Supplemental Materials

NF-κB Reporter Assay

TLR agonist activity was detected by assessing the activation of the transcription factor NF-κB in Human Embryonic Kidney 293 reporter cells stably expressing individual murine TLRs. In these cells activation of TLRs leads to translocation of NF-κB to the nucleus with subsequently induced expression of the reporter protein secreted alkaline phosphatase (SEAP). SEAP activity is quantified in culture supernatants by addition of a colorimetric substrate and monitoring the optical density at 650 nm on a Beckman Coulter AD 340C Absorbance Detector at the end of the incubation period. Mouse TLR-expressing reporter cells were plated at a density of 50,000 per well (96-well format) and incubated with the vehicle control, the respective TLR agonist positive controls or GS-9620 for 16-20 hours in a humidified 5% CO_2 incubator. The following control stimuli were provided by InvivoGen: TLR2: heat-killed *Listeria monocytogenes*; TLR3: poly I:C; TLR4: *Escherichia coli* K12 LPS; TLR5: *Salmonella typhimurium* flagellin; TLR7: CL097 (imidazoquinoline compound); TLR8: CL075 (thiazoquinoline compound); and TLR9: CpG oligodeoxynuclotide 1826. All compounds and controls were tested in duplicate for each TLR expressing cells.

In the receptor screening format of the assay, GS-9620 was tested at 100 µM on murine TLR2, TLR3, TLR4, TLR5, TLR7, TLR8 and TLR9 expressing reporter cells. Following initial identification of receptor agonist activity a subsequent experiment was then carried out to

determine the dose responsiveness of GS-9620 on murine TLR7-expressing reporter cells. In these assays, GS-9620 concentrations ranged from 10 µM with 10-fold serial dilutions to 10 pM.

Splenocyte and PBMC In Vitro Cytokine Release Assays

Fresh splenocytes were harvested and pooled from spleens (6 male mice per splenocyte isolation) or total PBMC were isolated from whole blood (2 per sex for cynomolgus monkeys) from healthy animals using standard Ficoll density gradient separation centrifugation and isolation techniques under sterile conditions. PBMCs or splenocytes were plated in duplicate at a density of 750,000 cells per well (96-well format) in 190 µL RPMI with Glutamax™ (Gibco), 10% heat inactivated fetal bovine serum (Hyclone, Logan, UT), Penicillin 100 Units/mL Streptomycin 100 µg/mL (Gibco, Carlsbad, CA). GS-9620 was serially diluted (0.5 $\log_{10}$ concentrations) into DMSO from a 10 mM DMSO stock solution onto a master plate to keep DMSO concentrations constant through the drug dilution series. Appropriate vehicle controls were included for each donor. After the incubation period culture supernatants were harvested and analyzed for levels of secreted proteins.

For in vitro cytokine induction studies, the following cytokines were evaluated by Luminex for cynomolgus monkeys: CCL3, CCL4, IL-1ra, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p40, IL-15, IL-17, IL-18, IFN-γ, sCD40L, and TNF-α, and for mice: CXCL2, CCL4 G-CSF, IL-1α, IL-1β, IL-6, IL-10, IL-12p40, IL-12p70, IL-17, IP-10, IFN-γ, and TNF-α. For detection of mouse and cynomolgus monkey IFN-α, ELISA kits were used. Murine IFN-α was determined using kits from PBL InterferonSource (Piscataway, NJ). Cynomolgus monkey IFN-α was quantified using a human IFN-α ELISA kit and a purified Rhesus/Cynomolgus IFN-α2 standard from PBL InterferonSource (Piscataway, NJ).

ELISA kits were used according to the manufacturer's instructions. Standard curves for each cytokine were generated using a nonlinear five-parameter curve fit. Median fluorescent intensities or OD (ELISA only) of experimental samples were interpolated against the standard curve to determine cytokine concentrations in cell culture supernatants.

Minimum effective concentration (MEC) values were defined as the concentration of GS-9620 that produced a cytokine concentration at least 3-fold above the DMSO control and that the next highest concentration of GS-9620 also produced a cytokine concentration at least 3-fold over background (ie, the beginning of a dose-response curve).

Animals

Naïve male and female Crl:CD1 mice and cynomolgus monkeys (*Macaca fascicularis*) were obtained from Charles River Laboratories or Covance Research Products Inc., respectively. Mice were approximately 7-9.5 weeks old and weighed between 19.9 and 44.6 g at initiation of dosing. Cynomolgus monkeys were approximately 2-6 years old and weighed between 2.3 and 7.3 kg at initiation of dosing. Animals were housed in temperature controlled rooms (18-26ºC or 20-26ºC mice and monkeys, respectively) with a 12-hour light/dark cycle ad libitum food and water.

Cytokine Analysis for in vivo studies

For in vivo cytokine induction studies, the following cytokines, or subset thereof, were evaluated by Luminex for cynomolgus monkeys: CCL2, CCL3, CCL4, CCL5, CD40L, CXCL9, G-CSF, GM-CSF, IFN-γ, IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IL-12p40, IL-12p70, IL-17, IL-18, IP-10, I-TAC, TGF-α and TNF-α and for mice: CCL2, CCL3, CCL4, CCL5 CCL11, CXCL1, CXCL2, CXCL9, G-CSF, GM-CSF, IFN-β, IFN-γ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-

5, IL-6, IL-7, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, IP-10, LIF, LIX, M-CSF, and TNF-α. Detection of mouse and cynomolgus monkey IFN-α were measured using VeriKine murine IFN-α serum ELISA kit and VeriKine Cynomolgus/Rhesus IFN-α serum ELISA kit, respectively (PBL InterferonSource, Piscataway, NJ). The lower limit of quantitation (LLOQ) for serum IFN-α ranged from 8 to 39 pg/ml for mouse and 25 to 130 pg/mL for cynomolgus monkey, depending on the dilution factor used.

ELISA kits were used according to the manufacturer's instructions. Standard curves for each cytokine were generated using a nonlinear five-parameter curve fit of fluorescent intensities (multiplex) or OD (ELISA). Median fluorescent intensities or OD values (ELISA only) of experimental samples were interpolated against the standard curve to determine cytokine concentrations in serum.

ISG Analysis for in vivo studies

1 mL of blood was pipetted into a polypropylene tube and centrifuged at $1300 \times$ g for 15 minutes within 1 hour of collection. Following centrifugation, the plasma layer was removed and discarded. The whole blood cell pellet was resuspended in 2.6 mL of RNAlater® (Qiagen Inc., Valencia, CA) solution and stored at -60 to -80℃, until shipped to the Southwest Foundation for Biomedical Research (SFBR; San Antonio TX), Covance Laboratories Inc (Madison, WI), or Gilead Sciences (Foster City, CA; mouse only) for analysis. After thawing, samples were centrifuged and the overlying RNAlater® was removed. The blood cell pellet was resuspended in up to 500 µL with PBS, and RNA was purified using Ambion RiboPure™ Kit (Applied Biosystems/Ambion, Austin, TX) according to the manufacturer's instructions.

All TaqMan$^{®}$ reactions were performed on an ABI 7900HT sequence detection system. Appropriate reactivity of the ABI Assays-on-Demand™ premixed primer/probes was verified in mouse or monkey prior to their use. The mRNA levels for the analyzed genes OAS1 and MX1 in blood were determined by the comparative C_T methodology using TaqMan$^{®}$ RNA-to-C_T 1-Step Kit (Applied Biosciences/Ambion) and specific probes.

LC/MS/MS Method

An aliquot of 50 µL of each serum sample was treated with 100 µL of cold acetonitrile containing internal standard. After the protein precipitation, the above solution was centrifuged at 3000 RPM for 20 minutes. An aliquot of 75 µL of the supernatant was mixed with 75 µL of water with 0.1% formic acid and 20 µL of the solution was injected into a HyPurity C18 HPLC column (30 × 2.1 mm; ThermoHypersil) using a TSQ Ultra Quantum LC/MS/MS system equipped with a Cohesive LX-2 multiplex system with two identical Agilent 1100 series binary pumps. The mass spectrometry was operating in positive ionization mode using selective reaction monitoring. The LLOQ for GS-9620 was 0.1 nM (or 0.04 ng/mL).

Mobile phases A and B contained 1% and 80% acetonitrile, respectively, in 10 mM ammonium formate aqueous solution containing 1% formic acid. The mobile phases program at an initial hold (0.0-1.5 min) was 0% B followed by a linear gradient 0-50% B (1.5-4.0 min); the conditions were then held at 100% B (4.0-7.0 min) and returned to the initial conditions (7.0-9.0 min). The total analysis duration was 9 min at a flow rate of 0.50 mL/min.

Pharmacokinetic Data Analysis

Pharmacokinetic parameters were calculated in individual animals by non-compartmental analysis using WinNonLin Professional (version 5.0.1; Pharsight, Mountain View, CA). The

maximum plasma/serum concentration (C_{max}) and time to reach C_{max} (T_{max}) were directly recorded from experimental observations. The area under the concentration-time curve (AUC) was calculated using the trapezoidal rule up to the last measurable concentrations. The bioavailability (F) was determined from the dose-corrected AUC after oral and i.v. administration. The components of GS-9620 availability were further predicted on the basis of the following equations: $F = F_{ABS} \cdot F_G \cdot F_H$, in which F_{ABS} is the fraction of the dose absorbed from the gut lumen, F_G is the fraction of the dose not metabolized by intestinal metabolic enzymes, and F_H is the fraction of the dose absorbed into the hepatic vein that escapes first-pass effect in the liver. F_H is defined as follows: $F_H = 1 - ER_H$, in which ER_H is the hepatic extraction ratio defined as the clearance of i.v. administered GS-9620 divided by the hepatic blood flow rate in corresponding species (Davies and Morris 1993). Bioavailability in dog portal vein ($F_{ABS} \cdot F_G$) can be simplified to absorption (F_{ABS}) assuming that gut metabolism is negligible ($F_G = 1$).

Metabolic Stability Assessment in Liver Microsomes

In this assay, mouse, rat, dog, monkey, and human liver microsomal incubations were conducted in duplicate. Reactions were commenced with the addition of NADPH and shaken in a water bath at 37°C. At t = 0 and at five time points ranging to 65 minutes, aliquots (25 μL) were removed and added to quenching solution (50 nM of internal standard with 0.2% formic acid in 95% acetonitrile/5% water). Final composition of the reaction mixture was: 0.25 mL of 2 uM compounds, 0.5 mg microsomal protein/mL, 1.25 mM NADPH (Phase I metabolism cofactor), 3.3 mM $MgCl_2$ in 50 mM phosphate buffer, pH 7.4. After quenching, the plates were centrifuged and 5 μL of the sample were injected into a LC/MS/MS.

Data (analyte to internal standard area ratios) are plotted on a semilog scale and fitted using an exponential fit:

$$C = C_o * \exp^{(-Kt)}.$$

Assuming first order kinetics, the $T_{1/2}$ and rate of metabolism are determined from the K values ($T_{1/2} = Ln(2)/K$ and rate = amount of drug/mg protein x K = 1000 pmol/mg x K).

The in vitro hepatic clearance, $CL_{in\ vitro}$, is calculated from in vitro $T_{1/2}$ data as described by Obach et al. (Obach et al 1997). Conversion of in vitro CL to hepatic CL uses the well-stirred model of hepatic clearance.

Estimation of Portal Vein Absorption In Vivo

Absorption of GS-9620 in vivo was estimated by measuring both portal vein and jugular vein concentrations in portal vein cannulated (PVC) dogs. In this study, surgical placement of portal vein catheter was performed to allow mesenteric access. Blood samples were collected from both the jugular vein and portal vein access port at the time points specified in the study design and processed to plasma. Concentrations of GS-9620 in plasma were determined by a LC/MS/MS method. The % of dose in portal vein was calculated based on similar method described in the previous section.

Pharmacokinetic Data Analysis. Total bioavailability (F) was estimated based on the exposure in jugular vein (AUC_{jv}) and the IV exposure from previous study with dose correction.

$F = (AUC_{jv}/PO\ Dose)/(AUC_{iv}/IV\ Dose)$

The fraction of the dose absorbed into the jugular vein that escapes first-pass effect is:

$F_H = AUC_{jv}/AUC_{pv}$ (AUC_{PV} is portal vein exposure).

The absorption in port vein is estimated as: $F_{ABS} = F/F_H$ (assuming $F_G = 1$).**Supplemental Fig. 1**

Chemical Structure of GS-9620
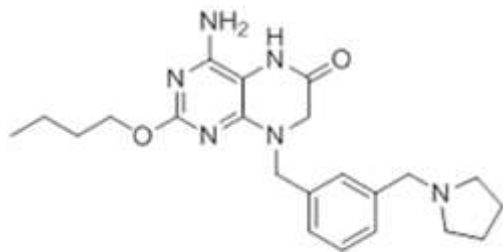
Supplement Figure 1

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TLR7_hu      MVFPMWTLKRLILILFNIILISKLLGARWFPKTLPCDVTLDVPKNHVIVDCTDKHLTEIP
TLR7_cy      MMFPVWTLKRQILILFNIILISKLLGARWFPKTLPCDVTLDVSKNHVIVDCTDKHLTEIP
TLR7_mo      MVFSMWTRKRQILIFLNMLLVSRVFGFRWFPKTLPCEVKVNIPEAHVIVDCTDKHLTEIP
TLR8_hu      MENMFLQSSMLTCIFLLISGSCELCAEENFSRSYPCDEKKQN--DSVIAECSNRRLQEVP
TLR8_cy      MENMFLQSSMLTCLFLLIPGSCELCPEENFSRSYPCEEKKQN--HCVIAECSNRRLREVP
TLR8_mo      MENMPPQSWILTCFCLLSSGTSAIFHKANYSRSYPCDEIRHN--SLVIAECNHRQLHEVP
             *               : :       . :       :.:: **:    .     **.:*..::* *:*

TLR7_hu      GGIPTNTTNLTLTINHIPDISPASFHRLDHLVEIDFRCNCVPIPLGSKNNMCIKRLQIKP
TLR7_cy      GGIPTNTTNLTLTINHIPDISPASFHRLVHLVEIDFRCNCVPIRLGSKSNMCPRRLQIKP
TLR7_mo      EGIPTNTTNLTLTINHIPSISPDSFRRLNHLEEIDLRCNCVPVLLGSKANVCTKRLQIRP
TLR8_hu      QTVGKYVTELDLSDNFITHITNESFQGLQNLTKINLNHNPNVQHQNGNPGIQSNGLNITD
TLR8_cy      QTVGKYVTELDLSDNFITHITNESFQGLQNLTKINLNHNPNVQRQNGNPGMQSNGLNITD
TLR8_mo      QTIGKYVTNIDLSDNAITHITKESFQKLQNLTKIDLNHNAKQQHPNEN----KNGMNITE
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TLR7_hu      RSFSGLTYLKSLYLDGNQLLEIPQGLPPSLQLLSLEANNIFSIRKENLTELANIEILYLG
TLR7_cy      RSFSGLTYLKSLYLDGNQLLEIPQGLPPSLQLLSLEANNIFSIRKENLTELANIEILYLG
TLR7_mo      GSFSGLSDLKALYLDGNQLLEIPQDLPSSLHLLSLEANNIFSITKENLTELVNIETLYLG
TLR8_hu      GAFLNLKNLRELLLEDNQLPQIPSGLPESLTELSLIQNNIYNITKEGISRLINLKNLYLA
TLR8_cy      GAFLNLKNLRELLLEDNQLPQIPSGLPESLTELSLIQNNIYNITKEGISRLINLKYLYLA
TLR8_mo      GALLSLRNLTVLLLEDNQLYTIPAGLPESLKELSLIQNNIFQVTKNNTFGLRNLERLYLG
             ::  .*   *    * *:.***  **  .** **   ***   ***:.: *:.     * *:: ***.

TLR7_hu      QNCYYRNPCYVSYSIEKDAFLNLTKLKVLSLKDNNVTAVPTVLPSTLTELYLYNNMIAKI
TLR7_cy      QNCYYRNPCYVSYSIEKDAFLNLTKLKVLSLKDNNVTTVPTVLPSTLTELYLYNNMIAEI
TLR7_mo      QNCYYRNPCNVSYSIEKDAFLVMRNLKVLSLKDNNVTAVPTTLPPNLLELYLYNNIIKKI
TLR8_hu      WNCYFNKVCEKTN-IEDGVFETLTNLELLSLSFNSLSHVPPKLPSSLRKLFLSNTQIKYI
TLR8_cy      WNCYFNKVCEKTN-IEDGVFETLTNLELLSLSFNSLSHVPPKLPSSLRKLFLSNTQIKYI
TLR8_mo      WNCYF-KCNQTFK-VEDGAFKNLIHLKVLSLSFNNLFYVPPKLPSSLRKLFLSNAKIMNI
             ***: :         :*...*   : :*::***. *.:   **. **..* :*:* *   *   *

TLR7_hu      QEDDFNNLNQLQILDLSGNCPRCYNAPFPCAPCKNNSPLQIPVNAFDALTELKVLRLHSN
TLR7_cy      QEDDFNNLNQLQILDLSGNCPRCYNAPFPCTPCKNNSPLQIPVNAFDALTELKVLRLHSN
TLR7_mo      QENDFNNLNELQVLDLSGNCPRCYNVPYPCTPCENNSPLQIHDNAFNSLTELKVLRLHSN
TLR8_hu      SEEDFKGLINLTLLDLSGNCPRCFNAPFPCVPCDGGASINIDRFAFQNLTQLRYLNLSST
TLR8_cy      GEEDFKGLINLTLLDLSGNCPRCFNAPFPCVPCDGGASINIDRFAFQNLTQLQYLNLSST
TLR8_mo      TQEDFKGLENLTLLDLSGNCPRCYNAPFPCTPCKENSSIHIHPLAFQSLTQLLYLNLSST
             ::**:.* :* :************:*.*:**.**. .:.::*    **: **:*  *.* *.

TLR7_hu      SLQHVPPRWFKNINKLQELDLSQNFLAKEIGDAKFLHFLPSLIQLDLSFNFELQVYRASM
TLR7_cy      SLQHVPPRWFKNINNLQELDLSQNFLAKEIGDAKFLHFLPNLIQLDLSFNFELQVYRASM
TLR7_mo      SLQHVPPTWFKNMRNLQELDLSQNYLAREIEEAKFLHFLPNLVELDFSFNYELQVYHASI
TLR8_hu      SLRKINAAWFKNMPHLKVLDLEFNYLVGEIASGAFLTMLPRLEILDLSFNYIKGSYPQHI
TLR8_cy      SLRKINAAWFKNMPHLKVLDLEFNYLVGEIASGEFLTMLPRLEILDLSFNYIKGSYPQHI
TLR8_mo      SLRTIPSTWFENLSNLKELHLEFNYLVQEIASGAFLTKLPSLQILDLSFNFQYKEYLQFI
             **: : .  **:*: :*: *.*. *:*. **  .. **   ** * **:***:     *      :

TLR7_hu      NLSQAFSSLKSLKILRIRGYVFKELKSFNLSPLHNLQNLEVLDLGTNFIKIANLSMFKQF
TLR7_cy      NLSQAFSSLKSLKILRIRGYVFKELKSFNLSPLHNLQNLEVLDLGTNFIKIANLSMFKQF
TLR7_mo      TLPHSLSSLENLKILRVKGYVFKELKNSSLSVLHKLPRLEVLDLGTNFIKIADLNIFKHF
TLR8_hu      NISRNFSKLLSLRALHLRGYVFQELREDDFQPLMQLPNLSTINLGINFIKQIDFKLFQNF
TLR8_cy      NISKNFSKLLSLRALHLRGYVFQELRKDDFQPLMQLPNLSTINLGINFIKQIDFNLFQNF
TLR8_mo      NISSNFSKLRSLKKLHLRGYVFRELKKKHFEHLQSLPNLATINLGINFIEKIDFKAFQNF
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TLR7_hu    KRLKVIDLSVNKISPSGDSSEVGFCSNARTSVESYEPQVLEQLHYFRYDKYARSCRFKNK
TLR7_cy    KRLKVIDLSVNKISPSGDSSEVGFCSNARTSVESYEPQVLEQLYYFRYDKYARSCRFKNK
TLR7_mo    ENLKLIDLSVNKISPSEESREVGFCPNAQTSVDRHGPQVLEALHYFRYDEYARSCRFKNK
TLR8_hu    SNLEIIYLSENRISPLVKDTRQSYANSSSFQRHIRKRRSTDFEFDPHSNFYHFTR-----
TLR8_cy    PNLEIIYLSENRISPLVKDTRQSYANSSSFQRHILKRRSTDFEFDPHSNFYHFTR-----
TLR8_mo    SKLDVIYLSGNRIASVLDGTDYSSWRN----RLRKPLSTDDDEFDPHVNFYHSTK-----
            .*.:* ** *:*:.  ..   .   .            :  .  : : *   :

TLR7_hu    E-ASFMSVNESCYKYGQTLDLSKNSIFFVKSSDFQHLSFLKCLNLSGNLISQTLNGSEFQ
TLR7_cy    E-ASFTSVNESCYKYGQTLDLSKNSIFFIKSSDFQHLSFLKCLNLSGNLISQTLNGSEFQ
TLR7_mo    EPPSFLPLNADCHIYGQTLDLSRNNIFFIKPSDFQHLSFLKCLNLSGNTIGQTLNGSELW
TLR8_hu    -----PLIKPQCAAYGKALDLSLNSIFFIGPNQFENLPDIACLNLSANSNAQVLSGTEFS
TLR8_cy    -----PLIKPQCAAYGKALDLSLNNIFFIGPNQFENLPDIACLNLSANSNAQVLSGTEFS
TLR8_mo    -----PLIKPQCTAYGKALDLSLNNIFIIGKSQFEGFQDIACLNLSFNANTQVFNGTEFS
                 ::  .*   **::**** *.**::  .:*: :  : ***** *   *.:.*:*:

TLR7_hu    PLAELRYLDFSNNRLDLLHSTAFEELHKLEVLDISSNSHYFQSEGITHMLNFTKNLKVLQ
TLR7_cy    PLAELRYLDFSNNRLDLLHSTAFEELRKLEVLDISSNSHYFQSEGITHMLNFTKNLKVLQ
TLR7_mo    PLRELRYLDFSNNRLDLLYSTAFEELQSLEVLDLSSNSHYFQAEGITHMLNFTKKLRLLD
TLR8_hu    AIPHVKYLDLTNNRLDFDNASALTELSDLEVLDLSYNSHYFRIAGVTHHLEFIQNFTNLK
TLR8_cy    AIPHVKYLDLTNNRLDFDNASALTELSDLEVLDLSYNSHYFRIAGVTHHLEFIQNFTNLK
TLR8_mo    SMPHIKYLDLTNNRLDFDDNNAFSDLHDLEVLDLSHNAHYFSIAGVTHRLGFIQNLINLR
            .: .::***::*****:    .*: :* .*****:* *:***   *:** * * ::: *

TLR7_hu    KLMMNDNDISSSTSR-TMESESLRTLEFRGNHLDVLWREGDNRYLQLFKNLLKLEELDIS
TLR7_cy    KLMMNDNDISSSTSR-TMESESLRTLEFRGNHLDVLWRDGDNRYLQLFKNLLKLEELDIS
TLR7_mo    KLMMNDNDISTSASR-TMESDSLRILEFRGNHLDVLWRAGDNRYLDFFKNLFNLEVLDIS
TLR8_hu    VLNLSHNNIYTLTDKYNLESKSLVELVFSGNRLDILWNDDDNRYISIFKGLKNLTRLDLS
TLR8_cy    VLNLSHNNIYTLTDKYNLESKSLVELVFSGNRLDILWNDDDNRYISIFKGLTNLTKLDLS
TLR8_mo    VLNLSHNGIYTLTEESELKSISLKELVFSGNRLDRLWNANDGKYWSIFKSLQNLIRLDLS
             * :..*.* : :..  ::* **   * * **:** **. .*.:* .:**.* :*  **:*

TLR7_hu    KNSLSFLPSGVFDGMPPNLKNLSLAKNGLKSFSWKKLQCLKNLETLDLSHNQLTTVPERL
TLR7_cy    KNSLSFLPSGVFDGMPPNLKNLSLAKNGLKSFIWEKLRYLKNLETLDLSHNQLTTVPERL
TLR7_mo    RNSLNSLPPEVFEGMPPNLKNLSLAKNGLKSFFWDRLQLLKHLEILDLSHNQLTKVPERL
TLR8_hu    LNRLKHIPNEAFLNLPASLTELHINDNMLKFFNWTLLQQFPRLELLDLRGNKLLFLTDSL
TLR8_cy    LNKLKHIPNEAFLNLPASLTELHINDNMLKFFNWTLLQQFPHLQLLDLRGNKLFFLTDSL
TLR8_mo    YNNLQQIPNGAFLNLPQSLQELLISGNKLRFFNWTLLQYFPHLHLLDLSRNELYFLPNCL
             * *. :*  .* .:* .* :* :  * * *: * *  *: : .*. ***  *:*  ::: *

TLR7_hu    SNCSRSLKNLILKNNQIRSLTKYFLQDAFQLRYLDLSSNKIQMIQKTSFPENVLNNLKML
TLR7_cy    SNCSRSLKNLILKNNQIRSLTKYFLQDAFQLRYLDLSSNKIQMIQKTSFPENVLNNLKML
TLR7_mo    ANCSKSLTTLILKHNQIRQLTKYFLEDALQLRYLDISSNKIQVIQKTSFPENVLNNLEML
TLR8_hu    SDFTSSLRTLLLLSHNRISHLPSGFLSEVSSLKHLDLSSNLLKTINKSALETKTTTKLSML
TLR8_cy    SDFTSSLQTLLLLSHNRISHLPSGFLSEVSSLMHLDLSSNLLKTINKSALETKTTTNLCIL
TLR8_mo    SKFAHSLETLLLLSHNHFSHLPSGFLSEARNLVHLDLSFNTIKMINKSSLQTKMKTNLSIL
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TLR7_hu    LLHHNRFLCTCDAVWFVWWVNHTEVTIPYLATDVTCVGPGAHKGQSVISLDLYTCELDLT
TLR7_cy    LLHHNRFLCTCDAVWFVWWVNHTEVTIPYLATDVTCVGPGAHKGQSVISLDLYTCELDLT
TLR7_mo    VLHHNRFLCNCDAVWFVWWVNHTDVTIPYLATDVTCVGPGAHKGQSVISLDLYTCELDLT
TLR8_hu    ELHGNPFECTCDIGDFRRWMDEHLNVKIPRLVDVICASPGDQRGKSIVSLELTTCVSDVT
TLR8_cy    ELHGNPFECTCDIGDFRRWMDEHLNVTIPRLVDVICASPGDQRGKSIVSLELTTCVSDVT
TLR8_mo    ELHGNYFDCTCDISDFRSWLDENLNITIPKLVNVICSNPGDQKSKSIMSLDLTTCVSDTT
            **  * * * *.**     *  *:::.          .:* *  .** :::.*::**:* ** * *

TLR7_hu    NLILFSLSISVSLFLMVMMTASHLYFWDVWYIYHFCKAKIKGYQRLISPDCCYDAFIVYD
TLR7_cy    NLILFSLSISVSLFLMVMMTASHLYFWDVWYIYHFCKAKIKGYQRLISPDCCYDAFIVYD
TLR7_mo    NLILFSVSISSVLFLMVVMTTSHLFFWDMWYIYYFWKAKIKGYQHLQSMESCYDAFIVYD
TLR8_hu    AVILFFFTFFITTMVMLAALAHHLFYWDVWFIYNVCLAKVKGYRSLSTSQTFYDAYISYD
TLR8_cy    AVILFFFTFFITTMVMLTALAHHLFYWDVWFIYNVCLAKVKGYRSLSTSQTFYDAYISYD
TLR8_mo    AAVLFFLTFLTTSMVMLAALVHHLFYWDVWFIYHMCSAKLKGYRTSSTSQTFYDAYISYD
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TLR7_hu        TKDPAVTEWVLAELVAKLEDPREKHFNLCLEERDWLPGQPVLENLSQSIQLSKKTVFVMT
TLR7_cy        TKDPAVTEWVLAELVAKLEDPREKHFNLCLEERDWLPGQPVLENLSQSIQLSKKTVFVMT
TLR7_mo        TKNSAVTEWVLQELVAKLEDPREKHFNLCLEERDWLPGQPVLENLSQSIQLSKKTVFVMT
TLR8_hu        TKDASVTDWVINELRYHLEESRDKNVLLCLEERDWDPGLAIIDNLMQSINQSKKTVFVLT
TLR8_cy        TKDASVTDWVINELRYHLEESQDKNVLLCLEERDWDPGLAIIDNLMQSINQSKKTVFVLT
TLR8_mo        TKDASVTDWVINELRYHLEESEDKSVLLCLEERDWDPGLPIIDNLMQSINQSKKTIFVLT
               **:.:**:**: **  :**:..:* . ******** ** .:::** ***: ****:**:*

TLR7_hu        DKYAKTENFKIAFYLSHQRLMDEKVDVIILIFLEKPFQKSKFLQLRKRLCGSSVLEWPTN
TLR7_cy        DKYAKTENFKIAFYLSHQRLMDEKVDVIILIFLEKPFQKSKFLQLRKRLCGSSVLEWPTN
TLR7_mo        QKYAKTESFKMAFYLSHQRLLDEKVDVIILIFLEKPLQKSKFLQLRKRLCRSSVLEWPAN
TLR8_hu        KKYAKSWNFKTAFYLALQRLMDENMDVIIFILLEPVLQHSQYLRLRQRICKSSILQWPDN
TLR8_cy        KKYAKSWNFKTAFYLALQRLMDENMDVIIFILLEPVLQHSQYLRLRQRICKSSILQWPDN
TLR8_mo        KKYAKSWNFKTAFYLALQRLMDENMDVIIFILLEPVLQYSQYLRLRQRICKSSILQWPNN
               .****: .** ****: ***:**::****:*:**  :* *::*:**:*:* **:*:** *

TLR7_hu        PQAHPYFWQCLKNALATDNHVAYSQVFKETV---
TLR7_cy        PQAHPYFWQCLKNALATDNHVAYSQVFKETV---
TLR7_mo        PQAHPYFWQCLKNALTTDNHVAYSQMFKETV---
TLR8_hu        PKAEGLFWQTLRNVVLTENDSRYNNMYVDSIKQY
TLR8_cy        PKAEGLFWQTLRNVVLTENDSRYNNMYVDSIKQ-
TLR8_mo        PKAENLFWQSLKNVVLTENDSRYDDLYIDSIRQY
               *:*.  *** *:*.: *:*.  *..:: :::
```

B

	TLR7_hu	TLR7_cy	TLR7_mo	TLR8_hu	TLR8_cy	TLR8_mo
TLR7_hu	100	97.8	80.5	40.8	40.5	40.4
TLR7_cy		100	80.2	40.8	40.6	41.8
TLR7_mo			100	40.4	40.6	39.5
TLR8_hu				100	96.8	70.4
TLR8_cy					100	70.1
TLR8_mo						100

Supplemental Fig. 2

Protein sequence comparison of TLR7 and TLR8 from the three species human (hu), mouse (mo), and cynomolgus (cy). Sequences were compared via (A) multiple sequence alignment, (B) % identity scores. In panel B, % identity scores are colored in blue when comparing TLR7 vs TLR7, in red when comparing TLR8 vs TLR8, and in gray when comparing TLR7 vs TLR8 sequences.

All sequences were downloaded from Genbank with the following accession numbers: TLR7_hu, AAZ9902.1; TLR8_hu, AAI01078.1; TLR7_mo, AAI32386.1; TLR8_mo, AAI32055.1; TLR7_cy, BAG55061.1; TLR8_cy, BAG55068.1.

Supplemental Table 1

Mean pharmacokinetic parameters of GS-9620 following oral administration of GS-9620 at 1 mg/kg in portal vein-cannulated male beagle dogs

Parameter	Sample Collection	
	Portal Vein	**Jugular Vein**
C_{max} (ng/mL)	248 ± 149	13.6 ± 1.6
T_{max} (hr)	0.83 ± 1.00	0.92 ± 0.95
AUC_{last} (ng•hr/mL)	302 ± 93	70.2 ± 6.6
F_{ABS}	82% ± 24%	N/A
F	N/A	20% ± 0%

AUC, area under the concentration-time curve; C_{max}, maximum plasma/serum concentration; F, bioavailability; F_{ABS}, absolute bioavailability; T_{max}, time to reach C_{max}.

Values are mean ± SD; n = 3.