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*N*H-1,2,3-Triazole-based Inhibitors of the VIM-2 Metallo-*β*-Lactamase: Synthesis and Structure-Activity Studies

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Abstract

Metallo-ß-lactamases (MBL) are an emerging cause of bacterial resistance to antibiotic treatment. The VIM-2 ß-lactamase is the most commonly encountered MBL in clinical isolates worldwide. Described here are potent and selective small molecule inhibitors of VIM-2 containing the arylsulfonyl-NH-1,2,3-triazole chemotype that potentiate the efficacy of the ß-lactam, imipenem, in *E. coli*.

Metallo-ß-lactamases (MBLs) are an emerging class of enzymes with the ability to degrade most ß-lactam antibacterial agents.[1] As a consequence of the rising rates of infections attributed to bacteria with acquired MBL genes, enzymes of this class represent new targets for adjuvant antibacterial therapy.[2,3] The VIM-2 enzyme is currently the most widespread MBL found in clinical isolates worldwide,[4-6] and as such small molecule inhibitors of this enzyme may prove useful in the treatment of resistant infections. Several MBL inhibitors have been reported,[7,8] with the most potent VIM-2 inhibitor, the thiol PhenylC-4SH, exhibiting a K_i of 190 nM.[9] However, apart from *p*-chloromercuribenzoic acid (*p*CMB), a nonspecific cysteine-reactive reagent, no VIM-2 inhibitor has been shown to potentiate the antibacterials effects of ß-lactams in VIM-2 expressing bacteria.[10]

We recently identified a family of 4,5-disubstituted NH-1,2,3-triazoles (Figure 1) of selective VIM-2 inhibitors through the biochemical screening of a focused NH-1,2,3-triazole library with 267 members.[10]

The most potent candidate from this study, compound **1**, N-((4-((but-3-ynyloxy)methyl)-1H-1,2,3-triazol-5-yl)methyl)-4-iodo-benzenesulfonamide, exhibited submicromolar activity (K_i = 0.41 ± 0.03 μM) against VIM-2 whilst being inactive towards the related MBL, IMP-1.[10] Unfortunately this compound did not potentiate the antibacterial effects of the ß-lactam, imipenem.

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Supporting information available: Method of enzyme inhibition, microbial inhibition, docking studies, and synthetic procedures and characterization are given. This material is available free of charge via the internet at http://pubs.acs.org.

Herein, we describe our efforts to examine structure-activity relationships (SAR) for this chemotype with the goal of improving the potency of the lead compound **1** and identifying VIM-2 inhibitors with the ability to potentiate ß-lactam antibacterials. In our study, substituents on the C-4 methyl of the triazole (R_1) or the arylsulfonamide (R_2) were systematically varied. *N*H-Triazole-containing sulfonamides were readily prepared as shown in Figure 2. The key transformation was the Banert cascade reaction of propargyl azides with various nucleophiles.[11] The sequence began with the synthesis of *N*-(4-chlorobut-2-ynyl)-sulfonamide derivatives (**b**) from propargyl amines (**a**) and sulfonyl chlorides under the Schotten-Baumann conditions. Nucleophilic substitution of the chloride with sodium azide gave the desired propargyl azides (**c**) which, upon heating, underwent a facile rearrangement to triazafulvenes (**d**), highly reactive intermediates that can be readily captured with different nucleophiles.

From our initial work with arylsulfonyltriazole VIM-2 inhibitors we noted that an alkoxy appendage on the C-4 methyl of the triazole moiety was important for activity.[10] We speculate that the oxygen of the alkoxy, a hydrogen-bond acceptor is important for effective inhibition of VIM-2, possibly through polar interactions between this atom and active site amino acid residues. Consequently, to expand the SAR around the VIM-2 inhibitor **1**, five new analogs bearing an alkoxy appendage on the C-4 methyl of the triazole moiety were synthesized (compounds **1**, **2**, **3**, **5** and **8** in Table 1) and examined in a nitrocefin biochemical assay[10] using VIM-2 cloned from a *Pseudomonas aeruginosa* clinical strain (COL-1). Three of the five new analogs exhibited improved potency compared to the original hit, **1**.

Next, to further explore the substitution requirements of the triazole moiety, and to show that VIM-2 inhibition was not unique to analogs containing a 4-iodobenzene group, a diverse series of 4-chlorobenzenesulfonyl derivatives was prepared and tested (Table 2). Of great importance, five inhibitors with sub-micromolar IC_{50} were identified from this group of candidates (compounds **12**-**16**); all of them contained an amine at the C-4 methyl of the triazole ring. As for SAR, the potency of the 4-chlorobenzene sulfonamides was strongly dependent on the nature of the C-4 methyl triazole substituent. In this series, hydrophobic amino (e.g. **12**, IC_{50} = 0.29 μM) and alkoxy derivatives (e.g. **17**, IC_{50} = 1.1 μM) were excellent inhibitors, while alkyl derivatives (i.e. **25** to **27**) were inactive towards VIM-2. A sulfide **30**, which is the thioether analog of **22**, was inactive. These findings lend further support to our hypothesis that a hydrogen-bond acceptor of the C-4 methyl of the triazole is important for inhibitor-protein interactions, with nitrogen being superior to oxygen. However, with respect to VIM-2 binding and inhibition, sulfur does not appear to be a good replacement, probably as a consequence of its larger size and more diffuse electronic properties leading to poor hydrogen-bond acceptor properties.

A series of unsubstituted arylsulfonamides was also examined (Table 3). Again, amino substitution on the C-4 methyl of the triazole resulted in the most potent compounds, with the adamantyl (**32**) and cyclohexyl (**33**) derivatives approaching 100 nM IC_{50}.

Next we looked at the effect of aromatic substituents. The equivalent unsubstituted, 4-iodobenzenesulfonyl and 4-chlorobenzenesulfonyl derivatives (Table 3) exhibited at most a 3-fold difference in potency, demonstrating that changes at the para-position provide minor improvements in inhibitor binding.

To further explore the contribution to binding provided by the arylsulfonamide moiety, we prepared analogs with the same triazole derivative but with differing aromatic substituents (Table 4). Again, halide aromatic substitution had discrete effects. For example, the potency of **8**, a 4-iodobenzenesulfonyl derivative and **36**, a 2,5-dichlorobenzenesulfonyl derivative,

which both share an isopropyl appendage on the triazole, differed by 2-fold. Similarly **42**, a 4-methoxybenzenesulfonyl derivative and **14**, a 4-chlorobenzenesulfonyl derivative, both sharing a dipropylamine appendage on the triazole, were equipotent. In contrast, derivatives with very bulky aromatic para-substituents (i.e. **38** to **40**) were poorly active or inactive against VIM-2.

During the course of lead optimization of **1**, we recognized that the most potent inhibitors contained a dichlorobenzene group (Table 5) and an amine substituent on the C-4 methyl of the triazole. Table 5 compares the most potent VIM-2 inhibitor **44** with other amino and alkoxy derivatives in the 2,5-dichlorobenzenesulfonyl series. Compounds **44** and **47** only differ by replacement of nitrogen by oxygen, and clearly demonstrate a 20-fold improvement in potency of the amino over the alkoxy derivative.

To ascertain the biochemical selectivity for this chemical class, all compounds reported here were tested against another class B1 MBL, IMP-1. Since VIM-2 and IMP-1 show considerable sequence divergence in their active sites (33% overall sequence identity), the arylsulfonyltriazoles were not expected to inhibit both enzymes unless behaving through promiscuous mechanisms. Indeed, all 47 compounds were inactive against IMP-1.

Insights from Docking 44 into VIM-2

Docking studies with **44**[12] suggest that this inhibitor binds to VIM-2 in the same mode as was predicted for the parent, **1**, which is described elsewhere.[10] SAR studies show that the best inhibitors (i.e. **41** and **44** to **46**) display hydrophobic groups emanating from the C-4 methyl of the triazole. Our docking and modeling studies have identified a cavity that can accommodate a compact hydrophobic group such as adamantyl or cyclohexyl.[10,12] Consistent with SAR findings, smaller triazole appendages cannot take full advantage of this cavity, while groups larger or more extended than adamantyl would be detrimental to binding due to steric clashes.

Potentiation effects of VIM-2 inhibitors

Currently only *p*CMB. a slowly reversible/irreversible VIM-2 inhibitor, has been shown to have a synergistic effect with ß-lactam antibacterials in VIM-2 expressing bacteria.[10] However this cysteine-reactive reagent is known to have several off-target activities, lessening its value for mechanistic studies. To demonstrate potentiation by the arylsulfonyltriazoles in bacteria we assessed the ability of these compounds to affect the growth of parental (BL21) and VIM-2 transformed (BL21/VIM-2) *E. coli*, in the presence or absence of the carbapenem, imipenem. Relative to the parental (BL21) strain the MIC (minimum inhibitory concentration) for imipenem increased approximately 9-fold when cells were transformed with the VIM-2 encoding plasmid (imipenem MIC = 0.21 μg/mL in BL21 *vs*. imipenem MIC = 1.85 μg/mL in BL21/VIM-2). This decrease in antibacterial potency reflects the ability of VIM-2 to degrade imipenem. When tested at 50 μM, 14 of the 47 arylsulfonyltriazoles potentiated imipenem and consequently were tested at lower doses (Table 6). Compound **45**, with the best potentiation improved the MIC of imipenem by 3-fold from 1.85 μg/mL to 0.617 μg/mL at 10 μM (Table 6). To ascertain whether this class of inhibitor can fully potentiate imipenem, compound **45** was tested at higher concentrations. Indeed, the MIC for imipenem was fully restored to that observed for the parental strain (i.e. lacking VIM-2) when **45** was tested at 150 μM. Furthermore, in parental (BL21) cells the MIC for imipenem was unaffected by the presence of **45** as high as 150 μM. It was also established that none of these 14 compounds exhibited intrinsic antibacterial activity since *E. coli* cell growth was unaffected when they were tested at 150 μM.

Inhibition kinetic studies

The mechanism of inhibition for the 14 VIM-2 inhibitors active at 50 μM in VIM-2 expressing *E. coli* was further evaluated through kinetic studies (Table 6). The best inhibitors exhibited K_i values as low as 10 nM (i.e. **46**), and all but one exhibited classical competitive inhibition and hence bind at the active site. Only **42** exhibits a mixed mode of inhibition with features of both competitive and non-competitive inhibition. While inhibition kinetics for **42** are somewhat different compared to the other 13 compounds, a competitive inhibition component still suggests that binding of **42** occurs at the active site.

In conclusion, among many factors that affect the successful outcome of medicinal chemistry optimization, two stand out: the degree of diversity of the building blocks and the speed of iterative synthesis. The fidelity and robustness of the Banert cascade has been used to rapidly assemble an NH-1,2,3-triazole library from diverse arrays of highly functionalized fragments. Medicinal chemistry efforts progressed *via* three iterative steps, and resulted in the synthesis of 320 unique compounds. Our starting point, **1**, exhibited moderate biochemical potency towards VIM-2 whilst being inactive towards IMP-1. Substitution at the arylsulfonamide produced subtle improvements in potency. On the other hand, analogs bearing amino substitution on the C-4 methyl of the triazole generated highly potent VIM-2 inhibitors that potentiate imipenem antibacterial activity. Our best inhibitors in biochemical assays exhibit as much as 40-fold improved potency over **1**, and represent the most potent VIM-2 inhibitors to date. Furthermore, these are the first reported inhibitors to be active against VIM-2 in bacteria (*E. coli*), with no apparent off-target effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The National Institutes of Health (NS059451, P.H.) and the Skaggs Institute for Chemical Biology (K.B.S and V.V.F.) supported this work. Pierre Baillargeon and Lina DeLuca (Lead Identification Division, Translational Research Institute, Scripps Florida) are thanked for their assistance with compound management. Moreno Galleni (d'Enzymologie & Centre d'Ingénierie des Protéines, Institut de Chimie) is thanked for helpful discussion.

References

1. Jacoby GA, Munoz-Price LS. The new beta-lactamases. N Engl J Med. 2005; 352:380–91. [PubMed: 15673804]
2. Cornaglia G, Akova M, Amicosante G, Canton R, Cauda R, Docquier JD, Edelstein M, Frere JM, Fuzi M, Galleni M, Giamarellou H, Gniadkowski M, Koncan R, Libisch B, Luzzaro F, Miriagou V, Navarro F, Nordmann P, Pagani L, Peixe L, Poirel L, Souli M, Tacconelli E, Vatopoulos A, Rossolini GM. Metallo-beta-lactamases as emerging resistance determinants in Gram-negative pathogens: open issues. Int J Antimicrob Agents. 2007; 29:380–8. [PubMed: 17223319]
3. Livermore DM. The threat from the pink corner. Ann Med. 2003; 35:226–34. [PubMed: 12846264]
4. Bebrone C. Metallo-beta-lactamases (classification, activity, genetic organization, structure, zinc coordination) and their superfamily. Biochem Pharmacol. 2007; 74:1686–701. [PubMed: 17597585]
5. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-beta-lactamases: the quiet before the storm? Clin Microbiol Rev. 2005; 18:306–25. [PubMed: 15831827]
6. Docquier JD, Lamotte-Brasseur J, Galleni M, Amicosante G, Frere JM, Rossolini GM. On functional and structural heterogeneity of VIM-type metallo-beta-lactamases. J Antimicrob Chemother. 2003; 51:257–66. [PubMed: 12562689]
7. Lienard MA, Strandh M, Hedenstrom E, Johansson T, Lofstedt C. Key biosynthetic gene subfamily recruited for pheromone production prior to the extensive radiation of Lepidoptera. BMC Evol Biol. 2008; 8:270. [PubMed: 18831750]

8. Olsen L, Jost S, Adolph HW, Pettersson I, Hemmingsen L, Jorgensen FS. New leads of metallo-beta-lactamase inhibitors from structure-based pharmacophore design. Bioorg Med Chem. 2006; 14:2627–35. [PubMed: 16378729]

9. Jin W, Arakawa Y, Yasuzawa H, Taki T, Hashiguchi R, Mitsutani K, Shoga A, Yamaguchi Y, Kurosaki H, Shibata N, Ohta M, Goto M. Comparative study of the inhibition of metallo-beta-lactamases (IMP-1 and VIM-2) by thiol compounds that contain a hydrophobic group. Biol Pharm Bull. 2004; 27:851–6. [PubMed: 15187432]

10. Minond D, Saldanha SA, Subramaniam P, Spaargaren M, Spicer T, Fotsing JR, Weide T, Fokin VV, Sharpless KB, Galleni M, Frère J-M, Hodder P. Inhibitors of VIM-2 by Screening Pharmacogically Active and Click-Chemistry Compound Libraries. Bioorg Med Chem. 2009; 15:5027–37. [PubMed: 19553129]

11. Loren JC, Sharpless KB. The banert cascade: A synthetic sequence to polyfunctional NH-1,2,3-triazoles. Synthesis-Stuttgart. 2005:1514–1520.

11. See supporting information for docking studies with compound **44**.

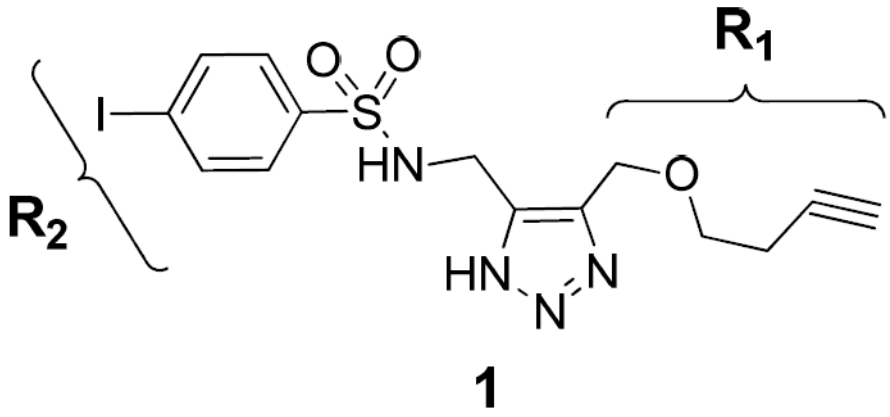


Figure 1.
4,5-Disubstituted NH-1,2,3-triazoles with substituents on C-4 methyl of the triazole (R_1) or the arylsulfonamide (R_2). Candidate **1** is shown.

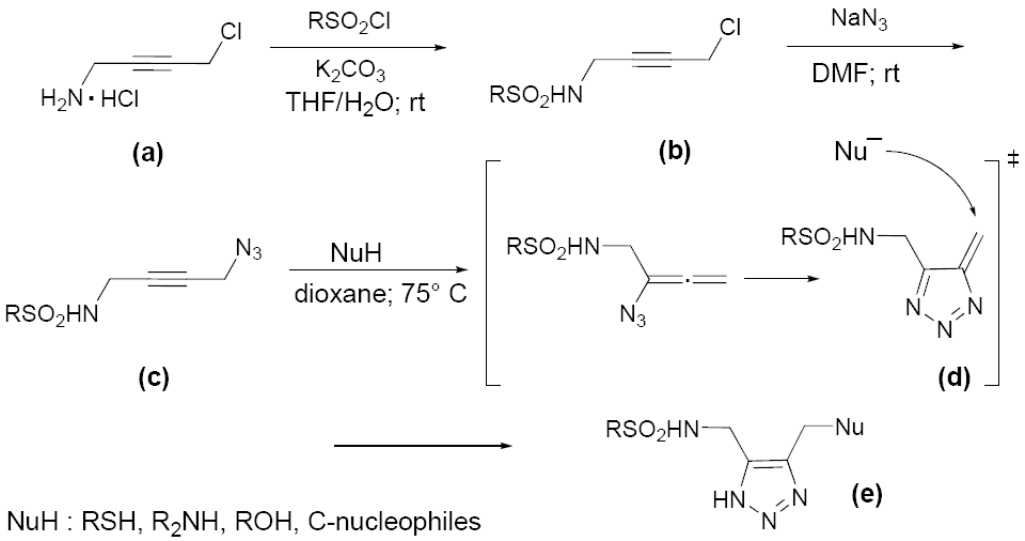
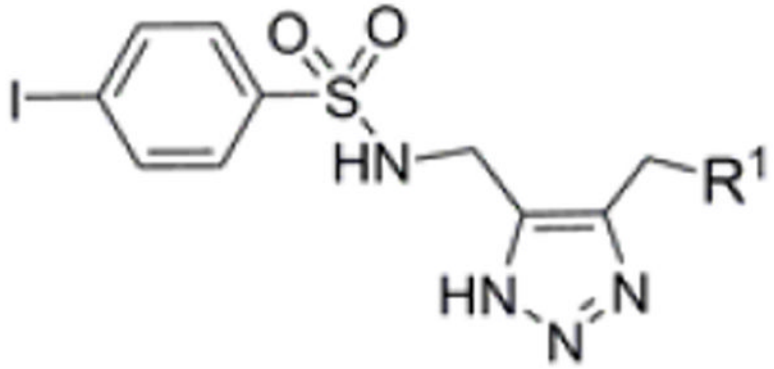
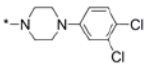
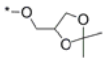
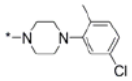
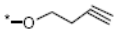


Figure 2.
Synthesis of candidate compounds.

Table 1

SAR of triazolylmethyl 4-iodobenzenesulfonamides with respect to the C-4 methyl substituent.



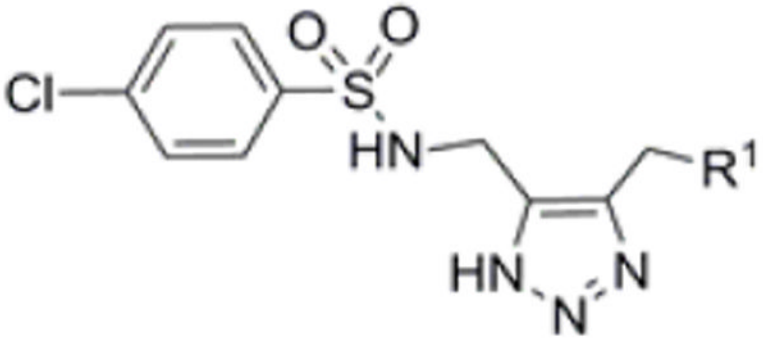
ID	R^1	IC_{50}[a]	ID	R^1	IC_{50}[a]	ID	R^1	IC_{50}[a]
1	O^{i}Pr	0.29 ± 0.03	**5**	-OBu	5.2 ± 0.7	**9**[b]		11%
2	-O^{t}Bu	1.1 ± 0.3	**6**[b]	-OMe	7.3 ± 1.9	**10**[b]		5.3%
3	-OEt	1.6 ± 0.3	**7**[b]		31%	**11**[b]		0%
4[b]		3.3 ± 0.4	**8**	-OBn	23%	-	-	-

[a] IC50 ± error [μM] reported as the standard deviation of 3 replicates. IC50 values obtained using a four parameter sigmoidal dose-response curve with adjustable baseline using GraphPad Prism. Maximum inhibition (at 56 μM) reported for compounds that do not achieve 50% inhibition;

[b] Compounds reported previously.[10]

Table 2

SAR of triazolylmethyl 4-chlorobenzenesulfonamides with respect to the C-4 methyl substituent.



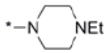
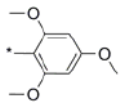
ID	R^1	IC_{50}[a]	ID	R^1	IC_{50}[a]	ID	R^1	IC_{50}[a]
12	-NHPh	0.29 ± 0.02	**19**	-O^{t}Bu	1.4 ± 0.1	**26**		34 %
13	-NHCyclohexyl	0.39 ± 0.02	**20**	-NPyrrolidine	3.0 ± 0.02	**27**		0 %
14	-NPr$_2$	0.45 ± 0.02	**21**	-NMorpholine	3.8 ± 0.6	**28**		0 %
15	-NIndoline	0.58 ± 0.05	**22**	-OBu	19 ± 6	**29**	-N(Bis(2-methoxyethyl))	0 %
16	-NHAdamantyl	0.67 ± 0.01	**23**	-OMe	21 ± 5	**30**	-SBu	0 %
17	-O^{i}Pr	1.1 ± 0.1	**24**	-N^{i}Pr$_2$	45 %	**31**	-N(Me)Bn	0 %
18	-NH^{t}Bu	1.1 ± 0.2	**25**		36 %	-	-	-

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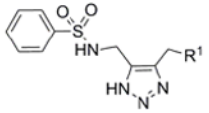
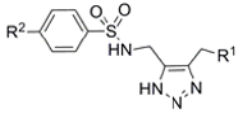
Table 3

SAR of triazolylmethyl arylsulfonamides with respect to the C-4 methyl substituent.





ID	R^1	IC_{50} [μM]	ID	R^1	R^2	IC_{50} [μM]	ID	R^1	R^2	IC_{50} [μM]
32	-NHAdamantyl	0.12 ± 0.01	**17**		-Cl	0.10 ± 0.10	**11**	-OBu	-I	5.20 ± 0.70
33	-NHCyclohexyl	0.13 ± 0.01	**18**	-O^{i}Pr	-I	0.29 ± 0.03	**22**		-Cl	18.9 ± 6.00
34	-NHPyrrolidine	0.19 ± 0.01	**35**		-H	0.85 ± 0.07	-	-	-	-
35	-O^{i}Pr	0.85 ± 0.07	**9**	-O^{t}Bu	-I	1.10 ± 0.03	**2**	-OMe	-I	7.30 ± 1.90
-	-	-	**19**		-Cl	1.40 ± 0.10	**23**		-Cl	21 ± 5

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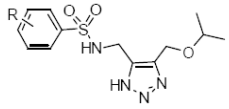
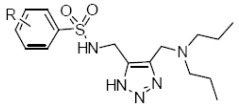
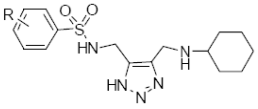
Table 4

SAR of triazolylmethyl arylsulfonamides with varying aromatic substituents.







ID	R	IC_{50}[a]	ID	R	IC_{50}[a]	ID	R	IC_{50}[a]
8	4-I	0.29 ± 0.03	**41**	2,5-Dichloro-	0.10 ± 0.01	**44**	2,5-Dichloro-	0.07 ± 0.003
36	2,5-Dichloro-	0.61 ± 0.09	**42**	4-OMe	0.33 ± 0.03	**45**	3,4-Dichloro-	0.07 ± 0.007
35	4-H	0.85 ± 0.07	**14**	4-Cl	0.45 ± 0.02	**34**	4-H	0.13 ± 0.01
17	4-Cl	1.10 ± 0.10	**43**	4-F	1.10 ± 0.10	**13**	4-Cl	0.39 ± 0.02
37	4-Br	1.10 ± 1.00						
38	4-NHAc	30 %						
39	4-CF_3	4.3 %						
40	4-^{t}Bu	0 %						

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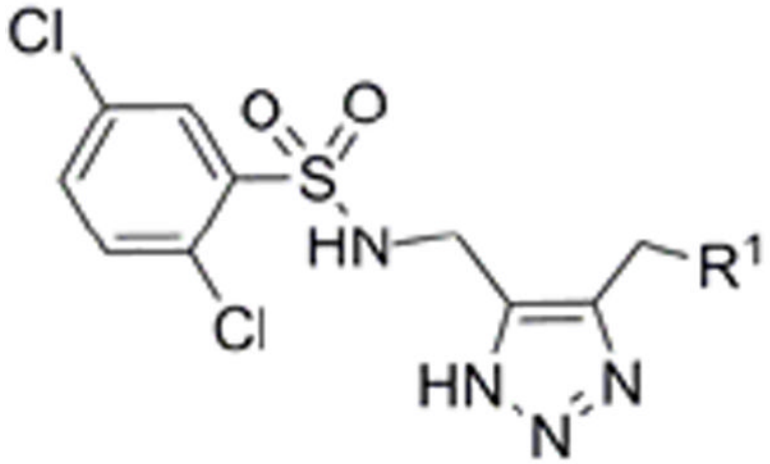
Table 5

SAR of triazolylmethyl 2,5-dichlorobenzenesulfonamides with respect to the C-4 methyl substituent.



ID	R^1	IC_{50} [μM]	ID	R^1	IC_{50} [μM]	ID	R^1	IC_{50} [μM]
44	-NHCyclohexyl	0.07 ± 0.003	**41**	$-NPr_2$	0.10 ± 0.01	**47**	-OCyclohexyl	1.40 ± 0.50
46	-NHAdamantyl	0.07 ± 0.01	**36**	$-O^iPr$	0.61 ± 0.09	-	-	-

Table 6

Results of K_i and MIC potentiation studies.

					Imipenem MIC (μg/mL)		
ID	**R_1**	**R_2**	**K_i (μM)[a]**	**Mechanism[b]**	**50 μM[c]**	**20 μM[c]**	**10 μM[c]**
46	-NHAdamantyl	2,5-Cl	0.01 ± 0.002	C	0.617	0.617	1.851
45	-NHCyclohexyl	3,4-Cl	0.02 ± 0.002	C	0.617	0.617	0.617
41	-NHDipropyl	2,5-Cl	0.02 ± 0.004	C	0.617	0.617	1.851
44	-NHCyclohexyl	2,5-Cl	0.03 ± 0.003	C	0.617	0.617	1.851
33	-NHCyclohexyl	H	0.03 ± 0.01	C	0.617	0.617	1.851
34	-NHPyrrolidine	H	0.03 ± 0.004	C	0.617	0.617	1.851
42	-NHDipropyl	Methoxy	0.06 ± 0.01	M	0.617	0.617	1.851
15	-NIndoline	Cl	0.06 + 0.01	C	0.617	0.617	1.851
12	-NHPh	Cl	0.08 ± 0.01	C	0.617	1.851	1.851
32	-NHAdamantyl	H	0.12 + 0.01	C	0.617	0.617	1.851
14	-NHDipropyl	Cl	0.12 + 0.03	C	0.617	1.851	1.851
47	-OCyclohexyl	2,5-Cl	0.17 ± 0.02	C	0.617	1.851	1.851
18	-NH*t* Butylamino	Cl	0.18 + 0.03	C	0.617	0.617	1.851
13	-NHCyclohexyl	Cl	0.39 ± 0.02	C	0.617	0.617	1.851

[a] K_i values calculated by non-linear regression (hyperbolic equation) using GraphPad Prism.

[b] C = Competitive inhibition; M = Mixed inhibition.

[c] Inhibitor concentration used in potentiation assay.