Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 80 (11) 1367–1374 (2015) UDC 547.496.2–327:542.913:615.282–188
JSCS–4803 *Original scientific paper*

Facile synthesis and antifungal activity of dithiocarbamate derivatives bearing an amide moiety

YU-WEN LI* and SHU-TAO LI

School of Chemistry and Pharmacy, Qingdao Agricultural University, Qingdao, P. R. China

(Received 14 January, accepted 24 May 2015)

Abstract: Two series of novel dithiocarbamate derivatives bearing an amide moiety, **3a**–**i** and **4a**–**i**, were synthesized by a facile method, and the structures of the derivatives were confirmed by elemental analysis and ^{1}H-NMR, ^{13}C--NMR and high-resolution mass spectrometry (HRMS). Their antifungal activity against five phytopathogenic fungi were evaluated, and the results showed that most of the target compounds displayed low antifungal activity *in vitro* against *Gibberella zeae, Cytospora* sp., *Colletotrichum gloeosporioides, Alternaria solani,* and *Fusarium solani* at a concentration of 100 mg L^{-1}. However, two compounds, **4f** and **4g**, exhibited significant activity against *A. solani* and *C. gloeosporioides,* respectively.

Keywords: antifungal activity; dithiocarbamate derivatives; amide moiety; synthesis.

INTRODUCTION

Plant disease arising from phytopathogenic fungi is one of the major causes of severe losses in agriculture and horticulture crop production worldwide, and poses a threat to global food security.[1] In the past years, fungicides have contributed enormously to reduce crop loss caused by phytopathogenic fungi. However, the main problem associated with the application of fungicides is the emergence of fungicide resistance. Therefore, it is necessary to develop efficient fungicides with novel structures to obviate this resistance.

Due to their multiple biological profiles, carboxylic acid amide compounds have found application not only in medicinal chemistry but also in pesticide chemistry, *i.e.*, as insectides,[2–4] fungicides,[5] herbicides[6] and plant growth regulators.[7] Dithiocarbamate derivatives, on the other hand, have played important roles in medicinal and pesticide chemistry because of their diverse activities.[8–11] In pesticide chemistry, dithiocarbamate derivatives have also served as fungicides.[12] An important strategy for drug discovery has emerged that consists of

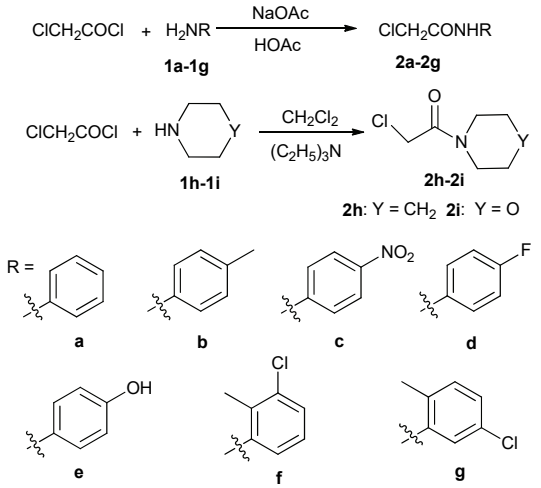
* Corresponding author. E-mail: ywli@qau.edu.cn
doi: 10.2298/JSC150114047L

hybridizing two bioactive molecules or pharmacophores to generate a novel class of molecules with a potentially stronger bioactivity profile.[13,14] Thus, inspired by the biological importance of carboxylic acid amides and dithiocarbamates as fungicides in the pesticide field, herein, the synthesis of novel carboxylic acid amide–dithiocarbamate hybrids **3a**–**i** and **4a**–**i** and their antifungal activities are reported.

RESULTS AND DISCUSSION

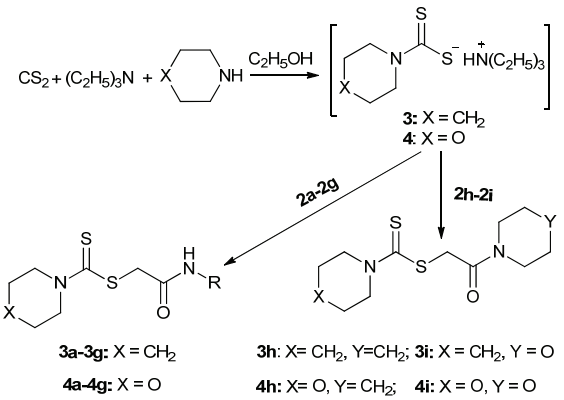
Chemistry

In this study, the starting materials, 2-chloroacetamides **2a**–**i**, were prepared according to the literature[15] with some modifications (Scheme 1). The intermediates **2a**–**g** were synthesized by treatment of 2-chloroacetyl chloride with **1a**–**g**, respectively, in a mixture of acetic acid and a saturated solution of sodium acetate. However, this procedure was unsuitable for the synthesis of intermediates **2h** and **2i** due to difficulties encountered in the separation and purification of the liquid **2h** and **2i** from the liquid mixture of HOAc and NaOAc. Thus, intermediates **2h** and **2i** were synthesized by reaction of 2-chloroacetyl chloride with **1h** and **1i**, respectively, in the presence of triethylamine as acid-scavenger and dichloromethane as solvent. With intermediates **2a**–**i** available, the target compounds **3a**–**i** and **4a**–**i** were synthesized. However, conventional synthesis of dithiocarbamates involves costly and toxic chemical reagents, such as thiophosgene.[16] Current strategies could partially alleviate the expense and toxicity for the synthesis by several one-pot syntheses *via* the reaction of carbon disulfide with amine and alkyl halides or acrylates.[17] Still, several drawbacks of these one-pot procedures remained inevitable, such as the use of strong bases, high reac-



Scheme 1. Synthesis of intermediates **2a**–**i**.

tion temperatures, long reaction time, and harmful organic solvents.[18] To circumvent the drawbacks associated with the previous procedures for the preparation of dithiocarbamates, the target compounds **3a**–**i** and **4a**–**i** were prepared according to Scheme 2.



Scheme 2. Synthesis of target compounds **3a**–**i** and **4a**–**i**.

As illustrated in Scheme 2, the synthesis method was improved such that the reactions of carbon disulfide, triethylamine and piperidine or morpholine were realized at 0 °C to obtain intermediates **3** and **4**, respectively, as white solids. To the thus-obtained intermediate**s 3** and **4**, absolute ethanol was added *in situ*, leading to the respective suspensions of **3** and **4** in absolute ethanol. Subsequently, reaction of the suspended **3** and **4** in ethanol with the intermediates **2a**–**i** at 50 °C led to the generation of the target compounds **3a**–**i** and **4a**–**i**, respectively. Upon completion of the reaction, **3a**–**i** and **4a**–**i** were *in situ* precipitated by cooling the corresponding reaction mixture down to 0 °C and collected by filtration. Since more or less **3a**–**i** or **4a**–**i** remained in the mother liquor, more sequential steps were indispensable to recover the residual **3a**–**i** and **4a**–**i** from their corresponding mother liquor. Firstly, evaporation of the mother liquor to dryness afforded a solid mixture containing the side-product triethylamine hydrochloride and residual **3a**–**i** and **4a**–**i**, respectively. Furthermore, given that triethylamine hydrochloride is not soluble in ether solvents while **3a**–**i** and **4a**–**i** are, the addition of 2-methyltetrahydrofuran to the thus-obtained solid mixtures left a white precipitate of triethylamine hydrochloride, a useful reagent in drug synthesis,[19] that was removed by filtration. The filtrates were then concentrated under reduced pressure to give the respective residuals **3a**–**i** and **4a**–**i**. Finally, recrystallization of the residuals **3a**–**i** and **4a**–**i** from 95 % ethanol led to the desired target compounds. The notable advantages of the present procedure over previous ones are that it is easy to conduct in terms of generation, separation, and purification of the target compounds **3a**–**i** and **4a**–**i** and is readily performed in one pot with only

one solvent by modulating the temperature in the range from 50 to 0 °C. In addition, taking the recovered **3a**–**i** and **4a**–**i** into account, the overall yields of the products were almost quantitative.

Antifungal activity: in vitro *screening of compounds **3a**–**3i** and **4a**–**4i***

All the newly synthesized target compounds **3a**–**i** and **4a**–**i** were evaluated *in vitro* for their antifungal activity against five phytopathogenic fungi, *i.e.*, *Gibberella zeae*, *Cytospora sp.*, *Colletotrichum gloeosporioides*, *Alternaria solani* and *Fusarium solani* at concentration of 100 mg L^{-1}. As summarized in Table I, most target compounds displayed low antifungal activities against these five phytopathogenic fungi at the indicated concentration with exception of compounds **4f** and **4g**. Compound **4f** displayed 77.26 % inhibition of *A. solani*, while compound **4g** exhibited 74.87 % inhibition of *C. gloeosporioides* at a concentration of 100 mg L^{-1}. To understand further the role of different groups of the compounds in conferring the antifungal activity, it is necessary to compare their structures. Structurally, compounds **3a**–**i** and **4a**–**i** are derived from same scaffold but with different substituents, leading to the difference in the antifungal activity. Generally, compounds **4a**–**4i** were superior to the corresponding compounds **3a**–**i** in terms of their antifungal activity, suggesting that the presence of morpholinyl group in **4a**–**i** conferred better antifungal activity than the corresponding piper-

TABLE I. Fungicidal activities of the target compounds **3a**–**i** and **4a**–**i** at a concentration of 100 mg L^{-1}; Hy – hymexazol

Compound	Antifungal activity (Inhibition rate, %)				
	G. zeae	*A. solani*	*C. gloeosporioides*	*Cytospora* sp.	*F. solani*
3a	33.41	24.52	20.25	5	26.75
3b	30.32	23.48	24.97	42.13	24.46
3c	34.42	21.47	14.81	36.29	34.79
3d	39.52	41.23	38.65	39.49	36.13
3e	21.78	30.14	23.41	31.73	35.47
3f	43.97	46.13	40.94	49.78	41.64
3g	40.27	43.94	39.08	46.66	39.35
3h	19.24	23.56	34.75	28.91	14.34
3i	27.88	29.76	30.51	32.14	30.09
4a	41.77	36.72	38.13	32.43	38.19
4b	46.18	39.76	30.67	51.09	32.08
4c	40.07	28.94	34.96	46.81	40.11
4d	47.14	45.97	47.08	52.13	43.93
4e	23.93	35.46	26.43	33.19	39.86
4f	58.11	77.26	59.04	57.89	53.98
4g	62.93	51.67	74.87	60.09	56.89
4h	30.08	32.77	47.69	38.06	45.77
4i	31.18	35.26	43.22	39.18	34.17
Hy	90.56	82.15	53.78	46.69	79.23

idinyl group in compounds **3a**–**i**. More interestingly, compounds **4f** and **4g**, bearing two substituents on the benzene ring, displayed significantly higher antifungal activities relative to the compounds with a single substituent on the benzene ring. In addition, the compounds with fluorine substituent on the benzene ring regardless of the substituent on the carbamic moiety, such as **3d** and **4d**, showed higher antifungal activities (although not dramatic) compared to other corresponding compounds without fluorine substituent on the benzene ring. Although most of the target compounds display low inhibition rate against mycelia growth of these five tested fungi at concentration of 100 mg/L, target compounds **4f**–**g** could be potential lead structures for further discovery of novel antifungal agrochemicals.

EXPERIMENTAL

Chemistry

All the employed chemicals were obtained from Qingdao Justness Reagent Company (China) and used without further purification. The melting points were measured using a WRS-1B digital melting point apparatus. The ^{1}H-NMR spectra were recorded on a Bruker DRX-400 Advance spectrometer at 400 MHz using TMS as an internal standard.

The physical, analytical and spectral data of the synthesized compounds are given in the Supplementary material to this paper.

*General procedure for the synthesis of compounds **2a**–**g***

A substituted aniline (34.3 mmol) was added to 12.5 mL of a saturated solution of sodium acetate, followed by 12.5 mL acetic acid. The suspension was cooled to 0 °C, and 2-chloroacetyl chloride (34.4 mmol, 2.75 ml) was added dropwise to the suspension at ≤5 °C. During the addition of 2-chloroacetyl chloride, the suspension dissipated and the mixture clarified. Before the addition of 2-chloroacetyl chloride was complete, a white precipitate began to form. Upon completion of the addition, the heterogeneous mixture was brought to 25 °C and stirred at room temperature for 2 h. The white precipitate was filtered, washed with distilled water (2×5 mL) and dried under vacuum to afford the crude product. Recrystallization of crude **2a**–**g** from absolute ethanol gave the desired **2a**–**g**, respectively. The physical, analytic and spectral data of **2a**–**g** are summarized in the Supplementary material to this paper.

*General procedure for synthesis of compounds **2h** and **2i***

To a stirred solution of triethylamine (17.2 mmol, 2.5 mL) and 17.2 mmol piperidine **1h** (or morpholine **1i**) in 10 mL CH_2Cl_2, 2-chloroacetyl chloride (17.2 mmol, 1.38 mL) was added dropwise at 0 °C. Upon completion of the dropwise addition, the solution was brought to room temperature and stirred for 2 h. Subsequently, the resulting solution was diluted with 20 mL CH_2Cl_2 and successively washed with 20 mL water and 20mL brine. The CH_2Cl_2 layer was separated and concentrated under reduced pressure to give crude **1h** (or **1i**) which was further purified by chromatography to afford the desired **1h** (or **1i**) as a yellowish oil. The physical, analytical and spectral data of **2h** and **2i** are summarized in the Supplementary material to this paper.

*General procedure for the synthesis of compounds **3a**–**i** and **4a**–**i***

To a solution of triethylamine (17.2 mmol, 1.5 mL) and 17.2 mmol piperidine **1h** (or morpholine **1i**), carbon disulfide (18.9 m mol, 1.2 mL) was added dropwise at 0 °C to form a white solid **3** (or **4**). Upon completion of the addition, 15 mL absolute ethanol was added to form a slurry of the white solid **3** (or **4**), to which 17.2 mmol respective intermediate **2a**–**i** was added. The mixture was heated to 50 °C to afford a clear solution. The clear solution was kept stirring at 50 °C for 3 h and then cooled to 0 °C. The cooled solution was kept for 2 h at 0 °C and the precipitate of **3a**–**i** (or **4a**–**i**) was collected by filtration and the corresponding filtrate was evaporated to dryness leading to a solid mixture of triethylamine hydrochloride and residual **3a**–**i** (or **4a**–**i**). Then, the addition of 2-methyltetrahydrofuran to the thus obtained solid mixture led to triethylamine hydrochloride by filtration. The corresponding filtrate was concentrated under vacuum to give the residual **3a**–**i** (or **4a**–**i**). Recrystallization of thus obtained residual **3a**–**i** (or **4a**–**i)** from 95 % ethanol led to the desired **3a**–**i** (or **4a**–**i**). Taking the recovered **3a**–**i** and **4a**–**i** into account, the overall yield of **3a**–**i** and **4a**–**i** was almost quantitative. The physical, analytical and spectral data for **3a**–**i** and **4a**–**i** are summarized in the Supplementary material to this paper.

Antifungal activity

Antifungal activities of target compounds **3a**–**i** and **4a**–**i** were evaluated *in vitro* against five phytopathogenic fungi (*Gibberella zeae*, *Cytospora* sp., *Colletotrichum gloeosporioides, Alternaria solani* and *Fusarium solani*) using the mycelium growth rate method.[20,21] All the fungi were provided by the Qingdao Agricultural University. The strains were retrieved from the storage tube and cultured for 2 weeks at 25 °C on potato dextrose agar (PDA).

The antifungal activity was assessed as follows: PDA medium was prepared in flasks and sterilized. The target compounds **3a**–**i** and **4a**–**i** were dissolved in acetone prior to mixing with molten agar at 55 °C, and the concentration of the target compounds **3a**–**i** and **4a**–**i** were 100 mg L^{-1}. The PDA medium was then poured into sterilized Petri dishes. The five fungi were incubated in PDA at 25 °C for 7 days to obtain new mycelium for the fungicidal assays, and a mycelia disk of 4 mm in diameter cut from culture medium was picked up with a sterilized inoculation needle and inoculated in the centre of the PDA Petri dishes. The inoculated Petri dishes were incubated at 25 °C ℃ for 3–4 days. Acetone was used as the control, and the commercially available agricultural fungicide hymexazol served as the positive control. Each compound was measured in three replicates, and each colony diameter of the three replicates was measured 4 times by the cross bracketing method. The inhibition rate (*IR*) was calculated according to the following formula:

$$IR(\%) = 100\frac{C-T}{C-4}$$

where *C* is the average diameter of mycelia in the blank test and *T* is the average diameter of mycelia on PDA treated with the target compounds.

CONCLUSIONS

In summary, a series of dithiocarbamates bearing an amide moiety **3a**–**i** and **4a**–**i** were synthesized in almost quantitative yield by a facile and convenient procedure. Especially, the synthesis, separation, and purification by recrystallization could be conducted in one-pot and in same medium by regulating the temperature from 50 to 0 °C. The results of the bioassay indicated that most target compounds

displayed low activities against *G. zeae*, *Cytospora* sp., *C. gloeosporioides*, *A. solani* and *F. solani* at a concentration of 100 mg L^{-1}. However, the compounds **4f** and **4g** gave significant inhibition rates against *A. solani* and *C. gloeosporioides*, respectively, at a concentration of 100 mg L^{-1}. Generally, the antifungal activities of compounds **4a**–**i**, which have a morpholinyl substituent, are superior to the corresponding compounds **3a**–**i** with the piperidinyl substituent. Additionally, the number of substituents on benzene ring influenced the antifungal activity as evidenced by the fact that the compounds bearing two substituents on the benzene ring, such as **3f** and **3g** and **4f** and **4g**, displayed better antifungal activities than the compounds with a single substituent on the benzene ring. Although most of the target compounds displayed low inhibition rates against mycelia growth of the five tested fungi at concentrations of 100 mg L^{-1}, the target compounds **4f** and **4g** could be potentially leading structures for further discovery of novel antifungal agrochemicals.

SUPPLEMENTARY MATERIAL

Physical, analytical and spectral data of the synthesized compounds are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

Acknowledgement. This work was financially supported by the Natural Science Foundation of Shandong Province (ZR2011BL003).

ИЗВОД

ЈЕДНОСТАВНА СИНТЕЗА АМИДНИХ ДЕРИВАТА ДИТИОКАРБАМАТА И ЊИХОВА АНТИФУНГАЛНА АКТИВНОСТ

YU-WEN LI и SHU-TAO LI

School of Chemistry and Pharmacy, Qingdao Agricultural University, Qingdao, P. R. China

Синтетисана је серија нових деривата дитиокарбамата који садрже амидну групу, **3a–i** и **4a–i,** применом олакшаног поступка и добијеним дериватима је структура потврђена ^{1}H-NMR, ^{13}C-NMR спектроскопијом, елементалном анализом и масеном спектрометријом високе резолуције (HRMS). Испитана је антифунгална активност према пет фитопатогених гљива. Резултати су показали да већина деривата показује *in vitro* активност према *Gibberella zeae, Cytospora sp., Colletotrichum gloeosporioides, Alternaria solani* и *Fusarium solani* при концентрацији 100 mg L^{-1}. Једињења **4f** и **4g** показују значајну активност према *A. solani* и *C. gloeosporioides*, редом.

(Примљено 14. јануара, прихваћено 24. маја 2015)

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