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ARTICLE

Synthesis and Insect Antifeedant Activity of Plumbagin Derivatives with the Amino Acid Moiety

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A series of plumbagin derivatives (**4a**−**4k**) containing an amino acid moiety were synthesized under mild esterification conditions in excellent yields (35%−80%) and screened for their antifeedant activities in tobacco caterpillar (*Spodoptera litura*) and castor semilooper (*Achaea janata*) using a no-choice laboratory bioassay. The parent compound plumbagin lacked significant activity, but the analogues were effective in reducing feeding by two insect species. The introduction of an *N*-acetyl-L-amino acid side chain to the Michael adduct of plumbagin at the third position of the quinone moiety significantly increased antifeedant activity. Several of the analogues were also toxic or caused developmental abnormalities following topical administration.

KEYWORDS: Plumbagin; amino acid esters; *Spodoptera litura*; *Achaea janata*; insect antifeedant

INTRODUCTION

The severe damage caused to the ecology and environment as well as human health due to the usage of complex chemical synthetic pesticides necessitated a shift to natural crop protection and nourishment. Plants produce a diverse range of secondary metabolites such as terpenoids, alkaloids, polyacetylenes, flavonoids, quinones, and sugars as part of their defense mechanism against insects. Among these classes, naphthaquinones, in particular, 5-hydroxy-1,4-naphthaquinones such as plumbagin and juglone, are an important class of natural products that are extremely useful for the development of potent agrochemicals in view of their high abundance and relatively nontoxic nature. A literature precedence revealed that plumbagin isolated from the *Plumbago capensis* possesses significant biological activities such as antimicrobial (*1*), cytotoxic (*2*), antimalarial (*3*), and antiprotozoal properties (*4*). Previously, it was illustrated that the ingestion of the crude aqueous methanol extracts of *P. capensis* and its derivatives caused the failure of the molting cycle in another lepidopteran insect, *Bombyx mori* (silk worm)(*5*).

Recently, Tokunaga et al. isolated certain plumbagin analogues from the carnivorous plant, *Dionae muscipula* Ellis, which were found to exhibit significant antifeedant activity (*6*). Further, they also suggested that the defined substituents on the 1,4-naphthaquinone backbone can increase the selectivity and potency of the desired action (*7*). The naphthaquinone skeletons with high abundance and favorable structural features form useful models for the development of potent antifeedants. As part of an ongoing effort to discover potential leads from Indian medicinal plants (*8*, *9*), we have isolated large quantities of plumbagin (**1**), which prompted us to synthesize derivatives and screen for antifeedant activity. In view of the fact that the 1,4-naphthaquinone backbone is essential for antifeedant activity, we focused on the synthesis of the new analogues, by exploiting the C-3 position.

In this context, we have designed analogues of plumbagin by introducing a peptidyl side chain at the 3-position of plumbagin and keeping the quinone moiety in tact. This was achieved by the Michael addition of 5-*O*-methylplumbagin with ethanolamine followed by condensation of a series of *N*-acetyl-L-amino acids with the Michael adducts. All of these derivatives along with plumbagin have been evaluated for their antifeedant, toxic, and growth regulatory activities against two major agricultural pests, castor semilooper, *Achaea janata*, and tobacco caterpillar, *Spodoptera litura*.

MATERIALS AND METHODS

General. ^{1}H and ^{13}C spectra were measured on a Bruker 300 MHz spectrometer using tetramethylsilane as an internal standard. Mass spectra were recorded on an Agilent LC/MSD trap SL 1100 series spectrometer with a 70 eV (ESI probe) and the infrared spectra on a Thermo Nicolet Nexus 670 FTIR spectrometer. Melting points were taken on a Fischer Scientific melting point apparatus and are uncorrected. The synthetic compounds were purified by column chromatography using 60−120 mesh size silica gel (Merck). Thin layer chromatography (TLC) involved the use of precoated silica gel 60 F_{254} TLC plates from Merck. The optical rotations were measured on a Jasco Dip 360 digital polarimeter.

Extraction and Isolation of Plumbagin. The roots of *Plumbago capensis* also known as *Nila chitramula* (*10*) were collected during August−September 2005 from Tirumala forest, Tirupati, Andhra Pradesh, India. A voucher specimen was deposited at the herbarium of Indian Institute of Chemical Technology, Hyderabad, India. The shade dried roots of *Plumbago capensis* were powdered in a pulvarizer (10 kg) and extracted with chloroform/methanol, 1:1, followed by concentration under reduced

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pressure. The resulting extract was (70 g) chromatographed over silica gel (60−120 mesh) and eluted with *n*-hexane/ethyl acetate combinations of increasing polarity. Plumbagin (12 g) was obtained by elution with *n*-hexane/ethyl acetate, 99:1.

General Procedure for the Synthesis of Compound 2. To a solution of plumbagin (**1**) (0.2 g, 1.0638 mmol) in dichloromethane (10 mL) at room temperature was added CH_3I (1.39 mmol) and freshly prepared Ag_2O (0.05 mmol). The resultant mixture was stirred for 10 h at room temperature. After completion of the reaction (monitored by TLC), the reaction mixture was filtered, and then the filtrate was concentrated to dryness. The residue thus obtained was purified by column chromatography with the elution of *n*-hexane/ethyl acetate, 90:10, to afford 5-*O*-methyl plumbagin (**2**) as yellow needles (*11*).

Procedure for the Synthesis of Compound 3. To a solution of 5-*O*-methyl plumbagin (**2**) (1 mmol) in dry dichloromethane (10 mL) under nitrogen atmosphere was added dropwise 2-amino ethanol (1.5 mmol) at room temperature. The resulting orange colored solution was stirred for 1 h at room temperature. The reaction was monitored by TLC, and after completion, the reaction mixture was concentrated to dryness. The residue was purified by column chromatography using dichloromethane/methanol (99:1) to yield the Michael adduct (**3**) as red needles. Yield: 68%. mp: 126 °C. IR (KBr) ν_{max}: 3312 (NH), 1662 (α,β unsaturated C═O), 1555, 1275, 1038 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 2.24 (3H, s, H-2, $-CH_3$), 3.74 (2H, dd, J = 6.8 Hz, 14.8 Hz, H-2′), 3.85 (2H, dd, J = 6.8 Hz, 14.8 Hz, H-3′), 3.98 (3H, s, -OMe), 7.15 (1H, dd, J = 1.1 Hz, 8.5 Hz, H-6), 7.62 (1H, t, J = 7.5 Hz, 8.5 Hz, H-7), 7.75 (1H, dd, J = 1.1 Hz, 7.5 Hz, H-8); ^{13}C NMR (75 M Hz, $CDCl_3$): δ 14.43, 50.95, 56.34, 64.35, 115.55, 118.06, 119.36, 128.65, 135.58, 135.64, 147.24, 161.02, 180.83, 183.34. ESIMS m/z (rel.int.): 284 [M^+23] (22), 261 [M^+H] ($C_{14}H_{15}NO_4$) (15), 201(100), 175 (7), 147 (34).

General Procedure for the Synthesis of Derivatives 4a−4k. To a cooled solution (0 °C) of **3** (1 equiv) in dry dichloromethane (10 mL) under nitrogen atmosphere was added *N*,*N*′-dicyclohexylcarbodimide and (1.5 equiv) and a catalytic amount of 3-hydroxy benzotriazole. *N*-Acetyl-L-amino acid (1.2 mmol) was added after stirring for 15 min, and stirring was continued at room temperature. After completion of the reaction (monitored by TLC), the reaction mixture was filtered to remove the precipitated dicyclohexylurea. The filtrate was evaporated under reduced pressure, and the residue was purified by silica gel (100−200 mesh) column chromatography using dichloromethane/methanol (98:2) as eluent to afford the corresponding plumbagin derivatives (**4a**−**4k**). The structures of the new derivatives were confirmed by the spectral analyses such as FTIR, ^1H NMR, ^{13}C NMR, and mass spectroscopy.

*2-(8-Methoxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalene-2-ylamino)ethyl-2-acetamidoacetate (**4a**).* The title compound was prepared by the reaction of compound **3** (0.050 g, 0.13 mmol) with *N*-acetyl-glycine (0.0243 g, 0.207 mmol). Yield: 78%. mp: 105 °C. IR (KBr) ν_{max}: 3312 (NH), 1747 (ester C═O), 1662 (α,β unsaturated C═O), 1555, 1275, 1038, 745 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.03 (s, 3H, 9′-CH_3), 2.24 (s, 3H, 2-CH_3), 3.14 (dd, 2H, J = 6.8 Hz, 14.8 Hz, H-2′), 3.98 (s, 3H, 5-OMe), 3.90 (dd, 1H, J = 5.4 Hz, 17.1 Hz, H-6′a), 4.03 (dd, 1H, J = 5.4 Hz, 17.1 Hz, H-6′b), 4.27 (dd, 2H, J = 6.8 Hz, 14.8 Hz, H-3′), 7.15 (dd, 1H, J = 1.1 Hz, 8.5 Hz, H-6), 7.62 (t, 1H, J = 7.5 Hz, 8.5 Hz, H-7), 7.75 (dd, 1H, J = 1.1 Hz, 7.5 Hz, H-8). ^{13}C NMR (75 MHz, $CDCl_3$,): δ 11.04, 14.03, 22.83, 50.95, 56.34, 64.35, 115.93, 118.06, 119.16, 128.80, 135.58, 135.69, 147.24, 159.61, 170.97, 173.04, 180.83, 183.34. ESIMS m/z (rel.int.): 383 [M^+23], 361 [M^+H] ($C_{18}H_{20}N_2O_6$) (15), 318 [M^+-$COCH_3$] (13), 300 [318-H_2O] (37), 273 (25), 260 (15), 244 (8) 225 (25), 212 (17), 149 (100), 121 (83), 99 (30).

*2-(8-Methoxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalene-2-ylamino)ethyl2-acetamidopropanoate (**4b**).* The title compound was prepared by the reaction of compound **3** (0.050 g, 0.13 mmol) with *N*-acetyl alanine (0.027 g, 0.207 mmol). Yield: 62%. mp: 109 °C; $[\alpha]_D^{20}$ +14.5° (*c* 1, H_2O). IR (KBr) ν_{max}: 3314 (NH), 1742 (ester C═O stretch), 1665 (α,β unsaturated C═O), 1552, 1270,1034, 742 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.46 (d, 3H, J = 6.8 Hz, 6′-CH_3), 2.03 (s, 3H, H-9′), 2.24 (s, 3H, 2-CH_3), 3.87 (dd, 2H, J = 6.8 Hz, 14.8 Hz, H-2′), 3.98 (s, 3H, 5-OMe), 4.35 (dd, 2H, J = 6.8 Hz, 14.8 Hz, H-3′), 4.45−4.46 (1H, m, H-6′), 7.15 (dd, 1H, J = 1.1, 8.5 Hz, H-6), 7.62 (t, 1H, J = 7.5 Hz, 8.5 Hz, H-7), 7.75 (dd, 1H, J = 1.1 Hz, 7.5 Hz, H-8). ^{13}C NMR (75 MHz, $CDCl_3$,): δ 11.02, 14.10, 22.83, 50.95, 53.34, 56.34, 64.31, 115.93, 118.08, 119.13, 130.39, 131.80, 135.60 147.12, 159.62, 169.94, 171.60, 180.80, 183.31. ESIMS m/z (rel.int.): 374 [M^+] ($C_{19}H_{22}N_2O_6$) (23), 331 [M^+-$COCH_3$] (4), 304 (11), 260 (63), 248 (47), 234 (79), 220 (9), 203 (100), 175 (33), 147 (11), 99.1 (15).

*2-(8-Methoxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalene-2-ylamino) ethyl 2-acetamido-3-mercaptopropanoate (**4c**).* The title compound was prepared by the reaction of compound **3** (0.050 g, 0.13 mmol) with *N*-acetyl cysteine (0.0426 g, 0.207 mmol). Yield: 42%. mp: 119 °C; $[\alpha]_D^{25}$ + 6.5° (*c*1, H_2O). IR (KBr) ν_{max}: 3329 (NH), 2923, 1740 (ester C═O), 1660 (α,β unsaturated C═O), 1575, 1449, 1273, 747 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.03 (s, 3H, 9′-CH_3), 2.24 (s, 3H, 2-CH_3), 2.32 (d, 2H, J = 7.5 Hz, H-1′′), 3.84 (dd, 2H, J = 6.8 Hz, 14.8 Hz, H-2′), 3.98 (s, 3H, 5-OMe), 4.32 (dd, 2H, J=6.8 Hz, 14.8 Hz, H-3′), 4.73 (t, 1H, J=7.3 Hz, H-6′), 7.15 (dd, 1H, J = 1.1, 8.5 Hz, H-6), 7.62 (t, 1H, J = 7.5 Hz, 8.5 Hz, H-7), 7.75 (dd, 1H, J = 1.1 Hz, 7.5 Hz, H-8). ^{13}C NMR (75 MHz, $CDCl_3$,): δ 11.03, 14.12, 22.83, 27.30, 31.37, 51.75, 56.34, 64.35, 115.82, 118.06, 119.16, 131.22, 135.58, 147.24, 159.61, 170.97, 173.04, 180.83, 183.34. ESIMS m/z (rel.int.): 407 [M^++H] ($C_{19}H_{22}N_2SO_6$) (23), 373 [M^+-SH] (8), 315 [373−58] (29), 301 (43), 288 (12), 260 (13), 185 (100), 155 (10), 93 (15).

*2-(8-Methoxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalene-2-ylamino) ethyl 2-acetamido-4-(methyl thio)butanoate (**4d**).* The title compound was prepared by the reaction of compound **3** (0.050 g, 0.13 mmol) with *N*-acetyl methionine (0.039 g, 0.204 mmol). Yield: 68%. mp: 122 °C; $[\alpha]_D^{25}$ +25.9° (*c* 1, H_2O). IR (KBr) ν_{max}: 3329 (NH), 2923, 1740 (ester C═O), 1660 (α,β unsaturated C═O), 1575, 1449, 1273, 747 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.49 (s, 3H, 4′′-CH_3), 1.95−1.97 (2H, m, H-1′′), 2.03 (s, 3H, 9′-CH_3), 2.24 (s, 3H, 2-CH_3), 2.49 (t, 2H, J = 7.2 Hz, H-2′′), 3.84 (dd, 2H, J = 6.8, 14.8 Hz, H-2′), 3.98 (s, 3H, 5-OMe), 4.44 (dd, 2H, J = 6.8 Hz, 14.8 Hz, H-3′), 4.63 (t, 1H, J = 7.3 Hz, H-6′), 7.15 (dd, 1H, J = 1.1 Hz, 8.5 Hz, H-6), 7.62 (t, 1H, J = 7.5 Hz, 8.5 Hz, H-7), 7.75 (dd, 1H, J = 1.1 Hz, 7.5 Hz, H-8). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.04, 14.12, 15.49, 22.68, 29.35, 31.37, 44.10, 51.73, 56.36, 64.52, 112.29, 115.93, 119.16, 130.95, 135.60, 147.13, 159.64, 170.12, 171.98, 180.86, 183.35. ESIMS m/z (rel.int.): 435 [M^++H] ($C_{21}H_{26}N_2SO_6$) (17), 419 [M^+-CH_3] (20), 408 [M^+-CO] (45), 301 (17), 288 (62), 260 (13), 185 (41), 155 (100), 93 (15).

*2-(8-Methoxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalene-2-ylamino) ethyl 2-acetamido-3-methyl Pentanoate (**4e**).* The title compound was prepared by the reaction of compound **3** (0.050 g, 0.13 mmol) with *N*-acetyl isoleucine (0.036 g, 0.208 mmol). Yield: 30%. mp: 98 °C; $[\alpha]_D^{25}$ +40.0 (*c*1, H_2O). ^1H NMR (300 MHz, $CDCl_3$): δ 0.89 (d, 3H, J= 5.8 Hz, 1′′-CH_3), 0.90 (t, 3H, J = 7.2 Hz, 3′′-CH_3), 1.25−1.28 (2H, m, H-2′′), 1.48−1.52 (1H, m, H-1′′), 2.03 (s, 3H, 9′-CH_3), 2.24 (3H, s, 2-CH_3), 3.84 (dd, 2H, J = 6.8 Hz, 14.8 Hz, H-2′), 3.98 (s, 3H, -OMe), 4.44 (dd, 2H, J = 6.8 Hz,14.8 Hz, H-3′), 4.63 (dd, 1H, J = 7.2 Hz, H-6′), 7.15 (dd, 1H, J = 1.1, 8.5 Hz, H-6), 7.62 (t, 1H, J = 7.5 Hz, 8.5 Hz, H-7), 7.75 (dd, 1H, J = 1.1 Hz, 7.5 Hz, H-8). ^{13}C NMR (75 MHz, $CDCl_3$,): δ 11.03, 14.10, 15.42, 22.83, 23.05, 29.35, 30.00, 38.65, 51.73, 56.34, 64.35, 115.90, 118.06, 119.16, 128.59, 135.58, 147.24, 159.61, 170.97, 173.04, 180.83, 183.34. ESIMS m/z (rel.int.): 417 [M^++H] ($C_{22}H_{28}N_2O_6$), 395 (16), 301 (12), 261 (38), 217 (100), 195 (15), 173 (27), 101 (39), 93 (18), 78 (21), 65 (34).

*2-(8-Methoxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalene-2-ylamino) ethyl 2-acetamido-4-methyl Pentanoate (**4f**).* The title compound was prepared by the reaction of compound **3** (0.050 g, 0.13 mmol) with *N*-acetyl leucine (0.036 g, 0.208 mmol). Yield: 80%. mp: 96 °C; $[\alpha]_D^{25}$ +14.5 (*c*1, H_2O). IR (KBr) ν_{max}: 3339 (NH), 2925, 1737(ester C═O), 1657 (α,β unsaturated C═O), 1576, 1452, 1275 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$,): δ 0.90 (d, 6H, J=9.3 Hz, 3′′-CH_3, 4′′-CH_3), 1.61−1.63 (1H, m, H-1′′), 1.78−1.82 (1H, m, H-2′′), 2.03 (s, 3H, H-9′), 2.24 (s, 3H, 2-CH_3), 3.84 (dd, 2H, J=6.8 Hz, 14.8 Hz, H-2′), 3.98 (s, 3H, -OMe), 4.44 (dd, 2H, dd, J= 6.8 Hz, 14.8 Hz, H-3′), 4.63 (t, 1H, J = 7.3 Hz, H-6′), 7.15 (dd, 1H, J = 1.1, 8.5 Hz, H-6), 7.62 (dd, 1H, J = 7.5 Hz, 8.5 Hz, H-7), 7.75 (dd, 1H, J = 1.1 Hz, 7.5 Hz, H-8). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.03, 14.10, 22.83, 22.99, 24.88, 31. 24, 42. 07, 41.06, 51.15, 56.34, 64.35, 115. 90, 118.06, 119.16, 128.59, 135.58, 147.24, 159.61, 170.97, 173.04, 180.83, 183.34. ESIMS m/z (rel.int.): 417 [M^++H] ($C_{22}H_{28}N_2O_6$) (18), 395 (16), 301 (12), 261 (38), 217 (100), 195 (15), 173 (27), 101 (39), 93 (18), 78 (21), 65 (34).

*2-(8-Methoxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalene-2-ylamino) ethyl 2-acetamido-3-phenyl Propanoate (**4g**).* The title compound was prepared by the reaction of compound **3** (0.050 g,

0.13 mmol) with *N*-acetyl phenyl alanine (0.054 g, 0.288 mmol). Yield: 52%. mp: 132 °C; $[\alpha]_D^{25}$ +10.0 (*c*1, H_2O). IR (KBr) ν_{max}: 3327 (NH), 2926, 2854, 1737 (ester C=O), 1662 (α,β unsaturated C=O), 1610, 1576, 1452, 1274 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.03 (s,3H, 9′-CH_3), 2.10 (s, 3H, 2-CH_3), 3.97 (s,3H, 5-OCH_3), 3.18 (d, 2H, *J* = 7.5 Hz, H-1″), 3.68 (dd, 2H, *J* = 6.8 Hz, 14.8 Hz, H-2′), 4.44 (dd, 2H, *J* = 6.8 Hz, 14.8 Hz, H-3′), 4.63 (t, 1H, *J* = 7.5 Hz, H-6′), 7.15 (dd, 1H, *J* = 1.1 Hz, 8.5 Hz, H-6), 7.17−7.20 (5H, m, H-1″, Ph), 7.62 (dd, 1H, *J* = 7.5 Hz, 8.5 Hz, H-7), 7.75 (dd, 1H, *J* = 1.1 Hz, 7.5 Hz, H-8). ^{13}C NMR (75 MHz, $CDCl_3$,): δ 11.41, 14.01, 22.98, 37.87, 44.11, 53.34, 56.34, 64.31, 115.93, 118.08, 119.13, 127.19, 128.59, 129.16, 129.40, 132.47, 135.57, 135.69, 147.12, 159.62, 167.77, 169.94, 171.60, 180.80, 183.31. ESIMS *m*/*z* (rel.int.): 451 [M^++H] ($C_{25}H_{26}N_2O_6$) (37), 433 (28), 413 (60), 391 [M^+-$NHCOCH_3$] (38), 358 [M^+- CH_2Ph] (21), 260 (15), 228 (38), 197 (100), 146 (28), 102 (20), 87 (40).

*2-(8-Methoxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalene-2-ylamino) ethyl 2-acetamido-3-methyl Butanoate (**4h**).* The title compound was prepared by the reaction of compound **3** (0.050 g, 0.13 mmol) with *N*-acetyl valine (0.033 g, 0.207 mmol). Yield: 60%. mp: 99 °C; $[\alpha]_D^{25}$ +15.0 (*c*1, H_2O). IR (KBr) ν_{max}: 3356 (NH), 2925, 2854, 1732 (ester C=O), 1658 (α,β unsaturated C=O), 1549, 1460, 1277, 747 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.91 (d, 3H, *J* = 10.3 Hz, 2″-CH_3), 0.94 (d, 3H, *J* = 10.3 Hz, 3″-CH_3), 2.00 (s, 3H, 9′-CH_3), 2.12 (s, 3H, 2-CH_3), 2.30−2.33 (1H, m, H-1″), 3.77 (dd, 2H, *J* = 6.8 Hz, 14.8 Hz, H-2′), 3.95 (s, 3H, 5-OCH_3), 4.44 (dd, 2H, *J* = 6.8 Hz, 14.8 Hz, H-3′), 4.63 (d, 1H, *J* = 7.5 Hz, H-6′), 7.15 (dd, 1H, *J* = 1.1, 8.5 Hz, H-6), 7.62 (t, 1H, *J* = 7.5 Hz, 8.5 Hz, H-7), 7.75 (dd, 1H, *J* = 1.1 Hz, 7.5 Hz, H-8). ^{13}C NMR (75 MHz, $CDCl_3$,): δ 14.09, 17.78, 18.95, 23.14, 30.17, 44.17, 56.36, 57.18, 68.15, 115.89, 117.62, 119.15, 123.96, 132.43, 135.56, 147.89, 157.09, 170.14, 172.08, 180.69, 183.70. ESIMS *m*/*z* (rel.int.): 402 [M^+] ($C_{21}H_{26}N_2O_6$) (31), 344 [M^+- $NHCOCH_3$] (16), 301 (12), 288 (23), 260 (9), 217 (100), 202 (58), 175 (42), 99 (8).

*2-(8-Methoxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalene-2-ylamino) ethyl 2-acetamido-2-(4-hydroxy phenyl) Acetate (**4i**).* The title compound was prepared by the reaction of compound **3** (0.050 g, 0.13 mmol) with *N*-acetyl tyrosine (0.046 g, 0.206 mmol). Yield: 40%. mp: 151 °C. $[\alpha]_D^{25}$ −12.0 (*c* 1, H_2O). IR (KBr) ν_{max}: 3356 (NH), 2925, 2854, 1732 (ester C=O), 1658 (α,β unsaturated C=O), 1549, 1460, 1277 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.98 (s, 3H, 9′-CH_3), 2.12 (s, 3H, 2-CH_3), 2.86 (dd, 1H, *J* = 5.0 Hz, 13.2 Hz, H-1″a), 3.15 (dd, 1H, *J* = 5.0 Hz, 13.2 Hz, H-1″b), 3.77 (dd, 2H, *J* = 6.8 Hz, 14.8 Hz, H-2′), 3.95 (s, 3H, 5-OCH_3), 4.44 (t, 2H, *J* = 6.8 Hz, 14.8 Hz, H-3′), 4.60 (t, 1H, *J* = 7.5 Hz, H-6′), 6.63 (d, 2H, *J* = 10.2 Hz, H-3″, H-7″), 7.15 (dd, 1H, *J* = 1.1, 8.5 Hz, H-6), 7.18 (d, 2H, *J* = 10.2 Hz, H-4″, H-6″) 7.62 (t, 1H, *J* = 7.5 Hz, 8.5 Hz, H-7), 7.75 (dd, 1H, *J* = 1.1 Hz, 7.5 Hz, H-8). ^{13}C NMR (75 MHz, $CDCl_3$,): δ 14.06, 22.94, 37.17, 44.17, 53.36, 57.18, 68.15, 115.14, 115.89, 117.62, 119.15, 127.96 (2C), 128.65(2C), 131.40, 133.67, 135.18, 147.89, 158.82, 159.62, 170.14, 172.08, 180.33, 183.14. ESIMS *m*/*z* (rel.int.): 466 [M^+] ($C_{25}H_{26}N_2O_7$) (15), 408 [M^+- $NHCOCH_3$] (21), 315 (49), 301 (12), 288 (23), 260 (9), 217 (100), 202 (58), 175 (42), 99 (8).

*2-(8-Methoxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalene-2-ylamino)ethyl-4-acetamido Butanoate (**4j**).* The title compound was prepared by the reaction of compound **3** (0.050 g, 0.13 mmol) with *N*-acetyl-4-amino butyric acid (0.030 g, 0.206 mmol) .Yield: 35%. mp: 101 °C. IR (KBr) ν_{max}: 3356 (NH), 2925, 2854, 1732 (ester C=O), 1658 (α,β unsaturated C=O), 1549, 1460, 1277 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.83 (dd, 2H, *J* = 6.8 Hz, 13.4 Hz, H-7′), 1.98 (s, 3H, 11′-CH_3), 2.16 (3H, s, 2-CH_3), 2.76 (t, 2H, *J* = 8.5 Hz, H-6′), 3.26 (dd, 2H, *J* = 7.0 Hz, 13.5 Hz, H-8′), 3.77 (dd, 2H, *J* = 5.4 Hz, *J* = 10.7 Hz, H-2′), 3.95 (s, 3H, 5-OCH_3), 4.44 (t, 2H, *J* = 6.8 Hz, H-3′), 4.26 (dd, 2H, *J* = 5.4 Hz, *J* = 10.7 Hz, H-3′), 7.16 (dd, 1H, *J* = 1.1, 8.5 Hz, H-6), 7.62 (t, 1H, *J* = 7.5 Hz, 8.5 Hz, H-7), 7.75 (dd, 1H, *J* = 1.1 Hz, 7.5 Hz, H-8). ^{13}C NMR (75 MHz, $CDCl_3$,): δ 14.33, 17.78, 18.95, 23.14, 30.17, 44.17, 56.36, 57.18, 68.15, 115.89, 117.62, 119.15, 123.96, 132.43, 147.32, 167.76, 170.14, 172.08, 180.69, 183.70. ESIMS *m*/*z* (rel.int.): 388 [M^+] ($C_{20}H_{24}N_2O_6$) (7), 330 [M^+-$NHCOCH_3$] (23), 301 (8), 288 (23), 260 (9), 217 (13), 202 (58), 169 (100), 97 (8).

*2-(8-Methoxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalene-2-ylamino)-ethyl-4-acetamido Benzoate (**4k**).* The title compound was prepared by the reaction of compound **3** (0.050 g, 0.13 mmol) with *N*-acetyl *para*-amino benzoic acid (0.037 g, 0.206 mmol). Yield: 58%. mp: 142 °C. IR (KBr) ν_{max}: 3356 (NH), 2925, 2854, 1732 (ester C=O), 1658 (α,β unsaturated C=O), 1549, 1460, 1277 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.00 (s, 3H, 9′-CH_3), 2.12 (s, 3H, 2-CH_3), 3.77 (dd, 2H, *J* = 6.8 Hz, 14.8 Hz, H-2′), 3.95 (s, 3H, 5-OCH_3), 4.44 (dd, 2H, *J* = 6.8 Hz, 14.8 Hz, H-3′), 7.15 (dd, 1H, *J* = 1.1 Hz, 8.5 Hz, H-6), 7.36 (d, 2H, *J* = 9 Hz, H-3″, H-5″), 8.02 (d, 2H, *J* = 9 Hz, H-2″, H-6″), 7.62 (dd, 1H, *J* = 7.5 Hz, 8.5 Hz, H-7), 7.75 (dd, 1H, *J* = 1.1 Hz, 7.5 Hz, H-8). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.94, 23.14, 44.17, 56.36, 64.15, 114.76, 115.89, 117.62, 119.15 (2C), 128.67, 132.43 (2C), 133.96, 135.55, 149.33, 157.76, 161.75, 167.76, 170.14, 172.08, 180.69, 183.70. ESIMS *m*/*z* (rel.int.): 420 [M^+] ($C_{23}H_{22}N_2O_6$) (31), 362 [M^+-$NHCOCH_3$] (16), 341 (12), 243 (23), 2231(9), 202 (58), 175 (42), 121 (32), 99 (8), 43 (24).

Bioassay. Antifeedant activity of the compounds was assessed on tobacco caterpillar larvae (*Spodoptera litura*) and castor semilooper (*Achaea janata*). The experiments were conducted according to the classical no-choice leaf-disk bioassay described earlier by Akhtar et al. (*12*). Castor semilooper, *Achaea janata* (L), and the tobacco cutworm, *Spodoptera litura* (Fab), were reared on fresh castor leaves (*Ricinus communis* (L)) grown in the laboratory at 28 ± 2 °C, relative humidity of 65 ± 5, and a 16:8 light/dark photo period. To study the antifeedant activity of the test compounds, a small circular disk of 5 cm diameter was cut from fresh castor leaves. The leaf discs were treated on their upper surface with individual concentrations of the compounds, and one leaf disk each was transferred to each Petri plate of 15 cm diameter containing moist filter paper. Control leaf discs were treated with the same volume of the solvent only.

In each Petri dish, prestarved healthy third instar larvae of *A. janata* and *S. litura* were introduced to assess antifeedant activity. Progress of the consumption of the leaf area was measured at 6, 12, and 24 h in both treated and control leaf disks. Areas of control and treated leaf discs consumed were measured after 6 h using an AM-300 leaf area meter (ADC, Bioscientific Limited, England). The antifeedant index was then calculated as $(C - T)/(C + T) \times 100$, where *C* is the consumption of control discs, and *T* is consumption of treated discs (*13*). For each concentration, 10 experimental sets were assayed. Each test was replicated three times. The mean of the 10 sets was taken for each compound, and the percentage of antifeedant activity with standard deviation was calculated.

Topical Bioassay. Toxicity of the compounds was determined by topical application to the fourth instar of test insects. The experiments were conducted according to the topical application method described earlier by Jamil et al. (*14*). Four micrograms of compounds was applied directly to the dorsum of the larva in a 1 μL drop of acetone using a micro applicator. The controls were treated with the solvent alone. The treated and control larvae were reared on fresh castor leaves. Mortality was determined daily until 3 days after treatment. Larvae that lost elasticity and showed no responses when their tails were pinched with forceps were regarded as dead. The abnormalities in the form of growth retardation symptoms were recorded at 4, 14, and 28 days after treatment. On the basis of three independent trials, each experiment was conducted in four replicates, leading to 12 replicates with each concentration of compounds. The mean of 12 replicates was taken for each compound, and the percentage of mortality with standard error was calculated.

Data Analysis. Antifeedant indices were calculated using five different concentrations of each compound, and data was subjected to probit analysis (*15*) to determine the ED_{50} value representing the concentrations that caused 50% feeding deterrence along with the 95% confidence intervals. Results from the topical and growth regulatory bioassays were analyzed by one way ANOVA. Post hoc testing was carried out using the Tukey test. A significant level of 0.05 was used for all statistical tests.

RESULTS AND DISCUSSION

Figure 1 outlines the general procedure used to synthesize plumbagin analogues, which were prepared in good overall yields via a three-step procedure. Initially, plumbagin was converted to its methyl ether **2** in 85% yield through a reaction with MeI in the presence of Ag_2O in dry dichloromethane. Michael addition of 5-*O*-methyl plumbagin with ethanolamine afforded the Michael adduct **3**. Thus, condensation of the Michael adducts with the *N*-acetyl-L-amino acids in the presence of *N*,*N*′-dicyclohexylcarbodimide and 3-hydroxy benzotriazole yielded the targeted products.

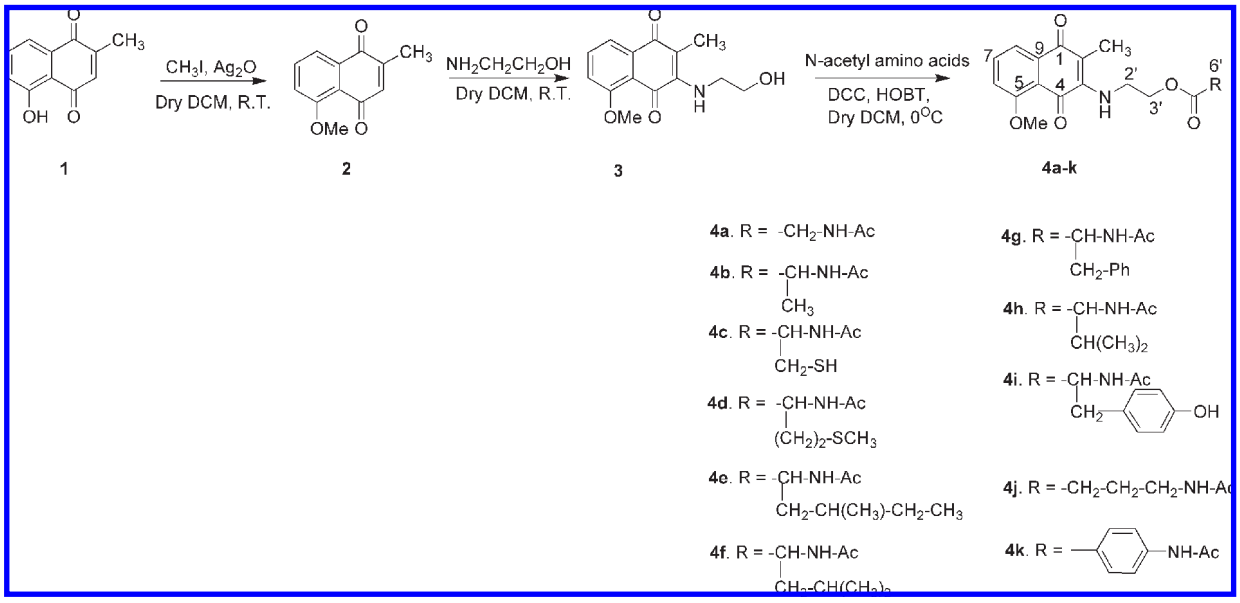

Figure 1. Synthesis of plumbagin derivatives.

Table 1. Antifeedant Effect of Plumbagin and Their Derivatives **4a**−**k** against *Achaea janata* and *Spodoptera litura* by the Leaf Disc Method

	LC_{50} (95% FL[a]) μg/cm^2	
compound	*A. janata*	*S. litura*
4a	**69.51**(35.02−92.45)	**36.87**(32.33−41.14)
4b	340.63(290.88−509.14)	>500
4c	>500	>500
4d	**37.37**(34.14−40.47)	**48.47**(16.77−77.36)
4e	292.00(256.89−392.71)	>500
4f	>500	466.28(335.42−2260.61)
4g	**51.96**(43.32−58.03)	105.93(94.21−119.48)
4h	104.59(65.71−134.12)	>500
4i	199.45(179.52−229.41)	461(298.04−4105.42)
4j	>500	247.59(210.62−348.87)
4k	268.88(238.17−327.93)	117.25 (79.03−170.46)
plumbagin	252.52(222.81−959.92)	335.52(246.41−701.22)
azadirachtin	21.30(17.70−24.80)	18.89 (16.77- 29.08)

[a] Fiducial limits.

Table 2. Toxicity Effects of Plumbagin and Their Derivatives **4a**−**4k** against *Achaea janata* and *Spodoptera litura* by a Topical Bioassay

	mean (%) toxicity ± SE[a]	
	A. janata	*S. litura*
4a	100.0 ± 0.0[a]	++
4b	++	++
4c	++	100 ± 0.0
4d	100.0 ± 0.0[a]	++
4e	++	++
4f	++	74.2 ± 0.4
4g	23.4 ± 0.4	++
4h	32.6 ± 0.3	84.5 ± 0.2
4i	24.6 ± 0.3	++
4j	++	++
4k	++	63.8 ± 0.2
plumbagin	++	++
azadirachtin	93.6 ± 1.2[a]	84.0 ± 0.5

[a] Percentage mean toxicity ± SE values are for doses tested at 4 μg/ insect for compounds **4a**−**4k** and plumbagin 0.1 μg/insect for azadirachtin. ++: moderately active (less than 20%).

With the 11 derivatives of plumbagin in hand, we next examined their antifeedant activities by using the conventional no-choice disk method. The results are summarized in **Table 1**. It is interesting to note that compared to the parent compound plumbagin, most of the derivatives synthesized displayed higher antifeedancy. Among the test compounds, plumbagin with glycine (**4a**), methionine (**4d**), valine (**4h**), and with the phenylalanine side chain (**4g**) displayed good antifeedancy against *A. janata* and *S. litura*. However, the introduction of the methionine (**4d**) side chain into plumbagin significantly enhanced activity against *S. litura* larvae. It is important to note that the derivatives **4c**, **4f**, and **4j** were totally inactive against *S. litura* larvae (**Table 1**).

In the topical bioassay, (**4a**) and (**4d**) were extremely toxic and produced 100% lethal activity in *A. janata*. In an analogous study, these derivatives did not show mortality against *S. litura*, but the treated larvae experienced prolonged molting and adult deformities with undeveloped wings swollen, abdomen, shortened and partial development of antennae (**Table 2**). Similar results were observed with **4i** and **4f**. The mortality rate in plumbagin derivatives with cysteine (**4c**), valine (**4h**), and the *para*-amino benzoic acid side chain (**4k**) against *S. litura* was high in comparison to that of *A. janata*. Plumbagin derivative **4k** caused abnormalities in the emerging adults by affecting their wing formation. Almost all of the abnormal adults with crumpled wings died shortly without mating and egg laying. The phenylalanine ester (**4g**) showed moderate toxic effects against *A. janata*, and the molting process of the survived larvae was prevented or was not carried out to completion. The defects intensified with the sequence of molts into the later instars. Insects went through normal apolysis but failed to complete ecdysis, resulting in larval pupal intermediates with exuvia adhering to the head capsule. The retention of the cuticular skin disrupts the excretory and locomotory functions and leads to death. It is possible that the compound acts as a chitin synthesis inhibitor, which needs verification. This indicates that the treatment of this derivative resulted in irreversible damage to physiological processes essential to the development of *A. janata*. Overall, these results imply that the mode of perception as well as the structure−activity relationship of the plumbagin analogues differs considerably between the insect species examined in this study. However, some of these compounds were highly promising as toxicants as well as antifeedants against the two prominent agricultural pests, and

future evaluations of the susceptibility of other pest species is likely to yield better pest management compounds.

It is intriguing that, though the test insects belong to the same order, i.e, Lepidoptera, the biological activity of the derivatives differed. The reason perhaps is the genetic variability of each genera. Both insects belong to different genera as well as different feeding habitats. *S. litura* is a polyphagous pest that can feed on any plant, and *A. janata* being monophagous has restricted feeding on a limited species of plants.

It is interesting to note that plumbagin was neither effective as a toxicant nor produced any growth inhibition in both test insects at the dosage employed. It is possible that the activity of the compound differed with the type of insect or that the dosage employed was too low to obtain visible effects. However, a dosage of 0.1% of plumbagin employed in the present investigation produced a moderate percentage of antifeedant activity against the third instar larvae of *S. litura* (26%) and *A. janata* (35.69%).

In conclusion, we have observed that the introduction of an amino acid side chain to the naphthaquinone moiety causes a significant increase in antifeedant activity. Esters of aliphatic amino acids were found to be more effective than those of aromatic amino acids. Analogues with sulfur containing amino acid side chains were also good antifeedants. On the basis of the results observed, glycine could be identified as the best possible side chain among the amino acids screened against the pests *S. litura* and *A. janata*. The work has been carried out only with neutral amino acids; further work in this regard with acidic and basic amino acids will throw more light on the efficacy of these compounds as potential antifeedants.

LITERATURE CITED

(1) Didry, N.; Dubrevil, L.; Pinkas, M. Activity of anthraquinonic and naphthoquinonic compounds on oral bacteria. *Die Pharmazie* **1994**, *49*, 681–683.

(2) Nguyen, A. T.; Malonne, H.; Duez, P.; Vanhaelen-Fastre, R.; Vanhaelen, M.; Fontaine, J. Cytotoxic constituents from *Plumbago zeylanica*. *Fitoterapia* **2004**, *75*, 500–504.

(3) Likhitwitayawuid, K.; Kaewamatawong, R.; Ruangrungsi, N.; Krungkrai, J. Antimalarial naphthaquinones from *Nepenthes thorelii*. *Planta Med*. **1998**, *64*, 237–241.

(4) Kayser, O.; Kiderlen, A. F.; Laatsch, H.; Croft, S. L. In vitro leishmanicidal activity of monomeric and dimeric naphthoquinones. *Acta Tropica* **2000**, *76*, 131–138.

(5) Kubo, I.; Uchida, M.; Klocke, J. A. An insect ecdysis inhibitor from African medicinalplant, *Plumbago capensis* (Plumbaginaceae) a naturally occurring chitin synthetase inhibitor. *Agric. Biol. Chem*. **1983**, *47*, 911–913.

(6) Tokunga, T.; Takada, N.; Ueda, M. Mechanism of antifeedant activity of plumbagin. a compound concerning the chemical defense in carnivorous plant. *Tetrahedron. Lett*. **2004**, *45*, 7115–7119.

(7) Tokunaga, T.; Dohmura, A.; Takada, N.; Ueda, M. Cytotoxic Antifeedant from *Dionaea muscipula* Ellis: a defensive mechanism of carnivorous plants against predators. *Bull. Chem. Soc. Jpn*. **2004**, *77*, 537–541.

(8) Babu, K. S.; Haribabu, T.; Srinivas, P. V.; Kishore, K. H.; Murthy, U. S. N.; Rao, J. M. Synthesis and biological evaluation of novel C (7) modified chrysin analogues as antibacterial agents. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 221–224.

(9) Haribabu, T.; Rao, V. R. S.; Tiwari, A. K.; Babu, K. S.; Srinivas, P. V.; Rao, J. M. Synthesis and Biological evaluation of novel 8-amino methylated oroxylin A analogues as α-glucosidase inhibitors. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 1659–1662.

(10) Nadkarni, K. M. In *Indian Materia Medica*; Popular Prakashan: Kolkata, India, 1994; Vol II, p 334.

(11) Dinda, B.; Hajra, A. K.; Das, S. K. Chemical constituents of *Plumbago indica* roots. *Indian J. Chem*. **1998**, *37B*, 672–675.

(12) Akhtar, Y.; Isman, M. B. Feeding responses of specialist herbivores to plant extracts and pure allelochemicals: effects of prolonged exposure. *Entomol. Exp. Appl*. **2004**, *111*, 201–208.

(13) Isman, M. B.; Koul, O.; Luczynski, A.; Kaminski, A. Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. *J. Agric. Food Chem*. **1990**, *38*, 1406–1411.

(14) Jamil, K.; Usha Rani, P.; Tyagarajan, G. Water hyacinth a potential new juvenile hormone mimic. *Int. Pest Cont*. **1984**, *26*, 106–108.

(15) Finney D. J. In *Probit Analysis*; Cambridge University Press: London, 1971; pp 68−72.

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