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Synthesis of pochoxime prodrugs as potent HSP90 inhibitors

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ABSTRACT

Pochoximes are potent inhibitors of heat shock protein 90 (HSP90) based on the radicicol pharmacophores. Herein we present a pharmacokinetics and pharmacodynamics evaluation of this compound series as well as a phosphate prodrug strategy to facilitate formulation and improve oral bioavailability.

The heat shock protein 90 (HSP90) has emerged as an extremely interesting therapeutic target in recent years.[1–3] Despite the seemingly ubiquitous functions of this highly expressed chaperone, its role in stabilizing conformationally labile proteins has implications in incredibly diverse pathologies. Inhibitors of HSP90 have been shown to be broadly effective for a number of cancer indications,[4,5] neurodegenerative diseases,[6–10] infectious diseases,[11] and inflammation-related disorders.[12]

Two natural products, radicicol and geldanamycin (**1** and **2**, Fig. 1), both of which disrupt the ATPase activity of Hsp90, have been instrumental in understanding the role of HSP90 in oncogenic processes and the therapeutic potential of its inhibition.[13–15] However, neither natural product has acceptable pharmacological properties for clinical application. Medicinal chemistry efforts have led to the discovery of novel scaffolds such as purines[16] and oxazoles[17–19] carbazolone[19] which are currently in clinical or preclinical development[5] however, improving the pharmacological properties and potency of the natural pharmacophores remains important. Indeed, the most advanced clinical candidate is a semi synthetic derivative of geldanamycin, 17AAG (**3**, Fig. 1).[20,21] Another semi synthetic derivative with a dimethoxyhydroquinone functionality has recently been reported to have better pharmacological properties than 17AAG while acting as a prodrug.[22]

We had previously shown[23] that pochonin D represents a simplified pharmacophore of radicicol which recapitulates its activity. Furthermore, significant improvements in cellular efficacy could be achieved through the formation of oximes.[24] In fact, pochoxime A–C, are amongst the most potent HSP90 inhibitors reported to date, inducing client protein degradation in SKBR3 cell lines at low nM concentration, and pochoxime A treatment leads to tumor regression in xenografts bearing BT474 breast tumor cells.

Further in depth analysis of pochoxime A's pharmacokinetics properties in mice demonstrated that while it was fairly rapidly cleared from plasma ($t_{1/2}$ 2.5 h), it accumulated in tumors and therapeutic concentration was maintained beyond 48 h following intraperitoneal administration (Table 1). As shown in Figure 2, a single dose was sufficient to achieve induction of Hsp90 client protein degradation, consistent with the observation that biweekly treatment was sufficient in reducing tumor growth.[24] A therapeutic limitation of 17AAG is its induction of hepatotoxicity which limits the dose schedule. To evaluate whether pochoxime A also induces liver toxicity, mice treated with 100 mg/kg of pochoxime A were compared to mice treated with the same dose of 17AAG three times per week for four weeks. While high levels of ALT (alanine aminotransferase) and AST (aspartate aminotransferase) were observed in the 17AAG treated group, the respective enzyme levels in the pochoxime A treated group were not elevated relative to (or, were similar to those in) the untreated group.

Pochoxime A–C exhibited very similar anti-proliferative activity, demonstrating effectiveness across a broad range of cancer cell lines (Table 2) including breast cancer (BT474 and MDA-MB468), leukemia (K562 CML and MV4; 11 AML), colon (HCT116), prostate cancer (PC-3), Tarceva- and Iressa-resistant non-small cell lung cancer (NSCLC, HCC829 and H1975), gastric cancer (N87) as well as glioblastoma (A172) cell lines. Interestingly, the pochoximes

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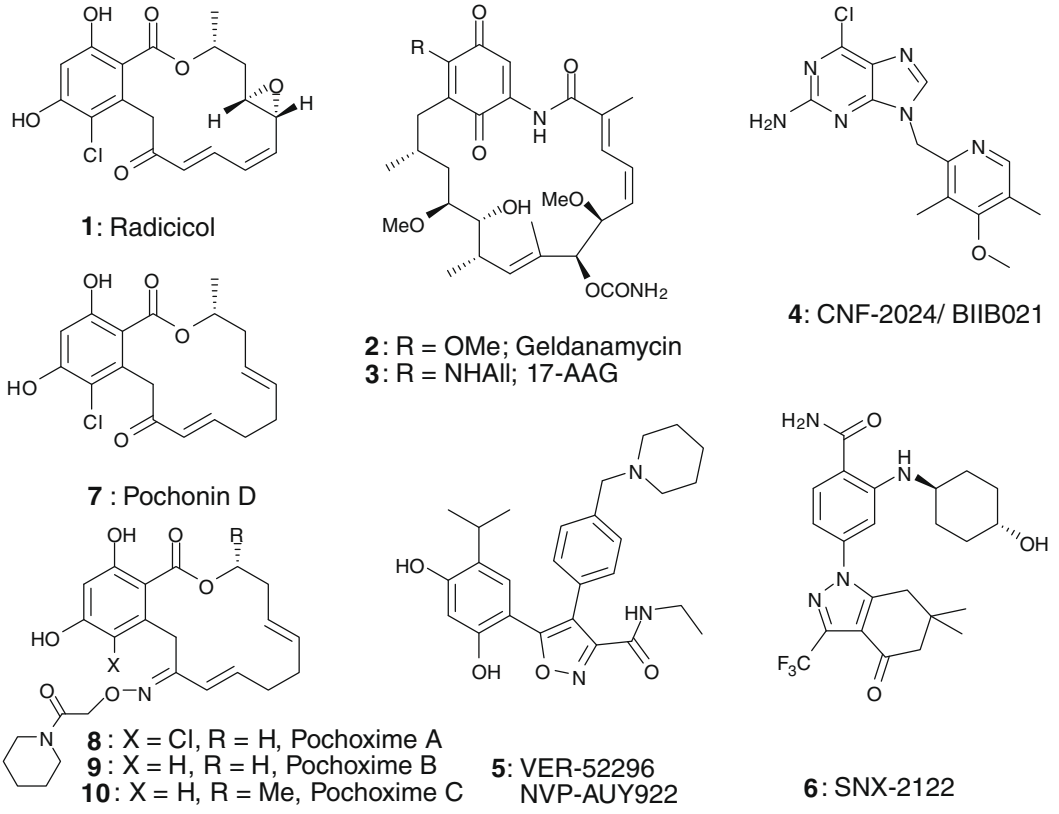


Figure 1. HSP90 inhibitors.

Table 1
Concentration of pochoxime A within tumor (BT474 xenoxraft) following IP injection

Time (h)	100 mg/kg	150 mg/kg	200 mg/kg
Pochoxime a concentration (ng/g) within tumor			
6	5491.7	6175.0	6525.0
12	5250.0	6500.0	8925.0
24	4025.0	7933.3	10,150.0
48	1497.5	4583.3	4025.0
AUC[a](0–48 h)	171,230	293,751	351041
AUC[a]/dose	1712	1958	1755

[a] AUC (area under time–concentration curve).

maintained anti-proliferative activity against MDA-MB468 which was significantly less sensitive to 17-AAG (**3**).

However, pochoxime A and related analogues have poor water solubility which limits the choices of formulations, and while pochoxime A has good bioavailability when administered intraperitoneally (F% = 60.5), its oral bioavailability in the tested formulation (10% DMSO, 9% PEG 400, 72% plurol oleate #497, 9% lauroglycol, and 0.09% Tween20) was insufficient (F% = 3.4). Closely related pochoxime B and C were found to have similar profiles of bioavailability (data not shown). An established strategy to improve solubility is the introduction of a phosphate group on a hydroxyl or phenol functionality.[25,26] The polar nature of this functionality makes the resulting prodrug more soluble enabling fast dissolution thus facilitating IV or oral formulation. For oral delivery, the high level of alkaline phosphatase on enterocytes lining the gastrointestinal track convert the prodrug to the drug while hepatic phosphatase or even phosphatase present in the target tissue can convert the prodrug when administered IV. Fosamprenavir (Fig. 3, HIV protease inhibitor), etoposide and fosphenytoin are prominent examples of alkoxyl, phenolic or methyloxyphosphate prodrugs respectively.[25] Many phosphate prodrugs are also currently in clinical trials.[27]

As the bioactive pochoximes bear two phenols, the structure lends itself to the application of a phosphate prodrug strategy. In the course of scaling up the synthesis of the pochoxime A or B macrocycle via the previously developed synthetic route[28] based on a ring closing metathesis (RCM), we noted contamination with small amounts of the macrocycle containing the *cis* alkene produced by RCM. We thus investigated an alternative strategy relying on a Mitsunobu macrolactonization with the geometry of the aforementioned alkene fixed. As shown in Scheme 1, esters **11**[24] were deprotonated with LDA and reacted with Weinreb amide **19** to afford, after oxime formation, the macrocycles **13**. Global silyl deprotection followed by macrocyclization yielded pochoxime A and B upon deprotection with sulfonic acid resin. While the compounds were obtained as an *E/Z* mixture of the oxime, the more active E isomer could be isolated by recrystallization to greater than 90% purity or as a single isomer by HPLC purification. The Weinreb amide **19** was obtained from the alkene **15** containing the required *trans* alkene via a sequence based on well established chemistry. Pochoxime A and B were thus obtained in 17% and 25% yield from **11a** and **b**, respectively. These syntheses could readily be scaled up to prepare over 10 g of the pochoxime A or B.

We next focused on the preparation of different permutations of phosphorylated pochoximes with a single phosphate on the *para* or *ortho* phenol, on both phenols or a phosphonooxymethyl on the *para* phenol. As shown in Scheme 2, owing to the greater acidity of the *para* phenol, pochoxime A–C could be selectively derivatized

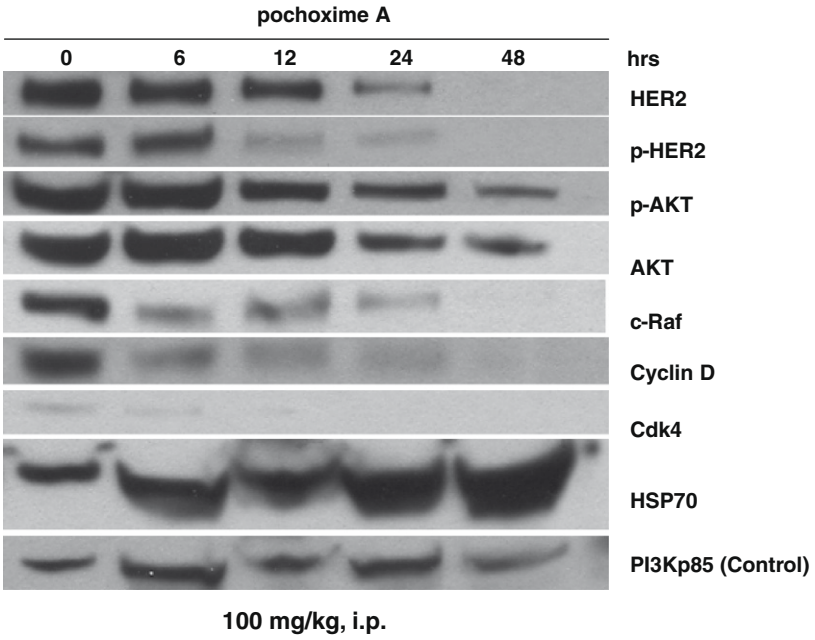


Figure 2. Western blot analysis of HSP90 clients proteins in vivo following a single dose of pochoxime A (100 mg/kg).

Table 2
Growth inhibition and HSP90α affinity of pochoxime derivatives[a]

Cell line	Pochoxime A (**8**)	Pochoxime B (**9**)	Pochoxime C (**10**)	17AAG (**3**)
Growth inhibition (GI_{50}) and HSP90 affinity of pochoxime derivative (nM)				
BT474 (breast)	7	2	6	5
MDA231 (breast)	7	2	6	NM
MDA-MB468 (breast)	9	2	6	780
N87 (gastric)	4	1	2	1
K562 (leukemia)	6	4	7	48
MV4;11 (leukemia)	3	2	3	11
HCT116 (colon)	9	11	NM	NM
HCC827 (lung)	31	NM	NM	56
H1975 (lung)	25	NM	NM	35
A172 (glioblastoma)	42	NM	NM	NM
HSP90α affinity	*21*	*15*	*18*	*32*

[a] The reported values are the average of three experiments performed in duplicates. Standard deviation for the assay was less than twofold for 2σ.

at that position using 1.0 equiv of bis(dimethylamino)phosphoryl chloride and 0.9 equiv of DBU. Hydrolysis of the phosphoramide bond with wet TFA in dichloromethane followed by neutralization using anion exchange resin (Amberlite) afforded the pochoxime derivative **21**–**23** in excellent yield. To access the *para* phosphate, the *ortho* phenol was first selectively protected with an EOM group followed by the same methodology to introduce the phosphate thus yielding pochoxime A derivative **24**. The bis-phosphate derivative **25** was prepared in the same way as the *ortho*-substitued analogue but using an excess of bis(dimethylamino)phosphoryl chloride and two equivalents of DBU. Finally, the phosphonooxymethyl derivative **26** could be accessed by the same methodology using di-*tert*-butyl chloromethylphosphonate[29] rather than bis(dimethylamino)phosphoryl chloride. In all cases, the acidic hydrolysis of the phosphoramide or *tert*-butyl deprotection resulted in an isomerization of the oxime (ca. 1:1 *E/Z*).

A single phosphate group on the pochoxime conferred an aqueous solubility of ⩾80 mg/ml while the bis phosphate analogue was soluble at >100 mg/mL (higher concentration not tested). When tested for their affinity to human HSP90α,[30] none of the prodrugs **21**–**26** showed any measurable affinity below 10 μM. This is not surprising considering that both phenols are important for binding to HSP90. Interestingly, pro-pochoxime A **21** showed comparable efficacy in depleting HSP90 client proteins to pochoxime A (data not shown) and had similar cytotoxicity against different cancer cell lines (Table 3), suggesting that these cancer cell lines have sufficient extracellular phosphatases to achieve the conversion. While the cytotoxicity values were nearly twofold higher than for pochoxime A, they were consistent with the fact that pochoxime A (**8**) is a single oxime isomer while pro-pochoxime A **21** is a mixture of the two oximes. We then evaluated the efficacy of prodrug conversion in vitro using tissue homogenates and a mimic of gastric fluid (pepsin solution at pH 1.1). As shown in Table 4, marginal conversion occurred in the gastric fluid and plasma, while significant conversion was afforded by both the liver and intestine homogenates. Interestingly, pro-pochoxime C was more efficiently converted than pro-pochoxime B and A with the best results for the bis-phosphate **25** reaching 43.5% conversion with intestine homogenate in 60 min. This data suggest that pro-pochoxime **25** would be the most suitable prodrug candidate and preliminary investigations in mice showed that high concentration in the liver was achieved via intravenous or oral administration (C_{max}: 3 838 ng/g by IV (4 mg/kg) and 12 469 ng/g by oral (30 mg/kg)).

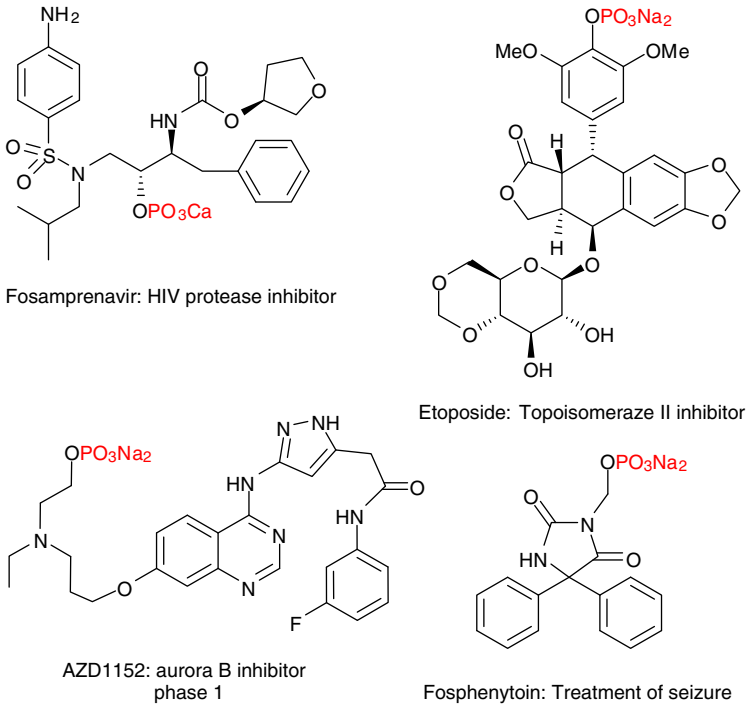
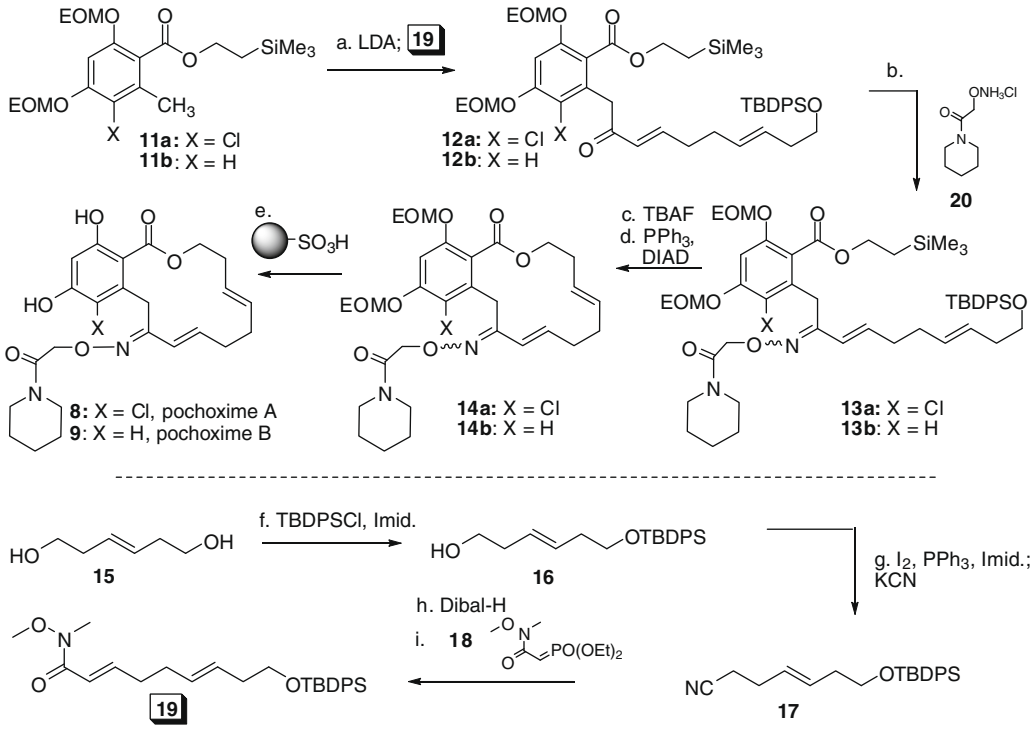
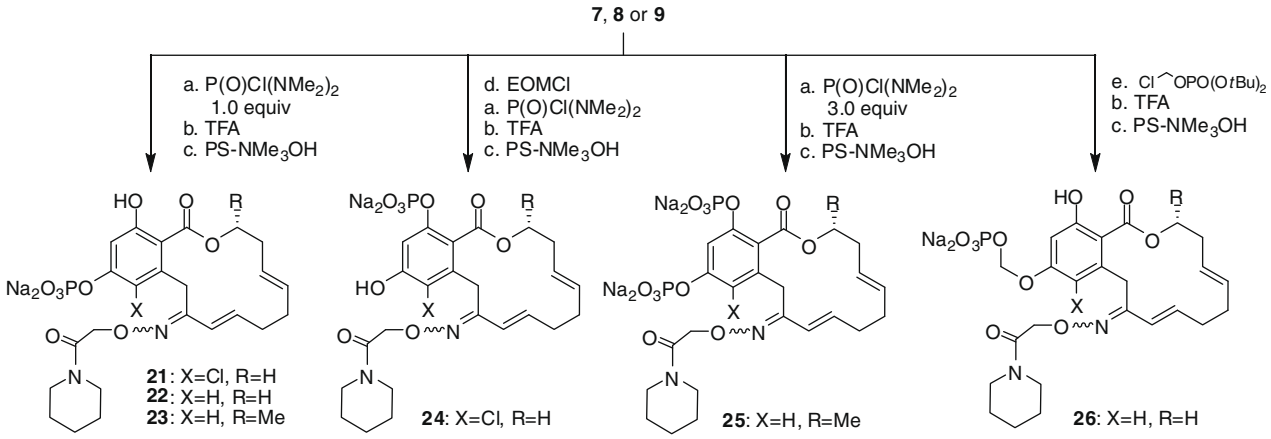


Figure 3. Selected examples of phosphate prodrugs in the market or in clinical trials.



Scheme 1. Altenative synthesis of pochoxime A and B. Reagents and conditions: (a) LDA (2.0 equiv), THF, −78 °C, 5 min; **19** (0.9 equiv), −78, 15 min, **12a** (65%) **12b** (54%); (b) **20** (2.5 equiv), pyridine, 40 °C, 24 h, **13a** (50%) **13b** (81%); (c) TBAF (2.5 equiv), 23 °C, 3 h; (d) Ph_3P (1.5 equiv), DIAD (1.5 equiv), toluene, 0–23 °C, 5 h, **14a** (60%) **14b** (61%); (e) PS-SO_3H (5.0 equiv, 3.0 mmol/g), MeOH, 23 °C, 2 h, **8** (90%) **9** (95%); (f) TBDPSCl (1.0 equiv), Imid (1.5 equiv), DMF, 23 °C, 5 h, 85%; (g) Imid (1.5 equiv), Ph_3P (1.1 equiv), iodine (1.1 equiv), 0 °C, 6 h; KCN (5.0 equiv), DMSO, 60 °C, 2 h, >90%; (h) Dibal-H (1.05 equiv), CH_2Cl_2, −78 °C, 30 min, 85%; (i) LiCl (1.2 equiv), **18** (1.2 equiv), DBU (1.0 equiv), CH_3CN, 23 °C, 45 min, 80%; DBU = 1,8-Diazabicyclo[5.4.0]undec-7-ene; Dibal-H = diisobutylaluminium hydride Imid = imidazole PS = polystyrene, TBDPSCl = *tert*-Butyl diphenyl chlorosilane.

In conclusion, pochoxime A–C are potent HSP90 inhibitors effective against a broad range of cancer cell lines. Phosphorylation of a single or both phenols of the pochoximes confers good aqueous solubility thus facilitating formulation. While the phospho-pochoxi-



Scheme 2. Synthesis of pochoxime A–C prodrugs. Reagents and conditions: (a) $P(O)Cl(NMe_2)_2$ (1–3.0 equiv), DBU (0.9–2.0 equiv), DMAP (0.01 equiv), CH_2Cl_2, 23 °C, 12 h, 50–60%; (b) TFA (5%), CH_3CN/H_2O 50/50; 23 °C, quantitative; (c) Amberlite IRA68, H_2O/CH_3CN 1/1, quantitative (d) EOMCl (1.0 equiv), iPr_2EtN (1.0 equiv), CH_2Cl_2, 0–23 °C, 12 h, 49%; (e) di-*tert*-butyl chloromethylphosphonate (1.2 equiv), DBU (1.0 equiv), Bu_4NI (0.2 equiv), CH_2Cl_2, 23 °C, 12 h, (68%); DBU = 1,8-Diazabicyclo[5.4.0]undec-7-ene; DMAP = 4-Dimethyl aminopyridine; EOMCl = ethoxymethyl chloride; TFA = trifluoroacetic acid.

Table 3
Growth inhibition and HSP90α affinity of pro-pochoxime

Cell line	Pro-pochoxime A (**21**)
Growth inhibition (GI_{50}) and HSP90 affinity of pro-pochoxime A (nM)	
BT474 (breast)	14
MDA-MB468 (breast)	37
N87 (gastric)	19
HSP90α affinity	*>10,000*

Table 4
Conversion (%) of phosphate prodrug to parent drug in tissue homogenates

	21	**22**	**23**	**24**	**25**	**26**
Liver	18.2	23.2	29.4	10.5	30.5	17.9
Intestine	18.9	26.6	41.6	11.6	43.5	18.7
Plasma	3.4	2.2	1.4	0	0	4.0
Gastric fluid	0.4	0.4	0	0	0	0.3

mes do not bind to HSP90 with notable affinity, they are rapidly converted to the corresponding pochoxime in the intestine or the liver and are effective in cancer cell cultures.

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Supplementary data

Supplementary data (experimental procedures and physical characterization) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.030.

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