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Part II. Development of novel colchicine-derived immunosuppressants with improved pharmacokinetic properties

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ABSTRACT

We have developed a new series of immunosuppressant with improved pharmacokinetic properties as the second-generation of colchicine analogs, which were designed based on the privileged structure derived from our previous work. In particular, we identified an analog (**14**), which exhibited a potent in vitro activity (IC_{50}: 5 nM) in MLR and excellent in vivo efficacy in the Zymosan A-induced arthritis model, in the Carrageenan-induced edema model and in the local lymph node assay (LLNA). Analog **14** also revealed a good oral bioavailability (*F*: 67.3%) in BALB/c mice.

Since the first organ-transplantation between human twins was successfully performed in 1954,[1] a large number of patients have received organ-transplants every year. However, they should be consistently treated with the immunosuppressive drugs to overcome rejection by their own immune system.[2] Generally, rejections caused by organ-transplantation and autoimmune diseases including rheumatoid arthritis, psoriasis, autoimmune pancreatitis and multiple sclerosis are mainly mediated by T cells with an assistance of B cells affecting the diseases through antibody production.[3] The T cell-mediated immune diseases are treated with a variety of therapies including medication of cyclosporine A (CsA)[4,5] and FK506.[6,7] Antibody therapy reduces a specific group of cells such as T cells or B cells and alter signal transduction pathways using effector cells.[8,9] CsA as one of the best immunosuppressant acts on calcineurin and selectively induces an inhibition of T cell proliferations.[10] Despite a great deal of therapeutic applications of immunosuppressant for organ-transplantation and autoimmune diseases, there still remains a need for more effective and less toxic immunosuppressant. Especially, many current immunosuppressive drugs revealed serious side effects such as renal toxicity, neurotoxicity and hypertension.[11–13] In addition, the most widely prescribed immunosuppressive drugs containing CsA or FK506 are not orally available due to high molecular weight and lipophilicity. Thus, a strong demand for the safe and orally available immunosuppressant for organ-transplantation and treatment of autoimmune diseases still remains.

Recently, we reported a colchicine-derived compound (**2**, Fig. 1), which exhibited strong immunosuppressive activities in the in vitro and in vivo models.[14] However, this lead compound turned out to have poor pharmacokinetic profiles including seriously poor bioavailability. The poor pharmacokinetic properties were likely due to the extremely low solubility in water. Thus, increase of aqueous solubility was considered for the improved oral bioavailability. The metabolically labile nitrate group seemed also to affect the bioavailability. We herein describe our recent effort to discover the second-generation of colchicine analogs that have the improved pharmacokinetic properties with maintaining the excellent immunosuppressive activity.

As shown in our preliminary work for the improved pharmacokinetics (Table 1), analog **4** possessing the thiomethoxy (R_1) and *N*-4-(nitrooxy)butanoyl (R_2) groups exhibited a threefold lower solubility in the media containing 20% of serum and a 10-fold higher activity compared to colchicine possessing the methoxy (R_1) and *N*-acetyl (R_2) groups. Analog **5** possessing the thiomethoxy (R_1) and 3-(nitrooxymethyl)benzoyl (R_2) groups showed a lower solubility and a higher activity compared to analog **4**. Thus, we assumed that the lower solubility of **5**, compared to those of colchicine and **4**, was due to a stacking effect of aryl moiety in the *N*-side chain of **5**. Based on our previous structure–activity relationship and the present structure–solubility relationship shown in Table 1, we designed the new analogs for optimization of the *N*-side chain of **2** as described in Figure 2. Our strategy

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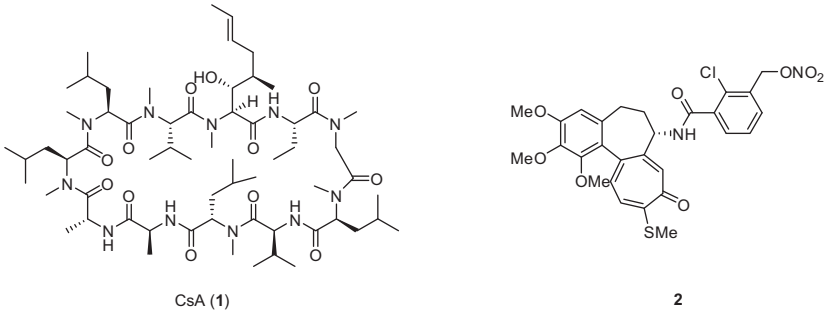
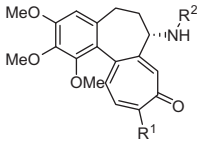


Figure 1. Structures of cyclosporine A (CsA, **1**) and **2**.

Table 1
Structure–solubility relationship of the previously reported colchicine derivatives



3, colchicine, R^1 = OMe, R_2 = acetyl
4, R^1 = SMe, R^2 = 4-(nitrooxy)butanoyl
5, R^1 =SMe, R^2 = *3*-(nitrooxymehyl)benzoyl

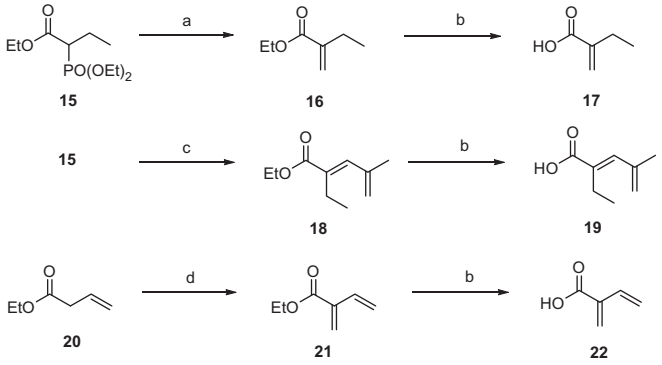
Compound	Solubility[a] (μM)	Activity[b] (IC_{50}, nM)
3, Colchicine	300	2300
4	100	210
5	10	68
2	—[c]	8

[a] Solubility of each compound was determined by evaluating both turbidity and light scattering using a UV/visible spectrophotometer and a Nepheloskan instrument. Solubility was assessed at 37 °C in the dosing media, which contained 20% serum.

[b] MLR assay. IC_{50} values are mean of three experiments, standard deviation below ±20%.

[c] Solubility of **2** was not determined.

involving incorporation of an aliphatic side chain, which consisted of alkenes or three-membered ring systems with sp^2 or relevant hybridizations, envisaged elevations of both solubility and activity. However, our initial analogs possessing the five- or six-membered heterocycles including pyrrole, thiophene, pyridine and pyrimidine instead of the 2-chlorobenzene moiety exhibited poor immunosuppressive activities (IC_{50}: >500 nM, data not shown). Thus, we



Scheme 2. Reagents and conditions: (a) $(CH_2O)_n$, NaH, DMF, rt, 66%; (b) NaOH, MeOH, H_2O, rt, **17**: 83%, **19**: 78%, **22**: 69%; (c) methacrolein, NaH, DMSO, rt, 91%; (d) $(CH_2O)_n$, NaH, DMF, rt, 91%.

designed alternative analogs avoiding the nitrate group, which is metabolically labile[15,16] because analog **2** devoid of the nitrate group exhibited an excellent immunosuppressive activity (IC_{50}: 22 nM).

As depicted in Scheme 1, most of the designed compounds were prepared by the EDC-mediated amide coupling reactions of 7-deacetylthiocolchicine **6** with various aliphatic carboxylic acids.[14] Some aliphatic carboxylic acids were synthesized from triethyl 2-phosphonobutyrate **15** or 3-butenoic acid **20** employing

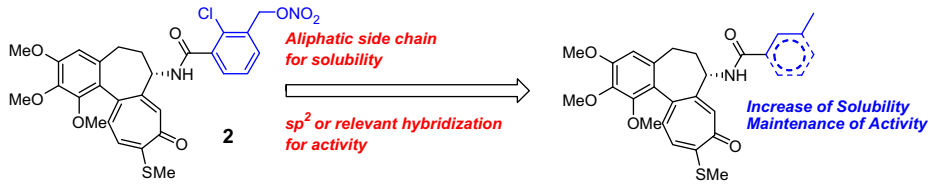
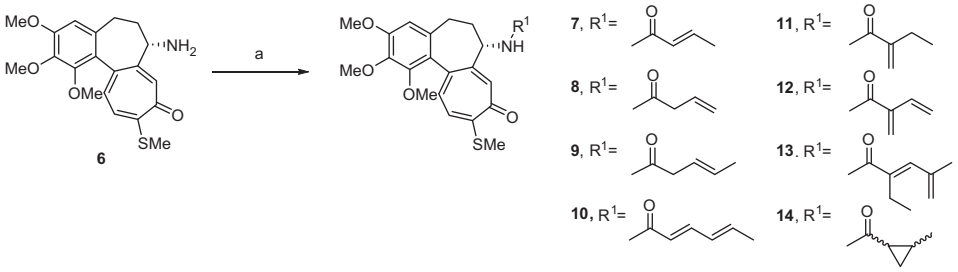
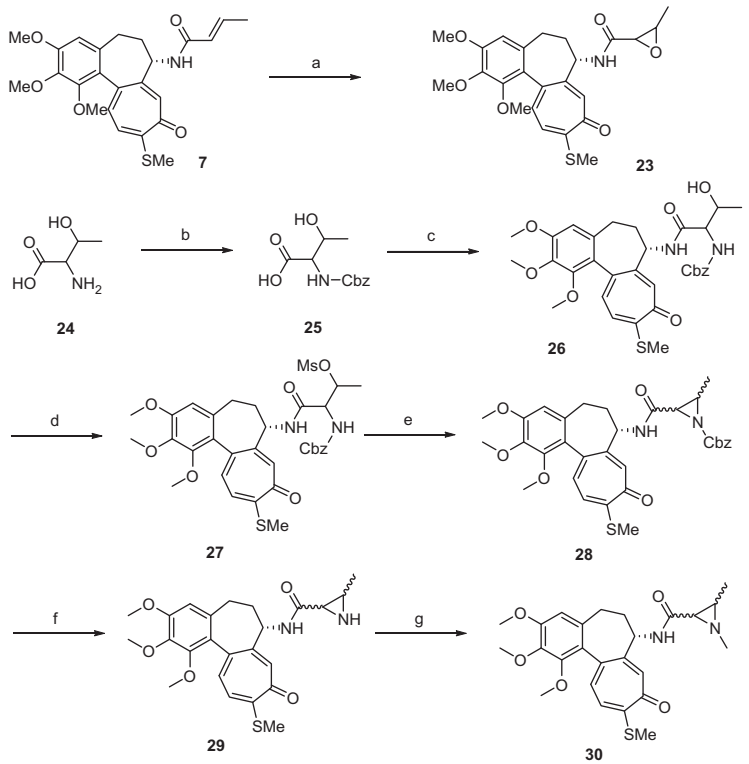


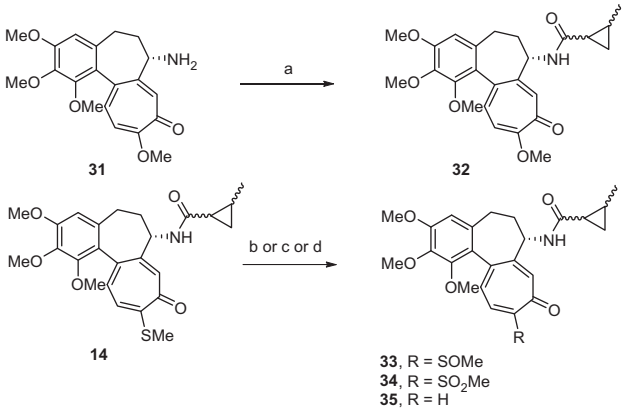
Figure 2. Strategy for design of the pharmacokinetically improved analogs.



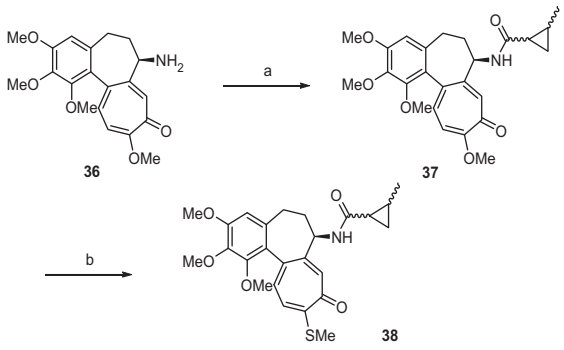
Scheme 1. Reagents and conditions: (a) carboxylic acids, EDC, CH_2Cl_2, rt, **7**: 92%,**8**: 96%, **9**: 78%, **10**: 62%, **11**: 63%, **12**: 53%, **13**: 71%, **14**: 98%.



Scheme 3. Reaction conditions and reagents: (a) *m*CPBA, CH_2Cl_2, rt, 82%; (b) CbzCl, $NaHCO_3$, THF, water, rt, 93%; (c) **6**, EEDQ, Et_3N, CH_3CN, 48%; (d) MsCl, Et_3N, CH_2Cl_2, rt, quant.; (e) *t*-BuOK, *t*-BuOH, CH_2Cl_2, 0 °C to rt, 38%; (f) KOH, THF, water, rt, 9%; (g) MeI, K_2CO_3, MeOH, 50 °C, 65%. EEDQ = *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline.

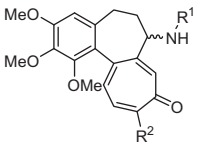


Scheme 4. Reagents and conditions: (a) 2-methylcyclopropanecarboxylic acid, EDC, CH_2Cl_2, rt, 71%; (b) R = SOMe:*m*CPBA (1.05 equiv), CH_2Cl_2, rt, 82%; (c) R = SO_2Me:*m*CPBA (2.5 equiv), CH_2Cl_2, rt, 84%; (d) R = H, (i) Raney Ni, EtOH, rt, 70%, (ii) Pd/C, toluene, reflux, 31%.



Scheme 5. Reagents and conditions: (a) 2-methylcyclopropanecarboxylic acid, EDC, CH_2Cl_2, rt, 86%; (b) NaSMe, THF, H_2O, 50 °C, 59%.

Table 2
Immunosuppressive activities of the prepared analogs in MLR



Compound[a]	R^1	R^2	IC_{50}[b] (nM)
CsA	—	—	30
2	2-Chloro-3-nitrooxy methyl benzoyl	SMe	8
7	2-Butenoyl	SMe	67
8	3-Butenoyl	SMe	702
9	3-Pentenoyl	SMe	1670
10	2,4-Hexadienoyl	SMe	4
11	2-Methylenebutanoyl	SMe	86
12	2-Methylene-3-butenoyl	SMe	112
13	2-Ethyl-4-methyl penta-2,4-dienoyl	SMe	31
14	2-Methylcyclopropane Carbonyl	SMe	5
23	3-Methyloxirane-2-carbonyl	SMe	119
29	3-Methylaziridine-2-carbonyl	SMe	46
30	1,3-Dimethylaziridine-2-carbonyl	SMe	38
32	2-Methylcyclopropane Carbonyl	OMe	1247
33	2-Methylcyclopropane Carbonyl	SOMe	N.A.
34	2-Methylcyclopropane Carbonyl	SO_2Me	N.A.
35	2-Methylcyclopropane Carbonyl	H	N.A.
38	2-Methylcyclopropane Carbonyl	SMe	N.A.

[a] All compounds were purified by column chromatography and then recrystallization (>95%).
[b] IC_{50} values are mean of three experiments, standard deviation below ±20%.

Table 3
Lymphoproliferation assay on T and B lymphocyte-activated cells

Compound	B cells (IC_{50}, ng/mL)[a]	T cells (IC_{50}, ng/mL)[a]
10	5.7	40.7
14	5.9	31.0

[a] IC_{50} values are mean of three experiments, standard deviation below ±20%.

Horner–Wadsworth–Emmons olefination or aldol condensation, followed by hydrolysis of the resulting ester as shown in Scheme 2. Synthesis of the analogs possessing the 3-membered heterocycles such as epoxide and aziridine are outlined in Scheme 3. Epoxidation of **7** with *m*CPBA produced epoxide **23** as a diastereomeric mixture in good yield. Cbz-protection of L-threonine followed by amide coupling reaction in the presence of EEDQ and Et_3N afforded **26**,[17] which was subsequently reacted with methanesulfonyl chloride to give **27**. Intramolecular cyclization of the Cbz-protected amine **27** in the presence of *t*-BuOK produced aziridine **28**, which was subjected to Cbz-deprotection and methylation to give **30**. We also introduced methoxy, sulfinyl and sulfonyl group instead of the thiomethoxy group of **2** because the sulfide group was likely to be easily oxidized via the phase I metabolism.[18] As shown in Scheme 4, oxidation of **14** using *m*CPBA produced the sulfoxide analog**33** and the sulfonyl analog **34**, respectively. Analog **35** devoid of the thiomethoxy group was prepared via a sequence of Raney Ni-mediated reduction and subsequent aromatization in the presence of Pd/C.[19] Finally, analog **38**, which lately turned out to be the best analog in terms of in vitro and in vivo activities, was prepared from **36**, an antipode of **31**, for analysis of the stereochemical effects of the *N*-side chain of **14**. Analog **38** was easily prepared from (+)-*N*-deacetylcolchicine **36**[20,21] by an EDC-mediated amide coupling reaction and NaSMe treatment as shown in Scheme 5.

Mixed-lymphocyte reaction (MLR) assay on splenocytes harvested from C57BL/6 and BALB/c mice was conducted to explore immunosuppressive activities of the prepared analogs as shown in Table 2.[22] Among the analogs with an alkene group, analog **10** possessing the 2,4-hexadienoyl substituent exhibited the most potent activity (4 nM), which is twofold more potent compared to the parent compound **2**. Generally, the analogs with a Michael acceptor such as **7**, **10**, **11** and **13**showed excellent immunosuppressive activities with IC_{50}s less than 100 nM. Interestingly, analogs **14**–**30** possessing the three-membered rings, of which sp^3-hybridization is known to have sp^2-characters,[23] revealed good or excellent immunosuppressive activities. In particular, analog **14** with a cyclopropane exhibited highly potent immunosuppressive activity with an IC_{50} of 5 nM. Replacement of the thiomethoxy substituent with methoxy, sulfinyl or sulfonyl group resulted in significant decrease (**32**) or loss (**33**, **34**) of activity as our previous report.[14] Removal of the thiomethoxy group (**35**) eliminated the activity, which supported a crucial role of the thiomethoxy group for immunosuppressive activity of the analogs. In addition, no immunosuppressive activity of the antipode (**38**) of **14** revealed the important stereochemical feature of the tested analogs.

Analogs **10** and **14**, which were most potent in MLR assay, were further evaluated using the lymphoproliferation assay on the B and T lymphocyte-activated cells.[24] Both analogs exhibited good suppressive activities against the activated B cells than T cells as shown Table 3. Analog **14** was finally selected because it exhibited higher potency in suppression of the activated T cells, which is important for treatment of the rejection in organ-transplantation and autoimmune diseases. Thus, we further evaluated analog **14** using the cell viability test on the cell lines including naive lymphocytes, activated lymphocytes, bone marrow-derived dendritic cells (BM-DC) and bone marrow-derived macrophages (BM-Mac). As shown in Figure 3, analog **14** exhibited potent inhibitory activity against activated lymphocytes while it did not affect

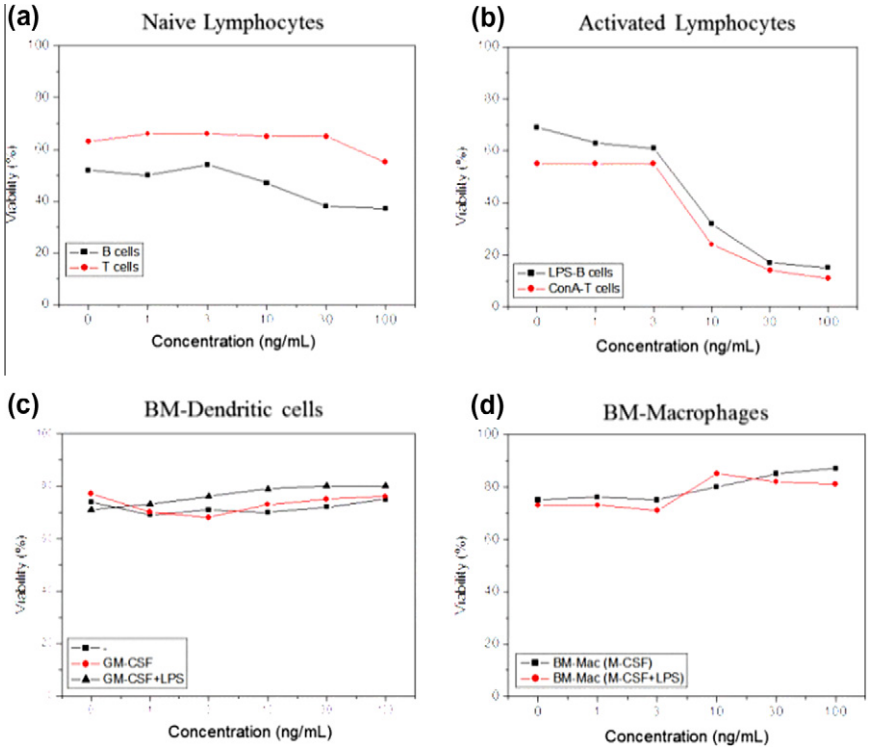


Figure 3. Cell viability of analog **14**. (a) Cell viability on naive lymphocytes, (b) cell viability on activated lymphocytes, (c) cell viability on bone marrow-derived dendritic cells, (d) cell viability on bone marrow-derived macrophages.

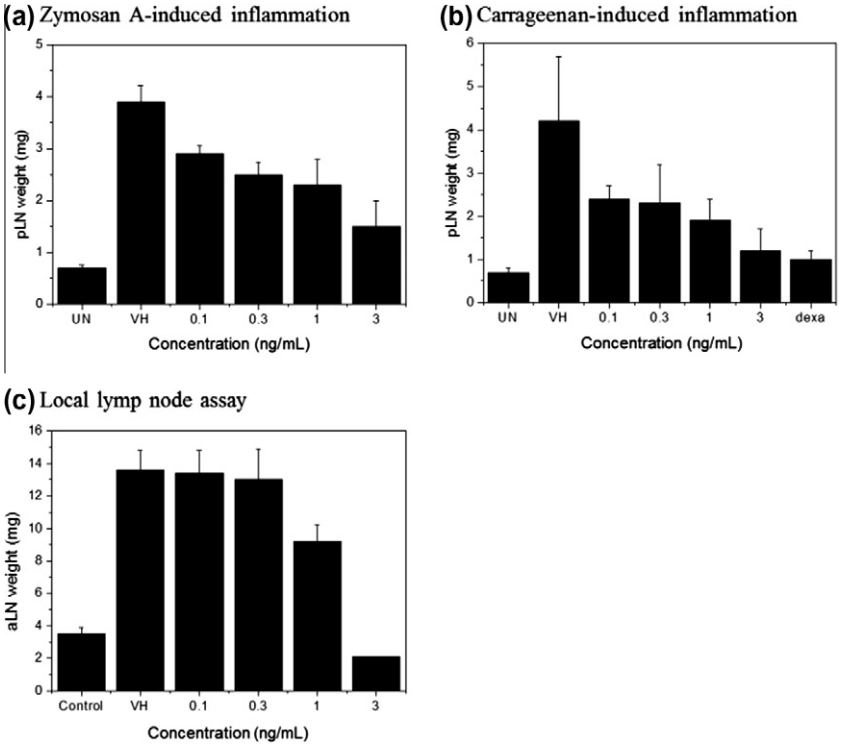


Figure 4. In vivo test of analog **14**. (a) Effect on Zymosan A-induced arthritis, (b) effect on Carrageenan-induced paw edema, (c) local lymph node assay (LLNA) induced by DNCB.

other cell lines, suggesting that analog **14** is much less toxic in normal cells.

In vivo immunosuppressive effect of **14**was evaluated using the arthritis, edema model and the local lymph node assay (LLNA). The C57BL/6 mice was treated with **14** after subcutaneous injection of Zymosan A (50 ml of 3 mg/ml) from *Saccharomyces cereviciae* (Sigma, St. Louis, MO) into the hind footpad.[25,26] As well, the C57BL/6 mice was treated with **14** after a paw edema was induced by a subcutaneous injection of 0.1 ml of 1% Carrageenan into the left hind paw.[25] Figure 4a and b illustrated that analog **14** reduced both arthritis and edema in a dose dependent manners. Considering that above in vivo models are mainly associated with activation of macrophages, we tested **14** using the in vivo local lymph node assay (LLNA), which envisioned an in vivo functional assay for immune reactivity to the T cell-dependent antigen like 2,4-dinitrochlorobenzene (DNCB).[27,28] Figure 4c also revealed that analog **14** effectively decreased the lymph node increased by DNCB. As mentioned in the introduction part, the final goal of our work focused on the discovery of immunosuppressive agents with improved pharmacokinetic profiles with the immunosuppressive activity maintained. Thus, we determined PK properties of analog **14**. As shown in Figure 5, analog **14** revealed systemic clearance (CL) with a half value of hepatic blood flow and a half-life ($t_{1/2}$) of 3 h, which seemed suitable for oral administration. Furthermore, analog **14** exhibited good oral bioavailability (*F*: 67.3%), which envisioned a good possibility for oral administration.

Parameter	Unit	IV	PO
Dose	(mg/kg)	0.5	1
T_{max}	(hr)	-	0.25
C_{max}	(ng/ml)	-	254.5
$AUC_{0\text{-}inf}$	(hr.ng/ml)	200.0	269.1
CL	(l/hr/kg)	2.50	-
V_{ss}	(l/kg)	1.60	-
$t_{1/2}$	(hr)	2.9	2.9
MRT_{inf}	(hr)	0.6	2.2
F	(%)	-	**67.3**

Notes : 1. Parameters were calculated by noncompartmental analysis using PK solutions 2.0 (Summit Research Services, Montrose, CO, USA). 2. Parameters were obtained using mean plasma-concentration time profiles of three (IV) or four (PO) animals per time points.

Figure 5. Pharmacokinetics of analog **14** in BALB/c mice.

In conclusion, we developed immunosuppressive agents with improved pharmacokinetic properties and strong immunosuppressive activity based on the structure–activity and structure–solubility relationships of **2**, which was identified as an immunosuppressive agent via our previous work. In particular, the alkene or three-membered ring-incorporated analogs **10** and **14** exhibited improved in vitro activities. In addition, analog **14** exhibited a good efficacy in both in vivo models mediated with T cell and macrophages. Analog **14** exhibited promising pharmacokinetic properties with bioavailability over 67%, which was significantly improved compared to the parent compound **2**. Currently, further studies on **14** including in vivo transplantation experiments are in progress.

Acknowledgement

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References and notes

1. Murray, J. E.; Merrill, J. P.; Harrison, J. H. *Ann. Surg.* **1958**, *148*, 343.
2. Smith, J. M.; Nemeth, T. L.; McDonald, R. A. *Pediatr. Clin. N. Am.* **2003**, *50*, 1283.
3. Duncan, M. D.; Wilkes, D. S. *Proc. Am. Thorac. Soc.* **2005**, *2*, 449.

4. Calne, R.; Rolles, K.; Thiru, S.; McMaster, P.; Craddock, G.; Aziz, S.; White, D.; Evans, D.; Dunn, D.; Henderson, R. *Lancet* **1979**, *314*, 1033.
5. Keown, P. A. *Drugs* **1990**, *40*, 315.
6. Kino, T.; Hatanaka, H.; Hashimoto, M.; Nishiyama, M.; Goto, T.; Okuhara, M.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot.* **1987**, *40*, 1249.
7. Liu, J.; Farmer, J. D., Jr.; Lane, W. S.; Friedman, J.; Schreiber, S. L. *Cell* **1991**, *66*, 807.
8. Klipa, D.; Mahmud, N.; Ahsan, N. *mABs* **2010**, *2*, 607.
9. Mahmud, N.; Klipa, D.; Ahsan, N. *mABs* **2010**, *2*, 148.
10. Handschumacher, R. E.; Harding, M. W.; Rice, J.; Drugge, R. J.; Speicher, D. W. *Science* **1984**, *226*, 544.
11. Smith, J. M.; Lajos Nemeth, T.; McDonald, R. A. *Pediatr. Clin. N. Am.* **2003**, *50*, 1283.
12. O'Shea, J. J.; Pesu, M.; Borie, D. C.; Changelian, P. S. *Nat. Rev. Drug Disc.* **2004**, *3*, 555.
13. Hong, J. C.; Kahan, B. D. *Semin. Nephol.* **2000**, *20*, 108.
14. Chang, D. J.; Yoon, E. Y.; Lee, G. B.; Kim, S. O.; Kim, W. J.; Kim, Y. M.; Jung, J. W.; An, H.; Suh, Y. G. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4416.
15. Fung, H. L. *Am. J. Cardiol.* **1987**, *60*, 4.
16. King, S.; Fung, H. *Drug Metab. Dispos.* **1984**, *12*, 353.
17. Belleau, B.; Martel, R. R.; Lacasse, G.; Menard, M.; Weinberg, N. L.; Perron, Y. G. *J. Am. Chem. Soc.* **1968**, *90*, 823.
18. Kerns, E. H.; Di, L. *Drug-like Properties: Concepts, Structure Design and Methods: from ADME to Toxicity Optimization*; Academic Pr, 2008.
19. Dumont, R.; Brossi, A.; Chignell, C. F.; Quinn, F. R.; Suffness, M. *J. Med. Chem.* **1987**, *30*, 732.
20. Dumont, R.; Brossi, A.; Silverton, J. V. *J. Org. Chem.* **1986**, *51*, 2515.
21. Shi, Q.; Verdier-Pinard, P.; Brossi, A.; Hamel, E.; Lee, K. H. *Bioorg. Med. Chem.* **1997**, *5*, 2277.
22. Bach, F. H.; Widmer, M. B.; Bach, M. L.; Klein, J. *J. Exp. Med.* **1972**, *136*, 1430.
23. Walborsky, H. M.; Motes, J. M. *J. Am. Chem. Soc.* **1970**, *92*, 2445.
24. Lillehoj, H. S. *Infect. Immun.* **1987**, *55*, 1616.
25. Calhoun, W.; Chang, J.; Carlson, R. *Inflamm. Res.* **1987**, *21*, 306.
26. Rocha, F. A. C.; Aragão, A. G. M., Jr.; Oliveira, R. C.; Pompeu, M. M. L.; Vale, M. R.; Ribeiro, R. A. *Inflamm. Res.* **1999**, *48*, 485.
27. Kimber, I.; Dearman, R. J.; Basketter, D. A.; Ryan, C. A.; Gerberick, G. F. *Contact Dermatitis* **2002**, *47*, 315.
28. van den Berg, F. A.; Baken, K. A.; Vermeulen, J. P.; Gremmer, E. R.; van Steeg, H.; van Loveren, H. *Toxicology* **2005**, *211*, 107.