Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 3593–3598

BIOORGANIC & MEDICINAL CHEMISTRY

# Determination of the Phamacophore of Penclomedine, a Clinically-Evaluated Antitumor Pyridine Derivative

Anita Tiwari,* William R. Waud and Robert F. Struck

*Drug Discovery and Drug Development Divisions, Southern Research Institute, 2000 Ninth Avenue South, Birmingham, AL 35255-5305, USA*

Received 14 December 2001; accepted 8 May 2002

**Abstract**—The main objective of this investigation was to identify the reactive pharmacophore in penclomedine (PEN, 3,5-dichloro-4,6-dimethoxy-2-(trichloromethyl) pyridine) for in vivo antitumor activity and also to discover related ring structures and sulfur analogues that might exhibit superior antitumor activity in vivo. Several new analogues of PEN and related structural variants have been synthesized and evaluated in vivo against MX-1 human breast tumor xenograft implanted subcutaneously (sc), although none of them demonstrated significant activity.


## Introduction

Penclomedine (PEN[1], 3,5-dichloro-4,6-dimethoxy-2-(trichloromethyl) pyridine), a lipophilic multichlorinated α-picoline derivative, is a novel antitumor agent, which was selected for clinical development by the National Cancer Institute based on its selective antitumor activity against subcutaneously (sc) implanted mouse $CD8F_1$ mammary adenocarcinoma and human MX-1 mammary carcinoma following ip administration of drug.[2,3] P388 leukemia cell lines that are resistant to the alkylating agents melphalan, cyclophosphamide, and carmustine were also resistant to PEN, but were not cross-resistant to antimetabolites and DNA intercalators, indicating that PEN is likely to be an alkylating agent.[2] PEN was also active against intracerebrally implanted MX-1 xenografts, suggesting that it penetrates the blood–brain barrier,[3] but in all clinical evaluations dose-limiting neurotoxicity was observed and was related to peak plasma levels of the parent drug.[4–6] Studies have been performed that suggest that PEN undergoes metabolism to yield reactive species that bind to DNA[7,8] in vivo. 4-DMPEN (demethylpenclomedine, 3,5-dichloro-4-hydroxy-6-methoxy-2-(trichloromethyl) pyridine), the major plasma metabolite of PEN in patients, has been synthesized and showed good activity when evaluated against MX-1 human breast tumor xenograft in vivo and was observed to be non-neurotoxic in comparison to PEN in a rat model of neurotoxicity.[9,10]

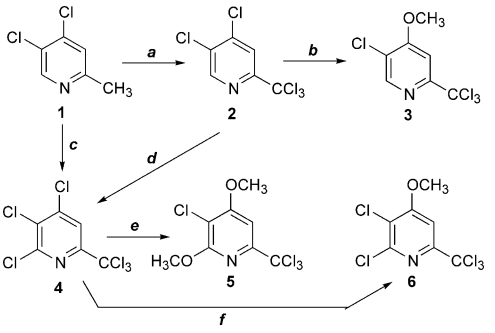
Based on these findings, we have synthesized sequentially dechlorinated and demethoxylated analogues of PEN as well as sulfur analogues of these derivatives, with the hope that a simpler structure would be identified for additional metabolism and SAR studies. Several structural variants were also designed and synthesized in an attempt to discover related ring structures and sulfur analogues that might exhibit better antitumor activity in vivo and provide additional lead compounds also worthy of SAR studies. These compounds were then evaluated against MX-1 human breast tumor xenografts in vivo.

## Chemistry

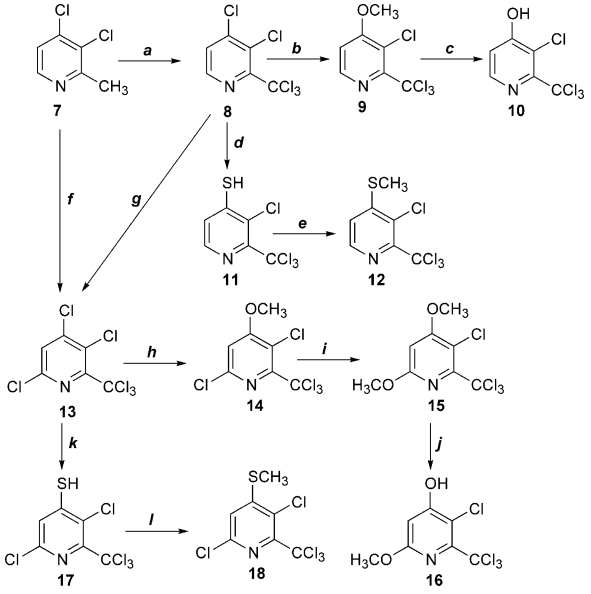
The photo-chlorination of **1**[11,12c] in refluxing $CCl_4$ for 2 h (Scheme 1) afforded **2** in 68% yield. The same reaction extended to 18 h provided **4** in 77% yield. These two compounds on reaction with methanol in the presence of sodium hydroxide gave the target compounds **3**, **5** and **6**. Another sequence of reactions as reported by Ife et al.[11] led to the preparation of **7**. Similarly, as described above for compound **1**, on chlorination, compound **7** (Scheme 2) afforded **8** and **13**. Reaction of **8** and **13** with methanol in the presence of sodium

*Corresponding author. Tel.: +1-205-581-2427; fax: +1-205-581-2877; e-mail: a.tiwari@sri.org

0968-0896/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0968-0896(02)00243-2

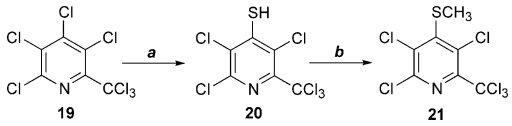


**Scheme 1.** Reagents and conditions: (a) $Cl_2$, $CCl_4$, hν, 2 h, reflux, 68%; (b) NaOH, MeOH, 2.5 h, reflux, 95%; (c) $Cl_2$, $CCl_4$, hν, 18 h, reflux, 77%; (d) $Cl_2$, $CCl_4$, hν, 12 h, reflux, 78%; (e) NaOH, MeOH, 2.5 days, reflux, 58%; (f) NaOH, MeOH, overnight, rt, 93%.

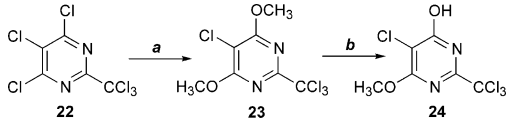


**Scheme 2.** Reagents and conditions: (a) $Cl_2$, $CCl_4$, hν, 1.5 h, reflux, 72%; (b) NaOH, MeOH, 2.5 h, reflux, 78%; (c) DMSO, 5 h, 150 °C, 25%; (d) NaSH, 12 h, rt, 21%; (e) $TMSCHN_2$, acetone, 30 min, rt, 83%; (f) $Cl_2$, $CCl_4$, hν, 24 h, reflux, 88%; (g) $Cl_2$, $CCl_4$, hν, 20 h, reflux, 88%; (h) NaOH, MeOH, 2.5 h, reflux, 94%; (i) NaOH, MeOH, 48 h, reflux, 27%; (j) $AlCl_3$, $CH_2Cl_2$, 3 h, rt, 25%; (k) NaSH, 5 h, rt, 44%; (l) $TMSCHN_2$, acetone, 30 min, rt, 51%.

hydroxide yielded **9** and **14**, respectively. Compound **14** was converted further to **15**. Demethylation of **9** and **15** with DMSO at 150 °C and with $AlCl_3$ at room temperature produced **10** and **16**, respectively. Compounds **11** and **17** were obtained by the treatment of **8** and **13** with freshly prepared sodium hydrogen sulfide solution. These compounds were treated with trimethylsilyldiazomethane to give **12** and **18**, respectively. The intermediate **19** (Scheme 3), prepared by a series of reactions from pentachloropyridine as reported,[12] was reacted with freshly prepared sodium hydrogen sulfide solution to yield **20**, which on methylation gave **21**. We attempted thiolation of both 4 and 6 positions but the reaction failed. Compound **22** (Scheme 4) was prepared by a reported method.[13] This intermediate on reaction with sodium hydroxide and methanol gave **23**, which on demethylation in DMSO afforded **24**. All of these samples were characterized by elemental analysis and mass and NMR spectroscopy prior to evaluation.



**Scheme 3.** Reagents and conditions: (a) NaSH, 5 h, rt, 93%; (b) $TMSCHN_2$, acetone, 30 min, rt, 87%.



**Scheme 4.** Reagents and conditions: (a) NaOH, MeOH, 4 h, reflux, 90%; (b) DMSO, 4 h, 150 °C, 59%.

## Results and Discussion

PEN was selected for phase I clinical trials based on its unique activity against a number of human and mouse tumors. 4-DMPEN, a major plasma metabolite of PEN showed good activity in vivo against the MX-1 human breast tumor xenograft. 6-DMPEN and DDM-PEN (3,5-dichloro-4,6-dihydroxy-2-(trichloromethyl) pyridine)[10] were subsequently synthesized and evaluated in vivo against MX-1 tumor, revealing inactivity for both structures. Previous studies from our laboratories had demonstrated the absolute dependence on the trichloromethyl substituent for antitumor activity in vivo. These results, coupled with microsomal studies[7] and our previous work[10,15] indicated that 4-DMPEN was on the metabolic activation pathway, and changes in the 4-position yielded more active compounds than PEN. We have proposed a mechanism of activation and action of 4-DMPEN and its acyl derivatives[14] in which these agents can be activated by free radical formation to an intermediate capable of alkylating tumor cell DNA to produce a DNA adduct, followed by crosslinking through the 5-chloro moiety, which can be activated via keto–enol tautomerism to produce an ∞-chloroketone moiety.

Based on these studies we have synthesized dechloro and demethoxy analogues of PEN and evaluated them in vivo against MX-1 human breast tumor xenograft in order to find a simpler pharmacophore for additional metabolism and SAR studies. The simplified analogues synthesized were 3-dechloro-PEN (**5**), 5-dechloro-PEN (**15**), 3-dechloro-6-demethoxy-PEN (**3**) and 5-dechloro-6-demethoxy-PEN (**9**). Although these structures could be activated similarly and yield a 2,5- or a 2,3-DNA crosslink, they did not demonstrate any activity as shown in Table 1. We also synthesized analogues of PEN in which sulfur replaced oxygen to determine whether changing to sulfur would yield better activity, but these congeners were similarly inactive. As a part of our SAR studies on PEN, pyrimidine analogues of PEN and 4-DMPEN were designed, synthesized and evaluated in vivo against MX-1 tumor with the hope of identifying other active ring structures, but neither was active. These studies revealed that the original, total structure of PEN is necessary for antitumor activity and represents the minimum pharmacophore, with only 4-demethylation being allowable for retention of activity.

**Table 1.** Response of subcutaneously-implanted MX-1 mammary tumor to treatment with PEN, 3-dechloro-PEN (**5**), 3-dechloro-6-demethoxy-PEN (**3**), 5-dechloro-PEN (**15**), and 5-dechloro-6-demethoxy-PEN (**9**)

| Treatment | | | | | Tumor regression | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Group no. | Agent | Dose (mg/kg) | Route | Schedule | Non specific deaths/total | Partial | Complete | Tumor free survival/total | Days to two doublings | Days delay (T-C) |
| 1 | Control | | IP | Q 1DX 5 Day 13 | | | | 0/10 | 7.0 | |
| 2 | PEN | 135 | IP | Q 1DX 5 Day 13 | 0/5 | 0 | 5 | 5/5 | >44.0 | >37.0 |
| 3 | PEN | 90 | IP | Q 1DX 5 Day 13 | 0/5 | 2 | 3 | 3/5 | >44.0 | >37.0 |
| 4 | PEN | 60 | IP | Q 1DX 5 Day 13 | 0/5 | 1 | 4 | 4/5 | >44.0 | >37.0 |
| 5 | 3-Dechloro-PEN | 135 | IP | Q 1DX 5 Day 13 | 0/5 | 0 | 0 | 0/5 | 8.2 | 1.2 |
| 6 | 3-Dechloro-PEN | 90 | IP | Q 1DX 5 Day 13 | 0/5 | 0 | 0 | 0/5 | 6.0 | −1.0 |
| 7 | 3-Dechloro-PEN | 60 | IP | Q 1DX 5 Day 13 | 0/5 | 0 | 0 | 0/5 | 6.2 | −0.9 |
| 8 | 3-Dechloro-6-Demethoxy-PEN | 135 | IP | Q 1DX 5 Day 13 | 0/5 | 0 | 0 | 0/5 | 6.6 | −0.4 |
| 9 | 3-Dechloro-6-Demethoxy-PEN | 90 | IP | Q 1DX 5 Day 13 | 0/5 | 0 | 0 | 0/5 | 8.2 | 1.2 |
| 10 | 3-Dechloro-6-Demethoxy-PEN | 60 | IP | Q 1DX 5 Day 13 | 0/5 | 0 | 0 | 0/5 | 8.0 | 1.0 |
| 11 | 5-Dechloro-PEN | 135 | IP | Q 1DX 5 Day 14 | 0/5 | 0 | 0 | 0/5 | 8.0 | 0.4 |
| 12 | 5-Dechloro-PEN | 90 | IP | Q 1DX 5 Day 14 | 0/5 | 0 | 0 | 0/5 | 9.5 | 1.9 |
| 13 | 5-Dechloro-PEN | 60 | IP | Q 1DX 5 Day 14 | 0/5 | 0 | 0 | 0/5 | 6.7 | −0.9 |
| 14 | 5-Dechloro-6-Demethoxy-PEN | 135 | IP | Q 1DX 5 Day 14 | 0/5 | 0 | 0 | 0/5 | 8.1 | 0.5 |
| 15 | 5-Dechloro-6-Demethoxy-PEN | 90 | IP | Q 1DX 5 Day 14 | 0/5 | 0 | 0 | 0/5 | 8.5 | 0.9 |
| 16 | 5-Dechloro-6-Demethoxy-PEN | 60 | IP | Q 1DX 5 Day 14 | 0/5 | 0 | 0 | 0/5 | 8.1 | 0.5 |

## Experimental

### Chemical synthesis

**General procedure.** TLC analysis was performed on Analtech precoated (250 μm) silica gel GF plates, and components were detected by UV light. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Purifications by flash chromatography were carried out on Merck silica gel (230–400 mesh). The mass spectral data were obtained with a Varian-MAT 311A mass spectrometer in the fast atom bombardment (FAB) mode. $^1$H NMR spectra were recorded on a Nicolet NT-300 NB spectrometer operating at 300.635 MHz. Chemical shifts are expressed in parts per million downfield from tetramethylsilane, and chemical shifts (δ) listed for multiplets were measured from the approximate centers; relative integrals of peak areas agreed with those expected for the assigned structures. Where analyses are indicated only by element symbols, analytical results obtained for those elements were within ±0.4% of the theoretical values, and where solvents were indicated in the formula, their presence was confirmed by $^1$H NMR. HPLC analyses were carried out on a Hewlett-Packard HP1084B liquid chromatograph with a Waters Associates μBondapak $C_{18}$ column (3.9 mm×30 cm) and UV monitoring (254 nm).

**4,5 Dichloro-2-methyl-pyridine (1).** This compound was prepared by a reported method.[11,12c]

**4,5-Dichloro-2-(trichloromethyl) pyridine (2).** A slow stream of $Cl_2$ gas was passed through a refluxing solution of **1** (290 mg, 1.78 mmol) in $CCl_4$ (50 mL) illuminated with a 500 W incandescent lamp for 2 h. Evaporation of the solvent afforded a residue which was chromatographed on a column (hexanes:dichloromethane 8:2 as eluent) to afford **2**: yield 322 mg (67.78%); mp 27–28 °C; MS *m*/*z* 264 $(M+H)^+$; $^1$H NMR ($CDCl_3$) δ 8.12 (s, 1H, 3-H), 8.68 (s, 1H, 6-H). Anal. calcd for $C_6H_2Cl_5N$: C, 27.15; H, 0.76; N, 5.27. Found: C, 27.29; H, 0.73; N, 5.12.

**5-Chloro-4-methoxy-2-(trichloromethyl) pyridine (3-dechloro-6-demethoxy-PEN) (3).** In a round bottomed flask were placed compound **2** (315 mg, 1.18 mmol) and sodium hydroxide (47 mg, 1.18 mmol). To this mixture was added 10 mL of anhydrous methanol, and the reaction mixture was heated to reflux for 2.5 h and concentrated to dryness. The residue was extracted with dichloromethane (2×20 mL). The organic layer was washed with water (2×20 mL), dried over $MgSO_4$ and concentrated to dryness. The residual oil was purified by column chromatography. The column (packed with silica gel 230–400 mesh) was eluted with 7:3 hexanes:dichloromethane. Desired fractions identified by TLC analysis were collected, concentrated and dried in vacuo over $P_2O_5$: yield 294 mg (95.14%); mp 49–51 °C; MS *m*/*z* 260 $(M+H)^+$; $^1$H NMR ($CDCl_3$) δ 4.05 (s, 3H, $OCH_3$), 7.57 (s, 1H, 3-H), 8.50 (s, 1H, 6-H). Anal. calcd for $C_7H_5Cl_4NO$: C, 32.22; H, 1.93; N, 5.36. Found: C, 32.26; H, 1.96; N, 5.28.

**4,5,6-Trichloro-2-(trichloromethyl) pyridine (4).** This compound was prepared the same way as described for **2** except the solution was refluxed for 18 h using **1** (920 mg, 5.67 mmol). After column chromatography (hexanes) a solid was obtained: yield 1.30 g (76.7%); mp 92–94 °C; MS *m*/*z* 298 $(M+H)^+$; $^1$H NMR ($CDCl_3$) δ 8.05 (s, 1H, 3-H). Anal. calcd for $C_6H_1Cl_6N$: C, 24.03; H, 0.33; N, 4.67. Found: C, 24.01; H, 0.30; N, 4.62.

**5 - Chloro - 4,6 - dimethoxy - 2 - (trichloromethyl) pyridine (3-dechloro-PEN) (5).** The same procedure was used as described earlier for the preparation of **3** using **4** (600 mg, 2.00 mmol) and NaOH (280 mg, 7.00 mmol). In this case the solution was refluxed for 2.5 days. After column chromatography (elution with 9:1 hexanes:di-

chloromethane): yield 338 mg (58.1%); mp 92–94 °C; MS $m/z$ 290 $(M+H)^+$; $^1H$ NMR ($CDCl_3$) δ 4.02 (s, 3H, 6-$OCH_3$), 4.07 (s, 3H, 4-$OCH_3$), 7.27 (s, 1H, 3-H). Anal. calcd for $C_8H_7Cl_4NO_2$: C, 33.02; H, 2.42; N, 4.81. Found: C, 33.17; H, 2.50; N, 4.82.

**5,6-Dichloro-4-methoxy-2-(trichloromethyl) pyridine (6).** This compound was prepared from **4** (180 mg, 0.60 mmol) and sodium hydroxide (24 mg, 0.60 mmol) as reported earlier for **3**, but in this case the reaction mixture was stirred at room temperature overnight. The compound was purified by column chromatography (9:1 hexanes:dichloromethane): yield 164 mg (92.65%); mp 88–90 °C; MS $m/z$ 294 $(M+H)^+$; $^1H$ NMR ($CDCl_3$) δ 4.07 (s, 3H, $OCH_3$), 7.51 (s, 1H, 3-H). Anal. calcd for $C_7H_4Cl_5NO$: C, 28.46; H, 1.36; N, 4.74. Found: C, 28.78; H, 1.26; N, 4.57.

**3,4-Dichloro-2-methyl-pyridine (7).** This compound was prepared by a reported method.[11]

**3,4-Dichloro-2-(trichloromethyl) pyridine (8).** The general procedure previously described for **2** was used to prepare **8** from **7** (1.00 g, 6.17 mmol). Refluxing time was 1.5 h. Purification was accomplished by column chromatography using hexanes as eluent: yield 1.18 g (72.4%); mp 50–52 °C; MS $m/z$ 264 $(M+H)^+$. Anal. calcd for $C_6H_2Cl_5N$: C, 27.15; H, 0.76; N, 5.27. Found: C, 27.11; H, 0.82; N, 5.16.

**3-Chloro-4-methoxy-2-(trichloromethyl) pyridine (5-dechloro-6-demethoxy-PEN) (9).** The procedure was the same as reported above for the preparation of **3** using **8** (1.18 g, 4.4 mmol). After column chromatography with 8:2 hexanes:dichloromethane: yield 900 mg (77.5%); mp 172–174 °C; MS $m/z$ 260 $(M+H)^+$; $^1H$ NMR ($CDCl_3$) δ 4.02 (s, 3H, $OCH_3$), 6.99 (d, 1H, 5-H), 8.41 (d, 1H, 6-H). Anal. calcd for $C_7H_5Cl_4NO$: C, 32.22; H, 1.93; N, 5.36. Found: C, 32.02; H, 1.85; N, 5.14.

**3-Chloro-4-hydroxy-2-(trichloromethyl) pyridine (10).** Compound **9** (1.94 g, 7.43 mmol) was dissolved in 20 mL of anhydrous DMSO, and the reaction mixture was heated at 150 °C for 5 h. The reaction did not go to completion and was getting dark. The reaction mixture was cooled and lyophilized, and the residue was purified by column chromatography (95:5 chloroform:methanol). The desired fractions were evaporated to dryness. The compound was dried in vacuo over $P_2O_5$ giving a syrup: yield 465 mg (25.40%); MS $m/z$ 246 $(M+H)^+$; $^1H$ NMR ($CDCl_3$) δ 7.15 (d, 1H, 5-H), 8.26 (d, 1H, 6-H), 12.65 (bs, 1H, OH); HPLC 99%, 95:5 ($H_2O$/MeCN). Anal. calcd for $C_6H_3Cl_4NO$: C, 29.19; H, 1.22; N, 5.67. Found: C, 28.96; H, 1.25; N, 5.78.

**3-Chloro-4-mercapto-2-(trichloromethyl) pyridine (11).** To compound **8** (2.00 g, 7.53 mmol) was added a freshly prepared solution of NaSH (20 mL), and the reaction mixture was stirred for 12 h at room temperature. The solution was poured into ice-water and acidified with concd HCl. The solid was filtered, washed with cold water and dried in vacuo. This crude product was purified by column chromatography, first eluted with 98:2 chloroform:methanol and then with 1:1 hexanes: dichloromethane. The desired fractions were collected, combined, concentrated and dried in vacuo over $P_2O_5$, yielding a white solid 416 mg (21.01%); mp 73–75 °C; MS $m/z$ 262 $(M+H)^+$; $^1H$ NMR ($CDCl_3$) δ 4.28 (s, 1H, SH), 7.39 (d, 1H, 5-H), 8.26 (d, 1H, 6-H). Anal. calcd for $C_6H_3Cl_4NS$: C, 27.40; H, 1.15; N, 5.32. Found: C, 27.62; H, 1.24; N, 5.38.

**3-Chloro-4-methylmercapto-2-(trichloromethyl) pyridine (12).** To a solution of **11** (416 mg, 1.58 mmol) in 10 mL of anhydrous acetone was added trimethylsilyldiazomethane (2.0 M solution in hexanes, 0.2 mL), and the reaction mixture was stirred for 30 min at room temperature. The solution was concentrated to dryness and the residue was purified by column chromatography. The column was eluted with 6:4 hexanes:dichloromethane. Desired fractions were combined, concentrated and dried in vacuo: yield 365 mg (83.33%); mp 108–110 °C; MS $m/z$ 262 $(M+H)^+$; $^1H$ NMR ($CDCl_3$) δ 2.54 (s, 3H, $SCH_3$), 7.17 (d, 1H, 5-H), 8.39 (d, 1H, 6-H). Anal. calcd for $C_7H_5Cl_4NS{\cdot}0.1C_2H_5OH$: C, 30.82; H, 2.01; N, 4.99. Found: C, 30.99; H, 2.11; N, 4.91.

**3,4,6-Trichloro-2-(trichloromethyl) pyridine (13).** The same procedure previously described for **2** was used to prepare **13** from **7** (560 mg, 3.45 mmol), and the reaction mixture was refluxed for 24 h. After column chromatography with hexanes, an oil was obtained that solidified in the freezer: yield 901 mg (87.5%); mp 80–82 °C; MS $m/z$ 298 $(M+H)^+$; $^1H$ NMR ($CDCl_3$) δ 7.62 (s, 1H, 5-H). Anal. calcd for $C_6H_1Cl_6N$: C, 24.03; H, 0.33; N, 4.67. Found: C, 23.97; H, 0.50; N, 4.89.

**3,6-Dichloro-4-methoxy-2-(trichloromethyl) pyridine (14).** Compound **14** was prepared from **13** (815 mg, 2.71 mmol) as reported for **3**. The product was purified by column chromatography (hexanes): yield 740 mg (93.67%); mp 194–196 °C; MS $m/z$ 294 $(M+H)^+$; $^1H$ NMR ($CDCl_3$) δ 4.03 (s, 3H, $OCH_3$), 6.99 (s, 1H, 5-H). Anal. calcd for $C_7H_4Cl_5NO$: C, 28.45; H, 1.36; N, 4.74. Found: C, 28.75; H, 1.38; N, 4.79.

**3 - Chloro - 4,6 - dimethoxy - 2 - (trichloromethyl) pyridine (5-dechloro-PEN) (15).** This compound was prepared from **14** (1.08 g, 3.65 mmol) and NaOH (438 mg, 10.95 mmol) by refluxing for 48 h using the procedure described for the preparation of **3**. Yield after column chromatography (8:2 hexanes:dichloromethane) 286 mg (26.98%); mp 180–182 °C; MS $m/z$ 290 $(M+H)^+$; $^1H$ NMR ($CDCl_3$) δ 3.95 (s, 3H, 6-$OCH_3$), 3.97 (s, 3H, 4-$OCH_3$), 6.35 (s, 1H, 5-H). Anal. calcd for $C_8H_7Cl_4NO_2$: C, 33.02; H, 2.42; N, 4.81. Found: C, 32.95; H, 2.51; N, 4.96.

**3-Chloro-4-hydroxy-6-methoxy-2-(trichloromethyl) pyridine (16).** To a solution of **15** (620 mg, 2.13 mmol) in anhydrous dichloromethane (20 mL) was added 620 mg of $AlCl_3$, and the reaction mixture was stirred for 3 h at room temperature. Starting material was still present. Since decomposition was occurring, the reaction mixture was concentrated to dryness. The residue was puri-

fied by column chromatography (chloroform) to give **16** as a solid: yield 150 mg (25.42%); mp 206–208 °C; MS *m/z* 276 $(M+H)^+$; $^1H$ NMR ($CDCl_3$) δ 3.95 (s, 3H, $OCH_3$), 6.21 (s, 1H, 5-H), 8.50 (s, 1H, OH). Anal. calcd for $C_7H_5Cl_4NO_2$: C, 30.36; H, 1.82; N, 5.05. Found: C, 30.53; H, 1.86; N, 4.96.

**3,6-Dichloro-4-mercapto-2-(trichloromethyl) pyridine (17).** Following the procedure for **11**, compound **13** (3.39 g, 11.30 mmol) and a solution of NaSH (40 mL) were stirred for 5 h to give **17**, which on purification by column chromatography (first with 98:2 chloroform:methanol and then with 1:1 hexanes:dichloromethane) yielded 1.48 g (44.04%); mp 65–67 °C; MS *m/z* 295 $(M+H)^+$; $^1H$ NMR ($CDCl_3$) δ 4.32 (s, 1H, SH), 7.44 (s, 1H, 5-H); HPLC 95%, 9:1 MeCN/$H_2O$. Anal. calcd for $C_6H_2Cl_5NS$: C, 24.23; H, 0.67; N, 4.70. Found: C, 24.29; H, 0.67; N, 4.65.

**3,6-Dichloro-4-methylmercapto-2-(trichloromethyl) pyridine (18).** The same procedure as described for the preparation of **12** was used to prepare **18** from **17** (493 mg, 1.65 mmol). After column chromatography (6:4 hexanes: dichloromethane), a solid was obtained: yield 265 mg (51.35%); mp 175–177 °C; MS *m/z* 310 $(M+H)^+$; $^1H$ NMR ($CDCl_3$) δ 2.54 (s, 3H, $SCH_3$), 7.13 (s, 1H, 5-H). Anal. calcd for $C_7H_4Cl_5NS$: C, 26.99; H, 1.29; N, 4.49. Found: C, 27.10; H, 1.26; N, 4.48.

**3,4,5,6-Tetrachloro-2-(trichloromethyl) pyridine (19).** This compound was synthesized from pentachloropyridine as reported in the literature.[12]

**3,5,6-Trichloro-4-mercapto-2-(trichloromethyl) pyridine (20).** This compound was prepared in good yield by the same procedure as reported above for **11** but starting from **19** (1.06 g, 3.17 mmol) and a solution of NaSH (15 mL), affording material (972 mg, 92.57%) suitable for the next step: MS *m/z* 330 $(M+H)^+$; $^1H$ NMR ($CDCl_3$) δ 7.56 (s, 1H, SH). Anal. calcd for $C_6H_1Cl_6NS$: C, 21.71; H, 0.30; N, 4.22. Found: C, 21.95; H, 0.39; N, 4.36.

**3,5,6-Trichloro-4-methylmercapto-2-(trichloromethyl) pyridine (21).** Treatment of **20** (1.4 g, 4.21 mmol) with trimethylsilyldiazomethane as reported above for **12** provided the desired product **21**. Purification of **21** was accomplished by column chromatography (hexanes): yield 1.27 g (86.7%); mp 61–63 °C; MS *m/z* 344 $(M+H)^+$; $^1H$ NMR ($CDCl_3$) δ 2.62 (s, 3H, $SCH_3$). Anal. calcd for $C_7H_3Cl_6NS$: C, 24.30; H, 0.87; N, 4.04. Found: C, 24.27; H, 0.88; N, 3.96.

**4,5,6-Trichloro-2-(trichloromethyl) pyrimidine (22).** This compound was prepared by a reported method.[13]

**5-Chloro-4,6-dimethoxy-2-(trichloromethyl) pyrimidine (23).** The same procedure previously described for **3** was used to prepare **23** from **22** (1.5 g, 4.98 mmol) and NaOH (490 mg, 12.25 mmol). The reaction mixture was refluxed for 4 h. After column chromatography (hexanes), a white solid was obtained: yield 1.31 g (90.34%); mp 100–102 °C; MS *m/z* 291 $(M+H)^+$; $^1H$ NMR ($CDCl_3$) δ 4.13 (s, 6H, 4,6-$OCH_3$). Anal. calcd for $C_7H_6Cl_4N_2O_2$: C, 28.79; H, 2.07; N, 9.59. Found: C, 28.79; H, 2.15; N, 9.75.

**5-Chloro-4-hydroxy-6-methoxy-2-(trichloromethyl) pyrimidine (24).** Compound **24** was prepared from **23** (180 mg, 0.61 mmol) over a period of 4 h using the procedure described for the preparation of **10**. Yield after column chromatography (first with chloroform and then with 7:1 chloroform:methanol) 100 mg (58.82%); mp 100–102 °C; MS *m/z* 277 $(M+H)^+$; $^1H$ NMR ($CDCl_3$) δ 4.04 (s, 3H, 6-$OCH_3$); HPLC 98.5%, 6:4 $NH_4H_2PO_4$ (0.01 M)-MeOH. Anal. calcd for $C_6H_4Cl_4N_2O_2 \cdot 1.0$ $CH_3OH$: C, 27.12; H, 2.60; N, 9.03. Found: C, 27.38; H, 2.45; N, 8.80.

## Biological evaluation

**Antitumor evaluation in vivo.** Athymic NCr-nu/nu mice were obtained from various suppliers under contract with NCI and housed in sterile, filtered-capped microisolater cages in a barrier facility. Human MX-1 breast tumor was obtained from the NCI Tumor Repository (Frederick, Md.). For intraperitoneal (ip) injection into mice, the analogues were prepared as a suspension in aqueous hydroxypropyl cellulose. For sc implants, tumor fragments (30–40 mg) from in vivo passage were implanted into the axillary region of the mice.

Treatment of groups of five 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 (T-C, i.e., the difference in the median time poststaging for tumors of the treated (T) group to double twice in mass compared to the median of the control (C) group), tumor regression (partial and complete), and tumor-free survivors, and experiments were terminated when the control tumors attained a mass of 1 g, which was typically 57–61 days.

## Acknowledgements

This investigation was supported by NIH grant No. CA34200. We thank Dr. James M. Riordan, Mark D. Richardson and Joan C. Bearden of the Molecular Spectroscopy Section of Southern Research Institute for elemental and spectroscopic determinations.

## References and Notes

1. Tobol, H. K. U.S. Patent 4 717 726, 1988; *Chem. Abstr.* **1988**, *108*, 204507.
2. Harrison, S. D., Jr.; Plowman, J.; Dykes, D. J.; Waud, W. R.; Griswold, D. P., Jr. *Cancer Res.* **1991**, *51*, 1979.
3. Plowman, J.; Harrison, S. D., Jr.; Dykes, D. J.; Paull, K. D.; Narayanan, V.; Tobol, H. K.; Griswold, D. P. *Cancer Res.* **1989**, *49*, 1909.

4. Berlin, J.; Stewart, J.; Tutsch, K.; Stover, V.; Arzoomanian, R.; Alberti, D.; Feierabend, C.; Wilding, G. *Proc. Am. Assoc. Cancer Res*. **1995**, *36*, 238.
5. Jodrell, D. I.; Bowman, A.; Stewart, M.; Dunlop, N.; French, R.; MacLellan, A.; Cummings, J.; Smyth, J. F. *Br. J. Cancer* **1998**, *77*, 808.
6. O'Reily, S.; Grochow, L. B.; Donehower, R. C.; Chan, T. L.; Bowling, K.; Hartman, N. R.; Struck, R. F.; Rowinsky, E. K. *J. Clin. Oncol*. **1997**, *15*, 1974.
7. Reid, J. M.; Mathiesen, D. A.; Benson, L. M.; Kuffel, M. J.; Ames, M. M. *Cancer Res*. **1992**, *52*, 2830.
8. Benvenuto, J. A.; Hittelman, W. N.; Zwelling, L. A.; Plunkett, W.; Pandita, T. K.; Farquhar, D.; Newman, R. A. *Biochem. Pharmacol*. **1995**, *50*, 1157.
9. Hartman, N. R.; O'Reilly, S.; Rowinsky, E. K.; Collins, J. M.; Strong, J. M. *Clin. Cancer Res*. **1996**, *2*, 953.
10. Waud, W. R.; Tiwari, A.; Schmid, S. M.; Shih, T.; Strong, J. M.; Hartman, N. R.; O'Reilly, S.; Struck, R. F. *Cancer Res*. **1997**, *57*, 815.
11. Ife, R. J.; Dyke, C. A.; Keeling, D. J.; Meenan, E.; Meeson, M. L.; Parsons, M. E.; Price, C. A.; Theobald, C. J.; Underwood, A. H. *J. Med. Chem*. **1989**, *32*, 1970.
12. (a) Chivers, G. E.; Suschitzky, H. A. *J. Chem. Soc. D* **1971**, 28. (b) Suschitzky, H.; Binns, F. *J. Chem. Soc. C* **1971**, *7*, 1223. (c) Binns, F.; Suschitzky, H. *J. Chem. Soc. D* **1970**, 750. (d) Julia, L.; Riera, J.; Teixido, R. *J. Chem. Soc., Perkin Trans. 1* **1991**, 1101.
13. Beitzke, B.; Blank, H. L. (Bayer A.-G.) Ger. Offen. DE 3,431,698 (Cl. C07D239/36), 13 March 1986, pp 16, Appl. 29 August 1984.
14. Struck, R. F.; Tiwari, A.; Friedman, H. S.; Morgan, L. R.; Waud, W. R. *Cancer Chemother. Pharmacol*. **2001**, *48*, 47.
15. Tiwari, A.; Riordan, J. M.; Waud, W. R.; Struck, R. F. *J. Med. Chem*. **2001**, *45*, 1079.