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# Discovery of novel sphingosine kinase-1 inhibitors. Part 2 ☆

Yibin Xiang [a,*], Bradford Hirth [a], John L. Kane Jr. [a], Junkai Liao [a], Kevin D. Noson [a], Christopher Yee [a], Gary Asmussen [b], Maria Fitzgerald [c], Christine Klaus [b], Michael Booker [b]

[a] Department of Medicinal Chemistry, Drug and Biomaterial R&D, Genzyme Corporation, 153 Second Avenue, Waltham, MA 02451, USA
[b] Department of In Vitro Biology, Drug and Biomaterial R&D, Genzyme Corporation, 153 Second Avenue, Waltham, MA 02451, USA
[c] Department of DMPK, Drug and Biomaterial R&D, Genzyme Corporation, 153 Second Avenue, Waltham, MA 02451, USA

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ABSTRACT

Building on our initial work, we have identified additional novel inhibitors of sphingosine kinase-1 (SK1). These new analogs address the shortcomings found in our previously reported compounds. Inhibitors **51** and **54** demonstrated oral bioavailability in a rat PK study.

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Sphingosine kinases (SKs), the enzymes that mediate the conversion of sphingosine (**1**) to sphingosine-1-phosphate (S1P, **2**), are important players in the sphingolipid metabolic pathway as they sit in a crucial position to regulate relative levels of S1P, sphingosine, and ceramide.[2] Two mammalian sphingosine kinases (SK1 and SK2) have been identified, and both are capable of phosphorylating sphingosine to produce S1P (Fig. 1).[3] However, there have been reports that indicate the enzymes may have different cellular functions. SK1 promotes cell growth and proliferation[4] whereas SK2 has the opposite effects.[5] Recently, it has been suggested that SK1 is potentially involved in a variety of pathological states, including various immune-mediated diseases, inflammation, and cancer.[6]

In a previous communication, we reported the identification of novel and potent SK1 selective inhibitors, as exemplified by the 3-hydroxyproline derivative **4**.[1] This compound is significantly more potent in SK1 inhibition than the widely studied pan-SK inhibitor DMS (**3**). Unfortunately, **4** suffers from poor aqueous solubility (~1 μg/mL in pH 7.4 buffered saline), making it difficult to evaluate the compound in an in vivo setting. In this communication, we re-

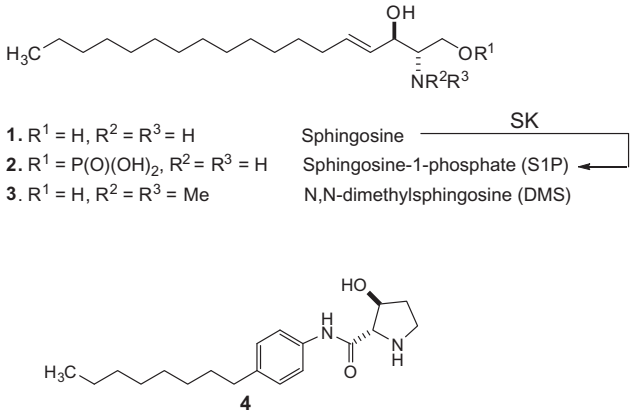
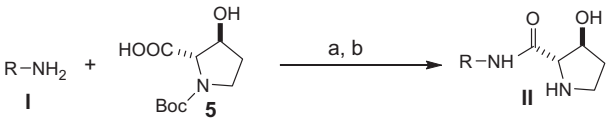



**Figure 1.**




**Scheme 1.** Reagents: (a) EDC, DMAP, $CH_2Cl_2$; (b) TFA, $CH_2Cl_2$ (72–84%, two steps).

☆ See Ref. 1.

* Corresponding author. Tel.: +1 781 434 3653; fax: +1 781 672 5823.
*E-mail address:* yibin.xiang@genzyme.com (Y. Xiang).

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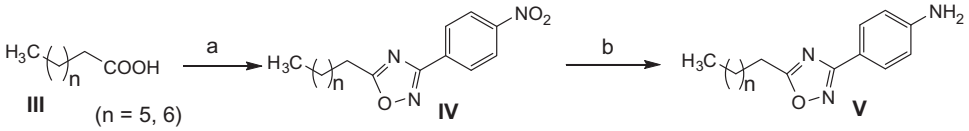
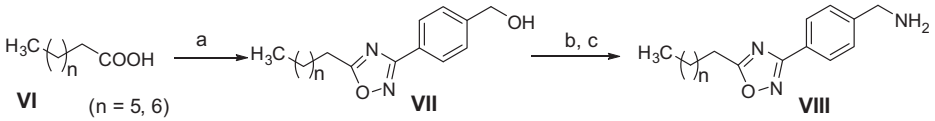
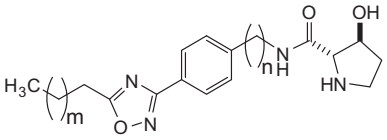


**Scheme 2.** Reagents and conditions: (a) (*Z*)-*N*′-hydroxy-4-nitrobenzimidamide, EDC, HOBT, $CH_3CN$, reflux (65–74%); (b) Zn, $NH_4Cl$, MeOH, 80 °C (77–85%).



**Scheme 3.** Reagents and conditions: (a) (*Z*)-*N*′-hydroxy-4-(hydroxymethyl)benzimidamide, EDC, DMF, 140 °C (67–76%); (b) DPPA, tol, rt; (c) $Ph_3P$, THF–$H_2O$, rt (62–74%).

**Table 1**
SKI inhibitory activity of initial *N*-(oxadiazolyl)benzylamide and *N*-(oxadiazolyl)phenylamide derivatives



| | $IC_{50}$[a] (μM) | Sol.[b] (μg/mL) |
|---|---|---|
| **4** | 0.062 | 1.5 |
| **6**, *n* = 0, *m* = 5, 530,745 | >10 | |
| **7**, *n* = 1, *m* = 5, 529,740 | 0.068 | 26.5 |
| **8**, *n* = 0, *m* = 6, 530,746 | >10 | |
| **9**, *n* = 1, *m* = 6, 529,743 | 0.086 | 4.0 |

[a] Concentration of the testing compounds to inhibit 50% activity of the enzyme.[14]
[b] Solubility measurement determined from DMSO stock at pH 7.4 in phosphate buffered saline, recorded as μg/mL.

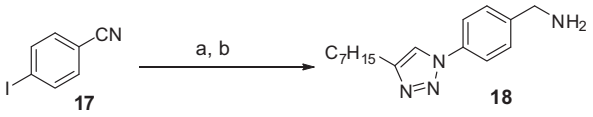
port our medicinal chemistry efforts to optimize this class of SK1 inhibitors.

We believe that the poor aqueous solubility of **4** could be the result of its highly lipophilic nature (log *D* > 3.5), due in large part to the presence of the *n*-octyl moiety. Thus, our plan was to insert an appropriate non-basic heterocycle into the hydrocarbon chain to reduce lipophilicity. Based on our previous experience with this series of compounds, we believed that the incorporation of a heterocycle would not dramatically impact activity against SK1 if it were properly located. Using this hypothesis, a series of oxadiazole-containing compounds was designed, synthesized, and profiled versus SK1.

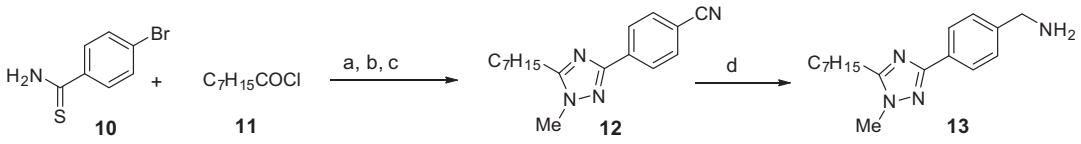
The compounds were prepared by the general method illustrated in Scheme 1. Briefly, an appropriate amine **I** was reacted with *N*-Boc protected 3-hydroxyproline (**5**) in the presence of EDC and DMAP in dichloromethane. The resulting amide was deprotected with trifluoroacetic acid in dichloromethane to produce the desired products **II**. The syntheses of the requisite amine coupling partners are outlined in Schemes 2 and 3. The aniline derivatives **V** were prepared from acids **III** via a two-step process involving EDC-mediated oxadiazole ring formation[7] followed by reduction of the aryl nitro group. Similarly, the benzylamine derivatives **VIII** were prepared by formation of the oxadiazole ring system[8] followed by conversion of the alcohol to the amine.

Table 1 summarizes the activity of some of the newly prepared oxadiazole-containing analogs. We were surprised to find that the compounds containing a phenylamide linkage (**6** and **8**) did not demonstrate activity versus SK1. However, potent inhibition was observed for the benzylamide analogs **7** and **9**. In fact, the activity of these two derivatives was comparable to our previously reported compound **4**. In addition, the aqueous solubility of **7** and **9** was significantly improved.

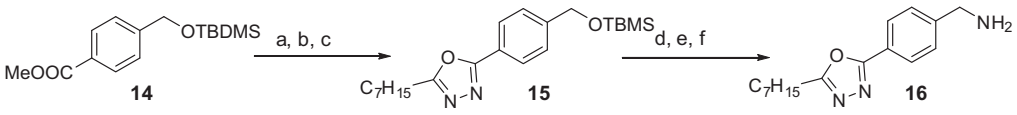
Encouraged by these results, additional heterocycle-containing derivatives of **II** were prepared. The required amines were synthesized by the methods illustrated in Schemes 4–9. As detailed in Scheme 4, triazole **13** was prepared from thioamide **10** via acylation/cyclization with the acid chloride **11**,[9] conversion of the aryl bromide to the corresponding nitrile **12**, and reduction to the desired amine. The synthesis of oxadiazole **16** was accomplished from **14**.



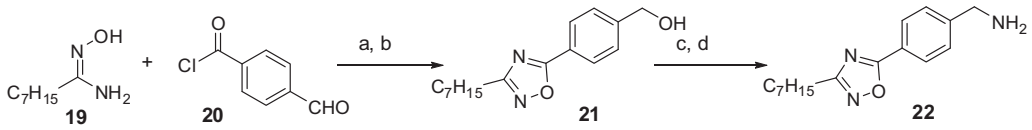
**Scheme 6.** Reagents and conditions: (a) $NaN_3$, 1-nonyne, sodium ascorbate *trans*-1,2-diaminocyclohexane, CuI, DMSO–$H_2O$, rt (63%); (b) LAH, THF, rt (48%).



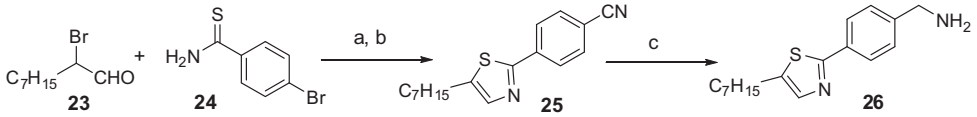
**Scheme 4.** Reagents and conditions: (a) Pyr, acetone, reflux (74%); (b) $MeNHNH_2$, AcOH, dioxane, reflux; (c) CuCN, NMP, reflux (40%, two steps); (d) LAH, THF, rt (99%).



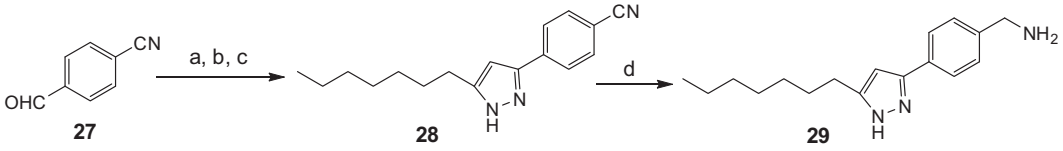
**Scheme 5.** Reagents and conditions: (a) $NH_2NH_2$, EtOH, rt (8%); (b) $C_7H_{15}COCl$, $Et_3N$, DCM, rt; (c) $Ph_3P$, $CCl_4$, $Et_3N$, DCM, reflux (54%, two steps); (d) TBAF, THF, rt; (e) DPPA, tol, rt; (f) $Ph_3P$, TFH–$H_2O$, rt (69%, three steps).



**Scheme 7.** Reagents and conditions: (a) Pyr, reflux; (b) $NaBH_4$, MeOH, rt (45%, two steps); (c) DPPA, tol, rt; (d) $Ph_3P$, THF–$H_2O$, rt (70%, two steps).



**Scheme 8.** Reagents and conditions: (a) $NaHCO_3$, THF, reflux (62%); (b) CuCN, NMP, reflux (9%); (c) LAH, THF, rt (60%).



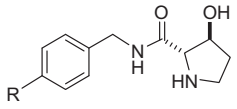
**Scheme 9.** Reagents and conditions: (a) 1-nonyne, LDA, THF, −78 °C to rt (69%); (b) idoxybenzoic acid, DMSO, rt; (c) $NH_2NH_2$, EtOH, reflux (37%, two steps); (d) LAH, THF, rt (60%).

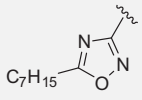
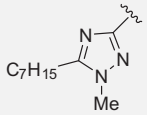
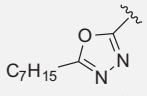
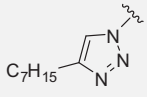
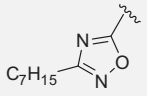
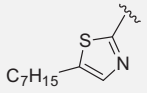
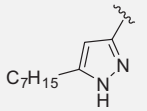
The ester was transformed to the oxadiazole-containing intermediate **15** under standard conditions[10] followed by conversion of the alcohol functionality to the amine (Scheme 5). As shown in Scheme 6, triazole **18** was prepared from iodide **17** utilizing a procedure described by Liang and co-workers[11] followed by nitrile reduction. The synthesis of oxadiazole **22** (Scheme 7) was carried out in a manner similar to the procedure described in Scheme 3. Thiazole **26** was prepared using standard Hantzsch chemistry[12] followed by a two-step cyanation/reduction protocol (Scheme 8). Finally, the pyrazole **29** was prepared via the multistep procedure described in Scheme 9. 4-Cyanobenzaldehyde (**27**) was condensed with the lithium anion of 1-nonyne followed by oxidation of the resulting alcohol with IBX to provide an acetylenic ketone. Reaction of this intermediate with hydrazine in ethanol provided pyrazolylbenzonitrile **28**.[13] Reduction of **28** afforded the desired amine **29**.

Modifying the nature of the heterocyclic group in the lipophilic sidechain has a dramatic impact on the observed $IC_{50}$ values of SK1 inhibition (Table 2).[14] Oxadiazole **33** and thiazole **34** have activity comparable to **7** while the other analogs are much weaker inhibitors. For the most part, the solubility of these compounds remains good, with the exception of **34**. Given the high *c* log *P* value for this derivative, this result is not surprising. Based on these findings, we decided that additional SAR studies would focus on further modification of **7**.

We suspected that both the alkyl chain of the oxadiazole and the unsubstituted benzylic position adjacent to the amide might be sites subject to metabolic degradation. Therefore, we set out to modify these two areas using the synthetic methods outlined in Scheme 3 and 10. The chiral oxadiazolylbenzyl amines **XII** used to synthesize compounds **47**–**54** (see Table 3) were prepared as shown in Scheme 10. The starting chiral amines (**IX**, R′ = Me, Et) were purchased from commercial sources. For the case where R′ = *i*Pr, the amine was prepared as described by Chan et al.[15] Protection of **IX** with trifluoroacetic anhydride followed by iodination and palladium-mediated cyanation afforded the corresponding nitriles **XI**. The nitriles were transformed to oxadiazoles via reaction with hydroxylamine followed by condensation with the appropri-

**Table 2**
SKI inhibitory activity of heterocycle-containing *N*-benzyl-pyrrolidine-2-carboxamides



| Compound | R | $IC_{50}$[a] (μM) | Sol.[b] (μg/mL) | *c* log *P* |
|---|---|---|---|---|
| **7** |  | 0.068 | 26.5 | 3.0 |
| **30** |  | 0.61 | >52 | 2.8 |
| **31** |  | 2.2 | >52 | 2.1 |
| **32** |  | 1.1 | 14 | 3.1 |
| **33** |  | 0.061 | 29.0 | 3.0 |
| **34** |  | 0.07 | <1.0 | 4.2 |
| **35** |  | 1.1 | 15 | 3.7 |

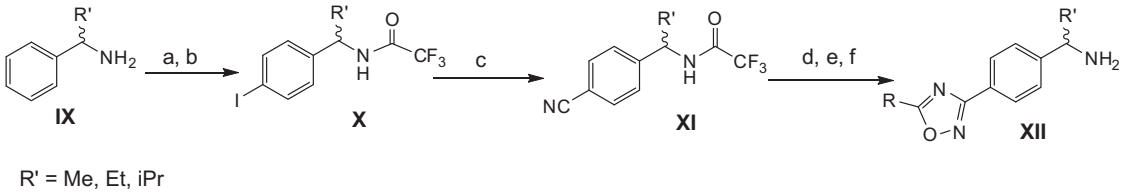
[a] Concentration of the testing compounds to inhibit 50% activity of the enzyme.[14]
[b] Solubility measurement determined from DMSO stock at pH 7.4 in phosphate buffered saline, recorded as μg/mL.

ate acid chloride. Removal of the amine protecting group under basic conditions afforded **XII**.

It is clear from the data presented in Table 3 that the straight alkyl chain found in **7** can be shortened by one carbon atoms without impacting activity (**37**). Further reductions to the chain length resulted in significant loss of potency (**38**). Replacing the alkyl chain with a carbocycle also resulted in decreased activity versus SK1 (**39**–**42**). Inserting a two-carbon alkyl linker between the oxadiazole ring and the carbocyclic moiety provided compounds with potency equivalent to or better than **7** and improved solubility (**43** and **45**). Interestingly, small substituents (i.e., methyl, ethyl) at the benzylic position adjacent to the amide were tolerated with the inhibitory activity associated with only the (*S*)-isomer (cf. **47**–**48**). Incorporation of a larger substituent (*iso*-propyl) resulted in a loss of potency (**50**). The most potent SK1 inhibitors obtained from the studies were **53** and **54**. It is worthy to note that all the compounds listed in Tables 3 and 4, including **53** and **54**, are not inhibiting SK2 at concentration of 10 μM.

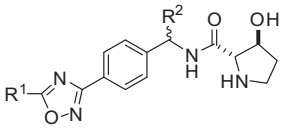
A number of these compounds were subjected to in vitro ADME profiling and some results are summarized in the Table4. Compound **36** contains an *n*-heptyl chain and demonstrated high intrinsic clearance in both rat and human liver microsomes. Compounds that possess a cyclopentylethyl or cyclohexylethyl off the oxadiazole ring and a small alkyl group at the benzylic position (i.e., **51**, **53**, and **54**) performed better in these assays and demonstrated significant improvement in the intrinsic clearance, particularly against human liver microsomes. Based on this data, **51** and **54** were selected for rat PK studies. Both compounds demonstrated modest in oral bioavailability with acceptable half-lives in blood circulation (Table 5).

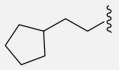
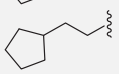
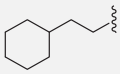
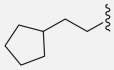
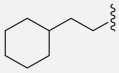
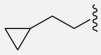
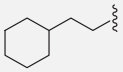
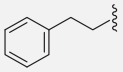
In conclusion, we have discovered a series of novel and potent sphingosine kinase-1 inhibitors based on an *N*-(5-alkyloxadiazol-3-yl)benzyl)-3-hydroxypyrrolidine-2-carboxamide scaffold. Optimization of the initial active hits resulted in the identification of compounds suitable for lead optimization. Further studies to elucidate the biological impact of these SK1 inhibitors in vivo are underway.




**Scheme 10.** Reagents and conditions: (a) TFAA, $Et_3N$, $CH_2CH_2$, 0 °C; (b) $I_2$, $PhI(O_2CCF_3)_2$, $CH_2Cl_2$, rt (42–48%, two steps); (c) NaCN, $Pd(Ph_3P)_4$, CuI, $CH_3CN$, reflux (74–92%); (d) $NH_2OH$–HCl, $NaHCO_3$, MeOH, reflux; (e) RCOCl, py, reflux; (f) LiOH–$H_2O$, aq THF (47–74%, three steps).

**Table 3**
SKI inhibitory activity of 3-hydroxy-*N*-(4-(oxadiazol-3-yl)benzyl)pyrrolidine-2-carboxamides containing modifications to the oxadiazole substituent and/or benzylic position



| Compound | $R^1$ | $R^2$ | $IC_{50}$[a] (μM) | Sol.[b] (μg/mL) | Compound | $R^1$ | $R^2$ | $IC_{50}$[a] (μM) | Sol.[b] (μg/mL) |
|---|---|---|---|---|---|---|---|---|---|
| **7** | $C_7H_{15}$– | H | 0.068 | 26.5 | **47** | $C_7H_{15}$– | Me (R) | 3.8 | 27 |
| **37** | $C_6H_{13}$– | H | 0.049 | >50 | **48** | $C_7H_{15}$– | Me (S) | 0.048 | 27 |
| **38** | $C_5H_{11}$– | H | 0.43 | >50 | **49** | $C_7H_{15}$– | Et (S) | 0.030 | 9 |
| **39** |  | H | 0.38 | >50 | **50** | $C_7H_{15}$– | *i*Pr (S) | 0.11 | 3 |
| **40** |  | H | 0.21 | >50 | **51** |  | Me (S) | 0.058 | >50 |
| **41** |  | H | 2.6 | >50 | **52** |  | Et (S) | 0.056 | 36 |
| **42** |  | H | 0.62 | >50 | **53** |  | Me (S) | 0.020 | 29 |
| **43** |  | H | 0.068 | >50 | **54** |  | Et (S) | 0.010 | 13 |
| **44** |  | H | 5.0 | >50 | | | | | |
| **45** |  | H | 0.025 | 26 | | | | | |
| **46** |  | H | 0.72 | >50 | | | | | |

[a] Concentration of the testing compounds to inhibit 50% activity of the enzyme.[14]
[b] Solubility measurement determined from DMSO stock at pH 7.4 in phosphate buffered saline, recorded as μg/mL.

**Table 4**
In vitro ADME data for selected SK1 inhibitors

| Compound | Sol., pH 7.4[a] | PAMPA pH 6.5–7.4[b] | *c* log *P*[c] | log *D*[d] | Microsomes rat $CL_{int}$[e] | Microsomes human $CL_{int}$[f] | 1A2 $IC_{50}$[g] (μM) | 2C9 $IC_{50}$[g] (μM) | 2C19 $IC_{50}$[g] (μM) | 2D6 $IC_{50}$[g] (μM) | 3A4 $IC_{50}$[g] (μM) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **7** | 27 | 46 | 3.0 | 3.7 | 60 | 45 | >5.0 | >5.0 | >5.0 | >5.0 | >5.0 |
| **51** | >74 | 49 | 2.7 | 2.9 | 49.5 | 12 | >5.0 | >5.0 | 1.4 | >5.0 | >5.0 |
| **53** | >75 | 58 | 3.3 | 3.6 | 50.2 | <4.5 | >5.0 | >5.0 | >5.0 | >5.0 | 4.3 |
| **54** | 13 | 44 | 3.8 | 4.3 | 38.8 | 10 | >5.0 | >5.0 | 1.2 | >5.0 | N/A[h] |

[a] Solubility measurement determined from DMSO stock at pH 7.4 in phosphate buffered saline, recorded as μg/mL.
[b] PAMPA (parallel artificial membrane permeability assay), pION Inc. Double Sink Method using donor (pH 6.5) and acceptor (pH 7.4 sink buffer) reported as 10–6 cm/s.
[c] *c* log *P* calculated log *P* (octanol–water partition coefficient).
[d] log *D* distribution coefficient measured at pH 7.4.
[e] Metabolic stability in rat liver microsomes. Intrinsic clearance reported as μL/min/mg protein.
[f] Metabolic stability in human liver microsomes. Intrinsic clearance reported as μL/min/mg protein.
[g] Cytochrome P450 inhibition using cDNA expressed isozymes and fluorescent probe substrates, reported as μM.
[h] Data not available.

**Table 5**
Rat PK data for selected SK1 inhibitors

| Compound | Dose | AUC[a] (ng h/mL) | $t_{1/2}$[b] (h) | $C_{max}$[c] (ng/mL) | CL[d] (mL/min/kg) | $V_{dss}$[e] (mL/kg) | $T_{max}$[f] (h) | *F*[g] (%) |
|---|---|---|---|---|---|---|---|---|
| **54** | 1 mg/kg (iv) | 204 | 1.64 | | 81.8 | 7860 | | |
| **54** | 3 mg/kg (po) | 94.8 | 1.64 | 37.5 | | | 0.75 | 15.3 |
| **51** | 1 mg/kg (iv) | 439 | 4.30 | | 37.1 | 5550 | | |
| **51** | 3 mg/kg (po) | 232 | 7.55 | 82.5 | | | 0.33 | 17.6 |

[a] Area under the plasma concentration versus time curve after drug administration.
[b] Half-life (time required for the plasma concentration to be reduced by 50%).
[c] Maximum plasma concentration observed after drug administration.
[d] Clearance (the volume of plasma from which the drug is completely removed per unit time).
[e] Volume of distribution at steady state.
[f] Time required to reach maximum concentration.
[g] Oral bioavailability.

## References and notes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