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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF BETULONIC ACID AMIDES WITH PIPERAZINE DERIVATIVES

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*New betulonic acid amides with piperazine derivatives were synthesized by the acid-chloride method and characterized using spectral data. It was shown that synthesized compounds **2**-**7** exhibited varied antibacterial activity. The most active of them was betulonic acid amide of 1-(3,4-dichlorophenyl)piperazine (**3**) and 1-(4-trifluoromethylphenyl)piperazine (**7**).*

Keywords: betulonic acid, amides, piperazine derivatives, antibacterial activity.

Betulonic acid is obtained via a one-step oxidation of betulin [1, 2] and has reactive functional groups in its structure. It is an available and convenient starting material for synthesizing new biologically active compounds [3–6]. Compounds with antioxidant, anti-inflammatory, antibacterial, hepatoprotective, antiviral, antitumor, and immunostimulant properties have been found among the synthesized derivatives of betulonic acid [4, 7–12]. Compounds with various substituents on C-28, in particular peptides [8] and amides [4, 8, 12], are well-known among them. It is also known that introduction into the triterpenoid acid structure of piperazine groups or its *N*-substituted derivatives expands the spectrum of their biological activity [4 ,13]. This can be viewed as one of the options for synthesizing new biologically active compounds based on betulonic acid.

Herein we present results for the synthesis of amides of betulonic acid with *N*-derivatives of piperazine and tests of their antibacterial activity.

Betulin for the synthesis of betulonic acid was obtained via extraction of *Betula platyphylla* bark. Its structure was confirmed by comparing PMR and ^{13}C NMR spectra with the literature data [14]. Oxidation of betulin by Jones reagent using the known method [1] produced betulonic acid, the PMR and ^{13}C NMR spectra of which were identical to those published [1, 12]. Betulonic acid chloride (**1**) was synthesized via reaction with oxalylchloride by the literature method [7]. The structure of **1** was confirmed by comparing its PMR and ^{13}C NMR spectra with the literature data [6]. Amides **2**–**7** were prepared via reaction of **1** with *N*-derivatives of piperazine in CH_2Cl_2 (1:2 mole ratio) in the presence of NEt_3 (4 eq). The yields of the target compounds were 74.9–89.8%.

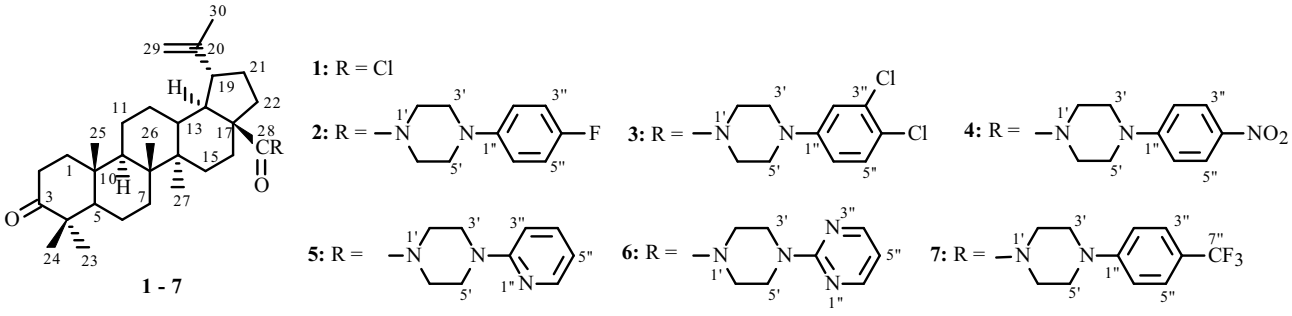

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TABLE 1. ^{13}C NMR Spectra of Amides **2**–**7** (δ, ppm)

C atom	**2**	**3**	**4**	**5**	**6**	**7**
1	39.89	39.88	39.88	39.88	39.87	39.89
2	34.39	34.38	34.36	34.37	34.37	34.39
3	218.50	218.50	218.42	218.47	218.45	218.54
4	47.57	47.57	47.56	47.55	47.54	47.58
5	55.27	55.26	55.25	55.26	55.26	55.26
6	19.84	19.83	19.83	19.84	19.83	19.83
7	33.88	33.87	33.88	33.88	33.87	33.87
8	40.81	40.81	40.81	40.81	40.80	40.81
9	50.41	50.40	50.38	50.41	50.40	50.40
10	37.15	37.15	37.14	37.14	37.14	37.15
11	21.87	21.86	21.85	21.87	21.87	21.86
12	25.85	25.83	25.82	25.86	25.85	25.83
13	37.19	37.19	37.21	37.19	37.19	37.19
14	42.16	42.16	42.18	42.16	42.16	42.17
15	30.01	30.01	30.03	30.01	30.01	30.01
16	33.88	33.87	33.88	33.88	33.87	33.87
17	54.76	54.76	54.80	54.80	54.82	54.77
18	52.78	52.75	52.73	52.80	52.81	52.74
19	45.83	45.82	45.83	45.85	45.84	45.84
20	151.41	151.32	151.19	151.42	151.41	151.34
21	31.50	31.48	31.46	31.50	31.50	31.47
22	36.19	36.18	36.17	36.21	36.22	36.20
23	26.79	26.79	26.79	26.79	26.78	26.78
24	21.24	21.24	21.22	21.22	21.22	21.24
25	16.23[a]	16.22[a]	16.21[a]	16.21[a]	16.24[a]	16.23[a]
26	16.13[a]	16.12[a]	16.12[a]	16.12[a]	16.20[a]	16.13[a]
27	14.82	14.82	14.82	14.81	14.81	14.83
28	173.73	173.79	173.99	173.85	173.90	173.83
29	109.50	109.56	109.63	109.47	109.48	109.57
30	19.88	19.85	19.82	19.86	19.87	19.86
2′	32.75	32.74	32.71	32.72	32.74	32.74
3′	50.81	49.29	47.37	45.82	44.15	48.56
5′	50.81	49.29	47.37	45.82	44.15	48.56
6′	32.75	32.74	32.71	32.72	32.74	32.74
1″	147.84	150.59	154.86			153.25
2″	118.38, d, (6.38)[b]	130.75	113.19	159.57	161.956	115.06
3″	115.87, d, (18.37)[b]	133.10	126.11	107.70		126.67, q, (3.25)[b]
4″	157.67, d, (199.1)[b]	123.13	139.25	137.90	157.94	121.43, q, (27.0)[b]
5″	115.87, d, (18.37)[b]	117.81	126.11	114.21	110.68	126.67, q, (3.25)[b]
6″	118.38, d, (6.38)[b]	115.91	113.19	148.06	157.94	115.06
7″						124.80, q, (224.38)[b]

[a]Chemical shifts denoted by the same letters may be switched within a single column.
[b]Splitting constants (in Hz) of C resonances by F atoms are given in parentheses.

The structures of the amides were established using IR and NMR spectra and a comparison with those for analogous compounds [5, 6, 12]. The ^{13}C NMR spectra of the synthesized compounds **2**–**7** contained resonances for the betulonic moiety and the corresponding number of C resonances for the amine component (Table 1). A strong-field shift of the C-28 resonance compared with that in betulonic acid (182.80 ppm) and the appearance in the IR spectra of **2**–**7** of a band for C=O vibrations of an amide at 1626–1634 cm^{-1} provided evidence that an amide bond had formed in these compounds.

The synthesized compounds **2**–**7** were tested for antibacterial activity, which was evaluated from the diameter of the growth-inhibition zone of colonies around paper disks with the applied compound (Table 2).

TABLE 2. Antibacterial Activity of Synthesized Compounds

Compound	Diameter of growth-inhibition zone, mm				
	O. anthropi	*O. intermedium*	*P. aeruginosa*	*S. maltophilia*	*S. nitritireducens*
2	–	–	–	–	11
3	15	14	13	13	15
4	12	13	–	11	13
5	12	10	–	10	11
6	13	10	–	10	11
7	15	14	14	14	16
Gentamicin	19	17	18	16	21

Table 2 shows that **2**–**7** differed in the spectrum of antibacterial activity. Whereas **2** inhibited growth of only *S. nitritireducens*, amides **3** and **7** exhibited activity against five of the studied test cultures. These bacteria were carriers of various infections and biological damage [15–17], the most dangerous of which was *P. aeruginosa*, which is known for its resistance to the action of various antibiotics [18]. The results indicated that the betulonic acid amides may contain potential antibacterial drugs. If it is assumed that the potential of the synthesized compounds is not limited to antibacterial activity, then the study of the biological activity of these compounds should be continued.

EXPERIMENTAL

^{13}C NMR and PMR spectra were recorded in $CDCl_3$ with TMS internal standard on a Varian Unity Inova 600 spectrometer at operating frequency 125 and 600 MHz, respectively. IR spectra were taken on a JASCO FT-IR 4100 FT-IR spectrometer using the total internal reflectance (TIR) method. Elemental analyses were carried out on an EA1110-Fisons instrument (ThermoQuest Italia SPA, CE Instruments). Melting points were determined on a Stuart Scientific SMP3 instrument. All piperazine derivatives were purchased from Sigma-Aldrich.

Betulin for the synthesis of betulonic acid was prepared by double extraction with EtOAc of *Betula platyphylla* bark collected in Chungbuk Province (South Korea) and recrystallization of the extract from $CHCl_3$:EtOH (82:18, v/v).

Betulonic acid was prepared via oxidation of betulin by Jones reagent as described before [1].

Betulonic acid chloride was synthesized via reaction of betulonic acid with oxalylchloride in anhydrous CH_2Cl_2 by the literature method [7].

General Method for Synthesizing 2–7. A solution of **1** (0.94 g, 2.0 mmol), piperazine derivative (4.0 mmol), and NEt_3 (1.12 mL, 8.0 mmol) in anhydrous CH_2Cl_2 (50 mL) was stirred at 25°C under a N_2 atmosphere for 48 h, washed successively with HCl solution (5%) and H_2O, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo. The solid was purified by preparative HPLC to afford highly pure amides **2**–**7** in 74.9–89.8% yields.

Preparative reversed-phase HPLC was performed over an OptimaPaK C_{18} column (250 × 21.1 mm, 10 μm) with isocratic elution by CH_3CN:H_2O (86:14, v/v) at flow rate 7 mL/min. Absorption of the effluents was estimated at 212 and 254 nm using a UV detector (UV730D, Young Lin Instrument, Korea).

Antibacterial activity was determined by the previously described paper-disk method [19]. Disks (8 mm diameter, Advantec Toyo Roshi Kaisha, Ltd., Japan) were treated with a solution (60 μL) of the compound in DMSO (1 mg/mL) and placed on the surface of inoculated agar in Petri dishes. All tests were carried out in triplicate. Pure solvent was used as the negative control; gentamicin, as the positive control. Test cultures of *Ochrobactrum anthropi* KCTC 22833^T, *Pseudomonas aeruginosa* KCTC 1750^T, and *Stenotrophomonas maltophilia* KCTC 1773^T were obtained from the Korean Collection for Type Culture (KCTC); *Ochrobactrum intermedium* KACC 11952^T and *Stenotrophomonas nitritireducens* KACC 10891^T, from the Korean Agricultural Culture Collection (KACC).

***N*-[3-Oxo-20(29)-lupen-28-oyl]-4-(4-fluorophenyl)-piperazine (2).** Yield 89.8%, white crystalline compound, 99.1% purity (HPLC analysis), mp 151–152°C. IR spectrum (TIR, ν, cm^{-1}): 2941, 1702 (C=O), 1634 (CONH), 1508, 1409, 1227, 1189, 1025, 880, 820.

PMR spectrum (600 MHz, $CDCl_3$, δ, ppm): 0.93, 1.02, 1.03, 1.06 (15H, 4s, CH_3-23, 24, 25, 26, 27), 1.68 (3H, s, CH_3-30), 2.39 (1H, m, H-2), 2.49 (1H, m, H-2), 2.98 (6H, m, H-13, 19, 3′, 3′, 5′, 5′), 3.76 (4H, m, H-2′, 2′, 6′, 6′), 4.74 and 4.57

(2H, both br.s, H-29), 6.89 (2H, m, H-2″, 6″), 6.98 (2H, m, H-3″, 5″) (for all compounds only characteristic proton resonances are given). $C_{40}H_{57}FN_2O_2$.

***N*-[3-Oxo-20(29)-lupen-28-oyl]-4-(3,4-dichlorophenyl)-piperazine (3).** Yield 86.7%, white crystalline compound, 99.2% purity (HPLC analysis), mp 182-183°C. IR spectrum (TIR, ν, cm^{-1}): 2941, 1700 (C=O), 1626 (CONH), 1593, 1483, 1411, 1232, 1192, 1026, 882, 801.

PMR spectrum (600 MHz, $CDCl_3$, δ, ppm): 0.93, 0.97, 1.01, 1.06 (15H, 4s, CH_3-23, 24, 25, 26, 27), 1.68 (3H, s, CH_3-30), 2.39 (1H, m, H-2), 2.49 (1H, m, H-2), 2.91 (1H, m, H-13), 2.99 (1H, m, H-19), 3.11 (4H, m, H-3′, 3′, 5′, 5′), 3.76 (4H, m, H-2′, 2′, 6′, 6′), 4.74 and 4.59 (2H, both br.s, H-29), 6.76 (1H, m, H-6″), 6.96 (1H, m, H-5″), 7.29 (1H, m, H-2″). $C_{40}H_{56}Cl_2N_2O_2$.

***N*-[3-Oxo-20(29)-lupen-28-oyl]-4-(4-nitrophenyl)-piperazine (4).** Yield 78.5%, yellow crystalline compound, 99.2% purity (HPLC analysis), mp 197–198°C. IR spectrum (TIR, ν, cm^{-1}): 2940, 1700 (C=O), 1626 (CONH), 1593, 1501, 1328, 1243, 1190, 1113, 1024, 879, 835.

PMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.93, 0.98, 0.99, 1.03, 1.08 (each 3H, s, CH_3-23, 24, 25, 26, 27), 1.69 (3H, s, CH_3-30), 2.39 (1H, m, H-2), 2.49 (1H, m, H-2), 2.90 (1H, m, H-13), 3.00 (1H, td, J_1 = 10.8, J_2 = 4.2, H-19), 3.41 (4H, m, H-3′, 3′, 5′, 5′), 3.80 (4H, m, H-2′, 2′, 6′, 6′), 4.74 and 4.60 (2H, both br.s, H-29), 6.85 (2H, d, J = 9.0, H-2″, 6″), 8.14 (2H, d, J = 9.0, H-3″, 5″). $C_{40}H_{57}N_3O_4$.

***N*-[3-Oxo-20(29)-lupen-28-oyl]-4-(2-pyridyl)-piperazine (5).** Yield 87.6%, white crystalline compound, 99.3% purity (HPLC analysis), mp 158–159°C. IR spectrum (TIR, ν, cm^{-1}): 2938, 1703 (C=O), 1633 (CONH), 1592, 1478, 1435, 1243, 1189, 1020, 980, 882, 773.

PMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.93, 0.98, 1.01, 1.06 (15H, 4s, CH_3-23, 24, 25, 26, 27), 1.69 (3H, s, CH_3-30), 2.39 (1H, m, H-2), 2.49 (1H, m, H-2), 2.94 (1H, m, H-13), 3.00 (1H, td, J_1 = 10.8, J_2 = 3.6, H-19), 3.51 (4H, m, H-3′, 3′, 5′, 5′), 3.73 (4H, m, H-2′, 2′, 6′, 6′), 4.74 and 4.59 (2H, both br.s, H-29), 6.68 (2H, m, H-3″, 5″), 7.52 (1H, m, H-4″), 8.20 (1H, m, H-6″). $C_{39}H_{57}N_3O_2$.

***N*-[3-Oxo-20(29)-lupen-28-oyl]-4-(2-pyrimidyl)-piperazine (6).** Yield 77.8%, white crystalline compound, 99.1% purity (HPLC analysis), mp 109–110°C. IR spectrum (TIR, ν, cm^{-1}): 2940, 1703 (C=O), 1633 (CONH), 1584, 1448, 1257, 1188, 982, 879, 797.

PMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.93, 0.98, 1.02, 1.06 (15H, 4s, CH_3-23, 24, 25, 26, 27), 1.69 (3H, s, CH_3-30), 2.39 (1H, m, H-2), 2.50 (1H, m, H-2), 2.94 (1H, m, H-13), 3.00 (1H, td, J_1 = 10.8, J_2 = 3.6, H-19), 3.69 (4H, m, H-3′, 3′, 5′, 5′), 3.76 (4H, m, H-2′, 2′, 6′, 6′), 4.74 and 4.59 (2H, both br.s, H-29), 6.55 (1H, m, H-5″), 8.33 (2H, m, H-4″, 6″). $C_{38}H_{56}N_4O_2$.

***N*-[3-Oxo-20(29)-lupen-28-oyl]-4-(4-trifluoromethylphenyl)-piperazine (7).** Yield 74.9%, white crystalline compound, 98.9% purity (HPLC analysis), mp 229–230°C. IR spectrum (TIR, ν, cm^{-1}): 2944, 1704 (C=O), 1626 (CONH), 1525, 1458, 1332, 1230, 1112, 1073, 1023, 885, 830.

PMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.93, 0.97, 0.98, 1.02, 1.06 (each 3H, s, CH_3-23, 24, 25, 26, 27), 1.69 (3H, s, CH_3-30), 2.39 (1H, m, H-2), 2.49 (1H, m, H-2), 2.92 (1H, m, H-13), 3.00 (1H, td, J_1 = 11.4, J_2 = 4.2, H-19), 3.24 (4H, m, H-3′, 3′, 5′, 5′), 3.78 (4H, m, H-2′, 2′, 6′, 6′), 4.74 and 4.60 (2H, both br.s, H-29), 6.94 (2H, d, J = 8.4, H-2″, 6″), 7.51 (2H, d, J = 8.4, H-3″, 5″). $C_{41}H_{57}F_3N_2O_2$.

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