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Modification of the side chain of micromolide, an anti-tuberculosis natural product

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ABSTRACT

This paper describes a series of modifications of the side chain of micromolide, an anti-tuberculosis natural product. Most of the synthesized compounds showed significantly decreased activities, which suggests that the long aliphatic side chain of micromolide and its double bond are essential to its activity.

Tuberculosis (TB), a chronic infectious disease caused by mycobacteria, primarily *Mycobacterium tuberculosis*, has threatened mankind for thousands of years and remains one of the deadliest diseases worldwide.[1] TB is also known as one of the major AIDS-associated infections. The morbidity caused by TB increases substantially when the immune system is impaired by HIV infection.[2] The current treatment for TB requires a regimen of three to four drugs for 6 to 9 months.[3,4] The lengthy treatment period required by the current therapies may cause significant toxic side effects and/or lead to relapse as a consequence of poor patient compliance. In addition, the emergence of drug-resistant TB, such as multi-drug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) has contributed to the resurgence of tuberculosis.[5] Despite the need for better TB therapies, no new TB drugs have been introduced over the last 40 years. Therefore, new anti-TB agents are urgently needed to shorten the current treatment protocol, to combat drug-resistant TB, as well as to be compatible with antiretroviral drugs for HIV patients.

Micromolide, ((−)-*Z*-9-octadecene-4-olide, (−)-**1**, Fig. 1), a natural product isolated from the stem bark of *Micromelum hirsutum* (Rutaceae), has been reported to show good in vitro anti-TB activity (MIC: 1.5 μg/mL).[6] While the anti-TB carbazole alkaloids of *Micromelum hirsutum* have already been followed-up in analog studies,[7,8] no efforts have been made thus far to utilize **1** as scaffold of a new drug. In addition, the high potency of micromolide against TB, as well as its low molecular weight and simple structure, makes it a promising lead compound. We first embarked on the synthesis of **1** in order to reconcile its reported biological data and provide larger quantities of **1** for additional animal tests. The total synthesis of racemic **1** has been carried out in our laboratories by following a literature procedure, with some modifications (Scheme 1).[9] The desired γ-lactone was obtained from the corresponding aldehyde and ethyl 3-bromoproionate by a SmI_2-induced Barbier-type reaction in the presence of hexamethylphosphoric triamide (HMPA). Both isomers **1** and **1a** in an approximate ratio of 95:5 were successfully separated by HPLC in the final step, which were created by the Wittig synthesis. The further Pd/C-catalyzed reduction of racemic **1** provided **1c**.

Possible modifications of **1** were then investigated. Despite its high in vitro anti-TB activity, the high lipophilicity (Clog P = 6.28)[10] of **1** is out of the range of Lipinski's Rule of Five and, therefore, may limit its therapeutic potential.[11] Micromolide is composed of a γ-lactone ring and a monounsaturated aliphatic side chain. The long aliphatic side chain of micromolide contributes significantly to its high lipophilicity. Therefore, a strategy to decrease the lipophilicity of micromolide and, consequently, to improve its bioavailability is to introduce polar groups into the aliphatic side chain. In this communication, a series of modifications of the side chain of micromolide are described.

Various functional groups were thus introduced into the side chain as displayed in Figure 2. The synthesized analogues include

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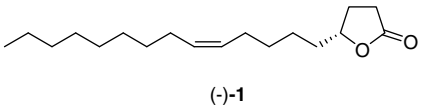
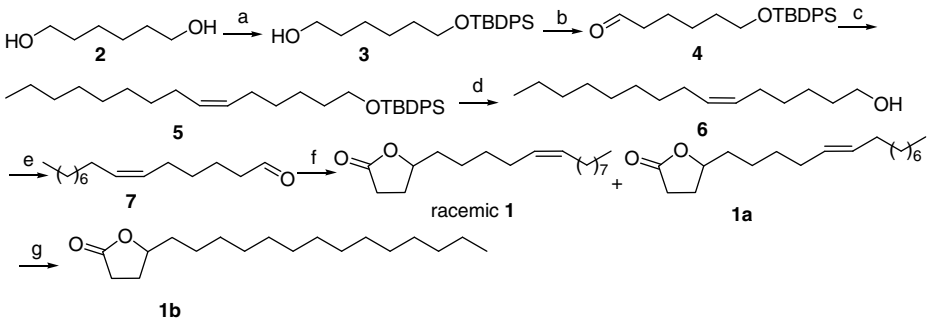


(-)-1

Figure 1. Structure of (−)-micromolide.

isoxazoline, isoxazole, amide, acetylene, and a shorter chain analogue. Compounds containing isoxazoline[12,13] or isoxazole[14–16] moieties have been previously reported as potential anti-TB agents. Therefore, we prepared **8a–d** and **10a** to study whether the isoxazoline or isoxazole rings can be combined with the lactone structure of micromolide. In addition to alkyl chains, esters and amides were attached to the isoxazoline and isoxazole rings to introduce more diversity (**9a–h**, **10b–c**). Amide analogues **11** and **12a–b** were selected for their simplicity in structure as well as lower Clog*P* values. Acetylenes **13a–b** were also prepared to study the importance of the double bond on activity. Another strategy we used to decrease the lipophilicity was to shorten the aliphatic chain as in compound **14**.

The synthesis of isoxazolines **8a–d** was carried out using a 1,3-dipolar cycloaddition (Scheme 2).[17] Condensation of aldehydes **15a–b** with hydroxylamine hydrochloride provided oximes **16a–b**, which were converted to oxime chlorides **17a–b** by treatment with *N*-chlorosuccinimide. Lactones **19a–b** were prepared from **18a–b** by treatment with 1-morpholino-2-trimethylsilyl acetylene followed by aqueous KHF_2.[18] Cycloaddition of **17a–b** with **19a–b** gave **8a–d**. A similar cycloaddition of **19a** with ethyl chlorooximidoacetate provided ethyl ester **9b**, which was subsequently hydrolyzed to give acid **9a** (Scheme 3). Compounds **9c–h** were obtained by coupling **9a** with corresponding amines and alcohols.



Scheme 1. Reagents and conditions: (a) Et_3N, *t*-$BuPh_2SiCl$, DMAP, CH_2Cl_2, rt; (b) PDC, CH_2Cl_2, rt; (c) $CH_3(CH_2)_8P(Ph_3)_3{}^+Br^-$, BuLi, THF, 78 °C; (d) Bu_4NF, THF, rt; (e) PDC, CH_2Cl_2, rt; (f) $BrCH_2CH_2COOEt$, SmI_2 in THF, HMPA; (g) Pd/C, CH_3OH, rt.

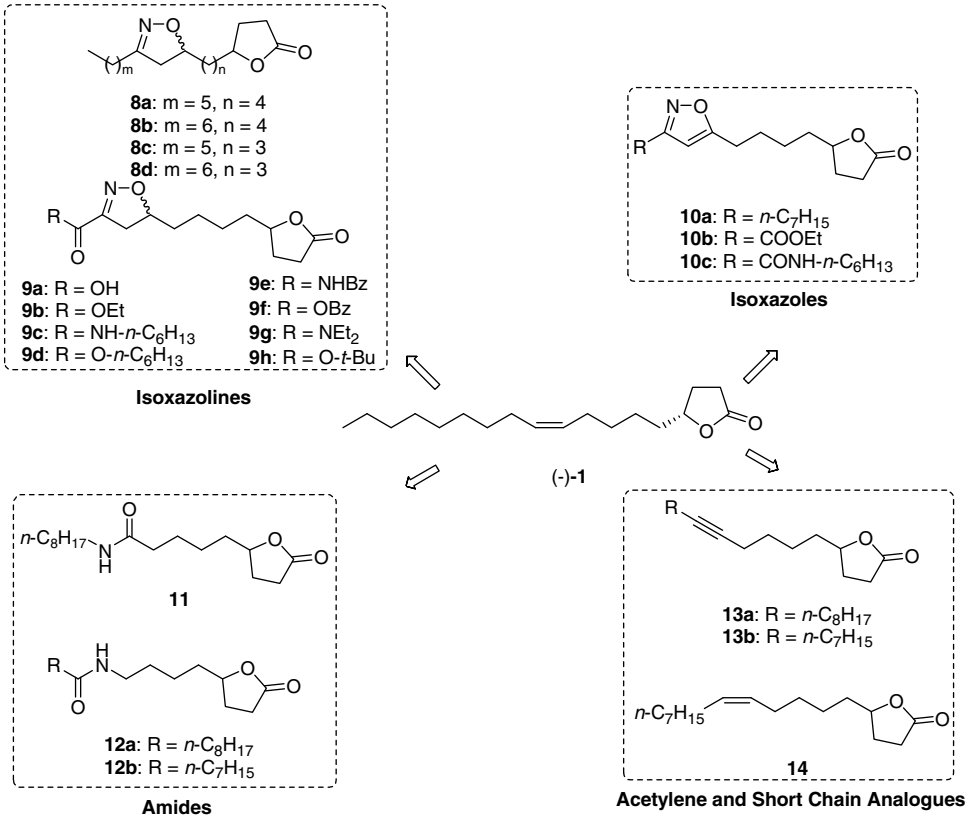


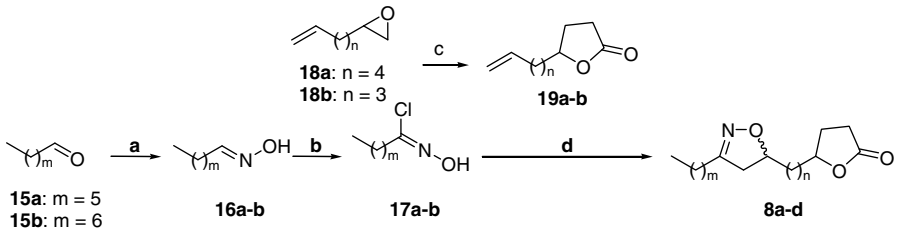
Figure 2. Analogues of micromolide selected for synthesis.

Isoxazoles **10a–c** were synthesized in a similar manner to that described for the preparation of the isoxazoline analogues (Scheme 4). The key intermediate **22** was prepared from **19a** via aldehyde **20**. Cycloaddition reactions of acetylene **22** with **17b** and ethyl chlorooximidoacetate provided **10a** and **10b**, respectively. Hexyl ester **10c** was made from **10b** via the acid **23**.

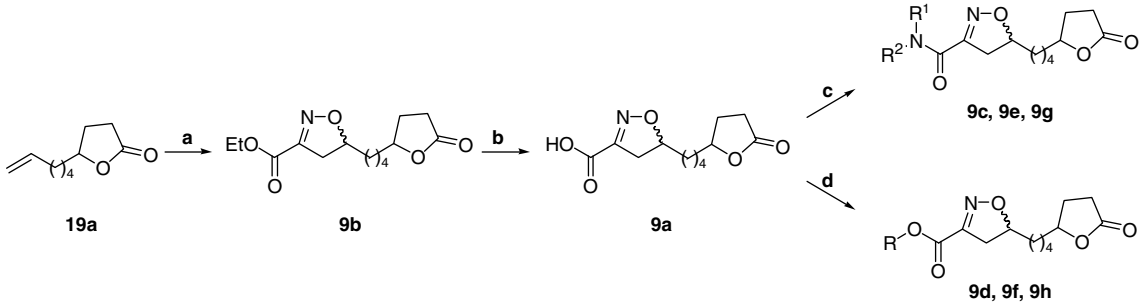
Amide **11** was synthesized from **24** (Scheme 5). Epoxidation of **24** with MCPBA provided **25**, which was then converted to lactone **26**. Hydrolysis of **26** with potassium hydroxide provided acid **27**. Compound **11** was obtained by coupling **27** with 1-octanamine.

The synthesis of **12a–b** is shown in Scheme 6. Compound **30** was prepared from bromide **28** via azide **29**. Epoxidation of **30** with MCPBA gave **31**, which was converted to lactone **32**. Deprotection of **32** with hydrochloric acid provided amine **33**. Amides **12a–b** were prepared by coupling **33** with nonanoic acid and octanoic acid, respectively.

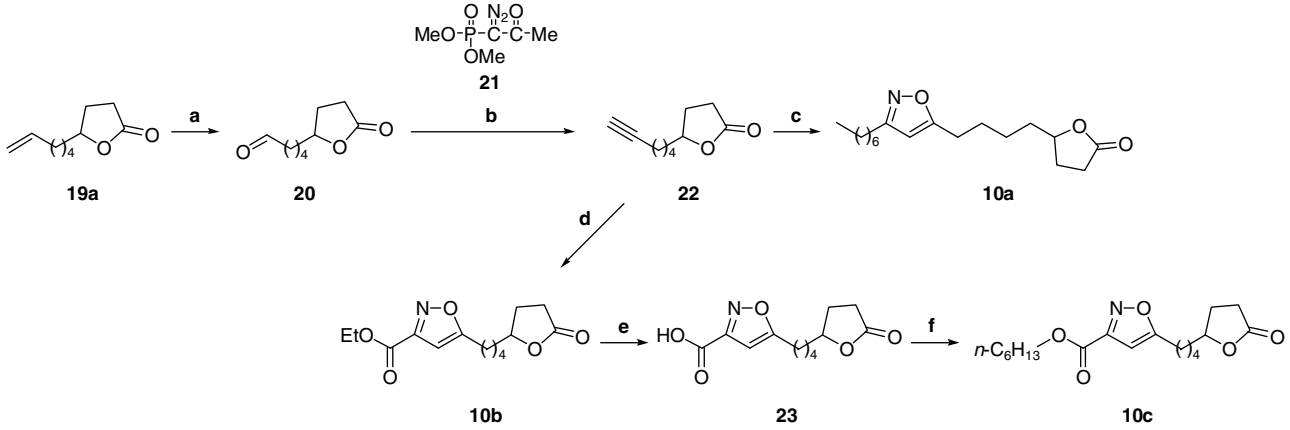
The synthesis of the acetylene analogues **13a–b** is shown in Scheme 7. Acetylenic coupling of **34a–b** with **28** provided **35a–b**. Epoxidation of **35a–b** followed by treatment with 1-morpholino-2-trimethylsilyl acetylene followed by aqueous KHF_2 gave



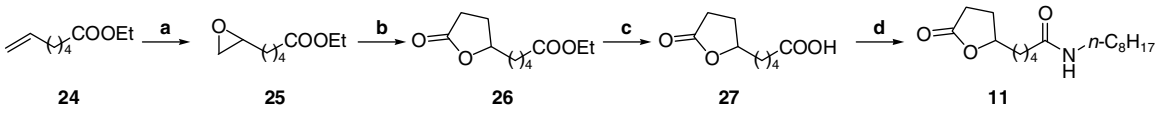
Scheme 2. Synthesis of **8a–d**. Reagents and conditions: (a) NH_2OH–HCl, Na_2CO_3, rt; (b) *N*-chlorosuccinimide, 40–50 °C; (c) i—1-morpholino-2-trimethylsilyl acetylene, $BF_3{\cdot}Et_2O$, 0 °C; ii—KHF_2, rt; (d) Et_3N, 0 °C to rt.



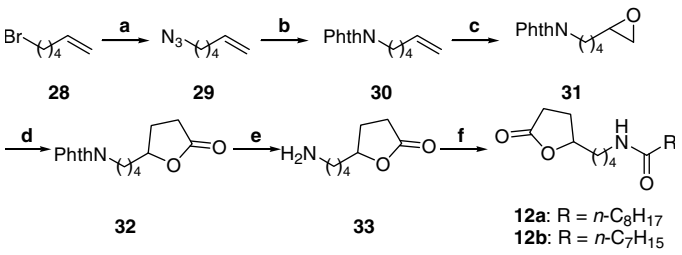
Scheme 3. Synthesis of **9a–h**. Reagents and conditions: (a) ethyl chlorooximidoacetate, Et_3N, 0 °C to rt; (b) NaOH, 0 °C to rt; (c) R^1R^2NH, BOP, DMAP, Et_3N, rt; (d) ROH, DCC, DMAP, rt.



Scheme 4. Synthesis of **10a–c**. Reagents and conditions: (a) OsO_4, 2,6-lutidine, KIO_4, rt; (b) **21**, K_2CO_3, rt; (c) **17b**, Et_3N, 0 °C to rt; (d) ethyl chlorooximidoacetate, Et_3N, 0 °C to rt; (e) NaOH, 0 °C to rt; (f) n-$C_6H_{12}OH$, DCC, DMAP, rt.



Scheme 5. Synthesis of **11**. Reagents and conditions: (a) MCPBA, 0 °C to rt; (b) i—1-morpholino-2-trimethylsilyl acetylene, $BF_3{\cdot}Et_2O$, 0 °C; ii—KHF_2; (c) KOH, rt; (d) $nC_8H_{17}NH_2$, BOP, DMAP, Et_3N, rt.



Scheme 6. Synthesis of **12a**–**b**. Reagents and conditions: (a) NaN_3, rt; (b) phthalic anhydride, $NBu_4^+CN^-$, rt; (c) MCPBA, 0 °C to rt; (d) i—1-morpholino-2-trimethylsilyl acetylene, $BF_3{\cdot}Et_2O$, 0 °C; ii—KHF_2, rt; (e) HCl, reflux; (f) RCOOH, BOP, DMAP, Et_3N, rt.

acetylene **13a**–**b**. Selective reduction of **13a**–**b** using Lindlar's catalyst under carefully controlled conditions provided (±)-**1** and the short chain analogue **14**. This is also an alternative pathway to synthesize (±)-**1**.

All of the synthesized compounds were evaluated by the MABA (microplate Alamar Blue assay)[19] method for their activities against *M. tuberculosis* strain $H_{37}Rv$. Some were also tested against non-replicating cultures using the LORA (low oxygen recovery assay) method, but showed no activity.[20] The MIC values using the MABA assay for these compounds are presented in Tables 1–4. *Clog P* values for these compounds were calculated using the program KOWWIN,[10] and the tPSA values were calculated using the program Molinspiration property calculator.[21]

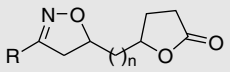
MIC values of synthesized (±)-**1**, **1a,** and **1b** are shown in Table 1 and were compared with that of (−)-**1** isolated from *M. hirsutum*. The *E* isomer **1a** is less active than the *Z* isomer **1**. Compound **1c** without a double bond in the side chain shows a dramatic loss of activity. These results suggest that the conformation and existence of the double bond in side chain is crucial to achieving good anti-TB activity. Compared with (−)-**1**, the racemic form of **1** still retains a reasonable level of activity, and has the advantage of being easier to synthesize. Based on these considerations, we prepared the lactone analogues **8**–**14** in racemic form instead of in optically pure form for SAR studies. Racemic micromolide (±)-**1** was used as a reference for activity.

Table 2 shows the MIC values for the isoxazoline analogues, including the alkyl side chain analogues, **8a**–**d**, as well as the carboxylic acid, esters, and amides, **9a**–**h**. Most of these compounds showed little or no activity against *M. tuberculosis* strain $H_{37}Rv$ except the hexyl ester **9d**, which exhibited a very modest MIC value of 63.1 μM in MABA. MIC values for isoxazole analogues **10a**–**c** are shown in Table 3. Compound **10a** showed a modest MIC value of 63.4 μM, while no significant activities were observed for esters **10b**–**c**. Amide analogies **11** and **12a**–**b** were also found to be inactive (Table 4). Both of the acetylene analogues **13a** and **13b** also showed weak activities (MIC values of 44.0 μM and 70.2 μM, respectively). The acetylene **13b** with a shorter side chain was less active than **13a**. This matches observations of a recent SAR study of anti-TB plant polyacetylenes, which showed that factors other than the triple bonds contribute significantly to the pharmacophore.[22] The short chain analogue **14** (MIC = 14.7 μM), although still less active than (±)-**1** (MIC = 6.90 μM), was shown to be the most active one among the synthesized analogues.

Table 1
In vitro activities for **1** analogues against *M. tuberculosis* $H_{37}Rv$

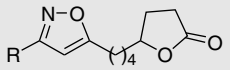
Compound	M_W	MIC (μM)		MIC (μg/ml)	*Clog P*	tPSA (Å^2)
		MABA	LORA			
(−)-**1**	280.45			1.5	6.1	26.3
(±)-**1**	280.45	6.90	124	1.93	6.1	26.3
1a	280.45	19.7		5.54	6.1	26.3
1b	282.46	>128	>128		6.6	26.3

Table 2
In vitro activities for isoxazoline analogues against *M. tuberculosis* $H_{37}Rv$



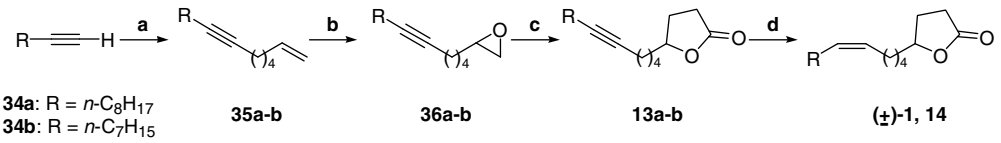
Compound	*n*	R	MIC (μM) MABA	*Clog P*	tPSA (Å^2)
8a	4	–*n*-C_6H_{13}	>128	4.59	47.9
8b	4	–*n*-C_7H_{15}	>128	5.08	47.9
8c	3	–*n*-C_6H_{13}	>128	4.10	47.9
8d	3	–*n*-C_7H_{15}	>128	4.59	47.9
9a	4	–COOH	>128	2.51	85.2
9b	4	–COOEt	>128	3.28	74.2
9c	4	–CONH-*n*-C_6H_{13}	>128	4.18	77.0
9d	4	COO-*n*-C_6H_{13}	63.1	5.25	74.2
9e	4	CONHBz	>128	3.43	77.0
9f	4	COOBz	>128	4.50	74.2
9g	4	$CONEt_2$	>128	3.41	68.2
9h	4	COO-*t*-Bu	>128	4.65	74.2

Table 3
In vitro activities for isoxazole analogues against *M. tuberculosis* $H_{37}Rv$



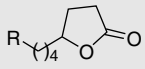
Compound	R	MIC (μM) MABA	*Clog P*	tPSA (Å^2)
10a	–*n*-C_7H_{15}	63.4	5.01	52.3
10b	–COOEt	>128	1.84	78.6
10c	–COO-*n*-C_6H_{13}	>128	3.81	78.6

According to Tables 2–4, most of the synthesized analogues showed considerably reduced activity against *M. tuberculosis* strain $H_{37}Rv$ compared with the activity of (±)-**1**. These results suggest that the double bond present in the side chain of **1** is essential for its activity. Replacement of this double bound with alternative functional groups results in reduced activity. In addition, in view of the comparison between **13a** and **13b**, as well as between (±)-**1** and **14**, it is suggested that decrease of the side chain length also leads to reduced activity. However, the chain length may not be as crucial for activity as the presence of a double bond.



Scheme 7. Synthesis of **13a**–**b**, (±)-**1** and **14**. Reagents and conditions: (a) i—*n*-BuLi, HMPA, 78 °C; ii—**28**, 78 °C to rt; (b) MCPBA, 0 °C to rt; (c) i—1-morpholino-2-trimethylsilyl acetylene, $BF_3{\cdot}Et_2O$, 0 °C; ii—KHF_2, rt; (d) H_2, Lindlar's cat, 10 °C.

Table 4
In vitro activities for amide, acetylene, and shorter chain analogues against *M. tuberculosis* $H_{37}Rv$



Compound	R	MIC (μM) MABA	*C*log*P*	tPSA (Å^2)
11	–CONH-*n*-C_8H_{17}	>128	3.49	55.4
12a	–NHCO-*n*-C_8H_{17}	>128	3.49	55.4
12b	–NHCO-*n*-C_7H_{15}	>128	3.00	55.4
13a	–C≡C-*n*-C_8H_{17}	44.0	5.78	26.3
13b	–C≡C-*n*-C_7H_{15}	70.2	5.29	26.3
14	–*cis*-CH=CH-*n*-C_7H_{15}	14.7	5.79	26.3

Among the synthesized compounds, **9d**, **10a**, **13a**–**b**, and **14** showed moderate activities, although they are less active than (±)-**1**. However, these compounds are less lipophilic. These five compounds were calculated to be 3- to 19-fold less lipophilic than (±)-**1** and comply better with Lipinski's Rule of Five. The decreased lipophilicities may result in better bioavailability and compensate for the lost in in vitro activities.

In summary, a series of modifications to the side chain of micromolide have been made. Most of the synthesized compounds showed significantly decreased activities, which suggests that the long aliphatic side chain of micromolide and its double bond contribute to its activity. Despite the reduced in vitro activities, some moderately active analogues showing lower lipophilicities and larger PSA values were identified, and these compounds may possess improved ADME parameters when studied in vivo.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.08.027.

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