Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 1705–1708

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Evaluation of *N*-Substituted 1,4-Oxazepanyl Sordaricins as Selective Fungal EF-2 Inhibitors

Satoru Kaneko,[a,*] Masami Arai,[a] Takuya Uchida,[a] Tamako Harasaki,[b] Takashi Fukuoka[b] and Toshiyuki Konosu[a]

[a]*Medicinal Chemistry Research Laboratories, Sankyo Co., Ltd., 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo 140-8710, Japan*

[b]*Biological Research Laboratories, Sankyo Co., Ltd., 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo 140-8710, Japan*

Received 13 March 2002; accepted 23 April 2002

Abstract—Sordaricin analogues possessing 6-methoxy-7-methyl-1,4-oxazepane moiety instead of the sugar part were synthesized and evaluated. It was found that *N*-substituents on the oxazepane ring had influence on biological activity. In particular, *N*-(2-methylpropenyl) derivative **12p** exhibited potent in vitro antifungal activity. Furthermore, **12p** maintained significant activity (MIC 0.25 μg/mL) against *Candida albicans* SANK51486 even in the presence of 20% horse serum.

Sordarin (**1**) is a diterpene glycoside isolated from *Sordaria araneosa* in the early 1970s (Fig. 1).[1] Acid-catalyzed hydrolysis of **1** affords sordaricin (**2**), which is a caged tetracyclic aglycon in common with the congeners of the sordarin family.[2a–e] In 1987, zofimarin (**3**) was isolated from *Zopfiella marina* SANK21274 as an antifungal natural product,[3] and showed moderate inhibitory activity in the growth of pathogenic fungi. Recently, a target molecule for the sordarin family has been disclosed. Compounds of this class interfere with fungal protein synthesis by means of selectively binding to fungal elongation factor 2 (EF-2).[4a,b,5,6] They contribute to form a stable EF-2-ribosome complex and prevent the release of EF-2 in the course of translation.[7] Since the mode of action was revealed, sordarin analogues have been a fascinating synthetic target for developing novel antifungal agents.

A number of sordaricin derivatives have been known to date,[8,9] and a few structure–activity relationships were reported during the preparation of this manuscript.[10–12] We also reported **4** as one of such potent compounds.[13] The only problem is that the activity of these compounds is generally diminished in the presence of serum.[14] Nevertheless, it was demonstrated by the Glaxo group that GW531920 (**5**) with a morpholine appendage instead of the sugar component exhibited good in vivo efficacy.[9,15]

Encouraged by this finding, we envisaged a new series of sordaricin derivatives bearing an oxazepane ring, anticipating efficient activity even in the presence of serum. Since the hydrophobicity of the side portion was very important to exhibit good antifungal activity,[16] our attention was focused on the introduction of different

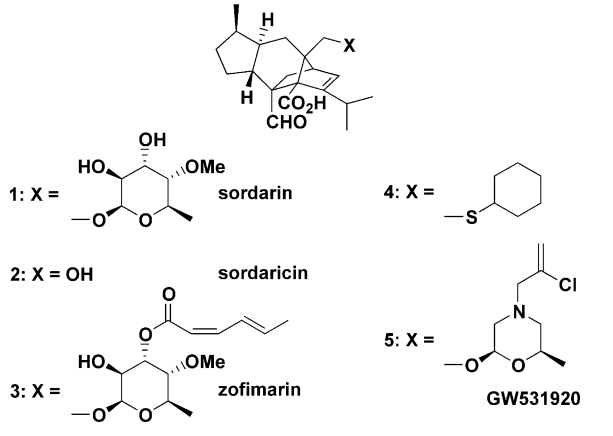
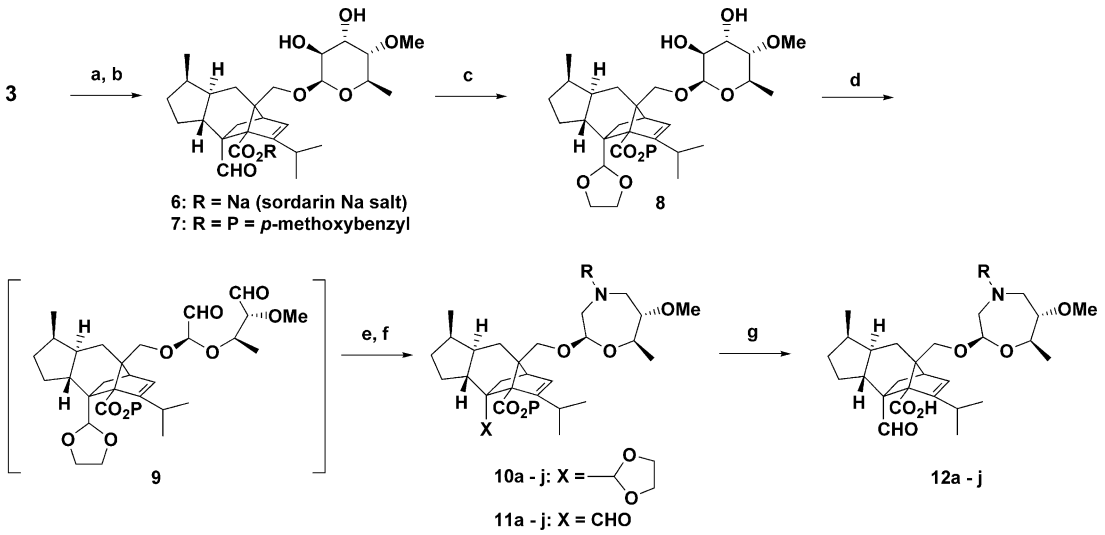


Figure 1. Chemical structures of sordarin **1** and its related compounds.

*Corresponding author. Tel.: +81-3-3492-3131; fax: +81-3-5436-8563; e-mail: skanek@shina.sankyo.co.jp

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00290-1



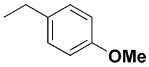
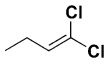
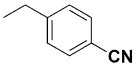
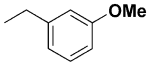
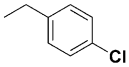
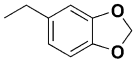
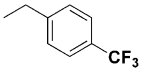
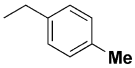
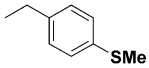
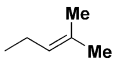
Scheme 1. Reagents and conditions: (a) NaOMe, MeOH, rt; (b) PMBCl, $NaHCO_3$, DMF, 70 °C; (c) $CH(OMe)_3$, $(CH_2OH)_2$, TsOH, MeOH, rt; (d) $NaIO_4$, $NaHCO_3$, MeOH–H_2O, rt; (e) primary amine, $NaBH_3CN$, AcOH, MeCN, rt; (f) 1 N-HCl, MeOH, rt; (g) TFA, CH_2Cl_2, rt.

lipophilic *N*-substituents. Herein, we describe the synthesis of *N*-substituted 1,4-oxazepan-2-yl sordaricin derivatives, and their in vitro antifungal activity under conditions with or without horse serum.

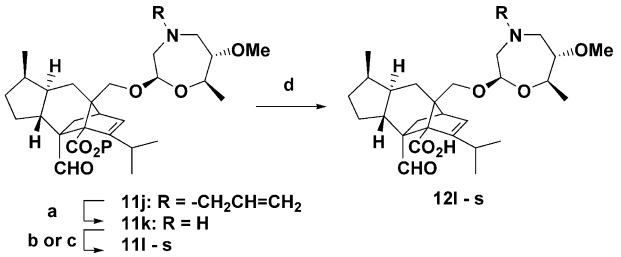
Chemistry

The synthetic route is outlined in Scheme 1. The synthesis commenced with the degradation of **3** produced by fermentation. Treatment of **3** with sodium methoxide afforded sodium salt **6** as a colorless powder in 86% yield. The resulting salt **6** was treated with *p*-methoxybenzyl chloride and $NaHCO_3$ in DMF to furnish PMB ester **7** in 99% yield. At this stage, the formyl group was protected by treatment with ethylene glycol and a catalytic amount of TsOH in MeOH, to provide ethylene acetal **8** in a quantitative yield. Oxidative cleavage of the vicinal diol **8** by employing aq $NaIO_4$ in MeOH gave rise to a quantitative amount of bis-aldehyde **9**, which was observed as diastereomeric cyclic hemiacetals in NMR experiments. Without purification, **9** was subject to the next step. Double reductive amination of **9** using different primary amines proceeded smoothly to afford reclosed oxazepane derivatives **10a–j** in moderate yields. *N*-Substituents are listed in Table 1. In some cases, undesired deprotection of 1,3-dioxolan-2-yl moiety occurred partially. Therefore, crude oxazepanes **10a–j**

Table 1. *N*-Substitutents on the 1,4-oxazepane ring and the chemical yields of **11** and **12**

	R	Yield[a] (%) of **11**	Yield (%) of **12**		R	Yield[a] (%) of **11**	Yield (%) of **12**		R	Yield[a] (%) of **11**	Yield (%) of **12**
a		58	48	**g**		64	77	**n**		76[c]	81
b		44	86	**h**		50	69	**o**		—[e]	78
c		46	68	**i**		48	86	**p**		91[d]	98
d		43	49	**j**		85	69	**q**		49[d]	87
e		48	65	**l**		52[c]	79	**r**		72[d]	96
f		40[b]	81	**m**		64[d]	76	**s**		72[c]	77

[a]Isolation yield in two steps from **9** unless otherwise noted.
[b]Compound **11f** was prepared by reductive amination of **11k** employing $NaBH_3CN$.
[c]*N*-Alkylation was performed with K_2CO_3 and KI in MeCN.
[d]*N*-Alkylation was performed with $NaHCO_3$ and NaI in EtOH.
[e]Compound **11o** was prepared by a different route as follows: (i) **11k**, ethyl 2-(bromomethyl)acrylate, K_2CO_3, MeCN, rt, 73%; (ii) $CH(OMe)_3$, $(CH_2OH)_2$, TsOH, MeOH, rt, 100%; (iii) DIBAL, THF, −60 °C, 61%; (iv) DAST, CH_2Cl_2, −60 to 0 °C, 50%; (v) 1 N-HCl, MeOH, rt, 84%.



Scheme 2. Reagents and conditions: (a) $Rh(PPh_3)_3Cl$, aq EtOH, reflux; (b) alkyl halide, K_2CO_3, KI, MeCN, rt; (c) alkyl halide, $NaHCO_3$, NaI, EtOH, rt; (d) TFA, CH_2Cl_2, rt.

were treated with 1 N-HCl in MeOH, to furnish aldehydes **11a**–**j**. Yields in two steps from **9** are shown in Table 1. Finally, the PMB groups of **11a**–**j** were removed to afford the desired products **12a**–**j** in good yields.[17]

When the primary amine was not commercially available, an alternative route using *N*-alkylation was adopted as shown in Scheme 2. *N*-Allyl derivative **11j** was dealkylated by employing Wilkinson's catalyst, to generate *N*-liberated derivative **11k** in 91% yield.[19] Then, *N*-alkylation of **11k** was performed with alkyl halides. Substituted alkyl groups and yields of **11l**–**s** are listed in Table 1. At last, deprotection of **11l**–**s** led to the desired products **12l**–**s**, respectively, in good yields.[17,18]

Biological Activity

The *N*-substituted oxazepane derivatives **12a**–**j**, **12l**–**s** were assayed for their in vitro antifungal activity under conditions with or without horse serum.[20] The results are summarized in Tables 2 and 3.

All of the *N*-benzyl substituted compounds **12a**–**i**, except **12d**, exhibited excellent antifungal activity (MIC $\leq 0.125\,\mu g/mL$) against *Candida albicans* including azole-low-susceptible strains. They also showed moderate activity (MIC 0.25–8 μg/mL) against *Candida glabrata* and *Candida tropicalis*. In addition, **12g**–**i** had somewhat potency (MIC 2 μg/mL) against *Cryptococcus neoformans*. In particular, **12i** is the most effective compound under the standard conditions used in our study. However, the activity of these compounds were decreased as well as that of **4** in the presence of 20% horse serum in the medium. In the case of **12i**, the MIC

Table 2. In vitro antifungal activity of sordaricin derivatives **12a**–**i**

Organism	MIC (μg/mL)										
	3	**4**	**12a**	**12b**	**12c**	**12d**	**12e**	**12f**	**12g**	**12h**	**12i**
Candida albicans ATCC24433	0.5	0.031	≤0.063	0.063	≤0.063	0.5	0.063	0.063	≤0.063	0.063	0.031
Candida albicans SANK51486	0.25	≤0.016	≤0.063	≤0.031	≤0.063	0.25	≤0.031	≤0.031	≤0.063	≤0.031	0.016
Candida albicans TIMM3164[a]	0.5	0.063	0.125	0.063	≤0.063	0.5	0.125	0.063	≤0.063	0.063	0.031
Candida albicans ATCC64550[a]	0.5	0.125	≤0.063	0.063	≤0.063	1	0.125	0.125	≤0.063	0.125	0.031
Candida parapsilosis ATCC90018	>4	>8	>32	>16	>32	>32	>16	>16	>32	>16	>16
Candida glabrata ATCC90030	>4	0.031	0.5	2	1	8	2	2	0.25	8	0.5
Candida tropicalis ATCC750	0.5	2	0.25	0.5	0.5	2	1	0.5	0.25	0.5	0.25
Cryptococcus neoformans TIMM1855	0.25	>8	4	8	4	>32	>16	>16	2	2	2
Aspergillus fumigatus ATCC26430	>4	>8	>32	>16	>32	>32	>16	>16	>32	>16	>16
Candida albicans ATCC24433[b]	4	8	1	2	8	32	8	4	1	2	1
Candida albicans SANK51486[b]	NT[c]	4	0.5	1	4	16	2	2	0.5	2	0.5

[a]Low susceptibility to fluconazole (MIC >4 μg/mL).
[b]In the presence of horse serum (20%).
[c]Not tested.

Table 3. In vitro antifungal activity of sordaricin derivatives **12j, l**–**s**

Organism	MIC (μg/mL)									
	5	**12j**	**12l**	**12m**	**12n**	**12o**	**12p**	**12q**	**12r**	**12s**
Candida albicans ATCC24433	0.031	0.063	0.125	0.25	≤0.031	≤0.031	0.031	0.031	0.031	0.125
Candida albicans SANK51486	0.016	0.031	0.063	0.125	≤0.031	≤0.031	0.016	0.016	0.016	0.063
Candida albicans TIMM3164[a]	0.031	0.063	0.125	0.25	≤0.031	≤0.031	0.031	0.031	0.031	0.125
Candida albicans ATCC64550[a]	0.031	0.063	0.25	0.5	0.063	≤0.031	0.031	0.031	0.031	0.125
Candida parapsilosis ATCC90018	8	>16	>16	>16	>16	>16	>16	16	>16	>16
Candida glabrata ATCC90030	0.125	0.25	4	16	1	0.5	0.25	0.125	0.25	4
Candida tropicalis ATCC750	0.125	0.5	1	2	0.5	0.25	0.125	0.25	0.125	1
Cryptococcus neoformans TIMM1855	16	>16	>16	>16	8	0.25	0.25	0.125	0.25	8
Aspergillus fumigatus ATCC26430	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Candida albicans ATCC24433[b]	0.5	0.25	1	2	2	0.5	0.5	0.5	1	8
Candida albicans SANK51486[b]	0.25	0.25	0.5	1	1	0.5	0.25	0.5	0.5	4

[a]Low susceptibility to fluconazole (MIC >4 μg/mL).

values were 1 and 0.5 μg/mL against *C. albicans* ATCC24433 and SANK51486, respectively.

The *N*-aliphatic substituted compounds **12j**, **12l–s** showed excellent activity (MIC $\leq$0.125 μg/mL) against *C. albicans* including azole-low-susceptible strains. They also showed moderate activity (MIC 0.25–4 μg/mL) against *C. glabrata* and *C. tropicalis*. Remarkably, compounds **12o–r** exhibited good activity (MIC 0.125–0.25 μg/mL) against *Cr. neoformans* unlike **5**.[21] In particular, **12p–r** showed broad spectrum and excellent activity among the sordarin family. Moreover, **12p** exhibited good activity (MIC 0.25–0.5 μg/mL) in the medium supplemented with 20% horse serum. Unfortunately, no compounds tested had any activity (MIC $\geq$16 μg/mL) against *Candida parapsilosis* or *Aspergillus fumigatus*.

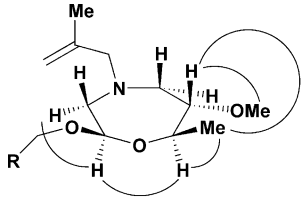
In conclusion, new sordarin derivatives which possess a 1,4-oxazepane ring moiety were synthesized by cyclization featuring successive reductive amination, and were evaluated as antifungal agents. The compound **12p** proved to exhibit excellent antifungal activity in the presence of serum. Thus, some *N*-substituted 1,4-oxazepanyl sordaricins, **12p** in particular, have been proved to be useful for treatment for systemic mycosis including infections with azole-resistant fungal strains. Further studies toward this therapeutic utility are under way.

Acknowledgements

The authors thank Dr. T. Honda (Sankyo Co., Ltd.) for helpful discussions.

References and Notes

1. Hauser, D.; Sigg, H. P. *Helv. Chim. Acta* **1971**, *54*, 1178.
2. (a) Coval, S. J.; Puar, M. S.; Phife, D. W.; Terracciano, J. S.; Patel, M. *J. Antibiot*. **1995**, *48*, 1171. (b) Kinsman, O. S.; Chalk, P. A.; Jackson, H. C. *J. Antibiot*. **1998**, *51*, 41. (c) Kennedy, T. C.; Webb, G.; Cannell, R. J. P.; Kinsman, O. S.; Middleton, R. F.; Sidebottom, P. J.; Taylor, N. L.; Dawson, M. J.; Buss, A. D. *J. Antibiot*. **1998**, *51*, 1012. (d) Okada, H.; Kamiya, S.; Shiina, Y.; Suwa, H.; Nagashima, M.; Nakajima, S.; Shimokawa, H.; Sugiyama, E.; Kondo, H.; Kojiri, K.; Suda, H. *J. Antibiot*. **1998**, *51*, 1081. (e) Daferner, M.; Mensch, S.; Anke, T.; Sterner, O. *Z. Naturforsch*. **1999**, *54c*, 474.
3. Ogita, T.; Hayashi, T.; Sato, A.; Furutani, W. JP Patent 62,040,292, 1987.
4. (a) Domínguez, J. M.; Kelly, V. A.; Kinsman, O. S.; Marriott, M. S.; Gómez, H. F.; Martin, J. *J. Antimicrob. Agents Chemother*. **1998**, *42*, 2274. (b) Domínguez, J. M.; Martin, J. J. *Antimicrob. Agents Chemother*. **1998**, *42*, 2279.
5. Capa, L.; Mendoza, A.; Lavandera, J. L.; Gómez, H. F.; García-Bustos, J. F. *Antimicrob. Agents Chemother*. **1998**, *42*, 2694.
6. Justice, M. C.; Hsu, M. J.; Tse, B.; Ku, T.; Balkovec, D.; Schmatz, D.; Nielsen, J. *J. Biol. Chem*. **1998**, *273*, 3148.
7. Domínguez, J. M.; Gómez-Lorenzo, M. G.; Martín, J. J. *J. Bio. Chem*. **1999**, *274*, 22423.
8. Gargallo-Viola, D. *Curr. Opin. AntiInfect. Invest. Drugs* **1999**, *1*, 297.
9. Odds, F. C. *Exp. Opin. Ther. Pat*. **2001**, *11*, 283, and references therein.
10. Arribas, E. M.; Castro, J.; Clemens, I. R.; Cuevas, J. C.; Chicharro, J.; Fraile, M. T.; García-Ochoa, S.; Heras, F. G.; Ruiz, J. R. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 117.
11. Bueno, J. M.; Cuevas, J. C.; Fiandor, J. M.; García-Ochoa, S.; Heras, F. G. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 121.
12. Serrano-Wu, M. H.; Laurent, D. R.; Mazzucco, C. E.; Stickle, T. M.; Barrett, J. F.; Vyas, D. M.; Balasubramanian, B. N. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 943.
13. Kaneko, S.; Uchida, T.; Shibuya, S.; Honda, T.; Kawamoto, I.; Harasaki, T.; Fukuoka, T.; Konosu, T. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 803.
14. It has been presumed that the sordarin family had a high binding affinity for serum protein. See: Aviles, P.; Falcoz, C.; Roman, R. S.; Gargallo-Viola, D. *Antimicrob. Agents Chemother*. **2000**, *44*, 2333.
15. Herreros, E.; Almela, M. J.; Lozano, S.; Heras, F. G.; Gargallo-Viola, D. *Antimicrob. Agents Chemother*. **2001**, *45*, 3132.
16. Tse, B.; Balkovec, J. M.; Blazey, C. M.; Hsu, M.-J.; Nielsen, J.; Schmatz, D. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 2269.
17. All new compounds gave satisfactory spectroscopic and analytical data. The representative data are shown as follows. **12i**: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.85 (1H, s), 6.91 (1H, s), 6.78 (1H, d, J=8.1 Hz), 6.74 (1H, d, J=8.1 Hz), 6.04 (1H, d, J=2.9 Hz), 5.94 (2H, s), 4.49 (1H, dd, J=8.8, 2.2 Hz), 4.29 (1H, d, J=9.5 Hz), 3.65 (1H, d, J=13.1 Hz), 3.59 (1H, t, J=6.6 Hz), 3.53 (1H, d, J=13.1 Hz), 3.30 (1H, d, J=9.5 Hz), 3.08 (3H, s), 2.99 (1H, d, J=13.9 Hz), 2.98 (1H, m), 2.96 (1H, d, J=11.7 Hz), 2.59 (1H, dd, J=13.9, 2.2 Hz), 2.47 (1H, t, J=3.7 Hz), 2.38 (1H, dd, J=11.7, 8.8 Hz), 2.33 (1H, quint., J=6.6 Hz), 2.12–0.96 (m), 1.25 (3H, d, J=6.6 Hz), 1.02 (3H, d, J=6.6 Hz), 0.97 (3H, d, J=6.6 Hz), 0.80 (3H, d, J=6.6 Hz); FABHRMS (m/z): calcd for $C_{35}H_{48}NO_8$ ($[M+H]^+$): 610.3380. Found: 610.3383. **12p**: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.85 (1H, s), 6.05 (1H, m), 4.89 (1H, s), 4.87 (1H, s), 4.47 (1H, dd, J=8.8, 2.2 Hz), 4.26 (1H, d, J=9.1 Hz), 3.35 (1H, quint., J=6.6 Hz), 3.33 (1H, d, J=9.1 Hz), 3.27 (3H, s), 3.06 (1H, d, J=13.2 Hz), 3.05 (1H, m), 2.98 (1H, d, J=13.2 Hz), 2.95 (1H, brd), 2.95 (1H, brd), 2.55 (1H, dd, J=14.6, 2.9 Hz), 2.49 (1H, t, J=4.0 Hz), 2.34 (1H, dd, J=12.5, 8.8 Hz), 2.32 (1H, quint., J=6.6 Hz), 2.12–0.96 (m), 1.76 (3H, s), 1.25 (3H, d, J=6.6 Hz), 1.02 (3H, d, J=6.6 Hz), 0.99 (3H, d, J=6.6 Hz), 0.81 (3H, d, J=6.6 Hz); FABHRMS (m/z): calcd for $C_{31}H_{48}NO_6$ ($[M+H]^+$): 530.3481. Found: 530.3459.
18. In NOE experiments, the stereochemistry of **12p** was determined as follows.



19. Moreau, B.; Lavielle, S.; Marquet, A. *Tetrahedron Lett*. **1977**, 2591.
20. In vitro antifungal activity was determined in RPMI1640 medium (for *Cr. neoformans*: yeast nitrogen base) buffered at pH 7.0. Microplates were incubated at 35 °C (for *A. fumigatus*: 30 °C). Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the test compound that inhibited the growth of the fungi by 80%. For experiments in the presence of horse serum, the medium was supplemented with 20% horse serum during incubation.
21. It has been reported that *Cr. neoformans* was resistant to all azasordarins such as GW531920 (MIC $\geq$16 μg/mL).[11].