Aromatic Quinolinecarboxamides as Selective, Orally Active Antibody Production Inhibitors for Prevention of Acute Xenograft Rejection

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The prevention of xenograft rejection is substantially dependent on inhibiting antibodies (Ab) produced by B-cells independently of T-cell signals (TI-1). Due to their ubiquitous biochemical mechanisms of action, the immunosuppressants currently employed not only fail to discriminate between B- and T-cells but also have a narrow therapeutic window and, thus, their prolonged use in complex immunosuppressive regimens is problematic. By capitalizing on the target enzyme-bound (DHODH) structure **1b** of one of these compounds, leflunomide, and modulating part of its multiple mechanisms of action to gain selectivity, the quinoline-8-carboxamide **3** was designed as a potentially weak enzyme inhibitor but effective immunosuppressant. Compound **3** fulfilled the mechanistic criteria set and had 10-fold B-cell over T-cell selectivity. Its pyridyl analogue **4** was found to be a highly potent and selective B-cell immunosuppressant with a 75-fold selectivity for B- over T-cells (as judged by the MLR data) and no general cytotoxicity at concentrations up to 160-fold higher than those required to inhibit B-cells. In the mouse, **4** effectively blocked TI-1 Ab production and suppressed Ab-mediated xenograft rejection in a xenotransplantation model under a once-daily dosing regimen, with efficacy down to 0.3 mg/kg/day po. These are the first data demonstrating the feasibility of the development of drugs specific for impeding Ab production.

Introduction

The very high success rate of allotransplantation and the resulting quality of life have led to the establishment of this technique as a standard therapeutic option for the treatment of end-stage organ failure in man. Due to the current shortage of donor organs, xenotransplantation, the transplantation of organs between two different species, has recently become a field of active research because it represents the means to solve the organ availability issue. Common to all innovative approaches, xenotransplantation faces many challenges, not least the immune response of the recipient against the transplant.[1] The pig-to-primate model is being extensively utilized as the most relevant preclinical model for the evaluation of immunosuppressive protocols that will enable the long-term acceptance of transgenic pig organs by man.[2]

The transplantation of a vascularized porcine organ into a nonhuman primate leads to an immediate and dramatic rejection process, known as hyperacute rejection (HAR). It is the result of the species disparities and is mediated by the binding of the naturally occurring, pre-existing antibody (Ab) of the host to the Galα1,3Gal epitopes found on the glycoproteins and glycolipids expressed on pig endothelium.[3] As a consequence, the host complement is activated and rapid destruction of the xenograft occurs. HAR can be reliably controlled by employing pig organs transgenic for human decay accelerating factor (hDAF), an endogenous complement cascade inhibitor found in primates.[4] Attention is currently turned toward an understanding of the immunological barrier mediating the next phase of xenograft rejection termed acute vascular rejection (AVR). This delayed rejection process is an Ab-mediated event that typical T-cell immunosuppressants such as cyclosporin A (CsA) cannot prevent, and it is likely that the control of such Ab synthesis by B-cells will play a pivotal role in overcoming it. With HAR and AVR suppressed, xenografts are expected to behave similarly to allografts and, consequently, be under the control of the currently existing T-cell immunosuppressants.[5]

Regimens aimed at the control of AVR have been extensively studied in rodent models of xenotransplantation as well as in the pig-to-primate model. They focus primarily on the inhibition of newly formed anti-Galα1,-3Gal Ab by host B-cells in response to the xenograft[6] and are based on the use of nonspecific agents such as the de novo nucleotide inhibitors leflunomide, brequinar, mycophenolic acid derivatives, and antiproliferative macrolides of the rapamycin class.[7] Despite their documented efficacy both in rodent models and in nonhuman primate recipients of pig organs transgenic for hDAF, the narrow therapeutic index of these compounds limits their use in regimens for AVR prevention.[8] In search of selective inhibitors of CsA-resistant Ab formation, we recently reported the discovery of pyrazole derivatives that had a novel biochemical mechanism of action as well as the desired in vitro profile but suffered from nonoptimal pharmacokinetic properties.[9] Pursuing our efforts in the field of the inhibition of CsA-resistant B-cell responses, we now report the design and biological evaluation of a novel class of B-cell inhibitors that

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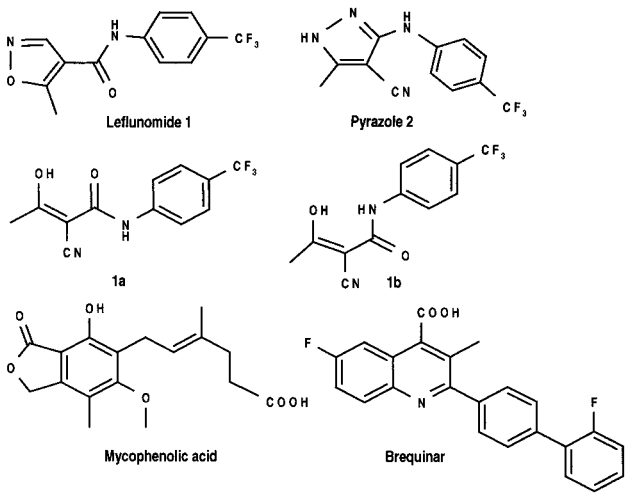


Figure 1. Structures of antibody inhibitors.

efficiently block Ab responses in vivo and prevent xenograft rejection upon daily oral treatment.

Design Concept

Two of the three current inhibitors of de novo nucleotide synthesis mediate their effects by a highly specific biochemical mechanism. Indeed, mycophenolic acid (MPA) and brequinar (BQR) block the enzymatic activities of inosine monophosphate dehydrogenase (IMPDH) and dihydrooroate dehydrogenase (DHODH), respectively.[10,11] The third inhibitor, leflunomide (**1**), is a prodrug having an isoxazole ring that is quickly and quantitatively opened in vivo as well as in vitro cellular systems. The resulting hydroxypropenamide inhibits DHODH at low concentrations but can operate by additional protein tyrosine kinase-related mechanisms at its therapeutically relevant concentrations.[11,12] Of the two hydroxypropenamide conformers **1a** and **1b** detected so far, the latter was demonstrated to inhibit DHODH (see below). However, because the contribution of each conformer to the various biological mechanisms of leflunomide is currently unknown, **1b** was chosen as a starting point for medicinal chemistry on the basis of the hypothesis that it could lead to lymphocyte selective substances via the modulation of only part of the operating mechanisms. The inhibition of de novo nucleotide biosynthesis not being a prerequisite for efficient B-cell inhibition,[13] it was expected to identify novel and potentially selective B-cell agents based on modifications of **1b** that would afford compounds devoid of the ubiquitous DHODH blockade but retaining the interactions of **1b** with its additional targets.

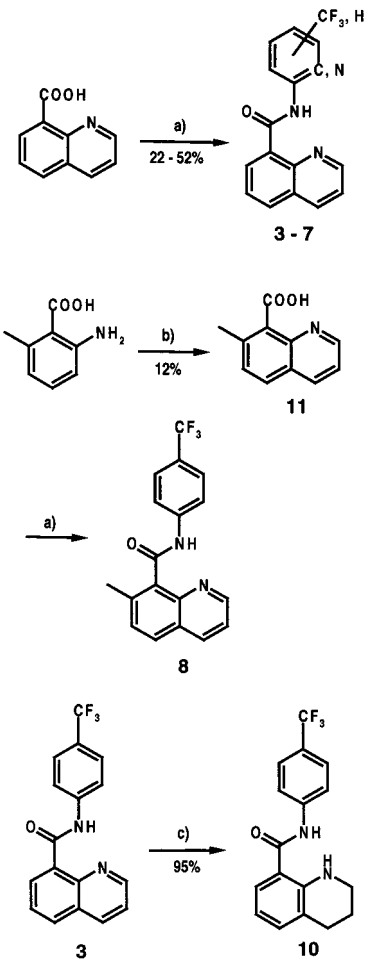
To translate these mechanistic considerations into synthetic targets, the corresponding free and enzyme-bound conformations **1a** and **1b** were taken into consideration, respectively (Figure 1). Both structures are planar and have a strong intramolecular hydrogen bond involving the enol and the amide functions. However, in **1a** the hydroxyl is the donor and the carbonyl the acceptor, whereas the corresponding interacting pair in **1b** are the amide NH and the hydroxyl oxygen.[14,15] The **1a** to **1b** conformational change is presumably the result of a keto−enol equilibrium leading to a torsional angle modification. With no information available on the DHODH-bound conformation of the hydroxypropenamide resulting from **1** during our previous investigations, we speculated that **1a** could reflect the bioactive conformation and, consequently, designed and obtained pyrazole **2** as a novel DHODH inhibitor having a biological activity similar to that of **1**.[9] In view of the disclosed X-ray structure **1b** of bioactive **1**,[15] the success of the design of **2** is puzzling. Retrospectively, it can be attributed either to binding to an allosteric position or to the remaining presence of a **1a** conformer population that is also inhibitory but failed to crystallize in complex with DHODH and was therefore not detected. We subsequently initiated a novel approach based on the structure of **1b** and designed quinoline **3** as a potential mimetic. Its probable conformation was deduced from substructure searches in the Cambridge Crystallographic Database, which indicated that an intramolecular hydrogen bond between the amide NH and the N(1) of quinoline should be formed, thus ensuring the planarity of the molecule. This speculative solid state conformation did not provide any information on the conformation of **3** in solution but only suggested that a similar folded structure could be energetically accessible. The CN and Me groups of **1b**, not being involved in interactions with the enzyme, were not specifically included in the designed molecule, but their electronic and steric properties were imitated by the phenyl ring of the quinoline. The only important functionality missing in **3** that exists in **1b** is a hydrogen bond donor allowing the O−H → Thr356 interaction. Given that no potent DHODH inhibition was sought, but only effects mediated by the additional mechanism of **1b**, and considering the straightforward synthetic access to **3** and derivatives, the study of aromatic quinoline-8-carboxamides was initiated.

Chemistry and Structure

The amides **3**−**6** were prepared, in moderate yields, via the coupling of the acid chloride of the commercially available quinoline-8-carboxylic acid with the corresponding anilines (Scheme 1). For the synthesis of **7**, the required 7-methyl-8-carboxylquinoline **11** was obtained on the basis of the Skraup reaction. The tetrahydroquinoline derivative **10** was quantitatively obtained upon catalytic hydrogenation of **3**. A different approach was followed for the synthesis of the 2-trifluoromethyl compound **9** (Scheme 2). The key step was the metalation (*n*-BuLi/TMEDA) of the 8-bromoquinoline **15** followed by quenching with (*p*-CF_3)phenyl isocyanate. In addition to **9** (30%), the reaction afforded a substantial amount (45%) of the debrominated quinoline. Compound **15**[16] was obtained in a very low overall yield (2.5%) from the highly moisture sensitive *N*-ethylidene-*tert*-butylamine **12**.[17]

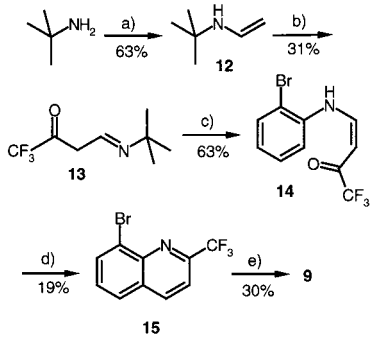
To verify structurally our working hypothesis and to gain insight for structure−activity result interpretation (see In Vitro Biological Results), the solid state conformation of two quinolines was determined crystallographically (Figure 2).[18] Molecules **4** and **9** adopt planar conformations that are stabilized by intramolecular hydrogen bonds between the amide NH and the quinoline nitrogen atom N(1) (Figure 3). Indeed, in both compounds the N···N distance is 2.7 Å and the N−H···N angle 143°. In agreement with 3D data on pyridyl amides, the nitrogen atom of the pyridine in **4**

Scheme 1[a]



(a) $(COCl)_2$, DMF (cat.), CH_2Cl_2, $ArNH_2$; (b) acrolein, dichlorobenzene, 140 °C, 3 h; (c) H_2, Pd/C, DMF/MeOH (1.5/98.5).

Scheme 2[a]

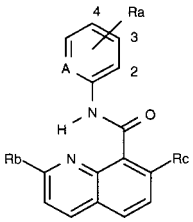


(a) CH_3CHO, 0 °C, pentane; (b) LDA, CF_3COOEt, −75 °C, 30 min; (c) Br−aniline, AcOH, CF_3COOH, 75 °C, 6 h; (d) $POCl_3$, heptane, 100 °C, 6 h; (e) *n*-BuLi, TMEDA, Et_2O, −78 °C then (*p*-CF_3)PhNCO.

is on the opposite side of the C═O group. This preferred conformation can be attributed to the weak intramolecular H-bond between the carbonyl and the ortho hydrogen of the pyridinyl moiety [*D* (C···O) = 2.86 Å, *θ* (C−H···O) = 119°]. Collectively, the data indicate that also quinolines **3**−**7** should be expected to have, if not identical, very closely related overall conformations.

In Vitro Biological Results

In the mouse, CsA-resistant responses are typically elicited by type I T-cell independent antigens (TI-1) like



Cpnd.	Ra	Rb	Rc	A
3	4-CF_3	H	H	C
4	4-CF_3	H	H	N
5	H	H	H	C
6	2-CF_3	H	H	C
7	3-CF_3	H	H	C
8	4-CF_3	H	Me	C
9	4-CF_3	CF_3	H	C

Figure 2. Structures of 8-quinolinecarboxamides.

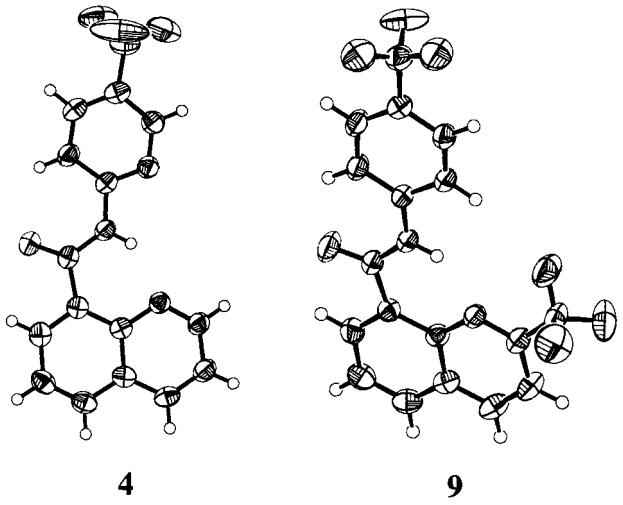


Figure 3. Solid state conformation of quinolines **4** and **9** (Ortep plot with 30% displacement ellipsoids).

Table 1. In Vitro Profile of Reference and Novel Compounds

compound	TNP-LPS[a,b]	MLR[a,c,e]	Jurkat[a,d,e]
CsA	>10 ± 0	0.006 ± 0.004	>10 ± 0
leflunomide **(1)**	5.1 ± 0.6	11.4 ± 1.4	34.6 ± 1.7
3	0.7 ± 0.1	6.7 ± 1.5	>44.7 ± 3.7
4	0.3 ± 0.1	>22.4 ± 5.9	>50 ± 0
5	12.7 ± 1.9	23.4 ± 3.5	>50 ± 0
6	4.4 ± 1.0	6.6 ± 0.9	5.2 ± 1.2
7	1.6 ± 0.4	20.2 ± 1.9	>50 ± 0
8	>50 ± 0	NT	NT
9	42.5 ± 3.8	NT	NT
10	19.5 ± 4.3	NT	NT

[a] IC_{50} ± SE values in μM. Mean of at least four independent determinations. [b] Inhibition of murine Ab responses to the T-cell independent B-cell antigen TNP-LPS (TI-1). [c] Inhibition of the mouse mixed lymphocyte reaction. [d] Inhibition of Jurkat cell proliferation. [e] NT, not tested.

the trinitrophenyl-lipopolysaccharide (TNP-LPS).[19,20] Our goal being the identification of novel agents that inhibit such TI-1 biological phenomena that are of relevance in xenotransplantation, a cellular assay based on the modulation of antibody production by TNP-LPS-stimulated murine B-cells was employed for the comparative screening of the compounds. In addition, the T-cell immunosuppressive activity of the compounds as well as their cytotoxicity was assessed in the mouse mixed lymphocyte reaction (MLR) and Jurkat proliferation assays, respectively, as an estimate of their lymphocyte selectivity and therapeutic window.[9] The biochemical mechanism of action of the compounds was compared to that of **1b** using the human DHODH enzymatic assay.[21]

The data are summarized in Table 1. As already alluded to, CsA failed to inhibit Ab formation from TNP-LPS-stimulated B-cells but potently and selectively

inhibited T-lymphocyte proliferation with an $IC_{50} = 6$ nM in the MLR. Leflunomide (**1**) inhibited both TNP-LPS responses and the MLR with IC_{50} values differing by 2-fold. Although less potently, it also inhibited Jurkat proliferation, and the resulting window relative to the TNP-LPS inhibition was ~7-fold. The overall profile of **1** is compatible with that of a mild antiproliferative agent and is the result of its ubiquitous mechanism of action. The prototype quinoline **3** was a potent B-cell inhibitor with 10- and >60-fold selectivities toward T-cells and Jurkat cells, respectively. The corresponding pyridyl derivative **4** inhibited the TNP-LPS responses with an $IC_{50} = 300$ nM, had a 73-fold selectivity toward T-cells, and was devoid of any effects on the Jurkat cell line. In line with their cellular profile and in contrast to **1** that inhibits DHODH with an $IC_{50} = 200$ nM, neither **3** nor **4** had any effects on the enzymatic activity at up to 50 μM concentrations. Moreover, they did not inhibit inosine monophosphate dehydrogenase, the target of the de novo nucleotide biosynthesis inhibitor MPA.[10] Because of the reported inhibition of EGFR- and PDGFR-Tyr kinases by leflunomide at high micromolar concentrations, the effects of **3** and **4** on these kinases as well as on cdk2 and the Src kinase family were assessed and found to be negligible up to a concentration of 30 μM.[12] Therefore, compounds **3** and **4** represent mechanistically novel, potent, and selective inhibitors of CsA-resistant Ab production by B-cells. The currently very limited information on the complex mechanisms of B-cell activation, proliferation, and Ab production does not permit a plausible explanation of the B- versus T-lymphocyte selectivity of these quinolines.[22]

Having demonstrated that **3** and **4** have the in vitro profile we were aiming at, a limited structure−activity investigation was undertaken to identify the key elements responsible for B-cell inhibition and selectivity. First, the presence and influence of the position of the trifluoromethyl substituent were assessed. In comparison to **3**, the corresponding phenyl derivative **5** was a weak B-cell inhibitor, whereas the ortho CF_3-substituted derivative **6**, although equipotent to **1**, had a cytostatic character because it was devoid of selectivity toward T-cells and Jurkat cells. In contrast, the meta isomer **7** was a highly selective compound with only 2 times weaker B-cell inhibitory activity as compared to that of **3**. Both derivatives having an additional substituent on the quinoline moiety were inactive in the TNP-LPS assay. In compound **8**, the 7-Me group most probably destroyed the planarity of the molecule by positioning the amide out of the quinoline plane. Indeed, molecular modeling inspection using both the CHARM and the Tripos force fields indicated that the distance between the carbonyl O and the 7-Me moiety is quite short ($D =$ 2.5 Å) for a nonbonded interaction. The 2-CF_3 substituted compound **9** was synthesized to probe the influence of the hydrogen bond between the N(1) and the amide NH on the biological activity. Indeed, the presence of the electron-withdrawing group was expected to decrease the electron density of N(1) and, thus, weaken its acceptor potential. Given the experimentally obtained structural data showing that **4** and **9** have the same conformation in the solid state, the lack of activity of **9** indicates that electron-rich groups are not tolerated at position 2. In agreement with our pharmacophore hypothesis requiring the intamolecular NH···N(1) H-bond, the nonplanar tetrahydroquinoline derivative **10** was only marginally inhibiting TNP-LPS responses.

Table 2. In Vivo Inhibition of Immunoglobulin (Ig) Production[a]

compound	dose[b]/route[c]	IgM[d]	IgG[d]
leflunomide **(1)**	50/sc	71 ± 10	85 ± 5.0
	30/sc	37 ± 15	39 ± 21
	3/sc	≤0	≤0
	10/po	5 ± 21	≤0
4	50/sc	82 ± 5	95 ± 5
	30/sc	86 ± 4	95 ± 6
	3/sc	73 ± 10	84 ± 4
	10/po	63 ± 12	89 ± 9

[a] Groups consisted of at least five mice per application. [b] mg/kg/day. [c] Vehicles for administration: (i) sc polyethoxylated castor oil 650 mg/mL and EtOH up to 1 mL; (ii) po Labrafil 330 mg/mL, EtOH 110 mg/mL, corn oil up to 1 mL. [d] Inhibition percent.

In Vivo Evaluation and Pharmacokinetics

The potency and selectivity of **4** in vitro prompted its comparative evaluation with leflunomide (**1**) in two relevant in vivo models of Ab-mediated immunity.

The effects of **1** and **4** on the in vivo TI-1 Ab production were assessed after immunization of OF1 mice with TNP-LPS and repetitive treatment with appropriate doses of compounds followed by the determination of antibody titers in the serum.[23] Upon subcutaneous (sc) administration, **4** was more effective than **1** already at the 30 mg/kg/day dose, which appears to be the dose leading to the maximum pharmacological efficacy in this model. Furthermore, **4** was highly active even at 3 mg/kg/day, as shown by the 73 and 84% inhibitions of TNP-specific immunoglobulin M (IgM) and G (IgG) titers, respectively. At this dose, leflunomide did not significantly inhibit either IgM or IgG (Table 2). Thus, the ED_{50} for the quinoline was estimated to be <3 mg/kg/day sc. Moreover, after oral application (10 mg/kg/day), **4** also blocked >60% of specific Ab formation, indicating therefore effect−bioavailability.

To correlate the in vitro activity of **4** with its pharmacodynamic (PD) effect, the time−concentration profile of the compound in the blood of OF1 mice was quantified by LC-MS after single-dose oral (30 mg/kg) and parenteral (10 mg/kg) applications (Figure 4). The maximum blood concentration was achieved 0.25 h after oral application, and 10-fold higher exposure levels (2.6 μM) than the IC_{50} value of 0.3 μM persisted for up to 2 h postdose. The dose-normalized AUC ratio (po/iv) pointed to an absolute bioavailability of 13% for **4**.

Having demonstrated that **4** is a potent inhibitor of TI-1 Ab responses in vivo, its efficacy in a xenotransplantation setting was investigated (Table 3). To assess the effect of compounds on Ab-mediated rejection of xenotransplants, the model of Syrian hamster hearts heterotopically transplanted into nude (athymic) mice was used.[24] The rejection process in these animals is restricted to the B-cell response due to the genetic deficiency of mature T cells. Hence, a xenograft can be rejected only by a T-cell-independent (TI) Ab response because a T-cell-dependent anti-xenograft Ab antibody formation cannot be mounted. The survival of the animals for 4 weeks with graft histology showing no Ab-mediated damage was considered to be a successful end point.

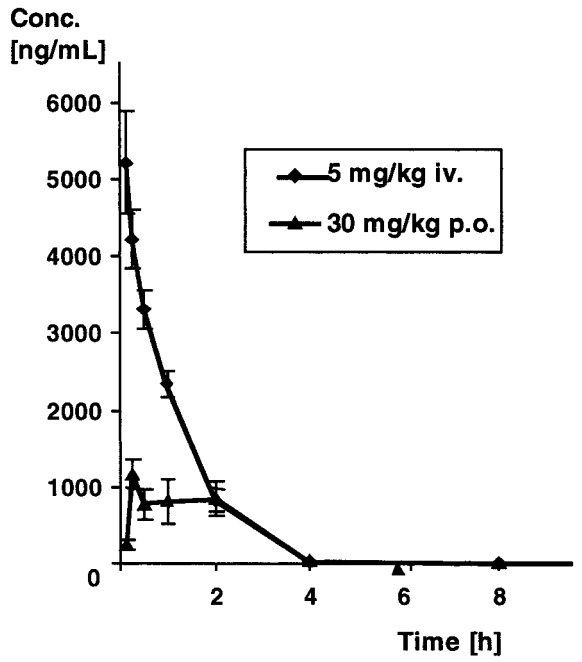


Figure 4. Concentration of **4** in mouse blood after oral and intravenous administration. Results are mean ± SE of four mice per time point.

Table 3. Prolongation of Xenoheart Survival and Histological Findings[a]

compound	dose[b]	survival[c]	histology[d]
placebo		5, 5, 6, 6, 6, 6	AbR ($n = 6$)
leflunomide (**1**)	50	>28 ($n = 6$)	no AbR ($n = 6$)
	30	7, 7, >28, >28	AbR ($n = 2$), ChR ($n = 2$)
	3	7 ($n = 3$)	AbR ($n = 3$)
4	30	>28 ($n = 6$)	no AbR ($n = 6$)
	10	11, >28 ($n = 4$)	CR ($n = 1$), no AbR ($n = 4$)
	1	>28 ($n = 4$)	no AbR ($n = 4$)
	0.3	>28 ($n = 4$)	no AbR ($n = 4$)
	0.1	6, 6, 7	AbR ($n = 3$)

[a] Hamster to athymic mouse xenotransplantation model. [b] mg/kg/day po in Labrafil 330 mg/mL, EtOH 110 mg/mL, corn oil up to 1 mL. [c] In days. > indicates mouse sacrificed with a beating graft. [d] AbR, antibody-mediated rejection; CR, cellular rejection; ChR, chronic rejection.

Daily oral administration of leflunomide for 4 weeks effectively controlled xenograft rejection only at the dose of 50 mg/kg/day. At 30 mg/kg/day only two of four animals had a beating graft at 28 days post-transplant, histology showing an acute Ab-mediated rejection in the two grafts rejected at day 7. The remaining two showed signs of chronic rejection at autopsy. The 3 mg/kg/day dose was clearly ineffective, leading to the rejection of all grafts within 7 days by Ab attack. The quinoline **4** showed efficacy from a daily oral dose of 30 mg/kg down to 0.3 mg/kg and, thus, was ~100-fold superior to leflunomide (**1**). At the highest dose tested, the six grafts survived to 28 days with no histological evidence of rejection. Likewise, at 10 and 1 mg/kg all mice were terminated after 4 weeks with beating grafts and normal heart histology except that of one animal of the former group that underwent cell-mediated rejection at day 11. The minimum effective dose of **4** in this model was established at ~0.3 mg/kg/day po with xenografts showing normal myocardium with no evidence of rejection. As a general tolerability parameter, body weight was recorded at the time of transplant and at autopsy. No adverse effect on normal body weight gain was seen in any treatment group.

Conclusions

On the basis of the nondependency of the inhibition of Ab production from ubiquitous mechanisms such as the well-established de novo nucleotide biosynthesis blockade and capitalizing both on the probable multiple mechanism of action of **1b** and on its enzyme-bound conformation, quinoline **3** was designed as a potentially weak inhibitor of DHODH but effective immunosuppressant. The compound not only fulfilled the mechanistic criteria set by showing no inhibition of DHODH but also provided additional evidence on the feasibility of achieving selective B-cell over T-cell inhibition and, hence, developing specific drugs impeding Ab production. In fact, the in vitro profile of the pyridyl derivative **4** sets a novel paradigm in the B-cell immunosuppression field because it has a 75-fold selectivity for B- over T-cells (as judged by the MLR data) and no general cytotoxicity up to >160-fold higher concentrations than those required for B-cell inhibition. In agreement with the in vitro potency, both subcutaneous and oral administration of quinoline **4** very effectively prevented Ab production in vivo as judged by the low IgM and IgG titers resulting from the immunization of mice with TNP-LPS antigen. IgG titers were less sensitive to the effects of the compound than IgM titers. Leflunomide, the reference compound, was clearly less potent in this setting.

Given the doses applied in the in vivo Ab pharmacological studies, the extrapolation of the pharmacokinetic (PK) results indicates that concentrations of **4** above its IC_{50} value in the corresponding cellular assay have been obtained. The persistence of such therapeutically relevant concentrations for at least 2 h postdose seems to be sufficient to prevent the onset of Ab against TNP-LPS for 24 h. Consequently, the data resulting from this mechanistic model indicate a PK/PD relationship with quinoline **4**.

Very importantly for our purpose, **4** also prevented graft rejection in the relevant mouse xenotransplantation model. Indeed, **4** is the most potent compound tested in suppressing Ab-mediated xenograft rejection under a once-daily dosing regimen with efficacy down to 0.3 mg/kg/day po. On the basis of the generated PK data, the exposure of the animals to the quinoline can be expected to be below the IC_{50} values at this dosing. However, such extrapolations are hazardous because not only were different mouse strains used for the xenotransplantation (athymic C57Bl/6) and PK (OF1) experiments but also discrepancies in the PK behavior of a compound between transplanted and healthy animals are not rare. In addition, no predictions can be made concerning the sensitivity to quinoline inhibition of anti-xenograft Ab versus TNP-LPS Ab.

Overall, the in vitro and in vivo results indicate that the quinolinecarboxamide **4** is a highly potent and selective agent for the blockade of Ab secretion in the xenotransplantation setting. Moreover, the lack of long-term adverse effects observed with 10 times higher

dosing emphasizes the substantial therapeutic potential of this compound in Ab-mediated diseases.

Experimental Section

(1) Chemistry. Compounds were characterized by 400 MHz proton NMR at room temperature using a Bruker MX-400 or DPX-400 spectrometer. Chemical shifts are expressed as parts per million downfield from tetramethylsilane, and *J* values are reported in hertz. Fast atom bombardment mass spectroscopy (Xe, 8 keV) on a VG70-SE mass spectrometer was used for the characterization of all reported compounds. C, H, and N analyses were carried out with all substances biologically evaluated, and ±0.4% was acceptable. For chromatographic purifications, the flash chromatography technique was applied using 230−400 mesh silica gel.

(a) Quinoline-8-carboxamides. General Procedure. To a solution of 8-carboxyquinoline (1.0 g, 5.78 mmol) in CH_2Cl_2 containing 2−3 drops of DMF was added dropwise oxalyl chloride (0.75 mL, 8.7 mmol). The resulting suspension was stirred for 1 h at room temperature and then cooled to 0 °C. At this temperature, a THF solution of the aniline (3.6 g, 17.32 mmol) was slowly added and the reaction stirred for 2 h at room temperature. The reaction mixture was subsequently poured onto 1 N aqueous $NaHSO_4$ solution and extracted with ethyl acetate. The crude amide was purified by silica gel chromatography (eluent of EtOAc/hexane = 4:6) to afford the title compound.

(i) *N*-[4-(Trifluoromethyl)phenyl]quinoline-8-carboxamide, 3: yield 47%; mp 111−112 °C; ^{1}H NMR (DMSO-d_6) δ 13.46 (1H, s), 9.12 (1H, d, J = 4.3), 8.58 (2H, m), 8.23 (1H, dd, J_1 = 8.2, J_2 = 1.5), 8.05 (2H, d, J = 8.5), 7.77 (2H, t, J = 7.9), 7.72 (2H, d, J = 7.9). Anal. ($C_{17}H_{11}F_3N_2O$): C, H, N.

(ii) *N*-[5-(Trifluoromethyl)pyridin-2-yl]quinole-8-carboxamide, 4: yield 45%; mp 170−172 °C; ^{1}H NMR (DMSO-d_6) δ 14.35 (1H, s), 9.16 (1H, dd, J_1 = 4.3, J_2 = 1.6), 8.80 (2H, m), 8.76 (1H, d, J = 7.4), 8.66 (1H, d, J = 8.3), 8.56 (1H, d, J = 8.7), 8.33 (1H, d, J = 8.1), 7.85 (1H, t, J = 7.8), 7.77 (1H, dd, J_1 = 8.3, J_2 = 4.3). Anal. ($C_{16}H_{10}F_3N_3O$): C, H, N.

(iii) *N*-(Phenyl)quinoline-8-carboxamide, 5: yield 52%; mp 132−134 °C; ^{1}H NMR (DMSO-d_6) δ 13.85 (1H, s), 9.11 (1H, d, J = 4.3), 8.68 (1H, d, J = 8.4), 8.52 (1H, d, J = 8.4), 8.22 (1H, d, J = 8.8), 7.89 (2H, d, J = 7.2), 7.72 (1H, t, J = 7.4), 7.67 (1H, d, J = 8.4), 7.45 (2H, t, J = 7.6), 7.15 (1H, t, J = 7.2). Anal. ($C_{16}H_{12}N_2O$): C, H, N.

(iv) *N*-[2-(Trifluoromethyl)phenyl]quinoline-8-carboxamide, 6: yield 42%; mp 128−130 °C; ^{1}H NMR (DMSO-d_6) δ 14.05 (1H, s), 9.08 (1H, m), 8.81 (1H, d, J = 7.4), 8.68 (1H, d, J = 7.4), 8.46 (1H, d, J = 7.4), 8.34 (1H, d, J = 7.5), 8.82 (4H, m), 7.37 (1H, t, J = 7.6). Anal. ($C_{17}H_{11}F_3N_2O$): C, H, N.

(v) *N*-[3-(Trifluoromethyl)phenyl]quinoline-8-carboxamide, 7: yield 22%; mp 115−116 °C; ^{1}H NMR ($CDCl_3$) δ 9.08 (1H, m), 8.97 (1H, d, J = 4.3), 8.36 (1H, d, J = 7.4), 8.16 (1H, s), 8.09 (1H, d, J = 7.9), 8.04 (1H, d, J = 7.4), 7.77 (1H, t, J = 8.4), 7.58 (1H, dd, J_1 = 8.4, J_2 = 4.3), 7.51 (1H, t, J = 7.9), 7.39 (1H, d, J = 7.9). Anal. ($C_{17}H_{11}F_3N_2O$): C, H, N.

(vi) 7-Methyl-*N*-[4-(trifluoromethyl)phenyl]quinoline-8-carboxamide, 8: yield 22%; mp 190−192 °C; ^{1}H NMR (DMSO-d_6) δ 13.40 (1H, s), 9.14 (1H, dd, J_1 = 4.3, J_2 = 1.6), 8.61 (1H, dd, J_1 = 8.3, J_2 = 1.6), 8.33 (1H, d, J = 8.2), 8.17 (2H, d, J = 8.1), 7.81 (2H, t, J = 7.9), 7.70 (2H, m), 2.53 (3H, s). Anal. ($C_{18}H_{13}F_3N_2O$): C, H, N.

(vii) *N*-[4-(Trifluoromethyl)phenyl]-2-(trifluoromethyl)quinoline-8-carboxamide, 9. To a cooled (−78 °C) solution of 8-bromo-2-(trifluoromethyl)quinoline **15**[15] (1.0 g, 3.6 mmol) in diethyl ether (50 mL) containing *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (2.0 mL, 13.5 mmol) was slowly added *n*-BuLi (3.12 mL of a 1.6 M solution in hexane, 4.6 mmol). After 30 min of stirring at this temperature, 4-(trifluoromethyl)-phenylisocyanate (0.71 mL, 4.9 mmol) was added dropwise, the cooling bath was removed, and the reaction was allowed to reach room temperature. After addition of water and extraction of the product with EtOAc, the organic phase was dried over $MgSO_4$ and concentrated. Chromatographic purification (eluent of hexane/EtOAc = 3:1) followed by recrystallization (hexane/EtOAc) afforded **9** (0.42 g, 30%): mp 155−157 °C; ^{1}H NMR (DMSO-d_6) δ 12.40 (1H, s), 8.98 (1H, d, J = 8.6), 8.62 (1H, d, J = 7.4), 8.47 (1H, d, J = 6.6), 8.21 (1H, d, J = 8.6), 8.03 (3H, m), 7.82 (2H, d, J = 8.6). Anal. ($C_{18}H_{10}F_6N_2O$): C, H, N.

(viii) *N*-[4-(Trifluoromethyl)phenyl]-1,2,3,4-tetrahydroquinoline-8-carboxamide, 10. A solution of **3** (0.05 g) in MeOH (30 mL) containing DMF (0.5 mL) was hydrogenated over Pd/C overnight. After removal of the catalyst, the crude was concentrated to give **10** (0.46 g, 95%): mp 252−254 °C; ^{1}H NMR (DMSO-d_6) δ 10.49 (1H, s), 7.98 (2H, d, J = 8.2), 7.80 (2H, d, J = 8.2), 7.55 (1H, d, J = 6.6), 7.15 (1H, m), 7.09 (1H, d, J = 6.6), 6.61 (1H, t, J = 6.6), 3.35 (2H, t, J = 4.5), 2.78 (2H, t, J = 5.2), 1.82 (2H, m). Anal. ($C_{17}H_{15}F_3N_2O$): C, H, N.

(ix) (7-Methyl)quinoline-8-carboxylic Acid, 11. To a suspension of 2-amino-6-methylbenzoic acid (10.0 g, 6.6 mmol) in 1,2-dichlorobenzene (150 mL) at 140 °C was slowly added acrolein (1.1 mL, 3.3 mmol) with stirring, and the reaction was further stirred at this tempereature for 3 h. After the mixture had cooled to room temperature., the solvent was evaporated, leaving a solid crude that mainly contained the starting benzoic acid. Chromatographic separaration (eluent of EtOAc) afforded (0.14 g, 12%): mp 142−143 °C; ^{1}H NMR (DMSO-d_6) 14.2 (1H, broad s), 8.94 (1H, m), 8.41 (1H, d, J = 7.2), 7.98 (1H, d, J = 7.2), 7.57 (2H, m), 2.47 (3H, s). ($C_{11}H_9NO_2$) C, H, N.

(2) Pharmacology. (a) Inhibition of Murine TNP-LPS Responses in Vivo. OF1 mice were immunized iv with approriate concentrations of TNP-LPS (eliciting a TI-1 antibody response). To determine the effect of compounds, mice received compounds on the day of immunization (day 0) and the three following days either by sc or by po application (total of four compound applications). On day 6, mice were bled and DNP-specific antibodies of the IgM and IgG class in the serum of individual mice were determined by ELISA using DNP−BSA-coated plates and appropriate developing reagents. Titers resulting in a defined OD at 405 nm were recorded.

(b) Heterotopic Hamster Heart Xenografting in Athymic Mice. The heart of a Syrian hamster was heterotopically transplanted in the abdomen of a congenitally athymic (nu/nu) C57Bl/6 mouse, with anastomoses between the donor and recipient's aorta and between the donor's right pulmonary artery and the recipient's inferior vena cava. Compounds were dissolved in Labrafil 330 mg/mL, absolute ethanol 110 mg/mL, and 1 mL of corn oil and diluted with pure corn oil (2:3 v/v). The graft was monitored daily by palpation of the abdomen. Rejection was concluded with cessation of heartbeat and confirmed histologically. Animals were terminated after 4 weeks, and the transplant was subjected to conventional histology.

(3) Pharmacokinetics. Male mice (OF1, ∼25 g) were administered a solution (Labrafil plus ethanol plus corn oil) of **4** either orally or by injection in the femoral vein with a solution (polyethoxylated castor oil plus ethanol plus aqueous NaCl). Blood was collected from the vena cava (four mice/time point) at 0.12, 0.25, 0.50, 1, 2, 4, 8, and 24 h after the beginning of the experiments. Electrospray mass spectroscopy was used for the detection of the parent compound, and quantification was based on integration of the peaks of the HPLC chromatogram corresponding to the ion *m*/*z* 317.

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