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Synthesis and biological activity of amide derivatives of nimbolide☆

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Abstract—Nimbolide (**1**), a limonoid isolated from *Azadirachta indica*, is the chief cytotoxic principle in Neem leaves extract. Using nimbolide as a lead compound for anti-cancer analogue design, a series of nimbolide derivatives have been synthesized and evaluated for in vitro cytotoxic activity against a panel of human cancer cell lines. Out of 10 compounds screened **2g**, **2h** and **2i** showed potent activity.

Cancer is a disease of striking significance in the world today. It is second leading disease cause of death in most of the countries after cardiovascular disease and it is projected to becoming the primary cause of death within the coming years.[1] *Azadirachta indica* popularly known as 'neem' or 'nimba' is indigenous to Indo-Pakistan subcontinent and almost all parts of the tree offer tremendous potential for medicinal, agricultural and industrial exploitation.

Among the various constituents of *A. indica*, limonoids are the major compounds. These compounds are identified to possess a wide range of biological activities. Nimbolide (**1**), the major component of leaves of *A. indica* (Neem), has been shown to exhibit numerous biological activities such as anti-feedent,[2] anti-malarial[3] and antimicrobial activities.[4] It has also exhibited significant anti-cancer activity.[5,6]

Chemically, nimbolide has a classical limonoid skeleton with α,β-unsaturated ketone system and δ-lactonic ring. However, literature search reveals that α,β-unsaturated ketone structural element is responsible for its anti-cancer activity.[7] On this basis, our studies on nimbolide have been targeted to prepare its derivatives without effecting the unsaturated ketone. So, we focused on lactone ring at C-28 position and prepared several derivatives by opening the lactone ring.

This was achieved by reacting nimbolide (**1**) with the primary amines for 24–36 h under reflux condition in anhydrous THF (Scheme 1). In order to understand the amine structural requirement, different amines with diverse structures are used in the reaction for studying the activity variations. In continuation of our ongoing research programme on natural product-based drug design,[8] we report here the synthesis of nimbolide derivatives and the evaluation of their biological activity in in vitro growth inhibition of cancer cell lines.

Nimbolide and its derived analogues were assayed for in vitro cytotoxicity against human cancer cell lines, HT-29, SW-620 (colon) and HOP-62, A-549 (lung), PC-3 (prostate), and OVCAR-5 (ovary) using sulforhodamine.[9] The cells were allowed to proliferate in the presence of test material for 48 h and results are reported in terms of their IC_{50} values (Table 2). It has been observed that all the compounds displayed negligible activity on A-549 cell line. All the tested compounds exhibited varying degrees of activity against the tested cell lines with each one showing some degree of cell specificity against particular cell lines. It is evident from Table 2, compounds **2g**, **2h** and **2i** showed potent cytotoxicity in comparison with the parent compound. Compound **2g** was highly active against HT-29, HOP-62 and PC-3 cell lines. Compound **2h** displayed remarkably high activity against all the tested cell lines except for A-549. Similarly, **2i** showed a high degree of cytotoxicity against SW-620, HT-29, OVCAR-5 and PC-3 cell lines.

Keywords: *Azadirachta indica*; Nimbolide analogues; Anti-cancer activity; Benzyl amines.
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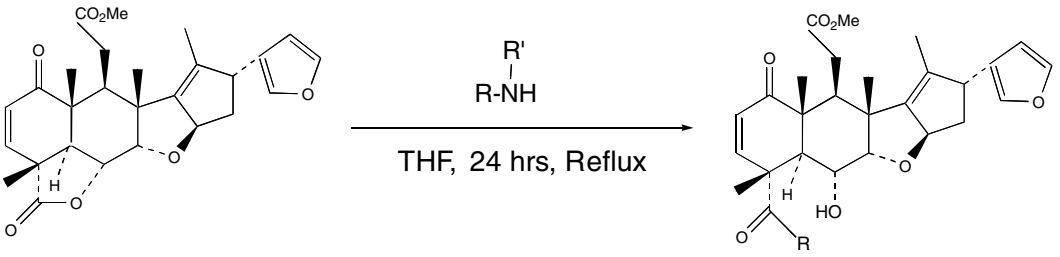

Scheme 1. Synthesis of amide derivatives of nimbolide.

Table 1. Synthesis of nimbolide analogues

Compound	R	Time (h)	Yield[a] (%)
2a	CH_2-NH-	24	80
2b	MeO, CH_2-NH-	24	90
2c	CH_2-NH-, MeO	24	86
2d	F, CH_2-NH-	26	92
2e	CF_3, CH_2-NH-	24	96
2f	CH_3, CH-NH-	30	80
2g	CH-CO_2Me, NH-	24	95
2h	NH-	24	96
2i	O, N-	24	80
2j	N-	36	70

[a] Isolated yields.

By comparing the cytotoxic potentials of the compounds with different substitutions as well as different positions the following conclusions were drawn: (i) the compounds that had a substitute at *para* position (**2b**, **2d**, and **2e**) of the benzene ring showed poor cytotoxicity than the hit compound **1**; (ii) derivatives with *meta* substituent (**2c**) weakened the activity towards all cell lines; (iii) introducing the amino acid group (**2g**) led to enhancement of the activity particularly and (iv) accordingly, introduction of cylic secondary amines also enhanced the activity to a greater extent. This result indicates that introduction of morpholine and methyl ester of phenyl glycine can lead to increasing the permeability and stability, which significantly improve inhibitory effect of cancer cell growth.

All the chemicals and solvents were used as purchased. The ^{1}H NMR spectra in $CDCl_3$ were recorded either on a Bruker AV-300 or Gemini 200 MHz spectrometers and all chemical shifts are reported in ppm. The IR spectra were recorded on Perkin-Elmer spectrophotometer. Mass spectral data were obtained on a Agilent 1100 series LC-MSD ion trap detector. ^{1}H NMR, IR and MS analyses characterized all the target compounds.[10]

In a typical experiment, nimbolide (**1**) (46 mg) was taken in anhydrous THF and to that benzyl amine (21 mg, 2 equiv) was added and the contents were refluxed under stirring for a period of 24 h. The reaction is monitored over TLC and at the end THF is removed under reduced pressure and the crude product is loaded on a silica gel column and eluted with 50% EtOAc in hexane to obtain pure **2a** in 80% yield. The various analogues prepared and their isolated yields are tabulated in Table 1.

Table 2. IC_{50} values (μM) for nimbolide and its analogues

Compound	HT-29	SW-620	HOP-62	A-549	PC-3	OVCAR-5
Nimbolide	6.94	8.25	10.37	15.56	4.59	4.17
2a	43.25	NA	26.28	NA	NA	82.40
2b	NA	NA	102.34	NA	21.50	93.13
2c	63.12	NA	79.17	NA	NA	NA
2d	78.14	NA	NA	NA	NA	84.11
2e	17.48	26.40	14.10	37.28	32.34	NA
2f	47.16	NA	NA	NA	43.14	89.18
2g	8.62	NA	13.48	NA	8.11	NA
2h	0.05	0.08	4.83	NA	7.43	1.53
2i	3.38	5.72	NA	NA	2.26	2.11
2j	91.46	NA	NA	78.34	48.18	62.34

NA, not active.

In summary, we prepared a novel class of nimbolide derivatives modified on lactone ring under catalyst-free conditions. The position and nature of the substituent seems to be crucial for the cytotoxic activity. We found that compounds **2g** and **2i** possessed strong inhibitory activities than nimbolide (**1**). In vivo studies and SAR studies are currently under investigation.

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10. *Spectral data of compound* ***2c***. IR (KBr) ν_{max}, cm^{-1}: 3525 (OH), 3335 (–NH), 2950, 1733 (COOMe), 1678 (C═O), 1610 (amide C═O), 1526, 1490, 1437, 1263, 1159, 1035, 873, 767, 665. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.34 (1H, d, J = 10 Hz, H-23), 7.31 (1H, s, H-3′), 7.24 (1H, s, H-21), 6.78–6.90 (3H, m, H-5′, -6′, -7′), 6.40 (1H, d, J = 9.6 Hz, H-3), 6.33 (1H, d, J = 1.0 Hz, H-22), 5.79 (1H, d, J = 9.6 Hz, H-2), 5.53 (1H, t, J = 6.6 Hz, H-15), 4.66 (1H, dd, J = 3.6, 12.6 Hz, H-6), 4,21 (1H, d, J = 3.5 Hz, H-7), 4.10 (2H, d, J = 14.5 Hz, H-1′), 3.92 (3H, s, Ar-OMe), 3.65 (1H, t, H-17), 3.55 (3H, s, OMe), 2.85 (1H, dd, J = 15.6, 5.8 Hz, H-11a), 2.68 (1H, t, H-6), 2.32 (1H, dd, J = 15.6, 5.8 Hz, H-11b), 2.21 (1H, dd, J = 12.0, 6.6 Hz, H-16a), 2.10 (1H, dd, J = 12.0, 6.0 Hz, H-16b), 1.73 (3H, s, H-18), 1.52 (3H, s, H-29), 1.27 (3H, s, H-19), 1.35 (3H, s, H-30). ^{13}C NMR (75 MHz, $CDCl_3$): δ 201.12 (C-1), 126.21 (C-2), 150.10 (C-3), 42.22 (C-4), 28.20 (C-5), 66.25 (C-6), 85.38 (C-7), 47.80 (C-8), 39.20 (C-9), 50.98 (C-10), 34.18 (C-11), 174.85 (C-12), 147.85 (C-13), 135.29 (C-14), 86.97 (C-15), 40.96 (C-16), 49.55 (C-17), 12.88 (C-18), 16.78 (C-19), 126.86 (C-20), 139.85 (C-21), 111.35 (C-22), 143.12 (C-23), 13.40 (C-29), 178.57 (C-28), 16.15 (C-30), 51.28 (OMe), 44.35 (C-1′), 138.78 (C-2′), 118.94 (C-3′), 129.52 (C-4′), 114.25 (C-5′), 160.11 (C-6′), 116.94 (C-7′), 56.98 (Ar-OMe). FABMS: 626 (M^++Na).
Compound ***2d***. IR (KBr) ν_{max}, cm^{-1}: 3530 (OH), 3328 (–NH), 2948, 1738 (COOMe), 1670 (C═O), 1640 (amide C═O), 1520, 1478, 1460, 1260. ^{1}H NMR 200 MHz, $CDCl_3$: δ 7.36 (1H, d, J = 1.0 Hz, H-23), 7.20 (1H, s, H-21), 7.13 (2H, d, J = 9.0 Hz, H-3′, H-7′), 6.40 (1H, d, J = 9.6 Hz, H-3), 6.35 (1H, d, J = 1, H-22), 5.85 (2H, d, J = 9.0 Hz, H-4′, -6′), 5.74 (1H, d, J = 9.8 Hz, H-2), 5.54 (1H, t, J = 6.6 Hz, H-15), 4.64 (1H, dd, J = 12.0, 3.5 Hz, H-6), 4.20 (1H, d, J = 3.5 Hz, H-7), 4.15 (2H, d, J = 14.0 Hz, H-1′), 3.65 (1H, t, J = 6.2 Hz, H-17), 3.53 (3H, s, OMe), 2.80 (1H, dd, J = 15.6, 5.3 Hz, H-11a), 2.68 (1H, t, J = 5.3 Hz, H-9), 2.65 (1H, d, J = 12.2 Hz, H-5), 2.32 (1H, dd, J, = 15.6, 5.8 Hz, H-11b), 2.21 (1H, dd, J = 12.0, 6.6 Hz, H-16a), 2.10 (1H, dd, J = 12.0, 6.5 Hz, H-16b), 1.75 (3H, s, H-18), 1.55 (3H, s, H-29), 1.33 (3H, s, H-30), 1.28 (3H, s, H-19). ^{13}C NMR (75 MHz, $CDCl_3$): δ 202.02 (C-1), 126.19 (C-2), 150.19 (C-3), 42.32 (C-4), 28.18 (C-5), 66.22 (C-6), 85.38 (C-7), 47.75 (C-8), 39.19 (C-9), 50.88 (C-10), 34.12 (C-11), 174.85 (C-12), 148.45 (C-13), 135.20 (C-14), 86.89 (C-15), 40.87 (C-16), 49.58 (C-17), 12.90 (C-18). 16.78 (C-19), 126.68 (C-20), 139.80 (C-21), 110.98 (C-22), 143.10 (C-23), 178.78 (C-28), 13.62 (C-29), 16.18 (C-30), 51.49 (OMe), 43.90 (C-1′), 134.10 (C-2′), 128.89 (C-3′, -7′), 115.14 (C-4′, -6′), 160.20 (C-5′). FABMS: 612 (M^+).
Compound ***2h***. IR (KBr) ν_{max}, cm^{-1}: 3559 (OH), 3370 (–NH), 2956, 1736 (COOMe), 1682 (C═O), 1636 (amide C═O), 1536, 1385, 1266, 1031, 778. ^{1}H NMR 200 MHz, $CDCl_3$: δ 7.30 (1H, d, J = 1.0 Hz, H-23),7.22 (1H, s, H-21), 6.42 (1H, d, J = 9.6 Hz, H-3), 6.34 (1H, d, J = 1.0 Hz, H-22), 5.78 (1H, d, J = 9.6 Hz, H-2), 5.52 (1H, t, J = 6.5 Hz, H-15), 4.64 (1H, dd, J = 12, 3.5 Hz, H-6), 4.20 (1H, d, J = 3.5 Hz, H-7), 3.20 (2H, t, J = 5.4 Hz, H-1′), 3.60 (1H, t, J = 6 Hz, H-17), 3.54 (3H, s, OMe), 2.80 (1H, dd, J = 15.6, 5.3 Hz, H-11a), 2.68 (1H, t, J = 5.3 Hz, H-11b), 2.20 (1H, dd, J = 12.0, 6.6 Hz, H-16a), 2.12 (1H, dd, J = 12.0, 6.5 Hz, H-16b), 1.74 (3H, s, H-18), 1.68 (1H, m, H-2′), 1.54 (3H, H-29), 1.35 (3H, s, H-30), 1.25 (3H, H-19), 0.98 (6H, s, H-3′, -4′). ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.75 (C-1), 126.18 (C-2), 150.05 (C-3), 42.08 (C-4), 28.19 (C-5), 66.15 (C-6), 85.34 (C-7), 47.96 (C-8), 39.00 (C-9), 50.88 (C-10), 34.08 (C-11), 174.98 (C-12), 148.02 (C-13), 135.26 (C-14), 86.97 (C-15), 40.96 (C-16), 49.78 (C-17), 12.76 (C-18), 16.78 (C-19), 126.75 (C-20), 139.60 (C-21), 111.14 (C-22), 143.19 (C-23), 178.05 (C-28), 13.74 (C-29), 15.98 (C-30), 51.55 (OMe), 48.01 (C-1′), 20.14 (C-2′), 18.15 (C-3′,4′). FABMS: 574 (M^++Na).
Compound ***2i***. IR (KBr) ν_{max}, cm^{-1}: 3497 (OH), 2956, 2851, 1760 (COOMe), 1700 (C═O), 1617 (amide C═O), 1540, 1353, 1219, 772. ^{1}H NMR 200 MHz, $CDCl_3$: δ 7.31 (1H, d, J = 1.0 Hz, H-23), 7.22 (1H, s, H-21), 6.42 (1H, d, J = 9.6, H-3), 6.30 (1H, d, J = 1 Hz, H-22), 5.74 (1H, d, J = 9.6 Hz, H-2), 5.55 (1H, t, J = 6.6, H-15), 4.64 (1H, dd, J = 12.2, 3.4 Hz, H-6), 4.20 (1H, d, J = 3.5 Hz, H-7), 3.70 (1H, t, J = 6.2 Hz, H-17), 3.62–3.68 (2H, m, H-2′, C-6′), 3.58 (3H, s, OMe), 3.55–3.50 (2H, m, H-3′, 5′), 2.85 (1H, dd, J = 15.6, 5.3 Hz, H-11a), 2.68 (1H, 1H, d, J = 5.3 Hz, H-9), 2.64 (1H, d, J = 12.0, H-5), 2.35 (1H, dd, J = 15.5, 5.4 Hz, H-11b), 2.20 (1H, dd, J = 12.0, 6.5 Hz, H-16a), 2.08 (1H, dd, J = 12.0, 6.5 Hz, H-16b), 1.75 (3H, s, H-18), 1.54 (3H, s, H-30), 1.25 (3H, s, H-19). ^{13}C NMR (300 MHz, $CDCl_3$): δ 200.15 (C-1), 126.33 (C-

2), 150.65 (C-3), 42.24 (C-4), 28.22 (C-5), 66.19 (C-6), 85.58 (C-7), 47.78 (C-8), 39.12 (C-9), 50.54 (C-10), 34.25 (C-11), 174.90 (C-12), 148.25 (C-13), 135.58 (C-14), 86.78 (C-15), 40.87 (C-16), 49.98 (C-17), 12.82 (C-18), 16.69 (C-19), 126.98 (C-20), 139.56 (C-21), 110.98 (C-22), 143.16 (C-23), 179.19 (C-28), 13.85 (C-29), 16.08 (C-30), 52.00 (OMe), 66.87 (C-2′, -3′), 43.65 (C-1′, -4′). FABMS: 576 (M^++Na).