RUTIN SEMISYNTHETIC DERIVATIVES WITH ANTIFUNGAL PROPERTIES

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Abstract

Among the often cited flavonoids with pharmacological properties, rutin is recently highlighted for its antifungal effect. On the other hand, the development ofazole based drugs represented a major advance in medical mycology; azoles are currently the most popular class of antifungals used in medicine ( clotrimazole, ketoconazole, fluconazole, itraconazole, a.o.). Starting from these facts, we synthesised some water soluble rutin derivatives containing imidazole and benzimidazole moieties in their chemical structure, treating rutin with 1,3-dichloro-2-propanol, 1-bromo-3-chloropropane, 2-dibromomethane and dibromomethane, and then with imidazole and benzimidazole, respectively. Molecular formula, weight, yield, melting points and solubility have characterized the new derivatives. Elemental analysis, IR and \textsuperscript{1}H-NMR spectral analysis have confirmed the structure of new the compounds. In vitro microbiological assays have been made. The tested compounds have shown a good antifungal activity (against \textit{Candida spp.}) and antibacterial effects also (against Gram- positive bacterial strains).

Rezumat

Din categoria flavonoidelor cu proprietăți farmacologice, rutozidul este recent investigat pentru proprietățile sale antifungice. Pe de altă parte, dezvoltarea medicamentelor ce conțin azoli în structura chimică a constituit un progres major al terapiei antifungice, la ora actuală azolii reprezând cea mai utilizată categorie de antimicotic e (clotrimazol, ketoconazol, fluconazol, itraconazol, a.a.). Pornind de la aceste premise, am sintetizat o serie de derivații hidrosolubilii a rutozidului, care conțin în structură fragmente de imidazol și benzimidazol, prin tratarea rutozidului cu 1,3-dicloro-2-propanol, 1-brom-3-cloropropan, 2-dibromomethan și dibromometan, în mediu de metoxid de sodiu, și apoi cu imidazol, respectiv, benzimidazol. Noi derivații au fost caracterizați prin formulă și masă moleculară, randament, punct de topire și solubilitate, iar structura a fost confirmată prin analiză elementală și analiză spectrală în IR și \textsuperscript{1}H-NMR. Au fost întreprinse teste microbiologice in vitro. Compuși testați prezintă bune proprietăți antifungice (impotriva \textit{Candida spp.}), precum și efecte antibacteriene (asupra bacteriilor gram- pozitive).

Keywords: flavonoids, rutin, imidazole, antifungal

Introduction

Several studies highlight antifungal properties of rutin or vegetal species with a high content of flavonoids (especially rutin); furthermore, there are some arguments that rutin is able to increase the antifungal activity of other compounds [2, 3, 5, 7, 8]. Unfortunately, rutin has a very low solubility in water, but our research team previously synthesized several rutin water-soluble derivatives [4]. On the other hand, it is well-known that azoles are important pharmacophores for antifungal effect, but also for antibacterial, anticancer, antiviral, and antiprotostomal actions. Imidazole ring is present in many antifungal existing drugs, like clotrimazole or miconazole. Besides, there are many recent studies concerning the design and synthesis of some new antifungal agents, incorporating azoles in their molecules [1, 6, 9, 10].

Starting from these premises, eight new rutin derivatives containing imidazole and benzimidazole moieties in their structures were synthesized and tested in antifungal and antibacterial assays.

Materials and Methods

All commercial chemicals and solvents are reagent grade and were used without further purification. Rutin (97 - 102%) was purchased from Acros Organics, Belgium; solvents (methanol, ethanol, isopropanol,) were purchased from Sigma-Aldrich, Germany, halogenated reagents (1,3-dichloro-2-propanol, 1-brom-3-chloropropane, 1,2-dibromoethane and dibromomethane) were purchased from Merck-
available discs containing Petri plates. 10 mm stainless steel cylinders (5 mm internal diameter; Hinton agar for yeasts (HiMedia) and then spread VIII (Oxoid) and McFarland standard no. 0.5 (106 CFU/mL). The in sterile 0.9% NaCl until the turbidity was equivalent A small amount of each microbial culture was diluted 1:10 in M to McFarland standard no. 0.5 (106 CFU/mL). The in sterile 0.9% NaCl until the turbidity was equivalent A small amount of each microbial culture was diluted 1:10 in M to McFarland standard no. 0.5 (106 CFU/mL). The in sterile 0.9% NaCl until the turbidity was equivalent A small amount of each microbial culture was diluted 1:10 in M to McFarland standard no. 0.5 (106 CFU/mL). Then, each microbial culture was diluted uncorrected and were measured in open capillary tubes on an Electrothermal Mel-Temp device; the elemental analysis was performed on an Exeter Analytical CE-440 elemental analyser. The IR spectra were recorded on a FT/IR Jasco 670 Plus spectrometer. The 1H-NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer using tetramethylsilane as internal standard and DMSO-d6 as solvent. The antimicrobial activity was studied using Gram- positive bacteria (Staphylococcus aureus ATCC 25923, Sarcina lutea ATCC 9341), Gram- negative bacteria (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853) and pathogenic yeasts (Candida albicans ATCC 90028, Candida glabrata ATCC MYA 2950); all these strains were obtained from the Culture Collection of the Department of Microbiology, Schuchardt, Germany, sodium was delivered by Riedel-de-Haen AG, Germany, and Silicagel was purchased from Fluka, Germany. Melting points are Antimicrobial tests of selected microorganisms were carried out using a disc-diffusion method [11, 12]. A small amount of each microbial culture was diluted in sterile 0.9% NaCl until the turbidity was equivalent to McFarland standard no. 0.5 (106 CFU/mL). The suspensions were further diluted 1:10 in Müller Hinton agar for bacteria (Oxoid) and Müller- Hinton agar for yeasts (HiMedia) and then spread on sterile Petri plates (25 mL/Petri plate). Sterile stainless steel cylinders (5 mm internal diameter; 10 mm height) were applied on the agar surface in Petri plates. Then, 0.1 mL of each compounds (I - VIII) were added into cylinders. Commercial available discs containing Ciprofloxacin (5 µg/disc), Fluconazole (25 µg/disc), Voriconazole (1 µg/disc) and Nystatin (100 µg/disc) were used as positive controls. The plates were incubated at 37°C for 24 h (bacteria) and at 24°C for 48 h (yeasts). After incubation, the diameters of inhibition zones were measured in mm, including disc size.

**Results and Discussion**

Eight new derivatives of rutin were synthesised and purified by column chromatography in good yield (72% - 86%); all these compounds are crystalline, hygroscopic, yellow powders, with no odour and having a slightly bitter taste, soluble in water,
alcohol, dimethylsulfoxide and dimethylformamide and insoluble in 2-propanol, dioxane, acetone, ether, benzene and chloroform. The chemical structures were proved by C, H, N elemental analysis and by IR and $^1$H NMR spectroscopy.

3-[6-0-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl-β-Oxoy]2-(3,4-dihydroxyphenyl]-5-hydroxy-7-(oxy-1-(β-hydroxy-propyl)-3-(imidazol-1-yl))-4H-I-benzopyran-4-one (I): yield 86%; mp 243 - 246°C; IR (KBr) (cm$^{-1}$): 3400 (OH), 2920 (CH), 1700 (C=N), 1660 (C=O on aromatic ring), 1630 (C=C on imidazole ring), 1600 (aromatic structure), 1500 (aromatic C=C), 1360, 1300, 1207 (C=O, C=C, 1220 (C-N), 780 (aromatic substituents); $^1$H NMR (DMso-d6): δ ppm: 7.74 (d, 1H, H-6'), 7.60 (d, 1H, N=CH-N), 7.44 (d, 1H, H-2'), 7.20 (d, 1H, N=CH-C), 6.90 (d, 1H, CH imidazole), 6.73 (d, 1H, H-5'), 6.46 (s, 1H, H-8), 6.31 (s, 1H, H-6), 5.52 (s, 1H, H1-glucoyl), 4.46 (s, 1H, H1-rhamnosyl), 4.32 (s, 2H, O2'-CH2), 4.1 (d, 1H, CH alkyll chain), 3.90 (s, 2H, CH2-N), 3.60, 3.20 (d, 4H, CH glu), 3.10, 3.03 (d, 4H, CH rha), 1.27 (s, 3H, CH3 rha); Molecular formula: C93H137N5O40; Molecular weight = 7346.66; Calculated = C (53.95%) H (5.21%) N (3.81%); Found = C (53.92%) H (5.20%) N (3.79%).

3-[6-0-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl-β-Oxoy]2-(3,4-dihydroxyphenyl]-5-hydroxy-7-(oxy-1-propyl-3-(imidazol-1-yl))-4H-I-benzopyran-4-one (II): yield 75.5%; mp 212 - 214°C; IR (KBr) (cm$^{-1}$): 3380 (OH), 2910 (CH), 1700 (C=N), 1650 (C=O on aromatic ring), 1630 (C=C on imidazole ring), 1600 (aromatic structure), 1520 (aromatic C=C), 1360, 1310, 1220, 1090 (C=C, C=N), 790 (aromatic substituents); $^1$H NMR (DMso-d6): δ ppm: 7.74 (d, 1H, H-6'), 7.57 (d, 1H, N=CH-N), 7.44 (d, 1H, H-2'), 7.18 (d, 1H, N=CH-C), 6.85 (d, 1H, CH imidazole), 6.74 (d, 1H, H-5'), 6.46 (s, 1H, H-8), 6.30 (s, 1H, H-6), 5.53 (s, 1H, H1-glucoyl), 4.46 (s, 1H, H1-rhamnosyl), 4.23 (s, 2H, O2'-CH2), 3.78 (s, 2H, CH2-N), 3.60, 3.20 (d, 4H, CH glu), 3.14, 3.02 (d, 4H, CH rha), 2.14 (m, 2H, C2H2-C2), 1.27 (s, 3H, CH3 rha); Molecular formula: C93H137N5O40; Molecular weight = 7346.66; Calculated = C (53.95%) H (5.21%) N (3.81%); Found = C (53.92%) H (5.20%) N (3.79%).

3-[6-0-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl-β-Oxoy]2-(3,4-dihydroxyphenyl]-5-hydroxy-7-(oxy-1-propyl-2-(imidazol-1-yl))-4H-I-benzopyran-4-one (III): yield 82%; mp 222 - 224°C; IR (KBr) (cm$^{-1}$): 3370 (OH), 2900 (CH), 1710 (C=C), 1655 (C=O on aromatic ring), 1640 (C=C on imidazole ring), 1600 (aromatic structure), 1510 (aromatic C=C), 1355, 1300, 1220, 1075 (C=C, C=N), 810 (aromatic substituents); $^1$H NMR (DMso-d6): δ ppm: 7.73 (d, 1H, H-6'), 7.50 (d, 1H, N=CH-N), 7.44 (d, 1H, H-2'), 7.27 (d, 1H, N=CH-C), 6.87 (d, 1H, CH imidazole), 6.73 (d, 1H, H-5'), 6.45 (s, 1H, H-8), 6.42 (s, 1H, H-6), 5.63 (d, 2H, O2'-CH2-N), 5.53 (s, 1H, H1-glucoyl), 4.46 (s, 1H, H1-rhamnosyl), 3.60, 3.20 (d, 4H, CH glu), 3.14, 3.02 (d, 4H, CH rha), 1.27 (s, 3H, CH3 rha); Molecular formula: C17H21N2O14; Molecular weight = 690.61; Calculated = C (53.91%) H (4.96%) N (4.05%); Found = C (53.85%) H (5.00%) N (4.00%).

3-[6-0-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl-β-Oxoy]2-(3,4-dihydroxyphenyl]-5-hydroxy-7-(oxy-1-(β-hydroxy-propyl)-3-(d-methyl-imidazol-1-yl))-4H-I-benzopyran-4-one (IV): yield 78.4%; mp 271 - 273°C; IR (KBr) (cm$^{-1}$): 3390 (OH), 3240 (CH hetareoarc ring in benzimidazole), 2924 (CH), 1700 (C=N), 1660 (C=O on aromatic ring), 1630 (C=C on benzimidazole ring), 1600 (aromatic structure), 1500 (aromatic C=C), 1360, 1305, 1205, 1080 (C-O-C), 804 (aromatic substituents); $^1$H NMR (DMso-d6): δ ppm: 8.04 (d, 1H, N=CH-N), 7.73 (d, 1H, H-6'), 7.65, 7.00 (m, 4H, Ar-H), 7.44 (d, 1H, H-2'), 7.46 (d, 1H, H-5'), 6.46 (s, 1H, H-8), 6.31 (s, 1H, H-6), 5.52 (s, 1H, H1-glucoyl), 4.46 (s, 1H, H1-rhamnosyl), 4.48 (s, 2H, CH2-N), 4.30 (s, 2H, O2'-CH2), 4.28 (m, 1H, CH alkyll chain), 3.60, 3.20 (d, 4H, CH glu), 3.10, 3.03 (d, 4H, CH rha), 1.27 (s, 3H, CH3 rha); Molecular formula: C17H18N2O14; Molecular weight = 784.72; Calculated = C (56.63%) H (5.13%) N (3.57%); Found = C (56.55%) H (5.15%) N (3.55%).

3-[6-0-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl-β-Oxoy]2-(3,4-dihydroxyphenyl]-5-hydroxy-7-(oxy-1-propyl-3-(benzimidazol-1-yl))-4H-I-benzopyran-4-one (V): yield 84.5%; mp 280 - 282°C; IR (KBr) (cm$^{-1}$): 3370 (OH), 3210 (CH hetareoarc ring in benzimidazole), 2930 (CH), 1680 (C=N), 1650 (C=O on aromatic ring), 1625 (C=C on benzimidazole ring), 1590 (aromatic structure), 1510
Antimicrobial activity of rutin derivatives I - VIII

<table>
<thead>
<tr>
<th>Compounds</th>
<th>S. aureus ATCC 25923</th>
<th>S. lutea ATCC 9341</th>
<th>E. coli ATCC 25922</th>
<th>Pseudomonas aeruginosa ATCC 27853</th>
<th>C. albicans ATCC 90028</th>
<th>C. glabrata ATCC MYA 2950</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>13.3 ± 0.57</td>
<td>20.0 ± 0.00</td>
<td>0</td>
<td>0</td>
<td>10.3 ± 0.57</td>
<td>11.0 ± 0.00</td>
</tr>
<tr>
<td>II</td>
<td>12.6 ± 0.57</td>
<td>20.3 ± 0.57</td>
<td>0</td>
<td>0</td>
<td>10.0 ± 0.00</td>
<td>10.0 ± 0.00</td>
</tr>
<tr>
<td>III</td>
<td>12.0 ± 0.00</td>
<td>20.0 ± 0.00</td>
<td>0</td>
<td>0</td>
<td>12.0 ± 0.00</td>
<td>11.0 ± 0.00</td>
</tr>
<tr>
<td>IV</td>
<td>13.5 ± 0.57</td>
<td>20.0 ± 0.00</td>
<td>0</td>
<td>0</td>
<td>10.3 ± 0.57</td>
<td>11.0 ± 0.00</td>
</tr>
<tr>
<td>V</td>
<td>13.0 ± 0.00</td>
<td>18.0 ± 0.00</td>
<td>0</td>
<td>0</td>
<td>10.0 ± 0.00</td>
<td>12.3 ± 0.57</td>
</tr>
<tr>
<td>VI</td>
<td>13.3 ± 0.57</td>
<td>20.0 ± 0.00</td>
<td>0</td>
<td>0</td>
<td>12.7 ± 0.06</td>
<td>10.3 ± 0.57</td>
</tr>
<tr>
<td>VII</td>
<td>13.0 ± 0.00</td>
<td>19.3 ± 0.57</td>
<td>0</td>
<td>0</td>
<td>10.3 ± 0.57</td>
<td>11.0 ± 0.00</td>
</tr>
<tr>
<td>VIII</td>
<td>13.0 ± 0.00</td>
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<td>0</td>
<td>0</td>
<td>10.3 ± 0.57</td>
<td>13.3 ± 0.57</td>
</tr>
</tbody>
</table>

The in vitro antimicrobial activity of rutin derivatives I - VIII were investigated against Gram-positive bacteria (Staphylococcus aureus ATCC 25923, Sarcina lutea ATCC 9341), Gram-negative bacteria (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853) and pathogenic yeasts (Candida albicans ATCC 90028, Candida glabrata ATCC MYA 2950) and were compared with commercial discs containing Ciprofloxacin (5 µg/disc), Fluconazole (25 µg/disc), Voriconazole (1 µg/disc) and Nystatin (100 µg/disc). The tested compounds showed good antibacterial activity on Gram-positive tested species and against all of the tested Candida spp. strains (Table I).
We have not registered antibacterial effect against Gram-negative species.

Conclusions

Eight new water-soluble rutin derivatives containing imidazole and benzimidazole moieties in their structures were synthesised; molecular formula, weight, yield, melting points and solubility have characterized the new derivatives. Elemental analysis and spectral analysis in IR and H-NMR have confirmed the structure of new compounds. The tested compounds have shown a good activity against Candida spp. and against Gram-positive bacterial strains and no activity against Gram-negative tested strains.

References