, thiono- and dithiocarbonate and thiocarbamate derivatives of demethylpenclomedine as novel anticancer agents

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Abstract *Purpose*: The purpose of this investigation was to synthesize a series of thiolo-, thiono- and dithiocarbonate and thiocarbamate derivatives of 4-demethylpenclomedine (DM-PEN), the major plasma metabolite of penclomedine (PEN) in patients observed subsequently to be an active antitumor agent and non-neurotoxic in a rat model, in order to compare their antitumor activity with that of DM-PEN. *Methods*: Derivatives were prepared from DM-PEN and evaluated in vivo against human MX-1 breast tumor xenografts implanted in the mammary fat pad, several of which were also evaluated against human brain tumor xenografts. *Results*: Thiolocarbonate and thiocarbamate derivatives were found to be superior to DM-PEN against MX-1 tumor and modestly active against glioblastoma. *Conclusion*: The activity of the thiolocarbonates and thiocarbamates against human tumor xenografts in vivo suggests consideration of these two series of derivatives of DM-PEN for clinical development.

Keywords Penclomedine · 4-demethylpenclomedine · Antitumor evaluation in vivo · Human tumor xenografts

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Introduction

Penclomedine (PEN) was evaluated in Phase I clinical trials at Johns Hopkins University Oncology Center, the University of Wisconsin Comprehensive Cancer Center and Western General Hospital in Edinburgh [1–5] for possible use in the treatment of breast cancer based on activity against human breast tumor xenografts and experimental mammary tumor models [6, 7], and in the treatment of brain tumors based on its activity against tumor xenografts in the brain [7]. In all of these clinical trials, dose-limiting neurotoxicity was observed after both intravenous and oral administration and was related to peak plasma levels of PEN [4]. 4-demethylpenclomedine (DM-PEN) was identified as the major plasma metabolite in patients and rodents [1, 2], and neuroanatomic studies of PEN and DM-PEN in rats revealed cerebellar damage only in the PEN-treated group [8].

These observations led to the synthesis and antitumor evaluation of DM-PEN, and the notable activity of DM-PEN against both s.c.- and i.c.-implanted human MX-1 breast tumor xenografts led to discussions with NCI and commercial clients about possible clinical development [9]. Subsequently, a series of acyl derivatives of DM-PEN was prepared and evaluated against MX-1 tumor xenografts, several other human tumor xenografts and murine P388 leukemia, revealing potent activity [10]. The results of the synthesis and antitumor evaluation of additional analogs are described in this report and they support consideration for clinical development of a representative derivative.

Materials and methods

DM-PEN was prepared by a modification of the reported method [1] and was obtained as a white, crystalline solid, which was characterized by mass spectral, NMR and elemental analysis.

Synthesis of thiolocarbonate, thionocarbonate, dithiocarbonate and thiocarbamate derivatives of DM-PEN

A general experimental procedure for the preparation of thiolo-, thiono-, dithiocarbonate and thiocarbamate derivatives of DM-PEN is as follows: DM-PEN (1 g) in 15 ml dry dichloromethane is treated with 0.5 ml triethylamine followed by one equivalent of a chlorothiolocarbonate, a chlorothionocarbonate, a chlorodithiocarbonate or a thiocarbamoyl chloride, respectively, added dropwise at room temperature in 5 ml dry dichloromethane. The solution is stirred 30 min at room temperature and evaporated to dryness *via* a water aspirator. The residue is triturated with 5 ml acetone and filtered to remove triethylamine hydrochloride. The acetone filtrate is concentrated to 1 ml and separated on an 8 inch, 2 mm silica gel plate containing a fluorescent indicator. The major UV–visible band is eluted with acetone and the solvent evaporated, resulting in high yield of the respective product . Characterization is provided by mass spectrometry, which reveals the appropriate mass number +1 corresponding to the expected structure, and thin-layer chromatography, which yields a single UV–visible component.

Antitumor evaluation in vivo

Antitumor evaluations were conducted as described previously [6, 7]. Athymic NCr-*nu*/*nu* and $CD2F_1$mice were obtained from various suppliers under contract with NCI and were housed in sterile, filter-capped microisolator cages in a barrier facility. Human tumors were obtained from the NCI Tumor Repository (Frederick, MD, USA). For i.p. injection into mice, DM-PEN and the derivatives were prepared as a suspension in aqueous hydroxypropyl cellulose. Tumor fragments (30–40 mg) from in vivo passage were implanted into the mammary fat pad of the mice.

Treatment of groups of 5 mice each was initiated when the tumors reached approximately 300 mg in mass and was continued for 5 days for all treatment groups. Each tumor was measured by caliper in two dimensions twice weekly and converted to tumor mass. Antitumor activity was assessed on the basis of tumor growth delay in comparison to a vehicle-treated control, tumor regressions (partial and complete), and tumor-free survivors; and experiments were terminated when the control tumors attained a size of 1 g, which is typically 57–61 days. For i.c. implants, 0.03 ml of an MX-1 tumor brei (containing 10^6 cells) was implanted into the right hemisphere of the mice.

Treatment of i.c. implants was initiated 1 day after tumor implantation and continued for 5 days. Mice were monitored daily for survival. Antitumor activity was assessed on the basis of the percentage increase in lifespan (ILS) in comparison to a vehicle-treated control, and long-term survivors.

"Principles of laboratory animal care" (NIH publication No. 85–23, revised 1985) were followed in all of the animal studies.

Results

Figure 1 shows the synthetic route and structures of the methyl derivatives of DM-PEN prepared as described in Materials and methods.

Each derivative was evaluated simultaneously with a DM-PEN control against MX-1 tumor implanted in the mammary fat pad with i.p. treatment. A range of dosages of 135, 90 and 60 mg/kg per dose was used, including the maximum tolerated dose which is defined as LD_{10}. All of the thiolocarbonate derivatives yielded superior activity to DM-PEN and produced one or two of five tumor-free survivors. The results are shown in Table 1.

The thionocarbonate and dithiocarbonate derivatives, however, demonstrated only low activity in this tumor model (data not shown).

The methyl thiolocarbonate derivative of DM-PEN, DM-SMTC-PEN was evaluated against intracranially implanted U251 human glioblastoma xenograft and was observed to be comparably active to the acyl derivatives against this tumor [10]. DM-SMTC-PEN was also evaluated against intracranially implanted D54 human glioblastoma multiforme, a highly resistant brain tumor, and has yielded an increase in life span of 18%, a modest response but one not greatly different from that produced by BCNU, the current drug of choice for clinical treatment of malignant gliomas, the major brain tumor in the USA.

The antitumor activity of the thiocarbamates is shown in Table 2. Against MX-1 human mammary tumor xenograft, potent antitumor activity greater than that observed for DM-PEN was observed for the dimethyl derivative (DM-DMTC-PEN) and the diethyl derivative (DM-DETC-PEN), with somewhat greater activity being observed for the dimethyl derivative, indicating possible clinical potential.

Evaluation of DM-DMTC-PEN against intracranially implanted U251 human brain tumor xenograft for comparison of its activity with that of DM-SMTC-PEN in a side-by-side experiment revealed activity of a 44% increase in life span (ILS), which was slightly inferior to DM-SMTC-PEN, and which yielded an ILS of 56%, but was identical to that of the ethyl and phenyl analogs of DM-SMTC-PEN.

A major concern for the penclomedine (PEN) series of derivatives is their possible neurotoxicity. PEN was removed from clinical development as a potential drug for treating breast cancer because of its dose-limiting neurotoxicity. Consequently, DM-SMTC-PEN was evaluated simultaneously with PEN in a behavioral test of neurotoxicity and was observed to be non-neurotoxic, as indicated by the absence of production of tremors in the DM-SMTC-PEN group in comparison to the PEN group.

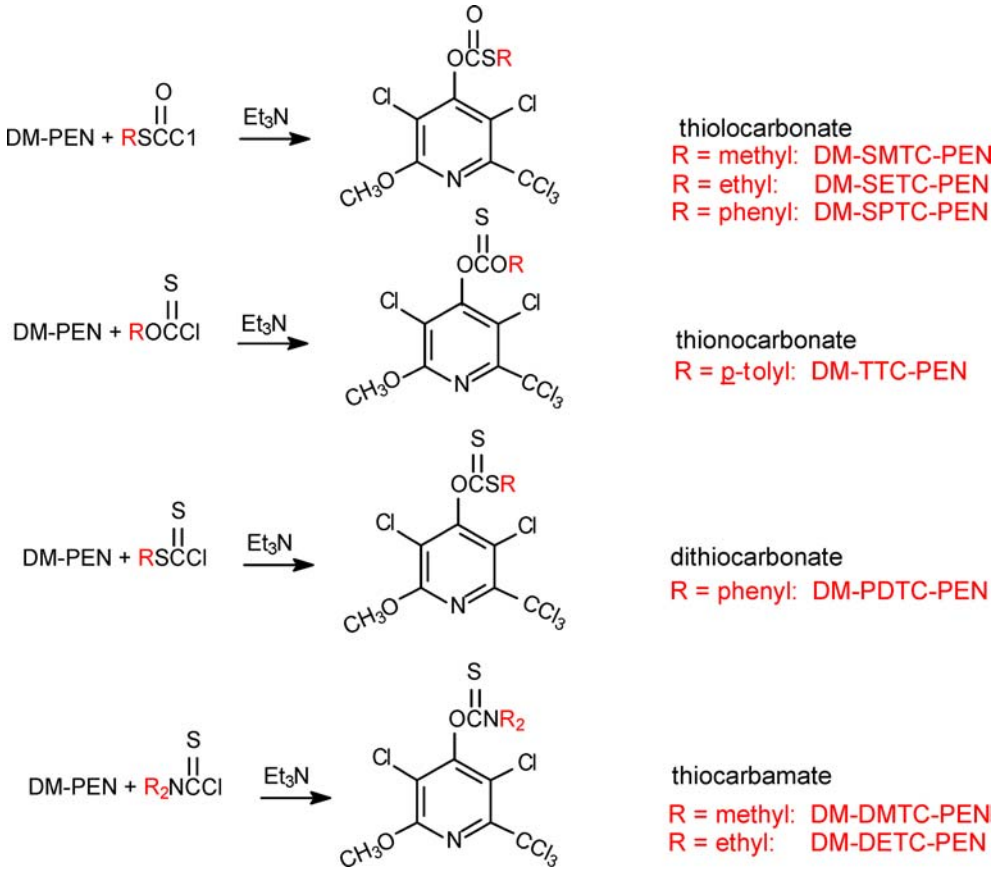


Fig. 1 Synthetic routes for thiolo-, thiono-, dithiocarbonate and thiocarbamate derivatives of DM-PEN

Response of MX-1 Mammary Tumor Implanted in the Mammary Fat Pad to Treatment with DM-PEN, DM-SETC-PEN, DM-SPTC-PEN, and DM-SMTC-PEN

Agent	IP Dosage (mg/kg/dose)	Schedule	Regressions		Growth Delay (T-C)	Tumor-free Survivors
			Partial	Complete		
DM-PEN	135	Days 15-19	2	0	32.8	0/5
DM-SETC-PEN	60	DAYS 15-19	3	2	>35.2	1/5
DM-SPTC-PEN	135	Days 15-19	2	2	>35.2	1/5
DM-SMTC-PEN	60	Days 15-19	2	2	>41.6	2/5

Thiolocarbonate Derivatives: R = methyl - DM - SMTC - PEN
R = ethyl - DM - SETC - PEN
R = phenyl - DM - SPTC - PEN

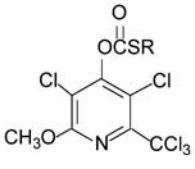


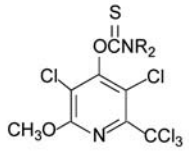
Table 1 Response of MX-1 mammary tumor implanted in the mammary fat pad to treatment with DM-PEN, DM-SETC-PEN, DM-SPTC-PEN, and DM-SMTC-PEN

Table 2 Response of MX-1 mammary tumor implanted in the mammary fat pad to treatment with DM-PEN, DM-DMTC-PEN, and DM-DETC-PEN

Response of MX-1 Mammary Tumor Implanted in the Mammary Fat Pad to Treatment with DM-PEN, DM-DMTC-PEN, and DM-DETC-PEN

Agent	IP Dosage (mg/kg/dose)	Schedule	Regressions		Growth Delay (T-C)	Tumor-free Survivors
			Partial	Complete		
DM-PEN	135	Days 15-19	2	0	32.8	0/5
DM-DETC-PEN	135	Days 15-19	1	1	>35.2	1/5
DM-DMTC-PEN	135	Days 13-17	1	4	>37.0	3/5

Thiocarbamate Derivatives: R = methyl - DM - DMTC - PEN
R = ethyl - DM - DETC - PEN



Antitumor Activity: MX-1 tumor

Discussion

Earlier studies demonstrated that DM-PEN was comparably active to PEN against MX-1 human tumor xenografts but that it lacked the neurotoxicity of PEN in a rat model [8], identifying it as a reasonable candidate for derivatization as a means of producing structures with increased, broad-spectrum antitumor activity in vivo and of diminished toxicity. Synthesis of a series of acyl derivatives was the first attempt to achieve this goal, and success was obtained as indicated by their improved activity against MX-1 human breast tumor xenografts implanted subcutaneously or intracranially, most notably in the latter model [10].

As a logical extension of this effort, a series of thiocarbonate and thiocarbamate derivatives of DM-PEN were prepared by reaction of DM-PEN with the appropriate chlorocarbonate or thiocarbamoyl chloride. Evaluation of the series against orthotopically implanted MX-1 tumor indicated curative activity for the thiolocarbonates and thiocarbamates but inactivity for the thiono- and dithio-carbonates, consistent with the stabilization of derivatized DM-PEN containing a thiocarbonyl moiety, preventing hydrolytic removal in vivo and concomitant inhibition of the alkylating functionality of DM-PEN.

Because of the activity of the acyl derivatives of DM-PEN against intracranially implanted MX-1 tumor, the methylthiolo derivative DM-SMTC-PEN and the dimethyl-thiocarbamate DM-DMTC-PEN were evaluated against U-251 human glioblastoma xenograft implanted intracranially, with activity being observed for both while DM-SMTC-PEN was slightly superior.

In summary, the activity of the thiolocarbonate and thiocarbamate derivatives of DM-PEN against both MX-1 tumor implanted orthotopically and U-251 tumor implanted intracranially identify these two classes of thio derivatives of DM-PEN as potential candidates for clinical development.

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