Green Synthesis of 5-Substituted-1,3,4-thiadiazole-2-thiols as New Potent Nitrification Inhibitors [1]

Ajoy Saha, Rajesh Kumar,* Rajendra Kumar, and C. Devakumar

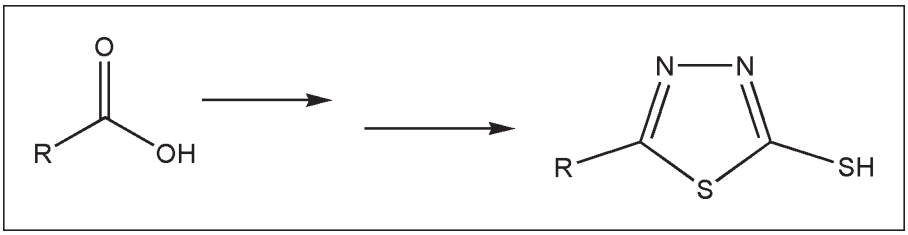
Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi 110 012, India

*E-mail: rkb_1973@yahoo.co.in or rkb1973@gmail.com.

Received June 20, 2009

DOI 10.1002/jhet.345

Published online 4 June 2010 in Wiley InterScience (www.interscience.wiley.com).



A fast, efficient synthesis of 5-substituted-1,3,4-thiadiazole-2-thiols was successfully developed, assessed using green chemistry matrices, and compounds were screened for their *in vitro* nitrification inhibitory activity. The greener method was superior with higher energy efficiency, E(nvironmental) factor, atom economy, atom efficiency, carbon efficiency, and reaction mass efficiency.

J. Heterocyclic Chem., **47**, 838 (2010).

INTRODUCTION

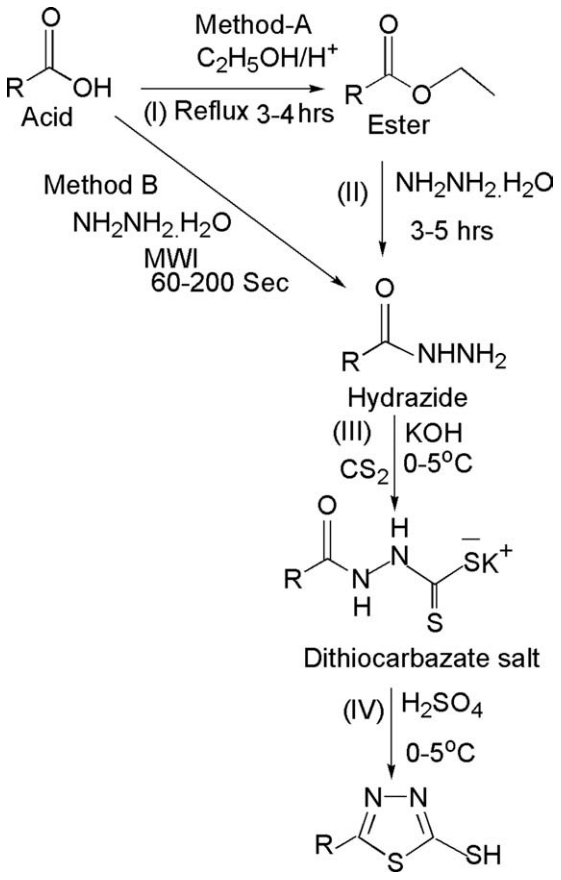
With increasing global population, demands have been increased to meet the requirements and consequently the industrial pollution. To control this detrimental phenomenon, the term ''green chemistry'' has come into existence [2]. Green chemistry is a package of technologies, design principles, and tools to reduce toxicity, resource energy use, and pollution of chemicals [2,3]. Due to environmental awareness [4], chemists have focused their attention to examine bioactive products such as heterocycles and processes in terms of environment friendliness. The 1,3,4-thiadiazoles are important bioactive heterocyclic moieties and have been reported to have wide range of bioactivities [5] such as neuroprotective, antibacterial, antidepressant, and antituberculour. One of the title compound, 5-methyl-1,3,4-thiadiazole-2-thiol is the side chain at C-3 position of a well known antibiotic Cefazolin sodium [6].

The low nitrogen use efficiency accounts to US$ 17 Billion annual nitrogen losses worldwide along with environmental and health hazards [7,8]. There is, thus, an urgent call for improving the efficiency of N-fertilizer use to achieve higher food production for catering the ever increasing population and also to minimize fertilizer related pollution of the environment [9]. Despite a great interest in the development and use of nitrification inhibitors to date, only a few compounds have been adopted for agricultural use [10]. The main problems associated with these inhibitors are the high cost involved in the development, subsequent registration of effective nitrification inhibitors, and the economics of their use in field conditions [11]. Various heterocyclics being an important bioactive class are used as nitrification inhibitors [12], but their use is restrained because of the complex synthetic procedures involving hazardous chemicals and subsequent cost. This problem can be solved by synthesizing potential nitrification inhibiting heterocyclics with minimum cost in an environment friendly manner. Keeping in view the aforementioned consideration, this study was taken up to design and synthesize 5-substituted-1,3,4-thiadiazole-2-thiols in a greener and cost effective way as potential nitrification inhibitors.

RESULTS AND DISCUSSION

5-Substituted-1,3,4-thiadiazole-2-thiols have been synthesized starting from carboxylic acids following the conventional (protocol 1) and improved greener synthetic protocol (protocol 2). Reaction pathway is depicted in Scheme 1. In protocol 1, carboxylic acids were esterified by refluxing with ethyl alcohol for 3–4 h in the presence of sulfuric acid. These ester yielded corresponding hydrazide on refluxing with hydrazine hydrate for 3–5 h [13]. The analytical and spectral data of hydrazides were in complete agreement with those given in literature [13–17]. Hydrazides on reaction with carbon disulphide and potassium hydroxide afforded thiocarbazate salts, which are cyclized in the presence of concentrated sulfuric acid to afford 5-substituted-1,3,4-

Scheme 1. Synthesis of 5-substituted-1,3,4-thiadiazole-2-thiols.



thiadiazole-2-thiols. In protocol 2, our new greener one-pot method was used for the synthesis of acid hydrazides [18], and hydrazides were further processed similarly as in protocol 1 to yield 5-substituted-1,3,4-thiadiazole-2-thiols. This innovation improved the overall synthetic protocol for 5-substituted-1,3,4-thiadiazole-2-thiols in terms of yield, time, energy, atom economy and efficiency, number of steps, and other green chemistry measures (Table 3). All these compounds were characterized by analytical and spectral data (Tables 1 and 2).

The structures of 5-substituted-1,3,4-thiadiazole-2-thiols were established on the basis of their IR, ^{1}H-NMR, ^{13}C-NMR, CHNS data. The analytical and spectral data of 5-substituted-1,3,4-thiadiazole-2-thiols are given in Tables 1 and 2. The structure was supported by the absence of IR bands at 3200–3300, 1650–1670 cm^{-1} due to $NHNH_2$ and CONH groups, respectively, and appearance of 1550–1590 cm^{-1} due to C=N groups. It was also supported by the ^{1}H-NMR data showing signals at δ 11.0–14.2 due to SH and ^{13}C-NMR data showing signals at δ 160.0–174.0 and 176–192 due to 5 C—N and 2 C—N, respectively.

Both conventional and greener synthetic protocols for 5-substituted-1,3,4-thiadiazole-2-thiols were assessed using various green chemistry measures [19–21]. The developed greener protocol showed 4.7–11.4% increase in overall yield (Table 1). 5-Phenyl-1,3,4-thiadiazole-2-thiol was taken as a case for all calculations and related data for both the protocols (1 and 2) are described in Table 3.

The overall yield, *i.e.*, starting from benzoic acid to 5-phenyl-1,3,4-thiadiazole-2-thiol, following protocol 2 was 69.3% as compared with 59.6% from conventional protocol. There was 9.7% increase in the yield. The number of steps was also reduced to three as compared with four in protocol 1. This resulted in the higher energy efficiency and reduction of time, as total heating time was just 60–200 s under microwaves in protocol 2 as compared with 6–9 h in protocol 1. Among the various green chemistry matrices, *E*-factor values for protocols 1 and 2 were 20.1 and 14.8 kg waste/kg product, respectively, showing the reduction of 26.4% in the waste produced over the production of 1 kg of the product. Atom economy was increased by 5% as it was 43.3 and 48.3% for protocols 1 and 2, respectively. Atom efficiency, which considers both atom economy and yield parameters, showed an improvement of 7.7%. Carbon efficiency was found to be 28.5 and 34.7% for protocols 1 and 2, respectively, and it was improved by 6.2%. Reaction mass efficiency possessed the values of 31.5 (protocol 1) and 36.1 (protocol 2) as well as an increase of 4.6% was observed.

Results obtained in the *in vitro* soil incubation study are described in the Table 4. All the test compounds showed significantly higher ammonium-N and lower nitrate-N content as compared with urea alone The nitrite-N content remained insignificant (<0.5 mg/kg) in all the samples on all the sampling days.

All the compounds have been found to be effective nitrification inhibitors showing 49.5–79.7%, 36.8–78.8%, 42.4–78.5%, and 24.6–76.85% nitrification inhibition (NI) at 7th, 14th, 21st, and 28th days, respectively (Table 4). 4-Amino-1,2-4-triazole (ATC), the reference inhibitor at 10% dose, showed 75.3%, 75.7%, 77.2%, and 73.7% inhibitory activity on 7th, 14th, 21st, and 28th days, respectively, whereas the corresponding data on 5% dose were 65.9%, 65.5%, 65.3%, and 59.2% as well as at 1% dose were 60.9%, 57.4%, 54.6%, and 39.0% on 7th, 14th, 21st, and 28th days, respectively.

Among the series, 5-heptyl-1,3,4-thiadiazole-2-thiol (**ii**), 5-(2-chloro phenyl)-1,3,4-thiadiazole-2-thiol (**vi**), 5-(2,4,-dichloro phenyl)-1,3,4-thiadiazole-2-thiol (**vii**), 5-(2-methyl phenyl)-1,3,4-thiadiazole-2-thiol (**viii**), 5-(3-methyl phenyl)-1,3,4-thiadiazole-2-thiol (**ix**), 5-(3,4,-dimethoxy phenyl)-1,3,4-thiadiazole-2-thiol (**xii**), 5-(2-hydroxy phenyl)-1,3,4-thiadiazole-2-thiol (**xiii**), and 5-(4-hydroxy-3-methoxy phenyl)-1,3,4-thiadiazole-2-thiol

Table 1

Physical and spectral data of 5-substituted-1,3,4-thiadiazole-2-thiols.

S. no.	Melting Point Obs./Lit.	% Yield Protocol 1 (Protocol 2)	IR (cm^{-1}) (C=N)	^{1}H NMR (DMSO-d_6 + $CDCl_3$ δ, ppm)	^{13}C NMR (DMSO-d_6 + $CDCl_3$ δ, ppm)
i.	182–184/184–187 [22]	53.1 (79.9)	1553	2.43 (s, 3H, CH_3), 14.16 (s,1H, 2-SH)	19.9 (CH_3), 160 (5 C=N), 190 (2 C=N)
ii.	97–98/95–96 [22]	58.4 (63.2)	1548	0.89 (t, 3H, 7′-CH_3), 1.29 (m, 8H, 4 × CH_2), 1.63 (m, 2H, 2′-CH_2), 2.35 (t, 2H, 1′-CH_2), 12.9 (s, 1H, 2-SH)	14.0 (CH_3), 23.1 (CH_2), 32.5 (CH_2), 30.0 (CH_2), 29.9 (CH_2), 32.1 (CH_2), 29.6, (CH_2) 155 (5 C=N), 187 (2 C=N)
iii.	105–108/105–107 [22]	49.5 (65.4)	1540	0.88 (t, 3H, 9′CH_3), 1.27 (m, 12H, 6 × CH_2), 1.61 (m, 2H, 2′-CH_2), 2.34 (t, 2H, 1′-CH_2), 12.8 (s, 1H, 2-SH)	14.2 (CH_2), 23.3 (CH_2), 32.6 (CH_2), 30.0 (CH_2), 29.3 (CH_2), 30.2 (CH_2), 31.2 (CH_2), 31.5 (CH_2), 34.1 (CH_2), 154 (5C=N), 182 (2 C=N)
iv.	123–126/123–125 [22]	51.7 (60.3)	1535	0.88 (t, 3H, 11′-CH_3), 1.26 (m, 16H, 8 × CH_2), 1.62 (m, 2H, 2′-CH_2), 2.34 (t, 2H, 1′ CH_2), 12.7 (s, 1H, 2-SH)	15.1 (CH_3), 24.1 (CH_2), 30.1 (CH_2), 31.2 (CH_2), 29.5 (CH_2), 31.2 (CH_2), 33.0 (CH_2), 31.2 (CH_2), 30.5 (CH_2), 32.1 (CH_2), 34.1 (CH_2), 151 (5 C=N), 180 (2 C=N)
v.	214–216/215–216 [23]	59.4 (77.0)	1572	7.51–7.55 (m, 1H, Ar—H), 7.59 (dd, 2H, Ar—H, J = 8.0 MHz), 7.94–7.96 (m, 2H, Ar—H) 11.0 (s, 1H, SH)	122.81 (Ar—C), 126.42 (Ar—C) 126.91(Ar—C), 129.82 (Ar—C), 132.0 (Ar—C), 160.90 (Ar—C), 177.8 (5 C=N), 192 (2 C=N)
vi.	212–214/210–212 [24]	57.9 (68.3)	1575	7.90 (d, 2H, Ar—H), 7.48 (d, 2H, Ar—H), 13.16 (s, 1H, SH)	129.1 (Ar—C), 130.0 (2 Ar—C), 131.5 (2 Ar—C), 138.2 (Ar—C), 166.9 (5 C=N), 179.23 (2 C=N)
vii.	197–201	57.1 (60.2)	1581	7.32–7.39 (m, 1H, Ar—H), 7.57 (d, 1H, Ar—H), 8.2 (d, 1H, Ar—H), 13.98 (s, 1H, SH)	126.58 (Ar—C), 127.20 (Ar—C), 131.47 (Ar—C), 133.55 (Ar—C), 136.02 (Ar—C), 139.62 (Ar—C), 169.60 (5 C=N), 182.46 (2 C=N)
viii.	142–143	54.5 (59.3)	1574	2.70 (s, 3H, CH_3), 8.12–8.31 (m, 1H, Ar—H), 7.92–8.1 (m, 1H, Ar—H), 7.46–7.49 (m, 1H, Ar—H), 7.29–7.32 (m, 1H, Ar—H), 12.28 (s, 1H, SH)	21.19 (CH_3), 125.59 (Ar—C), 129.71 (Ar—C), 131.98 (2Ar—C), 132.79 (Ar—C), 141.4 (Ar—C), 173.76 (5 C=N), 186.43 (2 C=N)
ix.	161–162	53.3 (61.2)	1569	2.43 (s, 3H,CH_3), 7.26 (d, H, Ar—H), 7.41 (dd, Ar—H, J = 9.6 MHZ) 7.49 (d, 1H, Ar—H), 7.94 (d, 1H, Ar—H), 12.68 (s, 1H, SH)	21.28 (CH_3), 127.39 (Ar—C), 128.39 (Ar—C), 129.22 (Ar—C), 130.72 (Ar—C), 134.63 (Ar—C), 138.32 (Ar—C), 172.65 (5 C=N), 183.24 (2 C=N)
x.	180–183	57.1 (67.4)	1561	2.28 (s, 3H, CH_3), 7.19 (d, 2H, Ar—H), 7.81 (d, 2H, Ar—H), 12.65 (s, 1H, SH)	21.42 (CH_3), 128.48 (Ar—C), 129.43 (2 Ar—C), 129.75 (2 Ar—C), 143.34 (Ar—C), 167.8 (5 C=N), 176.34 (2 C=N)
xi.	223–225/222–224 [24]	56.2 (65.3)	1573	3.72 (s, 3H, CH_3), 7.31 (d, 2H, Ar—H), 7.68 (d, 2H, Ar—H), 12.36 (s, 1H, SH)	48.24 (OCH_3), 111.30 (2 Ar—C), 121.35 (2 Ar—C), 139.75 (Ar—C), 147.34 (Ar—C), 164.8 (5 C=N), 178.34 (2 C=N)
xii.	240–242	53.6 (58.2)	1577	3.9 (s, 3H, OCH_3), 4.8 (s, 3H, OCH_3), 6.92 (d, 1H, Ar—H), 7.59 (s, 1H, Ar—H), 7.76–7.78 (dd, Ar—H, J = 6.81), 12.76 (s, 1H, SH)	55.98 (OCH_3), 56.04 (OCH_3), 110.30 (Ar—C), 112.22 (Ar—C), 121.70 (Ar—C), 124.59 (Ar—C), 148.61 (Ar—C), 153.45 (Ar—C), 172.03 (5C=N), 183.23 (2 C=N)
xiii.	232–235	55.5 (67.3)	1582	5.2 (s, 1H, OH), 6.83–6.91 (m, 1H, Ar—H), 7.41–7.45 (m, 1H, Ar—H), 7.76 (d, 1H, Ar—H), 7.78 (d, 1H, Ar—H), 11.38 (s, 1H, SH)	113.26 (Ar—C), 117.20 (Ar—C), 119.47 (Ar—C), 130.67 (Ar—C), 135.96 (Ar—C), 161.16 (Ar—C), 172.47 (5 C=N), 183.24 (2 C=N)

(*Continued*)

Table 1
(*Continued*)

S. no.	Melting Point Obs./Lit.	% Yield Protocol 1 (Protocol 2)	IR (cm^{-1}) (C=N)	^{1}H NMR (DMSO-d_6 + $CDCl_3$ δ, ppm)	^{13}C NMR (DMSO-d_6 + $CDCl_3$ δ, ppm)
xiv.	210–214	57.9 (59.3)	1579	3.89 (s, 1H, Ar—OH), 3.8 (s, 3H, OCH_3), 6.63 (d, 1H, Ar—H), 6.83–6.99 (m, 1H), 7.03 (d, 1H), 9.82 (s, 1H, OH), 12.43 (s, 1H, SH)	55.87 (CH_3), 113.05 (Ar—C), 115.46 (Ar—C), 122.03 (Ar—C), 151.45 (Ar—C), 167.72 (5C=N), 188.7 (2C=N)
xv.	167–169	53.3 (60.9)	1576	1.34 (s, 9H, *t*-C_4H_9), 7.46 (d, 2H, Ar—H), 8.03 (d, 2H, Ar—H), 13.39 (s, 1H, SH)	14.3 (Aliph-C), 29.7 (Aliph-C), 31.1 (Aliph-C), 35.2 (Aliph-C), 125.3 (Ar—C), 125.5 (Ar—C), 126.5 (Ar—C), 129.4 (Ar—C), 130.1 (Ar—C), 157.58 (Ar—C), 172.15 (5 C=N), 183.92 (2 C=N)

(**xiv**) were found to be the promising nitrification inhibitors.

Among the active series, 5-(3-methyl phenyl)-1,3,4-thiadiazole-2-thiol (**ix**) was most active at 10% dose with NI 78.2%, 77.1%, 77.3%, and 76.8% on 7th, 14th, 21st, and 28th days, respectively. The next active compound was 5-(2-chloro phenyl)-1,3,4-thiadiazole-2-thiol (**vi**) with activity at 10% was 79.7%,78.8%, 77.7%, and 75.1% on 7th, 14th, 21st and 28th days, respectively (Table 4).

Other active compounds (**vii**), (**xii**), (**xiii**), (**viii**), (**ii**), and (**xiv**) were statistically at par with ATC as evident from least significant difference (LSD) values. Compound (**xv**) showed 77.3%, 76.6%, 78.5%, and 68.4% NI on 7th, 14th, 21st, and 28th days, respectively. It performed statistically at par with ATC on 7th, 14th, 21st, but its activity was lower on 28th day. Rest of the compounds was less active at 10% doses than the reference inhibitor ATC. Among them, least active was compound (**iv**) with activity of 58% on the 28th day at 10% dose.

Among the active series compound (**vi**) was most active at 5% dose with NI 74.1, 73.5, 72.3, and 65.4 on 7th, 14th, 21st, and 28th days, respectively (Table 4). It performed better than ATC (5%) and statistically at par with ATC (10%) on 7th and 14th day. The next active compound was (**vii**) at 5% dose with NI 72.5%, 71.5%, 72.1%, and 63.1% on 7th, 14th, 21st, and 28th days, respectively. Other active compounds showed NI in the range of 56.1–73.8% during the entire period of incubation. All these compounds were statistically similar to ATC (5%). Remaining compounds were found to be inferior to reference inhibitor ATC (5%).

Compound (**vi**) at 5% dose performed best with NI 66.3%, 62.9%, 54.4%, and 41.4% on 7th, 14th, 21st, and 28th days, respectively. The next in performance were (**x**), (**xiv**), (**viii**), (**vii**), (**ii**), and (**xii**). All these

Table 2
Elemental-analytical data of 5-substituted-1,3,4-thiadiazole-2-thiols.

			C (%)		H (%)		N (%)		S (%)	
S. no.	Molecular formula	FW	Cal	Obs	Cal	Obs	Cal	Obs	Cal	Obs
i.	$C_3H_4N_2S_2$	132	27.25	28.2	3.04	2.99	21.19	20.83	48.51	47.21
ii.	$C_9H_{16}N_2S_2$	216	49.96	47.73	7.45	7.23	12.95	13.32	29.64	30.12.
iii.	$C_{11}H_{20}N_2S_2$	244	54.05	52.5	8.25	8.99	11.46	11.21	26.24	27.16
iv.	$C_{13}H_{24}N_2S_2$	272	57.30	59.29	8.88	7.59	10.28	9.73	23.54	22.24
v.	$C_8H_6N_2S_2$	194	46.46	44.70	3.11	2.97	14.42	13.63	33.01	31.42
vi.	$C_8H_5N_2S_2Cl$	228.5	42.01	40.26	2.20	2.09	12.25	12.85	28.04	26.52
vii.	$C_8H_4N_2S_2Cl_2$	263	36.51	34.76	1.53	1.60	10.64	9.83	24.37	23.56
viii.	$C_9H_8N_2S_2$	208	51.89	50.36	3.87	3.19	13.45	14.05	30.79	29.23
ix.	$C_9H_8N_2S_2$	208	51.89	49.12	3.87	3.64	13.45	12.86	30.79	31.03
x.	$C_9H_8N_2S_2$	208	51.89	49.64	3.87	3.61	13.45	12.32	30.79	31.63
xi.	$C_9H_8ON_2S_2$	224	48.19	46.21	3.59	3.32	12.49	12.93	28.59	26.35
xii.	$C_{10}H_{10}O_2N_2S_2$	254	47.23	45.06	3.96	3.41	11.01	12.51	25.22	24.89
xiii.	$C_8H_6ON_2S_2$	210	45.69	44.26	2.88	3.19	13.32	14.26	30.50	29.12
xiv.	$C_9H_8O_2N_2S_2$	240	44.98	42.91	3.36	2.98	11.66	12.59	26.69	25.36
xv.	$C_{12}H_{14}N_2S_2$	250	57.56	55.63	5.64	5.31	11.19	10.70	25.61	25.13

Table 3

Comparative assessment of protocols 1 and 2 using green chemistry measures.

Matrix	Protocol 1 (Conventional)	Protocol 2 (Greener)	Improvement
Overall yield (%)	59.6	69.3	9.7% increase
Number of steps	Four	Three	25% reduction
Heating time	6–9 h	60–200 s	162–360 times decrease
Energy consumption (KWh)	6–9	0.015–0.050	180–400 times reduction
E(nvironmental) factor (Kg waste/Kg product)	20.1	14.8	26.4% reduction
Atom economy (%)	43.3	48.3	5% increase
Atom efficiency (%)	25.8	33.5	7.7% increase
Carbon efficiency (%)	28.5	34.7	6.2% increase
Reaction mass efficiency (%)	31.5	36.1	4.6% increase

compounds were statistically at per with each other (Table 4). All the compounds showed an increase in NI with the increase in dose. Enhancement in NI is more from 1 to 5% increases in dose as compared with 5 to 10%.

Structure-activity relationship. In general, aryl substituted thiadiazoles performed better than the alkyl substituted 1,3,4-thiadiazole-2-thiols at all the doses. The 5-substitution in the 1,3,4-thiadiazole with aliphatic chains of 1, 7, 9, and 11 carbon atoms were used. The chain aliphatic seven of carbon atoms performed the best as evident from their performance on 28th day of incubation. The overall effect in minimizing NI was significantly higher with seven carbon atoms with 69.8 and 63.1% as compared with 58.1–64.4% and 52.9–56.2% with others at 10 and 5% doses, respectively (Fig. 1). At 1% dose, maximum NI, 36.2%, was observed with seven carbon atoms, but it was superior to NI with one carbon atom, 25.7% only and performed at par with others.

5-Aryl substitution in the 1,3,4-thiadiazole ring with chloro atoms in phenyl ring was used. Introduction of chlorine atoms in the phenyl ring resulted in the increase of activity and found to be significantly superior to phenyl substitution with no chlorine atom (Fig. 2). Their effect on NI was similar for both mono and dichloro phenyl derivatives (Fig. 2), which were superior to phenyl derivative. Both chloro derivative showed 36.2–41.4%, 63.8–65.4%, and 73.6–75.1% NI at 1, 5, and 10% dose, respectively. The phenyl derivative showed 24.6%, 43.6%, and 60.8% NI at respective 1, 5 and 10% doses.

5-(3-Methyl phenyl)-1,3,4-thiadiazole-2-thiol, 5-(4-chloro phenyl)-1,3,4-thiadiazole-2-thiol and 5-(2,4-dichlorophenyl)-1,3,4-thiadiazole-2-thiol emerged as potent nitrification inhibitors. The study revealed the cost effective and environment friendly synthesis of the potent 1,3,4-thiadizole-2-thiols. These compounds hold promise to be used as nitrification inhibitors and also as prototypes for the discovery of potent analogues through the process of compound library design and screening.

EXPERIMENTAL

Thin layer chromatography was performed on 200-μm thick aluminum sheets having silica gel 60 F_{254} as adsorbent. Different solvent systems were used for developing the TLC plates. Spots were visualized either by UV-light or iodine vapors. Melting points were determined by using electro thermal melting point apparatus and are uncorrected. Microwave irradiation was carried out by using Samsung microwave oven model CE118KF operating at 2450 MHz. A Christ Lypholizer model Alpha 1-2 LD was used for lyophilizing purpose. Nuclear magnetic resonance (NMR) spectra were recorded on a Brucker Abamce, 400 MHz instrument, after dissolving the samples in $CDCl_3$/DMSO(d_6) using tetramethylsilane (TMS) as an internal standard. Chemical shifts were reported in δ values relative to TMS and the notations used are s-singlet, d-doublet, t-triplet, m-multiplet, and brs-broad singlet. Infrared (IR) spectra were recorded on a Nicolet Fourier Transform Infrared Spectrophotometer, Model Impact 400 (FTIR) in either nujol mull or KBr disc. A Varian, Series 634, UV–Vis double beam spectrophotometer was used. Elemental analysis was carried out using EuroVector CHNS analyzer. All the chemicals and reagents were purchased from S D Fine Chemicals, Qualigens Fine Chemicals, and Merck India.

Synthesis of test chemicals. 5-Substituted-1,3,4-thiadiazole-2-thiols were synthesized following the Scheme 1 using the both conventional (protocol 1) and greener synthesis (protocol 2). Protocol 2 used the new greener method [18] for synthesizing the intermediate hydrazides which otherwise required 6–9 h heating under protocol 1 [13].

Conventional method of synthesis of carboxylic acid hydrazides (Method-A). The organic acids were esterified in the presence of ethyl alcohol and catalytic amount of sulfuric acid. The resulted esters were hydrazinolysed with hydrazine hydrate to afford the hydrazides [13].

Greener method for synthesis of carboxylic acid hydrazides (Method B). Carboxylic acid (0.01 mol) and hydrazine hydrate (0.012 mol) were irradiated under microwaves for 60–200 s at 900 W. Then, the reaction mixture was cooled to −20°C and lyophilized at −50°C. The product obtained was recrystallized from methyl alcohol. The hydrazides were characterized on the basis of physical and spectral data [13–17].

Preparation of 5-substituted-1,3,4-thiadiazole-2-thiols. Potassium hydroxide (0.11 mol) was dissolved in minimum amount of ethanol, and hydrazide (0.1 mol) was added to it. The reaction mixture was cooled to 0–5°C followed by

Table 4

Effect of 5-substituted-1,3,4-thiadiazole-2-thiols on nitrification inhibition (NI).

			Nitrification inhibition (%)			
Comp.	R	Dose	7th Day	14th Day	21st Day	28th Day
i.	CH_3—	1	54.7	36.8	44.1	25.7
		5	69.3	66.9	63.9	56.1
		10	76.8	74.1	75.4	64.4
ii.	$CH_3(CH_2)_5CH_2$—	1	59.1	53.9	51.2	36.2
		5	70.1	67.9	68.6	63.1
		10	76.1	76.0	77.0	69.8
iii.	$CH_3(CH_2)_7CH_2$—	1	52.7	46.3	46.9	32.8
		5	65.3	61.1	59.0	52.9
		10	73.9	69.4	68.0	63.0
iv.	$CH_3(CH_2)_9CH_2$—	1	49.5	40.6	42.4	32.8
		5	69.8	64.7	57.8	56.2
		10	75.8	73.0	74.7	58.1
v.	C_6H_5—	1	58.0	44.3	48.0	24.6
		5	61.9	58.4	56.3	43.6
		10	65.5	65.0	67.6	60.8
vi.	4-ClC_6H_4—	1	66.3	62.9	54.4	41.4
		5	74.1	73.5	72.3	65.4
		10	79.7	78.8	77.7	75.1
vii.	2,4-$(Cl)_2C_6H_3$—	1	61.3	54.1	53.5	36.2
		5	72.5	71.5	72.1	63.8
		10	78.4	76.6	76.7	73.6
viii.	2-$CH_3C_6H_4$—	1	65.8	61.2	54.6	37.9
		5	64.6	64.4	65.3	59.0
		10	72.4	71.2	72.4	70.4
ix.	3-$CH_3C_6H_4$—	1	63.4	60.1	52.7	39.1
		5	72.9	72.3	70.7	63.7
		10	78.2	77.1	77.3	76.8
x.	4-$CH_3C_6H_4$—	1	68.5	62.2	55.1	33.2
		5	73.7	72.3	68.7	61.7
		10	79.4	77.7	76.0	67.1
xi.	4-$CH_3C_6H_4$—	1	53.8	51.7	48.8	29.7
		5	62.6	64.2	63.0	52.8
		10	68.0	69.4	71.6	67.0
xii.	3,4-$(CH_3O)_2C_6H_3$—	1	58.9	52.9	51.3	34.4
		5	70.6	68.7	68.1	63.2
		10	77.3	75.1	75.7	71.9
xiii.	2-HOC_6H_4—	1	58.3	51.6	49.4	32.7
		5	72.3	69.9	67.0	59.2
		10	73.3	71.4	71.2	70.2
xiv.	4-HO, 3-$CH_3OC_6H_3$—	1	65.3	57.0	51.5	38.3
		5	68.8	67.7	61.3	62.5
		10	76.9	75.3	72.4	70.0
xv.	4-*t*-$C_4H_9C_6H_4$—	1	58.1	51.6	44.8	32.9
		5	73.8	71.5	69.8	60.1
		10	77.3	76.6	78.5	68.4
ATC	–	1	60.9	57.4	54.6	39.0
		5	65.9	65.5	65.3	59.2
		10	75.3	75.7	77.2	73.7
LSD (5%)		–	4.7	5.0	4.6	5.0

drop wise addition of carbon disulphide (0.11 mol). After addition, the reaction mixture was stirred for 30 min to afford solid potassium dithiocarbazate salt. It was filtered, washed with chilled acetone, dried, and used as such for further reaction. Potassium dithiocarbazate salt (0.1 mol) was added slowly in small lots to conc sulfuric acid (2.5 times of salt) at 5°C with constant stirring. The reaction mixture was stirred for 30 min, and the resulting viscous liquid was poured over crushed ice slowly. The solid obtained was filtered and washed with excess of water till the filtrate become neutral to litmus paper. The wet solid was suspended in water at 40°C and 25% sodium hydroxide solution was added slowly with stirring to adjust the pH of the solution in the range of 8.0–9.0. The resulting solution was filtered through a charcoal bed, and pH was adjusted

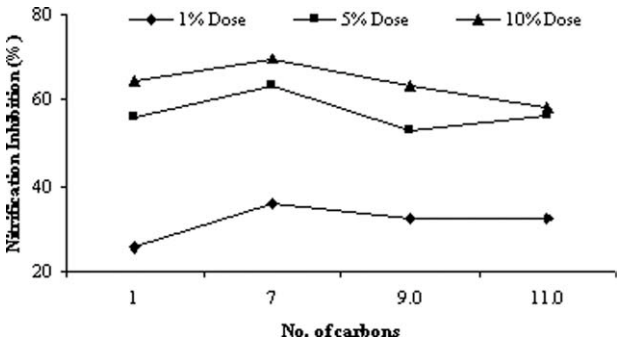


Figure 1. Effect of number of aliphatic C-atoms at 5-position of 1,3,4-thiadiazole-2-thiol on nitrification inhibition.

to 3.0–5.0 by drop wise addition of 50% hydrochloric acid. White solid obtained was filtered, washed with water, and dried. All the compounds were characterized on the basis of physical and spectral data as depicted in Tables 1 and 2.

Assessment by green chemistry matrices. The developed synthetic protocol for 5-substituted-1,3,4-thiadiazole-2-thiols was assessed by following green chemistry matrices, which were calculated as reported [19–21].

Nitrification inhibitory activity. The soil with following properties was collected from the farm of the institute for *in vitro* incubation experiments. Sand 60.8%, silt 18.7%, clay 20.5%, water holding capacity 35.5%, bulk density 1.51 mg/kg, organic C 0.5%, available N 553.72%, ammonium-N 3.2 mg/kg, nitrite-N traces, nitrate-N 8.54 mg/kg, pH (soil: water::1:2.5) 7.9, and EC at 25°C 0.35 dSm^{-1}.

The test chemicals (5-substituted-1,3,4-thiadiazole-2-thiols) and reference inhibitor, ATC, were tested at three doses (1, 5 and 10% of applied urea-N) along with urea alone control.

The experiments were laid following completely randomized design with three replicates. Fifty grams of air dried, finely ground, and sieved (10 mesh) soil was taken in 100 mL capacity plastic beakers. Calculated amount of the test chemical (0.1, 0.5, and 1.0 mg for 1, 5, and 10% dose of applied urea-N, respectively) in acetone was added to each beaker and mixed thoroughly. In all the treatments including control, same volume of acetone was added. After mixing, 10 mg urea-N (200 mg urea-N per kg of soil) in aqueous solution was added, mixed thoroughly, and distilled water was added to each beaker for maintaining the moisture at 50% of water holding capacity [25] of the soil. The experiment was conducted in triplicate with concomitant controls. For ATC, the soil was prepared in similar way. All the beakers were accurately weighed, labeled, and incubated in a BOD incubator at 28 ± 1°C, and 98% relative humidity. Soil moisture was maintained by adding distilled water every alternate day (if required) after taking the difference of weight. Samples (5 g) were drawn on 7th, 14th, 21st, and 28th day of incubation. Before sampling, distilled water was added to make up for the loss in weight because of evaporation of water and mixed thoroughly. Ammonium, nitrite, and nitrate-N were extracted in 50 mL 2*M* aqueous sodium sulfate solution. The soil with extracting solution was shaken for an hour on a reciprocal shaker and filtered. Ammonium, nitrite, and nitrate-N were estimated following Indophenol blue, sulfanilic acid, and phenol disulfonic acid method, respectively [26,27].

The contents of ammonium nitrate and nitrite-N were obtained from the standard curves and expressed in mg kg^{-1}. The nitrification rate for a constant period of incubation was calculated using Sahrawat's [28] formula. The data were statistically analyzed following the procedure laid out by Gomez and Gomez [29]. The analysis of variance was computed using Statistical Package for Social Services (SPSS version 10.0), and treatment means were compared by LSD at 5% levels.

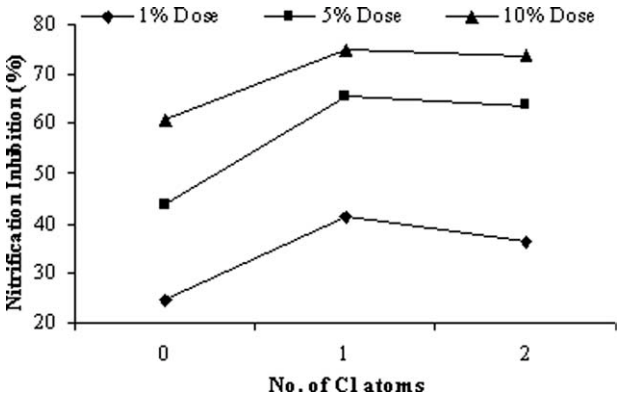


Figure 2. Effect of number of chlorine atoms in phenyl ring at 5-position of 1,3,4-thiadiazole-2-thiol on nitrification inhibition.

REFERENCES AND NOTES

[1] Contribution number 992.

[2] (a) Kidwai, M. Pure Appl Chem 2006, 78, 1983; (b) Anastas, P. T.; Warner, J. C. Green Chemistry: Theory and Practice; Oxford University Press: New York, 2000; p 135; (c) Ahluwalia, V. K.; Kidwai, M. New Trends in Green Chemistry; Kluwer Academic Publishers: Netherland, 2004; p 292; (d) Matlack, A. S. Introduction to Green Chemistry; Marcel Dekker Inc., CRC Press: New York, 2001; p 551; (e) Lancester, M. Green Chemistry: An Introductory Text; Royal Society of Chemistry: Cambridge, 2002; p 310; (f) Clark, J. H.; Macquarrie, D. Handbook of Green Chemistry and Technology; Blackwell Publishers: Oxford, 2002; p 540.

[3] Cusumano, J. A. J Chem Educ 1995, 72, 959.

[4] Iles, A. Bus Strat Env 2008, 17, 524.

[5] (a) Jaquith, J. B.; Villeneuve, G.; Boudreault, A.; Morris, S. J.; Durkin, J.; Gillard, J. W.; Hewitt, K. E.; Marsh, H. N. U.S. Patent 2009/0042953 A1, February 12, 2009; (b) Pattanayak, P.; Sharma, R.; Sahoo, P. K. Med Chem Res 2009, 18, 351; (c) Sainy, J.; Mishra, G. P.; Sharma, R.; Chaturvedi, S. C. Pharmaceut Chem J 2009, 43, 19; (d) Matysiak, J. Chem Pharm Bull (Tokyo) 2006, 54, 988; (e) Schenone, S.; Brullo, C.; Bruno, O.; Bondavalli, F.; Ranise, A.; Filippelli, W.; Rinaldi, B.; Capuano, A.; Falcone, G. Bioorg Med Chem 2006, 14, 1698. (f) Foroumadia, A.; Emamic, S.; Hassanzadehb, A.; Rajaeeb, M.; Sokhanvarb, K.; Hassan, M.; Shafieea, A. Bioorg Med Chem Lett 2005, 15, 4488; (g) Oruc, E. E.; Rollas, S.; Kandemirli, F.; Shvets, N.; Dimoglo, A. S. Bioorg Med Chem Lett 2004, 12, 5651; (h) Clerici, F.; Pocar, D. J. Med Chem 2001, 44, 931; (i) Chapleo, C. B.; Myers, M.; Myers, P. L.; Saville, J. F.; Smith, A. C. B.; Stillings, M. R.; Tulloch, I. F.; Walter, D. S.; Welbourn, A. P. J Med Chem 1986, 29, 2273; (j) Mullican, M. D.; Wilson, M. W.; Connor, D. T.; Kostlan, C. R.; Schrier, D. J. J Med Chem 1993, 36, 1090.

[6] Kariyone, H.; Harada, M.; Kurita, T.; Takana, J. J Antibiot 1970, 23, 131.

[7] Subbarao, G. V.; Ito, O.; Sahrawat, K. L.; Berry, W. L.; Nakahara, K.; Ishikawa, T.; Watanabe, T.; Suenaga, K.; Rondon, M.; Rao, I. M. Crit Rev Plant Sci 2006, 25, 305.

[8] Raun, W. R.; Johnson, G. V. Agron J 1999, 91, 357.

[9] Rao, E. V. S. P.; Puttana, K. Curr Sci 2006, 91, 1335.

[10] Prasad, R.; Power, J. F. Adv Agron 1995, 54, 233.

[11] Sahrawat, K. L.; Keeney, D. R. Commun Soil Sci Plant Anal 1985, 16, 517.

[12] McCarty, G. W. Biol Fertil Soil 1999, 29, 1.

[13] Furniss, B. S.; Rogers, V.; Smith, P. W. G.; Tatchell, A. R. Vogel's Textbook of Practical Organic Chemistry, 5th ed.; Longman Scientific and Technical: Essex England, 1989; pp 1342–1349.

[14] Jain, A. K.; Gupta, K. P.; Ganesan, K.; Pande, A.; Malhotra, R. C. Defence Sci J 2007, 57, 267.

[15] Toda, F.; Hyoda, S.; Okada, K.; Hirotsu, K. J Chem Soc Chem Commun 1995, 1531.

[16] Wagner, R. B.; Zook, H. D. Synthetic Organic Chemistry; Wiley: New York, 1953, p 569.

[17] Yale, H. L.; Losee, K.; Martins, J.; Holsing, M.; Perry, F. M.; Bernstein, J. J Am Chem Soc 1953, 75, 1933.

[18] Saha, A.; Kumar, R.; Kumar, R.; Devakumar, C. Towards green agrochemicals: Green synthesis of hydrazides. National Seminar on Green Chemistry and Natural Products, 26–27 November, Department of Chemistry, University of Delhi, Delhi, 2007, p 20.

[19] Constable, D. J. C.; Curzons, A. D.; Freitas dos Santos, L. M.; Geen, G. R.; Hannah, R. E.; Hayler, J. D.; Kitteringham, J.; McGuire, M. A.; Richardson, J. E.; Smith, P.; Webb, R. L.; Yu, M. Green Chem 2001, 3, 7.

[20] Constable, D. J. C.; Curzons, A. D.; Cunningham, V. L. Green Chem 2002, 4, 521.

[21] Curzons, A.; Constable, D.; Mortimer, D.; Cunningham, V. Green Chem 2001, 3, 1.

[22] Kidwai, M.; Kumar, R.; Goel, Y. Main Gp Met Chem 1997, 20, 367.

[23] Kidwai, M.; Bhushan, K. R. Indian J Chem 1998, 37B, 427.

[24] Baron, M.; Wilson, C. V. J Org Chem 1958, 28, 1021.

[25] Dastane, N. G. A Practical Manual for Water Use Research; Navbharat Prakashan: Poona (India), 1967; p 105.

[26] Keeney, D. R.; Nelson, D. W. anic forms. In Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties; Page, A. L., Ed.; Soil Science Society of America and American Society of Agronomy: Madison, WI, 1989; pp 643–698.

[27] Prasad, R. A Practical Manual for Soil Fertility; National Professor's Unit, Division of Agronomy, IARI, ICAR: New Delhi, India, 1998; pp 26–31.

[28] Sahrawat, K. L. Plant Soil 1980, 55, 487.

[29] Gomez, K. A.; Gomez, A. A. Statistical Procedures for Agricultural Research; Wiley: New York, 1984.