

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
28 August 2008 (28.08.2008)

PCT

(10) International Publication Number
WO 2008/103148 A1

(51) International Patent Classification:
A61K 31/70 (2006.01) *A61P 33/02* (2006.01)
A61K 47/48 (2006.01)

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(21) International Application Number:
PCT/US2007/005092

(22) International Filing Date:
24 February 2007 (24.02.2007)

(25) Filing Language: English

(26) Publication Language: English

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(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS,
JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS,
LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY,
MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS,
RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT,
RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA,
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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Published:

— with international search report

(54) Title: AN ORALLY-ABSORBED FORMULATION FOR PAROMOMYCIN

(57) Abstract: A composition for oral treatment of visceral leishmaniasis comprises paromomycin and a permeation enhancer that increases oral bioavailability of the paromomycin to at least 20%.

WO 2008/103148 A1

An Orally-Absorbed Formulation for Paromomycin

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[003] Statement regarding federally sponsored research: This work was supported by a Federal Grant from the National Institute of Allergy and Infectious Diseases (Contract No. NO1-AI-05414). The U.S. government may have rights in any patent issuing on this application

[004] Background of the Invention

[005] The field of the invention is oral formulations for paromomycin.

[006] Paromomycin sulfate (PAR) is an antibiotic indicated for intestinal amebiasis and as adjunctive therapy in hepatic coma when given orally. It is poorly absorbed after oral administration, with almost 100% of the drug recoverable in the stool (6). PAR is also used parenterally for treating Visceral Leishmaniasis (VL) in combination with sodium stibogluconate in developing countries. In 2004, an injectable form of PAR was tested in the largest prospective clinical trial ever performed for treatment of VL (1). PAR has also been effective in animal models of multiple-drug resistant tuberculosis (MDR TB) and mycobacterium avium complex (2, 3). However, poor oral absorption of PAR requiring parenteral administration limits its use as a drug of choice for treating these and other infectious diseases, including prophylactic use for opportunistic infections in HIV/AIDS patients (4).

[007] Summary of the Invention

[008] One aspect of the invention is a composition for oral treatment of visceral leishmaniasis, said composition comprising: paromomycin; and a permeation enhancer that increases oral bioavailability of the paromomycin, wherein the composition has a paromomycin bioavailability of at least 20% when orally administered to a mammal.

[009] In specific embodiments, the permeation enhancer increases oral bioavailability of the paromomycin by enhancing paracellular transport.

[010] In various embodiments, the permeation enhancer is selected from the group consisting of medium-chain glycerides, macrogolglycerides, polyglycols, sodium caprate, sodium caprylate, and mixtures thereof.

[011] In a specific embodiment the composition comprises 1-20 wt. % caprylocaproyl macrogol-8 glycerides, optionally with 1-10 wt. % d- α -tocopheryl polyethylene glycol 1000 succinate. In specific embodiments, the composition further comprises a Pgp inhibitor, which can be formulated as a shell surrounding the paromomycin.

[012] The composition can be prepared in unit dosage form, such as a liquid or powder form that is filled into capsules that is optionally enteric coated. In another embodiment, the composition is in a liquid form.

[013] In preferred embodiments, the paromomycin bioavailability is at least 30%.

[014] Another aspect of the invention is a method for treating leishmaniasis in a mammal, the method comprising: administering to the mammal a composition comprising: paromomycin; and a permeation enhancer that increases oral bioavailability of the paromomycin, wherein the composition has a paromomycin bioavailability of at least 20% when orally administered to a mammal. In a specific embodiment, prior to the administering step, the mammal is administered a Pgp inhibitor.

[015] A further aspect of the invention is a kit that contains a composition comprising paromomycin and a permeation enhancer that increases oral bioavailability of the paromomycin, wherein the composition has a paromomycin bioavailability of at least 20% when orally administered to a mammal. The kit may further comprise a Pgp inhibitor.

[016] Detailed Description of Specific Embodiments of the Invention

[017] The present invention provides compositions, kits, and methods for oral treatment of visceral leishmaniasis (Kala Azar) and other conditions amenable to systemic treatment with paromomycin. The composition comprises paromomycin, which has a molecular formula of $C_{23}H_{45}N_5O_{14}$, or pharmaceutically acceptable salts thereof (e.g. sulfate, phosphate, etc.). Paromomycin and salts thereof are referred to herein as paromomycin.

[018] The composition further comprises a permeation enhancer that increases oral bioavailability of the paromomycin to at least 20% when orally administered to a mammal. Enhancers that maximize paromomycin absorption at the jejunum are particularly preferred. Numerous intestinal permeation enhancers and their preferential sites of absorption are known (see Aungst et al, J. Pharm Sci (2000) 89:429-442). The preferential site of

absorption of a permeation enhancer/drug combination can also be determined using routine methods, such as the Ussing chamber method (see e.g. Gotoh et al, J Biomol Screen. (2005) 10:517-523; and Example 1 below).

[019] Suitable enhancers are selected from medium-chain glycerides, macroglycerides, polyglycols, glycerol esters of fatty acids, pegylated alcoholic esters of fatty acids, glyceryl monoesters, propylene glycol monoesters, medium chain fatty acids, chitosan and chitosan derivatives, and mixtures thereof. The term medium-chain glycerides (MCGs) generally refers to monoglycerides and diglycerides of fatty acid, and may contain triglycerides as well as monoglycerides and diglycerides of shorter and longer chain fatty acids. Examples of preferred MCGs include glyceryl monooleate (oleic acid acyl chain) and glyceryl monolinoleate (linoleic acid acyl chain). Examples of preferred macroglycerides are lauroyl macrogol-32 glycerides, sold as GELUCIRE 44/14 (Gattefosse Corporation, Paramus, NJ) and caprylocaproyl macrogol-8 glycerides, sold as LABRASOL (Gattefosse Corporation, Paramus, NJ). In a specific embodiment, the composition comprises 1-20 wt. %, and more preferably 2-10 wt. % caprylocaproyl macrogol-8 glycerides.

[020] Preferred combinations of enhancers include mixtures of d- α -tocopheryl polyethylene glycol 1000 succinate (TPGS) (Eastman Vitamin E TPGS, Eastman, Kingsport, Tennessee) with caprylocaproyl macrogol-8 glycerides and/or polysorbates. In a specific embodiment, the composition comprises 1-20 wt. % caprylocaproyl macrogol-8 glycerides and 1-10 wt. % d- α -tocopheryl polyethylene glycol 1000 succinate.

[021] Particularly preferred enhancers are those which enhance paracellular transport. Examples of such enhancers include the medium chain fatty acids caprate, laurate, caprylate, and derivatives thereof (e.g. sodium N-[8-(2-hydroxybenzoyl)amino]caprylate; see e.g. Hess et al., Eur J Pharm Sci. (2005) 25:307-12); and chitosan and chitosan derivatives (see e.g. Cano-Cebrian et al., Curr Drug Deliv. (2005) 2:9-22). Sodium caprate (also known as sodium decanoate), and sodium caprylate are particularly preferred enhancers.

[022] The permeation enhancer in the composition is provided in an effective amount, preferably at or above but proximate to a minimal effective amount, preferably less than about 80% by weight (wt. %) of the composition. More preferably, the amount of intestinal permeation enhancer is less than 60, 50, 40 or 30 wt. %. The amount of paromomycin in the composition is preferably at least 20% by weight of the composition. More preferably, the composition comprises at least 30, 40, 50, 60, 70, or 80 wt % of the paromomycin. In some embodiments, the composition consists essentially of the paromomycin and the

permeation enhancer, particularly in the case of liquid formulations. In other embodiments, the composition comprises additional excipients, binders, etc. for example to facilitate formulation into tablets, pellets, beads, etc.

[023] The particular form of the composition (i.e. solution, viscous gel, powder, tablet, etc.) will generally be dictated by the selected permeation enhancer. As one example, the composition can be prepared as a liquid formulation, suitable for drinking or filling into capsules, by dissolving paromomycin in HEPES buffer containing 5 wt. % Labrasol® and 5 wt. % Vitamin E TPGS. As another example, the composition can be prepared as a powder by mixing paromomycin with sodium caprate. The powder can then be combined with binders and pressed into tablets, or filled into capsules. Unit dosage forms (e.g. tablets, capsules, etc.) contain from about 5-450 mg of paromomycin, and are optionally enteric coated using known methods.

[024] Intestinal epithelial cells express transport systems that actively remove certain compounds from the cells, transporting them in the blood-to-lumen direction. We have found that these transport systems are operative against paromomycin. Accordingly, the composition may further comprise a secretory transport inhibitor. In specific embodiments, the transport inhibitor is a Pgp inhibitor such as Vitamin E TPGS, quinidine, digoxin, and verapamil. Numerous other suitable Pgp inhibitors are well-known (see e.g. Aungst, *supra*). The Pgp inhibitor may be administered as a predose in a liquid or solid form at a specified time interval (e.g. 5, 10, 15, 30, 60 minutes) prior to administration of the paromomycin. Alternatively, the Pgp inhibitor may be co-formulated with the paromomycin. In one co-formulation, the Pgp inhibitor, paromomycin, and enhancer are combined in a homogenous mixture or solution that can be filled into capsules. In another embodiment, the Pgp inhibitor is included as a solid in a solid/liquid matrix comprising the paromomycin and enhancer. In yet another formulation, the paromomycin and enhancer are formulated in a matrix with an outer sheathing of the Pgp inhibitor present as an outside shell. The capsule with the inner core of paromomycin/enhancer and the outer layer of Pgp inhibitor can be optionally coated by an enteric coating to promote delivery of both the inner core of drug and the outer covering of Pgp inhibitor at the target site.

[025] The compositions are formulated such that the bioavailability of the paromomycin when orally administered to a mammal is at least 20%, and is preferably at least 30, 40, or 50%. Oral bioavailability can be assessed using the Beagle dog, or equivalent model, wherein levels of plasma paromomycin from an orally administered composition are

compared to levels obtained after i.v. administration using the calculation: $BA = AUC_{PO} \times Dose_{iv} / AUC_{iv} \times Dose_{PO}$; where BA = bioavailability and AUC = area under the plasma concentration-time curve.

[026] The above-described composition is orally administered to a mammal that has a condition amenable to systemic treatment with paromomycin, such as visceral leishmaniasis (Kala Azar). In specific embodiments, the mammal is a human. In other embodiments, the mammal may be a livestock animal (horse, cow, pig, etc.) or a companion animal (e.g. dog, cat, etc.). For humans, the daily dose of absorbed paromomycin for effective treatment of visceral leishmaniasis is approximately 20 mg/kg. Thus, a composition of the invention that has a paromomycin bioavailability of approximately 50% will be administered at a dose of approximately 40 mg/kg/day paromomycin.

[027] In certain embodiments, a separately formulated Pgp inhibitor is administered prior to or together with the DTPA chelate composition. The composition may be provided in a kit with instructions on proper dosing. For example, the composition may be provided in a blister-pack kit, where one or more unit dosage forms are contained in a blister. The blister packaging may contain writing adjacent a blister or a row or column of blisters to indicate the proper timing of dosing. The kit may additionally contain a separately formulated Pgp inhibitor.

[028] Example 1: In Vitro Drug Transport Studies and Sample Analysis

[029] PAR transport across representative sections of harvested rat small intestinal segments and colon was initially measured in-vitro using an EasyMount Ussing System (Physiological Instruments Inc., CA, Item # EM-CSYS-8). To investigate the causes of poor oral absorption of PAR, both mucosal to serosal (M-to-S) transport and serosal to mucosal (S-to-M) transport across harvested rat jejunum segment was measured.

[030] PAR was dissolved in HEPES buffer (135mM NaCl, 5.4mM KCl, 1.2 mM CaCl₂, 1.2mM MgCl₂, 5.0mM NaOH, 10mM HEPES, 10mM dextrose), with or without additives, to obtain the predetermined drug concentrations. HEPES buffer was used as the base mucosal and serosal fluid. Transport of PAR across representative sections of the harvested rat small intestine (i.e. jejunum, duodenum, and ileum) and colon was monitored by mounting the harvested segments on sliders placed between the two chambers. First 5 ml of HEPES buffer was added to each of the two chambers (mucosal and serosal) and allowed to equilibrate for 20 min. Then the simple HEPES buffer either mucosal or serosal chamber

was replaced by HEPES buffer containing PAR and appropriate formulation additives to be tested (e.g. 5 mg/ml of PAR dissolved in HEPES buffer containing 10% w/w Labrasol®). Aliquots of sample solution were removed from the chamber on other side of the mounted intestinal segment periodically and replaced with an equal volume of fresh warm HEPES buffer.

[031] The effects of different additives, i.e. permeation enhancers, P-glycoprotein (Pgp) substrate and/or inhibitors on transport of PAR across different segments of intestine and colon were also determined using the Ussing system. These additives, either alone or in combinations, were dissolved in HEPES buffer on percent weight/weight basis of the HEPES medium. Owing to the expected variation in the *in-vitro* conditions and harvested rat intestinal segments, four replicates were used per experimental condition.

[032] The apparent permeability coefficients (P_{app}) were calculated according to:

[033] $P_{app} = (dQ / dt) / C_0 \times A$, where dQ/dt is the linear appearance rate of mass in receiver compartment, C_0 is the initial solute concentration in donor compartment, and A is the surface area.

[034] A modified HPLC method was used for analyzing PAR samples from *in-vitro* transport studies (5). The reverse phase method with a Zorbax sb C18 column, 5 μ m, 250 $\times$ 4.6 mm (maintained at 50°C) mobile phase 64% methanol and 36% water at a flow rate of 1.5 ml/min was used for quantifying the drug substance. The drug in post column eluant was derivatized with Dinitrofluorobenzene (DNFB) and monitored at 360 nm. The run time was 30 minutes.

[035] PAR transport into serosal chamber, based on calculated P_{app} values, was highest for jejunum and lowest for duodenum. Cumulative mean concentrations of PAR released into the serosal side as a function of time is shown in Table 1. P_{app} values for PAR transport are given in Table 2.

[036] **Table 1: Amount of PAR in HEPES buffer (pH 7.4) transported across intestinal segments**

Average (Ave) Cumulative Amount of Paromomycin Released (μ g) (5 mg/ml or 25 mg total of Paromomycin in HEPES Buffer); Exposed tissue area = 0.5 sq cm								
Time (hr)	Duodenum		Jejunum		Ileum		Colon	
	Ave	Std dev	Ave	Std dev	Ave	Std dev	Ave	Std dev
0.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.5	10.50	1.64	26.81	2.96	14.30	2.24	8.63	6.91
2	20.51	2.06	44.27	5.77	25.23	4.34	21.81	8.60
2.5	33.30	1.37	66.25	5.66	37.40	6.74	35.67	10.59

[037] Table 2: P_{app} Comparisons Across Various Intestinal Segments

HEPES Buffer with 5 mg/ml (~25 mg) of paromomycin in mucosal chamber; Exposed tissue area = 0.5 sq cm	P_{app} ($\times 10^{-6}$ cm/sec)	Std Dev ($\times 10^{-6}$)
Duodenum	1.94	0.30
Jejunum	3.93	0.53
Ileum	2.22	0.30
Colon	2.07	0.36

[038] Increasing the concentration of drug in the mucosal chamber resulted in an increase in M-to-S transport of PAR in a dose-dependent manner, as shown in Table A.

[039] Table A: Table A

Paromomycin: Average (Ave) Cumulative Amount of Paromomycin Released (μ g) (X mg/ml of Paromomycin in HEPES Buffer); Exposed tissue area = 0.5 sq cm								
Time (hr)	X = 5 mg/ml		X = 15 mg/ml		X = 50 mg/ml		X = 75 mg/ml	
	Ave	Std dev	Ave	Std dev	Av	Std dev	Ave	Std dev
0.5	0.0	0.0	0.0	0.0	40.8	29.6	48.6	22.5
1	0.0	0.0	24.1	2.9	155.9	66.8	235.2	82.6
1.5	26.8	3.0	52.9	5.5	284.5	103.5	495.7	60.1
2	44.3	5.8	94.6	8.0	468.5	189.5	776.5	46.1
2.5	66.2	5.7	142.5	11.2	680.0	225.1	1104.6	107.4

[040] Different permeation enhancers, i.e. Labrasol[®], Gelucire[®] 44/14, Vitamin E TPGS[®] and surfactants such as Polysorbate-80; either alone or in combination were evaluated for their effect on *in-vitro* transport of PAR from M-to-S side. Among the permeation enhancers used in this study, Labrasol[®] alone at low concentration of 5% w/w had

maximum permeation enhancement. The results are presented in Table B and C. P_{app} values for PAR transport, in the presence of different enhancers, are given in Table 3.

[041] Table B:

Time (hr)	Average Cumulative Amount of Paromomycin Released (μ g) (HEPES Buffer with 15 mg/ml of Paromomycin and enhancers); Exposed tissue area = 0.5 sq cm							
	HEPES buffer		5% w/w Labrasol		5% w/w Vitamin E TPGS		5% w/w Vitamin E TPGS, 5% w/w Labrasol	
	Ave	Std Dev	Ave	Std Dev	Ave	Std Dev	Ave	Std Dev
0.5	0.0	0.0	10.3	9.6	2.5	5.6	1.3	3.1
1	24.1	2.9	59.9	7.3	22.3	18.0	27.7	7.6
1.5	52.9	5.5	120.2	13.0	55.8	27.0	69.2	14.6
2	94.6	8.0	192.8	19.9	103.8	40.3	125.8	22.0
2.5	142.5	11.2	277.4	30.9	163.7	55.4	192.6	32.6

[042] Table C:

Time (hr)	Average Cumulative Amount of Paromomycin Released (μ g) (HEPES Buffer with 15 mg/ml of Paromomycin and enhancers); Exposed tissue area = 0.5 sq cm							
	HEPES buffer		5% w/w Labrasol		20% w/w bile salt		5% w/w Tween 80 + 5% w/w Captex 355	
	Ave	Std Dev	Ave	Std Dev	Ave	Std Dev	Ave	Std Dev
0.5	0.0	0.0	3.3	6.7	0.0	0.0	0.0	0.0
1	24.1	2.9	32.6	9.3	19.7	17.1	15.9	3.4
1.5	52.9	5.5	60.0	11.7	70.8	25.0	43.3	8.2
2	94.6	8.0	98.6	17.2	126.9	33.6	84.5	14.1
2.5	142.5	11.2	163.3	32.5	190.7	41.6	121.3	18.6

[043] Table 3: PAR Transport (P_{app} values) Across the Jejunum in Presence of Different Permeation Enhancers

Transport in Jejunum Segments	Paromomycin
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15 mg/ml of PAR in HEPES buffer containing enhancers. Exposed tissue area = 0.5 sq cm	P_{app} cm/sec)	($\times 10^{-6}$)	Std Dev ($\times 10^{-6}$)
only HEPES buffer	2.63		0.22
5% (w/w) Labrasol [®]	4.94		0.30
5% (w/w) Vitamin E TPGS [®]	2.99		0.34
5% (w/w) Vitamin E TPGS [®] + 5% (w/w) Labrasol [®]	3.56		0.35
5% (w/w) Polysorbate 80 + 5% (w/w) Labrasol [®]	2.89		0.23
5% (w/w) Gelucire [®] 44/14 + 5% (w/w) Labrasol [®]	2.86		0.32
20% (w/w) Bile salt	3.62		0.36
5% (w/w) Polysorbate 80 + 5% (w/w) Captex 355	2.31		0.21

[044] Increasing the concentration of PAR in the mucosal chamber resulted in an increase in the M-to S transport (P_{app} values) in a dose-dependent manner even in the presence of enhancers (5% w/w Vitamin E TPGS[®] + 5% w/w Labrasol[®]), as shown in Table D and Table 4.

[045] **Table D:**

Average Cumulative Amount Release (μ g) (X mg/ml of Paromomycin in HEPES Buffer containing 5% w/w Vitamin E TPGS + 5% w/w Labrasol)						
Time (hr)	X = 15 mg/ml		X = 50 mg/ml		X = 100 mg/ml	
	Average	Std Dev	Average	Std Dev	Average	Std Dev
0.5	1.26	3.10	10.89	3.25	57.33	26.17
1	27.66	7.63	65.61	18.40	267.65	105.42
1.5	69.24	14.57	142.86	48.06	555.38	181.11
2	125.77	22.04	261.53	68.01	943.28	205.27
2.5	192.59	32.58	505.68	51.62	1531.18	475.51

[046] Table 4: P_{app} Comparisons of Transport Across Different Intestinal Segments for PAR with Permeation Enhancers

X mg/ml of PAR in HEPES Buffer with 5% (w/w) Vitamin E TPGS [®] + 5% (w/w) Labrasol [®]	Paromomycin	
	P_{app} ($\times 10^{-6}$ cm/sec)	Std Dev ($\times 10^{-6}$)
x=15	3.56	0.35
x=50	2.63	0.48
x=100	4.03	0.47

[047] The results from studies evaluating the effect of Quinidine, a Pgp inhibitor added to serosal and mucosal chambers on the M-to-S transport of PAR; and in the presence or absence of permeation enhancers is presented in Table 5. Quinidine added to the mucosal chamber enhanced M-to-S transport of PAR. In presence of permeation enhancers, i.e. 5% w/w Vitamin E TPGS[®] + 5% w/w Labrasol[®], higher P_{app} values were obtained at 0.5 mg/ml Quinidine concentration, when compared to 0.05 mg/ml Quinidine concentration. Polysorbate-80 has shown a slightly negative effect on M-to-S transport (P_{app} values) of PAR in presence of Quinidine.

[048] Table 5: P_{app} Values for "Transport" Across Jejunum Segments – Comparisons Between PAR With and Without Quinidine (Pgp Inhibitor)

15 mg/ml of PAR in HEPES Buffer with and without Additives; Exposed tissue area = 0.5 sq cm		Paromomycin	
		P_{app} ($\times 10^{-6}$ cm/sec)	Std Dev ($\times 10^{-6}$)
HEPES Buffer	No Quinidine	2.63	0.22
	0.05 mg/ml Quinidine in mucosal	4.37	0.24
HEPES Buffer containing 5% w/w Labrasol [®]	No Quinidine	4.94	0.30
	0.05 mg/ml Quinidine in mucosal	5.00	0.43
HEPES Buffer containing 5% w/w Vitamin E TPGS [®] + 5% w/w Labrasol [®]	No Quinidine	3.56	0.35
	0.5 mg/ml Quinidine in mucosal. (1 st study)	7.20	0.50

	0.5 mg/ml Quinidine in mucosal. (2 nd study)	5.53	0.28
	0.05 mg/ml Quinidine in serosal	4.23	0.45
HEPES Buffer containing 5% w/w Polysorbate 80 + 5% w/w Labrasol [®]	No Quinidine	2.89	0.23
	0.05 mg/ml Quinidine in mucosal	2.79	0.28
HEPES Buffer containing 5% w/w Polysorbate 80 + 5% w/w Labrasol [®]	No Quinidine	2.86	0.32
	0.05 mg/ml Quinidine in mucosal	2.55	0.24
HEPES Buffer containing 5 % w/w Labrasol [®]	0.05 mg/ml Quinidine in mucosal	5.00	0.43
HEPES Buffer containing 15% w/w Labrasol [®]	0.05 mg/ml Quinidine in mucosal	3.50	0.42
HEPES Buffer containing 40 % w/w Labrasol [®]	0.05 mg/ml Quinidine in mucosal	5.83	1.39

[049] Table 6 gives the P_{app} values for PAR, in the absence of additives, in the M-to-S as well as S-to-M direction to be $2.63 \pm 0.22 \times 10^{-6}$ cm/sec and $2.75 \pm 0.22 \times 10^{-6}$ cm/sec, respectively. This is suggestive of the presence of efflux mechanisms for PAR. The net effective transport can be influenced by the presence of enhancers and Pgp inhibitors, as seen in Table 6. HEPES Buffer containing 5% w/w Vitamin E TPGS[®] + 5% w/w Labrasol[®] and 0.5 mg/ml Quinidine in mucosal gave the highest transport value for PAR in the M-to-S direction.

[050] Table 6: P_{app} Values for "Transport" of PAR in both M-to-S and S-to-M Directions

15 mg/ml (total 75 mg) of PAR in mucosal chamber Exposed tissue area = 0.5 sq cm	Paromomycin	
	P_{app}	Std

		($\times 10^{-6}$ cm/sec)	Dev ($\times 10^{-6}$)
M-to-S Transport	HEPES Buffer only	2.63	0.22
	HEPES Buffer containing 0.05 mg/ml Quinidine in mucosal	4.37	0.24
	HEPES Buffer containing (5% w/w Vitamin E TPGS® + 5% w/w Labrasol®) and 0.5 mg/ml Quinidine in mucosal.	7.20	0.50
S-to-M Transport	HEPES Buffer only	2.75	0.22
	HEPES Buffer containing 0.05 mg/ml Quinidine in mucosal	1.85	0.19
	HEPES Buffer containing (5% w/w Vitamin E TPGS® + 5% w/w Labrasol®) and 0.5 mg/ml Quinidine in mucosal.	1.30	0.21

[051] The results of *in vitro* transport studies using sodium decanoate, added to mucosal and serosal chambers in the presence or absence of enhancer are presented in Tables 7 and 8. Sodium decanoate added to the serosal chamber at higher concentrations of 1 mg/ml resulted in appreciable increase in M-to-S transport of Paromomycin.

[052] Table 7: P_{app} Values for "Transport" Across Jejunum Segments – Comparisons Between PAR With and Without Sodium Decanoate in the Absence of Other Permeation Enhancers

HEPES Buffer with 15 mg/ml (total 75 mg) of Paromomycin; Exposed tissue area = 0.5 sq cm		Paromomycin	
		P_{app} ($\times 10^{-6}$ cm/sec)	Std Dev ($\times 10^{-6}$)
Sodium Decanoate in Mucosal	No Sodium Decanoate	2.63	0.22
	0.2 mg/ml (equilibrated)*	1.48	0.24
	0.5 mg/ml (equilibrated)	2.22	0.14
	1 mg/ml (equilibrated)	4.40	0.33
	1 mg/ml (not equilibrated)	3.83	0.24
Sodium	No Sodium Decanoate	2.63	0.22

Decanoate in Serosal	0.2 mg/ml (not equilibrated)	3.86	0.34
	0.5 mg/ml (not equilibrated)	3.54	0.39
	1.0 mg/ml (not equilibrated)	7.20	0.58

* Equilibration is done by adding Sodium Decanoate 20 minutes prior to adding the drug

[053] Table 8: P_{app} Values for "Transport" Across Jejunum Segments – Comparisons Between PAR With and Without Sodium Decanoate in the Presence of Other Permeation Enhancers

HEPES Buffer with 15 mg/ml of Paromomycin in 5% w/w Vitamin E TPGS® + 5% w/w Labrasol® Exposed tissue area = 0.5 sq cm		Paromomycin	
		P _{app} ($\times 10^{-6}$ cm/sec)	Std Dev ($\times 10^{-6}$)
Sodium Decanoate in mucosal	No Sodium Decanoate	3.56	0.35
	0.2 mg/ml (equilibrated*)	3.99	0.17
	0.5 mg/ml (equilibrated)	2.43	0.10
	1.0 mg/ml (equilibrated)	2.55	0.12
	No Sodium Decanoate	3.56	0.35
Sodium Decanoate in serosal	0.2 mg/ml (equilibrated)	4.86	0.39
	0.2 mg/ml (not equilibrated)	2.01	0.13
	0.5 mg/ml (not equilibrated)	1.94	0.10
	1.0 mg/ml (not equilibrated)	6.01	0.46

* Equilibration is done by adding sodium decanoate 20 minutes prior to adding the drug.

[054] Example 2: Paromomycin In Vivo Absorption Studies in Rats

[055] Male rats (Sprague Dawley) were randomly divided into four groups with each group having three animals and were administered with PAR, either as an oral composition at a dose of 1000 mg /kg or an injection at a dose of 100 mg / kg dissolved in normal saline. Three different oral formulation compositions selected based on the *in vitro* transport studies were used in the *in vivo* absorption studies in rat animal model. Blood samples were collected and processed to obtain plasma at the following time points: intravenous (iv) dose group at 5, 10, 20, 30 min, and further at 1, 2, 3, 9, and 24 hr; oral dose groups at 15, and 30 min, and then at 1, 2, 4, 6, 8, 10, and 24 hr time-points after dose administration.

[056] Paromomycin was extracted from plasma samples (100 μ l volume) with phenylboronic acid solid phase extraction columns, and the bound drug was eluted from the

columns with 1% (v/v) formic acid in water. The acid eluates were evaporated under vacuum, and then the dry residues were reconstituted in 100 μ l of 1 mM heptafluorobutyric acid (HFBA) in water. The samples were then analyzed by LCMS using a C18 reversed phase column and a gradient from water to acetonitrile (both mobile phase constituents containing 1 mM HFBA), and detected by single ion recording on a Micromass Quattro LC triple quadrupole mass spectrometer set to monitor the M+2 ion of paromomycin at m/z 308.7.

[057] Experimental design for *in-vivo* studies is presented in Table 9. The plasma blood levels observed in the orally administered rats is shown in Table E. The serum drug levels for intravenous and oral groups are given in Tables 10 and 11, respectively.

[058] **Table 9: Pharmacokinetic study design for paromomycin formulations: intravenous and oral**

Group ^a	Route/ Formulation	Vehicle (% w/w in HEPES)	Dose level (mg/kg)	Dose conc. (mg/ml)	No. of Rats
1	IV	Sterile saline	100	10	3 M
2	Oral	5% Labrasol [®] and 5% Vitamin E TPGS [®]	1000	100	3 M
3	Oral	5% Labrasol [®] and 5% Vitamin E TPGS [®] + 10 mM sodium decanoate ^a	1000	100	3 M
4	Oral	5% Labrasol [®] + 5% Polysorbate 80	1000	100	3 M

^aSodium decanoate was administered orally 30 minutes prior to oral administration of Paromomycin.

[059] **Table E: Table E**

Average (Ave) Cumulative Amount Released (μ g) (HEPES Buffer containing X mg/ml of paromomycin and 5% w/w Vitamin E TPGS + 5% w/w Labrasol)						
Time (hr)	X = 15 mg/ml		X = 50 mg/ml		X = 100 mg/ml	
	Ave	Std	Av	Std	Ave	Std

		Dev		Dev		Dev
0.5	1.26	3.10	10.89	3.25	57.33	26.17
1	27.66	7.63	65.61	18.40	267.65	105.42
1.5	69.24	14.57	142.86	48.06	555.38	181.11
2	125.77	22.04	261.53	68.01	943.28	205.27
2.5	192.59	32.58	505.68	51.62	1531.18	475.51

[060] Table 10: Pharmacokinetic Analysis for PAR in Male Rats Dosed i.v. (100 mg/mL)

Group # 1					
Rat #	t _{1/2} (hr)	C _{max} (ng/ml)	AUC (hr·ng/ml)	V (L/kg)	Cl (L/hr/kg)
1	2.85	173931	93533	4.39	1.07
2	2.70	95651	55465	7.01	1.80
3	2.86	101318	85847	4.80	1.16
Mean	2.80	123633	78282	5.40	1.34
SD	0.09	43651	20130	1.41	0.40

[061] Table 11: Pharmacokinetic Analysis for PAR in Male Rats Dosed Orally (1000 mg/mL)

Group #2							
Formulation	Rat #	T _{max} (hr)	C _{max} (ng/ml)	AUC (hr·ng/ml)	V (L/kg)	Cl/F (L/hr/kg)	F (%)
5% w/w Labrasol Vitamin E TPGS®	4	1.0	137827	276965	3.44	0.72	20.10
	5	1.0	172924	344686	2.43	0.72	25.01
	6	1.0	371346	676064	0.96	0.73	49.05
	Mean	1.00	227366	432572	2.28	0.73	31.39
	SD	0.00	125919	213572	1.25	0.00	15.50
Group #3							
5% w/w Labrasol Vitamin E	7	0.5	80925	124258	7.53	0.72	9.02
	8	1.0	39958	72956	4.41	0.72	5.29
	9	0.3	56299	83462	14.72	0.66	6.06
	Mean	0.58	59061	93559	8.89	0.70	6.79

TPGS [®] + 10 mM Sodium Decanoate	SD	0.38	20623	27100	5.28	0.03	1.97
Group #4							
5% w/w	10	0.5	394550	497665	16.53	0.72	36.11
Labrasol + 5% w/w	11	0.5	132022	161879	4.66	0.76	11.75
Polysorbate 80	12	1.0	44004	95538	4.84	0.48	6.93
	Mean	0.67	190192	251694	8.68	0.65	18.26
	SD	0.29	182369	215584	6.80	0.15	15.64

[062] The formulation additives, i.e. the permeation enhancers, Pgp inhibitors and inducer of tight junctions opening, were well-tolerated by the rats at the given dose in this single dose studies. The bioavailability was also very good, especially because the oral bioavailability of this drug is historically considered to be close to zero (6).

[063] We have determined that PAR may be formulated as an oral dosage form with substantial systemic absorption for treating infections caused by susceptible microorganisms by co-formulating with combinations of permeation enhancers, Pgp inhibitors and/or inducers of tight junctions opening in the intestinal tract. The role Pgp plays in drug absorption and disposition has been studied. (7). Sodium decanoate has been used to improve bioavailability of certain drugs (8).

[064] We have determined that the oral absorption of PAR may be substantially improved in presence of permeation enhancers such as Labrasol® and Vitamin E TPGS®.

[065] Example 2: Oral Paromomycin Clinical Study

[066] This study assesses the efficacy and tolerability of oral paromomycin compared with injectable paromomycin for treating visceral leishmaniasis and is adapted from a previously published trial (Jha et al, BJM (1998) 316:1200-1205).

[067] **Design:** Randomized, unblinded, controlled trial with 180 days follow-up.

[068] **Setting:** Kala-Azar Research Centre, Brahmpura, Muzaffarpur, Bihar, India.

[069] **Subjects:** 120 patients of either sex aged 6-50 years with symptoms and signs suggestive of visceral leishmaniasis (fever, loss of appetite, enlarged spleen) with leishmania amastigotes detected in Giemsa stained aspirates of spleen or bone marrow.

[070] Interventions: Injectable paromomycin at 20 mg/kg administered intramuscularly for 21 days and oral paromomycin at 25, 50, and 100 mg/kg/day for 21 days (30 patients per treatment arm).

[071] Main outcome measures: Laboratory measures of efficacy: parasite count, haemoglobin concentration, white cell count, platelet count, serum albumin concentration. Clinical measures of efficacy: spleen size, fever, body weight, and liver size. Measures of safety: liver and renal function tests, reports of adverse events.

[072] Results: Cure rates of greater than 90% at end of follow-up demonstrate efficacy of oral paromomycin comparable to that of injectable paromomycin.

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WHAT IS CLAIMED IS:

1. A composition for oral treatment of visceral leishmaniasis, said composition comprising:
paromomycin; and
a permeation enhancer that increases oral bioavailability of the paromomycin,
wherein the composition has a paromomycin bioavailability of at least 20% when orally administered to a mammal.
2. The composition of claim 1 wherein the permeation enhancer increases oral bioavailability of the paromomycin by enhancing paracellular transport.
3. The composition of claim 1 wherein the permeation enhancer is sodium caprate.
4. The composition of claim 1 wherein the permeation enhancer is selected from the group consisting of medium-chain glycerides, macrogolglycerides, polyglycols, sodium caprate, sodium caprylate, and mixtures thereof.
5. The method of claim 1 wherein the permeation enhancer comprises caprylocaproyl macrogol-8 glycerides.
6. The method of claim 1 wherein the composition comprises 1-20 wt. % caprylocaproyl macrogol-8 glycerides.
7. The method of claim 1 wherein the permeation enhancer comprises caprylocaproyl macrogol-8 glycerides and d- α -tocopheryl polyethylene glycol 1000 succinate.
8. The method of claim 1 wherein the composition comprises 1-20 wt. % caprylocaproyl macrogol-8 glycerides and 1-10 wt. % d- α -tocopheryl polyethylene glycol 1000 succinate.
9. The composition of claim 1 further comprising a P glycoprotein (Pgp) inhibitor.
10. The composition of claim 1 further comprising a Pgp inhibitor, wherein the Pgp inhibitor is formulated as a shell surrounding the paromomycin.

11. The composition of claim 1 in an enteric-coated unit dosage form.
12. The composition of claim 1 in a liquid form.
13. The composition of claim 1 in a powder form filled in a capsule.
14. The composition of claim 1 wherein the paromomycin bioavailability is at least 30%.
15. A method for treating leishmaniasis in a mammal, the method comprising:
administering to the mammal the composition of claim 1.
16. The method of claim 15 wherein prior to the administering step, the mammal is administered a Pgp inhibitor.
17. A kit comprising the composition of claim 1.
18. The kit of claim 17 further comprising a Pgp inhibitor.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2007/005092**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 15-16
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 15-16 pertain to methods for treatment of the human or animal body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2007/005092**A. CLASSIFICATION OF SUBJECT MATTER***A61K 31/70(2006.01)i, A61K 47/48(2006.01)i, A61P 33/02(2006.01)i*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 8 as above

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Registry, CA(STN), eKIPASS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CROFT, S. L. et al., "Current scenario of drug development for leishmaniasis", Indian Journal of Medical Research, 2006, Vol.123, No.3, pp.399-410, CODEN: IMIREV; ISSN: 0971-5916 See the whole document.	1-14, 17-18
A	JHA, T. K., "Drug unresponsiveness & combination therapy for kala-azar", Indian Journal of Medical Research, 2006, Vol.123, No.3, pp.389-398, CODEN: IMIREV; ISSN: 0971-5916 See the whole document.	1-14, 17-18
A	THAKUR, C. P. et al., "A prospective randomized, comparative, open-label trial of the safety and efficacy of paromomycin (aminosidine) plus sodium stibogluconate versus sodium stibogluconate alone for the treatment of visceral leishmaniasis", Transactions of the Royal Society of Tropical Medicine and Hygiene, 2000, Vol.94, No.4, pp.429-431, CODEN: TRSTAZ; ISSN: 0035-9203 See the whole document.	1-14, 17-18
A	WO 1989010130 A2 (FARMITALIA CARLO ERBA S.R.L. et al.) 02 NOVEMBER 1989 See the whole document.	1-14, 17-18



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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"P" document published prior to the international filing date but later than the priority date claimed

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19 NOVEMBER 2007 (19.11.2007)

Date of mailing of the international search report

19 NOVEMBER 2007 (19.11.2007)

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2007/005092

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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