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SYNTHESIS OF NEW 1,8-NAPHTHALIMIDE DERIVATIVES WITH POTENTIAL BIOLOGICAL ACTIVITY

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Abstract: *This paper presents the synthesis of new 1,8-napthalimides by an interaction of 6-(10H-phenothiazin-10-yl)-1H,3H-naphtho[1,8-cd]pyran-1,3-dione with L-α-alanine, D-α-alanine and DL-α-alanine. The antimicrobial activity of the corresponding 2-(1,3-dioxo-6-phenothiazin-10-yl-benzo[de]isoquinolin-2-yl)propanoic acids against various microorganisms was investigated.*

Keywords: *1,8-napthalimides, IR, ^{1}H NMR, ^{13}C NMR, antimicrobial activity*

INTRODUCTION

Hypolipidemic drug agents are pharmaceutical products that are used to lower levels of bad cholesterol, LDL, to a greater extent than others can cautiously raise levels of good cholesterol, HDL. Other studies have shown that increasing high-density lipoprotein - HDL and lowering serum triglyceride - TGs levels are accepted measures for treating hyperlipidemias and atherosclerosis (*Steinberg et al.*, 1989; *Ginsberg*, 1990; *Larsen & Spilman*, 1993).

LDL and VLDL carry cholesterol to tissues and elevated levels of these lipoproteins are associated with atheroma formation. HDL, reverse transports cholesterol to the liver and is associated with protection against cardiovascular diseases (*Miller*, 2000).

The efficacy of hypolipidemic drugs is seen in the following effects:

- Decreased cholesterol levels and increased clearance of LDL from the bloodstream, which occurs by inhibiting cholesterol synthesis (*Boden*, 2000);
- Increased HDL and decreased triglycerides (TGs), e.g., first- and second-generation fibrates (*Staels et al.*, 1998; *Spieker et al.*, 2000; *Parsons*, 2003);
- Inhibition of cholesterol absorption or sequestration from food (*Rossi*, 2006).

EXPERIMENTAL

All used chemicals were purchased from Merck and Sigma-Aldrich. The melting points were determined by a SMP-10 digital melting point apparatus. The IR spectra were taken on Perkin-Elmer FTIR-1600 spectrometer in KBr discs. The NMR spectra were taken on a Bruker DRX-250 spectrometer, operating at 250.13 and 62.90 MHz for ^{1}H and ^{13}C, respectively, using the standard Bruker software. The chemical shifts were referenced to tetramethylsilane (TMS). The measurements in DMSO-d_6 solutions were carried out at ambient temperature (300 K).

SYNTHESIS OF PRODUCTS Va-c

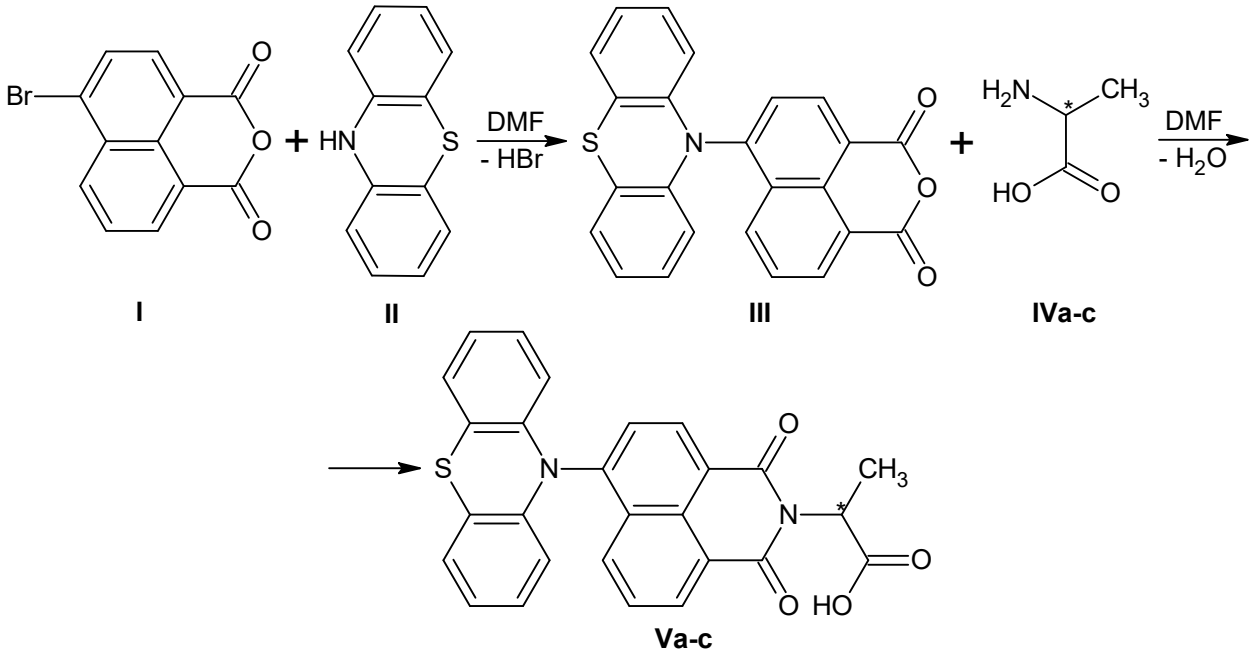
3.95 g (0.01 mol) of 6-(10*H*-phenothiazin-10-yl)-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione (III) and 0.89 g (0.01 mol) of *L*-α-alanine (IVa), *D*-α-alanine (IVb) and *DL*-α-alanine (IVc), respectively were refluxed in 30mL of DMF for 5h. The solvent was removed on a vacuum rotary evaporator. The residue was washed with EtOH and dried.

ANTIMICROBIAL STUDY

Agar diffusion method and test microorganisms: Gram-positive bacteria *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Bacillus cereus ATCC 10876*, Gram-negative bacteria *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NTCC 6017, yeast *Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 2601, molds *Aspergillus brasiliensis* ATCC 16404 and *Fusarium moniliforme*, were used to determine the antimicrobial action of the synthesized compounds. 1% solutions in solvent dimethyl sulfoxide (DMSO) were prepared from the compounds tested. The experiments were performed on nutrient medium Tryptic soy agar (Himedia) for bacteria and Sabouraud dextrose agar (Himedia) for yeast and molds. The agar media were melted in a Koch apparatus. They were cooled down to a temperature of 50-48°C and inoculated with 1% of the prepared suspensions of the test microorganisms, then mixed well. 20 mL of the inoculated media were poured into sterile Petri dishes (Ø = 90 mm). The agar was allowed to solidify. Cork borer was used to punch holes (Ø = 8 mm) in the agar plate. 50 μL of the prepared solutions were added dropwise to the wells, and after 30 min of prefusion at room temperature, the petri dishes were placed in a thermostat at 37°C for 24 h for bacteria; 28°C for 24 h for yeasts and for 72 h for molds (*Hussein et al.,* 2021). Diameters of the zones of growth inhibition were taken into account after cultivation as follows: up to 15 mm the microbial culture is weakly sensitive; from 15 to 25 mm – sensitive and over 25 mm – highly sensitive.

RESULTS AND DISCUSSION

The final products (Va-c) were obtained from the interaction of 6-(10*H*-phenothiazin-10-yl)-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione (III) with *L*-α-alanine (IVa), *D*-α-alanine (IVb) and *DL*-α-alanine (IVc) according to Scheme 1.



Scheme 1. Synthesis of compounds Va-c

Compound III was obtained according to our methodology (*Marinov et al.*, 2022).

Table 1. Physicochemical parameters of compounds Va-c

№	Systematic name	Yield (%)	M. p. (°C)
Va	(2*S*)-2-(1,3-dioxo-6-phenothiazin-10-yl-benzo[*de*]isoquinolin-2-yl)propanoic acid	69	265-266
Vb	(2*R*)-2-(1,3-dioxo-6-phenothiazin-10-yl-benzo[*de*]isoquinolin-2-yl)propanoic acid	64	264-265
Vc	(*R,S*)-2-(1,3-dioxo-6-phenothiazin-10-yl-benzo[*de*]isoquinolin-2-yl)propanoic acid	76	233-234

In the IR spectra, the vibrations for the hydroxyl group appear in a narrow range of 3425-3336 cm^{-1}, for carbonyl groups of the naphthalene nucleus – 1698 cm^{-1} and 1675 cm^{-1}, for acetyl carbonyl group – 1658 cm^{-1}, for aromatic core – 3068 cm^{-1} and for CH_3 group – 2887 cm^{-1}.

In ^{1}H NMR (DMSO-d_6, δ, ppm) the spectral data are as follows:

1.58-1.59 (*d*, 3H, CH_3), 5.62-5.65 (*q*, 1H, CH), 7.92-7.96 (*t*, 2H, Ar-H), 8.51-8.53 (*d*, 2H, Ar-H)

In ^{13}C NMR (DMSO-d_6, δ, ppm), the naphthalic anhydride ring C=O appears at 177.4 and 165.6 ppm, for acetyl C=O – 168.3 ppm, for CH_3 – 27.7 ppm and for *CH – 71.2 ppm.

Regarding their microbiological activity, compound Vb shows higher activity than Va and Vc.

Table 2. Antimicrobial activity of compounds Va-c

Test microorganism	Inhibition zone diameter (mm)		
	Va	Vb	Vc
Staphylococcus aureus	0	0	0
Staphylococcus epidermidis	13.8	17.2	11.3
Bacillus subtilis	16.5	19.4	14.7
Bacillus cereus	15.3	18.6	12.4
Pseudomonas aeruginosa	14.1	19.2	13.5
Escherichia coli	15.7	18.8	13.6
Salmonella abony	0	0	0
Candida albicans	16.3	19.9	15.4
Saccharomyces cerevisiae	0	0	0
Aspergillus brasiliensis	0	0	0
Fusarium moniliforme	0	0	0

The studied compounds Va-c showed antimicrobial activity against the Gram-positive bacteria *S. epidermidis, B. subtilis, B. cereus*, the Gram-negative bacteria *P. aeruginosa, E. coli* and the yeast *C. albicans*.

Compound Vb showed good antimicrobial activity against the Gram-positive bacteria *S. epidermidis* – 17.2 mm of growth inhibition zone; *B. subtilis* – 19.4 mm of growth inhibition zone; *B. cereus* – 18.6 mm of growth inhibition zone; The gram-negative bacteria *P. aeruginosa* – 19.2 mm of growth inhibition zone and *E. coli* – 18.8 mm of growth inhibition zone, and the yeast *C. albicans* – 19.9 mm of growth inhibition zone. The compound is not active against the Gram-positive bacterium *S. aureus*, the Gram-negative bacterium *S. abony*, the yeast *S. cerevisiae* and the molds used.

The antimicrobial activity of compound Va was lower than that of compound Vb. The diameters of the growth inhibition zones are: for the Gram-positive bacteria *S. epidermidis* – 13.8 mm; *B. subtilis* – 16.5 mm; *B. cereus* – 15.3 mm; Gram-negative bacteria *P. aeruginosa* – 14.1 mm and *E. coli* – 15.7 mm, and the yeast *C. albicans* – 16.3 mm. The compound was also inactive against the Gram-positive bacterium *S. aureus*, the Gram-negative bacterium *S. ebony*, the yeast *S. cerevisiae* and the molds used.

Compound Vc showed weak antibacterial activity against the Gram-positive bacteria *S. epidermidis* – 11.3 mm*; B. subtilis* – 14.7 mm; *B. cereus* – 12.4 mm; Gram-negative *bacteria P. aeruginosa* – 13.5 mm and *E. coli* – 13.6 mm, and the yeast *C. albicans* – 15.4 mm. The compound

was also inactive against the Gram-positive bacterium *S. aureus*, the Gram-negative bacterium *S. ebony*, the yeast *S. cerevisiae* and the molds used.

CONCLUSION

Three new derivatives of 6-(10*H*-phenothiazin-10-yl)-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione were synthesized. The compounds were proved by IR and NMR spectroscopy. Some of their physicochemical parameters have been determined. Their antimicrobial activity was studied and found to have such activity against *Staphylococcus epidermidis, Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans*. To other microorganisms they do not show activity.

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REFERENCES

Boden, W. E. (2000). High-density lipoprotein cholesterol as an independent risk factor in cardiovascular disease: assessing the data from Framingham to the veterans affairs high-density lipoprotein intervention trial. *Am. J. Cardiol.*, 86 (12), 19-22

Ginsberg, H. N. (1990). Lipoprotein physiology and its relationship to atherogenesis. *Endocrinol Metab Clin North Am.*, 19 (2), 211-228

Hussein, K. N., Molnár, T., Pinter, R., Toth, A., Ayari, E., Friedrich, L., Dalmadi, I. & Kiskó, G. (2021). *In vitro* antimicrobial activity of plant active components against *Pseudomonas lundensis* and *Listeria monocytogenes*. *Prog. Agric. Eng. Sci.*, 16 (S2), 163-172

Larsen, S. D., & Spilman, C. H. (1993). New potential therapies for the treatment of atherosclerosis. *Ann. Rep. Med. Chem.*, 28, 217-225

Marinov, M., Nikolova I., Kostova I., & Stoyanov N. (2022). Phenothiazine derivatives of 1,8-naphthalic anhydride: Synthesis, characterization and antimicrobial studies. *Phosphorus Sulfur Silicon Relat. Elem.*, 198 (2), 128–136

Miller, M. (2000). Differentiating the effects of raising low levels of high-density lipoprotein cholesterol versus lowering normal triglycerides: further insights from the veteran's affairs high-density lipoprotein intervention trial. *Am. J. Cardiol.*, 86 (12), 23-27

Parsons, W. B. (2003). Cholesterol control without diet! The niacin solution, 2nd edn, updated. *Lilac Press*, *Buena Vista*

Rossi, S. (2006). Australian medicines handbook.

Staels, B., Dallongeville, J., Auwerx, J., Schoonjans, K., Leitersdorf, E. & Fruchart, J.-C. (1998). Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation*, 98 (19), 2088-2093

Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C. & Witztum, J. L. (1989). Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.*, 320 (14), 915-924

Spieker, L. E., Noll, G., Hannak, M. & Lüscher, T. F. (2000). Efficacy and tolerability of fluvastatin and bezafibrate in patients with hyperlipidemia and persistently high triglyceride levels. *J. Cardiovasc. Pharmacol.*, 35 (3), 361-365