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Tetrahydrobenzotriazines as a new class of nematocide

Keiji Nishiwaki,[a,*] Azusa Okamoto,[a] Keizo Matsuo,[a,*] Yoshio Hayase,[b] Shunichiro Masaki,[b] Riichi Hasegawa[b] and Katsuaki Ohba[b]

[a]*Department of Pharmaceutical Sciences, School of Pharmacy, Kinki University, 3-4-1 Kowakae Higashiosaka, Osaka 577-8502, Japan*
[b]*Aburahi Laboratories, Shionogi and Co., Ltd, 1405 Gotanda, Koka-cho, Koka, Shiga 520-3423, Japan*

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Abstract—Tetrahydrobenzotriazines, a novel class of heterocyclic compounds, were synthesized. Examination of their biological activities resulted in the discovery that some of them possess potent nematocidal activity.

1. Introduction

We have recently reported that triazene compounds, possessing alkyl groups on the 2 and 6 positions of the benzene nuclei, produce tetrahydrobenzotriazines upon treatment with base.[1] The tetrahydrobenzotriazine has a novel framework that possesses a dearomatized six-membered ring and three successive nitrogen atoms. Because of this novel structure, there is interest in investigating biological activities of these compounds. We focused our attention on a potential nematocidal activity of the tetrahydrobenzotriazines.

Nematoda is the most ubiquitous species on the earth. In agricultural areas, soil nematodes are a serious problem at present. Although various pesticides have been used to eliminate the nematode, most of those that have been attempted are halogenated hydrocarbons including bromomethane, D–D (1,3-dichloropropene–1,2-dichloropropane), EDB (1,2-dibromoethane), etc. However, The Montreal protocol on substances that deplete the ozone layer requires developed countries to phase out bromomethane production and its nonquarantine use by 2005 and that developing countries do the same by 2015. For this reason, alternatives to bromomethane are currently being extensively developed.[2] Furthermore, nematode illnesses, for which there are no effective medical treatments, are found worldwide.[3] Thus, there is much impetus for the development of alternative nematocides. Therefore, we examined the nematocidal activity of the synthesized tetrahydrobenzotriazine compounds against the free-living nematode, *Caenorhabditis elegans*, which has become a popular model system for screening nematocidal activity since it is easy to maintain and has a rapid life-cycle.[4]

2. Results and discussion

We synthesized tetrahydrobenzotriazine derivatives possessing amide,[5] urea,[6] and thiourea[7] groups, using various electrophiles in the final step of the reaction, as we have previously reported.[1] Briefly, 2,6-xylidine was diazotized by sodium nitrite–hydrochloric acid to form the diazonium salt, which was trapped by dimethylamine in basic medium to give 1-(2,6-dimethylphenyl)-3,3-dimethyltriazene in an 85% yield, as previously described[8] (Scheme 1). The triazene was treated with *n*-butyllithium at 0 °C to generate the dearomatized anion.[1] Subsequently, electrophiles were added to produce the corresponding tetrahydrobenzotriazines (**1**–**10**) (Scheme 2). In preparing the acetamide derivative **1**, we used acetyl chloride as the electrophile at first, but the desire product **1** was not obtained. Therefore, we employed acetic anhydride instead of acetyl chloride to produce **1** in a 34% yield. We presumed that the hydrochloride or chloride ion decomposed the intermediate anion or the product. Consequently, acid anhydrides were used to

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* Corresponding authors. Tel.: +81 6 6721 2332; fax: +81 6 6730 1394; e-mail: k-nishi@phar.kindai.ac.jp

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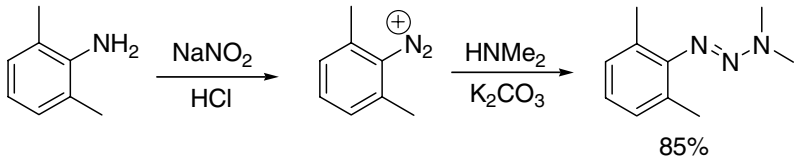
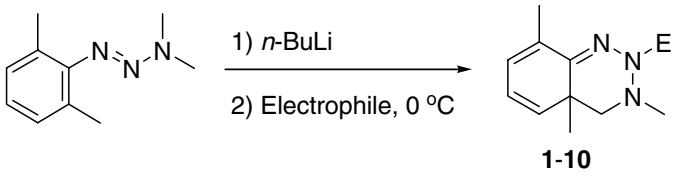


Scheme 1. Synthesis of triazene.



Scheme 2. Synthesis of tetrahydrobenzotriazine derivatives.

synthesize the amide derivatives. When octanoic anhydride was employed as the electrophile, **2** was obtained in a 34% yield. Similarly, nonanoic anhydride, decanoic anhydride, undecanoic anhydride, and dodecanoic anhydride were used as the electrophiles to give the amide derivatives (**3**–**6**) in 29%, 28%, 20% and 8% yields, respectively. Increasing the number of carbon in the acid anhydride decreased the yield of the products. These results were affected by the difficulty of extraction of the products from the reaction mixture after quenching of the reactions. When phenyl isocyanate and H_2O and phenyl isocyanate and iodomethane were used as the electrophiles, the urea derivatives (**7** and **8**) were obtained in 76% and 29% yields, respectively. Similarly, the thiourea derivatives (**9** and **10**) were synthesized by using phenyl isothiocyanate and H_2O and phenyl isothiocyanate and iodomethane as the electrophiles in 59% and 67% yields, respectively (Table 1). The yields of many compounds were low, because the reactions had not been fully optimised. In the case of acid anhydride as the electrophile, the yields was lower, as the carbon chains were longer. We attribute these results to the difficulty in post-treatment of the reaction.

Subsequently, evaluation of nematocidal activity of the synthesized compounds (**1**–**10**) was carried out. The nematocidal activity was tested against the third stage larvae of the free-living nematode *C. elegans*. Activity was shown as the revised death rate of the nematodes because they proliferated for test periods.[9] The percentage revised death rate was calculated by the following equation:

Revised death rate (%) = [(living rate in reference area - living rate in treated area)/living rate in reference area] × 100.

The results were summarized in Table 2.

All of the synthesized compounds tested possessed some degree of nematocidal activity. In a series of amide homologues (entries 1–6), the acetyl derivative **1** exhibited the strongest activity (100% of the revised death rate), while the octanoyl derivative **2** exhibited very low activity (10.3%). However, it is interesting that the decanoyl derivative **3**, which possessed the longer acyl chain, showed higher activity (97%), while the overall nematocidal activity of the compounds gradually decreased as the length of the acyl chains increased. Similar structure–activity tendencies were observed independently by Tsuda[5a] and Feldmesser[5e] in *N*-acyl cyclic amines and N-substituted amides, respectively. It is presumed that the hydrophobic/hydrophilic balance of the compounds controls the activity. In the phenylurea and phenylthiourea substituted tetrahydrobenzotriazines (entries 7–10), introduction of a methyl group on the nitrogen atom increased the nematocidal activity (entries 8 and 10). Particularly in the phenylthiourea derivatives, the activity of **10** was 5 times stronger than that of **9**. This may be due to the hydrophobic nature or bulkiness of the methyl group. It is worthwhile to note that **1** and **10**, as well as methyl isothiocyanate (a positive control), killed all the nematodes within 24 hours (entries 1, 10 and 11). These results indicated that these compounds (**1**, **3** and **10**) were potent nematocides.

3. Conclusion

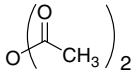
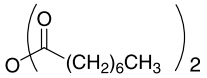
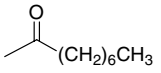
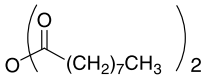
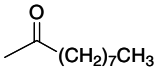
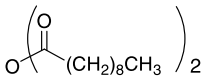
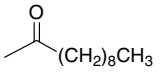
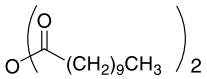
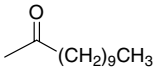
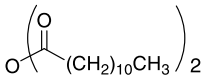
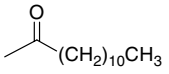
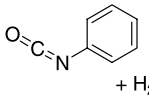
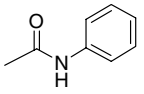
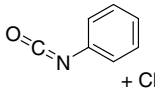
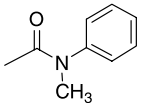
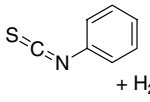
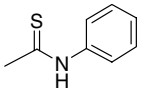
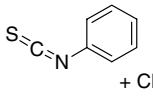
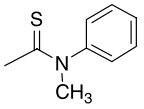
In conclusion, we synthesized new heterocyclic compounds, tetrahydrobenzotriazines, which possess a novel dearomatized framework. We subsequently evaluated their nematocidal activity. Of the compounds we tested, we determined that acetyl substituted (**1**), nonanoyl substituted (**3**) and *N*-methyl phenylthioamide substituted (**10**) tetrahydrobenzotriazines showed high nematocidal activity. We anticipate that these compounds are good candidates to become novel nematocides.

4. Experimental

4.1. General methods

NMR spectra were recorded on JEOL GSX-270 (^{1}H 270 MHz, ^{13}C 67.5 MHz) spectrometer in $CDCl_3$ or C_6D_6 with TMS as an internal standard. Mass spectra (EI) were recorded on a JMS-HX100 spectrometer.

Table 1. Yield of tetrahydrobenzotriazine derivatives (**1**–**10**)

Entry	Electrophiles	Products		Yield (%)
		Compound	E	
1		**1**		34
2		**2**		34
3		**3**		29
4		**4**		28
5		**5**		20
6		**6**		8
7		**7**		76
8		**8**		29
9		**9**		59
10		**10**		67

Infrared spectra were recorded on a Shimadzu IR-435 spectrophotometer. Unless otherwise noted, all chemicals were obtained from commercial suppliers and used without further purification. Tetrahydrofuran (THF) was freshly distilled under nitrogen from sodium benzophenone ketyl prior to use.

4.1.1. General procedure for transformation of triazenes into tetrahydrobenzotriazine derivatives (1–6). To a 1 M solution of 1-(2,6-dimethylphenyl)-3,3-dimethyltriazene in dry THF (2 mL) was added dropwise a solution of *n*-BuLi/hexane (1 equiv) at 0 °C. After stirring for 30 min at the same temperature, the corresponding acid anhydride (1 equiv) was added to the reaction mixture. The reaction was stopped by addition of H_2O, and the reaction mixture was extracted with ether. The combined extracts were washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure left the residue, which was purified on a silica gel flash column chromatography to afford tetrahydrobenzotriazine derivatives (**1**–**6**).

4.1.1.1. 1-(3,4a,8-Trimethyl-4,4a-dihydrobenzo[*d*] [1,2,3]triazin-2(3*H*)-yl)ethanone (1). Brown oil, 34%. ^{1}H NMR (C_6D_6) δ: 5.77 (dt, 1H, J = 7.0, 1.5 Hz), 5.62 (dd, 5H, J = 9.0, 6.0 Hz), 5.26 (m, 1H), 2.85 (d, 1H, J = 6.0 Hz), 2.77(d, 1H, J = 6.0 Hz), 2.52 (s, 3H), 2.26 (s, 3H), 1.94 (s, 3H), 0.92 (s, 3H). ^{13}C NMR (C_6D_6) δ: 175.31, 168.99, 136.96, 130.92, 126.52, 121.45, 61.21, 41.72, 30.08, 21.81, 21.24, 16.54. IR (neat) cm^{-1}: 3450 (s), 2900 (s), 1740 (w), 1670 (m), 1440 (w), 1400 (w), 1370 (w), 1330 (w), 1250 (w), 1030 (w), 1000 (w), 960

Table 2. Revised death rate of nematodes to tetrahydrobenzotriazine derivatives

Entry	Compound	Revised death rate (%)
1	**1**	100
2	**2**	10.3
3	**3**	97.0
4	**4**	64.6
5	**5**	20.3
6	**6**	17.2
7	**7**	43.8
8	**8**	74.2
9	**9**	20.9
10	**10**	100
11	Methyl isothiocyanate	100
12	Untreated	0

(w), 820 (w), 730 (s); MS (*m*/*z*, %): 219 (M^+, 11), 191 (2.6), 177 (13), 162 (53), 133 (36), 120 (23), 118 (22), 105 (100), 77 (21), 43 (35); HRMS (*m*/*z*): 219.1372 (Calcd for $C_{12}H_{17}N_3O$, 219.1372).

4.1.1.2. 1-(3,4a,8-Trimethyl-4,4a-dihydrobenzo[*d*] [1,2, 3]triazin-2(3*H*)-yl)octan-1-one (2). Brown oil, 34%. ^{1}H NMR (C_6D_6) δ: 5.77 (dt, 1H, J = 6.0, 1.5 Hz), 5.63 (dd, 1H, J = 6.0, 1.5 Hz), 5.26 (d, 1H, J = 9.0 Hz), 2.90 (d, 1H, J = 8.0 Hz), 2.80 (m, 1H), 2.56 (br s, 3H), 2.00 (s, 3H), 1.86 (quin, 2H, J = 7.2 Hz), 1.16–1.48 (m, 8H), 0.95 (s, 3H), 0.88 (t, 3H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$) δ: 170.93, 152.97, 136.78, 130.99, 126.29, 121.45, 61.16, 43.99, 41.77, 34.30, 31.69, 31.60, 29.40, 29.01, 25.31, 22.57, 16.57, 14.04. IR (neat) cm^{-1}: 3450 (m), 2900 (s), 1670 (s), 1560 (m), 1450 (w), 1400 (w), 1340 (w), 1160 (w), 1110 (w), 1020 (w), 960 (w), 720 (s); MS (*m*/*z*, %): 303 (M^+, 2.1), 275 (2.3), 262 (2.1), 230 (4.8), 177 (23), 162 (100), 133 (44) 105 (74), 77 (14), 57 (41), 44 (21); HRMS (*m*/*z*): 303.2287 (Calcd for $C_{18}H_{29}N_3O$, 303.2211).

4.1.1.3. 1-(3,4a,8-Trimethyl-4,4a-dihydrobenzo[*d*] [1,2, 3]triazin-2(3*H*)-yl)nonan-1-one (3). Brown oil, 29%. ^{1}H NMR (C_6D_6) δ: 5.77 (m, 1H), 5.63 (m, 1H), 5.26 (d, 1H, J = 9.0 Hz), 2.90 (d, 1H, J = 9.0 Hz), 2.80 (m, 1H), 2.56 (br s, 3H), 2.00 (s, 3H), 1.86 (m, 2H), 1.18–1.48 (m, 10H), 0.96 (s, 3H). ^{13}C NMR ($CDCl_3$) δ: 171.67, 136.78, 130.99, 126.30, 121.46, 61.16, 43.99, 41.78, 34.29, 31.80, 29.44, 29.39, 29.31, 29.15, 25.41, 25.31, 22.63, 16.59, 14.08. IR (neat) cm^{-1}: 3450 (s), 2850 (s), 1740 (w), 1670 (s), 1560 (m), 1440 (w), 1400 (w), 1340 (w), 1230 (m), 1150 (w), 1110 (w), 1030 (w), 960 (w), 920 (w), 870 (w), 720 (s); MS (*m*/*z*, %): 317 (M^+, 2.2), 244 (2.2), 177 (20), 162 (87), 134 (49), 105 (100), 58 (40); HRMS (*m*/*z*): 317.2466 (Calcd for $C_{19}H_{31}N_3O$, 317.2467).

4.1.1.4. 1-(3,4a,8-Trimethyl-4,4a-dihydrobenzo[*d*] [1,2, 3]triazin-2(3*H*)-yl)decan-1-one (4). Brown oil, 28%. ^{1}H NMR (C_6D_6) δ: 5.79 (dt, 1H, J = 6.0, 1.5 Hz), 5.63 (dd, 1H, J = 9.0, 6.0 Hz), 5.26 (d, 1H, J = 9.0 Hz), 2.90 (d, 1H, J = 9.0 Hz), 2.80 (m, 1H), 2.56 (br s, 3H), 2.00 (s, 3H), 1.88 (quin, 2H, J = 7.2 Hz), 1.18–1.48 (m, 14H), 0.98 (s, 3H), 0.90 (t, 3H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$) δ: 171.48, 158.30, 136.63, 130.80, 126.17, 121.34, 60.99, 43.85, 41.63, 34.19, 34.08, 33.94, 31.69, 29.29, 29.19, 29.07, 25.15, 22.48, 16.40, 13.91. IR (neat) cm^{-1}: 3450 (m), 2900 (s), 1670 (s), 1560 (m), 1450 (w), 1400 (w), 1340 (w), 1160 (w), 1110 (w), 1020 (w), 960 (w), 720 (s); MS (*m*/*z*, %): 331 (M^+, 1.9), 303 (6.5), 258 (5.2), 178 (26), 162 (100), 155 (35), 143 (53), 123 (39), 105 (81), 83 (30), 44 (27); HRMS (*m*/*z*): 331.2604 (Calcd for $C_{20}H_{33}N_3O$, 331.2624).

4.1.1.5. 1-(3,4a,8-Trimethyl-4,4a-dihydrobenzo[*d*] [1,2, 3]triazin-2(3*H*)-yl)undecan-1-one (5). Brown oil, 20%. ^{1}H NMR (C_6D_6) δ: 5.77 (dt, 1H, J = 6.0, 1.5 Hz), 5.62 (dd, 1H, J = 9.0, 6.0 Hz), 5.25 (d, 1H, J = 9.0 Hz), 2.89 (d, 1H, J = 9.0 Hz), 2.79 (d, 1H, J = 8.0 Hz), 2.56 (br s, 3H), 1.99 (s, 3H), 1.89 (quin, 2H, J = 7.2 Hz), 1.18–1.48 (m, 16H), 0.95 (s, 3H), 0.89 (t, 3H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$) δ: 171.62, 158.36, 136.80, 136.77, 134.93, 130.95, 126.23, 121.47, 61.11, 43.96, 41.76, 34.29, 31.83, 29.49, 29.46, 29.41, 29.36, 29.30, 29.24, 25.28, 22.59, 16.53, 14.03. IR (neat) cm^{-1}: 3450 (br w), 3020 (w), 2920 (s), 2850 (s), 1720 (w), 1650 (br s), 1560 (w), 1480 (w), 1475 (w), 1400 (m), 1365 (m), 1360–1340 (br m), 1300 (m), 1240 (m), 1210 (m), 1162 (m), 1105 (m), 1080 (m), 1030 (m), 970 (w), 930 (w), 870 (w), 730 (s); MS (*m*/*z*, %): 345 (M^+, 1.9), 316 (5.2), 304 (5.2) 272 (4.7), 177 (18), 162 (58), 123 (40), 105 (65), 44 (100); HRMS (*m*/*z*): 345.2791 (Calcd for $C_{21}H_{35}N_3O$, 345.2780).

4.1.1.6. 1-(3,4a,8-Trimethyl-4,4a-dihydrobenzo[*d*] [1,2, 3]triazin-2(3*H*)-yl)dodecan-1-one (6). Brown oil, 8%. ^{1}H NMR (C_6D_6) δ: 5.77 (dt, 1H, J = 6.0, 1.5 Hz), 5.63 (dd, 5H, J = 9.0, 6.0 Hz), 5.24 (d, 1H, J = 9.0 Hz), 2.89 (d, 1H, J = 9.0 Hz), 2.79 (m, 1H), 2.56 (br s, 3H), 1.99 (s, 3H), 1.88 (quin, 2H, J = 7.2 Hz), 1.18–1.48 (m, 18H), 0.95 (s, 3H), 0.87 (t, 3H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$) δ: 171.67, 158.00, 136.72, 130.97, 126.23, 121.45, 61.19, 43.97, 41.76, 34.28, 31.86, 29.57, 29.49, 29.43, 29.39, 29.33, 29.30, 26.59, 25.30, 22.63, 16.57, 14.07. IR (neat) cm^{-1}: 3450 (br w), 2900 (s), 2850 (s), 1680 (br s), 1580 (w), 1450 (m), 1400 (m), 1360 (br m), 1300 (w), 1210 (w), 1180 (w), 1110 (w), 1030 (w), 720 (s); MS (*m*/*z*, %): 359 (M^+, 2.4), 318 (7.3), 286 (4.8), 183 (25), 162 (70), 133 (56), 105 (87), 57 (43), 44 (100); HRMS (*m*/*z*): 359.2909 (Calcd for $C_{22}H_{37}N_3O$, 359.2937).

4.1.2. General procedure for transformation of triazenes into tetrahydrobenzotriazine derivatives (7–10). To a 1 M solution of 1-(2,6-dimethylphenyl)-3,3-dimethyltriazene in dry THF (2 mL) was added dropwise a solution of *n*-BuLi/hexane (1 equiv) at 0 °C. After stirring for 30 min at the same temperature, phenyl isocyanate and/or phenyl isothiocyanate (1 equiv) was added to the reaction mixture. After further stirring for 30 min at the same temperature, the reaction mixture was quenched with water and/or iodomethane (1 equiv). The reaction was stopped by addition of H_2O, and the reaction mixture was extracted with ether. The combined extracts were washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure left the residue, which was purified on a silica gel flash column chromatography to afford urea and thiourea derivatives (**7**–**10**).

4.1.2.1. 3,4a,8-Trimethyl-*N*-phenyl-4,4a-dihydrobenzo[*d*][1,2, 3]triazine-2(3*H*)-carboxamide (7). Brown oil, 76%. ^{1}H NMR ($CDCl_3$) δ: 8.49 (s, 1H), 7.00–7.55 (m, 5H), 6.23 (dt, 1H, J = 2.0, 0.5 Hz), 5.96 (dd, 1H, J = 3.0, 2.0 Hz), 5.85 (d, 1H, J = 3.0 Hz), 3.66 (d, 1H, J = 5.0 Hz), 3.35 (d, 1H, J = 5.0 Hz), 2.53 (s, 3H), 2.10 (s, 3H), 1.20 (s, 3H). ^{13}C NMR (C_6D_6) δ: 17.0.03, 151.37, 137.69, 129.30, 129.07, 128.29, 126.16, 123.21, 121.20, 119.16, 60.03, 40.19,34.32, 26.73, 16.66. IR (neat) cm^{-1}: 3350 (s), 3000 (s), 1700 (m), 1600 (m), 1500 (m), 1440 (m), 1300 (w), 1240 (w), 1040 (w), 750 (s); MS (*m*/*z*, %): 296 (M^+, 2.4), 162 (42), 133 (91), 120 (67), 105 (100), 91 (53), 77 (52), 65 (20); HRMS (*m*/*z*): 296.1608 (Calcd for $C_{17}H_{20}N_4O$, 296.1637).

4.1.2.2. *N*,3,4a,8-Tetramethyl-*N*-phenyl-4,4a-dihydrobenzo[*d*][1,2,3]triazine-2(3*H*)-carboxamide (8). Pale brown crystal, 29%; mp 93–96 °C (hexane). ^{1}H NMR ($CDCl_3$) δ: 7.54 (dt, 1H, J = 9.0, 1.5 Hz), 7.30 (tt, 2H, J = 9.0, 1.5 Hz), 7.05 (tt, 1H, J = 9.0, 1.5 Hz), 6.23 (dt, 1H, J = 6.0, 1.5 Hz), 5.99 (dd, 1H, J = 9.0, 6.0 Hz), 5.86 (d, 1H, J = 9.0 Hz), 3.68 (d, 1H, J = 13.0 Hz), 3.34 (d, 1H, J = 13.0 Hz), 2.53 (s, 3H), 2.15 (s, 3H), 1.70 (s, 3H), 1.20 (s, 3H). ^{13}C NMR ($CDCl_3$) δ: 162.0, 151.68, 138.34, 138.09, 130.34, 128.92, 126.82, 123.06, 121.19, 119.01, 64.30, 39.86, 34.33, 27.30, 16.60. IR (Nujol) cm^{-1}: 3200 (s), 2900 (s), 1670 (m), 1600 (m), 1500 (m), 1440 (m), 1300 (w), 1210 (w), 1120 (m), 1080 (m), 1020 (m), 920 (w), 900 (w), 860 (w), 720 (s); MS (*m*/*z*, %): 310 (M^+, 9.4), 277 (13), 221 (21), 194 (24), 135 (100), 91 (63), 77 (93), 44 (41); HRMS (*m*/*z*): 310.1780 (Calcd for $C_{18}H_{22}N_4O$, 310.1794).

4.1.2.3. 3,4a,8-Trimethyl-*N*-phenyl-4,4a-dihydro- benzo[*d*][1,2,3]triazine-2(3*H*)-carbothioamide (9). Brown oil, 59%. ^{1}H NMR (C_6D_6) δ: 9.44 (br s, 1H), 7.66 (d, 2H, J = 8.0 Hz), 7.37 (tt, 2H, J = 9.5, 2.0 Hz), 7.20 (tt, 1H, J = 7.0, 1.5 Hz), 6.36 (dt, 1H, J = 6.0, 1.5 Hz), 6.05 (dd, 1H, J = 9.0, 6.0 Hz), 5.95 (d, 1H, J = 9.0 Hz), 3.80 (d, 1H, J = 14.0 Hz), 3.44 (d, 1H, J = 14.0 Hz), 2.53 (s, 3H), 2.13 (s, 3H), 1.21 (s, 3H). ^{13}C NMR ($CDCl_3$) δ: 181.78, 176.30, 138.64, 130.06, 129.83, 128.60, 125.63, 124.57, 124.33, 121.16, 60.32, 42.29, 34.84, 27.88, 16.47. IR (neat) cm^{-1}: 3600 (s), 2900 (s), 1730 (m), 1600 (m), 1500 (w), 1450 (w), 1330 (w), 1200 (w), 1120 (w), 1030 (w), 980 (w), 720 (m); MS (*m*/*z*, %): 312 (M^+, 0.1), 209 (19), 162 (32), 134 (68), 119 (70), 105 (100), 91 (72), 77 (74), 51 (15); HRMS (*m*/*z*): 312.1418 (Calcd for $C_{17}H_{20}N_4S$, 312.1409).

4.1.2.4. *N*,3,4a,8-Tetramethyl-*N*-phenyl-4,4a-dihydrobenzo[*d*][1,2,3]triazine-2(3*H*)-carbothioamide (10). Brown oil, 67%. ^{1}H NMR ($CDCl_3$) δ: 7.18 (tt, 1H, J = 8.0, 2.0 Hz), 6.80–6.92 (m, 2H), 6.15 (dd, 1H, J = 6.0, 1.5 Hz), 5.90 (dd, 1H, J = 9.0, 6.0 Hz), 5.74 (d, 1H, J = 9.0 Hz), 3.48 (d, 1H, J = 13.0 Hz), 3.05 (d, 1H, J = 13.0 Hz), 2.34 (s, 3H), 2.28 (s, 3H), 1.65 (s, 3H), 1.06 (s, 3H). ^{13}C NMR ($CDCl_3$) δ: 160.64, 154.29, 151.43, 138.05, 130.65, 128.07, 126.03, 121.07, 120.89, 120.18, 65.15, 38.31, 33.95, 26.99, 16.14, 15.23. IR (neat) cm^{-1}: 2900 (s), 1610 (s), 1580 (s), 1480 (s), 1440 (s), 1410 (s), 1380 (m), 1280 (s), 1260 (s), 1190 (m), 1130 (m), 1060 (m); MS (*m*/*z*, %): 326 (M^+, 1.2), 266 (1.2), 208 (4.9), 150 (100), 135 (20), 77 (18), 57 (11); HRMS (*m*/*z*): 326.1560 (Calcd for $C_{18}H_{22}N_4S$, 326.1565).

4.2. Nematocidal activity test

The nematocidal activity was tested against the third stage larvae of free-living nematode *C. elegans*.

4.2.1. Preparation of the test solution. The 1% DMSO solution of synthetic compound (**1**–**10**) and methyl isothiocyanate as reference were prepared. Each solution was diluted with M-9 buffer[10] solution containing 100 ppm of Tween® 20 to make test solution of 500 ppm.

4.2.2. Assay method. To one well of three hole slide glass (15 mm in diameter, 1 mm in depth), M-9 buffer solution (40 μL) was added. After third stage larvae (10 worms) were added, 500 ppm test solution (40 μL) was added to each well and the mixtures were stirred. The final concentration of test solution was 250 ppm. After incubation periods of 24 and 48 h at ca. 22 °C, living nematodes were counted. Each experiment was repeated three times. The final concentration of reference solution was 200 ppm.

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