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Synthesis of Sub-Micromolar Inhibitors of MraY by Exploring the Region Originally Occupied by the Diazepanone Ring in the Liposidomycin Structure

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Abstract—The synthesis and inhibitory activity against MraY of a series of simplified analogues of liposidomycins are described. These compounds were mainly obtained by performing parallel synthesis in the 6′-position of a scaffold that gathers key features found necessary for the binding to MraY. Thus, inhibitory activity was improved from 5300 to 140 nM. This improvement was correlated with the length and lipophilicity of substituents, but was found to be independent of the nature of the chemical bond generated. In addition, some of these inhibitors presented encouraging antibacterial activities.

Translocase (MraY) is an essential enzyme for bacteria[1] and studied as a target of choice for the discovery of novel antibacterials in order to complement the current therapeutic arsenal, today weakened by the emergence of resistant bacterial strains.[2]

We have recently identified the *O*-β-D-ribofuranosyl nucleoside moiety of liposidomycins (LPMs) **I**[3] as the key fragment responsible for preserving a reasonable inhibitory activity of this family of naturally occurring inhibitors of MraY.

Further studies[4,5] have demonstrated the contribution of most of the substituents present in **I**, as well as the determinant nature of the chiral center (5′, *S*) found with its corresponding hydroxymethyl homologue **II** (Fig. 1).

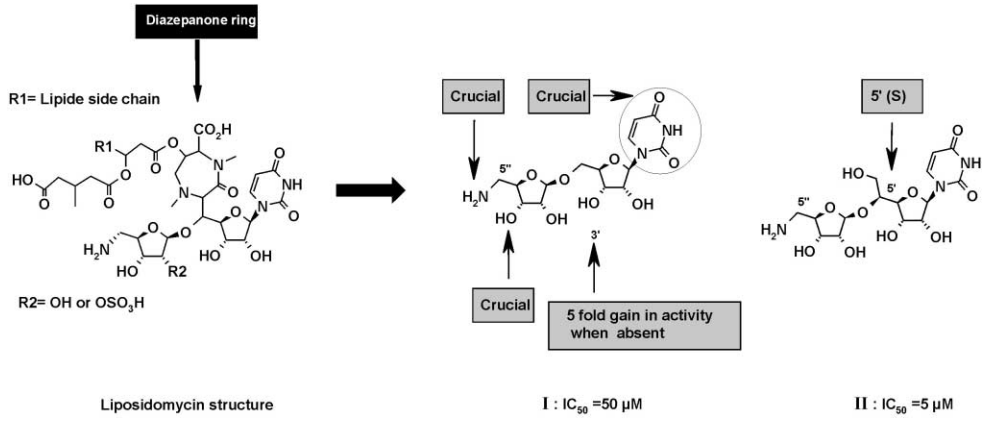

Figure 1.

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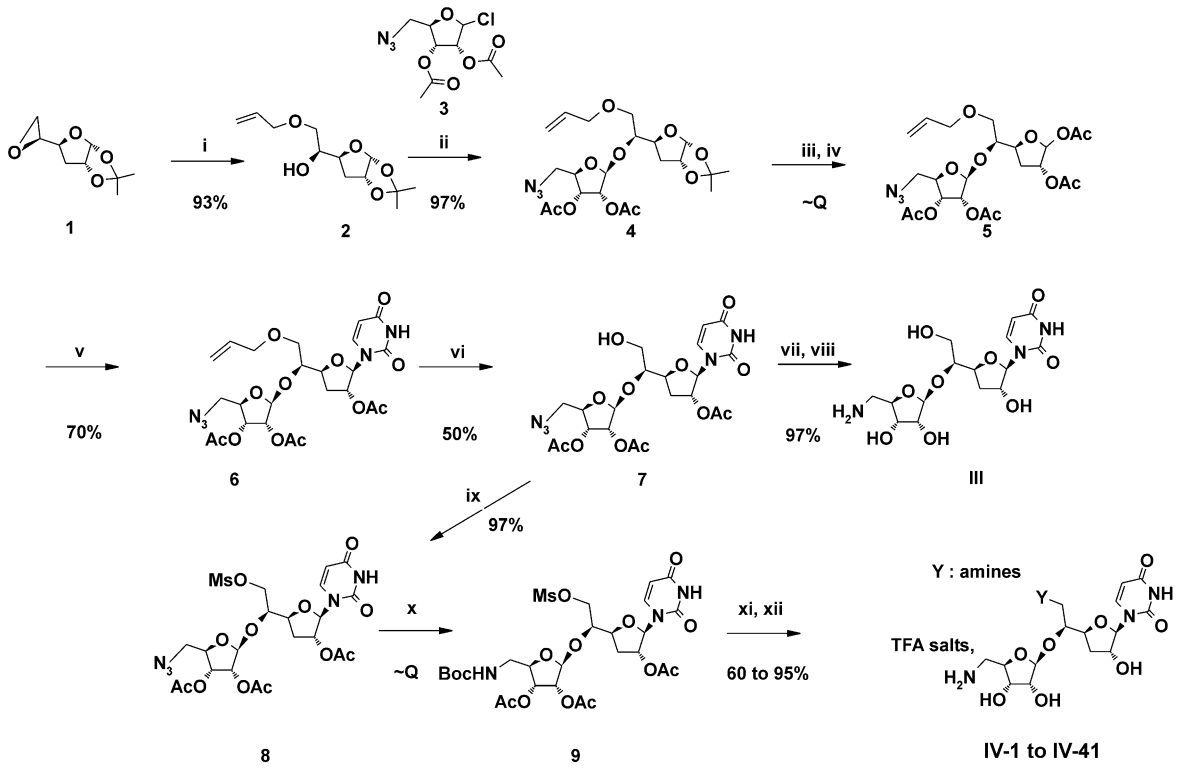


Scheme 1. (i) AllOH, NaH, DMF, 18 h, rt; (ii) $Hg(CN)_2$, $ClCH_2CH_2Cl$, 18 h, rt; (iii) 60% CH_3CO_2H aq, 10 h, 60 °C; (iv) Ac_2O, pyridine, 3 h, rt; (v) *O*,*O*′-bis(trimethylsilyluracil), TMSOTf, CH_3CN, 3 h, rt; (vi) $PdCl_2$, MeOH, 18 h, rt; (vii) MeONa, MeOH, 18 h, rt; (viii) Pd/C, H_2, MeOH, 30 min, rt; (ix) MsCl, pyridine, CH_2Cl_2, 24 h, rt; (x) Pd/C, H_2, $(BOC)_2O$, AcOEt, 3 h, rt; (xi) amines, DMSO, 18 h, 80 °C; (xii) IRA400 (OH−), MeOH; MeOH/AcOH then TFA/H_2O (70/30), 30 min, rt.

As a result of these SAR studies, we decided to synthesize compound **III** (Fig. 2) possessing all favorable features for obtaining of a good level of inhibitory activity against MraY: absence of hydroxyl in position 3′, a 5′(*S*) chiral center, a primary amine group in 5″ position and an unmodified uracil moiety.

The chemical strategy for its preparation was studied with the view of synthesizing further analogues bearing simple substituents in the 6′-position, corresponding to the location of the diazepanone moiety in LPMs. The aim of these efforts was the recovery of activity comparable to that of LPMs and other known inhibitors of MraY, namely tunicamycins (TCMs)[6] and mureidomycins (MRDs).[7]

Addition of sodium allylate to epoxide **1**,[8] gave **2**. Condensation of **2** with **3**,[3] in the presence of mercury (**II**) cyanide gave **4**. Hydrolysis of the acetonide group was accomplished by using 60% aqueous acetic acid. Acetylation of the crude compound gave **5**. The introduction of the uracil moiety was performed using bis-trimethylsilyl uracil and trimethylsilyl triflate giving rise to the nucleoside **6** as a unique stereoisomer. Deallylation was accomplished using $PdCl_2$ in methanol, giving **7**. Methanolysis of **7**, followed by palladium catalyzed reduction of the azido group led to **III** (Scheme 1).[9]

Despite combining a priori all favorable chemical features for improving inhibitory potency, the activity of **III** against MraY remained comparable to that of **II** (IC_{50} around 5 μM for both compounds).

Then, a series of amino derivatives was prepared in parallel in the following way.

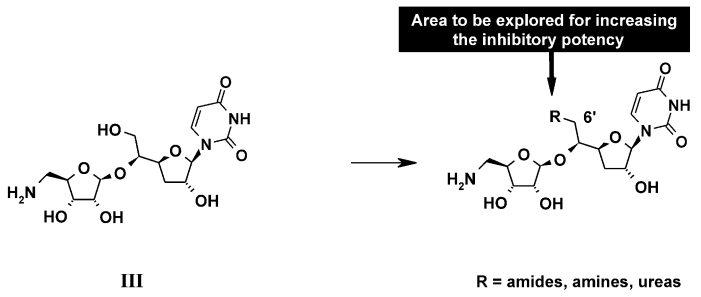
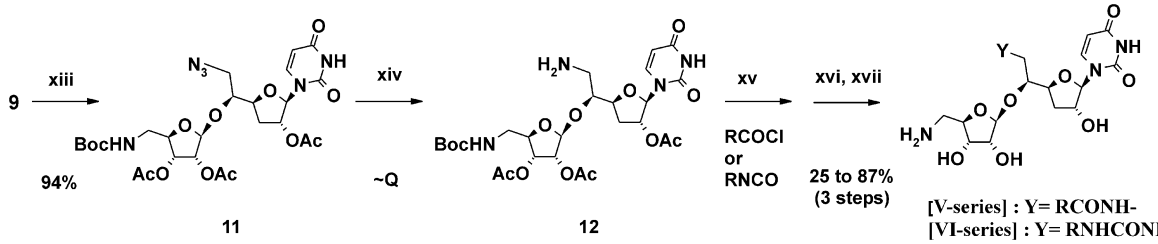


Figure 2.

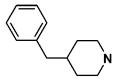
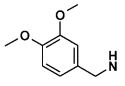
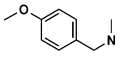
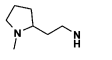
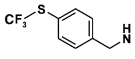
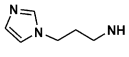
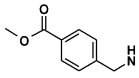
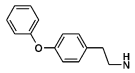
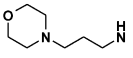
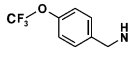
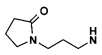
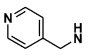
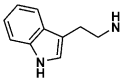
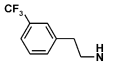
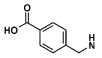
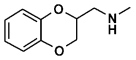
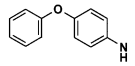
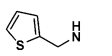
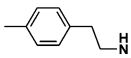
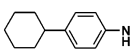
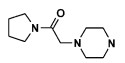
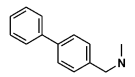
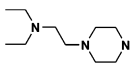
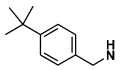
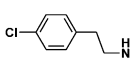


Scheme 2. (xiii) NaN_3, DMF, 60 °C, 17 h; (xiv) Pd/C, H_2, AcOEt, 6 h, rt; (xv) RCOCl/pyridine or RNCO/AcOEt; (xvi) IRA400 (OH^-), MeOH, then AcOH/MeOH; (xvii) TFA/H_2O (70/30), 30 min, rt.

Mesylation of the primary alcohol group of **7** was performed with methanesulfonyl chloride in the presence of pyridine providing **8**. Palladium catalyzed reduction of the azido group and concomitant protection of the resulting amine with di-*tert*-butyl dicarbonate, led to the intermediate **9**. The mesylate group of **9** was then substituted by various amines under identical reaction conditions and the remaining acetyl protective groups were cleaved off using Amberlyst resin (A-26; –OH form). After several washings with MeOH, the products were the released from the resin with methanolic acetic acid. Finally, 70% aqueous trifluoroacetic acid was used to remove the BOC protective group, leading to compounds **IV-1**–**IV-41**[10] in yields varying from 60 to 95% and purities over than 85%.[11]

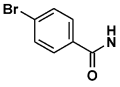
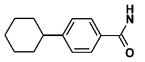
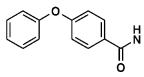
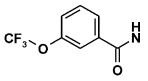
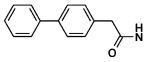
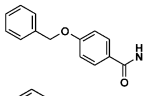
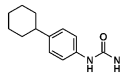
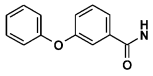
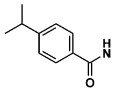
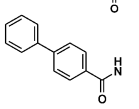
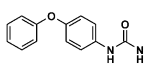
The inhibitory activities (IC_{50}) of compounds resulting from this parallel synthesis program (**IV-1**–**IV-41**) are summarized in Table 1 and ranked according their respective potency.[12]

Table 1. Amine library

Compd	Y	IC_{50} (μM)	Compd	Y	IC_{50} (μM)	Compd	Y	IC_{50} (μM)	Compd	Y	IC_{50} (μM)
TCMs[a]		0.48	**IV-9**		4.4	**IV-20**		1.6	**IV-31**		0.35
MRD-B[b]		0.065	**IV-10**		4.0	**IV-21**		1.4	**IV-32**	$CH_3(CH_2)_9NH$	0.33
III	OH	5.3	**IV-11**		3.9	**IV-22**		1.3	**IV-33**		0.31
IV-1	$CH_3(CH_2)_4N(CH_2)_4CH_3$	9.7	**IV-12**		3.8	**IV-23**		1.2	**IV-34**		0.31
IV-2		9.2	**IV-13**		3.8	**IV-24**	$CH_3(CH_2)_4NH$	0.90	**IV-35**		0.30
IV-3		7.0	**IV-14**		3.4	**IV-25**	$CH_3(CH_2)_7NH$	0.82	**IV-36**	$CH_3(CH_2)_{11}N(CH_3)$	0.23
IV-4		6.9	**IV-15**		2.9	**IV-26**	$CH_3(CH_2)_6NH$	0.74	**IV-37**		0.22
IV-5		6.5	**IV-16**		2.8	**IV-27**	$CH_3(CH_2)_{11}NH$	0.70	**IV-38**		0.20
IV-6		5.9	**IV-17**		2.3	**IV-28**		0.61	**IV-39**		0.18
IV-7		4.8	**IV-18**		1.9	**IV-29**	$CH_3(CH_2)_{13}NH$	0.59	**IV-40**		0.17
IV-8		4.6	**IV-19**		1.8	**IV-30**		0.58	**IV-41**		0.17

[a]TCMs (mixture of tunicamycins from Sigma, [CAS#11089-65-9].
[b]MRD-B: purified mureidomycin-B and compound **III** (see above).

Table 2.

Compd	R	IC_{50} (μM)	Compd	R	IC_{50} (μM)	Compd	R	IC_{50} (μM)	Compd	R	IC_{50} (μM)
V-1		14	**V-4**		0.44	**V-7**		0.39	**V-10**		0.20
V-2		1.0	**V-5**		0.43	**V-8**		0.32	**VI-1**		0.16
V-3		0.44	**V-6**		0.40	**V-9**		0.25	**VI-2**		0.14

As a result of this chemistry program, it turned out that inhibitory activity could be dramatically improved, up to 170 nM for compounds **IV-40** and **IV-41** (30-fold increase in potency, as compared to **III**). The level of activity was clearly dependent of the nature of substituents introduced: best compounds contain lipophilic features mainly located at their extremity. Along with this parameter, length of the substituent appears to be determinant: length equivalent or superior to *para*- or *ortho*-chloro substituted phenethyl amines is required to obtain sub-micromolar values: for instance, compounds **IV-37** and **IV-41** are more potent than **IV-20**. With comparable length and lipophilic properties, secondary (e.g., **IV-39**, **IV-41**), tertiary (e.g., **IV-31**, **IV-40**) amines give similar results. In the aliphatic secondary amine series (**IV-24**–**27**, **IV-29**, **IV-32**) potency of inhibitors is not fully correlated with length of the side chain: an increase in activity can be perceived from C5 to C14 with respect to the aliphatic length, excepted for the C10 derivative (**IV-32**), which turns out to be more active than any of the other. It is worth to note that the tertiary derivative **IV-36** generated with *N*-methyl dodecylamine displays similar activity to **IV-32**. In contrast, the *N*,*N*-dipentylamino derivative (**IV-1**) is 30 times less active than **IV-32** even though comparable in terms of the number of carbon atoms present in their respective substituents.

In order to assess a possible effect of the nature of the tether between the sugar unit and substituents generated, we have prepared a second intermediate **11** allowing the synthesis of amides and ureas bearing mostly substituents comparable to most promising ones identified in the 'amine library' program: substitution of the mesylate group of **9** with sodium azide in DMF gave **11**. Subsequent hydrogenation of the resulting azido function with palladium in ethyl acetate led to **12**. Compound **12** was reacted with some acyl halides and isocyanates to provide, after protecting groups removal, amides (**V-1** and **V-2**[13]) respectively in yields varying from 25 to 87% and purities over than 95% (Table 2).

Results obtained with amides and ureas turned out to be similar to those obtained in the 'amine library' with comparable substituents. It is worth noting that inhibitory activity was further improved to 140 nM with **VI-2** (Scheme 2).

All synthesized compounds were tested on whole bacterial cells. Some interesting antibacterial activities were found for compounds with long aliphatic side chains. The antibacterial activity appeared to be correlated with the chain length (Table 3).

Table 3. In vitro antibacterial activities were determined by microbroth dilution method against four clinical isolates ($5\times10^{+4}$ cfu)

	MICs (μg/mL)				
	IV-24	**IV-25**	**IV-32**	**IV-27**	**IV-29**
Staphylococcus aureus	>80	>80	40	5	2.5
Streptococcus pyogenes	>80	>80	20	2.5	2.5
Enterococcus faecium	>80	80	80	20	10
Escherichia coli	>80	>80	40	2.5	2.5

As a general conclusion, introduction of simple lipophilic substituents instead of the complex diazepanone ring present in the LPM structure, is sufficient to reach levels of inhibitory activity similar to those of MRDs and TCMs and presumably to those of LPMs (considered to fall between MRDs and TCMs, in potency[6,7]). The lipophilic character of best substituents found suggests a strong hydrophobic environment nearby the diazepanone-binding domain in MraY. According to recent findings concerning the overall organisation of MraY,[14] it is likely that these hydrophobic substituents are in interaction with the highly hydrophobic trans-membrane domains of MraY, while the *O*-β-D-ribofuranosyl nucleoside moiety might occupy the UDP-MurNAc-pentapeptide binding site, at the inner face of the bacterial cell membrane. In addition, sufficiently long aliphatic chains are required to obtain reasonable antibacterial activities.

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9. Analytical data for **III**: ^{1}H NMR (400 M Hz, DMSO-d_6) δ 1.85 (2H, m, H3′a, H3′b), 2.93 (1H, dd, $J=7$, 13.5 Hz, H5″a), 3.11 (1H, dd, $J=3$, 13.5 Hz, H5″b), 3.54 (1H, dd, $J=7$, 11.5 Hz, H6′a), 3.61 (1H, dd, $J=4.5$, 11.5 Hz, H6′b), 3.83 (1H, d, $J=4$ Hz, H2″), 4.00 (2H, m, H3″, H4″), 4.19 (1H, d, $J=4$ Hz, H2′), 4.33 (2H, m, H4′, H5′), 4.93 (1H, s, H1″), 5.55 (1H, s, H1′), 5.62 (1H, d, $J=8$ Hz, H5), 7.72 (1H, d, $J=8$ Hz, H6), 11.39 (1H, br s, CO–NH–CO), Other exchangeable hydrogens: 8.02 (3H, m, br) MS(FAB): 390+ (M+H+).
10. Analytical data for **IV-41**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.95 (2H, m, H3′a, H3′b), 2.80–3.40 (8H, m, H5″a, H5″b, H6′a, H6′b, NC$\underline{H_2}$C$\underline{H_2}$Ph), 3.84, (1H, br s, H2″), 4.00 (2H, m H4″, H3″), 4.11 (1H, t br, $J=6$ Hz, H5′), 4.27 (1H, s br, H2′), 4.42, (1H, t br, $J=7.5$ Hz, H4′), 5.03 (1H, s br, H1″), 5.65 (1H, d, $J=8$ Hz, H5), 5.66 (1H, s, H1′), 7.27 (2H, m, H–Ar), 7.37 (2H, m, H–Ar), 7.75 (1H, d, $J=8$ Hz, H6), 11.38 (1H, br, CO–N$\underline{H}$–CO). Other exchangeable hydrogens: 7.81 (1H, s br), 8.02 (2$\underline{H}$, s br), 8.42 (1H, s br), 8.60 (1H, s br). MS (FAB): 527+ (M+H+).
11. HPLC determination performed on a 250×4.6 mm column packed with Vydac C18 (5 μm particles) using a gradient method: from (A/B: 95/5) to A/B (0/100), (A = 0.1% aqueous TFA, B = CH_3CN), flow rate at 1 mL/min, (linear gradient analyses over 30 min, with UV detection at 254 and 210 nm, respectively.
12. The biochemical assay used was the same as mentioned in ref 4.
13. Analytical data for **VI-2**: ^{1}H NMR (400 MHz, CD_3OD) δ 1.98 (1H, ddd, $J=2$, 6, 13 Hz, H3′a), 2.02 (1H, ddd, $J=4.5$ Hz, 10, 13 Hz, H3′b), 3.11 (1H, dd, $J=9.5$, 13, H5″a), 3.33 (1H, dd, $J=2.5$, 13 Hz, H5″b), 3.54 (1H, dd, $J=7$, 14 Hz, H6′a), 3.61 (1H, dd, $J=4.5$, 14 Hz, H6′b), 3.91 (1H, ddd, $J=3$, 4.5, 7 Hz, H5′), 3.98, (1H, d, $J=4.5$ Hz, H2″), 4.10 (2H, m, H3″, H4″), 4.37 (1H, d br, $J=4.5$ Hz, H2′), 4.57 (1H, ddd, $J=3$, 6, 10 Hz, H4′), 5.11 (1H, s, H1″), 5.65 (1H, d, $J=8$ Hz, H5), 5.74 (1H, s, H1′), 6.90 (2H, d, $J=9$ Hz, H–Ar), 6.93 (2H, d, $J=8$ Hz, H–Ar), 7.06 (1H, t br, $J=8$ Hz, H–Ar), 7.32 (2H, m, H–Ar), 7.35 (2H, d, $J=9$ Hz, H–Ar), 7.94 (1H, d, $J=8$ Hz, H6), MS(FAB): 600+ (M+H+).
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