Synthesis of 1,3,4-thiadiazole chrysanthemamide derivatives promoted by phenyldichlorophosphate catalysis

Peng Yu, Rong Wan* Peng Wang, Jiang-Qiang Zhang and Qiu He

College of Sciences, Nanjing University of Technology, Nanjing 210009, P. R. China

A series of novel pro-pesticides with the activity of both 1,3,4-thiadiazole and chrysanthemic acid were synthesised using phenyldichlorophosphate catalysis. These 1,3,4-thiadiazole chrysanthemamides were identified by IR, ^{1}H NMR and elemental analyses.

Keywords: 1,3,4-thiadiazole, chrysanthemamide, phenyldichlorophosphate catalysis

The discovery of a new lead structure is an important aspect of research in medicinal and pesticide chemistry. Among various biological heterocyclic compounds, 1,3,4-thiadiazole derivatives have attracted considerable attention from chemists due to their promising biological activity. For example, some 1,3,4-thiadiazole derivatives have been reported to possess anti-inflammatory[1], antiproliferative[2], antibacterial[3–5], and fungicidal[6] activity. In addition, a wide spectrum of insecticidal[7] and herbicidal[8] activity was also found to be associated with 1,3,4-thiadiazole and their derivatives. Some compounds with broad spectrum of bioactivity have been commercialised. L 1215[9], for example, is highly effective against mosquito larvae[10], *Spodoptera eridania* larvae[11], and Western Spruce budworm[12].

Pyrethrins, naturally-occurring extracts of chrysanthemum flowers, have long been of interest as insecticides. A noteworthy advance in this area was the discovery by Elliott *et al*.[13] of certain highly active dihalovinylcyclopropanecarboxylates such as permethrin, which showed substantially improved photostability when compared with the previously available cyclopropanecarboxylates. Sudarshan have also reported[14] that Dowco 417 possessed low mammalian toxicity and offered a number of other significant advantages over conventional insecticides. Furthermore, Fendona (Alphamethrin) has been reported to be effective in in large scale control operations against the larvae and adults of *Culex quinquefasciatus* [15]. Cyhalothrin has been reported that it can be effectively used to control the major resistant strains of the cattle tick (*Boophilus microplus*) and the buffalo fly (*Haematobia irritans exigua*) on cattle [16].

In view of the above and our desire to develop new insecticidal agents of high potency, we combined the biologically active 1,3,4-thiadiazole and pyrethroid structures in order to obtain a series of new chrysanthemamide derivatives.

Results and discussion

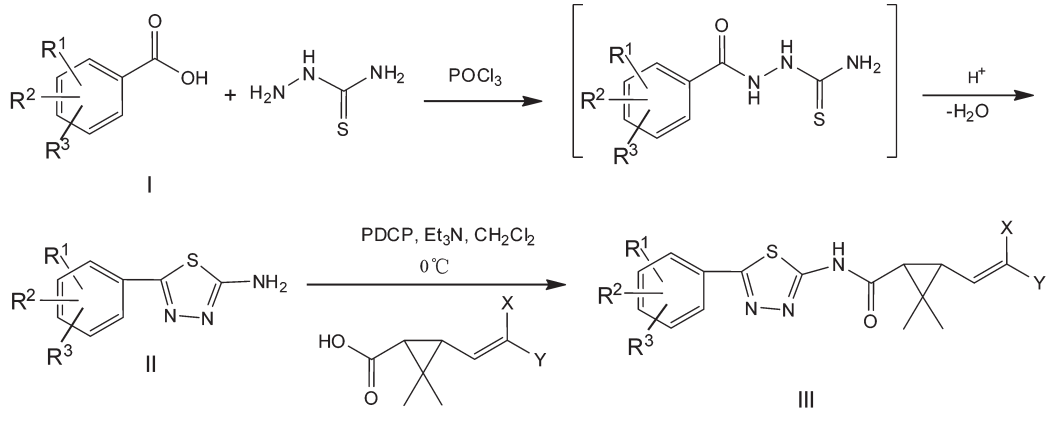
The title compounds **IIIa–p** were synthesised by the general routes as shown in Scheme 1. The 5-(substituted phenyl)-1,3,4-thiadiazol-2-yl-chrysanthemamides **IIIa–p** were synthesised by the reaction of 2-amino-5-substituted phenyl-1,3,4-thiadiazole **IIa–p** with triflurochrysanthemic acid or dichlorochrysanthemic acid in anhydrous dichloromethane using PDCP catalysis and Et_3N as the acid acceptor. The results are reported in Table 1.

Experimental

All nonaqueous reactions were carried out under a nitrogen atmosphere. Unless otherwise noted, all reagents and solvents were used as received. Reactions were monitored by TLC with visualisation by UV light. Melting points were recorded on an X-4 binocular microscope melting point apparatus (Beijing Tech Instruments Co., Beijing, China) and were corrected. Elemental analyses were performed on an Elementer Vario EL III elementary analysis instrument. ^{1}H NMR spectra were obtained in $CDCl_3$ and DMSO-d6, and were recorded on a Bruker DRX500 spectrometer and resonance were given in ppm (δ) relative to TMS, and peak multiplicity is reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or b (broad). IR spectra in KBr were recorded by a Perkin-Elmer PE-683 IR spectrometer. Yields were not optimised.

Preparation of 5-aryl-1,3,4-thiadiazol-2-amines (**II**): To a mixture of substituted benzoic acid I (0.1 mol) and thiosemicarbazide (0.1 mol) was added $POCl_3$ (0.3 mol) dropwise at 0–5 °C and maintained for 30 minutes. The reaction mixture was allowed to raise temperature until reflux and stirred for 4 h. After cooling, 50 mL water was added into the reaction mixture. The pH of the reaction solution was adjusted to the range of 8–9 with the solution of 50% NaOH. The crude product precipitated, filtered, washed with water, dried, and recrystallised from ethanol to afford compound **II**.

Preparation of 1,3,4-thiadiazole chrysanthemamide (**IIIa–p**): A solution of compound **II** (2.5 mmol), triflurochrysanthemic acid or



Scheme 1 Synthesis routine of compounds **IIIa–p**.

* Correspondent. E-mail: rwan@njut.edu.cn

Table 1 Synthesis of compounds **IIIa–p**

NO.	R^1	R^2	R^3	X	Y	Yield/%
IIIa	4-NO_2	H	H	Cl	CF_3	83.2
IIIb	4-OCH_3	H	H	Cl	CF_3	85.1
IIIc	3-OCH_3	4-OCH_3	5-OCH_3	Cl	CF_3	72.8
IIId	2-Cl	4-Cl	H	Cl	CF_3	84.1
IIIe	3-F	H	H	Cl	CF_3	80.5
IIIf	4-Br	H	H	Cl	CF_3	78.8
IIIg	2-Cl	4-NO_2	H	Cl	CF_3	84.2
IIIh	3-CH_3	5-CH_3	H	Cl	CF_3	76.6
IIIi	4-NO_2	H	H	Cl	Cl	78.6
IIIj	H	H	H	Cl	Cl	83.5
IIIk	4-OCH_3	H	H	Cl	Cl	76.6
IIIl	2-Cl	4-Cl	H	Cl	Cl	79.4
IIIm	3-CH_3	5-CH_3	H	Cl	Cl	77.9
IIIn	3-CH_3	H	H	Cl	Cl	85.3
IIIo	3-CF_3	H	H	Cl	Cl	82.9
IIIp	3-F	H	H	Cl	Cl	87.6

dichlorochrysanthemic acid (2.3 mmol) and phenyl dichlorophosphate (PDCP) (2.6 mmol) in 40 mL of anhydrous dichloromethane at 0 °C was treated with triethylamine (5.2 mmol) dropwise. The mixture was stirred at 0–5 °C and monitored by TLC. After the reaction was complete, the reaction mixture was washed with saturated sodium bicarbonate solution (25 mL) and extracted with dichloromethane (15 mL × 3). The organic layer was dried, concentrated and precipitated to give the crude product, which were crystallised from ethanol to give title compounds **IIIa–p**.

3-(2-Chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-N-[5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl]cyclopropanecarboxamide (**IIIa**): Yield 83.2%; m.p. 297–299 °C. ^{1}H NMR (500MHz, DMSO-d_6) δ(ppm): 1.29 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 2.40 (t, 1H, CH), 2.51 (d, 1H, CH), 7.16 (d, 1H, C=CH), 8.22–8.35 (m, 4H, Ph), 13.17(s, 1H, NH). IR (KBr) ν: 3446, 3151, 3080, 2960, 2904, 1683, 1652, 1554, 952, 852, 752, 686 cm^{-1}. Anal. Calcd for $C_{17}H_{14}ClF_3N_4O_3S$: C, 45.70; H, 3.16; N, 12.54; S, 7.18. Found: C, 45.60; H, 3.09; N, 12.22; S, 7.08%.

3-(2-Chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-N-[5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl]cyclopropanecarboxamide (**IIIb**): Yield 85.1%; m.p. >300 °C. ^{1}H NMR (500MHz, DMSO-d_6) δ(ppm): 1.27 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 2.37 (t, 1H, CH), 2.49 (d, 1H, CH), 3.83 (s, 3H, OCH_3), 7.17 (d, 1H, C=CH), 7.06–7.90 (m, 4H, Ph), 12.91(s, 1H, NH). IR (KBr) ν: 3566, 3168, 3078, 2966, 1683, 1652, 952, 833, 768, 692 cm^{-1}. Anal. Calcd for $C_{18}H_{17}ClF_3N_3O_2S$: C, 50.06; H, 3.97; N, 9.73; S, 7.42. Found: C, 49.92; H, 3.86; N, 9.56; S, 7.22%.

3-(2-Chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-N-[5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl]cyclopropanecarboxamide (**IIIc**): Yield 72.8%; m.p. 279–281 °C. ^{1}H NMR (500MHz, DMSO-d_6) δ(ppm): 1.42 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 2.43 (t, 1H, CH), 2.87 (d, 1H, CH), 3.87–3.89 (m, 9H, OCH_3), 7.15 (d, 1H, C=CH), 7.15–7.17 (m, 2H, Ph), 14.04 (s, 1H, NH). IR (KBr) ν: 3566, 3159, 3008, 2935, 2839, 1683, 1652, 952, 832, 775, 694 cm^{-1}. Anal. Calcd for C20H21ClF3N3O4S: C, 48.83; H, 4.30; N, 8.54; S, 6.52. Found: C, 48.63; H, 4.21; N, 8.36; S, 6.12%.

3-(2-Chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-N-[5-(2,4-dichlorophenyl)-1,3,4-thiadiazol-2-yl]cyclopropanecarboxamide (**IIId**): Yield 84.1%; m.p. 228–230 °C. ^{1}H NMR (500MHz, DMSO-d_6) δ(ppm): 1.37 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 2.41 (t, 1H, CH), 2.66 (d, 1H, CH), 7.10 (d, 1H, C=CH), 6.91–7.43 (m, 2H, Ph), 13.75 (s, 1H, NH). IR (KBr) ν: 3566, 3161, 3080, 2923, 2877, 1683, 1652, 952, 833, 748, 659 cm^{-1}. Anal. Calcd for $C_{17}H_{13}Cl_2F_3N_3OS$: C, 43.17; H, 3.02; N, 8.39; S, 6.40. Found: C, 43.02; H, 2.89; N, 8.18; S, 6.12%.

3-(2-Chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-N-[5-(3-fluorophenyl)-1,3,4-thiadiazol-2-yl]cyclopropanecarboxamide (**IIIe**): Yield 80.5%; m.p. >300 °C. ^{1}H NMR (500MHz, DMSO-d_6) δ(ppm): 1.41 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 2.47 (t, 1H, CH), 2.81 (d, 1H, CH), 7.45 (d, 1H, C=CH), 7.14–7.68 (m, 4H, Ph). IR (KBr) ν: 3566, 3151, 3029, 2964, 2894, 1683, 1652, 1276, 952, 831, 779, 680 cm^{-1}. Anal. Calcd for $C_{17}H_{14}ClF_4N_3OS$: C, 48.64; H, 3.36; N, 10.01; S, 7.64. Found: C, 48.58; H, 3.11; N, 9.85; S, 7.62%.

3-(2-Chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-N-[5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl]cyclopropanecarboxamide (**IIIf**): Yield 78.8%; m.p. >300 °C. ^{1}H NMR (500MHz, DMSO-d_6) δ(ppm): 1.27 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 2.38 (t, 1H, CH), 2.47 (d, 1H, CH), 7.16 (d, 1H, C=CH), 7.70–7.94 (m, 4H, Ph), 13.03 (s, 1H, NH). IR (KBr) ν: 3566, 3078, 3029, 2894, 2866, 1683, 1652, 1070, 827, 779, 636 cm^{-1}. Anal. Calcd for $C_{17}H_{14}BrClF_3N_3OS$: C, 42.47; H, 2.94; N, 8.74; S, 6.67. Found: C, 42.36; H, 2.72; N, 8.62; S, 6.37%.

3-(2-Chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-N-[5-(2-chloro-4-nitrophenyl)-1,3,4-thiadiazol-2-yl]cyclopropanecarboxamide (**IIIg**): Yield 84.2%; m.p. 258–261 °C. ^{1}H NMR (500MHz, DMSO-d_6) δ(ppm): 1.28 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 2.40 (t, 1H, CH), 2.52 (d, 1H, CH), 7.14 (d, 1H, C=CH), 8.29–8.49 (m, 3H, Ph), 13.18(s, 1H, NH). IR (KBr) ν: 3566, 3107, 3010, 2894, 2875, 1683, 1652, 1554, 839, 744 cm^{-1}. Anal. Calcd for $C_{17}H_{13}Cl_2F_3N_4O_3S$: C, 42.43; H, 2.72; N, 11.64; S, 6.66. Found: C, 46.42; H, 3.36; N, 13.51; S, 7.70%.

3-(2-Chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-N-[5-(3,5-dimethylphenyl)-1,3,4-thiadiazol-2-yl]cyclopropanecarboxamide (**IIIh**): Yield 76.6%; m.p. >300 °C. ^{1}H NMR (500MHz, DMSO-d_6) δ(ppm): 1.27 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 2.34 (s, 6H, CH_3), 2.38 (t, 1H, CH), 2.46 (d, 1H, CH), 7.15 (d, 1H, C=CH), 7.18–7.58 (m, 3H, Ph), 12.95 (s, 1H, NH). IR (KBr) ν: 3566, 3157, 3085, 2958, 2867, 1683, 1652, 844, 779, 688 cm^{-1}. Anal. Calcd for $C_{19}H_{19}ClF_3N_3OS$: C, 53.08; H, 4.45; N, 9.77; S, 7.46. Found: C, 52.78; H, 4.54; N, 9.68; S, 7.51%.

3-(2,2-Dichlorovinyl)-2,2-dimethyl-N-[5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl] cyclopropanecarboxamide (**IIIi**): Yield 78.6%; m.p. 279–281 °C. ^{1}H NMR (500MHz, DMSO-d_6) δ(ppm): 1.39 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 2.37 (t, 1H, CH), 2.61 (d, 1H, CH), 6.46 (d, 1H, C=CH), 8.07–8.38 (m, 4H, Ph), 13.70 (s, 1H, NH). IR (KBr) ν: 3442, 3145, 3046, 2956, 2898, 1691, 1652, 1558, 922, 852 cm^{-1}. Anal. Calcd for $C_{16}H_{14}Cl_2N_4O_3S$: C, 46.50; H, 3.41; N, 13.56; S, 7.76. Found: C, 46.42; H, 3.36; N, 13.51; S, 7.70%.

3-(2,2-Dichlorovinyl)-2,2-dimethyl-N-(5-phenyl-1,3,4-thiadiazol-2-yl) cyclopropanecarboxamide (**IIIj**): Yield 83.5%; m.p. 260–263 °C. ^{1}H NMR (500MHz, DMSO-d_6) δ(ppm): 1.37 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 2.33 (t, 1H, CH), 2.73 (d, 1H, CH), 6.50 (d, 1H, C=CH), 7.26–7.92 (m, 5H, Ph), 13.93 (s, 1H, NH). IR (KBr) ν: 3481, 3161, 3046, 2918, 2869, 1683, 1653, 921, 815 cm^{-1}. Anal. Calcd for $C_{16}H_{15}Cl_2N_3OS$: C, 52.18; H, 4.11; N, 11.41; S, 8.71. Found: C, 52.11; H, 4.09; N, 11.36; S, 8.65%.

3-(2,2-Dichlorovinyl)-2,2-dimethyl-N-[5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl] cyclopropanecarboxamide (**IIIk**): Yield 76.6%; m.p. 287–290 °C. ^{1}H NMR (500MHz, DMSO-d_6) δ(ppm): 1.37 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 2.31 (t, 1H, CH), 2.71 (d, 1H, CH), 3.87 (s, 3H, OCH_3), 6.51 (d, 1H, C=CH), 6.97–7.84 (m, 4H, Ph), 13.89 (s, 1H, NH). IR (KBr) ν: 3481, 3166, 3064, 2954, 2896, 2866, 2837, 1683, 1653, 921, 831 cm^{-1}. Anal. Calcd for $C_{17}H_{17}Cl_2N_3O_2S$: C, 51.26; H, 4.30; N, 10.55; S, 8.05. Found: C, 51.22; H, 4.28; N, 10.50; S, 8.00.

3-(2,2-dichlorovinyl)-2,2-dimethyl-N-[5-(2,4-dichlorophenyl)-1,3,4-thiadiazol-2-yl] cyclopropanecarboxamide (**IIIl**): Yield 79.4%; m.p. 293–298 °C. ^{1}H NMR (500MHz, DMSO-d_6) δ(ppm): 1.24 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 2.25 (t, 1H, CH), 2.30 (d, 1H, CH), 6.41 (d, 1H, C=CH), 7.60–8.16 (m, 3H, Ph), 12.91 (s, 1H, NH). IR (KBr) ν: 3566, 3155, 3064, 2896, 2866, 1683, 1652, 921, 821 cm^{-1}. Anal. Calcd for $C_{16}H_{13}Cl_4N_3OS$: C, 43.96; H, 3.00; N, 9.61; S, 7.33. Found: C, 43.93; H, 2.95; N, 9.57; S, 7.29%.

3-(2,2-Dichlorovinyl)-2,2-dimethyl-N-[5-(3,5-dimethylphenyl)-1,3,4-thiadiazol-2-yl] cyclopropanecarboxamide (**IIIm**): Yield 77.9%; m.p. 296–298 °C. ^{1}H NMR (500MHz, DMSO-d_6) δ(ppm): 1.37 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 2.31 (t, 1H, CH), 2.37 (s, 6H, CH_3), 2.70 (d, 1H, CH), 6.51 (d, 1H, C=CH), 7.10–7.53 (m, 3H, Ph), 13.84 (s, 1H, NH). IR (KBr) ν: 3566, 3151, 3062, 2956, 2854, 1679, 1652, 923, 831 cm^{-1}. Anal. Calcd for $C_{18}H_{19}Cl_2N_3OS$: C, 54.55; H, 4.83; N, 10.60; S, 8.09. Found: C, 54.50; H, 4.80; N, 10.00; S, 8.05%.

3-(2,2-dichlorovinyl)-2,2-dimethyl-N-[5-(3-methylphenyl)-1,3,4-thiadiazol-2-yl] cyclopropanecarboxamide (**IIIn**): Yield 85.3%; m.p. 205–253 °C. ^{1}H NMR (500MHz, DMSO-d_6) δ(ppm): 1.37 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 2.33 (t, 1H, CH), 2.41 (s, 3H, CH_3), 2.71 (d, 1H, CH), 6.51 (d, 1H, C=CH), 7.26–7.72 (m, 4H, Ph), 13.82 (s, 1H, NH). IR (KBr) ν: 3446, 3161, 3064, 2921, 2886, 1683, 1652, 923, 815 cm^{-1}. Anal. Calcd for $C_{17}H_{17}Cl_2N_3OS$: C, 53.41; H, 4.48; N, 10.99; S, 8.39. Found: C, 53.39; H, 4.40; N, 10.92; S, 8.31%.

3-(2,2-Dichlorovinyl)-2,2-dimethyl-N-{5-[3-(trifluoromethyl)phenyl]-1,3,4-thiadiazol-2-yl} cyclopropanecarboxamide (**IIIo**): Yield 82.9%; m.p. 200–202 °C. ^{1}H NMR (500MHz, DMSO-d_6) δ(ppm): 1.17 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 2.35 (t, 1H, CH), 2.68 (d, 1H, CH), 6.48 (d, 1H, C=CH), 7.61–8.04 (m, 4H, Ph), 13.92 (s, 1H, NH). IR (KBr) ν: 3155, 3066, 2960, 2898, 1683, 1652, 1338, 923, 819 cm^{-1}. Anal. Calcd for $C_{17}H_{14}Cl_2F_3N_3OS$: C, 46.80; H, 3.23; N, 9.63; S, 7.35. Found: C, 46.75; H, 3.18; N, 9.58; S, 7.29%.

3-(2,2-dichlorovinyl)-2,2-dimethyl-N-[5-(3-fluorophenyl)-1,3,4-thiadiazol-2-yl] cyclopropanecarboxamide (**IIIp**): Yield 87.6%; m.p. 230–232 °C. ^{1}H NMR (500MHz, DMSO-d_6) δ(ppm): 1.38 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 2.34 (t, 1H, CH), 2.68 (d, 1H, CH), 6.49 (d, 1H, C=CH), 7.16–7.66 (m, 4H, Ph), 13.89 (s, 1H, NH). IR (KBr) ν: 3566, 3151, 3064, 2956, 2912, 1683, 1652, 1149, 923, 813 cm^{-1}. Anal. Calcd for $C_{16}H_{14}Cl_2FN_3OS$: C, 49.75; H, 3.65; N, 10.88; S, 8.30. Found: C, 49.69; H, 3.60; N, 10.82; S, 8.22%.

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References

1 S. Silvia, B. Chiara, B. Olga, B. Francesco, R. Angelo, F. Walter, R. Barbara, C. Annalisa, F. Giuseppe, *Bioorg. Med. Chem.*, 2006, **14**, 1698.
2 M. Joanna, O. Adam, *Bioorg. Med. Chem.*, 2006, **14**, 4483.
3 F. Alireza, E. Saeed, H. Abdolreza, R. Majid, S. Kazem, H.M. Mohammad, S. Abbas, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 4488.
4 T. Sara, A. Tahmineh, R.F. Mohammad, J. Hossein, S. Abbas, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 1023.
5 M.T. Lisa, C.G. Robert, A.W. Elizabeth, M.O. Jason, W.F. Charles, E.Z. Gary, C.H. Judith, S. Douglas, K.M. Judy, D.S. Ronda, H.Y. Betty, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 4193.
6 X.J. Zou, L.H. Lai, G.Y. Jin, Z.X. Zhang, *J. Agric. Food Chem.*, 2002, **50**, 3757.
7 Y.P. Luo, G.F. Yang, *Bioorg. Med. Chem.*, 2007, **15**, 1716.
8 K. Hiroshi, S. Rokuro, H. Isao and O. Takuo, *J. Agric. Food Chem.*, 1970, **18**, 60.
9 J.S. Ward, US Patent 4141984, 1979.
10 C.H. Schaefer, T. Miura, W.H. Wilder, *J. Econ. Entomol.* 1981, **74**, 658.
11 Eli Lilly and Co., US Patent 55355 A 1982.
12 M. Robertson, L. Jacqueline, *J. Ga. Entomol. Soc.*, 1982, **17**, 413.
13 M. Elliott, N.F. Janes, D.A. Pulman, US Patent 4024163. 1977.
14 K.M. Sudarshan, C.V. John, L.L. Larry and J.R. Michael, *J. Agric. Food Chem.*, 1981, **29**, 1287.
15 D. Amalraj, K.D. Ramaiah, A.R. Rajavel, T. Mariappan, V. Vasuki, K.P. Paily, B.K. Tyagi, M. Kalyanasundaram and P.K. Das, *Indian J. Med. Res.*, 1987, **86**, 601.
16 V.K. Stubbs, C. Wilshire, L.G. Webber, *Aust. Vet. J.*, 1982, **59**, 152.