Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *77* (5) 599–605 (2012) UDC 547.433–304.2+547.789.1+542.913:57–188
JSCS–4293 *Original scientific paper*

Synthesis of 2-oxoazetidine derivatives of 2-aminothiazole and their biological activity

PUSHKAL SAMADHIYA*, RITU SHARMA, SANTOSH K. SRIVASTAVA and SAVITRI D. SRIVASTAVA

Synthesis Organic Chemistry Laboratory, Department of Chemistry, Dr. H.S. Gour University, Sagar, Madhya Pradesh-470003, India

(Received 5 January, revised 16 June 2011)

Abstract: A new series of 3-chloro-4-(substituted phenyl)-1-{[2-(2-thiazolylamino)ethyl]amino}-2-azetidinone, compounds **4a**–**m**, has been synthesized from 2-aminothiazole as the starting material. The structures of all the synthesized compounds were confirmed by chemical and spectral analyses, such as FTIR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. All the final synthesized compounds **4a**–**m** were screened for their antibacterial and antifungal activities against some selected bacteria and fungi and for their antitubercular activity against *Mycobacterium tuberculosis*, and their minimum inhibitory concentration (*MIC*) values were determined. The anti-inflammatory activities of the title compounds were screened using albino rats (either sex) and gave acceptable results.

Keywords: synthesis; 2-aminothiazole; azetidinone; antimicrobial; antitubercular; anti-inflammatory.

INTRODUCTION

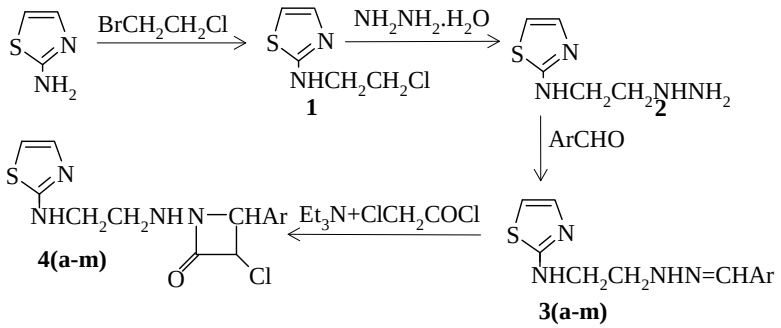
The azetidinone unit is a crucial structural feature of significant classes of antibiotics and its chemistry is very important to their biological activities, for instance, the penicillin and cephalosporin antibiotics possess *cis*-*β*-lactam units, whereas the thienamycins and trinems have *trans*-*β*-lactam moieties. The effective synthesis of *β*-lactam became a desirable goal based on the discovery of penicillin and cephalosporin. Although most penicillin and cephalosporin related compounds are obtained by biosynthesis, chemical modification of intermediates for bioassay of the antibacterial activity of the resulting compounds has become of utmost importance because of the growing resistance of bacteria against penicillin- and cephalosporin-like compounds and the need for medicines with more specific antibacterial activity.[1,2] The azetidinone derivatives have also been

* Corresponding author. E-mail: pushkalsamadhiya@rediffmail.com
doi: 10.2298/JSC110616002S

recognized as tumor necrosis factor-alpha (TNF-alpha) converting enzyme (TACE) inhibitors[3] and agents with new biological activities, such as anticancer,[4] anticoccidial,[5] cardiovascular,[6] antiviral,[7] mutagenic,[8] anticonvulsant and anti-inflammatory.[9,10] Condensed heterocyclic systems are of considerable interest not only because of their potential biological activity, but also because of their versatility as synthons in organic transformations.

The thiazole moiety is also an important component of the pharmacophores of a large number of molecules of medicinal significance and the evaluation of their biological activity, such as antiprotozoal,[11] antibacterial,[12] antifungal,[13,14] antitubercular[15] and anthelmintic,[16] with emphasis on their potential medicinal applications, is desirable. Moreover, thiazole derivatives have attracted a great deal of interest due to their wide applications in the field of pharmaceuticals.

In the present study, a series of *N*-[2-(2-aminothiazolyl)ethyl]-4-(substituted phenyl)-3-chloro-2-oxo-1-iminoazetidines, compounds **4a**–**m**, was synthesized as shown in Scheme 1. The structure of all the synthesized compounds was elucidated by FTIR, ^{1}H-NMR, ^{13}C-NMR, and chemical methods. All the final compounds **4a**–**m** were screened for their antibacterial, antifungal, antitubercular and anti-inflammatory activities.



Ar= substituted phenyl ring

Compound	Ar	Compound	Ar
3a and **4a**	C_6H_5	**3h** and **4h**	4-$NO_2C_6H_4$
3b and **4b**	4-ClC_6H_4	**3i** and **4i**	3-$NO_2C_6H_4$
3c and **4c**	3-ClC_6H_4	**3j** and **4j**	2-$NO_2C_6H_4$
3d and **4d**	2-ClC_6H_4	**3k** and **4k**	4-$CH_3OC_6H_4$
3e and **4e**	4-BrC_6H_4	**3l** and **4l**	4-$CH_3C_6H_4$
3f and **4f**	3-BrC_6H_4	**3m** and **4m**	4-HOC_6H_4
3g and **4g**	2-BrC_6H_4	–	–

Scheme 1. Synthesis of compounds **1**–**4**.

EXPERIMENTAL

Melting points were taken in open capillaries and are uncorrected. The progress of the reactions was monitored by silica gel-G coated TLC plates using MeOH:$CHCl_3$ (2:8) system.

The spot was visualized by exposing the dry plate to iodine vapors. The IR spectra were recorded in KBr discs on a Shimadzu 8201 PC FTIR spectrophotometer (ν_{max} in cm^{-1}) and the ^{1}H-NMR and ^{13}C-NMR spectra were measured on a Bruker DRX-300 spectrometer in $CDCl_3$ at 300 and 75 MHz, respectively, using TMS as an internal standard. All chemical shifts are reported on δ scales. Elemental analyses were realized on a Carlo Erba-1108 analyzer. The analytical data of all the compounds were satisfactory. For column chromatographic purification of the products, Merck silica gel 60 (230–400 mesh) was used. The reagent grade chemicals were purchased from commercial sources and further purified before use.

*Procedure for the synthesis of 2-[(2-chloroethyl)amino]thiazole, compound **1***

2-Aminothiazole and 1-bromo-2-chloroethane (1:1 mole) were dissolved in methanol. The reaction mixture was continuously stirred on a magnetic stirrer at 30–35 °C for about 8 h. The product was filtered and purified by column chromatography. The purified product was dried in the oven at 45–50 °C for 8 h and recrystallized from ethanol to yield compound **1**.

Procedure for the synthesis of N*-(2-hydrazinylethyl)-thiazolamine, compound **2***

Compound **1** and hydrazine hydrate (1:1 mole) were dissolved in methanol at room temperature. The reaction mixture was continuously stirred on a magnetic stirrer at 30–35 °C for about 5 h. The product was filtered off and purified by column chromatography. The purified product was dried in an oven at 45–50 °C for 9 h and recrystallized from ethanol to yield compound **2**.

*General procedure for the synthesis of substituted benzaldehyde, 2-[2-(thiazolylamino)ethyl]-hydrazone, compounds **3a**–**m***

Compound **2** and an appropriate substituted benzaldehyde (1:1 mole) were dissolved in methanol and allowed to reaction. The reaction mixture was first continuously stirred on a magnetic stirrer at 30–35 °C for about 3–5 h and then kept on a steam bath at 75–90 °C for about 3–4 h. The products were filtered and cooled to room temperature. The filtered products were purified by column chromatography. The purified products were dried in an oven at 55––60 °C for 6–10 h and recrystallized from ethanol to yield compounds **3a**–**m**.

*General procedure for the synthesis of 3-chloro-4-(substituted phenyl)-1-{[2-(2-thiazolylamino)ethyl]amino}-2-azetidinone, compounds **4a**–**m***

An appropriate compound **3a**–**m** and chloroacetyl chloride in the presence of Et_3N (1:1:1 mole ratio) were dissolved in methanol (50 ml) and allowed to react. The reaction mixture was first continuously stirred on a magnetic stirrer at 30–35 °C for about 3–4 h and then kept on a steam bath at 70–85 °C for about 3–5 h. The products were filtered and cooled to room temperature. The filtered products were purified by column chromatography. The purified products were dried in an oven at 60–65 °C for 4–8 h and recrystallized from ethanol to yield compounds **4a**–**m**, respectively.

Biological importance

The antibacterial, antifungal and antitubercular activities of compounds **4a**–**m** were assayed *in vitro* against selected bacteria: *Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae*, and fungi: *Aspergillus niger, A. flavus, Candida albicans* and *Fusarium oxysporum*, and *Mycobacterium tuberculosis* (H37Rv) strain. The minimal inhibitory concentration (*MIC*) values of compounds **4a**–**m** were determined using the filter paper disc diffusion method (antibacterial and antifungal activity) and the Lowenstein–Jensen (LJ) medium (conventional) method (antitubercular activity) at 100 μg mL^{-1} and lower concentrations. Streptomycin and griseofulvin, used as the standard for the antibacterial and antifungal

activity, respectively, showed MIC values in the range 1.25–3.25 μg mL^{-1} for all the bacterial strain and 6.25–12.5 μg mL^{-1} for all fungal strain. For the antitubercular activity, isoniazid and rifampicin were taken as standards (*MIC* range 1.25–2.50 μg mL^{-1}). All standards were also screened under similar condition for comparison.

Anti-inflammatory activity

The carageenan-induced rat paw edema method was employed for evaluating the anti-inflammatory activity of the compounds at a dose 50 mg kg^{-1} bw in albino rats (weighing 80–110 g, each group contained 5 animal) using phenylbutazone as the standard drug for comparison at a dose 30 mg kg^{-1} body weight. The rate paw edema was produced by the method of Winter *et al*.[17] The percentage inhibition of inflammation was calculated by applying the Newbould formula.[18]

RESULTS AND DISCUSSION

3-Chloro-4-(substituted phenyl)-1-{[2-(2-thiazolylamino)ethyl]amino}-2--azetidinone, compounds **4a**–**m**, were synthesized in four different steps. 2-Aminothiazole on reaction with $Cl(CH_2)_2Br$ at room temperature afforded 2-[(2-chloroethyl)amino]thiazole, compound **1**. The IR spectrum of compound **1** displayed absorptions at 1336 and 740 for (C–N) and (C–Cl), respectively, this clearly indicated the synthesis of compound **1**. Compound **1** on reaction with hydrazine hydrate at room temperature yielded *N*-(2-hydrazinylethyl)-2--thiazolamine, compound **2**. The IR spectrum of compound **2** showed absorptions for NH and NH_2 at 3378 and 3429 cm^{-1}, respectively, while the absorption for (C–Cl) in the IR spectrum of compound **1** had disappeared. The ^{1}H-NMR spectrum of **2** displayed signals at δ 7.63 and 5.56 ppm for NH and NH_2, respectively. Compound **2** on further reaction with several selected substituted aromatic aldehydes produced substituted benzaldehyde, 2-[2-(thiazolylamino)–ethyl]–hydrazone, compounds **3a**–**m**. The characteristic absorption for a Schiff base (N=CH) appeared in the range 1542–1579 cm^{-1} in the IR spectra of compounds **3a**–**m** and in the ^{1}H- and ^{13}C-NMR spectra signals appeared at δ 7.82–8.12 and δ 151.6–157.4 ppm, respectively. In the ^{1}H-NMR, the broad signal of NH_2 present in the spectrum of compound **2** had disappeared. Compounds **3a**–**m** on treatment with $ClCH_2COCl$ in the presence of Et_3N furnished the final products, compounds **4a**–**m**. In the IR spectra of compounds **4a**–**m**, the carbonyl group of the β-lactam ring showed a characteristic absorption in the range 1726––1752 cm^{-1} and the ^{1}H-NMR spectra of compounds **4a**–**m** showed two doublets for (N–CH) and (CH–Cl) in the range δ 4.72–4.98 and 4.13–4.28 ppm, respectively. In the ^{13}C-NMR spectra of compounds **4a**–**m**, three characteristic signals appeared for (N–CH), (CH–Cl) and (CO cyclic) in the δ ranges 60.8––65.7, 49.7–54.8 and 169.8–176.4 ppm, respectively. The IR absorption and ^{1}H- and ^{13}C-NMR signals of the N=CH group were absent. All these fact collectively suggest the successful synthesis of all the above compounds.

The analytic and spectral data of all the synthesized compounds are given in the supplementary material to this paper.

Biological testing

The results of all the described activities (antibacterial, antifungal, antitubercular and anti-inflammatory) are summarized in Tables I and II. The results of the antimicrobial screening data revealed that all the compounds **4a**–**m** showed considerable and varied activity against the selected microorganisms. The results shown in Tables I and II revealed that all the synthesized compounds **4a**–**m** have a structure activity relationship (SAR) because the activity of the compounds varied with substitution. The nitro group-containing compounds (**4h, 4i** and **4j**) showed higher activity than the chloro (**4c** and **4d**), or bromo group containing compounds (**4e** and **4f**). The chloro and bromo derivatives also had a higher activity than the other rested compounds. Based on the SAR, it can be concluded that the activity of the compounds depends on electron withdrawing nature of the substituent groups. The sequence of the activity is the following: NO_2 > Cl > Br > OH > > OCH_3 > CH_3.

TABLE I. Antibacterial, antifungal and antitubercular activities of compounds **4a**–**m**. The *MIC* values of the standard streptomycin for all bacteria strains and griseofulvin for all fungi strains were in the range of 1.25–3.25 and 6.25–12.5 μg ml^{-1}, respectively. Isoniazid and rifampicin were used as standards, *MIC* values in the range of 1.25–2.50 μg ml^{-1}, for *M. tuberculosis*

Compd.	Antibacterial activity				Antifungal activity				Antitubercular activity
	B. subtilis	*E. coli*	*S. aureus*	*K. pneumoniae*	*A. niger*	*A. flavus*	*F. oxisporum*	*C. albicans*	*M. tuberculosis*
4a	12.5	>6.25	12.5	6.25	>25	>25	>25	>25	>12.5
4b	>3.25	6.25	3.25	>3.25	>25	>12.5	>25	>25	>2.50
4c	6.25	>3.25	6.25	3.25	>12.5	25	>12.5	>12.5	>2.50
4d	>3.25	6.25	3.25	6.25	>12.5	>25	>12.5	>12.5	2.50
4e	6.25	>3.25	3.25	>3.25	>12.5	25	25	>25	>2.50
4f	6.25	3.25	6.25	>3.25	>12.5	>12.5	>12.5	>12.5	>2.50
4g	>3.25	6.25	>3.25	6.25	25	>12.5	>12.5	25	6.25
4h	3.25	>3.25	3.25	3.25	25	>12.5	>12.5	>25	2.50
4i	3.25	3.25	>3.25	3.25	>12.5	>12.5	>12.5	>12.5	2.50
4j	3.25	>3.25	3.25	3.25	>12.5	>12.5	>12.5	>12.5	>2.50
4k	>12.5	6.25	>12.5	6.25	25	25	>25	>25	12.5
4l	>12.5	>12.5	>12.5	>12.5	>25	>25	>25	>25	>12.5
4m	>3.25	>3.25	>6.25	>6.25	>25	25	>12.5	>12.5	6.25

The investigation of antimicrobial (antibacterial, antifungal and antitubercular) data revealed that the compounds **4c**, **4d**, **4e**, **4f, 4h**, **4i** and **4j** displayed high activity, the compounds **4b**, **4g** and **4m** showed moderate activity and the other compounds

showed low activity against all the strains compared with the standard drugs. In the anti-inflammatory activity test, compounds **4c**, **4d**, **4e**, **4f**, **4h**, **4i** and **4j** showed high activity while the other compounds displayed moderate to low activity.

TABLE II. Anti-inflammatory activity of compounds **4a**–**m**

Compound	Before carageenan administration (mean±*SEM*)	Total increase in paw volume after 5 h (mean±*SEM*)	Inhibition, %
4a	0.62±0.02	0.18±0.02	48.57
4b	0.65±0.02	0.16±0.02	54.29
4c	0.66±0.02	0.16±0.01	54.29
4d	0.64±0.02	0.17±0.02	51.43
4e	0.66±0.03	0.15±0.02	57.14
4f	0.67±0.02	0.14±0.01	60.00
4g	0.66±0.02	0.16±0.01	54.29
4h	0.65±0.03	0.14±0.01	60.00
4i	0.68±0.02	0.13±0.03	62.86
4j	0.67±0.03	0.12±0.02	65.71
4k	0.65±0.02	0.16±0.02	54.29
4l	0.64±0.02	0.18±0.02	48.57
4m	0.67±0.02	0.16±0.01	54.29
Control	0.68±0.02	0.35±0.01	–
Standard; phenylbutazone	0.66±0.03	0.10±0.02	71.43

CONCLUSIONS

The research study reports the successful synthesis of compounds **1**–**4**. The antimicrobial and antitubercular activity of the newly synthesized compounds bearing a 2-azetidinone moiety revealed that all the tested compounds showed moderate to good antibacterial, antifungal and antitubercular activities against the selected microbial strains. The results of the anti-inflammatory activity testing also showed positive results. Some of the compounds displayed promising activities and are of interest for further transformations towards more potent derivatives.

SUPPLEMENTARY MATERIAL

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

Acknowledgements. The authors are thankful to SAIF, Central Drugs Research Institute Lucknow (India) for providing the spectral and analytical data of the compounds. We are thankful to the Head, Department of Biotechnology, Dr. H. S. Gour, University, Sagar (India) for the antimicrobial (antibacterial and antifungal) activity determinations and to the Microcare Laboratory and the Tuberculosis Research Center Surat, Gujarat (India) for the antituberculosis activity results. We are also thankful to the Head, Department of Chemistry Dr. H. S. Gour, University, Sagar (India) for providing the facilities to carry out the work.

ИЗВОД

СИНТЕЗА И БИОЛОШКА АКТИВНОСТ 2-ОКСОАЗЕТИДИНСКИХ ДЕРИВАТА 2-АМИНОТИАЗОЛА

PUSHKAL SAMADHIYA, RITU SHARMA, SANTOSH K. SRIVASTAVA и SAVITRI D. SRIVASTAVA

Synthesis Organic Chemistry Laboratory, Department of Chemistry, Dr. H.S. Gour University, Sagar, Madhya Pradesh-470003, India

Синтетисана је серија нових деривата 3-хлоро-4-(супституисани фенил)-1-{[2-(тиазолиламино)етил]амино}-2-азетидинона, једињења **4a**–**m**, полазећи од 2-аминотиазола. Структура свих синтетисаних једињења потврђена је аналитичким и спектралним методама, IC, ^{1}H-NMR и ^{13}C-NMR спектроскопијом. Испитана је антибактеријска, антифунгална активност према одабраним ћелијским линијама бактерија и гљива, као и антитуберкулозна активност према *Mycobacterium tuberculosis* синтетисаних једињења **4a**–**m**. Антиинфламаторна активност је испитана према албино пацовима. Резултати показују задовољавајућу активност тестираних једињења.

(Примљено 5. јануара, ревидирано 16. јуна 2011)

REFERENCES

1. A. Upadhyay, S. K. Srivastava, S. D. Srivastava, R. Yadav, *Proc. Natl. Acad. Sci. India* **80** (2010) 131
2. Y. Ikee, K. Hashimoto, M. Nakashima, K. Hayashi, S. Sano, M. Shiro, Y. Nagao, *Bioorg. Med. Chem. Lett.* **17** (2007) 942
3. B. G. Rao, U. K. Bandarage, T. Wang, J. H. Come, E. Perola, Y. W. S.-K, Tian, J. O. Saunders, *Bioorg. Med. Chem. Lett.* **17** (2007) 2250
4. B. K. Banik, I. Banik, F. F. Becker, *Bioorg. Med. Chem.* **13** (2005) 3611
5. G.-B. Liang, X. Qian, D. Feng, M. Fisher, T. Crumley, S. J. Darkin-Rattray, P. M. Dulski, A. Gurnett, P. S. Leavitt, P. A. Liberator, A. S. Misura, S. Samaras, T. Tamas, D. M. Schmatz, M. Wyvratta, T. Biftu, *Bioorg. Med. Chem. Lett.* **18** (2008) 2019
6. S. Takai, D. Jin, M. Muramatsu, Y. Okamoto, M. Miyazaki, *Pharmaco* **501** (2004) 1
7. W. W. Ogilvie, C. Yoakim, F. Do, B. Hache, L. Lagace, J. Naud, J. A. Omeara, R. Deziel, *Bioorg. Med. Chem.* **7** (1999) 1521
8. H. Valette, F. Dolle, M. Bottlaender, F. Hinnen, D. Marzin, *Nucl. Med. Biol.* **29** (2002) 849
9. P. Kohli, S. D. Srivastava, S. K. Srivastava, *J. Indian Chem. Soc.* **85** (2008) 326
10. S. K. Srivastava, S. Srivastava, S. D. Srivastava, *Indian J. Chem., Sect B* **38** (1999) 183
11. R. A. Tapia, Y. Prieto, F. Pautet, N. Walchshofer, H. Fillion, B. Fenet, M.-E. Sarciron, *Bioorg. Med. Chem.* **11** (2003) 3407
12. C.-H. Oh, H.-W. Cho, D. Baek, J.-H. Cho, *Eur. J. Med. Chem.* **37** (2002) 743; b) S. K. Bharti, G. Nath, R. Tilak, S. K. Singh, *Eur. J. Med. Chem.* **45** (2010) 651
13. L. Joshi, S. K. Srivastava, *J. Sci Ind. Res.* **60** (2001) 331
14. S. K. Sonwane, S. D., Srivastava, *Proc. Natl. Acad. Sci. India* **78** (2008) 129
15. G. V. Suresh Kumar, Y. Rajendraprasad, B. P. Mallikarjuna, S. M. Chandrashekar, C. Kistayya, *Eur. J. Med. Chem.* **45** (2010) 2063
16. R. Yadav, S. K. Srivastava, S. D. Srivastava, *Chem. Indian J.* **1** (2003) 95
17. C. A. Winter, E. A. Risley, G. W. Nuss, *Proc. Soc. Exp. Biol.* **111** (1962) 544
B. B. Newbould, *Brit J Pharmacol.* **21** (1963) 127.