CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

In vitro growth-inhibitory effects of quinoline compounds and their derivatives against intestinal bacteria associated with colorectal cancer

MASTER'S THESIS

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DIPLOMA THESIS ASSIGNMENT

Štěpánka Rikanová

Tropical Forestry and Agroforestry

Thesis title

In vitro growth-inhibitory effects of quinoline compounds and their derivatives against intestinal bacteria associated with colorectal cancer

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Objectives of thesis

The aim of this thesis is to evaluate in vitro growth-inhibitory effects of quinoline compounds and their derivatives against intestinal bacteria associated with colorectal cancer (CRC).

Methodology

The antibacterial activity will be determined against the main representatives of colorectal cancer-causing bacteria (CRC). Strains that are going to be used in this research include: Clostridium septicum, Bacteroides fragilis, Escherichia coli, Fusobacterium necrophorum, Streptococcus bovis, and Peptostreptococcus anaerobius.

Broth microdilution method will be used for analysis, using 96-well microtiter plates according to Clinical and Laboratory Standards Institute (CLSI) guidelines, modified by Cos et al. (Cos et al. 2006).

Dimethyl sulfoxide will be used as a negative control and antibiotics, e.g.: ceftriaxone, ciprofloxacin, chloramphenicol, metronidazole, tetracycline, and vancomycine will be included in the as positive controls. Bacterial growth will be determined by measuring the optical density. All tests will be performed as at least three independent experiments, each in triplicate, with the results presented as median/modal values.

The proposed extent of the thesis

30-50

Keywords

the Antimicrobial activity, Quinoline compounds, MIC, In-vitro, Colorectal cancer

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Recommended information sources

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Dean

Prague on 21. 04. 2023

Declaration

I hereby declare that I have done this thesis entitled independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague

Date:

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Štěpánka Rikanová

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Abstract

Colorectal cancer (CRC) is one of the most common solid tumours of the colon or rectum. Recently the role of gut microbiome was observed to plays an important role in the development of CRC. Although no specific therapeutic measures have yet been established for the treatment of these pathogens in relation to the potential risk of CRC, chemotherapy based on conventional antibiotics of other agents (e.g. natural compounds) is being considered as a possible treatment. Current research is therefore focused on the discovery of new compounds, for example, derived from natural products. Quinolines are organic naturally occurring compounds in plants used in pharmacology and agriculture for their antimicrobial, anticancer and growth promoting properties. However, the effects of quinoline compounds on growth of bacteria association with CRC have not yet been investigated yet. In this study, six quinoline-based agents, namely carbadox, chloroxine, ferron, nitroxoline, oxyquinoline and olaquindox and six conventional antibiotics used to treat gastrointestinal diseases (ciprofloxacin, ceftriaxone, metronidazole, vancomycin, chloramphenicol, tetracycline) have been assayed for their *in vitro* growth-inhibitory effects against six pathogenic bacteria associate with CRC (*Bacteroides fragilis*, *Clostridium septicum, Escherichia coli, Fusobacterium necrophorum, Peptostreptococcus anaerobius, Streptococcus bovis*) using broth microdilution method. Antibacterial effect appeared to be strong for the fluoroquinoline antibiotic ciprofloxacin (MIC from 0.0625 to 2 μg/ml) and ceftriaxone (MIC from 0.025 to 32 μg/ml) from the cephalosporin group. The most effective quinoline compounds were the quinoxaline 1,4-dioxide carbadox (MIC ranging from 0.5 to 32 μg/ml) and the 8-hydroxyquinoline nitroxoline (MIC from 4 to 16 μg/ml), which were effective against all bacterial strains. These findings provide arguments for further investigation of quinoline compounds as prospective structures for modulation of gut microbiota composition in regard to suppressing of growth of bacteria associated with CRC.

Key words: *quinoline compounds, antibacterial, MIC, broth microdilution method*

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1. Introduction

1.1. Colorectal cancer (CRC)

The gastrointestinal (GI) system has several sophisticated and autonomous functions such as digestion, absorption, excretion, and protection (L. K. Cheng et al., 2010). The GI tract is divided into upper tract and lower tract with the small and large intestine, while the large intestine is divided into the appendix, ascending, transverse, descending and sigmoid colon, rectum, and anal canal (FitzPatrick & Keshav, 2020). Although, there are many diseases and conditions that can affect the gastrointestinal system, CRC, sometimes called large bowel cancer, is one of the most common solid tumours of the colon or rectum. Colon and rectal cancers therefore share many common features and are classified in one group (American Cancer Society, 2020). The causes of CRC are mainly poor lifestyle patterns and genetics. Another cause, chronic inflammation, is thought to be responsible for up to 20% of all tumours (Sánchez-Alcoholado et al., 2020).This disease develops from intestinal epithelial cells in the colon and rectum (Lichtenstern et al., 2020). The process begins with hyperproliferation of the colorectal mucosa and leads to adenomas, carcinoma and cancer itself in three main phases. The first phase is initiation, when irreversible changes in DNA occur and predispose the affected cells to subsequent neoplastic transformation. Consequently, promotion follows with abnormal tissue growth (neoplasm). In the final stage of progression, further genetic and epigenetic changes occur with expansive and transformative tendencies, when benign tumour cells (adenoma) turn into malignant tumour cells and acquire aggressive properties and metastatic potential (Keum & Giovannucci, 2019; Coleman et al., 2020). From "adenoma to carcinoma," genetic changes develop over the course of 10 years or more (Pitchumoni & Broder, 2020).

Specific groups of microbes or microbial communities have been implicated in the development of CRC. The process can start from microbiome alternations (Sears & Garrett, 2014), which could be in the initial phases of CRC employed in screening people for monitoring and prevention. Influences of microbiota have also been observed on therapeutic pharmaceutical efficacy, toxicity, and immunotherapies (Song et al., 2020). Recently, it has been demonstrated that gut microbiota can alter CRC

susceptibility and progression by modulating mechanisms such as inflammation and DNA damage, and by producing metabolites involved in tumour progression or suppression (Sánchez-Alcoholado et al., 2020). An example of a disorder caused by the microbial community is inflammatory bowel disease. In this disease, host genetics is probably the main factor that allows the development of a dysbiotic (in other words, dysfunctional, disease-causing) microbiome. The following cycle of host gene-microbiota interactions causes intestinal and potentially extraintestinal disease (Sears & Garrett, 2014).

1.1.1. Epidemiology

CRC is a major disease in developed countries, and its burden is increasing in middle- and low-income countries due to westernization (Xi & Xu, 2021). In 2019, CRC ranked as the 15th out of the top 20 causes of death worldwide, accounting for 1.7 % of global mortality and the fourth among all malignant neoplasms (World Health Organization, 2020). In 2020, it ranked third in incidence and second in mortality among the top 10 cancer types worldwide (Sung et al. 2021). In the same year, more than 5.25 million people worldwide (with 5-year prevalence) were living with CRC (Xi & Xu, 2021). The highest incidence of CRC is in European regions, Australia, New Zealand, and North America, whereas most regions in Africa and South and Central Asia tend to have a low prevalence. However, when considering rectal cancer only, the incidence rates in Eastern Asia rank among the highest (Sung et al. 2021). The future projections show an increase in incidence of 64% in China, from 0.56 million in 2020 to 0.91 million in 2040, and in the United States, from 0.16 million in 2020 to 0.21 million in 2040, implying that one in every 23 males and one in every 25 females will develop CRC (Siegel et al. 2020). Currently, CRC in men is the third most diagnosed cancer in the world (29 cases per 100 000 population), as well as in 11 countries (Saudi Arabia, Qatar, Kuwait, Bahrain, United Arab Emirates, Oman, Yemen, Ethiopia, Slovakia, Singapore, and Brunei). In women, it is the second most common globally, with high mortality rates in Japan, Spain, Croatia, Estonia, and Belarus (Sung et al., 2021).

The potential risk factor is likely related to a high human development index and western lifestyles (Sawicki et al., 2021). Accordingly, in native Africans, there was a scarce risk of CRC (1 case per 100,000 people). However, in African Americans, it accounted for 65 cases per 100,000 people (Coleman et al. 2020). Possible cause could

be due to the higher diversity of gut microbiome of endemic tribes caused by their traditional lifestyle and diet compared to the populations of Western countries (Martínez et al. 2015).

In the Czech Republic, CRC ranked third in incidence until 2014. However, its occurrence declined in the following years, and in 2019, it was already the eighth most common cause of death (World Health Organization, 2020). The decline or stabilization in CRC incidence is mainly due to the introduction of screening programs, improved treatment, and a healthier lifestyle (Xi & Xu, 2021). In the Czech Republic, the implementation of the screening programs started in 2000, when CRC was the fourth leading cause of death (Fidler et al., 2017).

1.1.2. Causes

The cause of CRC can be based on lifestyle patterns, family or personal medical history, or other factors. In the case of lifestyle, the main precursors of cancer risk are physical inactivity leading to overweight and obesity, cigarette smoking, and alcohol consumption. Dietary habits are another area where risks are mainly linked with high consumption of red and processed meat, a lack of fibre in the diet, or low consumption of fruit and vegetables (Sawicki et al., 2021). Unhealthy eating habits can increase the risk of CRC development by up to 70 % (Mármol et al., 2017).

Based on family and personal history, diseases such as diabetes mellitus, colon polyps, inflammatory bowel disease (Crohn's disease; ulcerative colitis), or cholecystectomy, as well as a previous family history of cancer, can lead to a higher risk of this malady. Other factors in the development of CRC that need to be considered include age, gender, race, and socioeconomic factors (Sawicki et al., 2021). It has recently been suggested that gut microbiome also plays an important role in the development of CRC. Microorganisms in GI tract are affected by the aforementioned risk factors, causing alterations in their composition and function leading to changes in specific metabolic and immunological processes (Song et al., 2020).

1.2. CRC-causing bacteria

Recently, numerous studies have demonstrated the presence and likely influence of specific gut microorganisms on the development of CRC. It enhances or mitigate the risk of this disease, either as individual microbes or as microbial communities with a collective effect (Brennan & Garrett, 2016). Thus, the goal of today's research is to better understand the relationships between the human gut microbiome as well as the positive and negative effects these relationships have on our health. In the case of the negative effects, there have been reports of gut bacterial influence on CRC development, specifically induced by dysbiosis, which is the disruption of the normal microbiome relationship between the host and the intestinal microbiota (Sánchez-Alcoholado et al., 2020). In the process of dysbiosis, pathogenic metabolites are produced by the microbiota, leading to higher levels of bile acids and, subsequently, to carcinogenesis. Moreover, the metabolites could trigger an inflammatory response and produce reactive forms of oxygen, toxins, or mediators (e.g., tumour necrosis factor alfa, interleukin 6, and cytokines). They can also damage epithelial cells or induce their dysfunction (Sánchez-Alcoholado et al., 2020). In addition, the influence of colon tumour location has been documented, with bacterial biofilms and pro-carcinogenic microorganisms being more common in right-sided carcinomas that are located closer to the flexure of the colon below the spleen, also known as the splenic flexure (Lee et al., 2017).

On the contrary, regarding the impact of CRC on the gut microbiome, CRC tissues reduce microbial diversity observed in bacterial genera such as *Clostridium* and *Bacteroides*. This reduction may be due to an inhospitable tumour environment in which rapidly growing tumour cells compete for nutrients and the immune cells produce inflammatory compounds toxic to microbes. However, tissues affected by CRC were specifically enriched with bacteria of the genus *Fusobacterium*, predominantly *Fusobacterium nucleatum*. Other bacteria frequently associated with CRC are *Streptococcus gallolyticus*, *Enterococcus faecalis*, colibactin-producing *Escherichia coli* or enterotoxigenic *Bacteroides fragilis* (Brennan & Garrett, 2016).

In the context of bacteria and the treatment of CRC, it is still unclear whether bacteria are one of the main causes or whether they merely accompany the disease (Sánchez-Alcoholado et al., 2020). Therefore, no specific therapeutic measures have yet

been established for the treatment of these pathogens in relation to the potential risk of CRC. Treatment of infections caused by bacteria therefore focuses mainly on the use of antibiotics. (Terlizzi et al., 2017; Legaria et al., 2021).

1.2.1. Bacteroides fragilis

Species of the genus *Bacteroides* are anaerobic gram-negative rod-shaped bacteria, accounting for approximately 25% of all anaerobes living in the oxygen-free environment of the human colon (Beytout et al., 1996; Wexler 2007). These bacteria are usually symbionts, such as non-enterotoxigenic *B. fragilis* (NTBF), which are thought to contribute to host nutritional status and mucosal and systemic immunity. Conversely, some species or strains can be detrimental, of which the most harmful is enterotoxigenic *B. fragilis* (ETBF), the leading anaerobe in intra-abdominal abscesses and bloodstream infections, with mortality reported as high as 20% (Wick & Sears, 2010). An emerging aspect of ETBF pathogenicity is in the toxin production. This strain have the ability to secrete a zinc-dependent metalloprotease toxin (Purcell, 2020) that can cause inflammatory diarrhoea in animals, children, and adults or the development of inflammatory bowel disease. Infection occurs due to disruption of the intestinal wall, diverticulum rupture, or other perforations caused by surgical intervention, malignancy, or inflammation of the appendix (appendicitis) (Wexler 2007). The intestinal inflammation caused by *B. fragilis* toxin (BFT) can result in abscess formation and bacteraemia and has been implicated in CRC (Purcell, 2020; Elsaghir & Reddivari, 2022). Another linkage between the ETBF and CRC, was observed in a four-case control meta-analysis, which found that these strains were consistently present in the intestinal microbiomes of CRC patients around the world (Song et al., 2020).

The treatment of ETBF infections consists mainly of antimicrobial therapy. Different classes of drugs, such as clindamycin, cephalosporins, carbapenems, metronidazole, and some new-generation fluoroquinolones, are used to treat infections caused by *B. fragilis* depending on the country and healthcare setting (Yekani et al., 2020). Imipenem, rifampin, and piperacillin have also shown strong efficacy against this bacterium. Nevertheless, the use of antibiotics has become difficult due to the increase in bacteria resistance. ETBF showed 100%, 60%, and 65% resistance to

ciprofloxacin, cefotaxime, and cefoxitin, respectively (Akhi et al., 2013). Another study showed that of the 58 original ETBF isolates tested, 74% were sensitive to tetracyclines and 90% were sensitive to ampicillin and clindamycin (Sears et al., 2008).

1.2.2. Clostridium septicum

Clostridium species are gram-positive non-pathogenic bacteria commonly present in the GI tract. Specifically, *C. septicum* is an anaerobic rod (D. Jain et al., 2017; Ramphal et al., 2018)., which differs from other *Clostridium* species in its absence of carbohydrate fermentation reactions, lack of lecithinase or lipase production, and the release of alpha toxin (Gabay et al., 1981; Tweten, 2001). This bacterium has been observed to occur in human faeces in less than 3% of cases, making it rather rare in the normal human gut. Unlike other *Clostridium* species, *C. septicum* can invade and infect healthy tissue and focuses on soft tissue with the low blood supply (Smith-Slatas et al., 2006). In most cases, diseases caused by this bacterium are always fatal without clinical intervention. It is the causative agent of traumatic (wound) and non-traumatic (endogenous) myonecrosis (malignant oedema, gangrene), which are rapidly fatal diseases. Furthermore, it can also cause various highly lethal intestinal infections in both humans and animals, such as necrotizing enteritis (Tweten, 2001). Its associated mortality of 50% to 60% is thus two to three times higher than infections caused by all the other *Clostridia* species (Santos et al. 2022).

In humans, it has been linked to CRC or other cancerous tumours (D. Jain et al., 2017; Manwani et al., 2019). This linkage is attributed to malignancy-related disturbance of the colonic mucosa, which results in a decreased oxygen level and promotes *C. septicum* growth with exotoxin production. Exotoxins induce capillary permeability, which causes increased translocation of this pathogen from the colon to the systemic circulation and the subsequent development of infection in nearby tissues (Santos et al. 2022).

The suggested treatment for infections caused by this bacterium is surgical intervention, but in most cases, antibiotic therapy is preferred. Usually, agents such as penicillin and clindamycin are used, but if patients are allergic to penicillin, it is recommended to use tetracycline, chloramphenicol, metronidazole, and vancomycin instead. For the not-defined diagnosis, broad-spectrum treatment with vancomycin plus

either piperacillin/tazobactam, ampicillin/sulbactam, or a carbapenem antimicrobial is advised. In case of clostridial myonecrosis, therapy with penicillin and clindamycin is recommended (Stevens et al., 2014). For vancomycin, mild resistance has been reported *in vitro* (Aldape et al., 2018). No significantly high resistance of this pathogen has been observed in case of other antibiotics so far.

1.2.3. Escherichia coli

This gram-negative rod-shaped bacterium is known to be a component of healthy intestinal flora. However, it can also cause intestinal and extraintestinal illness in humans due to toxin-producing or mucosa-inducing strains, inducing a wide range of diseases, from mild, self-limiting gastroenteritis to renal failure and septic shock (Jang et al., 2017). There are multiple *E. coli* strains causing intestinal illnesses such as enterotoxigenic (ETEC), enterohemorrhagic (EHEC), enteropathogenic *E. coli* (EPEC), or other strains (Estrada-Garcia & Tarr, 2022) with some of the enteropathogenic types increasing the susceptibility to CRC (Nouri et al., 2021). Human CRC and inflammatory bowel disease (IBD) samples were shown to have considerably more *E. coli* contamination than those from healthy individuals (Song et al., 2020). In CRC patients, it infiltrates the intestinal mucosa and grows intracellularly. The molecular research on the *E. coli* strains discovered the presence of pathogenic islands in their genomes that manage toxin production. A toxin mostly linked to *E. coli* populating CRC is Colibactin (pks), the genotoxic polyketide non-ribosomal peptide (PK-NRP) (Faïs et al., 2016), that influences cell cycle, crosslinks, and breaks of DNA strands (Sánchez-Alcoholado et al., 2020). This results in higher rates of mutations in affected cells (Coleman et al., 2020). Interestingly, the pks *E. coli* strains have been discovered more frequently in tumour tissue from late-stage CRC patients than in early-stage or non-tumour tissue (Song et al., 2020).

In case of the treatment, the constant development of novel antimicrobiotic substances is needed due to the bacterium high resistance reported in antibiotics such as cephalosporins, tetracyclines, macrolides, and fluoroquinolones (Terlizzi et al. 2017; Kakoullis et al. 2021). Newly developed compounds from the cephalosporin group include ceftobiprole, ceftaroline, cefiderocol, and ceftolozane, the last of which is used in combination with tazobactam. In addition, the newly developed tetracyclines,

omadacycline, and eravacycline, are active against bacteria that contain efflux pumps or ribosome-protecting proteins, which are mechanisms that usually confer resistance to older tetracyclines (Kakoullis et al., 2021).

1.2.4. Fusobacterium necrophorum

F. necrophorum is an obligate anaerobic, gram-negative, rod-shaped bacterium (Gebhardt et al., 2011). It does not make spores or pods, and its occurrence is mainly in humans and animal´s genitourinary tracts (F. F. Wang et al., 2022). Under the species *F. necrophorum* are two subspecies: *subsp.necrophorum*, mostly in animals, and *subsp. funduliforme,* a pathogen in humans, but also a commensal in the human pharynx (Bank et al., 2010). The *subsp. funduliforme* has been associated with Lemierre's syndrome, and the *subsp. Necrophorum* causes animal infections, such as liver abscess, foot rot, and endometritis (F. F. Wang et al., 2022). Additionally, *Fusobacterium* has been detected in cancer tumour samples, but its relationship with CRC is unknown. Generally, it is thought to be a passenger bacterium (Coleman et al., 2020). However, one of the possible mechanisms of bacterial tumorigenesis is the ability of its adhesin molecule (FadA) to bind to the cell adhesion protein (E-cadherin) on the colonic epithelium and activate the Wnt/β-catenin oncogenic pathway. The second mechanism may be caused by *Fusobacterium*'s capacity to limit T-lymphocyte growth and promote T-lymphocyte death, compromising host immunity. This mechanism is supported by the finding that tumours enriched in T-lymphocyte subsets are linked to a better prognosis (King et al., 2020). The presence of *Fusobacterium* in intestinal tumours has also been studied across nations, with a higher prevalence in Spain compared to America and Vietnam, possibly due to dietary differences (Sears & Garrett, 2014).

This bacterium is generally treated with antibiotics such as penicillin, tetracyclines, and macrolides, but it is also susceptible to beta-lactam antibiotics such as clindamycin, metronidazole, and chloramphenicol (Riordan, 2007; Yusuf et al., 2015). The resistance is observed to aminoglycosides and erythromycin (Tan et al. 1996). However, other authors have also reported *in-vitro* resistance to penicillin, chloramphenicol, clindamycin, and tetracycline (Riordan, 2007).

1.2.5. Peptostreptococcus anaerobius

P. anaerobius is a gram-positive, anaerobic cocci. It has been most commonly isolated from oral infections, skin and soft tissue infections, GI tract–associated infections, female genitourinary infections, bone and joint infections, and leg and foot ulcers (Wu et al., 2011). The bacterium has also been observed in implant-related, and respiratory infections. Generally, it is more associated with polymicrobial infections than monobacterial infections (Legaria et al., 2021). In addition, *P. anaerobius* has been found in mucosa and stool samples of CRC patients (Drewes et al., 2020; Dalal et al., 2021). According to research, it attaches to integrin-producing tumour cells of the intestinal epithelium with a protein called "*putative cell wall binding repeat 2*" (PCWBR2), causing tumour cell proliferation and a pro-inflammatory cascade (Long et al., 2019). The bacterium causes cell proliferation by supplying cells with increased levels of cholesterol synthesis observed in mice (Drewes et al., 2020). Long (2019) showed that the attachment of *P. anaerobius* and its mediating effects on tumour cells were suppressed by application of an integrin-inhibiting peptide. Based on these findings, it is not yet clear whether this pathogen has cancer-causing properties or causes CRC (Drewes et al., 2020).

In case of antibiotic treatment, the pathogen showed moderate susceptibility to ampicillin-sulbactam and amoxicillin-clavulanic acid. On the contrary, resistance was observed to penicillin, ampicillin, ciprofloxacin, levofloxacin, and imidazole. However, resistance has been shown to decrease when multiple antibiotics are used (Legaria et al., 2021).

1.2.6. Streptococcus bovis

The genus *Streptococci* includes non-pathogenic and pathogenic species of bacteria found on the skin, oral cavity, nasopharynx, upper respiratory tract, gastrointestinal tract, and urogenital tract of humans and animals. Pathogenic species can cause numerous maladies and disorders, including sore throat, pneumonia, meningitis, endocarditis, and necrotizing fasciitis. Species in the GI tract are facultatively anaerobic, with *S. bovis* being the most reported species. It is a gram-positive bacterium with cells of spherical or ovoid shape organized in chains or pairs. Due to its heterogeneity, it is divided into more strains. Some anaerobic strains can even

grow in broth with 6.5% NaCl and pH 9.6 or produce urease (Hardie & Whiley, 1995). Its abundance is high in the tracts of ruminants or faeces of pigs and humans, where it can occur in up to 11% of individuals (Smith et al. 2010; Krishnan & Eslick 2014). In humans, it is usually present in cases of septicaemia and infective endocarditis (Krishnan & Eslick 2014). Furthermore, the association with CRC has been reported in up to 50% of patients with *S. bovis* bacteraemia or endocarditis (A. H. Smith et al., 2010). A possible linkage between this bacterium and CRC is the induction of interleukin 8 (IL-8), an inflammation-inducing protein (Coleman et al., 2020).

The common treatment of the maladies caused by this pathogen are antibiotics such as penicillin, glycopeptides, vancomycin, and linezolid, with no evidence of substantial resistance (Pompilio et al., 2019). However, moderate resistance has been demonstrated to tetracycline, erythromycin, streptomycin, and clindamycin (Gerber et al., 2006; Tripodi et al., 2005). Moreover, the most antibiotic-resistant species within the whole genus have been observed to be a complex of *S. bovis* and *S. equinus* with varying degrees of resistance to clindamycin, erythromycin, tetracycline, and levofloxacin (Pompilio et al., 2019).

1.3. Management and conventional treatment

1.3.1. Prevention

CRC is preventable by early screening and polyp detection. The screening tests of stool are done for early stages of CRC without symptoms with high curability. Further examinations include colonoscopy, sigmoidoscopy, and CT colonoscopy, which can detect and remove polyps that have at least 10 years to fully develop into CRC before they become carcinoma. Prevention involves also dietary and lifestyle patterns, which may prevent sporadic CRC by at least 70 % and include lower or reduced consumption of high animal fat, no smoking, and no alcohol (Pitchumoni & Broder, 2020). Recently, the influence of the tumour microenvironment has gained attention in CRC (Van der Jeught et al., 2018). Specifically, the tumour microenvironment is critical for the initiation and maintenance of tumorigenesis through effects at both the molecular and cellular levels and involves interactions of incipient tumour cells with structural host cells as well as adaptive and innate immune cells (Casey et al., 2015). This finding has prompted extensive analysis of clinical trials aimed at evaluating human immune cell infiltration as prognostic and predictive markers of CRC (Van der Jeught et al., 2018).

1.3.2. Treatment

Colon cancer is typically treated with surgery, such as laparoscopy, targeted therapy or with radiotherapy and chemotherapy (Pitchumoni & Broder, 2020). In the 1970s, the focus on cancer treatment was limited to surgery, with a lack of efficacy and higher recurrence. However, in the last 40 years, there has been a decrease in local recurrence of 5% and mortality due to more efficient surgery with the use of radiotherapy alone or in combination with chemotherapy and increased use of preoperative histological tests to determine proper treatment and stage of carcinoma (Nicholls, 2014). When CRC is diagnosed, 80% of cases are local, and about 20 % are metastases. Surgery is utilised to remove local CRCs, while chemotherapy is used to treat metastases or invaded lymph nodes. Chemotherapy combined with surgery is used for more advanced stages of rectal cancer. Additionally, immunotherapy is a treatment option for CRC metastases (Thanikachalam & Khan, 2019).

Early detection and reduced surgical procedures are critical for minimising tissue loss for normal organ function and, as a result, lowering post-operative stress and death. Consequently, chemoradiotherapy has been employed as the primary treatment without or with little local surgery. Yet, in 30–50% of treated individuals, this technique contributes to reduced organ function (Nicholls, 2014). Another approach to cancer care is targeted therapy, in which the focus and goal are only cancer cells, proteins, or genes, with no harmful effects on healthy cells. One type of targeted treatment is anti-angiogenesis therapy, which uses blood flow to "starve" the tumour of nutrition (Pitchumoni & Broder, 2020). Another possible method of treatment turned out to be the use of quinoline derivatives (Ortiz et al., 2014). They represent a large number of substances with antiproliferative effects that exhibit cytotoxicity through interference with the replication process caused by altering the structure of cellular DNA (S. Jain et al., 2019). These derivatives are synthetically improved versions of quinoline alkaloids obtained usually from plants. For example, one of the representatives already used in the treatment of CRC is berberine used in combination with drugs or radiation. Specifically, it was found to be an adjuvant therapeutic agent in combination with taxol, a frequently used clinical chemotherapeutic drug, in HER2-overexpressing breast cancer cells (Ortiz et al., 2014). Recently, also, new 8-hydroxyquinoline and 4-quinoline derivatives were reported as potential antitumour agents displaying cytotoxic activity against cancer cell lines (Jain et al., 2019; Mathada, 2022).

1.4. Natural quinoline alkaloids

Quinolines are organic compounds that form the basis of the alkaloids from plants, trees, animals, or microorganisms (Marella et al., 2013). The use of these plant alkaloids began as early as 1820, when quinine was isolated from the tree *Cinchona officinalis*, replacing the raw bark of the tree in the treatment of malaria, thus expanding its use. Another important milestone was, when in the early 1966, the first isolate with antitumour and anticancer properties, campothecin, was obtained from the Chinese tree *Camptotheca acuminata* and its anticancer activity were reported (Shang et al., 2018; Mathada, 2022). Generally, natural isolates with a quinoline moiety have been shown as pharmacologically active and important due to their broad spectrum of biological activity. They have been found to have a wide range of actions such as antimalarial, antibacterial, antifungal, anti-inflammatory or analgesic activity (Marella et al., 2013). Currently, commercially used quinoline derivatives are for example bedaquiline and irinotecan for the treatment of multidrug-resistant tuberculosis and colorectal cancer, respectively (Chung et al., 2015). The research and development of discovered alkaloids is important for increasing their activity and drug development. However, research on new compounds is equally, if not more, important and should continuously contribute to the development of new drugs linked to the increasing problem of drug resistance. In case of quinoline compounds, nowadays the research focuses for example on the tropical plants of the family *Rutaceae*, which have been generally of great medicinal importance and provide numerous quinoline alkaloids with various biological activities. Some representatives of these plants include neotropical trees or small shrubs of the genus *Galiea* such as *Galipea longiflora*, *Galipea bracteata* and *Galipea officinalis* producing antileishmanial, molluscicidal and antimalarial activity, respectively (Shang, Morris-Natschke, Liu, et al., 2018). Another tree from the same family is the Chinese *Evodia rutaecarpa*containing alkaloid Rutaecarpine with extensive pharmacological effects namely cardiovascular protective, anti-cancer, anti-inflammatory and other activities (Shang, Morris-Natschke, Yang, et al., 2018).

The other group of Isoquinoline alkaloids are an extremely large group of alkaloids mostly occurring in plants with morphine and codeine being the major and widely studied isoquinoline alkaloids (Shang et al., 2020). Although many plants and other biological sources contain this group of alkaloids, the structural variety of

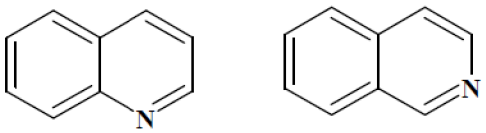
alkaloids in the genus *Corydalis* of the family *Papaveraceae* has been recently attractive for the researche of natural medicinal compounds. So far, several classes of isoquinoline alkaloids have been found in species of the genus *Corydalis*, including aporphine, protopin, protoberberine, tetrahydroprotoberberine, benzylisquinoline and morphinan. (Iranshahy et al., 2014). These extracts, pure compounds, and alkaloids derived from various *Corydalis sp*. show inhibitory efficacy against hepatitis virus and have anodyne and sedative activity. Namely *C. yanhusuo* has efficacy against central system disorders, *C. edulis* may impede the progress of Alzheimer's disease and *C. hendersonii* extract may effectively treat myocardial ischemia (Deng et al., 2021).

In this respect the compound of which derivatives are further observe in case of this work is a natural substance with a quinoline core 8-hydroxyquinoline. It is an organic compound that occurs as a natural product of the fungus *Cortinarius subtortus* (department *Basidiomycota*) and the plant *Allium stipitatum* of the family *Amaryllidacea* (PubChem, Bethesda, et al., 2023). This substance has also recently been extracted and purified from soil bacteria of the genus *Streptomyces spp* (Balthazar et al., 2022). In contrast, the second observed group are quinoxaline 1,4-dioxides a subgroup of quinoxaline-derived compounds that are usually synthetic. Natural derivatives of quinoxaline, such as echinomycin and triostin, are rare (Pereira et al., 2015).

In terms of chemical structure is quinoline a heterocyclic aromatic organic compound characterized by a double-ring structure that contains a benzene ring fused to pyridine at two adjacent carbon atoms. Isoquinoline, is a quinoline analogue differing in the presence of nitrogen at the second position of the double-ring. These two alkaloids show remarkable biological activities with relatively simple structures (S. Jain et al., 2019). The most important use of the quinoline structure is its antimalarial potential (Marella et al., 2013). The antibacterial mechanism of action of quinolines is due to inhibition of the bacteria's nucleic acid synthesis, which leads to the breakdown of the bacterial chromosome due to the interruption of enzymes such as DNA gyrase and topoisomerase IV (Kaur et al., 2022).

The antitumour and anticancer properties of the whole quinoline group have been investigated since 1966, when the first of them, camptothecin, and its anticancer activity were reported. Subsequently, other natural quinoline alkaloids have been found to possess anticarcinogenic activity, some of them also being antibiotics, e.g.,

streptonigrin, lophocerein, or lavendamycin (Mathada, 2022). These compounds are frequently investigated and further used in cases of breast, cervical, lung, liver and prostate cancer or CRC. In this respect, the effect of 8-HQ derivatives has been previously studied against CRC, other tumour tissues and against bacterial strains(S. Jain et al., 2019; Mathada, 2022). In addition, QdNOs have been reported to be another effective agent against bacteria observed in animals (Carta et al., 2005). However, there are no reports linking them to the treatment of CRC.



QUINOLINE ISOQUINOLINE

Figure 1 Chemical structure of quinoline and isoquinoline

1.4.1. Quinoxaline 1,4-dioxides

The quinoxaline 1,4-dioxides are heterocyclic aromatic A'-oxides of the quinoxaline ring, consisting of a benzene and a pyrazine ring, with nitrogen (Carta et al., 2005; G. Cheng et al., 2016; Gali-Muhtasib et al., 2001). They are a class of synthetic antibacterial agents with strong antimicrobial activity (Li et al., 2019). Oxidation of both nitrogens in the quinoxaline ring to obtain quinoxaline 1,4-dioxides enormously widens the biological properties, such as animal growth promotion, antibacterial, antimycobacterial, anticandidal, and antiprotozoal activities, hypoxia-selective action, and mutagenic properties (Carta et al., 2005). The quinoxaline 1,4-dioxides have also been shown to modulate the oxidative state in malignant melanocytes and brain tumour cell lines and exhibit a potential radio-sensitizing effect *in vitro* on the tested radioresistant cell lines (L. Silva et al., 2019). The derivatives from this group, which are being further observed, are carbadox and olaquindox, whose use in healthcare or possible association with CRC have not yet been extensively investigated.

Carbadox

It is a synthetically made antibiotic agent in the form of white powder or crystals. Its full name is "methyl *N*-[(*E*)-(1,4-dioxidoquinoxaline-1,4-diium-2-yl) methylideneamino] carbamate" (PubChem 2021). It is used commercially under the name Mecadox in pig feed as a growth promoter and for the treatment of dysentery in pigs caused by *Serpulina hyodysenteriae* or enteritis caused by *Salmonella* spp. (Practical Antimicrobial Therapeutics 2017; PubChem 2021).

It is mainly efficient against gram-positive bacteria and less so against gram-negative bacteria (Constable et al., 2017). Significant changes in the community structure and abundance of bacteria in the intestinal microflora of the pigs were observed within 4 days after administration of the drug to healthy animals, especially a relatively unchanged population of the *Prevotella* bacterium and a reduction in total bacteria in the medicated pigs. Specifically, a change in diet along with carbadox withdrawal was associated with an increase in *E. coli* in untreated pigs (Looft et al., 2014). In addition, preventive use of carbadox does not appear to increase the prevalence of antimicrobial resistance, at least in the case of *Salmonella*, in market-age pigs. However, in higher doses it is toxic due to its production of metabolites such as desoxycarbadox, quinoxaline-carboxylic acid, and methyl carbazate, which can form hydrazine. Because desoxycarbadox has been shown to be carcinogenic in rats and hydrazine to be tumorigenic in mice and rats, carbadox is prohibited in Europe and Canada ("PubChem" 2021). This substance adversely affects the human hormonal system. It affects the endocrine glands of the adrenal cortex through morphological changes that restrict the production of the steroid hormone aldosterone (Constable et al., 2017). This occurs in the case of prolonged exposure to concentrations higher than 25 ppm and causes adrenal degradation, a decrease in aldosterone, hyperkalaemia, and hyponatraemia (Kreutzer et al., 2008). There is not yet enough research on carbadox regarding its potential medical uses or effects on CRC or other cancers, perhaps because of its observed effects.

Olaquindox

N-(2-hydroxyethyl)-3-methyl-4-oxido-1-oxoquinoxalin-1-ium-2-carboxamide (PubChem 2019) is in the form of a yellow powder or crystals. It is used, sometimes also under the name Keyquindox or Bayo-n-oxo, as a feed additive in animal production

to promote growth and prevent bacterial infection in China (Croubels et al., 2004; Carta et al., 2005). Its antimicrobial effects have so far only been studied in animals, mostly in the gastrointestinal tract of pigs. Olaquindox has been shown to suppress inoculated *E. coli in vivo*, probably by inhibiting bacterial adhesion to the small intestine or its proliferation or both (Ding et al., 2006). No further studies on the effects of the substance on humans or on other species of pathogenic bacteria have been reported. In the case of adverse effects, higher doses of olaquindox have been found to leave residues in feed and may damage the immune system of animals, causing damage to the liver, kidneys, and protein metabolism (Shi et al., 2009). Moreover, it possesses highly phototoxic, mutagenic, genotoxic, and carcinogenic properties (Ray & Jana, 2017) and its toxicity causes degeneration or necrosis of tissues and organs in animals and potentially humans (Shi et al., 2009). Furthermore, the imino-N-oxide group makes olaquindox photoreactive with proteins and has photoallergic and phototoxic properties that have been observed in pigs (Beijersbergen Van Henegouwen, 1997). Due to these facts, it was banned in Europe in 1999, and its ongoing use is only in China (Zhao et al., 2013).

1.4.2. 8-Hydroxyquinolines

The 8-hydroxyquinolines are a quinoline-based group derived from plants and produced synthetically (Prachayasittikul et al., 2013a). They possess a pyrimidine ring that maintains its properties as an electron-deficient entity with a basic nitrogen. The pyrimidine ring is further fused to phenol with the hydroxyl group attached to position 8 (Saadeh et al. 2020). Their chemical structure is found in many biologically active compounds and several marketed drugs used for the treatment of infectious diseases, neuropathies, and cancers (Pape et al., 2022). Particularly in the case of cancer, there is ongoing research that shows promising results for its use in therapy, especially in the case of clioquinol (Oliveri et al., 2012). However, further research of other potential agents linked to this disease is also needed, and therefore other 8-hydroxyquinolines derivatives such as chloroxine, ferrone, nitroxoline, and oxyquinoline are being investigated here.

Chloroxine

The 5,7-dichloro-8-hydroxyquinoline is a bacteriostatic, fungistatic, and antiprotozoal agent that has antibacterial, antifungal, and antibiotic actions *in vivo*. It is in form of a white powder or crystals, it inhibits the growth and induces SOS-DNA repair of various gram-positive and gram-negative bacteria (PubChem, 2023; Shahabadi et al., 2021; Shahabadi & Zendehcheshm, 2020). In higher doses it causes acute toxicity when taken orally and may cause skin irritation or serious eye damage. A single exposure to a high dose may induce targeted organ toxicity and respiratory irritation (PubChem 2023). However, the *in vivo* testing showed that chloroxine nanoparticles with diameters of 600–800 nm demonstrated good tolerance in terms of skin irritation and antibacterial efficacy. As a result, the developed compound has promising potential for interventions in both dermatological infection control and prevention (Trousil et al., 2022). It is used topically in skin infections and as a cream in the treatment of dandruff and seborrheic dermatitis of the scalp under the commercial name Capitrol (Pérez-Ruiz et al., 1996). Moreover, chloroxine is also used as an antidiarrhea medication called Endiarone in the treatment of intestinal microflora disorders (Marquez-Gomez et al., 2022). Additionally, it showed slightly enhanced efficacy against *E. coli* and *S. aureus* when conjugated with silver nanoparticles. This conjugation inhibited bacterial growth by 100% at concentrations $\geq$ 0.25 μg/ml (Shahabadi et al., 2021). Furthermore, it possesses a high affinity for binding human serum albumin (Shahabadi & Zendehcheshm, 2020), which is among the most abundant proteins in blood plasma and transports hormones, fatty acids, and other substances throughout the body (P. Lee & Wu, 2015). Furthermore, a recent study demonstrated a novel mechanism of action of chloroxine on platinum-resistant cancer cells by overcoming their innate tolerance to DNA damage and facilitating cancer cell death by apoptosis, which was observed in case of ovarian cancer. Specifically, chloroxine showed synergy with various platinum agents used as treatment for this cancer that were no longer effective on their own due to resistance. This synergy showed a strong tumour-static effect *in vivo* (Silva et al., 2021). However, there is no further research on chloroxine in relation to cancer or directly to CRC.

Ferron

8-Hydroxy-7-Iodoquinoline-5-Sulfonic acid (ferron), also under the name Chiniofon, occurs in the form of white powder or crystals. It possesses antimycotic, antiamoebic, and antiprotozoal properties and can be used to treat dysentery, ulcers, urological, and gynaecological problems (Koseva et al. 1999; Quashie et al. 2017). Because of its ability to form complexes, it is used analytically as a selective colour reagent for iron detection (III) (G. Smith, 2012). The presence of the bulky 7-iodo substituent, however, compromises this complex formation capacity. As a result, ferron reacts only with iron (III) but not with iron (II) (G. Smith et al., 2004). This substance appears to cause severe skin burns and eye damage or, if inhaled, may result in severe corrosive damage to the upper respiratory tract and lungs (PubChem et al., 2023). No other adverse effects in terms of toxicity have been observed. Regarding its pharmaceutical application or its effectiveness in relation to CRC, there is a very limited or no record of its use.

Nitroxoline

The 5-nitroquinolin-8-ol, or 8-Hydroxy-5-nitroquinoline, is a halogenated derivative of 8-hydroxyquinoline in the form of a yellow powder or crystals (Jiang et al. 2011; PubChem et al. 2023b). Commercially, it is a previously used drug with numerous pharmacological properties. It was not used for many decades because it was considered ineffective, although its antibacterial activity was known (Wykowski et al., 2022a) Nowadays it is used in Russia as broad-spectrum antimicrobial agent against gram-positive microorganisms (Avexima JSC, 2023). Moreover, nitroxoline is also used in the treatment of urinary tract infections because of its effectiveness against the majority of Gram-negative bacteria, mycoplasmas and human pathogens *Candida spp*. However, its activity against *Acinetobacter spp., Enterococcus spp.* and *Serratia spp*. varies with resistance found in *Pseudomonas spp* (Mitrovic & Kos 2019). In contrast, nitroxoline showed no decrease in activity in the case of *E. coli*, despite *E. coli's* broad resistance profiles to other antimicrobial agents used in clinical practice for the treatment of urinary tract infections, which is probably caused by the low prescription rate of this drug (Wijma et al. 2018). Its *in vitro* efficacy was also observed in *S. aureus*, *E. faecalis*, *K. pneumoniae* and *P. mirabilis* (Sobke et al., 2018). Improper use of this

compound can result in acute toxicity and irritation (PubChem et al., 2023). No other adverse effects have been observed. In addition, its antitumour activity against human prostate cancer has been observed, as well as the possibility of immunotherapy in the case of multiple cancers (Xu et al. 2019).

Oxyquinoline

The quinolin-8-ol or 8-hydroxyquinoline is a heterocyclic phenol amine (Andersen 2016; PubChem et al. 2023). It is in the form of white powder or crystals. It is insoluble in water or ether and soluble in alcohol, acetone, chloroform, benzene, and aqueous mineral acids (Andersen, 2016). The compound acts as a biocide to eliminate bacteria and fungi and has been observed to be active against many gram-positive and gram-negative bacteria (Faizi et al. 1997; PubChem et al. 2023). OXQ and its salt exhibit little acute or sub-chronic toxicity in animal studies and were noncarcinogenic in several rodent feeding studies. However, it has been found to be genotoxic in certain *Salmonella typhimurium* strains. Moreover, it has been shown its strong inhibition observed for gastrointestinal bacteria *E. coli*, *C. difficile* and for *C. perfringens.* Its efficacy has also been reported against various strains of *S. aureus*. The inhibitory effect on *S. aureus* depends on chelating capacity of oxyquinoline and increases in the presence of Cu (Prachayasittikul et al., 2013). Furthermore, it is sold as a cosmetic biocide for use in cosmetic products, for example as part of hair colouring products such as Avalon Developer 6% or Moli Cosmetics Oxy 12%. (Andersen, 2016). Its 8-hydroxyquioline group has received a lot of attention in material sciences and bio-related sciences because of its simple structure, strong metal coordination properties, fluorescence properties, and extensive functionalization possibilities (Aoki et al., 2016). Oxyquinoline has strong coordination ability and good recognition properties for metals, and therefore it is widely used for analytical and separation purposes or for metal chelation (Prachayasittikul et al., 2013b).

2. Aims of the Thesis

The aim of this thesis is to evaluate *in vitro* growth-inhibitory effects of quinolines against intestinal bacteria associated with CRC.

The specific objectives are as follows:

- Determination of MIC values of 8-hydroxyquinolines and quinoxaline 1,4-dioxides and comparison their antibacterial action with six representatives of conventional antibiotics.
- Evaluation of relationship between chemical structure of quinoline compounds and their inhibitory effects against CRC associated bacteria.

3. Materials and Methods

3.1. Bacterial strains and culture media

The determination of antibacterial activity was performed against the main representatives of Gram-positive and Gram-negative, aerobic, and anaerobic bacteria associated with the development of CRC. The strains of Standard American Type Culture Collection (ATCC) were acquired from Oxoid [Basingstoke (UK)] and Czech Collection of Microorganisms (CCM) from Masaryk University, Brno. For experiments were used bacteria cultures commonly known for causing CRC. Strains used in this research follow: *B. fragilis* ATCC 25285, *C. septicum* ATCC 12464, *E. coli* ATCC 25922, *S. bovis* ATCC, 33317, *F. necrophorum* CCM 5981 and *P. anaerobius* CCM 3790.

Mueller-Hinton broth (MHB) was used as a growth medium for an aerobic group of bacteria, Wilkins-Chalgren broth (WCB) and Brain Heart Infusion (BHI) for anaerobic bacteria. Buffered versions of MHB, WCB and BHI were enriched by 0.2g KCl, 8.0g NaCl and 6.1g Trizma based on one litre of distilled water. After this, the pH of around 10.0 were adjusted to pH 7.6 by adding 35% HCl.

3.2. Antimicrobial testing method

Broth microdilution method were used for analysis, using 96-well microtiter plates according to Clinical and Laboratory Standards Institute (CLSI) guidelines, modified by Cos et al. (2006), where minimum inhibitory concentration (MIC) [μg/mL] was assessed and tested in anaerobic conditions, following the guidelines in Hecht (1999). The MIC, defined as "*the lowest concentration of antimicrobial compound inhibiting visible growth of the microorganism after incubation"*, was an important indicator for the antimicrobial resistance of microorganisms (Andrews, 2002) Inoculated microplates were incubated for 24 h at 37 °C according to their ability to resist oxygen.-Dimethyl sulfoxide was used as a negative control and antibiotics, e.g.: ceftriaxone, ciprofloxacin, chloramphenicol, metronidazole, tetracycline, and vancomycin as positive controls, were included in the experiments. Bacterial growth

was determined by measuring the optical density. The MICs were calculated as the lowest concentration that shows ≥80% reduction of microbial growth after 24 h of growth, and the comparison of average values were expressed by selectivity index. All tests were performed as at least three independent experiments, each in triplicate, with the results presented as median/modal values.

All bacterial cultures diluted to contain 1.5 × 108 CFU/ml were inoculated with the suspension in microtiter plate. The plates inoculated with aerobic bacteria were prepared in aerobic flow box workstation (Whitley A35, Don Whitley Scientific, Shipley, UK) and the plates with anaerobic bacteria in Anaerobic Workstation (Biological Thermostat BT 120) as well as incubated, with incubation for both aerobic and anaerobic for 24 h at 37 °C. The levels of antibacterial effect were classified according to the Manual of Clinical Microbiology (Jorgensen et al., 1999) as “strong” (MIC from 0.0625 to 32 μg/ml), “moderate” (MIC from 64 to 128 μg/ml), and “weak or no effect” (MIC equal or greater than 256 μg/ml).

4. Results and discussion

In this study, six quinoline-based agents, namely carbadox, chloroxine, ferron, nitroxoline, oxyquinoline and olaquindox and six conventional antibiotics used to treat gastrointestinal diseases (ceftriaxone, chloramphenicol, ciprofloxacin, metronidazole, vancomycin, tetracycline) produced *in vitro* antimicrobial effects against six pathogenic bacteria associate with CRC (*Bacteroides fragilis, Clostridium septicum, Escherichia coli, Fusobacterium necrophorum, Peptostreptococcus anaerobius, Streptococcus bovis*). In general, certain quinolines (carbadox and nitroxoline) produced inhibitory effect comparable with the most active conventional antibiotics (ciprofloxacin, ceftriaxone, and tetracycline).

Among all agents tested, ciprofloxacin produced the strongest growth-inhibitory effect against bacteria associated with CRC with MIC values ranging from 0.0625 to 2 μg/ml (see Table 1). This compound from the group of fluoroquinolones had the highest activity against *E. coli* (MIC = 0.0625 μg/ml), which was overall the lowest MIC value observed in the study. This finding is in link with the previous study conducted by Kudera et al. (2020). Additionally, ciprofloxacin exhibited high efficiency against *P. anaerobius* (MIC = 0.05 μg/ml), *F. necrophorum* (MIC = 0.025 μg/ml) and *C. septicum* (MIC = 0.05 μg/ml). Although MIC values for *B. fragilis* and *S. bovis* were slightly higher, both bacteria were susceptible to this fluoroquinolone antibiotic (MIC = 2 μg/ml). Third-generation cephalosporin antibiotic ceftriaxone and quinoline derivative carbadox also showed strong antibacterial activities with MIC values ranging from 0.25 to 16 μg/ml and from 0.5 to 32 μg/ml, respectively. Ceftriaxone showed the lowest MIC in case of *E. coli* (MIC = 0.25 μg/ml). Previously reported MIC values against *E. coli* strain of ceftriaxone were found to be 2.1 μg/ml (Anacona et al., 2021). The difference between these two results may be due to the use of different methods. In our case was used the broth microdilution method, while in the second study was used the agar dilution method. High activity of this agent was observed also against *S. bovis* (MIC = 1 μg/ml) with the MIC value lower than for ciprofloxacin. Other bacteria were less susceptible with MIC values ranging from 8 to 32 μg/ml with *F. necrophorum* as the most resistant bacterium. Among quinoline derivatives, carbadox produced highest activity against 4 out of 6 bacteria tested with the MIC ranging from 0.5 to 32 μg/ml (see Table 1). This is consistent with previous studies where this compound, as well as

several other quinoxaline 1,4-dioxides, equally affected the growth of both gram-positive and gram-negative species (Carta et al., 2005). Specifically, its activity was highest against *F. necrophorum* and *P. anaerobius* (for both MIC = 0.5 μg/ml) in which the values were lower or equal to those produced by ciprofloxacin (MIC = 0.25 and 0.5, respectively). In addition, *C. septicum* was also sensitive to carbadox (MIC = 4 μg/ml). The low activity of this compound was exhibited against *E. coli* (MIC = 32 μg/ml) and *S. bovis* (MIC = 32 μg/ml). Nitroxoline, another drug belonging to the quinoline derivatives, was as well as carbadox highly active against *B. fragilis* with the same MIC value (MIC = 4 μg/ml) and its activity against this bacterium was lower only in case of ciprofloxacin (MIC = 2 μg/ml). Generally, all pathogenic bacteria showed relatively high sensitivity to nitroxoline (MIC from 4 to 16 μg/ml). Three of them, namely *C. septicum*, *E. coli* and *S. bovis*, were susceptible at the same MIC of 8 μg/ml. These results are corresponding well with findings of Abouelhassan et al. (2017), who determined similar MIC values (12 μg/ml) clinical isolate of *E. coli* UAEC-1. Interestingly, nitroxoline is already used since the 1960s as broad-spectrum antimicrobial agent and is used also in treatment of urinary tract infections mainly against the *E. coli* and other gram- negative as well as gram-positive bacteria (Wykowski et al., 2022; Avexima JSC, 2023).

The rest of tested quinoline compound and antibiotics produced moderate or low antibacterial effects where MIC was equal or higher than 64 μg/ml at least for one bacterial strain tested. In this respect chloroxine, tetracycline and chloramphenicol exhibit moderate activity with the highest MIC values (MIC = 64 μg/ml) against *P. anaerobius*, *B. fragilis* and *E. coli*. Against some bacteria, however, these three compounds showed exceptionally strong activity. With exception of the low effect produced against *B. fragilis* (MIC = 64 μg/ml), the first-generation antibiotic tetracycline showed overall high activity with the MIC values ranging from 0.125 to 8 μg/ml. Surprisingly, this antibiotic possessed high activity against *C. septicum* (MIC = 0.125 μg/ml) and *S. bovis* (MIC = 1 μg/ml). In this context, the quinoline compound chloroxine along with chloramphenicol antibiotic had the same high activity against *S. bovis* (MIC = 4 μg/ml). Chloramphenicol also exhibited high activity against *C. septicum* and *F. necrophorum* (MIC = 8 μg/ml for both) compared to chloroxine (MIC = 32 μg/ml for both

Furthermore, antibiotics metronidazole and vancomycine produced rather moderate activity. Vancomycin showed rather strong activity against *F. necrophorum* (MIC = 2 μg/ml), *C. septicum* (MIC = 8 μg/ml) and *S. bovis* (MIC = 8 μg/ml). In contrast, it showed the lowest activity against B. fragilis (MIC = 34 μg/ml) followed by *E. coli* (MIC = 64 μg/ml). Compared to vancomycin, metronidazole showed exceptionally low inhibitory value in case of *E. coli* (MIC = 2 μg/ml). Against the rest of bacteria, metronidazole showed MIC 64 μg/ml, which clearly confirms its previously observed low efficacy (Schuetz, 2018).

As shown in Table 1, the lowest antimicrobial activity was observed in case of ferron, olaquindox and oxyquinoline. In case of olaquindox**,** this compound exhibited moderate activity against the most of the bacteria with MIC values ranging from 16 to 64 μg/ml. The highest activity of olaquindox was measured against *P. anaerobius* (MIC = 16 μg/ml). Interestingly, the activity against *S. bovis* (MIC = 64 μg/ml) was in contrary to previous research, which showed higher activity against *B. fragilis ATCC* 25285 (MIC = 2 μg/ml) (Jeong et al., 2009). The disparity in the two results can be accounted to the use of different bacterium cultivation conditions. Enriched thioglycollate medium was used as growth medium and incubation was done in an incubated in Gas-pak jar system in the previously published study. However, olaquindox exhibited ineffective only against *S. bovis* (MIC = 512 μg/ml). In contrast, oxyquinoline showed the high activity against *S. bovis* (MIC = 4 μg/ml) and *B. fragilis* (MIC = 32 μg/ml) and exhibited low or no antibiotic activity in almost all bacteria with MIC values ranging from 128 to 512 μg/ml. In addition, it was ineffective against *F. necrophorum* (MIC = 512 μg/ml) and *P. anaerobius* (MIC = 512 μg/ml). The least potent of all compounds was ferron with MIC values ranging from 128 to 512 μg/ml.

Table 1: *In vitro* growth-inhibitory effects of quinoline compounds and conventional antibiotics against intestinal bacteria associated with colorectal cancer

Compound	Microorganisms/ Minimum inhibitory concentration (μg/ml)					
	Bacteroides fragilis	*Clostridium septicum*	*Escherichia coli*	*Fusobacterium necrophorum*	*Peptostreptococcus anaerobius*	*Streptococcus bovis*
8-Hydroxyquinolines						
Ferron	128	256	512	512	512	256
Chloroxine	32	32	16	32	64	4
Nitroxoline	4	8	8	16	16	8
Oxyquinoline	32	128	128	512	512	4
Quinoxaline 1,4-dioxides						
Carbadox	4	4	32	0,5	0.5	32
Olaquindox	64	64	32	32	16	512
Antibiotics						
Ciprofloxacin	2	0.5	0.0625	0.25	0.5	2
Ceftriaxone	16	8	0.25	32	8	1
Tetracycline	64	0.125	2	1	8	1
Vancomycine	32	8	64	4	16	8
Metronidazole	64	64	2	64	64	64
Chloramphenicol	16	8	64	8	16	4

Footnote: Data are median or modal values of three independent experiments, each performed in triplicate.

4.1. Structure relationship study

Based on the chemical structure of each substance, we can distinguish eight basic groups. Specifically, the common antibiotics used as controls, consisting of the groups fluoroquinolines, cephalosporins, tetracyclines, miscellaneous agents (chloramphenicol), imidazoles and glycopeptides and then the two groups of quinoline compounds: quinoxaline 1,4-dioxides and 8-hydroxyquinolines. Tetracyclines, chloramphenicol and metronidazole belong to β-lactam antibiotics. Overall, the most effective antibiotics were from the fluoroquinolones (ciprofloxacine) and cephalosporin (ceftriaxone) groups, followed by two compounds from the quinoxaline 1,4-dioxides and 8-hydroxyquinoline groups, namely carbadox and olaquindox, respectively.

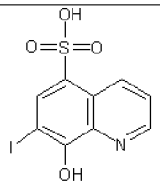
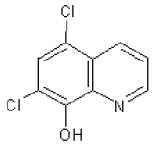
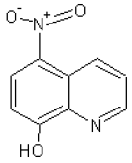
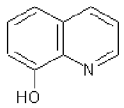
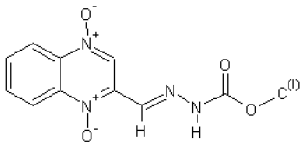
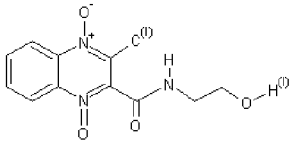
Regarding the previous findings the strongest growth-inhibitory effect has a fluoroquinolones ciprofloxacin. This antibiotic has shown to be far more effective than the other commonly used antibiotics. A major influence on their biological activity arises from the N-1-cyclopropyl substituent at the N-1 position, which enables a favourable combination of steric, spatial, and electronic interactions (Chu & Fernandest, 1989). According to the results, the third-generation cephalosporin antibiotic ceftriaxone was the second most effective agent. In general, cephalosporins consist of a compound β-lactam-Δ3-dihydrothiazine two-ring system and differ in their side chain substituents (see Table 3). These structures are divided into four generations based on their resistance to degradation by β-lactamases, which is often associated with bacterial resistance (Taherpour et al., 2015). Furthermore, two quinoline-based compounds, carbadox and nitroxoline from the group of quinoxaline 1,4-dioxides and 8-hydroxyquinolines, respectively, have strong antimicrobial activity. Both compounds are based on a quinoline ring consisting of two benzene atoms with a nitrogen in the first position. Crucial to their different effects are, among other things, the positions and substituents that define their properties. As shown in Table 2, carbadox has a large and bulky substituent at the second position with two additional nitro groups at the first and second positions of the quinoline nucleus, while nitroxoline has a nitro oxide at the fifth position and a hydroxide at the eighth position.

Compared to previously mentioned, the 8-hydroxyquinoline chloroxine, exhibited moderate activity along with the 4 representatives of antibiotics from classes of tetracyclines, imidazoles, glycopeptides and chloramphenicol. Concerning the chemical structure of chloroxine, it consists of two chloro-substituents at the fifth and seventh positions coupled with hydroxy group at the eighth position. In contrast tetracycline had incomparably better effects compared to chloroxine and other moderately active compounds. This antibiotic is a first-generation tetracyclines from natural product of species *Streptomyces*. The chemical structure consists of a four-ring naphthalene skeleton with many groups such as alkyl, hydroxyl, and amine on the top and bottom of the molecule (Askari 2018). In the case of chloramphenicol, higher potency was found compared to chloroxine, but low compared to other antibiotics. In terms of chemical structure, chloramphenicol consists of a benzene core with a nitro group attached to the first position, while the fourth position consists of a propanediol structure. This structure is further substituted with a hydroxy substituent at position one and a dichloroacetyl group at position two. The whole propanediol structure as its substituents are thought as the key to antimicrobial activity (Malik, 1972). Vancomycin, which is less effective than chloramphenicol, has completely different structure (see Table 3). In the structure of this antibiotic belonging to the group of glycopeptides, the removal of the important terminal amino acid leucine has been found as important in the antibacterial activity of vancomycin (Nagarajan, 1993). Overall, metronidazole exhibited the lowest activity among all antibiotics tested. This member of the imidazole antibiotics is substituted at position one, two and five with 2-hydroxyethyl, nitro and methyl groups respectively (PubChem et al., 2004). This drug is activated by reduction of the nitro group at low oxygen tension (Dingsdag & Hunter, 2018).

The weak antimicrobial activity was observed in case of quinoline compounds ferron, oxyquinoline and olaquindox. For the 1,4-dioxide quinoxaline olaquindox, almost all measured values, except one, were at similar levels to those of metronidazole (see Table 1). In terms of chemical structure, it possesses the N-oxide groups in the first- and fourth- positions, a methyl-nitrone in the second position and a carbon in the third position. The presence of N-oxide groups in positions one and four with methyl nitrone in second or third position is a major factor in the antibacterial activity of this group (Carta et al., 2005; X. Wang et al., 2015). However, we assume that the concomitant substitution in the second and third positions together with the absence of

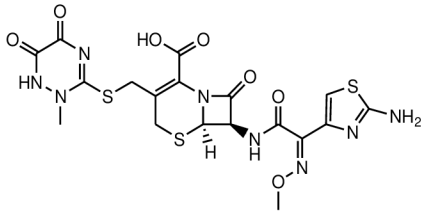
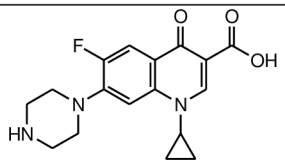
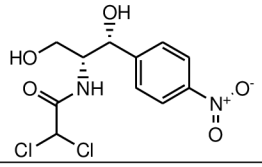
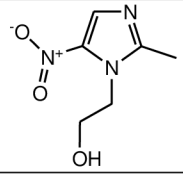
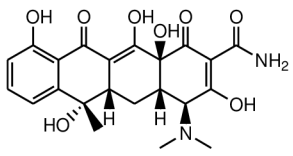
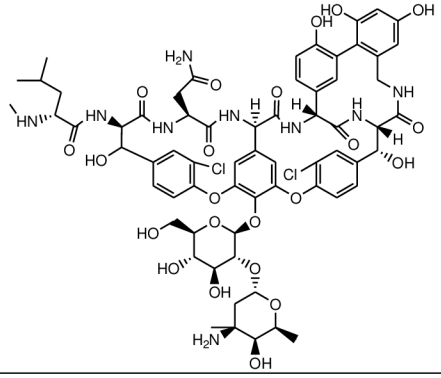
methyl nitrone in the third position may be a potential driver of the reduced activity of olaquindox. Eventually, oxyquinoline along with ferron as a representative of 8-hydroxyquinolines showed low or no antibiotic activity in almost all bacteria. The structure of oxyquinoline is simple with one hydroxy-group at the position eight of quinoline ring compared to ferron, which has the eighth, seventh and fifth position with the substituents of hydroxy-group, iodine, and sulfonic acid with an attached hydroxy group, respectively.

Table 2: Chemical structures of quinoline derivatives

Chemical formula	Name	Antimicrobial activity
8-Hydroxyquinolines		
	Ferron	Weak or no effect
	Chloroxine	Moderate
	Nitroxoline	Strong
	Oxyquinoline	Weak or no effect
Quinoxaline 1,4-dioxides		
	Carbadox	Strong
	Olaquindox	Weak or no effect

Footnote: The levels of antibacterial effect is defined as strong (MIC ranging from 0.0625 to 32 μg/ml), moderate (MIC ranging from 64 to 128 μg/ml), and weak or no effect (MIC equal or greater than 256 μg/ml).

Table 3: Antimicrobial activity of conventional antibiotics

Chemical formula	Name	Antimicrobial activity
Fluoroquinolines		
	Ceftriaxone	Strong
Cephalosporins		
	Ciprofloxacin	Strong
Miscellaneous agents		
	Chloramphenicol	Moderate
Imidazols		
	Metronidazole	Moderate
Tetracyclines		
	Tetracycline	Moderate
Glycopeptides		
	Vancomycine	Moderate

Footnote: The levels of antibacterial effect are defined as strong (MIC ranging from 0.0625 to 32 μg/ml), moderate (MIC ranging from 64 to 128 μg/ml), and weak or no effect (MIC equal or greater than 256 μg/ml).

5. Conclusion

In summary, quinoline compounds and conventional antibiotics tested in this study produced certain level of *in vitro* antimicrobial effects against pathogenic bacteria associate with CRC. Additionally, certain quinolines (carbadox and nitroxoline) produced inhibitory effect comparable with the most active conventional antibiotics (ciprofloxacin, ceftriaxone, and tetracycline). Specifically, carbadox showed to possess higher activity against 4 out of 6 bacteria even when compared to some antibiotics. According to this, it can be concluded that the quinoline derivatives of the 1,4-dioxide and 8-hydroxyquinoline groups, namely carbadox and olaquindox, appear to be antibacterial active chemical structures worthy of further investigation. Ciprofloxacin, conventional antibiotic belonging to the same class of compounds, together with ceftriaxone produced the strongest activity among all agents tested. According to our best knowledge, this is the first report on growth-inhibitory activity of antimicrobial agents against bacteria associated with CRC. Moreover, these findings provide arguments for further investigation of quinoline compounds as prospective structures for growth suppression of CRC associated bacterial strains.

6. References

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