

Disposition, Metabolism and Mass Balance of [¹⁴C]Apremilast, a Novel PDE4 Inhibitor, Following Oral Administration

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Abstract

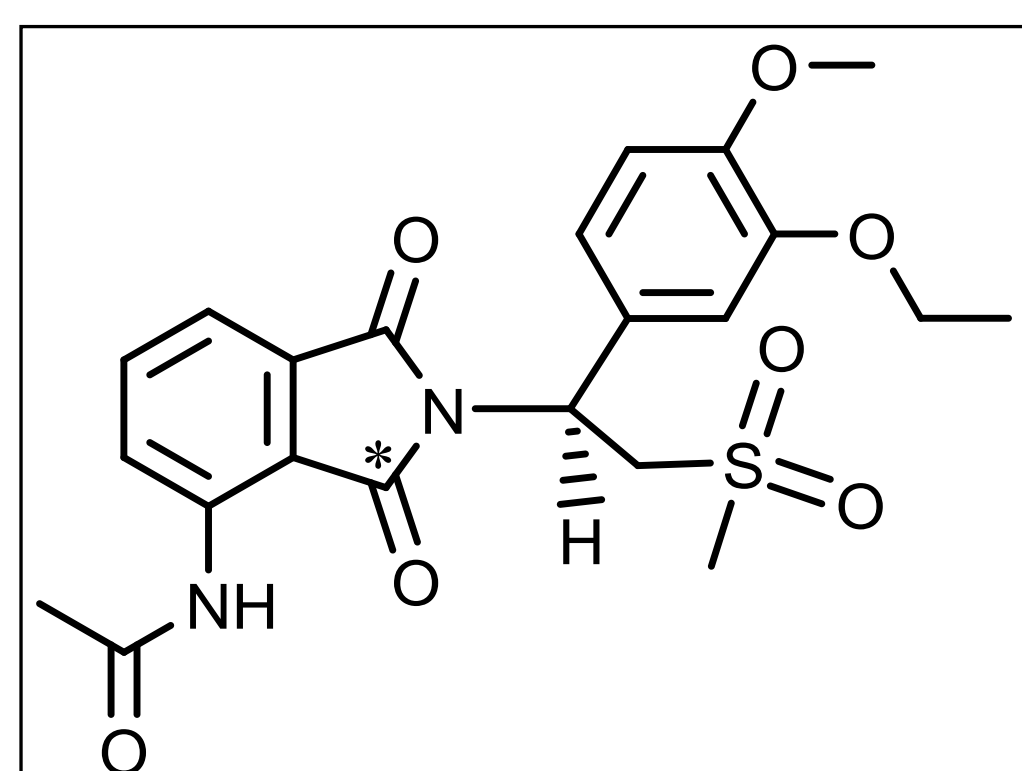
Apremilast is a novel, orally available small molecule that specifically inhibits PDE4 and thus modulates multiple pro- and anti-inflammatory mediators. Apremilast is currently under clinical development for the treatment of psoriasis, rheumatoid arthritis, and psoriatic arthritis. The pharmacokinetics, metabolism and excretion of [¹⁴C]apremilast were investigated following a single oral suspension dose (20 mg, 100 μCi) to six healthy male subjects. Mean C_{max}, AUC₀₋₄₈, t_{max}, and t_{1/2} values for apremilast in plasma were 333 ng/mL, 1970 ng*hr/mL, 1.5 hr and 6.8 hr. Approximately 58% of the radioactive dose was excreted in urine, while feces contained 39% of the radioactive dose. Apremilast was extensively metabolized via multiple metabolic pathways, with unchanged drug representing 45% of the circulating radioactivity and less than 7% of the excreted radioactivity. O-Demethylated apremilast glucuronide was the major circulating metabolite (39% of plasma radioactivity) and accounted for 34% of the excreted radioactivity. The only other radioactive components that represented >4% of the excreted radioactivity were O-demethylated apremilast and its hydrolysis product. Additional minor circulating and excreted compounds were formed via O-demethylation, O-deethylation, N-deacetylation, hydroxylation, glucuronidation, and/or hydrolysis. In conclusion, metabolic clearance of apremilast was the major route of elimination, while non-enzymatic hydrolysis and excretion of unchanged drug were involved to a lesser extent.

Introduction

Apremilast [CC-10004; (+)-N-[2-[(1S)-1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl]-1,3-dioxo-2,3-dihydro-1H-indol-4-yl]acetamide] is an oral agent that inhibits the activity of phosphodiesterase type 4 (PDE4) and the production of multiple pro-inflammatory cytokines and chemokines *in vitro*, including tumor necrosis factor (TNF)-α, interleukin (IL)-8, IL-12, IL-23, CXCL9, CXCL10, and interferon-γ.^{1,2} Apremilast has demonstrated anti-inflammatory effects *in vitro* and has shown efficacy in a pre-clinical mouse model for psoriasis.² Additionally, apremilast has shown clinical efficacy in subjects with moderate to severe psoriasis.³ Apremilast is also under clinical development for the treatment of other inflammatory autoimmune disorders that involve elevated cytokine levels such as psoriatic arthritis and Behçet disease.

The pharmacokinetics of apremilast in patients with severe plaque-type psoriasis following multiple daily doses showed rapid absorption (T_{max} = 2 hours) and a moderately long half-life (8.2 hours).⁴ Co-administration of apremilast with ketoconazole resulted in a 36% increase in apremilast AUC, indicating that CYP3A4/5 metabolism plays an important role in apremilast clearance but also suggesting that other clearance pathways are present. The current study was performed to evaluate the pharmacokinetics, metabolic disposition, and mass balance of a single oral suspension dose (20 mg, 100 μCi) of [¹⁴C]apremilast to healthy male subjects.

Materials and Methods



Apremilast, with the site of the ¹⁴C label indicated (*)

Study Design: This was an open-label, in-patient, single dose study conducted with six nonsmoking healthy male subjects, aged between 19 and 55 years, with body mass index between 19 and 29 kg/m². Before initiation of the study, the protocol and consent form were reviewed and approved by the institutional review board. All study participants gave written informed consent before the screening process was initiated.

Dose Administration: Each subject received a single oral suspension of 20 mg (100 μCi) of [¹⁴C]apremilast in distilled water. The residual radioactivity in the dosing vials was determined and accounted for approximately 0.3% of the total radioactivity.

Sample Collection. Blood samples for plasma radioactivity counting, whole blood radioactivity counting, plasma apremilast analysis, and metabolite profiling were collected at various time points up to 168 hr post-dose. Plasma was harvested by centrifugation and samples for apremilast analysis and metabolite profiling were stabilized with Sorensen's citrate buffer (pH 1.5) containing 20 μM amastatin. Urine and fecal samples were collected up to 216 hours (9 days) following dose administration and stabilized with 25 mM Sorensen's citrate buffer (pH 1.5).

Radioanalysis. Plasma and urine samples were directly analyzed by liquid scintillation counting (LSC). Fecal homogenates and blood samples were combusted prior to LSC. Using these criteria, the lower limits of quantification in plasma, blood, urine and feces were 2.07 ng equivalent/mL (ngEq/mL), 2.61 ngEq/g, 1.89 ngEq/mL, and 2.52 ngEq/g, respectively.

Measurement of Apremilast in Plasma. Plasma concentrations of apremilast were determined using a chiral LC-MS/MS assay validated for concentrations between 1.00 and 1000 ng/mL, with quality control (QC) samples prepared at 3.00, 50.0 and 750 ng/mL. For the QC samples, the accuracy ranged from 87.5% to 106.7%.

Metabolite Profiling Samples.

Plasma: Individual samples collected from all subjects up to 48 hr post-dose and a pooled 0-24 hr sample from subject 1.

Urine: 0-24 and 24-48 hr pools for each subject and a 0-168 hr pool from subject 1.

Feces: 0-48 and 48-96 hr pools for each subject and a 0-168 hr pool from subject 1.

PDE4 and TNF-α Activity of Apremilast Metabolites. PDE4 enzyme was isolated from U937 human monocytic cells and used for testing inhibition in a cAMP hydrolysis assay as previously described.² For the TNF-α production assay, human peripheral blood mononuclear cells (PBMC) were isolated from normal donor blood. Cells were plated in 96-well flat-bottom tissue culture plates in duplicate. Cells were stimulated with 1 ng/ml LPS in the absence or presence of compounds. The compounds were added to cells 1 hr before LPS stimulation. The cells were incubated for 16-18 hr at 37°C in 5% CO₂ and supernatants assayed for TNF-α levels by ELISA. IC₅₀ values for the assays were calculated by nonlinear regression.

Pharmacokinetic Analysis. Data were generated by non-compartmental analysis of plasma or whole blood vs. time profiles using WinNonlin (v. 4.1, Enterprise Ed.).

Figure 1

Cumulative elimination of radioactivity in urine and feces after a single oral 20 mg dose of [¹⁴C]apremilast in male healthy subjects. Values are mean ± SD.

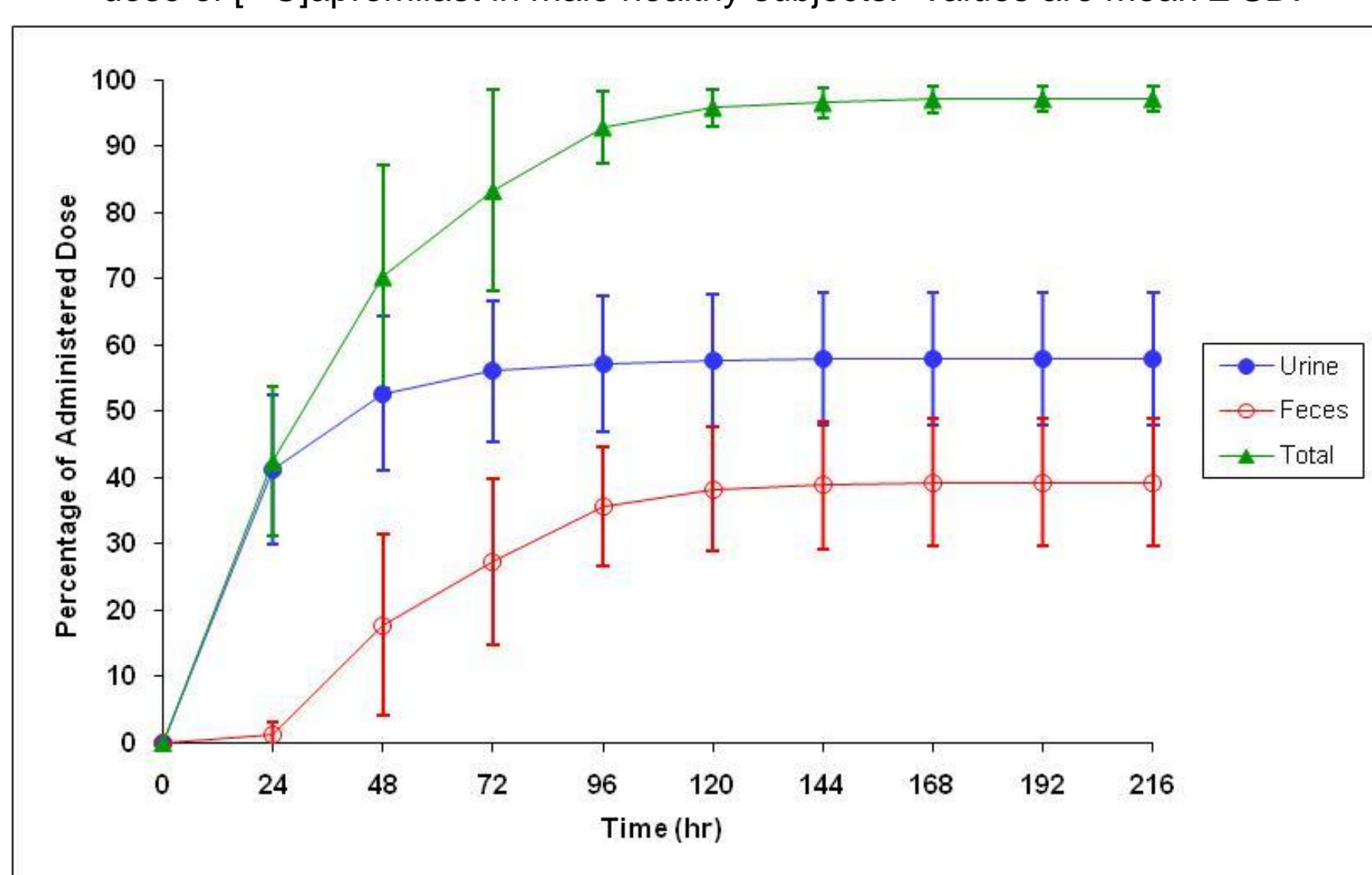


Figure 2

Concentration versus time curves for radioactivity in plasma, apremilast in plasma, and radioactivity in blood following a single oral 20 mg dose of [¹⁴C]apremilast in healthy male subjects. Values are mean ± SD.

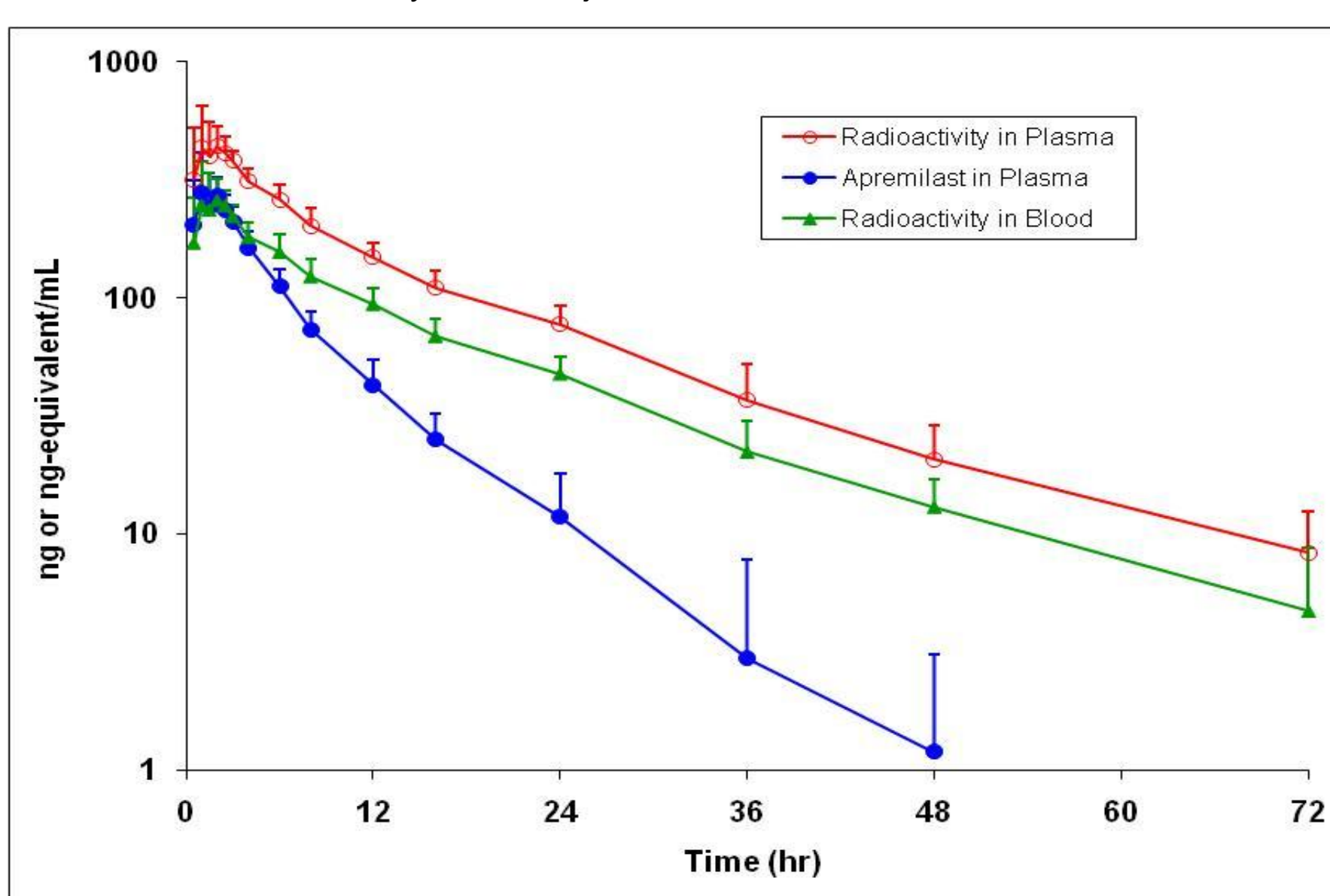


Table 1

Plasma and Whole Blood Total Radioactivity Pharmacokinetic Parameters following a Single Oral 20 mg Dose of [¹⁴C]Apremilast

PK Parameter	Whole Blood Total Radioactivity	Plasma Total Radioactivity	Blood to Plasma Ratio
C _{max}	303 ngEq/mL ± 77 ^a	527 ngEq/mL ± 127 ^a	0.57
T _{max}	2.0 hr (1.0-3.0)	1.5 hr (1.0-3.0)	NA
AUC ₀₋₄₈	3489 ngEq*hr/mL ± 509	6201 ngEq*hr/mL ± 937	0.56
AUC _{0-∞}	3664 ngEq*hr/mL ± 556	6632 ngEq*hr/mL ± 653	0.55
t _{1/2}	16.3 hr ± 5.2	50.4 hr ± 8.7	NA

a: Values are reported as mean ± SD except T_{max}, which is reported as median (min-max). NA: not applicable

Figure 3

Representative radiochromatograms of (A) 0-24 hour pooled plasma, (B) 0-24 hour pooled urine, and (C) 0-48 hour pooled feces after a single oral 20 mg dose of [¹⁴C]apremilast.

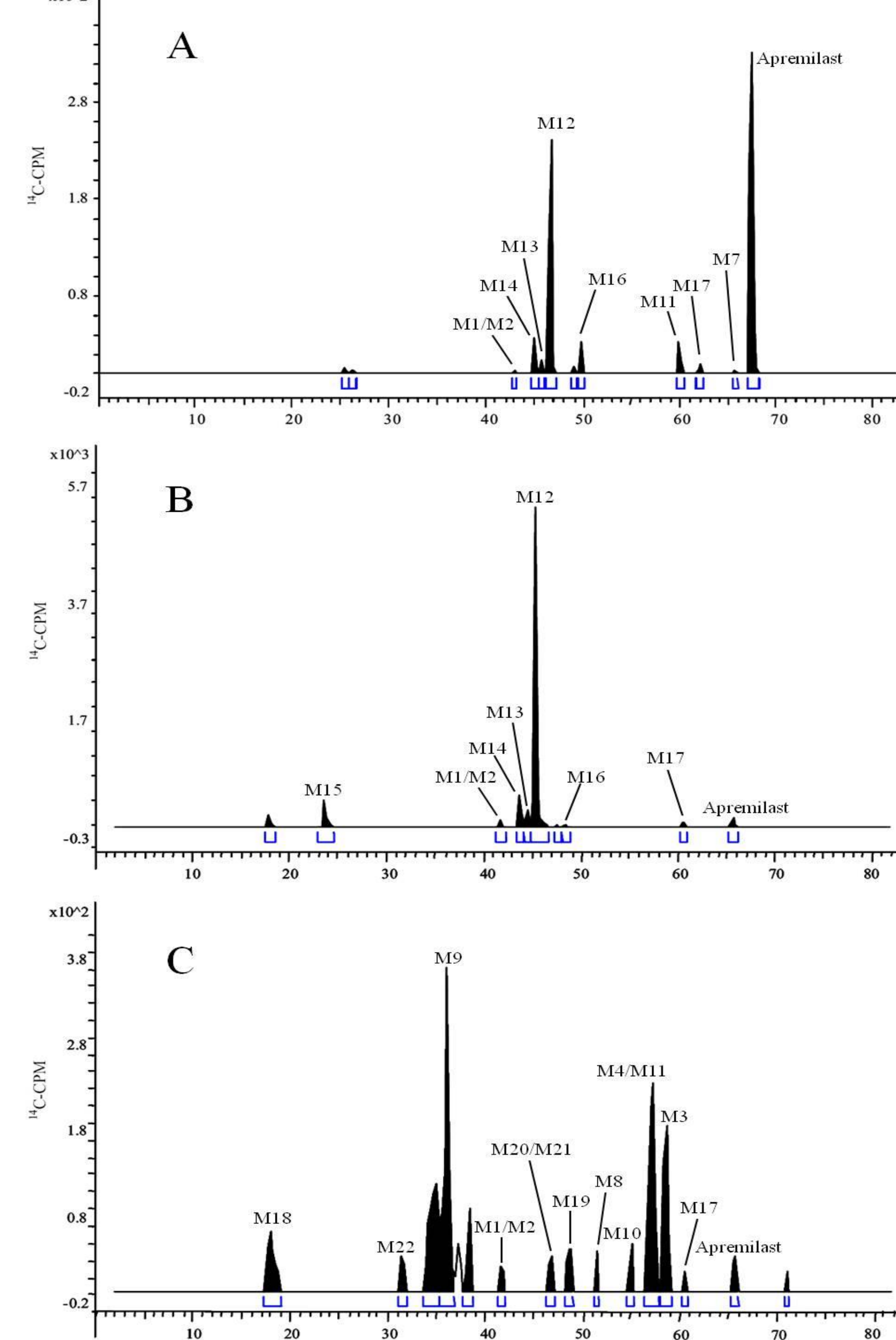


Table 2

Mean (SD) Plasma Pharmacokinetic Parameters for Apremilast and Circulating Metabolites after a Single Oral 20 mg Dose of [¹⁴C]Apremilast

	TRA	Apremilast ^a	Apremilast ^b	M11	M12	M13	M14	M16
C _{max} (ngEq/mL)	527 (127)	333 (76)	321 (134)	20.2 (7.6)	111 (36)	7.5 (6.8)	9.4 (4.3)	27.6 (26.0)
T _{max} (h)	1.5 (1-3)	1.5 (1-3)	1.8 (1-2.5)	1.0 (0.5-2.5)	2.5 (1-2.5)	2.5 (1-24)	2.5 (1-24)	5.3 (1-8)
AUC ₀₋₄₈ (ngEq*hr/mL)	5483 (825) ^c	1913 (339)	2455 (690)	139 (89)	2124 (331)	133 (125)	269 (146)	363 (54)
t _{1/2} (h)	50.4 (8.7)	6.8 (2.6)	7.1 (2.7)	10.7 (10.2)	15.8 (3.9)	n/c	n/c	11.0 (2.4)

a: Apremilast concentrations in plasma calculated using a LC-MS/MS assay.
b: Apremilast and metabolite concentrations in plasma calculated using plasma radioactivity concentrations and radiochromatography.
c: AUC₀₋₄₈ was used for TR AUC_{0-∞} for these calculations.
Tmax: median and range
n/c: not calculated

Table 3

Relative Amounts of Apremilast Metabolites Characterized in Plasma, Urine and Feces

Number	Metabolite Characterization	Plasma (% AUC relative to TR) ^d	% of Dose Excreted		
			Urine	Feces	
Apremilast	Unchanged	461	44.8	2.8	4.1
M1/M2	Hydrolysis products of apremilast	479	D	0.9	0.5
M3	O-Demethylation	447	D	D	4.6
M4	O-Demethylation, N-deacetylation	405	ND	ND	2.4
M7	N-Deacetylation	419	D	D	0.1
M9	Hydrolysis product of M3	465	ND	ND	7.7
M10	Hydroxylation, O-demethylation	463	ND	ND	1.3
M11	Hydroxylation, N-deacetylation	435	2.5	ND	1.4
M12	O-Demethylation, glucuronidation	623	38.7	33.7	ND
M13	O-Deethylation, glucuronidation	609	2.4	2.0	ND
M14	O-Demethylation, N-deacetylation, glucuronidation	581	4.9	4.0	ND
M15	Hydrolysis product of M12	641	ND	3.3	ND
M16	Hydroxylation, glucuronidation	653	6.6	0.6	ND
M17	Hydroxylation	477	D	1.2	0.9
M18	Hydrolysis	222 ^e	ND	ND	1.4
M23	Hydrolysis, hydroxylation	238 ^e	ND	ND	3.0

ND: not detected; D: detected by MS, but at concentrations too low to accurately quantify by radiochromatography.
Additional minor metabolites were observed accounted for less than 1% of the circulating radioactivity and less than 1% of the dose in excreta.
a: Many compounds were observed as [M+H]⁺ and [M+NH]₄⁺.
b: M20 and M21 co-eluted under the HPLC conditions used.
c: Characterized in negative mode, so values are [M-H].
d: AUC₀₋₄₈ (unchanged drug or metabolite)/AUC₀₋₄₈ (TRA) * 100%.

Figure 4

Metabolic scheme of apremilast in humans. For hydrolyzed phthalidomide ring products, only one of two possible forms is shown. [M5 was not observed in the current study and is a proposed intermediate metabolite] (GLU: glucuronic acid, * site of ¹⁴C label).

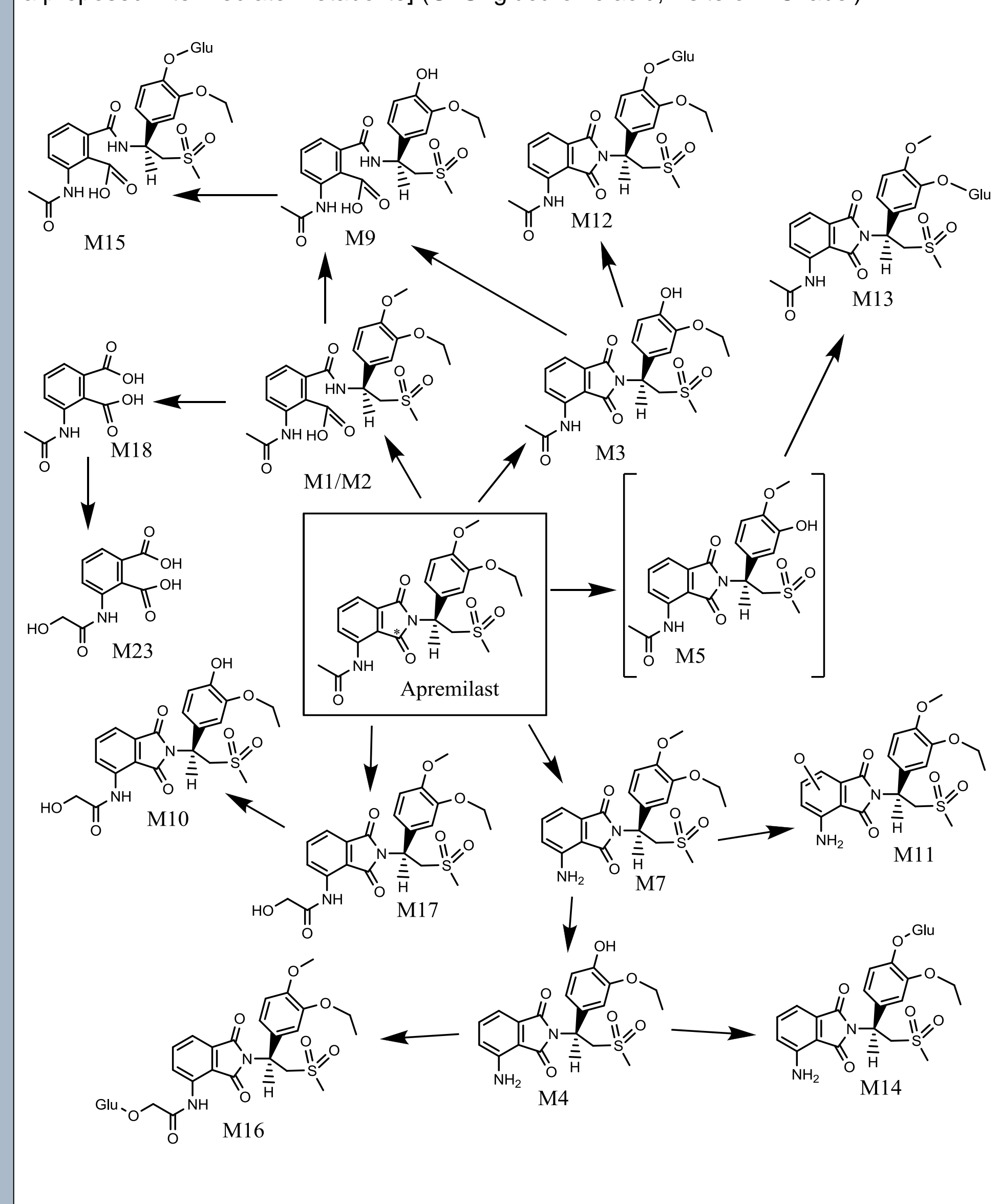


Table 4

Phosphodiesterase Type 4 and Tumor Necrosis Factor-α Inhibitory Activities of Apremilast and Its Metabolites

Compound	PDE4 IC ₅₀ (μM)	TNF-α IC ₅₀ (μM)
Apremilast (S-isomer)	0.074	0.077
M1/M2; Hydrolysis products of apremilast (S-isomer)	120	77
M3; O-Desmethyl apremilast (S-isomer)	8.3	5.6
M5; O-desethyl apremilast (racemate)	44	4.9
M7; N-deacetyl metabolite (S-isomer)	0.16	0.13
M12; O-Desmethyl apremilast glucuronide (S-isomer)	>100	>10
M14; O-Desmethyl, N-deacetyl apremilast glucuronide	>80	>10
M16; Acetamide hydroxylation glucuronide (S-isomer)	6.5	>10
M17; Acetamide hydroxylation (S-isomer)	0.094	0.021

Results and Conclusions

- Excretion of radioactivity was rapid and nearly complete, mean recovery of 97%; urine contained 58% of the radioactivity dose, with feces containing 39% (Fig 1).
- Mean blood/plasma AUC_{0-∞} ratio for radioactivity was 0.55, with ratios between 0.53 and 0.63 at all time points, indicating no preferential binding of radioactivity to whole blood constituents (Table 1).
- Absorption of [¹⁴C]apremilast was rapid and the plasma half-life was moderate, while plasma radioactivity had a much longer half-life (Table 1, Table 2, Fig 2), suggesting the presence of metabolites which are longer-lived than parent compound.
- Apremilast accounted for 45% of the circulating radioactivity and 7% of the dose was excreted as unchanged drug (Table 2, Table 3, Fig 2, Fig 3).
- The major metabolic pathway for apremilast was O-demethylation followed by glucuronidation (metabolite M12), with this metabolite accounting for 39% of the circulating radioactivity and 34% of the dose in excreta (Table 2).
- Additional minor metabolites were formed via O-demethylation, O-deethylation, N-deacetylation, hydroxylation, glucuronidation, and/or hydrolysis (Fig 4).
- Metabolic clearance of apremilast was major route of elimination, while non-enzymatic hydrolysis and excretion of unchanged drug were involved to a lesser extent.
- The major apremilast metabolites were at least 50-fold less active than apremilast with regard to their ability to inhibit PDE4 and TNF-α (Table 4). Metabolites M7 and M17, which were present at trace levels in plasma, did retain some PDE4 and TNF-α inhibition activity, with IC₅₀ values similar to those of apremilast.

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