Biological Activities of Emetine

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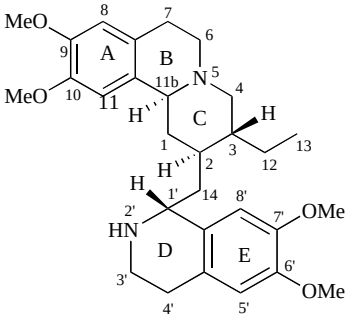
Abstract: Emetine is a natural product alkaloid from ipecac species. It is one of the main active ingredients in ipecac syrup used as emetic, and has been used extensively in phytomedicine as an antiparasitic drug. It inhibits both ribosomal and mitochondrial protein synthesis and interferes with the synthesis and activities of DNA and RNA. For this reason, it has been a vital tool to pharmacologists and has demonstrated many biological properties, such as antiviral, anticancer, antiparasitic and contraceptive activities. Also, it has been reported to cause the up-regulation and down-regulation of a number of genes. Some synthetic analogs with interesting biological activities have been prepared. This article reviews the biological activities of emetine and some emetine derived molecules.

Keywords: Alkaloid, biological activity, DNA, emetine, *Entamoeba histolytica*, ipecac, protein synthesis, RNA.

INTRODUCTION

Emetine (**1**) is a natural product alkaloid native to Brazil (Fig. **1**). Emetine and its analogs occur in three plant families including Alangiaceae, Icacinaceae, and Rubiaceae. One major source of emetine and its analogs is *Psychotria ipecacuanha* Stokes (Rubiaceae). This plant is also known as *Cephaelis ipecacuanha* A. Rich., and emetine is the principal alkaloid of ipecac root [1]. Garcia *et al.* reported that in a study involving the four major clusters of ipecac in the shady areas of the Atlantic Rain Forest of southeastern Brazil, the average concentration of emetine in the ipecac root varies significantly from one cluster to another [2]. The chemical structure and stereochemistry of emetine were studied and elucidated by chemical degradation experiments [3, 4]. Also, the biosynthesis of emetine and its closely related analog, cephaeline, have been studied extensively in various radiotracer experiments [5].



Emetine (1)

Fig. (1). Chemical Structure of Emetine.

Structure activity relationship (SAR) studies of emetine and its analogs and molecular modeling studies on emetine have helped in understanding the structural basis for its biological activities. In a study on protein synthesis inhibition, Grollman [6] reported that for emetine to be biologically active, C-1′ must have the *R* configuration and the 2′ position must be a secondary amine. This is partly because the epimer, **2** (isoemetine) (Fig. (**2**)) with the *S* configuration at C-1′ is inactive. Also, activity was lost in **3** (O-methylpsychotrine) with unsaturation at the 1′-2′ positions, and **4** (*N*-methylemetine) suggesting that the N2′ position must be a secondary amine. Asymmetry at carbons 2 and 3 is destroyed by the unsaturation at the 2-3 positions to give **5** (dehydroemetine) but this change does not affect protein synthesis inhibition. Further oxidation to **6** (1,2,3,4,5,11b-trisdehydroemetine) converts the tertiary nitrogen into a quaternary ammonium moiety and results in loss of activity [6]. In a more recent study, results obtained by Zhou *et al.* confirmed the need for the *R* configuration at the C-1′ position and also showed that the methoxy group at C-7′ is another essential structural requirement for the biological activities of emetine [7].

Further, Gupta *et al.* reported that the two aromatic rings and a planar molecular structure are part of the critical structural requirement for the biological activities of emetine [8]. However, molecular modeling studies on the structural requirements reported by Troconis *et al.* did not support the need for a planar structure, because emetine is not planar [9]. This study further showed that N-2′, N-5 and C-9 are critical positions that are essentially required for the biological activities of emetine [9].

Emetine has been shown to possess important biological activities that make it a vital tool to molecular biologists and pharmacologists. Further, both structure-activity relationship (SAR) and molecular modeling studies have proposed structural features necessary for many of the biological activities of emetine. The aim of this review is to present a concise account on the biological activities of the natural product emetine.

INHIBITION OF PROTEIN, DNA AND RNA SYNTHESIS

The earliest use of emetine in traditional medicine is as an emetic and expectorant [6]. Inhibition of the synthesis of

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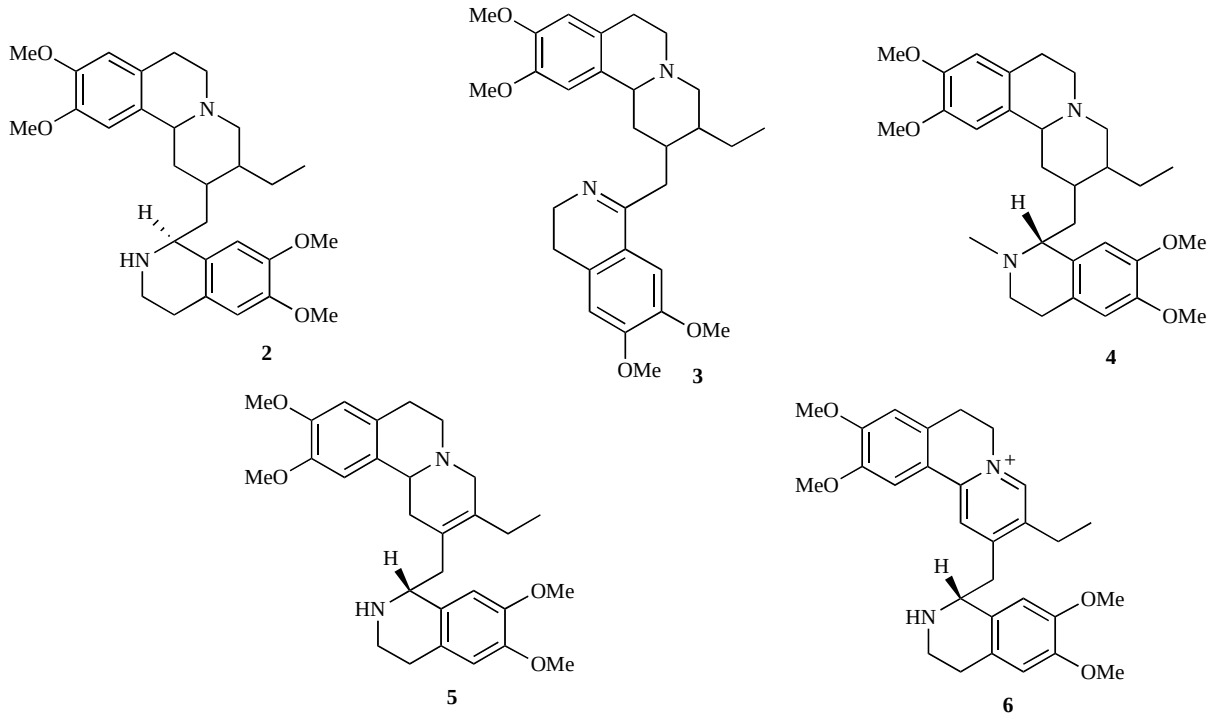


Fig. (2). Chemical structures of the analogs of emetine used in studying the structural requirements for its inhibition of protein synthesis.

certain macromolecules is partly its mode of action in biological systems. This was not understood until Grollman reported the inhibition of ribosomal protein synthesis in mammalian, yeast and plant cells by emetine in a concentration- and time-dependent manner. Extracts from bacteria however resisted protein synthesis inhibition by emetine [6]. The mechanism proposed was based on the inhibition of the aminoacyl-sRNA transfer reaction by emetine, a series of steps resulting in the incorporation of the aminoacyl moiety into polypeptide-bond form. This finding was used to investigate the mode of action of emetine on *Entamoeba histolytica* [6]. Emetine irreversibly inhibits protein synthesis in HeLa cells by depleting the number of free ribosomes and subsequently increasing the polyribosomes [10]. However, protein synthesis inhibition by emetine in Chinese hamster ovary (CHO) cells is reversible [11]. Jimenez *et al.* reported that the site of action of emetine is the 40S ribosomal subunit [12]. Moreover, emetine selectively inhibited mitochondrial protein synthesis in mouse liver [13]. Further studies showed that the natural product also inhibits DNA synthesis in HeLa cells irreversibly. The compound is also known to inhibit viral RNA synthesis in poliovirus-infected HeLa cells [10]. Inhibition of the biosynthesis of RNA in HeLa cell is dose dependent requiring different concentrations for different RNA species [14]. This RNA inhibition progresses from being reversible to irreversible [14].

These reports on the effects of emetine on protein and nucleic acid synthesis, led other scientists to further investigate how the compound affects cell physiology in different species. A study of the mechanism of action of emetine on *Tetrahymena pyriformis*, a ciliated protozoan, showed that at 4 μM, emetine inhibits the synthesis of protein directly and not by altering the functions of nucleic acids [15]. Also, investigation of the mechanism of action of emetine on myocardium showed that the incorporation of titrated leucine into soluble protein and actomyosin was inhibited with an IC_{50} value of 0.5 μM on treating rats with emetine for three days [16]. Protein concentrations of liver and kidney were reduced when rats were injected with an 11.8% lethal dose of emetine per day for 10 days, but RNA concentration was not affected [17].

Inhibition of protein synthesis by emetine was reported to completely block the initiation of DNA synthesis and mitosis in fertilized eggs of the sea urchin, *Anthocidaris crassispina* [18]. The ability of this natural product to inhibit protein synthesis was employed in inhibiting and normalizing the activity of alcohol dehydrogenase, and therefore suppressed pathological alcohol addiction [19]. Emetine also prevents induced autophagy in exocrine cells of mouse pancreas and seminal vesicle by stabilizing polyribosomes while inhibiting protein synthesis [20].

Emetine acts by blocking the early S phase of DNA replication [21]. At a single dose of 33 mg/Kg, administered subcutaneously to mice, DNA, RNA and protein synthesis of thymic cells were reduced by 90, 50 and 65%, respectively [22].

ANTIPARASITIC ACTIVITY OF EMETINE

Emetine has been employed in the treatment of amoebiasis and amebic dysentery caused by *Entamoeba histolytica*, an anaerobic parasitic protozoan, whose growth is inhibited by emetine [23-25]. The alkaloid was reported to be an effective treatment for amoebic liver [26] and a treatment of choice for perianal skin amoebiases [27], both of which are caused by the same parasite. Emetine was used as a standard drug for treating amoebiasis for about 50 years but its use was discouraged due to excessive toxicity [28]. In an *in vitro* experiment, it was recently shown that the drug works by inducing programmed cell death (PCD) in *Entamoeba histo-*

lytica [29]. Morphological effects of incubation with emetine include reduction of cytoplasmic volume, maintenance of plasma cell integrity, and production of nuclear condensation and DNA fragmentation. Further, overproduction of reactive oxygen species was found to be a significant biochemical change inside the trophozoites [29].

The antiparasitic activity of emetine has been investigated in some other parasites as well. Emetine showed potent *in vitro* antileishmanial activity against *Leishmania donavani* [30]. It has also been tested as a trypanocidal agent against *Trypanosoma cruzi* in search for drug against Chaga's disease [31, 32], and shown to display trypanocidal activity against *Trypanosoma brucei* [33-35]. In line with some of its mechanisms of action, emetine was found to induce apoptosis in *Trypanosoma b. brucei* by DNA intercalation, and inhibition of protein biosynthesis [34]. Emetine has been tested as anthelmintic in infected sheep and goat, and was effective against *Protostrongylus rufescens* at 1 mg/kg but toxic at 3 mg/kg. This dose was quite efficient (68%) against *Muellerius spp* [36].

ANTIVIRAL ACTIVITY OF EMETINE

Emetine has some interesting antiviral activities. A high throughput-screening of a chemical library of antagonists of vaccinia replication was done to develop new antipoxviral agents. Emetine emerged as an antipoxviral agent by inhibiting vaccinia virus replication at noncytotoxic doses (plaque inhibition at 0.25 μM; 48h yield IC_{99}: 0.1 μM) [37]. The compound was recently reported to also show significant antiviral activity against four serotypes of dengue virus (DENV) and a dose-dependent reduction of viral infection was observed at a noncytotoxic dose [38]. Post-treatment of cells with emetine at 0.1 μM shows 33% DENV II inhibition while at 0.5 μM to 10 μM the infection was strongly inhibited by more than 90%. It was reported that emetine inhibits DENV infection at an early stage of the viral replication cycle, either by affecting the viral RNA synthesis pathway or the viral protein translation pathway. Also, the production of positive-strand and negative-strand DENV RNA was significantly reduced by emetine [38].

ANTICANCER ACTIVITY OF EMETINE

A belief held by Lewisohn that neoplasm has a parasitic or amoebic origin resulted in the first clinical test of the activity of emetine on human tumors, with the observation of apparent tumor regression upon emetine administration. However, because Lewisohn could not observe the same effect in rats and mice, he concluded that emetine did not possess an antitumor property [39]. Van Hoosen also reported regression of malignancy in patients with a variety of malignancies after emetine therapy [40]. In addition, emetine was found effective in the treatment of murine L-1210 and P-388 leukemia [41]. Based on these reports, phase I trials were performed [42, 43]. Results observed in patient with lung cancer led to phase II clinical trials in a variety of solid tumors [44-46].

Emetine has been reported to cause apoptotic cytotoxicity in many human cancer cell lines: U937 (leukaemic cell line) [47], A549-S (lung adenocarcinoma) LD_{50}:55 μg/mL [48], Jurkat T cells (T cell leukemia) EC_{50}: 0.17 μM [49], CCRF-CEM (Human T cell lymphoblast-like cell line) EC_{50}: 0.05 μM [50], HL-60 (Human promyelocytic leukemia cells), EC_{50}: 0.09 μM [34], rat hepatocytes [50], and CEM/ADR5000 (leukemia cell line) EC_{50}: 2 μM [49]. The inhibition of protein biosynthesis and interaction with DNA are considered the sources of emetine cytotoxicity [6, 10].

Recent findings have shown that apart from protein synthesis inhibition and interaction with DNA, emetine induces apoptosis by regulation of pro-apoptotic factors. Emetine regulates alternative splicing of *Bcl-x* pre-mRNA by protein phosphatase I [51]. In all the cancer cells employed in this study, emetine down-regulates the levels of the anti-apoptotic variant Bcl-xL mRNA, and up-regulates the levels of the pro-apoptotic variant Bcl-xS mRNA, in a time and concentration-dependent manner [51]. The study reported a significant decrease of the Bcl-xL/Bcl-xS ratio in MCF-7 breast cancer, PC3-prostate cancer, C33A-cervical cancer and A549-lung cancer cell lines after emetine treatment [51].

Further, emetine has been reported to up-regulate some pro-apoptotic and anti-survival genes: *BAK1, CASP8* (caspase 8), CASP9 (caspase 9), *DAXX* (death-associated protein 6), *GZMB* (granzyme B) and *TNFRSF6* are reported to be up-regulated in Jurkat cells after treatment with emetine [52]. *BCL2*, *EGFR* (epidermal growth factor receptor), and *TNF* (tumor necrosis factor), are anti-apoptotic and pro-survival genes in Jurkat cells and are reported to be down-regulated after treatment with emetine [52]. In contrast, other anti-apoptotic and pro-survival genes (*AKT1*, *MST1*, *TNFRSF11B*, and *TNFSF13*) were also found to be up-regulated in Jurkat cells by emetine [52]. Emetine causes phosphatidylserine exposure, mitochondrial depolarization, and DNA fragmentation in Jurkat T-cells [52, 53]. It was further concluded that apoptosis induction in Jurkat T cells by emetine is mediated by the mitochondrial pathway, and p53 does not seem to play a role in it [53]. Apart from genes that are directly linked to apoptosis, emetine has been reported to affect the expression of other genes. The matrix metalloproteinase (MMP) gene family includes 24 genes involved in tissue remodeling and their dysregulation is observed in many pathological conditions, including cancer. Emetine was reported to block the induction of all genes except *MMP9* and *TIMP3* [54]. Increasing PGC-1α activity has been proposed to be beneficial in controlling muscular dystrophy, diabetes, neurodegenerative diseases and ovarian cancer [55, 56]. Increasing PGC-1α activity in the ovarian cancer cell line Ho-8910 has been shown to induce apoptosis [56]. Emetine was reported to be one of the most potent inducers of PGC-1α expression, as identified by high-throughput screening of 3,120 compounds [55].

It is noteworthy that studies have been performed to determine the effect of combining emetine with another therapeutic agent in cancer drug discovery research. The first study of this kind was a direct clinical use in which lung cancer patients were treated with emetine and cyclophosphamide, and some definite responses were observed [57]. A pronounced apoptosis not seen with either emetine or TNF-α alone was observed when HeLa cells were treated with a combination of TNF-α and emetine [58]. Similarly, induction of apoptosis in HeLa cells by purified TRAIL was 5-fold enhanced by emetine [59]. Also, synergism was observed when emetine was combined with either doxorubicin, etoposide, docetaxel, or oxaliplatin in treating human neu-

roendocrine tumor cell lines [60]. In synergy with 5-azacytidine, emetine was reported to restore sodium iodide symporter gene expression in the human thyroid adenoma cell line, KAK-1, by stimulating the expression of normalized luciferase [61]. Moreover, expression of *BAK1*, *BIK*, *CASP3*, *CASP8*, *CASP9*, *DAXX*, *GZMB*, *TNFRSF6*, *TNFRSF9*, which are pro-apoptotic and anti-survival genes was significantly up-regulated in Jurkat T cells after treatment with a combination of emetine and cisplatin ,whereas cisplatin alone induced the up-regulation of only *CASP8* and *TNFRSF6* [52]. These and other data point to the potential use of emetine in combination therapy for cancer treatment.

Adaptation of cancer cells to hypoxia and their survival under these conditions have been attributed to the critical role played by certain genes whose expression is induced by hypoxia-inducible factor-1 [62]. Emetine demonstrated significant inhibition of HIF-1 activation in T47D cells (IC_{50}: 0.11 μM) [7]. Putative hydrolase RBBP9 is an uncharacterized cancer-associated enzyme, and recently emetine was identified as its selective inhibitor *via* high-throughput screening with fluorescent activity-based probes [63]. Emetine was also reported to prevent heat shock response (HSR) in cancer cells [64].

REDUCTION OF T-2 TOXIN TOXICITY ASSOCIATION WITH CELLS BY EMETINE PRETREATMENT

T-2 toxin (trichothecene toxin) is a toxic metabolite produced by a number of *Fusarium* spp and it strongly inhibits protein synthesis by binding to a receptor and accumulating intra-cellularly [65]. The binding of T-2 toxin to chinese hamster ovary (CHO) cells and ribosomes derived from CHO cells was found to be reversible and emetine was used as a probe to disturb this interaction [65]. For cells pre-incubated with emetine before introducing T-2 toxin, interaction of T-2 toxin with cells was decreased by up to 90% at both 4 ^{o}C and 37^{o}C. Inhibition of the interaction of T-2 toxin with cell was reversible when exposure was brief (≤ 5 min) and irreversible over longer time periods (60min). An allosteric effect and competitive inhibition at the T-2 toxin ribosomal binding site were reported as the basis of the inhibition of the interaction of T-2 toxin with CHO cells [65]. Dissociation was initiated by emetine in cells pre-bound with the toxin [65]. Subsequently, inhibition of protein synthesis by T-2 toxin and its toxic effects were perturbed and reduced by emetine [66]. Further, interaction of other toxins such as diacetoxyscirpenol, verrucarin A and roridin with cells pre-incubated with emetine similarly caused a reduction in toxicity produced by these toxins [66].

CONTRACEPTIVE ACTIVITY OF EMETINE

The potency of emetine as a protein synthesis inhibitor inspired the idea of investigating its efficacy as a contraceptive agent when administered locally as a component of a medicated intrauterine delivery system (MIDS) [67]. This study was done in rabbit uterus, and the results demonstrated the antiimplantation effect of emetine dihydrochloride, which increased with concentration [67]. Another study examined the potential use of emetine ditartrate as an emergency contraceptive [68]. The study was carried out in five rodent species: hamster, rat, mouse, guinea pig and rabbit, by oral and intravaginal routes. The uterus and early embryos around implantation, possibly the trophoblast and endometrial cells at the attachment site, are the main target of the action of emetine ditartrate. This study concluded that emetine ditartrate could be used to terminate human pregnancy in its initial stages. It was also proposed that emetine ditartrate might be better than antiprogestin. Moreover, emetine ditartrate does not have the side effects associated with hormonal preparations [68].

EMETINE AND INHIBITION OF THE NONSENSE-MEDIATED MRNA DECAY (NMD) PATHWAY

Nonsense-mediated decay (NMD) is one of the mechanisms cells use to prevent the synthesis of truncated protein (gene expression) or mutant genes that lead to human diseases. Inhibiting NMD will stabilize the mutant transcripts and this has been used in identifying the genes containing the truncated mutant [69]. As a protein synthesis inhibitor, emetine inhibits NMD [69], and this approach was combined with microarrays to identify genes harboring nonsense codons that underlie human diseases in a strategy called *gene identification by NMD inhibition* (GINI) [70]. Emetine has been extensively employed in GINI to inhibit NMD. Huusko *et al*. applied the GINI strategy to prostate cancer cell lines (DU145, PC3, and LnCaP) using emetine as an NMD inhibitor, and identified mutations of *EPHB2* in human prostate cancer [71]. Using the same strategy, mutations in colon cancer cells [72] and melanoma [73] were identified.

TOXICITY OF EMETINE

Emetine is an alkaloid of great medicinal value. Its medicinal use has been discouraged because of toxicity. For instance, chronic usage has been reported to result in myopathy [74]. In addition, cardiotoxicity, including cardiomyopathy, is associated with chronic use of emetine [74-76]. In a study relating protein pharmacology to ligand chemistry, new targets were discovered for emetine and these were linked to some of the side effects of its pharmacological use [77]. In this study emetine was found to be an adrenergic (α2) blocker and an inhibitor of dipeptidyl aminopeptidase IV. It was also shown to be an antagonist of dopamine (D1 and D3) receptors, substance P, and neurokinin NK3 [77]. Understanding the targets responsible for the toxicity of this natural product will provide great insight into drug design efforts designed to afford less toxic and medicinally useful analogs.

BIOLOGICAL ACTIVITIES OF SYNTHETIC ANALOGS OF EMETINE

Activities of N2′-Derivatives of Emetine Against *Entamoeba histolytica*

One of the first emetine analogs to attract attention was dehydroemetine (**5**), which was reported to be effective in leukemia, rectal adenocarcinoma and other malignancies [78-80]. This compound was claimed to have reduced toxicity compared to emetine [81]. This outcome shows that a slight structural change in the emetine molecule could result in a lowering of its cytotoxicity.

With the goal of reducing the cardiotoxicity of emetine, Gradnik *et al*. reported the synthesis and biological activities of N2′-derivatives of emetine against *Entamoeba histolytica* [82]. The general structure of these analogs is shown in Fig.

(**3**). Specific structures and activities are shown in Table **1** below.

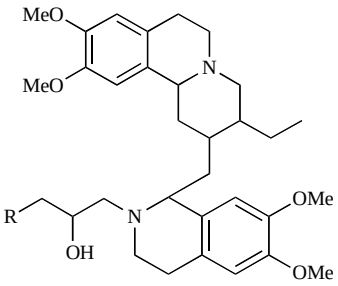


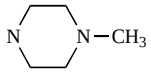
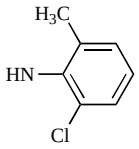
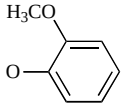
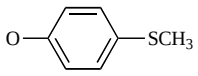
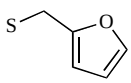
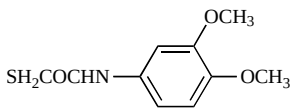
Fig. (3). General structure of the N2´-derivatives of emetine.

Cardiotoxicity was determined for **16** as a measure of the effect of the compound on the oxidation rate of pyruvates and lactases. *l*-Emetine decreases the oxidation rates of pyruvates and lactases by 19% and 20%, respectively; while **16** decreases them by 11% and 6%, respectively [82].

Bivalent Agents Based on Monomeric Emetine as Probes and Inhibitors of Human Multidrug Transporter P-Glycoprotein

To a large extent, growing resistance to chemotherapeutics in cancer has been linked to the up-regulation of a plasma membrane polypeptide multidrug transporter, P-glycoprotein [49, 83]. Through studies done in Chinese hamster ovary cell lines [83], *Entamoeba histolytica* [84], and human hepatocytes [85], it has been demonstrated that emetine is a substrate of P-glycoprotein and this is an efficient way by which certain mammalian cells and amoebae protect themselves against the cytotoxicity of emetine [83]. Emetine-resistant *Entamoeba histolytica* overexpresses P-

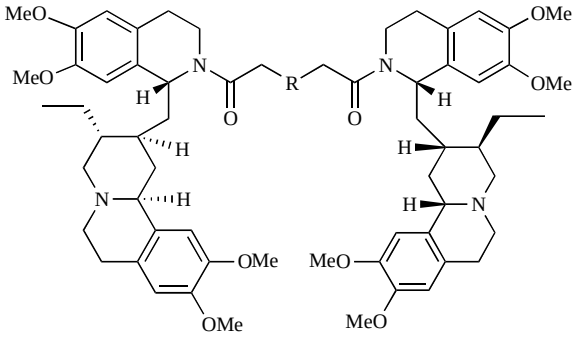
Table 1. Acute Toxicity of N2´-derivatives of Emetine Against *Entamoeba histolytica*

Compound	R	LD_{50}, mg/kg	Minimum Inhibitory Concentration (MIC), µg/mL
7	H	35	200
8	CH_3	51	100
9	$N[(CH_2)_4CH_3]_2$	31	100
10		37	40
11		28	40
12		25	100
13		17.5	40
14	OC_2H_5	20	40
15	$O(CH_2)_6CH_3$	27	20
16	$OCH_2CH(C_2H_5)(CH_2)_3CH_3$	31.5	40
17		9	40
18		34	100
19	$SCH(CH_3)C_2H_5$	83.5	40
20	$SC(CH_3)_3$	82.5	100
21	$SCH_2C_6H_5$	45	40
22		54	40
23		25	200
24	*l*-emetine	15.1	10-20

The structures, LD_{50} and MIC data are taken from reference [82].

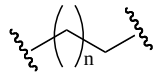
glycoprotein genes and consequently, emetine suffers from poor intracellular accumulation [86, 87]. Thus, Pires *et al.* proposed that a novel emetine-based inhibitor of P-glycoprotein would be both a potent drug for amoebiasis and a good probe of P-glycoprotein [88]. Since P-glycoprotein has more than one substrate binding site, a library of emetine homodimers was designed by varying the length, degree of hydrophobicity and polarity of the linkers [88]. Their general structures are shown in Fig. (**4**) [88].

Rhodamine 123 was the fluorescent agent used to measure the potency of these compounds. Compound **26** was the most potent analog; it had an IC_{50} value of 2.9 μM for inhibiting the efflux of rhodamine 123 and an IC_{50} of approximately 5 nM for inhibiting the binding of [^{125}I]-iodoarylazidoprazosin, a known P-glycoprotein substrate. Also, on treating MCF-7/DX1 cells with a combination of doxorubicin and a noncytotoxic concentration of **26**, the multidrug resistance phenotype of MCF-7/DX1 was reversed [88]. This and other data point to the potential use of emetine and its analogs in overcoming multidrug resistance (MDR) in cancer therapy.



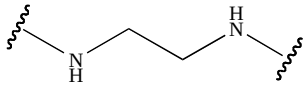
Emetine Homodimers

R =

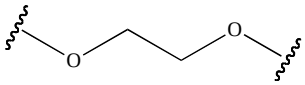


compounds	n
25	1
26	2
27	3
28	4
29	5
30	6
31	7

R=



32



33

Fig. (4). Chemical structures of emetine homodimers designed with varying cross-linking moieties (adapted from reference [88]).

CONCLUSIONS AND PERSPECTIVES

Emetine has been a vital tool to pharmacologists and molecular biologists. It has demonstrated more biological activities than most of the known natural products. Cardiotoxicity and cytotoxicity are the major problem discouraging the medicinal use of this vital drug. However, a closer look at the data of its biological activities indicates clearly that this toxicity is dose-dependent. Also, genetic makeup of the cell type and targets also determines the physiological effects of emetine. Thus, a well controlled use of emetine can help alleviate the symptoms of its toxicity.

Furthermore, a new drug with greater potency and significantly reduced cytotoxicity and cardiotoxicity can be designed and obtained from emetine. Chemical modification at the N2′ position of emetine has afforded analogs with reduced cytotoxicity. This suggests a good platform from which to start combating the problem of toxicity. Without any doubt, a properly diversified chemical modification of emetine at the N2′ position may likely produce a lead drug candidate with reduced toxicity, better selectivity, and potency in the treatment of one or more of diseases such as cancer, dengue fever, trypanosomiasis, amoebiasis, and other parasitic diseases.

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