New C-3′ hydroxamate-substituted and more lipophilic cyclic hydroxamate cephalosporin derivatives as a potential new generation of selective antimicrobial agents

Marvin J. Miller,*[a] Gaiying Zhao,[a] Sergei Vakulenko,[a] Scott Franzblau[b] and Ute Möllmann[c]

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Syntheses of a series of new C-3′ hydroxamate-substituted cephalosporin derivatives with potent antibacterial and media-dependent anti-TB activity are described.

Introduction

Tuberculosis (TB) is one of the leading causes of death and suffering worldwide. The increasing drug resistance, toxicity and side effects of currently used anti-tuberculosis drugs also emphasizes the urgent need for new, safer and more effective anti-tuberculosis agents.[1–5] Although mycobacteria produce β-lactamases and are intrinsically resistant to β-lactam antibiotics, there are reports that the addition of β-lactamase inhibitors to penems or cephems greatly improved their *in vitro* activity against tuberculosis.[6,7] Since the loss of activity of β-lactam antibiotics could also be attributed to the lack of cell envelope permeability and variations in certain peptidoglycan biosynthetic enzymes,[8,9] we initiated a study to identify structures with increased lipophilicity and/or improved anti-β-lactamase activity. Here, we report the syntheses and results of some biological assays of new C-3′ hydroxamate-substituted cephalosporins and their more lipophilic cyclic hydroxamate esters shown in Fig. 1.

Compared to the common cephalosporins, these 2,3-cyclic hydroxamate-fused cephalosporins were anticipated to be more hydrophobic and possibly able to diffuse through the lipid-rich cell wall of mycobacteria. As further shown in Fig. 1, the cyclic hydroxamate component is an "active ester" that was also anticipated to be able to hydrolytically generate the normal cephalosporin carboxylate subsequent to cellular uptake. The same intracellular hydrolytic process would also release a free hydroxamic acid that could influence either growth promotion or inhibition by affecting iron assimilation or metabolism since hydroxamates are iron binding components of iron sequestering agents (siderophores), including the mycobactins. Mycobactin T, a siderophore selective for growth promotion and virulence of *Mycobacterium tuberculosis* contains two hydroxamates and a phenolic oxazoline for iron chelation. We have previously shown that mycobactin analogs can have potent anti-tuberculosis activity and that, among other factors, activity does require a lipophilic component as does the natural mycobactin T with its long acyl

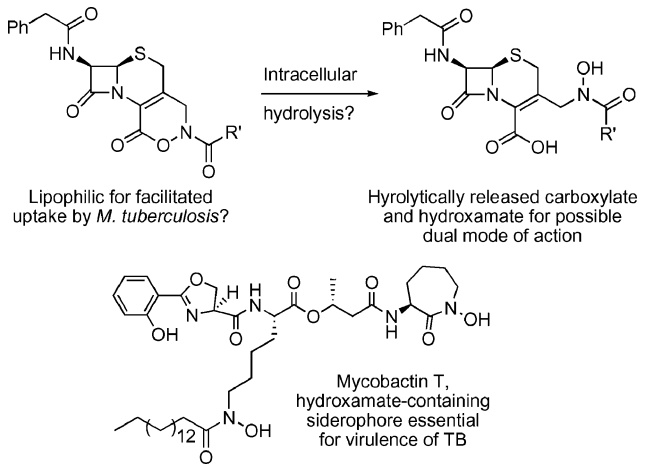
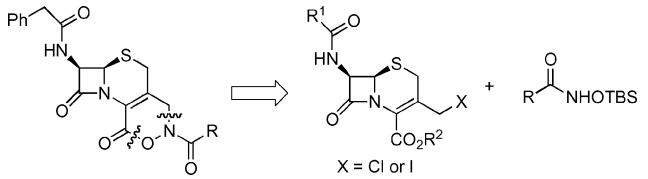


Fig. 1 New C-3′ hydroxamate-substituted cephalosporins (lipophilic cyclic form and hydrolytically generated hydrophilic free carboxylate/hydroxamate form) and mycobactin T.

moiety on the ε-amino group of the constituent linear *N*-hydroxy lysine residue.[10,11]

With these considerations in mind, our first goal was to synthesize representatives of our targeted structure (Fig. 1) and determine if this new class of cephalosporins has antibiotic activity. The retrosynthetic disconnections are shown in Scheme 1. A key point was the introduction of a C-3′ hydroxamate side chain by coupling of a cephalosporin to an *O*-protected hydroxamic acid.

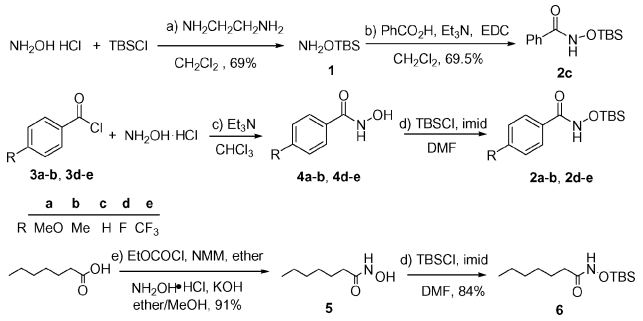


Scheme 1 Retrosynthetic analysis.

Results and discussion

The syntheses of the hydroxamic acids (**2a–e**, **6**) used for the alkylation of 3-chloromethyl-7-phenylacetylamino cephalosporanic acid *p*-methoxybenzyl ester (GCLE) are shown in Scheme 2. *O*-*tert*-Butyldimethylsilyl benzohydroxamic acid **2c** was obtained

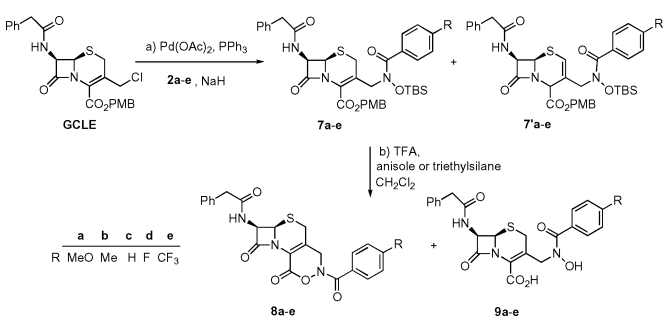
[a]*Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA. E-mail: mmiller1@nd.edu; Fax: +1 574-631-6652*
[b]*College of Pharmacy Institute for Tuberculosis Research, M/C 964, Rm 412, University of Illinois at Chicago, 833 S. Wood St., Chicago, Illinois, 60612-7231, (USA)*
[c]*Leibniz-Institute for Natural Product Research and Infection Biology-Hans Knoell Institute, Beutenbergstrasse 11a, D-07745, Jena, Germany*



Scheme 2 Syntheses of hydroxamic acids **2a–e**, **6**. a) NH_2OH hydrochloride (1.0 equiv.), 1,2-diaminoethane (1.0 equiv.), CH_2Cl_2, rt, 20 h; then TBSCl (1.0 equiv.), rt, 4 d, 69%; b) benzoic acid (1.0 equiv.), Et_3N (1.0 equiv.), EDC (1.1 equiv.), CH_2Cl_2, 16 h, 69%; c) NH_2OH hydrochloride (1.0 equiv.), Et_3N (2.0 equiv.), **3a–b**, **3d–e** (1.0 equiv.), $CHCl_3$, rt, 1 h; d) imidazole (1.3 equiv.), TBSCl (0.95 equiv.), DMF, rt, 16 h; e) octanoic acid (1.0 equiv.), EtOCOCl (1.2 equiv.), NMM (1.3 equiv.), Et_2O, 0 °C, 10 min; then NH_2OH (1.5 equiv.), ether–methanol, rt, 15 min, 91%. NMM = *N*-methyl morpholine.

by an EDC coupling reaction between benzoic acid and *O*-*tert*-butyldimethylsilyl hydroxylamine. The latter was prepared by treating hydroxylamine with *O*-*tert*-butyldimethylsilyl chloride in 1,2-diaminoethane and methylene chloride.[12] 4-Substituted benzohydroxamates **4a–b**, **4d–e**, which were easily obtained by reaction of hydroxylamine with the corresponding acyl chlorides **3a–b**, **3d–e**,[13] were treated with *O*-*tert*-butyldimethylsilyl chloride and imidazole in DMF to give 4-substituted *O*-*tert*-butyldimethylsilyl benzohydroxamates **2a–b**, **2d–e**. Octanoic acid was activated with ethyl chloroformate and *N*-methyl morpholine (NMM), followed by coupling with freshly-generated free hydroxylamine to give heptanohydroxamic acid (**5**) in 91% yield.[14] Then compound **5** was protected with TBSCl and imidazole (imid) to provide protected hydroxamic acid **6** in 84% yield.

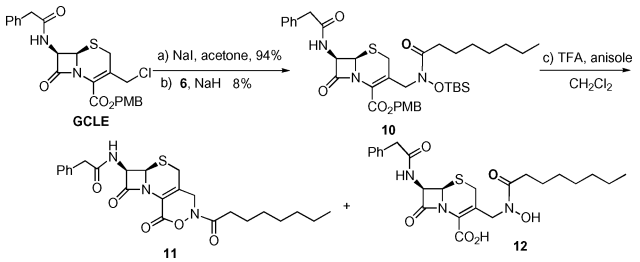
The syntheses of the target hydroxamate-containing cephalosporins are outlined in Scheme 3. After several methods were attempted, the approach that efficiently introduced the C-3′ hydroxamate side chain of cephalosporins was a palladium-catalyzed nucleophilic reaction.[15,16] A short survey of reaction conditions indicated that use of 0.12 equivalent of Pd(0) catalyst from 0 °C to rt for 3 h was most effective. Palladium-catalyzed nucleophilic reaction between GCLE and variously substituted



Scheme 3 Synthesis of **8a–e** and **9a–e**. a) GCLE (1.0 equiv.), $Pd(OAc)_2$ (0.12 equiv.), PPh_3 (0.60 equiv.), **2a–e** (1.1 equiv.), NaH (1.0 equiv.), 0 °C–rt, 3 h; b) TFA (14 equiv.), anisole (or triethylsilane) (4.0 equiv.), CH_2Cl_2, 0 °C–rt, 1.5 h.

protected hydroxamic acids (**2a–e**) provided key intermediates **7a–e** in 21–30% yield with 20–40% of GCLE recovered. Though some Δ-3 to Δ-2 double bond isomerization (**7′a–e**) was observed during these alkylation reactions, the desired products were isolated after purification by careful chromatography. Following deprotection with TFA in the presence of anisole[17,18] or triethylsilane with CH_2Cl_2 as the solvent, the desired products **8a–e** were obtained in 21–36% yield along with the corresponding ring-opened free carboxylic acid hydroxamates **9a–e** in 20–32% yield. Use of TFA alone as reactant and solvent led to isolation of only the free acids **9a–e**.

The syntheses of compounds **11** and **12**, with a more lipophilic acyl group that was expected to more effectively permeate the mycobacterial envelope, are outlined in Scheme 4. Direct substitution reaction between 3-iodomethyl cephalosporin and the corresponding hydroxamic acid **6** gave the desired product **10** in very low yield (8%). Pd(0)-catalyzed reaction only gave a trace of the desired product. However, using the direct alkylation route, enough material was generated to allow us to proceed with syntheses of the target molecules needed for biological studies. Following deprotection with TFA in the presence of anisole and CH_2Cl_2 as solvent, the desired product **11** was obtained in 31% yield, along with the corresponding ring opened free carboxylic acid hydroxamate **12** in 24% yield.



Scheme 4 Synthesis of **11** and **12**. a) GCLE (1.0 equiv.), NaI (1.25 equiv.), acetone, rt, 1.5 h, 94%; b) **6** (0.74 equiv.), NaH (0.70 equiv.), DMF, rt, 16 h, 8%; c) TFA (14 equiv.), anisole (4 equiv.), CH_2Cl_2, 0 °C–rt, 1.5 h.

All of the synthesized cephalosporins were tested for their antibacterial activities against various strains of Gram-positive and Gram-negative bacteria using standard MIC determinations.[19,20] The results showed that they are active only against Gram-positive strains such as *Micrococcus luteus* ATCC 10240, *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* SG 511 (Table 1). The assays also showed that the ring opened free carboxylic acid hydroxamates **9a–e**, **12** are superior in activity compared to the corresponding cyclic hydroxamate esters **8a–e**, **11**. This is consistent with the requirement of a free ionizable carboxyl acid for activity of classical β-lactam antibiotics. For the cyclic hydroxamate esters **8a–e**, **11**, the more lipophilic compound **11** with a long chain gave better activity against all the strains listed. Generally, both electron-donating and electron-withdrawing substituents on the phenyl ring retained or improved the biological activities. For *Micrococcus luteus* ATCC 10240, compound **9e** with a *p*-trifluoromethyl substituent on the phenyl ring gave the best activity with an MIC of 0.4 μM. Compound **9d** with a *p*-fluoro substituent on the phenyl ring and compound **12** with a long chain gave the best activity with an MIC of 0.2 μM

Table 1 Activity against representative bacteria (MIC in μM) and toxicity against VERO cells (IC_{50}[d] in μM)

Compound	Species/Strains					
	Micrococcus luteus ATCC 10240	*Bacillus subtilis* ATCC 6633	*Staphylococcus aureus* SG 511	*M. tuberculosis* H37Rv MABA[a]/GAS[b]	*M. tuberculosis* H37Rv MABA/GAST[c]	VERO cells
8a	6.25	0.4	1.56	31.16	3.2	>128
8b	6.25	0.4	1.56	12	2.8	NT
8c	12.5	0.78	1.56	>128	126	NT
8d	12.5	0.4	1.56	15.3	23.4	>128
8e	12.5	0.4	1.56	10.3	9.8	>128
11	1.56	0.2	0.4	124.1	10.1	19.5
9a	3.12	<0.1	0.78	61.6	46.2	>128
9b	1.56	<0.1	0.78	26.7	31.0	>128
9c	6.25	0.2	0.78	63.7	53.0	>128
9d	1.56	0.2	0.2	120.5	17.1	>128
9e	0.4	<0.1	0.4	86.0	126.6	NT[e]
12	1.56	0.2	0.2	69.3	9.1	14.6
GCLE	50	0.78	3.12	>128	13.9	NT

[a] Microplate Alamar Blue assay.[22] [b] GAS: glycerol-alanine-salts.[23] [c] GAST: GAS without added iron and containing Tween 80.[23] [d] Concentration resulting in 50% inhibition of the growth of VERO cells.[24] [e] NT = not tested.

against *Staphylococcus aureus* SG 511. Against *Bacillus subtilis* ATCC 6633, compound **9a** with a *p*-methoxy substituent, **9b** with a *p*-methyl substituent and compound **9e** with a *p*-trifluoromethyl on the phenyl ring showed excellent activity. Compounds **8c**, **9c** were also tested for their activity as β-lactamase inhibitors by methods described in the literature,[21] but were found to be inactive.

Compounds **8a–e**, **9a–e**, **11**, **12** were also tested against *M. tuberculosis* H_{37}Rv (Table 1). Consistent with our hypothesis, several of the more lipophilic cephalosporin derivatives were found to be more active against TB. Especially notable is the comparison of the results of assays of the cyclic hydroxamates **8** relative to the free carboxylate/hydroxamate series **9** in normal iron sufficient media (GAS) and in iron deficient media (GAST). In some cases (**8c**/**9c** and **8d**/**9d** in the GAST medium), there was little difference (± one dilution) of activity, but in others there were notable differences in activity. While the free carboxylate/hydroxamates would be able to bind iron, they are very polar and are not likely to be able to permeate the lipophilic outer layers of the mycobacterial cell. However, the cyclic hydrophobic forms **8** might effectively penetrate and then either have intrinsic activity themselves or, upon either enzymatic or general intracellular hydrolysis, convert to forms of **9**. Once generated in the targeted cells, the resulting new cephalosporins might cause cellular disruption by classical modes of action and/or by affecting critical iron assimilation and metabolism processes, especially under iron restricted conditions. The possible influence of iron regulation is suggested by the notably enhanced activity of some forms of **8** in the GAST ("iron deficient") medium. Thus, while additional studies are needed to determine details related to the possible reasons for differences in activity, the results are consistent with our initial hypotheses. Of additional interest was the observation that for the cyclic hydroxamate-containing cephalosporin compounds **8a–e**, the presence of either an electron-donating or an electron-withdrawing substituent on the phenyl ring (R′ of the generalized structure) seemed to notably increase anti-TB activity. This suggests that possible additional substitution studies will further help define important SAR relationships. The growth inhibitory activity of compound **11** with the lipophilic acyclic seven-carbon side chain was found to be more pronounced than that of compound **8c** without a substituent on the phenyl ring, but the activity was not as good as that of compounds **8a** or **8b** with electron-donating groups on the phenyl ring. The length and type of the carbon chain might need to be optimized in further SAR studies, especially, and not surprisingly, since use of aliphatic groups also reduced selectivity relative to VERO cells.

Conclusions

In summary, we have synthesized and studied several C-3′-hydroxamate substituted cephalosporins, including cyclic forms **8a–e**, **11** with enhanced lipophilicity and possible alternate modes of activity. For various Gram-positive strains listed in Table 1, the ring-opened free carboxylic acid hydroxamates **9a–e** and **12** are superior in activity compared to the corresponding cyclic hydroxamate esters **8a–e** and **11**. This is consistent with SAR of classical cephalosporins that seem to require a free carboxylate for activity and do not suffer from limited microbial permeability. Of the cyclic hydroxamate esters **8a–e** and **11**, compound **11** was more active against all of the non-mycobacterial strains listed and these cyclic, more lipophilic compounds were generally more active against TB. These studies have accomplished our first goal to demonstrate that novel hydroxamate-containing cephalosporins can have interesting and potentially useful antimicrobial activity. These results provide new leads for the possible generation of species-selective antimicrobial agents based on variation of peripheral functionality and substituents. Additional synthetic and antimicrobial studies are planned to further test this concept and to test our hypothesis related to alternate modes of action.

Experimental

General

All NMR spectra were recorded on a Varian instrument at 300 MHz (^{1}H), 75 MHz (^{13}C), or 500 MHz (^{1}H), 125 MHz (^{13}C)

unless otherwise noted. Chemical shifts are indicated in δ values (ppm) from internal reference peaks of TMS, $CDCl_3$, CD_3OD or DMSO-d_6. Melting points were taken on a Thomas Hoover melting point apparatus and are uncorrected. TLC was performed with aluminium backed Merck silica gel 60-F254 using 254 nm light, aq. $KMnO_4$, $FeCl_3$, or ninhydrin for visualization. Commercial reagents were used without purification. Flash chromatography was performed on Sorbent Technologies silica gel 60 (32–63 μm).

All reagents were purchased from Aldrich, Acros, Advanced ChemTech, and Fisher and used without purification unless otherwise indicated. THF was distilled from a mixture of sodium metal and benzophenone and CH_3CN and TEA were distilled from CaH_2. DI water was further purified through a mixed bed type II filter made by US Filter. All other solvents were used without purification. 3-Chloromethyl-7-phenylacetylamino cephalosporanic acid *p*-methoxybenzyl ester (GCLE) was generously provided by Otsuka Chemical Co., Ltd.

O-tert-Butyldimethylsilyl benzohydroxamic acid (2c)

To a stirred solution of benzoic acid (610 mg, 5.0 mmol) in methylene chloride (10 mL), a solution of *O-tert*-butyldimethylsilyl hydroxylamine[12] (735 mg, 5.0 mmol) in methylene chloride (20 mL) and triethylamine (0.7 mL, 5.0 mmol) were added. Then a suspension of EDC·HCl (1054 mg, 5.5 mmol) in methylene chloride (25 mL) was added. The reaction mixture was allowed to stir overnight at room temperature. Then the mixture was diluted with methylene chloride (60 mL), washed with saturated $NaHCO_3$ solution (60 mL), water (60 mL) and brine (60 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure at room temperature. The residue was purified by flash column chromatography (hexanes–EtOAc: 5 : 1) to give (872 mg, 3.47 mmol) (69.5%) of compound **2c** as a white solid. Mp 138–140 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.26 (s, 3H), 0.38 (s, 3H), 1.02 (s, 9H), 7.46 (m, 5H).

Representative procedure for the syntheses of hydroxamic acids starting from the corresponding acyl chloride

***p*-Methoxybenzohydroxamic acid (4a).** To a suspension of hydroxylamine hydrochloride (815 mg, 0.012 mol) in chloroform (20 mL), was added triethylamine (3.3 mL, 0.024 mol) and *p*-methoxybenzoyl chloride (2.0 g, 0.012 mol) in chloroform (10 mL) dropwise. The resulting mixture was stirred for 1 h. Then the solvent was removed under vacuum. The residue was dissolved in water and cooled in an ice bath. Then 1 N HCl was added. The resulting precipitate was filtered, washed with cold water and dissolved in dilute NaOH. Small pieces of dry ice were added to the alkaline solution and a precipitate was obtained, which was recrystallized from acetone–ether to give (996 mg, 5.96 mmol) (51%) of compound **4a** as colorless crystals. Mp 159–159.5 °C. ^{1}H NMR (CD_3OD, 300 MHz) δ: 3.85 (s, 3H), 6.98 (dd, $J = 2.1$ Hz, $J = 9.0$ Hz, 2H), 7.83 (dd, $J = 2.1$ Hz, $J = 9.0$ Hz, 2H), 8.27 (br, 1H), 10.65 (br, 1H). ^{13}C NMR (CD_3OD, 75 MHz) δ: 55.78, 114.08, 125.38, 129.10, 161.95.

***p*-Methylbenzohydroxamic acid (4b).** 44% yield. Mp 148–149.5 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.35 (s, 3H), 7.26 (d, $J = 8.1$ Hz, 2H), 7.67 (d, $J = 8.1$ Hz, 2H), 8.98 (s, 1H), 11.19 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 21.41, 127.34, 129.36, 130.44, 141.46, 164.69.

***p*-Fluorobenzoylhydroxamic acid (4d).** 34% yield. Mp 161–162 °C. ^{1}H NMR (CD_3OD, 300 MHz) δ: 7.17 (t, $J = 8.7$ Hz, 2H), 7.79 (dd, $J = 5.1$ Hz, $J = 8.7$ Hz, 2H). ^{13}C NMR (CD_3OD, 75 MHz) δ: 116.52, 116.81, 130.75, 130.87, 164.67, 168.00.

***p*-Trifluorobenzoylhydroxamic acid (4e).** 37% yield. Mp 134–136 °C. ^{1}H NMR (CD_3OD, 300 MHz) δ: 7.18 (t, $J = 8.4$ Hz, 2H), 7.81 (dd, $J = 5.1$ Hz, $J = 8.4$ Hz, 2H).

O-tert-Butyl-dimethylsilyl-*p*-methoxybenzohydroxamic acid (2a)

To a stirred solution of *p*-methoxybenzohydroxamic acid **4a** (1.06 g, 0.064 mol) in DMF (10 mL), imidazole (566 mg, 0.083 mol) and *tert*-butyldimethylsilyl chloride (961 mg, 0.061 mol) were added successively. The resulting mixture was allowed to stir overnight at room temperature. Then 10 mL of water was added followed by extraction with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexanes–EtOAc: 5 : 1) to give 812 mg (86% based on recovered starting material) of compound **2a** as a white solid. 500 mg of *p*-methoxybenzohydroxamic acid was recovered. Mp 132–134 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.24 (s, 6H), 1.00 (s, 9H), 3.83 (s, 3H), 6.90 (d, $J = 9.0$ Hz, 2H), 7.69 (d, $J = 9.0$ Hz, 2H). ^{13}C NMR (CD_3OD, 75 MHz) δ: −5.50, 18.29, 25.96, 55.37, 113.87, 128.41, 205.28.

***O-tert*-Butyl-dimethylsilyl-*p*-methylbenzohydroxamic acid (2b).** 82% yield (based on recovered starting material). Mp 128–130 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.25 (s, 6H), 1.01 (s, 9H), 2.39 (s, 3H), 7.31 (m, 4H). ^{13}C NMR (CD_3OD, 75 MHz) δ: −5.42, 17.79, 20.94, 25.75, 126.86, 128.87, 129.84, 140.94, 164.19.

***O-tert*-Butyl-dimethylsilyl-*p*-fluorobenzoylhydroxamic acid (2d).** 61% yield (based on recovered starting material). Mp 152–153 °C. ^{1}H NMR (CD_3OD, 300 MHz) δ: 0.06 (s, 6H), 0.89 (s, 9H), 7.17 (t, $J = 8.7$ Hz, 2H), 7.79 (dd, $J = 5.1$ Hz, $J = 9.0$ Hz, 2H). ^{13}C NMR (CD_3OD, 75 MHz) δ: −5.28, 26.48, 116.49, 116.78, 130.73, 130.85, 164.64, 167.96.

***O-tert*-Butyl-dimethylsilyl-*p*-trifluoromethylbenzoylhydroxamic acid (2e).** 53% yield (based on recovered starting material). Mp 129.5–131 °C. MS (FAB) $(M + H)^+$ m/z 320. ^{1}H NMR (CD_3OD, 300 MHz) δ: 0.02 (s, 6H), 1.02 (s, 9H), 7.78 (d, $J = 8.1$ Hz, 2H), 7.90 (d, $J = 8.1$ Hz, 2H).

Heptanohydroxamic acid (5)

To a solution of octanoic acid (2.88 g, 0.02 mol) in diethyl ether (60 mL) at 0 °C, ethyl chloroformate (2.60 g, 0.024 mol) and *N*-methyl morpholine (2.63 g, 0.026 mol) were added and the mixture was stirred for 10 min. The solid was filtered off and the filtrate was added to freshly-prepared hydroxylamine (0.03 mol) in methanol (8 mL). The resulting mixture was stirred for 15 min at room temperature. Then the solvent was removed and the residue was purified by iron-free silica gel column chromatography (hexanes–EtOAc: 1 : 2) to give 2.92 g (91.7%) of desired compound **5** as a white solid. Mp 76–78 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.89 (t,

J = 7.2 Hz, 3H), 1.30 (m, 8H), 1.63 (m, 2H), 2.16 (t, J = 7.5 Hz, 2H), 4.90 (br, 1H), 7.50 (br, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.06, 22.60, 25.44, 28.95, 29.11, 31.67, 33.03, 172.02.

O-*tert*-Butyl-dimethylsilyl heptanohydroxamic acid (6)

To a stirred solution of **5** (500 mg, 3.2 mmol) in DMF (7 mL), imidazole (279 mg, 4.1 mmol) and *O*-*tert*-butyldimethylsilyl chloride (468 mg, 3.1 mmol) were added successively. The resulting mixture was allowed to stir overnight at room temperature. Then water (10 mL) was added followed by extraction with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was recrystallized from hexanes–EtOAc twice to give 520 mg (84%) of compound **6** as white solid. Mp 58.5–61 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.18 (s, 6H), 0.95 (m, 12H), 1.27 (m, 8H), 1.63 (m, 2H), 2.09 (m, 2H), 7.65 (br, 1H).

3-[*tert*-Butyldimethylsilyloxy-(4-methoxybenzoyl)amino]methyl-7-phenylacetamido-3-cephem-4-carboxylic acid *p*-methoxybenzyl ester (7a)

To a suspension of GCLE (1.302 g, 2.675 mmol) in THF (20 mL) was added a solution of $Pd(OAc)_2$ (72.1 mg, 0.321 mmol) and PPh_3 (420.9 mg, 1.605 mmol) in THF (30 mL) under N_2 over 15 min. Then a solution of **2a** (790.0 mg, 2.809 mmol) and sodium hydride (60% oil suspension) (107.1 mg, 2.675 mmol) in THF (20 mL) was added at 0 °C. The resulting mixture was stirred at room temperature for 3 h under N_2. Then 70 mL of water was added and the resulting mixture was extracted with EtOAc (3 × 150 mL). The organic layers were combined and washed with saturated NaCl solution (400 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexanes–EtOAc: 3 : 1) to give 453 mg (30.2%) of compound **7a** as a light yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: −0.101 (s, 3H), −0.03 (s, 3H), 0.81 (s, 9H), 3.22 (d, J = 18.3 Hz, 1H), 3.58 (d, J = 16.0 Hz, 1H), 3.59 (d, J = 18.3 Hz, 1H), 3.65 (d, J = 16.0 Hz, 1H), 3.79 (s, 3H), 3.83 (s, 3H), 4.81 (d, J = 16.2 Hz, 1H), 4.89 (d, J = 4.8 Hz, 1H), 4.94 (d, J = 16.2 Hz, 1H), 5.10 (dd, J = 11.7 Hz, 2H), 5.80 (dd, J = 4.8 Hz, J = 9.0 Hz, 1H), 6.16 (d, J = 9.0 Hz, 1H), 7.31 (m, 13H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: −5.18, −5.15, 17.84, 25.71, 26.27, 43.18, 55.29, 55.42, 57.64, 59.16, 68.04, 113.57, 113.95, 125.08, 125.90, 126.76, 127.53, 127.77, 129.01, 129.38, 130.72, 130.84, 133.99, 159.93, 161.47, 161.84, 164.72, 171.33.

3-[*tert*-Butyldimethylsilyloxy-(4-methylbenzoyl)amino]methyl-7-phenylacetamido-3-cephem-4-carboxylic acid *p*-methoxybenzyl ester (7b)

29.1% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: −0.08 (s, 3H), −0.02 (s, 3H), 0.81 (s, 9H), 2.38 (s, 3H), 3.59 (dd, J = 18 Hz, 2H), 3.62 (dd, J = 16 Hz, 2H), 3.79 (s, 3H), 4.89 (dd, J = 16 Hz, 2H), 4.90 (d, J = 4.8 Hz, 1H), 5.10 (dd, J = 12 Hz, 2H), 5.80 (dd, J = 4.8 Hz, J = 9.0 Hz, 1H), 6.10 (d, J = 9.0 Hz, 1H), 7.31 (m, 13H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: −5.91, −5.11, 17.87, 21.55, 25.68, 26.16, 43.29, 55.29, 57.51, 59.13, 68.05, 113.97, 125.13, 126.73, 127.70, 127.74, 128.62, 128.99, 129.14, 129.44, 130.83, 130.93, 133.76, 141.59, 159.95, 161.47, 164.66, 171.18.

3-[(*tert*-Butyldimethylsilyloxy)benzamido]methyl-7-phenylacetamido-3-cephem-4-carboxylic acid *p*-methoxybenzyl ester (7c). 29.3% yield. MS (FAB) (M + H)$^+$ m/z 702. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: −0.07 (s, 3H), 0.01 (s, 3H), 0.83 (s, 9H), 3.28 (d, J = 18 Hz, 1H), 3.64 (d, J = 18 Hz, 1H), 3.66 (dd, J = 16 Hz, 2H), 3.83 (s, 3H), 4.93 (dd, J = 15 Hz, 2H), 4.93 (d, J = 5 Hz, 1H), 5.13 (dd, J = 16 Hz, 2H), 5.85 (dd, J = 5 Hz, J = 9 Hz, 1H), 6.08 (d, J = 9 Hz, 1H), 7.30 (m, 14H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: −4.99, 18.01, 25.83, 26.31, 43.57, 55.49, 57.64, 59.31, 68.28, 92.03, 114.16, 125.41, 126.87, 127.69, 128.01, 128.57, 128.66, 129.44, 129.67, 131.04, 131.29, 133.78, 134.08, 160.15, 161.64, 164.79, 171.24.

3-[*tert*-Butyldimethylsilyloxy-(4-fluorobenzoyl)amino]methyl-7-phenylacetamido-3-cephem-4-carboxylic acid *p*-methoxybenzyl ester (7d). 27% yield. MS (FAB) (M + H)$^+$ m/z 720. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: −0.12 (s, 3H), −0.02 (s, 3H), 0.82 (s, 9H), 3.30 (d, J = 18.6 Hz, 1H), 3.61 (d, J = 18.6 Hz, 1H), 3.64 (dd, J = 15.9 Hz, 2H), 3.82 (s, 3H), 4.93 (dd, J = 15.0 Hz, 2H), 4.93 (d, J = 4.8 Hz, 1H), 5.14 (dd, J = 12.0 Hz, 2H), 5.83 (dd, J = 4.8 Hz, J = 9.0 Hz, 1H), 6.08 (d, J = 9.3 Hz, 1H), 7.30 (m, 13H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ: −5.13, 17.80, 25.62, 26.21, 43.30, 55.29, 57.60, 59.17, 68.13, 113.97, 115.27, 115.57, 125.30, 126.66, 127.26, 127.70, 129.15, 129.43, 129.90, 130.86, 131.02, 131.14, 133.74, 159.98, 161.44, 162.52, 164.64, 165.86, 171.18.

3-[*tert*-Butyldimethylsilyloxy-(4-trifluoromethylbenzoyl)amino]-methyl-7-phenylacetamido-3-cephem-4-carboxylic acid *p*-methoxybenzyl ester (7e). 21% yield. MS (FAB) (M + H)$^+$ m/z 770. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: −0.13 (s, 3H), −0.02 (s, 3H), 0.80 (s, 9H), 3.32 (d, J = 18.0 Hz, 1H), 3.63 (d, J = 17.0 Hz, 1H), 3.67 (dd, J = 16.0 Hz, 2H), 3.84 (s, 3H), 4.95 (dd, J = 15.0 Hz, 2H), 4.95 (d, J = 4.8 Hz, 1H), 5.16 (dd, J = 12.0 Hz, 2H), 5.85 (dd, J = 4.8 Hz, J = 9.0 Hz, 1H), 6.07 (d, J = 9.0 Hz, 1H), 7.30 (m, 13H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: −5.07, 17.78, 25.53, 26.15, 43.32, 55.29, 57.61, 59.21, 68.17, 94.78, 113.98, 125.24, 126.63, 126.90, 127.74, 129.00, 129.17, 129.43, 130.85, 133.71, 160.00, 161.44, 164.65, 171.17.

N-[6-(4-Methoxybenzoyl)-1,8-dioxo-2,2a,4,5,6,8-hexahydro-1*H*-7-oxa-3-thia-6,8b-diazacyclobuta[*a*]naphthalen-2-yl]-2-phenylacetamide (8a)

To a mixture of compound **7a** (60 mg, 0.082 mmol) and triethylsilane (0.01 mL) in methylene chloride (0.5 mL), was added TFA (0.1 mL) at 0 °C. The resulting mixture was stirred at room temperature for 1.5 h. Then the TFA was removed under reduced pressure and the residue was taken up in EtOAc–H_2O (4 mL of a 1 : 1 mixture). The pH value of the aqueous layer was adjusted to 7.5 using saturated $NaHCO_3$. The aqueous layer was separated, washed with EtOAc (3 × 1 mL) (The aqueous layer was used for the synthesis of compound **9a**). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated to give a yellow oil. The residue was purified by flash column chromatography on silica gel (hexanes–EtOAc: 2 : 1) to give 14 mg (35.6%) of compound **8a** as a light yellow solid. MS (FAB) (M + H)$^+$ m/z 480. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.31 (d, J = 18.6 Hz, 1H), 3.64 (dd, J = 16.2 Hz, 2H), 3.69 (d, J = 18.6 Hz, 1H), 3.84 (s, 3H), 4.65 (dd, J = 18.0 Hz, 2H), 4.98 (d, J = 5.0 Hz,

1H), 5.84 (dd, $J = 5.0$ Hz, $J = 0.9$ Hz, 1H), 6.22 (d, $J = 9.0$ Hz, 1H), 7.32 (m, 9H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 25.43, 43.26, 46.42, 55.48, 57.26, 59.66, 113.77, 121.03, 122.55, 127.81, 129.23, 129.47, 132.02, 133.60, 134.27, 157.75, 163.23, 163.68, 170.20, 171.27.

***N*-[6-(4-Methylbenzoyl)-1,8-dioxo-2,2a,4,5,6,8-hexahydro-1*H*-7-oxa-3-thia-6,8b-diazacyclobuta[*a*]naphthalen-2-yl]-2-phenylacetamide (8b).** 23% yield. MS (FAB) $(M + H)^+$ m/z 464. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.39 (s, 3H), 3.31 (d, $J = 19.2$ Hz, 1H), 3.64 (dd, $J = 16.0$ Hz, 2H), 3.68 (d, $J = 19.2$ Hz, 1H), 4.67 (dd, $J = 18.0$ Hz, 2H), 4.98 (d, $J = 4.8$ Hz, 1H), 5.84 (dd, $J = 4.8$ Hz, $J = 8.4$ Hz, 1H), 6.20 (d, $J = 8.4$ Hz, 1H), 7.41 (m, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 21.67, 25.42, 43.27, 46.32, 57.29, 59.65, 121.05, 127.69, 127.83, 129.15, 129.24, 129.49, 129.65, 133.61, 134.18, 143.56, 157.68, 163.74, 170.53, 171.29.

***N*-[6-Benzoyl-1,8-dioxo-2,2a,4,5,6,8-hexahydro-1*H*-7-oxa-3-thia-6,8b-diazacyclobuta[*a*]naphthalen-2-yl]-2-phenylacetamide (8c).** 24% yield. MS (FAB) $(M + H)^+$ m/z 450 decompose at 122 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.34 (d, $J = 18.6$ Hz, 1H), 3.67 (dd, $J = 16.2$ Hz, 2H), 3.75 (d, $J = 18.6$ Hz, 1H), 4.72 (dd, $J = 18$ Hz, 2H), 5.01 (d, $J = 4.8$ Hz, 1H), 5.88 (dd, $J = 4.8$ Hz, $J = 9$ Hz, 1H), 6.10 (d, $J = 9$ Hz, 1H), 7.45 (m, 10H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 25.66, 43.55, 46.38, 57.53, 59.90, 121.33, 128.10, 128.69, 129.52, 129.71, 129.73, 130.86, 132.93, 133.78, 134.23, 157.70, 163.95, 170.56, 171.44.

***N*-[6-(4-Fluorobenzoyl)-1,8-dioxo-2,2a,4,5,6,8-hexahydro-1*H*-7-oxa-3-thia-6,8b-diazacyclobuta[*a*]naphthalen-2-yl]-2-phenylacetamide (8d).** 21% yield. MS (FAB) $(M + H)^+$ m/z 486. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.37 (d, $J = 19.2$ Hz, 1H), 3.68 (dd, $J = 16.2$ Hz, 2H), 3.75 (d, $J = 19.2$ Hz, 1H), 4.73 (dd, $J = 18.0$ Hz, 2H), 5.03 (d, $J = 5.1$ Hz, 1H), 5.90 (dd, $J = 4.8$ Hz, $J = 8.7$ Hz, 1H), 6.10 (d, $J = 8.7$ Hz, 1H), 7.12–7.88 (m, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 25.44, 43.28, 46.07, 57.29, 59.68, 115.57, 115.86, 121.01, 127.86, 129.27, 129.49, 132.22, 132.35, 133.54, 134.16, 163.60, 163.74, 166.97, 169.15, 171.27.

***N*-[6-(4-Trifluoromethylbenzoyl)-1,8-dioxo-2,2a,4,5,6,8-hexahydro-1*H*-7-oxa-3-thia-6,8b-diazacyclobuta[*a*]naphthalen-2-yl]-2-phenylacetamide (8e).** 21% yield. MS (FAB) $(M + H)^+$ m/z 518. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.39 (d, $J = 19.2$ Hz, 1H), 3.69 (dd, $J = 16.0$ Hz, 2H), 3.77 (d, $J = 19.0$ Hz, 1H), 4.78 (dd, $J = 18.0$ Hz, 2H), 5.04 (d, $J = 5.1$ Hz, 1H), 5.91 (dd, $J = 5.1$ Hz, $J = 8.7$ Hz, 1H), 6.19 (d, $J = 8.4$ Hz, 1H), 7.29–7.89 (m, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 25.63, 29.91, 43.45, 46.03, 57.52, 59.92, 121.21, 125.63, 125.68, 128.06, 129.37, 129.46, 129.67, 129.99, 133.71, 134.18, 157.22, 163.97, 168.75, 171.54.

3-[Hydroxy-(4-methoxybenzoyl)-amino]methyl-7-phenylacetamido-3-cephem-4-carboxylic acid (9a)

The aqueous layer from initial part of the workup during the synthesis of compound **8a** was adjusted to pH 5.5 using diluted HCl and extracted with EtOAc (3 × 2 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated to give 8 mg (20.3%) of compound **9a** as a light yellow solid. MS (FAB) $(M + H)^+$ m/z 498, 520 (its sodium salt). ^{1}H NMR (CD_3OD, 300 MHz) δ: 3.46 (d, $J = 18.0$ Hz, 1H), 3.59 (dd, $J = 14.4$ Hz, 2H), 3.67 (d, $J = 18.0$ Hz, 1H), 3.83 (s, 3H), 4.88 (overlap with the solvent peak, 2H), 5.06 (d, $J = 4.8$ Hz, 1H), 5.70 (d, $J = 4.8$ Hz, 1H), 7.34 (m, 9H). ^{13}C NMR (CD_3OD, 75 MHz) δ: 25.91, 41.75, 50.24, 54.47, 57.54, 59.23, 112.86, 125.39, 126.59, 128.17, 128.81, 130.53, 131.43, 135.04, 161.92, 164.74, 170.40, 173.21.

3-[Hydroxy-(4-methylbenzoyl)-amino]methyl-7-phenylacetamido-3-cephem-4-carboxylic acid (9b). 29% yield. MS (FAB) $(M + H)^+$ m/z 482, 504 (M + Na). ^{1}H NMR (CD_3OD, 300 MHz) δ: 2.37 (s, 3H), 3.45 (d, $J = 18.0$ Hz, 1H), 3.59 (dd, $J = 15.0$ Hz, 2H), 3.67 (d, $J = 18.0$ Hz, 1H), 4.89 (overlap with the solvent peak, 2H), 5.06 (d, $J = 4.8$ Hz, 1H), 5.70 (d, $J = 4.8$ Hz, 1H), 7.42 (m, 9H). ^{13}C NMR (CD_3OD, 75 MHz) δ: 21.61, 27.44, 43.29, 51.70, 59.08, 60.77, 128.14, 129.71, 129.78, 129.84, 130.36, 132.29, 136.58, 142.62, 165.25, 166.25, 172.39, 174.75.

3-[Hydroxy(benzoyl)amino]methyl-7-phenylacetamido-3-cephem-4-carboxylic acid (9c). 31% yield. MS (FAB) $(M + H)^+$ m/z 468. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.34 (d, $J = 18$ Hz, 1H), 3.67 (dd, $J = 16.2$ Hz, 2H), 3.77 (d, $J = 18$ Hz, 1H), 4.75 (dd, $J = 15$ Hz, 2H), 5.08 (d, $J = 5$ Hz, 1H), 5.69 (dd, $J = 5$ Hz, $J = 8$ Hz, 1H), 7.43 (m, 10H), 9.13 (d, $J = 8$ Hz, 1H), 10.05 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) δ: 27.29, 43.57, 49.68, 55.52, 57.61, 59.44, 68.39, 95.05, 114.21, 119.60, 124.95, 125.21, 126.91, 128.02, 128.19, 128.54, 129.43, 129.46, 129.70, 130.67, 131.06, 131.59, 133.11, 133.85, 136.41, 151.41, 160.19, 161.81, 164.89, 171.31.

3-[Hydroxy-(4-fluorobenzoyl)-amino]methyl-7-phenylacetamido-3-cephem-4-carboxylic acid (9d). 31% yield. MS (FAB) $(M + H)^+$ m/z 486. ^{1}H NMR (CD_3OD, 300 MHz) δ: 3.48 (d, $J = 18.0$ Hz, 1H), 3.60 (dd, $J = 14.1$ Hz, 2H), 3.68 (d, $J = 18.0$ Hz, 1H), 4.92 (s, 2H), 5.07 (d, $J = 4.8$ Hz, 1H), 5.71 (d, $J = 4.8$ Hz, 1H), 7.13–7.78 (m, 9H). ^{13}C NMR (CD_3OD, 75 MHz) δ: 27.50, 43.28, 51.48, 59.08, 60.79, 115.89, 116.18, 127.96, 128.13, 129.70, 130.34, 131.44, 132.42, 132.54, 136.57, 163.96, 166.25, 167.27, 171.16, 174.76.

3-[Hydroxy-(4-trifluoromethylbenzoyl)-amino]methyl-7-phenylacetamido-3-cephem-4-carboxylic acid (9e). 31% yield. MS (FAB) $(M + H)^+$ m/z 536. ^{1}H NMR (CD_3OD, 300 MHz) δ: 3.52 (d, $J = 18.0$ Hz, 1H), 3.63 (dd, $J = 15.0$ Hz, 2H), 3.74 (d, $J = 18.0$ Hz, 1H), 4.96 (s, 2H), 5.11 (d, $J = 4.8$ Hz, 1H), 5.75 (d, $J = 4.8$ Hz, 1H), 7.26–7.87 (m, 9H). ^{13}C NMR (CD_3OD, 75 MHz) δ: 26.01, 41.75, 49.72, 57.55, 59.27, 122.13, 124.56, 124.61, 125.80, 126.60, 126.77, 128.17, 128.71, 128.81, 131.54, 131.97, 135.04, 137.87, 163.67, 164.69, 169.37, 173.23.

3-[*tert*-Butyldimethylsilyloxyl(octanoyl)amino]methyl-7-phenylacetamido-3-cephem-4-carboxylic acid *p*-methoxybenzyl ester (10)

To a solution of GCLE (900 mg, 1.80 mmol) in acetone (18 mL) was added NaI (346.5 mg, 2.25 mmol). The mixture was stirred at room temperature for 1.5 h. Then 18 mL of water was added and the resulting mixture was extracted with methylene chloride (2 × 18 mL). The combined organic layers were washed with 5% aqueous $Na_2S_2O_3$ (12 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give 1.0 g (94%) of the 3-iodomethyl cephalosporin intermediate as a yellow solid. Then to a solution of *O*-*tert*-butyl-dimethylsilyl heptanohydroxamic acid (300 mg, 1.156 mmol) in DMF (3 mL) cooled in an ice bath, sodium hydride (61% oil dispersion) (44 mg, 1.098 mmol) was

added. After stirring for 30 min, a solution of the 3-iodomethyl cephalosporin intermediate (900 mg, 1.556 mmol) in DMF (3 mL) was then added and the mixture was stirred at rt overnight. Then 6 mL of water was added. The resulting mixture was extracted with EtOAc (3 × 6 mL). The organic layers were combined and washed with brine (20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexanes–EtOAc: 5 : 1) to give 70 mg (8%) of compound **10** as a yellow solid. MS (FAB) $(M + H)^+$ *m/z* 724. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.07 (s, 3H), 0.19 (s, 3H), 0.94 (m, 12H), 1.30 (m, 8H), 1.63 (m, 2H), 2.38 (m, 2H), 3.31 (dd, J = 18.0 Hz, 2H), 3.67 (dd, J = 16.2 Hz, 2H), 3.84 (s, 3H), 4.66 (d, J = 16.5 Hz, 1H), 4.90 (d, J = 4.8 Hz, 1H), 5.00 (d, J = 16.5 Hz, 1H), 5.83 (dd, J = 4.8 Hz, J = 9.3 Hz, 1H), 6.11 (d, J = 9.3 Hz, 1H), 6.90–7.42 (m, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: −4.78, −4.64, 14.09, 17.80, 22.63, 24.36, 25.67, 25.75, 29.06, 29.39, 29.74, 31.70, 32.59, 43.39, 55.30, 59.14, 68.04, 113.98, 124.82, 126.79, 127.83, 127.97, 129.25, 129.48, 130.87, 133.62, 159.95, 161.57, 164.55, 171.13.

N-(6-Octanoyl-1,8-dioxo-2,2a,4,5,6,8-hexahydro-1*H*-7-oxa-3-thia-6,8b-diazacyclobuta[*a*]naphthalene-2-yl)-2-phenylacetamide (11)

To a mixture of compound **10** (50 mg, 0.069 mmol) and anisole (29.8 mg, 0.276 mmol) in methylene chloride (0.5 mL), was added TFA (110 mg, 0.967 mmol) at 0 °C. The resulting mixture was stirred at room temperature for 0.5 h. Then TFA was removed under reduced pressure and the residue was purified by flash column chromatography on silica gel (hexanes–EtOAc: 2 : 1) to give 10 mg (30.8%) of compound **11** as a light yellow solid. MS (FAB) $(M + H)^+$ *m/z* 472. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.87 (t, J = 6.6 Hz, 3H), 1.28 (m, 8H), 1.61 (t, J = 6.9 Hz, 3H), 2.51 (m, 2H), 3.28 (d, J = 18.9 Hz, 1H), 3.66 (dd, J = 16.0 Hz, 2H), 3.65 (d, J = 18.6 Hz, 1H), 4.59 (dd, J = 18.0 Hz, 2H), 4.98 (d, J = 5.0 Hz, 1H), 5.88 (dd, J = 4.8 Hz, J = 8.7 Hz, 1H), 6.10 (d, J = 8.7 Hz, 1H), 7.25–7.40 (m, 5H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.10, 22.60, 24.15, 25.30, 28.94, 29.09, 31.63, 32.51, 43.30, 44.72, 57.26, 59.68, 121.02, 127.89, 129.29, 129.50, 133.52, 134.28, 157.54, 163.86, 171.26, 173.77.

3-[Hydroxy(octanoyl)amino]methyl-8-oxo-7-phenylacetamido-3-cephem-4-carboxylic acid (12)

The column from the chromatography described for the preparation of **11** was eluted with 5% MeOH in CH_2Cl_2 to give 8 mg (23.7%) of compound **12** as a light yellow solid. MS (FAB) $(M + H)^+$ *m/z* 490. ^{1}H NMR (CD_3OD, 300 MHz) δ: 0.94 (t, J = 6.9 Hz, 3H), 1.35 (m, 8H), 1.64 (t, J = 6.6 Hz, 3H), 2.52 (t, J = 7.5 Hz, 2H), 3.38 (d overlap with solvent peak, J = 18.6 Hz, 1H), 3.55 (d, J = 18.6 Hz, 1H), 3.62 (dd, J = 14.1 Hz, 2H), 4.78 (dd, J = 15.6 Hz, 2H), 5.08 (d, J = 4.5 Hz, 1H), 5.73 (d, J = 4.5 Hz, 1H), 7.26–7.34 (m, 5H). ^{13}C NMR (CD_3OD, 75 MHz) δ: 14.57, 23.84, 25.99, 27.29, 30.31, 30.60, 33.04, 33.31, 43.28, 59.07, 60.77, 128.13, 128.62, 129.70, 130.34, 136.57, 165.06, 166.25, 174.76, 177.09.

Antibacterial activity

Antibacterial activity of the compounds was studied by determination of minimal inhibitory concentrations (MIC) according to the NCCLS guidelines[19,20] using the broth micro dilution method.

Anti-tuberculosis and cytotoxicity assays

Activity against replicating *Mycobacterium tuberculosis* H_{37}Rv (American Type Culture Collection, Rockville, MD) was determined using a fluorescence readout in the Microplate Alamar Blue Assay (MABA)[22,24] following incubation for one week with test compounds in glycerol-alanine-salts medium (GAS) as well as in medium without added iron but with Tween 80 (GAST).[23] The MIC was defined as the minimum concentration inhibiting fluorescence by 90% relative to bacteria-only controls. Toxicity to African green monkey kidney cells (VERO) was determined using a colorimetric assay as previously described.[24]

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