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# 2-Phenylimidazo[1,2-*a*]pyridine-containing ligands of the 18-kDa translocator protein (TSPO) behave as agonists and antagonists of steroidogenesis in a mouse leydig tumor cell line

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ABSTRACT

Ligands of 18-kDa translocator protein (TSPO) are known for their ability to potently and dose-dependently stimulate steroid biosynthesis in steroidogenic cells. In this study, we investigated a number of 2-phenyl-imidazo[1,2-*a*]pyridine acetamide derivatives, analogs of alpidem, for their ability to bind TSPO and to affect steroidogenesis in a mouse Leydig tumor cell line. We observed that not only some compounds behaved as agonists, stimulating steroidogenesis (e.g., **3** and **4**) with $EC_{50}$ values (15.9 and 6.99 μM) close to that determined for FGIN-1-27 used as positive control (7.24 μM), but two compounds, namely **5** and **6**, which on the other hand are the most lipophilic ones in the investigated series, behaved as antagonists, by significantly inhibiting steroid production at concentrations at least twenty times lower than the cytotoxic ones. To our surprise, the newly synthesized compound **3**, which is a strict analog of alpidem bearing at the *para* position of the 2-phenyl group a methoxy group instead of chlorine, achieved a ten-fold stimulation of the steroid production (for comparison FGIN-1-27 achieved 1.6-fold stimulation). Within the limits of the examined property space, some unprecedented SARs were unveiled, which can help in understanding the key molecular factors underlying the transition from agonism to antagonism in the steroidogenesis process. Besides the substitution pattern and the physicochemical features (mainly hydrogen bonding potential) of the substituents at the positions C(6) and C(8) of the imidazo[1,2-*a*]pyridine nucleus, and at the *para* position of the 2-phenyl group, the structure–activity relationship analysis suggested lipophilicity, whose increase seems to be generally related to steroidogenesis inhibition, and steric hindrance, which appeared as a stimulation-limiting factor, as two main properties to control in the design or optimization of novel imidazo[1,2-*a*]pyridine-based TSPO ligands endowed with potential in modulating the steroidogenesis process.



## 1. Introduction

The 18-kDa translocator protein (TSPO), previously known as the peripheral benzodiazepine receptor (PBR), is a high affinity cholesterol and drug-binding protein located in the outer mitochondrial membrane (Papadopoulos et al., 2006). Discovered for its ability to bind benzodiazepine drugs outside of the central nervous system (CNS), the biological function of this protein has remained enigmatic, though a body of evidence implicates TSPO in steroid biosynthesis. The rate-limiting step in steroid biosynthesis by steroidogenic cells is the movement of cholesterol from the outer to the inner mitochondrial membrane, where it is metabolized to the steroid pregnenolone by the cytochrome P450 enzyme CYP11A1 on the matrix side of the inner mitochondrial membrane. A number of structurally divergent TSPO ligands have been shown to potently and dose-dependently stimulate intramitochondrial cholesterol transport and steroid biosynthesis, suggesting that TSPO plays an important role in this process (Papadopoulos et al., 1990; Krueger and Papadopoulos, 1990; Romeo et al., 1992). Moreover, the benzodiazepine flunitrazepam was shown to potently inhibit hormone-mediated steroidogenesis in model cell systems, suggesting that small molecule TSPO ligands may be useful modulators (stimulators or inhibitors) of steroidogenesis. This pharmacological tactic has been of recent interest, as

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small molecule TSPO ligands, operating through a neurosteroid biosynthetic capacity, exhibit anxiolytic effects in rodents and humans, making TSPO an attractive drug target.

Numerous classes of small molecules with high affinity and selectivity for TSPO have been identified (Fig. 1), including isoquinoline carboxamides (e.g., PK 11195; Le Fur et al., 1983), benzodiazepines (e.g., RO5-4864; Marangos et al., 1982), 2-aryl-3-indoleacetamides (e.g., FGIN-1-27; Romeo et al., 1992), and 2-phenylimidazo[1,2-*a*]pyridine acetamides (e.g. alpidem; Langer et al., 1990). The TSPO ligand alpidem has attracted interest as it exhibits an anxioselective anxiolytic pharmacological profile *sans* benzodiazepine-like sedative side effects in preclinical animal models and clinically in humans; alpidem has been withdrawn from human use, however, owing to hepatoxicity (Skolnick, 2012). Alpidem has been shown to act on both TSPO and the central benzodiazepine receptor (CBR), with a preference toward TSPO. In efforts to differentiate, some of us have synthesized a series imidazo[1,2-*a*]pyridine-based potent TSPO ligands by introducing on the alpidem structure various substituents at the positions 6 and 8 of imidazo[1,2-*a*]pyridine nucleus and at the position *para* of the 2-phenyl group. Since the pioneering work by Trapani et al. in the late 1990s on 2-phenylimidazo[1,2-*a*]pyridine derivatives as TSPO ligands (Trapani et al.,1997) a number of promising candidates have been proposed (Denora et al., 2008, 2013, 2014; Piccinonna et al., 2013; Trapani et al., 1999, 2005). In the present study we investigated the ability of a number of imidazo[1,2-*a*]pyridine-containing TSPO ligands (**3**–**10**, Fig. 1) to affect steroidogenesis in a mouse Leydig tumor cell line.

## 2. Materials and methods

### 2.1. Chemistry

The 2-[6,8-*X*,*Y*-substituted-2-(4-*Z*-substituted-phenyl)imidazo-[1,2-*a*]pyridin-3-yl]-*N*,*N*-dipropylacetamide derivatives **4**–**10** were synthesized using previously reported procedures (Denora et al., 2008, 2012, 2013; Piccinonna et al., 2013; Trapani et al., 1997, 1999, 2005). Solvents, chemicals and reagents of analytical grade were purchased from Sigma–Aldrich (Milan, Italy) and used without further purification. Reactions were monitored by TLC carried out on 250 μm silica gel plates 60F-254 (E. Merck) using UV light for visualization. Silica gel 60, particle size 15–40 μm (E. Merck), was used for flash column chromatography. Elemental analyses were carried out with a Eurovector (Milan, Italy) model EA 3000 CHN and the results agreed to within ±0.40% of the theoretical values. Mass spectrometry: electrospray ionization mass spectrometry (ESI-MS) was performed with an electrospray interface and an ion trap mass spectrometer (1100 Series LC/MSD Trap system Agilent, Palo Alto, CA). FT-IR spectra were carried out on a PerkinElmer 1600 FT-IR spectrometer (Spectrum One); for each spectrum 40 scans from 4000 to 400 $cm^{-1}$ were performed. $^1H$ NMR spectra were recorded on Varian Mercury 300 MHz instrument. Chemical shifts are expressed in $\delta$ (ppm) and the coupling constants $J$ in Hz. $^1H$ chemical shifts were referenced by using the residual protic peak of the solvent as internal reference (2.50 ppm for DMSO-$d_6$, 4.80 ppm for $D_2O$, 7.24 ppm for $CDCl_3$). Abbreviations are as follows: s, singlet; d, doublet; t, triplet; q,

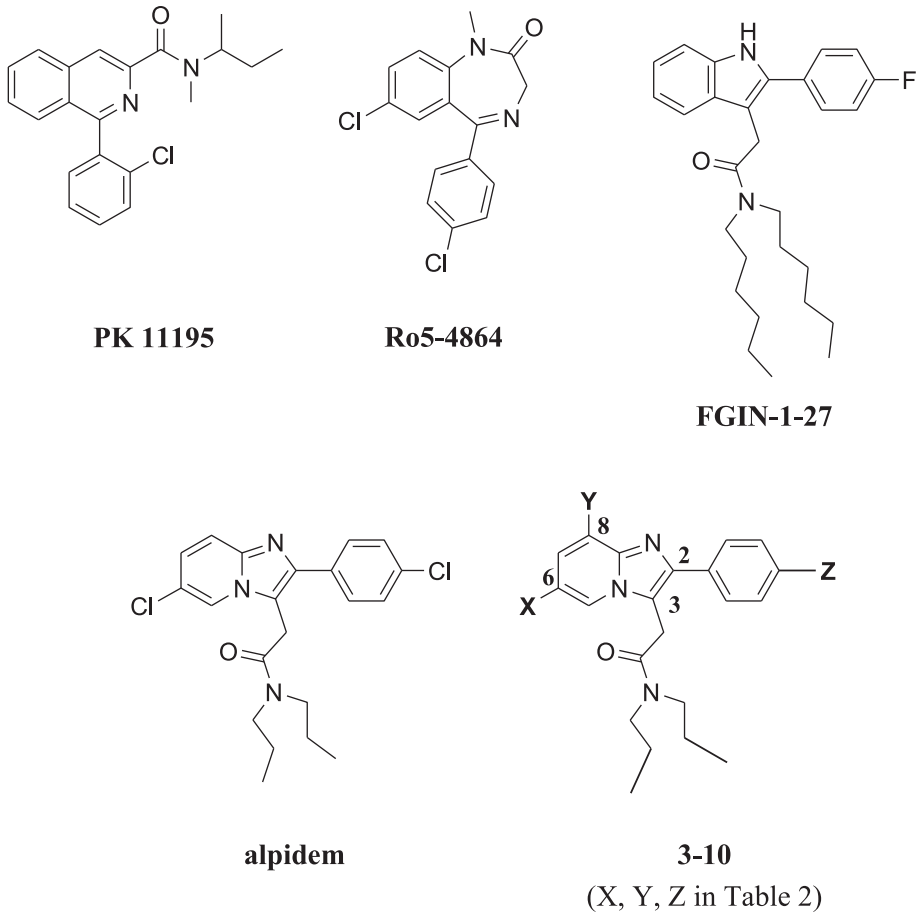


**Fig. 1.** Known and herein investigated synthetic TSPO ligands.