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A novel photoaffinity ligand for the dopamine transporter based on pyrovalerone

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ABSTRACT

Non-tropane-based photoaffinity ligands for the dopamine transporter (DAT) are relatively unexplored in contrast to tropane-based compounds such as cocaine. In order to fill this knowledge gap, a ligand was synthesized in which the aromatic ring of pyrovalerone was substituted with a photoreactive azido group. The analog 1-(4-azido-3-iodophenyl)-2-pyrrolidin-1-yl-pentan-1-one demonstrated appreciable binding affinity for the DAT (K_i = 78 ± 18 nM), suggesting the potential utility of a radioiodinated version in structure-function studies of this protein.

1. Introduction

Despite decades of committed research, no FDA approved medications are clinically available to combat psychostimulant abuse and addiction.[1] Pharmacological and behavioral studies indicate that the dopamine transporter (DAT) is the brain receptor chiefly responsible for the reward/reinforcing properties associated with amphetamines[2] and cocaine.[3] There are a plethora of structurally heterogeneous ligands that are known to bind to the DAT and inhibit the uptake of dopamine;[4] however, details regarding the transport inhibition mechanism and the discrete ligand binding pockets remain poorly understood. As a result, the synthesis of compounds towards elucidating conformational states and structural elements associated with the DAT, namely via probing the interactions of substrates and inhibitors with this protein, remains an important objective in the search for psychostimulant abuse therapeutics.

Results from structure–activity relationship (SAR) studies and site-directed mutagenesis experiments imply that structurally disparate inhibitors bind to different domains or binding sites within the DAT.[5–7] Additionally, it has been suggested that the binding of inhibitors to distinct DAT domains could affect their behavioral profile in cocaine abuse animal models.[6] As a result, radiolabeled (^{3}H, ^{125}I) affinity (–NCS) and photoaffinity (–N_3) irreversible ligands continue to remain pertinent towards mapping substrate and inhibitor DAT binding sites at the amino acid level. The chemical development of DAT irreversible ligands to date has predominantly focused on tropane-based ligands[8–16] and their conformationally flexible piperidine[17] and piperazine[18–22] analogs. [^{125}I]-MFZ-2-24 (**1**, Fig. 1), an irreversible tropane-based cocaine analog, demonstrated covalent ligation via photoaffinity labeling to the more intracellular-proximal half of transmembrane domain (TM) 1 within a 13-amino acid sequence.[23] However, [^{125}I]-RTI-82 (**2**), which possesses the same tropane pharmacophore found in MFZ-2-24 but features the photoreactive azide moiety anchored off the ester rather than the tropane nitrogen, demonstrated

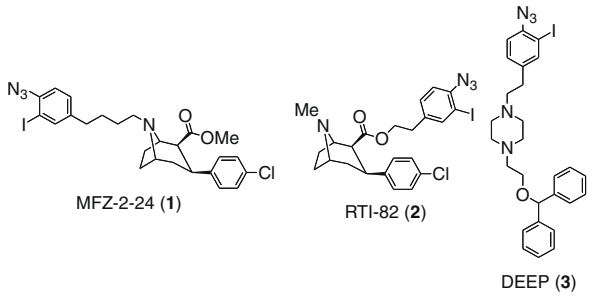

Figure 1. Representative examples of tropane- and piperazine-based DAT photoaffinity ligands.

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incorporation into TM6.[24] Collectively these probes provide evidence of the close 3-D proximity of DAT TMs 1 and 6. Additionally, piperazine analog [^{125}I]-DEEP (**3**), a conformationally flexible analog of the tropane-based benztropine class of DAT inhibitors,[6] also demonstrated incorporation into TM 1-2.[25]

Structurally heterologous non-tropane-based compounds have received significantly less attention versus tropane-based ligands in terms of their development into DAT irreversible probes. In an effort to expand the arsenal of complementary chemical probes useful for characterizing DAT 3-D structure, we report herein the design, synthesis, and preliminary labeling studies of a photoaffinity probe based on pyrovalerone (**5**, Fig. 2), a modestly selective inhibitor of the dopamine transporter over the norepinephrine transporter with little effect upon serotonin trafficking.

Our interest in pyrovalerone stems from its structural resemblance to bupropion (**4**), a well-known drug marketed as a smoking-cessation agent (Zyban) and antidepressant (Wellbutrin). More recently, bupropion as a dual norepinephrine and dopamine reuptake inhibitor has attracted significant attention as a pharmacotherapeutic for methamphetamine dependence.[1,26] However, determination of the DAT conformational states and binding sites for bupropion and structurally related compounds is in its infancy.[27] With respect to development of this structural class of inhibitors into potential DAT photoaffinity probes, it is demonstrated herein that pyrovalerone displays markedly higher binding affinity than bupropion at the DAT. Additionally, structure–activity relationship studies performed by Meltzer and colleagues indicate that pyrovalerone's aromatic ring is able to tolerate a wide range of substitutions in terms of retaining appreciable DAT affinity.[28] Thus, the present study seeks to develop pyrovalerone into a compact DAT photoaffinity probe for providing high resolution structural information regarding its binding site. This work also further explores the therapeutic potential of its analogs.

2. Results and discussion

2.1. Chemistry

Target compound **6** was prepared in six overall steps via synthetic methodology common to the construction of DAT photoaffinity ligands (Scheme 1). First, *N*-[4-(2-pyrrolidin-1-yl-pentanoyl)phenyl acetamide (**7**) was synthesized via Friedel–Crafts acylation, α-bromination, and pyrrolidine displacement as previously described.[28] Amide hydrolysis of **7** under acidic conditions provided the aniline pyrovalerone derivative **8** in moderate yield (43%). Regioselective electrophilic aromatic iodination was then achieved using ICl in acetic acid as previously described.[10] Finally, conversion of **9** to target azido–iodo analog **6** was accomplished in good yield (93%) via diazotization with nitrous acid and displacement with sodium azide.

2.2. Pharmacology

With pyrovalerone derivatives **6**–**9** in hand, ligand affinities (K_i values) were determined for inhibition of [^{3}H]-WIN-35,428 (a cocaine analog) binding to hDAT in N2A neuroblastoma cells. [^{3}H]-Dopamine uptake inhibition potencies in the same cells under the same conditions were also determined (Table 1). Racemic

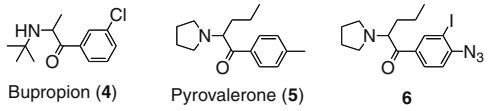
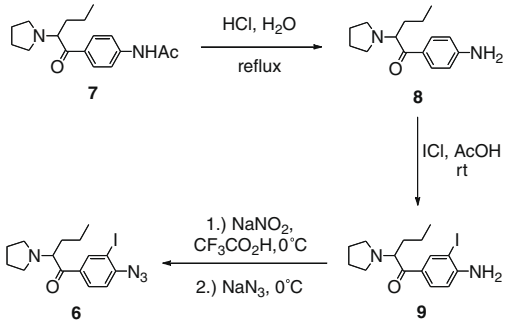


Figure 2. Structural relationship between bupropion (**4**), pyrovalerone (**5**), and target photoaffinity compound **6**.



Scheme 1. Synthesis of target compound **6**.

bupropion (**4**) and pyrovalerone (**5**) were also synthesized[28,29] and pharmacologically evaluated for comparison to the novel compounds. Replacement of the 4-Me group in pyrovalerone with 4-NHAc slightly reduced DAT binding affinity.[28] However, hydrolysis of the amide to the corresponding aniline results in a compound (**8**) with high DAT affinity comparable to pyrovalerone. Addition of the 3-I group resulted in ~6-fold decrease in binding affinity for the DAT while replacing the aniline NH_2 with the N_3 group further decreased affinity by 2.5-fold. The 78 nM DAT affinity for target compound **6** was sixfold higher than bupropion yet 10-fold less than pyrovalerone, retaining substantial DAT affinity that justified its further development into a potential DAT photoaffinity probe. Uptake inhibition IC_{50} values (Table 1) were typically 3–4-fold higher than the binding K_i values for each compound (using the Cheng–Prusoff equation, conversion of uptake inhibition constants from IC_{50} to K_i did not significantly change the value, allowing for direct comparison of binding and uptake results). This 3–4-fold shift was previously observed with rDAT/CHO cells in this laboratory for WIN 35,428, cocaine, mazindol, and methylphenidate.[7] Interestingly, compound **8** displayed essentially the same value for binding and uptake inhibition (Table 1), a pattern previously seen for benztropine and the related compounds GBR-12,909 and rimcazole.[7] Cocaine and benztropine have been suggested to occupy nonidentical DAT sites or conformations;[5–7] the present result may imply that compound **8** also interacts with the DAT in a fashion different from the other compounds in Table 1.

2.3. Radiosynthesis

Given that **6** demonstrated reasonably high DAT affinity, and that wash-resistant binding experiments on nonradioactive azido compounds frequently give false positives in the assessment of covalent attachment,[14] [^{125}I]-**6** was directly synthesized. The radioiodo compound could then be used to determine if photoactivation produced covalent ligation to the DAT. As shown in Scheme 2, a one-flask synthesis of [^{125}I]-**6** was performed using methodology previously described in detail for the preparation of radioiodinated cocaine analogs as DAT photoaffinity labels.[11] Briefly, electrophilic radioiodination of **8** with [^{125}I]-NaI (1.67 mCi) under no-carrier-added conditions using Chloramine-T as the oxidant was followed by diazotization and subsequent treatment with sodium azide. Although this sequence ending with reversed-phase HPLC isolation provided [^{125}I]-**6** in only 20% isolated yield, high purity (>99%) and high specific activity (1946 mCi/μmol) were achieved. The radioligand exhibited a chromatographic profile identical to that of non-radioactive **6** (Fig. 3) and showed good stability upon prolonged storage at −20 °C in the dark (92% radiochemical purity after 25 days). Figure 3A shows the preparative HPLC trace where [^{125}I]-**6** (t_R = 20.0 min) was well resolved from radioactive and non-radioactive side-products. The major non-radioactive materials are assigned as the azide (t_R = 6.2 min) and chloroazide (t_R = 13.0 min) congeners based upon model studies