Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 2000–2005
Bioorganic & Medicinal Chemistry Letters

Reduced cardiac side-effect potential by introduction of polar groups: Discovery of NIBR-1282, an orally bioavailable CCR5 antagonist which is active in vivo

Gebhard Thoma,* Christian Beerli, Marc Bigaud, Christian Bruns, Nigel G. Cooke, Markus B. Streiff and Hans-Guenter Zerwes

Novartis Institutes for BioMedical Research, Forum 1, Novartis Campus, CH-4002 Basel, Switzerland

Received 19 December 2007; revised 26 January 2008; accepted 29 January 2008
Available online 2 February 2008

Abstract—Introduction of polar groups in a series of potent CCR5 antagonists which are very likely to adversely affect the conduction system in the heart led to the identification of **NIBR-1282** which did not show adverse effects when tested in an isolated rabbit heart ex vivo model. Administration of **NIBR-1282** in combination with a non-efficacious dose of CsA led to significant prolongation of kidney allograft survival in cynomolgus monkeys.

The chemokine receptor CCR5 interacts with several high affinity ligands (i.e., RANTES, MIP-1α, MIP-1β, MCP-2) and plays an important role in inflammatory and autoimmune disorders by affecting the trafficking of effector T cells and monocytes to diseased tissues.[1] In addition, CCR5 can function as a co-receptor for macrophage-infecting strains of HIV. A mutation in the human CCR5 gene (i.e., the CCR5Δ32 mutation) which leads to CCR5 deficiency in homozygous carriers confers protection against HIV infection without being associated with increased morbidity.[2,3] Furthermore, it has been demonstrated that renal transplant recipients homozygous for CCR5Δ32 show significantly prolonged graft survival compared to CCR5 wild-type or heterozygous individuals.[4,5] As all patients have received full immunosuppressive treatment this finding indicates that a CCR5 inhibitor on top of standard medication may result in an additional beneficial effect.

Recently, we have reported on the SAR of a new series of highly potent and selective competitive CCR5 antagonists.[6] While all compounds are inactive on both rat and murine CCR5, this series includes examples, in contrast to CCR5 inhibitors from other series,[7] which cross-react with the cynomolgus monkey (cyno) receptor. Compound **1** (Table 1) had good PK properties in cynos and an overall favorable profile making it a potential candidate for in vivo profiling in transplantation and other disease models. However, to our disappointment we found that most of these compounds even at concentrations below 1 μM showed adverse cardiac side-effects (such as action potential duration prolongation, triangulation, reverse use dependence, and instability) in an ex vivo isolated rabbit heart model (SCREENIT).[8] Antagonist **1** was safe up to 1.5 μM but this was considered insufficient for a development candidate in man. Therefore, we decided not to evaluate compound **1** further in cynos but rather to improve the cardiac safety profile.[9]

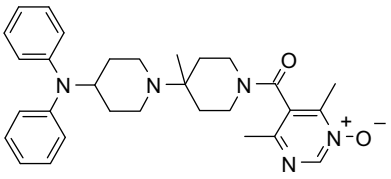
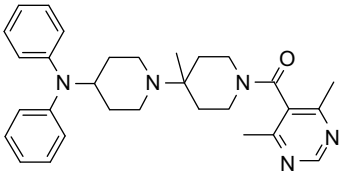
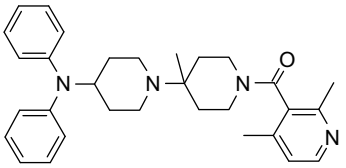
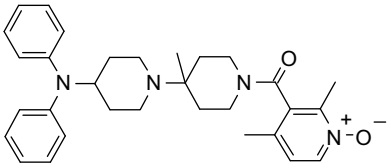
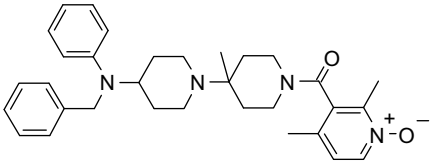
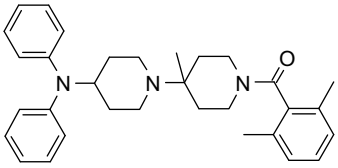
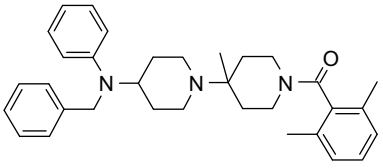
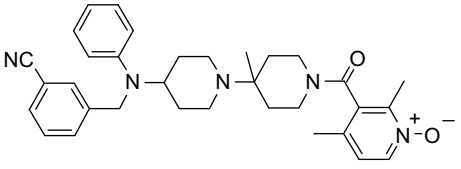
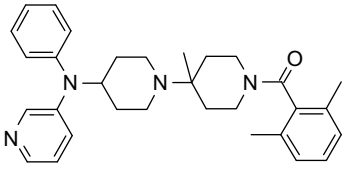
Here we disclose **NIBR-1282** (=**18**), a selective, competitive CCR5 antagonist with good oral bioavailability in cynos and dogs, which did not show any potential for cardiac side-effects even at 10 μM. The compound was discovered by modifying the distribution of polar groups within our series. In combination with a subtherapeutic dose of cyclosporine A it was efficacious in a life-supporting kidney transplantation model in cynos.

Several representatives of our series (**1**–**8**) were tested in the SCREENIT model (Table 1). With the exception of **1**, all compounds with polar groups in the right-hand portion but lacking polarity in the left-hand portion

Keywords: Chemokines; Chemokine receptors; CCR5; Cardiac side-effects; hERG.
* Corresponding author. Tel.: +41 61 3243342; fax: +41 61 3246735; e-mail: gebhard.thoma@novartis.com

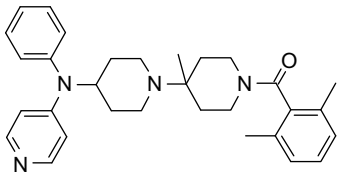
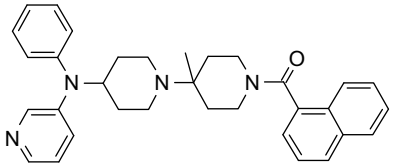
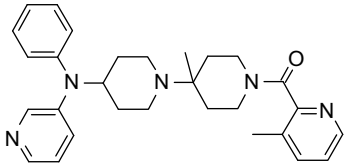
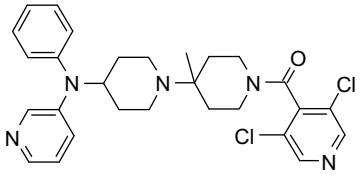
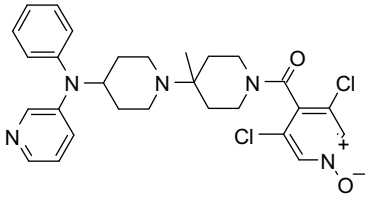
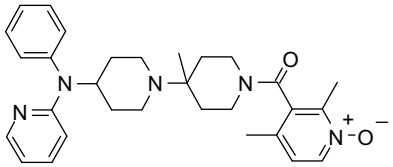
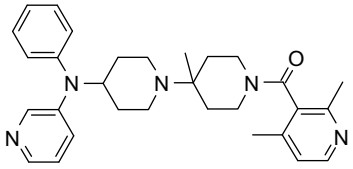
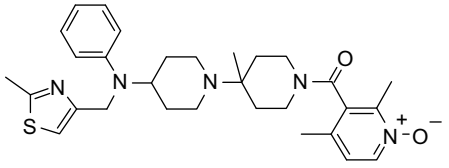
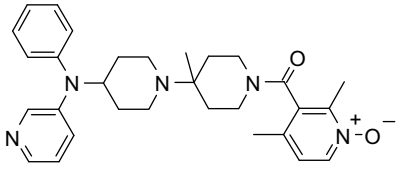
0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.01.108

Table 1. CCR5 antagonists

Compound		Binding (CCR5) hu/cy IC_{50}[a] [nM]	Rabbit heart model safe up to[b] [μM]	Binding (hERG)[c] IC_{50} [μM]	clog *D*
1		2.9/12	1.5	1.2	2.3
2		1.0/2.2	<0.5	n.t.	2.9
3		2.3/1.1	<0.5	0.57	3.8
4		3.3/3.4	<0.5	n.t.	2.5
5		2.0/5.1	<0.5	0.55	2.4
6		2.6/0.7	1.0	0.15	4.9
7		1.4/3.0	1.5	0.67	4.8
8		0.6/2.8	1.0	1.3	1.9
9		0.5/1.1	<0.5	n.t.	4.5

(*continued on next page*)

Table 1 (*continued*)

Compound		Binding (CCR5) hu/cy IC_{50}[a] [nM]	Rabbit heart model safe up to[b] [μM]	Binding (hERG)[c] IC_{50} [μM]	clog *D*
10		2.0/9.8	<0.5	n.t.	4.5
11		1.3/3.6	1.0	n.t.	4.2
12		6.5/39	<0.5	0.41	2.4
13		0.8/3.2	<0.5	2.0	3.5
14		1.0/4.2	1.0	4.4	2.3
15		1.8/12	1.0	n.t.	1.7
16		0.9/0.8	1.0	>30	3.0
17		1.7/107	5.0	12.2	1.4
18		5.1/8.1	>10	>30	1.7

[a] Average of at least three independent measurements; for details on the assays see Ref. 7.

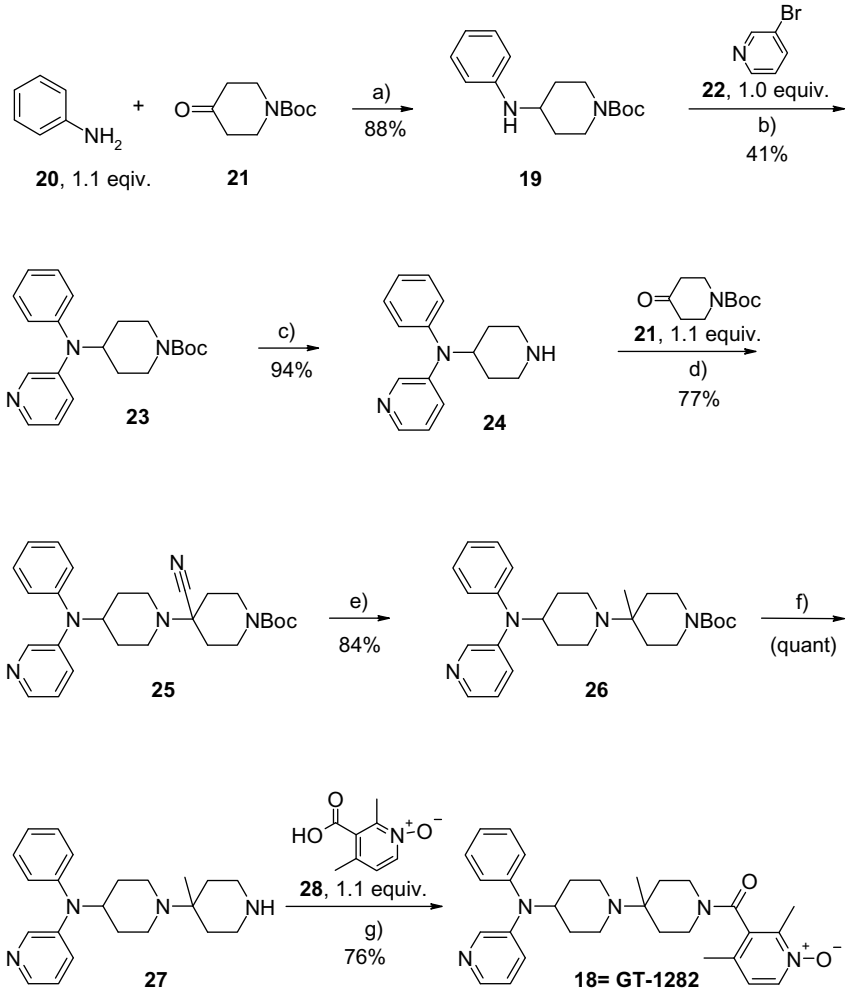
[b] See Ref. 8 for details on the SCREENIT model which measures adverse cardiac side-effects such as action potential duration prolongation, triangulation, reverse use dependence and instability.

[c] Test compounds compete for [^{3}H] dofetilide binding to a preparation of HEK-293 cell membranes stably transfected with hERG channels.

(**2**–**5**) showed adverse effects below 0.5 μM whereas compounds **6** and **7** without any polar groups appeared to be superior. Compound **8** with polar groups in both portions also showed an improved profile. To further explore the effects of polarity and distribution of polar groups on the cardiac side-effect potential, we prepared highly potent antagonists (**9**–**18**, Table 1) according to the previously established procedures,[6] and tested them in the SCREENIT model. Compounds **9**–**11** with polar groups in the left-hand portion but lacking polarity in the right-hand portion showed adverse effects at low concentrations mirroring the properties of compounds **2**–**5** with a polar group only in the right-hand portion. Compounds **12**–**18** with polar groups in both the left- and right-hand portion gave mixed but best results. Two representatives (**12**, **13**) showed adverse effects below 0.5 μM whereas compounds **14**–**16** were safe up to 1 μM. Antagonist **17** was safe up to 5 μM but showed unsatisfying cross-reactivity with cyno CCR5. Compound **18** (**NIBR-1282**) was safe even at 10 μM and was selected for further profiling both in vitro and in vivo. Most compounds were also tested in a biochemical assay measuring competitive inhibition of [^{3}H] dofetilide binding to a preparation of HEK-293 cell membranes stably transfected with hERG channels. As the SCREENIT model is not solely dependent on hERG inhibition, it is not unexpected that some compounds showed a poor correlation between ex vivo model and hERG binding assay (e.g., **6**, **13**, **16**).[10]

NIBR-1282 was prepared in seven steps in an overall yield of 16.6% (Scheme 1). Intermediate **19** was obtained from aniline (**20**) and the protected piperidone **21**. Aminoheteroarylation with bromopyridine **22** gave **23**, which was deprotected (→ **24**), reacted with **21** in the presence of Ti(O-*i*-Pr)$_4$ and, subsequently, with Et$_2$AlCN to give **25**. Treatment with CH$_3$MgBr gave **26** which upon removal of the protecting group gave **27**. Condensation of **27** with **28** yielded the final compound **18** = **NIBR-1282**.[11]

NIBR-1282 was tested in a series of CCR5-dependent in vitro assays (see Table 2). It was highly potent in radioligand binding assays using membranes from



Scheme 1. Reagents and conditions: (a) NaBH(OAc)$_3$, AcOH (CH$_2$Cl)$_2$, 25 °C, 16 h; (b) Pd(OAc)$_2$ (3 mol%), xantphos (4.5 mol%), NaO*t*-Bu (4 equiv), toluene, 110 °C, 16 h; (c) TFA/CH$_2$Cl$_2$/H$_2$O (8:5:1), 25 °C, 2 h; (d) Ti(O-*i*-Pr)$_4$ (1.1 equiv) (CH$_2$Cl)$_2$, 25 °C, 16 h, then Et$_2$AlCN (2.2 equiv 1.0 M solution in toluene), 25 °C, 4 h; (e) CH$_3$MgBr (5.0 equiv 3.0 M solution in ether), THF; (f) TFA/CH$_2$Cl$_2$/H$_2$O (8:5:1), 25 °C, 2 h, HBTU (1.1 equiv), DMF/EtN(*i*-Pr)$_2$ (5:3).

CHO cells transfected with human or cyno CCR5 and human MIP-1α as ligand but inactive on murine CCR5. In a functional assay it effectively inhibited agonist-induced Ca^{2+}-mobilization. Compound **NIBR-1282** was also highly potent in CCR5 mediated migration assays with CCR5 transfected L1.2 cells. The Schild–Gaddum analysis with **NIBR-1282** using the radioligand binding assay indicated a competitive mode of inhibition.[12] The compound did not inhibit cytochrome P450 enzymes with IC_{50} values below 5300 nM indicating a low potential for drug–drug interactions which is particularly important for an application in transplantation where generally several immunosuppressive drugs are being applied at the same time. The compound was highly stable when incubated with liver microsomes (low intrinsic clearance), exhibited excellent aqueous solubility and was only weakly bound by human and rat plasma proteins. **NIBR-1282** is a selective antagonist of CCR5 and did not inhibit other chemokine receptors (CCR1, CCR2, CCR3, CCR4, CCR6, CCR7, CXCR1, CXCR2, CXCR3, CXCR4, CXCR6) when tested at concentrations up to 1 μM. Furthermore, screening against a broad panel of non-chemokine GPCRs (including muscarinic acetylcholine receptors) and various ion channels did not show inhibition of any of these receptors or channels at 1 μM.

Table 2. Properties of compound **NIBR-1282** (**18**)

Assay	Result
hu CCR5 (binding)	IC_{50} = 5.1 ± 1.7 nM
cy CCR5 (binding)	IC_{50} = 8.1 ± 1.5 nM
MIP-1α-induced Ca^{2+}-mobilization (hu)	IC_{50} = 43.6 ± 3.2 nM
MIP-1α-induced Ca^{2+}-migration (cy)	IC_{50} = 64 ± 7.7 nM
MIP-1α-induced migration of transfectants (hu)	IC_{50} = 16.2 ± 3.1 nM
CYP1A2	IC_{50} > 10,000 nM
CYP2C9	IC_{50} > 10,000 nM
CYP2C19	IC_{50} > 10,000 nM
CYP2D6	IC_{50} > 10,000 nM
CYP3A4	IC_{50} = 5300 nM
hERG (binding)	IC_{50} > 30,000 nM
hERG (patch clamp)	23% inh. at 22,000 nM
pK_a	8.3, 5.5
log *P*	2.2
log *D* (pH 6.8)	0.8
Intrinsic clearance (hu)	<15 μL min^{-1} mg^{-1}
Intrinsic clearance (rat)	<15 μL min^{-1} mg^{-1}
Solubility (water)	1.0 g/L
Solubility (pH 7.4)	>12.5 g/L
Caco2	Efflux
	P_{AP-BL}: 4×10^{-5} cm/min
	P_{BL-AP}: 65×10^{-5} cm/min
Protein binding (hu)	Free fraction: 62%
Protein binding (rat)	Free fraction: 48%

The oral exposure of **NIBR-1282** in rats was not favorable (Table 3). However, satisfactory PK profiles were obtained both in cynos (*F* 43%; MRT 6 h) and dogs (*F* 56%; MRT 8 h) making **NIBR-1282** an interesting candidate for further in vivo evaluation.

NIBR-1282 was tested in a model of life-supporting kidney transplantation in cynomolgus monkeys.[13] In this model, untreated recipients rejected their graft within 7–10 days (entry 1 in Table 4) and high doses of cyclosporine A (CsA; 100 mg/kg d, po) were required to achieve long term survival (>100 q.d.). A lower dose of CsA (20 mg/kg q.d., po) did not affect graft survival (entry 2 in Table 4). **NIBR-1282** as monotherapy was also non-efficacious (entry 3 in Table 4). However, the combination of non-efficacious doses of CsA and **NIBR-1282** led to statistically significant prolongation of graft survival (entries 4, 5 in Table 4; $p < 0.05$). Importantly, combining **NIBR-1282** and CsA did not result in significant increase in CsA exposure, as revealed by monitoring the CsA blood levels that all varied within 30–140 ng/ml, strongly indicating that the efficacy of the CsA/**NIBR-1282** combination was due to a pharmacodynamic interaction between both drugs rather than a pharmacokinetic interaction.

In conclusion, **NIBR-1282** is a selective, competitive CCR5 antagonist with promising oral bioavailability in dogs and cynos. It did not show adverse effects when tested in an isolated rabbit heart ex vivo model. It was discovered modifying both the polarity (log *D*) and the distribution of polar groups within a series of potent CCR5 antagonists which were very likely to adversely affect the conduction system in the heart. Administration of **NIBR-1282** in combination with a non-efficacious dose of CsA led to significant prolongation of

Table 4. **NIBR-1282** tested in a kidney transplantation model in cynomolgus monkeys[13]

Entry	**NIBR-1282** (mg/kg)	CsA (mg/kg, po)	Graft survival (days)
1	—	—	7–10[a]
2	—	20 q.d.	7, 7, 7, 7, 11
3	20 b.i.d.; sc	—	6, 6
4	20 b.i.d.; sc	20 q.d.	8, 37, 66, >100
5	40 q.d.; po	20 q.d.	9, 20, 36, 39

[a] Range.

Table 3. Pharmacokinetic parameters of **NIBR-1282** (**18**) following po administration

Species	AUC[a] [ng/ml h]	C_{max}[b] [ng/ml]	T_{max} [h]	$T_{1/2}$ [h]	F[d] [%]
Rat	41 ± 9.2	5.8 ± 1.7	0.4–4.0[c]	8.1	22
Cyno	503 ± 208	93 ± 38	0.5–4.0[c]	6.3	43
Dog	278 ± 79	84 ± 35	0.9 ± 0.6	7.9	56

[a] Area under the curve normalized to a dose of 1 mg/kg.
[b] Maximal concentration normalized to a dose of 1 mg/kg.
[c] Range.
[d] Oral bioavailability obtained from ratio AUC (oral administration)/AUC (iv administration).

kidney allograft survival in cynomolgus monkeys. Inhibition of CCR5 may offer new therapeutic opportunities for transplant patients.[14]

Acknowledgment

We thank the members of the Novartis non-human primate transplantation group for their valuable contributions.

References and notes

1. (a) Murphy, P. M.; Baggiolini, M.; Charo, I. F.; Hebert, C. A.; Horuk, R.; Matsushima, K.; Miller, L. H.; Oppenheim, J. J.; Power, C. A. *Pharmacol. Rev.* **2000**, *52*, 145; (b) Moser, B.; Loetscher, P. *Nat. Immunol.* **2001**, *2*, 123; (c) Loetscher, P.; Uguccioni, M.; Bordoli, L.; Baggiolini, M.; Moser, B.; Chizzolini, C.; Dayer, J. M. *Nature* **1998**, *391*, 344; (d) Kunkel, E. J.; Boisvert, J.; Murphy, K.; Vierra, M. A.; Genovese, M. C.; Wardlaw, A. J.; Greenberg, H. B.; Hodge, M. R.; Wu, L.; Butcher, E. C.; Campbell, J. J. *Am. J. Pathol.* **2002**, *160*, 347.
2. Berger, E. A.; Murphy, P. M.; Farber, J. M. *Annu. Rev. Immunol.* **1999**, *17*, 657.
3. For small molecule CCR5 antagonists as potential anti-HIV agents see (a) Gao, Z.; Metz, W. A. *Chem. Rev.* **2003**, *103*, 3733; (b) Kazmierski, W.; Bifulco, N.; Yang, H.; Boone, L.; DeAnda, F.; Watson, C.; Kenakin, T. *Bioorg. Med. Chem.* **2003**, *11*, 2663, 4155; (c) Palani, A.; Tagat, J. R. *J. Med. Chem.* **2006**, *49*, 2851; (d) Mills, S. G.; DeMartino, J. A. *Curr. Top. Med. Chem.* **2004**, *4*, 1017; (e) Maeda, K.; Nakata, H.; Ogata, H.; Koh, Y.; Miyakawa, T.; Mitsuya, H. *Curr. Opin. Pharmacol.* **2004**, *4*, 447; (f) Wood, A.; Armour, D. *Prog. Med. Chem.* **2005**, *43*, 239; (g) Cumming, J. G.; Brown, S. J.; Cooper, A. E.; Faull, A. W.; Flynn, A. P.; Grime, K.; Oldfield, J.; Shaw, J. S.; Shepherd, E.; Tucker, H.; Whittaker, D. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3533; (h) Habashita, H.; Kokubo, M.; Hamano, S.-I.; Hamanaka, N.; Toda, M.; Shibayama, S.; Tada, H.; Sagawa, K.; Fukushima, D.; Maeda, K.; Mitsuya, H. *J. Med. Chem.* **2006**, *49*, 4140; (i) Imamura, S.; Ichikawa, T.; Nishikawa, Y.; Kanzaki, N.; Takashima, K.; Niwa, S.; Iizawa, Y.; Baba, M.; Sugihara, Y. *J. Med. Chem.* **2006**, *49*, 2784; (j) Nishizawa, R.; Nishiyama, T.; Hisaichi, K.; Matsunaga, N.; Minamoto, C.; Habashita, H.; Takaoka, Y.; Toda, M.; Shibayama, S.; Tada, H.; Sagawa, K.; Fukushima, D.; Maeda, K.; Mitsuya, H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 727; (k) Lu, S.-F.; Chen, B.; Davey, D.; Dunning, L.; Jaroch, S.; May, K.; Onuffer, J.; Phillips, G.; Subramanyam, B.; Tseng, J.-L.; Wei, R. G.; Wei, M.; Ye, B. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1833.
4. (a) Fischereder, M.; Luckow, B.; Hocher, B.; Wuthrich, R. P.; Rothenpieler, U.; Schneeberger, H.; Panzer, U.; Stahl, R. A.; Hauser, I. A.; Budde, K.; Neumayer, H.; Kramer, B. K.; Land, W.; Schlondorff, D. *Lancet* **2001**, *357*, 1758; (b) Strieter, R. M.; Belperio, J. A. *Lancet* **2001**, *357*, 1725.
5. For reviews on the role of chemokines and their receptors in transplant rejection see (a) DeVries, M. E.; Hosiawa, K. A.; Cameron, C. M.; Bosinger, S. E.; Persad, D.; Kelvin, A. A.; Coombs, J. C.; Wang, H.; Zhong, R.; Cameron, M. J.; Kelvin, D. J. *Semin. Immunol.* **2003**, *15*, 33; (b) Nelson, P. J.; Krensky, A. M. *Immunity* **2001**, *14*, 377; (c) Hancock, W. W.; Wang, L.; Ye, Q.; Han, R.; Lee, I. *Curr. Opin. Immunol.* **2003**, *15*, 479.
6. Thoma, G.; Nuninger, F.; Schaefer, M.; Akyel, K. G.; Albert, R.; Beerli, C.; Bruns, C.; Francotte, E.; Luyten, M.; MacKenzie, D.; Oberer, L.; Streiff, M. B.; Wagner, T.; Walter, H.; Weckbecker, G.; Zerwes, H.-G. *J. Med. Chem.* **2004**, *47*, 1939.
7. Strizki, J. M.; Xu, S.; Wagner, N. E.; Wojcik, L.; Liu, J.; Hou, Y.; Endres, M.; Palani, A.; Shapiro, S.; Clader, J. W.; Greenlee, W. J.; Tagat, J. R.; McCombie, S.; Cox, K.; Fawzi, Ahmad B.; Chou, C.-C.; Pugliese-Sivo, C.; Davies, L.; Moreno, M. E.; Ho, D. D.; Trkola, A.; Stoddart, C. A.; Moore, J. P.; Reyes, G. R.; Baroudy, B. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 12718.
8. (a) Valentin, J.-P.; Hoffmann, P.; De Clerck, F.; Hammond, T. G.; Hondeghem, L. *J. Pharmacol. Toxicol. Methods* **2004**, *49*, 171; (b) Hondeghem, L. M.; Hoffmann, P. *J. Cardiovasc. Pharmacol.* **2003**, *41*, 14.
9. (a) Jamieson, C.; Moir, E. M.; Rankovic, Z.; Wishart, G. *J. Med. Chem.* **2006**, *49*, 5029; (b) Price, D. A.; Armour, D.; De Groot, M.; Leishman, D.; Napier, C.; Perros, M.; Stammen, B. L.; Wood, A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4633.
10. For models of the hERG channel to predict how compounds might bind see (a) Cavalli, A.; Poluzzi, E.; De Ponti, F.; Recanatini, M. *J. Med. Chem.* **2002**, *45*, 3844; (b) Perry, M.; de Groot, M. J.; Helliwell, R.; Leishman, D.; Tristani-Firouzi, M.; Sanguinetti, M. C.; Mitcheson, *J. Mol. Pharmacol.* **2004**, *66*, 240.
11. Analytical data for **NIBR-1282**. ^{1}H NMR ($CDCl_3$, 20 °C) δ = 8.14 (1H, d), 8.11 (1H, d), 8.11 (1H, d), 7.38 (2H, t), 7.20 (1H, t), 7.11 (1H, dd), 6.98 (1H, d), 6.96 (2H, d), 6.94 (1H, m), 4.04 (1H, m), 3.82 (1H, m), 3.43 (1H, m), 3.22 (1H, m), 2.99 (1H, m), 2.94 (1H, m), 2.85 (1H, m), 2.38 (3H, s), 2.28 (1H, t), 2.26 (1H, t), 2.24 (3H, s), 2.02 (1H, m), 1.98 (1H, m), 1.94 (1H, m), 1.75 (1H, m), 1.43 (1H, m), 1.41 (1H, m), 1.35 (1H, m), 1.26 (1H, m), 0.97 (3H, s), HRMS/ESI $C_{30}H_{38}N_5O_2$ Calcd: 500.3026. Found: 500.3028.
12. Gaddum, J. H. *Pharmacol. Rev.* **1957**, *9*, 211.
13. Details on the transplantation experiments will be published elsewhere. For details on the model see Schuler, W.; Bigaud, M.; Brinkmann, V.; di Padova, F.; Geisse, S.; Gram, H.; Hungerford, V.; Kleuser, B.; Kristofic, C.; Menninger, K.; Tees, R.; Wieczorek, G.; Wilt, C.; Wioland, C.; Zurini, M. *Transplantation* **2004**, *77*, 717.
14. Very recently, a study of the role of CCR5 blockade on the modulation of inflammation and alloimmunity in primates has been published Schroeder, C.; Pierson, R. N.; Nguyen, B.-N. H.; Kawka, D. W.; Peterson, L. B.; Wu, G.; Zhang, T.; Springer, M. S.; Siciliano, S. J.; Iliff, S.; Ayala, J. M.; Lu, M.; Mudgett, J. S.; Kathy Lyons, K.; Mills, S. G.; Miller, G. G.; Singer, I. I.; Azimzadeh, A. M.; DeMartino, J. A. *J. Immunol.* **2007**, *179*, 2289.