Synthesis and Biological Studies of a Novel CB1 Antagonist *

Authors
K. Banerjee[1, 2], M. Jain[1], A. Vallabh[2], B. Srivastava[1], A. Joharapurkar[1], H. Patel[1]

Affiliations
[1] Zydus Research Centre, Ahmedabad, India
[2] Department of Chemistry, Faculty of Science, M. S. University of Baroda, Vadodara, India

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- cannabinoid CB1 and CB2 receptors
- neutral antagonists
- anti-obesity drugs
- piperidin-1-ylamide derivatives

Abstract

This paper describes the synthesis, early process development, salt selection strategies and pre clinical evaluation of novel, potent and selective CB1 antagonist, 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide **1**. The CB1 antagonism of compound **1** is also confirmed by reversal of CB1 agonist-induced hypothermia in Swiss albino mice. The process for the preparation of the compound **1** as a crystalline solid is also described. The crystalline form of the compound is found to be low bioavailable, therefore attempts have been made to improve its bioavailability through polymorphic transformation and salt formation. None of the salts prepared were found to be suitable for further development. The amorphous form of the compound **1** is found to be better suited. In vivo efficacy study of the amorphous form of compound **1** in 5% sucrose solution intake model in female Zucker fa/fa rats at single oral dose of 10 mg/kg demonstrates better reduction in the sucrose solution consumption than the corresponding crystalline form. The plasma concentration C_{max} at AUC exposure of the amorphous form of the compound **1** is significantly improved and better than the C_{max} of the corresponding crystalline form of the compound **1**. On the basis of the efficacy, pharmacokinetic and toxicological evaluations, the compound **1** in the amorphous form is selected as a pre-clinical lead candidate.

Introduction

Obesity is widely recognised as the largest and fastest growing public health problem in the developed and developing countries [1] and considered as a global epidemic. The International Obesity Task Force estimates that more than 300 million individuals world wide are obese and an additional 800 million are over-weight [2, 3]. Sedentary life style, advent of junk foods, modern lifestyles with their various stresses and increasing disposable income play a part in this problem [4]. Obesity is associated with substantial increases in morbidity, premature mortality, impaired quality of life and large healthcare costs [5]. The problem with obesity are compounded due to the major comorbidities associated with it that include type 2 diabetes, metabolic syndrome, hypertension, dyslipidaemia, myocardial infarction, stroke, certain types of cancers, sleep apnea and osteoarthritis [6]. Lifestyle changes in the form of dieting and/or exercise per se do not generally produce marked or sustainable weight loss [7, 8], whereas psychological therapies are difficult to deliver on a mass scale [9] and long-term results are disappointing. Hence, pharmacotherapy for obesity has become a popular choice, especially among the younger generations [10].

The role of cannabinoid receptor system in the regulation of appetite and food intake in animals and humans intrigued the scientific community to develop drugs targeted towards cannabinoid receptor type 1 (CB1) [11–13]. Rimonabant hydrochloride (Compound-**2**, **Fig. 1**) has been the first therapeutically relevant, potent and selective CB1 receptor inverse agonist, which was approved in Europe as an anti-obesity drug [14]. However, reports of serious psychiatric problems (such as anxiety, depression and suicide) led to withdrawal of Rimonabant and termination of several other CB1 receptor-antagonist-based anti-obesity drug development programmes (such as taranabant, otenabant, surinabant and ibipinabant) [15, 16].

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Correspondence
K. Banerjee
Zydus Research Centre
Sarkhej-Bavla N.H 8A Moraiya
Ahmedabad-382210
India
Tel.: +91/2717/665 555
Fax: +91/2717/665 355
kaushik.banerji@zyduscadila.com

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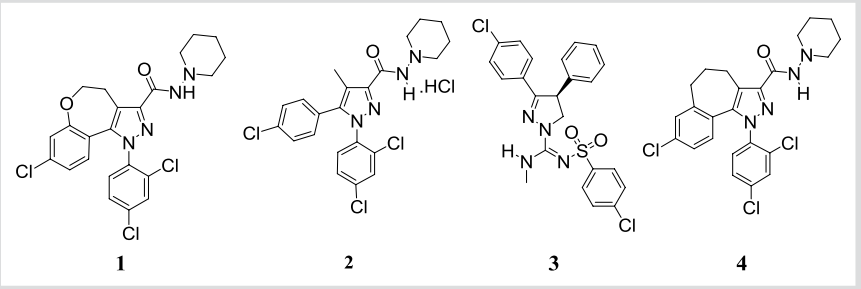

Fig. 1 CB1 receptor antagonist with diverse chemical structures.

Researchers have tried to develop several classes of CB1 receptor antagonist with diverse chemical structures [17–19]. Thus, for example, Solvay Pharmaceuticals has disclosed the 3,4-diaryl dihydropyrazole class of compounds (Compound-**3**, **Fig. 1**) as a CB1 antagonist, which has elicited potent in vitro [20] and in vivo activities [21]. 8-Chloro-1-(2,4-dichlorophenyl)-*N*-piperidin-1-yl-1,4,5,6-tetrahydrobenzo-[6, 7]-cyclohepta-[1, 2–c]-pyrazole-3-carboxamide (Compound-**4**, **Fig. 1**) was found to be a very potent CB1 antagonist in cell-based in vitro assays, and ex vivo screens [22]. However, the compound **4** had poor in vivo efficacy and oral bioavailability [23]. Despite the withdrawal of Rimonabant and the demise of several CB1 receptor antagonist development programmes, researchers believe that one has not yet reached the end of the line for anti-obesity treatments targeting the CB1 receptor [24,25]. It is now believed that neutral antagonists might retain the weight loss advantages and will be devoid of adverse effects [26]. Another promising approach is to develop agents which restricts their function through the periphery and do not reach to brain [27]. In continuation of our drug-discovery efforts to develop novel therapeutic agents to treat obesity, we have discovered a series of novel compounds as CB1 modulators [28] and compound **1** (**Fig. 1**) has been selected as a lead compound for pre-clinical evaluation. Though the compound **1** showed good pharmacological profile, it had poor bioavailability. The aim of the present study is to develop a new synthetic process for the preparation of compound **1** in high yield suitable for pre-clinical development and improve its bioavailability through salt selection strategies as well as through polymorphic transformations. Herein, the efficacy, pharmacokinetic evaluations of novel CB1 antagonist, 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e] azulene-3-carboxylic acid piperidin-1-ylamide **1** are described.

Chemistry

The compound **1** is synthesized as described in **Fig. 2**. 4-(3-chlorophenyl)-butanoic acid **5** is reacted with oxalyl chloride in dichloromethane at room temperature (27–30 °C) followed by Friedel-craft's acylation to provide 8-Chloro-3,4-dihydro-2H-benzo[b]oxepin-5-one **6**. Treatment of **6** with diethyl oxalate in ethanol followed by 2N HCl afforded 8-Chloro-5-oxo-2,3,4,5-tetrahydro-benzo[b]oxepin-4-yl)-oxo-acetic acid ethyl ester **7**. The reaction of **7** with 2,4-Dichlorophenyl hydrazine hydrochloride in ethanol at 5–7 °C provided 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid ethyl ester **8**, which upon hydrolysis using KOH in methanol yielded 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e] azulene-3-carboxylic acid **9**. The reaction of **9** with $SOCl_2$/toluene followed by reaction with 1-Amino piperidine under usual amide bond formation chemistry yielded 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e] azulene-3-carboxylic acid piperidin-1-ylamide as an oil which was treated with ethereal HCl to get the HCl salt **10** as a brown solid . Trituration with ethyl acetate and subsequent drying yielded the pure compound **10** as a white solid. The neutralization of **10** with NaOH provided the compound **1** in crystalline form. The crystalline compound **1** is dissolved in dimethyl formamide by heating at 40 °C and the resulting solution is cooled to 27–30 °C over a period of 48 h to give needle shaped crystals of the DMF solvate **11**. The different salts of **1** were prepared by dissolving **1** in acetone and adding appropriate mineral/organic acids at 40–50 °C [**12** (a–e)]. Stirring the compound **1** in dichloromethane at room temperature (27–30 °C), followed by removal of solvents under reduced pressure, afforded the amorphous form of the compound **1**.

Results and Discussions

The compound **1** in its crystalline form is synthesized according to the scheme described in **Fig. 2**. The crystalline nature of the compound **1** was confirmed by XRD (**Fig. 3b**). The binding affinity of compound **1** was tested in an in vitro cAMP assay. The compound **1** exhibits an EC_{50} 14.5 µM in human CB1 receptor forskolin-induced cAMP assay. In the human CB2 receptor (cannabinoid receptor 2) binding assay, the compound **1** showed Ki 3.19 µM, IC_{50} 4.75 µM at 30 µM concentration, indicating favorable CB1 selectivity. In vitro CB1 antagonism was measured using a binding assay in CHO cells expressing human CB1 receptors (hCB1). Interestingly, the compound **1** did not change the forskolin-stimulated cAMP accumulation in CB1-transfected HEK cells up to 10 µM concentration indicating the possible neutral antagonist nature. The CB1 antagonism of compound **1** was also confirmed by reversal of CB1 agonist-induced hypothermia in Swiss albino mice [29].

However, the crystalline form of the compound **1** like other promising molecules in the CB1 class suffers from poor oral bioavailability and pharmacokinetic profile. In order to improve the oral bioavailability of compound **1**, attempts were made to change the polymorphic form of the compound **1**. However, these attempts were limited due to poor solubility of compound **1** in most of the organic solvents. In one of the attempts, the compound **1** was dissolved in large excess of dichloromethane, heated to reflux and the volume of the resulting solution was reduced to minimum, which upon cooling to 27–30 °C over a period of 48 h did not yield any solid. In another attempt, the compound **1** was dissolved in large excess of acetone, heated to reflux and the volume of the solution reduced to minimum. The residue obtained was cooled to 27–30 °C and kept over a period of 48 h, but did not yield any solid. The compound **1** was found to

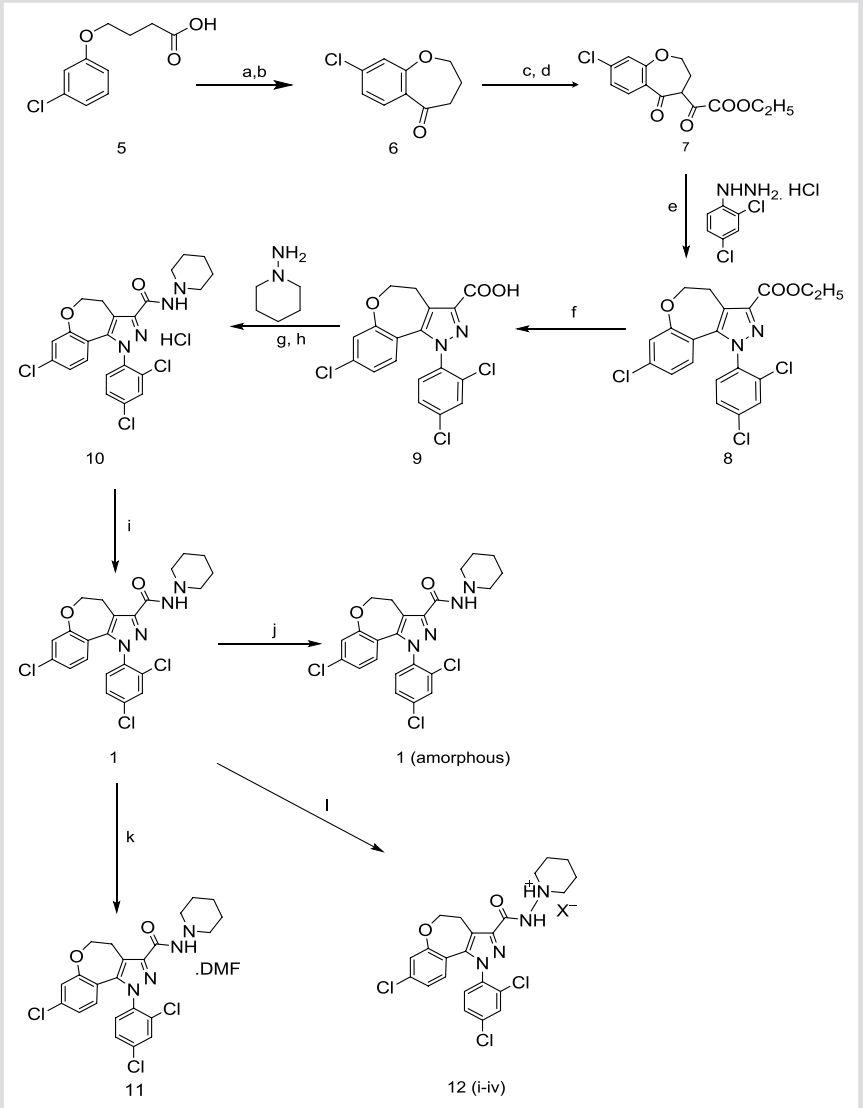

Fig. 2 Synthetic scheme for preparing compound **1**. Reagents and conditions: **a** $(COCl)_2$, anhydrous CH_2Cl_2, stirred at 0 °C for 30 min and 26–28 °C for 1.5 h; **b** anhydrous $AlCl_3$, CH_2Cl_2, stirred at 0–5 °C for 20–25 min and at 26–27 °C for 30 min; **c** diethyl oxalate, Na metal, ethanol; **d** 2N HCl, $CHCl_3$; **e** ethanol, IPA/HCl, refluxed at 75–77 °C for 2 h; **f** KOH/MeOH; **g** $SOCl_2$, toluene, refluxed at 107–108 °C for 30 min; **h** anhydrous methanol, ethereal HCl, 0–5 °C; **i** NaOH; **j** CH_2Cl_2, reflux under reduced pressure at 50 °C; **k** DMF, heat at 40–50 °C for 2 h, cooled and kept for 48 h; **l** acetone, heat at 40 °C till clear solution, added acids corresponding to X = Cl−, HSO4−, $(COO)_2^{-2}$ & $-CH_3I$ in acetone, and the mixture was cooled when necessary and filtered.

be more soluble in solvents such as dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO). The hot solution of compound **1** in DMSO upon cooling at 27–30 °C over a period of 48 h afforded a pasty material, which was found to be the solvate of DMSO. In a subsequent attempt, the solution of compound **1** was prepared in DMF by heating and subsequent cooling at 27–30 °C over a period of 48 h yielded crystals. The crystals of compound **1** in DMF were characterized as solvates of DMF **11**. The acute exposure to dimethyl formamide is reported to cause liver toxicity in humans [30], thus the crystalline form of the DMF solvate of compound **1** was not evaluated further in any pharmacological studies.

Furthermore, we synthesized the stable oxalate **12b**, benzenesulfonate **12c**, bisulfate **12d**, and methyl iodide **12e** salts of compound **1**. None of the salts altered the in vitro binding mode, potency and selectivity as initially exhibited by the crystalline form of compound **1**. The hydrochloride salt **12a** upon storage gets hydrolysed wherein the piperidine ring gets cleaved to form the corresponding methyl ester **13,** ▷ **Fig. 4**. The structure of compound **13** was confirmed through single crystal analysis.

The oxalate **12b**, benzenesulfonate **12c**, bisulfate **12d**, and methyl iodide **12e** salts of compound **1** significantly improved the pharmacokinetic parameters; however, they were found to be associated with accumulation in tissues after acute exposure, thereby limiting their use for repeated dose medication.

Development of amorphous form of pharmaceutical compounds represents both an opportunity and a necessity in pharmaceutical development. The opportunity arises from the potential of the amorphous form to improve the pharmacokinetic properties and bioavailability rather than the corresponding crystalline form.

The compound **1** was made into amorphous form and confirmed through XRD spectra (▷ **Fig. 3a**). The amorphous form of compound **1** was evaluated for its pharmacokinetic properties and its efficacy was evaluated using sucrose (5% w/v) consumption model in Zucker fa/fa rats.

The results are provided in ▷ **Tables 1 and 2**:

The crystalline form of the compound **1** showed significant appetite suppression in rodent model (▷ **Table 1**), however its pharmacokinetic profile was poor (▷ **Table 2**).

The in vivo efficacy study of the amorphous form of compound **1** in 5% sucrose solution intake model in female Zucker fa/fa rats at single oral dose of 10 mg/kg (▷ **Table 1**) demonstrates better reduction in the sucrose solution consumption than the corresponding crystalline form.

The pharmacokinetic parameters of both the crystalline and amorphous form of compound **1** were evaluated (▷ **Table 2**). The

plasma concentration C_{max} at AUC exposure of the amorphous form of the compound **1** is significantly improved and better than the C_{max} of the corresponding crystalline form of the compound **1**. Further, compound **1** exhibited acceptable safety profile upto a dose 30 times of the efficacy dose. No changes in body weight and weights of the organs (kidney, heart and liver) were observed upto 300 mg/kg dose in a 14 day repeat dose study. The compound also did not bind to hERG upto 3 micromolar concentrations.

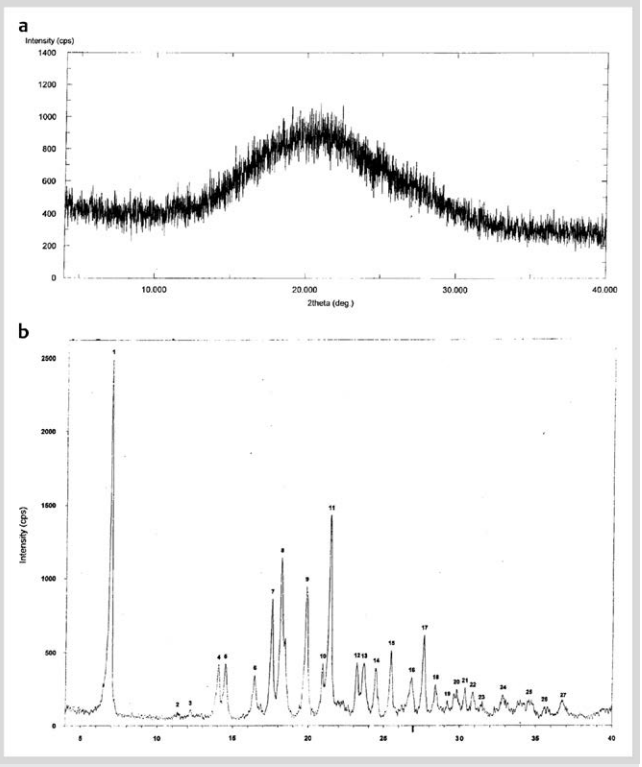

Fig. 3 XRD patterns of amorphous form **a** and crystalline form **b** of compound **1**.

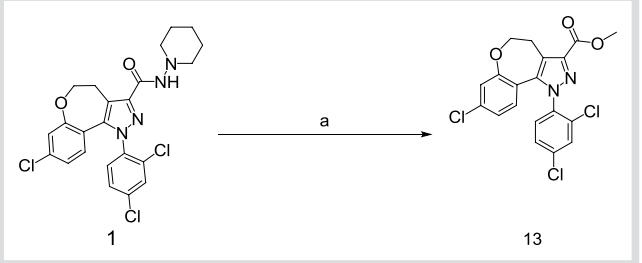

Fig. 4 Structure of compound **13**. Reagents and conditions: **a** acetone, heat at 40 °C till clear solution, HCl.

Based on the impressive in vitro, in vivo and pharmacokinetic parameters of the amorphous form of the compound **1** as a possible neutral CB1 antagonist, the compound has been selected for further development.

In order to prepare sufficient quantities of the compound **1** to support the development program a novel process for preparation of the compound **1** was developed as described in **Fig. 2**. The process described in **Fig. 2** is scalable and gives the compound **1** in high yield (77.6%).

Conclusion

Early process development, synthesis and identification of the amorphous form of the potent CB1 antagonist compound **1** is described, The improved process described herein is scalable, gives the stable amorphous form in high yield and is suitable for meeting the needs of long term development. The compound **1** demonstrated favourable safety profile in rodent models. Initial pharmacological data of compound **1** are indicative that the compound may be a neutral antagonist and it may not be accompanied by behavioral signs of nausea etc. during the feeding suppression induced by compound **1.** Additional research will be necessary to confirm this hypothesis.

Experimental Section

Synthesis

Synthetic materials and methods

Reagents and solvents were obtained from commercial suppliers and used without further purification. Flash chromatography was performed using commercial silica gel (230–400 mesh). Melting points were determined on a capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu FT IR 8 300 spectrophotometer (Vmax in cm^{-1}, using KBr pellets or Nujol). The ^1H NMR spectra were recorded on a Brucker Avance-300 spectrometer (300 MHz). The chemical shifts (δ) are reported in parts per million (ppm) relative to TMS, either in $CDCl_3$ or DMSO-d_6 solution. Signal multiplicities are represented by s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), bs (broad singlet), and m (multiplet). ^{13}CNMR spectra were recorded on Brucker Avance-400 at 100 MHz either in $CDCl_3$ or DMSO-d_6 solution. Mass spectra (ESI-MS) were obtained on Shimadzu LC-MS 2010-A spectrometer. HPLC analysis were carried out at λ_{max} 220 nm using column ODS C-18, 150 nm × 4.6 nm × 4 µ on AGILENT 1 100 series.

8-Chloro-3,4-dihydro-2H-benzo[b]oxepin-5-one(6): 4-(3-chlorophenyl)-butanoic acid (128.0 g, 596.74 mmol) was taken in a round bottom flask and anhydrous dichloromethane (1L) was

Compound	in-vivo (in 5% sucrose solution intake)		
	Total consumption in 4 h	% Change vs. Control (Sucrose intake)	5% Change vs. Control (per 100 g body wt.)
Control	48.00 ± 2.20		
Rimonabant	8.4 ± 3.3	−66.4 ± 11.0	−62.0 ± 15.3
Crystalline Form of 1	32.2 ± 6.4	−38.7 ± 12.2	−30.2 ± 16.4
Amorphous Form of 1	31.9 ± 5.2	−54.7 ± 11.5	−24.2 ± 15.3

[a] Values indicate Mean ± SEM for n = 6 in 4 h

Table 1 In vivo efficacy of the crystalline and amorphous form of the compound 4 in 5% sucrose solution intake model in female Zucker fa/fa rats at single oral dose of 10 mg/kg.[a]

Table 2 Mean pharmacokinetic parameters of the crystalline and amorphous form of the 1 in fasted female Zucker fa/fa rats p.o. at 10 mg/kg.

Compd.	Route	dose (mg/kg)	T_{max} (h)	C_{max} (*n*g/mL)	$T_{1/2}$ (h)	AUC(0–∞) (h.*n*g/mL)
1 (crystalline)	Oral	10	4.5 ± 0.7	223 ± 13	23.9 ± 6.8	3241 ± 125
1 (Amorphous)		10	4.2 ± 1.2	575 ± 32	22.3 ± 2.9	6338 ± 234

[a] Values indicate mean ± SD for n = 6

added to it. The solution was stirred and cooled to −20 °C. To this solution oxalyl chloride (74.06 mL, 835.43 mmol, 1.4 eq) was added drop wise at −20 °C over a period of 15–20 min. The resulting solution was stirred at 0 °C for 30 min and 26–28 °C for 1.5 h. The progress of the reaction was monitored by TLC until all starting material was consumed. The reaction was quenched with ethanol and TLC was checked using mobile phase 5% methanol in chloroform. The solvents were removed on a rotatory evaporator under reduced pressure to afford brown oil. Separately, in a 4-necked round bottomed flask, anhydrous $AlCl_3$ (95.6 g, 716.08 mmol, 1.2 eq) was taken and to it was added anhydrous dichloromethane (1 L). The suspension was stirred and cooled to 0–5 °C. To this cooled suspension, a solution of acid chloride obtained above in anhydrous CH_2Cl_2 (200 mL) was added dropwise at 0–5 °C over a period of 20–25 min. The resulting solution was stirred at 26–27 °C for 30 min. The progress of the reaction was monitored by TLC using mobile phase 5% methanol in chloroform. The reaction mixture was poured into mixture of demineralized water (DM water) and crushed ice (3 L) in 5 L round bottom flask followed by CH_2Cl_2 (1 L). The mixture was stirred at 26–27 °C for 16 h. The organic layer was separated, washed with DM water (3 × 1 L). The organic layer was separated, dried over anhydrous Na_2SO_4 and treated with activated charcoal (3-tea spoon) at 38–39 °C for 15–20 min and filtered hot through Hyflow. The solvents were removed on a rotatory evaporator under reduced pressure to yield a brown oil (118 g, 100%).

IR (KBr cm^{-1}): 2970, 2887, 1685, 1595, 1087, 821, 767; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.70 (dd, J = 8.72 Hz, J = 0.77, 1H), 7.06 (m, 2H), 4.25 (t, J = 6.60 Hz, 2H), 2.88 (t, J = 6.97 Hz, 2H), 2.22 (m, 2H); ESI-MS m/z (Relative intensities) (+ve mode) 256.8 $(M+K)^+$.

(8-Chloro-5-oxo-2,3,4,5-tetrahydro-benzo[b]oxepin-4-yl)-oxo-acetic acid ethyl ester (7): Anhydrous ethanol (1500 mL) was taken in a round bottom flask and small pieces of sodium metal (31.10 g, 1526.71 mmol, and 2.0 eq) were added to it with stirring. The solution was stirred till all Na metal was dissolved. The solution was cooled to 25–27 °C. To this diethyl oxalate (103.67 mL, 763.335 mmol, 1.0 eq) was added drop wise at 25–27 °C over a period of 15–20 min and stirred for 25–30 min. at the same temperature. To this was added a solution 8-Chloro-3,4-dihydro-2H-benzo[b]oxepin-5-one (150.0 g, 763.335 mmol) in ethanol (2000 mL) drop wise at 25–27 °C over a period of 15–20 min. The resulting yellow solution was stirred at 25–26 °C for 3–4 h and the progress of reaction was monitored by TLC using mobile phase 10% ethyl acetate in petroleum ether. The reaction mixture was diluted with DM water (2500 mL), acidified with 2 N HCl to pH 4 and extracted with $CHCl_3$. The chloroform layer was separated, washed with DM water, dried over anhydrous Na_2SO_4 and evaporated on a rotatory evaporator under reduced pressure to get yellow oil, which solidifies upon standing. The solid was slurried in diethyl ether (1 L) and filtered to get yellow solid (110 g, 48.6%).

IR (KBr cm^{-1}) 3417, 3109, 1830, 1714, 1614, 1595, 1544, 1207, 1089, 661, 617, 565, 447; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.93 (d, J = 9.3 Hz, 1H), 7.26 (m, 2H), 4.37 (m, 2H), 2.88 (m, 2H).

8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid ethyl ester (8): (8-Chloro-5-oxo-2,3,4,5-tetrahydro-benzo[b]oxepin-4-yl)-oxo-acetic acid ethyl ester (22.0 g, 74.199 mmol) **7** was taken in a round bottom flask and to it was added anhydrous ethanol (225 mL). The suspension was stirred and cooled to 5–7 °C. To this suspension, 2,4-Dichlorophenyl hydrazine hydrochloride (17.90 g, 83.845 mmol, 1.13 eq) was added portion wise at 5–7 °C. The resulting suspension was stirred at 5–7 °C for 15–20 min and brought to 25–26 °C. Isopropyl alcohol: HCl (2.2 mL) was added to this mixture and refluxed at 75–77 °C for 2 h. The progress of the reaction was monitored by TLC using mobile phase 20% ethyl acetate in petroleum ether. The reaction mixture was cooled to 25–27 °C and the solid separated out was filtered on a Buchner funnel under reduced pressure and dried to afford orange solid (38.0 g, 100%).

IR (KBr cm^{-1}) 3425, 1724, 1679, 1564, 1535, 1454, 1083, 906, 813; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.40 (t, 1H), 7.34 (d, J = 2.25 Hz, 2H), 6.83 (dd, J = 8.58 Hz, 2H), 6.66 (d, J = 8.58 Hz, 1H), 4.50-4.31 (m, 4H), 3.46 (d, 4H), 1.44 (t, 3H); ESI-MS m/z (Relative intensities) (+ve mode) 458.5 $(M+H)^+$.

8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylicacid(9): 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid ethyl ester (38.0 g, 86.857 mmol) **(8)** was taken in a round bottom flask and to it was added methanol (300 mL). To this, a solution of KOH (9.73 g, 173.174 mmol, 2.0 eq) in methanol: water (1:1, 200 mL) was added and reaction mixture was refluxed at 65–68 °C for 2.0 h. The progress of the reaction was monitored by TLC using 10% methanol in chloroform as mobile phase. The reaction mixture was cooled to 25–26 °C, poured into ice cold water and acidified to pH 4 using 10% HCl solution. The solid separated out was filtered on a Buchner funnel under suction, washed with water, dried under suction. The solid was further taken in isopropyl alcohol (IPA), stirred for 10–15 min and filtered to afford an off white solid (20.2 g, 56.8%).

IR (KBr cm^{-1}) 3450, 3082, 1687, 1596, 1568, 1431, 1386, 1035, 985, 567, 549, 455; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 13.08 (bs, 1H), 7.88 (d, J = 2.04 Hz, 1H), 7.72-7.64 (m, 2H), 7.23 (d, J = 2.13 Hz, 1H), 7.01 (dd, J = 2.19 Hz, J = 8.58 Hz, 1H), 6.71 (d, J = 8.58 Hz, 1H), 4.04 (m, 4H); ESI-MS m/z (Relative intensities) (+ve mode) 410.5 $(M+H)^+$.

8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide (1), crystalline: 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid (5.0 g, 12.25 mmol) was taken in a round bottom flask and toluene (30 mL) was added to it. To this solution, thionyl chloride (2.91 g,

24.5 mmol) was added and the mixture was refluxed at 107–108 °C for 30 min. The complete conversion of acid to acid chloride was confirmed by TLC. Subsequently, the reaction mixture was cooled to 30–35 °C and transferred into single neck round bottom flask. The solvents were evaporated on a rotatory evaporator under reduced pressure to afford an oil. The oil was further diluted in anhydrous CH_2Cl_2 (20 mL), cooled to 0–5 °C in an ice bath and treated with 1-amino piperidine (1.83 g, 18.38 mmol). The resulting mixture was stirred at 25–26 °C for 15–20 min. The progress of the reaction was monitored by TLC using mobile phase 50% EtOAc in hexane. The reaction mixture was diluted with DM water (200 mL) and extracted with toluene. The organic layer was separated, washed with DM water (100 mL), brine solution (100 mL), dried over anhydrous Na_2SO_4 and solvents were removed on a rotatory evaporator under reduced pressure to afford an oil. The oil was diluted with anhydrous methanol (25 mL), cooled to 0–5 °C in an ice bath and treated with ethereal HCl (5–6 mL). The solvents were evaporated on a rotatory evaporator under reduced pressure to afford brown solid. The crude solid was triturated in ethyl acetate, filtered on a Buchner funnel under suction and dried to afford an off white solid (5.0 g, 77.6%).
^{1}HNMR: ($CDCl_3$, 300 MHz): δ 10.35 (s, 1H), 7.51 (d, J = 1.74 Hz, 1H), 7.40 (m, 2H), 7.34 (m, 1H), 7.14 (d, J = 1.95 Hz, 1H), 6.62 (d, J = 8.55 Hz, 1H), 4.38 (m, 2H), 4.06 (bs, 3H), 3.48 (bs, 3H), 3.26 (m, 2H), 3.11 (m, 2H), 1.67(bs, 4H), 1.41(m, 1H), 1.18 (m, 1H);
^{1}HNMR: (DMSO-D_6, 300 MHz): δ 10.53 (s, 1H), 7.89 (d, J = 2.01 Hz, 1H), 7.78 (d, J = 8.49 Hz, 1H), 7.68 (dd, J = 10.47, 1.95 Hz, 1H), 7.26 (d, J = 1.95 Hz, 1H), 7.03 (dd, J = 10.56, 1.95 Hz, 1H), 6.72 (d, J = 8.61 Hz, 1H), 4.45 (bs, 1H), 4.25 (bs, 1H), 3.23 (bs, 4H), 3.06 (m, 3H), 1.72 (bs, 4H), 1.42(bs, 2H), 1.16(t, 1H), 0.98 (t, 1H).
The basification of the HCl salt of compound **1** with NaOH gave the compound of **1** in the crystalline form.
^{1}HNMR: ($CDCl_3$, 300 MHz) δ 7.8 (d, J = 7.5 Hz, 2H), 7.4 (d, J = 7.5 Hz, 2H), 7.39 (d, J = 8.4 Hz, 1H), 7.34 (d, J = 1.8 Hz, 1H), 7.3 (m, 2H), 7.0 (d, J = 8.4 Hz, 1H), 6.5 (d, J = 5.4 Hz, 1H), 3.27 (d, J = 5.1 Hz, 2H), 3.18 (d, J = 5.1 Hz, 1H), 2.6 (m, 3H), 1.5 (s, 3H); Melting point (by DSC): Onset = 195.3 °C, peak = 196.6 °C.
XPRD: 6.97, 16.42, 17.53, 18.19, 18.35, 19.8, 20.93, 21.4, 23.69, 27.62° ± 0.2° degrees 2θ.

8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide (1), dimethyl formamide solvate (11): Placed crystalline compound **1** (0.50 g, 1.017 mmol) into round bottom flask, followed by addition of dimethyl formamide (15 mL). The solution was heated to 60–70 °C for 2 h to obtain a clear solution. The solution was cooled to 27–30 °C for 48 h whereby white crystals were obtained.
Weight of White solid substance = 0.258 g
IR (KBr cm^{-1}) 3412, 2941, 1678, 1595, 1564, 1492, 1290, 1265, 1209, 1153, 1026, 983, 956, 869, 817, 810;
1H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.01 (s, 1H), 7.63 (s, 1H), 7.53 (d, J = 2.4 Hz, 1H), 7.39 (dd, J = 8.6 Hz, J = 2.0 Hz, 1H), 7.33 (dd, J = 9.2 Hz, J = 4.4 Hz, 1H), 7.14 (d, J = 2.0 Hz, 1H), 6.79 (dd, J = 8.8 Hz, J = 2.0 Hz, 1H), 6.60 (d, J = 8.4 Hz, 1H), 4.38 (bs, 2H), 3.49 (bs, 2H), 2.95 (s, 3H), 2.85(s, 3H), 2.95-2.85 (m, 4H), 1.78-1.72 (m, 4H), 1.45-1.43 (m, 2H).
ESI-MS m/z (Relative intensities) (+ve mode) 493.0 $(M+H)^+$ (100%).

8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide oxalate (12 b): Compound **1** (0.500 g (1.017 mmol) was taken in a round bottomed flask, followed by 10 mL acetone. The suspension was warmed upto 45–50 °C to get clear solution. To the solution of **1** in acetone a solution of oxalic acid (0.075 g, 1.017 mmol) in 3 mL acetone was added slowly. The resulting clear solution was cooled to 0–5 °C and stirred for 35–40 min. The solid separated out which was filtered, washed with acetone and dried under vacuum to afford white solid (0.267 g, 45.9%).
IR (KBr cm^{-1}) 3412, 2951, 1899, 1691, 1597, 1564, 1492, 1236, 817, 704;
^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.19 (s, 1H), 7.90 (d, J = 2.4 Hz, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.71 (dd, J = 8.4 Hz, J = 2.4 Hz, 1H), 7.27 (d, J = 2.4 Hz, 1H), 7.04 (dd, J = 8.6 Hz, J = 2.4 Hz, 1H), 6.74 (d, J = 8.4 Hz, 1H), 4.49 (bs, 1H), 4.26 (bs, 1H), 3.40 (bs, 2H), 3.28 (bs, 2H), 2.82-2.80 (m, 4H), 1.64-1.58 (m, 4H), 1.4-1.3 (m, 2H).
ESI-MS m/z (Relative intensities) (+ve mode) 493.05 (M+H)+ (80%).

8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide benzene sulfonate salt (12c): Placed **1** (0.500 g, 1.017 mmol) in a round bottomed flask and to it was added acetone (10 mL). The suspension was warmed upto 45–50 °C to get clear solution. To the solution of **1** in acetone, benzene sulfonic acid (0.160 g, 1.017 mmol) in acetone (3 mL) was added slowly. The resulting clear solution was cooled to 0–5 °C and stirred for 35–40 min. The solid separated out was filtered, washed with acetone and dried under vacuum to afford a white solid (0.350 g, 53%).
IR (KBr cm^{-1}) 3383, 3153, 2945, 1896, 1701, 1697, 1606, 1552, 1219, 1151, 981, 956, 896, 731; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 11.05 (bs, 1H), 7.94 (d, J = 2.4 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.73 (dd, J = 8.6 Hz, J = 2.4 Hz, 1H), 7.65-7.61 (m, 2H), 7.37-7.30 (m, 4H), 7.07 (dd, J = 8.6 Hz, J = 2.4 Hz, 1H), 6.77 (d, J = 8.8 Hz, 1H), 4.55-4.52 (m, 1H), 4.30-4.29 (m, 1H), 3.46-3.43 (m, 1H), 3.33-3.32 (m, 5H), 1.83-1.80 (m, 4H), 1.5-1.4 (m, 2H). ESI-MS m/z (Relative intensities) (+ve mode) 493.0 $(M+H)^+$ (100%).

8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide bisulfate (12d): Placed **1** (1.000 g, 2.034 mmol) in a round bottomed flask and to it was added acetone (15 mL). The suspension was warmed upto 45–50 °C to get clear solution. To the solution of **1** in acetone was added conc. H_2SO_4 (0.108 mL, 2.034 mmol) dropwise. The resulting clear solution was cooled to 0–5 °C and stirred for 15–20 min. The solid separated was filtered, washed with acetone and dried under vacuum to afford a White solid (1.03 g, 85.87%) IR (KBr cm^{-1}) 3404, 3153, 2951, 1708, 1599, 1550, 1369, 1215, 1049, 987, 958, 868, 812;
^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 10.85 (bs, 1H), 7.93 (d, J = 2.4 Hz, 1H), 7.83 (d, J = 8.8 Hz, 1H), 7.71 (dd, J = 8.6 Hz, J = 2.4 Hz, 1H), 7.29 (d, J = 2.4 Hz, 1H), 7.07 (dd, J = 8.6 Hz, J = 2.4 Hz, 1H), 6.76 (d, J = 8.8 Hz, 1H), 4.55-4.52 (m, 1H), 4.29-4.28 (m, 1H), 3.45-3.3.42 (m, 1H), 3.33-3.31 (m, 1H), 3.35-3.15 (m, 4H), 1.80-1.77 (m, 4H), 1.5-1.4 (m, 2H). ESI-MS m/z (Relative intensities) (+ve mode) 493.0 $(M+H)^+$ (99%).

8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide methyl iodide [13e]: Placed **1** (5.000 g, 10.17 mmol) in a round bottomed flask and to it was added acetone (15 mL). The suspension was warmed to 45–50 ° to get clear solution. To this solution methyl iodide (14.435 g, 6.322 mmol) was added. The resulting clear solution was refluxed over a period of 16 h. The solid precipitated was dissolved in distilled acetone and resulting solution was concentrated under reduced pressure at 45 °C to get a yellow solid. The solid was filtered, washed with acetone and dried under vacuum to yield off-white solid (5.3 g, 82.81 %) IR (KBr cm^{-1}) 3 419, 2 958, 1 593, 1 564, 1 496, 1 309, 1 294, 1 209, 1 030, 954, 920, 869, 812; 1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.83 (d, J = 2.4 Hz, 1H), 7.62 (dd, J = 8.6 Hz, J = 2.0 Hz, 1H), 7.57 (d, J = 8.8 Hz, 1H), 7.18 (d, J = 2.0 Hz, 1H), 6.96 (dd, J = 8.8 Hz, J = 2.0 Hz, 1H), 6.66 (d, J = 8.4 Hz, 1H), 4.40 (bs, 1H), 4.22 (bs, 1H), 4.12 (bs, 2H), 3.41 (s, 3H), 3.22-3.14 (m, 4H), 2.01-1.98 (m, 2H), 1.66-1.58 (m, 3H), 1.42-1.36 (m, 1H). ESI-MS m/z (Relative intensities) (+ve mode) 507.0 $(M+H)^+$ (100 %).

8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide (1), amorphous: The crystalline 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-diaza-benzo[c]azulene-3-carboxylic acid-piperidin-1-ylamide (5 g, 10.17 mmol) was placed in a round bottom flask and added of dichloromethane (100 mL). The resulting solution was stirred at 27–29 °C for 10 min. The solvents were removed under reduced pressure at 50 °C to afford white solid. The solid obtained was analyzed by XRD pattern, established it to be in the amorphous form. The DSC thermogram showed 2 peaks one at 182.9 °C and the other at 196.3 °C.

In vitro cAMP assay

Fatty acid-free BSA, IBMX (isobutyl methyl xanthine), RO20-1724 {4-[(3-butoxy-4-methoxyphenyl) methyl]-2-imidazololidinone}, forskolin and DMSO (hybrimax) were purchased from Sigma Chemical Co. cAMP detection ELISA kit was from Assay Designs, USA. Tissue culture reagents were purchased from Sigma and Hi-media. Other reagents used were all of analytical grade. The cAMP assay was carried out in Chinese Hamster Ovarian (CHO) cells (CHOK1) stably expressing human CB1 receptor following the method of Rinaldi-Carmona et al [31]. Cells grown to 80 % confluence were maintained in HAM'S F12 medium containing 10 % heat inactivated dialyzed fetal bovine serum and 0.8 mg/mL G-418. Cells were seeded at a density of 50 000 cells/well in 24-well plate, grown for 16–18 h, washed once with PBS and incubated for 30 min at 37 °C in plain HAM'SF12 containing 0.25 % free fatty acid BSA, IBMX (0.1 mM) and RO20-1724 (0.1 mM). IBMX, the pan phosphodiesterase inhibitor and RO20-1724, the specific phosphodiesterase-4 inhibitor were added to restore cAMP up to the detection limit. After 5 min incubation with the drugs, forskolin was added at a final concentration of 10 mM and incubation was carried out for another 20 min at 37 °C. The reaction was terminated by washing once with PBS and adding 200 lL lysis buffer comprising 0.1 N HCl and 0.1 % Trition X-100. The lysates were centrifuged and aliquotes from supernatants were used for detection of cAMP by ELISA as per the manufacturer's protocol.

5 % Sucrose solution intake in Zucker fa/fa rats

All the animals used in the study were procured from the Animal Breeding Facility of Zydus Research Center. Institutional Animal Ethical Committee approved all the study protocols. Female Zucker fa/fa rats (age of 10–12 weeks and 300–350 g of weight) were used for in vivo experiments. Compounds were suspended with 0.5 % carboxymethyl cellulose sodium salt in distilled water. The test compounds were administered at the dose of 10 mg/kg and by oral route in a volume of 2 mL/kg body weight. The obese Zucker fa/fa rats were housed individually and subjected to training for consuming 5 % sucrose solution over a period of 4 h, by allowing access to the 5 % sucrose solution in the bottles. Food and water were withdrawn during this time. This training was given for 6 consecutive days, at the same time of the day. On seventh day, the animals were randomized into groups of 6 animals each and treated with the test compounds. After 1 h of treatment, the animals were exposed to the 5 % sucrose solution for 4 h as that of the training schedule. The amount of sucrose solution consumed by each animal was calculated. Difference between the control and treatment groups were analyzed by performing one way ANOVA followed by Dunnett's test on sucrose solution consumption using Graph pad Prism software.

Pharmacokinetics experiment

Pharmacokinetics of the test compound was studied *via* per-oral route of administration in *Zucker fa/fa rats* of 8–10 weeks of age (6 animals). Animals were fasted for 18 h and food was supplied after 4 h of administration of the test compound. There was free access to water throughout the study. A homogenous suspension of the test substance was prepared in 0.5 % w/v CMC in normal saline and a per-oral dose of 10 mg/kg was administered. After the administration of the test compounds, blood samples were withdrawn at various time intervals through retro-orbital plexus and collected into heparinized micro centrifuge tubes. Plasma was separated by centrifugation at 4 000 rpm for 5 min at ambient temperature and analyzed immediately. Remaining samples were stored at −20 °C until analyzed.

Analysis was carried out by taking an aliquots of 180 μL plasma and 20 μL of internal standard (Atorvastatin) and was extracted with 2.5 mL of extracting solvent (ethyl acetate: acetonitrile 80:20, v/v) in glass test-tube by vortexing with spinix vortex mixture for a minute. This was then centrifuged at 2 000 rpm for 2.0 min. The supernatant was transferred to another glass test-tube and the solvent was evaporated under nitrogen using Zymark evaporator at 40 °C. Finally, the tubes were reconstituted with 0.1 mL diluent (acetonitrile: methanol: water 40:40:20, v/v/v). The reconstituted samples were analyzed on Agilent 1 100 Series HPLC system with a mobile phase of 0.05 % v/v trifluoroacetic acid in water: acetonitrile (32:68, v/v); flowing at a flow rate of 1.0 mL/min through a Kromasil 250 mm × 4.6 mm × 5 μ column maintained at 30 °C. Chromatographic separation was achieved within 15 min. Agilent software version Chemstation Rev.A.09.01. (1206) was used to acquire and process all chromatographic data. Quantification was based on a series of calibrators ranging from 0.031 to 32 μg/mL, prepared by adding test compound to drug free rat plasma. Quality control samples were analyzed in parallel to verify that the system performs in control. Pharmacokinetic parameters namely; maximum plasma concentration (C_{max}), time point of maximum plasma concentration (t_{max}), area under the plasma concentration – time curve from 0 h to infinity ($AUC_{0-\infty}$) and half-life of drug elimination during the terminal phase ($t_{1/2}$) were calculated from plasma concentration vs. time data, by standard non-compartmental

methods, using the WinNonLin software version 4.0.1 procured from Pharsight Corporation, USA.

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Conflict of Interest

None

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