Tropical Journal of Pharmaceutical Research December 2019; 18 (12): 2617-2621
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v18i12.22

Original Research Article

Novel sulfamoylamino-containing cephalosporin derivatives, and their in vitro antibacterial properties

Xudong Jiang[1], Li Chen[1], Xiaoling Lu[1]*, Lan Liao[1], WeiGuang Wang[1], Xiaocheng Huang[2]
[1]School of Medicine, Guangxi University of Science and Technology, [2]College of Biological and Chemical Engineering, Guangxi University of Science and Technology, Liuzhou 545005, China

For correspondence: **Email: v0964y@163.com*

Sent for review: 21 September 2019 *Revised accepted: 28 November 2019*

Abstract

Purpose: *To prepare and develop new antibacterial agents with novel molecular structures.*
Method: *A series of novel sulfamoylamino-containing cephalosporin derivatives were synthesized. The in vitro antibacterial effects of the derivatives against Gram-positive bacteria (S. aureus, S. pneumonia and S. epidermidis), and Gram-negative bacteria (E. coli, P. aeruginosa, and K. pneumonia) were investigated.*
Results: *Compounds 13a and 13b exhibited excellent antibacterial effects against all the Gram-positive and Gram-negative bacteria tested, when compared with other cephalosporin derivatives.*
Conclusion: *Of these new cephalosporin derivatives, compounds 13a and 13b show the most potent antibacterial activity and would need to be further investigated.*

Keywords: *Cephalosporin, Sulfamoylamino derivatives, Gram-positive, Gram-negative, Antibacterial*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

The β-lactams are antibiotics with a β-lactam ring nucleus, and include carbapenems, cephalosporins, monobactams, penicillin, and β-lactamase inhibitors [1,2]. Generally, their mechanism of action is associated with inhibition of the biosynthesis of bacterial cell wall via formation of covalent bonds with penicillin-binding proteins (PBPs) involved in cell wall biosynthesis [3-5]. These antibiotics have the unique advantages of strong antibacterial effect, low toxicity and wide application as broad-spectrum antibiotics in clinical practice [6,7]. However, the global problem of resistance to antibiotics has been rapidly exacerbated by the problem of widespread use of antibiotics [8,9]. One effective way to solve this problem is to evolve new antibacterial agents with novel molecular structures, with a view to developing new classes of antibiotics [10,11].

Cephalosporins are a group of semi-synthetic β-lactam-derived antibiotics. Five generations of cephalosporins (more than 60 different types) have been developed and used to treat human diseases since the first cephalosporin

cephalothin become available in 1964, due to their broad-spectrum antibacterial effects and excellent penicillinase-resistance, when compared with penicillin [12-14]. Cephalosporin has been used as first-line antibiotic in anti-infective treatment in recent years. Modification of the structure of existing cephalosporin is an effective method for improving its antibacterial effect through the introduction of heterocyclic or quaternary ammonium group at 3-position of the cephalosporin nucleus [15-18].

Ceftobiprole has a pyrrolidone-methylidene structure at 3-position of cephalosporin nucleus, which provides a multi-site binding stable complex with penicillin binding protein 2a (PBP2a), resulting in significant antibacterial effect against Gram-positive bacteria [19]. Ceftaroline has a unique structure bearing a 1,3-thiazole ring side chain linked to cephalosporin nucleus through sulfur atom at 3-position, resulting in high affinity for PBP2a and enhanced anti-MRSA activity [20].

Studies have shown that antibiotic compounds containing sulfamoylamino derivatives have strong antibacterial effects against *Staphylococcus aureus* [21]. Results from previous studies showed that carbapenem derivatives containing sulfamoylamino group exerted strong antibacterial effects against *S. aureus*, *Enterococcus* and *E. coli* [22]. In this study, novel cephalosporin derivatives were prepared by the introduction of sulfonamide heterocyclic group linked to cephalosporin nucleus through sulfur atom at 3-position, and the antibacterial effects of these compounds were evaluated.

EXPERIMENTAL

General

Compound 1, compound 9 and a series of tert-butyl (piperidine or pyridinylmethyl)sulfamoyl-carbamate compounds (2a-2e) were synthesized in the laboratory. The other reagents and solvents were purchased from commercial sources, and used without further purification. The ^{1}H-NMR spectra (400 MHz) of cephalosporin compounds were measured on a DRX-400 spectrometer using D_2O as solvent and tetramethylsilane (TMS) as internal standard. Chemical shifts were expressed in ppm. Multiples were recorded as singlet (s), broad singlet (brs), doublet (d), (triplet (t), quartet (q) and multiplet (m). Mass spectra were obtained on an LC-MSD1100 spectrometer with ESI.

Synthesis of sulfamoylamino-containing cephalosporin derivatives

The outline of route of synthesis of the novel sulfamoylamino-containing cephalosporin derivatives (8a-8e) is shown in Figure 1.

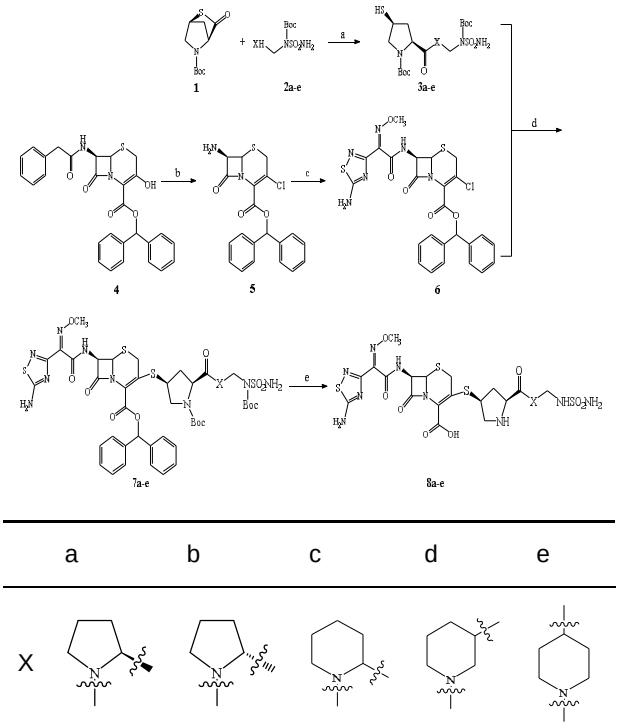

Figure 1: Route of synthesis of novel cephalosporin derivatives 8a-8e. Reagents and conditions: (a) DIPEA, rt; (b) (1) PCl_5/Py, 0-5°C; (2) PCl_5, rt; (c) CAS: 89604-91-1, Et_3N, rt; (d)DIPEA, THF, rt; (e)HCl(conc.), rt

Compounds 1 and 2 were dissolved in dichloromethane (DCM) at a volume ratio of 1:1.2. Next, the resultant solution was refluxed with diisopropylethylamine (DIPEA) for 6 h under nitrogen atmosphere to yield compound 3 as a crude oil without further purification. The synthesis of compound 5 was in accordance with a known route [23]. Phosphorus pentachloride (PCl_5) was suspended in dry DCM under controlled temperature (0 to 5°C), and pyridine (Py) was introduced drop-by-drop, followed by incubation for 0.5 h, with stirring at 0 to 5°C for 1 h, after which 3-hydroxycephem (compound 4) was added. A white solid was precipitated out during the reaction.

The white solid (pyridine hydrochloride and phenylacetic acid) was removed through filtration, and the filtrate was introduced drop-by-drop slowly to a solution of PCl_5 and N,N-dimethyl-formamide (DMF) at 0 to 5°C, and stirred for 3h at 20 - 25°C. Compound 5, a pale yellow solid was recrystallized from isobutanol.

Compound 5 and (Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-methoxyimino thioacetate were added to tetrahydrofuran (THF) and water (1:1 volume ratio), followed by addition of triethylamine (Et_3N). The reaction mixture was stirred overnight to obtain compound 6 in the form of a pale-yellow solid. Compound 7 was prepared by condensation reaction between compounds 3 and 6 in the presence of DIPEA. Finally, diphenylmethyl and tert-butoxycarbonyl groups were treated with concentrated hydrochloric acid to give the target sulfamoyl-amino-containing cephalosporin derivatives (8a-8e) in the form of off-white solids.

The relationship between antibacterial effect and molecular basicity was investigated by designing and synthesizing compounds 13a-13e using the route shown in Figure 2.

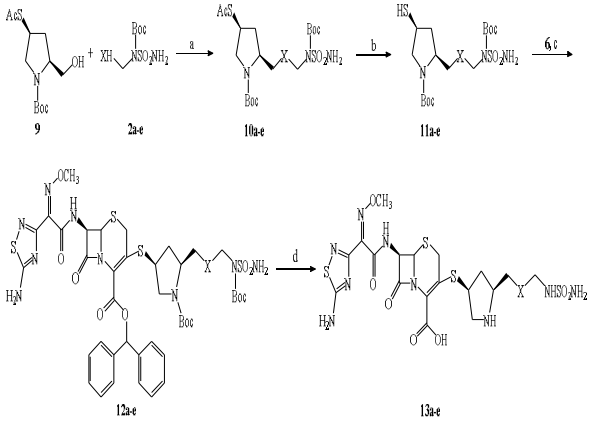

Figure 2: Route of synthesis of cephalosporin derivatives13a-13e. Reagents and conditions: (a) (1) Et_3N, MsCl; (b) $NaOCH_3/HOCH_3$, rt; (c)DIPEA, THF, rt; (d)HCl(conc.), rt

Compound 9 and triethylamine (Et_3N) were dissolved in DCM and stirred at -5 - 0°C for 10 min, followed by dropwise addition of methane sulfonyl chloride (MsCl), and stirring for 2 h. Compound 2 was dissolved in THF and introduced drop-by-drop to the Et_3N-MsCl solution. Following stirring for 12 h without further purification, compound 10 was produced as crude oil. This was dissolved in methanol, and the acetyl group was deprotected by addition of sodium methoxide under nitrogen atmosphere to obtain compound 11 as a crude oil without further purification. Similarly, compound 12 was prepared through condensation reaction between compounds 11 with 6 in the presence of DIPEA. Finally, the diphenylmethyl and tert-butoxycarbonyl groups were treated with concentrated hydrochloric acid to give the target sulfamoylamino-containing cephalosporin derivatives 13a-13e as off-white solid.

Evaluation of antibacterial activity

The minimum inhibitory concentrations (MICs) of these compounds against the selected bacterial strains were determined using disc diffusion method as previously described by Ye *et al* [24]. Six Gram-positive bacteria and Gram-negative bacteria (*P. aeruginosa, S. aureus*, *S. pneumonia*, *S. epidermis, E. coli*, and *K. pneumonia)* were evaluated, with cefpirome as standard drug.

RESULTS

Sulfamoylamino-containing cephalosporin derivatives –spectral characteristics

A series of compounds (8a-8e) containing thiol group and sulfamoylamino group was first synthesized. The thiol group was linked to pyrrolidine ring at the 4-position, while the sulfamoylamino-methyl-piperidinyl or sulfamoyl-amino-methyl- pyrrolidinyl group was linked to pyrrolidine ring with 2-carbamoyl group. Next, compounds 13a-13e were synthesized. These compounds differed slightly from compounds 8a-8e with respect to molecular structure, and they were used to investigate the effect of molecular basicity on antibacterial effect. Compounds 13a-13e were stronger in alkalinity than compounds 8a-8e and sulfamoylamino-methyl-piperidinyl or sulfamoylamino-methyl-pyrrolidinyl group linked to pyrrolidine ring by 2-methylamino group. These compounds were confirmed based on their spectral characteristics.

Antibacterial effects

The *in vitro* antibacterial effects of the novel sulfamoylamino-containing cephalosporin derivatives were evaluated in terms of inhibitory capacity against some Gram-positive bacteria and Gram-negative bacteria. The results are shown in Table l.

DISCUSSION

As shown in Table 1, the test compounds and positive control displayed significant antibacterial effects. The antibacterial effect of compound 13b against *S. aureus* was comparable to that of the standard drug cefpirome, but it produced superior antibacterial effect against *S. pneumonia* and *S. epidermidis*. Compounds 13a, 8a and 8b showed the same antibacterial effects against *S. epidermidis* as cefpirome, but exerted superior antibacterial effect against *S. aureus* and *S. pneumonia*.

Table 1: *In vitro* antibacterial activity (MIC, µg/mL) of the cephalosporin derivatives

Compound	*S. aureus*	*S. pneumonia*	*S. epidermidis*	*E. coli*	*P. aeruginosa*	*K. pneumonia*
8a	1.56	0.78	0.78	3.13	6.25	0.39
8b	1.56	0.78	0.78	3.13	6.25	0.39
8c	1.56	1.56	1.56	6.25	6.25	0.39
8d	3.13	1.56	3.13	6.25	6.25	0.39
8e	1.56	1.56	1.56	6.25	6.25	0.78
13a	1.56	0.78	0.78	0.78	3.13	0.195
13b	0.78	0.78	1.56	1.56	3.13	0.195
13c	3.13	1.56	1.56	3.13	6.25	0.39
13d	3.13	1.56	3.13	3.13	6.25	0.39
13e	3.13	1.56	1.56	3.13	6.25	0.39
Cefpirome	0.78	0.39	0.78	3.13	3.13	0.195

However, compounds 13a and 13b exhibited better antibacterial effects against Gram-negative bacteria than the other cephalosporin derivatives, and their effects were comparable to, or better than that produced by cefpirome.

Previous studies have demonstrated that the side chain of β-lactamantibiotics generally have two functional groups i.e. lipophilic and alkaline groups [25]. In the present study, with increase in lipophilicity, the antimicrobial effect against Gram +ve organisms was enhanced, while with increase in alkalinity, antimicrobial effect against Gram-ve bacteria became enhanced. The sulfur atom in the side chain of the compounds is a lipophilic center, while the pyrrolidine or piperidine enhances alkalinity. Therefore, these cephalosporin derivatives have high bacteriostatic effects on Gram +ve and Gram-ve species. Compounds 13a and 13b showed better antibacterial effects against Gram-negative bacteria than compounds 8a and 8b with similar structures, due to increased alkalinity.

CONCLUSION

A series of sulfamoylamino-containing cephalosporin derivatives has been prepared by a series of substitution, condensation and deprotection reactions. Similarly, compounds containing unique structural composition of sulfamoylamino-methyl-pyrrolidin-1-yl-methyl have been obtained which exhibit excellent and comprehensive antibacterial effects against Gram +ve and Gram-ve bacteria, relative to the other compounds. Moreover, the compounds 13a and 13b produced higher antibacterial effect on Gram-ve bacteria than those with similar structures containing cephalosporin derivatives due to the increased alkalinity of the former. Compounds 13a and 13b also showed better inhibitory effects on Gram-ve bacteria, as well as similar or superior antibacterial effect on Gram-positive species than cefpirome. Therefore, compounds 13a and 13b merit further investigations.

DECLARATIONS

Acknowledgement

This research was supported by grants from Guangxi Natural Science Foundation Program (no. 2016GXNSFAA380118).

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication. Xiaoling Lu conceived and designed the study; Xudong Jiang, Li Chen, Xiaoling Lu, Lan Liao, WeiGuang Wang and Xiaocheng Huang collected and analyzed the data, while Xudong Jiang wrote the manuscript.

REFERENCES

1. Li GL, Cao RY, Gu JF. Progress in research on new antibiotic targets. Chin Med Biotechnol 2013; 8(4): 303-307.
2. Zhang T, Ni MX, Shao L, Chen SJ. Research progress in mechanism of β-lactam antibiotics. J Chin Antibiot 2015; 40(10):785-790.
3. Miragaia M. Factors contributing to the Evolution of mecA-Mediated β-lactam Resistance in Staphylococci: Update and New Insights from Whole Genome Sequencing (WGS). Front Microbiol 2018; 9: 2723.
4. Chovanová R, Mikulášová M, Vaverková Š. Modulation of mecA Gene Expression by Essential Oil from Salvia sclarea and Synergism with Oxacillin in Methicillin Resistant Staphylococcus epidermidis Carrying Different Types of Staphylococcal Chromosomal Cassette mec. Int J Microbiol 2016; 2016: 6475837.
5. Noto MJ, Fox PM, Archer GL. Spontaneous deletion of the methicillin resistance determinant, meca, partially compensates for the fitness cost associated with high-level vancomycin resistance in staphylococcus aureus. Antimicrob Agents Chemother 2008; 52(4): 1221-1229.
6. O'Connell KM, Hodgkinson JT, Sore HF, Welch M, Salmond GP, Spring DR. Combating multidrug-resistant bacteria: current strategies for the discovery of novel antibacterials. Angew Chem Int Ed Engl 2013; 52(41): 10706-10733.
7. Domalaon R, Idowu T, Zhanel GG, Schweizer F. Antibiotic hybrids: the next generation of agents and adjuvants against gram-negative pathogens. Clin Microbiol Rev 2018; 31(2):77.
8. Foster TJ. Antibiotic resistance in staphylococcus aureus. current status and future prospects. FEMS Microbiol Rev 2017; 41(3):430-449.
9. Bush K, Bradford PA. β-Lactams and β-lactamase inhibitors: an overview. Cold Spring Harb Perspect Med 2016; 6(6):1-22.
10. Watkins RR, Papp-Wallace KM, Drawz SM, Bonomo RA. Novel β-lactamase inhibitors: a therapeutic hope against the scourge of multidrug resistance. Front Microbiol 2013; 4(392): 392.
11. Wright PM, Seiple IB, Myers AG. The evolving role of chemical synthesis in antibacterial drug discovery. Angew Chem Int Ed Engl 2014; 53(34): 8840-8869.
12. Li JY, Gu JF. Research progress and market trend of the fifth-generation cephalosporin antibiotics. Prog Pharm Sci 2014; 38(8): 741-746.
13. Wang QQ, Wang DX, Wang JQ. Recent progress and novel perspectives of electrochemical sensor for cephalosporins detection. Int J Electrochem Sci 2019; 14 (9):8639-8649.
14. Xue Y, Chen YY. New development of cephalosporin antibiotics. Chin J Antibiotic 2011; 36(2):86-92.
15. Yokoo K, Yamawaki K, Yoshida Y, Yonezawa S, Yamano Y, Tsuji M, Hori T, Nakamura R, Ishikura K. Novel broad-spectrum and long-acting parenteral cephalosporins having an acyl cyanamide moiety at the C-3 terminal: synthesis and structure-activity relationships. Eur J Med Chem 2016; 124: 698-712.
16. Cui SF, Addla D, Zhou CH. Novel 3-aminothiazolquinolones: design, synthesis, bioactive evaluation, SARs, and preliminary antibacterial mechanism. J Med Chem 2016; 59(8):3635-3649.
17. Majewski MW, Tiwari R, Miller P, Cho S, Franzblau SG2, Miller MJ. Design, syntheses, and anti-tuberculosis activities of conjugates of piperazino-1,3-benzothi-azin-4-ones (pBTZs) with 2,7-dimeth- ylimidazo [1,2-a] pyridine-3-carboxylic acids and 7-phenylacetyl cephalosporins. Bioorg Med Chem Lett 2016; 26(8):2068-2071.
18. Verdino A, Vigliotta G, Giordano D, Caputo I, Soriente A, De Rosa M, Marabotti A. Synthesis and biological evaluation of the progenitor of a new class of cephalosporin analogues, with a particular focus on structure-based computational analysis. PLoS One 2017; 12:181563.
19. Daniel DG, Robert RE, Taketa DC. Ceftobiprole: a novel, broad-spectrum cephalosporin with activity against methicillin-resistant staphylococcus aureus. Am J Health Syst Pharm 2010; 67:983-993.
20. Duplessis C, Crum-Cianflone NF. Ceftaroline: anew cephalosporin with activity against methicillin-resistant staphylococcus aureus (MRSA). Clin Med Rev Ther 2011;3: 2466.
21. Yang N, Teng D, Mao R, Hao Y, Wang X, Wang ZL, Wang XM, Wang JH. A recombinant fungal defensin-like peptide-P2 combats multidrug-resistant Staphylococcus aureus and biofilms. Appl Microbiol Biotechnol 2019; 103(13): 5193-5213.
22. Chu WC, Bai PY, Yang ZQ, Cui DY, Hua YG, Yang Y, Yang QQ, Zhang E, Qin S. Synthesis and antibacterial evaluation of novel cationic chalcone derivatives possessing broad spectrum antibacterial activity. Eur J Med Chem 2018; 143: 905-921.
23. Guo X, Zhang NH, Bao GL, Zhang ZK, Zhang GM. A novel synthetic process for ceftaroline fosamil. Chin J Pharmaceut2018; 49(11):1518-1523.
24. Ye XL, Cao D, Song FY, Fan GR, Wu FH. Preparative separation of nine flavonoids from Pericarpium Citri Reticulatae by preparative-HPLC and HSCCC. Separationence Technol 2016; 51(5): 807-815.
25. Fan Z, Shi J, Bao X. Synthesis and antimicrobial evaluation of novel 1,2,4-triazole thioether derivatives bearing a quinazoline moiety. Mol Divers 2018; 22(3): 657-667.