

Synthesis and antibacterial activity evaluation of novel rhodanine based amide derivatives

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Abstract

A novel series of rhodanine based amide derivatives have been prepared through multi-component reaction of rhodanine-*N*-acetic acid with aromatic aldehydes and alkyl isocyanides in the presence of aniline. The products were isolated in 85–95% yields by easy workup procedure. The synthesized compounds have also been evaluated for their antibacterial effects against four pathogenic bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. On the basis of the obtained results, some compounds showed moderate growth inhibitory effect against all tested bacteria.

Introduction

A major dispute of modern chemistry is the plan of new chemical reaction successions that supply novel compounds in high yields. Multicomponent reactions (MCRs) [1] have become precious tools for the synthesis of highly functionalized organic molecules and pharmacologically important heterocyclic compounds because of their convergence, simplicity and synthetic efficiency, atom economy, and other suitable characteristics from the point of view of green chemistry [2]. MCRs are quicker and cheaper than conventional reactions, since they are performed by mixing compounds together in one pot, without separating any intermediate. Isocyanide-based MCRs (IMCRs) are especially important, because they are more diverse and flexible than other MCRs [3,4]. Amides are an incredibly vital group of organic compounds with a variety of functions. Some derivatives of amides reveal biological properties such as vermifuge [5], antihistamine [6], fungicide and antibacterial [7,8]. The conventional approach for the synthesis of amides is the reaction of carboxylic acids with amines at high temperature. Because of carboxylic acid's low activity, several procedures for their activation have been reported in the literature [9]. Negative aspects of these procedures consist of modest yields, by-products, costly coupling reagents and difficulty in removal of surplus reagents. Consequently, the growth of a new and uncomplicated synthetic procedure for the preparation of amides has become an interesting challenge.

On the other hand, rhodanine (2-thioxothiazolidin-4-one) as a privileged scaffold is found in a variety of biologically active compounds especially with antiviral [10], antibacterial [11], antifungal [12], antitubercular [13], anticancer [14], anticonvulsant [15], and hypnotic activities [16]. Therefore, following our interest in isocyanide-based reactions and our studies towards the development of new directions for the synthesis of novel organic compounds with biological activities [17–19], we herein report the multi-component reaction of aromatic aldehydes 1 with aniline 2, rhodanine-*N*-acetic acid 3 and isocyanides 4 in THF as a suitable procedure affording rhodanine-based amides 5 in good yields (Figure 1). The synthesized amides were also screened for their antibacterial activity by the disc diffusion method.

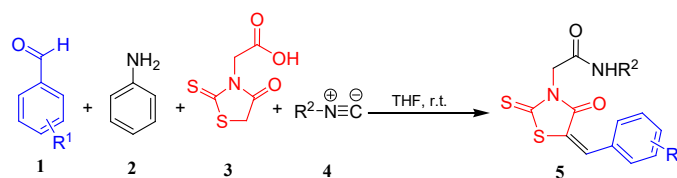


Figure 1. Multi-component reaction of rhodanine-*N*-acetic acid with aromatic aldehydes and alkyl isocyanides in the presence of aniline

Experimental

General information

All chemicals and reagents were purchased from Merck (Darmstadt, Germany) or Aldrich (Missouri, United States), and used without purification. Melting points were measured on an Electrothermal 9100 (Staffordshire, United Kingdom) apparatus. ¹H and ¹³C NMR spectra were recorded on a Bruker DRX-400 AVANCE (Massachusetts, United States) spectrometer at 400.13 and 100.61 MHz, respectively. Chemical shifts are given in parts per million (δ) relative to internal tetramethylsilane standard and coupling constants (J) are reported in hertz (Hz). IR spectra were recorded on a Bruker Tensor 27 (Massachusetts, United States) spectrometer. Mass spectra were determined on a Finnigan-Matt 8430 (Waltham, United States) mass spectrometer operating at an ionization potential of 70 eV. Elemental analyses were carried on a Perkin-Elmer 2400II CHNS/O Elemental Analyzer (Massachusetts, United States).

General procedure for the synthesis of compounds (5a–5j)

A mixture of aromatic aldehydes 1 (1 mmol), aniline 2 (0.1 mL, 1 mmol), rhodanine-*N*-acetic acid 3 (0.2 g, 1 mmol) and alkyl isocyanides

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4 (1 mmol) in 5.0 mL THF was stirred at 25 °C for the definite time period. After completion of the reaction, checked by TLC, the solvent was removed under reduced pressure to provide desired product. The structures of products 5a-j were determined on the basis of their elemental analysis, ^1H and ^{13}C NMR, IR and mass spectra.

General procedure for evaluation of antibacterial activity

The *in vitro* biocidal screening, antibacterial activities of the synthesized 2-(5-arylidene-4-oxo-2-thioxothiazolidin-3-yl)-*N*-alkylacetamide derivatives 5a-j was assayed using Kirby-Bauer disc diffusion method where a filter disc was impregnated with the compounds and placed on the surface of inoculated agar plates [20]. The synthesized compounds were dissolved into DMSO to achieve 20 mg mL^{-1} solution then filter sterilised using a 0.22 m Ministart (Sartorius).

The antibacterial activity of the compounds was investigated against four bacterial species. Test organisms included *Escherichia coli* PTCC 1330, *Pseudomonas aeruginosa* PTCC 1074, *Staphylococcus aureus* ATCC 35923 and *Bacillus subtilis* PTCC 102 [21]. Late exponential phase of the bacteria was prepared by inoculating 1% (v/v) of the cultures into the fresh Muller-Hinton broth (Merck) and incubating on an orbital shaker at 37°C and 100 rpm overnight. Before using the cultures, they were standardized with a final cell density of approximately 10^8 cfu mL^{-1} . Muller-Hinton agar (Merck) were prepared and inoculated

from the standardized cultures of the test organisms then spread as uniformly as possible throughout the entire media. Sterile paper discs (6 mm diameter, Padtan, Iran) were impregnated with 20 μL of the compound solution then allowed to dry. The impregnated disc was introduced on the upper layer of the seeded agar plate and incubated at 37°C for 24 hours. The antibacterial activities of the synthesized compounds were compared with known antibiotic gentamicin (10 μg /disc) and chloramphenicol (30 μg /disc) as positive control and DMSO (20 μL /disc) as negative control. Antibacterial activity was evaluated by measuring the diameter of inhibition zone (mm) on the surface of plates and the results were reported as Mean \pm SD after three repeats.

Results and discussion

The desired product 5a, confirmed by NMR and Mass spectra, was obtained in 90% yield (Figure 1) [22]. To illustrate the role of aniline, the reaction was checked in the absence of aniline. Interestingly, this reaction miscarried under reflux conditions, even after 24 h, and starting materials were recovered. Additionally, the three-component reaction of benzilidenerhodanine-*N*-acetic acid (1.0 mmol), benzaldehyde (1.0 mmol) and *tert*-butyl isocyanide (1.0 mmol) without aniline showed no progress in boiling THF even after long reaction time. Obviously, these results prove that aniline is a vital component for this reaction. Afterward the effect of solvent on this reaction was screened. The

Table 1. Optimization of reaction conditions for the synthesis of amide 5a^a

Entry	Solvent	Temp/°C	Time/h	Yield/% ^b
1	THF	25	13	90
2	1,4-Dioxane	25	46	25
3	CH_2Cl_2	25	40	20
4	EtOH	25	48	5
5	H_2O	25	48	5
6	THF	70	13	91 ^c

^aReaction conditions: benzaldehyde (1 mmol), aniline (1 mmol), rhodanine-*N*-acetic acid (1 mmol), *tert*-butylisocyanide (1 mmol); ^bYields refer to isolated products; ^cbenzaldehyde (2 mmol)

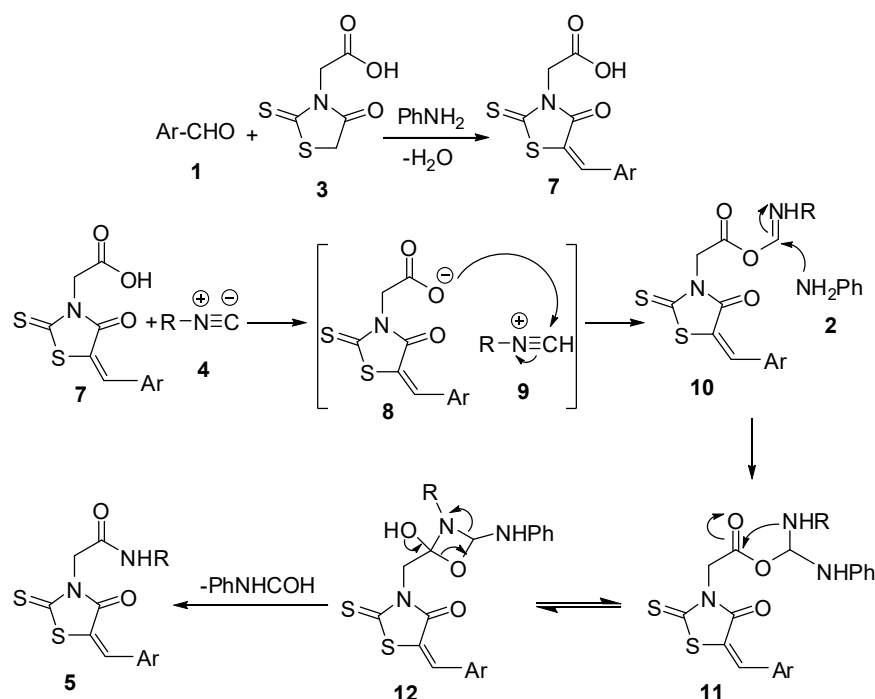
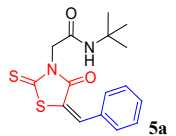
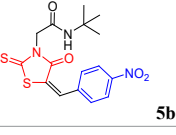
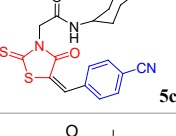
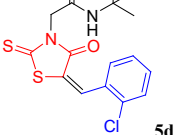
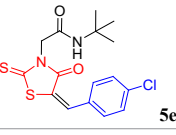
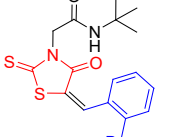
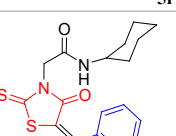
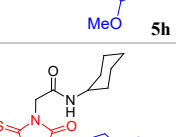
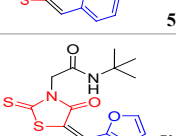


Figure 2. Proposed mechanism for the synthesis of rhodanine-based amides 5

Table 2. Synthesis of rhodanine-based amides 5^a

	R ¹	R ²	Product	M.p. (°C)	Time (h)	Yield (%) ^b
1	H	<i>tert</i> -Butyl	 5a	225-227	13	90
2	4-NO ₂	<i>tert</i> -Butyl	 5b	250-252	12	95
3	4-CN	Cyclohexyl	 5c	240-242	12	95
4	2-Cl	<i>tert</i> -Butyl	 5d	215-218	12.5	90
5	4-Cl	<i>tert</i> -Butyl	 5e	249-251	12.5	95
6	2-Br	<i>tert</i> -Butyl	 5f	230-232	12	90
7	H	Cyclohexyl	 5g	247-250	13	90
8	2-MeO	<i>tert</i> -Butyl	 5h	262-264	14	85
9	4-MeO	Cyclohexyl	 5i	250-252	14	85
10	2-furyl	<i>tert</i> -Butyl	 5j	205-208	12	90

^aReaction and conditions: aldehydes (1 mmol), aniline (1 mmol), rhodanine-*N*-acetic acid (1 mmol), isocyanides (1mmol), THF (5 mL), room temperature, stirring; ^b Yields refer to isolated products.

model reaction was carried out in different solvents including CH₂Cl₂, 1,4-dioxane, EtOH and H₂O under similar conditions at ambient temperature (Table 1). As revealed in table 1, the maximum yield was observed in THF (Table 1). Therefore, the best result is achieved by the use of substrates in equimolar ratio in THF at room temperature.

To explore the substrate scope of this novel procedure, the condensation reactions of various aromatic and heteroaromatic aldehydes 1a-1i with rhodanine-*N*-acetic acid 3, and alkyl-isocyanides (*tert*-butyl isocyanide 4a and cyclohexylisocyanide 4b) in the presence of aniline 2 were studied in THF at room temperature. Under optimum reaction conditions, a novel series of rhodanine based amides were synthesized, the results are listed in table 2. In all cases, aromatic aldehyde substituted with either electron-donating or electron-withdrawing groups underwent the reaction smoothly and gave desired products in excellent yields (85%-95%). The reaction products were isolated by simple workup procedure and did not need any further purification steps. As can be seen from table 2, the presence of electron-withdrawing substituents on the aromatic aldehyde accelerated the reaction rate, whereas electron-releasing substituent reduced the rate of reaction. However, the nature of the substituents did not affect the yield of the products.

The structure of rhodanine-based amides 5 was deduced from ¹H and ¹³C NMR, IR and mass spectra as well as elemental analyses. The physical properties, MS data and elemental analysis results for these compounds are listed in table 2, and the spectral data are shown in table 2. For example, the mass spectrum of 5a displayed the molecular ion (M⁺) peak at *m/z* 334, which is consistent with the structure of compound. The ¹H NMR spectrum of 5a showed two sharp signals at 1.22 and 4.39 ppm for the *tert*-butyl and methylene groups, a multiplet at 7.52-7.59 ppm and a doublet at 7.66 ppm corresponding to five aromatic protons, and two singlets at 7.81 and 8.16 ppm for vinyl proton and NH, respectively. The ¹³C NMR spectrum of 5a demonstrated 12 signals in agreement with the anticipated structure.

Although a mechanism for the formation of product 5 has not yet been established experimentally, a possible explanation is anticipated in figure 2. First, the Knoevenagel condensation between aldehyde 1 and rhodanine-*N*-acetic acid 3 in the presence of aniline 2 generates benzilidenerhodanine-*N*-acetic acid 7. According to the well-established chemistry of the reaction of isocyanides with acids [23], it is sensible to suppose that protonation of the isocyanide 4 by the carboxylic acid 3. Then nucleophilic attack of carboxylate 8 to nitrilium 9 produces *O*-acyl-imine 10 which on quenching with aniline rearranges to intermediate 12. Finally, removal of *N*-phenyl from this intermediate gives rhodanine-based amide 5 [24].

Antibacterial activity

The antibacterial activity of synthesized compounds 5a-5j was evaluated against gram positive (*S. aureus* and *B. subtilis*) and gram negative bacteria (*E. coli* and *P. aeruginosa*) by the disc diffusion method; the results are illustrated in table 3. In addition, the finding towards inhibition of microorganisms was compared with that of positive controls, gentamicin and chloramphenicol, and DMSO as a negative control. As presented in the table 3, compounds 5a, 5e, 5f and 5j exhibited moderate growth inhibitory effect against all tested bacteria, whereas compound 5i showed no activity. Compounds 5b, 5c and 5h displayed good activity against Gram positive bacteria while these compounds remained inactive against two Gram-negative bacteria. Moreover, compound 5d showed good activity against all bacteria except *B. subtilis*. In general, the antibacterial activities of these

Table 3. Antibacterial activity of the compounds 5a-5j using Kirby-Bauer technique (zone of growth inhibition, mm)^a

Compound	<i>E. coli</i> /mm	<i>P. aeruginosa</i> /mm	<i>S. aureus</i> /mm	<i>B. subtilis</i> /mm
5a	8.5 ± 0.7	8.5 ± 0.7	12.5 ± 0.7	10.5 ± 0.7
5b	NE ^b	NE	16.5 ± 0.7	10.5 ± 0.7
5c	NE	NE	12.5 ± 0.7	10.5 ± 0.7
5d	10.5 ± 0.7	10.0 ± 1.4	15.5 ± 0.7	NE
5e	11.5 ± 0.7	11.0 ± 0.0	10.5 ± 0.7	12.5 ± 0.7
5f	10.0 ± 1.4	9.0 ± 1.4	14.5 ± 0.7	15.0 ± 1.4
5g	10.0 ± 1.4	9.5 ± 0.7	12.5 ± 0.7	NE
5h	NE	NE	15.5 ± 0.7	8.0 ± 0.0
5i	NE	NE	NE	NE
5j	9.5 ± 0.7	9.5 ± 0.7	12.5 ± 0.7	14.5 ± 0.7
Gentamicin (10 µg/disc)	19.6 ± 1.1	15.6 ± 0.5	20.3 ± 1.5	26.0 ± 1.7
Chloramphenicol (30 µg/disc)	20.7 ± 1.5	NE	21.7 ± 0.6	22.3 ± 1.2

^aConcentration of compounds 5a-5j: 20 mg mL⁻¹; ^bNo effect

compounds tended to be more potent against Gram-positive species than Gram-negative bacteria, which can be related to the variation in their cell wall structure.

Conclusions

In summary, we have reported an efficient approach for the synthesis of rhodanine-based amides *via* multi-component reaction of aromatic aldehydes, rhodanine-*N*-acetic acid and alkyl isocyanides in the presence of aniline in THF at ambient temperature. The advantages of the present procedure include good functional group tolerance, high yields of products, simple operation, and absence of any tedious workup or purification. On the basis of the obtained results from antibacterial test, compounds 5a, 5e, 5f and 5j showed moderate growth inhibitory effect against all tested bacteria. Among compounds, 5b exhibited the most antibacterial activity against *S. aureus*.

Acknowledgements

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Supplementary data

Electronic Supplementary Material associated with this article can be found in the online version of this paper.

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