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Design, synthesis and cytotoxicity studies of dithiocarbamate ester derivatives of emetine in prostate cancer cell lines

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ABSTRACT

A small library of emetine dithiocarbamate ester derivatives were synthesized in 25–86% yield via derivatization of the N2′- position of emetine. Anticancer evaluation of these compounds in androgen receptor positive LNCaP and androgen receptor negative PC3 and DU145 prostate cancer cell lines revealed time dependent and dose-dependent cytotoxicity. With the exception of compound **4c**, all the dithiocarbamate ester analogs in this study showed appreciable potency in all the prostate cancer cell lines (regardless of whether it is androgen receptor positive or negative) with a cytotoxicity IC_{50} value ranging from 1.312 ± 0.032 μM to 5.201 ± 0.125 μM by day 7 of treatment. Compared to the sodium dithiocarbamate salt **1**, all the dithiocarbamate ester analogs (**2** and **4a**–**4g**) displayed lower cytotoxicity than compound **1** (PC3, IC_{50} = 0.087 ± 0.005 μM; DU145, IC_{50} = 0.079 ± 0.003 μM and LNCaP, IC_{50} = 0.079 ± 0.003 μM) on day 7 of treatment. Consequently, it appears that S-alkylation of compound **1** leads to a more stable dithiocarbamate ester derivative that resulted in lower anticancer activity in the prostate cancer cell lines.

1. Introduction

Prostate cancer is the most common non-cutaneous malignancy among American men. Patients diagnosed at stage III and IV of the disease, are usually treated with androgen-deprivation therapy (surgical or chemical castration) and virtually all patients progress to the castration resistant stage and ultimately resulting in mortality once it escapes the confines of the gland. It was estimated that more than 29,000 men in the United States would die from prostate cancer in 2014.[1] Recently, several new agents have been approved for the treatment of castration resistant disease based on a few months median survival improvement over placebo. These include anti-androgen therapy abiraterone acetate and enzalutamide, second-line chemotherapy with cabazitaxel, bone-targeted denosumab, radiation therapy with radium-223 and immunotherapy vaccine, sipuleucel-T.[2,3] All these agents only extend patient life by several months and there remains an urgent need to develop new therapies for castration resistant prostate cancer.[3] Currently, efforts are being directed along several frontlines to develop more efficacious therapy with unique anticancer mechanisms, improved survival benefit and relatively low systemic toxicity to normal cells.[3,4] Natural products continue to be valuable source of compounds with tremendous biological and medicinal importance including compounds with excellent anticancer activities. Thus, natural products remain a vital source of scaffolds and compounds for the development of useful anticancer agents. In our efforts to develop clinically useful anticancer agents based on natural products scaffold, we find emetine to be an attractive scaffold for chemical transformation.

Akinboye and Bakare recently reviewed the biological activities of emetine; this account shows the versatility of emetine as a bioactive natural product.[5] Among other biological activities, it is a

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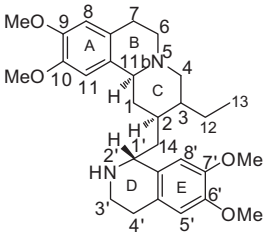
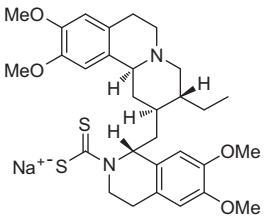


Emetine



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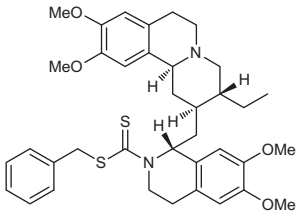

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well known anti-cancer alkaloid from *Psychotria ipecacuanha*.[6–8] Its anticancer activity was evaluated in several clinical studies up to Phase II for treating solid tumor, however narrow therapeutic index and dose dependent side effects stopped further studies.[9] Recently, some studies reported novel mechanisms of action for the cytotoxicity of emetine. For instance, among other alkaloids screened for the inhibition of the activation of Hypoxia-inducible Factor-1 (HIF-1) in breast cancer, emetine demonstrated an appreciable potency.[6] It regulates the alternative splicing of Bcl-x Pre-mRNA, producing an up-regulation of the levels of the pro-apoptotic variant, Bcl-xS, and down-regulation of the levels of the anti-apoptotic variant Bcl-xL in different cancer cell lines.[10] In a separate study on leukemia cells, emetine itself induced apoptosis, and also improved drug-induced apoptosis when combined with other chemotherapeutic agents.[11] Smukste et al., in a study on the use of small molecules to overcome drug resistance induced by a viral oncogene, also reported emetine and other protein synthesis inhibitors to potentiate the lethality of doxorubicin in RKO-E6 cell.[12] More interestingly, emetine has been found to be a substrate of permeability glycoprotein (P-gp).[13] The glycoprotein (P-gp) is a membrane protein that pumps drug molecules out of cells so that they cannot elicit their cytotoxic effects, therefore, contributing significantly to multidrug resistance (MDR). Consequently, a small library of emetine homodimers was designed and synthesized to probe the substrate binding sites of P-gp.[14] One of these homodimers was reported to reverse the MDR phenotype of MCF-7/DX1 cells at a non-cytotoxic concentration when co-administered with cytotoxic agent such as doxorubicin.[14] Thus, it appears that modification of emetine could lead to anticancer drug candidates with better therapeutic index.

In our approach to obtain emetine derivatives without acute systemic toxicity, but still retaining anti-cancer potency, we designed the derivatization of the N2′- position of emetine into biologically interesting compounds for bioactivity studies. We initially synthesized and evaluated the anti-prostate cancer activities of a diverse library of hydrolysable analogs and prodrugs of emetine involving a variety of functional moieties. Among the compounds studied in a 7 day drug treatment, the emetine dithiocarbamate salt **1** showed considerable potency in the prostate cancer cell lines studied (PC3, $IC_{50} = 0.087 \pm 0.005$ μM; LNCaP, $IC_{50} = 0.079 \pm 0.003$ μM) compared to the rest of the analogs studied. Upon alkylation of **1** to form the benzyl dithiocarbamate ester **2**, the cytotoxic activity was reduced (PC3, $IC_{50} = 1.560$ μM; LNCaP, $IC_{50} = 1.970$ μM).[15] In order to establish the effect of alkylation of **1** on the anticancer properties of the resulting dithiocarbamate ester and establish structure activity relationship (SAR) studies that would allow us to identify a lead emetine dithiocarbamate ester derivative for further studies, we designed the synthesis and anticancer studies of a small library of emetine dithiocarbamate ester derivatives. In addition, incorporating the dithiocarbamate moiety into a library of emetine analogs is of interest to us because the presence of this moiety in a number of compounds has been associated with anti-carcinogenic, anti-mutagenic, and cancer chemo-preventive activities.[16–19] In the present paper, we have carried out a time-dependent cytotoxicity study of compound **2** and seven other dithiocarbamate ester analogs in androgen receptor positive LNCaP and androgen receptor negative PC3 and DU145 prostate cancer cell lines. We herein report the synthesis, characterization and preliminary anti-prostate cancer activities of a small library of emetine dithiocarbamate ester analogs.

2. Results and discussion

2.1. Chemistry

As we previously[15] described for compound **2**, the synthesis of the dithiocarbamate analogs of emetine (**4a**–**4g**) commenced with the conversion of emetine dihydrochloride salt to the dithiocarbamate salt **1** by treating a solution of emetine dihydrochloride salt in ethanolic NaOH with carbon disulfide (Scheme 1). Compound **1** was obtained in almost quantitative yield. Subsequent reaction of **1** with different alkylating agents **3a**–**3g** (Fig. 1) in acetonitrile furnished the dithiocarbamate ester analogs of emetine (**4a**–**4g**) in 25% to 86% yield. The resulting crude product was subjected to a short flash chromatography on silica gel column using different ratios of methanol and ethyl acetate as eluent. The main impurity is believed to result from decomposition of the dithiocarbamate salt, which resulted in a more polar by-product than the dithiocarbamate ester analog as shown by thin-layer chromatography (TLC) on silica. No attempt was made to further isolate and characterize this by-product. All compounds were characterized by infrared, ^{1}H and ^{13}C NMR spectroscopy as well as by electrospray ionization mass spectrometry.

2.2. Biology

In a seven-day exposure, we initially compared the cytotoxicity of emetine, the dithiocarbamate salt **1** and a benzyl substituted dithiocarbamate ester analog **2** of emetine in PC3 and LNCaP.[15] In this study, emetine was about three folds more potent than the dithiocarbamate salt **1** while it was about 50 folds more potent than the dithiocarbamate ester analog in PC3. We established in this initial study that the dithiocarbamate salt **1** served as a prodrug of emetine and was hydrolyzed to emetine in the slightly acidic cancer cell growth medium mimicking the cancerous tumor microenvironment, whereas the dithiocarbamate ester **2** was more stable as a result of S-alkylation of the dithiocarbamyl group. More importantly, a preliminary evaluation of the safety of compound **1** in healthy mice compared to emetine suggested that the dithiocarbamate salt **1** is safer than emetine in vivo. Specifically, mice that received a single dose of emetine at 100 mg/kg body weight all died within 48 h. On the other hand, 100 mg/kg body weight of compound **1** did not produce observable lethality. Further, when 33 mg/kg body weight of emetine or compound **1** was administered, mice that received emetine were lethargic and showed about four times weight loss compared with control; while, mice