SYNTHESIS AND *IN VITRO* ANTIMICROBIAL EVALUATION OF A NEW METRONIDAZOLE DERIVATIVE

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ABSTRACT

INTRODUCTION: For decades there has been active work on the synthesis, identification, and exploration of heterocyclic compounds. However, the imidazole nucleus is the main scaffold in molecules with various pharmacological properties such as antibacterial, antifungal, antineoplastic, antiviral, antidiabetic, etc. This work describes a synthesis of new metronidazole amide derivative by condensation of -(2-methyl-5-nitro-1H-imidazole-1-yl)acetic acid with 2-(diethylamino)ethyl 4-aminobenzoate (procaine). The chemical structure of the new compound was confirmed by its IR and UV-VIS spectral data and was evaluated for *in vitro* potential antimicrobial activity against standard bacterial strains of *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231.

AIM: The present study aimed to synthesize, characterize, and evaluate the potential antimicrobial activity of a new metronidazole amide compound against standard bacterial strains of *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Candida albicans* 10231.

MATERIALS AND METHODS: The new amide metronidazole derivative 2-(diethylamine) ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide)benzoate was tested for in vitro antibacterial activity by the following described methods: cup-plate technique, minimum inhibitory concentration determination (MIC), and minimum bactericidal concentration (MBC) determination.

RESULTS: A synthesis of a new metronidazole amide derivative was made by condensation of -(2-methyl-5-nitro-1H-imidazole-1-yl)acetic acid with 2-(diethylamino)ethyl 4-aminobenzoate (procaine). The chemical structure of the new compound was confirmed by its IR and UV-VIS spectral data and was evaluated for *in vitro* potential antimicrobial activity against standard bacterial strains of *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231.

CONCLUSION: The obtained data assess the same antimicrobial activity of the new compound and metronidazole against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231. The highest sensitivity was observed against *Candida albicans*.

Keywords: *metronidazole, antimicrobial, amide, synthesis*

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INTRODUCTION

For decades there has been active work on the synthesis, identification, and exploration of heterocyclic compounds. However, the imidazole nucleus is the main scaffold in molecules with various pharmacological properties such as antibacterial, antifungal, antineoplastic, antiviral, antidiabetic, etc. On the other hand, 5-nitroimidazole drug derivatives are the most efficient antimicrobial agents. They have a great variety of therapeutic applications (1,2,3,4). The main target of 5-nitroimidazoles is bacteria and protozoa. The most widely known agent from this group is metronidazole—a drug that, for the past 70 years, has been the most efficient in severe anaerobic infections (5,6,7). However, metronidazole is becoming increasingly inefficient because of drug resistance due to the microbial variability and its long-term use (8,9,10). Therefore, it is a suitable object for different chemical transformations and the synthesis of modified metronidazole derivatives. On the other hand, designing hybrid drugs by covalently linking two distinct chemical moieties with multiple effects is a common strategy in today's search for new potential drugs (11). For example, current research in this field seems to endorse hybrid molecules as the next-generation antimalarial drugs. If the selective toxicity of hybrid prodrugs can be demonstrated *in vivo* with good bioavailability at the target site in the parasite, it would offer various advantages including dosage compliance, minimized toxicity, ability to design better drug combinations, and cheaper preclinical evaluation while achieving the ultimate object of delaying or circumventing the development of resistance (12). The goal is to achieve better pharmacokinetic properties, reduced toxic effects, and extend the biological activity. This work describes a synthesis of new metronidazole amide derivative by condensation of -(2-methyl-5-nitro-1H-imidazole-1-yl)acetic acid with 2-(diethylamino)ethyl 4-aminobenzoate (procaine). The chemical structure of the new compound was confirmed by its IR and UV-VIS analysis and was evaluated for *in vitro* potential antimicrobial activity against standard bacterial strains of *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231.

AIM

The present study aimed to synthesize, characterize, and evaluate the potential antimicrobial activity of new metronidazole amide compound against standard bacterial strains of *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Candida albicans 10231.*

MATERIALS AND METHODS

2-methyl-5-nitroimidazole-1-ethanol (Fluorochem); sulfuric acid (99,99% Chem Lab); sodium bichromate (≥99.5%, Sigma-Aldrich); ethyl-4-aminobenzoate (98% Sigma Aldrich); procaine **(2-(diethylamine)ethyl-4-aminobenzoate,** Sigma-Aldrich)**;** tetrahydrofuran (anhydrous, ≥99.9%, inhibitor-free Sigma Aldrich); methanol (for HPLC ≥ 99.9%); N,N1-diciclohexylcarbodiimide (DCC) (Sigma Aldrich); ethanol (≥99.5%, for HPLC, Sigma-Aldrich); DMSO (®, ≥99%, Sigma-Aldrich); 0.9% sodium chloride (Baxter); *Bacteroides fragilis* ATCC 25285—lyophilized strains 25285 (Mecconti, MicroSwabs®); Metronidazole 5 mcg MT—antibiotic discs; Mueller-Hinton broth—agar (HiEncap); Sabouraud dextrose agar—growth medium in capsules (HiEncap); Wilkins-Chalgren agar—petri dish, 90 mm (HiEncap); Infusion Agar—growth medium (HiEncap), granulated; Soyabean Casein Digest Agar—growth medium; (HiEncap™); Brain Heart Broth—growth medium in capsules (HiEncap); Brain Heart Infusion broth—growth medium; (HiEncap).

The new amide metronidazole derivative 2-(diethylamine)ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide)benzoate was tested for *in vitro* antibacterial activity by following described methods.

The **cup-plate technique** includes dense seed of 0.5 MF standardized microbial culture on Mueller–Hinton agar (HiMedia®). After the surfaces of the culture media have dried, we made 6 mm wells in the agar with a sterile borer and filled them with 40 µL of the appropriate concentration of the test compound 2-(diethylamine)ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide) benzoate and metronidazole as a control sample. Solutions corresponded to 1.5, 3, 6, 12.5, and 25 µg/mL. One more control was set for DMSO 1% (V/V). All samples were made in triplicate. The Petri dishes were incubated aerobical-

ly for 24 hours at 37°C for bacterial cultures and 48 hours at 35°C for *Candida albicans*.

Minimum Inhibitory Concentration (MIC) Determination

For the testing of the MIC of metronidazole and its derivative, we used Brain Heart Infusion broth (HiEncapTM). We made serial dilutions of the tested antimicrobial agents in the range from 1.5 to 25 μg/mL. We placed 0.1 mL of standardized microbial culture in each tube and cultured the samples aerobically for 24 hours at 37°C for bacterial cultures and 48 hours at 35°C for *Candida albicans*. Minimum inhibitory concentrations are defined as the lowest concentration of the testing antimicrobial agents that inhibit the visible growth of a microorganism after overnight incubation.

Minimum Bactericidal Concentration (MBC) Determination

After determining the MICs of the tested solutions from all tubes in which no visual turbidity was reported, bacterial seeds were made on Blood Agar (HiMedia®). The volume of the suspensions taken with one bacterial loop was transferred on the agar media and placed in an incubator at 37°C/24 hours for *E. coli* and *S. aureus* and at 35°C/48 hours for *C. albicans*. The lowest concentration at which bacterial growth is inhibited to 99.9% is reported as MBC.

RESULTS

The condensation of metronidazole by one local anesthetic procaine was processed in the presence of N,N′-Dicyclohexylcarbodiimide (DCC). The synthetic procedure involves two steps, as illustrated on Fig. 1. The first stage of the synthesis includes oxidation of the initial metronidazole in the presence of Jones reagent to obtain as an intermediate the corresponding 2-(2-methyl-5-nitro-1H-imidazole-1-yl) acetic acid (13).

The next step involved the classical amidation approach of direct interaction of the initial 2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetic acid with the corresponding 2-(diethylamino) ethyl 4-aminobenzoate in the catalytical presence of DCC to obtain the target 2-(diethylamine)ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide)benzoate (13).

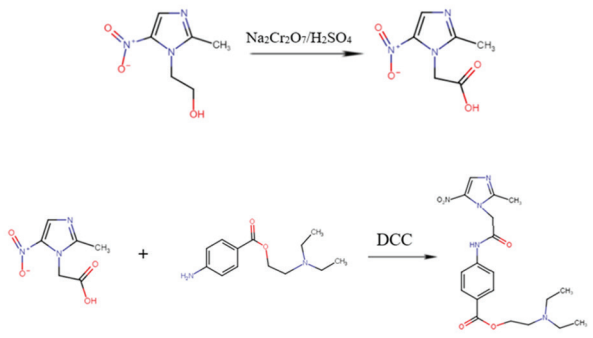

Fig. 1. Reaction scheme of synthesis of the targeted derivative.

FTIR spectroscopy

The captured spectrum of 2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetic acid is similar to the metronidazole spectrum because only one structural change was done—the transformation of hydroxyl into carboxyl group. The vibrations that appeared at 3140 cm^{-1} confirm the presence of the newly formed carboxyl group. The strong absorption at 1716 cm^{-1} is assigned to the carboxylic group too. The presence of a carboxyl group (-COOH) is also confirmed by the composite bands at 1275 and 1222 cm^{-1}, corresponding to C-O valence fluctuations, regarded to the single peak at 1264 cm^{-1} in the metronidazole spectrum. The spectra of 2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetic acid and 2-(diethylamine)ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide)benzoate are presented on Fig. 2.

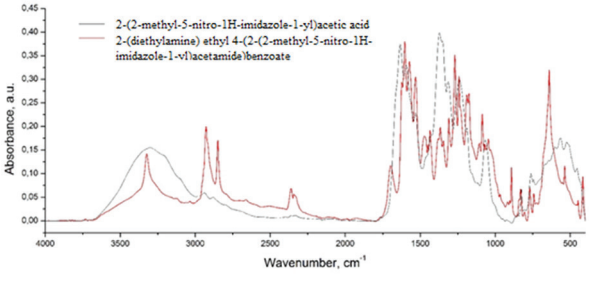

Fig. 2. IR spectra of 2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetic acid and 2-(diethylamine) ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide)benzoate.

A careful assessment of the 2-(diethylamine) ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl) acetamide)benzoate spectrum shows significant

differences compared to 2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetic acid. The set of bands in the region 3500-2500 cm^{-1} registered more complex spectral imaging compared to mother structures. The major bands observed at 3323 and 2927 cm^{-1} are associated with the presence of a new amide bond. The strong absorption band at 1697 cm^{-1} can rely on the carbonyl group. The frequency of the nitro group is at 1513 cm^{-1}. The oscillation in the 1600 to 1000 cm^{-1} region is consistent with C-N, C-C, C-C-H bonds. The peaks at 1190 and 1174 cm^{-1} cannot definitely be relied on by the ester group because in these frequencies C-N bonds have absorption too.

UV-Vis spectroscopy

UV-Vis spectroscopic analysis of 2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetic acid and 2-(diethylamine)ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide)benzoate was performed to identify the spectral behavior and absorption properties of the test compound. In the spectrum of 2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetic acid, close values of the reported absorption maximum λ_{max} was observed at 310 nm. The result is similar for the new amide derivative of metronidazole and its absorption maximum was reported at λ_{max} 270 nm. We can conclude that the hypsochromic shift in the spectrum of 2-(diethylamine)ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide)benzoate is due to the newly introduced aromatic substituents.

Antimicrobial activity of 2-(diethylamine) ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl) acetamide)benzoate against Staphylococcus aureus ATCC 29213, Escherichia coli ATCC 25922, and Candida albicans ATCC 10231

In the study with agar media–cup–plate method, zones of inhibition were not reported. The reason was that the antimicrobial agents in the wells did not show visible diffusion in the agar. When conducting studies to determine the minimum inhibitory and minimum bactericidal concentration, we found that there is an antimicrobial activity of 2-(diethylamine)ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide)benzoate and metronidazole against the tested microorganisms, but apparently the diffusion method is not suitable for its reporting.

Determination of minimum inhibitory concentration of metronidazole and 2-(diethylamine) ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl) acetamide)benzoate Against Staphylococcus aureus ATCC 29213, *Escherichia coli* ATCC 25922, *and Candida albicans ATCC 10231 by a modified method*

Solutions with dilutions of metronidazole and 2-(diethylamine)ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide)benzoate and metronidazole corresponding to 1.5, 3, 6, 12.5, and 25 μg/mL, were prepared. Again, DMSO 1% was used as a diluent. The demonstrated minimal inhibitory concen-

Table 1. Determination of MIC of metronidazole and 2-(diethylamine)ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl) acetamide)benzoate against Staphylococcus aureus ATCC 29213, Escherichia coli ATCC 25922, and Candida albicans ATCC 10231.

	Concentrations of DMSO Solutions				
μg/mL	**1.5**	**3**	**6**	**12.5**	**25**
	Staphylococcus aureus				
M	+	+	-	-	-
M1	+	+	-	-	-
	Escherichia coli				
M	+	+	-	-	-
M1	+	+	-	-	-
	Candida albicans				
M	+	+	-	-	-
M1	+	+	-	-	-

Legend: *(+) - bacterial growth is observed; (-) - lack of bacterial growth;*
M—metronidazole; M1—2-(diethylamine)ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide)benzoate.

trations of M and M1 against *E. coli*, *S. aureus*, and *C. albicans* were determined to be 6 μg/mL (Table 1 and Fig. 3).

Determination of the minimum bactericidal concentration of metronidazole and 2-(diethylamine) ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide)benzoate against Staphylococcus

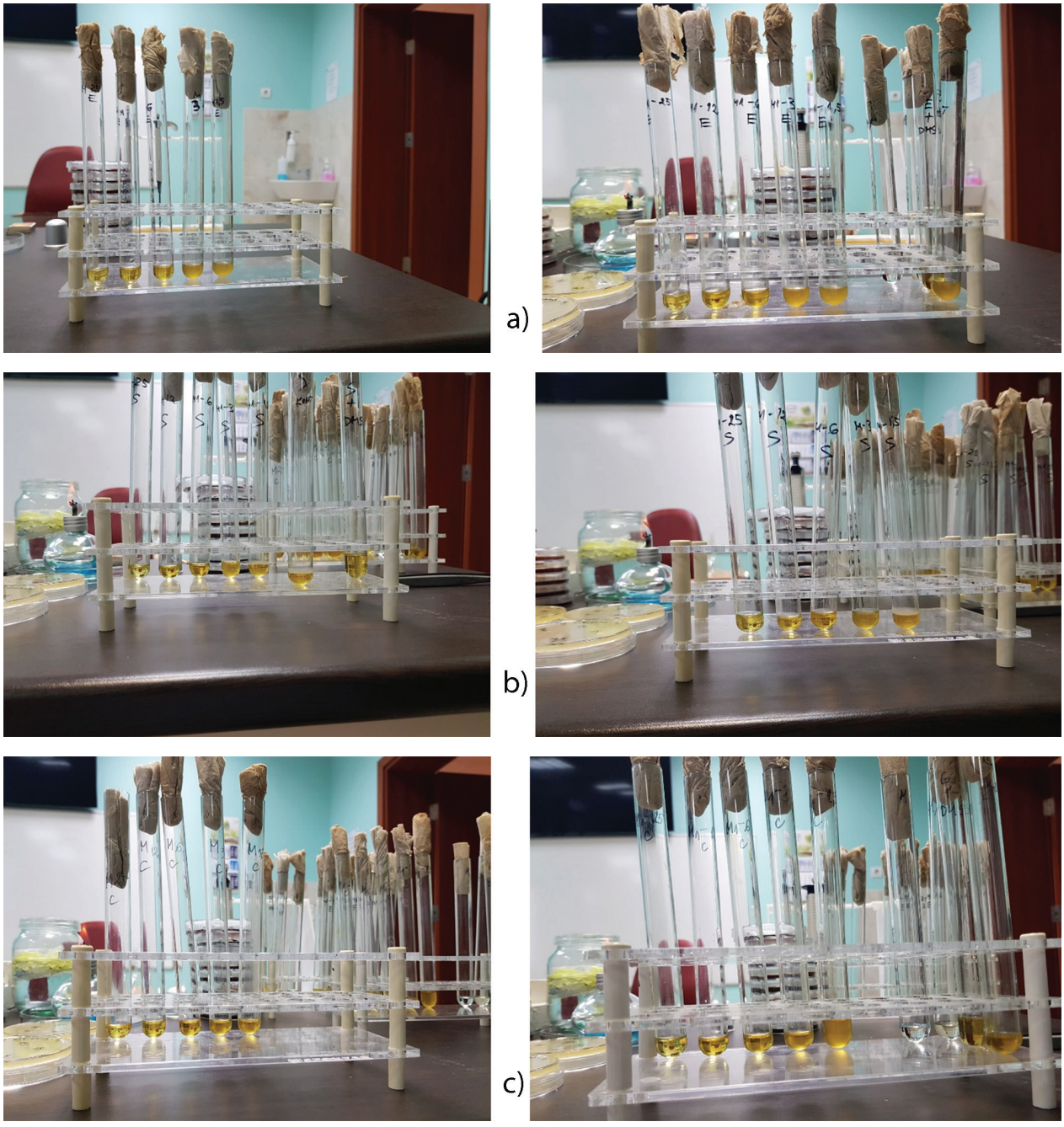

Fig. 3. a) determination of MIC of 2-(diethylamine)ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide)benzoate (left photo) and metronidazole (right photo) against E.coli; b) determination of the MIC of 2-(diethylamine)ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide)benzoate (left photo) and metronidazole (right photo) against S. aureus; c) determination of the MIC of 2-(diethylamine)ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide) benzoate (left photo) and metronidazole (right photo) against Candida albicans ATCC 10231.

aureus ATCC 29213, Escherichia coli ATCC 25922, and Candida albicans ATCC 10231

After determining the MICs of the test solutions, from all tubes without visible growth, we transferred from the suspension into Blood Agar. After an incubation period, the following minimum bactericidal concentrations were obtained: metronidazole—25 μg/mL; 2-(diethylamine)ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide)benzoate—25 μg/mL against *S. aureus*; metronidazole —25 μg/mL; 2-(diethylamine)ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide)benzoate—25 μg/mL against *E.coli*, and metronidazole—12 μg/mL; 2-(diethylamine)ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide)benzoate—12 μg/mL against *Candida albicans,* shown in Fig. 4.

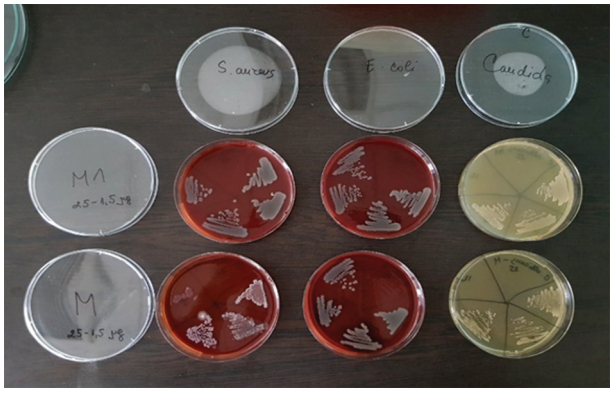

Fig. 4. Determination of minimum bactericidal concentration of metronidazole and 2-(diethylamine)ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide) benzoate against S. aureus, E.coli, and Candida albicans.

CONCLUSION

Despite advances in medicine, we remain vulnerable to infections with limited or non-standard therapies. It is important to look at the situation in the context of AMR as well, as resistant pathogens are evolving and leading us to the era of incurable infections. There is a need to develop newer functional antimicrobial agents. One new metronidazole derivative was successfully synthesized and its antimicrobial properties were evaluated by agar and broth dilution assays. The obtained data evaluate the same antimicrobial activity of the new compound and metronidazole against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231. The highest sensitivity was observed against *Candida albicans.*

Acknowledgement

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