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Brief Article

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Synthesis and Biological Evaluation of 4-Phenylquinazoline-2-carboxamides Designed as a Novel Class of Potent Ligands of the Translocator Protein

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Supporting Information

ABSTRACT: A series of novel 4-phenylquinazoline-2-carboxamides (**1**−**58**) were designed as aza-isosters of PK11195, the well-known 18 kDa translocator protein (TSPO) reference ligand, and synthesized by means of a very simple and efficient procedure. A number of these derivatives bind to the TSPO with K_i values in the nanomolar/subnanomolar range, show selectivity toward the central benzodiazepine receptor (BzR) and exhibit structure−affinity relationships consistent with a previously published pharmacophore/topological model of ligand−TSPO interaction.

INTRODUCTION

The human 18 kDa translocator protein (TSPO) is a highly hydrophobic protein mainly located at the contact sites between the outer and the inner mitochondrial membranes. TSPO is expressed in many peripheral tissues, including heart, kidney, and lung, with higher expression levels in steroid producing tissues. Low levels are expressed in brain, where it is limited to glial cells (astrocytes and microglia).[1]

The TSPO is involved in a variety of important physiological processes, including cholesterol transport, steroidogenesis, calcium homeostasis, lipid metabolism, mitochondrial oxidation, cell growth and differentiation, apoptosis induction, and regulation of immune functions. This makes TSPO an intriguing target to develop novel potential therapeutic tools for the treatment of a variety of diseases.[1,2] In addition, the alteration of its expression levels in a number of human pathologies involving a perturbation of cell survival/death processes, such as cancer (particularly solid colon, breast, and prostate cancers), cerebral injury, including neurodegenerative disorders (Huntington's and Alzheimer's diseases), or inflammatory and neoplastic brain disorders, highlights TSPO as a valid diagnostic marker for related-disease state and progression.[3]

However, the exact physiopathological role of TSPO is not completely defined, encouraging a continued research in this field to develop new potent and selective ligands as suitable tools to deepen the knowledge about this protein functioning. Since its identification by means of the benzodiazepines diazepam and Ro5−4864 (Figure 1),[4] structurally different classes of high affinity and selectivity ligands have been reported,[5,6]

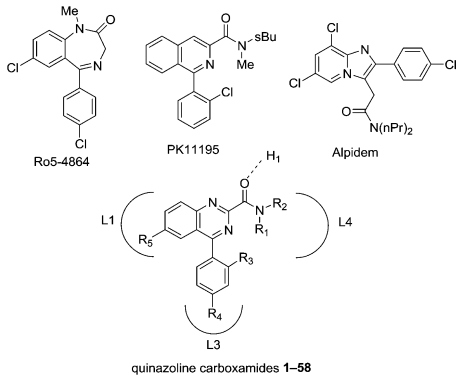

Figure 1. Structure of known TSPO ligands and newly synthesized quinazoline derivatives within the framework of our pharmacophore/topological model of ligand−TSPO interaction.[13]

including the isoquinolinecarboxamides, of which the 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-1-isoquinoline carboxamide (PK11195, Figure 1) is widely considered as a prototypical TSPO ligand, used as reference compound in biological experiments[7] and radiolabeled for imaging studies.[8,9] Nevertheless, PK11195 shows several drawbacks, principally caused by its high lipophilicity, such as, for example, high level of nonspecific binding and a poor signal-to-noise ratio in PET studies.[9]

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To pursue our interest in the medicinal chemistry of TSPO ligands,[7,10,11] and with the aim to identify novel chemotype ligands, we now report the study of a series of quinazoline-2-carboxamide derivatives (**1**–**58**, Figure 1), straightforwardly obtained with a simple and efficient synthetic procedure.

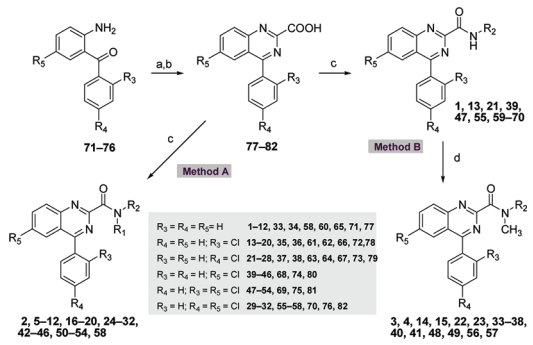
Although structurally related to the isoquinolinecarboxamide PK11195 (Figure 1), these quinazoline derivatives were assumed to have a better drug-like character, possessing a higher hydrophilicity and water solubility than their quite lipophilic isoquinoline counterpart. Indeed, preliminary calculations of several physicochemical and pharmacokinetic parameters conducted on the structures of PK11195 and of its aza-isoster **14** supported our hypotheses (see Results and Discussion). In a 1985 patent,[12] reporting on the synthesis and the affinity for TSPO of arene- and heteroarene-carboxamides, a couple of quinazoline compounds were described but without any investigation of their pharmacological properties.

Taking into account the structural requirements needed for high TSPO affinity and selectivity,[6] compounds **1**–**58** featuring different combinations of R_1–R_5 substituents were designed. Specifically, symmetrically or asymmetrically *N*,*N*-disubstituted quinazolines bearing linear, branched, or alicyclic alkyl chains were synthesized. In addition, to investigate the role of a double substitution on the amide nitrogen to gain high TSPO affinity in this class, a number of *N*-monosubstituted derivatives were studied. Finally, a chlorine atom was inserted at different positions (2′, 4′, 6) of the basic 4-phenylquinazoline scaffold. All novel compounds (**1**–**58**) have been tested for their affinity at TSPO in rat kidney membranes, and SARs were discussed in light of a previously published pharmacophore/topological model of ligand–TSPO interaction.[10,13] In addition, a preliminary in vitro biological characterization has been performed on compounds **9** and **14** using PK11195 as a standard reference.

CHEMISTRY

The key intermediates in the synthesis of phenylquinazoline-2-carboxamides **1**–**58** are the carboxylic acids **77**–**82**, prepared by condensation of the appropriate 2-aminobenzophenones **71**–**76** with glyoxylic acid in the presence of ammonium acetate, followed by light irradiation with 20 W halogen tungsten lamp (Scheme 1; see Supporting Information (SI) for details).

Scheme 1. Synthesis of Quinazoline Derivatives 1–58[a]



[a](a) CHOCOOH, NH_4OAc, EtOH, RT, 0.5 h; (b) light irradiation, DMF, RT, 12 h, 62–86% (two steps); (c) $SOCl_2$, reflux, 2 h, then monoalkyl- or dialkylamine, NEt_3, THF, RT, 36–48 h, 43–88%; (d) NaH, CH_3I, DMF, 0 °C to RT, 0.5 h, 67–93%.

Noncommercially available 2-aminobenzophenones **72**, **73**, and **76** were synthesized according to literature procedures.[14] After activation of the acids **77**–**82** with thionyl chloride, direct coupling with the proper commercially available *N*,*N*-dialkylamine yielded *N*,*N*-dialkylquinazolinecarboxamides **2**, **5**–**12**, **16**–**20**, **24**–**32**, **42**–**46**, **50**–**54**, and **58** (method A, Scheme 1). When the required *N*-methyl-*N*-alkylamine was not available, the intermediate carboxylic acids **77**–**82** were activated with thionyl chloride and reacted with the appropriate primary amines to give the secondary amides **1**, **13**, **21**, **39**, **47**, **55**, and **59**–**70**, which were subsequently *N*-methylated with methyl iodide in the presence of sodium hydride to furnish compounds **3**, **4**, **14**, **15**, **22**, **23**, **33**–**38**, **40**, **41**, **48**, **49**, **56**, and **57** (method B, Scheme 1).

BIOLOGY

The TSPO binding affinities of compounds **1**–**58** were determined by competition experiments against [^{3}H]-PK11195,[7,10] carried out in rat kidney membranes (Table 1). The TSPO/central benzodiazepine receptor (BzR) selectivity of compounds **1**–**58** was evaluated by experiments against [^{3}H]flumazenil in rat cerebral cortex membranes (Table S6, SI).[7,10] In addition, to explore the effect of this new class of compounds on TSPO function, a representative subset of TSPO ligands was examined for the ability to stimulate pregnenolone formation from rat C6 glioma cells (**2**, **7**, **9**, **14**, **22**, and **25**)[7,10] and for the effect on the proliferation/viability of U87MG glioma cells (**9** and **14**).[15,16]

RESULTS AND DISCUSSION

Many of the synthesized compounds show high affinity for TSPO in the nanomolar/subnanomolar range, with a potency higher than that of the lead PK11195 (Table 1) and a high selectivity for TSPO over BzR, as they did not significantly inhibit the binding of [^{3}H]flumazenil in membranes from rat brain tissues (inhibition percentages at 10 μM concentration ranging from 0% to 48%, Table S6, SI). The SARs emerging from this series are herein discussed in light of a previously published pharmacophore/topological model of ligand–TSPO interaction made up by three lipophilic pockets (L_1, L_3, and L_4) and an H-bond donor group (H_1) (Figure 1).[13]

All the *N*-monosubstituted quinazolines tested (**1**, **13**, **21**, **39**, **47**, **55**) are devoid of affinity. These compounds miss hydrophobic contacts which would otherwise take place between an *N*-alkyl group and the L_4 pocket. Moreover, it is hypothesized that they adopt a conformation unsuited for optimal interaction with the receptor. In fact, as detailed in the SI, molecular mechanics (MM) calculations revealed that the *N*-monosubstituted and the *N*,*N*-disubstituted derivatives are predicted to adopt low energy conformations characterized by the O=C-*N*< moiety laying within the plane (torsion angle N1–C2–C=O about 0°) and out of the plane (torsion angle N1–C2–C=O about 30°) of the quinazoline ring (Figure 2), respectively. Such a difference in the conformational properties of the two subsets of compounds might depend on the fact that each of the two quinazoline nitrogens attracts electrostatically the *N*-hydrogen while repelling sterically any *N*-alkyl group. We could speculate that only the carbonyl group of the *N*,*N*-disubstituted derivatives is correctly oriented within the binding cleft to make a strong H-bond with the H_1 site. Our hypothesis is consistent with the results of the excellent work by Cappelli and co-workers, who mapped the TSPO binding site using conformationally restrained analogues of PK11195.[17] According to these authors, the TSPO-bound conformations of the most potent

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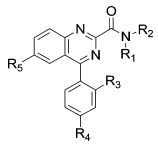
Table 1. TSPO Affinity of Quinazoline Derivatives 1−58



N	R_1	R_2	R_5	R_3	R_4	I% (1 μM) or K_i (nM)[a]
1	H	$CH(CH_3)CH_2CH_3$	H	H	H	0%
2	CH_3	$(CH_2)_3CH_3$	H	H	H	46.0 ± 5.1
3	CH_3	$CH(CH_3)CH_2CH_3$	H	H	H	3.00 ± 0.30
4	CH_3	$CH_2CH(CH_3)_2$	H	H	H	66.5 ± 6.2
5	CH_3	$CH(CH_3)_2$	H	H	H	70.3 ± 6.2
6	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	H	H	H	36.8 ± 3.0
7	$(CH_2)_3CH_3$	$(CH_2)_3CH_3$	H	H	H	3.30 ± 0.30
8	$(CH_2)_4CH_3$	$(CH_2)_4CH_3$	H	H	H	2.48 ± 0.21
9	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	H	H	H	0.690 ± 0.070
10	$(CH_2)_4$		H	H	H	36%
11	$(CH_2)_5$		H	H	H	61%
12	$(CH_2)_6$		H	H	H	79%
13	H	$CH(CH_3)CH_2CH_3$	H	Cl	H	63%
14	CH_3	$CH(CH_3)CH_2CH_3$	H	Cl	H	3.06 ± 0.30
15	CH_3	$CH_2CH(CH_3)_2$	H	Cl	H	6.98 ± 0.71
16	CH_3	$CH(CH_3)_2$	H	Cl	H	12.5 ± 1.3
17	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	H	Cl	H	65.1 ± 7.0
18	$(CH_2)_4$		H	Cl	H	70%
19	$(CH_2)_5$		H	Cl	H	46.0 ± 5.3
20	$(CH_2)_6$		H	Cl	H	8.77 ± 0.82
21	H	$CH(CH_3)CH_2CH_3$	H	H	Cl	37%
22	CH_3	$CH(CH_3)CH_2CH_3$	H	H	Cl	2.67 ± 0.30
23	CH_3	$CH_2CH(CH_3)_2$	H	H	Cl	20.7 ± 2.3
24	CH_3	$CH(CH_3)_2$	H	H	Cl	50.4 ± 4.9
25	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	H	H	Cl	3.42 ± 0.29
26	$(CH_2)_4$		H	H	Cl	644 ± 60
27	$(CH_2)_5$		H	H	Cl	493 ± 50
28	$(CH_2)_6$		H	H	Cl	84.3 ± 8.5
29	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	Cl	H	Cl	4.31 ± 0.50
30	$(CH_2)_4$		Cl	H	Cl	64%
31	$(CH_2)_5$		Cl	H	Cl	76%
32	$(CH_2)_6$		Cl	H	Cl	77%
33	CH_3	(*S*)-$CH(CH_3)CH_2CH_3$	H	H	H	6.09 ± 0.52
34	CH_3	(*R*)-$CH(CH_3)CH_2CH_3$	H	H	H	6.41 ± 0.51
35	CH_3	(*S*)-$CH(CH_3)CH_2CH_3$	H	Cl	H	2.20 ± 0.20
36	CH_3	(*R*)-$CH(CH_3)CH_2CH_3$	H	Cl	H	1.55 ± 0.19
37	CH_3	(*S*)-$CH(CH_3)CH_2CH_3$	H	H	Cl	2.51 ± 0.27
38	CH_3	(*R*)-$CH(CH_3)CH_2CH_3$	H	H	Cl	1.57 ± 0.14
39	H	$CH(CH_3)CH_2CH_3$	Cl	H	H	0%
40	CH_3	$CH(CH_3)CH_2CH_3$	Cl	H	H	22%
41	CH_3	$CH_2CH(CH_3)_2$	Cl	H	H	842 ± 75
42	CH_3	$CH(CH_3)_2$	Cl	H	H	0%
43	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	Cl	H	H	38.5 ± 4.0
44	$(CH_2)_4$		Cl	H	H	43%
45	$(CH_2)_5$		Cl	H	H	46%
46	$(CH_2)_6$		Cl	H	H	38%
47	H	$CH(CH_3)CH_2CH_3$	Cl	Cl	H	0%
48	CH_3	$CH(CH_3)CH_2CH_3$	Cl	Cl	H	43%
49	CH_3	$CH_2CH(CH_3)_2$	Cl	Cl	H	77%
50	CH_3	$CH(CH_3)_2$	Cl	Cl	H	0%
51	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	Cl	Cl	H	30.4 ± 3.1
52	$(CH_2)_4$		Cl	Cl	H	73%
53	$(CH_2)_5$		Cl	Cl	H	273 ± 30
54	$(CH_2)_6$		Cl	Cl	H	415 ± 40
55	H	$CH(CH_3)CH_2CH_3$	Cl	H	Cl	49%
56	CH_3	$CH(CH_3)CH_2CH_3$	Cl	H	Cl	179 ± 18
57	CH_3	$CH_2CH(CH_3)_2$	Cl	H	Cl	60.7 ± 5.7
58	CH_3	$CH(CH_3)_2$	Cl	H	Cl	39%
Ro5-4864						23.0 ± 3.0
PK11195						9.30 ± 0.5
Alpidem						0.5−70

[a]The concentration of test molecules that inhibited [^{3}H]PK11195 binding to rat kidney mitochondrial membranes by 50% (IC_{50}) was determined with six concentrations of the compounds, each performed in triplicate. K_i values and inhibition percentages at 1 μM are the mean ± SEM of three determinations.

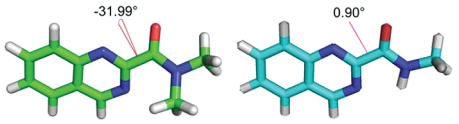


Figure 2. Low energy conformations as calculated by MM simulations of the phenyl-truncated *N*,*N*-disubstituted and the *N*-monosubstituted quinazolines represented as green and cyan sticks, respectively. The N1−C2−C═O dihedral angle value is also labeled.

isoquinoline-3-carboxamides are characterized by the carbonyl group oriented out of the plane of the isoquinoline ring system.

In an attempt to explain the total lack of affinity shown by the *N*-monosubstituted derivatives compared with their potent *N*,*N*-disubstituted counterparts, we also pairwise examined their IR and ^{1}H NMR spectra to verify whether the O═C-NH− moiety was switched to the corresponding HO-C═N− tautomer. Yet, no data supported such a hypothesis.

Noticeably, the introduction of a second nitrogen atom in the isoquinoline nucleus of PK11195 produces a favorable effect on TSPO affinity, as product **14** shows a 3-fold gain in potency with respect to its parent compound. Compound **9**, with $R_1 = R_2 =$ *n*-hexyl, $R_3 = R_4 = R_5 = H$, is the most potent one (K_i 0.7 nM) and also slightly less potent compounds (**3**, **7**, **8**, **14**, **15**, **22**, **25**, **29**, **33**−**38**) display K_i values lower than PK11195. It is noteworthy that all these molecules share the following features: (i) they are *N*,*N*-disubstituted, (ii) one of the two *N*-alkyl groups contains a number of carbon atoms comprised between 4 and 6. Taken together, the above data suggest that an optimal ligand−TSPO interaction requires full occupation of the L_4 hydrophobic pocket by at least one bulky *N*-alkyl group. The shape of such *N*-alkyl group is, in some instances, a key determinant of affinity: compare the *N*-*s*-butyl derivatives **3** (K_i 3.00 nM) and **22** (K_i 2.67 nM) with their much less potent *N*-*i*-butyl isomers **4** (K_i 66.5 nM) and **23** (K_i 20.7 nM), respectively. In contrast, the *N*-*s*-butyl and the *N*-*i*-butyl derivatives **14** (K_i 3.06 nM) and **15** (K_i 6.98 nM) are practically equipotent, and the *N*-*i*-butyl derivatives **41** (K_i 842 nM) and **57** (K_i 60.7 nM) are more potent than their *N*-*s*-butyl counterparts **40** (inhib 22%) and **56** (K_i 179 nM). Chirality does not impact on the affinity within the subset of *N*-*s*-butyl derivatives assayed as pure enantiomers (compare **33** (K_i 6.09 nM) vs **34** (K_i 6.41 nM), **35** (K_i 2.2 nM) vs **36** (K_i 1.55 nM) and **37** (K_i 2.51 nM) vs **38** (K_i 1.57 nM)), differently from what happens with PK11195, for which the *R*-enantiomer is reported to show higher TSPO affinity than the racemic mixture in rats.[18]

Affinity is worsened to different extents by any of the following features: (i) anellation of the side chain to give the cyclic pyrrolidinyl- piperazinyl- and azepinyl-amides **10**−**12**, **18**−**20**, **26**−**28**, **30**−**32**, **44**−**46**, and **52**−**54**, (ii) insertion of a chlorine at the 6-position of the quinazoline ring (**29**−**32** and **39**−**58**), with a few exception (compare **51** and **17**). These data suggest that a rigid carboxamide side chain and a substituent at 6-position of the quinazoline ring cannot fit into the L_4 pocket or the L_1 pocket for sterical reasons, respectively.

Mixed results are associated with the absence/presence of a chlorine at the 2′- or 4′-position of the pendant phenyl: the *N*-*s*-butyl derivatives **3** (K_i 3.00 nM), **14** (K_i 3.06 nM), and **22** (K_i 2.67 nM), differing in the above substitution pattern, are essentially equipotent; the *N*-*i*-butyl and *N*-*i*-propyl derivatives **4** (K_i 66.5 nM) and **5** (K_i 70.3 nM) are significantly less potent than their 2′-chloro derivatives **15** (K_i 6.98 nM) and **16** (K_i 12.5 nM) and are slightly less potent than their 4′-chloro derivatives **23** (K_i 20.7 nM) and **24** (K_i 50.4 nM). These data,

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characterized by interdependent (nonadditive) effects of the R_2, R_3, and R_4 substituents on affinity, are difficult to be rationalized using a "static" pharmacophore/topological model. It is tempting to hypothesize that a strong hydrophobic interaction of R_2 = *s*-butyl with the L_4 pocket may pose the ligand in the binding cleft so as to weaken the beneficial effects of R_3 or R_4 = Cl within the L_3 pocket (e.g., **3**, **14**, and **22**). Otherwise, a less strong interaction of R_2 = *i*-butyl or *i*-propyl with the L_4 pocket may allow a better fit of R_3 and R_4 into the L_3 pocket (e.g., **4** and **15** or **5** and **16**). When the interaction with the L_4 pocket is optimized (R_1 = R_2 = *n*-hexyl), the presence of one or even two chlorine atoms is tolerated (e.g., **17**, **25**, **29**, and **51**).

Some physicochemical and pharmacokinetic properties of **14** and its parent compound PK11195 were calculated and compared as a rough assessment of the drug-like character of our quinazoline derivatives. For such a purpose, we employed the Qikprop program (Schrödinger. LLC New York). The results are summarized in Table S5, SI. QSAR studies on CNS active drugs and their analogues, together with retrospective analyses based on marketed CNS drugs, have suggested the physical and chemical properties that CNS drugs must possess. They are: molecular weight (MW) less than 450, ClogP less than 5, number of H-bond acceptor atoms less than 7, polar surface area (PSA) less than 90 Å, number of rotatable bonds (RB) less or equal to 10. Thus, for CNS penetration, the physical properties, usually, have a smaller range than general therapeutics (the latter ranges are reported in Table S5, SI).

On the basis of these premises, compound **14** is predicted to have a good probability of entering the CNS, as all its estimated physicochemical parameters fall in the aforementioned ranges. Furthermore, it is worth mentioning that, despite the structural similarity between the two compounds resulting in comparable structure-derived physicochemical properties, compound **14** scores better than PK11195 if we consider the Lipinski's Rule-of-Five and the Jorgensen's Rule-of-Three, both aimed at identifying drug-like molecules.

To explore the effect of the compounds on steroid biosynthesis, a representative subset of TSPO quinazoline ligands (**2**, **7**, **9**, **14**, **22**, and **25**) and PK11195 and Ro5−4864 as standards, were examined for their ability to stimulate pregnenolone formation from rat C6 glioma cells.[7,10] In this assay, all tested derivatives, with the exception of the practically inactive **9**, exhibited low/medium percentage increase in pregnenolone production vs control, with an efficacy lower than that of PK11195 and Ro5−4864 (Table S7, SI), independently from their K_i values. The lack of any correlation between affinity and steroidogenic activity showed by these quinazoline ligands is in line with data from other known classes of highly affine TSPO ligands, including 2-phenylindolglyoxylamides, imidazo-pyridines, and pyrazolo-pyrimidines.[6] Furthermore, to investigate whether the newly synthesized compounds affected another well-known TSPO function, which is the regulation of life−death cell processes, compounds **9** and **14** were preliminarily evaluated for their effect on the proliferation/viability of U87MG glioma cells. PK11195 was also tested as reference compound. As detailed in the SI, the colorimetric viability MTS assay[16] showed that PK11195 produced effects in agreement with those reported in the literature[19] on the same cell line, namely a statistically significant reduction of U87MG cell proliferation/viability (Figure S1, SI) only at the highest concentration tested (100 μM). Compound **14** showed comparable results with those obtained with PK11195 (Figure S2, SI), whereas compound **9** was more effective than the reference standard at inhibiting the U87MG cell survival (Figure S3, SI). On the basis of these preliminary data, it may be speculated that these quinazoline ligands possess the ability to bind the TSPO with high affinity, leading to protein conformational changes that do not significantly affect the steroidogenic TSPO function, whereas they produce a slight modulation on cell proliferation/viability.

CONCLUSION

A simple and efficient synthetic procedure has been developed to obtain quinazoline azaisosters of PK11195, a potent TSPO ligand widely used as reference compound. This strategy allowed us to easily prepare a great number of related derivatives (**1**−**58**), many of which show nanomolar/subnanomolar K_i values for the TSPO, with improved affinity with respect to the lead PK11195. Compound **9** (*N*,*N*-di-*n*-hexyl-4-phenylquinazoline-2-carboxamide) stands out as the most potent ligand of the series with a K_i of 0.7 nM, and high selectivity toward BzR.

SARs emerging from this series were rationalized in light of a previously published pharmacophore/topological model of ligand−TSPO interaction, allowing to define the main structural requirements for an optimal interaction with the target protein, that is: (i) *N*,*N*-disubstitution on the carboxamide moiety, (ii) at least one of the two *N*-alkyl groups with a number of carbon atoms comprised between 4 and 6. The comparison of some calculated physicochemical and pharmacokinetic properties of compound **14** and of the lead PK11195, evidenced a better drug-like character for our quinazoline derivative.

In a preliminary biological in vitro investigation, the effects exerted by these new derivatives on steroidogenesis and life−death cell processes, with respect to those produced by the lead PK11195, suggested that even little structural modifications may affect ligand-mediated modulation of TSPO functions.

Taken together, all these findings highlighted the quinazoline nucleus as a suitable scaffold to further expand the chemical diversity in TSPO ligands and provided SARs data for the design of new TSPO modulators useful to deepen the knowledge about this protein, even by means of the development of imaging tracers with improved specificity.

EXPERIMENTAL SECTION

Chemistry. General directions are in the SI. Purity of tested compounds is ≥95% (combustion analysis).

General Procedure for the Synthesis of *N*-alkyl-4-phenylquinazoline-2-carboxamides 1, 2, 5−13, 16−21, 24−32, 39, 42−47, 50−55, and 58−70. A solution of the appropriate 2-carboxylic acid **77**−**82** (2.0 mmol) in thionyl chloride (15 mL) was refluxed for 2 h under nitrogen atmosphere. After cooling at room temperature, the excess thionyl chloride was removed at reduced pressure and the crude material dried under vacuum. To the residue, dissolved in dry THF (10 mL) and cooled to 0 °C, a mixture of the proper amine (2.0 mmol) and triethylamine (2.0 mmol) in dry THF (5 mL) was added dropwise. The mixture was stirred at room temperature for 36−48 h (TLC analysis), filtered, and evaporated. The crude residue was dissolved in DCM (20 mL), washed with HCl 1N, saturated $NaHCO_3$, and water, dried, and concentrated in vacuum. Purification by column chromatography on silica gel (DCM−EtOAc) provided title compounds (yields, physical, and spectral data are reported in Tables S2 and S3, SI).

General Procedure for the Synthesis of *N*-Alkyl-*N*-methyl-4-phenylquinazoline-2-carboxamides 3, 4, 14, 15, 22, 23, 33−38, 40, 41, 48, 49, 56, and 57. Sodium hydride (5.5 mmol) was added portionwise, under nitrogen atmosphere, to an ice-cooled solution of the appropriate *N*-alkyl-4-phenylquinazoline-2-carboxamide (5.0 mmol) in dry DMF (5 mL). The mixture was stirred for 30 min and treated with an excess of methyl iodide (0.68 mL, 11.0 mmol). The mixture

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was stirred for 1 h at 25 °C, and an ice cooled solution of HCl 1N and chloroform were added. The organic layer was separated, washed with brine, dried, and concentrated in vacuum. Purification by column chromatography on silica gel (DCM−EtOAc) provided title compounds (yields, physical, and spectral data are reported in Table S4, SI).

■ ASSOCIATED CONTENT

Supporting Information

General chemistry directions, general procedure for the synthesis of 4-phenylquinazoline-2-carboxylic acids **77**−**82**, yields, physical, and spectral data of compounds **1**−**70**, and **77**−**82**, computational chemistry, calculated physicochemical and pharmacokinetic properties of compound **14** and PK11195, biological methods, BzR binding data of compounds **1**−**58**, increase in pregnenolone production of selected quinazoline derivatives, effect on proliferation/viability of U87MG glioma cells of compounds **9** and **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

BBB, blood−brain barrier; BzR, central benzodiazepine receptor; CNS, central nervous system; PBR, peripheral benzodiazepine receptor; PET, positron emission tomography; SARs, structure−activity relationships; TSPO, 18 kDa translocator protein

■ REFERENCES

(1) Papadopoulos, V.; Baraldi, M.; Guilarte, T. R.; Knudsen, T. B.; Lacapere, J. J.; Lindemann, P.; Norenberg, M. D.; Nutt, D.; Weizman, A.; Zhang, M. R.; Gavish, M. Translocator protein (18 kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. *Trends Pharmacol. Sci.* **2006**, *27*, 402−409.

(2) Rone, M. B.; Fan, J.; Papadopoulos, V. Cholesterol transport in steroid biosynthesis: role of protein−protein interactions and implications in disease states. *Biochim. Biophys. Acta* **2009**, *1791*, 646−658.

(3) Chen, M. K.; Guilarte, T. R. Translocator protein 18 kDa (TSPO): molecular sensor of brain injury and repair. *Pharmacol. Ther.* **2008**, *118*, 1−17.

(4) Braestrup, C.; Squires, R. F. Specific benzodiazepine receptors in rat brain characterized by high-affinity (^{3}H)diazepam binding. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 3805−3809.

(5) James, M. L.; Selleri, S.; Kassiou, M. Development of ligands for the peripheral benzodiazepine receptor. *Curr. Med. Chem.* **2006**, *13*, 1991−2001.

(6) Taliani, S.; Pugliesi, I.; Da Settimo, F. Structural Requirements to Obtain Highly Potent and Selective 18 kDa Translocator Protein (TSPO) Ligands. *Curr. Top. Med. Chem.* **2011**, *11*, 860−886.

(7) Primofiore, G.; Da Settimo, F.; Taliani, S.; Simorini, F.; Patrizi, M. P.; Novellino, E.; Greco, G.; Abignente, E.; Costa, B.; Chelli, B.; Martini, C. *N*,*N*-Dialkyl-2-phenylindol-3-ylglyoxylamides. A new class of potent and selective ligands at the peripheral benzodiazepine receptor. *J. Med. Chem.* **2004**, *47*, 1852−1855.

(8) Matarrese, M.; Moresco, R. M.; Cappelli, A.; Anzini, M.; Vomero, S.; Simonelli, P.; Verza, E.; Magni, F.; Sudati, F.; Soloviev, D.; Todde, S.; Carpinelli, A.; Kienle, M. G.; Fazio, F. Labeling and evaluation of *N*-[^{11}C]-methylated quinoline-2-carboxamides as potential radioligands for visualization of peripheral benzodiazepine receptors. *J. Med. Chem.* **2001**, *44*, 579−585.

(9) Chaveau, F.; Boutin, H.; Van Camp, N.; Dolle, F.; Tavitian, B. Nuclear imaging of neuroinflammation: a comprehensive review of [11C]PK11195 challengers. *Eur. J. Nucl. Med. Mol. Imaging* **2008**, *35*, 2304−2319.

(10) Da Settimo, F.; Simorini, F.; Taliani, S.; La Motta, C.; Marini, A. M.; Salerno, S.; Bellandi, M.; Novellino, E.; Greco, G.; Cosimelli, B.; Da Pozzo, E.; Costa, B.; Simola, N.; Morelli, M.; Martini, C. Anxiolytic-like effects of *N*,*N*-dialkyl-2-phenylindol-3-ylglyoxylamides by modulation of translocator protein promoting neurosteroid biosynthesis. *J. Med. Chem.* **2008**, *51*, 5798−5806.

(11) Cosimelli, B.; Simorini, F.; Taliani, S.; La Motta, C.; Da Settimo, F.; Severi, E.; Greco, G.; Novellino, E.; Costa, B.; Da Pozzo, E.; Bendinelli, S.; Martini, C. Tertiary amides with a five-membered heteroaromatic ring as new probes for the translocator protein. *Eur. J. Med. Chem.* **2011**, *46*, 4506−4520.

(12) Dubroeucq, M. C.; Renault, C.; Le Fur, G. Derivatives of arene and hetero-arene carboxamides and their use as medicaments. US 1983/482082, US19830482082, 19830405, Feb 12, 1985.

(13) Campiani, G.; Nacci, V.; Fiorini, I.; De Filippis, M. P.; Garofalo, A.; Ciani, S. M.; Greco, G.; Novellino, E.; Williams, D. C.; Zisterer, D. M.; Woods, M. J.; Mihai, C.; Manzoni, C.; Mennini, T. Synthesis, biological activity, and SARs of pyrrolobenzoxazepine derivatives, a new class of specific "peripheral-type" benzodiazepine receptor ligands. *J. Med. Chem.* **1996**, *39*, 3435−3450.

(14) Sugasawa, T.; Toyoda, T.; Adachi, M.; Sasakura, K. Aminohaloborane in organic synthesis. 1. Specific ortho substitution reaction of anilines. *J. Am. Chem. Soc.* **1978**, *100*, 4842−4845.

(15) Chelli, B.; Lena, A.; Vanacore, R.; Da Pozzo, E.; Costa, B.; Rossi., L.; Salvetti, A.; Scatena, F.; Ceruti, S.; Abbracchio, M. P.; Gremigni, V.; Martini, C. Peripheral benzodiazepine receptor ligands: mitochondrial transmembrane potential depolarization and apoptosis induction in rat C6 glioma cells. *Biochem. Pharmacol.* **2004**, *68*, 125−134.

(16) Ruan, S.; Okcu, M. F.; Pong, R. C.; Andreeff, M.; Levin, V.; Hsieh, J. T.; Zhang, W. Attenuation of WAF1/Cip1 expression by an antisense adenovirus expression vector sensitizes glioblastoma cells to apoptosis induced by chemotherapeutic agents 1,3-bis(2-chloroethyl)-1-nitrosourea and cisplatin. *Clin. Cancer Res.* **1999**, *5*, 197−202.

(17) Cappelli, A.; Anzini, M.; Vomero, S.; De Benedetti, P. G.; Menziani, M. C.; Giorgi, G.; Manzoni, C. Mapping the peripheral benzodiazepine receptor binding site by conformationally restrained derivatives of 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3- isoquinolinecarboxamide (PK11195). *J. Med. Chem.* **1997**, *40*, 2910−2921.

(18) Shah, F.; Hume, S. P.; Pike, V. W.; Ashworth, S.; McDermott, J. Synthesis of the enantiomers of [*N*-methyl-^{11}C]PK11195 and comparison of their behaviours as radioligands for PK binding sites in rats. *Nucl. Med. Biol.* **1994**, *21*, 573−581.

(19) Kugler, W.; Veenman, L.; Shandalov, Y.; Leschiner, S.; Spanier, I.; Lakomek, M; Gavish, M. Ligands of the mitochondrial 18 kDa translocator protein attenuate apoptosis of human glioblastoma cells exposed to erucylphosphohomocholine. *Cell. Oncol.* **2008**, *30*, 435−450.