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Synthesis and Biological Activity of Novel 1β-Methylcarbapenems with Oxyiminopyrrolidinylamide Moiety

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Abstract—The synthesis and antibacterial activity of novel 1β-methylcarbapenems **1a**–**f** bearing oxyiminopyrrolidinylamide moiety at C-5 position of pyrrolidine are described. Most compounds exhibited comparable antibacterial activity to meropenem against a wide range of Gram-positive and Gram-negative organisms including *Pseudomonas aeruginosa* isolates. Of these carbapenems, **1a** showed potent and broad spectrum of antibacterial activity and similar stability to DHP-I to meropenem. Against clinical isolates of 40 Gram-negative bacterial species including MDR and ESBL-producing strains, the selected carbapenem **1a** possessed excellent in vitro activity except for MDR *P. aeruginosa*, and was comparable in potency to meropenem.

Introduction

The clinical importance of carbapenem antibiotics increases in proportion as appearance of highly resistant strains to cephalosporins such as multi-drug resistant (MDR) or extended-spectrum β-lactamase (ESBL)-producing strains. 1β-Methylcarbapenems are potent drugs against a variety of pathogens, including MDR bacteria. Meropenem[1] has a broad spectrum of antibacterial activity against clinically significant Gram-positive, Gram-negative aerobic and anaerobic pathogens, and high stability to dehydropeptidase-I (DHP-I). Ertapenem[2,3] is new long-acting 1β-methylcarbapenem antibiotic with excellent activity against most common Gram-positive and Gram-negative isolates, except for *Pseudomonas aeruginosa*.

In our previous work,[4] we described the synthesis and biological evaluation of novel 1β-methylcarbapenems having pyrrolidinylamide group at C-5 position of pyrrolidine. Most carbapenems showed potent and well-balanced antibacterial activity. The selected diol compound **2** exhibited superior or similar profiles to meropenem for pharmacokinetics and in vivo therapeutic efficacy in systematic infections in mice.

Based on these results and the structural feature of meropenem and ertapenem with an amide linkage, we attempted the introduction of pyrrolidinylamide moiety containing oxyiminoalkyl substituent as a polar basic site.

Herein, we wish to describe the synthesis of new 1β-methylcarbapenems **1a**–**f** having oxyiminopyrrolidinylamide moiety and their antibacterial activity against clinically important pathogens including MDR and ESBL-producing strains (Fig. 1).

Chemistry

Boc-protected 3-oxyiminoalkyl-4-substituted pyrrolidines **8a**,**b**,**e**,**f** were prepared by linear route as shown in Scheme 1.

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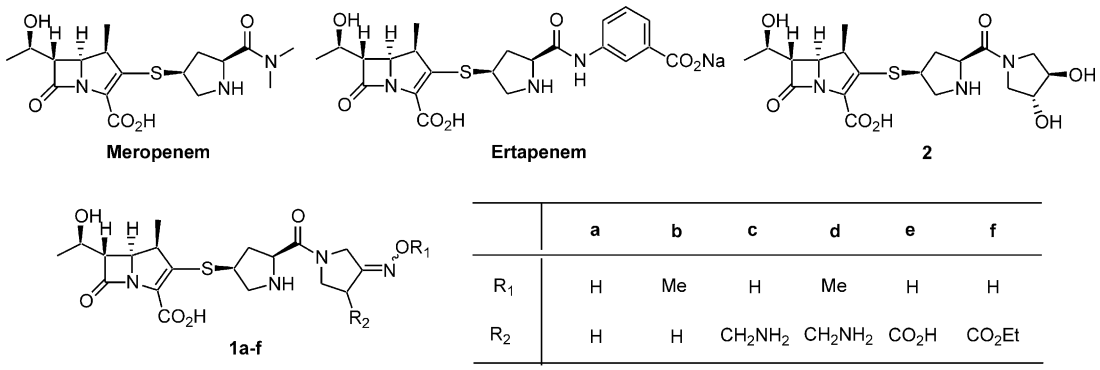
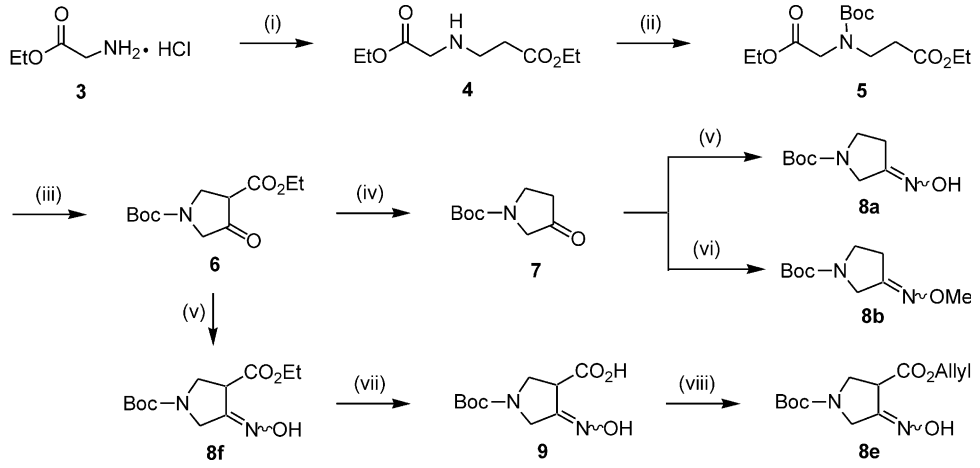


	a	b	c	d	e	f
R_1	H	Me	H	Me	H	H
R_2	H	H	CH_2NH_2	CH_2NH_2	CO_2H	CO_2Et

Figure 1.



Scheme 1. Reagents and reaction conditions: (i) CH_2CHCO_2Et, H_2O, 5 N NaOH, 0 °C to rt, 19 h (70%); (ii) $(Boc)_2O$, CH_2Cl_2, rt, 1 h; (iii) NaOEt, EtOH, reflux, 3 h (88% from **4**, two steps); (iv) DMSO/H_2O (10:1), 120–130 °C, 4 h (70%); (v) $NH_2OH{\cdot}HCl$, $NaHCO_3$, EtOH/THF (2:1), rt, 1 h (90% for **8a**, 93% for **8f**); (vi) $NH_2Ome{\cdot}HCl$, $NaHCO_3$, EtOH/THF (2:1), 40 °C, 1 h (90%); (vii) Claisen alkali, MeOH, 0 °C, 1 h (98%); allyl bromide, TEA, DMF, 65 °C, 1 h (76%).

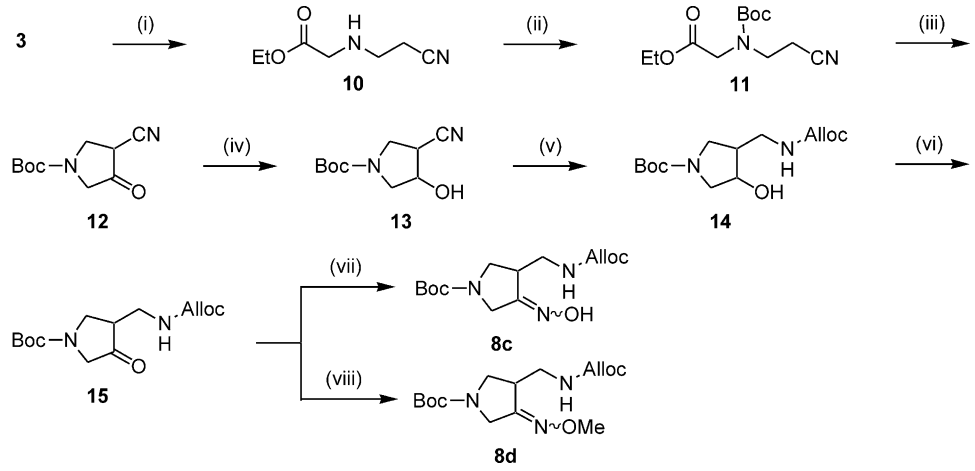
Michael addition to glycine ethyl ester hydrochloride (**3**) with ethyl acrylate under basic condition followed by Boc-protection of amino group gave diethyl 2-azabutane-1,4-dicarboxylate (**5**), which was cyclized by Dieckmann reaction to afford 4-ethoxycarbonyl-3-pyrrolidinone **6**.[5] Treatment of hydroxylamine and methoxylamine with **7**, which was prepared from **6** by Krapcho synthesis,[6] led to the corresponding oxyiminoalkylpyrrolidines **8a** and **8b**,[7] respectively. **8f** was prepared from **6** in similar manner and converted then to **8e** by base-catalyzed hydrolysis and subsequent Allyl-protection of the resulting carboxylic acid **9**.

On the other hand, **8c**,**d** were prepared starting from **3** according to Scheme 2. 4-Cyano-3-pyrrolidine **12**, which was prepared by similar procedure as described for the preparation of **6**, was reduced with sodium borohydride to give the alcohol **13**.[7] Reduction of cyano group of **13** with lithium aluminum hydride followed by Alloc-protection of the resulting amine provided **14**. **14** was oxidized using pyridine sulfur trioxide complex in the presence of triethylamine to give 3-pyrrolidinone **15**, which was converted to the desired oxyiminoalkyl compounds **8c** and **8d**, respectively. The pyrrolidines **8a–f** were obtained as a mixture of geometric isomers of *syn* and *anti,* which were difficult to separate by column chromatography.

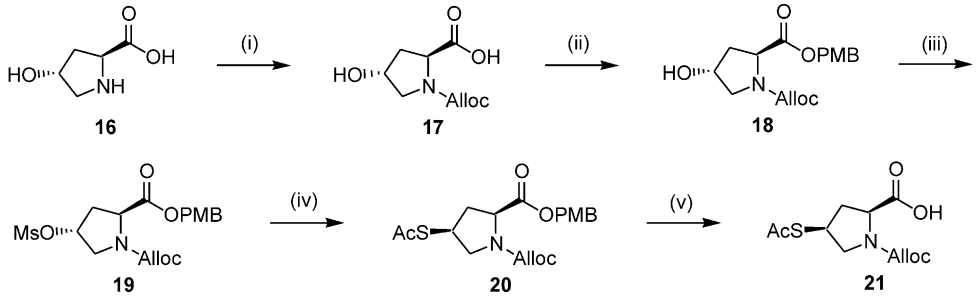
The synthesis of 5-carboxy thioacetate **21** is described in Scheme 3. *trans*-4-Hydroxy-L-proline (**16**) was treated with allyl chloroformate to give Alloc-protected **17**,[8] which was esterified to afford benzoate **18**. Mesylation of hydroxyl group of **18** followed by conversion of the resulting mesylate **19** with potassium thioacetate gave thioacetyl proline **20**, which upon hydrolysis with trifluoroacetic acid provided the desired thioacetate **21**.

In the presence of *N,N*-carbonyldiimidazole, treatment of thioacetate **21** with 3-oxyiminoalkylpyrrolidines, which were generated from Boc-protected **8a–f** by trifluoroacetic acid, afforded pyrrolidinylamido thioacetates **22a–f** (Scheme 4).

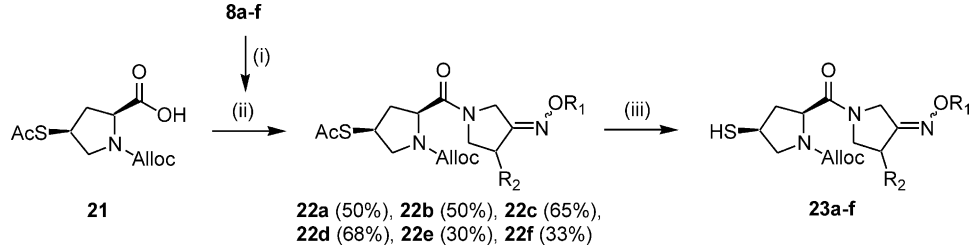
Thiols **23a–f**, applicable for the coupling with carbapenem enolphosphate **24**,[9] were prepared from **22a–f** by deacetylation under basic condition.



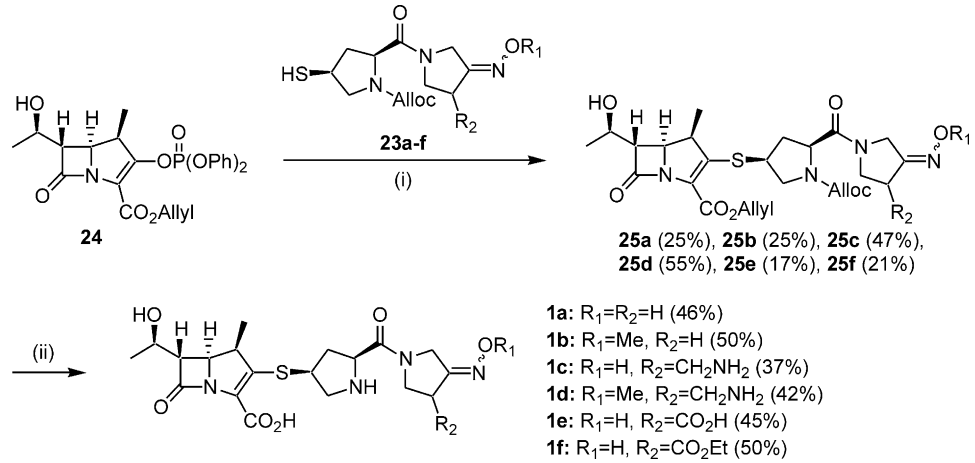
Scheme 2. Reagents and reaction conditions: (i) acrylonitrile, 5 N NaOH, H_2O, 0 °C, 19 h (45%); (ii) $(Boc)_2O$, CH_2Cl_2, rt, 1 h; (iii) NaOEt, EtOH, reflux, 3 h (86% from **10**, two steps); (iv) $NaBH_4$, EtOH, 0 °C, 0.5 h (95%); (v) $LiAlH_4$, THF, −5 °C then 15% NaOH, H_2O, 1 h/allyl chloroformate, dioxane (76%); (vi) DMSO, Et_3N, pyridine–SO_3, 0 °C, 3 h (83%); (vii) $NH_2OH{\cdot}HCl$, $NaHCO_3$, EtOH–THF (2:1), rt, 1 h (85%); (viii) $NH_2OMe{\cdot}HCl$, $NaHCO_3$, EtOH–THF (2:1), 40 °C, 1 h (92%).



Scheme 3. Reagents and reaction conditions: (i) alloc-Cl, TEA, CH_2Cl_2, rt, 3 h (95%); (ii) *p*-methoxybenzyl chloride, TEA, DMF, 70 °C, 10 h (78%); (iii) MsCl, TEA, CH_2Cl, rt, 1 h (95%); (iv) AcSK, CH_3CN, reflux, >90 °C; (v) TFA, anisole, rt, 0.5 h (72%).



Scheme 4. Reagents and reaction conditions: (i) CF_3COOH, CH_2Cl_2, 0 °C, 1 h/Et_3N, CH_3CN; (ii) *N*,*N*-carbonyldiimidazole, CH_3CN, rt, 3 h; (iii) 1 N NaOH, MeOH, 0 °C, 1 h (for **23a–d**), NaSMe, allyl alcohol, 0 °C, 1 h (for **23e**), 1 N NaOH, EtOH, 0 °C, 1 h (for **23f**).



Scheme 5. Reagents and reaction conditions: (i) *N*,*N*-diisopropylethylamine, CH_3CN, 0 °C, 3 h; (ii) $(n\text{-}Bu)_3SnH$, cat. $(PPh_3)_4Pd(0)$, CH_2Cl_2, 0 °C, 2 h.

Table 1. In vitro antibacterial activity and DHP-I stability of **1a–f**

Orgamism	MIC (μg/mL)[a]							
	1a	**1b**	**1c**	**1d**	**1e**	**1f**	IPM[b]	MPM[c]
S. pyogenes 308A	0.013	—	0.002	0.004	0.013	0.013	0.007	0.013
S. aureus SG 511	0.195	—	0.098	0.098	0.098	0.195	0.025	0.195
S. aureus 285	0.195	—	0.195	0.195	0.195	0.391	0.025	0.195
S. aureus 503	0.098	—	0.098	0.049	0.098	0.195	0.013	0.098
E. coli 078	0.025	0.049	0.025	0.025	0.013	0.013	0.195	0.025
E. coli 1507E	0.025	0.049	0.049	0.049	0.025	0.049	0.195	0.025
P. aeruginosa 9027	0.391	0.391	0.195	0.391	0.391	6.250	0.781	0.195
P. aeruginosa 1592E	0.391	3.125	0.098	0.195	0.391	3.125	0.781	0.195
P. aeruginosa 1771M	0.195	0.781	0.098	0.098	0.195	0.391	0.195	0.049
S. typhymurium	0.025	0.098	0.098	0.098	0.049	0.049	0.781	0.049
K. aerogenes 1522E	0.025	0.049	0.098	0.098	0.049	0.049	0.391	0.049
E. cloacae 1321E	0.013	0.049	0.049	0.025	0.025	0.025	0.195	0.025
DHP-I stability[d]	1.03	—	0.31	0.25	0.54	0.26	0.17	1.00

[a]MIC was determined by agar dilution method using Mueller–Hinton.
[b]IPM = imipenem.
[c]MPM = meropenem.
[d]Relative $t_{1/2}$ of hydrolysis to meropenem by partially purified porcine renal DHP-I.

Table 2. Comparative in vitro antibacterial activity of **1a**, meropenem, and ertapenem against 40 Gram-negative clinically isolated pathogens (MIC, μg/mL)[a]

Organism	**1a**	MPM[b]	EPM[c]	Organism	**1a**	MPM[b]	EPM[c]
E.c. DC 0	0.03	0.03	≤0.008	*S.t.*	0.03	0.03	0.016
E.c. DC 2	0.016	0.03	0.016	*S.t.* 1	0.016	0.03	≤0.008
E.c. TEM	0.016	0.016	0.016	*S.t.* 2	0.016	0.016	≤0.008
E.c. ATCC25922	0.016	0.016	≤0.008	*K.o.* 1082E	0.13	0.06	0.03
E.c. 0157:H7	0.016	0.03	≤0.008	*En.c.* P99	0.13	0.03	0.06
E.c. CCARM 1108 *MDR*	0.03	0.03	0.016	*S.e.*	0.016	0.016	≤0.008
E.c. CCARM 1109 *MDR*	0.06	0.03	0.06	*S.e.* 1	0.016	0.016	≤0.008
E.c. CCARM 1110 *MDR*	0.06	0.016	0.03	*S.e.* 2	≤0.008	0.016	≤0.008
E.c. CCARM 1111 *MDR*	0.016	0.016	≤0.008	*S.e.* 3	0.016	0.03	≤0.008
E.c. CCARM 1112 *MDR*	0.016	0.016	0.016	*S.f.* ATCC9199	0.016	0.03	≤0.008
E.c. CCARM 1113 *MDR*	0.06	0.03	0.06	*S.s.* ATCC9290	0.016	0.03	≤0.008
E.c. CCARM 1114 *MDR*	0.13	0.03	0.25	*K.p.* 1 *ESBL*	0.03	0.03	0.016
E.c. CCARM 1115 *MDR*	0.03	0.016	0.06	*K.p.* 2 *ESBL*	0.03	0.03	0.016
E.c. CCARM 1116 *MDR*	0.016	0.03	≤0.008	*K.p.* 3 *ESBL*	0.13	0.06	0.5
E.c. CCARM 1117 *MDR*	0.03	0.03	0.03	*K.p.* 4 *ESBL*	0.03	0.016	0.06
P.a. ATCC27853	1	0.5	4	*K.p.* 5 *ESBL*	0.016	0.016	≤0.008
P.a. 1 *MDR*	1	1	8	*K.p.* 6 *MDR*	0.03	0.03	0.06
P.a. 2 *MDR*	8	8	8	*K.p.* 7 *MDR*	0.06	0.06	0.25
P.a. 3 *MDR*	8	8	8	*K.p.* 8 *MDR*	0.25	0.06	1
				K.p. 9 *MDR*	0.03	0.03	0.016
				K.p. 10 *MDR*	0.016	0.016	0.016

E.c., *Escherichia coli*; *P.a.*, *Pseudomonas aeruginosa*; *S.t.*, *Salmonella typhimurium*; *K.o.*, *Klebsiella oxytoca*; *En.c.*, *Enterobacter cloacae*; *S.e.*, *Salmonella enteritidis*; *S.f.*, *Shigella flexneri*; *S.s.*, *Shigella sonnei*; *K.p.*, *Klebsiella pneumoniae*.
[a]MIC was determined by agar dilution method using Mueller–Hinton.
[b]MPM, meropenem.
[c]EPM, ertapenem.

Coupling reaction of enolphosphate **24** with freshly prepared thiols **23a–f** in the presence of diisopropylethylamine afforded the protected 1β-methylcarbapenems **25a–f** (Scheme 5).

Deprotection of **25a–f** with tributyltin hydride in the presence of catalytic amount of tetrakis(triphenylphosphine)palladium(0) provided the title 1β-methylcarbapenems **1a–f**[10] as an amorphous solid by lyophilization, after purification by column chromatography on Diaion HP-20, respectively.

Biological Properties

Table 1 shows the in vitro antibacterial activity and stability to porcine renal DHP-I of new 1β-methylcarbapenems prepared above, together with those of imipenem and meropenem as reference compounds.

Most compounds exhibited potent and well-balanced antibacterial activity against both Gram-positive and Gram-negative bacteria including *P. aeruginosa* isolates, and were comparable to that of meropenem. However, their stabilities to DHP-I were poorer than meropenem,

with the exception of **1a**. In our series, the simple hydroxyimino compound **1a** ($R_1 = R_2 = H$) exhibited potent and broad spectrum of antibacterial activity including *P. aeruginosa* and similar stability to DHP-I to meropenem. As to the oxyiminoalkyl substituents, hydroxyimino compounds **1a**,**c** showed better activities against Gram-negative bacteria than methoxyimino compounds **1b**,**d**. There was no significant difference among the activities of hydroxyimino derivatives **1a**, **1c**, and **1e**. 4-Aminomethyl compound **1c** had comparable antibacterial activity to meropenem against *P. aeruginosa*, whereas it did not possess good stability to DHP-I. Compared to the parent acid **1e**, the ester **1f** displayed 2-fold inferior activity against Gram-positive bacteria and very poor activity against *P. aeruginosa*. Among the compounds prepared, based on its balanced in vitro antibacterial activity and stability to DHP-I, **1a** was selected for further evaluation.

Comparative in vitro activities of **1a**, meropenem, and ertapenem against clinical isolates of Gram-negative 40 bacterial species including MDR and ESBL-producing strains were summarized in Table 2.

The selected carbapenem **1a** possessed excellent in vitro activity against 40 target pathogens except for MDR *P. aeruginosa,* and was almost equal to that of meropenem. Among Gram-negatives, ertapenem had excellent activity against *E. coli* and *K. pneumoniae* (MICs, $\leq$0.008–1 μg/mL) including MDR and ESBL-producing strains, but did not have good in vitro activity against *P. aeruginosa*. Antibacterial activity of **1a** against clinical isolates of *P. aeruginosa* was similar to that of meropenem, and superior to that of ertapenem. Against MDR *K. pneumoniae*, **1a** was 2–4 times more active than the compared ertapenem. As a whole **1a** was comparable in potency to meropenem against Gram-negative species including MDR and ESBL-producing strains.

Acknowledgements

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10. Selected spectral data: **1a**: ^{1}H NMR (300 MHz, D_2O) δ 1.09 (d, 3H, J = 7.2 Hz), 1.17 (d, 3H, J = 6.3 Hz), 1.78–1.84 (m, 1H), 2.69–2.76 (m, 2H), 2.79–2.86 (m, 1H), 3.25–3.29 (m, 2H), 3.33–3.36 (m, 1H), 3.48–3.52 (m, 1H), 3.57–3.80 (m, 2H), 3.82–3.90 (m, 1H), 4.09–4.17 (m, 3H), 4.29–4.35 (m, 1H), 4.43–4.49 (m, 1H); FABHRMS m/z calcd for $C_{19}H_{27}N_4O_6S$ $(M+H)^+$ 439.1651, Found 439.1649. **1b**: ^{1}H NMR (300 MHz, D_2O) δ 1.10 (d, 3H, J = 7.2 Hz), 1.18 (d, 3H, J = 6.3 Hz), 1.78–1.84 (m, 1H), 2.69–2.76 (m, 2H), 2.79–2.86 (m, 1H), 3.25–3.29 (m, 2H), 3.33–3.36 (m, 1H), 3.48–3.52 (m, 1H), 3.57–3.80 (m, 2H), 3.77 (s, 3H), 3.82–3.90 (m, 1H), 4.09–4.17 (m, 3H), 4.29–4.35 (m, 1H), 4.43–4.49 (m, 1H); FABHRMS m/z calcd for $C_{20}H_{29}N_4O_6S$ $(M+H)^+$ 453.1808, found 453.1799.