



## Possible use of antimicrobial agents in cancer therapy

Možnosti uporabe protimikrobnih učinkovin pri zdravljenju raka

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### Abstract

Cancer represents a major burden for health systems and is the second leading cause of death in the developed world. Despite major progress during past years, a lot of resources are still directed towards the identification of new antitumour drugs that would allow more efficient treatment, and prolonged patients' survival, lower cancer recurrence rate, and decreased side effects associated with cancer therapy, thus increased quality of life for cancer patients. However, the introduction of new drugs to clinical practice is associated with high costs. To keep the health system sustainable, the search for new indications of existing drugs has attracted a lot of attention in the field of oncology. Among others, antitumour activity has been shown for some antibiotics and other antimicrobial agents, including doxycycline, chloroquine, nitroxoline, and certain fluoroquinolones. In this review, we summarize various molecular mechanisms and tumour models used to define their antitumour activity. The latter has been demonstrated in a number of independent studies both *in vitro* as well as *in vivo*. Moreover, structures of existing compounds were used as lead compounds for the development of new derivatives and the identification of structural elements that improve antitumour activity. Together, these studies point to new possibilities for the use of already well-established drugs, which could expand their range, and, either, alone or in combination with existing therapy, improve the effectiveness of antitumour therapy.

### Izveček

Rak je veliko breme za zdravstvene sisteme in še vedno drugi najpogostejši vzrok smrti v razvitem svetu. Zato se, kljub velikem napredku v zadnjih letih, še vedno veliko sredstev usmerja v iskanje novih učinkovitejših protitumorskih zdravil, ki bi omogočila bolj učinkovito zdravljenje, daljše preživetje bolnikov z rakom, preprečila ponovni pojav raka in zmanjšala stranske učinke, povezane z zdravljenjem raka, s tem pa povečala kakovost življenja bolnikov z rakom. Uvajanje novih zdravil v klinično uporabo pa je hkrati povezano z vse večjimi stroški. Zato je zaradi velike potrebe po prepoznavanju novih protitumorskih zdravil iskanje novih indikacij za že obstoječa zdravila na področju onkologije pritegnilo veliko pozornosti. Pri tem se je med drugim protitumorsko delovanje pokazalo tudi za nekatere antibiotike in druge protimikrobne učinkovine. Slednje se je med drugim izkazalo za doksiciklin, klorokin, nitroksolin in nekatere predstavnike fluorokinolonov. V

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preglednem članku povzemamo različne molekulske mehanizme, uporabljene tumorske modele in raziskave, ki opredeljujejo njihovo protitumorsko delovanje. To so prikazale številne neodvisne študije tako *in vitro* kot *in vivo*, strukture obstoječih učinkovin pa so se hkrati uporabile kot spojine za razvoj novih derivatov in identificiranje strukturnih elementov, ki izboljšajo protitumorsko delovanje. Te raziskave tako kažejo na nove možnosti za uporabo že dobro znanih učinkovin, ki bi lahko razširile svoj spekter uporabe in samostojno ali v kombinaciji z že obstoječim zdravljenjem izboljšale uspešnost protitumorskega zdravljenja.

## 1 Introduction

After cardiovascular diseases, cancer is one of the biggest health problems and the second leading cause of death in the developed world, including Slovenia. According to the World Health Organization (WHO), 9.6 million people died of cancer worldwide in 2018 (1). In addition to being a major health problem, cancer also presents a major social and economic burden in today's society (1). Despite major advances in cancer treatment in recent years, we still face many challenges (2). The key challenges in developing more effective therapies are to prevent cancer recurrence, avoid therapy resistance and reduce the many side-effects associated with most of the therapeutic approaches currently used in clinical practice, which further reduce the quality of life for cancer patients (3). The development of new drugs is extremely costly and the costs only continue to increase, which is why the number of new drugs on the market has declined over the last decade, despite high investment in research and development (4).

In response to these high costs and the small number of drugs that successfully pass all stages of testing and reach the market, more and more attention is being dedicated to finding new indications and uses for drugs already on the market (so-called drug repurposing, or repositioning) (5,6). The toxicological profiles, tolerability, pharmacokinetic and pharmacodynamic properties of these drugs are already well known, and these are the main advantages that increase their potential for use in cancer treatment. For existing drugs, their dosage regimen, pharmacological properties and their interactions with other drugs are known (2,5-7). Due to the large amount of known data, the use of existing active substances for new indications is therefore associated with shorter time and lower costs required for the substances' successful translation into clinical use (2,5,6).

When looking for new indications for existing drugs, the first step is to investigate in detail their mechanism of action on new targets and to test their efficacy and safety in clinical studies, as the use of a drug for a new indication may be associated with new side-effects that are not yet known (5,6). However, for existing drugs, it

is possible to move more quickly into phases 2 and 3 of clinical trials due to the large amount of information already obtained in previous pre-clinical and clinical trials (2). In addition to drugs that are in clinical use, active substances that have been shown to be safe in clinical trials but have not entered clinical use for other reasons are also well suited to the search for new indications (6).

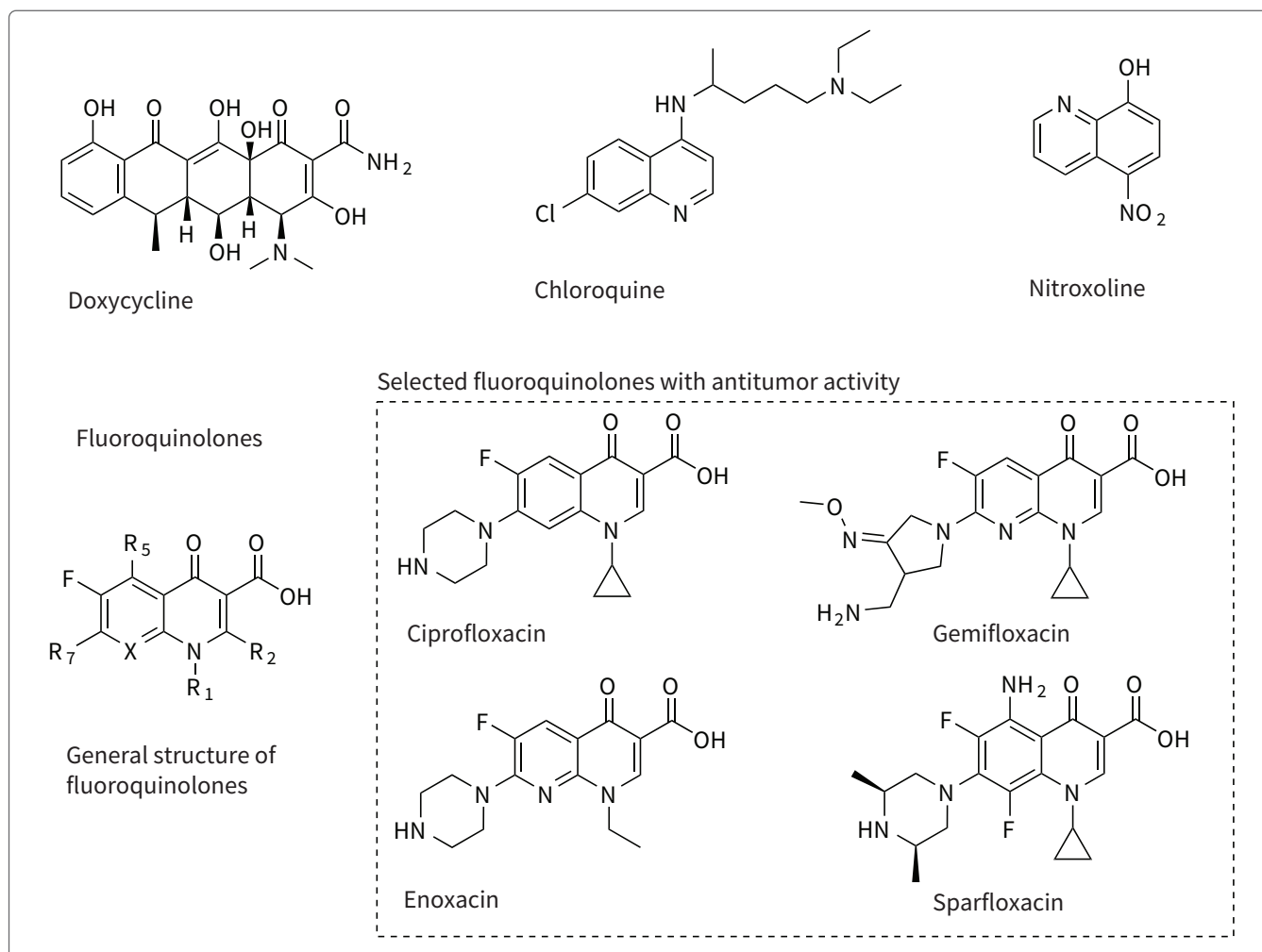
The drug repurposing has attracted particular attention in the field of oncology, due to the great need to identify new, more effective active substances (3,5). It is now known that many active substances that are used to treat other indications and differ in both their structure and mechanism of action, can also show antitumour activity. Antitumour activity has already been confirmed for thalidomide, metformin, acetylsalicylic acid, statins, raloxifene, tamoxifen, certain antidepressants and antipsychotics, and many other drugs (2,5).

In addition to these, some antibiotics and antimicrobials have also shown antitumour activity, including doxycycline, chloroquine, nitroxoline and fluoroquinolones (Figure 1). In this review article, we will focus on the potential use of some antimicrobial agents for cancer treatment and present possible mechanisms for their antitumour activity.

## 2 Doxycycline

Doxycycline (Figure 1) is a tetracycline antibiotic used to treat a wide range of bacterial infections (5,8). It is also used prophylactically in malaria prevention and is effective in the treatment of malaria in combination with quinine. Doxycycline is rapidly absorbed and rapidly crosses the blood-brain barrier following oral administration, its pharmacokinetic properties and half-life are known, and it is generally well tolerated by patients following administration (8).

In recent years, several studies have shown that, in addition to its potent antibacterial activity, doxycycline has antitumour activity. Antitumour activity of doxycycline has been demonstrated in various types of cancer, including breast, cervical, ovarian, prostate, lung, oral,



**Figure 1:** Structures of antimicrobial agents exhibiting antitumour activity.

pancreatic and duodenal cancer, colorectal cancer, melanoma and leukaemia (5,8). More than 30 years ago, in leukaemia cells, doxycycline treatment was shown to inhibit T-cell proliferation and lead to their complete destruction, indicating that the mechanism of action of doxycycline depends on its concentration and the degree of tumour progression (9).

Several mechanisms by which doxycycline inhibits tumour formation and progression have been described (8). As one of its mechanisms of action, doxycycline inhibits the matrix metalloproteinases MMP-2 and MMP-9, which are important players in various processes of cancer onset and progression. It further inhibits MMP activity by increasing the expression of tissue inhibitor MMP-2 (TIMP-2) (5,10-12). In animal models, doxycycline has been shown to inhibit metastases by inhibiting MMP-2 and MMP-9 (5,13,14). Furthermore, doxycycline inhibits cancer progression through a number of signalling pathways (5,8). Among others, it directly

inhibits protease-activated receptor 1 (PAR-1) signalling and downregulates VEGF signalling (10,15,16). Doxycycline also inhibits tumour cell adhesion, migration and invasion by inhibiting the expression and phosphorylation of adhesion molecules such as focal adhesion kinase (FAK) in leukaemia cells and melanoma cells (11,12). It also reduces angiogenesis and cancer cell migration by inhibiting the expression of interleukin 8 (IL-8) (17). By inhibiting doxycycline signalling pathways, it inhibits tumour cell invasion, epithelial-mesenchymal transition of tumour cells and metastasis formation (5,8,14).

Doxycycline induces apoptosis and inhibits cell proliferation (8,17). Inhibition of apoptosis involves both the mitochondria-dependent and caspase-dependent pathways (8,18). Doxycycline has been shown to induce cell cycle arrest in the G2/M phase in prostate cancer cells (19). According to the results in different types of cancer, doxycycline promotes apoptosis and inhibits cell proliferation by inhibiting various molecular targets that

are importantly involved in these processes (8).

Doxycycline is as an antitumour agent of particular interest for its action against cancer stem cells (8). In cervical cancer, doxycycline has been shown to reduce the expression of cancer stem cell markers such as SOX-2, OCT-4, NANOG and NOTCH (20).

The anti-tumour activity of doxycycline has also been evaluated in clinical studies. However, in a phase 2 clinical study in patients with breast cancer and bone metastases, doxycycline did not show significant effects (21), while in a small pilot clinical study involving 15 patients, it showed a positive effect in almost 90% of persons with early forms of breast cancer, who received doxycycline 14 days prior to surgery. The expression of cancer stem cell markers (22) decreased in tumour samples following doxycycline treatment, showing promising possibilities for its use in cancer therapy. However, this needs to be further evaluated in a larger clinical study.

Doxycycline is also effective in combination with other known chemotherapeutic agents, making it a promising active substance already in clinical use that could be used in oncology to treat cancer alone or in combination with conventional therapy to treat cancer and prevent its recurrence (8).

### 3 Chloroquine

Chloroquine (7-Chloro-4-(4-Diethylamino-1-Methylbutylamino)-Quinoline) (Figure 1) and its analogue hydroxychloroquine are used for the prevention and treatment of malaria (23-25). Chloroquine is an old drug, synthesised as early as 1934. It was used as the compound of choice in the treatment of malaria until the emergence of resistant strains (24). Later, its use was extended to the treatment of rheumatoid arthritis and lupus erythematosus (23,25). Chloroquine's anti-tumour activity was first observed in the 1970s when, during a study of its effect against malaria, the incidence of Burkitt's lymphoma was reduced in a group receiving chloroquine. However, it was only later that the latter attracted more interest (26). To date, the antitumour activity of chloroquine and hydroxychloroquine has been demonstrated in several *in vitro* and *in vivo* studies in mouse models with different types of cancer, including breast, liver and colon cancer, glioblastoma and melanoma. It reduced tumour progression and slowed tumour growth (25). In a mouse model of liver cancer, a reduction in the number and size of metastases in the lungs was also shown (27).

As antitumour agents, chloroquine and hydroxychloroquine can be used individually or as supportive

therapies to improve the effect of chemotherapy and radiotherapy in different types of cancer. They are currently involved in several clinical studies (23-25). As of February 2021, 21 clinical trials for chloroquine in cancer and 89 clinical trials for hydroxychloroquine have been registered on clinicaltrials.gov, which are at various stages, many of the trials still ongoing, with results available for only some of the trials (28). In clinical trials, patients mainly received chloroquine and hydroxychloroquine in combination with other antitumour agents (25,28). Results to date show that for some types of cancer, patients receiving chloroquine or hydroxychloroquine as supportive therapy have prolonged survival compared to the control group (25,29). A significant improvement in survival of patients receiving chloroquine in addition to radiotherapy and chemotherapy was already observed in one of the first clinical trials, which started in May 1998, in 18 patients with glioblastoma (30). A similar improvement in survival rate of glioblastoma patients who received chloroquine in addition to conventional chemotherapy and radiation, when compared to those who received placebo, was also observed in a clinical study conducted in October 2000. The study included 15 patients in each group (31). The retrospective study pooled all data obtained over a 5-year period from 41 glioblastoma patients who received chloroquine as adjuvant therapy and were not included in the aforementioned study (31). In two independent phase 2 clinical studies (32) and a prospective study, in one group (33) of patients with brain metastases, chloroquine was shown to increase the sensitivity of cells to radiotherapy in patients with brain metastases. It prolongs both the metastasis progression-free survival and overall survival, suggesting the possibility of its use as a supportive treatment for radiation therapy (25). Furthermore, several clinical studies of different types of cancer have shown that the addition of hydroxychloroquine to conventional therapy improves response to therapy by inhibiting autophagy (25).

Due to their long-standing use in clinical practice, the side-effects of chloroquine and hydroxychloroquine are well known. Serious side effects rarely occur after their short-term use. However, adverse toxic effects, in particular nephrotoxicity, may occur after prolonged use at higher doses and when taken concomitantly with other antitumour agents (23,25,34). The presence of an additional hydroxy group in hydroxychloroquine significantly reduces toxicity and affects the pharmacokinetic properties of the compound, while the differences in antitumour activity of chloroquine and hydroxychloroquine are not fully known (25,35).

Chloroquine and hydroxychloroquine are highly soluble and are rapidly and well absorbed after oral administration (24,25). Chloroquine is a weak base; at physiological pH 7.4, it is found in an unprotonated or partially protonated form, which allows it to pass well through cell membranes and enter acidic cell compartments. As a weak base, chloroquine is protonated and retained in lysosomes after entering the acidic environment of lysosomes. This also results in an increase in the pH of the lysosomes, which reduces their function, as most of the proteins in lysosomes have their optimum activity at acidic pH (23-25).

The retention of chloroquine inside cells leads to inhibition of autophagy, one of the key mechanisms of its antitumour activity (23-25,34). Chloroquine and hydroxychloroquine inhibit autophagy by inhibiting autophagosome fusion and degradation within lysosomes due to an increase in pH (25,36). It also affects autophagy via the PI3K/Akt/mTOR signalling pathway, where it has a synergistic effect with inhibitors of the AMPK signalling pathway and the activated Janus kinase-2 (JAK2)/STAT3 signalling pathway (23,25,37). In addition to early autophagy, chloroquine also inhibits late stages of autophagy and can induce cell death even under conditions where inhibitors of early stages of autophagy cannot (23).

Several independent studies have also shown an antitumour activity of chloroquine, independent of inhibition of autophagy (24). One of the mechanisms is modulation of cellular metabolism, affecting amino acid metabolism, glucose metabolism and mitochondrial metabolism (23,24). Chloroquine and hydroxychloroquine also affect apoptosis and cell cycle arrest by regulating molecules importantly involved in these processes (25). Moreover, chloroquine can also induce cell death via lysosome-mediated apoptosis by acting on cathepsins (24). Chloroquine is also involved in the regulation of cellular stress and modulators of inflammation by regulating proteostasis via the ubiquitin/proteasome system (23). Next, it affects cell proliferation and survival by acting on glutamate dehydrogenase activity (25). Chloroquine also exerts antitumour effects by normalising tumour vasculature by reducing vascular density and increasing tight junction formation (25,38).

Several molecular targets of the antitumour activity of chloroquine and hydroxychloroquine are known, including the NF- $\kappa$ B transcription factor, p53 tumour suppressor factor and CXCL12/CXCR4 signalling pathways (24,25). By inhibiting the CXCL12/CXCR4 signalling pathway, chloroquine reduces phosphorylation of the signalling molecules ERK and STAT3 (25) and thereby

also acts on cancer stem cells (23). Additionally, in a bile duct cancer cell line, chloroquine inhibits tumour cell metastasis by affecting hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ), VEGF and the epithelial-mesenchymal transition process (25).

By inhibiting autophagy, chloroquine can bypass the limitations of existing chemotherapy, as autophagy is, among other things, one of the key processes for survival and resistance to existing chemotherapy in cancer stem cells and facilitates the epithelial-mesenchymal transition of tumour cells. This is one of the processes by which cells can acquire the properties of cancer stem cells (23,25). In cancer stem cells, chloroquine at a concentration of 20  $\mu$ M was shown to affect stemness by specific action on autophagy (23,39). It was further shown that in cancer stem cells, low micromolar concentrations of chloroquine affect JAK2/STAT3, Hedgehog and CXCR4 signalling pathways, DNA methyl transferase expression and expression of stemness markers (23,25,40). Chloroquine has also been shown in several studies to be involved in regulating the immune system and the inflammatory response through various mechanisms (23,24).

When in concomitant use, chloroquine improves the effect of chemotherapy and radiotherapy. By inhibiting autophagy, it increases the sensitivity of tumour cells to radiotherapy, which has been shown in various types of cancer including breast cancer, lung cancer, and glioma. A meta-analysis of studies has shown that addition of chloroquine to chemotherapy or radiotherapy increases patient survival and prolongs disease-free survival (23). Chloroquine and hydroxychloroquine have a synergistic effect with BET inhibitors already at low concentrations and induce cancer stem cell apoptosis in pancreatic cancer and acute myeloid leukaemia (23,41,42). Chloroquine also increases the efficiency and killing of tumour cells in combination with cell cycle inhibitors and leads to apoptosis together with proteasome inhibitors, as shown in a mouse model of liver cancer (23,43). Furthermore, inhibitors of the AMPK signalling pathway and the STAT3 signalling pathway have also been shown to have a synergistic effect on cell death (23).

Despite promising results from pre-clinical studies, chloroquine significant antitumour effect in clinical studies was not yet shown. Key reasons for this could be that chloroquine is less able to enter tumour cells due to the acidic extracellular environment of tumours, and that chloroquine has an important role in regulating the immune response by inhibiting autophagy in lysosomes (23,25). In addition, the different dependence of tumours on autophagy results in different responses to

chloroquine therapy in different types of tumours (25). At the same time, it was shown that high concentrations of chloroquine are required to achieve a therapeutic effect on autophagy inhibition in leukaemia, which limits its use. Nevertheless, chloroquine has been shown to affect metastatic tumour cells that are not in an acidic tumour environment and are particularly sensitive to lysosomal inhibition. This reduces the possibility of metastasis formation (23). In triple-negative breast cancer, chloroquine successfully kills cancer stem cells and reduces the ability of tumours to metastasise in both *in vitro* and *in vivo* mouse models (44). Chloroquine has been shown to act primarily on tumour cells with cancer stem cell properties. Therefore, its use is particularly appropriate in the context of concomitant use of agents targeting differentiated tumour cells (23,25).

#### 4 Nitroxoline

Nitroxoline (5-nitro-8-hydroxyquinoline) (Figure 1) is a well-known antimicrobial agent used in the treatment of urinary tract infections. Nitroxoline's antimicrobial activity was discovered in the 1950s and it was fast used in clinical practice (45). Despite it being used for a long time, bacteria have still not developed resistance to nitroxoline (45,46). Nitroxoline is effective against most Gram-positive and Gram-negative pathogenic urinary tract bacteria, mycoplasmas (*M. hominis*, *Ureaplasma urealyticum*) and human pathogens *Candida spp.* (45). In addition, nitroxoline showed efficacy against most other Gram-negative bacteria and was also effective against Gram-positive bacteria, suggesting potential use as an antimicrobial agent for other indications in addition to the treatment of urinary tract infections (47). Nitroxoline exerts its antimicrobial activity mainly by chelation of various bivalent ions (45,48,49). In this way, it stabilises lipopolysaccharides on the bacterial surface, which increases the hydrophobicity of the bacterial surface and reduces their adhesion (45,48,49). By this nitroxoline also inhibits RNA synthesis during yeast cell division (45,50), while at lower concentrations, it inhibits the formation of bacterial biofilms (45,46,51). As an established antimicrobial agent, nitroxoline has well-known pharmacokinetic and pharmacodynamic properties (45,52-54). Nitroxoline is completely and rapidly absorbed in the urinary tract after oral administration (52,54), and only minor and tolerable side effects have been observed, suggesting that nitroxoline administration is safe (45).

Antitumour activity of nitroxoline has been demonstrated by several studies to date. Its antitumour activity

was first identified in the search for methionine aminopeptidase type 2 (MetAP2) inhibitors, which could be used as new anti-angiogenic agents (55). Nitroxoline has been shown as potent inhibitor of MetAP2 *in vitro*, inhibiting endothelial cell proliferation and angiogenesis both *in vitro* and *in vivo*. In addition, nitroxoline induces senescence and inhibits HUVEC endothelial cell proliferation by simultaneously inhibiting MetAP2 and Sirtuin 1 (Sirt1). By Sirt1 inhibition, nitroxoline increases the amount of acetylated tumour suppressor p53, resulting in reduced angiogenesis. At the same time, nitroxoline significantly reduced the size of breast tumours in a mouse model and inhibited the growth of bladder cancer in an orthotopic mouse model (55). Nitroxoline was later shown to reduce the expression of proteins associated with the epithelial-mesenchymal transition of tumour cells and the amount of myeloid-derived suppressor cells (MDSCs) in peripheral blood in bladder cancer (56). Furthermore, nitroxoline was identified in a second, independent study by high throughput compound library search and biochemical evaluation of the best hits as a potent, reversible and non-covalent inhibitor of the lysosomal cysteine peptidase cathepsin B (57), which plays an important role in the initiation and progression of cancer, its invasion and metastasis, and is known to be a promising target for the development of novel antitumour agents (58-60). The crystal structure of the nitroxoline-cathepsin B complex shows that nitroxoline binds to the active site cleft of cathepsin B and thereby selectively inhibits its endopeptidase activity at low micromolar concentrations (57). In various functional assays in cell lines and murine tumour models, nitroxoline significantly reduced extracellular matrix degradation, tumour cell invasion, metastasis and endothelial tube formation in an *in vitro* model of angiogenesis (58).

Nitroxoline acts as an antitumour agent and inhibits tumour cell migration by reducing the expression of the tumour transcription factor FOXM1, an important regulator of cancer progression. In the same study, nitroxoline was also shown to reduce the expression of MMP-2 and MMP-9, which are important players in tumour migration and invasion and whose expression is controlled by, among others, FOXM1. In addition to inhibition of MMP-2 and MMP-9 by inhibiting FOXM1, molecular interaction studies suggest that nitroxoline also directly interacts with the catalytic domains of MMP-2 and MMP-9 (61).

Furthermore, several studies have shown that nitroxoline inhibits tumour cell proliferation. This has been shown in cells from a variety of cancer types, including malignant glioma, multiple myeloma, glioblastoma,

leukaemia, prostate cancer, bile duct cancer (cholangiocarcinoma), pancreatic cancer, small cell lung cancer and bladder cancer (56,62). The antitumour activity of nitroxoline has been shown to be dose-dependent, as well as dependent on the period of administration, with lower doses leading mainly to cell cycle arrest in the G0/G1 phase, while higher doses promote apoptosis. Nitroxoline promotes apoptosis and cell cycle arrest in the G0/G1 phase by acting on various molecular targets that are importantly involved in these processes (62). The antitumour activity of nitroxoline on pancreatic cell lines was further improved when nitroxoline was co-administered together with nelfinavir, a competitive inhibitor of HIV aspartate peptidase, and other antiviral agents for the treatment of HIV infection (63). However, according to data available at [clinicaltrials.gov](http://clinicaltrials.gov), the antitumour activity of nitroxoline has not yet been evaluated in clinical studies (28).

To further improve the antitumour activity of nitroxoline, several new derivatives with different structural modifications have been prepared by structure-based chemical synthesis, based on the structure of nitroxoline (62). By modifying various substituents, a large number of nitroxoline derivatives was prepared with the aim to improve cathepsin B inhibition (57,64–66), its anti-angiogenic action by inhibiting MetAP2 and SIRT1 (67) and inhibition of BET proteins, which bind competitively to BRD-BD1 and are involved in maintaining chromatin stability, thereby controlling cell cycle progression (62,68,69). The preparation of new derivatives also revealed the structural features needed to improve activity on a particular target (62). Additionally, the antitumour activity of nitroxoline and the inhibition of cathepsin B were also improved by the preparation of organoruthenium complexes with nitroxoline and its derivatives (70). The antitumour activity of nitroxoline, and in particular its uptake into lysosomes, was also improved by its incorporation into nanoparticles composed of bovine serum albumin, copper ions and nitroxoline (BSA/Cu/NQ) (71).

Nitroxoline is a promising, known active substance that could be used to treat various types of cancer. Nitroxoline is also an interesting compound for the development of new derivatives with improved antitumour activity and selectivity for specific targets.

## 5 Fluoroquinolones

Fluoroquinolones (Figure 1) are the largest group of antimicrobial agents. Fluoroquinolones inhibit bacterial DNA duplication and transcription, either by inhibiting

bacterial DNA gyrase or topoisomerase II (4). Nalidixic acid was discovered as the first quinolone with antimicrobial activity in the 1960s. The discovery was followed by further development and optimization of quinolones. They can be divided into four generations based on their pharmacokinetic and antimicrobial properties. Initially, quinolones were only effective against Gram-negative bacteria, but as their structure has been optimized, their spectrum of activity has broadened. Fourth-generation fluoroquinolones are effective against Gram-negative and Gram-positive bacteria, anaerobes *Pseudomonas sp.* and atypical bacteria (72). In addition to their antibacterial activity, some fluoroquinolones have been shown to be effective in combination with chloroquine in the treatment of malaria. They have also been shown to work against some other parasites, such as *Trypanosoma brucei* and *Toxoplasma gondii* (4). In addition to their direct effect on bacterial growth, fluoroquinolones contribute to antimicrobial activity by their immunomodulatory properties. Fluoroquinolones, especially those of the newer generation such as ciprofloxacin, norfloxacin and ofloxacin, inhibit cytokine synthesis even at low concentrations. They have also displayed an anti-inflammatory response and a protective role against lipopolysaccharide-induced liver injury (4,73).

Several independent studies have demonstrated antitumour activity of fluoroquinolones, with multiple different mechanisms of antitumour action. Different fluoroquinolones have been shown to stop tumour progression with cell cycle arrest, mainly in the S/G2 phase, and by inducing tumour cell apoptosis (4). Ciprofloxacin has been shown as the most potent inducer of apoptosis and has induced apoptosis in cells from a variety of cancers, including prostate, bladder, pancreatic, colorectal cancer and others (4,74,75). In contrast, a more recent study of ciprofloxacin showed the opposite effect on cancer progression, showing it promotes the formation of cells with a cancer stem cell phenotype in human small cell lung cancer cells. Here, ciprofloxacin was shown to increase the expression of stemness markers and proteins required for cell self-renewal (76). Among fluoroquinolones, only gemifloxacin has been shown to inhibit tumour cell metastasis by inhibiting migration and invasion through inhibition of TNF $\alpha$ -stimulated NF $\kappa$ B activation (77,78). Additionally, sparfloxacin was shown to have an impact on invasion and migration of colon cancer tumour cells (79). Enoxacin, due to its unique structure, shows antitumour effects by inhibiting the production of microRNA (miRNA), because it is oncogenic and contributes to tumour progression (4,80,81). Several independent studies showed fluoroquinolones, including

ciprofloxacin, fleroxacin, moxifloxacin and enoxacin, improve the antitumour effects of chemotherapeutic agents when co-administered with existing chemotherapeutic agents used in clinical practice (4). Several clinical trials are also currently underway to test the anti-tumour activity of fluoroquinolones in combination with established therapies in different types of cancer, such as bladder cancer and acute myeloid leukaemia. The final results have yet to be published (28).

The antitumour activity of fluoroquinolones can be further enhanced by the formation of complexes with metal ions. The latter was first demonstrated for complexes of norfloxacin with copper, which showed improved antitumour activity against leukaemia cells (82). This led to the preparation of metal complexes with other fluoroquinolones, further improving the anti-tumour activity against different cancer cell lines compared to the initial compound. The gold-ruthenium complexes proved to be particularly effective in this regard, with antitumour activity mainly against metastatic tumour cells, but without toxic effects on normal, healthy cells. The antitumour activity of ruthenium complexes is thought to be due to action on multiple targets, while the antitumour activity of gold complexes is thought to be mainly due to alteration of mitochondrial function and inhibition of protein synthesis. Additionally, the introduction of nitrogen adducts into metal complexes with fluoroquinolones inhibits their elimination from cells, thus allowing for increased retention of active compounds in tumours (4).

Moreover, research on fluoroquinolones is also focused on finding structural modifications to the molecules of existing fluoroquinolones that could further improve their antitumour activity. The presence of major functional groups at the C-7 site has been shown to be beneficial for enhancing antitumour activity (4,83).

The large group of fluoroquinolones represents a promising group of antimicrobial drugs whose use could be extended to the treatment of cancer due to their antitumour activity. At the same time, structural modifications of existing fluoroquinolones could lead to the

development of new molecules with improved antitumour activity.

## 6 Conclusion

Successful cancer treatment still faces many challenges. One of the important steps is to develop new drugs to avoid the limitations of current therapeutic approaches, such as resistance to therapy and the many side effects, associated with existing chemotherapeutics. However, the high costs associated with bringing a new drug to market have recently led to an increasing focus on finding new indications for existing drugs. It was shown that some antimicrobial drugs that have long been used successfully in clinical practice have antitumour activity. Among the latter, antitumour activity was also demonstrated for doxycycline, chloroquine, nitroxoline and fluoroquinolones that are discussed in detail in this review article. Their antitumour activity has been demonstrated in a number of tumour models and on different cancer types, and different mechanisms of antitumour action have been described. Known antimicrobial agents with antitumour activity also represent interesting compounds for the development of new derivatives with improved antitumour activity. Despite the numerous studies confirming the antitumour activity of anti-microbial agents, further clinical studies are needed to confirm their efficacy and allow their successful introduction into clinical practice. Attention should also be paid to possible interactions with existing therapies, in particular immune therapies and antibiotic therapy, which cancer patients often receive because of infections that result from their weakened immune system. Drug repurposing is therefore a promising approach that will allow faster and more efficient development of new anti-tumour therapies and improve the success of cancer treatment.

## Conflict of interest

None declared.

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