Supporting Information:

Structure-Based Scaffold Repurposing for for G Protein-Coupled Receptors (GPCRs): Transformation of Adenosine Derivatives into $5HT_{2B}$/$5HT_{2C}$ Serotonin Receptor Antagonists

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Figure S1. IE profile obtained during 30 ns of MD simulations of the **23**-h5HT$_{2B}$R complex starting from the docking pose. The electrostatic (IE$_{ele}$) and van der Waal (IE$_{vdW}$) contributions and the total IE (IE$_{tot}$) are represented as blue, green and pink lines, respectively. IE values are in Kcal/mol.

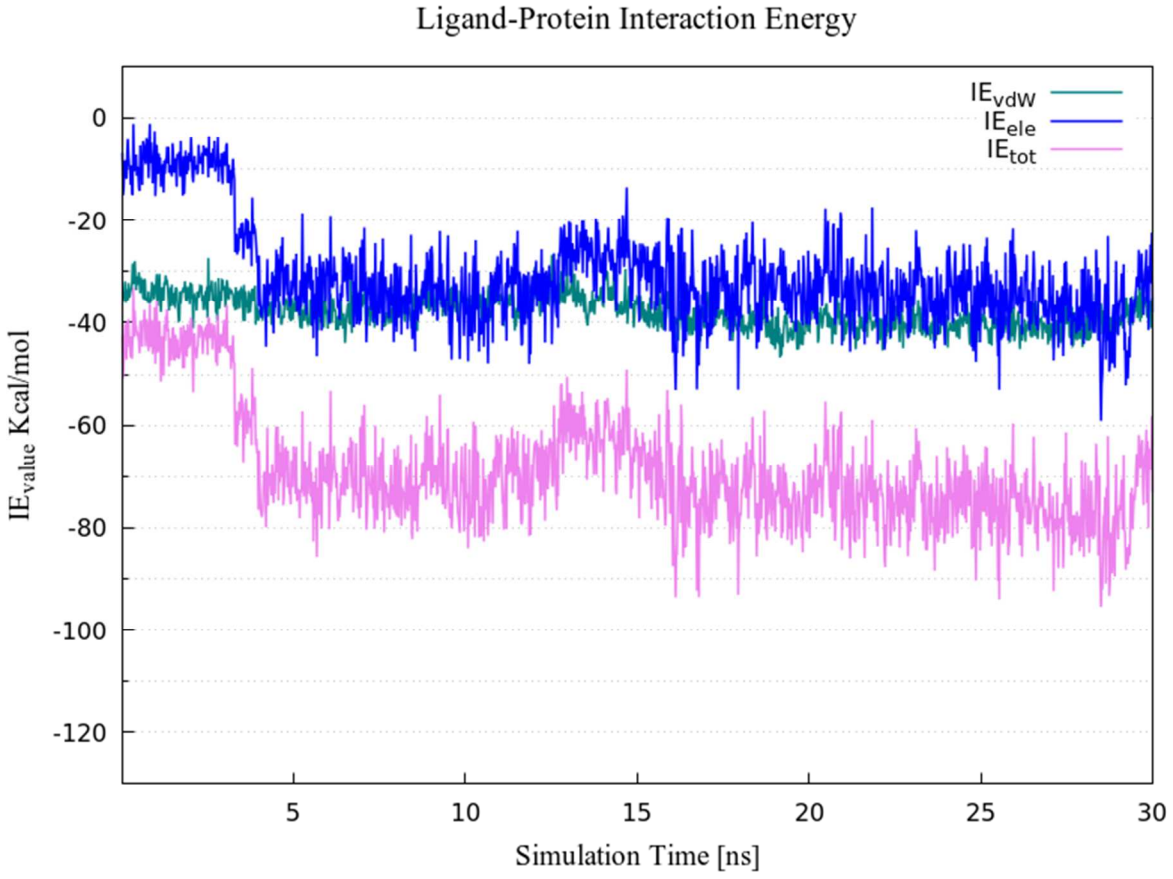


Figure S2. Panel A: superimposition between initial (pink) and MD refined (green) **23**-h5HT$_{2B}$R complex structure. Panel B: average water density during 30 ns of MD simulation of **23**-h5HT$_{2B}$R complex. Structural alignment was based upon alpha carbon atoms of TM domains. Ligand and selected residues are represented as van der Waals spheres.

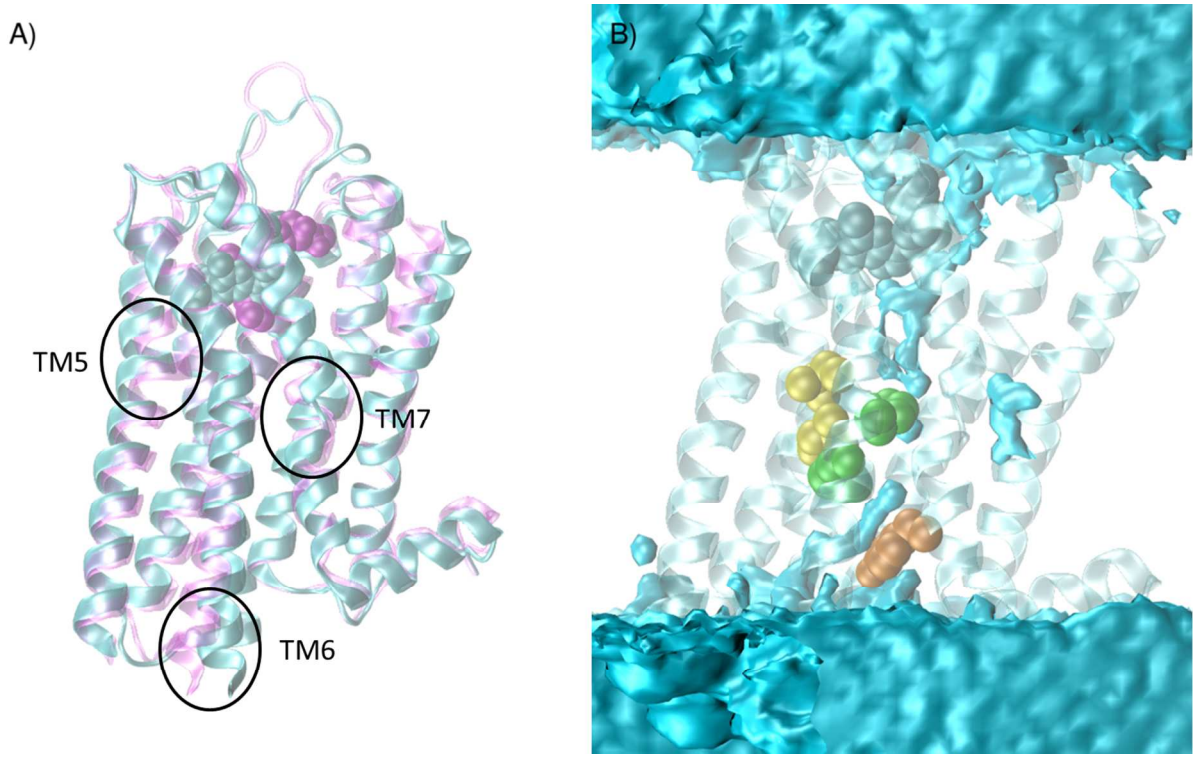


Figure S3. Heteroatoms distance distribution in selected pairs of conserved residues during 30 ns of MD simulations of the 23-h5HT$_{2B}$R (green line) and the h5HT$_{2B}$R-apo (magenta line) structures.

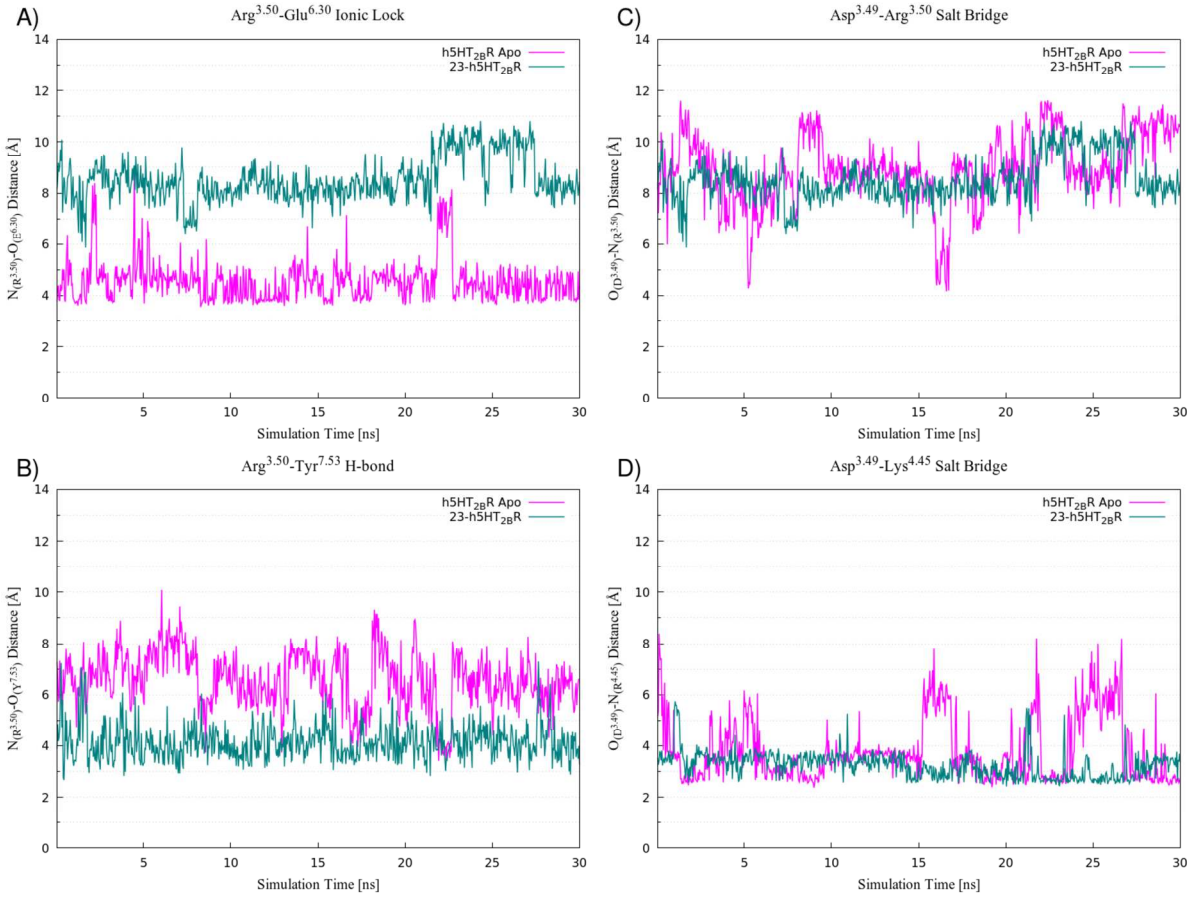


Table S1. Docking scores obtained at the h5HT$_{2B}$R. Values are in Kcal/mol.

Compound	SP scoring function	XP scoring function
14	**-8.284**	**-10.496**
23	**-7.713**	**-9.823**
25	**-8.012**	**-9.342**
26a	**-7.908**	**-10.286**
26b	**-7.771**	**-9.577**
27	**-7.965**	**-9.471**
35	**-7.892**	**-11.481**

Table S2. Structure based sequence alignment of TM4 (up to conserved 4.50 residue) of aminergic and nucleotide class A GPCRs.

Receptor	4.35	4.36	4.37	4.38	4.39	4.40	4.41	4.42	4.43	4.44	4.45	4.46	4.47	4.48	4.49	4.50
5-HT1A receptor	-	-	-	-	P	R	R	A	A	A	L	I	S	L	T	W
5-HT1B receptor	-	-	-	-	P	K	R	A	A	V	M	I	A	L	V	W
5-HT1D receptor	-	-	-	-	A	G	H	A	A	T	M	I	A	I	V	W
5-HT1E receptor	-	-	-	-	A	K	R	A	A	L	M	I	L	T	V	W
5-HT1F receptor	-	-	-	-	P	K	H	A	G	I	M	I	T	I	V	W
5-HT2A receptor	-	-	-	-	R	T	K	A	F	L	**K**	I	I	A	V	W
5-HT2B receptor	-	-	-	-	R	A	T	A	F	I	**K**	I	T	V	V	W
5-HT2C receptor	-	-	-	-	R	T	K	A	I	M	**K**	I	A	I	V	W
5-HT4 receptor	-	-	-	-	P	L	R	I	A	L	M	L	G	G	C	W
5-HT5A receptor	-	-	-	-	K	C	V	S	N	V	M	I	A	L	T	W
5-HT6 receptor	-	-	-	-	P	L	R	A	L	A	L	V	L	G	A	W
5-HT7 receptor	-	-	-	-	G	K	C	M	A	K	M	I	L	S	V	W
M1 receptor	-	-	-	-	P	R	R	A	A	L	M	I	G	L	A	W
M2 receptor	-	-	-	-	T	K	M	A	G	M	M	I	A	A	A	W
M3 receptor	-	-	-	-	T	K	R	A	G	V	M	I	G	L	A	W
M4 receptor	-	-	-	-	T	K	M	A	G	L	M	I	A	A	A	W
M5 receptor	-	-	-	-	P	K	R	A	G	I	M	I	G	L	A	W
α1A-adrenoceptor	-	-	-	-	Q	R	R	G	L	M	A	L	L	C	V	W
α1B-adrenoceptor	-	-	-	-	R	R	K	A	I	L	A	L	L	S	V	W
α1D-adrenoceptor	-	-	-	-	E	R	K	A	A	A	I	L	A	L	L	W
α2A-adrenoceptor	-	-	-	-	P	R	R	I	K	A	I	I	I	T	V	W
α2B-adrenoceptor	-	-	-	-	P	R	R	I	K	C	I	I	L	T	V	W
α2C-adrenoceptor	-	-	-	-	P	R	R	V	K	A	T	I	V	A	V	W
β1-adrenoceptor	-	-	-	-	R	A	R	A	R	G	L	V	C	T	V	W
β2-adrenoceptor	-	-	-	-	K	N	K	A	R	V	I	I	L	M	V	W
β3-adrenoceptor	-	-	-	-	K	R	C	A	R	T	A	V	V	L	V	W
D1 receptor	-	-	-	-	P	K	A	A	F	I	L	I	S	V	A	W
D2 receptor	-	-	-	-	K	R	R	V	T	V	M	I	S	I	V	W
D3 receptor	-	-	-	-	C	R	R	V	A	L	M	I	T	A	V	W
D4 receptor	-	-	-	-	S	R	R	Q	L	L	L	I	G	A	T	W
D5 receptor	-	-	-	-	Q	R	M	A	L	V	M	V	G	L	A	W
H1 receptor	-	-	-	-	K	T	R	A	S	A	T	I	L	G	A	W
H2 receptor	-	-	-	-	P	V	R	V	A	I	S	L	V	L	I	W
H3 receptor	-	-	-	-	T	R	R	A	V	R	**K**	M	L	L	V	W
H4 receptor	-	-	-	-	V	L	K	I	V	T	L	M	V	A	V	W
TA1 receptor	-	-	-	-	I	L	V	I	C	V	M	I	F	I	S	W
A1 receptor	-	-	-	-	P	R	R	A	A	V	A	I	A	G	C	W
A2A receptor	-	-	-	-	G	T	R	A	K	G	I	I	A	I	C	W
A2B receptor	-	-	-	-	G	T	R	A	R	G	V	I	A	V	L	W
A3 receptor	-	-	-	-	H	R	R	I	W	L	A	L	G	L	C	W
P2Y1 receptor	G	R	L	K	K	K	N	A	I	C	I	S	V	L	V	W
P2Y2 receptor	R	W	G	R	A	R	Y	A	R	R	V	A	G	A	V	W

P2Y4 receptor	R	W	G	R	P	R	L	A	G	L	L	C	L	A	V	W
P2Y6 receptor	-	R	G	G	R	R	A	A	W	L	V	C	V	A	V	W
P2Y11 receptor	S	H	L	R	P	K	H	A	W	A	V	S	A	A	G	W
P2Y12 receptor	-	P	K	N	L	L	G	A	K	I	L	S	V	V	I	W
P2Y13 receptor	-	L	K	K	P	V	F	A	K	T	V	S	I	F	I	W
P2Y14 receptor	-	I	Q	S	V	S	Y	S	K	L	L	S	V	I	V	W
CONSENSUS	R	+	G	R	P	R	R	A	A	L	**M**	I	A	L	V	W

Table S3. Parameters considered for the selection of a representative trajectory among three replicas: protein alpha carbon atoms (Cα) average RMSD, ligand average RMSD, average Area per lipid headgroup (APL_{ave}), and slope of the dynamic scoring function (DSF). RMSD and RMSF values are in Å, APL is in $Å^2$, and DSF_{tot} is adimensional.

	Run1	**Run2**	**Run3**
Cα RMSD	1.681	2.083	1.828
Ligand RMSD	3.381	3.500	3.247
APL	66.717	66.584	67.084
DSF_{tot}	-59.162	-54.036	**-70.179**

Video S1. Video of the trajectory for the classical all-atom MD simulations (30 ns) of membrane-embedded **23**-h5HT_{2B}R: Side view facing TM2, TM3 and TM4 (upper left panel); Side view facing TM5, TM6, and TM7 (upper right panel); Top view (lower left panel); IE profile (lower right panel). Receptor is represented in cyan ribbons with selected residues depicted in lines and water molecules in balls and sticks. H-bond interactions are represented as orange dashed lines. Lipids are represented as tan dotted surface.

[separate file, available as Supporting information)

Figure S4. Correlation plot of affinity of 2-Cl (N)-methanocarba adenosine derivatives (compounds **13** – **35**) at the $5HT_{2B}R$ and $5HT_{2C}R$.

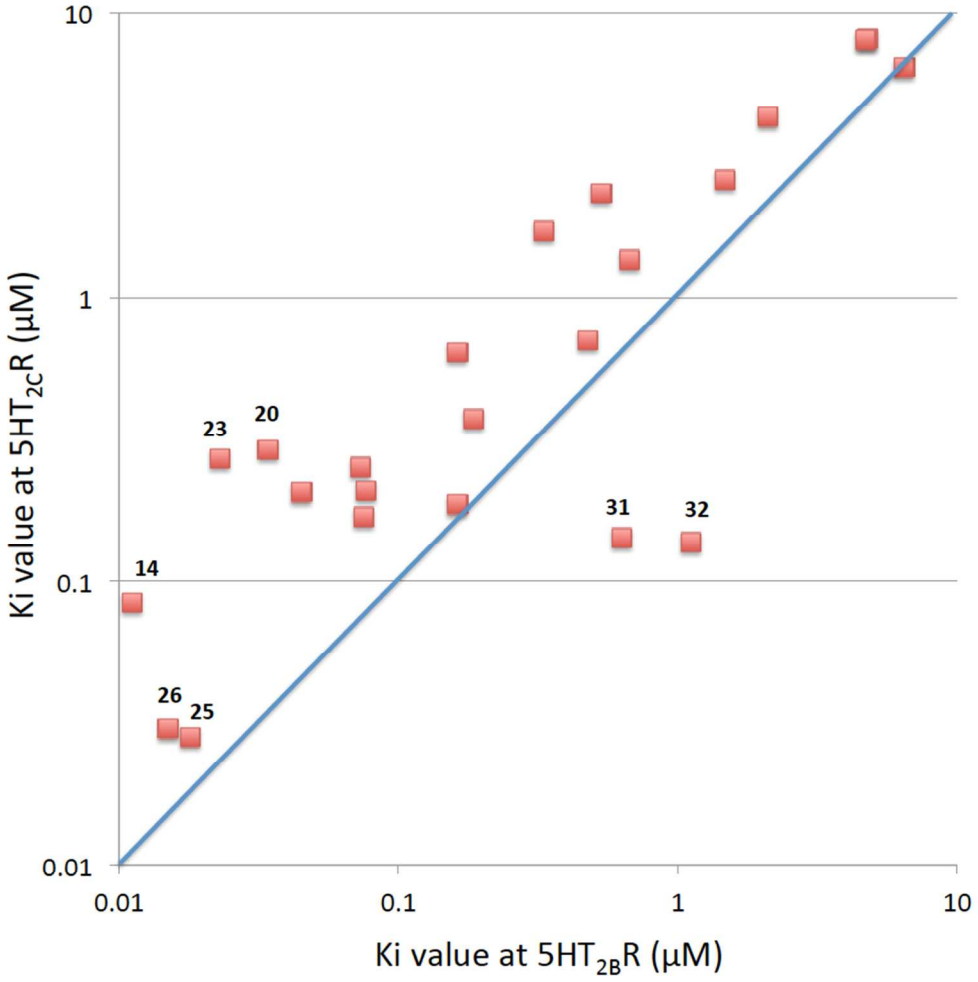
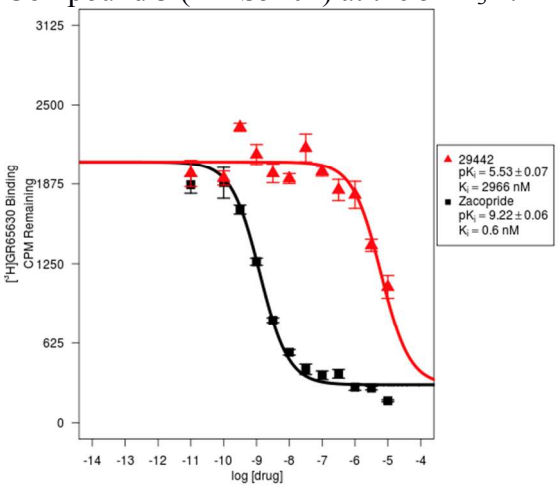
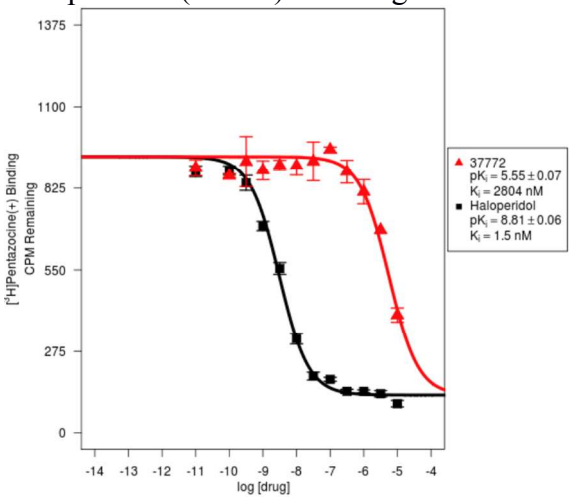


Figure S5. Other off-target activities.

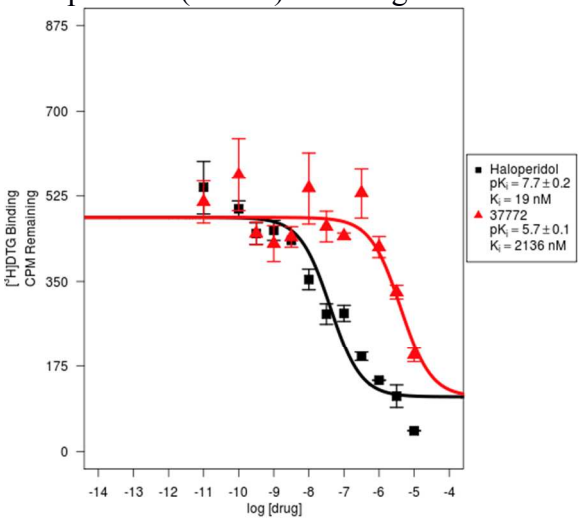
Compound **3** (MRS5202) at the $5HT_3R$:



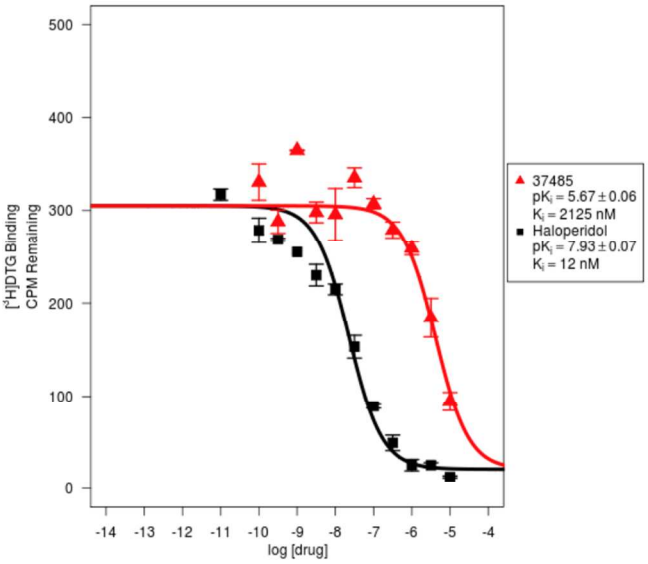
Compound **9** (CPCA) at the sigma1:

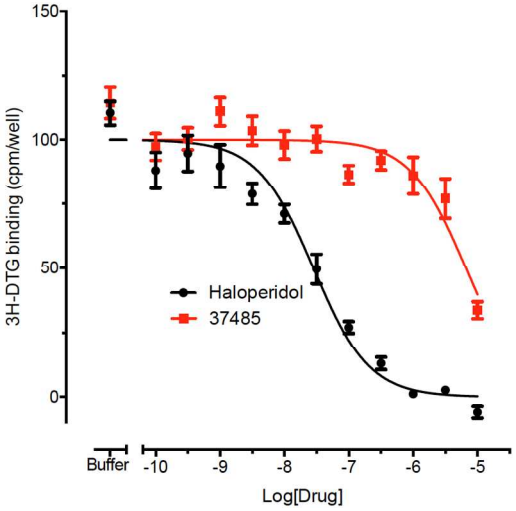


Compound **9** (CPCA) at the sigma2:

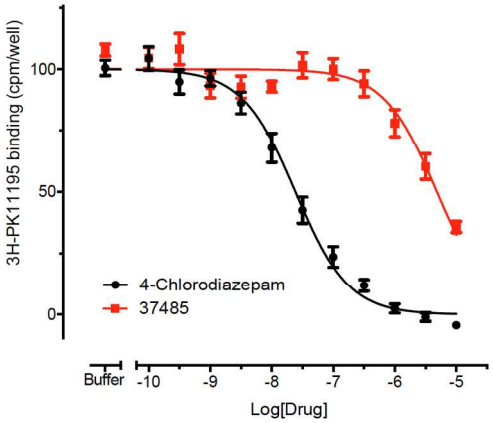


Compound **13** (MRS1947) at the sigma2:



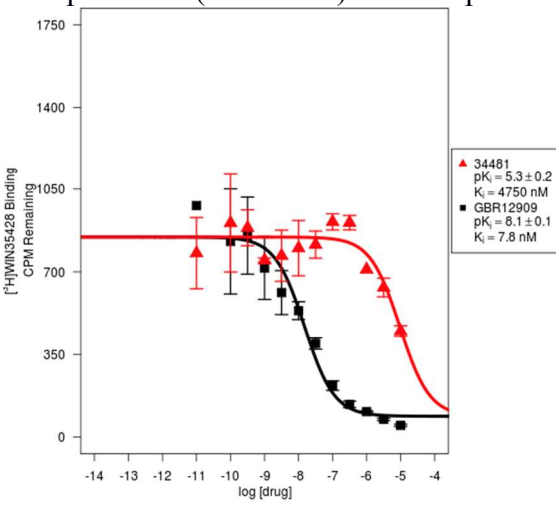


Compound **13** (MRS1947) at TSPO:

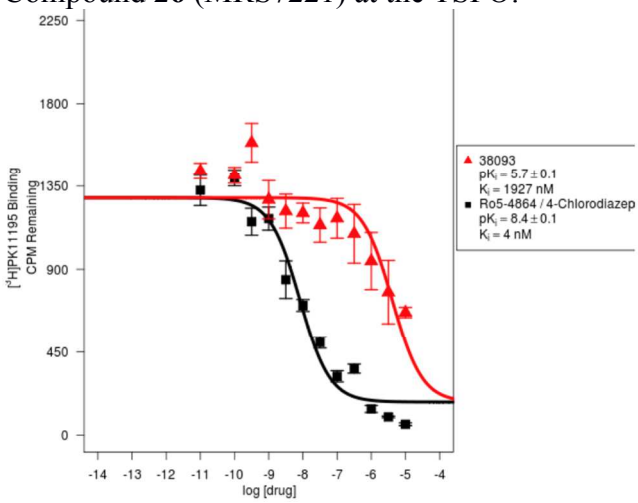


The curves for compounds **13** at sigma2 and TSPO were best fit using a monophasic isotherm with a variable Hill slope (F-test, $P < 0.05$ was considered as statistically significant).

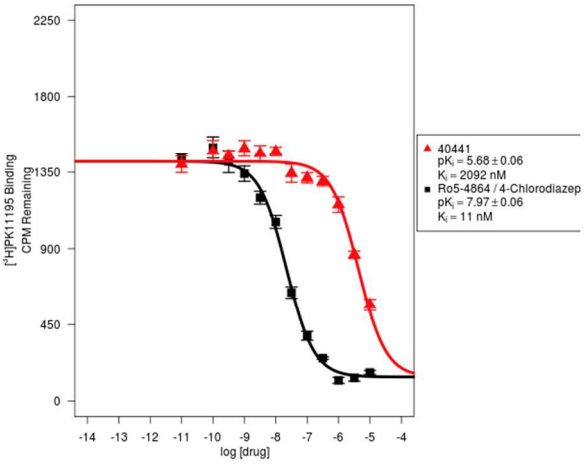
Compound **14** (MRS7134) at the dopamine transporter:



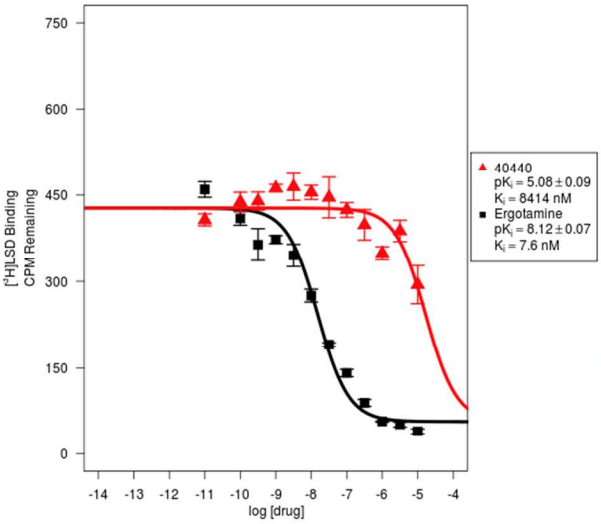
Compound **26** (MRS7221) at the TSPO:



Compound **27** (MRS7249) at the TSPO:



Compound **30** (MRS7248) at the $5HT_{5A}R$:



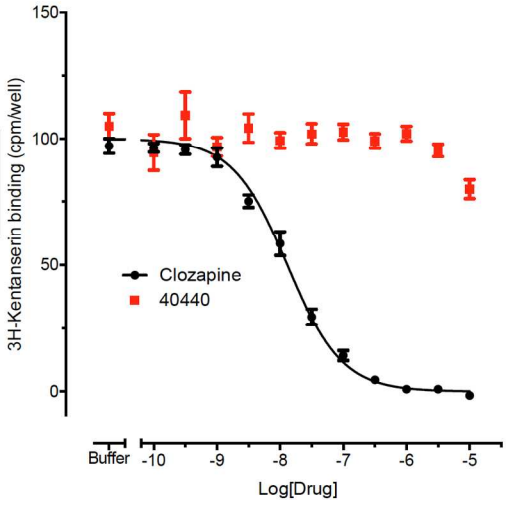


Figure S6. Functional assays of PDSP38093 (MRS7221, **26**)

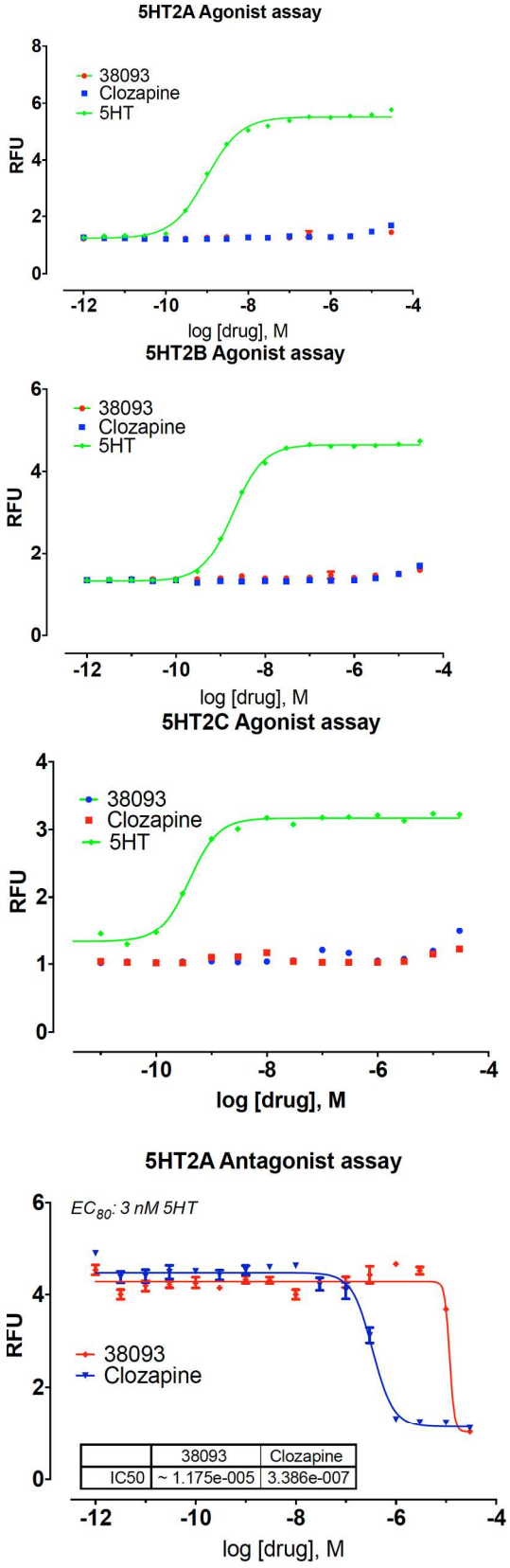
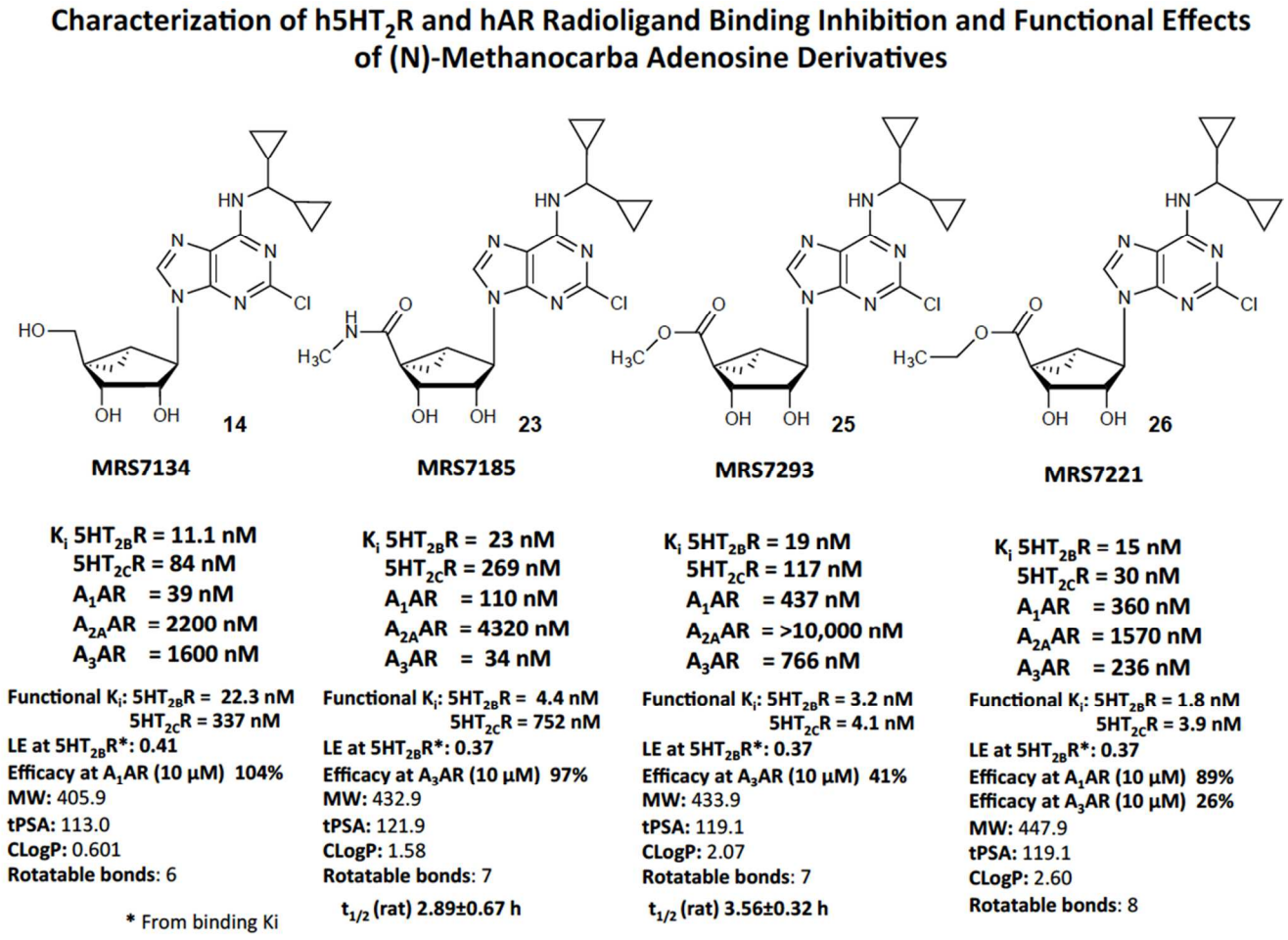
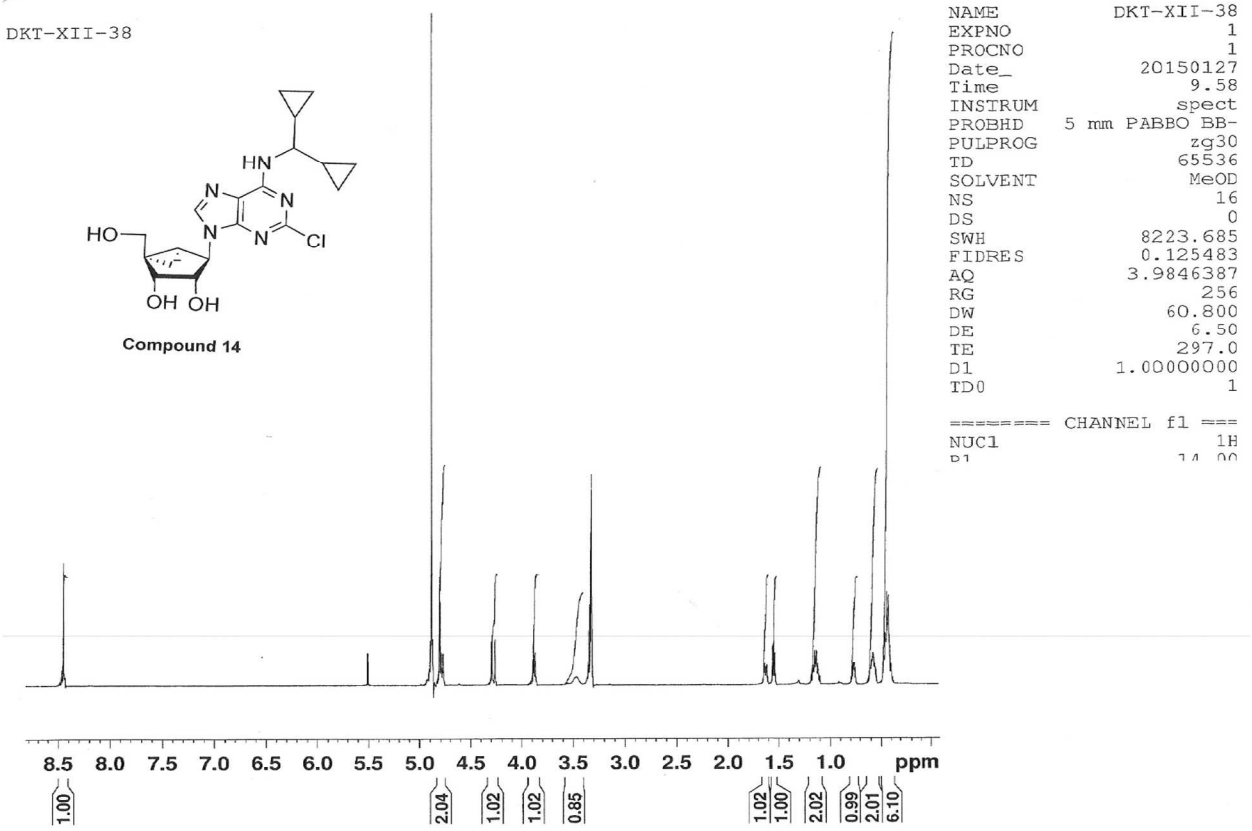


Figure S7. Summary of $5HT_2R$ data, AR data and in vivo half-life for key nucleoside derivatives.



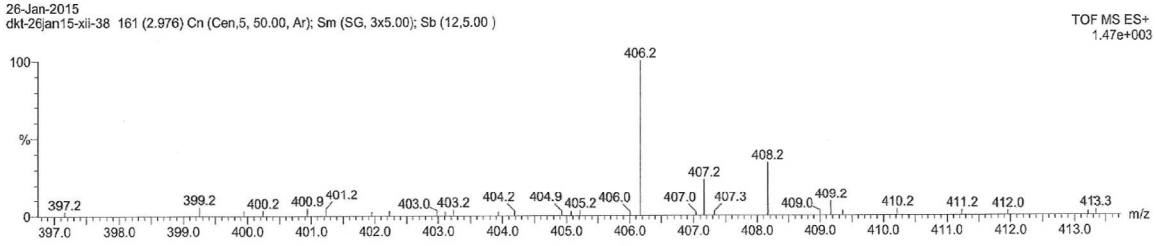
tPSA (total polar surface area) and CLogP calculated using ChemDraw Professional (PerkinElmer Informatics, Inc., Boston, MA), v. 15.0.
LE = $1.4(-\log K_i)/N$, where N is the number of non-hydrogen atoms.

Figure S8. NMR and mass spectra of selected compounds.

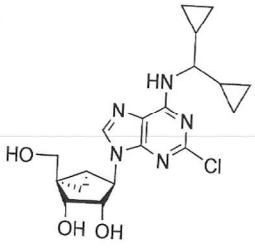


Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -50.0, max = 500.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

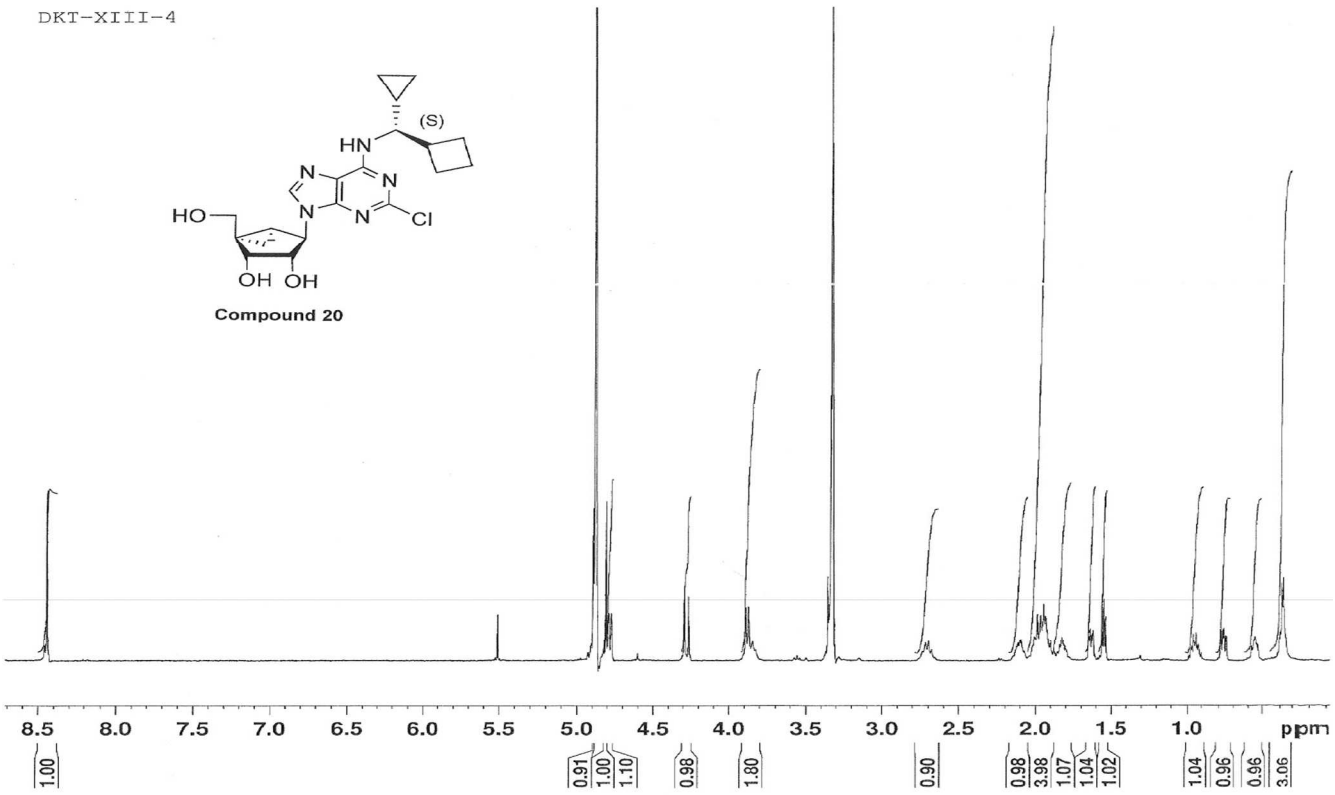
Monoisotopic Mass, Even Electron Ions
169 formula(e) evaluated with 3 results within limits (up to 10 closest results for each mass)
Elements Used:
C: 0-100 H: 0-200 N: 5-5 O: 0-30 35Cl: 1-1
26-Jan-2015
dkt-26jan15-xii-38 161 (2.976) Cn (Cen,5, 50.00, Ar); Sm (SG, 3x5.00); Sb (12,5.00)



Minimum:				-50.0		
Maximum:		10.0	10.0	500.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
406.1653	406.1646	0.7	1.7	9.5	206.3	C19 H25 N5 O3 35Cl
	406.1611	4.2	10.3	-12.5	320.7	C H33 N5 O16 35Cl
	406.1705	-5.2	-12.8	0.5	227.4	C12 H29 N5 O8 35Cl

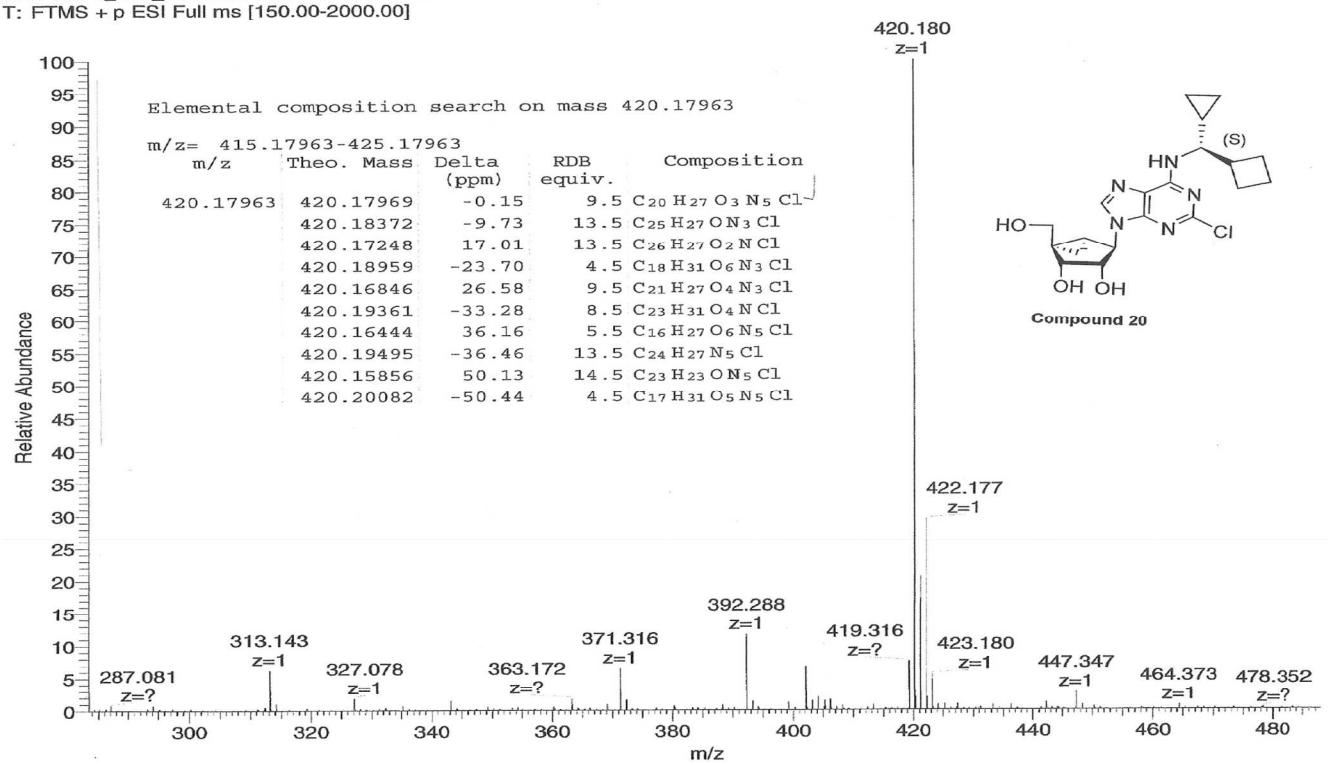


Compound 14



DKT-XIII-4_419_150520113539 5/20/2015 12:07:25 PM

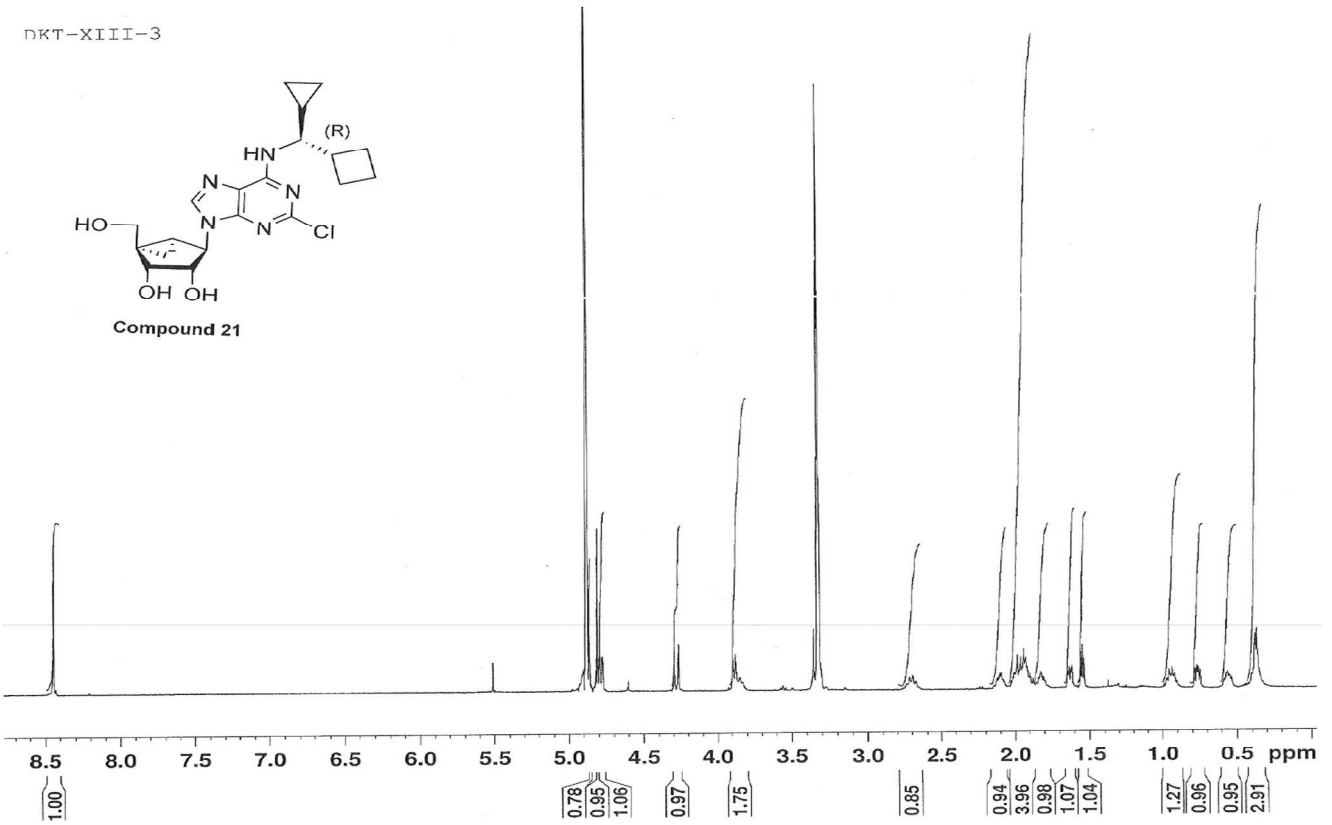
DKT-XIII-4_419_150520113539 #84 RT: 1.21 AV: 1 NL: 1.09E6
T: FTMS + p ESI Full ms [150.00-2000.00]



Elemental composition search on mass 420.17963

m/z= 415.17963-425.17963

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
420.17963	420.17969	-0.15	9.5	$C_{20}H_{27}O_3N_5Cl$
	420.18372	-9.73	13.5	$C_{25}H_{27}ON_3Cl$
	420.17248	17.01	13.5	$C_{26}H_{27}O_2NCl$
	420.18959	-23.70	4.5	$C_{18}H_{31}O_6N_3Cl$
	420.16846	26.58	9.5	$C_{21}H_{27}O_4N_3Cl$
	420.19361	-33.28	8.5	$C_{23}H_{31}O_4NCl$
	420.16444	36.16	5.5	$C_{16}H_{27}O_6N_5Cl$
	420.19495	-36.46	13.5	$C_{24}H_{27}N_5Cl$
	420.15856	50.13	14.5	$C_{23}H_{23}ON_5Cl$
	420.20082	-50.44	4.5	$C_{17}H_{31}O_5N_5Cl$

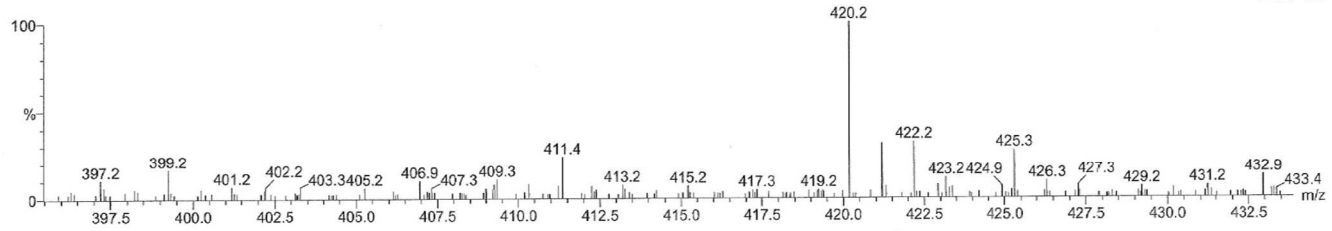


Compound 21

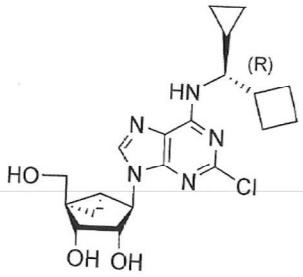
Single Mass Analysis
Tolerance = 25.0 mDa / DBE: min = -2.0, max = 500.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
69 formula(e) evaluated with 6 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-100 H: 0-200 N: 5-5 O: 0-30 35Cl: 1-1
19-May-2015
dkt-19may15-xiii-3 90 (1.664) Cn (Cen,7, 50.00, Ar); Sm (SG, 3x5.00); Sb (12,5.00)

TOF MS ES+
7.43e+002

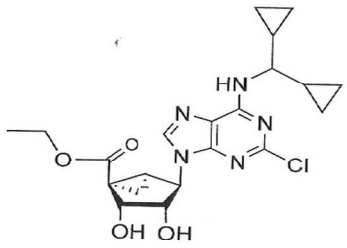


Minimum:				-2.0		
Maximum:		25.0	10.0	500.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
420.1799	420.1802	-0.3	-0.7	9.5	97.9	C20 H27 N5 O3 35Cl
	420.1861	-6.2	-14.8	0.5	119.5	C13 H31 N5 O8 35Cl
	420.1650	14.9	35.5	5.5	111.2	C16 H27 N5 O6 35Cl
	420.1955	-15.6	-37.1	13.5	97.0	C24 H27 N5 35Cl
	420.1591	20.8	49.5	14.5	98.8	C23 H23 N5 O 35Cl
	420.2014	-21.5	-51.2	4.5	115.8	C17 H31 N5 O5 35Cl



Compound 21

DKT-XIV-16

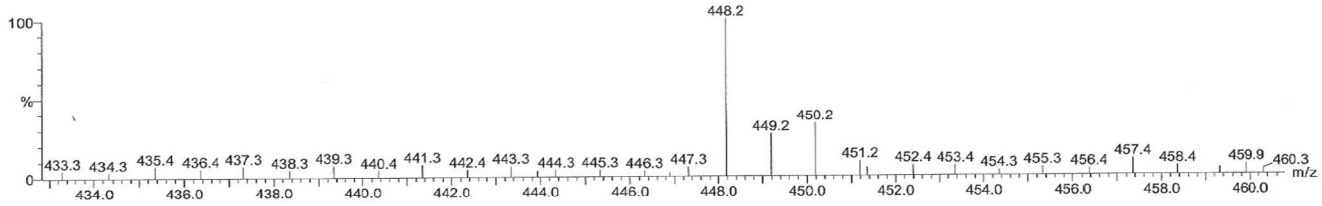


Compound 26

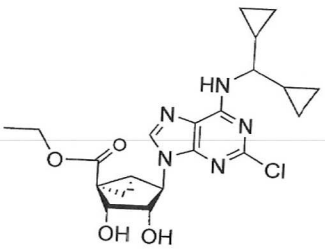
Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -2.0, max = 500.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
78 formula(e) evaluated with 2 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-120 H: 0-200 N: 5-5 O: 0-40 35Cl: 1-1
13-Nov-2015
dkt-13nov15-xiv-16 118 (4.145) Cn (Cen,5, 50.00, Ar); Sm (SG, 3x5.00); Sb (12,5.00)

TOF MS ES+
9.35e+003

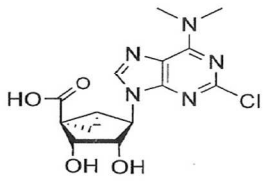


Minimum:				-2.0		
Maximum:		10.0	10.0	500.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
448.1745	448.1752	-0.7	-1.6	10.5	1201.5	C21 H27 N5 O4 35Cl
	448.1810	-6.5	-14.5	1.5	1353.1	C14 H31 N5 O9 35Cl



Compound 26

DKT-XIV-2

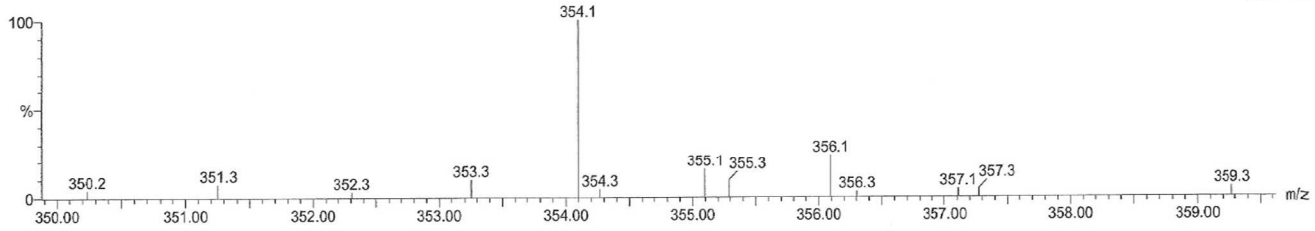


Compound 30

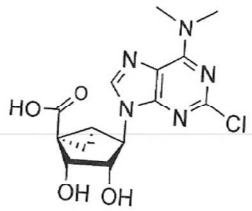
Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -2.0, max = 500.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
45 formula(e) evaluated with 2 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-120 H: 0-200 N: 5-5 O: 0-50 35Cl: 1-1
20-Oct-2015
dkt-20oct15-xiv-2 64 (2.249) Cn (Cen,5, 50.00, Ar); Sm (SG, 3x5.00); Sb (12,5.00)

TOF MS ES+
3.09e+003



Minimum:				-2.0		
Maximum:		10.0	10.0	500.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
354.0966	354.0969	-0.3	-0.8	8.5	299.8	C14 H17 N5 O4 35Cl
	354.1028	-6.2	-17.5	-0.5	321.3	C7 H21 N5 O9 35Cl

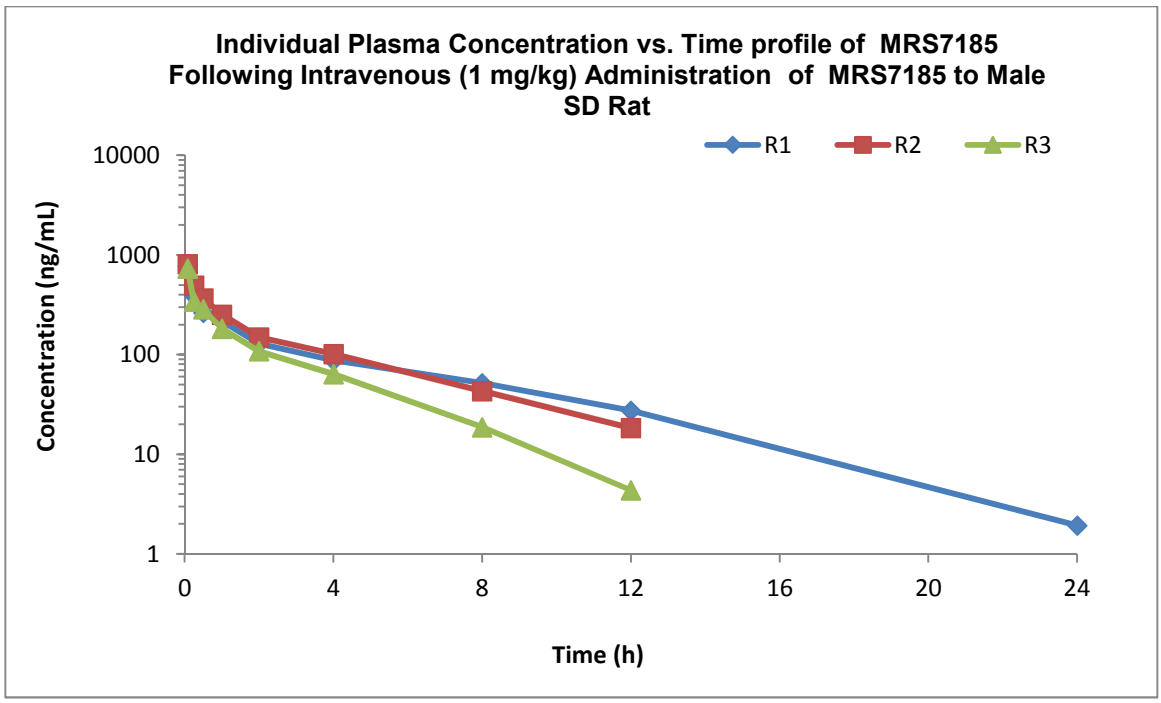


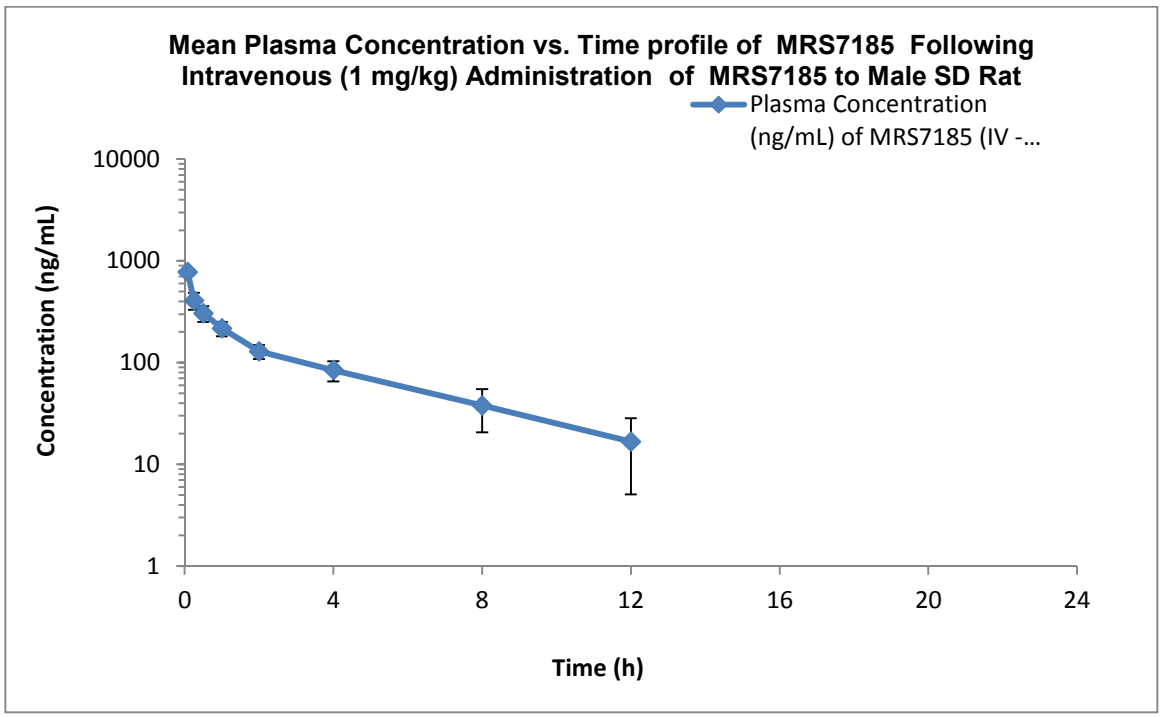
Compound 30

Pharmacokinetic measurements of MRS7185 (23)

Study Title:	PHARMACOKINETICS OF MRS7185 INTRAVENOUS ADMINISTRATION IN MALE SPRAGUE DAWLEY RATS					
Study Number	1324-16-DMPK					
Compound	MRS7185					
Formulation IV solution	DMSO: 20% HPBCD (10:90)					
Species	Male SD rat (Fed for IV group)					
Study Design	Single dosing for IV in non-anesthesized rats					
Matrix	Plasma					
Bioanalytical Details						
Analyte	MRS7185					
LLOQ	1.04 ng/mL		**ULOQ**		1040.00 ng/mL	
Plasma Concentration (ng/mL) of MRS7185 (IV - 1mg/kg)						
Time (h)	**R1**	**R2**	**R3**	**Mean**	**Std Dev**	**% CV**
0.08	772.65	811.92	731.51	**772.03**	40.21	5.21
0.25	389.51	491.84	339.95	**407.10**	77.46	19.03
0.50	263.75	365.83	284.37	**304.65**	53.98	17.72
1.00	216.99	251.75	181.78	**216.84**	34.99	16.13
2.00	128.94	149.38	108.22	**128.85**	20.58	15.97
4.00	87.67	101.50	63.83	**84.33**	19.06	22.60
8.00	51.85	43.13	18.65	**37.88**	17.21	45.44
12.00	27.46	18.30	4.35	**16.70**	11.64	69.67
24.00	1.93	BLQ	BLQ	**BLQ**	NC	NC
Dose (mg/kg)	1.00	1.00	1.00	**1.00**	0.00	0.00
Co(ng/mL)	1085.99	1041.61	1070.60	**1066.07**	22.53	2.11
$t_{1/2}$ (h)	3.31	3.24	2.11	**2.89**	0.67	23.32
Vdss(L/kg)	3.63	2.86	3.00	**3.16**	0.41	12.92
Vd (L/kg)	3.66	3.43	3.54	**3.54**	0.12	3.26
Cl (mL/min/kg)	12.78	12.25	19.35	**14.80**	3.96	26.75
$AUC_{0\text{-last}}$ (ng·h/mL)	1295.05	1274.82	847.87	**1139.24**	252.54	22.17
$AUC_{0\text{-inf}}$ (ng·h/mL)	1304.27	1360.27	861.11	**1175.22**	273.46	23.27
AUC_{Extra}(%)	0.71	6.28	1.54	**2.84**	3.01	105.82
$MRT_{0\text{-last}}$ (h)	4.56	3.04	2.39	**3.33**	1.12	33.53
Rsq	0.9965	0.9999998	0.9966	**1.00**	0.00	0.20
	0.900034518	0.968292499	0.975566819			

*BLQ : Below level of Quantification, #n.c. : Not calculated



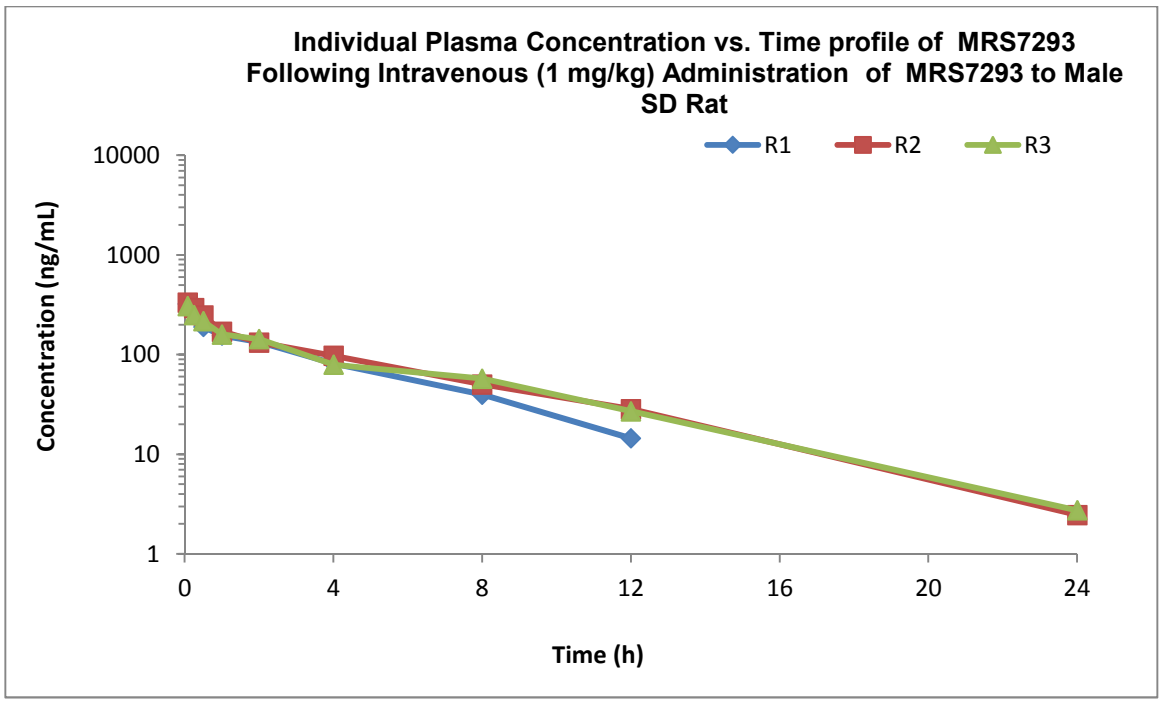


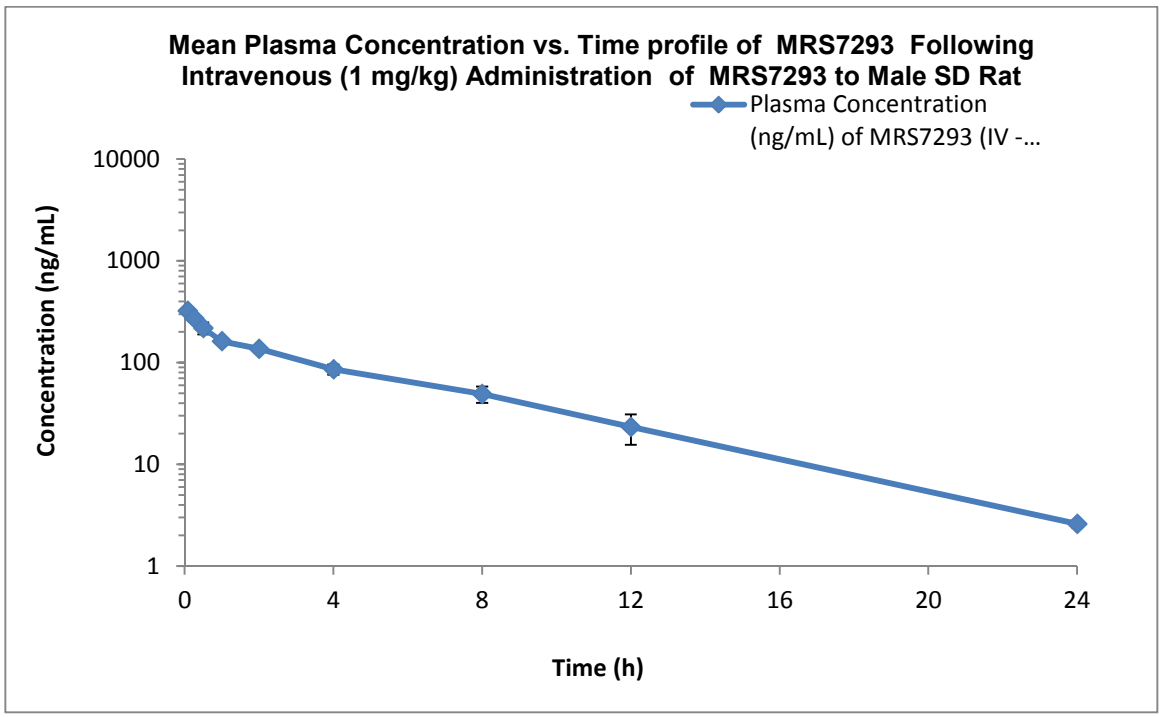
All measurements of ADME-tox parameters on MRS7185 (23) and MRS7293 (25) were performed by GVK BIO Sciences, Pvt Ltd., Hyderabad, India. Web:www.gvkbio.com.

Pharmacokinetic measurements of MRS7293 (25)

Study Title:	PHARMACOKINETICS OF MRS7293 INTRAVENOUS ADMINISTRATION IN MALE SPRAGUE DAWLEY RATS					
Study Number	1325-16-DMPK					
Compound	MRS7293					
Formulation IV solution	DMSO: 20% HPBCD (10:90)					
Species	Male SD rat (Fed for IV group)					
Study Design	Single dosing for IV in non-anesthesized rats					
Matrix	Plasma					
Bioanalytical Details						
Analyte	MRS7293					
LLOQ	2.08 ng/mL		**ULOQ**	1040.00 ng/mL		
Plasma Concentration (ng/mL) of MRS7293 (IV – 1 mg/kg)						
Time (h)	**R1**	**R2**	**R3**	**Mean**	**Std Dev**	**% CV**
0.08	323.95	332.87	307.75	**321.52**	12.73	3.96
0.25	277.03	292.93	249.57	**273.18**	21.94	8.03
0.50	189.89	248.84	216.76	**218.50**	29.51	13.51
1.00	155.05	170.52	159.43	**161.67**	7.97	4.93
2.00	134.05	132.30	143.45	**136.60**	6.00	4.39
4.00	81.38	97.49	79.19	**86.02**	9.99	11.62
8.00	39.68	50.23	57.41	**49.11**	8.92	18.16
12.00	14.48	28.45	27.01	**23.31**	7.68	32.96
24.00	BLQ	2.46	2.74	**2.60**	0.20	7.61
Dose (mg/kg)	1.00	1.00	1.00	**1.00**	0.00	0.00
Co(ng/mL)	350.15	354.70	341.53	**348.79**	6.69	1.92
$t_{1/2}$ (h)	3.21	3.83	3.64	**3.56**	0.32	9.03
Vdss(L/kg)	4.56	4.49	4.83	**4.62**	0.18	3.88
Vd (L/kg)	4.74	4.57	4.51	**4.60**	0.12	2.59
Cl (mL/min/kg)	17.07	13.78	14.29	**15.05**	1.77	11.79
$AUC_{0\text{-last}}$ (ng·h/mL)	909.24	1196.24	1151.83	**1085.77**	154.48	14.23
$AUC_{0\text{-inf}}$ (ng·h/mL)	976.20	1209.84	1166.23	**1117.42**	124.23	11.12
AUC_{Extra}(%)	6.86	1.12	1.23	**3.07**	3.28	106.74
$MRT_{0\text{-last}}$ (h)	3.55	5.15	5.33	**4.68**	0.98	20.96
Rsq	0.9961	0.9957	1.0000	**1.00**	0.00	0.24
	0.900034518	0.968292499	0.975566819			

*BLQ : Below level of Quantification, #n.c. : Not calculated

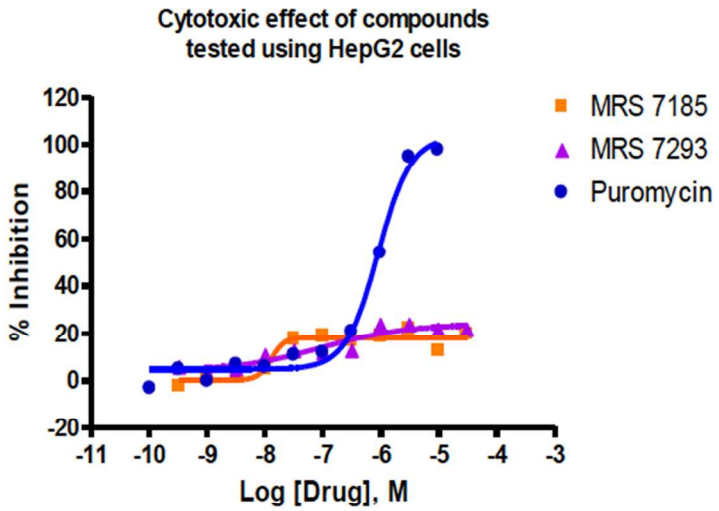

Individual Plasma Concentration vs. Time profile of MRS7293 Following Intravenous (1 mg/kg) Administration of MRS7293 to Male SD Rat
R1
R2
R3
Concentration (ng/mL)
10000
1000
100
10
1
0
4
8
12
16
20
24
Time (h)


Mean Plasma Concentration vs. Time profile of MRS7293 Following Intravenous (1 mg/kg) Administration of MRS7293 to Male SD Rat
Plasma Concentration (ng/mL) of MRS7293 (IV -...
Concentration (ng/mL)
10000
1000
100
10
1
0
4
8
12
16
20
24
Time (h)

Summary of PK parameters

IV	MRS7185	MRS7293
Dose (mg/kg)	**1.00**	**1.00**
Co(ng/mL)	**1066.07**	**348.79**
$t_{1/2}$ (h)	**2.89**	**3.56**
Vdss(L/kg)	**3.16**	**4.62**
Vd (L/kg)	**3.54**	**4.60**
Cl (mL/min/kg)	**14.80**	**15.05**
$AUC_{0\text{-last}}$ (ng·h/mL)	**1139.24**	**1085.77**
$AUC_{0\text{-inf}}$ (ng·h/mL)	**1175.22**	**1117.42**
AUC_{Extra}(%)	**2.84**	**3.07**
$MRT_{0\text{-last}}$ (h)	**3.33**	**4.68**
Rsq	**1.00**	**1.00**

Dose response curves of cytotoxicity assay in HepG2 cells



Compound ID	CC50, µM	pCC50	CC50_Lower 95% Confidence interval	CC50_Upper 95% Confidence interval	Max Response	Max response concentration, µM	HillSlope
MRS 7185	>30	<4.52	-	-	20	30	-
MRS 7293	>30	<4.52	-	-	22	30	-
Puromycin	1.1	6	0.77	1.6	99	10	1.5

STABILITY: Mouse liver microsomes

Compound Name	Half life (min)	% Rem @ 60 min	% Rem @ 60 min W/O Cofactor	CL int protein (µL/min/mg protein)	CL int invivo (mL/min/kg BW)	CL invivo (mL/min/kg BW)-Well stirred model	%QH (Well stirred model)	Classification
MRS-7185	130.49	70.06	102.84	5.42	21.48	17.29	19.21	Low
MRS-7293	144.53	81.53	95.04	2.76	10.93	9.72	10.80	Low
Verapamil	2.24	0.00	102.08	308.84	1223.02	83.83	93.14	High

Rat liver microsomes

Compound Name	Half life (min)	% Rem @ 60 min	% Rem @ 60 min W/O Cofactor	CL int protein (µL/min/mg protein)	CL int invivo (mL/min/kg BW)	CL invivo (mL/min/kg BW)-Well stirred model	%QH (Well stirred model)	Classification
MRS-7185	144.53	73.72	103.46	4.75	8.55	7.61	10.88	Low
MRS-7293	144.53	81.07	100.46	3.66	6.58	6.01	8.59	Low
Verapamil	2.93	0.00	95.21	236.59	425.86	60.12	85.88	High

Human liver microsomes

Compound Name	Half life (min)	% Rem @ 60 min	% Rem @ 60 min W/O Cofactor	CL int protein (µL/min/mg protein)	CL int invivo (mL/min/kg BW)	CL invivo (mL/min/kg BW)-Well stirred model	%QH (Well stirred model)	Classification
MRS-7185	144.53	86.85	100.04	1.75	1.46	1.36	6.48	Low
MRS-7293	144.53	93.59	91.89	0.77	0.64	0.61	2.93	Low
Verapamil	3.09	0.11	96.46	224.44	186.74	18.88	89.88	High

Classification criteria	%QH
Low clearance	<30
Medium clearance	30-70
High clearance	>70

Plasma stability (Mean% Remaining at 120 Min)

Compound ID	Mouse Plasma	Rat Plasma	Human Plasma
MRS-7185	95.04	88.68	103.91
MRS-7293	61.06	70.91	95.29
Propantheline	11.10	54.78	2.46

Stability in simulated gastric fluid and intestinal fluid ($t_{1/2}$, Mean% Remaining at 120 min)

	FaSSGF (pH 1.60)	
Compound Name	**$t_{1/2}$, min**	**% remaining**
MRS-7185	131.85	53.18
MRS-7293	125.07	48.27
Erythromycin	87.77	38.66
	FaSSIF (pH 6.50)	
Compound Name	**min**	**%**
MRS-7185	>480	96.62
MRS-7293	>480	96.79
Erythromycin	>480	100.23

CACO-2 cell permeability

Compound Name	**Average Values**					
	Papp (10^{-6} cm/sec)		**Efflux Ratio**	**% Recovery (A2B)**	**% Recovery (B2A)**	**Classification**
	A2B Papp (10^{-6} cm/sec)	**B2A Papp (10^{-6} cm/sec)**				
MRS-7185	4.73	46.21	11.70	86.00	98.14	Medium
MRS-7293	18.47	30.59	1.67	88.00	101.54	High
Propranolol	32.20	27.79	0.87	79.50	97.06	High
Atenolol	0.00	1.37	#DIV/0!	90.00	97.39	Low

Result of permeability Papp (10^{-6}cm/s)	Range
<1.5	Low permeable
1.5 to 10	Medium permeable
>10	High permeable

Conclusions

MRS-7185 is moderately permeable with significant efflux in Caco-2 cell system.
MRS-7293 is high permeable compound with slight efflux in Caco-2 cell system.

Inhibition of cytochrome P450 isozymes

Compound	**IC50 (μM)**									
	1A2		**2C9**		**2C19**		**2D6**		**3A4**	
MRS-7185	>	30.00	>	30.00	>	30.00	>	30.00	>	30.00
MRS-7293	>	30.00	=	20.11	>	30.00	>	30.00	>	30.00
QC	=	0.0078	=	0.45	=	0.50	=	0.02	=	0.03

Bioanalytical method for pharmacokinetic study of 23 and 25 (quality control shown for **23**):

Study Number		1324-16-DMPK			
Study Type		IVPK study			
Instrument ID		API 4000			
COMPOUND DETAILS:					
Name of Compound		**Molecular weight Free Form**	**Molecular weight Salt Form**	**Purity/potency/ assay**	**Diluents-Master stock preparation**
Analyte	MRS7185	432.90	432.90	100.00%	DMSO
Internal Standard	Telmesartan	514.50	514.50	98.00%	DMSO
CHROMATOGRAPHY:					
Generic Gradient:					
Time (min)		%A		%B	
0.01		90		10	
1.00		5		95	
2.60		5		95	
2.70		90		10	
3.50		90		10	
Mobile Phase (A)		0.01% Formic acid in water			
Mobile Phase (B)		100 %Acetonitrile			
Column		Kinetics EVO C18, 50*4.6 mm, 5 µ			
Injection Volume (µL)		10			
Flow Rate (mL/min)		1			
Run Time(min)		3.5			
Sample Cooler Temperature (°C)		10			
Column Oven Temperature (°C)		40			
Rinsing Solution		Acetonirile:Methanol: water::20:60:20,V/V			
SAMPLE PREPARATION:					
Extraction Technique		**Protein Precipitation**			
Extraction Solvent		**Acetonitrile Containing IS**			
Calibration Curve & QC preparation: 2.0 µL of calibration curve standards added to 48 µL of blank matrix and precipitated with 200 µL of acetonitrile containing internal standard at 200ng/mL conc. then vortexed for 5 min at 850 rpm, centrifuged at 4000 rpm for 5 min at 4 °C, from this 110 µL of supernatant was separated and diluted with 130 µL of methanol:water(1:1,v/v)					
Sample Preparation: 50 µL of sample was taken and precipitated with 200 µL of Acetonitrile containing internal standard at 200 ng/mL conc. then vortexed for 5 min at 850 rpm, centrifuged at 4000 rpm for 5 min at 4 °C, from this 110 µL of supernatant was separated and diluted with 130 µL of methnol:water(1:1,v/v) .					

MASS SPECTROMETRIC CONDITION:							
Ionization Mode-Polarity		**ESI-Positive**					
Name of Compound		**Retention Time (Min)**	**MRM Transitions**		**Declustering Potential (DP)**	**Entrance Potential (EP)**	**Collision Energy (CE)**
			Q1-mass	**Q3-mass**			
Analyte	MRS7185	1.47	433.10	264.10	112	10	30
Analyte	MRS7293	1.57	434.20	66.90	120	10	49
Internal Standard	Telmesartan	1.37	515.30	276.10	60	10	64
Collision Cell Exit Potential (CXP)		12					
Collision Gas (CAD)		12					
Curtain Gas (CUR)		25					
Nebulizer Gas (GS1)		50					
Heater Gas (GS2)		55					
Nebulizer current (NC)		5					
Temperature (TEM)		600^0 C					
Interface Heater (ihe)		Off					

Calibration Curve Standards of MRS7185 in SD rat plasma			
Standard	**Concentration [ng/mL]**		**% Accuracy**
	Nominal	**Calculated**	
STD 1 (LLOQ)	1.04	1.08	103.38
STD 2	2.08	1.89	90.97
STD 3	10.41	11.53	110.80
STD 4	52.07	54.10	103.89
STD 5	208.26	199.34	95.72
STD 6	520.65	525.03	100.84
STD 7	833.04	785.68	94.32
STD 8	936.00	918.94	98.18
STD 9 (ULOQ)	1040.00	1059.79	101.90
Correlation (r)	0.998		
Regression equ.	Y=0.000591X+0.000129		

Regression Fit	Linear with 1/x2 weighting factor

Quality Controls of MRS7185 in SD rat plasma

QC Set		**Concentration (ng/mL)**		**% Accuracy**
		Nominal	**Calculated**	
HQC	HQC-1	920	829.28	90.14
	HQC-2		929.89	101.07
MQC	MQC-1	552	545.61	98.84
	MQC-2		555.58	100.65
LQC	LQC-1	4.42	5.3	119.88
	LQC-2		5.5	124.45*