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Digest

Doxycycline, salinomycin, monensin and ivermectin repositioned as cancer drugs

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ABSTRACT

Chemotherapy is one of the standard methods for the treatment of malignant tumors. It aims to cause lethal damage to cellular structures, mainly DNA. Noteworthy, in recent years discoveries of novel anticancer agents from well-known antibiotics have opened up new treatment pathways for several cancer diseases. The aim of this review article is to describe new applications for the following antibiotics: doxycycline (**DOX**), salinomycin (**SAL**), monensin (**MON**) and ivermectin (**IVR**) as they are known to show anti-tumor activity, but have not yet been introduced into standard oncological therapy. To date, these agents have been used for the treatment of a broad-spectrum of bacterial and parasitic infectious diseases and are widely available, which is why they were selected. The data presented here clearly show that the antibiotics mentioned above should be recognised in the near future as novel agents able to eradicate cancer cells and cancer stem cells (CSCs) across several cancer types.

Introduction

Chemotherapy is one of the most powerful treatments for cancer and benefits patients in the form of decreased relapse and metastasis and longer overall survival. It is therefore unsurprising that medicinal chemists continue the search for new anticancer agents or develop new uses for existing drugs. The development of molecular biology has promoted the identification of some mechanisms that control the growth, division and metastases of tumor cells. Targeted therapy is aimed at eradicating these mechanisms. Theoretically, targeted therapy should not affect healthy tissues or unaffected organs and therefore in comparison to standard chemotherapy it is less toxic.[1,2] Natural cytotoxic agents are extremely specific to their targets. Chemists and biologists have focused on discovery of novel anticancer agents by exploring the anticancer properties of new agents (naturally occurring, semi-synthetic or synthetic compounds) or by assessing drugs used for the treatment of other non-malignant diseases.[3]

Over the last several years, antibiotics with cytostatic activity have been registered as antineoplastic therapy agents. The most commonly applied include: doxorubicin, actinomycin, mitoxantron, bleomycin and mitomycin.[4] DNA is the most common molecular target. These drugs affect DNA synthesis and replication through interference in a DNA sequence, interaction with DNA by intercalation and through the inhibition of topoisomerase all of which prevent the cancer cells from further division.[5–9]

Recently, novel anticancer agents have been identified among numerous antibiotics, including those selected for this study. We are of the opinion that such discoveries can pave the way for new appealing treatments for neoplastic disease. Therefore, in this short review article we discuss the possibility of the off-label use of doxycycline (**DOX**), salinomycin (**SAL**), monensin (**MON**) and ivermectin (**IVR**), which are currently used for the treatment of a broad-spectrum of bacterial and parasitic infectious diseases. *In vitro* and *in vivo* studies of the anticancer activity of these drugs suggest promising clinical implications for their use as new anticancer agents.

Doxycycline

Doxycycline (**DOX**, Fig. 1) introduced in 1967, is a semisynthetic derivative of oxytetracycline. There are, however, many differences between **DOX** and natural tetracyclines, including markedly different pharmacokinetic properties, resulting in both lower doses and less frequent administration. **DOX** shows a broad spectrum of antibacterial activity. It is well tolerated, almost 100% is absorbed in the alimentary tract and it effectively penetrates the blood-brain barrier.[10,11] The major target of antibiotics such as tetracyclines is the ribosome and

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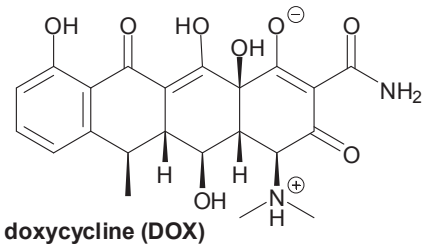
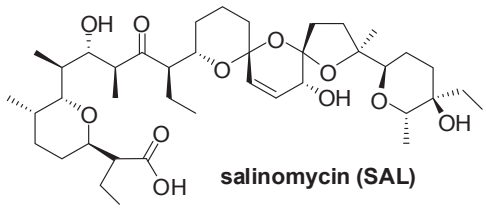


doxycycline (DOX)

Fig. 1. The chemical formula of **DOX**.



salinomycin (SAL)

Fig. 2. The chemical formula of **SAL**.

protein synthesis within the cell resulting in the prevention of the binding of aminoacyl *t*-RNA to the 30S ribosomal subunit.[5,9–12]

DOX has a broad spectrum of antimicrobial activity that includes many respiratory pathogens and an apparent excellent safety profile. It was initially widely used to treat young children with respiratory tract infections. Moreover, **DOX** has a good safety record when used for a long time and is well-tolerated in humans. **DOX** is also safe to use in pregnancy and early childhood because no correlation was noted between its use and teratogenic effects during pregnancy.[13]

In addition to its antibacterial activity **DOX** has cytotoxic and antiproliferating properties in various cancer cells.[14,15] Current studies have demonstrated that **DOX** is also a pluripotent drug that affects a wide range of pleiotropic therapeutic properties such as the control of invasive and metastatic cancer cells including anti-tumor growth effect and the inhibition of migration of cancer cells. It also exhibits interesting potential regarding enhanced therapeutic activity of several cancer therapies.[16–19] **DOX** inhibits the growth of tumor cells and metastases.[20–22] Using established cervical cancer cell lines (HeLa), Yang *et al.*[20] documented that **DOX** inhibits proliferation, induces apoptosis and reduces invasion of tumor cells. It also decreases cancer stem cell markers: SOC-2, OCT-4, NANOG, NOTCH and BMI-1 in cell culture.[21] In an animal model **DOX** reduces the cell proliferation markers: Ki67 and PCNA. This observation is significant as cancer stem cells (CSCs) are linked to resistance to treatment with cytometastatics, thought to be responsible for the relapse of the disease.

The antineoplastic effect of **DOX**, synergistic with the commonly applied anticancer drug doxorubicin, is also noted in prostate cancer cells: cancer cells underwent apoptosis due to an increase in the antiapoptotic Bax protein and a reduction in the antiapoptotic Bcl-2 protein. A similar mechanism of **DOX** action was noted in an animal model of pancreatic cancer: a reduction in the antiapoptotic protein as well as lowered expression of angiogenic IL-8.[20–22]

Another mechanism involves the inhibition of leukemic cell migration due to a lower expression of matrix metalloproteinase MMP2 and MMP9 associated with cellular migration and the inhibition of adhesive phosphorylation FAK (focal adhesion kinase).[23] Lee *et al.*[5] studied the effect of **DOX** on cell lines of fibroneuroma that developed on the basis of neurofibromatosis (NF-1) (malignant peripheral sheath tumor). DOX was additionally applied alongside photodynamic therapy (PDT) and 5-aminolevulinic acid (ALA) in the treatment of this disease. **DOX** was associated with reduced toxic symptoms that occur in photodynamic therapy. The authors recommend further clinical studies with oral **DOX** during local PDT.

Salinomycin

Salinomycin (**SAL**, Fig. 2), isolated from *Streptomyces albus*, is an antibiotic belonging to polyether antibiotics which show a broad spectrum of bioactivity, including antibacterial, antiparasitic, antifungal, antiviralactivity.[24] Specifically, **SAL** induces cell apoptosis disturbing the equilibrium of Na^+/K^+ ions in cellular membranes including mitochondria and cytoplasm.[25,26]

SAL reduces cell proliferation in various types of cancer, including those resistant to cytostatic drugs, it inhibits MDR (multidrug resistance), signalling along Akt, Wnt/β-catenin, hedgehog and Notch pathways participating in cancer progression. **SAL** has been also shown to destroy the CSCs of several malignant tumors and to promote the action of radio- and chemotherapy.[27–29] As mentioned above, the presence of stem cells – CSCs is considered to be involved in failures in standard antineoplastic therapy. In 2009, Gupta *et al.* [30] showed that when **SAL** was applied it eradicated CSCs 100 times more effectively as compared to paclitaxel. Lu *et al.*[31] described the positive effects of **SAL** on chronic lymphocytic leukaemia cells.

Several recent review articles have reported on the activity of **SAL** and its new derivatives (obtained only during last decade) in many types of cell lines, in animal and human models.[28,29,32–34] In another review, the effects of **SAL** on ovarian cancer have been described.[35,36]

Norouzi *et al.*[37] described the efficacy of nanofibers containing **SAL** in reducing human glioblastoma cells – more than 50% of tumor cells manifested apoptosis. The action of **SAL** reduced the activity of the Wnt signalling pathway that is indispensable for the survival of stem cells (CSCs). Additionally, **SAL** augmented the activity of tumor suppressors (Rb 1 and Rb 2) and the activity of caspase 3 – the tumor death enzyme. The authors suggest the application of **SAL** to the brain following resection of the tumor.[37] **SAL** is currently widely used as a growth promoter in animal husbandry, but despite the long history of use of this compound as an anticoccidial drug for poultry and as a growth promoter for ruminants, it has not been used as an antimicrobial compound for humans.[24,25] The treatment of tumors with **SAL** was initiated by Naujokat and Stainhart, in the case of a 40-year old woman with breast tumor metastases to the bones and an 82-year old woman with widespread perineal cancer, showing encouraging treatment results.[38] **SAL** is an antibiotic of negligible side-effects (transient tachycardia, hand tremor), acting alongside several already-mentioned mechanisms.[38] Apart from acting on CSCs, **SAL** activates death receptor 5 and caspase 8, demonstrating the enzymatic mechanism of cell death.[34,36] Furthermore, **SAL** also acts on the nuclear transcription factor NF-κB, which controls the function of several genes. Therefore, **SAL** could potentially be applied in the treatment of humans with neoplastic disease.[39,40] Promising lead compounds such as **SAL** tend to generate considerable interest among scientists including bioorganic chemists and biologists. Over the last decade **SAL** has been modified by several research groups from all over the world, giving rise to more than 200 new derivatives.

The cytotoxic activities of a number of **SAL** derivatives were stronger than that of **SAL** in the treatment of neoplastic diseases.[29,34,41–45] SAL and its new derivatives against human cancer stem cells, their confirmed activity *in vitro* and *in vivo* against numerous types of cancer including those displaying multi-drug resistance (MDR), together with low toxicity levels, seem promising options as new antineoplastic agents.

Monensin

Monensin (**MON**, Fig. 3) is an ionophoric antibiotic isolated from *Streptomyces cinnamonensis* with pronounced antibacterial[24,46] and antiparasitic potential.[47] It is commonly used in veterinary practice for the control of coccidiosis in poultry.[48] Numerous recently published results have shown that this antibiotic also manifests very interesting antineoplastic action described below.

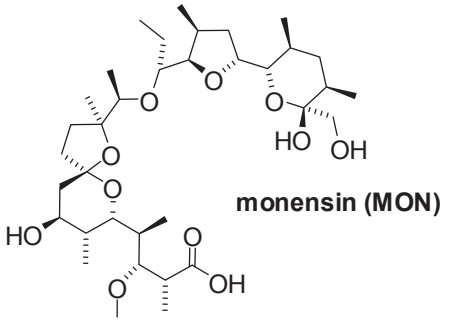


Fig. 3. The chemical formula of **MON**.

MON inhibits the signalling pathways linked to development of cancer – *e.g.* NF-κB and STAT, and also reduces the expression of EGFR (epidermal growth factor receptor).[49,50] The inhibitory effect of **MON** regards the proliferation, migration of ovarian cancer cells and induction of apoptosis. It has been shown to act synergistically with oxaliplatin.[50] In vivo, a reduced signalling of Wnt/β-catenin was detected in cases of multiple intestinal tumors. Due to the absence of side-effects on normal intestinal mucosa the antibiotic might be tested in clinical trials in humans.[51] Chemo-resistance of cancers is frequently associated with a dysfunction of apoptotic mechanisms in the cell. Apoptosis in neoplastic cells might be induced by the activation of TRAIL (tumor necrosis factor – TNF related apoptosis, inducing ligand). This shows that TRAIL could represent the effectors' mechanism in the elimination of tumor cells, without damage to normal body cells. **MON** sensitizes cerebral glioma cells (but not normal astrocytes) to the action of TRAIL and so apoptosis develops leading to an effective therapy.[52] Similarly, the combination of TRAIL/monensin has been proposed as a novel strategy for the treatment of chemo-resistant neoplasms.[53]

In the study performed by Wang and co-workers, it has been demonstrated that **MON** may be repurposed to treat chemo-resistant pancreatic cancer and that this antibiotic may act synergistically with other anticancer drugs, such as gemcitabine or erlotinib, for the treatment of drug-resistant pancreatic cancer.[54] Pancreatic cancer has a poor 5-year survival rate of less than 5%, to date only modest improvement in effective systemic chemotherapy of this type of cancer has been attained.

The effect of **MON** on multiple cancer-related pathways has been proven and there is evidence that **MON** can inhibit the E2F/DP1, STAT1/2, NFκB, AP-1 and Elk-1/SRF pathways. Furthermore, the expression of EGFR and its downstream genes, such as RAF1 and BRAF, is effectively suppressed by **MON**. In summing up their studies, Wang et al. have suggested that **MON** may exert its potent proliferation suppression effect through the inhibition of multiple growth factor-induced signal pathways, especially EGFR.[55]

Other mechanism-directed studies performed by Park et al.[56–60] demonstrated that **MON** can reduce the expression of cyclin A, CDK6, and cyclin D1, inducing programmed cell death-related gene activity in, for example, caspase-3, caspase-8 and Bax, as well as stimulating mitochondria transmembrane potential in some types of human cancer lines.

The effectiveness of erlotinib, an epidermal growth factor receptor inhibitor, or rapamycin, a mammalian target of rapamycin (mTOR) inhibitor, in a combination therapy with **MON** has been tested on non-small cell lung cancer cells (NCI-H1299). It has been shown that a 50 nM concentration of monensin can enhance apoptosis induced by rapamycin or erlotinib in NCI-H1299 cells.[60] Compared to their application alone, in cases where rapamycin or erlotinib were used together with monensin, the result was increased levels of pro-apoptotic proteins, including Bax, cleaved caspase 3 and cleaved PARP, and decreased the levels of anti-apoptotic proteins, including Bcl-2 and Bcl-xL. DNA content analysis shows that the cotreatment with monensin increased the numbers of cells in the G_1 cycle phase, as compared to the treatment with rapamycin or erlotinib alone. **MON** can also induce accumulation of cells in the G_1 phase by modulating cell cycle regulators.[60]

From high-throughput cell-based screening performed using a library of 4910 drug-like compounds, for different prostate cancer cell lines, **MON** emerged as a new selective active agent that can inhibit prostate cancer cell proliferation at nanomolar concentrations.[61] Ketola *et al.*[63] demonstrated that **MON** effects at nanomolar concentrations are linked to the induction of apoptosis and a potent reduction of androgen receptor mRNA and protein in prostate cancer cells. **MON** also elevated intracellular oxidative stress in prostate cancer cells as evidenced by the increased generation of intracellular reactive oxygen species and by induction of a transcriptional profile characteristic of an oxidative stress response. Overall, **MON** can be a new potential drug, well-tolerated, *in vivo* compatible with strong proapoptotic effects that are specific to prostate cancer cells, and synergistic effects with antiandrogens.[62]

In 2019 Vanneste et al.[63] tested 2640 compounds and demonstrated that both salinomycin and monensin displayed a potent and selective cytotoxic effect against EMT-like cells. Epithelial-to-mesenchymal transition (EMT) is implicated in cancer metastasis and drug resistance. EMT-like cells also exhibit resistance to a variety of therapeutic modalities and therefore selectively killing cells in an EMT-like state is expected to be useful in combination with conventional therapies to prevent the development of therapeutic resistance. **MON** (at only 10 nM concentrations) induced apoptosis, cell cycle arrest, and an increase in reactive oxygen species (ROS) production in the sensitive sub-population of prostate cancer cell line (TEM 4–18). In addition, **MON** rapidly induced subcellular effects by disruption of the Golgi apparatus in EMT-like cells characterized by the accumulation of swollen vesicles in the cytoplasm and observed also early effects of **MON** on mitochondria—including an increase in mitochondrial membrane potential.[63]

In summary, results obtained so far evidenced an interesting anticancer activity of polyether antibiotic – monensin (**MON**) which could represent a candidate worthy of further investigation.

Ivermectin

Ivermectin (**IVR**, Fig. 4) is a 22,23-dihydro derivative of avermectin B1 (Fig. 4) from macrocyclic lactone produced by the *Streptomyces avermitilis* bacterium. **IVR,** recognised in the 2015 Nobel Prize in Physiology or Medicine, is a strong antiparasitic agent and almost four decades after its remarkable commercial introduction in 1981 for the control of endoparasitic nematodes and ectoparasitic arthropods in livestock, **IVR** was FDA-approved for human use in 1987.[64,65]

IVR consists of a mixture of two homologues containing at least 80% 5-O-demethyl-22,23-dihydroavermectin and less than 20% 5-O-demethyl-25-de(1-methylpropyl)-22,23-dihydro-25-(1-methylethyl) avermectin, generally referred to as 22,23-dihydroavermectin B1a and B1b, respectively (Fig. 4), and it is obtained through selective, catalytic hydrogenation of the *cis*-22,23-double bond of the avermectins B1a and B1b.[66,67]

IVR belongs to the group of broad-spectrum antiparasitic agents which have a unique mode of action and is currently authorized to use for the treatment of onchocerciasis, lymphatic filariasis, strongyloidiasis, scabies and head lice.[67] Recently, it has been shown that **IVR** can also exhibits a lot of new interesting activities such as antibacterial, antiviral and anticancer. **IVR** acts as a positive allosteric regulator of several channels including the glutamate-gated chloride channel (GluCl), γ-aminobutyric acid type-A receptor, glycine receptor, neuronal α7-nicotinic receptor and purinergic P2X4 receptor. In most of the **IVR**-sensitive channels, the effects of **IVR** include the potentiation of agonist-induced currents at low concentrations and channel opening at higher concentrations.[68] **IVR** also acts as a positive allosteric regulator of several ligand-gated ion channels in vertebrates. Submicromolar concentrations of **IVR** activate or modulate the γ-

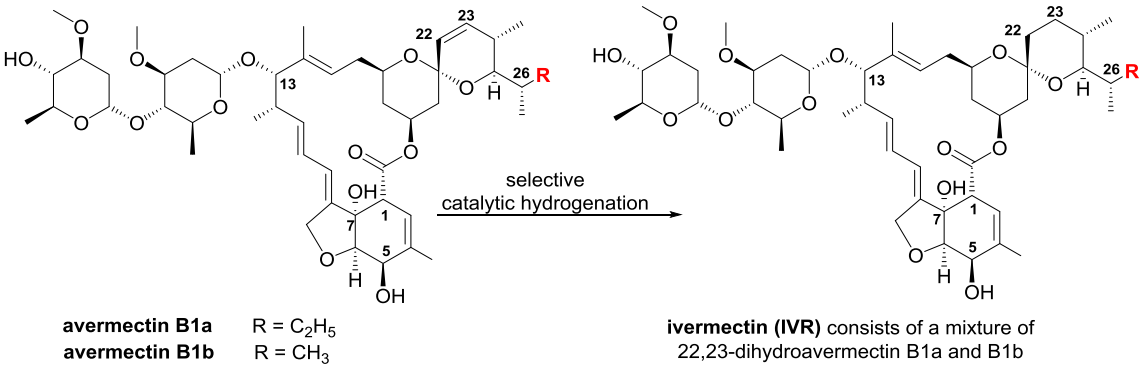


Fig. 4. The chemical structure of avermectins and **IVR**. The two component chemicals are extremely similar, ivermectin B1a has an ethyl group at the C-26 position, while ivermectin B1b has a methyl group. IVR is composed of at least 80% of B1a and not more than 20% of B1b.

aminobutyric acid type-A receptor (GABAAR), glycine receptor (GlyR) and neuronal α7-nicotinic receptor (nAChR).[68] Its anti-parasitic activity is strictly connected with selective binding and high affinity of this compound to the GluCl channels which occur in invertebrate nerve and muscle cells. This leads to an increase in the permeability of the cell membrane to chloride ions with hyperpolarization of the nerve or muscle cell, resulting in paralysis and death of the parasite. The basis of **IVR** activity is the fact that some mammals do not have GluCl channels, that **IVR** has a very low affinity to mammalian GluCl channels and that it does not cross the blood-brain barrier in humans.[69,70] **IVR** appears safe for human use, though there have been reports describing parasympathetic disturbances linked to the drug (salivation, dilation of pupils).[65,69,70] In addition to well-known anti-parasitic activity of **IVR,** this compound has been recently shown to exhibit potent anti-cancer activities and may have substantial value for the treatment of a variety of cancers.[6] **IVR** manifests antineoplastic activity related to its ability to inhibit multidrug resistance (MDR) proteins, the AKT/mTOR pathway and blocking the Wnt/TCF pathway (transcription factor of *T*-cells). **IVR** causes the degradation of PAK-1 (p21 – activated kinase), a main oncogenic kinase.[6,71] It is effective in colon cancer, *glioma multiforme* and melanoma as well as skin and lung cancer by Wnt-TCF blocking.[72]

IVR also increases the level of intracellular ROS (reactive oxygen species) in tumor cells, associated with oxidative stress and DNA damage.[72] Additionally, it preferentially inhibits cells resembling breast cancer stem cells (CSCs). Exposure to **IVR** reduces the expression of markers typical of stem cells: NANOG, OCT-4 and SOX2 (also the transcription factor of stem cells on the levels of mRNA and protein). This action is similar to that of **SAL** described earlier.[72]

Dou *et al.* have shown that **IVR** induces autophagy, a self-degrading effect in breast cancer.[73] Studies conducted on cell lines of breast cancer and on animal models, plus breast cancers of 20 patients have demonstrated reduced autophagy of breast cancer cells linked to reduced expression of PAK-1 due to the ubiquitin mediated degradation. The inhibition of PAK-1 reduced the phosphorylation of Akt, leading to the Akt/mTOR signalling pathway blocking, with the resulting decrease in tumor growth.

In other studies, **IVR** has been demonstrated to selectively inhibit SIN 3 – a protein that is associated with the pathogenesis of triple negative breast cancer.[74] Using stabilized cell lines of ovarian cancer, Hashimoto *et al.* have proved that **IVR** induces the inactivation of PAK-1 kinase, intensely inhibiting the growth of ovarian cancer.[75] The same authors have also observed an inhibition of malignant neuroma growth with no effect on the normal cell line. **IVR** was found to inhibit the YAP1 protein (yes-associated protein 1), whose the nuclear accumulation is linked to poor prognosis in gastric cancer. Using *in vitro* proliferation and animal model tests, **IVR** was shown to manifest a promising therapeutic potential in the inhibition of gastric cancer due to blocking of YAP1.[76]

Conclusions

Anticancer antibiotics have made an important contribution to the area of antitumor chemotherapeutics. Different classes of antibiotics such as anthracyclines (daunorubicin, doxorubicin, epirubicin), glycopeptides (bleomycins), indolocarbazoles (staurosporine), exhibit anticancer properties. The antibiotics which are currently used in anticancer chemotherapy act in various ways, influencing molecules and signalling pathways.

The last decade has seen increased awareness of known drugs including antibiotics repositioned as antineoplastic agents and the results of further clinical trials regarding their efficacy are awaited. Research in this field should not only focus on developing newer safer derivatives of known derivatives of doxycycline (**DOX**), salinomycin (**SAL**), monensin (**MON**) and ivermectin (**IVR**) but also investigate novel drug delivery systems (NDDS). The evidence presented in this short review article indicates that the discussed compounds (**DOX**, **SAL**, **MON**, **IVR**) could be promising scaffolds in the development of new therapeutic strategies in cancer therapy in the near future. Significant changes in the parent chemical structures of these compounds can also lead to progress in this field. Future clinical trials for testing the efficacy of these cancer-targeted antibiotics, in multiple cancer types, are now clearly clinically warranted. The use of antibiotics in anti-cancer therapy can also be cost-effective therefore making treatment more accessible in the developing world.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.04.045. These data include MOL files and InChiKeys of the most important compounds described in this article.

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