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New iodinated quinoline-2-carboxamides for SPECT imaging of the translocator protein

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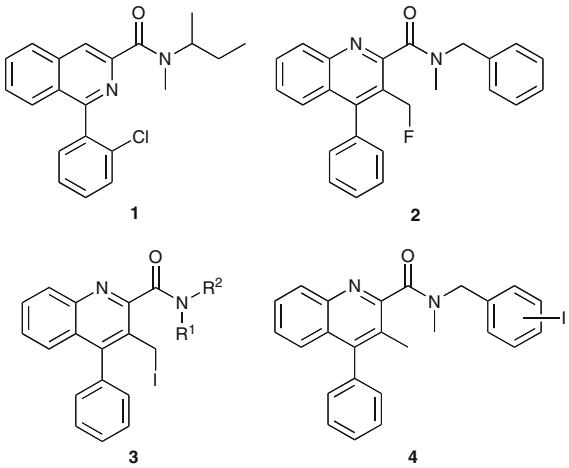
ABSTRACT

With the aim of developing new SPECT imaging agents for the translocator protein (TSPO), a small library of iodinated quinoline-2-carboxamides have been prepared and tested for binding affinity with TSPO. *N*,*N*-Diethyl-3-iodomethyl-4-phenylquinoline-2-carboxamide was found to have excellent affinity (K_i 12.0 nM), comparable to that of the widely used TSPO imaging agent PK11195.

The translocator protein 18 kDa (TSPO, formerly known as the peripheral benzodiazepine receptor) was initially characterised as a high affinity binding site for diazepam and has been shown to be functionally and structurally different from the central benzodiazepine receptor (CBR).[1] TSPO is found in lung, heart, spleen, liver, kidney, brain, adrenals, macrophages and mast cells and its function while tissue specific, revolves primarily around steroid synthesis.[2] Within the brain, TSPO is expressed by reactive glial cells and is implicated in a number of nervous system disorders such as cerebral ischaemia, epilepsy, nerve injury, and neurodegenerative diseases as well as immune system diseases such as cancer.[3] Expression of brain TSPO is markedly increased during chronic neurodegeneration and following acute brain injury[4] and thus, is an attractive target for molecular imaging of neuroinflammation in human neurodegenerative diseases such as Alzheimer's disease[5] and in stroke-induced brain injury.[6]

A number of positron emission tomography (PET) and single positron emission computed tomography (SPECT) ligands for the molecular imaging of TSPO have been developed.[7] The most widely used ligand is the isoquinoline carboxamide PK11195 **1**.[7a] However, there are a number of limitations in using radiolabelled analogues of PK11195 **1** such as high nonspecific binding, high plasma protein binding and relatively low brain uptake.[8] In the search for new radioligands for the molecular imaging of TSPO, the Cappelli group identified a family of quinoline-2-carboxamides (e.g., **2**) which have exceptionally high affinity for TSPO and showed promise in biodistribution experiments.[9] This work led to the development of various [^{11}C]-quinoline-2-carboxamides for PET imaging of TSPO.[9b,e] As yet, there are no reported examples of radioiodinated quinoline-2-carboxamides of this type developed for SPECT imaging of TSPO. In this Letter, we report the preparation of a small library of iodinated quinoline-2-carboxamides (e.g., **3** and **4**) and the screening of these compounds for affinity with TSPO.



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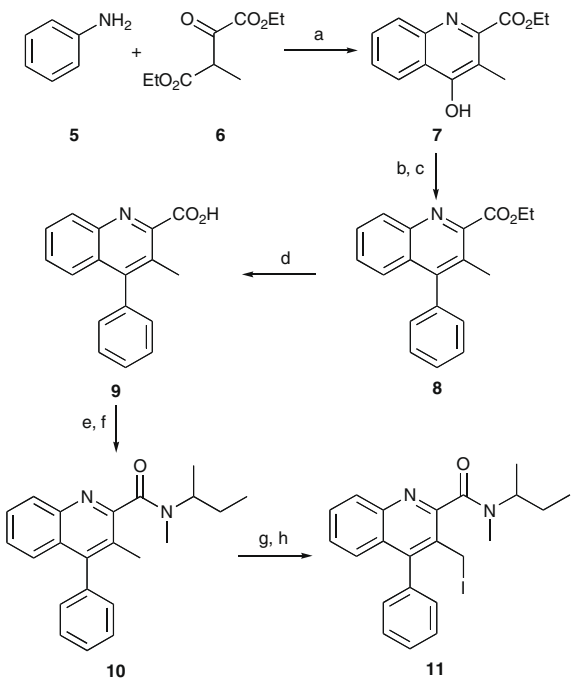
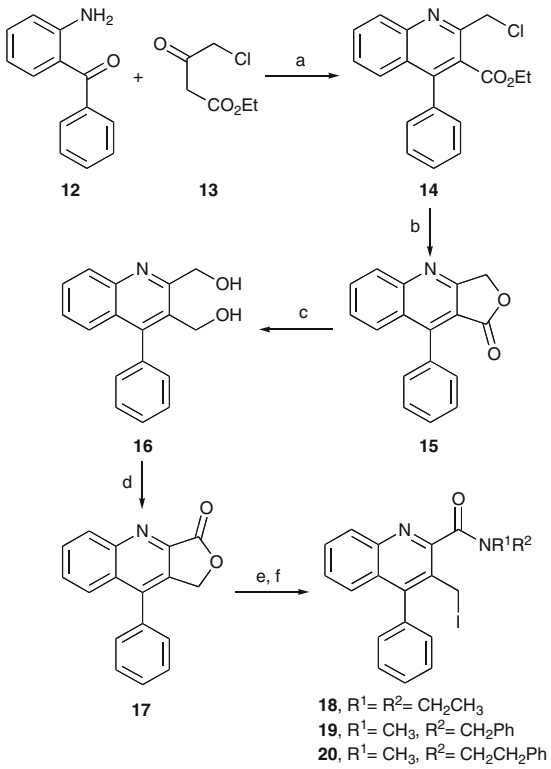
Our first aim was the synthesis of 3-iodomethylquinoline-2-carboxamide **11**, an analogue bearing the same side-chain as PK11195 **1** (Scheme 1). The quinoline carboxamide core was prepared in two steps by condensation of aniline **5** with diethyl oxalpropionate **6** followed by acid-mediated ring closure of the resulting imine which gave the 4-hydroxyquinoline **7** in good yield over the two steps.[10] The phenyl group was then introduced by bromination of **7** with phosphorus oxybromide followed by a Suzuki reaction with phenylboronic acid which gave **8** in excellent yields.[11] The PK11195 side-chain was then introduced using a standard series of transformations involving hydrolysis of the ester, formation of the acid chloride, reaction with *sec*-butyl amine and finally methylation which gave quinoline-2-carboxamide **10**. The final stage involved bromination of **10** with NBS in the presence of dibenzoyl peroxide followed by nucleophilic substitution with sodium iodide to give 3-iodomethylquinoline-2-carboxamide **11** in good overall yield.[9e]

While this initial approach allowed the synthesis of 3-iodomethylquinoline-2-carboxamide **11**, problems were encountered during the NBS bromination stage of quinoline-2-carboxamides with more bulky amide side-chains resulting in either low yields or no reaction. Thus, for the general synthesis of 3-iodomethylquinoline-2-carboxamides of this type a second approach was developed as shown in Scheme 2. A chlorotrimethylsilane-mediated Friedländer condensation was used for the efficient synthesis of quinoline **14**.[12] Lactonisation was then followed by reduction with lithium aluminium hydride which gave diol **16**.[13] Regioselective oxidation with manganese dioxide gave the key lactone intermediate **17** in 80% yield. Lactone **17** was then reacted with a series of secondary amines in the presence of trimethylaluminium which gave the corresponding 3-hydroxymethylquinoline-2-carboxamides in good yields. Chlorination of the hydroxymethyl group with thionyl chloride followed by reaction with sodium iodide gave 3-iodomethylquinoline-2-carboxamides **18**, **19** and **20**.



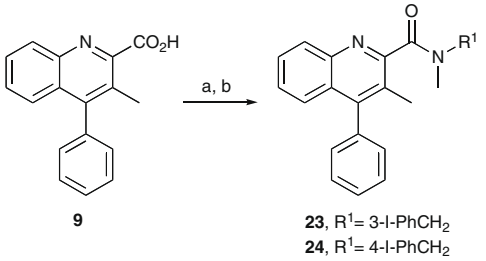
Scheme 1. Reagents and conditions: (a) (i) *p*-TsOH, cyclohexane, Δ; (ii) PPA, 120 °C, 55%; (b) $POBr_3$, K_2CO_3, MeCN, Δ, 98%; (c) phenylboronic acid, $Pd(PPh_3)_4$, K_3PO_4, DMF, Δ, 99%; (d) NaOH, EtOH/H_2O (1:1), 80 °C, 93%; (e) (i) $(COCl)_2$, DMF, CH_2Cl_2, 50 °C; (ii) *sec*-butyl amine, CH_2Cl_2, 50 °C, 70%; (f) MeI, NaH, THF, Δ, 82%; (g) NBS, dibenzoyl peroxide, CCl_4, Δ, 45%; (h) NaI, 18-crown-6, MeCN, 76%.



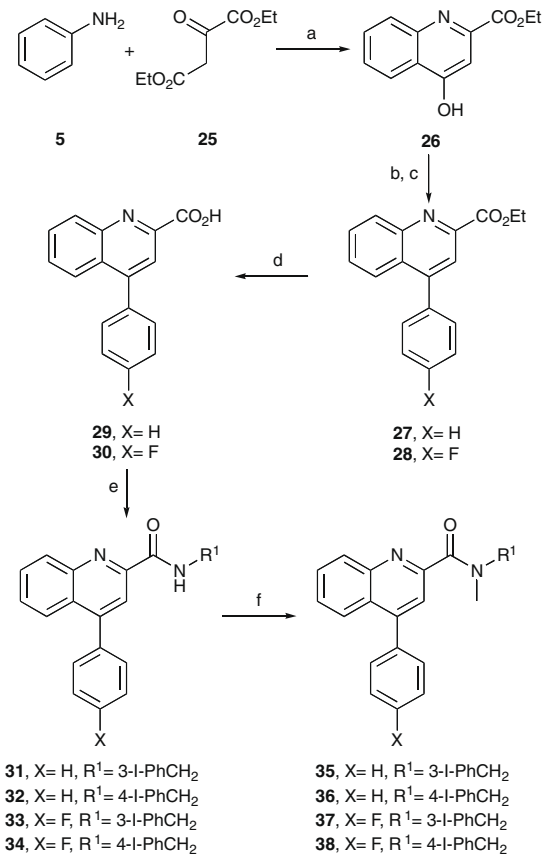
Scheme 2. Reagents and conditions: (a) TMSCl, DMF, 100 °C (sealed tube), 85%; (b) 6 M HCl, EtOH, Δ, 91%; (c) (i) $LiAlH_4$, THF; (ii) 10% Pd/C, MeOH, 67%; (d) MnO_2, $CHCl_3$, 80%; (e) diethylamine, *N*-methylbenzylamine or *N*-methylphenethylamine, Me_3Al, CH_2Cl_2, Δ, (65%, 59%; 53%, respectively); (f) (i) $SOCl_2$, CH_2Cl_2; (ii) NaI, MeCN, Δ, (**18**, 56%; **19**, 39%; **20**, 53%).

Having prepared a series of 3-iodomethylquinoline-2-carboxamides with various amide side-chains, we wanted to explore the TSPO binding affinity of 3-methylquinoline-2-carboxamides with iodine incorporated in the amide side-chain. Accordingly, two analogues containing a 3- and 4-iodobenzyl amide side-chain were prepared (Scheme 3). The previously prepared quinoline carboxylate **9** was reacted with oxalyl chloride to give the acid chloride and this was then reacted with either 3- or 4-iodobenzyl amine. Methylation using iodomethane and sodium hydride gave targets **23** and **24**.

Finally, a series of desmethyl quinoline-2-carboxamides were prepared to investigate the affinity of less rigid compounds (Scheme 4). 4-Hydroxyquinoline **26** was prepared in two steps by condensation of aniline **5** with diethyl oxalacetate **25**. Bromination followed by Suzuki reaction with phenylboronic acid gave **27** in excellent yield. Formation of the amide side-chain was then carried out incorporating either 3- or 4-iodobenzyl groups as described above to give targets **35** and **36** in good overall yields.



Scheme 3. Reagents and conditions: (a) (i) $(COCl)_2$, DMF, CH_2Cl_2, 50 °C; (ii) 3-iodobenzylamine or 4-iodobenzylamine, CH_2Cl_2, 50 °C, (**21**, R^1 = 3-I–$PhCH_2$, 80%; **22**, R^1 = 4-I–$PhCH_2$, 53%); (b) MeI, NaH, THF, Δ, (**23**, 76%; **24**, 90%).

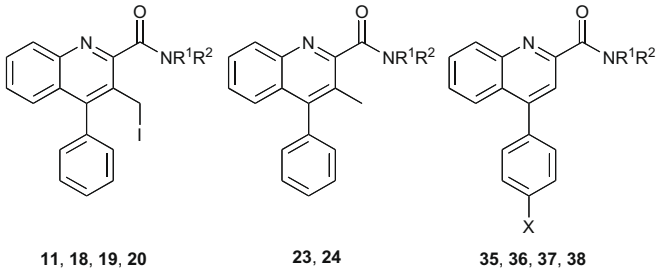


Scheme 4. Reagents and conditions: (a) (i) *p*-TsOH, cyclohexane, Δ; (ii) PPA, 120 °C, 48%; (b) $POBr_3$, K_2CO_3, MeCN, Δ, 99%; (c) phenylboronic acid or 4-fluorophenylboronic acid, $Pd(PPh_3)_4$, K_3PO_4, DMF, Δ, (**27**, 92%; **28**, 69%); (d) NaOH, EtOH/H_2O (1:1), 80 °C, (**29**, 90%; **30**, 96%); (e) (i) $(COCl)_2$, DMF, CH_2Cl_2, 50 °C; (ii) 3-iodobenzylamine or 4-iodobenzylamine, CH_2Cl_2, 50 °C, (**31**, 94%; **32**, 73%; **33**, 84%; **34**, 79%); (f) MeI, NaH, THF, Δ, (**35**, 74%; **36**, 56%; **37**, 66%; **38**, 64%).

A further series of compounds were prepared incorporating fluorine at the *para*-position of the phenyl ring. These analogues have the potential to be used for either SPECT (^{123}I) or PET (^{18}F) imaging. Compounds **37** and **38** were prepared as described for **35** and **36** except using 4-fluorophenylboronic acid during the Suzuki reaction.

The binding affinity of the library of compounds at TSPO was evaluated in vitro using homogenates of rat brain (Table 1).[14] The binding affinity of PK11195 **1** was also determined so that a direct comparison could be made with the new compounds. Two compounds from the 3-iodomethylquinoline-2-carboxamide series were found to have excellent affinity for TSPO. Benzyl analogue **19** was found to have a K_i value of 26.1 nM,[15] while diethyl analogue **18** has binding affinity (K_i 12.0 nM) comparable to that of PK11195 **1**. As shown by the results for compounds **20**, **23**, **24** and **35**–**38**, substantially increasing the size of the side-chain of the amide prevents these compounds from effectively binding to TSPO resulting in poor affinity. The higher affinity of 3-methyl analogue **23** (K_i 455 nM) compared to the desmethyl analogue **35** (K_i >1000 nM) confirms the observations of others, that semi-rigid compounds (restricted rotation of the amide carbonyl) bind with higher affinity to TSPO.[9a] The log *P* value for these compounds was also determined using an HPLC method involving C18 chromatography.[16] The analogues bearing a benzyl group in the amide side-chain all have log *P* values too high to be considered as suitable imaging agents. While diethyl analogue **18** also has a relatively high log *P* value (4.76), this value is comparable to other SPECT imaging agents known to cross the blood brain barrier.[17]

Table 1
Binding affinity of quinoline-2-carboxamide analogues **11**, **18**, **19**, **20**, **23**, **24** and **35**–**38** with the translocator protein



11, **18**, **19**, **20** **23**, **24** **35**, **36**, **37**, **38**

Compound	R^1	R^2	X	log *P*	K_i (nM)[a]
PK11195 **1**	—	—	—	3.85	9.8 ± 1.6
11	Me	*sec*-Butyl	—	5.17	173 ± 35
18	Et	Et	—	4.76	12.0 ± 1.3
19	Me	$PhCH_2$	—	5.39	26.1 ± 4.7
20	Me	$PhCH_2CH_2$	—	5.41	411 ± 62
23	Me	3-I–$PhCH_2$	—	5.03	455 ± 51
24	Me	4-I–$PhCH_2$	—	5.12	>1000
35	Me	3-I–$PhCH_2$	H	5.12	>1000
36	Me	4-I–$PhCH_2$	H	5.11	>1000
37	Me	3-I–$PhCH_2$	F	5.16	>1000
38	Me	4-I–$PhCH_2$	F	5.21	>1000

[a] Calculated from three independent experiments.

In summary, a small library of quinoline-2-carboxamides have been synthesised and their affinity for TSPO determined. *N*,*N*-Diethyl-3-iodomethyl-4-phenylquinoline-2-carboxamide **18** was found to have excellent affinity for TSPO with a K_i value comparable to that of PK11195 **1**. It should be noted that diethyl analogue **18** contains an iodomethyl moiety, a well-known alkylating group. However, due to bulky substitution at both positions *ortho* to the iodomethyl group, diethyl analogue **18** is relatively stable under physiological conditions.[18] In general, the binding affinity results and the log *P* values determined for this library of compounds show that for the successful development of a SPECT imaging agent for TSPO, quinoline-2-carboxamides with small amide groups are required. Further pharmacological analysis of **18** to determine its potential as a SPECT imaging agent for TSPO is currently underway.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures and spectroscopic data for all compounds synthesised as well as details for competition binding assays) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.061.

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14. A [^{3}H]-PK11195 displacement assay was used for TSPO. See Supplementary data for full experimental details.
15. Compound **19** has been prepared previously and shown to have an IC_{50} value of 0.4 nM with TSPO. See Ref. 9e for details.
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17. The log *P* value for CNS 1261, a known SPECT imaging agent for the NMDA receptor (Owens, J.; Tebbutt, A. A.; McGregor, A. L.; Kodama, K.; Magar, S. S.; Perlman, M. E.; Robins, D. J.; Durant, G. J.; McCulloch, J. *Nucl. Med. Biol.* **2000**, *27*, 557) was determined using our HPLC method and was found to be 4.94.
18. Compound **18** was incubated in phosphate buffered saline at 37 °C. The decomposition of **18** was then monitored using HPLC. Decomposition was only observed at around 24 h. See Supplementary data for full experimental details. A patient, on average is normally scanned 2–4 h after injection of a SPECT imaging agent.