A-770041, a Novel and Selective Small Molecule Inhibitor of Lck, Prevents Heart Allograft Rejection


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Abstract

Lck, one of eight members of the Src-family of tyrosine kinases, is activated following T cell stimulation and is required for T cell proliferation and IL-2 production. Inhibition of Lck has been a target to prevent lymphocyte activation and acute rejection. Here, we report the pharmacologic characterization of A-770041; an orally bioavailable pyrazolo[3,4-d]pyrimidine with increased selectivity for Lck compared to previously reported compounds. A-770041 is a 147 nM inhibitor of Lck (1 mM ATP) and is 300-fold selective against Fyn, the other Src-family kinase involved in T cell signaling. Concanavalin A stimulated IL-2 production in whole blood is inhibited by A-770041 with an EC₅₀ of about 80 nM. A-770041 is orally bioavailable (F=34.1 ± 7.2% at 10 mg/kg) and has a t₁/₂ 4.1 ± 0.1 h. Concanavalin A induced IL-2 production in vivo is inhibited by oral administration of A-770041 (in vivo EC₅₀ = 78 ± 28 nM). Doses of A-770041 at or above 10 mg/kg/day prevent rejection of hearts transplanted heterotopically in rats from Brown Norway donors to Lewis recipients across a major histocompatibility barrier for least 65 days. Grafts from animals treated with 20 mg/kg/day A-770041 or 10 mg/day Cyclosporin A had minimal microvascular changes or multifocal mononuclear infiltrates. However, mineralization in myocytes from the grafts from A-770041 treated animals was less than animals treated with Cyclosporin A. Lck inhibition is an attractive target to prevent acute rejection.
Introduction

One of the most proximal signaling proteins downstream of the T cell receptor (TCR) is the Src-family tyrosine kinase Lck (Straus and Weiss, 1992). T cells are activated when they contact antigen associated with MHC proteins inducing multimerization of CD4 or CD8 co-receptors present on the T cell. Autophosphorylation of Lck associated with these receptors occurs, resulting in increased kinase activity. CD4/CD8 co-receptors associate with the TCR zeta chain resulting in the phosphorylation of its ITAMs (Barber et al., 1989). ZAP-70 binds to phosphorylated zeta chain through its tandem SH-2 domains where it is phosphorylated by Lck and subsequently undergoes autophosphorylation (Duplay et al., 1994). These events ultimately lead to PLCγ activation and the release of calcium from intracellular stores generating the calcium signal required for calcineurin activation and production of cytokines such as IL-2 and interferon-γ (Weiss and Littman, 1994).

Lck plays a crucial role in the maturation of lymphocytes in the thymus and in mature T cell activation and proliferation (Molina et al., 1992; Straus and Weiss, 1992). Recently, recruitment of Lck to the cell membrane by CD4 has been postulated to play a crucial role to enhance T-cell sensitivity and may regulate the amount of antigen required to activate the T-cell (Li et al., 2004). Lck -/- mice have very few circulating mature T-cells since Lck is required for thymocytes to progress to the double negative stage (Molina et al., 1992). Mice with an inducible Lck transgene on an Lck -/- background are reported to have normal numbers of circulating T-cells but the cells do not produce IL-2 or proliferate in response to CD3/CD28 stimulation (Legname et al., 2000; Seddon et al., 2000).

Knockout studies support the notion that inhibition of Lck should prevent acute rejection of transplanted organs. Skin grafts transplanted from wild-type mice to Lck -/- mice across a major histocompatibility barrier survive indefinitely (Wen et al., 1995). Knockout mice are useful in the study of T-cell development but are less valuable in understanding the kinase inhibitory effects and function on mature T-cells, hence, inhibitors of Lck are being investigated to prevent rejection of transplanted solid organs. A non-selective Lck inhibitor discovered by Abbott Laboratories, A-420983, has been shown to limit rejection in a non-vascularized neonatal heart.
transplantation model as well as prevent acute rejection of pancreatic beta cells transplanted across major histocompatibility barriers (Waegell et al., 2002; Borhani et al., 2004).

Although A-420983 is a 37 nM Lck inhibitor, it lacks selectivity within the Src family. In particular, it exhibits only 8-fold selectivity against Fyn, a kinase that also plays a role in T cell signaling (Borhani et al., 2004). Besides the role of Fyn in T cell maturation and activation, it plays a role in myelination of neurons (Umemori et al., 1994; Umemori et al., 1999), sertoli cell maintenance of germ line stem cells in the testicle (Maekawa et al., 2002) and degranulation of mast cells (Parravicini et al., 2002). A-420983 is also equipotent against Src and only 10-fold selective against Fgr. Src plays a role in bone formation and osteoclast function (Lowe et al., 1993) and Src -/- mice develop osteopetrosis as a result of the decreased resorption of bone by osteoclasts (Soriano et al., 1991). Hck/Fgr double knockout mice show defects in neutrophil adhesion, and activation leading to defective respiratory burst and degranulation that could have consequences innate immunity (Lowell et al., 1996; Lowell and Berton, 1998). We continued our chemistry effort to find a molecule with greater selectivity for Lck to minimize the possibility that efficacy was driven by co-inhibition of both Fyn and Lck. We also wanted to find a molecule with greater selectively against Src and Fgr to avoid the potential detrimental effects that have been implied from studies from knockout mice for these Src family members.

Subsequent synthetic chemistry efforts resulted in the discovery of A-770041 (Figure 1); a compound with increased selectivity for Lck over other Src family members compared to A-420983. The purpose of these studies was to determine whether a more selective Lck inhibitor that does not inhibit Fyn, A-770041, could prevent T-cell activation, IL-2 production \textit{in vivo} and rejection of vascularized transplanted heart in a rodent model. In the present studies, it is demonstrated that A-770041 abolishes the production of IL-2 \textit{in vitro} and \textit{in vivo}. Furthermore, A770041 prevents the rejection of fully mismatched vascularized heart allografts in rats and results in prolonged survival of grafts similar to treatment with Cyclosporin A.
Materials and Methods

Animals. Lewis and Brown Norway rats (male, 200-300 g) were obtained from Charles River Laboratories (Wilmington, MA), maintained on a 12 h light/dark cycle and provided food and water ad libitum. All animal studies were reviewed by the Abbott Bioresearch Center IACUC and complied with AAALAC guidelines and the “Guide for the Care and Use of Laboratory Animals”.

Compounds. A-770041 was synthesized by Abbott Bioresearch Center, Worcester MA. Compound was dissolved in a 5% ethanol in sterile water for all in vivo studies. CsA (Neoral) was purchased from Novartis (East Hanover, NJ). CsA was diluted in 5% ethanol in sterile water was used for all in vivo studies.

HTRF-Kinase Assay. The purified recombinant tyrosine kinase to be tested was mixed with biotynylated Lck peptide substrate and varying inhibitor concentrations with 1 mM ATP, 10 mM Mg^{2+} and 2 mM Mn^{2+}. After incubation for 60 minutes, a europium cryptate labeled anti-phosphotyrosine and streptavidin labeled allophycocyanin were added to the well. The ratio of the signal of 620 nm and 665 nm were used to calculate IC_{50}.

Anti-CD3-Induced IL-2 in Whole Blood. Heparinized human whole blood was stimulated with αCD3 monoclonal antibody and PMA in the presence of A-770041 (0-30 µM). IL-2 release into plasma was determined 2 hours after stimulation by an ELISA.

Concanavalin A-Induced Cytokine Production. Male Lewis Rats were dosed with 2.5 mg/kg A-770041 or vehicle (1 mL/kg). At 2, 6, 10 and 22 hours after dosing of the test compound, rats were administered 5 mg/kg concanavalin A (0.5mL in saline, Amersham Pharmacia Biotech AB, #2000-06) intravenously via the tail vein. Two hours after concanavalin A administration, (i.e., 4, 8, 12 and 24 hours after test compound dosing) rats were euthanized with CO_{2} and blood was collected by cardiac puncture into heparinized tubes and plasma was separated. The plasma
samples were divided into two aliquots, one for the assay of A-770041 concentration (200 µL) and one for the detection of IL-2 by ELISA (100 µL).

**Heterotopic Heart Transplantation.** Hearts were transplanted between Brown Norway (donor) and Lewis (recipient) rats essentially as described by Korecky and Masika (Korecky and Masika, 1990) with modifications. Recipient animals were treated with compounds beginning on Day –1 and the day of surgery was denoted as Day 0. The donor and recipient were anesthetized by inhalation of isoflurane and the dorsal fur was shaved. The graft harvest in the donor and preparation of the recipient for implantation occurred simultaneously using one surgeon for each procedure to minimize ischemic time. In the donor, the superior and inferior vena cava, the pulmonary veins and the vena azygous were ligated with 5-0 silk sutures. The pulmonary artery and aorta were isolated and transected just proximal to the first branch of the pulmonary artery. The organ was immediately flushed with 5 mL of ice-cold, sterile, lactated Ringer’s solution and submerged in the solution. The aorta and pulmonary artery were dissected and surrounding adipose tissue were removed. The recipient was prepared by making an incision along the midline. The abdominal contents were warped in a gauze soaked with warm (37°C) Ringer’s solution and moved to the left. The inferior vena cava and abdominal aorta were dissected and separated. The heart was implanted by end to side anastomoses of the graft aorta to the recipient abdominal aorta and the graft pulmonary artery to the vena cava with 7-0 synthetic suture. The clamp was released and the anastomoses were compressed with a collagen pad to stop bleeding. Once the heart began to beat and the bleeding was stopped, the abdomen was closed and anesthesia was withdrawn. The abdomen was palpated daily to determine if the graft was beating. Blood was collected either via the tail vein (200 µL) during survival studies or from the vena cava upon scheduled sacrifice, allowed to clot and serum was separated and kept at –70°C until analysis.

**Detection of A-770041.** A-770041 concentrations in plasma or serum were analyzed by protein precipitation and liquid chromatography/mass spectrometry/mass spectrometry (LC-MS/MS)
analysis. The drug precipitation protocol was performed using robotic sample preparation on a Tecan Genesis workstation. To 50µL of plasma or serum was added 200 µL of internal standard solution the resultant precipitate was filtered using a 96 well format 3M filter plate. The filtrate was diluted 1:5 mL with 35% acetonitrile in water, and 10 µL was injected onto a 3.5 µM Xterra C18 high performance liquid chromatography column, eluted at 0.85 mL/min using a gradient mobile phase consisting of acetonitrile and 0.2% formic acid. The concentrations were determined by LC-MS/MS using an API 4000 instrument in positive electrospray mode. The calibration curve ranged from 1nM to 3 µM.

**Histology.** On Day 65 post transplantation, the animal was euthanized with CO₂ and the graft was removed and placed in formalin. The tissue was embedded, cut and stained with hematoxyllin and eosin. All sections were assessed by a pathologist blinded to the treatments.

**Statistics.** Data presented as the mean ± SD of at least n=4 measurements. Data from the survival studies are presented as percentages with at least n=6 per treatment group. Differences in survival rates were determined with the Mann-Whitney rank sum test.

**Results**

**Structure, Physical and Chemical Characteristics and Selectivity of A-770041**

The structure, molecular weight and ClogP of A-770041, an ATP-competitive inhibitor of Lck, are shown in Figure 1. A-770041 is a pyrazolo[3,4-d]pyrimidine with an IC₅₀ of 147 nM against recombinant human Lck (64-509) in the presence of 1 mM ATP (Kₘ= 12 µM). Inhibition of the kinase activity of other Src-family members by A-770041 is shown in Table 1. A-770041 is 60-fold selective against Src, 95-fold selective against Fgr and 300-fold selective against Fyn. A-770041 was also greater than 200-fold selective against a secondary battery of about 20 serine/threonine and tyrosine kinases outside of the Src family and IC₅₀ values greater than 10 µM in a CEREP panel of about 70 molecular targets (data not shown).
Effect of A-770041 on of T cell Activation In Vitro

The effect of A-770041 on anti-CD3 stimulated IL-2 production in whole blood was determined (Figure 2). IL-2 production was decreased in a dose-dependent manner. The EC$_{50}$ for inhibition of anti-CD3 induced IL-2 was 80 nM and IL-2 was suppressed greater than 90% at all concentrations above 1 µM.

Single Dose Pharmacokinetics of A-770041

Lewis rats were dosed intragastrically with A-770041 at 10 mg/kg or intravenously at 5 mg/kg. Blood was collected from the tail vein at several different time points throughout a 36-hour period. The plasma concentration of A-770041 was determined and is shown in Figure 3. A-770041 has a t$_{1/2}$ of about 4.2 hours and a bioavailability of 34% with a C$_{max}$ of 2186± 410 nM at 5.3 hours at a dose of 10 mg/kg. Single dose pharmacokinetic studies showed that the AUC scaled linearly with dose up to 30 mg/kg when the compound was given orally (data not shown). Pharmacokinetic modeling of the exposures from this single oral dose studies was completed to determine an acceptable dosing regime of A-770041 that would be expected to maintain C$_{min}$ blood concentrations above 1 µM with repeated dosing which was expected to significantly inhibit Lck throughout the dosing period. It was determined that twice daily oral dosing in rats is required to maintain C$_{min}$ above 1 µM.

Effect of A-770041 on Concanavalin A-Induced IL-2 Release In Vivo

Compounds that inhibit Lck in vivo should prevent the activation of T cells by Concanavalin A and the subsequent release of IL-2 in a dose-dependent manner. Animals were dosed intragastrically with 2.5 mg/kg A-770041, Concanavalin A was administered intravenously via the tail vein at various times after drug treatment and blood was collected two hours after Concanavalin A, as described in Methods. The correlation of Concanavalin A-induced IL-2 levels to A-770041 concentrations in the serum is shown in Figure 4. Inhibition of Concanavalin A-induced IL-2 was shown to be dependent upon plasma concentration of A-770041 (open circles).
with an in vivo EC$_{50}$ of 78 ± 28 nM. The inhibition of IL-2 in vivo by A-770041 fits to a sigmoidal curve ($r^2=0.79$, Figure 4, line).

**The Effect of A-770041 on Rejection of Heterotopically Transplanted Hearts**

Recipient animals were treated with vehicle control, or 2.5-20 mg/kg/day of A-770041 in equally divided doses 12 hours apart beginning the day before surgery. Hearts were transplanted heterotopically from a Brown Norway donor to a Lewis recipient, as described in Methods. Animals were treated for 14 days and graft viability was assessed by abdominal palpation each day. As expected, hearts transplanted into animals receiving the vehicle control ceased beating between Days 6 and 7 (Figure 5). A-770041 dosed at 2.5 mg/kg/day did not lengthen graft survival time. There was a dose-dependent increase in survival with doses of 5 and 10 mg/kg/day. At doses of 10 and 20 mg/kg/day of A-770041, 100% of transplanted grafts were still beating at 14 days.

Plasma concentrations of A-770041 (Table 2) were determined 12 hours after compound administration on Days 3, 7 and 14 (C$_{12h}$) just prior to the administration of the next dose or termination of the study. As expected, plasma concentration of A-770041 increased almost linearly with dose and had reached steady state by Day 3.

With the positive result from the 14-day study, a subsequent experiment was completed to determine the effect of A-770041 on long-term survival of heart allografts and comparison to Cyclosporin A. Recipients were treated beginning the day before heterotopic heart transplantation with 10 or 20 mg/kg/day of A-770041 in equally divided doses 12 hours apart or 10 mg/kg/day of Cyclosporin A once per day for 65 days. All grafts (n=6/group) in animals receiving 10 or 20 mg/kg/day A-770041 or 10 mg/kg/day Cyclosporin A survived to 65 days after transplantation. C$_{12h}$ serum concentrations of A-770041 from both groups at on Day 7, 34, 60 and 65 days were comparable to the concentrations seen in the 14-day study (data not shown).

Animals were sacrificed at Day 65 to harvest the transplanted graft for histological analysis. Representative photomicrographs of allografts are shown in Figure 6. Allografts from rats dosed with 10 mg/kg/day A-770041 (A and B) or 10 mg/kg/day Cyclosporin A (E and F) had
minimal multifocal mononuclear infiltrates in allografts (arrows). The allografts from animals dosed with 10 mg/kg/day A-770041 had an increased incidence of minimal vascular changes characterized by tunica media hypertrophy, vacuolated myocytes, and reactive endothelial cells (vessel, Panel B) compared to the Cyclosporin A or 20 mg/kg/day A-770041 groups. In addition, one graft from the 10 mg/kg/day A-770041 group had minimal vasculitis (not shown). There was also increased incidence of minimal edema (e) within the 10 mg/kg/day A-770041 and 10 mg/kg/day Cyclosporin A groups. Mineralization was present in allografts from the Cyclosporin A group, with minimal to mild scores in three of five allografts but was not seen in grafts from animals treated with A-770041 (not shown). Additionally, allografts from the Cyclosporin A group had neovascularization extending intramurally from the pericardium.

Discussion

A-770041 is a selective inhibitor of Lck that shows comparable efficacy to Cyclosporin A in preventing acute transplant rejection in solid organs. This compound has prototypical activity of an immunosuppressive agent by blocking T-cell activation and IL-2 production in vitro and in vivo. Pharmacokinetic data show that A-770041 is amenable to chronic, oral, twice daily dosing in rats with compound reaching a steady state $C_{12h}$ in blood by Day 3 and maintaining it through Day 65. Oral doses of A-770041 at 10 and 20 mg/kg/day prevented acute rejection for 65 days after transplantation; at which point the experiment was concluded. The 10 mg/kg/day dose limited rejection but did not completely prevent the infiltration of mononuclear cells into the allograft. These data show that a specific inhibitor of Lck is efficacious in preventing acute rejection in a rodent model.

The serum concentrations of A-770041 measured in the dose-response experiments of Concanavalin A-mediated IL-2 production in vivo compared to the results of 14 day and 65 day transplantation studies provide some interesting predictions concerning the efficacious plasma concentrations of A-770041 needed to prevent acute rejection. One hundred percent survival of grafts at either 14 days or 65 days after transplantation only occurred when the $C_{\text{min}}$ concentrations for A-770041 were maintained above the EC$_{90}$ for inhibiting IL-2 production in
vivo. This point is most clearly illustrated by the lack of efficacy of A-770041 when dosed at 2.5 mg/kg/day and 5 mg/kg/day. In both of these treatment groups, the $C_{\text{min}}$ serum concentrations are well below the EC$_{90}$ for inhibition of Concanavalin-A induced IL-2. These data suggest, similar to the data published with Cyclosporin A, that IL-2 production must be nearly maximally inhibited to prevent acute rejection of transplanted organs.

Lck is an attractive target to block T cell activation for a number of reasons (Tsutsui et al., 2003). Primarily, Lck is crucial in the activation of T cells causing clonal expansion and generation of a cytotoxic T cell response (Weiss and Littman, 1994). Secondly, it is expected that inhibitors of Lck should prevent rejection of transplanted organs as evidenced by prolonged skin allograft survival in Lck -/- mice (Wen et al., 1995). The data presented here and in a previous publication with a first generation Lck inhibitor (A-420893) shows the potential of an inhibitor of Lck to prevent acute rejection (Waegell et al., 2002; Borhani et al., 2004).

Additionally, the expression of Lck is thought to be limited to T cells, NK cells, B1 cells, heart, brain and retinal neurons (Omri et al., 1998; Ping et al., 2002). While expression of Lck in the brain and heart has been reported, Lck -/- mice have not been reported to have any cognitive or cardiovascular deficits. The only reported detrimental effect of knocking out Lck in non-immune tissues is retinal dysplasia and retinal detachment (Omri et al., 1998). Whether this is a developmental or functional defect from the lack of Lck expression will need to be explored with potent, selective inhibitors of Lck such as A-770041.

Lck -/- mice show thymic atrophy from a reduction in double positive thymocytes and profound lymphopenia with a 100-fold reduction in mature CD4 cells and a 3-fold reduction in mature CD8 cells (Molina et al., 1992). While the lack of Lck expression through development of the knockout mouse certainly affects thymopoiesis, lack of Lck expression in peripheral mature T-cells does not alter survival (Legname et al., 2000; Seddon et al., 2000). In a doxycycline-inducible Lck-transgenic mouse on an Lck-/- background, when doxycycline is fed through development, there is a relatively normal repertoire of mature T cells. Cessation of doxycycline treatment and loss of Lck expression results in decreased thymopoiesis but no effects on the numbers of circulating mature T-cells for several months. Circulating T cells from the Lck -/- mice...
have a significantly decreased proliferative response, calcium mobilization and IL-2 production (Straus and Weiss, 1992; Trobridge and Levin, 2001). These data suggest that the inhibition of Lck with a small molecule should have no effect on the number of mature circulating T cells or survival of these cells but should be expected to block activation and proliferation.

Inhibitors of Lck may also be efficacious in other inflammatory diseases, including rheumatoid arthritis, multiple sclerosis, inflammatory bowel diseases, type 1 diabetes, systemic lupus erythematosus and psoriasis (Kamens et al., 2001). In conclusion, we have discovered A-770041, a selective inhibitor of Lck that is orally active, inhibits T cell activation and proliferation and prevents allograft rejection in transplanted hearts similar to Cyclosporin A for up to 65 days. These data show that selective inhibitors of Lck have the potential to be efficacious in preventing acute rejection. These inhibitors would be expected to avoid the renal effects of long-term therapy with Cyclosporin A.

Acknowledgements

Special thanks are extended to Ms. Elizabeth O’Connor and Mr. Jamie Erickson for expert processing of the tissue sections from this study and ABC Bioresources for help with dosing and professional care of animals on this study.
References

Barber EK, Dasgupta JD, Schlossman SF, Trevillyan JM and Rudd CE (1989) The CD4 and CD8 antigens are coupled to a protein-tyrosine kinase (p56lck) that phosphorylates the CD3 complex. Proc Natl Acad Sci U S A 86:3277-3281.


Figure Legends

Figure 1. Structure, Molecular Weight and ClogP of A-770041.

Figure 2. Effect of A-770041 CD3-mediated IL-2 Production in Whole Blood. IL-2 released from human whole blood was determined as described in Materials and Methods. A-770041 was added (0-30 μM) at time 0. IL-2 was determined at 24 hours. Data are mean ± SD (n=4).

Figure 3. Single Dose Pharmacokinetics of A-770041 in the Rat. A-770041 was dosed intravenously (5 mg/kg) or orally (10 mg/kg) and blood samples were collected from the tail vein as described in Materials and Methods. Data are mean ± SD from three animals for each route of administration.

Figure 4. Inhibition of Concanavalin A-Induced IL-2 Release In Vivo. Rats were dosed orally with 2.5 mg/kg A-770041 and Concanavalin A was given intravenously at 2, 6, 10 and 22 hours after A-770041. Two hours after Concanavalin A, blood was collected and IL-2 and A-770041 concentrations were determined in serum. Data points (circles) are representative of one blood sample each.

Figure 5. Effect of A-770041 on 14 Day Survival of Heterotopically Transplanted Heart Allografts. Recipient Lewis rats were treated with A-770041 (0-20 mg/kg/day) starting the day before surgery and for 14 days after transplantation of a heart from a Brown Norway donor. The abdomen was palpated daily to assess graft viability. Data are plotted as percentage of grafts surviving daily (n=6/group). Statistical differences in survival between groups were determined by Mann-Whitney rank sum test.

Figure 6. Histology of Transplanted Heart Grafts at 65 Days after Transplantation. Recipient Lewis rats were treated with A-770041 (10-20 mg/kg/day) or Cyclosporin A (10 mg/kg/day) starting the day before surgery and for 65 days after transplantation of a heart from a
Brown Norway donor. Sections of the transplanted graft were collected and processed as described in Materials and Methods. A) and B), A-770041, 10 mg/kg/day; C) and D), A-770041, 20 mg/kg/day; E) and F) Cyclosporin A, 10/mg/kg/day. A), C) and E) are low magnification and B), D), and F) are high magnification. >, representative areas of inflammation; e, representative areas of edema.
Table 1. Activity of A-770041 Against Selected Src-family kinases

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<tr>
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<th>IC₅₀ (µM)</th>
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<tbody>
<tr>
<td>Lck</td>
<td>0.147</td>
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<tr>
<td>Src</td>
<td>9.1</td>
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<tr>
<td>Fgr</td>
<td>14.1</td>
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<tr>
<td>Fyn</td>
<td>44.1</td>
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*Activity against Src-family kinases was determined as described in Materials and Methods. The ATP concentration was 1 mM.*
Table 2. Serum Concentrations of A-770041 in the 14-day Transplantation Study\textsuperscript{a}

<table>
<thead>
<tr>
<th>Study Day</th>
<th>A-770041 (mg/kg/day) C\textsubscript{12h} (nM)</th>
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<tbody>
<tr>
<td></td>
<td>2.5</td>
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<tr>
<td>Day 0</td>
<td>128 ± 18</td>
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<td>Day 3</td>
<td>222 ± 63</td>
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<tr>
<td>Day 7</td>
<td>278 ± 58</td>
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<tr>
<td>Day 14</td>
<td>191 ± 29</td>
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\textsuperscript{a}Serum concentrations of A-770041 were measured as described in Materials and Methods. Data are presented as mean ± SD (n=6).
Molecular Weight = 621.74
ClogP = 2.958

A-770041

Figure 1
Figure 2

Graph showing the effect of A-770041 (M) on IL-2 production (% of control). The x-axis represents the concentration of A-770041 in M, ranging from 0 to 10^-4 M. The y-axis shows the percentage of control IL-2 production, ranging from 0 to 100%.
Figure 3
Figure 4
Figure 5