Design, synthesis, biological assessment, and molecular docking studies of complexes based on chromium(iii) and metformin

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Abstract
The design, synthesis, computational and experimental assessment of the antibacterial activity, as well as docking investigations of newly synthesized chromium based metal complexes, are all included in this paper. E. coli and B. cereus were successfully inhibited by all three compounds (1, 2, and 3). Compound 2 outperformed reference medications ampicillin and streptomycin, which were inert or only shown bacteriostatic activity against MRSA, P. aeruginosa, and E. coli, respectively, in the evaluation of antibacterial activity against the three resistant pathogens. The antifungal properties of all the compounds were present, with compound 2 being the most potent. Almost all chemicals outperformed ketoconazole in their ability to combat different fungus strains. In order to elucidate the mechanism of antibacterial and antifungal activities, molecular docking studies on E. coli Mur B and C.
Keywords: chromium, antibacterial, antifungal, docking; MurB; CYP51

1. Introduction

The formation of biofilms is one of the bacterial growth strategies, and it may be viewed as a fundamental survival tactic in unfavourable situations [1,2]. The development of biofilms is crucial for both bacterial infection and antibiotic resistance. Bacteria buried in biofilms are often more resilient to conventional antimicrobial agents and host defence mechanisms than planktonic bacteria [3]. Chronic and persistent infections result from established biofilms' reduced sensitivity to immune system clearance [4]. Pseudomonas aeruginosa is the main bacterium that causes biofilm-associated infections. On a variety of surfaces, it can develop biofilm [5,6]. Similar results in the literature suggest that Candida's biofilm may be up to 1000 times more resistant to antifungal medications than its planktonic cell [7]. The increasing resistance to the current antimicrobial treatment has resulted in a crucial need for the discovery and development of novel entities for the treatment of infections with different modes of action that could target both sensitive and, in particular resistant strains [8]. This need is even greater for patients suffering from chronic inflammatory bowel diseases as well as cystic fibrosis (CF). During an inflammatory response in the gut, some commensal microorganisms, such as E. coli and C. albicans, can thrive and contribute to illness [9]. While Pseudomonas aeruginosa is an
opportunistic pathogen that commonly infects the CF lung, promoting an accelerated decline of pulmonary function [10]. Although new drugs were introduced in the battle against bacteria and fungi, such as echinocandin derivatives [11,12], nevertheless, some fungal species are still resistant. Consequently, the major clinical challenge is to overcome the need for further new antimicrobial agents that can simultaneously combat the resistance dilemma by designing powerful new drugs being less prone to multiresistance or to toxic side effects.

2. Biological Evaluation

2.1. Antibacterial Action

The derivatives were evaluated for their inhibitory action on the growth of eight bacterial strains in addition to eight fungi using the microdilution method with the aim of disclosing minimal inhibitory (MIC), minimal bactericidal (MBC) and minimal fungicidal (MFC) concentrations. All compounds demonstrated antibacterial efficiency, but their potency was different. MIC were in the range of 26.3–378.5 μM and MBCs ranged between 52.6–757.0 μM. The antibacterial activity of these compounds can be presented in the following order: 2 > 1 > 3 > AMP. Compound 2 exhibited the highest antibacterial activity. Its bacteriostatic activity was detected in concentrations of 43.3–86.7 μM and its bactericidal activity at concentrations of 86.7–173.4 μM. This compound is 6 times more potent than ampicillin and 3 times than streptomycin against all bacteria. Compound 3 was the less active (MIC ranging from 125.4 to 344.8 μM and MBC ranging from 250.7 to 689.6 μM).

2.2. Antifungal Activity

The compounds were then studied for their antimycotic activity, and results are all three compounds showed very good antifungal activity. Particularly, the ranges of MIC and MFC were 27.7–578 μM and 55.4–1156.0 μM, respectively. The activity sequence can be presented as follows: 3 > 2 > 1 > bifonazole > ketoconazole. The most active compound was 3 with MIC 59.6–119.2 μM and MFC 119.2–238.4 μM, while the minimum effect was recorded for derivative 1 (MIC = 156.7–501.5 μM and MFC = 313.4–1003.0 μM

2.3. In silico molecular docking study

Discovery Studio 2.1 software was used to perform docking between the metal complex (ligand) and BDNA (receptor) sequence. Input used for the docking is B-DNA sequence 5′-D (*AP * CP * CP * GP * AP * CP *GP * TP * CP * GP * GP * T)-3′ retrieved from protein data bank (PDB ID: 423D) at a resolution of 1.6 A° and the 3D models of the metal complexes. Receptor was prepared bydeleting all the hetero atoms including water, and by adding polar hydrogen atoms, docking energy (CDocker Interaction energy) of title derivatives were explored towards target. From the docking analysis of B-DNA sequence with the synthesized complexes, unveiled theirdocking scores and interaction patterns. The docked complexes **1, 2, 3** exhibited fitness scores with a range of 26.936, 30.251, 26.337 as compared to metformin 28.961 (Table 4). Among all the compounds, Compound 2 was ranked highest docking score of 30.251. It also showed good interaction with the binding site of DNA as metformin. The protein–ligand interaction visualization of the compounds is shown below.

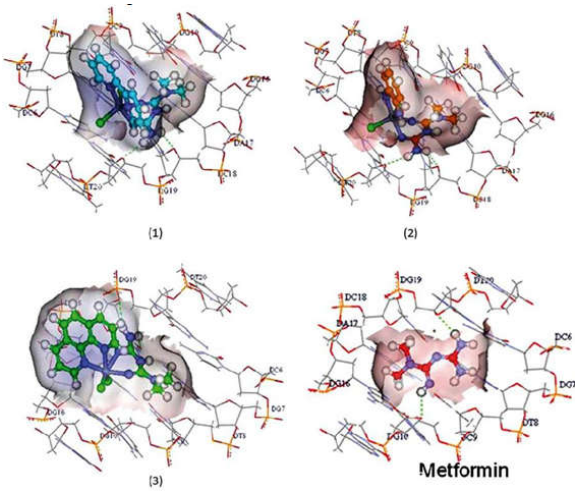

Fig1: Receptor-ligand hydrogen bonds (green colour) and bumps (pink colour) of complexes (1, 2, 3) and Metformin with B-DNA sequence (PDB: 423D).

3. Materials and Methods

3.1. Synthesis of metal complexes

Metformin (0.166 g, 1 mmol) was dissolved in 5 ml 0.1 N KOH solution. $CrCl_3.6H_2O$ (0.266 g, 1 mmol) in 5 ml water was added to the metformin solution, in the presence of bipyridine (0.156 g, 1 mmol) or orthophenylenediamine (0.108 g,1 mmol) or 1,10-phenanthroline (0.198 g, 1 mmol) at room temperature.The reaction mixture was heated for 1–2 hr on water bathand the complex separated out was filtered, dried. The purity of the complexes was tested by thin layer chromatography using different solvent mixtures. Yield: 70–90% for all the three complexes. The melting points of complexes are above 320^0c.

$[Cr(Cl)_2(Hmet)(bipy)]1.5H_2O$: Yield:85%; Colour: green; M.wt:434; Anal.cal. for $CrC_{14}H_{21}N_7O_{1.5}Cl_2$: C 38.70%, H 4.83%, N 22.58%, Cl 16.35%, Cr 11.98% found: C 38.0%, H 4.60%, N 22.21%, Cl 16.74%, Cr 11.73%; Λm(Ω−1 cm2 mol−1) 4.2; FT IR (KBR disc cm−1): 3250–3400, 1566, 1494, 1442, 1421, 940, 522, 453, 345; LC– MS:434 (M)m/z.

$[Cr(Cl)_2(Hmet)(opda)]0.5H_2O$: Yield:80%; Colour: brown; M.wt:368; Anal. cal. For $CrC_{10}H_{19}N_7O_{0.5}Cl_2$: C 32.60%, H 5.16%, N 26.63%, Cl 19.29%, Cr 14.13% found: C 32.15%, H 5.24%, N 26.63%, Cl 19.34%, Cr 14.00%; Λm(Ω−1 cm2 mol−1) 4.5; FT IR (KBR disc cm−1): 3250– 3400, 1498, 1413, 1664, 947, 522, 437, 345; LC–MS:369(M + 1) m/z.

$[Cr(Cl)_2(Hmet)(ophen)]H_2O$: Yield:75%; Colour: brown M.wt:449; Anal. cal. For $CrC_{16}H_20N_7OCl_2$:C 42.76%, H 4.45%, N 21.82%, Cl 15.81%, Cr 11.11% found: C 42.32%, H 4.47%, N 21.87%, Cl 15.75%, Cr 11.11%; Λm(Ω−1 cm2 mol−1) 4.3; FT IR (KBR disc cm−1): 3250–3400, 1514, 1450, 1508, 1417, 947, 540, 422, 331; LC–MS:449(M) m/z.

3.2. Biological Evaluation

3.2.1. Antibacterial Action

Bacterial strains utilized include Gram-negative: Salmonella typhimurium, (ATCC 13311) Pseudomonas aeruginosa (ATCC 27853), Escherichia coli (ATCC 35210), Enterobacter cloacae (ATCC 35030) and Gram-positive bacteria: Micrococcus flavus (ATCC 10240), Bacillus cereus (isolated clinically), Staphylococcus aureus (ATCC 6538) and Listeria monocytogenes (NCTC 7973) bacteria. Pathogens were provided from the Mycological Laboratory, Institute for Biological Research “Siniša Stankovic” Belgrade. Resistant strains used were MRSA, E. coli and P. aeruginosa. The MIC/MBC were effectuated utilizing microdilution assay as previously described [13,14].

3.2.2. Methicillin-resistant Staphylococcus aureus (MRSA)

This strain is isolated from cows with subclinical mastitis. Milk samples were streaked onto Columbia agar plates (Torlak, Beograd, Serbia) containing 5% sheep blood, BairdParker agar plates (HiMedia, Mumbai, Maharashtra, India) and chromogenic culture media (chromID MRSA, bioMérieux, Marcy l'Etoile, France). After incubation at 37 ◦C for 24 h, the colonies were presumptively identified according to morphological features, pigment production, Gram staining results, catalase and oxidase tests, type of hemolysis and characteristic growth on Baird-Parker agar plates (HiMedia, Mumbai, Maharashtra, Antibiotics 2021, 10, 309 14 of 19 India), BP agar and chromogenic culture media (chromID MRSA, bioMérieux, Durham, NC, USA) chromID MRSA. Suspected colonies of S. aureus on the blood agar and green colonies on chromogenic media were transferred to individual plates to obtain pure cultures. The identification was confirmed using a BBL Crystal G/P ID kit (Becton Dickinson, Nairobi, Kenya). Antimicrobial susceptibility testing was performed by the disk diffusion method with 30 µg cefoxitin discs (Rosco, Taastrup, Denmark) in accordance with the Clinical and Laboratory Standard Institute recommendations. All isolated strains of S. aureus were tested for the presence of penicillin-binding protein (PBP2) with latex agglutination tests (Slidex MRSA detection, bioMérieux, Zhujiang, New Town, China). Staphylococcus aureus ATCC 25923 was used as the control strain. All isolates were tested for the presence of the mecA gene by PCR [15].

3.2.3. E. coli

Samples of rectal swabs, feces and intestines from diseased pigs were taken. In order to isolate E. coli strains, the following nutrition media were used: MacConkey agar (Torlak, Beograd, Serbia), Columbia agar (Torlak, Beograd, Serbia) with 5% defibrinated sheep blood and brilliant green agar (Torlak). For the identification of the isolated strains, laboratory tests with the following nutritious media and reagents were performed: Simmons citrate agar (Torlak), MR/VP broth (Torlak), Christensen urea agar (Torlak, Beograd, Serbia), peptone water for the indole test (Torlak), catalase and oxidase, triple sugar agar (Torlak), as well as identification systems BBL crystal entero/nonfermenter ID kit (Becton Dickinson Nairobi, Kenya,). Sensitivity studies on the isolated bacteria were completed by the disc diffusion method on Mueller–Hinton agar with the use of antibiogram discs (Bioanalyse, Ankara, Turkey) and tablets (Torlak) for the following antibiotics: penicillin, ampicillin, amoxicillin, tetracycline, neomycin, gentamicin, colistin, ceftriaxone, sulfamethoxazole with trimethoprim, enrofloxacin and florfenicol. All isolated E. coli strains were resistant to all tested antibiotics with the exception of enrofloxacin, colistin and florfenicol [16].

3.2.4. Pseudomonas aeruginosa

The strains were isolated from cats and dogs. Samples were inoculated on Columbia agar plates (Torlak, Serbia) containing 5% sheep blood, nutrition agar (HiMedia) and MacConkey agar (Torlak) and incubated under aerobic conditions at temperatures of 37 ◦C and 42 ◦C for 24 h. Pure cultures were identified on the basis of morphological and biochemical characteristics. For identification of pigment production, subcultivation on the corresponding medium was carried out. Identification was confirmed using a BBL crystal entero/nonfermenter ID kit (Becton Dickinson). Sensitivity studies were completed by the disc diffusion method on Mueller–Hinton agar with the use of antibiogram discs (Bioanalyse) and tablets (Torlak) for the following antibiotics: penicillin G, ampicillin, amoxicillin, tetracycline, neomycin, gentamicin, ceftriaxone, sulfamethoxazole with trimethoprim, enrofloxacin and florfenicol. All isolated Pseudomonas aeruginosa strains were resistant to all tested antibiotics with the exception of enrofloxacin and florfenicol [16].

3.2.5. Inhibition of Biofilm Formation

The method was performed as described by us [17] with some modifications. Briefly, P. aeruginosa resistant strain was incubated with MIC and subMIC of tested compounds in tryptic soy broth enriched with 2% glucose at 37 ◦C for 24 h. After 24 h, each well was washed twice with sterile PBS (phosphate-buffered saline, pH 7.4) and fixed with methanol for 10 min. Methanol was then removed, and the plate was air-dried. Biofilm was stained with 0.1% crystal violet (Bio-Merieux, Marcy l'Etoile, France) for 30 min. Wells were washed with water, air dried, and 100 µL of 96% ethanol (Zorka, Sabac, Serbia) was added. The absorbance was read at 620 nm on a Multiskan™ FC microplate photometer, Thermo Scientific™. The percentage of inhibition of biofilm formation was calculated by the formula: [(A620 control − A620 sample)/A620 control) × 100] Antibiotics 2021, 10, 309 15 of 19

3.2.6. Antifungal Activity

For the antifungal bioassays, eight fungi were used: Aspergillus niger (ATCC 6275), Aspergillus ochraceus (ATCC 12066), Aspergillus fumigatus (human isolate), Aspergillus versicolor (ATCC 11730), Penicillium funiculosum (ATCC 36839), Penicillium ochrochloron (ATCC 9112), Trichoderma viride (IAM 5061), Penicillium verrucosum var. cyclopium (food isolate). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stankovic", Belgrade, Serbia. All experiments were performed in duplicate and repeated three times [18].

3.3. Statistical Analysis

All tests were performed three times, and the values were determined as standard deviation (SD) and mean values. A one-way ANOVA test was allowed to determine variance analysis with Tukey HSD Test (0.05 levels). The analysis was executed with the help of SPSS statistics software (version 18).

3.4. Cytotoxicity

CellTiter 96® aqueous nonradioactive cell proliferation assay (Promega, Madison, WI, USA) was performed to evaluate the in vitro effects of evaluated compounds in breast adenocarcinoma MCF7/S0.5 (parental MCF7 cells adapted to low-sera conditions) and human kidney immortalized cell line HK-2. The employed method uses the bioreduction of tetrazolium salt of
MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4- sulfophenyl)-2H-tetrazolium) into a colored formazan with an absorbance peak maximum at wavelength 490 nm. Only viable cells are able to metabolize the compound. Experiments were conducted in accordance with manufacturer guidelines. Briefly, cells were treated with the test compounds, negative control (SDS 10%) or vehicle (DMSO 0.1%) for 48 h in 96- wells plates. At the end of the treatment, 20 µL of MTS reagent was added to each well and incubated for a further 3 h prior to absorbance measurement using a plate reader (Hidex Sense Beta Plus plate reader, Hidex, Turku, Finland). MCF7/S0.5 cell line was cultivated in DMEM/F-12 media w/o phenol red supplemented with 1% FBS and insulin 6 ng/mL. HK-2 cells were cultivated in DMEM with high-glucose and L-glutamine, supplemented with 10% FBS. Results are expressed as the relative cell viability, considering the vehicle to have 100% viability.

Conclusions

A wide range of Gram-positive, Gram-negative, and fungi were significantly inhibited in their development by three newly created and manufactured [Cr Cl (met) opda/bipy/ophen]H2O compounds. The bulk of the compounds were more effective than the antibiotic ampicillin, which is the gold standard. Moreover, compounds 1, 2, and 3 showed greater efficacy than streptomycin. E. coli was the most susceptible bacteria, whereas P. aeruginosa showed the highest levels of resistance. Moreover, several of the compounds had greater or equal efficacy to streptomycin, with compound 3 being the most powerful of them all. It was found that S. typhimurium was the most resistant Gram-negative bacterium, and E. coli was the most

susceptible to the chemicals examined. B. cereus was the most delicate Gram-positive bacterium, and L. monocytogenes was found to be the most resistant bacterium. Furthermore, all compounds exhibited antibacterial potency against the tested resistant bacterial strains displaying better efficacy than ampicillin and some of them (12 and 15) even higher than streptomycin. Compounds 1, 3 appeared to be more potent than streptomycin and ampicillin against the resistant strain of E. coli, which was the most sensitive, while some of them exhibited better activity than reference drugs against methicillin-resistant Staphylococcus aureus, the most resistant strain. Compounds 1,2 and 3 exhibited significant antibiofilm activity. Antibiotics 2021, 10, 309 16 of 19 As regards antifungal activity, most of the examined compounds displayed better potency than reference drugs, ketoconazole and bifonazole. Thus, the most active compound 2 was found more active than bifonazole and ketoconazole, respectively. Docking analysis to E. coli MurB indicated a probable involvement of MurB inhibition in the antibacterial mechanism of compounds tested, while docking to 14α-lanosterol demethylase (CYP51) and tetrahydrofolate reductase of Candida albicans indicated a probable implication of CYP51 reductase at the antifungal activity of the compounds. Finally, toxicity prediction revealed that compounds are not toxic. In addition, according to the prediction of physicochemical parameters for the passage through biological membranes, they will likely be absorbed via passive diffusion when given orally. Anyway, their toxicity toward a human cell line was experimentally assessed at a relatively high concentration of 50 μM. Several active compounds were not toxic even at this high concentration.

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