SUPPORTING INFORMATION

Novel tetrahydroquinazolinamines as Selective Histamine 3 Receptor Antagonists for the Treatment of Obesity

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Key words: Histamine 3 receptor (H3R), GPCR, obesity, GloSensor, Quinazolinamines
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**Sample Information**

Sample Name: S-013-1596-02/08/18
Sample Type: Unknown
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Date Processed: 8/3/2018 3:40:13 PM IST

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Channel Name: 280.0 nm

**Auto-Scaled Chromatogram**

**Peak Results**

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Report Method ID: 1354
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Date Printed: 8/3/2018 3:40:41 PM Asia/Calcutta
$^{1}$H-NMR, $^{13}$C-NMR, HRMS & HPLC of 4b
$^{1}H$-NMR, $^{13}C$-NMR, HPLC & HRMS of 4c
S9

CDRI SAIF GLC & HPLC LAB
Lucknow

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Auto-Scaled Chromatogram

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Acquired By: System
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Auto-Scaled Chromatogram

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Auto-Scaled Chromatogram

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$^{1}$H-NMR, $^{13}$C-NMR, HRMS & HPLC of 4f
Sample Information

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Acquired By: System
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Channel Name: 280.0nm

Auto-Scaled Chromatogram

Peak Results

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$^1$H-NMR, $^{13}$C-NMR, HPLC & HRMS of 4g
$^1$H-NMR, $^{13}$C-NMR, HRMS & HPLC of 4h
Sample Information

Sample Name: S-013-0332-02/08/18 - Unknown
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Acquired By: System
Sample Name: S-013-0332-02/08/18 - Unknown
Acq. Method Set: GENRAL HPLC
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Channel Name: 280.0nm

Auto-Scaled Chromatogram

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$^1$H-NMR, $^{13}$C-NMR, HPLC & HRMS of 4i
CDRI SAIF GLC & HPLC LAB
Lucknow

SAMPLE INFORMATION

Sample Name: S-013-0333-02/08/18 -
Sample Type: Unknown
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Acq. Method Set: GENRAL HPLC
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Channel Name: 280.0nm

Auto-Scaled Chromatogram

Peak Results

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$^{1}$H-NMR, $^{13}$C-NMR, HRMS & HPLC of 4j
Sample Information

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Injection Volume: 5.00 ul
Run Time: 30.0 Minutes
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Sample Name: S-013-0334-02/08/18 -
Acq. Method Set: GENRAL HPLC
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Channel Name: 254.0nm

Auto-Scaled Chromatogram

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$^1$H-NMR, $^{13}$C-NMR, HRMS & HPLC of 4l
Sample Information

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Acquired By: System
Sample Name: S-013-0357-03/08/18 -
Acq. Method Set: GENRAL HPLC
Processing Method: S-013 0357 280nm
Channel Name: 280.0nm

Auto-Scaled Chromatogram

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Auto-Scaled Chromatogram

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Auto-Scaled Chromatogram

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Project Name: RKP2017/RKP2017A
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$^1$H-NMR, $^{13}$C-NMR, HPLC & HRMS of 4o
SAMPLE INFORMATION

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Channel Name: 280.0nm

Auto-Scaled Chromatogram

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Automatically Chromatogram

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Page: 1 of 1
$^{1}H$-NMR, $^{13}C$-NMR, HPLC & HRMS of 4q
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**Auto-Scaled Chromatogram**

**Peak Results**

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**Project Name:** RKP20171RKP2017A

**Date Printed:** 8/3/2018 5:24:42 PM Asia/Calcutta
\( ^1H\text{-NMR, }^{13}C\text{-NMR, HRMS & HPLC of } 4r \)
$\text{H-NMR, }^{13}\text{C-NMR, HPLC & HRMS of 4s}$
$^1$H-NMR, $^{13}$C-NMR, HRMS & HPLC of 4t
CDRI SAIF GLC & HPLC LAB
Lucknow

SAMPLE INFORMATION

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Date Processed: 8/6/2018 4:17:30 PM IST

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Processing Method: S 015 2318 280nm
Channel Name: 280.0nm

Auto-Scaled Chromatogram

Peak Results

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Reported by User: System
Report Method: CDRI_Report
Report Method ID: 1354
Page: 1 of 2

Project Name: RKP2017/RKP2017A
Date Printed: 8/6/2018 4:17:58 PM Asia/Calcutta
$^{1}H$-NMR, $^{13}C$-NMR, HPLC & HRMS of 4u
$^{1}$H-NMR, $^{13}$C-NMR, HRMS & HPLC of 4v
CDRI SAIF GLC & HPLC LAB
Lucknow

SAMPLE INFORMATION

Sample Name: SO-13-2321-20/09/18-PNY
Sample Type: Unknown
Visit: 1
Injection #: 11
Injection Volume: 10.00 ul
Run Time: 35.0 Minutes
Date Acquired: 9/20/2018 4:49:24 PM IST
Date Processed: 9/20/2018 5:53:49 PM IST

Acquired By: System
Sample Name: SO-13-2321-20/09/18-PNY
Acq. Method Set: GLYCOSIDE
Processing Method: 90 13 2321 290.0nm
Channel Name: 290.0nm

Auto-Scaled Chromatogram

Peak Results

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Reported by User: System
Report Method: CDRI_Report
Report Method ID: 1354
Project Name: RKP2017/RKP2017A
Date Printed: 9/20/2018 5:54:22 PM Asia/Calcutta
$^{1}$H-NMR, $^{13}$C-NMR, HPLC & HRMS of 4w
**SAMPLE INFORMATION**

Sample Name: S-015-2324-06/08/18 -
Sample Type: Unknown
Vial: 1
Injection #: 2
Injection Volume: 5.00 ul
Run Time: 30.0 Minutes
Date Acquired: 8/6/2018 10:59:28 AM IST
Date Processed: 8/7/2018 3:37:44 PM IST

**Auto-Scaled Chromatogram**

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Reported by User: System
Report Method: CDRI_Report
Report Method ID 1354
Page: 1 of 1

Project Name: RKP2017/RKP2017A
Date Printed: 8/7/2018
3:38:23 PM Asia/Kolkata
$^1$H-NMR, $^{13}$C-NMR, HRMS & HPLC of 4x
$^1$H-NMR, $^{13}$C-NMR, HRMS & HPLC of 4y
$^1$H-NMR, $^{13}$C-NMR & HRMS of 4a'

![NMR Spectra](image-url)
Sample Information

Sample Name: S015-1148-02/12/2018-SYC-3
Sample Type: Unknown
Vial: 1
Injection #: 3
Injection Volume: 10.00 ul
Run Time: 25.0 Minutes
Date Acquired: 12/3/2018 11:12:47 AM IST
Date Processed: 12/3/2018 11:57:38 AM IST

Acquired By: System
Acq. Method Set: GENRAL HPLC
Processing Method: so15 1148 254nm
Channel Name: 254.0nm

Auto-Scaled Chromatogram

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Reported by User: System
Report Method: CDRI_Report
Report Method ID: 1354
Page: 1 of 1

Project Name: RKP2017/RKP2017A
Date Printed: 12/3/2018
11:59:19 AM Asia/Calcutta
$^1$H-NMR, $^{13}$C-NMR & HRMS of 4b'
**Sample Information**

- **Sample Name:** S015-2322-02/12/2018-SYC-2
- **Sample Type:** Unknown
- **Injection #:** 2
- **Injection Volume:** 10.00 ul
- **Run Time:** 25.0 Minutes
- **Date Acquired:** 12/3/2018 10:42:39 AM IST
- **Date Processed:** 12/3/2018 11:47:45 AM IST

### Auto-Scaled Chromatogram

![Chromatogram Image]

**Peak Results**

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**Reported by User:** System

**Report Method:** CDRI_Report

**Report Method ID:** 1354

**Page:** 1 of 1

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**Project Name:** RKP2017/RKP2017A

**Date Printed:** 12/3/2018

11:48:12 AM Asia/Calcutta
$^{1}$H-NMR, $^{13}$C-NMR & HRMS of 4c'
CDRI SAIF GLC & HPLC LAB
Lucknow

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Auto-Scaled Chromatogram

Peak Results

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Report Method: CDRI_Report
Report Method ID 1354
Page: 1 of 1

Project Name: RKP2017/RKP2017A
Date Printed: 12/3/2018 1:16:56 PM Asia/Calcutta
$^1$H-NMR, $^{13}$C-NMR & HRMS of 4d'
SAMPLE INFORMATION

Sample Name: S015-2317-03/12/2018-SYC-3
Sample Type: Unknown
Vial: 1
Injection #: 5
Injection Volume: 10.00 ul
Run Time: 26.0 Minutes
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Date Processed: 12/3/2018 12:43:13 PM IST

Acquired By: System
SampleName S015-2317-03/12/2018-SYC-3
Acq. Method Set: GENRAL HPLC
Processing Method: so15 2317 254nm
Channel Name: 254.0nm

Auto-Scaled Chromatogram

Peak Results

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Reported by User: System
Report Method: CDRI_Report
Report Method ID 1354
Page: 1 of 1

Project Name: RKP2017/RKP2017A
Date Printed: 12/3/2018 12:43:41 PM Asia/Calcutta
Chiral Separation: Chromatogram of Chiral separation of 4c enantiomers

**Figure:** HPLC chiral separation of 4c into its E1 and E2 enantiomers

**Figure:** HPLC chromatogram of 4c
Figure: HPLC chromatogram of enantiomer of E1

Figure: HPLC chromatogram of enantiomer E2
Supplementary methods

1. Chiral separation of 4c enantiomers E1 and E2

The HPLC system (Shimadzu, Kyoto, Japan) equipped with a binary gradient pump (Prominence LC-20 AD, Shimadzu), an auto sampler injector (Prominence SIL-20A HT), and a photo diode array (PDA) detector (Prominence SPDM20A), system controller (CTO-20A). Shimadzu HPLC interface and Lab Solutions Lite software and CLASS-VP workstation was used for data acquisition. A Rheodyne (Cotati, CA, USA) model 7125 injector with a 20 µL loop for analytical estimation and 100 µL loop for preparative isolation was used. CLASS-VP software (version 6.14, Shimadzu, Japan) was utilized for data acquisition. All solvents employed in the study were of HPLC grade supplied by Merck India Ltd., Mumbai, India or Spectrochem Pvt Limited, Mumbai, India.

Chromatographic conditions for chiral separation:

Column : Chiral Pak IA (5 um) [250 × 10 mm] chiral column
Detection : PDA (280 nm)
Flow Rate : 2.0 ml /min
Injection Volume : 100 µl
Column temperature : 25 ± 3°C
Mobile Phase : Mixture of Hexane and ethanol (90:10 v/v)
Diluents : Ethanol
Concentration : 10 mg/ml

Chromatographic conditions for peak purity check:

Column : C18 (5 µ) Merck (Col No. 834095) LichroCART (250 mm – 4 mm)
Detection : PDA (266 nm)
Flow Rate : 1.0 ml/min
Injection Volume : 20 µl
Column temperature : 25 ± 3°C
Mobile Phase : Mixture of KH$_2$PO$_4$ buffer and Acetonitrile (40:60 v/v)
Diluents : Methanol
Concentration : 200 mcg/ml
Figure S1 Representative dose-response curve of H3R antagonist and H2R agonist mediated cAMP response: Concentration response curve of 4b', 4d', and 4c' at H3R for antagonist activity in transiently transfected HEK293T cells (A). Concentration response curve of 4a, 4d, 4e and 4f (B), Dimaprit (reference agonist of H2R), 4k, 4o, 4u and 4w (C) and 4b', 4d', and 4c' (D) at H2R for agonist activity in transiently transfected HEK293T cells
Figure S2 Representative dose-response curve of enantiomers E1 and E2 with recemate 4c at H3R for antagonist mediated cAMP response: Concentration response curve of E1, E2 and 4c at H3R for antagonist activity in transiently transfected HEK293T cells.

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<th>E2</th>
<th>4c</th>
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<tr>
<td>LogIC50</td>
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Figure S3. Dose-response curve of 4c to determine hERG liability: Concentration response curve of positive control E4031 and compound 4c with hERG membrane
Figure S4. Chronic treatment of compound 4c did not show any effect on body weight loss in lean mice: Bar graph showing net body weight change after chronic treatment of H3R antagonist 4c (20mg/kg) or vehicle p=0.6361; N=4-5, t-test.

Figure S5. H3R antagonist 4c did not show any effect on glucose homeostasis in HFD fed mice:(A) Line graph shown glucose level relative to time after glucose administration (2g/kg body weight) in 16h fasted HFD fed obese mice treated with compound 4c (20mg/kg and 10mg/kg; oral) or vehicle (B) Change in blood glucose AUC analysis of ipGTT results. *p<0.05 (n=4-6), p value determined by two-way ANOVA or one-way ANOVA.
Figure S6. H3R antagonist 4c did not show any effect on lipid parameters in HFD fed mice or db/db mice: Bar graph showing lipid parameters (i.e. triglycerides, total cholesterol, HDL and LDL) (A) Chow and HFD fed obese mice treated with compound 4c (20mg/kg and 10mg/kg; oral) or vehicle (B) db/db mice treated with 4c (20mg/kg) or vehicle.

Figure S7. Chronic treatment of H3R antagonist 4c significantly reduced fasting glucose level in db/db mice: Bar graph showing glucose level (mg/dl) in 16 h fasted db/db mice treated with 4c or vehicle.
## Supplementary Table S1: Used reference ligand for off target receptor activity

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