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Reactions of nitroxides. Part X: Antifungal activity of selected sulfur and selenium derivatives of 2,2,6,6-tetramethylpiperidine ☆

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ABSTRACT

The antifungal activity of nitroxyl radicals—derivatives of 2,2,6,6-tetramethylpiperidine-1-oxyl with reactive substituents 4-isothiocyanato-, 4-isocyano-, and 4-isoselenocyanato- and of *N*-formyl-, *N*-thioformyl-, *N*-selenoformyl-derivatives of 2,2,6,6-tetramethylpiperidine was investigated. Those of the above compounds, which contain a sulfur or selenium atom are the most active against four fungus plant patogens: *Botrytis cinerea*, *Fusarium culmorum*, *Phytophthora cactorum*, *Rhizoctonia solani*. 4-Isoselenocyanato-2,2,6,6-tetramethylpiperidine-1-oxyl proved to be the most active compound.

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Organothio and organoselenium compounds exhibit many interesting biological properties. Aliphatic isothiocyanates are present as glucosinolates in food in cruciferous vegetables (horseradish, mustard, cabbage, broccoli, Brussels sprouts). The breakdown of glucosinolates liberates dietary isothiocyanates: sulforaphane ($CH_3S(=O)(CH_2)_4NCS$), allyl isothiocyanate (mustard oil), benzyl and phenethyl isothiocyanates. The chemistry and mechanisms of anticarcinogenic activities of dietary isothiocyanates are reviewed.[2] In particular, the activities of sulforaphane (present in broccoli)[3] and aralkyl isothiocyanates[4,5] were reported. Lower alkyl isothiocyanates, allyl isothiocyanate, benzyl and phenethyl isothiocyanates were tested as protective compounds against gastric lesions induced by ethanol or acetylosalicylic acid. Allyl isothiocyanate exhibits strong protective activity against such lesion factors.[6] Sulforaphane and other isothiocyanates exhibit direct antibacterial activity against *Helicobacter pylori* and other bacterial and fungal pathogens,[7] and may prevent gastric cancer.[8] The effect of 2-(3,4-dihydroxyphenyl)ethyl isothiocyanate on a bacterial virus K was described.[9]

Aromatic isothiocyanates were tested in a wide spectrum of biological activities. Anthelmintic properties of a series of isothiocyanatophenyl derivatives of 1,2,4-oxadiazoles were reported.[10] A symmetric aryl diisothiocyanate ($(CH_2)_n(NHC(=S)NHC_6H_4NCS)_2$) and diisothiocyanatostilbenedisulfonic acid derivatives (DIDS and H_2DIDS) were examined as inhibitors of phospholipase C.[11] Aryl isothiocyanates were investigated as potential anticancer drugs due to their capability to create covalent bonds with bioreceptors.[12]

Selenium plays an important role as a trace element in living organisms (reviews[13]). Traces of selenium are important in oxygen metabolism. Organoselenium compounds are considered to be inhibitors of free radicals, as well as anti-inflammatory and antioxidant agents. Recently, selenoureas have been found to be effective superoxide radical scavengers.[14] Heterocyclic compounds containing the selenium atom may restrain cancer cell growth.[15] More generally, selenium and its relationship to cancer was reviewed.[16] The capability of organoselenium compounds to undergo biotransformations was also described.[17]

In general, isoselenocyanates are more active than their dietary thio analogs described above. The comparison of the same series of aralkyl isoselenocyanates with analogous aralkyl isothiocyanates shows that the antitumor activity of selenium compounds is higher than the activity of thio analogs.[18] Aralkyl isoselenocyanates ($C_6H_5(CH_2)_nNCSe$ $n = 1, 2, 4, 6$) are therapeutically effective for inhibiting melanoma tumor development by inducing the apoptosis in melanoma cells.[19]

The selenium analog of sulforaphane [$CH_3S(=O)(CH_2)_4NCSe$, 'selenoraphane'] seems to be more toxic to cancer cells than sulforaphane itself, but less toxic to normal cells.[20]

In our earlier papers, we described the synthesis and some pesticidal properties of thio- and seleno-analogs of some commonly known *N*,*N*-dimethyl-*N*′-aryl urea herbicides,[21] as well as thio and selenoureas bearing 2,2,6,6-tetramethylpiperidine-1-oxyl (nitroxyl) moiety.[22,23] In this Letter, we present the antifungal activity of reactive nitroxyl radicals with isothiocyanato, isocyano, and

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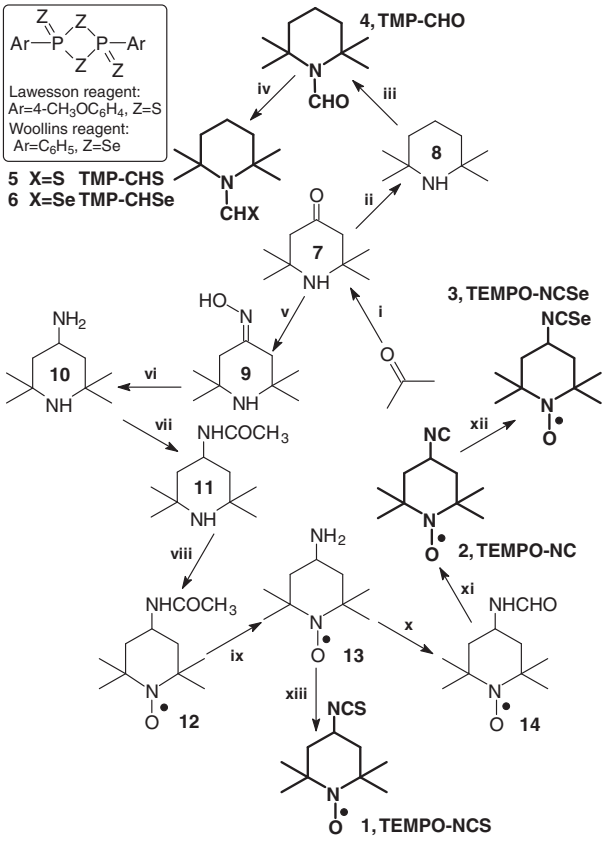
isoselenocyanato substituents, as well as of *N*-formyl, *N*-thioformyl, and *N*-selenoformyl derivatives of 2,2,6,6-tetramethylpiperidine.

4-Isothiocyanato-, 4-isocyano-, 4-isoselenocyanato-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO-NCS (**1**), TEMPO-NC (**2**), TEMPO-NCSe (**3**), respectively), and *N*-formylo-, *N*-thioformylo-, *N*-selenoformylo-2,2,6,6-tetramethylpiperidine (TMP-CHO (**4**), TMP-CHS (**5**), TMP-CHSe (**6**), respectively) were synthesized according to the methods presented in Scheme 1.

2,2,6,6-Tetramethyl-4-piperidinone (triacetonamine, **7**) was obtained from acetone, concentrated aqueous ammonia solution and ammonium chloride.[24–26] The Wolf–Kishner reduction of **7** with hydrazine in the presence of potassium hydroxide in diethylene glycol affords 2,2,6,6-tetramethylpiperidine (**8**).[25–27] Formylation of **8** with chloroform using PTC conditions affords TMP-CHO (**4**).[22] **4** was either thionized with Lawesson reagent to TMP-CHS (**5**),[22] or selenized with either P/Se or Woollins reagent to TMP-CHSe (**6**).[23] **7** was converted in a multistep synthesis to 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO-NH_2, **13**).[1,29–34] Reaction of **13** with thiophosgene affords TEMPO-NCS (**1**).[34] Formylation of **13** with ethyl formate[35] followed by dehydration of **14** with phosgene or diphosgene results in TEMPO-NC (**2**).[35] Selenization of **2** with metallic selenium affords TEMPO-NCSe (**3**).[23,36] The details of the synthetic protocols are presented in the above-mentioned references.



Scheme 1. Synthesis of compounds **1**–**14**. Reagents and conditions: (i) NH_3 (aq), NH_4Cl, three days, 26–33%;[24–26] (ii) 50% aqueous NH_2NH_2, potassium hydroxide, diethylene glycol, 127–200 °C, 75–82%;[25–27] (iii) $CHCl_3$, 50% NaOH aq, TEBACl, 50 °C, 5.5 h, 45%;[22] (iv) X = S: Lawesson reagent, toluene, reflux, 3.5 h, rt overnight, 28%;[22] X = Se: P/Se, xylene, reflux, 18% or Woollins reagent, toluene, rt, 18 h, 83%;[23] (v) $NH_2OH \times HCl$, CH_3COONa, H_2O, 18%;[28–30] (vi) Na, *n*-C_3H_7OH, reflux, 1 h, 80%;[30–33] (vii) $(CH_3CO)_2O$, diethyl ether, 5–10 °C, 3.5 h, 100%;[1,34] (viii) H_2O_2, WO_4^{2-}, rt, six days, 41%;[1,34] (ix) H_2O, KOH, reflux, 30 h, 93%;[1,34] (x) $HCOOC_2H_5$ (excess), reflux, 5 h, 96%;[35] (xi) $COCl_2$, $N(C_2H_5)_3$, CH_2Cl_2, 5–8 °C, 84%[35] or $ClCOOCCl_3$ (diphosgene), $N(C_2H_5)_3$, CH_2Cl_2, <10 °C, 78%;[23] (xii) Se, $CHCl_3$ reflux, 70 h, 38%;[23,36] (xiii) $CSCl_2$, $N(C_2H_5)_3$, benzene, 10–17 °C, 67%.[34]

Table 1
Evaluation of antifungal activity of **1**–**6**

Compd	*B. cinerea*, 200 mg/L in vitro	*F. culmorum*, 200 mg/L in vitro	*P. cactorum*, 200 mg/L in vitro	*R. solani*, 200 mg/L in vitro	*B. graminis*, 1000 mg/L in vivo
1	60	67	97	100	35
2	5	0	35	60	37
3	94	100	97	100	40
4	0	0	10	13	12
5	77	44	100	100	17
6	80	62	100	86	43

Reduction of colonies growth (%).

Table 2
IC_{50} values in mg/L of sulfur and selenium derivatives **1**, **3**, **5**, **6**

Compd	*B. cinerea*	*F. culmorum*	*P. cactorum*	*R. solani*
1	102.2	95.3	41.7	61.8
3	19.3	17.5	6.3	5.1
5	110.1	221.3	48.4	65.1
6	121.5	132.7	59.4	68.7

The pesticidal activity of TEMPO-NCS (**1**), TEMPO-NC (**2**), TEMPO-NCSe (**3**), TMP-CHO (**4**), TMP-CHS (**5**), TMP-CHSe (**6**) was investigated. The results of the antifungal activity against *Botrytis cinerea*, *Fusarium culmorum*, *Phytophthora cactorum*, *Rhizoctonia solani*, *Blumeria graminis* are presented in Table 1. The detailed procedure was presented in the previous paper.[22] The values of the inhibition index IC_{50} for the most active compounds **1**, **3**, **5**, **6** are presented in Table 2. The inhibition index IC_{50} (the concentration that inhibits 50% of mycelial growth) was calculated by the probit analysis.

All synthesized compounds (**1–6**) were inactive as herbicides and insecticides, but some of them showed antifungal activity. TEMPO-NCSe (**3**) proved to be the most active compound: it showed the maximum inhibitory effect on the mycelium growth for *B. cinerea*, *F. culmorum*, *P. cactorum*, *R. solani*. In particular, the strongest activity was found against two fungi *P. cactorum* and *R. solani* with IC_{50} values of 6.3 and 5.1, respectively.

TEMPO-NCS (**1**), TMP-CHS (**5**) and TMP-CHSe (**6**) exhibited similar activities: they induced a good inhibitory effect against *P. cactorum* and *R. solani*, whereas the growth of *B. cinerea* and *F. culmorum* was inhibited by approximately 44–80%. Generally, all compounds tested showed weak to moderate activity against powdery mildew on wheat *(B. graminis)* at concentration of 1000 mg/L.

In conclusion, 2,2,6,6-tetramethylpiperidine derivatives containing sulfur and selenium—nitroxyl radicals: 4-isothiocyanato-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO-NCS, **1**), 4-isoselenocyanato-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO-NCSe, **3**), and *N*-substituted compounds: 1-thioformyl-2,2,6,6-tetramethylpiperidine (TMP-CHS, **5**), and 1-selenoformyl-2,2,6,6-tetramethylpiperidine (TMP-CHSe, **6**) were found to exhibit the antifungal activity in vitro against four fungus species. In particular, TEMPO-NCSe (**3**) proved to be the most active among all compounds tested. The comparison of the antifungal activity of **4**–**6** indicates that incorporating a selenium or sulfur atom instead of the oxygen one may have a positive influence on the enhanced antifungal activity of the tested molecules.

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