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

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Abstract

The histamine 3 receptor (H3R) is a presynaptic receptor, which modulates several neurotransmitters including histamine and various essential physiological processes, such as feeding, arousal, cognition and pain. The H3R is considered as a drug target for the treatment of several central nervous system disorders. We have synthesized and identified a novel series of 4-Aryl-6-methyl-5,6,7,8-tetrahydroquinazolinamines that act as selective H3R antagonist. Among all the synthesized compounds, *in vitro* and docking studies suggested that 4-methoxy-phenyl substituted tetrahydroquinazolinamine compound **4c** has potent and selective H3R antagonist activity (IC₅₀<0.04 μ M). Compound **4c** did not exhibit any activity on hERG ion channel and Pan-assay interference compounds (PAINS) liability. Pharmacokinetic studies showed that 4c crosses the blood brain barrier and *in vivo* studies demonstrated that **4c** induces anorexia, and weight loss in obese, but not in lean mice. These data reveal the therapeutic potential of **4c** as an anti-obesity candidate drug via antagonizing the H3R.

Introduction

The histamine 3 receptor (H3R) is an inhibitory G-protein coupled receptor (GPCR), which is abundantly expressed in various brain regions, but modestly expressed in periphery.¹ Activation of presynaptic H3Rs expressed on histaminergic neurons (autoreceptor) inhibits the synthesis and release of histamine, while presynaptic hetero H3Rs (expressed on other neurons) negatively regulates several other neurotransmitters such as dopamine, serotonin, and norepinephrine.^{1, 2} Several rodent studies suggest that antagonists or inverse agonists of the H3R increase the levels of various neurotransmitters, including histamine, dopamine, serotonin, acetylcholine and norepinephrine.³⁻⁵ Multiple lines of evidence indicate that histaminergic neurons originate from the tuberomammilary nucleus of hypothalamus and project to major brain area including the satiety centre of the hypothalamus that play a crucial role in eating behavior and energy homeostasis.^{2,3, 6-8} Indeed, several preclinical studies showed that H3R antagonists (i.e. NNC38-1049, NNC38-1202, JNJ-5207852, GT-2394, A-423579 and A-331440) reduced the food intake, body weight and blood glucose level in obese animals.⁷⁻¹³ On the basis of several preclinical observations two H3R antagonists such SCH-497079 (NCT00642993, NCT00673465) and HPP-404 (NCT01540864) were evaluated in clinical trials for the treatment of obesity and diabetes, but both of these molecules were stopped from further development due to the lack of desired level of efficacy.¹⁴ A recent study also suggests that Pitolisant (a selective inverse agonist of the H3R) treatment reduces body weight in obese mice.¹⁵ Moreover, Betahistine, a partial inverse H3R agonist and H1R partial agonist, induced modest weight loss with minimal adverse events in women under 50 years of age and significant weight loss in animals that developed obesity after olanzapine treatment.¹⁶⁻¹⁸ A recent study mentioned several melanin-concentrating hormone receptor 1 (MCHR1) antagonist with anti-obesity effects that also exhibited H3R antagonist activity and suggested a polypharmacology approach for better therapeutics for obesity.¹⁹

Besides metabolic conditions, H3R antagonists have also been evaluated for neuropathic pain, sleeping disorder, cognitive impairments, Tourette syndrome and attention deficit hyperactivity disorder (ADHD).^{14, 20-22} For example, GSK239512 (NCT01009255), MK-0249 (NCT00420420), ABT-288 (NCT01018875) and SAR110894 (NCT01266525) were evaluated in Phase 2 clinical trials for the treatment of Alzheimer's disease, but none of them were approved due to lack of efficacy or side effects.¹⁴ Nevertheless, recently one H3R antagonist-Pitolisant has been approved by the European Medicines Agency for the treatment of narcolepsy.²³ Furthermore, the positive effect of betahistine (H3R antagonist and modest H1R agonist) on weight gain induced by atypical antipshycotics, ¹⁸ but not robust and consistent effect in obese subjects, suggest that variations in basal histaminergic tone in the brains of obese populations might be responsible for inconsistencies between the preclinical and clinical effects of H3R antagonist. Therefore, despite several setbacks in the development of H3R antagonists for the treatment of obesity, more potent and selective H3R antagonist might still be useful to treat obesity, especially when this disease has become a global epidemic with severe CNS related co-morbidities.11,24

Pyrimidines are considered a versatile scaffold from a medicinal chemistry point of view because they display a broad spectrum of biological activities, including antihistaminic activity.²⁵⁻²⁹ Examples of biologically active pyrimidine derivatives include 4-(4'-chlorophenyl)-6-(4"-methylphenyl)-2-hydrazinepyrimidine that show antidepressant properties ³⁰ and dihydropyrimidine containing calcium channel blockers ²⁶ Aminopyrimidine moieties are also found to show a wide variety of biological activity.³¹⁻³⁴ Further, aminoquinazolines and

tetrahydro-aminoquinazolines are also considered as aminopyrimidine derivatives, which have been shown to exhibit analgesic, narcotic, sedative and hypoglycemic activities.^{35, 36} We have also shown that 2-aminopyrimidine derivatives exhibit antitubercular and antidiabetic activities.^{37, 38}



Figure 1: Designed prototype based on known tetrahydroquinazolinamine derived GPCRs ligands.

Interestingly, Savall *et al.* have described a tricyclic aminopyrimidine derivative as a histamine 4 receptor (H4R) antagonists (JNJ 40279486).³⁹ Previous reports also suggest

aminopyrimidine moiety is active at other GPCRs including H3R.⁴⁰⁻⁴⁴ Other previously reported pyrimidine compounds include the potent anti-obesity compound **6** agonist of human cholecystokinin-1 receptor (CCK-1R), compound **7** antagonist of MCHR1, H3R antagonists compounds **4** and **5**, and H4R antagonists compounds **1-3** (Figure 1). With these structures in mind we designed and synthesized a novel series of tetrahydroquinazolinamine derivatives and evaluated them for agonist as well as antagonist actions at histamine receptors.^{39, 40, 42, 43, 45-47} Interestingly, we discovered a very selective and potent H3R antagonist that induced anorexia and weight loss in rodent models of obesity.

Results and discussion

Chemistry

Synthesis of 4-aryl-tetrahydroquinazolin-2-amines. This study was mainly focused on the synthesis of various substituted tetrahydroquinazolinamines and their biological evaluations at histamine receptors in vitro followed by in vivo efficacy in mouse models of obesity. We first synthesized various arylidenylcyclohexanones 3 as the important precursors, following our earlier protocol ⁴⁸ by employing the Claisen-Schmidt condensation reaction of cyclohexanone (1a-1d) and hexanone (1e) with different aromatic aldehydes 2 in the presence of 10% aqueous KOH in ethanol at ambient temperature (Scheme 1). The oily crude products thus obtained were purified with column chromatography to give the required arylidenylcyclohexanone derivatives in good to moderate yields (55-68%). These molecules were characterized on the basis of their spectroscopic data and are supported by previous literature.⁴⁹⁻⁵⁷ The appropriately substituted tetrahydroquinazolinamines were accessed by condensation of respective arylidenvlcvclohexanones 3 with guanidine hydrochloride in the presence of NaH in DMF as the solvent following earlier reported protocols ^{37, 38} (Scheme 2). The reaction involves conjugate 1,

4 addition of guanidine to the olefinic bond of the enone followed by cyclization and condensation at the carbonyl end, tautomerization and aromatization by aerial oxidation resulting into the formation of 2-aminopyrimidine nucleus. The targeted compounds were thus obtained in moderate yield and their characterization was done by ¹H, ¹³C NMR and mass spectrometry. Furthermore, purity of all compounds (4a-4y, 4a'-4c'and 4d') were evaluated by general HPLC and found to be \geq 95% pure.



Scheme 1: Synthesis of different substituted 2-arylidene-cyclohexanones (3a-3z) & 2arylheptenones (3a')

Two of the final compounds **4h** and **4t**, having benzyloxy substitution at 4 and 3 position of phenyl ring were subjected to debenzylation using 10% Palladium over charcoal and hydrogen



gas atmosphere, to get the debenzylated analogues 4x and 4y, respectively (Scheme 2).

Scheme 2: Synthesis of various substituted 4-aryl-tetrahydroquinazolin-2-amines (4a-4y, 4a'-4c') and 4-aryl-2-aminopyrimidine (4d')

Biology

In vitro activity of compounds at human histamine receptors. The newly synthesized 25 tetrahydroquinazolinamines 4a-4y (Scheme 2) were evaluated for their agonist and antagonist activity at the human H3R by the GloSensor assay in which we measured cAMP production in live cells. First, all compounds were tested at 10 μ M concentration in triplicate for agonist or antagonist activity, and if any compound exhibited a minimum of 50% inhibition or stimulation in comparison to control (for agonist activity- forskolin 10 μ M and for antagonist activity-histamine 100 nM + forskolin 10 μ M), then that particular compound was further evaluated for

concentration-dependent (10 μ M to 10 pM) response to determine the EC₅₀ or the IC₅₀. After the primary screening, we found 14 compounds active as antagonist at H3R, and therefore further tested in a concentration dependent manner to determine IC₅₀ value at H3R (**Table 1** and **figure 2**). All 25 compounds were also evaluated for activity at the other histamine receptors H1R, H2R and H4R. None of them showed any activity at H1R and H4R, while 8 compounds exhibited agonist activity at H2R (**Table 1** and **Fig S1**).



Figure 2. Representative concentration-response curve of H3R antagonist mediated blockade of HA response (cAMP): Concentration response curve (A) GSK334429 (known H3R antagonist) and compounds 4c, (B) 4a, 4b, 4d, 4e and 4f, (C) 4g, 4k, 4o and 4q and (D) 4s, 4u, 4y and 4w at human H3R for antagonist activity in transiently transfected HEK293T cells. Data are expressed as mean \pm S.E.M. of normalized result of two individual experiments in triplicates.

Structure-activity relationship (SAR). Among all of these, compound 4c having 4-methoxyphenyl substituted tetrahydroquinazolinamine, exhibited very good antagonist activity at H3R with IC₅₀ 0.04 μ M (Fig 2A and Table 1), while no activity at any other HA receptors. Compounds 40 having 3-methoxy-phenyl substitution and 4k having 3-bromo-phenyl substitution also exhibited antagonist activity at H3R with IC₅₀ values of 0.07 μ M and 0.09 μ M, respectively (Fig 2C and Table 1), almost similar to compound 4c but both compounds also displayed moderate agonist activity at human H2R with EC₅₀ value 0.25 μ M and 0.84 μ M (Table 1 and Fig S1), respectively. Compound 4w having cyclohexyl substituted at 4-position of tetrahydroquinazolinamine showed modest H3R antagonist activity (IC₅₀ =0.24 μ M, Fig 2D) and also have similar agonist activity at H2R with an EC₅₀ of 0.36 μ M (Fig S1). On the other hand, compound 4a with an unsubstituted phenyl ring and compounds 4e and 4f having 4-chloro and 4-fluoro substituted phenyl ring, exhibited almost similar antagonist action with IC₅₀ 1.30 µM, 0.90 µM and 0.56 µM, respectively (Fig 2B and Table 1). Compounds 4a, 4e and 4f also showed better agonist activity at H2R with EC_{50} 0.10 μ M, 0.08 μ M and 0.08 μ M, respectively (Table 1 and Fig S1).

Table 1: Summary of hit compounds screened at human histamine receptor:

S.No	Compounds		hH2R		hH3R	
	-					
	Structure	Code	LogEC ₅₀	EC_{50}	LogIC ₅₀	IC_{50}
			0 50		6 30	50
				$(\mathbf{u}\mathbf{M})$		$(\mathbf{u}\mathbf{M})$
				(µ111)		(μινι)
	NIL I					
1		4a	-7.02 ± 0.21	0.10	-5.89 ± 0.13	1.30
	N ^{//⊂} N					

2		4b	NA	-	-5.51 ± 0.13	3.1
3		4c	NA	-	-7.35 ± 0.12	0.04
4		4d	-6.05 ± 0.20	0.88	-5.53 ± 0.18	2.93
5		4e	-7.11 ± 0.23	0.08	-6.04 ± 0.07	0.90
6	NH2 N N F	4f	-7.12 ± 0.21	0.08	-6.26 ± 0.08	0.56
7	NH ₂ N N	4g	NA	-	-5.57 ± 0.14	2.71
8	NH2 N N Br	4k	-6.50 ± 0.22	0.84	-7.07 ± 0.10	0.09
9		40	-6.61±0.06	0.25	-7.18 ± 0.07	0.07
10	NH ₂ N N N	4q	NA	-	-5.17 ± 0.16	6.80
11		4s	NA	-	-5.26 ± 0.15	5.50
12		4u	-5.27 ± 0.32	5.29	-4.91 ± 0.21	12.30

13		4y	NA	-	-5.34 ± 0.17	4.61
14	NH ₂ N N	4w	-6.44±0.05	0.36	-6.61 ± 0.80	0.24
15	NH2 NNN OMe	4b'	-5.67 ± 0.13	2.12	-5.33 ± 0.10	4.68
16	NH ₂ N N OMe	4c'	-5.89 ± 0.28	1.39	-5.56 ± 0.09	2.75
17		4d'	-5.28 ± 0.25	5.18	-5.35 ± 0.07	4.45

NA= Not Active

The H3R antagonist potential of compounds **4d** (IC₅₀= 2.93 μ M), **4b** (IC₅₀= 3.1 μ M), **4s** (IC₅₀= 5.50 μ M) and **4u** (IC₅₀= 12.30 μ M) were reduced H3R activity with corresponding -CH₃, Br, –CN and -NO₂ groups substitutions at para-position of phenyl ring. While compound **4d** and **4u** also showed H2R agonist activity with EC₅₀ value 0.88 μ M and 5.29 μ M (**Table 1 and Fig S1**). The effect of -OH group at both meta- and para- position of phenyl ring was also checked, compound **4y** with meta-position substitution showed no activity at H3R. Substitution of the aryl group with a naphthyl (**4g**, IC₅₀= 2.71 μ M) and pyridinyl (**4q**, IC₅₀= 6.80 μ M) ring also reduced antagonist activity at H3R. The other compounds with substitutions at para position of phenyl ring such as - OCH2Ph, -dimethylamino, -isopropyl, -methylthio, -CF3 and -OCF3 did not exhibit any antagonistic potential at H3R. Homology modelling showed that some of the active compounds (**4e**, **4b**, and **4b'**) show similar binding orientation and comparable docking score (**4e**= -7.25, **4b**=

-7.25, **4b'=** -7.68) like the most potent compound **4c** having methoxy group in the phenyl ring, but no TYR115 hydrogen bonding with oxygen atom of methoxy group, suggesting the critical nature of this molecular interaction for high affinity binding. Furthermore, the compound 4v having ethoxy group docks in different orientation, resulting in the loss of hydrogen bond interaction with TYR115 and did not show any antagonistic activity at H3R at all, again underlining the importance of hydrogen bond interaction at TYR115 for desired activity. We also determined the role of methyl group at tetrahydroquinazolin ring compounds with 8,8-dimethyl substitution (4a') and found that this modification lead to loss of activity at all HA receptors, including H3R. However, compounds with 6-methyl substitution (4b'), without methyl substitution (4c'), and an open chain 2-aminopyrimidine derivative (4d') showed reduced antagonist activity at H3R as compared to 4c having same 4-methoxy-phenyl group (Table 1, Fig S1 A). Compounds 4b', 4c' and 4d' also showed modest agonist activity at H2R with EC_{50} 2.12 μ M, 1.39 μ M and 5.18 μ M, respectively (**Table 1, Fig S1 D**). This signifies the importance of monosubstituted 8-methyl group and *para*-methoxy group in the most potent compound 4c to act as an efficient antagonist at H3R and negligible activity at other histamine receptors.

Selectivity profiling of 4c. *In vitro* activity of 4c, 4k and 4o show approximately similar IC₅₀ as H3R antagonist but compounds 4k and 4o have agonist activity at H2R, while 4c has no activity towards H2R. On the basis of histamine receptor functional activity data we considered 4c as a lead compound with potent antagonist activity against human H3R, while lacking any noticeable activity in the functional assays to other subtypes of histamine receptors- H1R, H2R, and H4R. Furthermore, pre-treatment with compound 4c (10 μ M) significantly blocks the H3R agonists (i.e. histamine or imitet; 1 μ M) induced inhibition of cAMP response, which suggest that 4c selectively work through H3R and did not show receptor independent activity (Fig. 3).



Figure 3. Compound 4c selectively inhibits H3R agonist response. Bar graph showing cAMP dependent relative luminescence unit (RLU) induced by forskolin (10 μ M) treatment and pretreatment with compound 4c or reference ligand GSK (GSK334429, a known H3R antagonist) at 10 μ M concentration significantly block the H3R agonists (i.e. histamine or imitet; 1 μ M) induced inhibition of cAMP response. ^{\$SS}p<0.001 denote forskolin treatment significantly increased RLU compare to control cells, ^{***}p<0.001 denote histamine or imitet (IMT) significantly decreased the cAMP compared to forskolin alone, ^{###}p<0.001 and ^{\$886}p<0.001 denote significant blockade of cAMP by pretreatment with 4c or GSK compared to histamine and imitet, respectively.

Next, we evaluated the off target activity of lead compound **4c** at other families of GPCRs such as dopamine receptors (D1-D5), muscarinic receptors (M1 and M5), G-protein-coupled bile acid receptor 1, G-protein coupled receptor 40, serotonin receptor (5- HT_{2C}) and kappa opioid receptor (KOR). Lead compound 4c did not exhibit noticeable activity at GPCRs of other families (**Table 2**). These *in vitro* results demonstrate that compound 4c exhibits selective antagonist activity at H3R, over the panel of GPCRs tested.

Since we were interested to discover and develop a selective H3R antagonist with desirable safety profile for the treatment of obesity, we preferred to evaluate compound **4c** for

the binding at the protein of the Human Ether-a-go-go-related Gene (hERG), a potassium ion channel in heart responsible for long QT syndrome using a predictor hERG assay kit (Invitrogen Corp, USA). We observed that test compound **4c** did not bind to the hERG up to 10 μ M , while reference compound E-4031 (well known hERG ligand) showed activity with IC₅₀ < 0.30 μ M (**Table 2** and **Fig S2**). We also found that all 25 compounds including **4c** (**Table 1**) have no Panassay interference compounds (PAINS) liability as determined by PAINS- Remover online web server.⁵⁸ Although evaluation at CEREP panel targets would have yielded better clarity on the safety profile of **4c**, our data for 15 targets indicate a reasonable selectivity and specificity for this compound.

S. No	Targets	Ac	Activity		
		Agonist	Antagonist		
1	Dopamine D1 receptor (D1R)	NA	NA		
2	Dopamine D2 receptor (D2R)	NA	NA		
3	Dopamine D3 receptor (D3R)	NA	NA		
4	Dopamine D4 receptor (D4R)	NA	NA		
5	Dopamine D5 receptor (D5R)	> 10 µM	NA		
6	Histamine H1 receptor (H1R)	NA	NA		
7	Histamine H2 receptor (H2R)	> 10 µM	NA		
8	Histamine H4 receptor (H4R)	NA	NA		
9	Muscarinic M1 receptor (M1R)	NA	NA		
10	Muscarinic M5 receptor (M5R)	NA	NA		
11	Opioid κ receptor (KOR)	NA	>10 µM		

 Table 2. Off-targets activity of 4c

12	Serotonin 5-HT2 _C receptor (5-HT2 _C R)	NA	NA
13	G-protein-coupled receptor40 (GPR40)	NA	NA
14	G-protein-coupled bile acid receptor 1 (GPBAR1)	NA	NA
15	hERG liability	No	
16	PAINS liability	No	

Characterization of 4c enantiomers activity at H3R. First we evaluated activity of both (R and S) enantiomer of **4c** at H3R by *in silico* docking experiment with homology modelling. We first generated 50 models, and the top 10 models were selected on the basis of DOPE score and validated with PROCHECK and QMEAN. Models having more than 90% residues in the most favoured regions of the Ramachandran plot were considered good quality models.^{59, 60}. Models having QMEAN score close to 1 indicate a better model quality.⁶¹ Visual inspection was done for the correct orientation of side chains of conserved residues favourable for binding of antagonists and stabilization of receptor in the inactive state.^{62,63, 64}



Figure 4 Ramachandran Plot of human H3R model protein and Predicted binding mode of R- and S- isomer of the compound 4c: (A) 96.9% residues in most favoured region and 0.0%

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residues in disallowed region. **(B)** Binding pose of R-isomer (blue) and (C) S-isomer (dark green) within the H3R model constructed from 4U15. Amino acid residue interacted with both the isomers of the compound **4c** via hydrogen bond showing in green.

Previous studies showed that GLU206, TYR115 and TYR374 lining the orthosteric site are the gripping points for agonists, including histamine. Binding of more complex ligands were described as extending to the allosteric site residues, TYR189, ALA190, ARG381, PHE193, TYR194 and TYR394.^{62, 65-68} A Ramachandran plot was generated using a PROCHECK server and the best model showed 96.9% residues in the maximum allowed region. Further, the calculated QMEAN was 0.600 (**Fig 4A**). Both R- and S- isomers showed relatively similar molecular interactions with the surrounding binding site residues of the protein with highest docking score of -7.642 and -7.518, respectively (**Fig 4B and 4C**). Interactions involved in the binding of R- and S- isomer of the compound **4c** were shown in figure 5A and 5B and summarized in **Table 3**. Aliphatic ring **I** formed π -alkyl interactions with TYR394. In the extracellular loop 2 (ECL2) on the top edge of the binding site interacted with R-isomer, PHE193 and TYR194 interacted with the R-isomer by forming two conventional hydrogen bonds with amine group **IV** of the R-isomer. Aromatic ring **II** participated in a π -lone pair interaction with TYR394 and a π -alkyl interaction with ALA190.



Figure 5. Interaction of R and S isomer of compound 4c with the human H3R model protein. (A) Amino acid residues of H3R involved in interaction with R-isomer (blue) and (B) S-isomer (dark green). Amino acid residues involved in hydrogen bonding showed in green. (C) Interacting groups of compound **4c** with the human H3R.

The methoxy oxygen atom of interacting group **III** forms hydrogen bond with TYR115. In addition, charged residues ASP114, ARG381 and ASP391 formed ionic interaction with the R-isomer. Moreover, residues LEU111, TRP110, PHE398, TYR91, TYR194, TRP196 and LEU199 formed hydrophobic interactions with the R-isomer. The S-isomer formed relatively similar molecular interactions with binding site residues that were observed for the R-isomer except interaction between TYR194 and ASP114. TYR194 was observed to form both hydrogen bonding and hydrophobic interactions with the R-isomer, whereas TYR194 formed only hydrophobic interactions with the S-isomer. These direct and indirect interactions stabilized the R-and S-isomers of compound **4c** in the H3R model. Docking experiments suggest that both R

and S enantiomer exhibits equal activity at H3R with almost similar docking score and interaction. Table 3 Summary of interactions between R- and S-isomers of the 4c and the human H3R **Molecule feature Interacting residue Interaction type** Ι TYR394 (R,S) π-Alkyl Π TYR394 (R,S) π -Lone Pair ALA190 (R,S) π-Alkyl Ш TYR115 (R,S) Hydrogen Bond IV PHE193 (R,S) Hydrogen Bond

	TYR194 (R)	Hydrogen Bond
V	HIS387 (R,S)	π-Alkyl
Furthermor	e, enantiomers of compoun	d 4c were separated as pure enantiomers E1 and
E2 by chiral HP	LC using polysaccharide	based chiral column known as Chiralpak IA
(Supporting inform	nation: Chiral separation se	ection). After separation, both enantiomers were
evaluated for their	in vitro antagonist activity	v at H3R. We found that E1 and E2 have almost
similar activity at I	H3R with IC_{50} 0.008 and 0.0	003 μ M, slightly more than racemate (4c) IC ₅₀ (Fig
S2). These data sug	ggest that IC ₅₀ of enantiome	ers (E1 and E2) and racemate (4c) have almost the
same in vitro activ	ity. Therefore, we did not pe	erform further studies with the pure enantiomers of

4c (Fig S2).

Pharmacokinetic profile of compound 4c. In mice, levels of compound 4c and its metabolite in brain and blood serum were determined using LC-MS/MS. Following a 20 mg/kg oral dose, 4c showed a peak serum concentration (C_{max} , 3356.7 ± 343.8 ng/mL) at 0.25 h indicating rapid

absorption (Fig 6). Its demethylated metabolite (4x) showed C_{max} (3333.3 ± 249.4 ng/mL) at 0.5





Figure 6. Mean serum concentration-time profile of **4c** and its metabolite after single 20 mg/kg oral dose in mice. Bar represents SEM (n=4)

The concentration-time profile was analyzed using both a non-compartmental and compartmental approach. The pharmacokinetic models were compared according to the maximal correlation between the observed and predicted concentrations, the minimal sum of squared residuals, Akaike's Information Criterion and Schwarz Bayesian Criterion.^{69, 70} The concentration-time profile was best described by a two-compartmental open model and the pharmacokinetic parameters are shown in **Table 4**. The volume of distribution (28.5 L/kg) was larger than the total blood volume of mice (0.085 L/kg)⁷¹ and systemic clearance (10.3 L/h/kg) was higher than the total hepatic blood flow in mice (5.4 L/h/kg)⁷¹ indicating extra-vascular distribution along with the extra hepatic elimination of the compound. Following oral administration of test compound (**4c**; 20 mg/kg), both **4c** and its metabolite crossed the blood

brain barrier but the availability was lower than that in serum (Fig 6). Compound 4c showed a peak brain concentration (C_{max} , 322.8 ± 80.5 ng/mL) at 0.25 h, while concentration of metabolite (C_{max} , 101.4 ± 31.7ng/mL) was lower than the parent compound (Fig 6 and Table 4). Furthermore, plasma protein binding (PPB) and unbound fraction in brain (F_u) of compound 4c was estimated in plasma and brain tissue homogenate by an equilibrium dialysis method. We found high PPB in plasma (95.76±0.94%) of compound 4c, whereas F_u was 28.41±3.51% of 4c in mouse brain tissue. Thus, freely available 4c to antagonize H3R in brain (unbound fraction, F_u) at C_{max} would be around 90 ng/ml (equivalent to 334.7 nM, 8.3 times of IC₅₀ of 4c), and level of 4c at 4h in brain would be 5.6 ng/ml (equivalent to 20.8 nM). Thus, we believe that this brain concentration of 4c might be enough to antagonize the H3R in brain and produce CNS specific beneficial effects mediated by H3R.

Parameters	4c (20mg/kg, PO)		Metabolite (4x)	
	Serum	Brain	Serum	Brain
C _{max} (ng/mL)	3356.7 ± 343.8	322.8 ± 80.5	3333.3 ± 249.4	101.4 ± 31.7
t _{max} (h)	0.25	0.5	0.25	0.5
AUC(ng h/mL)	1919.3	310.4	1646.9	100.7
T _{1/2} (h)	1.9	1.1	2.1	1.2
Cl/F(L/h/kg)	10.3	-	-	-
V _d /F (L/kg)	28.5	28.3	-	-
$AUC_{M}/AUC_{P} (\%)$	85.8		32.4	
$AUC_{B}/AUC_{S}(\%)$	16.2		6.1	

Table 4. Pharmacokinetic pa	arameters of 4c and its n	netabolite (4x) i	in C57BL/6J mice
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Each value represents the average of five mice. Concentrations are mean \pm SEM from five mice.

Abbreviations: AUC = area under the serum concentration-time curve up to last observation, AUC_M/AUC_P= metabolite to 4c AUC ratio, AUC_B/AUC_S= brain to serum AUC ratio, Cl/F = clearance, C_{max} = peak concentration, t_{max} = time to C_{max} , V_d/F = volume of distribution, $T_{1/2}$ = elimination half-life.

Acute treatment with 4c reduced food intake via H3R in obese mice. Considering the encouraging pharmacokinetic profile, the selectivity profile and the IC₅₀ of 4c at H3R, we investigated the acute anorexic (suppression of food intake) effect of 4c in a High Fat diet (HFD) and the db/db mouse model of obesity. We observed that oral administration of 4c and lorcaserin significantly reduced cumulative food intake during the four hour night phase (9:00 pm to 1:00 am) (Fig 7). Compound 4c at 20 mg/kg oral dose reduced food intake by 28% compared to vehicle treated HFD fed mice $(0.9693 \pm 0.04339 \text{ g}, \text{N}=15 \text{ vs } 0.6967 \pm 0.03972 \text{ g}, \text{N}=12)$, while an oral dose of 10 mg/kg reduced food intake by approximately 26% in comparison to the vehicle group (0.9693 \pm 0.04339 g, N=15 to 0.7150 \pm 0.07869 g, N=6 (Fig 7 A, **p<0.01, ***p<0.001, One Way ANOVA). We used lorcaserin (10 mg/kg) as a standard anti-obesity drug for comparison, which showed robust anorexic effect (40% decrease) better than 4c treated $(0.9693 \pm 0.04339 \text{ g N}=15 \text{ to } 0.5830 \pm 0.06178 \text{ g N}=11)$ (Fig 7 A, *** p<0.001, One Way ANOVA). These data suggest that temporary blockade of H3R by 4c in brain might lead to increase histamine secretion like the previously reported antagonist and concomitant activation of various downstream effectors that induced anorexia.7, 11, 72-74



Figure 7. Compound 4c produced an anorexic effect in Diet-induced and db/db obese mice, via H3R inhibition: Representative bar graph shown 4h cumulative food intake 30 minutes after oral administration, of test compound 4c (20 mg/kg and 10 mg/kg body weight) or LRC (lorcaserin,-reference drug, 10 mg/kg body weight) or vehicle in diet-induced obese C57BL/6J mice (A), *db/db* obese mice (B), lean C57BL/6J mice treated with compound 4c (20 mg/kg, PO) or vehicle (C) and pre-treatment with Imitet (H3R selective agonist, 5mg/kg, *i.p.* 30 min before 4c) or HDC inhibitor (α -MDLH, 20 mg/kg, *i.p.* 2 h before 4c) significantly blocked the anorexic effect (i.e. reduced food intake) of 4c (20 mg/kg, oral) in *db/db* mice (D). **p<0.01, ***p<0.001 denote significant decrease in food intake after the treatment with 4c and #p<0.05 and ###p<0.001 denote significant blocked anorexic effect of 4c (20 mg/kg, *i.p.* 2 h before 4c), p value calculated by One Way ANOVA followed by Newman-Keuls multiple comparison test.

We next investigated the anorexic effect of 4c in a genetically obese db/db mice model to explore if the mode of satiety is a leptin dependent or a leptin independent mechanism. The db/db obese mice lack the leptin system due to a point mutation in lpr gene.⁷⁵ Oral administration of compound 4c (20 mg/kg) in 12 hour fasted db/db mice significantly reduced cumulative food intake (33%) in comparison to vehicle treated mice (1.956 \pm 0.1431g, N=10 vs 1.315 ± 0.1183 g, N=13), while we did not find any change at a dose of 10 mg/kg dose (Figure **7B**, ** p<0.01, n=8-12, One Way ANOVA followed by Newman-Keuls multiple comparison test). The anorexic effect of 4c in *db/db* mice suggests that H3R antagonists exhibit anorexia in a leptin independent manner. Next, we examined the anorexic activity of compound 4c in db/dbmice is H3R dependent or not. Pre-treatment with an H3R selective agonist (Imitet, 5mg/kg, *i.p.* 30 min before 4c) or the irreversible inhibitor of histidine decarboxylase (histamine synthesizing enzyme) α -Methyl-DL-histidine dihydrochloride (α -MDLH, 20 mg/kg, *i.p.* 2 h before 4c) significantly reversed the anorexic effect of compound 4c (20 mg/kg, oral) and alone treatment with imitet and α -MDLH did not exhibits any effects (Fig 7D, #p<0.05 and ###p<0.001, n=4-7, One Way ANOVA followed by Newman-Keuls multiple comparison test). These results suggest that compound **4c** probably increased the brain histamine level via inhibition of H3R and this increased histamine level produced the anorexic effect observed in the obese db/db mice. We also determined the food intake in normal healthy lean mice after 12 hours of food deprivation. Healthy lean mice acutely treated with vehicle or 4c at 20 mg/kg dose, did not display any effect on food intake (Fig 7C), suggesting no adverse effects in normal healthy mice after treatment with 4c.

Acute administration of 4c induce c-FOS expression in hypothalamic nuclei of db/db mice. Since 4c enters in to the brain where H3Rs are expressed and induces the suppression of food intake, we sought to determine the activation of c-FOS (a surrogate marker of neuronal activation) in the hypothalamus. We observed that administration of 4c (20 mg/kg; *i.p.*) significantly increased c-Fos expression compared to vehicle treated db/db mice in the paraventricular region of the hypothalamus (PVN), which is considered a satiety centre (Fig 8). This result is similar to results reported for other H3R antagonists.⁷⁴



Figure 8. H3R antagonist 4c activates c-FOS in the PVN. Representative immuno-stained fluorescent image showing c-Fos induction in the para-ventricular nucleus (PVN) of hypothalamus 90 minutes after intraperitoneal administration of the test compound 4c (20 mg/kg) or vehicle in db/db mice. After treatment with 4c, the number of c-Fos positive neurons increased in the PVN as compare to vehicle treated mice (Scale bar 50µm)

Compound 4c ameliorates body weight gain in HFD fed mice. Several previous reports

clearly suggest that treatment with H3R antagonist shows anti-obesity effect by reducing body weight in rodent model of obesity.^{7, 9, 10} **Figure 9A** shows the percent body change of normal chow fed mice and high-fat diet fed mice treated with vehicle or **4c** or lorcaserin in a time dependent manner. Chronic oral administration of **4c** at a dose of 20 mg/kg resulted in a significant reduction in percent body weight gain evident from day 20, compared to vehicle treated HFD fed mice (N=5-6, *p<0.05, **p<0.01 and ***p<0.001; two-way ANOVA). The positive control, the known anti-obesity drug, Lorcaserin, also shows significant reduction in body weight gain at day 20 (N=5-6, *p<0.05, **p<0.01 and ***p<0.001; two-way ANOVA). This anti-obesity effect of test compound **4c** consistently increased during the course of treatment and reached an effect equal to that of lorcaserin by day 27. Although **4c** at dose 10 mg/kg also modestly reduced the percent body weight gain, this effect was not statistically significant in comparison to vehicle treated HFD mice.



Figure 9. H3R antagonist 4c act as anti-obesity agent in diet-induced obese mice: Line graph showing % change in body weight relative to the treatment days **(A)** and bar graph showing net body weight change after six weeks of treatment (B) with 4c (10 mg/kg and 20 mg/kg body weight), or lorcaserin (10 mg/ kg body weight), or vehicle. #p<0.05, ##p<0.01 and ###p<0.001;

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n=5-6 [#] Denotes significant body weight increase of HFD mice compared to chow mice and *p<0.05, **p<0.01 and ***p<0.001; n=5-6, * denote significant body weight decrease treated with 4c or lorcaserin mice compare to HFD vehicle mice. p values were determined by two-way ANOVA or by one-way ANOVA followed by Newman-Keuls Multiple Comparison Test.

Thus, six weeks of chronic administration of compounds 4c significantly reduced the net body weight gain compared to vehicle treated HFD fed mice (3.86 ± 0.99) vs 0.80 ± 0.70 g, Fig. **9B**, *p<0.05; one way ANOVA), while **4c** at a dose of 10 mg/kg did not show significant body weight reduction (3.86 ± 0.99) vs 2.6 ± 0.06). As expected, the positive control drug lorcaserin significantly reduced the body weight gain (3.867 ± 0.99) vs 0.95 ± 0.34 ; Fig 10B, *p<0.05; one way ANOVA). Furthermore, we also investigated the chronic effect of 4c in healthy lean C57BL/6J mice. We found that chronic administration of 4c did not show any significant effect on body weight in normal lean mice (Fig S4). We next analyzed the body composition of mice after the six weeks of treatment with 4c by EchoMRI live scanning of mice. EchoMRI data suggested that high fat diet fed mice have significantly higher amount (63.2%) of fat mass compared to normal chow fed mice (13.8%) and did not have any significant difference in lean mass weight (Fig 10). Chronic administration of compounds 4c at 20 mg/kg dose significantly reduced the body fat mass (27.1%) compared to HFD mice treated with vehicle (Fig 10 A and C, N=5-6; p<0.05, one way ANOVA). This effect was similar to the anti-obesity drug lorcaserin, which reduced the fat mass approximately 22.4% compared to HFD mice (Fig 10C, N=5-6; *p < 0.05, one way ANOVA). However, compound **4c** at dose 10 mg/kg showed a very minimal effect on fat mass. Compound 4c treatment did not alter lean mass of HFD mice compared to vehicle treated mice (Fig 10B). We did not find any change in blood glucose and lipid

parameters (**Fig S5** and **S6A**) after the chronic treatment with **4c** (dosage) in HFD fed mice. These observations confirm that **4c** abrogated body weight gain via suppressing food intake and consequently reducing body fat mass in obese mice.



Figure 10. H3R antagonist 4c treatment reduced body fat composition in diet-induced obese mice: Representative bar graph shows body fat mass (**A**), lean mass (**B**) and % body fat mass (**C**) of normal chow fed mice and HFD fed mice treated with 4c (10 mg kg and 20 mg/kg body weight) or lorcaserin (10 mg/kg body weight) or vehicle. ###p<0.001 (n=5-6, chow vs HFD), *p<0.05 (n=5-6) denote significant decrease of body fat after treatment with 4c or lorcaserin compared to vehicle treated HFD fed mice. p values were determined by one-way ANOVA followed by Newman-Keuls Multiple Comparison Test.

Compound 4c reduces the body weight and blood glucose in *db/db* **mice.** Since previous studies reported that concentration of hypothalamic histamine is significantly lower in leptin signaling disrupted rodents such as *ob/ob*, *db/db* and Zucker fatty (*fa/fa*) rats ⁷⁶ and chronic i.c.v. infusion of histamine decreases food intake and body weight in leptin signaling disrupted obese mice⁷³, we also evaluated the effect of chronic treatment with **4c** (20 mg/kg body weight, oral)

on body weight loss in db/db mice. As expected, we observed that four weeks of chronic oral administration of 4c significantly reduced the body weight of db/db mice compared to vehicle treated db/db mice (Fig 11A, *p<0.05; N=4, t-test). Food intake and chronic studies data suggested that compounds 4c shows anti-obesity effect in db/db mice via leptin independent signalling (Fig 7 and 11). These results suggest that 4c shows anti-obesity effect in db/db mice by increasing histaminergic signaling in satiety neurons.

Curiously, we also measured the fasting blood glucose levels and glucose tolerance which is highly impaired in *db/db* mice, and found that chronic administration of **4c** significantly reduced fasting glucose (**Fig S7**, N= 4-5; *p<0.05; t-test) and improve glucose homeostasis, observed in an *intra peritoneal* glucose tolerance test (ipGTT; **Fig11 B** and **C**, N= 4-5; *p<0.05 one way ANOVA and t-test). However, we did not found any change in blood lipid parameters after the chronic treatment with **4c** in *db/db* mice (**Fig S6B**).



Figure 11. H3R antagonist 4c acts as anti-obesity agent and improves glucose homeostasis in *db/db* **obese mice: (A)** Net body weight change after four weeks treatment with **4c** (20mg/kg) in *db/db* mice, **(B)** Line graph shows glucose level relative to time after glucose administration (1 g/kg body weight) in 16 hour fasted *db/db* mice and **(C)** Change in blood glucose AUC analysis of ipGTT results. *p<0.05 (n=4), p value were determined by two-way ANOVA or student's t test.

Conclusion

A small series of tetrahydroquinazolinamine derivatives containing the versatile 2aminopyrimidine scaffold was synthesized and tested for agonist and antagonist activity at human H3R. Three compounds 4c, 4k and 4o of this series showed the reasonable H3R antagonist activity with almost similar IC₅₀ 0.04 μ M, 0.09 μ M and 0.07 μ M, respectively. Another compound 4w also showed moderate antagonist activity H3R with IC_{50} 0.24 μ M. The SAR studies revealed that the 4-methoxy group in the phenyl ring attached to the tetrahydroquinazolinamine is responsible for the higher binding affinity of 4c to the H3R. Selectivity profile of 4c revealed that it has no activity at several families of GPCRs (Dopamine receptor, Serotonin receptors, and opioid receptors) as well as hERG ion channels. Furthermore, docking studies and *in vitro* activity suggests both enantiomers of 4c have almost similar antagonist activity like the racemate (4c) at H3R. Pharmacokinetic analysis 4c showed good plasma level (C_{max} =3356.7 ng/mL) and have sufficient brain availability (concentration 322.8 ng/mL $\sim 1.2 \,\mu$ M). Follow-up comprehensive *in vivo* studies in two rodent models of obesity revealed that 4c is highly efficacious, and induces c-Fos in ventral hypothalamus, suggesting target engagement. Notably, 4c did not induce weight loss in lean mice. These results support the essential role of H3R in obesity with novel tetrahydroquinazolinamine derivative 4c being a good starting point for further optimization.

Experimental Section

General Methods. Commercially available reagent grade chemicals and solvents were used as received. All reactions were monitored by TLC on E.Merck Kieselgel 60 F_{254} , with detection by UV light, spraying Dragendorff's reagent or exposure to I₂ vapours. Column chromatography was performed on 100-200 mesh E. Merck silica gel using hexane/EtOAc as mobile phase. IR spectra were recorded as thin films or on KBr pellets with a PerkinElmer Spectrum RX-1 (4000-450 cm⁻¹) spectrophotometer. The ¹H NMR (400 MHz) and ¹³C NMR (100, 75 and 50 MHz) spectra were recorded on a Bruker Avance-400, Bruker DRX-300 and Bruker DRX-200 in CDCl₃ or DMSO-*d*₆. Chemical shift values are reported in ppm relative to TMS (tetramethylsilane) as an internal reference or residual solvent peak. Unless stated otherwise; s (singlet), d (doublet), t (triplet), dd (doublet of doublet), bs (broad singlet), m (multiplet); *J* in hertz. The ESI-MS were recorded on ION TRAP LCQ ADVANTAGE MAX mass spectrometer of Thermo Electron Corporation, and HRMS spectra were performed using a mass spectrometer Q-TOF (Agilent 6520). The purities of all compounds were \geq 95% as determined by HPLC.

General experimental procedure for the compounds (3a-3z, 3a'). To the mixture of 2methylcyclohexanone 1 (1 mmol) and respective aromatic aldehyde (2a-2w, 1.2 mmol) in ethanol (20 mL), pellets of KOH (1.2 mmol) were added. The reaction mixture was stirred for 3-4 hours at an ambient temperature, followed by neutralization with 3NHCl. The precipitate thus obtained was filtered and re-crystallized from ethanol to give the desired arylidenylcyclohexanones **3a-3w**, identical in all respects to those reported earlier.⁴⁸

General procedure for the synthesis of target compounds (4a-4w, 4a'-4d'). Slurry of

1.1mmol of NaH was prepared in 1 mL of DMF at 0 °C, and then 1.1mmol of guanidine hydrochloride (dissolved in the minimum amount of DMF) was added drop wise. After a while, 1 mmol of respective 2-arylidene-6-methylcyclohexanones (3a-3w) dissolved in DMF, was added drop wise into the stirring reaction mixture. Stirring was continued at 0 °C for 20 minutes and then it was heated up to 90 °C for 24 hours. After the reaction was complete (on TLC), the reaction mixture was diluted with ethyl acetate and washed 3 times with water. Organic layer was collected and dried in vacuum. The viscous crude mass thus obtained was purified by column chromatography using ethyl acetate/hexane as eluent in the 1:3 ratio to give the titled compounds 4 as detailed below.

8-methyl-4-phenyl-5,6,7,8-tetrahydroquinazolin-2-amine (4a). It was obtained by the reaction of 3a (0.200 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 25% yield (0.059 g) as white solid ; mp 124-126 °C; IR (neat) v_{max} cm⁻¹: 3409, 1601, 1069, 669; ¹H NMR (CDCl₃, 400 MHz) δ: 7.47-7.38 (m, 5H , Ar), 5.05 (brs, 2H, NH₂), 2.83-2.78 (m, 1H), 2.57-2.47 (m, 2H), 2.02-1.96 (m, 1H), 1.78-1.71 (m, 1H), 1.59-1.56 (m, 2H), 1.36 (d, *J*=7.14 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ: 171.5, 166.7, 161.0, 139.0, 128.7, 128.5 (2C), 128.3 (2C), 117.1, 35.9, 30.9, 26.8, 21.1, 20.7; ESI-HRMS calcd for C₁₅H₁₇N₃ [M + H]⁺240.1495 found 240.1443. HPLC purity: ≥95%.

4-(4-bromophenyl)-8-methyl-5,6,7,8-tetrahydroquinazolin-2-amine (4b). It was obtained by the reaction of 3b(0.278 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 21% yield (0.066 g) as white solid ;mp144-146 °C; IR (neat) v_{max} cm⁻¹: 3399, 1638, 928, 626; ¹H NMR (CDCl₃, 400 MHz) δ : 7.57 (d, J = 8.45 Hz,

2H, Ar), 7.37 (d, J=8.45 Hz, 2H, Ar), 4.89 (br s, 2H, NH₂), 2.85-2.78 (m, 1H), 2.58-2.46 (m, 2H), 2.05-1.97 (m, 1H), 1.80-1.72 (m, 1H), 1.63-1.56 (m, 2H), 1.36 (d, J=7.13 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ : 171.9, 165.3, 161.0, 137.8, 131.5 (2C), 130.3 (2C), 123.1, 117.0, 35.9, 30.9, 26.8, 21.1, 20.7; ESI-HRMS calcd for C₁₅H₁₆BrN₃ [M + H]⁺ 318.06 found 318.0531. HPLC purity: \geq 95%.

4-(4-methoxyphenyl)-8-methyl-5,6,7,8-tetrahydroquinazolin-2-amine(4c). It was obtained by the reaction of **3c** (0.230 g, 1.0 mmol), NaH (0.026 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 21% yield (0.063 g) as white solid ; mp 104-106 $^{\circ}$ C;IR (neat) v_{max} cm⁻¹: 3411, 1599, 1069, 669; ¹H NMR (CDCl₃, 400 MHz) δ : 7.47 (d, *J* = 8.74 Hz, 2H), 6.96 (d, *J* = 8.74 Hz, 2H), 4.88 (brs, 2H, NH₂), 3.84 (s, 3H), 2.85-2.78 (m, 1H), 2.62-2.51 (m, 2H), 2.03-1.97 (m, 1H), 1.79-1.78 (m, 1H), 1.64-1.54 (m, 2H), 1.36 (d, J = 7.17 Hz, 3H) ; ¹³C NMR (CDCl₃, 100 MHz) δ : 171.3, 166.2, 161.0, 160.1, 131.3, 130.1 (2C), 117.0, 113.6 (2C), 55.4, 35.9, 31.0, 27.1, 21.2, 20.9; ESI-HRMS calcd for C₁₆H₁₉N₃O[M + H]⁺ 270.1601 found 270.1619. HPLC purity: ≥95%.

8-methyl-4-(p-tolyl)-5,6,7,8-tetrahydroquinazolin-2-amine (4d). It was obtained by the reaction of 3d(0.214 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 26% yield (0.065 g) as white solid ;mp 146-148 °C; IR (neat) v_{max} cm⁻¹: 3409, 1625, 1069, 669; ¹H NMR (CDCl₃, 400 MHz) δ: 7.38 (d, *J* = 7.96 Hz, 2H, Ar), 7.24 (d, *J*=7.85 Hz, 2H, Ar), 5.05 (brs, 2H, NH₂), 2.84-2.79 (m, 1H), 2.55-2.52 (m, 2H), 2.38 (s, 3H, CH₃), 2.02-1.96 (m, 1H), 1.78-1.68 (m, 1H), 1.59-1.53 (m, 2H), 1.35 (d, *J*=7.13 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ: 171.3, 166.8, 160.9, 138.7, 136.0, 128.9 (2C), 128.5 (2C),

117.2, 35.8, 30.9, 26.9, 21.4, 21.2, 20.7; ESI-HRMS calcd for $C_{16}H_{19}N_3$ [M + H]⁺ 254.1652 found 254.1660. HPLC purity: \geq 95%.

4-(4-chlorophenyl)-8-methyl-5,6,7,8-tetrahydroquinazolin-2-amine (4e). It was obtained by the reaction of **3e** (0.234 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 24% yield (0.065 g) as white solid ; mp 136-138 °C; IR (neat) v_{max} cm⁻¹: 3417, 1598, 1090, 669; ¹H NMR (CDCl₃, 400 MHz) δ: 7.43-7.38 (m, 4H, Ar), 5.03 (brs, 2H, NH₂), 2.84-2.78 (m, 1H), 2.56-2.46 (m, 2H), 2.02-1.96 (m, 1H), 1.79-1.71 (m, 1H), 1.59-1.57 (m, 2H), 1.35 (d, *J*=7.13 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ: 171.8, 165.3, 161.0, 137.3, 134.9, 130.0 (2C), 128.5 (2C), 117.1, 35.9, 30.9, 26.8, 21.1, 20.7; ESI-HRMS calcd for C₁₅H₁₆ClN₃ [M + H]⁺274.1106 found 274.1090. HPLC purity: ≥95%.

4-(4-fluorophenyl)-8-methyl-5,6,7,8-tetrahydroquinazolin-2-amine (4f). It was obtained by the reaction of **3f** (0.218 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 24% yield (0.061 g) as white solid ; mp 146-148 °C; IR (neat) v_{max} cm⁻¹: 3416, 1601, 1069, 626; ¹H NMR (CDCl₃, 400 MHz) δ : 7.49-7.45 (m, 2H , Ar),7.12-7.08 (m, 2H , Ar),4.98 (brs, 2H, NH₂), 2.83-2.78 (m, 1H), 2.55-2.50 (m, 2H), 2.03-1.96 (m, 1H), 1.81-1.72 (m, 1H), 1.62-1.56 (m, 2H), 1.36 (d, *J*=7.12 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ : 171.7, 165.5, 164.3, 161.0, 135.0 (d,*J*=3.33 Hz),130.6,130.5, 117.0, 115.4, 115.2, 35.9, 31.0, 27.0, 21.1, 20.9; ESI-HRMS calcd for C₁₅H₁₆FN₃ [M + H]⁺258.1401 found 258.1369. HPLC purity: ≥95%.

8-methyl-4-(naphthalen-1-yl)-5,6,7,8-tetrahydroquinazolin-2-amine (4g). It was obtained by the reaction of 3g (0.250 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride

(0.105 g, 1.1 mmol) in anhydrous DMF in 22% yield (0.063 g) as white solid ; mp 136-138 °C; IR (neat) v_{max} cm⁻¹: 3400, 1644, 1069, 669; ¹H NMR (CDCl₃, 400 MHz) δ : 7.89 (d, *J*=7.96, 2H, Ar), 7.53-7.36 (m, 5H, Ar), 4.94 (br s, 2H, NH₂), 2.88-2.87 (m, 1H), 2.23-2.17 (m, 2H), 2.01-1.96 (m, 1H), 1.59-1.56 (m, 3H), 1.41 (s, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ : 171.4, 166.9, 161.0, 136.6, 133.7, 130.5, 128.7, 128.5, 126.6, 126.1, 125.6, 125.4, 125.3, 118.6, 35.9, 30.8, 25.6, 20.9, 20.2; ESI-HRMS calcd for C₁₉H₁₉N₃ [M + H]⁺290.1652 found 290.1652. HPLC purity: ≥95%.

4-(4-(benzyloxy)phenyl)-8-methyl-5,6,7,8-tetrahydroquinazolin-2-amine (**4h**). It was obtained by the reaction of **3h** (0.306 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 23% yield (0.079 g) as white solid ; mp 144-146 °C; IR (neat) v_{max} cm⁻¹: 3410, 1645, 1069, 669; ¹H NMR (CDCl₃, 400 MHz) δ : 7.50-7.45 (m, 4H, Ar), 7.43-7.39 (m, 2H, Ar), 7.37-7.33 (m, 1H, Ar), 7.06-7.03 (m, 2H, Ar), 5.13 (s, 2H, -OCH₂) 4.85 (brs, 2H, NH₂), 2.86-2.80(m, 1H), 2.66-2.55 (m, 2H), 2.05-2.00 (m, 1H), 1.82-1.75 (m, 1H), 1.63-1.59 (m, 2H), 1.38 (d,*J*=7.11 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ : 171.0, 166.4, 160.6, 159.5, 136.9, 131.4, 130.2 (2C), 128.8, 128.7, 128.2, 127.5 (2C), 117.2, 114.7 (2C), 70.2, 35.8, 30.9, 27.1, 21.2, 20.8; ESI-HRMS calcd for C₂₂H₂₃N₃O [M + H]⁺346.1914 found 346.1928. HPLC purity: ≥95%.

4-(4-(dimethylamino)phenyl)-8-methyl-5,6,7,8-tetrahydroquinazolin-2-amine (4i). It was obtained by the reaction of 3i (0.243 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 23% yield (0.064 g) as yellow solid ; mp160-162 °C; IR (neat) v_{max} cm⁻¹: 3412, 1610, 1068, 668; ¹H NMR (CDCl₃, 400 MHz) δ : 7.48

(d, J = 8.84 Hz, 2H, Ar), 6.75 (d, J = 8.84 Hz, 2H, Ar), 5.01 (brs, 2H, NH₂), 2.99 (s, 6H, -N(CH₃)₂), 2.85-2.78 (m, 1H), 2.66-2.62 (m, 2H), 2.04-1.97 (m, 1H), 1.79-1.71 (m, 1H), 1.59-1.57 (m, 2H), 1.36 (d, J = 7.12 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ : 170.6, 166.6, 160.7, 151.0, 130.1 (2C), 126.3, 117.0, 111.6 (2C), 40.5 (2C), 35.8, 31.1, 27.4, 21.3, 21.1; ESI-HRMS calcd for C₁₇H₂₂N₄ [M + H]⁺283.1917 found 283.1920. HPLC purity: \geq 95%.

4-(4-isopropylphenyl)-8-methyl-5,6,7,8-tetrahydroquinazolin-2-amine (4j). It was obtained by the reaction of **3j** (0.242 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 26% yield (0.073 g) as white solid ; mp 140-142 °C; IR (neat) v_{max} cm⁻¹: 3417, 1600, 1055, 626; ¹H NMR (CDCl₃, 400 MHz) δ: 7.42 (d, *J* = 8.21 Hz, 2H, Ar), 7.29 (d, *J*=8.13 Hz, 2H, Ar), 5.04 (brs, 2H, NH₂), 2.99-2.89 (m, 1H), 2.87-2.78 (m, 1H), 2.64-2.51 (m, 2H), 2.03-1.97 (m, 1H), 1.79-1.71 (m, 1H),1.62-1.56 (m,2H), 1.37 (d, *J*=7.12 Hz, 3H, CH₃), 1.27 (d, *J*=6.92 Hz, 6H); ¹³C NMR (CDCl₃, 100 MHz) δ: 171.1, 166.9, 160.7, 149.8, 136.3, 128.5 (2C), 126.4 (2C), 117.2, 35.8, 34.1, 30.9, 26.9, 24.0 (2C), 21.1, 20.8; ESI-HRMS calcd for C₁₈H₂₃N₃ [M + H]⁺282.1965 found 282.1969. HPLC purity: ≥95%.

4-(3-bromophenyl)-8-methyl-5,6,7,8-tetrahydroquinazolin-2-amine (4k). It was obtained by the reaction of **3k** (0.278 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 19% yield (0.060 g) as white solid ; mp 144-146 °C; IR (neat) v_{max} cm⁻¹: 3411, 1602, 1069, 669; ¹H NMR (CDCl₃, 400 MHz) δ: 7.63(t, *J* = 1.70 Hz, 1H, Ar), 7.54-7.52 (m, 1H, Ar), 7.41-7.39 (m, 1H, Ar), 7.29 (t, *J*=7.83 Hz, 1H, Ar), 4.89 (brs, 2H, NH₂), 2.85-2.78 (m, 1H), 2.57-2.46 (m, 2H), 2.04-1.97 (m, 1H), 1.80-1.72 (m, 1H), 1.63-1.56 (m, 2H), 1.36 (d, *J*=7.13 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ: 172.0, 165.0,

160.9, 141.0, 131.8, 131.6, 129.9, 127.1, 122.5, 117.2, 35.9, 30.9, 26.7, 21.0, 20.7; ESI-HRMS calcd for C₁₅H₁₆BrN₃ [M + H]⁺ 318.06 found 318.0557. HPLC purity: ≥95%.

8-methyl-4-(4-(methylthio)phenyl)-5,6,7,8-tetrahydroquinazolin-2-amine (41). It was obtained by the reaction of 31 (0.246 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 21% yield (0.059 g) as white solid ; mp 138-140 °C; IR (neat) v_{max} cm⁻¹: 3415, 1599, 1068, 669; ¹H NMR (CDCl₃, 400 MHz) δ: 7.45 (d, *J*=8.40 Hz, 2H, Ar), 7.31 (d, *J*=8.40 Hz, 2H, Ar), 5.06 (brs, 2H, NH₂), 2.87-2.80 (m, 1H), 2.61-2.54 (m, 2H), 2.51 (s, 3H, -SCH₃), 2.04-1.97 (m, 1H), 1.80-1.72 (m, 1H), 1.64-1.54 (m, 2H), 1.38 (d, *J*=7.13 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ: 171.1, 166.2, 160.5, 140.1, 135.2, 129.1 (2C), 126.0 (2C), 117.2, 35.7, 30.9, 26.9, 21.1, 20.8, 15.7; ESI-HRMS calcd for C₁₆H₁₉N₃S [M + H]⁺286.1372 found 286.1381. HPLC purity: ≥95%.

8-methyl-4-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydroquinazolin-2-amine (4m). It was obtained by the reaction of 3m(0.290 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 19% yield (0.062 g) as white solid ; mp170-172 °C; IR (neat) v_{max} cm⁻¹: 3416, 1600, 1128, 669; ¹H NMR (CDCl₃, 400 MHz) δ : 6.68 (s, 2H, Ar), 4.87 (brs, 2H, NH₂), 3.88-3.86 (m, 9H, 3X-OCH₃) 2.84-2.79 (m, 1H), 2.56-2.55 (m, 2H), 2.04-1.98 (m, 1H), 1.80-1.74 (m, 1H), 1.60-1.59 (m, 2H), 1.37 (d, *J*=7.05 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ : 171.7, 166.6, 160.8, 153.2 (2C), 138.6, 134.4, 117.2, 105.8 (2C), 61.0, 56.3 (2C), 36.0, 30.9, 26.9, 21.1, 20.8; ESI-HRMS calcd for C₁₈H₂₃N₃O₃ [M + H]⁺330.1812 found 330.1802. HPLC purity: \geq 95%.

4-(3,4-dimethoxyphenyl)-8-methyl-5,6,7,8-tetrahydroquinazolin-2-amine (**4n**). It was obtained by the reaction of **3n** (0.260 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 23% yield (0.068 g) as white solid ; mp162-164 °C; IR (neat) v_{max} cm⁻¹: 3399, 1603, 1026, 669; ¹H NMR (CDCl₃, 400 MHz) δ: 7.08-7.06 (m, 2H, Ar), 6.92 (d, *J*=8.81 Hz, 1H, Ar), 4.86 (brs, 2H, NH₂), 3.92-3.91 (m, 6H, 2X-OCH₃), 2.84-2.79 (m, 1H), 2.63-2.52 (m, 2H), 2.04-1.98 (m, 1H), 1.81-1.73 (m, 1H), 1.62-1.56 (m, 2H), 1.37 (d, *J*=7.12 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ: 171.4, 166.2, 160.9, 149.6, 148.8, 131.6, 121.5, 117.1, 112.0, 110.7,56.0 (2C),35.9, 31.0, 27.1, 21.1, 20.9; ESI-HRMS calcd for C₁₇H₂₁N₃O₂ [M + H]⁺300.1707 found 300.1706. HPLC purity: ≥95%.

4-(3-methoxyphenyl)-8-methyl-5,6,7,8-tetrahydroquinazolin-2-amine (40). It was obtained by the reaction of **30** (0.230 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 19% yield (0.051 g) as white solid ; mp 142-144 °C; IR (neat) v_{max} cm⁻¹: 3417, 1601, 1068, 669; ¹H NMR (CDCl₃, 400 MHz) δ : 7.33 (t, *J* =7.85 Hz, 1H, Ar), 7.04 (d, *J*=7.64 Hz, 1H, Ar), 7.00-6.99 (m, 1H, Ar), 6.95-6.93 (m, 1H, Ar), 4.90 (brs, 2H, NH₂), 3.83 (s, 3H, -OCH₃), 2.84-2.79 (m, 1H), 2.55-2.51 (m, 2H), 2.04-1.97 (m, 1H), 1.79-1.73 (m, 1H), 1.62-1.56 (m, 2H), 1.37 (d, *J*=7.11 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ : 171.5, 166.5, 160.9, 159.5, 140.3, 129.3, 120.8, 117.0, 114.6, 113.8,55.4, 35.9, 30.9, 26.7, 21.1, 20.7; ESI-HRMS calcd for C₁₆H₁₉N₃O [M + H]⁺270.1601 found 270.1596. HPLC purity: ≥95%.

8-methyl-4-(4-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinazolin-2-amine (4p). It was obtained by the reaction of 3p(0.268 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 21% yield (0.064 g) as white solid ;

mp132-134 °C; IR (neat) v_{max} cm⁻¹: 3400, 1602, 1066, 669; ¹H NMR (CDCl₃, 400 MHz) δ: 7.70 (d, *J* = 8.01 Hz, 2H, Ar), 7.60 (d, *J*=8.01 Hz, 2H, Ar), 4.94 (brs, 2H, NH₂), 2.87-2.80 (m, 1H), 2.56-2.46 (m, 2H), 2.04-1.98 (m, 1H), 1.81-1.75 (m, 1H), 1.63-1.55 (m, 2H), 1.37 (d, *J*=7.17 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ: 172.1, 165.1, 161.0, 142.5, 131.32 (q, *J*=33.28), 128.9 (2C), 125.35,125.32, 122.8, 117.1, 35.9, 30.8, 26.6, 21.0, 20.7; ESI-HRMS calcd for C₁₆H₁₆F₃N₃ [M + H]⁺308.1369 found 308.1368. HPLC purity: ≥95%.

8-methyl-4-(pyridin-3-yl)-5,6,7,8-tetrahydroquinazolin-2-amine (4q). It was obtained by the reaction of 3q (0.201 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 24% yield (0.057 g) as white solid ; mp152-154 °C; IR (neat) v_{max} cm⁻¹: 3416, 1600, 1025, 669;; ¹H NMR (CDCl₃, 400 MHz) δ : 8.76 (d, *J* =1.54 Hz, 1H, Ar), 8.64 (dd, *J*=4.74, 1.54 Hz, 1H, Ar), 7.85-7.82 (m, 1H, Ar), 7.39-7.36 (m, 1H, Ar), 4.93 (brs, 2H, NH₂), 2.87-2.79 (m, 1H), 2.63-2.50 (m, 2H), 2.05-1.98 (m, 1H), 1.83-1.77 (m, 1H), 1.65-1.58 (m, 2H), 1.37 (d, *J*=7.16 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ : 172.1, 163.4, 161.1, 149.8, 149.5, 136.1, 134.7, 123.2, 117.4, 35.9, 30.8, 26.7, 21.0, 20.7; ESI-HRMS calcd for C₁₄H₁₆N₄ [M + H]⁺241.1448 found 241.1444. HPLC purity: ≥95%.

8-methyl-4-(4-(trifluoromethoxy)phenyl)-5,6,7,8-tetrahydroquinazolin-2-amine (4r). It was obtained by the reaction of **3r**(0.284 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 21% yield (0.067 g) as white solid ; mp108-110 °C; IR (neat) v_{max} cm⁻¹: 3417, 1600, 1020, 626; ¹H NMR (CDCl₃, 400 MHz) δ : 7.54 (d, J = 8.77 Hz, 2H, Ar), 7.29 (d, J=8.00 Hz, 2H, Ar), 4.92 (brs, 2H, NH₂), 2.86-2.78 (m, 1H), 2.60-2.48(m, 2H), 2.04-1.98 (m, 1H), 1.82-1.76 (m, 1H), 1.66-1.55 (m, 2H), 1.37 (d, J=7.12 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ : 171.9, 165.2, 161.0, 149.6, 137.6, 131.5, 130.2 (2C),

120.8 (2C), 117.1, 35.9, 30.9, 26.8, 21.1, 20.7; ESI-HRMS calcd for $C_{16}H_{16}F_3N_3O [M + H]^+$ 324.1318 found 324.1317. HPLC purity: \geq 95%.

4-(2-amino-8-methyl-5,6,7,8-tetrahydroquinazolin-4-yl)benzonitrile (4s). It was obtained by the reaction of **3s** (0.225 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 22% yield (0.058 g) as pale yellow solid ; mp184-186 °C; IR (neat) v_{max} cm⁻¹: 3417, 1602, 1069, 669; ¹H NMR (CDCl₃, 400 MHz) δ : 7.74 (d, *J* = 8.40 Hz, 2H, Ar), 7.61 (d, *J*=8.40 Hz, 2H, Ar), 4.93 (brs, 2H, NH₂), 2.87-2.79 (m, 1H), 2.56-2.43 (m, 2H), 2.04-1.98 (m, 1H), 1.82-1.74 (m, 1H), 1.65-1.57 (m, 2H), 1.37 (d, *J*=7.12 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ : 172.4, 164.4, 161.0, 143.4, 132.1 (2C), 129.4 (2C), 118.6, 117.0,112.5, 35.9, 30.7, 26.6, 21.0, 20.6; ESI-HRMS calcd for C₁₆H₁₆N₄ [M + H]⁺265.1448 found 265.1440. HPLC purity: ≥95%.

4-(3-(benzyloxy)phenyl)-8-methyl-5,6,7,8-tetrahydroquinazolin-2-amine (4t). It was obtained by the reaction of **3t** (0.306 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 19% yield (0.065 g) as white solid ; mp 142-144 °C; IR (neat) v_{max} cm⁻¹: 3414, 1599, 1026, 671; ¹H NMR (CDCl₃, 400 MHz) δ : 7.44-7.32 (m, 6H, Ar), 7.07-7.04 (m, 2H, Ar), 7.03-7.00 (m, 1H, Ar), 4.89 (brs, 2H, NH₂), 2.85-2.76 (m, 1H), 2.49-2.45 (m, 2H), 2.02-1.95 (m, 1H), 1.77-1.73 (m, 1H), 1.59-1.54 (m, 2H), 1.35 (d, *J*=7.11 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ : 171.5, 166.4, 160.9, 158.6, 140.3, 137.0, 129.4, 128.7 (2C), 128.0, 127.5 (2C), 121.1, 117.1, 115.5, 114.9, 70.1, 35.9, 30.9, 26.7, 21.1, 20.7; ESI-HRMS calcd for C₂₂H₂₃N₃O [M + H]⁺ 346.1914 found 346.1914. HPLC purity: ≥95%.

8-methyl-4-(4-nitrophenyl)-5,6,7,8-tetrahydroquinazolin-2-amine (4u). It was obtained by the reaction of **3u**(0.245 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 19% yield (0.053 g) as pale yellow solid ; mp162-164 °C; IR (neat) v_{max} cm⁻¹: 3417, 1600, 1023, 671; ¹H NMR (CDCl₃, 400 MHz) δ : 8.30 (d, J = 8.72 Hz, 2H, Ar), 7.67 (d, J=8.72 Hz, 2H, Ar), 4.94 (brs, 2H, NH₂), 2.85-2.80 (m, 1H), 2.56-2.45 (m, 2H), 2.03-2.00 (m, 1H), 1.83-1.74 (m, 1H), 1.64-1.58 (m, 2H), 1.37 (d, J=7.13 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ : 172.5, 164.1, 161.0, 148.0, 145.3, 129.7 (2C), 123.6 (2C), 117.2, 36.0, 30.8, 26.7, 21.0, 20.7; ESI-HRMS calcd for C₁₅H₁₆N₄O₂[M + H]⁺285.1346 found 285.1348. HPLC purity: \geq 95%.

4-(4-ethoxyphenyl)-8-methyl-5,6,7,8-tetrahydroquinazolin-2-amine (4v). It was obtained by the reaction of **3v** (0.244 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 24% yield (0.067 g) as white solid ; mp152-154 °C; IR (neat) v_{max} cm⁻¹: 3409, 1608, 1068, 669; ¹H NMR (CDCl₃, 400 MHz) δ : 7.46 (d, *J* = 8.74 Hz, 2H, Ar), 6.94 (d, *J*=8.77 Hz, 2H, Ar), 4.88 (brs, 2H, NH₂), 4.08 (q, *J*=7.00 Hz, 2H), 2.84-2.76 (m, 1H), 2.64-2.51 (m, 2H), 2.03-1.97 (m, 1H), 1.77-1.73 (m, 1H), 1.63-1.53 (m, 2H), 1.43 (t, *J*=7.00 Hz, 3H,)1.36 (d, *J*=7.12 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ : 171.3, 166.2, 161.0, 159.5, 131.2, 130.1 (2C), 117.0, 114.2 (2C), 63.6,35.9, 31.0, 27.1, 21.2, 20.9, 14.9; ESI-HRMS calcd for C₁₇H₂₁N₃O[M + H]⁺284.1757 found 284.1748. HPLC purity: ≥95%.

4-cyclohexyl-8-methyl-5,6,7,8-tetrahydroquinazolin-2-amine (4w). It was obtained by the reaction of **3w** (0.206 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 26% yield (0.063 g) as white solid ; mp104-106 °C; IR (neat) v_{max} cm⁻¹: 3411, 1599, 1069, 669; ¹H NMR (CDCl₃, 400 MHz) δ : 4.69 (brs, 2H, NH₂),

2.76-2.63 (m, 2H), 2.62-2.50 (m, 2H), 1.95-1.81 (m, 4H), 1.69-1.62 (m, 4H), 1.60-1.50 (m, 3H), 1.38-1.32 (m, 3H), 1.29 (d, *J*=7.06 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ : 173.3, 169.7, 161.1, 116.2, 41.0, 35.7, 31.1, 31.0, 30.5, 26.6 (2C), 26.1, 24.3, 20.8, 20.2; ESI-HRMS calcd for C₁₅H₂₃N₃ [M + H]⁺246.1965 found 246.1958. HPLC purity: \geq 95%.

8,8-Dimethyl-4-(4-methoxyphenyl)-5,6,7,8-tetrahydroquinazolin-2-amine (4a'). It was obtained by the reaction of 3x (0.245 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 28% yield (0.079 g) as white solid ; mp 140-142 °C; IR (neat) v_{max} cm⁻¹: 3406, 1605, 1070, 669; ¹H NMR (CDCl₃, 400 MHz) δ: 7.45 (d, *J* = 8.28 Hz, 2H), 6.94 (d, *J* = 8.28 Hz, 2H), 4.82 (brs, 2H, NH₂), 3.84 (s, 3H), 2.58-2.55 (m, 2H), 1.70-1.66 (m, 4H), 1.30 (s, 6H) ; ¹³C NMR (CDCl₃, 100 MHz) δ: 174.4, 166.2, 161.2, 160.0, 131.7, 130.1 (2C), 116.3, 113.6 (2C), 55.4, 38.7, 36.9, 30.0, 27.8, 19.8 (2C); ESI-HRMS calcd for C₁₇H₂₂N₃O [M + H]⁺ 284.1757 found 284.1750. HPLC purity: ≥95%.

4-(4-methoxyphenyl)-)-6-methyl-5,6,7,8-tetrahydroquinazolin-2-amine (4b'). It was obtained by the reaction of **3y** (0.230 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 24% yield (0.064 g) as white solid; mp 184-186 °C; IR (neat) v_{max} cm⁻¹: 3396, 1640, 1050, 626; ¹H NMR (CDCl₃, 400 MHz) δ: 7.46 (d, *J* = 8.80 Hz, 2H, Ar), 6.96 (d, *J*=8.80 Hz, 2H, Ar), 4.94 (br s, 2H, NH₂), 3.84 (s, 3H), 2.82-2.75 (m, 2H), 2.63-2.58 (m, 1H), 2.28-2.22 (m, 1H), 1.94-1.88 (m, 1H), 1.73-167 (m, 1H), 1.53-1.45 (m, 1H), 1.01 (d, *J*=6.56 Hz, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ: 167.2, 166.1, 160.8, 160.2, 131.1 (2C), 130.1, 116.9, 113.7(2C), 55.43, 34.9, 32.3, 30.7, 29.6, 21.7; ESI-HRMS calcd for C₁₆H₂₀N₃O [M + H]⁺ 270.1601 found 270.1593 . HPLC purity: ≥95%.

4-(4-methoxyphenyl)-5,6,7,8-tetrahydroquinazolin-2-amine(4c'). It was obtained by the reaction of **3z** (0.216 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g,

1.1 mmol) in anhydrous DMF in 22% yield (0.056 g) as white solid ; mp 144-146 °C; IR (neat) v_{max} cm⁻¹: 3410, 1601, 1069, 669; ¹H NMR (CDCl₃, 400 MHz) δ : 7.46 (d, *J* = 8.70 Hz, 2H), 6.95 (d, *J* = 8.70 Hz, 2H), 5.04 (brs, 2H, NH₂), 3.84 (s, 3H), 2.75 (t, *J* = 6.48 Hz, 2H), 2.59 (t, *J* = 6.06 Hz, 2H), 1.88-1.82 (m, 2H), 1.71-1.67 (m, 2H); ¹³C NMR (CDCl₃, 100 MHz) δ : 167.27, 166.1, 160.6, 160.1, 131.0, 130.0 (2C), 117.4, 113.6 (2C), 55.3, 32.4, 26.4, 23.3, 22.5; ESI-HRMS calcd for C₁₅H₁₈N₃O[M + H]⁺ 256.1444 found 256.1442. HPLC purity: ≥95%.

4-butyl-6-(4-methoxyphenyl)pyrimidin-2-amine (4d'). It was obtained by the reaction of **3a'** (0.218 g, 1.0 mmol), NaH (0.025 g, 1.1 mmol), guanidine hydrochloride (0.105 g, 1.1 mmol) in anhydrous DMF in 30% yield (0.077 g) as white solid ;mp 128-130 °C; IR (neat) v_{max} cm⁻¹: 3350, 1620, 1066, 669; ¹H NMR (CDCl₃, 400 MHz) δ: 7.96 (d, *J* = 8.92 Hz, 2H, Ar), 7.97 (d, *J*=8.92 Hz, 2H, Ar), 6.86 (s, 1H), 5.04 (brs, 2H, NH₂), 3.86 (s, 3H), 2.63-2.59 (m, 2H), 1.72-1.62 (m, 2H), 1.45-1.36 (m, 2H), 0.95 (t, *J*=7.32 Hz, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ: 172.5, 166.8, 164.9, 163.4, 161.6, 130.2 (2C), 128.6, 114.1, 105.9, 55.4, 37.9, 31.2, 22.7, 14.0; ESI-HRMS calcd for C₁₅H₂₀N₃O [M + H]⁺ 258.1601 found 258.1592. HPLC purity: ≥95%.

General procedure for the synthesis of compounds 4x, 4y. To the solution of the above synthesized compounds 4h or 4t in methanol, 10% palladium on carbon was added and stirred at room temperature under a hydrogen atmosphere, connected to a double layer balloon of hydrogen for 8 hours. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to get the desired debenzylated products4x and 4y, respectively as detailed below.

4-(2-amino-8-methyl-5,6,7,8-tetrahydroquinazolin-4-yl)phenol (4x). It was obtained by the reaction of **4h** (0.35 g, 1.0 mmol), 10% palladium on charcoal (0.010 g), in methanol in 66% yield (0.168 g) as white solid ; mp 216-218 °C; IR (neat) v_{max} cm⁻¹: 3392, 1636, 1068, 771; ¹H

NMR (DMSO, 400 MHz) δ : 9.63 (brs, 1H, OH), 7.35 (d, *J*=8.50 Hz, 2H, Ar), 6.79 (d, *J*=8.50 Hz, 2H, Ar), 6.12 (brs, 2H, NH₂), 2.68-2.63 (m, 1H), 2.54-2.51 (m, 2H), 1.94-1.88 (m, 1H), 1.70-1.62 (m, 1H), 1.49-1.43 (m, 2H), 1.26 (d, *J*=7.08 Hz, 3H, CH₃); ¹³C NMR (DMSO, 400 MHz) δ : 169.8, 165.2, 161.3, 157.7, 130.1 (2C), 129.5, 114.6, 114.5 (2C), 35.2, 30.6, 26.5, 20.8, 20.7; ESI-HRMS calcd for C₁₅H₁₇N₃O[M + H]⁺256.1444 found 256.1442. HPLC purity: \geq 95%.

3-(2-amino-8-methyl-5,6,7,8-tetrahydroquinazolin-4-yl)phenol (4y). It was obtained by the reaction of **4t** (0.35 g, 1.0 mmol),10% palladium on charcoal (0.010 g), in methanol in 63% yield (0.16 g) as white solid ; mp 200-202°C; IR (neat) v_{max} cm⁻¹: 3403, 1629, 1076, 758; ¹H NMR (DMSO, 400 MHz) δ : 9.54 (brs, 1H), 7.21 (s, 1H, Ar), 6.85-6.80 (m, 3H, Ar), 6.21 (brs, 2H), 2.68 (s, 1H), 2.49-2.45 (m, 2H), 1.92-1.49 (m, 4H), 1.27 (s, 3H); ¹³C NMR (DMSO, 400 MHz) δ : 170.0, 165.5, 161.3, 156.9, 140.1, 128.8, 119.0, 115.3 (2c), 114.6, 35.2, 30.5, 26.2, 20.7, 20.5; ESI-HRMS calcd for C₁₅H₁₇N₃O[M + H]⁺256.1444 found 256.1431. HPLC purity: ≥95%.

Biology

GloSensor cAMP Assay. We used GloSensor (a cAMP biosensor) assay as described previously to evaluate *in vitro* activity of tetrahydroquinazolinamines compounds at human H2R, H3R and H4R.⁷⁷ This assay was also used for off-target activity of **4c** at other family of GPCRs, which couples with either G_i or G_s proteins (D1R, D2R, D5R, KOR and GPBAR1). In brief, 5 million HEK293 T cells (mention source) were plated into optically bottom 96-well tissue culture plate containing high glucose Dulbecco's Modified Eagle's medium (DMEM) containing 5% foetal bovine serum (FBS) and transiently transfected with 5 µg of GloSensor-22F cAMP plasmid DNA (Cat. No. E2301, Promega Corp) and 5 µg of desired human GPCR plasmid DNA followed by incubation at 37° C for 15 to 18 h in a humidified incubator with 5% CO₂. Following overnight incubation, cell culture medium was aspirated and cells were incubated with sodium luciferin (1 mg/ml) for 90 min and treated with test compounds or reference ligands at 10 μ M to 0.01 nM for determination of IC₅₀. After 10 to 15 min of treatment with test compounds, in case of agonist mode of G_s-coupled receptor (H2R, D1R, D5R and GPBAR1) directly measured luminescence whereas in case of antagonist mode cells further treated with 100 nM selective agonist according receptors and measured luminescence after 10 minutes incubation. For G_i-coupled receptor (H3R, H4R, D2R and KOR), in agonist mode cells were stimulated with 10 μ M forskolin after treatment with test compound or reference ligand and measured luminescence/well whereas in case of antagonist, finally cells were stimulated with 10 μ M forskolin and luminescence/well was measured by using multimode plate reader (BMG, Labtech). Relative luminescence units (RLU) were plotted as percent inhibition of histamine response using nonlinear regression fit analysis equation of GraphPad Prism 5.0.

NFAT-RE luciferase assay. All active compounds were also evaluated at H1R and off-target activity of 4c evaluated at M1R, M5R, GPR40 and 5-HT2_C receptors by using NFAT-RE luciferase assay as described previously.⁷⁷ In brief 5 million of HEK293T cells were transiently transfected with 5 μ g of NFAT-RE luciferase (luc2P/NFAT-RE/Hygro, Promega Corp) plasmid DNA and 5 μ g receptor plasmid DNA. Transfected cells were plated in 96 well tissue culture plate with DMEM and incubated overnight in CO₂ incubator (37°C, 5% CO₂). Next day cells were treated with test compounds and reference ligands for 6-8 h. Following treatment with test compounds, luciferase activity was measured by addition of Bright-Glo substrate solution (1 mg/ml, final concentration) in multimode plate reader (BMG, Labtech).

Homology modeling of H3R. Protein sequence of the Human histamine receptor 3 (hH3R) was retrieved from UniProt⁷⁸ with accession number (Q9Y5N1). BLASTP suite within NCBI was used against PDB database to obtain best template for homology modeling. Muscarinic acetylcholine receptor M3 (PDB: 4U15) having 29% identity and 91% query coverage with target sequence, bound with antagonist tiotropium was selected as template. All models were built using MODELLER9.17 software.⁷⁹ Total 50 models were generated. Pre-selected models on the basis of DOPE score were assessed with PROCHECK ⁸⁰ and QMEAN ⁸¹ functions for validation.

Schrodinger suite⁸² was used for the protein and ligand preparation as well as for docking experiments. Protein preparation wizard ⁸³ was used for the preparation of the hH3R models. It assigned correct bond order, checked disulfide bonds and added all hydrogens. Hydrogen bonds were incorporated at physiological pH using PROPKA and further minimized with OPLS2005 force field. Ligand was prepared using LigPrep.⁸³ Default settings were used for protein and ligand preparation. Receptor Grid of 22 X 22 X 22 A^o dimensions was generated with centroid at ASP114. All the docking experiment was performed with Glide XP module.⁸⁴ Visualization and interaction analysis was carried out in Schrodinger suite and UCSF Chimera package.⁸⁵

hERG assay. To determine the hERG binding activity of **4c**, we used Predictor hERG assay kit (Invitrogen Corp, USA) as per manufacturer's instruction. Briefly, in this assay we used a membrane fraction expressing hERG ion channel protein and a high-affinity red fluorescent hERG channel ligand also called as "tracer" (Predictor hERG Tracer Red), in a homogenous, fluorescence polarization (FP)-based format. Compounds that bind to the hERG channel protein (competitors) are identified by their ability to displace the tracer, resulting in a lower

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fluorescence polarization. We incubated the test compound at seven concentrations (33 μ M-33 pM) with hERG expressing membranes in presence of 1 nM of tracer red, for 3 hrs at room temperature. Thereafter, FP was measured at excitation bandwidth 544/10 nm and emission bandwidth 590/10 nm using multimode plate reader (BMG Omega Polarstar) and difference in polarization (mP) was analyzed using in-built software. Further, to validate the assay, we used known hERG ligand E-4031 at the same concentrations.

PAINS. All tested compound checked for Pan Assay Interference (PAINS) substructure by using online web server False Positive Remover (http://www.cbligand.org/PAINS/).

Animals. The *in vivo* experiments with mice in this study were approved by the Institutional Animal Ethics Committee (IAEC) of CSIR-Central Drug Research Institute, Lucknow, India. Male C57BL/6J mice (6–8 weeks old) weighing 22-25 g and *db/db* (C57BLKS/J background) mice (Jackson laboratory; 35-45 g) were used in this study. Animals were housed on a 12 h light/ dark cycle (lights on at 8.00 a.m.). Food and water were provided ad libitum.

In vivo pharmacokinetic activity. The pharmacokinetic studies of 4c were carried out in 6-8 week old male C57BL/6J mice weighing 20 ± 5 g. The mice were acclimatized to this environment for at least five days before conducting the experiment. The study was conducted in overnight fasted (12-16 h) mice (n = 5 per time point). All experiments, euthanasia and disposal of carcasses were carried out as per the guidelines of Local Ethics Committee for animal experimentation. Suspension formulation containing 2.5 mg/mL of 4c was prepared by triturating 4c, gum acacia (1% w/v) and water (drop wise addition) in a mortar and pestle. A single 20 mg/kg oral dose was given to conscious mice using feeding needle. Brain and blood samples were withdrawn at various predefined times up to 24 h post dose. Serum samples were harvested from blood. All samples were stored at -80 °C until analysis.

A Shimadzu UFLC pump (LC-20AD) with online degasser (DGU-20A3), an autosampler (SIL-HTc) with a temperature-controlled peltier-tray and a triple quadrupole API 4000 mass spectrometer (Applied Biosystems, Toronto, Canada) was used for analysis. Chromatographic separation was made on a Discovery HS C-18 column (5 µm, 50 x 4.6 mm id) preceded with a guard column (5 µm, 20 x 4.0 mm, id) packed with the same material with mobile phase [acetonitrile: aqueous ammonium acetate buffer (0.01M, 4.5 pH) (90:10, % v/v)] pumped at a flow rate of 0.7 mL/min under isocratic condition. The mobile phase was degassed by ultrasonication for 15 min before use. LC-MS/MS system was equilibrated for approximately 20 min before commencement of analysis. The column oven temperature was 40°C. Total analysis time was 4 min per sample. The mass spectral analysis was performed in positive ionization mode at 5500 V using multiple reactions monitoring technique to monitor the transitions m/z 270.4 \rightarrow 253.8 for 4C, m/z 256.1 \rightarrow 240.1 for metabolite and m/z 180.1 \rightarrow 138.0 for phenacetin (internal standard). Data acquisition and quantitation were performed using analyst software (version 1.4.2; AB Sciex, Toronto, Canada). The method utilizes 50 µL of serum. Liquid-liquid extraction was used for sample clean up. The method showed linearity over the range of 0.25-200 ng/mL with recovery of >50% and acceptable accuracy and precision [FDA, Guidance for Industry: Bioanalytical Method Validation. http://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf [15 July 2015]. Brain tissues were homogenized in homogenization solution (phosphate buffer saline; 1:6, w/v) and the homogenate was processed as described above.

Development of obesity in C57BL/6J mice. To develop diet induced obesity (DIO) in C57BL/6J mice (6-8 weeks old, 22–25 g), male mice were fed with high fat diet (HFD- 60%

kcal from fat, Research diet Inc; D12492) for 10 weeks and an experimental control group fed with normal chow diet (10% kcal from fat, Research diets Inc; Cat no. D12450). Obesity was determined by measurement of body weight and associated hyperglycemia validated by glucose tolerance test.

Evaluation of cumulative food intake. All food intake study performed during the night phase (9:00 pm to 1:00 am) according previously described methods.⁷⁷ For food intake studies, mice were starved for 12 h (8:00 am to 8:00 pm) while water remained available. Starved mice housed in one mouse/cage and treated by oral administration of test compound **4c** (10 mg/kg or 20 mg/kg with 5% Gum acacia in normal saline) or lorcaserin (10 mg/kg with 5% Gum acacia normal saline solution) or vehicle (5% Gum acacia in normal saline). After 30 min of treatment, a weighed amount of food pellets were placed on food rack. Four hour post presentation of food, remaining food was collected and weighed the amount. Cumulative food intake determined by difference between initial food and remaining food.

Intra peritoneal glucose tolerance test (ipGTT). Glucose tolerance test performed in HFD fed mice and *db/db* mice was performed according to previously described method.²⁴ In brief, mice were starved for 16 h and baseline blood glucose level was measured by glucose meter (Accu-Chek Active Kit of Roche Products India Pvt. Ltd.) by taking blood from tail vein. After taking baseline blood glucose level, mice were treated with glucose (2 g/kg; C57BL/6J and 1 g/kg; *db/db* mice) by *intra peritoneal* injection and blood glucose level was measured at various time points 15, 30, 60, 90, and 120 minutes post injection.

Blood Lipid profiling. At the end of drug treatment (six weeks for HFD fed mice and four weeks for *db/db* mice), mice were euthanized using avertin (350 mg/kg) and blood was collected in 3.8 % sodium citrate buffer by cardiac puncture. Blood plasma was isolated by centrifugation

at 4000 rpm and stored at -80°C for biochemical analysis. Blood plasma triglycerides (TG), total cholesterol (TC), high density lipid cholesterol (HDLc) and low density lipid cholesterol (LDLc) were estimated using Merck selectra junior bio-analyzer (Merck Millipore).

Measurement of body fat mass. Body composition including fat mass and lean mass of HFD fed experimental mice were determined in live animal at the end of experiment by using specialized nuclear magnetic resonance (NMR)-Magnetic resonance imaging- Echo MRI (Echo Medical Systems).⁸⁶

Immunohistochemistry for cFos expression. For the cFos expression db/db mice treated with test compound **4c** (20 mg/kg; *i. p*) or vehicle (saline; *i.p.*). Anesthetized animals (as described above) were transcardially perfused with 4% paraformaldehyde (PFA). Perfused brains were kept overnight in 4% PFA solution and later in 30% sucrose solution for dehydration. Brains were cryosectioned at 30 µm thickness using a cryotome (FSE, Thermo Scientific, USA) and free-floating brain sections were processed for immunohistochemistry as previously described using mouse specific anti-cFos antibody (ab7963; Abcam).⁸⁷ Fluorescence signals of hypothalamic area were captured under Leica DMI6000 microscope using 20 X objective.

Data Analysis. All statistical analysis were performed by using GraphPad Prism 5.01 software

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Additional Information

Competing financial interests: The authors declare no competing financial interests.

Abbreviations Used

H3R, histamine 3 receptor; PAINS, pan-assay interference compounds; hERG, human Ether-ago-go-related Gene; GPCR, G-protein coupled receptor; MCHR1, melanin-concentrating hormone receptor 1; ADHD, attention deficit hyperactivity disorder; CCK-1R, cholecystokinin-1 receptor; SAR, structure-activity relationship; RLU, relative luminescence unit; IMT, imitet dihydrobromide; D1-D5, dopamine receptors 1-5; M1 and M5, muscarinic receptors 1 and 5; GPBA1, G-protein-coupled bile acid receptor 1; GPR40, G-protein coupled receptor 40; 5-HT_{2C}, serotonin receptor 2c ; KOR, kappa opioid receptor; ECL2, extracellular loop 2; PPB, plasma protein binding; F_u , unbound fraction; HFD, high fat diet; LRC, lorcaserin; GSK, GSK333329; HDC, histidine decarboxylase; α -MDLH, α -Methyl-DL-histidine dihydrochloride; PVN, paraventricular nucleus; ipGTT, *intra peritoneal* glucose tolerance test; DMEM, dulbecco's modified eagle's medium; FBS, foetal bovine serum; FP, fluorescence polarization; DIO, diet induced obesity; TG, triglycerides; TC, total cholesterol; HDLc, high density lipid cholesterol; LDLc, low density lipid cholesterol; PFA, paraformaldehyde;

Supporting Information

Following Figures, Tables and methods provided as Supporting information: Spectroscopic data (¹H-NMR, ¹³C-NMR, HPLC &HRMS), Chromatogram of chiral separation of **4c** enantiomers, Chiral separation methods, **Figure S1**: *in vitro* activity of **4b'**, **4d'**, and **4c'** at H3R and **4a**, **4d**, **4e**, **4f 4k**, **4o**, **4u**, **4w 4b'**, **4d'** and **4c** at H2R, **Figure S2**: enantiomer activity at H3R, **Figure S3**: **4c** hERG liability, **Figure S4**: body weight of lean mice, **Figure S5**: blood glucose data of DIO mice, **Figure S6**: lipid parameters, **Figure S7**: fasting glucose data of db/db mice, **Table S1**: List of reference ligands and Molecular Formula Strings.

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