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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and insect antifeedant activity of plumbagin derivatives

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Abstract Napthaquinones have received considerable interest in agricultural chemistry because of a novel action mode, extremely high activity against a broad spectrum of insects, low acute toxicity to mammals, and environmentally benign characteristics. A series of plumbagin derivatives (**3a–3o**) were synthesized under mild esterification conditions in straightforward procedure. The structures of all new compounds were confirmed by NMR, IR, MS, and HREIMS analyses. The plumbagin derivatives were screened for their insecticidal activities against tobacco caterpillar (*Spodoptera litura*) and castor semilooper (*Achaea janata*) using a no-choice laboratory bioassay. The results show that some of the title compounds exhibit excellent antifeedant activities against 3rd instar larvae of *A. janata* and *S. litura*. The improvement in antifeedant activity requires a reasonable combination of substituents in the parent structure, which provides some hints for further investigation on structure modification.

Keywords Plumbagin · Amines · *Spodoptera litura* · *Achaea janata* · Insect antifeedant activity

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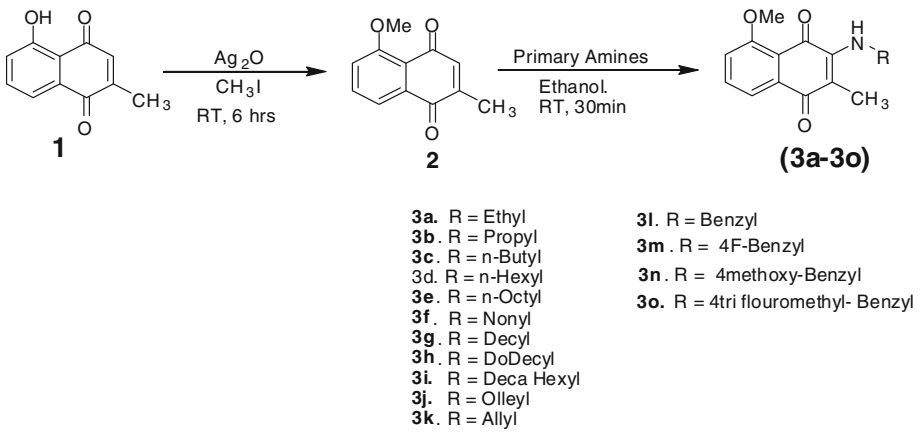
Introduction

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 a part of their defence mechanism against insects. Among these classes, naphthaquinones, in particular, 5-hydroxy-1,4-naphthaquinones such as plumbagin and juglone, are important class of natural products that are extremely useful for the development of potent agrochemicals in view of their high abundance and relatively non toxic nature. A literature precedence revealed that plumbagin isolated from the *Plumbago zeylanica* possesses significant biological activities such as antimicrobial (Didry *et al.*, 1994), cytotoxic (Nguyen *et al.*, 2004), antimalarial (Likhitwitayawuid *et al.*, 1998), antifilaricidal (Mathew *et al.*, 2002), and antiprotozoal properties (Kayser *et al.*, 2000). The naphthaquinone skeletons with high abundance and favorable structural features form the useful models for the development of the potent antifeedants. Previously, it was illustrated that the ingestion of the crude aqueous methanol extracts of *Plumbago zeylanica* and its derivatives caused failure of moulting cycle in another lepidopteran insect, *Bombyx mori* (silk worm) (Kubo *et al.*, 1983).

As part of the ongoing program in the field of insecticidal drugs (Sreelatha *et al.*, 2010), the authors recently reported a series of plumbagin derivative, with an amino acid moiety as a new class of insecticidal agents (Sreelatha *et al.*, 2009). Encouraged by these reports, the authors developed an idea that the introduction of an amine by substituting the hydrogen 3rd position could improve biological properties and decrease resistance. Therefore, in a

Scheme 1 Synthesis of plumbagin derivatives



search for new insecticides with low environmental impact and improved profiles, a series of novel plumbagin derivatives containing long chain and aromatic amines were designed and synthesized (Scheme 1). In the present investigation, quinine moiety was kept unchanged because it is the characteristic structural requirement for activity. In this article, the authors describe the synthesis and evaluation of insect antifeedant activity of plumbagin derivatives in the continuation of the search for new potent insecticidal agents for *Achaea janata* and *Spodoptera litura*.

Results and discussion

The reaction sequence employed for the synthesis of the title compounds is shown in Scheme 1. Initially, plumbagin was converted to its methyl ether **2** in 85% yield through reaction with MeI in the presence of Ag_2O in dry dichloromethane. Michael addition of 5-*O*-methyl plumbagin with various amines at room temperature using ethanol as solvent afforded the targeted products. It should be noted that previous studies have yielded a limited series of plumbagin derivatives with amino acid moiety as the core structure. In the current studies, it was decided to examine the importance of the aliphatic amines as well as the aromatic amines substitution pattern of at 3rd position in plumbagin. Thus, all the 15 derivatives of plumbagin were examined for their antifeedant activities against *A. janata* and *S. litura* using the conventional no-choice disk method. The results of the bioassays are given in Table 1, which indicate that one of the title compounds **3a–3o** exhibited moderate antifeedant activity against the test insects, *A. janata* and *S. litura*, when compared to the plumbagin and displayed 10–500 times less potency than the standard, azadirachtin. The title compounds **3k** and **3o** have shown potent antifeedant activity against *A. janata*. Similarly, compounds **3f**, **3g**, **3i**, and **3k** displayed higher antifeedancy than the plumbagin against *S. litura* larvae. It

Table 1 Antifeedant effect of plumbagin and their derivatives **3a–3o** against *Achaea janata* and *Spodoptera litura* by leaf disk method

Compound	ED_{50} (95% FL[a]), μg/cm^2	
	A. janata	*S. litura*
3a	24.41 (21.05–27.78)	>500
3b	>500	>500
3c	>500	64.08 (59.71–69.65)
3d	73.31 (59.29–104.54)	278.34 (165.39–1209.07)
3e	44.76 (35.59–63.39)	>500
3f	24.04 (22.95–25.08)	32.50 (28.46–68.42)
3g	30.25 (24.61–36.82)	31.109 (26.69–236.620)
3h	59.62 (50.81–71.02)	>500
3i	64.23 (48.86–61.00)	16.77 (5.32–29.37)
3j	26.49 (22.65–31.68)	180.36 (121.64–860.72)
3k	13.51 (7.300–18.19)	31.22 (18.68–79.42)
3l	>500	>500
3m	51.091 (42.73–62.86)	302.32 (174.78–1509.03)
3n	>500	>500
3o	21.82 (19.43–23.58)	>500
Plumbagin	23.97 (12.33–34.12)	43.71 (28.42–72.36)
Azadirachtin	0.72 (0.64–0.79)	0.89 (0.54–1.39)

[a] Fiducial limits, ED_{50} (concentrations causing 50% AFI)

was noticed that in the series of aliphatic amine derivatives, compound with decahexyl chain displayed potent activity against *S. litura*, and replacing the aliphatic chain with the aromatic amines (compounds **3k–3o**) resulted in a drastic loss of antifeedant activity against both insect species, i.e., *A. janata* and *S. litura*. Overall, these results imply that the mode of perception as well as the structure–activity relationship of the plumbagin analogues differs considerably among the insect species examined in this study. However, some of these compounds were highly promising as antifeedants against the two prominent agricultural pests, and the future evaluation of the other pest species susceptibility 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 limited species of plants.

Conclusion

In conclusion, it was observed that the introduction of the aliphatic and aromatic amines to the naphthaquinone moiety causes a significant increase in the antifeedant activity. Aliphatic amines were found to be more effective than those of aromatic amines. On the basis of the results observed, hexadecyl amine could be identified as the best possible side chain among the amines screened to the pests, *S. litura* and *A. janata*. This study has been carried out only with simple amines; further study in this regard with various substituted amines will throw more light on the efficacy of these compounds as potential antifeedants.

Experimental

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 Agilent LC/MSD trap SL 1100 series with a 70 eV (ESI probe) and the infrared spectra on a Thermo Nicolet Nexus 670 FTIR spectrometer. Melting points were measured on a Fischer Scientific melting point apparatus and were 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 of Merck. The optical rotations were measured on Jasco Dip 360 digital Polarimeter.

Extraction and isolation of plumbagin

The roots of *Plumbago zeylanica* also known as *Nila chitramula* (Isman *et al.*, 1990) were collected during the 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 zeylanica* were powdered in a pulvarizer (10 kg) and extracted with chloroform/methanol, 1:1 followed by the concentration under reduced 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 6 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 (Dinda *et al.*, 1998).

General procedure for the synthesis of compound (**3a–3o**)

To a solution of 5-*O*-methyl plumbagin (**2**) (1 mmol) in ethanol (10 ml) was added dropwise primary amines (1 mmol) at room temperature. The resulting red-colored solution was stirred for 30 min 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 *n*-hexane/ethyl acetate, 85:15, to yield the Michael adduct (**3a–3o**) as red color powder with the yields of 75–90%.

3-(Ethyl amino)-5-methoxy-2-methylnaphthalene-1,4-dione (**3a**): red color liquid. IR(KBr)ν_{max}: 3435 (NH), 2966, 2925, 1664 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (t, 3H, $J = 6.9$ Hz, 7.1 Hz, 3′-CH_3), 1.50–1.70 (brs, NH, 1H), 2.21 (s, 3H, 2-CH_3), 3.59 (q, 2H, $J = 7.1$ Hz, H-2′), 3.99 (s, 3H, 5-OCH_3), 7.14 (d, 1H, $J = 8.5$ Hz, H-8), 7.61 (t, 1H, $J = 7.9$ Hz, 8.1 Hz, H-6), 7.78 (d, 1H, $J = 7.5$ Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.88, 16.10, 40.22, 56.34, 110.10, 115.51, 118.02, 119.08, 135.49, 135.96, 147.13, 159.55, 181.16, 183.19. HR-ESIMS Calcd. for $C_{14}H_{15}NO_3$ (M + H): 246.1125. Found: 246.1132.

5-Methoxy-2-methyl-3-(propylamino)naphthalene-1,4-dione (**3b**): mp: 60°C. IR(KBr)ν_{max}: 3439, 2924, 2358, 1658 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 (t, 3H, $J = 7.3$ Hz, 4′-CH_3), 1.26 (t, 2H, $J = 7.1$ Hz, H-3′), 1.70–1.75 (brs, NH, 1H), 2.20 (s, 3H, 2-CH_3), 3.51 (t, 2H, $J = 6.9$ Hz, 7.1 Hz, H-2′), 3.99 (s, 3H, 5-OCH_3), 7.14 (d, 1H, $J = 8.3$ Hz, H-8), 7.62 (t, 1H, $J = 7.9$ Hz, 8.3 Hz, H-6), 7.78 (d, 1H, $J = 7.5$ Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.99, 14.41, 24.05, 47.14, 56.33, 109.93, 115.45, 117.52, 119.07, 135.00, 135.52, 147.13, 159.53, 181.12, 183.14. HR-ESIMS Calcd. for $C_{15}H_{18}NO_3(M^+ + H)$: 260.1281. Found: 260.1267.

3-(Butylamino)-5-methoxy-2-methylnaphthalene-1,4-dione (**3c**): mp: 63°C. IR(KBr)ν_{max}: 3467, 2922, 1648 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H, $J = 7.1$ Hz, 7.3 Hz, 5′-CH_3), 1.36–1.48 (m, 2H, H-4′), 1.56–1.65 (m, 2H, H-3′), 1.71–1.73 (brs, NH, 1H), 2.21 (s, 3H, 2-CH_3), 3.54 (t, 2H, $J = 6.7$ Hz, 6.9 Hz, H-2′), 3.99 (s, 3H, 5-OCH_3), 7.14 (d, 1H, $J = 8.4$ Hz, H-8), 7.62 (t, 1H, $J = 7.7$ Hz, 8.3 Hz, H-6), 7.78 (d, 1H, $J = 7.7$ Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.99, 13.71, 19.80, 32.84, 45.14, 56.34, 109.95, 115.48, 117.55, 119.08, 135.51, 136.00, 147.18, 159.56, 181.14, 183.15. HR-ESIMS Calcd. for $C_{16}H_{20}NO_3(M^+ + H)$: 274.1438. Found: 274.1433.

3-(Hexylamino)-5-methoxy-2-methylnaphthalene-1,4-dione (**3d**): mp: 58°C. IR(KBr)ν_{max}: 3446, 2926, 2359, 1663 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, 3H, $J = 6.0$ Hz, 6.9 Hz, 7′-CH_3), 1.25–1.45 (m, 6H, H-4′–6′), 1.56–1.66 (m, 2H, H-3′), 1.70–1.73 (brs, NH, 1H), 2.21 (s, 3H, 2-CH_3), 3.53 (t, 2H, $J = 6.9$ Hz, H-2′), 3.99 (s, 3H, 5-OCH_3), 7.14 (d, 1H, $J = 8.1$ Hz, H-8), 7.62 (t, 1H, $J = 7.9$ Hz, 8.3 Hz, H-6), 7.78 (d, 1H, 7.5 Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.97, 13.96, 22.51, 26.31, 30.77, 31.43, 45.43, 56.33, 109.83, 115.45, 117.54, 119.05, 135.00, 135.51, 147.13, 159.53, 181.12, 183.13. HR-ESIMS Calcd. for $C_{18}H_{24}NO_3$ ($M^+ + H$): 302.1752. Found: 302.1751.

5-Methoxy-2-methyl-3-(octylamino)naphthalene-1,4-dione (**3e**): red color liquid. IR(KBr)ν_{max}: 3455, 1640 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 6.0$ Hz, 6.9 Hz, 9′-CH_3), 1.23–1.34 (m, 10H, H-4′–8′), 1.56–1.62 (m, 2H, H-3′), 2.20 (s, 3H, 2-CH_3), 3.53 (q, 2H, $J = 6.7$ Hz, H-2′), 3.99 (s, 3H, 5-OCH_3), 5.87–5.95 (brs, NH, 1H), 7.14 (d, 1H, $J = 8.3$ Hz, H-8), 7.62 (t, 1H, $J = 7.9$ Hz, 8.3 Hz, H-6), 7.78 (d, 1H, $J = 7.5$ Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.99, 14.05, 22.61, 26.64, 29.15, 29.23, 30.83, 31.74, 45.46, 56.35, 109.90, 115.48, 118.03, 119.09, 135.51, 136.02, 147.17, 159.56, 181.15, 183.15. HR-ESIMS Calcd. for $C_{20}H_{28}NO_3$ ($M^+ + H$): 330.2064. Found: 330.2077.

5-Methoxy-2-methyl-3-(nonylamino)naphthalene-1,4-dione (**3f**): mp: 78°C. IR(KBr)ν_{max}: 3458, 2923, 2856, 1663 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 6.2$ Hz, 6.7 Hz, 10′-CH_3), 1.20–1.44 (m, 12H, H-4′ and 9′), 1.55–1.67 (m, 2H, H-3′), 2.21 (s, 3H, 2-CH_3), 3.53 (q, 2H, $J = 6.6$ Hz, H-2′), 3.99 (s, 3H, 5-OCH_3), 5.87–5.95 (brs, NH, 1H), 7.14 (d, 1H, $J = 8.3$ Hz, H-8), 7.62 (t, 1H, $J = 7.9$ Hz, 8.1 Hz, H-6), 7.78 (d, 1H, $J = 7.5$ Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.94, 14.04, 22.58, 26.59, 29.15, 29.23, 29.39, 30.76, 31.76, 45.38, 56.27, 109.75, 115.38, 117.85, 119.00, 135.46, 135.91, 147.05, 159.46, 181.04, 183.04. HR-ESIMS Calcd. for $C_{21}H_{29}NO_3(M^+ + H)$: 344.2220. Found: 344.2226.

3-(Decylamino)-5-methoxy-2-methylnaphthalene-1,4-dione (**3g**): mp: 78°C. IR(KBr)ν_{max}: 3451, 2923, 2853, 1663 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 6.2$ Hz, 6.7 Hz, 11′-CH_3), 1.18–1.45 (m, 14H, H-4′–10′), 1.55–1.69 (m, 2H, H-3′), 2.20 (s, 3H, 2-CH_3), 3.53 (q, 2H, $J = 6.7$ Hz, H-2′), 4.00 (s, 3H, 5-OCH_3), 5.87–5.95 (brs, NH, 1H), 7.14 (d, 1H, $J = 8.4$ Hz, H-8), 7.62 (t, 1H, $J = 7.9$ Hz, 8.1 Hz, H-6), 7.78 (d, 1H, 7.5 Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.00, 14.10, 22.65, 26.64, 29.26, 29.48, 30.82, 31.84, 45.44, 56.34, 109.84, 115.44, 117.93, 119.08, 135.52, 135.99, 147.13, 159.53, 181.13, 183.13. HR-ESIMS Calcd. for $C_{22}H_{32}NO_3$ ($M^+ + H$): 358.2377. Found: 358.2370.

3-(Dodecylamino)-5-methoxy-2-methylnaphthalene-1,4-dione (**3h**): mp: 82°C. IR(KBr)ν_{max}: 3451, 2921, 2853, 1662 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 5.8$ Hz, 6.9 Hz, 12′-CH_3), 1.20–1.36 (m, 18H, H-4′–11′), 1.55–1.66 (m, 2H, H-3′), 2.20 (s, 3H, 2-CH_3), 3.53 (q, 2H, $J = 6.6$ Hz, H-2′), 3.99 (s, 3H, 5-OCH_3), 5.88–5.95 (brs, NH, 1H), 7.14 (d, 1H, $J = 8.3$ Hz, H-8), 7.62 (t, 1H, $J = 7.9$ Hz, 8.3 Hz, H-6), 7.78 (d, 1H, 7.5 Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.98, 14.10, 22.65, 26.62, 29.26, 29.30, 29.47, 29.52, 29.58, 30.80, 31.87, 45.42, 56.32, 109.80, 115.42, 117.91, 119.05, 135.51, 135.96, 147.11, 159.51, 181.10, 183.10. HR-ESIMS Calcd. for $C_{24}H_{36}NO_3(M^+ + H)$: 386.2690. Found: 386.2697.

3-(Hexadecylamino)-5-methoxy-2-methylnaphthalene-1,4-dione (**3i**): mp: 84°C. IR(KBr)ν_{max}: 3430, 2921, 2851, 1662 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 6.0$ Hz, 6.9 Hz, 17′-CH_3), 1.21–1.39 (m, 26H, H-4′–16′), 1.53–1.62 (m, 2H, H-3′), 2.20 (s, 3H, 2-CH_3), 3.53 (t, 2H, $J = 6.9$ Hz, H-2′), 3.99 (s, 3H, 5-OCH_3), 5.85–5.98 (brs, NH, 1H), 7.14 (d, 1H, $J = 8.4$ Hz, H-8), 7.62 (t, 1H, $J = 7.7$ Hz, 8.3 Hz, H-6), 7.78 (d, 1H, 7.5 Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.00, 14.11, 22.68, 26.66, 29.29, 29.35, 29.49, 29.55, 29.66, 30.84, 31.92, 45.47, 56.36, 109.91, 115.49, 118.01, 119.10, 135.52, 136.03, 147.17, 159.57, 181.16, 183.16. HR-ESIMS Calcd. for $C_{28}H_{44}NO_3$ ($M^+ + H$): 442.3316. Found: 442.3317.

(E)-5-Methoxy-2-methyl-3-(octadec-9-enylamino)naphthalene-1,4-dione (**3j**): red color liquid. IR(KBr)ν_{max}: 3460, 1643 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 6.2$ Hz, 6.7 Hz, 19′-CH_3), 1.22–1.36 (m, 22H, H-4′–8′, H-13′–18′), 1.55–1.65 (m, 3H, H-3′,12′), 1.94–2.04 (m, 3H, H-9′,12′), 2.21 (s, 3H, 2-CH_3), 3.53 (q, 2H, $J = 6.7$ Hz, H-2′), 4.00 (s, 3H, 5-OCH_3), 5.31–5.39 (m, 2H, H-10′,11′), 5.87–5.95 (brs, NH, 1H), 7.14 (d, 1H, $J = 8.3$ Hz, H-8), 7.62 (t, 1H, $J = 8.1$ Hz, H-6), 7.78 (d, 1H, 7.7 Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.99, 14.09, 22.67, 26.65, 27.16, 27.20, 29.18, 29.30, 29.38, 29.50, 29.69, 29.75, 30.83, 31.89, 45.46, 56.35, 109.90, 115.48, 117.99, 119.10, 129.74, 129.98, 135.52, 136.02, 147.16, 159.57, 181.15, 183.15. HR-ESIMS Calcd. for $C_{30}H_{46}NO_3$ ($M^+ + H$): 468.3472. Found: 468.3492.

3-(Allylamino)-5-methoxy-2-methylnaphthalene-1,4-dione (**3k**): mp: 93°C. IR(KBr)ν_{max}: 3454, 2924, 1615, 1577 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.18 (s, 3H, 2-CH_3), 3.99 (s, 3H, 5-OCH_3), 4.11–4.18 (m, 2H, H-2′), 5.22–5.30 (m, 2H,

H-4′), 5.86–5.90 (m, 1H, H-3′), 6.00–6.07 (brs, NH, 1H), 7.14 (d, 1H, $J = 8.3$ Hz, H-8), 7.61 (t, 1H, $J = 7.9$ Hz, 8.3 Hz, H-6), 7.76 (d, 1H, $J = 7.5$ Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.65, 47.21, 56.24, 110.57, 115.55, 116.64, 117.81, 118.96, 134.61, 135.42, 135.65, 146.85, 159.46, 180.80, 183.15. HR-ESIMS Calcd. for $C_{15}H_{16}NO_3$ ($M^+ + H$): 258.1125. Found: 258.1108.

3-(Benzylamino)-5-methoxy-2-methylnaphthalene-1,4-dione (**3l**): mp: 92°C. IR(KBr)ν_{max}: 3432, 2924, 2857, 2306, 1661 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.20 (s, 3H, 2-CH_3), 4.00 (s, 3H, 5-OCH_3), 4.73 (d, 2H, $J = 5.4$ Hz, H-2′), 6.17–6.23 (brs, NH, 1H), 7.16 (d, 1H, $J = 8.4$ Hz, H-8), 7.26–7.36 (m, 5H, H-1″, Ph), 7.63 (t, 1H, $J = 7.9$ Hz, 8.1 Hz, H-6), 7.79 (d, 1H, $J = 7.5$ Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.99, 49.33, 56.35, 110.96, 115.65, 119.11, 127.04, 127.72, 128.57, 128.76, 128.90, 135.56, 135.77, 146.97, 159.59, 180.97, 183.92. HR-ESIMS Calcd. for $C_{19}H_{18}NO_3$($M^+ + H$): 308.1281. Found: 308.1287.

3-(4-Fluorobenzylamino)-5-methoxy-2-methylnaphthalene-1,4-dione (**3m**): mp: 100°C. IR(KBr)ν_{max}: 3432.0, 2923.9, 2370.9, 1664 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.19 (s, 3H, 2-CH_3), 4.00 (s, 3H, 5-OCH_3), 4.69 (s, 2H, H-2′), 6.07–6.17 (brs, NH, 1H), 7.01–7.08 (m, 2H, H-2″, H-6″), 7.17 (d, 1H, $J = 8.3$ Hz, H-8), 7.24–7.29 (m, 2H, H-3″, H-5″), 7.64 (t, 1H, $J = 7.7$ Hz, 8.3 Hz, H-6), 7.78 (d, 1H, $J = 7.5$ Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.02, 48.72, 56.38, 111.32, 115.68, 115.76, 115.97, 117.98, 119.17, 128.74, 128.85, 135.61, 135.74, 146.87, 159.65, 180.94, 183.41. HR-ESIMS Calcd. for $C_{19}H_{16}FNO_4$ ($M^+ + H$): 326.1207. Found: 326.1247.

5-Methoxy-3-(4-methoxybenzylamino)-2-methylnaphthalene-1,4-dione (**3n**): red color liquid. IR(KBr)ν_{max}: 3424, 2924, 2858, 1630 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.21 (s, 3H, 2-CH_3), 3.81 (s, 3H, 4″-OCH_3), 3.99 (s, 3H, 5-OCH_3), 4.66 (s, 2H, H-2′), 6.03–6.19 (brs, NH, 1H), 6.88 (d, 2H, $J = 8.6$ Hz, H-2″, H-6″), 7.15 (d, 1H, $J = 8.3$ Hz, H-8), 7.21 (d, 2H, $J = 8.4$ Hz, H-3″, H-5″), 7.62 (t, 1H, $J = 7.7$ Hz, 8.3 Hz, H-6), 7.78 (d, 1H, $J = 7.5$ Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.04, 48.96, 55.27, 56.33, 110.81, 114.26, 115.89, 115.61, 119.08, 123.93, 124.42, 128.47, 135.53, 147.01, 159.14, 159.55, 180.97, 183.33. HR-ESIMS Calcd. for $C_{20}H_{20}NO_4$($M^+ + H$): 338.1387. Found: 338.1389.

5-Methoxy-2-methyl-3-(4-(trifluoromethyl)benzylamino) naphthalene-1,4-dione (**3o**): mp: 104°C. IR(KBr)ν_{max}: 3426, 2929, 2299, 1663 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.15 (s, 3H, 2-CH_3), 4.01 (s, 3H, 5-OCH_3), 4.79 (s, 2H, $J = 6.4$ Hz, H-2′), 6.22–6.30 (brs, NH, 1H), 7.18 (d, 1H, $J = 8.3$ Hz, H-8), 7.41 (d, 2H, $J = 7.7$ Hz, H-2″, H-6″), 7.59–7.66 (m, 3H, H-6, H-3″, H-5″), 7.79 (d, 1H, $J = 7.5$ Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.93, 48.67, 56.39, 111.67, 115.84, 119.19, 125.85, 125.90, 127.12, 127.48, 128.05, 135.59, 135.69, 142.92, 146.69, 159.67, 180.85, 183.44. HR-ESIMS Calcd. for $C_{20}H_{17}F_3NO_3$ ($M^+ + H$): 376.1155. Found: 376.1164.

Bioassay

Antifeedant activity of the compounds was assessed on the larvae of tobacco caterpillar (*S. litura*) and castor semilooper (*A. janata*). The experiments were conducted according to the classical no-choice leaf-disk bioassay described earlier by Akhtar and Isman (2004). The castor semilooper, *A. janata* (L.) and the tobacco cutworm, *S. litura* (Fab.) were reared on fresh castor leaves (*Ricinus communis* (L.)) grown in the laboratory at 28 ± 2°C, relative humidity 65 ± 5, and 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 disks 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 disks were treated with the same volume of the solvent only.

In each Petri dish, prestarved healthy 3rd instar larvae of *A. janata* and *S. litura* were introduced for assessing their feeding 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 disks consumed were measured after 6 h using AM-300 leaf area meter (ADC, Bioscientific Limited, England, UK). The antifeedant index was then calculated as $(C - T)/(C + T) \times 100$, where C is consumption of control disks, and T is consumption of treated disks (Isman *et al.*, 1990). For each concentration, 10 experimental sets were assayed. Each test was replicated three times. The mean of the 10 sets were taken for each compound, and the percentage of antifeedant activity with standard deviation was calculated.

Data analysis

Antifeedant indices were calculated using five different concentrations of each compound, and data were subjected to probit analysis (Finney, 1971) to determine ED_{50} representing the concentrations that caused 50% feeding deterrence along with the 95% fiducial limits.

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