



# A NOVEL AMPHETAMINE-RELATED PHOTOAFFINITY PROBE

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## Abstract

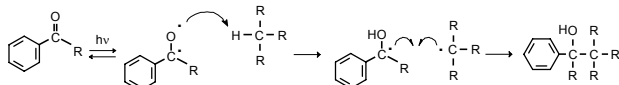
We have synthesized a novel methcathinone analog as a potential photoaffinity label for amphetamine binding sites. This compound, 1-(4-iodophenyl)-2-(methylamino)propan-1-one (4-IMAP), was synthesized "cold" for *in vitro* pharmacological analysis and in radioactive form for labeling purposes. The iodinated compound was synthesized from a tributylstannyl intermediate derived from 1-(4-bromophenyl)-2-(methylamino)propan-1-one (4-BMAP). We screened 4-IMAP for its ability to inhibit [<sup>3</sup>H]monoamine uptake via the plasma membrane uptake transporters SERT, DAT, and NET, and via the vesicle monoamine transporter VMAT2, and for its affinity for [<sup>3</sup>H]pentazocine-labeled sigma-1 receptors. At the SERT, DAT, NET, and VMAT2, 4-IMAP exhibited IC<sub>50</sub> values of 1.00 ± 0.16 μM, 1.54 ± 0.21 μM, 318 ± 40 nM, and 3.41 ± 0.53 μM, respectively. At sigma-1 receptors, the calculated K<sub>d</sub> was in the low micromolar range. [<sup>125</sup>I]4-IMAP was synthesized carrier-free and was used to photolabel human platelet membranes in pilot studies. In these experiments, [<sup>125</sup>I]4-IMAP labeled a protease-sensitive ~60 kDa protein that is protectable by some amphetamine-related compounds (methcathinone, 4-BMAP, MDMA) and fluoxetine. Further studies are underway with [<sup>125</sup>I]4-IMAP to identify and characterize amphetamine binding sites in the purified sigma-1 receptor.

## Introduction and Rationale

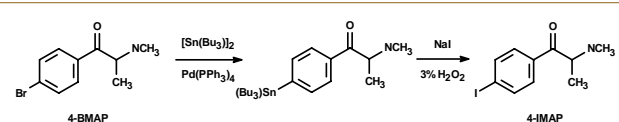
•Amphetamine and related compounds increase locomotor activity and alertness, elevate mood, decrease food intake, and enhance athletic performance. These effects are mediated through activity at serotonin, norepinephrine, and dopamine transporters and receptors, among others. Recently, it has been reported that amphetamine and other phenylalkylamine drugs bind to sigma-1 receptors, whose endogenous ligand(s) has not yet been discovered.

•Identification and characterization of known and unknown amphetamine binding sites will broaden understanding of how amphetamine-like compounds produce their effects and may identify new drug targets to treat mood disorders, substance abuse, eating disorders, learning disabilities, and others.

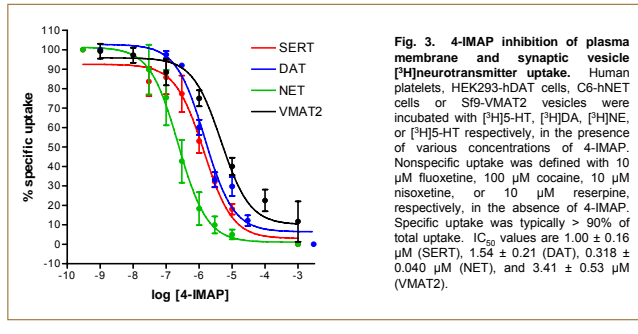
•Photoaffinity labeling can be used to identify and characterize known and unknown protein drug targets by covalently labeling the proteins with light-sensitive radioactive probe molecules. Arylketones, including the amphetamine-like compound methcathinone, are intrinsically photoactivable and can form covalent bonds with proteins to which they bind. It is thus feasible to synthesize radioactive analogs of methcathinone which will be photoactivable and which can be used to covalently label and identify amphetamine binding proteins.



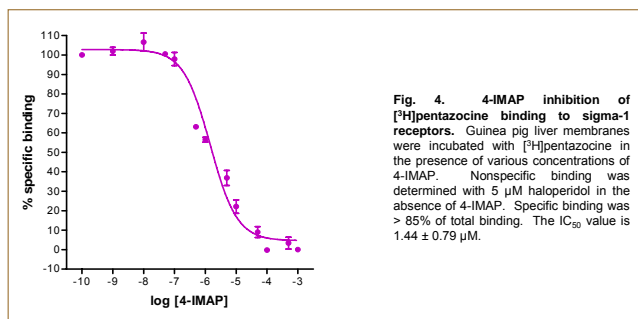
**Fig. 1. Proposed mechanism of arylketone photolysis.** The carbonyl bond of arylketones absorbs light in the 300-360 nm range to form an excited triplet. The triplet can react with nearby C-H bonds, but not with water; the triplet relaxes back to the ground state if no reaction occurs. Norman, R.O.C. Photochemical Reactions. In *Principles of Organic Synthesis*. Chapman and Hall Ltd.: London, pp. 507-533 (1978)



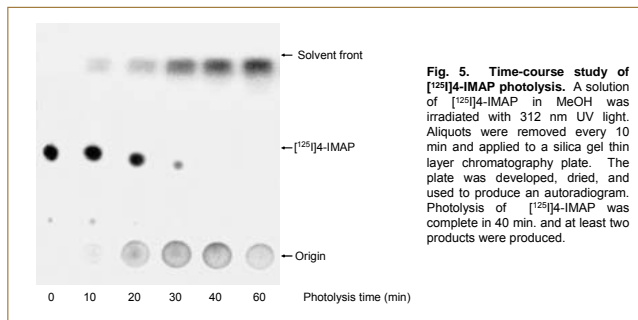
**Fig. 2. Synthesis of 4-IMAP.** 4-BMAP was synthesized as described (Foley and Cozzi, *Drug Dev. Res.*, 60, 252-260 (2003)). Stannylation was performed in refluxing toluene. After 90 min, the reaction mixture was cooled and concentrated *in vacuo* then the tributylstannyl intermediate was purified by column chromatography. Iodination was performed with NaI under oxidative conditions in NaOAc buffer, pH = 4.2. After quenching, the final product was isolated by acid-base extraction and was crystallized as the hydrochloride salt from diethyl ether. Structures were confirmed with <sup>1</sup>H-NMR and elemental analysis. Carrier-free [<sup>125</sup>I]4-IMAP was synthesized using Na<sup>125</sup>I and was purified using semi-preparative TLC.



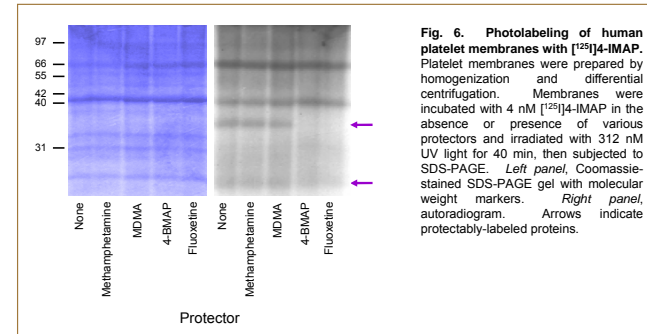
**Fig. 3. 4-IMAP inhibition of plasma membrane and synaptic vesicle [<sup>3</sup>H]neurotransmitter uptake.** Human platelets, HEK293-hDAT cells, C6-hNET cells or Sf9-VMAT2 vesicles were incubated with [<sup>3</sup>H]5-HT, [<sup>3</sup>H]DA, [<sup>3</sup>H]NE, or [<sup>3</sup>H]5-HT respectively, in the presence of various concentrations of 4-IMAP. Nonspecific uptake was defined with 10 μM fluoxetine, 100 μM cocaine, 10 μM nisoxetine, or 10 μM reserpine, respectively, in the absence of 4-IMAP. Specific uptake was typically > 90% of total uptake. IC<sub>50</sub> values are 1.00 ± 0.16 μM (SERT), 1.54 ± 0.21 (DAT), 0.318 ± 0.040 μM (NET), and 3.41 ± 0.53 μM (VMAT2).



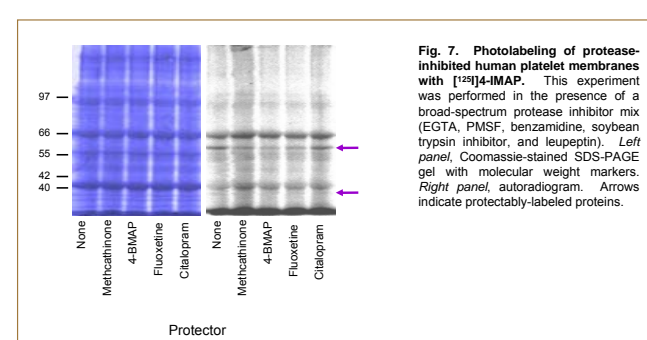
**Fig. 4. 4-IMAP inhibition of [<sup>3</sup>H]pentazocine binding to sigma-1 receptors.** Guinea pig liver membranes were incubated with [<sup>3</sup>H]pentazocine in the presence of various concentrations of 4-IMAP. Nonspecific binding was determined with 5 μM haloperidol in the absence of 4-IMAP. Specific binding was > 85% of total binding. The IC<sub>50</sub> value is 1.44 ± 0.79 μM.



**Fig. 5. Time-course study of [<sup>125</sup>I]4-IMAP photolysis.** A solution of [<sup>125</sup>I]4-IMAP in MeOH was irradiated with 312 nm UV light. Aliquots were removed every 10 min and applied to a silica gel thin layer chromatography plate. The plate was developed, dried, and used to produce an autoradiogram. Photolysis of [<sup>125</sup>I]4-IMAP was complete in 40 min. and at least two products were produced.



**Fig. 6. Photolabeling of human platelet membranes with [<sup>125</sup>I]4-IMAP.** Platelet membranes were prepared by homogenization and differential centrifugation. Membranes were incubated with 4 nM [<sup>125</sup>I]4-IMAP in the absence or presence of various protectors and irradiated with 312 nm UV light for 40 min, then subjected to SDS-PAGE. *Left panel*, Coomassie-stained SDS-PAGE gel with molecular weight markers. *Right panel*, autoradiogram. Arrows indicate protectably-labeled proteins.



**Fig. 7. Photolabeling of protease-inhibited human platelet membranes with [<sup>125</sup>I]4-IMAP.** This experiment was performed in the presence of a broad-spectrum protease inhibitor mix (EGTA, PMSF, benzamide, soybean trypsin inhibitor, and leupeptin). *Left panel*, Coomassie-stained SDS-PAGE gel with molecular weight markers. *Right panel*, autoradiogram. Arrows indicate protectably-labeled proteins.

## Conclusions

•4-IMAP inhibits serotonin and catecholamine accumulation via plasma membrane and vesicle transporters and inhibits haloperidol-sensitive pentazocine binding to the sigma-1 receptor. The IC<sub>50</sub> values of 4-IMAP for these sites are in the nanomolar-to-low micromolar range. The values are similar to previously reported values for amphetamine.

•Carrier-free [<sup>125</sup>I]4-IMAP was synthesized with a specific activity of 2200 Ci/mmol and this molecule undergoes time-dependent photodecomposition upon exposure to UV light. At least two products were obtained from photolysis in methanol.

•[<sup>125</sup>I]4-IMAP photolabels several proteins in human platelet membranes. The labeling is partially or completely protectable by the amphetamine-related drugs methcathinone, 4-BMAP, and MDMA, and by fluoxetine, indicating that these drugs and 4-IMAP share common protein binding sites. 4-IMAP will be useful in identifying additional drug binding sites in other tissues, including brain.