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# Pyrazolo[1,5-*a*]pyrimidine acetamides: 4-Phenyl alkyl ether derivatives as potent ligands for the 18 kDa translocator protein (TSPO)

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ABSTRACT

Herein, we report the synthesis of four new phenyl alkyl ether derivatives (**7**, **9**–**11**) of the pyrazolo[1,5-*a*]pyrimidine acetamide class, all of which showed high binding affinity and selectivity for the TSPO and, in the case of the propyl, propargyl, and butyl ether derivatives, the ability to increase pregnenolone biosynthesis by 80–175% over baseline in rat C6 glioma cells. While these compounds fit our in silico generated pharmacophore for TSPO binding the current model does not account for the observed functional activity.



The translocator protein (TSPO), formerly known as the peripheral-type benzodiazepine receptor (PBR),[1] is ubiquitously expressed in most peripheral organs, with the highest densities in steroidogenic tissues[2] as well as in heart, kidney, lung and testis,[3] but only minimally expressed in the healthy brain.[4] Increased levels of TSPO expression have been noted in a number of neuroinflammatory and neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, and stroke, coinciding with the activation of microglia, the principle immune effector cells in the central nervous system (CNS).

The TSPO is a highly lipophilic 18 kDa protein comprising 169 amino acids.[5] Primarily located on the outer mitochondrial membrane[6] with five trans-membrane domains, the TSPO is believed to be closely associated with the voltage-dependant ion channel (VDAC) and the adenine nucleotide transporter (ANT) as part of the mitochondrial permeability transition pore (MPTP).[7] While its exact physiological role remains unknown, the TSPO is implicated in several biochemical processes including cell proliferation,[8] apoptosis,[9] steroidogenesis,[3,10–12] porphyrin transport and heme synthesis,[13] and immunomodulation.[14] Among these, the rate-limiting translocation of cholesterol from the outer to inner mitochondrial membrane and subsequent enzymatic side chain cleavage to form pregnenolone, the precursor to all mammalian steroids, is perhaps the best documented.

The relationship between neuroinflammation/microglial activation and TSPO expression has resulted in the development of a number of structurally diverse TSPO ligands which have been described in reviews by James et al.[15] and Scarf et al.[16] Some of these ligands have been shown to mediate non-sedating anxiolytic-like effects in both rodent models of anxiety[17] and in humans[18] related to the production of neurosteroids which enhance γ-aminobutyric acid-mediated neurotransmission.[19]

The synthesis and biological evaluation of the arylpyrazolo[1,5-*a*]pyrimidine acetamides, a novel and selective class of high affinity ligands for the TSPO, was first reported by Selleri et al.[20] The authors concluded that substitution on the pyrimidine ring is important in determining selectivity for the TSPO over the central

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benzodiazepine receptor (CBR) and that substituents at the 4′-position of the phenyl ring had an effect on TSPO binding affinity. Some of these compounds were shown to increase pregnenolone biosynthesis in rat C6 glioma cells with similar potency to that of known TSPO ligand, PK11195.

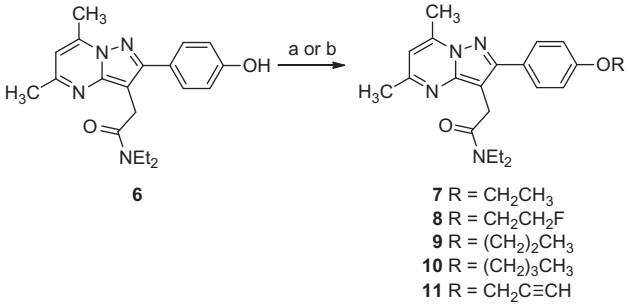
We aimed to explore the effect on TSPO binding affinity and pregnenolone biosynthesis of various length 4′-phenyl alkyl ethers derived from the arylpyrazolo[1,5-*a*]pyrimidine acetamide **6**, which we have previously reported.[21]

The synthetic route (Scheme 1) commenced with commercially available methyl 4-methoxybenzoate (**1**) which underwent nucleophilic attack in the presence of the stabilized carbanion derived from acetonitrile to form a tetrahedral intermediate which, upon loss of methoxide, gave the 3-oxopropanenitrile **2** in modest yield (58%). C-alkylation of **2** occurred upon treatment with sodium hydroxide to form the corresponding enolate followed by addition of commercially available *N*,*N*-diethylchloroacetamide and sodium iodide to afford **3** (80%). Aminopyrazole **4** was obtained in 80% yield following cyclization of a hydrazone intermediate derived from **3** and hydrazine hydrate. Treatment of **4** with 2,4-pentanedione in refluxing ethanol generated the pyrazolo[1,5-*a*]pyrimidine **5** in 93% yield via a double condensation. Cleavage of the methyl ether **5** to give the corresponding phenol **6** was achieved in the presence of refluxing 48% v/v aqueous hydrobromic acid and catalytic tetra-*n*-butylphosphonium bromide (0.10 equiv) in 54% yield.

The phenol **6** was elaborated to give the desired 4′-phenyl alkyl ethers (**7**–**11**) by Mitsunobu alkylation or by simple O-alkylation of the corresponding in situ generated phenoxide (Scheme 2). We have previously reported the binding of compounds **5** and **8** and their labeling with the short-lived positron-emitters carbon-11 and fluorine-18, respectively, as radioligands[22] for in vivo positron emission tomography (PET) imaging studies.[21,23–30]

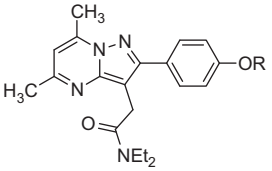
The TSPO binding of compounds **5**, **7**–**11** and PK11195 was evaluated in a membrane binding assay using [$^3$H]PK11195 as the radioligand and mitochondrial fractions from rat kidney as the receptor source. To determine the selectivity for the TSPO, binding to the CBR was also assessed using [$^3$H]Ro15,1788 and rat brain tissue. The results are summarized in Table 1.

All of the new compounds (**7**, **9**–**11**) displayed high affinity and complete selectivity for the TSPO over the CBR as well as a large range of CNS receptors and transporters (see Supplementary data). Increasing the length of the alkyl ether had little effect on TSPO binding affinity, suggesting the possible existence of a lipophilic binding pocket that can accommodate the steric bulk of an *n*-butyl group.



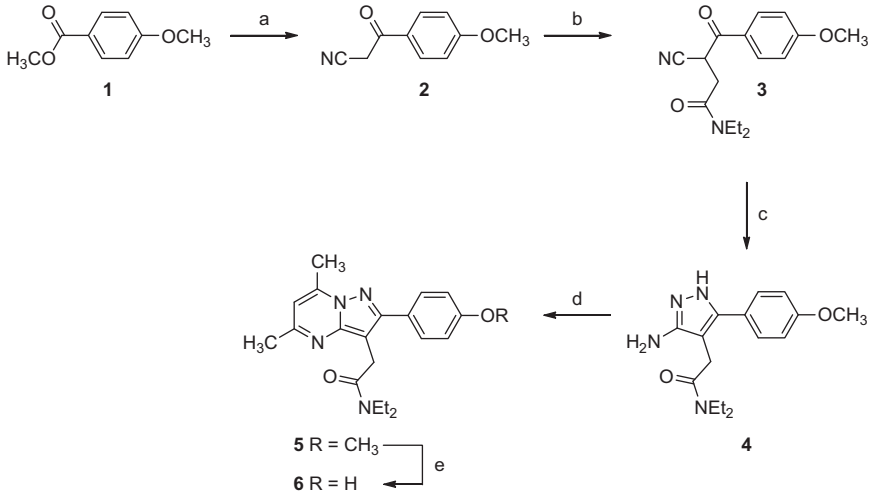

**Scheme 2.** Reagents and conditions: (a) DIAD (2.2 equiv), $PPh_3$ (2.2 equiv), $NEt_3$ (0 or 2.2 equiv), ROH (2.2 equiv), THF or DMF, 0 °C → RT, 72 h; (b) NaH or $K_2CO_3$, DMF, 0 °C, then ROTs or RBr, rt.

**Table 1**
Binding affinity of compounds **5**, **7**–**11** and PK11195 for the TSPO and CBR



| Compound | R | $K_i$ (TSPO) (nM) | $K_i$ (CBR) (nM) |
|---|---|---|---|
| PK11195 | | 9.3 ± 0.5 | >10,000 |
| **5** | $CH_3$ | 4.7 ± 0.2 | >10,000 |
| **7** | $CH_2CH_3$ | 5.7 ± 0.5 | >10,000 |
| **8** | $CH_2CH_2F$ | 7.0 ± 0.4 | >10,000 |
| **9** | $(CH_2)_2CH_3$ | 1.4 ± 0.2 | >10,000 |
| **10** | $(CH_2)_3CH_3$ | 1.1 ± 0.1 | >10,000 |
| **11** | $CH_2C{\equiv}CH$ | 4.8 ± 0.5 | >10,000 |

Each of the newly synthesized compounds was assessed, alongside PK11195, for its ability to increase pregnenolone biosynthesis in rat C6 glioma cells using a well developed steroidogenic assay.[20] Each TSPO ligand was used at the same concentration (40 μM) and at the end of the incubation period (2 h) the amount of pregnenolone was quantified by radio-immunoassay (RIA). The results are shown in Figure 1 (the values are the mean of three determinations). The known 4′-phenyl (2″-fluoroethyl) ether **8** stimulated pregnenolone biosynthesis at levels 80% above baseline, displaying significantly greater potency than PK11195 whereas the known




**Scheme 1.** Reagents and conditions: (a) NaOMe, MeCN, reflux, 32 h, 58%; (b) *N*,*N*-diethylchloroacetamide, NaI, NaOH, EtOH/$H_2O$ (80:20), rt, 7 h, 80%; (c) $NH_2NH_2{\cdot}H_2O$, AcOH, EtOH, reflux, 4 h, 86%; (d) 2,4-pentanedione, EtOH, reflux, 12 h, 93%; (e) 48% v/v HBr, tetra-*n*-butylphosphonium bromide (0.10 equiv), 100 °C, 7 h, 54%.