Supplementary File 2:

Radioligand binding assays: Methods employed in this study have been adapted from the scientific literature to maximize reliability and reproducibility. Reference standards were run as an integral part of each assay to ensure the validity of the results obtained.

Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
Adenosine A_1	443228	hum	2	10 µM	9
Adenosine A_{2A}	443229	hum	2	10 µM	10
Adenosine A_{2B}	443528	hum	2	10 µM	-21
Adenosine A_3	443150	hum	2	10 µM	1
Adrenergic α_{1A}	443231	hum	2	10 µM	1
Adrenergic α_{1B}	443232	hum	2	10 µM	29
Adrenergic α_{1D}	443199	hum	2	10 µM	6
Adrenergic α_{2A}	443233	hum	2	10 µM	18
Adrenergic α_{2B}	443268	hum	2	10 µM	-3
Adrenergic α_{2C}	443269	hum	2	10 µM	26
Adrenergic β_1	443234	hum	2	10 µM	2
Adrenergic β_2	443235	hum	2	10 µM	7
Adrenergic β_3	443365	hum	2	10 µM	-25
Aldosterone	443311	hum	2	10 µM	32
Androgen (Testosterone)	443169	hum	2	10 µM	-2
Angiotensin AT_1	443327	hum	2	10 µM	8
Angiotensin AT_2	443328	hum	2	10 µM	1
APJ	443143	hum	2	10 µM	3
Atrial Natriuretic Factor (ANF)	443352	gp	2	10 µM	9
Bombesin BB1	443366	hum	2	10 µM	-10
Bombesin BB2	443367	hum	2	10 µM	-17
Bombesin BB3	443366	hum	2	10 µM	-3
Bradykinin B_1	443260	hum	2	10 µM	-18
Bradykinin B_2	443261	hum	2	10 µM	20
Calcitonin	443148	hum	2	10 µM	13
Calcitonin Gene-Related Peptide $CGRP_1$	443149	hum	2	10 µM	-4
Calcium Channel L-Type, Benzothiazepine	443204	rat	2	10 µM	16
Calcium Channel L-Type, Dihydropyridine	443237	rat	2	10 µM	58
Calcium Channel L-Type, Phenylalkylamine	443534	rat	2	10 µM	47
Calcium Channel N-Type	443501	rat	2	10 µM	-3
Cannabinoid CB_1	443238	hum	2	10 µM	-22
Cannabinoid CB_2	443239	hum	2	10 µM	10
Chemokine CCR1	443348	hum	2	10 µM	-15
Chemokine CCR2B	443372	hum	2	10 µM	-1
Chemokine CCR5 (Rhesus Macaque)	443379	rm	2	10 µM	-6

Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
Chemokine CX3CR1	443385	hum	2	10 µM	17
Chemokine CXCR1 (IL-8R$_A$)	443379	hum	2	10 µM	9
Chemokine CXCR2 (IL-8R$_B$)	443317	hum	2	10 µM	-10
Chemokine CXCR3	443379	hum	2	10 µM	6
Chemokine CXCR4	443381	hum	2	10 µM	2
Cholecystokinin CCK$_1$ (CCK$_A$)	443532	hum	2	10 µM	-1
Cholecystokinin CCK$_2$ (CCK$_B$)	443273	hum	2	10 µM	-15
Colchicine	443368	rat	2	10 µM	6
Corticotropin Releasing Factor CRF$_1$	443366	hum	2	10 µM	-2
CysLT$_2$ (LTC$_4$)	443356	hum	2	10 µM	19
Dopamine D$_1$	443200	hum	2	10 µM	28
Dopamine D$_{2S}$	443201	hum	2	10 µM	5
Dopamine D$_3$	443200	hum	2	10 µM	34
Dopamine D$_{4.4}$	443432	hum	2	10 µM	11
Dopamine D$_5$	443580	hum	2	10 µM	16
Endothelin ET$_A$	443155	hum	2	10 µM	23
Endothelin ET$_B$	443163	hum	2	10 µM	-2
Epidermal Growth Factor (EGF)	443223	hum	2	10 µM	6
Estrogen ERα	443304	hum	2	10 µM	12
Estrogen ERβ	443305	hum	2	10 µM	3
GABA$_A$, Chloride Channel, TBOB	443188	rat	2	10 µM	52
GABA$_A$, Flunitrazepam, Central	443253	rat	2	10 µM	6
GABA$_A$, Muscimol, Central	443252	rat	2	10 µM	13
Gabapentin	443459	rat	2	10 µM	21
γ-Hydroxybutyric Acid (GHB) Receptor	443380	rat	2	10 µM	3
Glucagon	443355	hum	2	10 µM	2
Glucocorticoid	443162	hum	2	10 µM	1
Glutamate, AMPA	443320	rat	2	10 µM	16
Glutamate, Kainate	443191	rat	2	10 µM	28
Glutamate, Metabotropic, mGlu$_2$	443318	hum	2	10 µM	10
Glutamate, Metabotropic, mGlu$_5$	443319	hum	2	10 µM	-3
Glutamate, NMDA, Agonism	443189	rat	2	10 µM	-4
Glutamate, NMDA, Glycine	443190	rat	2	10 µM	2
Glutamate, NMDA, Phencyclidine	443254	rat	2	10 µM	58
Glutamate, NMDA, Polyamine	443322	rat	2	10 µM	13
Glycine, Strychnine-Sensitive	443214	rat	2	10 µM	8
Gonadotropin-Releasing Hormone	443372	hum	2	10 µM	-1

Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
Growth Hormone Secretagogue (GHS, Ghrelin)	443150	hum	2	10 µM	-3
Histamine H_1	443245	hum	2	10 µM	13
Histamine H_2	443336	hum	2	10 µM	-12
Histamine H_3	443161	hum	2	10 µM	-19
Histamine H_4	443412	hum	2	10 µM	28
Imidazoline I_2, Central	443209	rat	2	10 µM	11
Inositol Trisphosphate IP_3	443350	rat	2	10 µM	-7
Insulin	443170	rat	2	10 µM	2
Interleukin IL-1 R1	443314	hum	2	10 µM	-6
Interleukin IL-6	443315	hum	2	10 µM	0
IP (PGI2)	443426	hum	2	10 µM	5
Leukotriene, BLT (LTB_4)	443147	hum	2	10 µM	-8
Leukotriene, Cysteinyl $CysLT_1$	443324	hum	2	10 µM	2
Melanin-Concentrating Hormone MCH1 (SLC1)	443402	hum	2	10 µM	-9
Melanocortin MC_1	443196	hum	2	10 µM	2
Melanocortin MC_3	443384	hum	2	10 µM	4
Melanocortin MC_4	443197	hum	2	10 µM	2
Melanocortin MC_5	443384	hum	2	10 µM	7
Melatonin MT_1	443382	hum	2	10 µM	4
Melatonin MT_2	443383	hum	2	10 µM	15
Motilin	443154	hum	2	10 µM	-14
Muscarinic M_1	443240	hum	2	10 µM	11
Muscarinic M_2	443241	hum	2	10 µM	1
Muscarinic M_3	443242	hum	2	10 µM	9
Muscarinic M_4	443243	hum	2	10 µM	0
Muscarinic M_5	443243	hum	2	10 µM	2
Muscarinic, Oxotremorine M	443413	rat	2	10 µM	-10
Neuropeptide Y Y_1	443194	hum	2	10 µM	3
Neuropeptide Y Y_2	443419	hum	2	10 µM	-17
Neurotensin NT_1	443326	hum	2	10 µM	-13
Nicotinic Acetylcholine α1, Bungarotoxin	443255	hum	2	10 µM	4
Nicotinic Acetylcholine α3β4	443258	hum	2	10 µM	-4
Nicotinic Acetylcholine α4β2, Cytisine	443420	rat	2	10 µM	8
Nicotinic Acetylcholine α7, Bungarotoxin	443373	rat	2	10 µM	4
Nicotinic Acetylcholine α7, Methyllycaconitine	443415	hum	2	10 µM	2

Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
Opiate δ_1 (OP1, DOP)	443172	hum	2	10 µM	6
Opiate κ (OP2, KOP)	443172	hum	2	10 µM	5
Opiate µ (OP3, MOP)	443389	hum	2	10 µM	38
Orexin OX_1	443367	hum	2	10 µM	10
Orexin OX_2	443374	hum	2	10 µM	11
Orphanin ORL_1	443386	hum	2	10 µM	6
Oxytocin	443407	hum	2	10 µM	17
Phorbol Ester	443256	mouse	2	10 µM	-22
Platelet Activating Factor (PAF)	443265	hum	2	10 µM	9
Platelet-Derived Growth Factor (PDGF)	443144	mouse	2	10 µM	-6
Potassium Channel [K_A]	443194	rat	2	10 µM	5
Potassium Channel [K_{ATP}]	443104	ham	2	10 µM	18
Potassium Channel [SK_{CA}]	443421	rat	2	10 µM	-2
Potassium Channel hERG	443395	hum	2	10 µM	-35
Progesterone PR-B	443168	hum	2	10 µM	20
Prostanoid CRTH2	443370	hum	2	10 µM	12
Prostanoid DP	443387	hum	2	10 µM	-74
Prostanoid EP_1	443592	hum	2	10 µM	19
Prostanoid EP_2	443387	hum	2	10 µM	17
Prostanoid EP_3	443387	hum	2	10 µM	19
Prostanoid EP_4	443247	hum	2	10 µM	17
Prostanoid FP	443276	hum	2	10 µM	5
Purinergic P2X	443581	rat	2	10 µM	-12
Purinergic P2Y, Non-Selective	443156	rat	2	10 µM	32
Retinoid X Receptor RXRα	443165	hum	2	10 µM	2
Rolipram	443533	rat	2	10 µM	5
Ryanodine RyR3	443377	rat	2	10 µM	-4
Serotonin (5-Hydroxytryptamine) 5-HT_{1A}	443193	hum	2	10 µM	14
Serotonin (5-Hydroxytryptamine) 5-HT_{1B}	443371	rat	2	10 µM	-8
Serotonin (5-Hydroxytryptamine) 5-HT_{2B}	443251	hum	2	10 µM	45
Serotonin (5-Hydroxytryptamine) 5-HT_{2C}	443325	hum	2	10 µM	19
Serotonin (5-Hydroxytryptamine) 5-HT_3	443342	hum	2	10 µM	-2
Serotonin (5-Hydroxytryptamine) 5-HT_4	443375	gp	2	10 µM	17
Serotonin (5-Hydroxytryptamine) 5-HT_{5A}	443208	hum	2	10 µM	25
Serotonin (5-Hydroxytryptamine) 5-HT_6	443406	hum	2	10 µM	10
Serotonin (5-Hydroxytryptamine) 5-HT_7	443370	hum	2	10 µM	19
Sigma σ_1	443244	hum	2	10 µM	41

Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
Sigma σ_2	443425	rat	2	10 µM	34
Sodium Channel, Site 2	443427	rat	2	10 µM	47
Somatostatin sst1	443211	hum	2	10 µM	2
Somatostatin sst2	443212	hum	2	10 µM	1
Somatostatin sst3	443211	hum	2	10 µM	17
Somatostatin sst4	443213	hum	2	10 µM	15
Somatostatin sst5	443213	hum	2	10 µM	-1
Tachykinin NK_1	443153	hum	2	10 µM	19
Tachykinin NK_2	443418	hum	2	10 µM	-10
Tachykinin NK_3	443414	hum	2	10 µM	-3
Thyroid Hormone	443312	rat	2	10 µM	3
Thyrotropin Releasing Hormone (TRH)	443430	hum	2	10 µM	15
Transforming Growth Factor-β (TGF-β)	443145	mouse	2	10 µM	-11
Transporter, Adenosine	443202	gp	2	10 µM	16
Transporter, Choline	443353	rat	2	10 µM	10
Transporter, Dopamine (DAT)	443272	hum	2	10 µM	49
Transporter, GABA	443275	rat	2	10 µM	18
Transporter, Glycine	443152	rat	2	10 µM	-16
Transporter, Norepinephrine (NET)	443236	hum	2	10 µM	44
Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	443343	hum	2	10 µM	-91
Transporter, Vesicular Monoamine (Non-Selective)	443412	hum	2	10 µM	11
Urotensin II	443419	hum	2	10 µM	9
Vasoactive Intestinal Peptide VIP_1	443376	hum	2	10 µM	6
Vasoactive Intestinal Peptide VIP2/PACAP VPAC2	443379	hum	2	10 µM	-7
Vasopressin V_{1A}	443270	hum	2	10 µM	-18
Vasopressin V_{1B}	443424	hum	2	10 µM	1
Vasopressin V_2	443161	hum	2	10 µM	-16
Vitamin D_3	443349	hum	2	10 µM	19

Significant responses (≥ 50% inhibition or stimulation for Biochemical assays) were noted at 10 µM in the primary assays listed below,

Cat #	Assay Name	Species	Conc.	% Inh.	IC_{50}*
214600	Calcium Channel L-Type, Dihydropyridine	rat	10 µM	58	
226810	$GABA_A$, Chloride Channel, TBOB	rat	10 µM	52	
233000	Glutamate, NMDA, Phencyclidine	rat	10 µM	58	
268060	Prostanoid DP	hum	10 µM	-74	
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	hum	10 µM	-91	

Therefore, the IC50/EC50 were calculated in a secondary assay confirming that these activities are only at concentration not reached in PK studies.

Assay Name	Species	Conc.	% Inh.	IC_{50}*	K_i	n_H
Calcium Channel L-Type, Dihydropyridine	rat	10 µM	58	8.99 µM	5.78 µM	0.78
$GABA_A$, Chloride Channel, TBOB	rat	100 µM	82	34.9 µM	31.3 µM	1.37
Glutamate, NMDA, Phencyclidine	rat	100 µM	70	39.1 µM	26.5 µM	0.78
Calcium Channel L-Type, Dihydropyridine	rat	10 µM	58	8.99 µM	5.78 µM	0.78
Glutamate, NMDA, Phencyclidine	rat	100 µM	70	39.1 µM	26.5 µM	0.78
$GABA_A$, Chloride Channel, TBOB	rat	100 µM	82	34.9 µM	31.3 µM	1.37

In vitro Micronucleus Assay with VCE-003.2 in Cultured Peripheral Human Lymphocytes: The objective of this study was to evaluate VCE-003.2 for its ability to induce micronuclei in cultured human lymphocytes, either in the presence or absence of a metabolic activation system (S9-mix). The possible clastogenicity and aneugenicity of VCE-003.2 was tested in two independent experiments. Whole blood samples obtained from young healthy subjects were treated with an anti-coagulant (heparin) and cultured in the presence of a mitogen (phytohaemagglutinin). Briefly, stimulated lymphocytes were exposed to selected doses of VCE-003.2. Prior to the mitosis (during or after exposure of VCE-003.2) the chemical cytochalasin B was added to the cultures. Cytochalasin B arrests the formation of actin filaments. Consequently, the cell is not able to divide, but nuclear division continues. In this way, cytochalasin B allows discrimination between cells that have undergone nuclear division (binucleated) and cells that have not (mononucleated). Cells were harvested, stained and interphase cells (bi-nucleated cells) were analyzed microscopically for the presence of micronuclei. Results from cultures treated with VCE-003.2 were compared with control (vehicle) treated cultures.

In the first cytogenetic assay, the test material was tested up to 40 and 45 µg/mL for a 3 hours exposure time with 27 hours harvest time in the absence and presence of S9-fraction, respectively. Appropriate toxicity was reached at this dose level (Table 1).

In the second cytogenetic assay, the test material was tested up to 20 µg/mL for a 24 hours exposure time with 24 hours harvest time in the absence of S9-mix. Appropriate toxicity was reached at this dose level (Table 2).

VCE-003.2 did not induce a statistically significant or biologically relevant increase in the number of binucleated cells with micronuclei in the absence and presence of S9-mix, in either of the two experiments.

Table 1: Number of Binucleated Cells with Micronuclei of Human Lymphocyte Cultures Treated with VCE-003.2 in the First Cytogenetic Assay

Without metabolic activation (-S9-mix)

3 hours exposure time, 27 hours harvest time

Concentration (µg/mL)	Cytostasis (%)	Number of binucleated cells with micronuclei [1)]		
		1000	1000	2000
		A	B	A+B
0	0	1	1	2
10	3	0	0	0
30	32	1	2	3
40	56	2	1	3
0.20 MMC-C	33	3	7	10*
0.25 MMC-C	61	8	7	15**
0.05 Colch	35	6	8	14**
0.1 Colch	87	11	14	25****

With metabolic activation (+S9-mix)
3 hours exposure time, 27 hours harvest time

Concentration (µg/mL)	Cytostasis (%)	Number of binucleated cells with micronuclei [1)]		
		1000	1000	2000
		A	B	A+B
0	0	1	1	2
10	12	2	2	4
40	20	1	1	2
45	47	1	2	3
7.5 CP	60	13	13	26****

*) Significantly different from control group (Fisher's exact test), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ or **** $P < 0.0001$.

1) 1000 binucleated cells were scored for the presence of micronuclei. Duplicate cultures are indicated by A and B.

Table 2: Number of Binucleated Cells with Micronuclei of Human Lymphocyte Cultures Treated with VCE-003.2 in the Second Cytogenetic Assay

Without metabolic activation (-S9-mix)
24 hours exposure time, 24 hours harvest time

Concentration (µg/mL)	Cytostasis (%)	Number of binucleated cells with micronuclei [1)]		
		1000	1000	2000
		A	B	A+B
0	0	3	2	5
5	-25	1	0	1
10	-9	1	2	3
20	52	3	5	8
0.125 MMC-C	23	10	8	18**
0.01 Colch	7	4	5	9
0.05 Colch	100	4[2)]	2[2)]	6**

*) Significantly different from control group (Fisher's exact test), * P < 0.05, ** P < 0.01, *** P < 0.001 or **** P < 0.0001.

1) 1000 binucleated cells were scored for the presence of micronuclei. Duplicate cultures are indicated by A and B.

2) 213 and 94 binucleated cells were scored for the presence of micronuclei, respectively.

In vivo Micronucleus Test in Bone Marrow Cells of the Rat with VCE-003.2: male animals were dosed twice by oral gavage with vehicle (sesame oil) or with 500, 1000 or 2000 mg VCE-003.2 per kg body weight. As the positive control, slides were used from three animals dosed once by oral gavage with 19 mg cyclophosphamide (CP) per kg body weight. In total 4 treatment groups were used, each consisting of 5 animals. No treatment related clinical signs or mortality were noted in any animal treated with VCE-003.2 or control animals receiving vehicle or cyclophosphamide. Bone marrow was sampled 48 hours after the first dosing. A drop of the cell suspension was spread in clean slides and after Giemsa staining the number of micronucleated polychromatic erythrocytes was counted in at least 4000 polychromatic erythrocytes (with a maximum deviation of 5%). The ratio of polychromatic to normochromatic erythrocytes was determined by counting and differentiating at least the first 1000 erythrocytes at the same time. Micronuclei were only counted in polychromatic erythrocytes. Averages and standard deviations were calculated.

The mean number of micronucleated polychromatic erythrocytes per group and the mean ratio of polychromatic to normochromatic erythrocytes are presented in Table 3. The mean

number of micronucleated polychromatic erythrocytes scored in VCE-003.2 treated groups were compared with the corresponding solvent control group.

Table 3. Mean Number of Micronucleated Polychromatic Erythrocytes and Ratio of Polychromatic/Normochromatic Erythrocytes

Group	Treatment	Number of Animals	Dose (mg/kg body weight)	Number of micronucleated polychromatic erythrocytes (mean ± S.D.) [1]	Ratio polychromatic / normochromatic erythrocytes (mean ± S.D.) [2]
	MALES				
1	Vehicle Control	5	0	2.4 ± 1.1	0.92 ± 0.05
2	VCE-003.2	5	500	3.4 ± 2.5	0.94 ± 0.08
3	VCE-003.2	5	1000	2.6 ± 1.9	0.85 ± 0.13
4	VCE-003.2	5	2000	2.6 ± 1.1	0.89 ± 0.06
5	CP	3	19	72.0 ± 19.3 [3]	0.31 ± 0.08 [3]

Vehicle control = sesame oil
CP = Cyclophosphamide.
(1) At least 4000 polychromatic erythrocytes were evaluated with a maximum deviation of 5%.
(2) The ratio was determined from at least the first 500 erythrocytes counted.
(3) Significantly different from corresponding control group (Students t test, $P < 0.001$).

No biological relevant increase in the mean frequency of micronucleated polychromatic erythrocytes was observed in the bone marrow of animals treated with VCE-003.2 compared to the vehicle treated animals. Cyclophosphamide, the positive control material, induced a statistically significant increase in the number of micronucleated polychromatic erythrocytes.

Comparative Metabolism in Cryopreserved Hepatocytes from Mouse, Rat, Dog, Minipig, Monkey and Human: Incubations with VCE-003.2 at 1 μM were performed in triplicate at 0, 5, 15, 30, 60 and 120 minutes for all species while incubations with the test item at 10 μM were performed singularly at 0, 5, 15, 30, 60 and 120 minutes.

The metabolic activity of the hepatocytes was confirmed for each species by measuring cytochrome P450 (CYP)-dependent enzymatic activity (phenacetin metabolism) and phase II enzymatic activity (7-hydroxycoumarin [HC] metabolism). All hepatocyte batches used metabolized phenacetin and HC, indicating that they were metabolically active. Following incubations of VCE-003.2 with mouse, rat, dog, minipig, monkey and human hepatocytes, samples were analyzed by LC-PDA-MS for metabolic stability assessment and for metabolite profiling purposes.

Twenty-six metabolites of VCE-003.2 were found in the hepatocyte incubations of the six species investigated. Based on the MS peak intensities, the major metabolites of VCE-003.2 in the 120 minutes hepatocyte incubation samples were M13, M15, M17, M19 and M20 which were present in at least four of the six species tested. No human specific metabolites were observed. Five metabolites (M13, M15, M17, M19 and M20) were selected for identification purposes. Metabolic reactions observed included oxidations, loss of C3H6 in combination with two oxidations, and a desaturation in combination with one or two oxidations. The identification and presence of metabolites detected in the hepatocyte incubations of the different species are presented in table 4.

Table 4. Interspecies Comparison of the Metabolite profile of VCE-003.2

Code	Rt (min)[1)]	*m/z* of $[M+H]^+$	Mass shift (Da)[2)]	Proposed Metabolic Reaction(s)[3)]	Detected in					
					M	R	D	Mi	Cm	H
M1	7.1	538.3375	164.0689	Reduction and glucoside conjugation	-	-	+	-	-	-
M2	7.6-8.0	552.3166	178.0480	Oxidation and glucoside conjugation	-	+	+	-	-	-
M3	13.6-13.9	679.3374	305.0688	GSH Conjugation	+	+	+	+	+	+
M4	13.6-14.1	663.3422	289.0736	Dehydration and GSH conjugation	-	+	+	+	+	+
M5	14.9-15.8	566.2959	192.0273	Oxidation and glucuronide conjugation	-	+	+	+	+	+
M6	14.9-15.4	520.3395	146.0709	Hydration and glutamine conjugation	+	+	+	+	+	+
M7	15.4-15.8	520.3395	146.0709	Hydration and glutamine conjugation	+	+	+	+	+	+
M8	15.5-16.1	258.2429	-116.0257	unknown	+	+	+	+	+	+
M9	15.4	388.2479	13.9793	Desaturation and oxidation	-	-	+	-	-	-
M10	15.4-15.9	548.2851	174.0165	Desaturation and glucuronide conjugation	+	+	+	+	+	+
M11	15.9-16.3	548.2851	174.0165	Desaturation and glucuronide conjugation	+	-	+	-	+	+
M12	15.8-16.2	408.2743	34.0057	Hydration and oxidation	+	+	+	+	+	+
M13	**16.2-16.8**	**364.2120**	**-10.0566**	**Loss of C_3H_6 and two oxidations**	-	+	+	+	+	+
M14	17.4-17.6	404.2431	29.9745	Desaturation and two oxidations	+	-	+	-	-	+
M15	**18.3-18.8**	**404.2431**	**29.9745**	**Desaturation and two oxidations**	+	+	-	+	+	+
M16	18.5-18.9	372.2534	-2.0152	Desaturation	-	+	+	-	-	-
M17	**18.5-19.0**	**390.2638**	**15.9952**	**Oxidation**	+	+	+	+	+	+
M18	19.0-19.4	372.2534	-2.0152	Desaturation	-	+	+	-	-	-
M19	**19.5-19.9**	**390.2638**	**15.9952**	**Oxidation**	+	+	+	+	+	+
M20	**19.6-20.1**	**388.2481**	**13.9795**	**Desaturation and oxidation**	+	+	-	+	-	+
M21	19.4-20.1	390.2638	15.9952	Oxidation	-	+	+	+	+	+
M22	20.4-21.0	372.2532	-2.0154	Desaturation	+	+	+	+	+	+
M23	20.4	388.2481	13.9795	Desaturation and oxidation	+	+	-	-	-	-
M24	20.3-20.8	390.2638	15.9952	Oxidation	+	+	+	+	+	+
M25	22.1-22.6	346.2377	-28.0309	Loss of C_2H_4	+	+	+	+	+	+
M26	23.8-24.3	372.2534	-2.0152	Desaturation	+	+	+	+	+	+
Test item	**25.1-25.5**	**374.2685**	**-0.0001**	**NA**	+	+	+	+	+	+

Rt: retention time; min: minutes; M: mouse; R: rat; D: dog; Mi: minipig; Cm: Cynomolgus monkey H: human; NA: not applicable
Bold = peak intensity ≥8.1E4 in at least one of the species; tentatively identified metabolites.
[1)] Range of retention time mentioned as observed in different runs and different species
[2)] Mass shift compared to parent compound
[3)] Based on mass shift compared to parent compound

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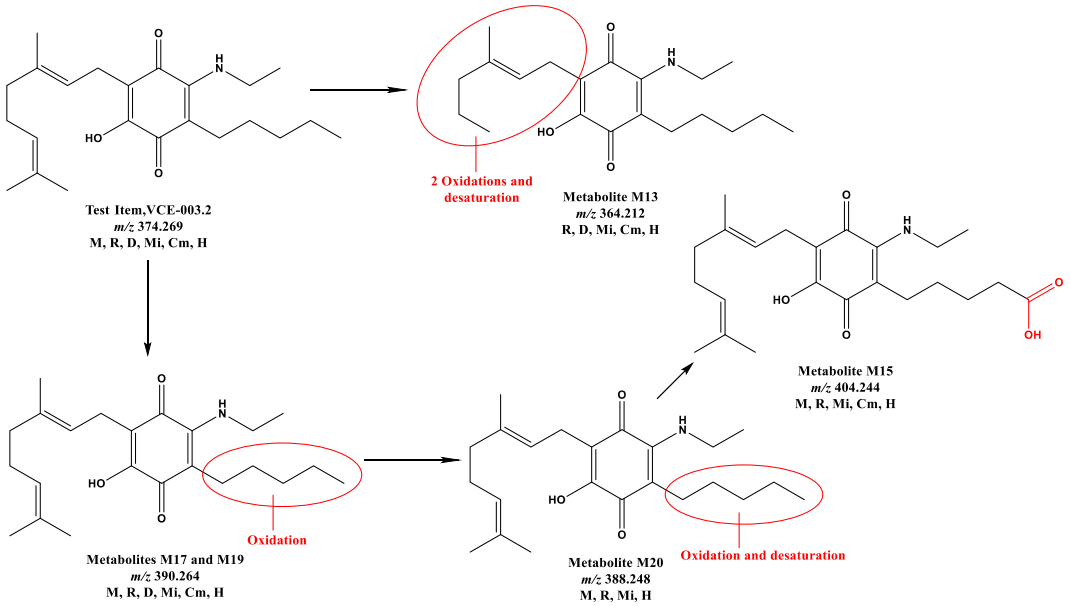
Figure 1: The postulated major metabolic pathway for the test item (based on the id



M: mouse, R: rat, D: dog, Mi: minipig, Cm, Cynomolgus monkey, H: human

In vitro Interaction Studies of VCE-003.2 with human BCRP, BSEP and MDR1 Efflux (ABC) Transporters, and with human MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1 and OCT2 Uptake Transporters: The purpose of this study was to evaluate VCE-003.2 as an inhibitor and/or substrate of the human ABC (efflux) transporters: BCRP, BSEP and MDR1, and the human SLC (uptake) transporters: MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1 and OCT2 (Table 5).

Table 5: Test article (VCE-003.2) and transporter assays

Test article	Transporter	Assay	Applied nominal concentrations
VCE-003.2	BCRP MDR1	Monolayer substrate	0.1, 1, 10 and 22.5 µM
	BCRP BSEP MDR1	VT inhibition	2.25 and 22.5 µM
	MATE1 MATE2-K	Uptake inhibition	1 and 5 µM
	OAT1 OAT3 OATP1B1 OATP1B3 OCT1 OCT2		1.125 and 11.25 µM
	MATE1 MATE2-K OAT1 OCT1	Uptake substrate	0.1, 1, 10 and 20 µM
	OAT3 OATP1B1 OATP1B3 OCT2		0.1, 1, 10 and 22.5 µM

VT: vesicular transport assay

The concentration range tested *in vitro* was selected based upon solubility limit in assay buffers and cytotoxic effects of VCE-003.2.

Tabulated summary of results and methods is provided in Table 6 and Table 7.

Table 6. Summary of the obtained results – ABC transporter assays

Pharmacokinetics: Inhibition of BCRP, BSEP and MDR1 and/or substrate of BCRP and MDR1.

Type of Study:	A non-GLP in vitro study to determine whether VCE-003.2 is an inhibitor of BCRP, BSEP, and MDR1 transporters using inside-out membrane vesicles prepared from cells overexpressing human ABC (efflux) transporters. The study design also included solubility and non-specific binding assessments. A non-GLP in vitro study to determine whether VCE-003.2 is a substrate of BCRP or MDR1 transporters using BCRP or MDR1 transfected MDCKII cell monolayers. The study design also included solubility, recovery and cytotoxicity assessments.
Method:	For inhibition assessment, vesicles were incubated in the respective assay buffer containing the probe substrate in the presence of VCE-003.2 (2.25 and 22.5 µM). The reaction was initiated with the addition of ATP or AMP to the solution; following the incubation time, the reaction was stopped by the addition of 200 µL of ice-cold washing buffer and immediately filtered via glass fiber filters mounted to a 96-well plate (filter plate). The filters were washed, dried and the amount of substrate inside the filtered vesicles was determined by liquid scintillation counting. E3S (1 µM) for BCRP, TC (0.2 µM) for BSEP and NMQ (1 µM) for MDR1 were used as positive control substrates. Bidirectional transport of VCE-003.2 was determined through control, MDCKII-BCRP and Abcb1KO-MDCKII-MDR1 cell monolayers. Assay buffer containing VCE-003.2 at four concentrations (0.1, 1, 10 and 22.5 µM) with and without reference inhibitor was added to the appropriate apical or basolateral chamber. Samples were taken from the helper plate before the incubation and from the donor chambers after the incubation to determine the initial concentration (C0) and recovery (R) of the test compound and the controls. After incubation the receiver chambers were sampled to determine the amount of translocated VCE-003.2 and controls. Samples were analyzed using LC-MS/MS. Prazosin (1 µM) for BCRP and Digoxin (1 µM) for MDR1 were used as positive control
Analysis:	Radiodetection / LC-MS/MS

Inhibition assessment of ABC transport by VCE-003.2

Transporter	Maximum % inhibition	IC50 (µM)
BCRP	89 %	NA
BSEP	26 %	NA
MDR1	81 %	NA

Bidirectional permeability of VCE-003.2 for ABC transporters

Transporter	Maximum net ER	Substrate
BCRP	NA	ND
MDR1	NA	ND

NA: not applicable; ND: not determined.

Table 7. Summary of the obtained results – SLC transporter assays

Pharmacokinetics:Inhibition of MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1 and OCT2 and/or substrate of MATE1, MATE2-K, OAT1, OAT3, OATP1B1,OATP1B3, OCT1 and OCT2

Type of Study:	A non-GLP in vitro study to determine whether VCE-003.2 is an inhibitor of or substrate of MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1 and OCT2 transporters using Madin-Darby canine kidney mammalian (MDCKII) and human embryonic kidney mammalian (HEK293) cells expressing MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1 and OCT2. The study design also included solubility, non-specific binding and cytotoxicity assessments.
Method:	For inhibition assessment, cells were pre-incubated for 30 minutes in the respective assay buffer in the presence of VCE-003.2 (1 and 5 or 1.125 and 11.25 µM). Then the probe substrate was added in a fresh dosing solution of VCE-003.2, following the incubation time, the reaction was stopped by washing the cells with ice-cold buffer. The cells were lysed with 0.1 M NaOH solution and the accumulation of the probe substrat in the cells was determined by liquid scintillation counting. For substrate assessment, cells were incubated for 10 minutes in the respective assay buffer in the presence of VCE-003.2 (0.1, 1, 10 µM and 20 or 0.1, 1, 10 and 22.5 µM) with and without the respective reference inhibitor. Following the incubation time, the reaction was stopped by washing the cells with ice-cold buffer. The cells were lysed wit methanol:buffer mixture (2:1) and the accumulation of VCE-003.2 in the cells was determined by mass-spectrometry. Metformin (10 µM) for MATE1, MATE2-K, OCT1 and OCT2, Tenofovir (5 µM) for OAT1, E3S (1 µM) for OAT3, E217βG (1 µM) for OATP1B1 and CCK-8 (1 µM) for OATP1B3 were used as positive control substrates.
Analysis:	Radiodetection / LC-MS/MS

Inhibition assessment of SLC transport by VCE-003.2

Transporter	Maximum % inhibition	IC50 (µM)
MATE1	NIO	NA
MATE2-K	NIO	NA
OAT1	NIO	NA
OAT3	NIO	NA
OATP1B1	NIO	NA
OATP1B3	NIO	NA
OCT1	NIO	NA
OCT2	NIO	NA

Substrate assessment of VCE-003.2 for SLC transporters

Transporter	Maximum transporter-specific fold accumulation	Substrate
MATE1	< 2	NO
MATE2-K	< 2	NO
OAT1	< 2	NO
OAT3	< 2	NO
OATP1B1	< 2	NO
OATP1B3	< 2	NO
OCT1	< 2	NO
OCT2	< 2	NO

NIO: no inhibition observed; NA: not applicable