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Development of Candidates for PET Imaging of Ghrelin Receptor in Disease: Design, Synthesis and Evaluation of Fluorine-bearing Quinazolinone Derivatives

Jinqiang Hou[1,2], Michael S. Kovacs[2,3], Savita Dhanvantari [2,3,4], Leonard G. Luyt[1,2,5,]*

[1] London Regional Cancer Program, London, Canada, N6A 4L6 [2] Lawson Health Research Institute, London, Canada, N6C 2R5 [3] Department of Medical Biophysics, University of Western Ontario, London, Canada, N6A 3K7 [4]Department of Pathology and Laboratory Medicine [5] Departments of Chemistry, Oncology and Medical Imaging, University of Western Ontario, London, Canada, N6A 3K7.

Abstract

Molecular imaging with PET (Positron Emission Tomography) is an attractive platform for non-invasive detection and assessment of disease. The development of a PET imaging agent targeting the ghrelin receptor (growth hormone secretagogue receptor type 1a or GHS-R1a) has the potential to lead to the detection and assessment of the higher than normal expression of GHS-R1a in diseases such as prostate, breast, and ovarian cancer. To enable the development of ^{18}F radiopharmaceuticals, we have designed and synthesized three series of quinazolinone derivatives, resulting in the identification of two compound (**5i, 17**) with sub-nanomolar binding affinity and one fluorine-bearing compound (**10b**) with picomolar binding affinity (20 pM), representing the highest binding affinity for GHS-R1a reported to date. Two lead compounds (**5b**, IC_{50} = 20.6 nM; **5e**, IC_{50} = 9.3 nM) were successfully ^{18}F-radiolabeled with radiochemical purity

of greater than 99%. Molecular modelling studies were performed to shed light on ligand-receptor interactions.

Introduction

Molecular imaging techniques, such as positron emission tomography, single photon emission computed tomography, and magnetic resonance imaging, can allow for visualizing, characterizing, and measuring important biological processes at the molecular and cellular levels in vivo. Molecular imaging is rapidly being recognized as an effective method for non-invasive detection and assessment of disease. Moreover, molecular imaging is believed to hold the promise of personalized medicine due to its roles in choosing the right treatment for an individual patient, guiding targeted therapies, and developing and optimizing new therapeutics.[1] Among all of the imaging modalities, PET is one of the most promising imaging methods for use in the clinic due to its high sensitivity and specificity, and has already provided a benefit to the fields of oncology, neurology, and cardiology.[2–4] In addition to patient care, PET imaging is capable of providing important insights for medicinal chemists in drug discovery, in order to reduce side effects arising from off-target binding of a drug candidate.[5,6] However, successful PET imaging depends on a validated radiopharmaceutical for clinically relevant targets. The development of effective, specific, validated radiotracers targeting clinically relevant biomarkers and their preparation methods are the most critical components to the expanding clinical application of PET imaging.[7,8]

One such clinically relevant target, the growth hormone secretagogue receptor type 1a (GHS-R1a), has potential therapeutic application towards a range of diseases due to its wide spectrum

of biological functions.[9–13] GHS-R has been identified in two isoforms thus far. GHS-R1a (also referred to as ghrelin receptor) is one isoform and is known to be activated by its endogenous ligand, ghrelin, to induce growth hormone release,[14] while GHS-R1b is a truncated version that doesn't bind ghrelin and is functionally inactive.[15] In addition to its therapeutic potential, GHS-R1a has been found to be highly expressed in prostate[16,17], breast[18], and ovarian cancer[19] and has been identified as a biomarker related to cardiovascular disease[20]. Therefore, the development of a PET imaging probe targeting GHS-R1a has the potential to detect and assess the GHS-R1a expression in diseases such as cancer and cardiovascular disease. Our group previously reported on fluorine and rhenium containing ghrelin analogues for potential use in the imaging of GHS-R1a.[21] The Beck-Sickinger group also reported on ^{68}Ga-labelled peptidomimetics as radiotracers for PET Imaging of GHS-R1a.[22]

Other than peptides and peptidomimetics, a class of small molecules, based upon a quinazolinone scaffold, was initially developed by Bayer Pharmaceuticals for the treatment of diabetes and obesity by targeting GHS-R1a[23] and offers a high affinity template as a starting point for the development of PET imaging probes. The most potent compound synthesized by Bayer (Figure 1B) possesses a Ki of 0.9 nM. A similar class of small molecules called azaquinazolinone derivatives was further optimized by Prosidion Ltd (UK) to achieve better lipophilicity. As a result, bioavailability was improved but the binding affinity for GHS-R1a was reduced as compared to quinazolinone derivatives.[24] Recently, Moldovan et.al. reported the synthesis of fluorinated azaquinazolinone derivatives.[25] In 2011, Potter et.al. reported on a ^{11}C radiolabeled quinazolinone derivative for PET imaging of GHS-R1a in the brain of mice. The studies showed that the imaging properties of the radioligand was not sufficient for GHS-R1a imaging and radiotracers with stronger binding affinity and moderate lipophilicity are required in

order to effectively image GHS-R1a in vivo.[26] In the present study, we report on an extensive structure-activity relationship investigation in order to develop fluorine-bearing azaquinazolinone derivatives with low nanomolar binding affinity and moderate cLogD. Molecular modelling studies were employed to develop a rationale for the SAR and provide guidance for future development of quinazolinone-based molecules for GHS-R1a. Furthermore, a ^{18}F-radiolabeling strategy was explored for the lead molecules in order to develop PET tracers for potential use in the molecular imaging of GHS-R1a in disease.

Results and Discussion

Design, Synthesis and Evaluation of Quinazolinone Derivatives

The structure activity relationship (SAR) investigation on quinazolinone derivatives (Figure 1 A) previously demonstrated that, (1) the piperidine substituent is essential for the binding affinity,[23] (2) a bulky group in R^1 position is essential for binding affinity,[23] (3) alkyl groups in R^2 position affects the functional profiles of the small molecules,[23] (4) decreasing the size of the R^3 substituent gradually reduces binding affinity,[23] (5) (S)-stereoisomers are significantly more favorable than the (R)-isomers.[23] The most potent compound (**5f**) previously discovered from this class of small molecules possesses a Ki of 0.9 nM (Figure 1B).[23] Hanrahan et al. focused on the modification of the lipophilicity of the central core by replacing the quinazolinone type structure with the azaquinazolinone as exemplified in Figure 1 C.[24] The SAR showed that adding one or two nitrogen atoms to the quinazolinone core at any position resulted in decreased binding affinity, while modification in the R^1 position provided the opportunity to improve the binding affinity, resulting in ligand (**20**) with a Ki of 10 pM (Figure 1 D). For comparative purposes, we prepared and evaluated **20** in our lab and determined the IC_{50} to be 0.71 nM, as determined by radioligand binding assay.

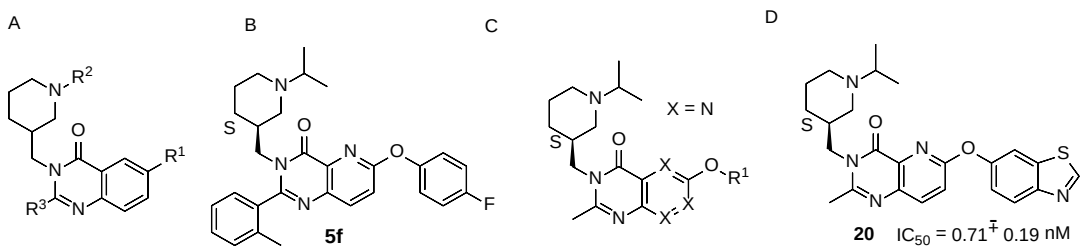
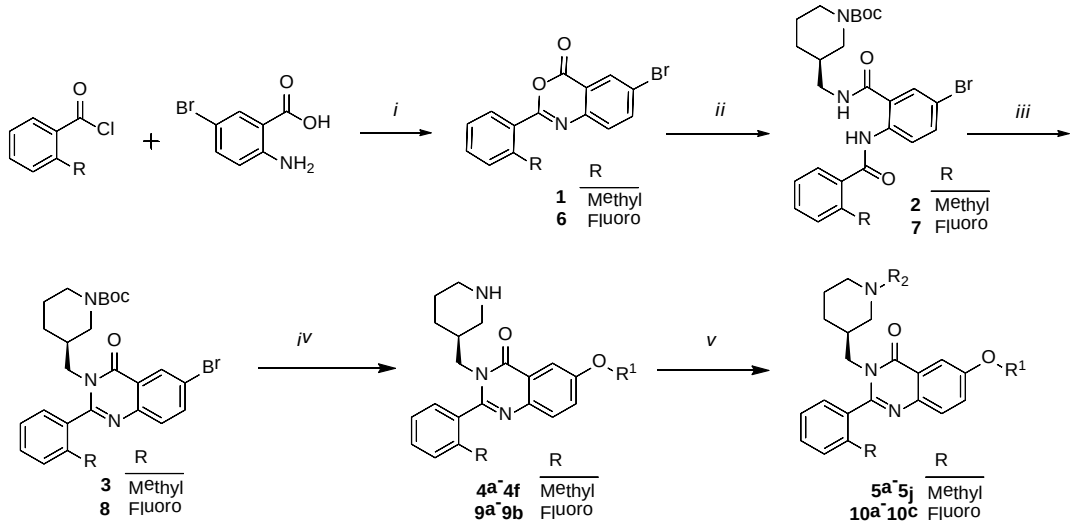


Figure 1. Various modifications to quinazolinone derivatives.

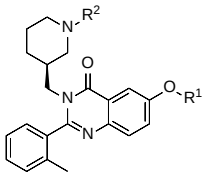
Our aim was to design and synthesize fluorine-containing compounds with strong binding affinity for GHS-R1a that would be suitable for application as PET agents. Candidate molecules that exhibit strong binding affinity proceeded to radiolabelling using fluorine-18 (^{18}F), a commonly used radioisotope with superior characteristics for PET imaging.[27] Our initial effort was directed towards modifying the R^1 and R^2 substituents (Scheme 1) as suggested by the above mentioned SAR information. In addition to the effort of improving the binding affinity, we have considered the lipophilicity which is indicated by $cLogP_{7.4}$. Generally speaking, less lipophilic imaging agents result in more favorable biodistribution characteristics due to fast clearance and less blood protein binding.[28] In addition, it has been shown that radiotracers with cLogD lower than 5 are able to cross the blood-brain barrier[29] and thus we decided to maintain the lipophilicity of the small molecules to cLogD < 5.

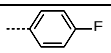
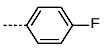
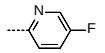
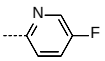
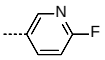
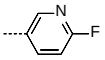
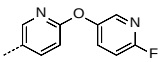
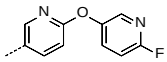
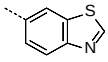
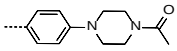
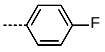
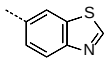
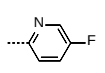
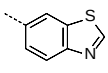
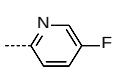


Scheme 1. Synthesis of quinazolinone derivatives **4a**-**4f**, **5a**-**5j**, **9a**-**9b** and **10a**-**10c**. (*i*) a, Triethylamine, dichloromethane, rt.; b, Acetic anhydride, 50°C. (*ii*)(R)-1-Boc-3-(aminomethyl) piperidine, toluene, reflux. (*iii*) Ethylene glycol, LiOH, 150°C, microwave. (*iv*) a, HO- R^1, CuI, TMHD, Cs_2CO_3, NMP, 120°C; b, 10% TFA, dichloromethane, rt.. (*v*); R^2-I, K_2CO_3, 70°C.

Compounds **4a** and **5f**, reported previously,[23] were synthesized and used as reference compounds. Following scheme 1, a number of quinazolinone analogues were synthesized. 2-Amino-5-bromobenzoic acid was treated with o-toluoyl chloride to form benzoxazinone **1**. The addition of BOC-protected aminomethylpiperidine led to uncyclized product **2**, which was cyclized under microwave irradiation to give compound **3**. Ullmann coupling reactions were then performed with aromatic hydroxy building block to form the intermediates, followed by BOC-deprotection to furnish compound **4a**-**4f**. Further N-alkylations on the piperidine nitrogen atoms using nucleophilic substitution conditions furnished compound **5a**-**5j.** The compounds were evaluated using radioligand binding assay and the IC_{50} values are listed in Table 1.

Table 1. 1st series of quinazolinone derivatives with IC_{50} and $cLogD_{7.4}$ values.



Compound	R^1	R^2	IC_{50} (nM)	pIC_{50} [a]	cLog $D_{7.4}$ [b]
4a		H	5.6 (0.9)[c]	8.26 ± 0.06	2.22
5a			29.5	7.63 ± 0.31	4.50
4b		H	52.0	7.31 ± 0.14	0.90
5b			20.6	7.69 ± 0.07	3.40
4c		H	68.5	7.17 ± 0.05	1.24
5c			64.2	7.18 ± 0.03	3.53
4d		H	82.9	7.09 ± 0.08	1.24
5d			65.2	7.19 ± 0.08	2.48
4e		H	5.3	8.30 ± 0.12	1.89
5e			9.3	8.06 ± 0.15	4.25
4f		H	587.0	6.24 ± 0.06	1.62
5f			3.7(0.9)[c]	8.62 ± 0.43	3.32
5g			1.8	8.87 ± 0.33	2.25
5h			18.2	7.74 ± 0.03	2.08
5i			0.4	9.46 ± 0.24	2.62
5j			>1000		3.43

Ghrelin (1-28)				3.3	8.53 ± 0.13

[a], mean values ± SEM; [b], calculated using ACD Labs; [c],the Ki values reported in the literature.[23]

The binding affinities of **4a** and **5f**, which both contain a fluorine atom, were determined to be 5.6 nM and 3.7 nM. However, the radiolabeling of these compounds would be challenging as the fluorine is attached to the non-activated arene in the absence of any electron-withdrawing group. However, compound **4a** has a secondary amine which could be used for the incorporation of a fluoroethyl group. A two-step radiolabeling approach is commonly used for amine bearing small molecules. Firstly, ethylene ditosylate is treated with [$^{18}F^-$] to generate radioactive compound [^{18}F]2-fluoroethyl tosylate. In the second step, the amine bearing small molecules is reacted with [^{18}F]2-fluoroethyl tosylate via nucleophilic substitution to provide ^{18}F-radiolabelled product (Scheme S1 method A). Therefore, compound **5a** with a fluoroethyl group incorporated onto the piperidine was synthesized. This compound can be readily radiolabeled using the two-step approach, but the addition of the fluoroethyl group caused the IC_{50} to increase to 29.5 nM. To reduce the cLog $D_{7.4}$, compound **5b** and **5c** were synthesized by replacing the fluorophenyl with fluoropyridinyl group. The IC_{50} of **5b** was decreased to 20.6 nM while **5c** was increased to 64.2 nM. A comparison of the IC_{50} values between **4b**, **4c** and **5b**, **5c** suggested that the incorporation of fluoroethyl group on the piperidine has a slightly positive effect on the binding affinity. A bulkier substituent than fluorophenyl in the R^1 position is not beneficial for the binding affinity as suggested by compound **4d**, **5d, 4f**. However, the benzothiazolyl group in the same position improved the IC_{50} to 5.3 nM (**4e**). The incorporation of a fluoroethyl group on the piperidine increased the IC_{50} of **5e** to 9.3 nM. It has been demonstrated that a free amine on every studied GHS-R1a ligand played an important role in binding with GHS-R1a. The positive charge at the

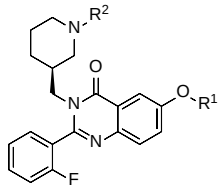
amine being protonated in physiological condition is believed to establish ionic interactions with Glu124, thus anchoring the ligands to the binding pocket of GHS-R1a.[30–32] Consistently, the introduction of the 2-fluoropropionyl group into piperidine diminished the binding affinity (**5j**). This is probably due to the formation of an amide bond preventing the molecule from protonation, thus not being able to anchor the ligand to Glu124 in the orthostatic binding pocket GHS-R1a. Since previous studies suggest that incorporation of an isopropyl group on the piperidine always provides a positive effect on the binding affinity, compound **5g** was made and was determined to possess an IC_{50} of 1.8 nM. In addition, compound **5h** bearing a fluoroethyl group has an IC_{50} of 18.2 while **5i** has an IC_{50} of 0.4 nM, providing a first sub-nanomolar binding affinity ligand.

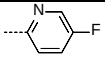
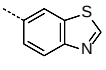
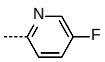
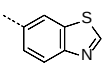
The first round of modification resulted in two fluorine-bearing lead compounds **5b** and **5e**. Both compounds then proceeded to the radiolabelling stage as described later, in the radiochemistry section. These optimization steps also provided high-quality compounds **5g** and **5i** with low nanomolar to sub-nanomolar binding affinities and optimal $cLogD_{7.4}$. Unfortunately, these two compounds don't possess a fluorine atom in a position suitable for ^{18}F radiolabelling. The second round of structural modification then focused on incorporating a fluorine atom into the molecule, while maintain the sub-nanomolar binding affinity with GHS-R1a.

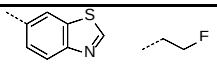
Given the fact that the size of a methyl group is similar to that of fluorine, we decided to replace the methyl group with a fluorine. Following the synthesis route as depicted in Scheme 1, compounds **9a-b**, **10a-c** were made. Surprisingly, as compared to the methyl analog (**4b**, IC_{50} = 52.0 nM), the fluorine replacement dramatically reduced the binding affinity when there is fluoropyridine (**9a**, IC_{50} = 935.2 nM) group in the R^1 position (Table 2), but the effect was minimal when there is benzothiazolyl group in the R^1 position (**9b**, IC_{50} = 16.0 nM), as compared

to the corresponding methyl analog (**4e**, IC_{50} = 5.3 nM). Upon incorporation of an isopropyl group in the R^2 position, the binding affinity was significantly enhanced for compounds **10a** (IC_{50} = 82.3 nM) and **10b** (IC_{50} = 0.02 nM). The fluorine bearing compound **10b**, with picomolar binding affinity and a $cLogD_{7.4}$ suitable for brain penetration (2.39), appears to be the best candidate for developing into a radiotracer. We thus synthesized the respective radiolabeling precursor, with a nitro group available for substitution, as described in Scheme S2. However, the radiolabeling was unsuccessful (Figure S1), probably due to the quinazolinone scaffold being a weak electron withdrawing group and the existence of steric hindrance. To enable radiolabeling, compound **10c** was made with fluoroethyl group in the R^2 position, but the IC_{50} increased to 8.1 nM. These modifications provided one fluorine bearing compound (**10b**) with picomolar binding affinity. To the best of our knowledge, this is the highest affinity ligand for GHS-R1a reported to date.

Table 2. The 2nd series of quinazolinone derivatives with IC_{50} and $cLogD_{7.4}$ values.



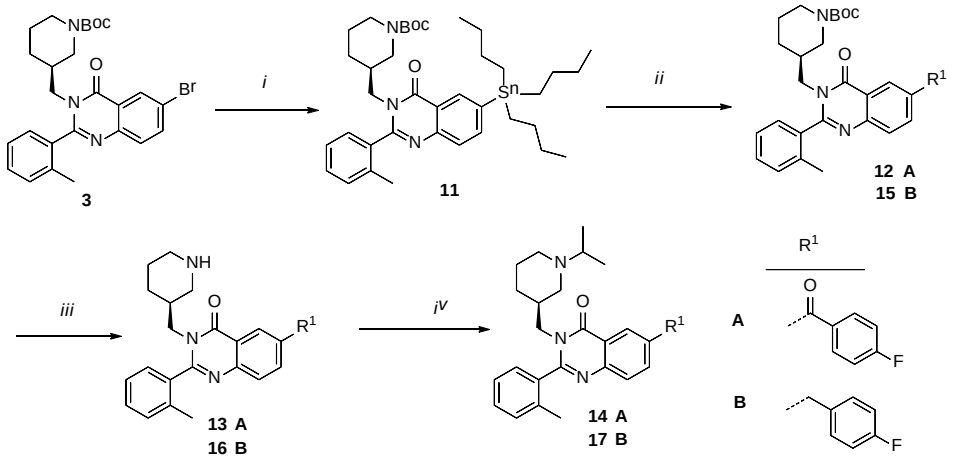
Compds.	R^1	R^2	IC_{50} (nM)	pIC_{50} [a]	cLog $D_{7.4}$ [b]
9a		H	935.2		0.53
9b		H	16.0	7.86 ± 0.23	1.57
10a			70.5	7.16 ± 0.07	1.53
10b			0.02	10.92 ± 0.32	2.39

10c		8.1	8.15 ± 0.23	3.99

[a], mean values ± SEM; [b], calculated using ACD Labs

Nucleophilic aromatic substitution (as described in Scheme S1, Method B) has been commonly used for ^{18}F-radiolabeling of aromatic systems, but it requires sufficient activation of the aromatic ring, which can be achieved by electron withdrawing group such as carbonyl group in the ortho or para position to the leaving group.[6,33] The nitro group has been reported to be an efficient leaving group for ^{18}F nucleophilic aromatic substitution and a number of clinically used ^{18}F-radiopharmaceuticals (*e.g.*,^{18}F-altanserin,[34] ^{18}F-fluorodopamine[35]) have been synthesized by aromatic nucleophilic substitution using nitro as a leaving group. Compound **4a** and **5f** have similar binding affinity to that of natural ghrelin and a cLogD$_{7.4}$ suitable for brain penetration as listed in Table 1. The compound possesses fluorine, which can potentially be substituted by radioactive ^{18}F. To make the radiolabeling of the compounds feasible, the 4-fluorophenoxy was replaced with fluorobenzoyl group. We hypothesized that the carbonyl would serve as electron withdrawing group, thus facilitating the nucleophilic aromatic substitution with ^{18}F anion. The precursor with a nitro group in replacement of fluorine can be made readily for ^{18}F radiolabelling. Based on these considerations, compounds **13** and **14** were made following the synthesis route as described in Scheme 2. Starting from compound **3**, the aryl stannane **11** was made by treating **3** with bis(tributyltin), which was followed by a Stille coupling reaction to give compound **12**. Boc-deprotection was then performed to furnish compound **13**, which was followed by alkylation to provide compound **14**. As expected, compound **14** has an IC$_{50}$ of 3.8 nM, which is equivalent to that of compound **5f** (Table 3). Compound **13** also showed very strong binding affinity (4.0 nM). Both compounds have a cLogD$_{7.4}$ suitable for brain penetration as listed in Table 3. We next sought to develop the radiolabeling precursor for compound **14**. The precursor

with the nitro group in replacement of fluorine was synthesized (Scheme S3), but the radiolabeling proceeded in extremely low yield (Figure S2). Given the similarity of a methylene group to an ether linker, the methylene analog **17** was also synthesized (Scheme 2). Surprisingly, the IC_{50} of **17** was determined to be 0.32 nM.



Scheme 2. Synthesis of quinazolinone derivatives **14** and **17**. (*i*) $(Bu_3Sn)_2$, toluene, 110°C. (*ii*) $PdCl_2(pph_3)_2$, 4-fluorobenzoyl chloride/4-fluorobenzyl bromide, toluene, 110°C. (*iii*) TFA, dichloromethane, rt.. (*iv*) 2-bromopropane, K_2CO_3, 70°C.

Table 3. The 3rd series of quinazolinone derivatives with IC_{50} and $cLogD_{7.4}$ values.

Compds.	**IC_{50} (nM)**	**pIC_{50}** [a]	**cLog $D_{7.4}$** [b]
13	4.0	8.40 ± 0.01	1.82
14	3.8	8.43 ± 0.03	2.76
17	0.3	9.59 ± 0.29	4.80

[a], mean values ± SEM; [b], calculated using ACD Labs

Ligand conformation analysis

We noticed that the methyl attached to the phenyl exhibited double peaks on ^{1}HNMR spectrum for all of the quinazolinone derivatives. However, VT (variable-temperature) NMR studies showed that the double peak merged to one single peak when the temperature was increased to 100 °C as demonstrated by compound **4e** (Figure S3), suggesting that the quinazolinone derivatives may have more than one conformation. We also noticed that the methyl hydrogens did not show a double peak until after the cyclization reaction, as demonstrated by compound **2** showing a single peak while compound **3** had double peaks for the methyl hydrogens. By examining the 3D structure of the small molecule, we found that the piperidine encountered steric clashes with the carbonyl in the quinazolinone main core as well as the phenyl group (Figure 2A), resulting in the single bond b_1 not being fully rotatable. Therefore, the piperidine was trapped into two conformations during the cyclization step. Moreover, the steric clash between the piperidine and the phenyl group resulted in the single bond b_2 not being fully rotatable, thus the methyl attached to the phenyl group may also be trapped into to two opposite directions. Therefore, four conformers were proposed as shown in Figure 2B. These conformers are referred to as C1, C2, C3 and C4. To investigate if the proposed conformers are stable ones, an *ab* initio calculation was performed. The result showed that all proposed conformers are stable with similar total energies (Table S1). Although the ^{1}HNMR spectrum showed only two peaks for the methyl, it is possible that these four conformers may exist at the same time, while not being visible in the NMR spectrum.

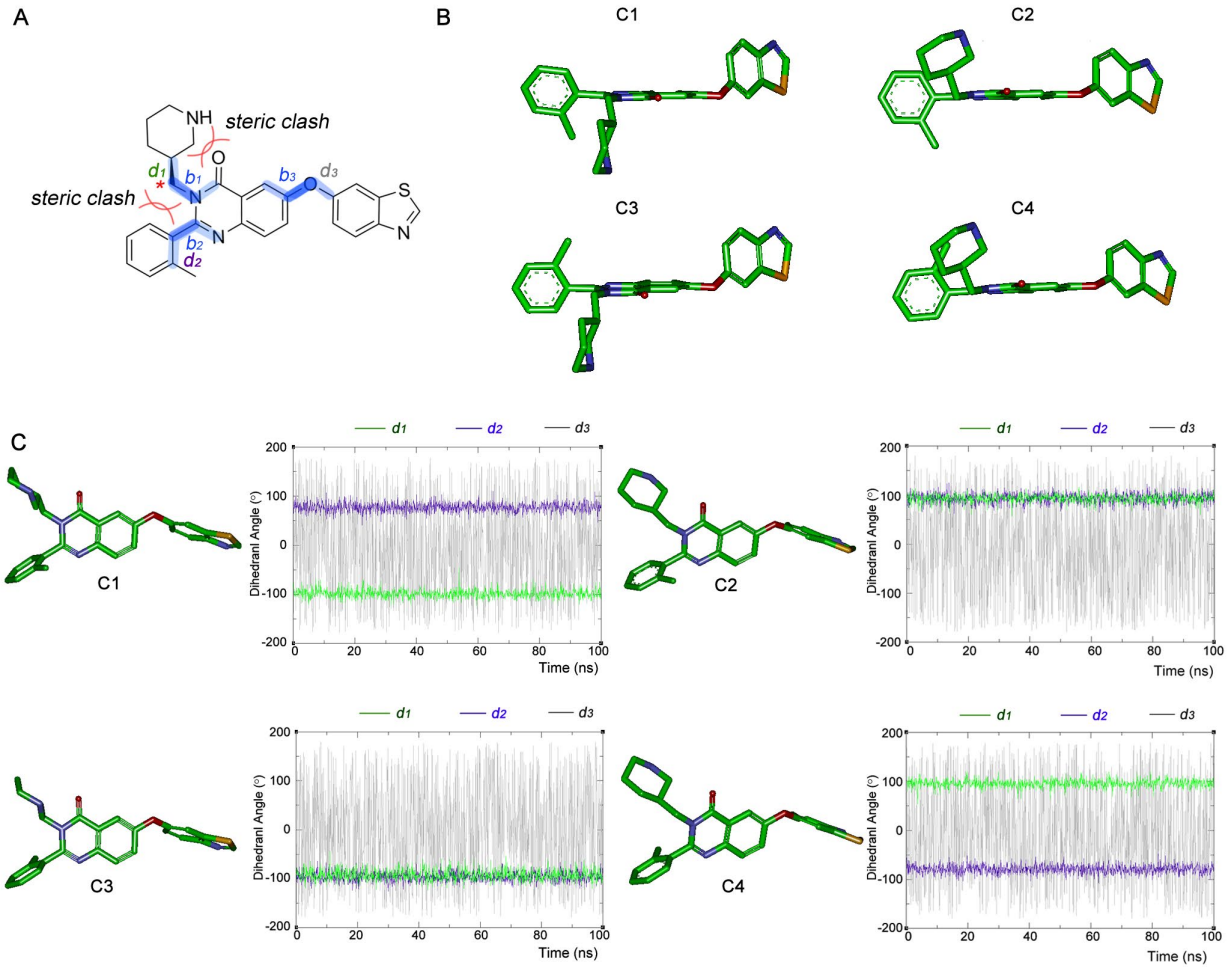


Figure 2. The conformation study on compound **4e**. A: The structure of compound **4e** with single bond b_1, b_2, b_3 and dihedral angle d_1, d_2, d_3 indicated; B: Four stable conformers C1, C2, C3 and C4 of **4e** calculated by *ab* initio method; C: The front view of the conformers and the plots of dihedral angle d_1 (green), d_2 (purple) and d_3 (grey) versus MD simulation time**.**

To further demonstrate how the steric clashes among the piperidine, the carbonyl in the quinazolinone main core, and the methyl phenyl group constrain the single bond b_1 and b_2 (Figure 2A), molecular dynamics (MD) simulation was performed. To demonstrate the rotatability of the single bond b_1, b_2 and b_3, the dihedral angles (d_1, d_2 and d_3) over the course of simulation were plotted and depicted in Figure 2C. For C1 and C3, the dihedral angle d_1

fluctuates from roughly from -80 to -110 °, while for C2 and C4 conformers, the dihedral angle d_1 fluctuates from roughly 80 to 110 °. The dihedral angle d_2 also experienced similar fluctuation in a shallow range. The result indicates that the bond b_1 and b_2 are highly constrained due to the steric hindrance. As a reference, the dihedral angle d_3 fluctuates from -180 to 180 °, which is what one would expect to see as the single bond b_3 is freely rotatable. The MD study clearly showed that the bonds b_1 and b_2 are highly constrained due to the steric hindrances and the four conformers are not convertible at room temperature.

Molecular docking study

In an attempt to understand the binding mode of quinazolinone derivatives, molecular docking studies for compound **4e**, **5e**, **5g**, **5i**, and **10b** were performed using a homology model of GHS-R1a created recently based on the homologous GPCR structures (PDB ID: 4BWB,[36] 3ZEV,[36] 4BUO,[36] 4BV0,[36] 4GRV,[37] 4EA3[38]).[39] To include all the possible conformations of the small molecules in the docking study, the single bond b_1 and b_2 are treated as freely rotatable bonds. A large body of literature has reported that the amine on GHS-R1a ligands is important for binding affinity as well as functional activity.[30–32] An ionic interaction between the positively charged terminal amine of the agonist and Glu124 on the receptor serves as an anchor point for binding. The SAR of quinazolinone derivatives clearly demonstrated that the amine on the quinazolinone derivatives played an important role in binding as evidenced by the conversion of amine to the amide resulting in a dramatically reduced binding affinity (**5j**, IC_{50} > 1μM). The result is consistent with the previous findings.[30–32] Given the importance of the interaction between the amine and Glu124, the docked poses where the positively charged amine of quinazolinone derivatives is within 5 Å of the carboxylate of Glu124 were selected.

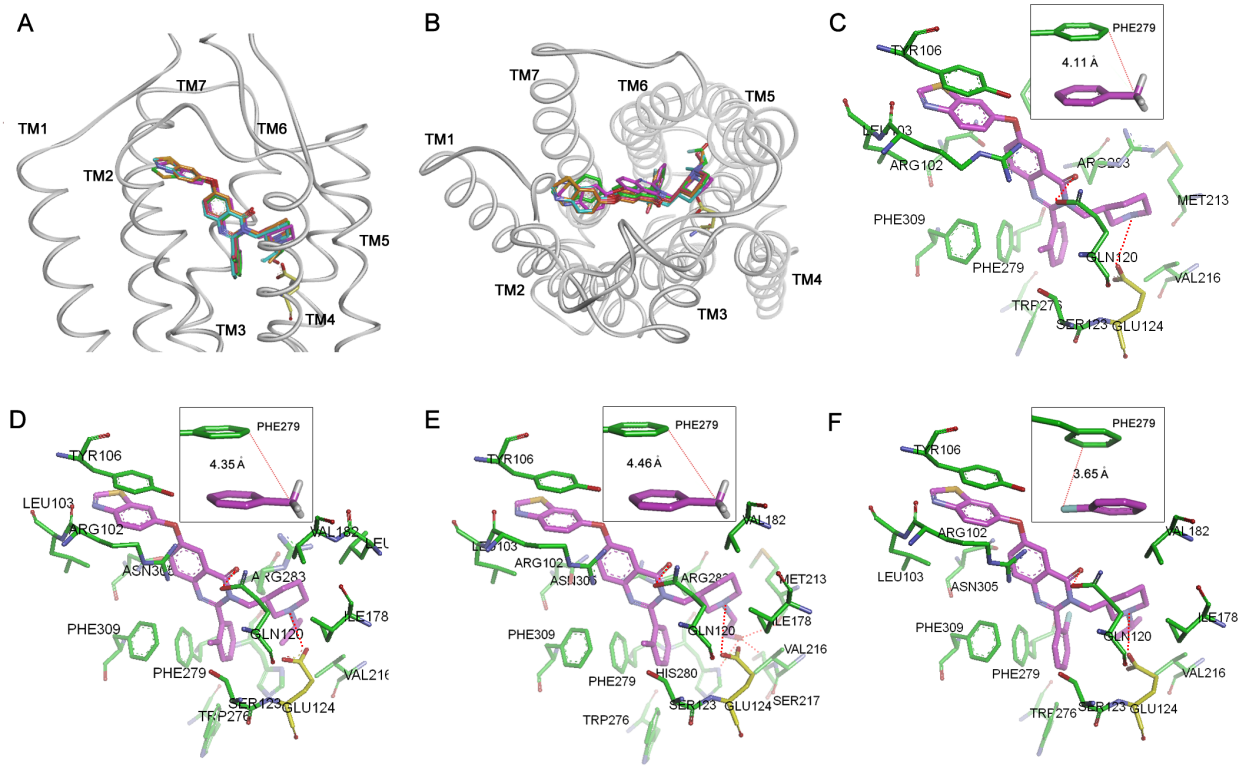


Figure 3. The complex structures from molecular docking studies using a homology model of GHS-R1a created based on the homologous GPCR structures (PDB ID: 4BWB,[36] 3ZEV,[36] 4BUO,[36] 4BV0,[36] 4GRV,[37] 4EA3[38]). A: **4e** (purple), **5e** (red), **5g** (yellow), **5i** (green) and **10b** (cyan) are superimposed and viewed from extracellular side; B: Side view of **4e** (purple), **5e** (red), **5g** (yellow), **5i** (green) and **10b** (cyan) in the GHS-R1a; 3D depiction of the binding mode and surrounding residues for **4e** (C), **5g** (D), **5i** (E), **10b** (F). Ligands are shown in purple sticks; the residues are shown in green sticks except for Glu123 that is shown in yellow. The insert figures show the closest distances between the methyl carbon/fluorine of quinazolinone derivatives and phenyl ring in Phe279.

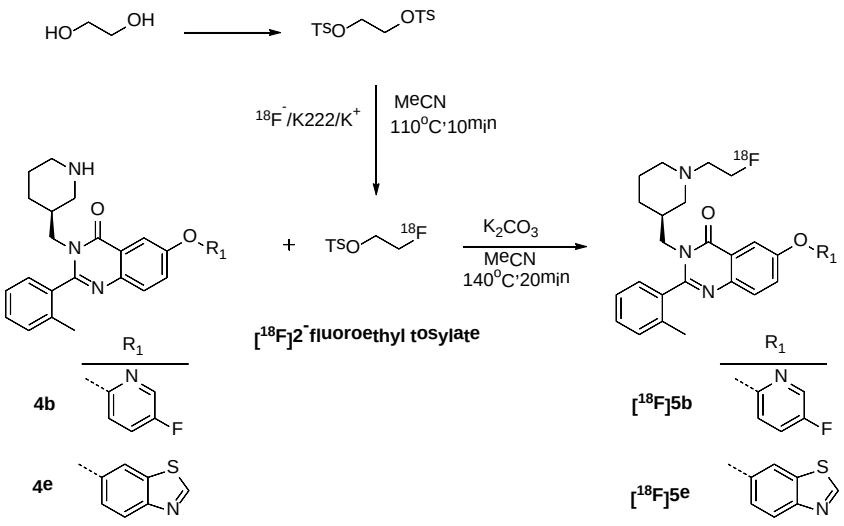
In an attempt to investigate the binding modes of the small molecules with the benzothiazolyl group in the R^1 position (i.e., **4e**, **5e**, **5g**, **5i**, **10b**), we found that all of these small molecules displayed similar binding poses, as indicated in Figure 3A,B. Their docking poses are aligned

well with the amine on the piperidine ring pointing to the carboxylate of Glu124. The small molecules established strong pi-pi stacking interactions with Tyr106 and Phe279 (Figure 3C,D,E,F). It is worth mentioning that the hydroxyl group of **5i** established three H-bonds with His280, Met213 and Ser217, which may explain its sub-nanomolar binding affinity (Figure 3E). For compound **10b** with fluorophenyl in replacement of methylphenyl group, the fluorine is pointing at the opposite direction as compared to methyl group in ligand **4e**, **5e**, **5g** and **5i**. As shown in Figure 3C,D,E (insert figures), the methyl groups in all models are pointing away from Phe279, implying steric hindrances posed by three hydrogens of the methyl group, while fluorine is involved in the pi-pi stacking interaction with Phe279, as evidenced by a distance of 3.64 Å (Figure 3F). The enhanced pi-pi stacking interaction with Phe279 may partially contribute to a 90-fold boost in the binding affinity of compound **10b** (0.02 nM) as compared to **5g** (1.8 nM). It has been reported that, in many cases, fluorine substitution resulted in a dramatically increased binding affinity.[40,41] Although the contributions of fluorine substitution to the energetics of ligand binding are poorly understood, it has been suggested that the boost in binding affinity may have to do with the changes in desolvation or binding to a "fluorophilic" pocket.[42]

^{18}F Radiochemistry

Compounds **5b** and **5e** obtained from the first round of optimization were also identified as lead candidates as they have low nanomolar binding affinity and moderate cLogD$_{7.4}$ values. The radiolabelling through a two-step approach is described in Scheme 3. The bitosylate was first made from tosylation of ethylene glycol. Nucleophilic substitution of the bi-tosylate with the [^{18}F^{-}] anion acquired from the PET cyclotron led to the formation of radioactive compound [^{18}F]2-fluoroethyl tosylate. The reaction mixture was diluted with hexane/diethyl ether (3:1) and

passed through a Sep-Pak Silica Plus column (Waters) to obtain purified product. The non-decay corrected radiochemical yield of [^{18}F]2-fluoroethyl tosylate was around 50%.



Scheme 3. Radio-synthesis of **[^{18}F]5b** and **[^{18}F]5e**.

Next, conditions for radiolabeling of **4b** were explored including variables reaction solvent (DMSO, MeCN), base ($KHCO_3$, K_2CO_3, Cs_2CO_3), reaction time (15−30 min), reaction temperature (100−150 °C), and precursor quantity (0.5-3 mg). The best conditions were identified. The [^{18}F]fluoroalkylation of amine precursor **4b** using intermediate [^{18}F]2-fluoroethyl tosylate yielded the desired ligand **[^{18}F]5b** with overall decay-corrected yield of 10.3 ± 0.8% and high purity (≥99) (Table 4). Further evidence for successful labeling of **4b** to **[^{18}F]5b** was provided by a co-injection of a pre-mixed solution of non-radioactive standard **5b** and radioactive ligand **[^{18}F]5b** (Figure 4). Conditions for radiolabeling of **5e** was also explored; radioligand **[^{18}F]5e** was synthesized with overall decay-corrected yields of 7.0 ± 0.6% and greater than 99% purity. Further evidence for successful labeling of **4e** to **[^{18}F]5e** was also provided by a co-injection of a pre-mixed solution of the non-radioactive standard **5e** and radioactive ligand **[^{18}F]5e** (Figure 5).

Table 4. Radiosynthesis Data

Radioligand	[^{18}F]5b	[^{18}F]5e
Radiochemical yield (%)	10.3 ± 0.8	7.0 ± 0.6
Radiochemical purity (%)	≥99	≥99
Total synthesis time (min)	105 ± 1.7	127 ± 7.1

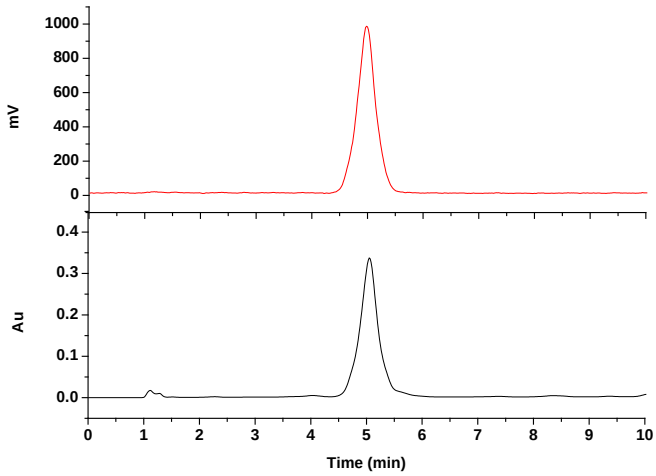


Figure 4, Stacked HPLC chromatograms (λ = 254 nm) (bottom line) and radio chromatograms (top line) for **5b** and **[^{18}F]5b**

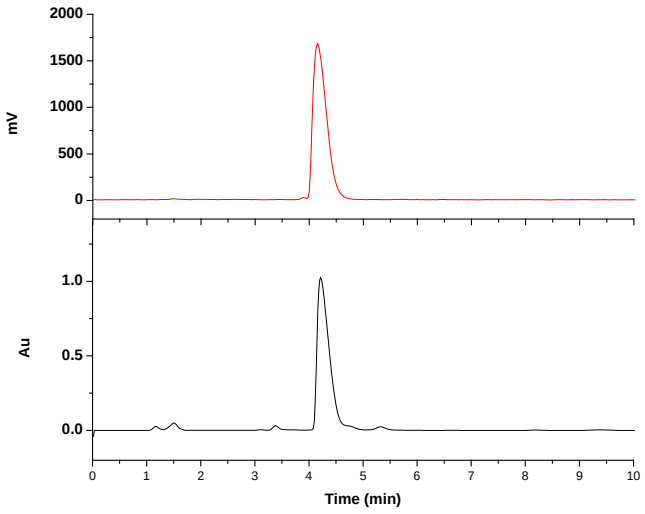


Figure 5, Stacked HPLC chromatograms (λ = 254 nm) (bottom line) and radio chromatograms (top line) for **5e** and **[^{18}F]5e**

Conclusion

We have developed three series of fluorine-bearing quinazolinone derivatives with potential for imaging of GHS-R1a in disease by PET. Their binding affinities with GHS-R1a were evaluated using a radio ligand competitive binding assay. The SAR investigation resulted in the identification of two compound (**5i, 16)** with sub-nanomolar binding affinity and one fluorine-bearing compound (**10b**) with picomolar binding affinity (20 pM), representing the highest binding affinity for GHS-R1a reported to date. A molecular modelling study was carried out to provide insights into structure-activity relationships with GHS-R1a. Although there are many reports in the literature of the nitro group being an efficient leaving group for ^{18}F nucleophilic aromatic substitution in the presence of an electron withdrawing group, our attempts to radiolabel compound **14** and **10b** using this strategy were not successful. Two other lead compounds (**5b**, IC_{50} = 20.6 nM; **5e**, IC_{50} = 9.3nM) with low nanomolar binding affinity for

GHS-R1a were successfully ^{18}F-radiolabeled with high radiochemical purity. Preliminary in vivo studies are planned with a murine cancer model for both radiolabeled compounds. Further development of ^{18}F-radiolabeled compounds (**10b**) is currently underway in our laboratories and will be reported in due course.

Experimental Section

All reagents were obtained from Sigma-Aldrich, Fisher Scientific, Ark Pharm Inc or Oakwood Chemicals. The purity of the compounds was analyzed using analytical UHPLC. These results are summarized in Table S2 and Figure S4, with all compounds determined to have ≥95% purity. Acquity UHPLC H-Class system (Waters, Inc.) combined with a Xevo QTof mass spectrometer was used for analytical UHPLC-MS work. Waters Acquity UHPLC BEH C18 column (2.1 x 50 mm, 1.7 μm) was used. High resolution mass spectra were obtained on a Thermo Scientific DFS (Double Focusing Sector) mass spectrometer. NMR data was obtained on the Varian Inova 400 MHz in methanol-*d*$_4$ with the chemical shifts referenced to solvent signals. [^{125}I]-Ghrelin for binding assay was purchased from Perkin Elmer. Cyclotron produced [^{18}F]$H_2{}^{18}O$ was obtained from the Nordal Cyclotron & PET Radiochemistry Facility at Lawson Health Research Institute, London, Ontario, Canada. All Sep-Pak cartridges for radiochemistry were purchased from Waters. Analytical radio-RP-HPLC was performed on a Waters 1525 Binary HPLC pump containing a Waters 2487 dual λ absorbance detector and gamma detector. Agilent^{TM} RP-C18 column (4.6 x 150 mm, 5 μm) was used for analytical analysis. Agilent RP-C18 column (19 x 150 mm, 5 μm) was used for semi-preparative HPLC purification.

Synthesis of quinazolinone derivatives

6-bromo-2-(o-tolyl)-4H-benzo[d][1,3]oxazin-4-one (1)

The compound was made according to literature procedures with some modifications.[23] Toluoyl chloride (10.0 g, 64.7 mmol) was added slowly into a solution of 5-bromoanthranilic acid (12.7 g, 58.8 mmol) and triethylamine (24.6 mL, 176.4 mmol) in dichloromethane (120 mL) at 0 °C. The reaction mixture was stirred at rt for 16 h. After the solvent was removed under reduced pressure, water (80ml) and dichloromethane (80ml) were added into the resulting product (yellow oil). The organic layer was washed with additional 80ml of water, dried over Na_2SO_4 and filtered. Acetic anhydride (50 mL) was added in to the filtrate and the solution was heated at 50 °C for 3 h. The reaction mixture was cooled to rt, diluted with dichloromethane. Saturated $NaHCO_3$ was added slowly. The layer was separated and the organic layer was washed with brine, dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the crude product was treated with ethanol and stirred for 10mins. The solid was filtered and washed with additional ethanol, and then air dried for 3 h to give 13 g of the product in 70% yield. UHPLC-MS (waters) method: 5-90% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 2.58, calcd m/z 316.0 (MH^+), found m/z 316.9.

(R)-tert-butyl 3-((5-bromo-2-(2-methylbenzamido)benzamido)methyl)piperidine-1-carboxylate (2)

The compound was made according to literature procedure with some modifications.[23] Compound **1** (3g, 9.5mmol) was dissolved in toluene (20ml). (R)-1-Boc-3-(aminomethyl) piperidine (2.4 g, 11.4 mmol) was added and the solution was refluxed at 110 °C for 8h. The

resulting solution was cooled down to rt. The solvent was removed by around 90% and hexane (20ml) was added. The precipitate was filtered out and washed with additional 10ml hexane to give 4.9g pure product in 97% yield. UHPLC-MS (waters) method: 50-90% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 2.19, calcd m/z 530.2 (MH^+), found m/z 530.1.

(R)-tert-butyl 3-((6-bromo-4-oxo-2-(o-tolyl)quinazolin-3(4H)-yl)methyl)piperidine-1-carboxylate (3)

Compound **2a** (2.50 g, 4.72 mmol) was added to microwave reaction vessel followed by ethylene glycol (12 mL) and LiOH (0.22mg, 9.17 mmol). The resulting mixture was subjected to microwave irradiation with stirring for 30 minutes at 150 °C, cooled to rt, and diluted with dichloromethane (50ml) and water (50ml). The organic layer was washed with brine (50 mL) twice. The combined organic layer was dried over Mg_2SO_4 and then concentrated under reduced pressure. Purification via silica gel column chromatography using a gradient elution from 25 to 50% ethyl acetate in hexanes to yield 1.93g the product in 82% yield. UHPLC-MS (waters) method: 50-90% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.86, calcd m/z 512.2 (MH^+), found m/z 512.1.

(S)-6-(4-fluorophenoxy)-3-(piperidin-3-ylmethyl)-2-(o-tolyl)quinazolin-4(3H)-one (4a)

The compound was made according to literature procedure with some modifications.[23] Under a nitrogen atmosphere, compound **3a** (512 mg, 1.00 mmol), copper(I) iodine (95 mg, 0.05 mmol), 2,2,6,6-tetramethylheptane-3,5-dione (TMHD) (46 mg, 0.25 mmol), 4-fluorophenol (168mg, 1.5mmol) and Cs_2CO_3 (652 mg, 2.00 mmol) were added to 1-methyl-2-pyrrolidone (2 mL). The reaction mixture was stirred for 16 h at 120 °C, cooled, and filtered through Celite. The filtrate

was concentrated under reduced pressure and purified via silica gel column chromatography using a gradient elution from 10 to 20% ethyl acetate in hexanes to give 300mg of the product in 55% yield. The above product (300 mg, 0.55 mmol) was dissolved in dichloromethane (5 mL), followed by TFA (2 mL). The solution was stirred for 4 h at rt. The solvent was removed and saturated aqueous $NaHCO_3$ solution was added. The product was then extracted with EtOAc (2×). The combined organic layers were dried with Na_2SO_4 and filtered, and the solvent was removed under reduced pressure, and the crude compound was purified by reverse phase chromatography (Isolera One, HS-C18-30g cartridge) with a gradient from 5 to 60 % MeOH in water. It was lyophilized to give desired product as white powder in 85% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.65. HRMS (ES^+) for $C_{27}H_{27}FN_3O_2$ (MH^+), calcd 444.2087, found 444.2094. ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 7.69 (d, J = 8.9 Hz, 1H), 7.60 (d, J = 2.7, 1H), 7.54 (dd, J = 8.9, 2.7 Hz, 1H), 7.46-7.50 (m, 2H), 7.41-7.37 (m, 2H), 7.22 – 7.10 (m, 4H), 4.19 – 3.93 (m, 1H), 3.64 – 3.35 (m, 1H), 2.86-2.83 (m, 1H), 2.80-2.62 (m, 1H), 2.46-2.37 (m, 1H), 2.23 – 2.07 (m, 4H), 1.80-1.72 (m, 1H), 1.60-1.27 (m, 3H), 1.06 – 0.90 (m, 1H). ^{19}F NMR (376 MHz, methanol-d_4) δ ppm -118.76 – -118.66 (m)

(S)-6-((5-fluoropyridin-2-yl)oxy)-3-(piperidin-3-ylmethyl)-2-(o-tolyl)quinazolin-4(3H)-one (4b)

The method similar for the preparation of compound **4a** was used, except replacing 4-fluorophenol with 5-fluoro-2-pyridinol. The Boc-protected intermediate was purified via silica gel column chromatography using a gradient elution from 20 to 30% ethyl acetate in hexane and gave 44% yield. After Boc deprotection, the crude compound was purified by reverse phase

chromatography (Isolera One, HS-C18-30g cartridge) with a gradient from 30 to 100 % MeOH in water. It was lyophilized to give the product in 67% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 2.01. HRMS (ES^+) for $C_{26}H_{26}FN_4O_2$ (MH^+), calcd 445.2040; found 445.2046. ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 8.04 (d, J = 3.2 Hz, 1H), 7.90 (d, J = 2.7 Hz, 1H), 7.75 – 7.68 (m, 2H), 7.62 (dd, J = 8.9, 2.7 Hz, 1H), 7.53 – 7.46 (m, 2H), 7.44 - 7.37 (m, 2H), 7.15 (dd, J = 8.9, 3.2 Hz, 1H), 4.23 – 3.99 (m, 1H), 3.66 – 3.37 (m, 1H), 2.90 – 2.65 (m, 2H), 2.41 (qd, J = 11.9, 2.8 Hz, 1H), 2.27 – 2.07 (m, 4H), 1.85 – 1.72 (m, 1H), 1.64 – 1.24 (m, 3H), 1.10 – 0.90 (m, 1H).

(S)-6-((6-fluoropyridin-3-yl)oxy)-3-(piperidin-3-ylmethyl)-2-(o-tolyl)quinazolin-4(3H)-one (4c)

The method similar for the preparation of compound **4a** was used, except replacing 4-fluorophenol with 6-fluoropyridin-3-ol. The Boc-protected intermediate was purified via silica gel column chromatography using elution of 30% ethyl acetate in hexane and gave 44% yield. After Boc deprotection, the crude compound was purified via aluminum oxide column chromatography using a gradient elution from 2 to 5% methanol in dichloromethane to give the product in 95% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.25. HRMS (ESI^+) for $C_{26}H_{26}FN_4O_2$ (MH^+), calcd 445.2040, found 445.2047. ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 8.07 (d, J = 2.9 Hz, 1H), 7.79 – 7.72 (m, 2H), 7.70 (d, J = 2.9 Hz, 1H), 7.63 (dd, J = 8.9, 2.9 Hz, 1H), 7.53 – 7.46 (m, 2H), 7.44 – 7.37 (m, 2H), 7.17 (dd, J = 9.2, 2.9 Hz, 1H), 4.22 – 4.00 (m, 1H), 3.65 – 3.38 (m, 1H), 2.86 (d, J = 12.0 Hz, 1H), 2.82 – 2.64 (m, 1H), 2.43 (q, J = 12.0 Hz, 1H), 2.28 – 2.08 (m, 4H), 1.83 – 1.73 (m, 1H), 1.62 – 1.24 (m, 3H), 1.06 – 0.93 (m, 1H).

(S)-6-((6-((5-fluoropyridin-2-yl)oxy)pyridin-3-yl)oxy)-3-(piperidin-3-ylmethyl)-2-(o-tolyl)quinazolin-4(3H)-one (4d)

Compound **4d** was synthesized as a side product during the preparation of compound **4c** with a yield of 34%. UHPLC-MS (waters) method: 10-90% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.48. HRMS (ESI^+) for $C_{31}H_{29}FN_5O_3$ (MH^+), calcd 538.2254, found 538.2255. ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 8.10 (s, 1H), 7.99 (d, J = 2.9 Hz, 1H), 7.86 – 7.79 (m, 1H), 7.72 (d, J = 8.8 Hz, 2H), 7.64 (d, J = 2.9 Hz, 1H), 7.61 (dd, J = 8.8, 2.9 Hz, 1H), 7.52 – 7.45 (m, 2H), 7.44 – 7.36 (m, 2H), 7.20 (d, J = 8.8 Hz, 1H), 7.13 (dd, J = 8.8, 2.9 Hz, 1H), 4.20 – 3.96 (m, 1H), 3.65 – 3.36 (m, 1H), 2.84 (d, J = 11.9 Hz, 1H), 2.81 – 2.62 (m, 1H), 2.47 – 2.34 (m, 1H), 2.25 – 2.06 (m, 4H), 1.81 – 1.69 (m, 1H), 1.60 – 1.24 (m, 3H), 1.04 – 0.92 (m, 1H).

(S)-6-(benzo[d]thiazol-6-yloxy)-3-(piperidin-3-ylmethyl)-2-(o-tolyl)quinazolin-4(3H)-one (4e)

The method similar for the preparation of compound **4a** was used, except replacing 4-fluorophenol with 6-bezothiazolol. The Boc-protected intermediate was purified via silica gel column chromatography using an elution of 25% ethyl acetate in hexane and gave 14% yield. After Boc deprotection, the crude compound was purified via reverse phase chromatography (Isolera One, HS-C18-30g cartridge) with a gradient from 30 to 100 % MeOH in water. The product was lyophilized to give white power in 90% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.43. HRMS (ESI^+) for $C_{28}H_{27}N_4O_2S$ (MH^+), calcd 483.1855, found 483.1855. ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 9.23 (s, 1H), 8.12 (d, J = 8.9 Hz, 1H), 7.82 (d, J = 2.3 Hz, 1H), 7.75 (dd, J = 8.9, 1.4 Hz, 1H), 7.71 – 7.68 (m, 1H), 7.67 – 7.61 (m, 1H), 7.54 – 7.47 (m, 2H), 7.46 – 7.39 (m, 2H), 7.35 (dd, J =

8.9, 2.3 Hz, 1H), 4.34 – 4.03 (m, 1H), 3.78 – 3.32 (m, 1H), 3.26 – 3.09 (m, 2H), 2.89 – 2.78 (m, 1H), 2.77 – 2.51 (m, 1H), 2.29 – 2.18 (m, 3H), 2.10 – 1.94 (m, 1H), 1.88 – 1.76 (m, 1H), 1.63 – 1.31 (m, 2H), 1.23 – 1.05 (m, 1H).

(S)-6-(4-(4-acetylpiperazin-1-yl)phenoxy)-3-(piperidin-3-ylmethyl)-2-(o-tolyl)quinazolin-4(3H)-one (4f)

The method similar for the preparation of compound **4a** was used, except replacing 4-fluorophenol with 1-acetyl-4-(4-hydroxyphenyl)piperazine. The Boc-protected intermediate was purified via reverse phase chromatography (Isolera One, HS-C18-30g cartridge) with a gradient from 0 to 100 % MeOH in water and gave 7% yield. After Boc deprotection, the crude compound was purified via reverse phase chromatography (Isolera One, HS-C18-30g cartridge) with a gradient from 8 to 80 % MeOH in water. The product was lyophilized to give white power in 90% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.38. HRMS (ESI^+) for $C_{33}H_{38}FN_5O_3$ (MH^+), calcd 552.2975, found 552.2968. ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 7.68 (d, J = 9.3 Hz, 1H), 7.59 – 7.52 (m, 2H), 7.52 – 7.46 (m, 2H), 7.46 – 7.38 (m, 2H), 7.13 – 7.02 (m, 4H), 4.34 – 4.01 (m, 1H), 3.78 – 3.34 (m, 5H), 3.25 – 3.07 (m, 6H), 2.89 – 2.51 (m, 2H), 2.27 – 2.18 (m, 3H), 2.18 – 2.13 (m, 3H), 2.02 (s, 1H), 1.89 – 1.76 (m, 1H), 1.63 – 1.32 (m, 2H), 1.23 – 1.06 (m, 1H).

(S)-3-((1-(2-fluoroethyl)piperidin-3-yl)methyl)-6-(4-fluorophenoxy)-2-(o-tolyl)quinazolin-4(3H)-one (5a)

Compound **4a** (160 mg, 0.36 mmol), 1-fluoro-2-idoethane (313 mg, 1.80 mmol), and K_2CO_3 (200 mg, 1.45 mmol) were combined in acetonitrile (10ml) and heated to 70 °C for 8 h. The solid was filtered off, and the filtrate was concentrated under reduced pressure. Saturated aqueous $NaHCO_3$ solution was added, and the product was extracted with EtOAc (2×). The combined organic layers were dried with Na_2SO_4 and filtered. The solvent was removed under reduced pressure, and the crude product was purified via silica gel column chromatography (2% MeOH in dichloromethane) to give 80mg of the product in 45% yield. UHPLC-MS (waters) method: 20-60% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.93. HRMS (ES^+) for $C_{29}H_{30}F_2N_3O_2$ (MH^+), calcd 490.2306, found 490.2308. ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 7.70 (d, J = 8.9 Hz, 1H), 7.62 (d, J = 2.9 Hz, 1H), 7.56 (dd, J = 8.9, 2.9 Hz, 1H), 7.51 – 7.46 (m, 2H), 7.42 – 7.37 (m, 2H), 7.23 – 7.11 (m, 4H), 4.56 – 4.46 (m, 1H), 4.44 – 4.35 (m, 1H), 4.14 – 4.04 (m, 1H), 3.57 – 3.43 (m, 1H), 2.84 – 2.51 (m, 4H), 2.23 (d, J = 2.5 Hz, 3H), 2.00 – 1.84 (m, 2H), 1.82 – 1.22 (m, 4H), 0.93 – 0.76 (m, 1H).

(S)-3-((1-(2-fluoroethyl)piperidin-3-yl)methyl)-6-((5-fluoropyridin-2-yl)oxy)-2-(o-tolyl)quinazolin-4(3H)-one (5b)

The method similar for the preparation of compound **5a** was used, except replacing compound **4a** with **4b**. The compound was purified by reverse phase chromatography (Isolera One, HS-C18-30g cartridge) with a gradient from 30 to 100 % MeOH in water. The product was lyophilized to give white power in 72% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.46. HRMS (ESI^+) for $C_{28}H_{29}F_2N_4O_2$ (MH^+), calcd 491.2259, found 491.2260. ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 7.99 (d, J = 3.2 Hz, 1H), 7.86 (d, J = 2.7 Hz, 1H), 7.71 – 7.63 (m, 2H), 7.57 (dd, J = 8.8, 2.7 Hz, 1H), 7.50 –

7.42 (m, 2H), 7.39 – 7.33 (m, 2H), 7.10 (dd, J = 9.0, 3.2 Hz, 1H), 4.48 (dd, J = 11.5, 5.3 Hz, 1H), 4.36 (dd, J = 11.5, 5.3 Hz, 1H), 4.14 – 4.01 (m, 1H), 3.58 – 3.41 (m, 1H), 2.75 (t, J = 11.0 Hz, 1H), 2.71 – 2.45 (m, 3H), 2.20 (d, J = 2.1 Hz, 3H), 1.90 (t, J = 11.0 Hz, 2H), 1.80 – 1.21 (m, 4H), 0.92 – 0.71 (m, 1H). ^{19}F NMR (376 MHz, methanol-d_4) δ ppm -134.69 – -135.69 (m, 1F), -217.01 – -218.50 (m, 1F)

(S)-3-((1-(2-fluoroethyl)piperidin-3-yl)methyl)-6-((6-fluoropyridin-3-yl)oxy)-2-(o-tolyl)quinazolin-4(3H)-one (5c)

The method similar for the preparation of compound **5a** was used, except replacing compound **4a** with **4c**. The crude compound was purified by reverse phase chromatography (Isolera One, HS-C18-30g cartridge) with a gradient from 5 to 100 % MeOH in water. The product was lyophilized to give white power in 45% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.39. HRMS (ESI$^+$) for $C_{28}H_{29}F_2N_4O_2$ (MH^+), calcd 491.2259, found 491.2259. ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 8.07 (d, J = 3.0 Hz, 1H), 7.78 – 7.72 (m, 2H), 7.69 (d, J = 2.9 Hz, 1H), 7.62 (dd, J = 8.9, 2.9 Hz, 1H), 7.52 – 7.46 (m, 2H), 7.44 – 7.37 (m, 2H), 7.16 (dd, J = 8.8, 3.0 Hz, 1H), 4.56 – 4.46 (m, 1H), 4.46 – 4.33 (m, 1H), 4.11 (dt, J = 13.9, 7.0 Hz, 1H), 3.64 – 3.43 (m, 1H), 2.86 – 2.53 (m, 4H), 2.23 (d, J = 2.9 Hz, 3H), 2.01 – 1.84 (m, 2H), 1.85 – 1.23 (m, 4H), 0.93 – 0.75 (m, 1H). ^{19}F NMR (376 MHz, methanol-d_4) δ ppm -73.85 (s, 1F), -217.04 – -218.61 (m, 1F)

(S)-6-((6-((5-fluoropyridin-2-yl)oxy)pyridin-3-yl)oxy)-3-((1-(2-hydroxyethyl)piperidin-3-yl)methyl)-2-(o-tolyl)quinazolin-4(3H)-one (5d)

Compound **5d** was purified by flash column chromatography (Al_2O) with a gradient elution from 1 to 2 % methanol in dichloromethane and was lyophilized to give the desire product with 23% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.46. HRMS (ESI^+) for $C_{33}H_{33}FN_5O_4$ (MH^+), calcd 582.2517, found 582.2512. ^1H NMR (400 MHz, methanol-d_4) δ ppm 8.10 (dd, *J* = 2.8, 1.4 Hz, 1H), 7.99 (d, *J* = 3.0 Hz, 1H), 7.86 – 7.79 (m, 1H), 7.74 – 7.69 (m, 2H), 7.68 – 7.64 (m, 1H), 7.64 – 7.59 (m, 1H), 7.54 – 7.47 (m, 2H), 7.44 – 7.38 (m, 2H), 7.20 (dd, *J* = 8.8, 4.4 Hz, 1H), 7.14 (dd, *J* = 8.9, 3.1 Hz, 1H), 4.24 – 4.03 (m, 1H), 3.70 – 3.41 (m, 3H), 3.08 – 2.77 (m, 2H), 2.73 – 2.59 (m, 2H), 2.31 – 1.90 (m, 6H), 1.72 – 1.61 (m, 1H), 1.59 – 1.24 (m, 2H), 1.00 – 0.86 (m, 1H).

(S)-6-(benzo[d]thiazol-6-yloxy)-3-((1-(2-fluoroethyl)piperidin-3-yl)methyl)-2-(o-tolyl)quinazolin-4(3H)-one (5e)

The method similar for the preparation of compound **5a** was used, except replacing compound **4a** with **4e**, for synthesis of compound **5e** as a white power with a yield of 47%. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.49. HRMS (ESI^+) for $C_{30}H_{30}FN_4O_2S$ (MH^+), calcd 529.2074, found 529.2073. ^1H NMR (400 MHz, methanol-d_4) δ ppm 9.21 (s, 1H), 8.10 (d, J = 8.9 Hz, 1H), 7.80 (d, J = 2.4 Hz, 1H), 7.72 (d, J = 8.8 Hz, 1H), 7.69 (d, J = 2.8 Hz, 1H), 7.60 (dd, J = 8.8, 2.8 Hz, 1H), 7.52 – 7.45 (m, 2H), 7.43-7.37 (m, 2H), 7.35 (dd, J = 8.9, 2.4 Hz, 1H), 4.58 – 4.49 (m, 1H), 4.47 – 4.35 (m, 1H), 4.15 – 4.03 (m, 1H), 3.58 – 3.45 (m, 1H), 2.88 – 2.55 (m, 4H), 2.22 (d, J = 3.8 Hz, 3H), 2.04 – 1.22 (m,

6H), 0.95 – 0.75 (m, 1H). ^{19}F NMR (376 MHz, methanol-d_4) δ ppm -218.50 (s).-217.80 – -219.41 (m, 1F)

(S)-6-(4-fluorophenoxy)-3-((1-isopropylpiperidin-3-yl)methyl)-2-(o-tolyl)quinazolin-4(3H)-one (5f)

The compound was made according to literature procedure.[23] UHPLC-MS (waters) method: 20-80% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.86. HRMS (ESI$^+$) for $C_{30}H_{33}FN_3O_2$ (MH^+), calcd 486.2557, found 486.2556. ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 7.70 (d, J = 8.9 Hz, 1H), 7.63 (d, J = 2.9 Hz, 1H), 7.57 (dd, J = 8.9, 2.9 Hz, 1H), 7.53 – 7.45 (m, 2H), 7.44 – 7.38 (m, 2H), 7.23 – 7.12 (m, 4H), 4.25 – 4.04 (m, 1H), 3.62 – 3.41 (m, 1H), 2.97 – 2.59 (m, 3H), 2.30 – 1.88 (m, 6H), 1.73 – 1.58 (m, 1H), 1.58 – 1.27 (m, 2H), 1.14 – 0.97 (m, 6H), 0.97 – 0.77 (m, 1H). ^{19}F NMR (376 MHz, methanol-d_4) δ ppm -120.24 – -120.39 (m, 1F)

(S)-6-(benzo[d]thiazol-6-yloxy)-3-((1-isopropylpiperidin-3-yl)methyl)-2-(o-tolyl)quinazolin-4(3H)-one (5g)

The method similar for the preparation of compound **5e** was used, except replacing compound 1-fluoro-2-iodoethane with 2-bromopropane. Compound **5e** was purified by reverse phase chromatography (Isolera One, HS-C18-30g cartridge) with a gradient elution from 5 to 80 % methanol (0.1% TFA) in water (0.1% TFA), and was lyophilized to give the desire product in 90% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.95. HRMS (ESI$^+$) for $C_{31}H_{33}N_4O_2S$ (MH^+), calcd 525.2324, found 525.2327. ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 9.23 (s, 1H), 8.13 (d, J = 8.9 Hz, 1H), 7.84 (d, J = 2.4 Hz, 1H), 7.74 (d, J = 8.9 Hz, 1H), 7.71 (d, J = 2.9 Hz, 1H), 7.64 (dd, J = 8.9, 2.9 Hz, 1H), 7.53 – 7.47

(m, 2H), 7.45 – 7.39 (m, 2H), 7.38 (dd, J = 8.9, 2.4 Hz, 1H), 4.21 – 4.03 (m, 1H), 3.56 – 3.41 (m, 1H), 2.91 – 2.52 (m, 3H), 2.24 (d, J = 4.0 Hz, 3H), 2.18 – 2.05 (m, 1H), 2.00 – 1.72 (m, 2H), 1.67 – 1.58 (m, 1H), 1.44 – 1.27 (m, 2H), 1.06 – 0.94 (m, 6H), 0.93 – 0.81 (m, 1H).

(S)-6-((6-fluoropyridin-3-yl)oxy)-3-((1-(2-hydroxyethyl)piperidin-3-yl)methyl)-2-(o-tolyl)quinazolin-4(3H)-one (5h)

Compound **4c** (200mg, 0.45mmol) was dissolved in dichloromethane (60ml), 2-bromoethanol (288mg, 2.25mmol) was added, followed by TEA (227mg, 2.25mmol). The solution was stirred at room temperature for 8 days. Saturated $NaHCO_3$ was added into reaction solution and the organic layer was washed, collected, dried over Mg_2SO_4. The crude product was purified by reverse phase chromatography (Isolera One, HS-C18-30g cartridge) with a gradient elution from 5 to 50 % acetonitrile in water, and was lyophilized to give the desire product in 73% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.42. HRMS (ESI^+) for $C_{28}H_{30}FN_4O_3$ (MH^+), calcd 489.2302, found 489.2312. ^1H NMR (599 MHz, methanol-*d*$_4$) δ ppm 8.04 (d, J = 3.2 Hz, 1H), 7.91 (d, J = 2.7 Hz, 1H), 7.72 (d, J = 8.8 Hz, 2H), 7.62 (dd, J = 8.8, 2.7 Hz, 1H), 7.52 – 7.46 (m, 2H), 7.43 – 7.38 (m, 2H), 7.15 (dd, J = 9.0, 3.2 Hz, 1H), 4.18 – 4.08 (m, 1H), 3.61 – 3.47 (m, 3H), 2.83 – 2.76 (m, 1H), 2.74 – 2.61 (m, 1H), 2.47 – 2.41 (m, 2H), 2.24 (d, J = 5.5 Hz, 3H), 1.97 – 1.79 (m, 2H), 1.68 – 1.28 (m, 4H), 0.92 – 0.79 (m, 1H). 19F NMR (376 MHz, methanol-*d*$_4$) δ ppm -135.13 – --135.29 (m, 1F)

(S)-6-(benzo[d]thiazol-6-yloxy)-3-((1-(2-hydroxyethyl)piperidin-3-yl)methyl)-2-(o-tolyl)quinazolin-4(3H)-one (5i)

The method similar for the preparation of compound **5h** was used, except replacing compound **4b** with **4e**. Compound **5i** was purified by reverse phase chromatography (Isolera One, HS-C18-30g cartridge) with a gradient elution from 0 to 50 % acetonitrile in water, and was lyophilized to give the desire product in 80% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.48. HRMS (ESI^+) for $C_{30}H_{31}FN_4O_3S$ (MH^+), calcd 527.2117, found 527.2112. ^{1}H NMR (400 MHz, methanol-*d*$_4$) δ ppm 9.19 (s, 1H), 8.09 (d, J = 8.9 Hz, 1H), 7.79 (d, J = 2.4 Hz, 1H), 7.70 (d, J = 8.9 Hz, 1H), 7.67 (d, J = 2.8 Hz, 1H), 7.59 (dd, J = 8.9, 2.8 Hz, 1H), 7.50 – 7.44 (m, 2H), 7.40 – 7.35 (m, 2H), 7.33 (dd, J = 8.9, 2.4 Hz, 1H), 4.18 – 3.99 (m, 1H), 3.62 – 3.39 (m, 3H), 2.94 – 2.66 (m, 2H), 2.58 – 2.45 (m, 2H), 2.20 (d, J = 5.7 Hz, 3H), 2.13 – 1.91 (m, 2H), 1.80 – 1.18 (m, 4H), 0.93 – 0.75 (m, 1H)

3-(((3S)-1-(2-fluoropropanoyl)piperidin-3-yl)methyl)-6-((5-fluoropyridin-2-yl)oxy)-2-(o-tolyl)quinazolin-4(3H)-one (5j)

2-Fluoropropionic acid (18.4mg, 0.22mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (38.4mg, 0.22mmol) and hydroxybenzotriazole (HOBt) (5mg,0.033mmol) were added into dichloromethane (5ml). The solution was stirred for 5 min. Compound **4b** (44mg, 0.099mmol) was then added. The resulting solution was stirred at room temperature for 24 hours. The solvent was removed and the product was subjected to reverse phase chromatography (Isolera One, HS-C18-30g cartridge) with a gradient elution of 0-75% acetonitrile in water. The purified compound was lyophilized to give 35mg white power in 69% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 2.09.

HRMS (ESI$^+$) for $C_{29}H_{28}F_2N_4O_3Na$ (M+Na$^+$), calcd 541.2027, found 541.2031. ^{1}H NMR (400 MHz, methanol-*d*$_4$) δ ppm 7.94 (d, J = 2.9 Hz, 1H), 7.81 (d, J = 2.7 Hz, 1H), 7.65 – 7.56 (m, 2H), 7.52 (dd, J = 8.8, 2.7 Hz, 1H), 7.45 – 7.36 (m, 2H), 7.34 – 7.26 (m, 2H), 7.05 (dd, J = 8.9, 2.9 Hz, 1H), 5.37 – 4.98 (m, 1H), 4.23 – 3.87 (m, 2H), 3.75 – 3.32 (m, 2H), 2.97 – 2.23 (m, 2H), 2.19 – 2.09 (m, 3H), 1.74 – 1.00 (m, 8H). 19F NMR (376 MHz, methanol-*d*$_4$) δ ppm -134.46 – -135.77 (m, 1F), -177.27 – -179.90 (m, 1F)

6-bromo-2-(2-fluorophenyl)-4H-benzo[d][1,3]oxazin-4-one (6)

To a solution of 2-amino-5-bromobenzoic acid (5g, 23mmol) and triethylamine (10ml; 70mmol) in dichloromethane (50ml) was added 2-fluorobenzoyl chloride (4.4g, 28mmol) drop wise at room temperature. After the suspension was stirred for 12 hours, the solvent was removed and acetic acid anhydride (45ml) was added. The resulting suspension was stirred at 50 ^{o}C for 2 hours. After cooling down, the precipitate was collected by filtration and washed with ethanol (100ml). The product was dried under vacuum for an hour to give 5.9g light yellow powder in 80% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run; calcd m/z 320.0 (MH$^+$), found m/z 320.0, RT (min) 2.32.

(R)-tert-butyl 3-((5-bromo-2-(2-fluorobenzamido)benzamido)methyl)piperidine-1-carboxylate (7)

Compound **7** (2g, 6.3mmol) was dissolved in toluene (20ml). (R)-1-Boc-3-(aminomethyl) piperidine (1.62 g, 7.6 mmol) was added and the solution was refluxed at 125 °C for 16 h. The solvent was removed and the crude product was purified via silica gel column chromatography using 100% ethyl acetate to give the product as yellow oil in 89% yield. UHPLC-MS (waters)

method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 2.57, calcd m/z 534.1 (MH^+), found m/z 534.2.

(R)-tert-butyl 3-((6-bromo-2-(2-fluorophenyl)-4-oxoquinazolin-3(4H)-yl)methyl)piperidine-1-carboxylate(8)

Compound **7** (1.50 g, 2.80 mmol) was added to microwave reaction vessel, followed by ethylene glycol (6 mL) and LiOH (0.14g, 5.60 mmol). The resulting mixture was subjected to microwave irradiation with stirring for 30 minutes at 130°C, cooled to room temperature, and diluted with dichloromethane (50ml) and water (50ml). The organic layer was washed with brine (50 mL) twice. The combined organic layer was dried over Mg_2SO_4 and then concentrated under reduced pressure. The crude compound was purified via silica gel column chromatography using an elution of 25% ethyl acetate in hexanes to give 0.95g of the product as yellow oil in 66% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 2.81, calcd m/z 516.1(MH^+), found m/z: 516.1.

(S)-2-(2-fluorophenyl)-6-((5-fluoropyridin-2-yl)oxy)-3-(piperidin-3-ylmethyl)quinazolin-4(3H)-one (9a)

Under a nitrogen atmosphere, compound **8** (307 mg, 0.60 mmol), copper(I) chloride (30 mg, 0.30 mmol), 2,2,6,6-tetramethylheptane-3,5-dione (TMHD) (55 mg, 0.30 mmol),5-fluoro-2-pyridinol (81mg, 0.72mmol) and Cs_2CO_3 (391 mg, 1.20 mmol) were added to 1-methyl-2-pyrrolidone (1 mL). The reaction mixture was stirred for 16 h at 110 °C, cooled, and filtered through Celite. The filtrate was concentrated under reduced pressure and purified via silica gel column chromatography using a gradient elution from 20 to 25% ethyl acetate in hexanes to give 103mg

of Boc-protected intermediate with 31% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run; Calculated m/z 549.2313(MH^+), Found m/z: 549.2064; RT (min) 2.56. The Boc-protected intermediate (103 mg, 0.19 mmol) was dissolved in dichloromethane (10 mL). TFA (2 mL) was added. The solution was stirred for 3 hours at room temperature. The solvent was removed and saturated aqueous $NaHCO_3$ solution was added. The product was then extracted with EtOAc (2×). The combined organic layers were dried with Na_2SO_4 and filtered, and the solvent was removed under reduced pressure, and the crude compound was purified by reverse phase chromatography (Isolera One, HS-C18-30g cartridge) with a gradient from 20 to 90 % methanol in water. The product was lyophilized to yield 76 mg white powder in 90% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.39. HRMS (ESI^+) for $C_{25}H_{23}F_2N_4O_2$ (MH^+), calcd 449.1789, found 449.1780. ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 8.05 (d, J = 3.1 Hz, 1H), 7.90 (d, J = 2.7 Hz, 1H), 7.77 – 7.62 (m, 5H), 7.46 – 7.40 (m, 1H), 7.36 (t, J = 9.2 Hz, 1H), 7.16 (dd, J = 9.0, 3.1 Hz, 1H), 4.27 – 4.01 (m, 1H), 3.79 – 3.52 (m, 1H), 2.89 – 2.66 (m, 2H), 2.46 – 2.31 (m, 1H), 2.27 – 2.02 (m, 1H), 1.84 – 1.69 (m, 1H), 1.61 – 1.50 (m, 1H), 1.46 – 1.21 (m, 2H), 1.07 – 0.85 (m, 1H). 19F NMR (376 MHz, methanol-d_4) δ ppm -114.29 – -115.32 (m, 1F), -134.92 – -135.09 (m, 1F)

(S)-6-(benzo[d]thiazol-6-yloxy)-2-(2-fluorophenyl)-3-(piperidin-3-ylmethyl)quinazolin-4(3H)-one (9b)

The method similar for the preparation of compound **9a** was used, except replacing 5-fluoro-2-pyridinol with 6-benzothiazolol. The Boc-protected intermediate was purified via silica gel column chromatography using a gradient elution from 25 to 50% ethyl acetate in hexane and

gave 18% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 2.62, calcd m/z 587.2 (MH^+), found m/z: 587.1. After Boc deprotection, the crude compound was purified by reverse phase chromatography (Isolera One, HS-C18-30g cartridge) with a gradient from 0 to 100 % methanol in water. The product was lyophilized to give the product as white powder in 57% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.43. HRMS (ESI^+) for $C_{27}H_{24}FN_4O_2S$ (MH^+), calcd 487.1604, found 487.1605. ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 9.22 (s, 1H), 8.12 (d, J = 8.8 Hz, 1H), 7.83 (d, J = 2.4 Hz, 1H), 7.75 (d, J = 8.8 Hz, 1H), 7.71 – 7.59 (m, 4H), 7.46 – 7.39 (m, 1H), 7.39 – 7.31 (m, 2H), 4.23 – 3.98 (m, 1H), 3.78 – 3.52 (m, 1H), 2.87 – 2.65 (m, 2H), 2.43 – 2.31 (m, 1H), 2.22 – 2.00 (m, 1H), 1.81 – 1.68 (m, 1H), 1.58 – 1.49 (m, 1H), 1.44 – 1.20 (m, 2H), 1.04 – 0.86 (m, 1H). ^{19}F NMR (376 MHz, methanol-d_4) δ ppm -114.05 – -114.49 (m, 1F)

(S)-2-(2-fluorophenyl)-6-((5-fluoropyridin-2-yl)oxy)-3-((1-isopropylpiperidin-3-yl)methyl)quinazolin-4(3H)-one (10a)

Compound 9a was treated with 2-bromopropane and potassium carbonate in acetonitrile at 70°C for 16h. The crude compound was purified by reverse phase chromatography (Isolera One, HS-C18-30g cartridge) with a gradient from 5 to 70 % methanol in water. The product was lyophilized to give the product as white powder in 47% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.59. HRMS (ESI^+) for $C_{28}H_{29}F_2N_4O_2$ (MH^+), calcd 491.2259, found 491.2262. ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 8.05 (d, *J* = 3.1 Hz, 1H), 7.93 (d, *J* = 2.7 Hz, 1H), 7.78 – 7.63 (m, 5H), 7.49 – 7.34 (m, 2H), 7.17 (dd, *J* = 9.0, 3.5 Hz, 1H), 4.33 – 4.02 (m, 1H), 3.97 – 3.65 (m, 1H), 3.52 – 3.38 (m, 1H),

3.27 – 3.18 (m, 1H), 2.86 – 2.60 (m, 2H), 2.28 – 2.12 (m, 1H), 1.91 (t, *J* = 13.4 Hz, 1H), 1.69 – 1.41 (m, 2H), 1.39 – 1.01 (m, 8H). ^{19}F NMR (376 MHz, methanol-*d*$_4$) δ ppm -114.55 – -115.80 (m, 1F), -136.10 – -136.17 (m, 1F)

(S)-6-(benzo[d]thiazol-6-yloxy)-2-(2-fluorophenyl)-3-((1-isopropylpiperidin-3-yl)methyl)quinazolin-4(3H)-one (10b)

The method similar for the preparation of compound **10a** was used, except replacing **9a** with **9b**. The crude compound was purified by reverse phase chromatography (Isolera One, HS-C18-30g cartridge) with a gradient from 25 to 80 % methanol in water. The product was lyophilized to give the product as white powder in 52% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.54. HRMS (ESI$^+$) for $C_{30}H_{30}FN_4O_2S$ (MH^+), calcd 529.2074, found 529.2071. ^{1}H NMR (400 MHz, methanol-*d*$_4$) δ ppm 9.21 (s, 1H), 8.11 (d, J = 8.9 Hz, 1H), 7.82 (d, J = 2.3 Hz, 1H), 7.74 (d, J = 8.9 Hz, 1H), 7.69 (d, J = 2.8 Hz, 1H), 7.68 – 7.63 (m, 2H), 7.61 (dd, J = 8.9, 2.8 Hz, 1H), 7.42 (t, J = 7.5 Hz, 1H), 7.38 – 7.32 (m, 2H), 4.17 – 4.03 (m, 1H), 3.75 – 3.61 (m, 1H), 2.86 – 2.59 (m, 3H), 2.22 – 2.05 (m, 1H), 1.99 – 1.75 (m, 2H), 1.68 – 1.56 (m, 1H), 1.55 – 1.32 (m, 2H), 1.04 – 0.95 (m, 6H), 0.91 – 0.73 (m, 1H). ^{19}F NMR (376 MHz, methanol-*d*$_4$) δ ppm -113.78 – -114.26 (m, 1F)

(S)-6-(benzo[d]thiazol-6-yloxy)-3-((1-(2-fluoroethyl)piperidin-3-yl)methyl)-2-(2-fluorophenyl)quinazolin-4(3H)-one (10c)

The method similar for the preparation of compound **10b** was used, except replacing 2-bromopropane with **9b**. The crude compound was purified by reverse phase chromatography (Isolera One, HS-C18-30g cartridge) with a gradient (0.1%TFA) from 5 to 80 % methanol in

water. The product was lyophilized to give the product as TFA salt in 48% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.55. HRMS (ESI^+) for $C_{29}H_{27}F_2N_4O_2S$ (MH^+), calcd 533.1823, found 533.1824. ^1H NMR (400 MHz, methanol-d_4) δ ppm 9.23 (s, 1H), 8.13 (d, J = 8.9 Hz, 1H), 7.83 (d, J = 2.4 Hz, 1H), 7.76 (d, J = 8.9 Hz, 1H), 7.71 – 7.61 (m, 4H), 7.43 (t, J = 7.3 Hz, 1H), 7.40 – 7.33 (m, 2H), 4.74 – 4.64 (m, 1H), 4.61 – 4.52 (m, 1H), 4.31 – 4.02 (m, 1H), 3.87 – 3.58 (m, 1H), 3.21 – 2.93 (m, 4H), 2.60 – 1.98 (m, 3H), 1.82 – 1.31 (m, 3H), 1.14 – 0.84 (m, 1H). ^{19}F NMR (376 MHz, methanol-d_4) δ ppm -114.77 – -116.91 (m, 1F), -219.89 – -222.07 (m, 1F)

(R)-tert-butyl 3-((4-oxo-2-(o-tolyl)-6-(tributylstannyl)quinazolin-3(4H)-yl)methyl)piperidine-1-carboxylate (11)

Tetrakis(triphenylphosphine)palladium(0) (127mg, 0.13mmol) and Bis(tributyltin) (1.50g, 2.56 mmol) was added to compound **3** (655mg, 1.28mmol) in in toluene (50ml). The resulting mixture was heated to 110°C for 18h. The reaction mixture was cooled down and passed through Celite. The solvent was removed under reduced pressure. The crude compound was purified via silica gel column chromatography using an elution of 10% ethyl acetate in hexanes to give 0.42g of the product as oil in 56% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min): 1.74.

(R)-tert-butyl 3-((6-(4-fluorobenzoyl)-4-oxo-2-(o-tolyl)quinazolin-3(4H)-yl)methyl)piperidine-1-carboxylate (12)

4-Fluorobenzoyl chloride (115 mg, 0.72 mmol) and bis(triphenylphosphine)palladium(II) dichloride (35.1 mg, 0.05 mmol) were added to compound **11** (347 mg, 0.48 mmol) in toluene (5ml). The resulting mixture was heated to 110^{o}C for 6h. The reaction mixture was cooled down and passed through Celite. The solvent was removed under reduced pressure. The crude product was purified by reverse phase chromatography (Isolera One, HS-C18-30g cartridge) with a gradient from 20 to 90 % acetonitrile in water. The product was lyophilized to give 110 mg of the product as white powder in 42% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min): 2.73, calcd m/z 556.3 (MH^+), found m/z 556.3.

(S)-6-(4-fluorobenzoyl)-3-(piperidin-3-ylmethyl)-2-(o-tolyl)quinazolin-4(3H)-one (13)

Compound **12** (50 mg, 0.09 mmol) was dissolved in dichloromethane (5 mL). TFA (1 mL) was added. The solution was stirred for 3 hours at room temperature. The solvent was removed and saturated aqueous $NaHCO_3$ solution was added. The product was then extracted with EtOAc (2×). The combined organic layers were dried with Na_2SO_4 and filtered, and the solvent was removed under reduced pressure, and the crude compound was purified by reverse phase chromatography (Isolera One, HS-C18-30g cartridge) with a gradient from 10 to 95 % acetonitrile (0.1% TFA) in water (0.1% TFA). The product was lyophilized to yield 34 mg white powder in 83% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min): 1.55. HRMS (ESI^+) for $C_{28}H_{27}FN_3O_2$ (MH^+), calcd 456.2087, found 456.2075. ^{1}H NMR (400 MHz, methanol- d_4) δ ppm 8.61 (d, J = 1.8 Hz, 1H), 8.28 – 8.21 (m, 1H), 7.95 – 7.88 (m, 2H), 7.81 (dd, J = 8.5, 1.3 Hz, 1H), 7.56 – 7.50 (m, 2H), 7.49 – 7.40 (m, 2H), 7.35 – 7.28 (m,

2H), 4.37 – 4.07 (m, 1H), 3.84 – 3.40 (m, 1H), 3.27 – 3.14 (m, 2H), 2.91 – 2.55 (m, 2H), 2.28 (d, J = 13.3 Hz, 3H), 2.16 – 2.00 (m, 1H), 1.92 – 1.78 (m, 1H), 1.68 – 1.36 (m, 2H), 1.27 – 1.09 (m, 1H). ^{19}F NMR (376 MHz, methanol- *d*$_4$) δ ppm -106.97 – -107.12 (m, 1F)

(S)-6-(4-fluorobenzoyl)-3-((1-isopropylpiperidin-3-yl)methyl)-2-(o-tolyl)quinazolin-4(3H)-one (14)

Compound **13** (10 mg, 0.02 mmol), 2-bromopropane (20 mg, 0.22 mmol), and K_2CO_3 (21 mg, 0.22 mmol) were combined in acetonitrile (2 ml) and heated to 70 °C for 8 h. The solid was filtered off, and the filtrate was concentrated under reduced pressure. Purification was performed by preparative reverse-phase C18 HPLC (gradient 35-95% acetonitrile in water, 0.1% TFA). The product was lyophilized to yield 9 mg white powder in 75% yield. UHPLC-MS (waters) method: 5-95% acetonitrile (0.1% TFA) in water (0.1% TFA), 3 min run, RT (min) 1.66. HRMS (ESI$^+$) for $C_{31}H_{33}FN_3O_2$ (MH^+), calcd 498.2557, found 498.2559. ^{1}H NMR (400 MHz, methanol- *d*$_4$) δ ppm 8.66 – 8.59 (m, 1H), 8.25 (dd, J = 8.5, 2.0 Hz, 1H), 7.96 – 7.88 (m, 2H), 7.86 – 7.78 (m, 1H), 7.59 – 7.49 (m, 2H), 7.49 – 7.38 (m, 2H), 7.36 – 7.27 (m, 2H), 4.35 – 4.13 (m, 1H), 3.73 – 3.34 (m, 2H), 3.26 – 3.08 (m, 1H), 2.88 – 2.53 (m, 2H), 2.28 (d, J = 17.2 Hz, 3H), 2.23 – 2.12 (m, 1H), 1.97 – 1.84 (m, 1H), 1.68 – 1.55 (m, 1H), 1.44 – 1.33 (m, 1H), 1.33 – 1.03 (m, 8H). ^{19}F NMR (376 MHz, methanol- *d*$_4$) δ ppm -106.96 – -107.30 (m, 1F).

(S)-6-(4-fluorobenzyl)-3-(piperidin-3-ylmethyl)-2-(o-tolyl)quinazolin-4(3H)-one (16)

The method similar for the preparation of compound **12** was used, except replacing 4-Fluorobenzoyl chloride with 4-Fluorobenzyl bromide. The crude product was purified by reverse

phase chromatography (Isolera One, HS-C18-30g cartridge) with a gradient from 20 to 90 % acetonitrile in water to give compound **15**. It was treated with TFA in dichloromethane for 3 hours. The crude compound was purified by HPLC with a gradient from 20 to 80 % acetonitrile (0.1% TFA) in water (0.1% TFA). HPLC-MS (waters) method: 30-95% acetonitrile (0.1% TFA) in water (0.1% TFA), RT (min): 9.83. HRMS (ESI^+) for $C_{28}H_{29}FN_3O$ (MH^+), calcd 442.2295, found 442.2280. ^1H NMR (400 MHz, methanol- d_4) δ ppm 8.11 (s, 1H), 7.77 (d, J = 8.4 Hz, 1H), 7.65 (d, J = 8.4 Hz, 1H), 7.56 – 7.47 (m, 2H), 7.48 – 7.39 (m, 2H), 7.33 – 7.25 (m, 2H), 7.10 – 7.00 (m, 2H), 4.40 – 4.26 (m, 1H), 4.17 (s, 2H), 3.46 – 3.37 (m, 1H), 3.27 – 3.22 (m, 1H), 2.92 – 2.82 (m, 1H), 2.81 – 2.56 (m, 1H), 2.26 (d, J = 12.6 Hz, 3H), 2.12 – 1.99 (m, 1H), 1.93 – 1.81 (m, 1H), 1.62 – 1.55 (m, 1H), 1.46 – 1.39 (m, 1H), 1.25 – 1.12 (m, 1H), 0.98 (d, J = 3.4 Hz, 1H).

(S)-6-(4-fluorobenzoyl)-3-((1-isopropylpiperidin-3-yl)methyl)-2-(o-tolyl)quinazolin-4(3H)-one (17)

The method similar for the preparation of compound **14** was used. The crude compound was purified by HPLC with a gradient from 10 to 95 % acetonitrile (0.1% TFA) in water (0.1% TFA). HPLC-MS (waters) method: 40-95% acetonitrile (0.1% TFA) in water (0.1% TFA), RT (min): 8.52. HRMS (ESI^+) for $C_{31}H_{35}FN_3O$ (MH^+), calcd 484.2764, found 484.2762. ^1H NMR (400 MHz, methanol- d_4) δ ppm 8.12 (d, J = 2.0 Hz, 1H), 7.77 (dt, J = 8.4, 1.8 Hz, 1H), 7.65 (dd, J = 8.4, 1.8 Hz, 1H), 7.57 – 7.49 (m, 2H), 7.50 – 7.39 (m, 2H), 7.31 – 7.26 (m, 2H), 7.06 (t, J = 8.4 Hz, 2H), 4.32 – 4.07 (m, 3H), 3.71 – 3.40 (m, 3H), 3.15 – 2.56 (m, 3H), 2.26 (d, J = 17.2 Hz, 3H), 2.23 – 2.12 (m, 1H), 2.00 – 1.87 (m, 1H), 1.69 – 1.60 (m, 1H), 1.47 – 0.97 (m, 8H).

Radiochemistry

Production of [^{18}F]Fluoride

The [$^{18}F^-$]anion was produced by a GE Healthcare PETtrace 880 cyclotron (16.5 MeV) (St. Joseph's Health Care London Ontario, Canada) as a result of the $^{18}O(p,n)^{18}F$ reaction involving proton bombardment of [^{18}O]H_2O. A Waters Sep-Pak® Accell™ PlusLight (46 mg) QMA Carbonate cartridge was pre-activated by slowly treating with EtOH (10 ml) and Milli-Q® water (10 ml) and then flushing with air. The radioactive [$^{18}F^-$] anion was then trapped by drawing up the [^{18}O]H_2O solution containing it through the Sep-Pak.

Synthesis of [^{18}F] fluoroethyl tosylate

To potassium carbonate (3.0 mg) and Kryptofix 222 (10.0 mg) was added water (200 µl) and MeCN (800 µl) and the resulting solution used to elute the Sep-Pak® containing [^{18}F]fluoride into a glass vial. The mixture was dried azeotropically (120 °C). The drying step was repeated twice more after the drop-wise addition of anhydrous MeCN (1 ml). Ethylene glycol ditosylate (6.5 mg in 0.5 mL of MeCN) was added to the aforementioned mixture, and the mixture was heated at 125^{o}C for 10 min under sealed conditions. The reaction mixture was cooled, diluted with 6 ml hexane/diethyl ether (3:1) and loaded onto a Sep-Pak Silica Plus column (Waters). The last 3ml of eluate was collected, and the cartridge was then eluted with 10 mL of hexane/diethyl ether (3:1). Eluate was collected and evaporated on a spin-vap (HI volatile, pressure: 100 psi, temp.: 36^{o}C).

Synthesis of [^{18}F]5b

The [^{18}F] fluoroethyl tosylate in 0.5ml MeCN was transferred to the second reactor, which had been preloaded with **4b** (1 mg) and Cs_2CO_3(15 mg). The reaction mixture was heated at 150^{o}C for 20 min. After cooling, saturated K_2CO_3 solution (10ml) was added and it was passed through sep-pak C18 plus cartridge, followed by 5ml water. The product was eluted out with 1.5ml MeCN. Azeotropically removed 0.5ml MeCN and 0.5ml water (0.5ml,0.1%TFA) was added. The radiolabelled compound was purified by semi-prep HPLC using 35-50% MeCN in Water (0.1% TFA, flow rate: 4.5ml/min, 15min run, 2min wash).

Synthesis of [^{18}F]5e

The [^{18}F] fluoroethyl tosylate in 0.5ml MeCN was transferred to the second reactor, which had been preloaded with **4e** (1 mg) and Cs_2CO_3(15 mg). The reaction mixture was heated at 140^{o}C for 20 min. After cooling, saturated K_2CO_3 solution (10ml) was added and it was passed through sep-pak C18 plus cartridge, followed by 5ml water. The product was eluted out with 1.5ml MeCN. Azeotropically removed 0.5ml MeCN and 0.5ml water (0.5ml,0.1%TFA) was added. The radiolabelled compound was purified by semi-prep HPLC using 40% MeCN in Water (0.1% TFA, flow rate: 4.5ml/min, 17min run, 2min wash).

Radioligand binding assay

The affinity for GHS-R1a was determined using a radioligand binding assay. Assays were performed using GHS-R1a transfected HEK293 cells as the receptor source and human [^{125}I-His^{9}]ghrelin(1-28) (PerkinElmer Inc.) as the competitive radioligand. Human ghrelin(1-28)

(purchased from Abcam) was used as a reference to ensure the validity of the results. Test peptides at various concentrations and [^{125}I-His^{9}]ghrelin (approximately 15 pM per assay tube) were mixed in binding buffer (25 mM HEPES, 5 mM magnesium chloride, 1 mM calcium chloride, 2.5 mM EDTA, and 0.4% BSA, pH 7.4). The HEK293 cells (50,000 cells per assay tube) was added to the assay tube containing test peptides and [^{125}I]-ghrelin. The resulting suspension was incubated for 20 min with shaking (550 rpm). Unbound [^{125}I]-ghrelin was washed off and the amount of [^{125}I-His^{9}]ghrelin bound to GHS-R1a was measured by a Gamma counter (1470 WIZARD gamma counter/Wallac). All experiments (except for 5j and 9a) were performed in triplicate and repeated at least two times independently. The IC_{50} was determined using a logistic nonlinear regression analysis.

Ligand conformation analysis

All structures were initially constructed by DS viewer 3.5 (http://accelrys.com/). The structure calculation was performed using *ab* initio method using HF/6-31G(g) basis set. The results with no calculated negative energies ensure a global minimum has been established. The calculations were performed with Gaussian 09.[43] MD simulations were performed using the AMBER 14.[44] The partial atomic charges for ligands were calculated by using AM1-BCC method. Other parameters for ligands were taken from the Generalized Amber Force field (GAFF). The final systems were subjected to initial minimization and were then maintained at a temperature of 300 K. Langevin thermostat was used for temperature regulation. Generalized Born implicit solvent model was used with a distance cut-off of 12 Å. The calculation was carried out with the SANDER module. A total of 1000 snapshots were taken from the simulation trajectory for the

dihedral angle analysis. All calculations were performed on high-performance computing (HPC) facilities within the Compute Canada network.[45]

Molecular docking study

All docking studies were performed using Autodock Vina (ver. 1.1.2).[46] The homology model of GHS-R1a created recently in our lab based on the homologous GPCR structures (PDB ID: 4BWB, 3ZEV, 4BUO, 4BV0, 4GRV, 4EA3) was used as the receptor in the docking study.[32] The 3D structures of the small molecules were sketched using DS viewer 3.5. Autodock Tools (ver. 1.5.6) was used to convert all the structure file to pdbqt format.[47] The key residues surrounding the orthostatic binding pocket, including Glu124, Arg102, Phe286, Phe119, Arg283, Gln120, Phe309, Phe279, Asp99, Tyr313, Ser123, Tyr106, Gln105 and Asn305, were treated as fully flexible during the docking calculation. The docking parameters were set as follow: size_x = 60; size_y = 60; size_z = 60; exhaustiveness = 20. Other parameters were left as default. The docked poses with the most favorable binding free energy and where the positively charged amine of quinazolinone derivatives is within 5 Å of the carboxylate of Glu124 were selected.

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ABBREVIATIONS

PET, positron emission tomography; SPECT, single photon emission computed tomography; MRI, magnetic resonance imaging; GHS-R1a, growth hormone secretagogue receptor subtype 1a; GHS-R1b, growth hormone secretagogue receptor subtype 1b; [^{125}I]-ghrelin. Gly-Ser-Ser(n-Octanoyl)-Phe-Leu-Ser-Pro-Glu-[^{125}I]His-Gln-Arg-Val-GlnGln-Arg-Lys-Glu-Ser-Lys-Lys-Pro-Pro-Ala-Lys-Leu-Gln-Pro-Arg; HEPES, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; K_{222}, kryptofix 2.2.2; SHARCNET, The Shared Hierarchical Academic Research Computing Network;

SUPPORTING INFORMATION

Supporting Information is available free of charge on the ACS Publications website.

Scheme S1: Proposed radiolabeling approachs for secondary amine bearing molecules and aromatics

Scheme S2: Synthesis of nitro-bearing precursor **23** for radiolabeling of **10b**

Figure S1: The unsuccessful radiolabeling of **10b**

Scheme S3: Synthesis of nitro-bearing precursor **25** for radiolabeling of **14**

Figure S2: The unsuccessful radiolabeling of **14**

Figure S3: VT NMR spectrum of compound **4e**

Table S1: Calculated gas phase total energies

Table S2: Compound purities determined by U-HPLC chromatogram

Table S3: Representative binding curves

Figure S4: UHPLC Chromatograms

Figure S5: 1H and 19F NMR spectra

Atomic coordinates of the GHS-R1a-4e, GHS-R1a-5e, GHS-R1a-5g, GHS-R1a-5i, GHS-R1a-10b complex. Authors will release the atomic coordinates upon article publication.

Molecular formula strings is available.

Corresponding Author Information

∗ Corresponding author. London Regional Cancer Program, Lawson Health Research Institute, 790 Commissioners Road East, London, Ontario N6A 4L6, Canada. Email address: lluyt@uwo.ca (L.G. Luyt)

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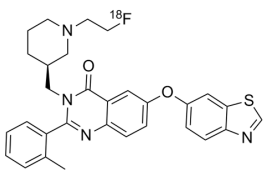
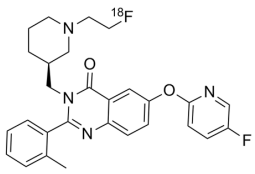
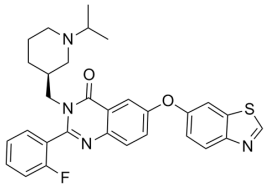
TOC



5e IC_{50} = 9.3 nM
RCY = 7.0 ± 0.6 %
Radio-purity > 99%



5b IC_{50} = 20.6 nM
RCY = 10.3 ± 0.8 %
Radio-purity > 99%



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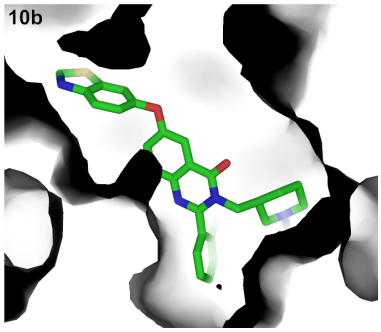

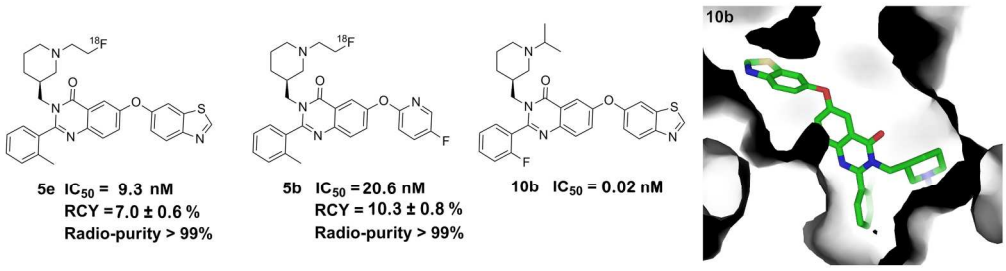

TOC

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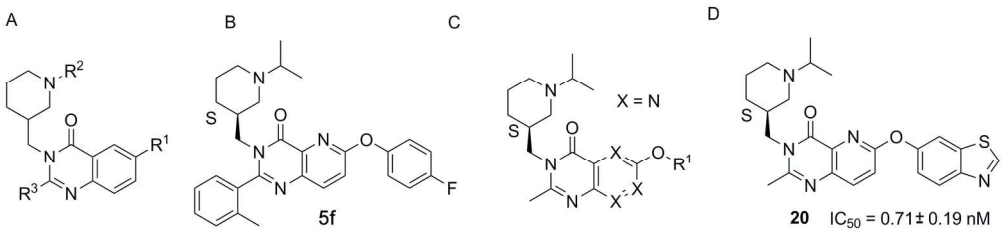

Figure 1

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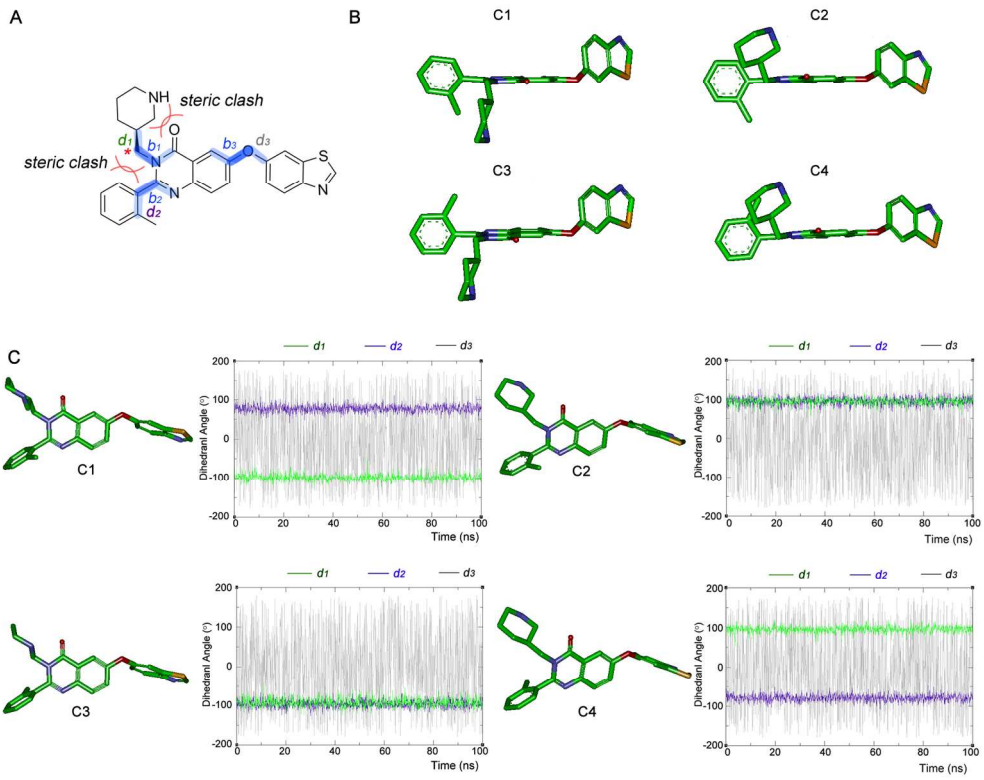

Figure 2

176x139mm (300 x 300 DPI)

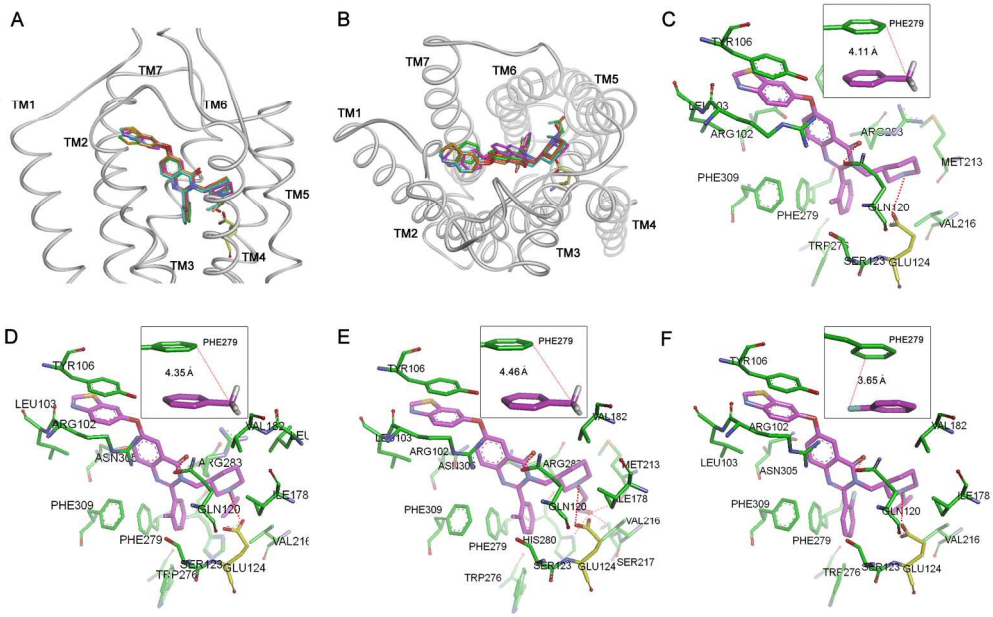

Figure 3

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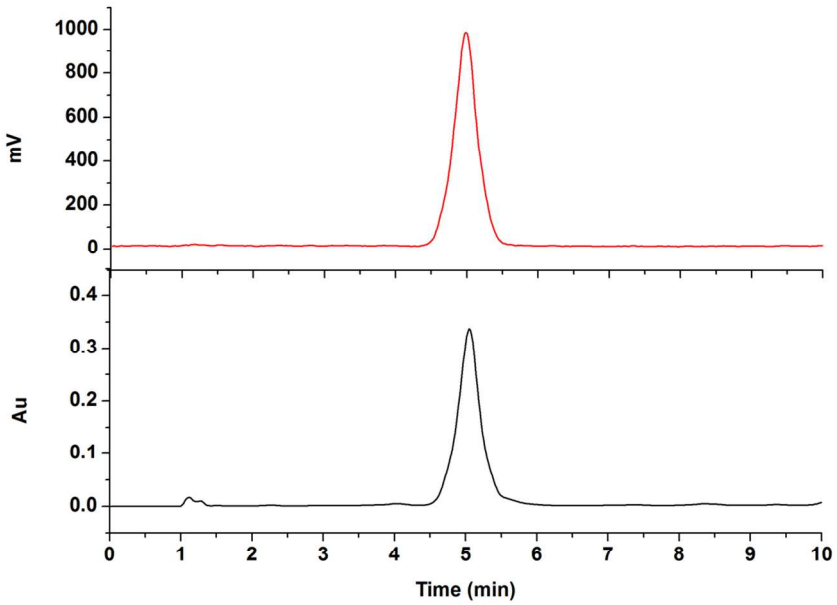

Figure 4

115x80mm (300 x 300 DPI)

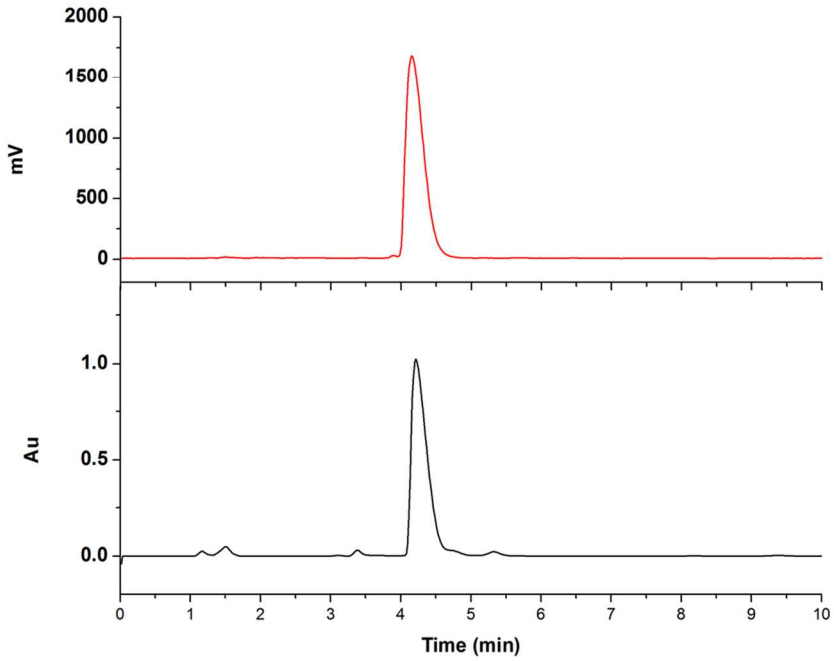

Figure 5

115x80mm (300 x 300 DPI)