In Vitro Antimicrobial Activity and Structure-activity Relationship of C-2 Triazolylthio and Pyridinylmethylthio Carbapenems

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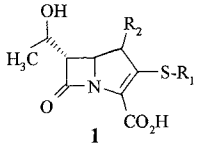
The carbapenems are an important group of β-lactam antibiotics with unique nuclear structure that differs from the penam and cephem nuclei of penicillins and cephalosporins, respectively. Because of their unrivaled antibacterial spectrum and remarkable stability towards a wide range of β-lactamases as embodied in imipenem (**1a**) and the fact that even imipenem has certain drawbacks, it is not surprising that there has been a great deal of interest amongst medicinal chemists in finding new and improved carbapenems[1~5]. Despite the inherent problem of chemical and metabolic instability combined with poor semi-synthetic methods for this class of compounds, considerable progress has been made. Two new carbapenems, meropenem (**1b**) and biapenem (**1c**) have been shown to possess better microbiological and pharmacokinetic profiles[6,7]. Meropenem is a stable to renal DHP and it has recently been approved for clinical use in some countries. In recent years, a few other analogs such as DX-8739, GV-104326, BO-2727 and S-4661 have been reported to possess some advantages over the known derivatives[8~11].

Fig. 1



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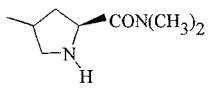
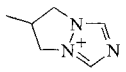
a, Thienamycin, $R_1 = CH_2CH_2NH_2$, $R_2 = H$

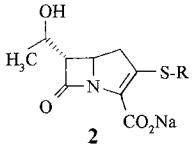
b, Imipenem, $R_1 = CH_2CH_2NHCH{=}NH$, $R_2 = H$

c, Meropenem, $R_1 =$ 

$R_2 = CH_3$

d, Biapenem, $R_1 =$ 

$R_2 = CH_3$



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Although the low molecular weight, excellent β-lactamase stability and high affinity for bacterial PBPs of carbapenems provide exceptional antibacterial spectrum, the nature of C-2 substitution has also been recognized as major contributor to carbapenems antibacterial properties. Presence of basic or quaternary amino moieties at C-2 considerably improves the chemical stability and extends the antibacterial spectrum relative to compounds with less basic side chain at the same position[5,12].

In recent years, more attempts were made to improve the antimicrobial activity against clinically resistant gram negative strains including *Pseudomonas* spp. by changing the position 2 substituents with more basic or cationic substituents. There are a limited reports[13] on the effect of position 2 substituents in carbapenem class of compounds towards the gram positive strains including highly resistants *S. aureus*. In this paper we have focused our attention to study the effect of groups such as 3-substituted-1,2,4-triazol-5-yl-thio and halosubstituted pyridin-3-yl-methylthio at position 2 of carbapenems towards antimicrobial activity especially against Gram-positive strains including highly resistant *S. aureus*.

Chemistry

The synthetic routes employed for the synthesis of title compounds are similar to those reported in the literature[14~16] and the typical procedure is shown in scheme-1. The bicyclic keto compound **3** was treated with diphenyl chlorophosphate in the presence of diisopropylamine to obtain a common intermediate enolphosphate **4** *in situ* which was then reacted with freshly prepared substituted 1,2,4-triazole-5-thione or halosubstituted pyridinyl-methylthiol afforded 2-substituted carbapenems **5**. **5a**: yield 51%; IR (nujol) cm^{-1}: 1779, 1718, 1694; ^1H NMR (200 MHz, $CDCl_3$) δ: 1.28 (3H, d, $J=6.2$ Hz), 2.0 (1H, br s), 2.90 (2H, m), 3.2 (1H, dd, $J_1=6.7$ Hz, $J_2=2.8$ Hz), 3.95 (3H, s), 4.14 (2H, m), 4.85 (2H, m), 5.35 (2H, m), 6.0 (1H, m), 7.40 (3H, m), 8.15 (2H, m). Deprotection of allyl group with tetrakis triphenylphosphine palladium (0) in presence of triphenyl phosphine and sodium 2-ethyl hexanoate gave crude sodium salt of carbapenem derivative **2** which could be purified by either on a reverse phase (Whatman $MKC_{18}F$) preparative plates using acetonitrile-water (94:6) mixture as developing solvent or column chromatography on dianion HP-20 afforded the foamy product after lyophilization. **2a**: yield 21%; ^1H NMR (200 MHz, D_2O) δ: 1.3 (3H, d, $J=6$ Hz), 2.52 (1H, m), 2.85 (1H, m), 3.41 (1H, dd, $J_1=5.6$ Hz, $J_2=2.7$ Hz), 3.87 (3H, s), 4.18 (2H, m), 7.54 (3H, m), 7.95 (2H, m); HPLC purity 92%. Similarily compounds **2b~2j** were synthesized, purified by chromatography and characterized by

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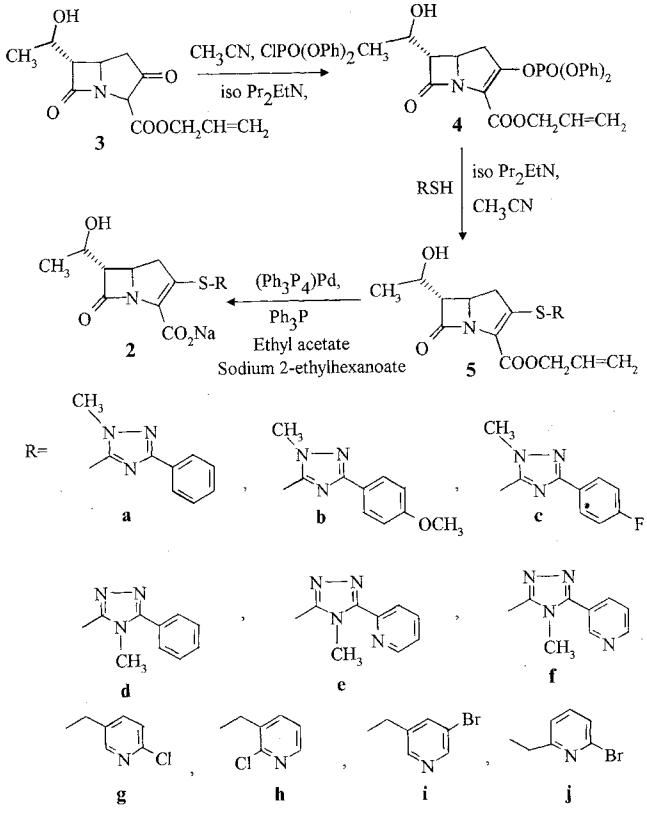
Scheme 1.



NMR and HPLC. The synthesis of substituted 1,2,4-triazole-5-thione[17] and halosubstituted pyridinyl-methylthiol from halosubstituted pyridinyl-methanol[18,19] were done by following the synthetic procedure as described in literature.

Results and Discussion

Structure-activity relationship studies on a series of novel carbapenems **2** with triazolylthio and pyridinylmethylthio substituents at position 2 were studied. The *in vitro* antibacterial activities against a panel of Gram-positive and Gram-negative bacteria were determined by the agar dilution method as recommended in the National committee for Clinical Laboratory Standards[20]. The MICs are reported in Table 1. Out of two classes triazolylthio carbapenem (**2a**~**2f**) and pyridinylmethylthio carbapenem (**2g**~**2j**) derivatives, the compounds **2g**~**2j** showed similar or slightly inferior activity than imipenem against most of the Gram-positive and Gram-negative bacteria with the exception of *Pseudomonas aeruginosa* isolates. The compounds **2a** and **2b** showed very poor activity against Gram-negatives but good activity against Gram-positives. Compound **2a** exhibited 1~2 fold better activity against MRSA (Sa 157-399 and Sa Amp81-30 than imipenem. Compounds **2c**~**2f** exhibited several fold inferior activity against

Table 1. Antibacterial activity of C-2 triazolylthio and pyridylthiomethyl carbapenems **5**.

Test organisms	Imipenem	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**	**2h**	**2i**	**2j**
E.c. ATCC 25922	0.06	128	>128	32	16	8.0	1.0	0.5	8.0	4.0	2.0
E.c. DCO	0.25	128	>128	32	8	4.0	1.0	0.5	8.0	4.0	1.0
E.c. DC2	0.25	0.25	0.50	1.0	0.25	0.5	0.5	<0.06	0.5	0.12	0.12
E.c. SHV1	0.06	128	>128	16	8	4.0	1.0	0.25	4.0	2.0	1.0
E.c. TEM1	0.06	128	>128	16	8	4.0	2.0	0.25	4.0	2.0	1.0
E.c. TEM2	0.06	32	128	8.0	4.0	2.0	2.0	<0.06	2.0	0.5	0.25
E.c. OXA1	0.12	>128	>128	32	16	4.0	2.0	0.5	8.0	4.0	2.0
E.c. OXA3	0.12	128	>128	16	8.0	4.0	1.0	0.5	8.0	2.0	1.0
E.cl. ATCC 23355	0.25	>128	>128	32	32	16	8.0	2.0	32	16	4.0
E.cl. (Multi Resist)	1.0	>128	>128	>128	128	64	64	32	>128	64	32
K.p. ATCC 13833	0.5	>128	>128	32	8.0	4.0	1.0	1.0	16	8.0	4.0
K.ox. ATCC 15764	0.12	128	128	8.0	1.0	0.5	0.5	0.12	2.0	1.0	0.25
Sh.so. ATCC 11060	0.12	128	>128	32	8.0	2.0	1.0	1.0	4.0	1.0	1.0
C.f. (R-ceph)	0.25	>128	>128	128	32	8.0	16	2.0	16	8.0	2.0
P.a. ATCC 27853	2.0	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
P.a. PSE1	1.0	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
P.a. PSE2	1.0	>128	>128	>128	>128	>128	>128	>128	8.0	2.0	1.0
P.a. PSE3	1.0	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
P.a. PSE4	1.0	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
P.ret. ATCC 29944	0.5	128	>128	16	4.0	1.0	2.0	0.50	4.0	1.0	0.25
P.m. ATCC 2675	0.5	>128	>128	8.0	4.0	1.0	2.0	1.0	2.0	0.5	0.25
S.mar. ATCC 2982	1.0	>128	>128	>128	>128	>128	>128	4.0	32	16	8.0
S.a. ATCC 29213	<0.015	0.12	0.12	0.5	0.12	0.25	0.25	<0.06	0.25	0.06	0.12
S.a. JHHD078	<0.015	0.06	0.12	0.5	0.12	0.25	0.25	<0.06	0.25	0.06	0.12
S.a. JHHM241	0.06	0.12	0.25	2.0	0.25	0.5	0.5	0.12	0.5	0.06	0.25
S.a. 157-399	8.0	2.0	2.0	32	8.0	64	64	8.0	64	8.0	32
S.a. AMP81-3	2.0	1.0	4.0	8.0	2.0	8.0	8.0	8.0	16	2.0	1.0

Agar dilution method; medium, Mueller Hinton agar; inoculum, 10^4 cfu/spot; incubation, 18 hours at 35°C. *E.c.*: *Escherichia coli*, *E.cl.*: *Enterobacter cloacae*, *K.p.*: *Klebsiella pneumoniae*, *K. ox.*: *Klebsiella oxytoco*, *Sh.so.*: *Shigella sonnei*, *C.f.*: *Citrobacter freundii*, *P.a.*: *Pseudomonas aeruginosa*, *P.ret.*: *Providencia rettgeri*, *P.m.*: *Proteus mirabilis*, *S.ma.*: *Serratia marcescens*, *S.a.*: *Staphylococcus aureus*.

Table 2. Permeability profile of C-2 triazolyl thio and pyridyl thiomethyl carbapenems **5**.

Compound No.	MIC (μg/ml)			
	DCO/DC2	*E. coli* DCO	*E. coli* DC2	*E. coli* ATCC 25922
2a	512	128	0.25	128
2b	512	>128	0.5	>128
2c	32	32	1.0	32
2d	32	8.0	0.25	16
2e	8.0	4.0	0.5	8.0
2f	2.0	1.0	0.5	1.0
2g	8.0	0.5	<0.06	0.50
2h	16	8.0	0.5	8.0
2i	32	4.0	0.12	4.0
2j	8.0	1.0	0.12	2.0
Imipenem	1.0	0.25	0.25	0.06

Gram-negative but moderate activity against Gram-positive bacteria as compared to imipenem. In carbapenem derivatives (**2g**~**2j**), the halogen substitution at pyridine and linkage between pyridine and methylthio had moderate effect on the antimicrobial activity. Compound **2g** having 6-chloro-pyridin-3-yl-methylthio substituent at position 2 showed similar antimicrobial activity whereas the other compounds are less active than the imipenem.

Keeping the favorable MRSA activity of **2a** in mind several other analogs were prepared and evaluated. None of these compounds showed improved activity over compound **2a**. It appears that the position of methyl and the nature of the aryl group on the triazole has a predominant effect on the activity against Gram-negative strains but has only a minor effect on the activity against the Gram-positives. The methyl substitution at N-4 and pyridin-3-yl at C-3 of triazole in the compounds were favorable for activity against both Gram-positive and Gram-negative bacteria.

The nature of the substitutent at C-2 position has considerable effect on the permeability of compounds through the outer membrane of Gram-negative bacteria[5,12]. We tested our compounds against two strains of *E. coli*, *E. coli* DCO (with normal permeability barrier) and *E. coli* DC2 (permeability mutant of *E. coli* DCO having no permeability barrier). The MICs of C-2 triazolyl thio and pyridinyl thiomethyl carbapenems **2** against these strains and their ratio (MIC DC0/MIC DC2) are given in Table 2. The ratio of MICs of these two strains are indicative of permeability profile of each derivatives. When a compound is equally active against both strains the MIC DC0/MIC DC2 ratio approaches to 1, as seen in the case of imipenem. Low values indicate that the compound has no problem of crossing the outer membrane. Higher values suggest permeability problem as seen in the case of **2a**~**2d** and **2h**~**2i** (Table 2).

Acknowledgments

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