Development of N-Methyl-(2-arylquinolin-4-yl)oxypropanamides as Leads to PET Radioligands for Translocator Protein (18 kDa)

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ABSTRACT: Translocator protein (18 kDa), known as TSPO, is a recognized biomarker of neuroinflammation. Radioligands with PET accurately quantify TSPO in neuroinflammatory conditions. However, the existence of three human TSPO genotypes that show differential affinity to almost all useful TSPO PET radioligands hampers such studies. There is an unmet need for genotype-insensitive, high-affinity, and moderately lipophilic TSPO ligands that may serve as leads for PET radioligand development. To address this need, we varied the known high-affinity TSPO ligand (1)-N,N-diethyl-2-methyl-3-(2-phenylquinolin-4-yl)propanamide in its aryl scaffold, side chain tether, and pendant substituted amido group while retaining an N-methyl group as a site for labeling with carbon-11. From this effort, oxygen-tethered N-methyl-arylxypropanamides emerged as new high-affinity TSPO ligands with attenuated lipophilicity, including one example with attractive properties for PET radioligand development, namely N-methyl-N-phenyl-2-[(2-(pyridin-2-yl)quinolin-4-yl)oxy]propanamide (22a; rat $K_i = 0.10 \text{ nM}$; human TSPO genotypes $K_i = 1.4 \text{ nM}; \text{clogD} = 4.18$).

INTRODUCTION

Translocator protein 18 kDa (TSPO), formerly known as the peripheral benzodiazepine receptor,° is located predominantly at the mitochondrial membrane³ in association with a voltage-dependent anion channel and an adenine nucleotide transporter. TSPO is present in several major organs, and is particularly dense in adrenal gland, heart, kidney, and testis.³ Low amounts are present in normal human brain,⁵ primarily in microglia where TSPO plays a crucial role in membrane biogenesis,⁶ and in steroid⁷ and heme⁸ biosynthesis. Activated microglia upregulate TSPO in instances of neuronal damage⁹⁻¹¹ as seen in many neurological disorders including cerebral ischemia,¹² Alzheimer’s disease,¹³¹⁴ Parkinson’s disease,¹⁵ and multiple sclerosis.¹⁶ TSPO can therefore serve as an important biomarker for neuroinflammation.¹⁷

$[^{11}C]PK 11195 ([^{11}C]1)$, first as racemate¹⁸ and then as the higher affinity (R)-enantiomer ($[^{11}C]((R)-1)$,¹⁹ has long been used to detect human TSPO in vivo with PET.¹⁰¹¹ However, accurate quantification of TSPO density with $[^{11}C]((R)-1$ is confounded by limited brain uptake,²⁰ a low ratio of specific to nonspecific binding,²¹ and an unfavorable metabolic profile.²² In view of these deficiencies, new TSPO radioligands have been developed from other structural classes with superior imaging characteristics (Chart 1).²³⁻²⁵ These include, for example, $[^{11}C]{}^{28}{}^{28}$[^{11}C]DRB28 ($[^{11}C]12),[^{11}C]DA1106 ([^{11}C]3),[^{11}C]DPA713 ([^{11}C]4),[^{18}F]FBR ([^{18}F]5),[^{18}F]PBR111 ([^{11}C]6), and[^{18}F]FEPPA ([^{18}F]7).

With the advent of the more sensitive radioligand, $[^{11}C]2$,²⁶ heterogeneity in human TSPO binding to PET radioligands has been discovered. Thus, $[^{11}C]2$ fails to image TSPO in some human subjects.²⁷⁻²⁹ Furthermore, in a study of deceased individuals diagnosed with multiple sclerosis, 46% had brain TSPO showing high affinity ($K_i \sim 4 \text{ nM}$) for 2, 23% low affinity ($K_i \sim 200 \text{ nM}$), and 31% intermediate affinity.³⁰ This heterogeneity in binding affinity was found to derive from a genetic polymorphism among individuals of European ancestry, namely an Ala147Thr³¹⁻³² mutation in TSPO. Three populations exist: those homozygous for Ala147, homozgyous for Thr147, and heterozygous for Ala147/Thr147, now dubbed high-affinity binders (HABs), low-affinity binders (LABs), and mixed-affinity (MABs), respectively. Several other second-generation TSPO ligands are also genotype-sensitive to different extents.³³⁻³⁶ PET measurements of TSPO density in studies of neuroinflammation assume that radioligand binding affinity is the same in all subjects because the output measure in PET experiments is usually a function of binding potential ($B_{max}/K_i$), the product of TSPO density ($B_{max}$) to radioligand affinity ($1/K_i$). Therefore, PET radioligands with heterogeneous binding affinity may impair data interpretation, especially comparisons of binding potentials for patient populations with those of normal subjects, unless all subjects are characterized for TSPO genotype.³⁷ However, genotyping is resource demanding and would be unnecessary if a genotype-insensitive TSPO radioligand could be employed.

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Pharmuka Laboratories, who introduced the prototypical TSPO ligand $1$, later reported $N,N$-diethyl-2-methyl-3-(2-phenylquinolin-4-yl)propanamide ($\pm Q; 10a$) as a potent inhibitor of $[^{3}H]1$ in rat brain cortex ($IC_{50} = 13.7$ nM).\textsuperscript{38} TSPO strongly bound the $l$-enantiomer ($-10a$ (PK 14067; $IC_{50} = 5.4$ nM), and not the $d$-enantiomer, ($+$)-10a (PK 14068; $IC_{50} = 4000$ nM). The binding affinity of ($-10a$ was also found to be high in human cerebral cortex ($K_i = 44$ nM)\textsuperscript{5} although clearly much lower than in rat. Thus, the (2-arylquinolinyl-4-yl)propanamide ($-10a$ represents a unique structural class of TSPO ligand that has so far been neglected for PET radioligand development. In this study, we explored this structural class for potential to generate high-affinity, acceptably lipophilic and human genotype-insensitive TSPO ligands to serve as leads for PET radioligand development.

Results and Discussion

Successful PET radioligands for imaging proteins in brain are required to display a wide array of properties.\textsuperscript{39–41} Among these properties are (i) high affinity and selectivity for the target protein, (ii) low molecular weight and intermediate polar surface area for blood–brain barrier penetration, (iii) moderate lipophilicity for adequate brain entry in the absence of excessive nonspecific binding, and (iv) amenability to labeling with a positron-emitter. In addition, PET radioligands for imaging TSPO in humans should ideally be insensitive to genotype. This study aimed to develop TSPO ligands as leads with a desirable combination of properties for PET radioligand development. Ligands were developed by modifying the 2-(arylquinolinyl-4-yl)propanamide 10a and initially assessed for binding affinity toward rat TSPO. Lipophilicities (clogD) were estimated by computation. The lipophilicity cost for high ligand affinity may be indexed as a lipophilicity efficiency parameter ($\text{LipE}$), defined\textsuperscript{42–44} as ligand $pIC_{50}$ (or $pK_i$) minus clogD. We sought ligands with improved LipE scores in addition to other desirable properties. Several ligands that we found to have appealing properties were also assayed against human HAB and LAB TSPOs to assess genotype sensitivity. Many new high-affinity TSPO ligands emerged from this effort and a few of these are promising new leads to PET radioligands.

Chemistry. Ligands were synthesized in one of three general ways, depending on the tether $X$ in the general structure (Figure 1).

For ligands with $X = CH_2$ (10a–w), a propargyl aniline (9a–t), prepared in situ by copper(I)-catalyzed addition of a terminal butynamide to an aldimine, was subjected to intramolecular cyclization to the dihydroquinoline. A second equivalent of aldimine (or adventitious oxygen) enabled oxidation of the dihydroquinoline to the desired quinoline, with the entire sequence conducted in one pot (method C).\textsuperscript{45} The requisite butynamides were prepared either by PyBroP-mediated amidation (9a–9k; method A)\textsuperscript{46} or by $\alpha$-alkylation of amides with propargyl bromide (9a, 9l–9t; method B)\textsuperscript{47} (Scheme 1).

A similar reaction with palladium catalysis\textsuperscript{50} was used to prepare the analogous ligand with $X = NH$ (14) (Scheme 3). The required amide 13 was readily prepared from 2-bromo-$N$-methyl-$N$-phenylpropanamide by conversion into the azide followed by addition of the product to 4-chloro-2-phenylquinoline in the presence of base\textsuperscript{59} (Scheme 2).

A similar reaction with palladium catalysis\textsuperscript{50} was used to prepare the analogous ligand with $X = NH$ (14) (Scheme 3). The required amide 13 was readily prepared from 2-bromo-$N$-methyl-$N$-phenylpropanamide by conversion into the azide followed by reduction.\textsuperscript{51}

For the analogous ligand with $X = O$ (15a), 2-phenyl-4-quinolone was alkylated with 2-bromo-$N$-methyl-$N$-phenylpropanamide in the presence of base\textsuperscript{52} (Scheme 4). Two
Scheme 1. Synthesis of Ligands 10a−w

Reagents and conditions: (i) PyBroP, DIPA, DCM, rt; (ii) propargyl bromide, LDA, THF, −78 °C; (iii) HCl(g), toluene, rt; (iv) 4% Pd(OAc)2, 8% DPEPhos, K3PO4, dioxane, 85 °C.

Scheme 2. Synthesis of S-Tethered Ligand 12

Reagents and conditions: (i) PhNHMe, 190 °C; (ii) t-BuOK, t-BuOH, DMF, 135 °C.

Scheme 3. Synthesis of N-Tethered Ligand 14

Reagents and conditions: (i) NaN3, DMF, 70 °C; (ii) Ph3P, H2O, THF, rt; (iii) HCl(g), toluene, rt; (iv) 4% Pd(OAc)2, 8% DPEPhos, K3PO4, dioxane, 85 °C.

Scheme 4. Syntheses of O-Tethered Ligands 15a−c

Reagents and conditions: (i) Cs2CO3, acetone, rt.

truncated versions of 15a in which either the phenyl group (15b) or the benzo fusion was absent (15c), were made similarly (Scheme 4). A series of 1,2-naphthyridine analogues of 15a (x = 5−8; 19a−d) and a quinazoline analogue (x = 3; 19e) were also prepared similarly (Scheme 5).

The α-benzamidocaptopyrindines 17a,b,d were made by acylation of α-benzamidobromopyrindines (16a,b,d) according to Weinreb’s method (Scheme 5), whereas 17c was made by benzyolation of 4-acetyl-3-aminoopyrindine (Scheme 5). 17a−d were then subjected to Camps cyclization54,55 to give the respective 1,2-naphthyridones (18a−d). The quinazoline 18e was prepared by hydrolysis of 4-chloro-2-phenylquinazoline. The naphthyridones and quinazolinone (18a−e) were then alkylated in the same manner (method D) used to make 15a to give the ligands 19a−e (Scheme 5). The 2-pyridinylquinolines 22a−c were synthesized similarly to round out the series of regioisomeric nitrogen-substituted ligands (Scheme 5).

Determination of Absolute Configuration of (−)−10a.

Initially, the absolute configuration of (−)−10a was unknown. We considered that this information could be valuable in subsequent TSPO ligand design. Therefore, compound 10a was resolved by chiral HPLC and the optical rotations of the separate enantiomers were measured. We confirmed that (−)−10a was the higher affinity enantiomer (Table 1). (−)−10a is a thick syrup, and attempts to crystallize the picrate salt failed. This precluded X-ray crystallography for determination of absolute configuration, and so we resorted to VCD.56 The solvent-corrected IR and VCD spectra for (−)−10a were obtained (Supporting Information Figure S1). A conformational search of the R-enantiomer at the molecular mechanics level was performed on the entire molecule followed by optimizations using a B3LYP “functional” on a 6-31G(d) basis set with Gaussian 09. These calculations revealed 12 conformers that were all within 1.5 kcal/mol of the lowest-energy conformer (Supporting Information Figure S2). VCD and IR spectra were calculated on the optimized geometries of these conformers. Their Boltzmann summation was compared with the observed spectra of (−)−10a (Supporting Information Figure S3). Given the agreement between calculation and experiment, the absolute configuration of (−)−10a was assigned to be R.

Effect of Structural Changes to 10a on Rat TSPO Binding Affinity and LipE.

The binding affinities (Kd) of all TSPO ligands, including 10a and its enantiomers, were determined on rat brain homogenates (Tables 1−5). Except for 10a, ligands were tested as racemates only. Assuming that all these ligands bind TSPO enantioselectively, as observed for 10a, the high-affinity enantiomer is expected to have about 2-fold higher affinity than that recorded for the racemate. Altering the chiral center of 10a by either removing the methyl group entirely (10b) or by lengthening this group from methyl to ethyl (10c) had a substantial detrimental effect on TSPO affinity. Therefore, a chiral center incorporating a methyl group appeared necessary for high TSPO affinity. With few exceptions, we retained this methyl group in all subsequently prepared ligands.

A tertiary amide is present in almost all high-affinity TSPO ligands (e.g., see Chart 1). Previous analogues of 1 that have had rotation about the amide locked or restricted have significantly reduced affinity for TSPO.57 Nonetheless, we found that complete restriction of amide bond rotation in the pyrrolidinyl ligand 10d had almost no adverse effect on affinity (cf. 10e; Table 1). The ability of the pyrrolidinyl ring to rotate...
with respect to the isoquinolinyl group perhaps compensates for absence of amide bond rotation. Creation of this pyrrolidinyl ring appreciably improved the LipE score (Table 1).

Methylation at a secondary amido nitrogen with [11C]methyl iodide has been a successful strategy for preparing useful PET radioligands. Therefore, we retained an N-methyl group in subsequently prepared ligands to provide a potential site for rapid labeling with carbon-11 ($t_{1/2} = 20.4$ min). We found that progressive lengthening of the remaining N-alkyl substituent from N-methyl to N-butyl (10e–h) dramatically increased TSPO affinity, yet this effect reached a plateau at N-butyl, as the N-pentyl compound (10i) offered no further improvement in affinity (Table 2). The N-propyl analogue offered the highest LipE score in this series but still with a quite high clogD value. Chain branching effects were not as predictable. Thus, TSPO affinity increased on replacing N-ethyl (10f) with N-isopropyl (10j) yet decreased on replacing N-propyl (10g) with N-sec-butyl (10k) or N-iso-butyl (10l). The N-isopropyl analogue (10j) gave the best LipE score among analogues with branched N-alkyl groups and the lowest clogD value among 10e–l.

An N-phenyl (10m) or N-benzyl (10n) group was well-tolerated, with 10m showing subnanomolar TSPO affinity (Table 2). TSPO showed variable sensitivity to substitution of

Table 1. TSPO Ligands Based on 10a: Dependence of Binding Affinity, Lipophilicity, and Genotype Sensitivity on Side Chain Alkyl Substituents

| ligand | $R^1$ | $R^2$ | $R^3$ | aff (nM)$^a$ | cLogD | LipE | LAB aff (nM)$^b$ | HAB aff (nM)$^c$ | (LAB aff)/(HAB aff) |
|--------|------|------|------|-------------|-------|------|-----------------|-----------------|-----------------|
| 1 (PK 11195) | Me | Et | Et | 0.5 ± 0.3 | 3.97 | 4.3 ± 0.2 | 4 ± 1 | 4 ± 1 | 1.0 ± 0.5 |
| 10a | Me | Et | Et | 2.1 ± 1.6 | 4.3 | 4.4 ± 0.1 | 0.90 ± 0.09 | 4.33 | 4.72 ± 0.04 | 743 ± 190 | 10 ± 2 | 76 ± 27 |
| (R)-10a (PK 14067) | (R)-Me | Et | Et | 0.90 ± 0.09 | 4.33 | 4.72 ± 0.04 | 743 ± 190 | 10 ± 2 | 76 ± 27 |
| (S)-10a (PK 14068) | (S)-Me | Et | Et | 73 ± 36 | 4.33 | 4.72 ± 0.04 | 743 ± 190 | 10 ± 2 | 76 ± 27 |
| 10b | H | Et | Et | 32 ± 11 | 4.84 | 2.7 ± 0.2 | 32 ± 11 | 4.84 | 2.7 ± 0.2 | 32 ± 11 | 4.84 | 2.7 ± 0.2 |
| 10c | Et | Et | Et | 14 ± 6 | 5.26 | 2.6 ± 0.2 | 32 ± 11 | 4.84 | 2.7 ± 0.2 | 32 ± 11 | 4.84 | 2.7 ± 0.2 |
| 10d | CH$_2$-R$^1$ | CH$_2$-R$^1$ | Me | 53 ± 22 | 3.69 | 3.6 ± 0.2 | 32 ± 11 | 4.84 | 2.7 ± 0.2 | 32 ± 11 | 4.84 | 2.7 ± 0.2 |
| 10e | Me | Me | Me | 40 ± 8 | 4.58 | 2.8 ± 0.1 | 32 ± 11 | 4.84 | 2.7 ± 0.2 | 32 ± 11 | 4.84 | 2.7 ± 0.2 |

$^a$Mean ± SD for $n = 6$, except for 1 ($n = 60$), and (R)-10a ($n = 5$). $^b$Mean ± SD for $n = 6$, except for 1 ($n = 12$). $^c$Mean ± SD for $n = 6$, except for 1 ($n = 14$).
the N-phenyl group in 10m with a pyridinyl or fluoro phenyl group. Binding affinity varied with nature and position of the new heteroatom. Thus, replacement of a phenyl group with an o-pyridinyl group (10o) left binding affinity unchanged at subnanomolar. However, there was a 10-fold loss of affinity in the m-pyridinyl analogue (10p) and a further 10-fold loss in the p-pyridinyl analogue (10q). In contrast to the pyridinyl analogues, all three fluoro isomers (10r–t) showed almost identical subnanomolar affinity. The p-fluorophenyl ligand 10t had the highest LipE score but still possessed high computed lipophilicity.

Chlorophenyl groups have featured in examples of high-affinity TSPO ligands from other structural classes such as the isouquinoline carbamazide 1. Therefore, we also prepared the chlorophenyl isomers 10u–w of the N-methyl-N-isopropyl ligand 10j (Table 3). All three ligands showed lower TSPO affinity than the phenyl analogue 10j, with the o-isomer (10u) showing lowest affinity.

Having explored how TSPO affinity was affected by structural change at the amido nitrogen, we next explored changes to the methylene group that bridges the amide to the quinolinyl ring. We replaced the methylene tether of 10m with an oxygen (15a), sulfur (12), or NH tether (14). In each case, rat TSPO binding affinity was greatly increased, and particularly so for the oxygen-tethered compound (15a) which showed a Ki value of 70 nM (Table 4). Increased affinity may be due to improved spatial relations of the amido group relative to the quinolinyl ring via altered bond lengths and dihedral angle. Further benefits of the heteroatom tethers were decreased lipophilicities, resulting in higher LipE scores. We chose to focus on preparing further analogues of the very high affinity O-tethered lead (15a). We next considered whether high TSPO affinity might be retained in less lipophilic analogues in which the phenyl-quinoline scaffold was modified by removing an aromatic ring or by inserting a nitrogen atom. Compounds that lacked either the pendant 2-phenyl group (15b) or the benzo fusion (15c) illustrated that these two rings, and especially the phenyl group, were quite important for very high TSPO affinity (Table 5). Nonetheless, 15b and 15c still exhibited affinity in the low nanomolar range, with radically reduced cLogD values and greatly enhanced LipE scores. All nitrogen substitutions in the quinoline core resulted in ligands that retained near- or subnanomolar affinity for TSPO, with affinities slightly increasing in the following order 19a < 19b < 19c ∼ 19d < 19e. Like the quinazoline (19e), the 2-(o-pyridyl)quinoline (22a) had very high affinity, approaching that of 15a, but with much lower computed lipophilicity and hence a much higher LipE score. The other pyridylquinolines (22b,c) showed virtually equal subnanomolar affinity.

Overall, the presence of a heteroatom tether strikingly increased LipE score, as may be readily appreciated from a plot of pKi versus cLogD for ligands with each type of tether (Figure 2). In particular, ligands with oxygen tethers clearly cluster into

Table 2. TSPO Ligands Based on 10a: Dependence of Binding Affinity, Lipophilicity, and Genotype Sensitivity on Amido Substituent

| ligand | R³ | rat Kᵢ (nM) | cLogD | LipE | LAB Kᵢ (nM) | HAB Kᵢ (nM) | (LAB Kᵢ)/(HAB Kᵢ) |
|--------|----|-------------|-------|------|------------|------------|------------------|
| 10e    | Me | 40 ± 8      | 4.58  | 2.8 ± 0.1 |            |            |                  |
| 10f    | Et | 9 ± 2       | 4.28  | 3.8 ± 0.1 |            |            |                  |
| 10g    | Pr | 1.2 ± 0.4   | 4.74  | 4.2 ± 0.1 |            |            |                  |
| 10h    | Bu | 0.4 ± 0.2   | 5.53  | 3.9 ± 0.2 | 89 ± 3     | 1.0 ± 0.6   | 89 ± 50          |
| 10i    | Pen | 0.6 ± 0.1  | 6.61  | 2.6 ± 0.1 |            |            |                  |
| 10j    | 1-Pr | 2.9 ± 0.9  | 4.22  | 4.3 ± 0.1 | 973 ± 409 | 15 ± 2      | 64 ± 28          |
| 10k    | sec-Bu | 5 ± 3    | 5.20  | 2.9 ± 0.2 |            |            |                  |
| 10l    | Bu | 5 ± 2       | 5.00  | 3.3 ± 0.1 | 185 ± 81   | 6 ± 2       | 32 ± 18          |
| 10m    | Ph | 0.9 ± 0.2   | 5.57  | 3.5 ± 0.1 | 53 ± 22    | 1.9 ± 0.5   | 27 ± 14          |
| 10n    | Bn | 5 ± 3       | 6.53  | 1.8 ± 0.2 |            |            |                  |
| 10o    | o-Py | 1.6 ± 0.6  | 5.26  | 3.6 ± 0.2 | 119 ± 56   | 4 ± 1       | 30 ± 18          |
| 10p    | m-Py | 10 ± 3     | 5.35  | 2.7 ± 0.1 |            |            |                  |
| 10q    | p-Py | 106 ± 24   | 5.33  | 1.7 ± 0.1 |            |            |                  |
| 10r    | o-F-Ph | 0.9 ± 0.2  | 5.54  | 3.5 ± 0.1 |            |            |                  |
| 10s    | m-F-Ph | 0.8 ± 0.2  | 5.64  | 3.5 ± 0.1 |            |            |                  |
| 10t    | p-F-Ph | 0.4 ± 0.1  | 5.32  | 4.1 ± 0.2 |            |            |                  |

Mean ± SD for n = 6, except for 10h (n = 9).

Table 3. TSPO Ligands Based on 10a: Dependence of Rat Binding Affinity and Lipophilicity on Pendant Aryl Substituent

| ligand | Ar | rat Kᵢ (nM) | cLogD | LipE |
|--------|----|-------------|-------|------|
| 10j    | Ph | 2.9 ± 0.9   | 4.22  | 4.3 ± 0.1 |
| 10u    | o-CI-Ph | 22 ± 6    | 4.50  | 3.2 ± 0.1 |
| 10v    | m-CI-Ph | 4 ± 1     | 4.71  | 3.7 ± 0.1 |
| 10w    | p-CI-Ph | 4 ± 1     | 4.83  | 3.6 ± 0.1 |

Mean SD for n = 6, except for 10u (n = 9).
Assessment of Ligand Sensitivity to Human TSPO Genotype. Compound 1, the lead compound (R)-10a, examples of methylene-tethered ligands (10h,j,l,m,o), and the heteroatom-tethered compounds (12, 14, 15a,c, 19a–e, 22a–c) were selected to evaluate their human genotype sensitivities by measurement of their $K_i$ values for binding to leukocytes from HABs and LABs. Our measurements confirmed that 1 has low genotype sensitivity (Table 1). (R)-10a showed about 10-fold lower affinity to HAB TSPO than to rat TSPO (Table 1). Affinity for LAB TSPO was about 76-fold lower than for HAB TSPO. All the methylene-tethered ligands had similar or lower affinity to HAB TSPO than to rat TSPO and had high genotype sensitivities (Table 2). Thus, overall, variation in amide substituents had little impact on genotype sensitivity.

For the group of ligands in which only the tether atom differed (10m, 12, 14, 15a) affinity for HAB TSPO was again somewhat lower than for rat TSPO, and genotype sensitivity reduced progressively across the tether series CH$_2$, S, NH, and O (Table 4). The oxygen-tethered ligand 15a showed very low sensitivity (2.5) in addition to subnanomolar affinity and high LipE score. We surmise this improvement may relate to altered bond lengths and torsional angles at the oxygen atom.

Finally, we looked at the effects of introducing a second nitrogen into the 2-phenylquinoline scaffold (Table 5). Of the eight compounds tested, four (19a,b,d, 22b) displayed greater than 10-fold lower binding affinity to LAB TSPO than to HAB TSPO. The remaining four compounds were either much less sensitive (19d, 22c) or insensitive (19e, 22a) to TSPO.

**Table 4.** TSPO Ligands Based on 10a: Dependence of Rat Binding Affinity, Lipophilicity, and Genotype Sensitivity on Tether for Pendant Alkyl Carboxamido Group

| ligand | X     | rat $K_i$ (nM)$^a$ | cLogD | LipE | LAB $K_i$ (nM)$^b$ | HAB $K_i$ (nM)$^b$ | (LAB $K_i$)/(HAB $K_i$) |
|--------|-------|-------------------|-------|------|-------------------|-------------------|-------------------------|
| 10m    | CH$_2$| 0.9 ± 0.2         | 5.57  | 3.5 ± 0.1 | 53 ± 22          | 1.9 ± 0.5         | 27 ± 14                 |
| 12     | S     | 0.39 ± 0.04       | 5.31  | 4.10 ± 0.05 | 26 ± 5          | 2.5 ± 0.6         | 10 ± 3                  |
| 14     | NH    | 0.19 ± 0.01       | 4.11  | 5.62 ± 0.03 | 4 ± 1           | 0.44 ± 0.08       | 9 ± 3                   |
| 15a    | O     | 0.070 ± 0.004     | 4.73  | 5.42 ± 0.03 | 1.3 ± 0.2       | 0.5 ± 0.2         | 2.5 ± 0.9               |

$^a$Mean SD for $n = 6$, except for 14 $(n = 5)$. $^b$Mean ± SD for $n = 6$, except for 10m $(n = 5)$. $^c$Mean ± SD for $n = 6$, except for 10m $(n = 5)$.

**Table 5.** Oxygen-Tethered TSPO Ligands Based on 15a: Dependence of Binding Affinity, Lipophilicity, and Genotype Sensitivity on Scaffold Aryl Groups

| ligand | Ar  | A     | B | C | D | E | rat $K_i$ (nM)$^a$ | cLogD | LipE | LAB $K_i$ (nM)$^b$ | HAB $K_i$ (nM)$^b$ | (LAB $K_i$)/(HAB $K_i$) |
|--------|-----|-------|---|---|---|---|-------------------|-------|------|-------------------|-------------------|-------------------------|
| 15a    | Ph  | CH    | CH | CH | CH | CH | 0.070 ± 0.004     | 4.73  | 5.42 ± 0.03 | 1.3 ± 0.2         | 0.5 ± 0.2         | 2.5 ± 0.9               |
| 15b    | H   | CH    | CH | CH | CH | CH | 0.57 ± 0.5        | 2.96  | 5.29 ± 0.04 | 66 ± 22           | 3.3 ± 0.9         | 20 ± 9                  |
| 15c    | Ph  | H    | CH | CH | CH | CH | 3.3 ± 0.6         | 3.52  | 5.3 ± 0.3  | 62 ± 11           | 2.0 ± 0.6         | 31 ± 11                 |
| 19a    | Ph  | N    | CH | CH | CH | CH | 1.4 ± 0.5         | 4.27  | 4.6 ± 0.1  | 23 ± 7            | 1.1 ± 0.3         | 21 ± 8                  |
| 19b    | Ph  | N    | CH | CH | CH | CH | 0.6 ± 0.1         | 4.15  | 5.0 ± 0.1  | 25 ± 8            | 1.3 ± 0.5         | 19 ± 9                  |
| 19c    | Ph  | CH   | N  | CH | CH | CH | 0.3 ± 0.2         | 4.15  | 5.4 ± 0.3  | 47 ± 26           | 1.4 ± 0.4         | 33 ± 20                 |
| 19d    | Ph  | CH   | N  | CH | CH | CH | 0.29 ± 0.03       | 3.91  | 5.63 ± 0.04 | 4.7 ± 0.7         | 0.9 ± 0.2         | 5 ± 2                   |
| 19e    | Ph  | CH   | CH | CH | CH | CH | 0.13 ± 0.08       | 5.66  | 4.4 ± 0.5  | 2 ± 1             | 2 ± 1             | 1 ± 1                   |
| 22a    | o-Py| CH   | CH | CH | CH | CH | 0.10 ± 0.05       | 4.18  | 5.9 ± 0.2  | 1.4 ± 0.5         | 1.4 ± 0.2         | 1.0 ± 0.4               |
| 22b    | m-Py| CH   | CH | CH | CH | CH | 0.20 ± 0.03       | 4.06  | 5.6 ± 0.1  | 12 ± 3            | 0.90 ± 0.09       | 13 ± 4                  |
| 22c    | p-Py| CH   | CH | CH | CH | CH | 0.22 ± 0.02       | 4.06  | 5.60 ± 0.04 | 7 ± 3             | 1.0 ± 0.5         | 7 ± 5                   |

$^a$Mean ± SD for $n = 6$, except for 19d, 22b $(n = 5)$. $^b$Mean ± SD for $n = 6$, except for 15a,b, 19c $(n = 5)$, and 15c $(n = 4)$. $^c$Mean ± SD for $n = 6$, except for 15c $(n = 5)$. 

Figure 2. Plot of rat p$K_i$ versus clogD for ligands having different side chain tethers.
genotype. Notably, compounds having the second nitrogen in nearest proximity to the quinolinyl nitrogen had the least sensitivity to genotype. Ligand 22a offered the most appealing combination of properties as a lead for PET radioligand development, including high HAB TSPO affinity, genotype insensitivity, and high LipE score for binding to rat and human TSPO, which are all improved over corresponding values for 10a.

An important consideration in attempts to develop genotype-insensitive PET radioligands for TSPO, is whether genotype sensitivity is likely to increase with ligand affinity. Generally, we observed that genotype sensitivity tended to decrease with HAB TSPO affinity among the tested ligands (Figure 3).

**CONCLUSIONS**

Judicious structural modifications to 10a led to several TSPO ligands with affinity in the nanomolar or subnanomolar range plus an enhanced LipE score. Truncation of the phenylisoquinoline scaffold as in ligands 15b and 15c was particularly effective in reducing LipE score, and these ligands may serve as leads for further PET radioligand development. Introduction of an oxygen tether in place of the methylene tether was as genotypic sensitivity as leads for PET radioligand development.

**EXPERIMENTAL SECTION**

**Materials and Methods.** Reagents and solvents were purchased unless stated otherwise. Air-sensitive reagents were stored under N2 in a PureLab HE glovebox (Innovative Technology; Amesbury, MA). Melting points were determined on an SMP10 apparatus (Stuart; Staffordshire, UK). Boiling point vacuum pressures were determined on a DVR-200 apparatus (J-Kem Scientific Inc.; St. Louis, MO). Optical rotations were determined on a P-1010 instrument (JASCO Inc.; Easton, MD). IR-VCD spectra were recorded on a Chiral IR-2X apparatus (J-Kem Scientific Inc.; St. Louis, MO). 1H and 13C NMR (100 MHz), and 19F NMR (376 MHz) spectra were recorded on an Avance 400 instrument (Bruker; Billerica, MA). Chemical shifts for 19F are reported relative to neat TFA in a coaxial insert (δf = 0). HRMS were obtained at the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois Urbana—Champaign using a Micromass Q-ToF Ultima instrument for ESI (Waters Corp.; Columbia, MD) or a GCT Premier instrument for EI (Waters Corp.). Preparative HPLC was performed with either a Luna PFP(2) (5 μm; 100 Å; 30 mm × 250 mm), Gemini C18 (10 μm; 110 Å; 30 mm × 250 mm), or a Lux Amylose-2 (3 μm; 10 mm × 250 mm) column. HPLC separation conditions are given in parentheses and refer to column type, flow rate (mL/min), and organic phase ‘O’/‘A’ phase ratio, as follows: O = MeOH, O = MeCN, A = H2O, A = NH4CO3H (10 mM), A = Et3NH (0.1%). The chemical purities of all compounds were established by HPLC on either a Luna PFP(2) (5 μm; 100 Å; 4.6 mm × 250 mm), or a Lux Amylose-2 (3 μm; 4.6 mm × 250 mm) column. Chemical purities were all >95% and typically >99%, as monitored by absorbance at 220 nm. cLogD was computed with Pallas for Windows software version 3.8 in default option (CompuDrug; Bal Harbor, FL).

**Method A: N,N-Diethyl-2-methylpent-4ynamide (9a).** DIPA (1.0 mL, 6.0 mmol) was added dropwise to a solution of 2-methylpent-4-yonic acid32 (0.33 mL, 3.0 mmol), diethylamine (0.34 mL, 3.3 mmol), and PyBroP (1.4 g, 3.0 mmol) in DCM (3 mL) at rt. This mixture was stirred for 2 h and then the solvent removed. The residue was taken up in EtOAc (30 mL) and washed successively with 5% KH2SO4 (30 mL × 3), brine (30 mL), 5% NaHCO3 (30 mL × 3), and brine (30 mL), and finally dried (Na2SO4). FC (hexanes/EtOAc, 1:1) of the residue gave 9a as a colorless oil (0.33 g, 66%). HRMS–ESI (m/z): [M + H]+ calcd for C9H15NO, 168.1388; found, 168.1397. Melting point was determined on an SMP10 apparatus (Stuart; London, UK).

As described in the Supporting Information, this method also gave: N2-dimethyl-N-propylpent-4ynamide (9b), N2-butyl-N2-dimethylpent-4ynamide (9c), N2-dimethyl-N2-pentylpent-4ynamide (9d), N2-butan-2-yl-N2-dimethylpent-4ynamide (9e), N2-dimethyl-N2-(2-methylpropyl)pent-4ynamide (9f), N2-benzyl-N2-dimethylpent-4ynamide (9g), N2-dimethyl-N2-(pyridine-2-yl)pent-4ynamide (9h), N2-dimethyl-N2-(pyridine-3-yl)pent-4ynamide (9i), N2-dimethyl-N2-(pyridin-4-yl)pent-4ynamide (9j), N2-(fluorophenyl)N2-dimethylpent-4ynamide (9k), N2-(2-fluorophenyl)N2-dimethylpropanamid (9sa), N2-(3-fluorophenyl)-N2-methylpropanamid (9ta), N2-(4-acetylpyridin-3yl)benzamid (17c), N2-(2-acetylphenyl)pyridine-2-carboxamid (20a), and N2-(acetylpyridin)pyridine-4-carboxamid (20c).

**Method B: N,N-Diethyl-2-methylpent-4ynamide (9a).** N-Butyl lithium in hexanes (1.6 M, 28 mL, 44 mmol) was added dropwise to a solution of 2-methylpent-4-yonic acid (39 mL, 39 mmol) in toluene (9.4 mL) at −75 °C. Propargyl bromide (87 mmol) in toluene (9.4 mL) was added dropwise. This solution slowly was warmed to −20 °C and then cooled back to −75 °C. N,N-Diethylpropanamide (5.6 mL, 39 mmol) was added dropwise. This solution was slowly warmed to −20 °C and then cooled back to −75 °C. Propargyl bromide (87 mmol) in toluene (9.4 mL) was added dropwise. This solution slowly was warmed to rt and stirred for several hours. Brine (200 mL) was added, the organic layer separated, and the aqueous phase extracted with ether (100 mL × 2). The combined extracts were washed with brine (100 mL) and dried (MgSO4). Fractional distillation of the residue gave 9a as a colorless oil (bp 53–54 °C at 5.8 mmHg; 4.8 g, 71%). A small quantity was further purified by HPLC (Gemini, 30, 0.1%ACN, 55:45; d = 0.90 g/mL).

As described in the Supporting Information, this method also gave: N,N-diethylpent-4ynamide (9l), N,N2-diethylpent-4ynamide (9m), 1-methyl-3-(prop-2-yn-1-yl)pyrrolidin-2-one (9n), N2,N2-trimethylpent-4ynamide (9o), N,N2-ethyl-N2-dimethylpent-4ynamide (9p), N2,N2-dimethyl-N2-(propan-2-yl)pent-4ynamide (9q), N2,N2-dimethyl-N,N2-phenylpent-4ynamide (9r), N2-(fluorophenyl)N2-dimethylpent-4ynamide (9s), and N2-(3-fluorophenyl)N2-dimethylpent-4ynamide (9t).

**Method C: N,N-Diethyl-2-methyl-3-(2-phenylquinolin-4-yl)propanamid (10a).** Copper(1) chloride (10 mg, 0.10 mmol) and then silver triflate (26 mg, 0.10 mmol) were added to a solution of N-benzylidenemamine (362 mg, 2.00 mmol) and 9a (167 mg, 1.00 mmol) in DCA (1.0 mL) under Ar. The solution was heated to 100 °C for 17

![Figure 3](image-url)

**Figure 3.** Plot of genotype sensitivity versus HAB TSPO binding affinity for tested ligands.
h, cooled to rt, and filtered through diatomaceous earth. The mixture was taken up in EtOAc (10 mL) and washed with aqueous NH4Cl/NaOH (pH 8, 10 mL). The aqueous phase was extracted with EtOAc (10 mL × 2). The combined extracts were washed with brine (15 mL) and dried (MgSO4). HPLC (PE, 30, O2/A, 65:35) of this material gave 10a as a dark-yellow syrup (0.12 g, 36%). HRMS—ESI (m/z): [M + H]+ calcd for C25H25N2O2, 383.1760; found, 383.1755. 1H NMR (CDCl3): δ 8.02 (bs, 1H), 8.15 (m, 2H), 8.04 (dd, J = 8.4, 0.8 Hz, 1H), 7.74 (s, 1H), 7.70 (dt, J = 7.8, 1.2 Hz, 1H), 7.55 (dd, J = 7.8, 1.2 Hz, 1H), 7.53–7.48 (2H), 7.44 (tt, J = 7.2, 1.6 Hz, 1H), 3.52 (dd, J = 13.6, 9.2 Hz, 1H), 3.36–3.24 (m, 2H), 3.19–3.09 (m, 1H), 2.87 (dq, J = 7.2, 2.4 Hz, 1H), 1.31 (d, J = 6.4 Hz, 3H), 0.88 (t, J = 7.2 Hz, 3H), 0.75 (t, J = 7.2 Hz, 3H). 13C NMR (CDCl3): δ 174.4, 156.9, 148.4, 146.7, 139.5, 130.6, 129.3, 128.8, 127.5, 126.5, 126.2, 123.2, 120.0, 41.7, 40.6, 37.0, 36.7, 19.0, 14.5, 13.0.

As described in the Supporting Information, this method also gave: N,N-diethyl-3-(2-phenylquinolin-4-yl)propionamide (10b), N,N-diethyl-2-[(2-phenylquinolin-4-yl)methyl]butanamide (10c), 1-methyl-3-(2-phenylquinolin-4-yl)methyl)pyrrolidin-2-one (10d), N,N-(2-triethylammonium)-2-(2-phenylquinolin-4-yl)propanamide (10e), N-ethyl-N,N-diethyl-3-(2-phenylquinolin-4-yl)propionamide (10f), N,N-diethyl-N-(2-phenylquinolin-4-yl)propionamide (10g), N-butyl-N,N-diethyl-3-(2-phenylquinolin-4-yl)propionamide (10h), N,N-diethyl-N-pentyl-3-(2-phenylquinolin-4-yl)propionamide (10i), N,N-diethyl-3-(2-phenylquinolin-4-yl)-N-propan-2-yl)propionamide (10j), N-butyl-N,N-diethyl-3-(2-phenylquinolin-4-yl)propionamide (10m), N-benzyl-N,N-diethyl-3-(2-phenylquinolin-4-yl)propionamide (10n), N,N-diethyl-3-(2-phenylquinolin-4-yl)propionamide (10o), N,N-diethyl-3-(2-phenylquinolin-4-yl)propionamide (10p), N,N-diethyl-3-(2-phenylquinolin-4-yl)propionamide (10q), N,N-diethyl-3-(2-phenylquinolin-4-yl)propionamide (10r), N,N-diethyl-3-(2-phenylquinolin-4-yl)propionamide (10s), N,N-diethyl-3-(2-phenylquinolin-4-yl)propionamide (10t), N,N-diethyl-3-(2-phenylquinolin-4-yl)propionamide (10u), N,N-diethyl-3-(2-phenylquinolin-4-yl)propionamide (10v), and 9H,10H-phenyl-2-[(2-phenyl-1,6-naphthyridin-4-yl)filterted through diatomaceous earth, and extracted under Ar for 2 d. The mixture was cooled to rt, diluted with water (500 mL), and extracted into ether (200 mL × 3). The combined extracts were washed successively with water (100 mL × 5) and brine (100 mL) and dried (Na2SO4). The solvent was removed to yield the azide as a yellow oil (1.98 g, 97%), which was taken up in THF (100 mL). Ph3P (2.54 g, 9.70 mmol) and water (260 μL, 15 mmol) were added, and the mixture stirred overnight at rt. The solvent was removed, the residue taken up in benzene (or toluene), and HCl (g) bubbled through for 10 min. The hydrochloride salt was washed with ether to give 13 as a cream powder (1.78 g, 83%); mp 230–233 °C dec. HRMS—ESI (m/z): [M + H]+ calcd for C13H19N2O, 197.1364; found, 197.1361.

2-Amino-N-methyl-N-phenylpropanamide hydrochloride (13). NaNO2 (2.58 g, 39.8 mmol) and 2-bromo-N-methyl-N-phenylpropanamide (424.2 g, 1.00 mmol) in DMF (50 mL) were heated to 70 °C under Ar for 2 h. The solution was cooled to rt, diluted with water (500 mL), and extracted into ether (200 mL × 3). The combined extracts were washed successively with water (100 mL × 5) and brine (100 mL) and dried (Na2SO4). The solvent was removed to afford the azide as a yellow oil (1.98 g, 97%), which was taken up in THF (100 mL). Ph3P (2.54 g, 9.70 mmol) and water (260 μL, 15 mmol) were added, and the mixture stirred overnight at rt. The solvent was removed, the residue taken up in benzene (or toluene), and HCl (g) bubbled through for 10 min. The hydrochloride salt was washed with ether to give 13 as a cream powder (1.78 g, 83%); mp 230–233 °C dec. HRMS—ESI (m/z): [M + H]+ calcd for C13H19N2O, 197.1364; found, 197.1361.

N-Methyl-N-phenyl-2-[(2-phenylquinolin-4-yl)-oxoxy]propanamide (15a). A mixture of 13 (322 mg, 1.50 mmol), 4-chloro-2-phenylquinoline (240 mg, 1.00 mmol), Pd(OAc)2 (9 mg, 0.04 mmol), DPEPhos (43 mg, 0.080 mmol), and K2PO4 (742 mg, 3.50 mmol) in dioxane (4.0 mL) was heated to 85 °C under Ar for 2 d. The mixture was cooled to rt and filtered through diatomaceous earth, which was then washed with EtOAc (20 mL). The organic phase was washed with water (20 mL) and the water extracted with EtOAc (20 mL × 2). The combined extracts were washed with brine (20 mL) and then dried (MgSO4). The product was isolated by FC (CHCl3/MeOH/TEA, 90:1:1) and recrystallized (cyclohexane/EtOAc) to give 14 as pale-yellow chunks (0.13 g, 34%); mp 190–191 °C. HRMS—ESI (m/z): [M + H]+ calcd for C20H18N2O2, 350.1627; found, 350.1628.

Method D: 2-N-Methyl-N-phenyl-2-[(2-phenylquinolin-4-yl)-oxoxy]propanamide (15a). 2-Phenylquinolin-4(1H)-one hydrochloride (144 mg, 0.56 mmol), 2-bromo-N-methyl-N-phenylpropanamide (203 mg, 0.84 mmol), and Cs2CO3 (736 mg, 2.26 mmol) were stirred in aceton (5.0 mL) at rt for 19 h. Solid was filtered off and washed with a little aceton. Solvent was then removed, and the residue recrystallized (DCM/ether) to give 15a as a colorless solid (123 mg, 57%); mp 158–160 °C. HRMS—ESI (m/z): [M + H]+ calcd for C19H15N2O2, 339.1352; found, 339.1352. 1H NMR (CDCl3): δ 8.15 (dd, J = 8.0, 0.8 Hz, 2H), 7.98 (dd, J = 6.8, 1.6 Hz, 2H), 7.72 (dt, J = 7.4, 1.2 Hz, 1H), 7.54–7.46 (7H), 7.19 (t, J = 2.0 Hz, 3H), 6.94 (2H), 4.12 (q, J = 6.8 Hz, 2H), 3.23 (s, 3H), 1.58 (d, J = 6.8 Hz, 3H). 13C NMR (CDCl3): δ 171.5, 156.8, 148.3, 144.7, 145.2, 139.5, 130.4, 130.3, 130.0, 129.7, 129.0, 128.3, 127.8, 127.2, 126.8, 124.6, 119.7, 42.8, 38.0, 19.9.

As described in the Supporting Information, this method also gave: N,N-dimethyl-2-[(2-phenylquinolin-4-yl)-oxoxy]propanamide (15b), N-methyl-N-phenyl-2-[(2-phenylquinolin-4-yl)-oxoxy]propanamide (15c), N-ethyl-N-phenyl-2-[(2-phenylquinolin-4-yl)-oxoxy]propanamide (15d), and N,N-dimethyl-N-phenyl-2-[(2-phenylquinolin-4-yl)-oxoxy]propanamide (15e).
This method also gave 2-phenyl-1,4-dihydro-1,6-naphthyridin-4-one (18b), 2-phenyl-1,4-dihydro-1,7-naphthyridin-4-one (18c), and 2-phenyl-1,4-dihydro-1,8-naphthyridin-4-one (18d) (see Supporting Information).

2-Phenyl-1,4-dihydroquinazolin-4-one (18e). A slurry of 4-chloro-2-phenylquinazoline (5.18 g, 21.5 mmol) and NaOH (860 mg, 21.5 mmol) was heated to 110 °C in DMSO (200 mL) for 1 h. This slurry was then cooled to rt, whereupon fine colorless crystals appeared. These were collected and washed with water (500 mL x 2) to give 18e (1.28 g, 27%); mp 238–239 °C (lit. mp 232–233 °C). HRMS – EI (m/z): [M + H]+ calcd for C17H13NO3, 220.0793; found, 220.0792. 1H NMR (HFPi-d6): δ 8.40 (d, J = 8.0 Hz, 1H), 8.24 (d, J = 8.8 Hz, 1H), 8.16 (dt, J = 7.2, 0.8 Hz, 1H), 7.95 (d, J = 7.6 Hz, 2H), 7.83 (t, J = 8.0 Hz, 1H), 7.60 (t, J = 7.6 Hz, 1H), 7.53 (t, J = 8.0 Hz, 2H), 4.95 (br, s, 1H). 13C NMR (HFPi-d6): δ 166.3, 161.8, 151.6, 136.1, 135.6, 130.9, 128.6, 123.3, 127.6, 125.2, 122.7, 114.6. Addition of water to the mother liquor precipitated unreacted starting material (1.37 g).

Method G: N-Methyl-N-phenyl-2-[2-(pyridin-2-yl)quinolin-4-yl]propanamide (22a). 2-(Pyridin-2-yl)-1,4-dihydroquinazolin-4-one (18f) (222 mg, 1.00 mmol), 2-bromo-N-methyl-N-propyranopropamide (270 mg, 1.12 mmol), and K2CO3 (834 mg, 6.03 mmol) in MeCN (35 mL) were heated to 55 °C (solution temp) under Ar for 16 h. The mixture was then cooled to rt and poured into stirring water (175 mL). After several min, the precipitate was collected and recrystallized (cyclohexane/EtOAc) to give 22a as colorless plates (267 mg, 70%); mp 185–187 °C. HRMS – ESi (m/z): [M + H]+ calcd for C21H17NO4, 383.1708; found, 383.1712. 1H NMR (CDCl3): δ 8.77 (dd, J = 4.8, 0.8 Hz, 1H), 6.89 (d, J = 7.6 Hz, 1H), 8.28 (dd, J = 8.4, 0.8 Hz, 1H). 13C NMR (CDCl3): δ 170.2, 160.8, 157.0, 156.3, 149.2, 140.7, 142.5, 136.3, 129.2, 127.2, 148.4, 111.0.

**Method E: N-(2-Acetylpyridin-3-yl)benzamide (17a).** n-BuLi in hexanes (24 mL, 33 mmol) was added dropwise to a solution of N-(2-acetylpyridin-3-yl)benzamide (1.95 g, 24%), mp 99–101 °C. HRMS – ESi (m/z): [M + H]+ calcd for C20H19NO2, 276.0976; found, 276.0974. 1H NMR (CDCl3): δ 8.54 (br s, 1H), 8.49 (dd, J = 4.8, 1.6 Hz, 1H), 7.97–7.94 (3H, J 60), (tt, J = 7.2, 1.2 Hz, 1H), 7.52 (tt, J = 7.6, 1.2 Hz, 2H), 7.05 (dd, J = 8.0, 4.8 Hz, 1H). 13C NMR (CDCl3): δ 165.0, 148.8, 147.5, 141.5, 134.2, 132.4, 128.9, 127.5, 121.6, 112.7.

**Method F: N-(3-Acetophenin-4-yl)benzamide (17b).** This method also gave N-phenyl-2-[2-(pyridin-2-yl)quinolin-4-yl]propanamide (22c). Journal of Medicinal Chemistry
ASSOCIATED CONTENT

Supporting Information

Syntheses of compounds not described in main text and determination of absolute configuration of (−)-10a. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

This paper was written with contributions from all authors. All authors approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

DCA, 1,2-dichloroethane; DIPA, diisopropylethylamine; DPE-Phos, bis-[(2-diphenylphosphino)phenyl]ether; HAB, high-affinity binder; LAB, low-affinity binder; MAB, mixed-affinity binder; HFIP, hexafluoroisopropanol; PFP, pentafluorophenol; PyBroP, bromotripropylidinophosphonium hexafluorophosphate; TEA, triethylamine; TSPO, translocator protein (18 kDa)

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