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Synthesis and biological activity of 5-aminopyrimidineterpenes

Zbigniew Rykowski[a], Jerzy Cieplik[a], Katarzyna Paulus[a]
Janusz Pluta[b], Olaf Gubrynowicz[b]

[a] Department of Organic Chemistry, Medical Academy, 9 Grodzka Str., 50-137 Wrocław, Poland
[b] Department of Applied Pharmacy, Medical Academy, 38 Szewska Str., 50-137 Wrocław, Poland

Abstract

The synthesis of 4-arylamine-6-methyl-2-phenyl-5- methylamine-terpene derivatives was presented and an antibacterial activity of the obtained compounds was investigated.

Introduction

The previous studies on the synthesis and biological activity of the pyrimidine ring [1-2] and 5-aminomethylpyrimidine derivatives showed that there was an enormous potential in the area of biological activity [3-6]. Another group of interesting compounds includes terpene amines which also present considerable biological activity [7-9]. This encouraged us to carry out the synthesis of 5-terpeneaminomethyl derivatives of pyrimidine and to investigate this microbiological activity.

Chemistry

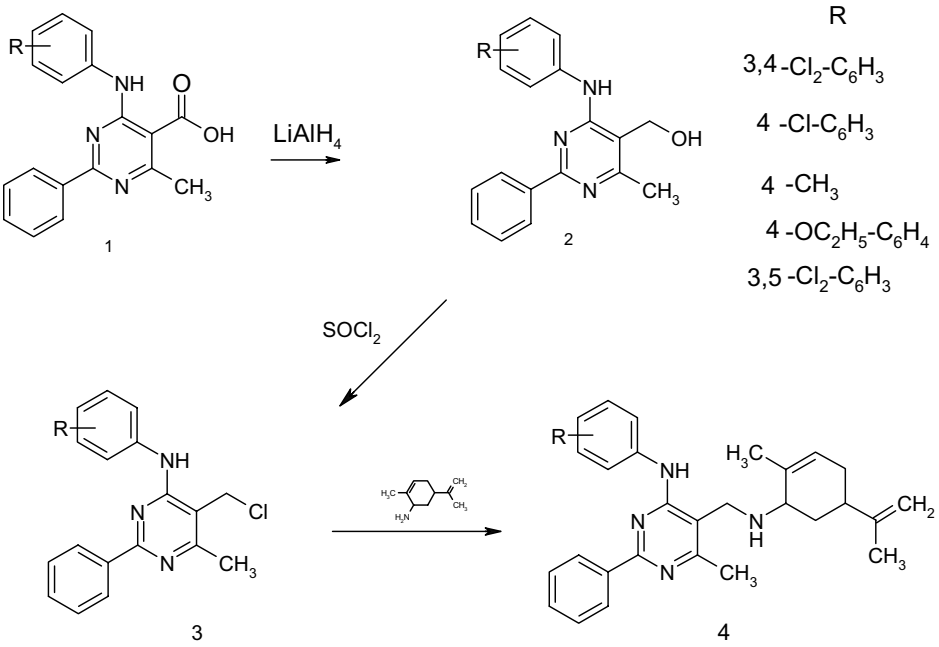
The substrate of our synthesis was 2-phenyl-4-phenylamino-6-methyl-5-pyrimidine carboxylic acid (**1**) which underwent $LiAlH_4$ reduction, in 2-phenyl-4-phenylamino-6-methyl-5-hydroxymethylpyrimidine[3] (**2**). The obtained pyrimidine hydroxyderivative 2 was exposed to $SOCl_2$ which resulted in 5-chloromethyl-6-methyl-2-phenyl-2-phenylaminopyrimidines [10] (**3**).

The obtained aminoacid **1** was reduced by $LiAlH_4$ **2**. to obtained 4-arylamino-6-methyl-2-phenyl-5-hydroxymethylpyrimidine (**2**) which reacted with $SOCl_2$ to give 2-arylamino-6-methyl-2-phenyl-5-chloromethylpyrimidine (**3**).

The starting materials in our study were 5-chloromethyl-6-methyl-2-phenyl-2-phenylaminopyrimidines (**3**). The chloroderivatives were treated with terpene amines giving 5-terpeneaminomethyl-6-methyl-2-phenyl-4-phenyloaminopyrimidines (**4**).

The terpene amines were synthesized from carvone and menthol (**4**) which gave dihydrocarvylamine, carvylamine and menthylamine.

The obtained derivatives **4a-4r** were investigated microbiologically on ten standard strains were used in order to evaluate their antibacterial and antifungal activity (Tables 1, 2).

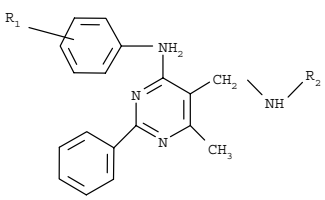


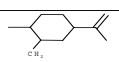
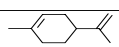
Scheme 1

Results an Discussion

The obtained products were tested microbiologically on the selected bacterial strains in order to estimate their bioactivity. The included tables show the results for the most active compounds. The investigation is based on m-7, a-5 standards (MIC Testing). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – Fifth Edition NCCLS. The fungal stains were also cultivated on the broth recommended by these standards – Mueller Hinton Broth II. Sample bacteria cultures were suspended in 3 ml of a sterile solution of PBS according to 0,5 Mc Farland's standard (corresponding to 1 to $2x10^8$ CUF/ml) and then diluted with a sterile 1:10 PBS solution (giving $1x10^7$ CFU/ml). 0.01 ml of suspension was inoculated into the 0.2 ml of sterile final dilution of the investigated substances obtaining $5x10^4$ concentration of bacteria in the investigated samples. Results of these tests are presented in Table 2 and 3.

Table 1. Physical properties of the tested compounds



Co. mp.	R_1	R_2	Brutto formula (molar mass)	mp. (C^0)	Yield (%)
4a	4-H		$C_{29}H_{36}N_4$ (428.53)	121-123	70.2
4b	4-H		$C_{28}H_{32}N_4$ (424.50)	158-160	68.1

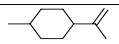
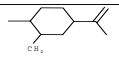
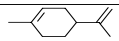
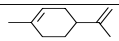
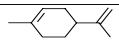
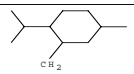
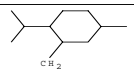
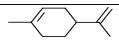
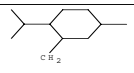
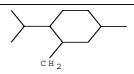
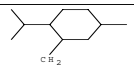
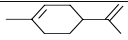
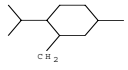
4c	4-H		$C_{28}H_{34}N_4$ (426.52)	212-214	68.3
4d	4-CH_3		$C_{30}H_{38}N_4$ (454.56)	106-108	70.9
4e	4-CH_3		$C_{29}H_{34}N_4$ (438.53)	97-98	83.3
4f	4-CH_3O		$C_{29}H_{34}N_4O$ (454.52)	133-135	60.4
4g	4-C_2H_5O		$C_{30}H_{36}N_4O$ (468.54)	136-138	70.3
4h	4-C_2H_5O		$C_{31}H_{41}N_4O$ (485.59)	137-139	76.9
4i	4-CH_3		$C_{30}H_{39}N_4$ (455.57)	211-215	76.5
4j	4-F		$C_{28}H_{31}FN_4$ (442.49)	217-220	75.1
4k	3-F		$C_{29}H_{36}FN_4$ (459.53)	190-192	69.5
4l	4-F		$C_{29}H_{36}FN_4$ (459.53)	175-177	73.8
4m	4-Cl		$C_{29}H_{31}ClN_4$ (458.95)	201-202	74.2
4n	4-Cl		$C_{28}H_{30}ClN_4$ (470.96)	188-190	73.9
4o	3,4-Cl_2		$C_{28}H_{30}Cl_2N_4$ (493.39)	118-120	80.2
4p	2,4-Cl_2		$C_{29}H_{35}Cl_2N_4$ (510.43)	207-209	62.2

Table 2. Minimal Inhibitory Concentration (MIC) (g/ml) Testing M-7, A-5

		Chloramphenicol	**1a**	**1b**	**1d**	**1f**	**1h**	**Erythromycin**
Bacillus subtilis ATCC 6633	G(+)	2	128	64	128	256	64	0.25
Eschrichia coli ATCC 25922	G(-)	8	64	256	64	128	128	32
Klebsiella pneumoniae ATCC 13886	G(-)	1	32	128	128	256	256	1
Proteus vulgaris NCTC 4635	G(-)	2	32	128	64	256	128	64
Seratia marcescens ATCC 274	G(-)	8	64	64	128	256	128	64
Pseudomonas aeruginosa ATCC 27853	G(-)	128	64	32	128	64	64	64
Enterococcus faecalis PCM 896	G(-)	4	32	64	128	128	128	4
Staphylococcus epidermidis ATCC 14990	G(+)	16	64	32	128	256	128	32
Staphylococcus aureus ATCC 6538 P	G(+)	2	32	64	64	64	128	0,25
Candida albicaus ATCC 10231		128	32	32	128	256	256	128

Table 3. Minimal Inhibitory Concentration (MIC) (g/ml) Testing M-7, A-5

		Chloramphenicol	**4i**	**4j**	**4k**	**4m**	**4p**	**Erythromycin**
Bacillus subtilis ATCC 6633	G(+)	2	32	64	128	128	64	0.25
Eschrichia coli ATCC 25922	G(-)	8	64	128	64	128	256	32
Klebsiella pneumoniae ATCC 13886	G(-)	1	32	64	32	64	128	1
Proteus vulgaris NCTC 4635	G(-)	2	16	128	32	64	128	64
Seratia marcescens ATCC 274	G(-)	8	32	64	16	128	256	64
Pseudomonas aeruginosa ATCC 27853	G(-)	128	32	128	16	32	64	64
Enterococcus faecalis PCM 896	G(-)	4	64	64	32	128	128	4
Staphylococcus epidermidis ATCC 14990	G(+)	16	128	64	64	128	128	32
Staphylococcus aureus ATCC 6538 P	G(+)	2	32	64	32	128	128	0.25
Candida albicaus ATCC 10231		128	128	64	32	64	128	128

Experimental

Melting points were determined in Kofler apparatus. ^{1}H NMR spectra were recorded on Brucker 300.14 MHz. Infra-red (IR) spectra were recorded in nujol with a Specord spectrophotometer, at the Elemental Analytical Laboratory, Medical Academy in Wrocław. Results of the elemental analysis indicated by the symbols were within ±0.4% of the theoretical values.

5-Terpeneaminomethyl-6-methyl-2-phenyl-4-phenylaminopyrimidine (4b)

5 g (0.016 mole) of 5-chloromethyl-6-methyl-2-phenyl-4- phenylamino - pyrimidine (**3**) were dissolved in 20 ml of chloroform and 5.5 g (0.032 mole) carvylamine was added. The mixture was left for 2 hours and then poured into 100 ml of water. The solution was extracted three times with chloroform and the combined extracts were dried by $MgSO_4$ and then filtrated condensed under reduced pressure. The oily residue was purified by column chromatography with chloroform as eluent. The product in the amount of 5. 9 g had Rf. 0. 7 fraction and m.p. 158-160^{0}C . IR (KBr): v 3280 cm^{-1}(NH). ^{1}H-NMR ($CDCl_3$/TMS_{int}): δ = 1.15 (s 1H Alkil-NH); 2.58 (s, 3H CH_3); 3.04 (s, 3H CH_3); 3.35 (s, 3H CH_3); 3.75 (s 2H, CH_2); 3.90 (s 2H, CH_2); 4.15 (t 2H, CH_2); 4.25 (t 2H, CH_2); 5.20 (s 1H $Aryl_2$–NH) 6.20-8.15 (m 10 H, atomat). Compounds **4a-4p** were obtained by the same procedure.

Conclusion

In order to reveal a potential activity of sixteen newly obtained compounds, they were tested on ten bacterial species: Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Seratia marcescens, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus epidermidis, Staphylococcus aureus, Candida albicans. The obtained results are shown in Table 2 and 3. Many of the investigated derivatives presented interesting antibacterial activity. The mentioned 5-terpeneaminomethylpyrimidine derivatives ensure considerable

antibacterial activity especially when they include a fragment coming from menthyl amin. Also the electronegative atoms, such as chlorine and fluorine, increased antibacterial activity.

References

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