1,2,4-TRIAZOLE-3-THIONE COMPOUNDS POTENTLY INHIBIT VIM AND NDM-1 METALLO-β-LACTAMASES AND RE-SENSITIZE MULTI-RESISTANT CLINICAL ISOLATES TO MEROPENEM

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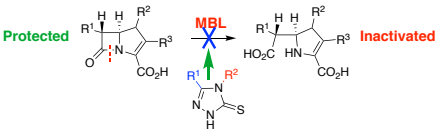
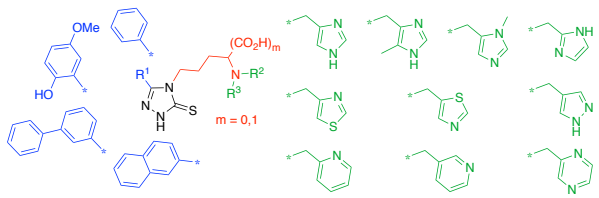
Introduction

Dizinc metallo-β-lactamases (MBLs) are increasingly involved as a major mechanism of resistance to carbapenems in relevant opportunistic Gram-negative pathogens. Unfortunately, clinically efficient MBL inhibitors still represent an unmet medical need. We are developing compounds containing a 1,2,4-triazole-3-thione scaffold as an original zinc ligand and few promising series were already reported.[1-3] Here, we present a new series possessing an α-amino acid moiety at the 4-position of the heterocycle where the amine was mono- or disubstituted by diverse heteroaryl groups.

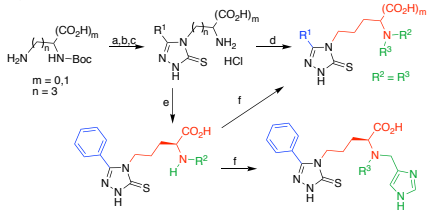


General structure

R^3 = H: monosubstituted; $R^2 = R^3 \neq$ H: homodisubstituted; $R^2 \neq R^3 \neq$ H: heterodisubstituted



Synthesis



Reagents and conditions: (a) *i*, DPT, Na_2CO_3, DMF, 55 °C, 3h; *ii*, R^1-$CONHNH_2$, DMF, 55 °C, 3h; (b) aqueous $NaHCO_3$, 100 °C, 3h; (c) conc. HCl/dioxane (1:2), anisole (3 eq.), 45 °C, 1h ; (d) R^2-CHO (5 eq.), TEA (2 eq.), $NaBH_3CN$ (5 eq.), EtOH, rt; (e) R^2-CHO (2-5 eq.), anhydrous DMF, $NaBH_4$, rt, 15h; (f) R^2-CHO or R^3-CHO (3 eq.), $NaBH_3CN$ (3 eq.), DMF/AcOH (98:2) rt,15h.

Antibacterial synergistic activity of compounds on MBL-producing clinical isolates with meropenem determined by the broth microdilution method

Cpd (32 µg/mL)	Meropenem MIC (µg/mL)			
	K. pneumoniae		*P. aeruginosa*	*E. coli*
	7023 (bla_{VIM-1}+)	VA-416/02 (bla_{VIM-4}+)	182/00 (bla_{VIM-2}+)	MO-287 (bla_{NDM-1}+)
None	8	16	64	32
1	0.03	0.03	4	0.03
2	0.03	ND	4	0.03
3	4	ND	64	16
5	2	ND	64	32
6	0.06	ND	8	0.03
7	4	ND	64	32
8	4	2	64	32
9	1	ND	ND	ND

Cpd (32 µg/mL)	Meropenem MIC (µg/mL)			
	K. pneumoniae		*P. aeruginosa*	*E. coli*
	7023 (bla_{VIM-1}+)	VA-416/02 (bla_{VIM-4}+)	182/00 (bla_{VIM-2}+)	MO-287 (bla_{NDM-1}+)
10	1	ND	64	16
11	2	ND	64	32
12	0.06	0.06	4	0.03
14	2	ND	64	32
15	0.06	ND	4	0.03
16	4	ND	64	32
17	4	1	64	8
18	0.12	ND	8	4
19	1	ND	64	32

- The CO_2H group and R^2/R^3 = heterocycles with an unsubstituted nitrogen at the *ortho* position are essential for potent microbiological activity.

Meropenem MIC (µg/mL) alone or with compound 1 (JMV7061, 32 µg/mL)						
K. pneumoniae	*E. coli*			*P. aeruginosa*		
SI-518 (bla_{NDM-1}+)	SI-G001 (bla_{NDM-4}+)	NTBC102.1 (bla_{NDM-5}+)	NTBC103 (bla_{NDM-7}+)	ARS243 (bla_{VIM-1}+)	VR-193/98 (bla_{VIM-2}+)	FAPL-B64 (bla_{VIM-4}+)
32	64	64	128	128	16	128
0.12	0.5	2	1	8	0.5	8

Inhibitory potencies of homo- (1-10) and hetero-disubstituted (11-19) analogues[a] against clinically relevant MBLs

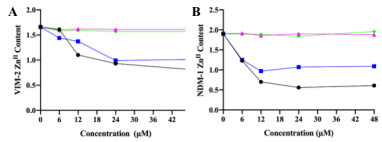
Cpd	Structure $R^2 = R^3$	K_i (µM) or (% inhibition at 100 µM)				
		VIM-1	VIM-2	VIM-4	NDM-1	IMP-1
1 (JMV7061)		0.15 ± 0.01	0.41 ± 0.02	0.20 ± 0.01	0.030 ± 0.004	(40%)
2[b] (D)		0.17 ± 0.09	0.19[d]	0.11 ± 0.01	0.02[d]	7.4 ± 0.2
3[c] (m = 0)		0.80[d]	1.5 ± 0.2	0.90 ± 0.06	9.2 ± 1.4	NI
4		0.69 ± 0.08	0.90 ± 0.09	0.66 ± 0.05	0.020 ± 0.004	(61%)
5		3.66 ± 0.32	(67%)	0.29 ± 0.01	NI	NI
6		1.7 ± 0.2	0.96 ± 0.12	0.76 ± 0.05	0.57 ± 0.08	NI
7		2.6 ± 0.1	0.42 ± 0.03	0.52 ± 0.02	(67%)	NI
8		2.5 ± 0.3	1.2 ± 0.1	0.63 ± 0.04	17.9 ± 1.1	(51%)
9		2.4 ± 0.2	(63%)	2.0 ± 0.2	(66%)	(48%)
10		0.30 ± 0.02	0.10 ± 0.01	0.14 ± 0.01	(57%)	NI

Cpd	Structure R^3	K_i (µM) or (% inhibition at 100 µM)				
		VIM-1	VIM-2	VIM-4	NDM-1	IMP-1
11		4.36 ± 0.48	3.24 ± 0.45	3.81 ± 0.60	(66%)	(50%)
12		0.45 ± 0.04	3.34 ± 0.12	0.35 ± 0.02	2.66[d]	(49%)
13		4.59 ± 0.43	(53%)	1.96 ± 0.20	(37%)	(34%)
14		4.14 ± 0.22	2.07 ± 0.21	1.01 ± 0.09	(60%)	(45%)
15		0.95 ± 0.11	0.64 ± 0.08	0.56 ± 0.08	0.38 ± 0.05	NI
16		5.18 ± 0.51	1.84 ± 0.20	1.73 ± 0.15	34.8 ± 7.9	NI
17		3.29 ± 0.20	2.36 ± 0.20	1.93 ± 0.15	0.89 ± 0.11	(43%)
18		0.82 ± 0.06	0.31[d]	0.35[d]	0.030 ± 0.002	(59%)
19		2.49 ± 0.11	1.09 ± 0.09	0.77 ± 0.05	13.1 ± 2.0	(39%)

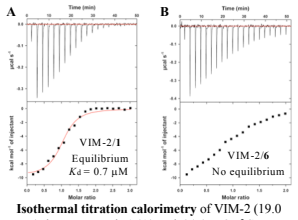
[a]All compounds have CO_2H, *L* stereochemistry, excepted [b]D stereochemistry, [c]No CO_2H. [d]Apparent K_i value, not determined due to non linear v_0/v_i vs [I] plot. Kinetics were monitored at 30 °C by following the absorbance variation observed upon substrate hydrolysis. K_i's were determined when inhibition > 75% at 100 µM. Assays were performed in triplicate. NI: no inhibition (< 30% inhibition at 100 µM).

- Monosubstituted analogues are poor inhibitors (not shown).
- JMV7061 (Cpd **1**) analogues with a different R^1 group are similarly potent (not shown).
- The CO_2H group and R^2/R^3 = heterocycles with an unsubstituted nitrogen at the *ortho* position are essential for potent NDM-1 inhibition.
- Two exceptions: compounds **8** and **9**: may be due to differences in the pK_a values of the non-protonated nitrogen of heterocycle: 6.9 and 5.2 for imidazole and pyridine *vs* 2.5 and 0.6 for thiazole and pyrazine, respectively (values for unsubstituted heterocycles).

Inhibition mode



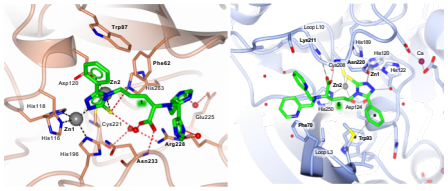
Equilibrium dialysis study with VIM-2 (A) and NDM-1 (B) in the presence of cpds **1** (black), **6** (blue), **7** (magenta) and **10** (green). The metal content of VIM-2 (3 µM) and NDM-1 (3 µM) was measured after overnight dialysis with various inhibitor concentrations.



Isothermal titration calorimetry of VIM-2 (19.0 µM) by compounds **1** (A) and **6** (B) at 25 °C. No equilibrium detected in the case of NDM-1.

Native ESI-MS study of 10 µM VIM-2 and NDM-1 with 50 µM compounds **1**, **6**, and **7**.

Sample	Protein MW (Da)	Suspected species
VIM-2 + **1**	26,359	VIM-2 with 1 eq Zn^{II} + 1 eq **1**
VIM-2 + **6**	25,842	apo-VIM-2
VIM-2 + **7**	25,972	VIM-2
	26,446	VIM-2 + 1 eq **7**
NDM-1 + **1**	25,255	apo-NDM-1
NDM-1 + **6**	25,385	NDM-1
	25,859	NDM-1 + 1 eq **6**
NDM-1 + **7**	25,385	NDM-1
	25,859	NDM-1 + 1 eq **7**



VIM-2/Cpd 1 complex (8A4M.pdb) **NDM-1/Cpd 6 complex (8A76.pdb)**

The triazole-thione moiety binds the two Zn by its N^2 and S atoms.

Co-crystallization of NDM-1 with cpd **1** yielded crystals of the apo-enzyme.

- The *N,N*-diheteroarylmethyl-amino acid part of **1** and **6** behaves as a Zn^{II} chelating moiety ($K_d \approx 10$ nM).
- Compounds **1** and **6** at least partially inhibited one or both MBLs by zinc stripping.

Characterization of compound 1 (JMV7061)

- Cytotoxicity against HeLa cells (membrane integrity assay): $IC_{50} > 250$ µM.

- ADME profile *in vitro*[4]

Cpd	Solubility (µM)[a]	LogD[b]	Plasma[c] $t_{1/2}$ (h)	Microsomes[d] $t_{1/2}$ (min)	Microsomes[d] Cl_{int} µl/min/mg	Plasma proteins binding (%)[e]
1	≥ 200	-2.1 ± 0.1	> 6	> 40	Stable	67.6

[a]In 10 mM PBS, pH 7.4; [b]Measured by mixing in 10 mM PBS pH 7.4/n-octanol (1:1); [c]Stability at 37 °C in mouse plasma; [d]Stability at 37 °C using mouse microsomes; [e]Mouse plasma.

- Selectivity profile toward mammalian metalloenzymes[4]

Cpd	IC50 (µM) or (% inhibition at 100 µM)			
	IDE[a]	APN[b]	ERAAP[c]	HDAC6[d]
1	20	30	(51%)	NI[e]

[a]Insulin Degrading Enzyme; [b]Aminopeptidase N; [c]Endoplasmic Reticulum Aminopeptidase associated with Antigen Processing; [d]Histone De-Acetylase 6; [e]NI, No Inhibition.

- Pharmacokinetic study in Wistar Rats (iv):[5] 1 mg/kg: plasmatic $T_{1/2} \approx 40$ min, CL ≈ 29 mL/min/kg

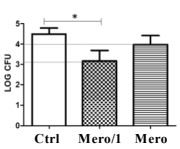
1, 10, 30 mg/kg co-injected with meropenem (30 mg/kg): $65 < T_{1/2} < 75$ min. No adverse effect.

- *In vivo* efficacy of meropenem/Cpd 1 (both 30 mg/kg, iv, twice) in a murine pneumonia model[6]

Infection with NDM-1-producing *K. pneumoniae*

Treatments at 2.5 and 18h, euthanasia at 24h post-infection

Log CFU measured in harvested lungs (*p = 0.0121)



Mero MIC = 128 µg/mL

Ctrl: 17 mice

Mero/1: 10 mice

Mero: 12 mice

Conclusions

- ❑ Critical role of certain di-heteroaryl-substituted amino acid moieties in both NDM-1 inhibition and potentializing activity.
- ❑ Compounds inhibited both VIM-2 and NDM-1 at least partially by stripping their catalytic zinc ions.
- ❑ The broad spectrum MBL inhibition, its remarkable microbiological activity and favourable properties in terms of ADME, toxicity, selectivity profiles and *in vivo* behaviour make compound **1** (JMV7061) highly promising.

References

(1) Legru A. et al. *Eur. J. Med. Chem.* 226 (2021) 113873
(2) Gavara L. et al. *Bioorg. Chem.* 113 (2021) 105024
(3) Verdirosa F. et al. *ChemMedChem* 17 (2022) e202100699

Acknowledgments

(4) Done by C. Piveteau, F. Leroux, R. Deprez-Poulain, B. Deprez, Plateforme Ariadne-ADME, Institut Pasteur Lille, France
(5) Done by Bienta, Enamine, Kiev, Ukrainia
(6) Done by C. Falciani (Dpt of Medical Biotechnology, Siena University, Italy)