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Synthesis and biological evaluation of 5-substituted derivatives of benzimidazole

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Abstract: A series of eight novel 5-substituted derivatives of benzimidazole was synthesized by condensation of the corresponding diamine with ethyl 4-[4--(2-chlorophenyl)piperazin-1-yl]butanoate in refluxing 4 M hydrochloric acid. *In vitro* antibacterial activity against ten strains, namely *Bacillus subtilis*, *Clostridium sporogenes*, *Streptosporangium longisporum*, *Micrococcus flavus*, *Sarcina lutea*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis* and *Proteus vulgaris* and antifungal activity against two fungal strains, namely *Candida albicans* and *Saccharomyces cerevisiae*, were evaluated. Of all the compounds screened for activity, 2-{3-[4-(2-chlorophenyl)piperazin-1-yl]propyl}-5-iodo-1*H*-benzimidazole and 2-{3-[4-(2-chlorophenyl)piperazin-1-yl]propyl}-5-methyl-1*H*-benzimidazole were associated with higher antifungal activity than commercial drugs.

Keywords: arylpiperazines; benzimidazoles; antibacterial activity; antifungal activity.

INTRODUCTION

One of the main objectives of organic and medicinal chemistry is the design, synthesis and production of molecules having value as human therapeutic agents.[1–3] The synthesis of nitrogen-containing heterocyclic systems has been attracting interest over the past decade because of their utility in various applications.[4–7] Substituted benzimidazoles and arylpiperazines derivatives have been one of the most extensively studied classes of heterocyclic compounds, receiving much attention from synthetic organic chemists because of their broad spectrum of biological properties, such as antiviral, anticancer, antibacterial, antifungal and many others.[8–11]

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These results were an inspiration to synthesize compounds containing a system that involves the combination of these pharmacophores in one molecular framework to give the title structure in order to screen their antimicrobial activities. Resent observations showed that the antimicrobial activities of 4-substituted piperazines varied significantly depending of the substituent group at the 4-piperazine position, suggesting the necessity of an H-bond acceptor and/or donor group at the 4N-position.[12] Prompted by these observations and in continuation the search for bioactive molecules, a series of novel 5-substituted benzimidazoles was designed and synthesized. The design emphasized the strategy of combining two chemically different but pharmacologically compatible molecules, benzimidazole and arylpiperazine, with an alkyl chain that provides hydrophobic interactions and the 2-chlorophenyl group in the piperazine part of the molecule that ensured H-bonding. The aim was to determine the influence of the substituent in the benzimidazole part of the molecule on the antimicrobial activities of the molecules.

EXPERIMENTAL

General

The ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 200 and 50 MHz, respectively, on a Gemini 2000 (Varian, Oxford). The spectra were recorded in deuterochloroform with tetramethylsilane as the internal standard; the chemical shifts (δ) are reported in parts per million (ppm). LC/MS was performed on a 6210 Time-of-Flight LC-MS system (Agilent Technologies, Germany). For data analysis, MassHunter Workstation Software was used.

The infrared (IR) spectra were run on a Thermo Scientific spectrometer. For analytical thin-layer chromatography (TLC), POLYGRAM SIL G/UV$_{254}$ plastic-backed thin-layer silica gel plates were used (Macherey-Nagel, Germany). Chromatographic purifications were performed on Merck-60 silica gel columns (diameter 70 mm, h = 45 mm; the same for all compounds), 230–400 mesh ASTM, under medium pressure (dry column flash chromatography). All reagents and solvents used in this work were obtained from Alfa–Aesar and used without further purification. Solvents were routinely dried over anhydrous Na_2SO_4 prior to evaporation.

Analytic and spectral data for compounds **3** and **20**–**27** are given in the Supplementary material to this paper.

Chemistry

*Synthesis of ethyl 4-[4-(2-chlorophenyl)piperazin-1-yl]butanoate (**3**).* Suspension of 1-(2-chlorophenyl)piperazine monohydrochloride (**2**) (25 g, 107.3 mmol), triethylamine (10.84 g, 107.3 mmol), K_2CO_3 (30 g, 214.6 mmol) and ethyl 4-bromobutanoate (**1**) (20.93 g, 107.3 mmol) in 2-butanone (120 mL) was stirred for 24 h at 80 °C. After cooling, the mixture was poured into cold water and the organic layer was extracted with CH_2Cl_2 and concentrated *in vacuo*. The resulting ester was purified by silica gel column chromatography using a gradient of methanol (0–5 %, predicted by TLC) in dichloromethane. Yield: 22.6 g (68 %).

*General procedure for the reduction of 2-nitroaniline (**4**) and 4-substituted 2-nitroanilines **5**–**11***

Ra/Ni (0.4–0.5 g) was added in small portions to a stirred solution of 6.5 mmol of the required nitro compound (**4**–**11**) in 12 mL EtOH, 12 mL 1,2-dichloroethane and 2 mL (20

mmol) hydrazine hydrate at 30 °C. After completion of the Ra/Ni addition, the mixture was heated in a water bath (50 °C, 60 min) and filtered through celite. The filtrate was evaporated *in vacuo* and crude product used for further syntheses.

*General procedure for the synthesis of 1*H*-benzimidazoles **20**–**27***

Diamines **12**–**19** (5.56 mmol), ester **3** (4.2 g, 13.5 mmol) and 4 M HCl (28 mL) were heated at100 °C for 24 h. After cooling to ambient temperature, the reaction mixture was poured into ice-cold water and neutralized with a saturated solution of sodium hydroxide. The product was extracted with CH_2Cl_2 and concentrated *in vacuo*. The resulting 1*H*-benzimidazoles were purified by silica gel column chromatography using a gradient of methanol (0–5 %) in dichloromethane.

Antimicrobial activity

Sterile 96-well polystyrene microtiter plates with well capacities of 300 µL were used and 100 µL of fresh Mueller Hinton broth were added to each well of the plate. One hundred microliters of a stock solution (10 mg/mL) of the compound in DMSO were added to each well of the first column. Then, 100 µL of the solution were removed from the first column and mixed thoroughly with the broth in the corresponding wells of the second column. Subsequently, a 100 µL aliquot was removed from each well in this column and mixed with contents of the corresponding well of the next column. This doubling dilution was performed in all rows across the plate. Two rows in each plate were used as controls. One row was used as a positive control and contained a broad-spectrum antibiotic, chloramphenicol, to determine the sensitivity of Gram-positive and Gram-negative bacterial species, and the antimycotics nystatin and fluconazole, to determine the sensitivity of the fungal species. The other row contained the solvent (DMSO) as a negative control. In each well of the plate, 10 µL of bacterial cultures (10^6 cells per mL) for antibacterial activity and 10 µL of fungi-cultures (10^5 spores per mL) were inoculated. The microtitre plate was incubated at 37 °C for 24 h for bacteria or at 30 °C for 48 h for the fungi. Subsequently, the bacterial and fungi growth were measured. The *MIC* was determined as the lowest concentration that resulted in inhibition of bacterial and fungal growth.

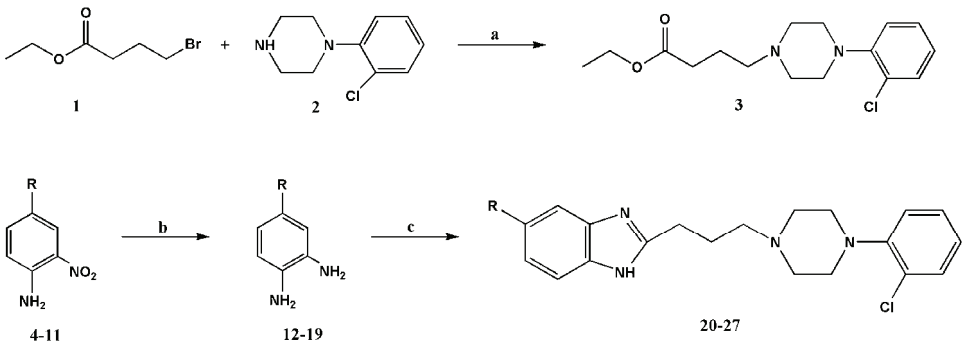
RESULTS AND DISCUSSION

Synthesis of 5-substituted derivatives of benzimidazole, depicted in Scheme 1, started with the preparation of ethyl 4-[4-(2-chlorophenyl)piperazin-1-yl]butanoate (**3**) from 1-(2-chlorophenyl)piperazine monohydrochloride (**2**) and ethyl 4-bromobutanoate (**1**) in 2-butanone. 2-Nitroaniline (**4**) and 4-substituted 2-nitroanilines **5**–**11** were reduced by Ra-Ni/hydrazine to give the diamines **12**–**19**, which were converted to the benzimidazoles **20**–**27** by condensation with the starting ester **3** in refluxing 4 M HCl.

The micro-broth dilution assay was used to evaluate the antimicrobial efficacy of all newly synthesized compounds against Gram-positive and Gram-negative bacteria and fungal cells. The results are presented in Tables I and II for the antibacterial and antimycotic activities, respectively.[13]

The Gram-positive bacteria used were *Bacillus subtilis* (ATCC 6633), *Clostridium sporogenes* (ATCC 19404), *Streptosporangium longisporum* (ATCC 25212), *Micrococcus flavus* (ATCC 10240), *Sarcina lutea* (ATCC 9341) and *Staphylo-*

coccus aureus (ATCC 6538). The Gram-negative bacteria used were *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enteritidis* (ATCC 13076) and *Proteus vulgaris* (ATCC 13315). The fungi tested were *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 9763).



No	20	21	22	23	24	25	26	27
R	H	F	Cl	Br	I	CH_3	CF_3	OCH_3

Scheme 1. Synthesis of 5-substituted derivatives of benzimidazole. Reagents: a) Et_3N, K_2CO_3 and 2-butanone; b) 1,2-dichloroethane, EtOH, NH_2NH_2 and Raney Ni; c) 4 M HCl, ester **3**.

TABLE I. Antibacterial activity (*MIC* / mg mL^{-1}) of compounds **20**–**25**

Bacteria	Compound						
	20	**21**	**22**	**23**	**24**	**25**	Chloramphenicol
B. subtilis	1.250	1.250	1.250	1.250	0.625	0.312	0.015
C. sporogenes	1.250	2.500	1.250	1.250	1.250	1.250	0.250
S. longisporum	1.250	1.250	1.250	1.250	0.625	0.625	0.066
M. flavus	1.250	2.500	1.250	1.250	0.625	0.625	0.031
S. lutea	1.250	1.250	1.250	1.250	0.625	1.250	0.125
S. aureus	1.250	2.500	2.500	0.625	0.312	0.312	0.015
P. vulgaris	1.250	2.500	1.250	1.250	0.625	2.500	0.125
P. aeruginosa	1.250	2.500	2.500	1.250	0.312	2.500	0.250
S. enteritidis	1.250	2.500	2.500	1.250	1.250	1.250	0.043
E. coli	1.250	–	–	0.312	0.625	0.312	0.043

TABLE II. Antifungal activity (*MIC* / mg mL^{-1}) of compounds **20**–**25**

Fungi	Compound							
	20	**21**	**22**	**23**	**24**	**25**	Nystatin	Fluconazole
C. albicans	1.250	1.250	1.250	1.250	0.625	0.078	2.250	0.313
S. cerevisiae	1.250	1.250	1.250	1.250	0.625	0.313	1.250	

Results revealed that, among all the synthesized and tested compounds, compounds **26** and **27** did not show antibacterial activity. The most potent

against the Gram-positive and Gram-negative bacteria were 2-{3-[4-(2-chlorophenyl)piperazin-1-yl]propyl}-5-iodo-1*H*-benzimidazole (**24**) and 2-{3-[4-(2-chlorophenyl)piperazin-1-yl]propyl}-5-methyl-1*H*-benzimidazole (**25**).

A comparison of activity data of all tested compounds and antifungal activity of nystatin and fluconazole indicated that all synthesized derivatives showed the same or better activity against *C. albicans* and *S. cerevisiae* than nystatin (except for 5-(trifluoromethyl) and 5-methoxy derivatives, **26** and **27**, respectively). The 5-methyl derivative **25** was four times more biologically active against *C. albicans* than fluconazole.

CONCLUSIONS

The synthesis of a series of eight novel 2-{3-[4-(2-chlorophenyl)piperazin-1-yl]propyl}-5-substituted-1*H*-benzimidazoles is presented, emphasizing the strategy of combining two chemically different but pharmacologically compatible heterocyclic molecules (benzimidazole and arylpiperazine) in one frame. The synthesized compounds were tested *in vitro* for their antibacterial and antifungal activities. The results indicate that, although the length of the aliphatic chain affects lipophilicity and 2-chlorophenyl group in the piperazine part of molecule provides for H-bonding (properties which are mandatory for antibacterial and antifungal activity), only the substituent in benzimidazole part of the molecule was crucial for the activity. Although the title compounds did not exhibit significant antibacterial activity, all of them (except **26** and **27**) exhibited the same or better antifungal activity than commercial nystatin and fluconazole. In some case of the fungi *C. albicans*, this activity was 4 times higher. More specifically, 5-iodo **24** and 5-methyl **25** derivatives exhibited the best activities against both fungal species.

SUPPLEMENTARY MATERIAL

Analytic and spectral data for compounds **3** and **20**–**27** are available electronically from http:// //www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА И БИОЛОШКО ИСПИТИВАЊЕ 5-СУПСТИТУИСАНИХ ДЕРИВАТА БЕНЗИМИДАЗОЛА

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Синтетисана је серија од осам нових, 5-супституисаних бензимидазола, конденза-цијом одговарајућег диамина са етил-4-[4-(2-хлорфенил)пиперазин-1-ил]-бутаноатом у 4 M HCl, на температури рефлуктовања. Одређена је *in vitro* антибактеријска активност

на десет сојева, *Bacillus subtilis*, *Clostridium sporogenes*, *Streptosporangium longisporum*, *Micrococcus flavus*, *Sarcina lutea*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis* и *Proteus vulgaris,* и антифунгална активност на два соја, *Candida albicans* и *Saccharomyces cerevisiae*. Од свих новосинтетисаних једињења за 2-{3-[4-(2--хлорфенил)пиперазин-1-ил]пропил}-5-јод-1*H*-бензимидазол (**24**) и 2-{3-[4-(2-хлорфенил)пиперазин-1-ил]пропил}-5-метил-1*H*-бензимидазол (**25**) се може истаћи да поседују бољу антифунгалну активност од комерцијалних лекова.

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