# CHARACTERIZATION OF THE HIGHLY SELECTIVE 5-HT$_{2C}$ AGONISTS LORCASERIN AND CP-809101 ON FOOD AND NICOTINE MOTIVATED BEHAVIORS IN THE RAT

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# Introduction

**The therapeutic potential of a drug is governed by both its clinical efficacy and side effect property. Experimental evidence supports 5-HT$_{2C}$ agonists as offering therapeutic potential in indications such as obesity and nicotine dependence although relatively few members of this class have been evaluated. Agonist specific features such as intrinsic efficacy, functional and target selectivity and pharmacokinetics property highlight the need to evaluate multiple 5-HT$_{2C}$ agonists in terms of behavioural profiles predictive of clinical efficacy and side effect liability. Therefore in this study, we have evaluated the highly selective and chemically diverse 5-HT$_{2C}$ agonists CP-809101 and lorcaserin, and the prototypic 5-HT$_{2C}$ agonist Ro 60-0175 on (1) behaviours motivated by either food or nicotine reinforcement, (2) a nicotine drug discrimination procedure, (3) against the somatic signs of a precipitated nicotine withdrawal, and (4) side effect profiles. Furthermore plasma levels of lorcaserin were determined at a dose (1 mg/kg SC) effective against food and nicotine motivated behavior, and a dose (3 mg/kg SC) that produces side effects.**

Summary profile of Ro 60-0175, lorcaserin and CP-809101 across h5-HT$_2$ receptors

| | h5-HT$_{2C}$ | h5-HT$_{2A}$ | h5-HT$_{2B}$ | Ratio 2C/2A | Ratio 2C/2B |
|---|---|---|---|---|---|
| **Lorcaserin (1)** | 7.9 (1.0) | 6.7 (1.0) | 6.0 (1.0) | 16 | 80 |
| **Ro 60-0175 (2)** | 7.5 (0.84) | 6.4 (0.69) | 9.1 (0.79) | 13 | 0.03 |
| **CP-809101 (3)** | 10.0 (0.93) | 6.8 (0.67) | 7.2 (0.57) | 1585 | 630 |

The table summarises agonist potency (pEC50) and efficacy relative to a supramaximal concentration of 5-HT. Because of differences between cell lines, receptor density and functional readout, these data are best used for within drug comparisons across h5-HT2 receptors. Data from: (1) Thomsen et al (2008) JPET 325: 577-587; (2) Porter et al (1999) BJP 128: 13-20; (3) Siuciak et al (2007) Neuropharm. 52: 279-290.

# Methods

*Nicotine self-administration (FR5TO20s schedule)*: Test subjects were male, Long Evans rats. All rats were singly housed and maintained at ~85% of free feeding body weight. Following a brief period of food magazine training rats were implanted with jugular catheters. Initially rats were trained to self-administer nicotine (0.03 mg/kg/infusion) on an FR1 schedule. Each infusion (2s duration) was accompanied by a 2s tone and 20s stimulus light/20s offset houselight (i.e CS). Schedule requirements were gradually increased to a final FR5 until a stable pattern of responding was reached. To study the effect of test drug on nicotine self-administration, separate groups of 12-16 rats per group were used for each to test the effect of varying doses according to a repeated measures design, with each animal receiving each dose and vehicle in a counterbalanced sequence, with a 2-4 day interval between each cycle.

*Food maintained responding (FR5TO20s schedule)*: Test subjects were male, Sprague-Dawley rats, trained to respond for food (45mg pellets) under an identical schedule to that used for intravenous nicotine, i.e FR5 schedule requirement with reinforcement delivery accompanied by a 2s tone and 20s stimulus light/20s offset houselight (i.e CS). Drug treatment as above.

*Nicotine discrimination studies:* Male, Sprague-Dawley rats were trained to discriminate nicotine (0.3 mg/kg SC) from saline using a standard 2 lever choice procedure (FR10 schedule). After stable discrimination, substitution tests were conducted. Next, the effect of each drug to modify the dose related nicotine (0.03 – 0.3 mg/kg SC) generalisation was examined. Varenicline was also included in these studies.

*Nicotine withdrawal studies*: Male, Wistar rats were implanted s.c. with osmotic minipumps containing either saline or nicotine (bitartrate salt) primed to deliver drug at either 6mg/kg/day or 9mg/kg/day for 9 days. Blood levels of nicotine attained by the 9mg/kg/day regimen are considered equivalent to the range associated with heavy smokers (see Malin & Goyarzu, 2009). A precipitated withdrawal model was used by injecting rats with either mecamylamine (1mg/kg SC) or saline (control) before each test procedure.

*Feeding tests:* Separate cohorts of male, Sprague-Dawley rats were trained to either (1) consume a sweetened wet mash (palatability-induced feeding), (2) 45mg food pellets under a progressive ratio schedule of reinforcement, or (3) standard lab chow made available for 2h each day, i.e deprivation-induced feeding.. After stable levels of intake, the effect of each drug on intake was determined using a within subjects design.

*Drugs and injections :* Lorcaserin, Ro 60-0175 and CP-809101 were prepared in saline in a dose volume of 1ml/kg and administered by the SC route. Doses are expressed as base. Pretreatment times are 10min unless identified as otherwise under a specific test.

# Results

## A. Effect of lorcaserin, Ro 60-0175 and CP-809101 against responding maintained by intravenous nicotine or food

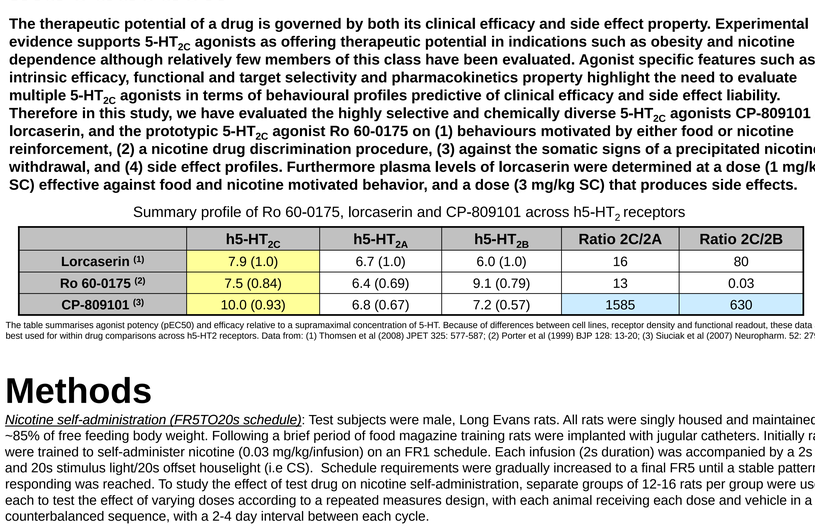


***Figure 1.*** Effect of Lorcaserin (0.3-1 mg/kg SC), Ro 60-0175 (0.3-1 mg/kg SC) and CP-809101 (1-3 mg/kg SC) pretreatment on behaviour motivated by food (45mg Bioserve pellet) or intravenous nicotine (0.03mg/infusion) reinforcement each made available under an FR5TO20s schedule. Upper panels show the number of reinforcements received over the 60min session, and the lower panel the number of active lever presses recorded over the same test session. In each study rats received all treatments according to a randomised design. * p<0.05 vs. vehicle pretreatment. With each 5-HT2C agonist, despite markedly different rates of responding, there was very little difference in the magnitude of effect against each reinforcer.

## B. Characterisation of lorcaserin, Ro 60-0175 and CP-809101 against a nicotine cue.

**Tests of generalisation to a nicotine cue**

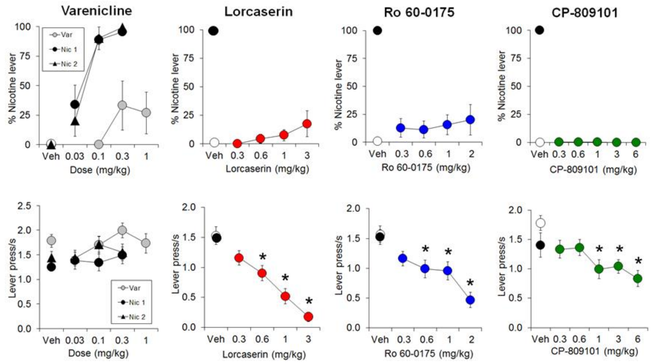


***Figure 2.*** Upper panel: Effect of nicotine (0.03-0.3 mg/kg SC), varenicline (0.1-1 mg/kg SC), lorcaserin (0.3-3 mg/kg SC), Ro 60-0175 (0.3-2 mg/kg SC) and CP-809101 (0.3-6 mg/kg SC) to substitute for a nicotine (0.3 mg/kg SC) cue in a 2-lever food reinforced discrimination procedure. Data are presented as % nicotine lever responding. Nic 1 and Nic 2 refer to nicotine dose response curves run at the start and completion of these studies, respectively. Lower panel: Effect of nicotine (0.03-0.3 mg/kg SC), varenicline (0.1-1 mg/kg SC), lorcaserin (0.3-3 mg/kg SC), Ro 60-0175 (0.3-2 mg/kg SC) and CP-809101 (0.3-6 mg/kg SC) on response rate from the same study as (A). Data expressed as number of responses/s. * p<0.05 vs. vehicle group (Tukey's test).

**Tests of antagonism to a nicotine cue**

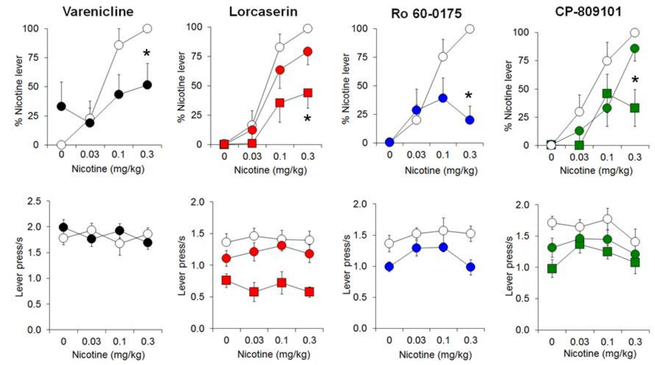


***Figure 3.*** Upper panel: Effect of varenicline (0.3 mg/kg SC), lorcaserin (● =0.3 mg/kg, ■ = 0.6 mg/kg SC), Ro 60-0175 (● = 0.6 mg/kg SC), CP-809101 (● =0.3 mg/kg, ■ = 1 mg/kg SC), or vehicle (○) pretreatment prior to nicotine (0.03 – 0.3 mg/kg SC) administration. Data are presented as % nicotine lever responding. * p<0.05 vs. vehicle/nicotine (Tukeys test). Lower panel: Effect of test drug and nicotine combinations on response rate from the same study. Data expressed as number of responses/s. All data presented as means+SEM..

## C. Effect of lorcaserin and Ro 60-0175 against somatic signs of nicotine withdrawal

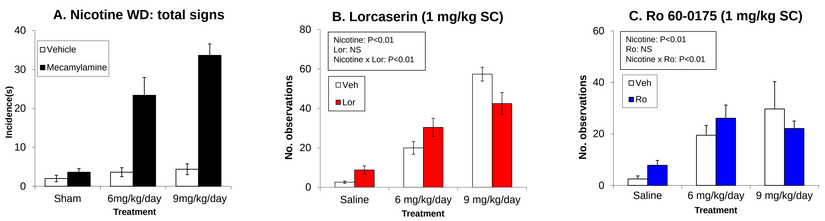


***Figure 4.*** **(A)** Male Wistar rats were either implanted SC with osmotic minipump primed to deliver saline (Sham) or nicotine at 6 mg/kg/day or 9 mg/kg/day. Some change in weight gain was noted, particulary in the 9 mg/kg/day group. On day 9, withdrawal precipitated by an acute injection of mecamylamine (1 mg/kg SC) or vehicle control. Somatic withdrawal signs (e.g chewing, ptosis, wet dog shakes, twitches) were measured over the subsequent 30min period – these were only evident in rats treated with the nicotine + mecamylamine combination. * P<0.05 vs. respective vehicle treated group. In separate groups of rats prepared with minipumps primed to deliver Saline, nicotine at 6 or 9 mg/kg/day, the effect of acute pretreatment with either **(B)** lorcaserin (1mg/kg SC; 30min ptt.), or **(C)** Ro 60-0175 (1mg/kg SC; 30min ptt.) was investigated against a mecamylamine (1 mg/kg) precipitated withdrawal in Wistar rats. Testing was conducted 9 days post minipump implantation. Neither treatment significantly affected overall incidence of withdrawal signs in each nicotine group.

## D. Characterisation of lorcaserin, Ro 60-0175 and CP-809101 in three feeding tests

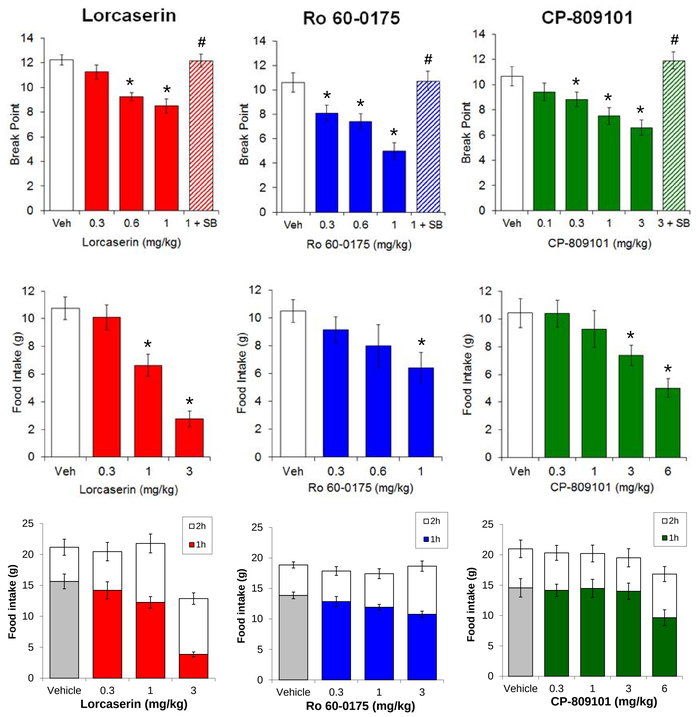


***Figure 5.*** Upper panel: Effect of Lorcaserin (0.1-1 mg/kg SC), Ro 60-0175 (0.1-1 mg/kg SC) and CP-809101 (0.1-3 mg/kg on responding for food under a progressive ratio schedule of reinforcement. In each experiment a drug + SB-242084 (0.5 mg/kg IP) arm was also included. * p<0.05 vs. vehicle, # p<0.05 vs. drug alone (Tukeys test). Middle panel: Effect of lorcaserin (0.3-3 mg/kg SC), Ro 60-0175 (0.3-1 mg/kg SC), CP-809101 (0.3-6 mg/kg SC), on food consumption in rats given access to a sweetened wet mash diet. * p<0.05 vs. vehicle (Dunnetts t-test). Lower panel: Effect of lorcaserin (0.3-3 mg/kg SC), Ro 60-0175 (0.3-1 mg/kg SC), CP-809101 (0.3-6 mg/kg SC), on food consumption induced by 22h deprivation, i.e 2h daily access. Intakes at 1h and 2h are shown.

## E. Characterisation of lorcaserin and CP-809101 on spontaneous behaviour

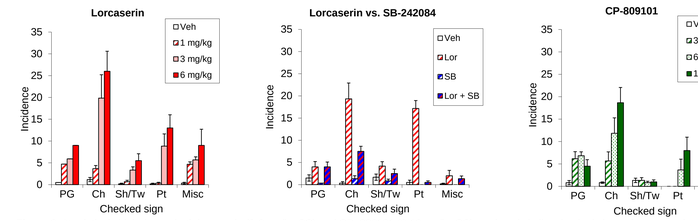


***Figure 6.*** At relatively high doses, both lorcaserin (3-6 mg/kg SC) and CP=809101 (6-12 mg/kg SC) produced similar checked signs of vacuous chewing, ptosis, penile grooming and some salivation. The effects of lorcaserin were blocked by SB-242084 (0.5 mg/kg IP). Miscellaneous (Misc) items scored in the lorcaserin study included flat body posture, chin rubbing and back muscle contracture. PG = penile grooming, Sh/Tw = shakes/twitches, Ch = vacuous chewing, Pt = ptosis.

## F. Pharmacokinetic study of lorcaserin

| Lorcaserin dose | Strain | $T_{max}$ (h) | $C_{max}$/Dose (kg*ng/mL/mg) | Half-life (h) | $AUC_{0-inf}$/Dose (h*kg*ng/mL/mg) | $MRT_{0-inf}$ (h) |
|---|---|---|---|---|---|---|
| 1 mg/kg | SD | 0.55±0.27 | 232±94 | 3.5±1.1 | 541±156 | 3.11±0.63 |
| 3 mg/kg | SD | 0.80±0.27 | 148±39 | 3.1±0.3 | 540±152 | 3.69±0.33 |

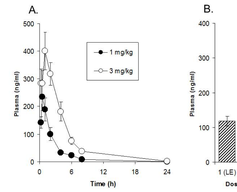


***Figure 7.*** **(A)** Time course of lorcaserin plasma concentration following SC administration at a dose of either 1 or 3 mg/kg in male, Sprague-Dawley (SD) rats. Plasma samples were collected at 0.25, 0.5, 1, 2, 4, 6, 8, 24 h and lorcaserin was quantified by LC-MS/MS analysis. N=5 rats per treatment. **(B)** Comparison of mean plasma levels measured at 0.5 and 1 h post dose in Sprague-Dawley (SD) and Long-Evans (LE) rats. Note the plasma levels are similar between the LE and SD rats following an equivalent 1 mg/kg dose. These time points were selected as they correspond to when the majority of behavioural investigations were made following lorcaserin pretreatment.

# Summary and conclusions

1. **The 5-HT$_{2C}$ receptor agonists lorcaserin, Ro 60-0175 and CP-809101 reduced responding for both intravenous nicotine and food made available under an identical FR5TO20s schedule of reinforcement. Despite markedly differing response rates, there was no trend to identify any differential selectivity towards each reinforcer type by these drugs.**
2. **The 5-HT$_{2C}$ receptor agonists lorcaserin, Ro 60-0175 and CP-809101 did not generalise to a nicotine cue at pharmacologically relevant doses. However each did reliably attenuate a nicotine cue, a feature similar to the nicotine partial agonist varenicline (see also Quarta et al, 2007; Zaniewska et al, 2007).**
3. **In rats chronically exposed to nicotine, acute administration of mecamylamine produced somatic behavioural signs such as ptosis, wet dog shakes, chewing and a decrease in spontaneous motor activity. Pretreatment with either lorcaserin or Ro 60-0175 (both 1 mg/kg SC) failed to attenuate the somatic withdrawal signs to a mecamylamine precipitated W/D. Interestingly, Zaniewska et al (2010) recently reported that Ro 60-0175 may attenuate certain affective nicotine W/D signs although this has not been investigated yet using the present methods.**
4. **Plasma levels of lorcaserin (1 mg/kg SC) at a timepoint producing robust efficacy against both nicotine and food maintained behaviour was approximately 120-150 ng/ml. Assuming linearity, extrapolation to lower doses (0.3-0.6 mg/kg) effective against nicotine maintained behaviour including reinstatement (Higgins et al, 2012), suggests similar exposure to that reported in human obesity trials (~50 ng/ml; see NDA 22-529).**
5. **At a higher dose of lorcaserin (3 mg/kg SC) equivalent to a plasma level in the range of 300-400 ng/ml, side effects such as salivation, flat body posture, ptosis, vacuous chewing become predominant behaviours. Many of these are shared by emetogans such as rolipram implying they may reflect malaise. Nausea and vomiting are described as the most frequent AE in lorcaserin trials and these preclinical findings may reflect this side effect.**
6. **Overall the effects of 5-HT$_{2C}$ agonists against a variety of nicotine behaviours which contribute to its abuse liabilty overlap with doses effective in feeding tests. PK studies using lorcaserin conducted in SD and Long Evans rats support this bioequivalence. These experiments support the consideration of 5-HT$_{2C}$ agonists, as treatments to support smoking cessation. Studies suggest an equivalent dose regimen of lorcaserin could be used in smoking cessation trials to that used in obesity trials.**
7. **Despite potential agonist differences between lorcaserin, CP809101 and the prototypic agonist Ro 60-0175, there was no obvious differences in efficacy profile suggesting a broad class effect. Side effect profiles may differ.**

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