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(±)-2-(*N-tert*-Butylamino)-3′-[^{125}I]-iodo-4′-azidopropiophenone: A dopamine transporter and nicotinic acetylcholine receptor photoaffinity ligand based on bupropion (Wellbutrin, Zyban)

David J. Lapinsky [a,*], Shaili Aggarwal [a], Tammy L. Nolan [a], Christopher K. Surratt [a], John R. Lever [b,c,d], Rejwi Acharya [e], Roxanne A. Vaughan [e], Akash Pandhare [f], Michael P. Blanton [f]

[a] Division of Pharmaceutical Sciences, Duquesne University, 600 Forbes Avenue, Pittsburgh, PA 15282, United States
[b] Department of Radiology, One Hospital Drive, University of Missouri, Columbia, MO 65212, United States
[c] Department of Medical Pharmacology and Physiology, One Hospital Drive, University of Missouri, Columbia, MO 65212, United States
[d] Harry S. Truman Veterans Administration Medical Center, 800 Hospital Drive, Columbia, MO 65201, United States
[e] Department of Biochemistry and Molecular Biology, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND 58202, United States
[f] Department of Pharmacology and Neuroscience, Texas Tech University Health Sciences Center, Lubbock, TX 79430, United States

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ABSTRACT

Towards addressing the knowledge gap of how bupropion interacts with the dopamine transporter (DAT) and nicotinic acetylcholine receptors (nAChRs), a ligand was synthesized in which the chlorine of bupropion was isosterically replaced with an iodine and a photoreactive azide was added to the 4′-position of the aromatic ring. Analog (±)-**3** (SADU-3-72) demonstrated modest DAT and α4β2 nAChR affinity. A radioiodinated version was shown to bind covalently to hDAT expressed in cultured cells and affinity-purified, lipid-reincorporated human α4β2 neuronal nAChRs. Co-incubation of (±)-[^{125}I]-**3** with non-radioactive (±)-bupropion or (−)-cocaine blocked labeling of these proteins. Compound (±)-[^{125}I]-**3** represents the first successful example of a DAT and nAChR photoaffinity ligand based on the bupropion scaffold. Such ligands are expected to assist in mapping bupropion-binding pockets within plasma membrane monoamine transporters and ligand-gated nAChR ion channels.

Bupropion (Wellbutrin, Zyban) ((±)-**1**, Fig. 1) is a well-established antidepressant in use for more than two decades.[1] With respect to treating nicotine abuse, a sustained release formulation of bupropion displays significant benefits as a smoking cessation agent.[2] More recently, (±)-**1** has garnered noteworthy attention as a treatment for both cocaine and methamphetamine dependence, showing greater efficacy in the latter.[3] Given its clinical versatility, a number of analogs of (±)-**1** and its major active metabolite, (2*S*,3*S*)-hydroxybupropion, have been recently disclosed in the search for improved pharmacotherapies for smoking cessation and cocaine addiction.[4–7] Despite these well-documented therapeutic effects, the neurochemical mechanisms underlying bupropion's action are still not well-defined, and the molecular determinants of how (±)-**1** interacts with its major drug targets remain unknown.

Unlike many other antidepressants, (±)-**1** has very little effect on serotonin reuptake. Instead, bupropion is traditionally described as a norepinephrine-dopamine reuptake inhibitor (NDRI) showing eight-fold selectivity for binding to the dopamine transporter (DAT).[4] Additionally, (±)-**1** non-competitively inhibits a number of nicotinic acetylcholine receptors (nAChRs) including the α3β4*, α3β2, α4β2, and α7 subtypes.[8] Multiple molecular targets are employed by (±)-**1** in modulating the behavioral effects of nicotine and psychostimulants. Slight adjustments to the nAChR activity of

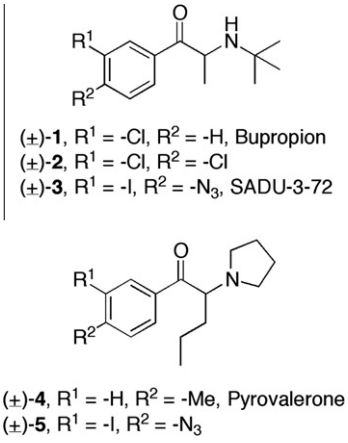

Figure 1. Structural comparison of aminoketone DAT inhibitors and photoprobe analogs.

* Corresponding author. Tel.: +1 (412) 396 6069; fax: +1 (412) 396 5593.
E-mail address: lapinskyd@duq.edu (D.J. Lapinsky).

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an analog with a NDRI profile may be the key to developing more efficacious smoking cessation aids.[7]

With respect to the DAT, details regarding the transport inhibition mechanism and the discrete ligand-binding pockets remain poorly understood. Results from site-directed mutagenesis experiments and structure–activity relationship (SAR) studies imply that structurally heterogeneous inhibitors bind to different domains or binding sites within the DAT.[9–11] Additionally, it has been suggested that the binding of inhibitors to distinct DAT domains or conformations could affect their behavioral profile in psychostimulant abuse animal models.[12] As a result, molecular probes based on bupropion would represent important pharmacological tools for structure–function studies of nAChRs and the DAT towards developing enhanced addiction and depression therapeutics. Given the emergence of 3D molecular models of these proteins, photoaffinity ligands based on (±)-**1** are expected to aid in determining protein conformational states and mapping of bupropion-binding sites. This information might reveal how the DAT and nAChRs discriminate therapeutic versus abused compounds at the molecular level.

To date, the chemical development of DAT photoaffinity ligands has predominantly focused on tropane-based ligands and their conformationally flexible piperidine and piperazine analogs (see references within Ref. [13]). In contrast, structurally heterologous non-tropane compounds have received significantly less attention in terms of their development into DAT irreversible probes.[13,14] In particular, determination of the DAT and nAChR conformational states and binding sites for bupropion and structurally related compounds is in its early stages.[15,16]

Our interest in developing a photoaffinity ligand based on bupropion stemmed from initial DAT labeling success with photoprobe (±)-[^{125}I]-**5**, a derivative of pyrovalerone ((±)-**4**).[13] Bupropion and pyrovalerone belong to the aminoketone class of DAT inhibitors, bearing similar structures to cathinone and diethylpropion as stimulants, and to phenethylamines (e.g., amphetamines) in general (Fig. 1). In addition to their chemical differences, (±)-**1** and -**4** differ substantially in abuse potential, with the latter being a Schedule V controlled substance in the United States. This is in sharp contrast to bupropion, which is classified as 'non-abusable' or having low abuse potential. Pyrovalerone (hDAT K_i = 8 ± 2 nM) displays 55-fold higher DAT affinity versus bupropion (hDAT K_i = 441 ± 174 nM) in N2A neuroblastoma cells.[13] Although the notably lower DAT affinity represents a risk in developing a DAT photoprobe based on bupropion, pursuit of target photoprobe (±)-**3** was justified based on its clinical significance and possible utilization in both DAT and nAChR photoaffinity labeling studies.

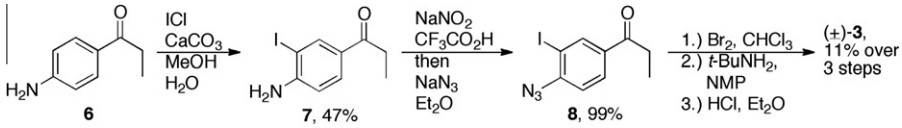
The design of pyrovalerone photoprobe (±)-[^{125}I]-**5** resulted from structure–activity relationships (SAR) indicating pyrovalerone's aromatic ring is able to tolerate a wide range of substitutions with respect to retaining appreciable DAT affinity.[17] As a result, photoprobe (±)-[^{125}I]-**5** was designed by replacing the 4′-methyl group of pyrovalerone with a photoreactive azide ($-N_3$) and including an iodine at the 3′ position of the aromatic ring to facilitate future incorporation of ^{125}I as a radiotracer tag.[13] These substitutions resulted in a ~9.8-fold loss in DAT affinity for photoprobe (±)-**5** (hDAT K_i = 78 ± 18 nM) when compared to pyrovalerone (hDAT K_i = 8 ± 2 nM). A ^{125}I version of this compound was shown to bind covalently to rDAT and hDAT expressed in cultured cells.[13]

Target bupropion photoprobe (±)-**3** was rationally designed in an analogous manner. Similar to pyrovalerone, monoamine transporter and nAChR SAR have been recently reported for bupropion analogs featuring a variety of substituents on the aromatic ring.[4,5] In particular, analog (±)-**2** (hDAT K_i = 472 ± 81 nM) containing hydrophobic chlorine atoms at positions 3′ and 4′ of the aromatic ring displayed improved DAT affinity (1.8-fold higher) versus bupropion (hDAT K_i = 871 ± 126 nM) in HEK cells.[4] Thus, target photoprobe (±)-**3** was designed by isosteric replacement of the 3′-chlorine in bupropion ((±)-**1**) with the larger hydrophobic halogen iodine, plus addition of a hydrophobic photoreactive azide to the 4′-position of the aromatic ring. Support for designation of an aryl azide as a hydrophobic functional group stems from work of Petukhov et al. in the area of histone deacetylase inhibitor photoprobes.[18]

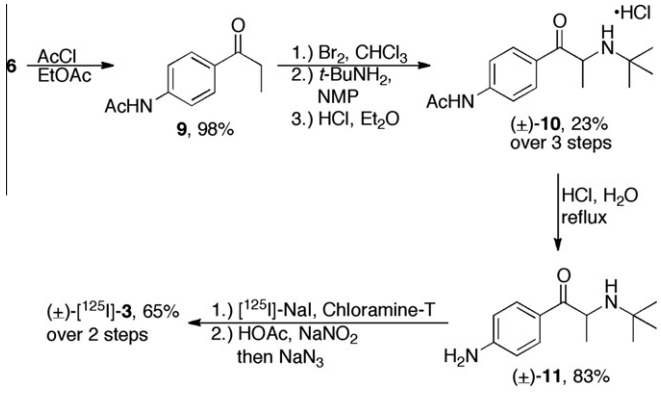
The synthesis of target bupropion photoprobe (±)-**3** is depicted in Scheme 1. 4′-Aminopropiophenone (**6**) was first iodinated using ICl and $CaCO_3$ analogous to Fujita et al.[19] to provide iodo-aniline **7** in moderate yield (47%). The aniline was subsequently converted into an aryl azide (**8**) in excellent yield via diazotization and displacement with sodium azide. Access to target (±)-**3** in its HCl salt form was then achieved using standard methodology for the synthesis of bupropion and its derivatives.[4,5,20] First, ketone **8** was brominated to provide an α-bromo ketone derivative, followed by displacement of the bromide with *tert*-butyl amine and subsequent HCl salt formation. Although this strategy proved successful, it provided target photoprobe (±)-**3** in rather low yield, 11% over three steps.

Another synthetic route to target photoprobe (±)-**3** was explored that also allowed formation of a desired ^{125}I derivative (Scheme 2). This strategy featured introduction of the photoreactive azide group as the final step versus earlier in the synthetic sequence. Aniline **6** was first converted to its corresponding amide derivative (**9**) in essentially quantitative yield using acetyl chloride. The amide was then transformed into (±)-**10** in 23% yield over three steps using the same chemistry previously described for synthesizing bupropion analogs. Amide hydrolysis then provided aniline bupropion analog (±)-**11**, which could be utilized to prepare a radioactive version of probe (±)-**3** to determine if photoactivation produced covalent ligation to the DAT or a selected nAChR. In order to accomplish this task, a one-flask synthesis of (±)-[^{125}I]-**3** was performed using methodology previously described for the preparation of radioiodinated cocaine analogs as DAT photoaffinity labels.[21] Briefly, electrophilic radioiodination of (±)-**11** with [^{125}I]-NaI (1.86 mCi) under no-carrier-added conditions using Chloramine-T as the oxidant was followed by diazotization and subsequent treatment with sodium azide. This sequence ending with reversed-phase HPLC isolation provided (±)-[^{125}I]-**3** in 65% yield, high purity (>99%), and high specific activity (2057 mCi/μmol). The ligand exhibited a chromatographic profile identical to that of non-radioactive (±)-**3** (Supplemental Fig. 1). Supplemental Figure 1A shows the preparative HPLC trace where (±)-[^{125}I]-**3** (t_R = 16.6 min) was well resolved from radioactive and non-radioactive side products. The major non-radioactive materials are assigned as the azide (t_R = 5.1 min) and chloroazide (t_R = 10.6 min) congeners based upon model studies conducted in the presence and in the absence of Chloramine-T. Supplemental Figure 1B shows purified (±)-[^{125}I]-**3**, while Fig. 1C shows HPLC co-elution of purified (±)-[^{125}I]-**3** with a fully characterized sample of non-radioactive (±)-**3**.

With both non-radioactive (±)-**3** and (±)-[^{125}I]-**3** in hand, preliminary DAT and nAChR pharmacology and photoaffinity labeling experiments were initiated. DAT ligand affinities of (±)-**3** and synthesized (±)-**1**[20] were determined via inhibition of [^{3}H]-WIN-35,428 (a cocaine analog) binding to hDAT in N2A neuroblastoma cells. The DAT affinity for target compound (±)-**3** (hDAT K_i = 3071 ± 497 nM) was sevenfold lower than bupropion (hDAT K_i = 441 ± 174 nM), but still bioactive in the range of parent compound (±)-**1** such that further experimentation was justified. To determine if the DAT underwent irreversible labeling with (±)-[^{125}I]-**3**, LLCPK$_1$ cells expressing 6Xhis-hDAT were photoaffinity labeled with (±)-[^{125}I]-**3** in the absence or presence of 10 μM or 100 μM (±)-bupropion or (−)-cocaine. The cells were then deter-



Scheme 1. Synthesis of bupropion photoprobe (±)-**3** (SADU-3-72).



Scheme 2. Synthesis of (±)-[^{125}I]-**3** ((±)-[^{125}I]-SADU-3-72).

gent-solubilized and the lysates were immunoprecipitated with anti-his antibody and analyzed by SDS–PAGE/autoradiography analogous to previously described procedures.[22] Labeled proteins of ~80 kDa were obtained from LLCPK$_1$ hDAT cells (Fig. 2), demonstrating the incorporation of (±)-[^{125}I]-**3** into the DAT. Incorporation of the ligand was blocked by 40–70% by either (±)-bupropion or (−)-cocaine in a dose-dependent manner, demonstrating the appropriate pharmacological specificity of (±)-[^{125}I]-**3** attachment to the DAT. Similar to results previously reported for tropane, GBR, and benztropine DAT photoaffinity ligands,[23,24] analysis of total cell lysates showed that several proteins undergo adduction with (±)-[^{125}I]-**3** (not shown). However, these do not represent the DAT because they do not immunoprecipitate with DAT antibody as shown for the protein in Figure 2.

Likewise, nAChR pharmacology and photoaffinity labeling experiments were performed with (±)-[^{125}I]-**3**. The affinity of (±)-[^{125}I]-**3** was approximated via inhibition by non-radioactive (±)-bupropion for binding to HEK-α4β2 nAChR cell membranes. (±)-Bupropion inhibited (±)-[^{125}I]-**3** binding to human α4β2 nAChRs with an IC$_{50}$ value of 8.3 μM, a value consistent with those previously reported.[8] Affinity-purified and lipid-reincorporated (DOPC/DOPA/CH-3:1:1) human α4β2 neuronal nAChRs (~45 μg) were photolabeled with 78 nM (±)-[^{125}I]-**3** ([^{125}I]-SADU-3-72) in the absence or presence of 160 μM (±)-bupropion. Polypeptides were then gel-fractionated, visualized by Coomassie Blue staining, and processed for autoradiography (Fig. 3). As evident from the significant reduction in (±)-[^{125}I]-SADU-3-72 labeling in the presence of an excess of (±)-bupropion, (±)-[^{125}I]-**3** specifically photoincorporates into the α4β2 nAChR.

In summary, a photoaffinity ligand based on the well-known antidepressant and smoking cessation agent bupropion was designed, synthesized, and pharmacologically evaluated. Analog (±)-[^{125}I]-**3** represents the first successful example of a DAT and nAChR photoaffinity ligand based on the bupropion scaffold, thus representing an important contribution to the growing arsenal of probes useful for characterizing the function and 3D structure of the DAT and nAChRs as therapeutically significant proteins. The binding affinities of compound (±)-**3** for the DAT and α4β2 neuronal nAChR are reasonably similar to bupropion, suggesting this probe is accessing the biologically relevant site(s). In particular, the affinity of photoprobe (±)-**3** for neuronal α4β2 nAChRs is similar to that of bupropion and other ligands that bind within the nAChR ion channel.[8] In fact, recent pharmacological studies suggest the

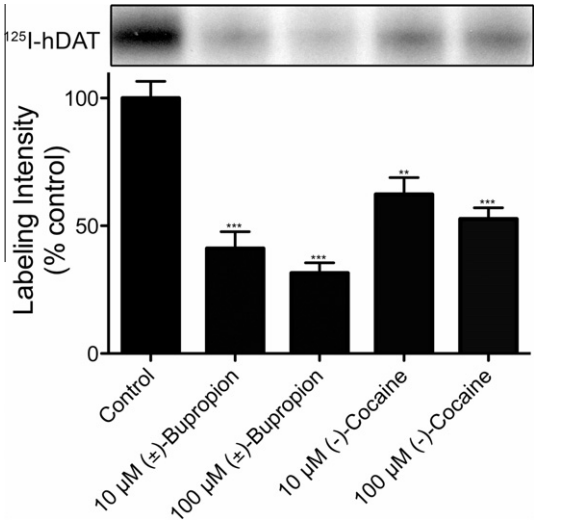


Figure 2. Photoaffinity labeling of hDAT with (±)-[^{125}I]-**3**. LLCPK$_1$ cells expressing 6Xhis-hDAT were photoaffinity labeled with 10 nM (±)-[^{125}I]-**3** in the absence or presence of 10 or 100 μM (±)-bupropion or (−)-cocaine. Cells were solubilized and DATs were immunoprecipitated followed by analysis by SDS–PAGE and autoradiography. The relevant portion of a representative autoradiograph is pictured followed by a histogram that quantitates relative band intensities (means ± SE of three independent experiments; $^{***}P < 0.0001$ versus control; $^{**}P < 0.001$ versus control).

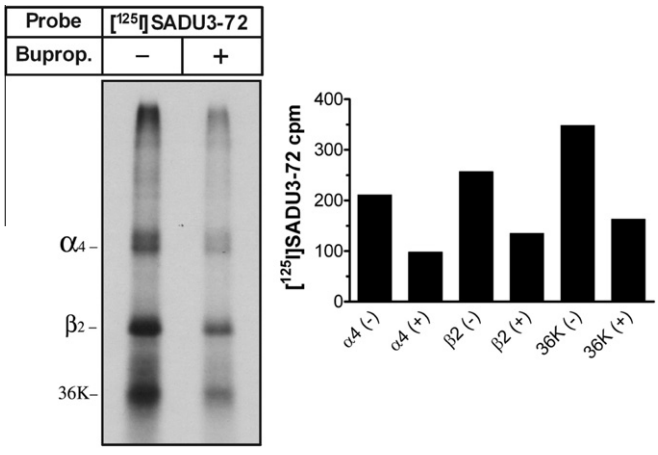


Figure 3. Photoincorporation of (±)-[^{125}I]-**3** ((±)-[^{125}I]-SADU-3-72) into the human α4β2 neuronal nAChR. UV irradiation at 365 nM proceeded for 10 min. Left panel, autoradiograph of an 8% SDS–polyacrylamide gel (1-week exposure) showing (±)-[^{125}I]-**3** photoincorporation into the α4 and β2 subunits, and into an ~36 kDa proteolytic fragment of the β2 subunit, in the absence (−) or presence (+) of 160 μM (±)-bupropion. Right panel, (±)-[^{125}I]-**3** photoincorporation of each band was quantified by gamma counting where inclusion (+) of (±)-bupropion (160 μM) inhibited labeling of each band by ~50%.

bupropion-binding site lies within the nAChR ion channel and not at the agonist-binding site[8,16]; thus, probe (±)-**3** is pharmacologically distinct from nAChR agonist photoprobes such as 5-azidoepibatidine. Furthermore, the modest nAChR binding affinity of (±)-**3** does not preclude identification of the bupropion-binding site within neuronal nAChR ion channels.[25] Iodine-125-labeled (±)-**3** was shown to bind covalently to hDAT expressed in cultured cells and affinity-purified, lipid-reincorporated human α4β2 neuronal nAChRs. Successful adduction of (±)-[^{125}I]-**3** to these proteins suggests that this non-tropane ligand tolerates direct substitution of a photoreactive azido group on the aromatic ring of the inhibitor scaffold. This contrasts with the design of tropane-based DAT photoaffinity ligands, wherein the azide is placed at a distance (usually via a linker) from the inhibitor pharmacophore in order to achieve successful DAT labeling.[26] As a result, compact photoaffinity ligands based on bupropion offer the advantage of a shorter tether between probe functional groups and protein amino acid residues in or near the inhibitor-binding site. Given the evidence that both DAT and nAChR inhibitors bind to non-identical sites or conformations,[8–12,16] this suggests that novel irreversible ligands based on bupropion may yield new nAChR and monoamine transporter structure–function information. Future directions include additional photoprobes based on bupropion and its major active metabolite, (2*S*,3*S*)-hydroxybupropion, their pharmacological characterization, binding site prediction via docking within DAT and nAChR computational models, and detailed elucidation of the binding domains within the DAT and selected nAChRs.

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Supplementary data

Supplementary data (Experimental section and spectral data.) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.10.086.

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