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Studies of the Biogenic Amine Transporters. 15. Identification of Novel Allosteric Dopamine Transporter Ligands with Nanomolar Potency

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List of non-standard abbreviations:

BATs	Biogenic amine transporters
DAT	Dopamine transporter
DAT binding	Binding assay for the dopamine transporter using a standard radioligand such as [¹²⁵ I]RTI-55 or [³ H]WIN35428
DAT-mediated [³ H]MPP ⁺ release	The dopamine release assay conducted using [³ H]MPP ⁺ .
DAT-mediated [³ H]DA release	The dopamine release assay conducted using [³ H]DA.
DAT uptake	A standard [³ H]DA uptake assay conducted with rat brain caudate synaptosomes
EC ₅₀	Molar drug concentration producing 50% of maximal release
E _{max}	Maximal response (efficacy measure)
IC ₅₀	Molar drug concentration producing 50% inhibition of maximal uptake or binding
NET	Norepinephrine transporter
NSS	Neurotransmitter/sodium symporter
RTI-55	3β-(4'-iodophenyl)tropan-2β-carboxylic acid methyl ester. Also identified as β-CIT
[¹²⁵ I]RTI-55	3β-(4'- ¹²⁵ iodophenyl)tropan-2β-carboxylic acid methyl ester. Also identified as [¹²⁵ I]β-CIT
SERT	Serotonin transporter
SRI-20040 (formerly SoRI-20040)	<i>N</i> -(2,2-Diphenylethyl)-2-phenyl-4-quinazolinamine
SRI-20041 (formerly SoRI-20041)	<i>N</i> -(3,3-Diphenylpropyl)-2-phenyl-4-quinazolinamine
SRI-9804 (formerly SoRI-9804)	<i>N</i> -(Diphenylmethyl)-2-phenyl-4-quinazolinamine
T _{1/2}	Time to half-maximal accumulation

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Abstract. Novel allosteric modulators of the dopamine transporter (DAT) have been identified. We have shown previously that *N*-(diphenylmethyl)-2-phenyl-4-quinazolinamine (SRI-9804), *N*-(2,2-diphenylethyl)-2-phenyl-4-quinazolinamine (SRI-20040), and *N*-(3,3-Diphenylpropyl)-2-phenyl-4-quinazolinamine (SRI-20041) partially inhibit [¹²⁵I]RTI-55 binding and [³H]dopamine ([³H]DA) uptake, slow the dissociation rate of [¹²⁵I]RTI-55 from the DAT, and allosterically modulate *d*-amphetamine-induced DAT-mediated dopamine (DA) release. We synthesized and evaluated the activity of over 500 analogs of these ligands and report here on 36 selected compounds. Using synaptosomes prepared from rat caudate, we conducted [³H]DA uptake inhibition assays, DAT binding assays with [³H]WIN35428, and DAT-mediated release assays with either [³H]MPP⁺ or [³H]DA. We observed three groups of [³H]DA uptake inhibitors: 1) full efficacy agents with a one-site fit, 2) full efficacy agents with a two-site fit and 3) partial efficacy agents with a one-site fit – the focus of further studies. These agents partially inhibited DA, serotonin, and norepinephrine uptake, yet were much less potent at inhibiting [³H]WIN35428 binding to DAT. For example, SRI-29574 partially inhibited DAT uptake with an IC₅₀ = 2.3±0.4 nM, without affecting binding to DAT. These agents did not alter DAT-mediated release of [³H]MPP⁺ in the absence or presence of 100 nM *d*-amphetamine. SRI-29574 had no significant effect on the *d*-amphetamine EC₅₀ or E_{max} value for DAT-mediated release of [³H]MPP⁺. These studies demonstrate the existence of potent DAT ligands that partially block [³H]DA uptake, without affecting DAT binding or *d*-amphetamine-induced [³H]MPP⁺ release. These compounds may prove to be useful probes of biogenic amine transporter function as well as novel therapeutics.

Introduction

The biogenic amine transporters (BATs) are members of the neurotransmitter/sodium symporter (NSS) protein superfamily. These membrane-spanning proteins co-transport neurotransmitters and Na⁺ ions from the extracellular space into the cytoplasm, utilizing the potential energy inherent to the inwardly-directed transmembrane Na⁺ gradient (Gether et al., 2006; Forrest et al., 2011). Under normal circumstances, BATs tightly control the extracellular concentrations of previously released biogenic amine transmitters (dopamine [DA], norepinephrine [NE], serotonin [5-HT]) by translocating these molecules back into the nerve terminal, a process termed “uptake.” Dopaminergic signaling is involved in several aspects of brain function such as cognition, movement, motivation, affect, behavioral reinforcement and economic analysis (reward prediction and valuation) (Greengard, 2001; Montague and Berns, 2002; Salamone et al., 2009). Perturbation of dopamine transporter (DAT) function is implicated in a number of neuropsychiatric disorders: ADHD, Parkinson’s disease, depression, anhedonia and addictive/compulsive disorders (Gainetdinov and Caron, 2003; Felten et al., 2011; Kurian et al., 2011). Moreover, the DAT is a target of several important medications and a number of recreational drugs (Reith et al., 2015; Sitte and Freissmuth, 2015). For example, clinically used DAT ligands include psychostimulants (e.g. *d*-amphetamine, methylphenidate and modafinil), antidepressants (e.g. bupropion) and certain anorectics (e.g. phendimetrazine, a prodrug that is converted to the DAT ligand phenmetrazine *in vivo* (Rothman et al., 2002)). Interaction with the DAT also contributes to the powerful reinforcing and locomotor stimulant effects of cocaine, one of the most prominent drugs of addiction (reviewed in (Gainetdinov and Caron, 2003; Schmitt and Reith, 2010)).

Drugs that interact with the BATs are typically classified into two categories: (1) ligands that bind to the BAT but are not transported (i.e., uptake inhibitors), and (2) ligands that bind to the BAT and are translocated through the transporter into the intracellular medium (i.e., substrates). Cocaine and the widely used antidepressant,

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fluoxetine, are examples of uptake inhibitors. A variety of psychoactive drugs are substrates for the BATs, and these compounds are often called “releasers”, since they induce the release of neurotransmitters by reversing the normal direction of flux through the transporter. Using the DAT as an example, the DA release process occurs via a mechanism classically called “carrier-mediated exchange.” According to this mechanism, the inward transport of an exogenous substrate, like amphetamine, releases cytoplasmic DA via reverse transport. Reverse transport by the DAT depends upon increased concentration of intracellular Na^+ (Khoshbouei et al., 2003), which accompanies translocation of amphetamine-like substrates, thereby promoting DA efflux (Sitte et al., 1998). The molecular basis for this reverse transport process is complex and still under investigation (see (Schmitt et al., 2013) for a review).

With regard to DAT ligands, one simple hypothesis is that all uptake inhibitors will interact with the DAT in a similar manner, and therefore produce similar in vitro and behavioral effects. By extension, all DAT substrates will also interact with the DAT in a similar manner to produce similar in vitro and behavioral effects. Recent studies, reviewed by Schmitt et al. (Schmitt et al., 2013) are not compatible with this hypothesis. For example, atypical DA uptake inhibitors, based on the bztropine structure and developed primarily by the Katz group (Tanda et al., 2009), block DA uptake but do not produce the expected cocaine-like behavioral effects. The efforts of our laboratory have entailed the screening of an extensive chemical library to identify potential BAT ligands. These efforts identified three quinazolinamine DAT allosteric modulators: *N*-(2,2-Diphenylethyl)-2-phenyl-4-quinazolinamine (SRI-20040), *N*-(3,3-Diphenylpropyl)-2-phenyl-4-quinazolinamine (SRI-20041) and *N*-(Diphenylmethyl)-2-phenyl-4-quinazolinamine (SRI-9804) (see Fig 1A, Fig 1B and Supplemental Fig. 1 for structures) (Pariser et al., 2008). A key finding with these compounds is that they are partial inhibitors of both DA uptake and [^{125}I]3 β -(4'-iodophenyl)tropan-2 β -carboxylic acid methyl ester ([^{125}I]RTI-55) binding to the DAT. Unlike cocaine, which can inhibit DA uptake and DAT binding with 100% efficacy, these compounds display E_{max} values ranging from 40-60%. These allosteric modulators increase the K_D value and decrease the B_{max} value for

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[¹²⁵I]RTI-55 binding to DAT, and also slow the dissociation rate of bound [¹²⁵I]RTI-55, further suggesting that these ligands do not compete for the same binding site as phenyltropane ligands (Pariser et al., 2008). Perhaps most importantly, while two of the quinazolinamine modulators (SRI-9804 and SRI-20040) partially inhibit both uptake of [³H]DA (forward transport) and DAT-mediated release of preloaded [³H]DA (reverse transport), a third compound (SRI-20041) inhibits substrate uptake, without appreciable effects on efflux (Rothman et al., 2009). This latter compound appeared to be the first DAT ligand to differentially affect substrate uptake versus transmitter release, suggesting that it may be possible to design compounds which selectively influence a single component of the NSS translocation cycle.

These aforementioned first generation allosteric modulators were limited by their weak potency (low micromolar range) in affecting DAT function. This low potency made in vivo study of their possible behavioral effects difficult. We therefore continued this effort by additional structure-activity studies, which are still ongoing, in order to develop allosteric ligands with greater potency. At the current time, over 500 analogs have been synthesized and evaluated in various in vitro assays (see Methods) for possible allosteric modulation of the DAT. We report here the initial results with 36 second generation compounds, some of which allosterically modulate the DAT with nanomolar potency.

Methods

Animals. Male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 300-400 g were used as subjects in these experiments. Rats were housed in standard conditions (lights on from 0700 to 1900 h) with food and water freely available. Animals were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC), and experiments were performed in accordance with the Institutional Care and Use Committee of the National Institute on Drug Abuse (NIDA), Intramural Research Program (IRP).

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Neurotransmitter uptake assays. Uptake inhibition assays for the DAT, and transporters for norepinephrine (NET) and serotonin (SERT), were conducted in rat brain synaptosomes as described elsewhere with minor modifications (Rothman et al., 2001). Freshly removed caudate (DAT), or whole brain minus cerebellum and caudate (NET and SERT), was homogenized in 10% ice-cold sucrose with 12 strokes of a hand-held Potter-Elvehjem homogenizer followed by centrifugation at 1000 x g for 10 min. The supernatants were saved on ice and used immediately. Transporter activity at DAT, NET, and SERT was assessed using 5 nM [³H]DA, 10 nM [³H]NE and 5 nM [³H]5-HT, respectively. The assay buffer was Krebs-phosphate buffer (pH 7.4) containing 126 mM NaCl, 2.4 mM KCl, 0.5 mM KH₂PO₄, 1.1 mM CaCl₂, 0.83 mM MgCl₂, 0.5 mM Na₂SO₄, 11.1 mM glucose, 13.7 mM Na₂HPO₄, 1 mg/ml ascorbic acid, and 50 μM pargyline. For NET uptake assays, 50 nM GBR12935 was added to the sucrose solution and assay buffer to prevent uptake of [³H]NE by DAT. For SERT uptake assays, the sucrose solution and assay buffer contained 100 nM nomifensine and 50 nM GBR12935 to prevent uptake of [³H]5-HT by NET and DAT, respectively. Uptake inhibition assays were conducted at 25°C (DAT and SERT) or 37°C (NET), and were initiated by adding 100 μl of tissue to 900 μl assay buffer containing test drug and [³H]neurotransmitter. Test drugs were diluted in assay buffer containing 1 mg/ml bovine serum albumin prior to addition. Nonspecific uptake was measured by incubating in the presence of 1 μM indatraline. The reactions were stopped after 15 min (DAT), 10 min (NET), or 30 min (SERT) by rapid vacuum filtration with a cell harvester (BRANDEL) over GF/B filters (Whatman) presoaked in wash buffer maintained at 25° C (10 mM Tris-HCl, pH 7.4/150 mM NaCl). Filters were rinsed with 6 ml wash buffer and retained tritium was measured with a MicroBeta liquid scintillation counter (PerkinElmer) after overnight extraction in 0.6 mL of liquid scintillation cocktail (Cytoscint, ICN).

Neurotransmitter Release Assays. DAT-mediated release assays were carried out as previously described with minor modifications (Rothman et al., 2003). Synaptosomes

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were prepared from rat caudate tissue as described for uptake inhibition assays, except that the sucrose solution contained 1 μ M reserpine to block vesicular uptake of substrates. Synaptosomal preparations were incubated to steady state with 9 nM [3 H]MPP $^+$ (60 min, 25 $^\circ$ C) in uptake assay buffer containing 1 μ M reserpine to block vesicular uptake of substrates and 100 nM citalopram and 100 nM desipramine to block uptake of [3 H]MPP $^+$ by SERT and NET. Subsequently, 850 μ l of synaptosomes preloaded with [3 H] MPP $^+$ were added to polystyrene test tubes that contained 150 μ l of test drug in assay buffer plus 1 mg/ml BSA. After 30 min at 25 $^\circ$ C, the release reaction was terminated by rapid vacuum filtration as described for uptake assays. Nonspecific values were measured by incubations in the presence of 10 μ M tyramine. The retained tritium was measured as described for uptake assays.

[3 H]WIN35428 binding assays. The ability of test drugs to inhibit [3 H]WIN35428 binding to DAT in rat caudate membranes was assessed as follows. For each experiment, caudates from four rat brains were suspended in 15 ml ice cold assay buffer (50 mM sodium phosphate pH 7.4) and homogenized using a polytron (setting 6, 20 sec). The homogenate was centrifuged at 30,000 x g for 10 min at 4 $^\circ$ C. The pellet was resuspended with vigorous vortexing in 15 ml fresh ice cold assay buffer, and the centrifugation was repeated. The pellet was resuspended in 15 ml fresh ice cold assay buffer with vigorous vortexing followed by six strokes with a glass-on-glass hand-held homogenizer and was diluted to a final volume of 235 ml in ice cold assay buffer. [3 H]WIN35428 was diluted to 10 nM in assay buffer that contained 25 μ g/ml chymostatin, 25 μ g/ml leupeptin, 0.1 mM EDTA, and 0.1 mM EGTA. Each assay tube contained 0.75 ml membrane preparation, 0.15 ml test drug diluted in assay buffer containing 1 mg/ml bovine serum albumin, and 0.1 ml [3 H]WIN35428 preparation (final concentration of 1 nM). Assays were initiated by the addition of membranes and were terminated after 2h at 25 $^\circ$ C by rapid vacuum filtration as described above. Retained tritium was measured as described above.

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Chemical Synthesis. The details of the chemical synthesis will be described in another publication (in preparation).

Screening Methods. Test compounds were evaluated in a step-wise manner. First, eight-point dose-response curves were generated for each compound (1 – 20,000 nM) in the [³H]DA uptake assay. The data from three experiments were pooled and fit to a dose-response curve equation (using Kaleidagraph), to yield an E_{max} and IC₅₀ value. Next, since we were interested in characterizing potent partial DAT inhibitors, compounds were selected for further evaluation only if they had an IC₅₀ ≤ 20 nM (high potency), and an E_{max} ≤ 70% (partial efficacy). Compounds that displayed properties of full efficacy inhibitors of [³H]DA uptake were not tested further. A subset of potent partial inhibitors was then tested for dose-response effects in the [³H]NE and [³H]5-HT uptake inhibition assays. In addition, these same compounds were tested for their ability to alter DAT-mediated release of [³H]MPP⁺ in the absence and presence of 100 nM *d*-amphetamine. Selected compounds were also tested for their ability to inhibit [³H]WIN35428 binding to rat caudate DAT.

Data analysis and statistics. For release experiments, dose-response curves were generated using eight concentrations of test drug. In order to describe the method for calculating the release dose-response curves, the following definitions are necessary

Total Binding (TB) = cpm in the absence of any drug.

Nonspecific Binding (NS) = cpm in the presence of 10 μM tyramine.

Maximal Release (MR) = TB-NS

Specific Release (SR) = (cpm in the presence of drug) – NS

% MAX Release = 100 – SR/MR*100.

The data from three experiments, expressed as % MAX Release, were then fit to a dose-response curve equation: $Y = E_{max} \times ([D]/([D] + EC_{50}))$ for the best fit estimates of

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the E_{\max} and EC_{50} using either KaleidaGraph version 3.6.4 or MLAB-PC (Nightingale et al., 2005). In some cases, dose response curves were fit to a two-component equation: $Y = E_{\max 1} \times ([D]/([D] + EC_{50-1})) + E_{\max 2} \times ([D]/([D] + EC_{50-2}))$. Statistical significance of the one-site versus two-site fits was based on F-test results. In “shift” experiments, a substrate dose-response curve was generated in the absence and presence of a test drug. Apparent K_e values were calculated according to the equation: $[Test\ Drug]/(EC_{50-2}/EC_{50-1} - 1)$, where EC_{50-2} is the EC_{50} value in the presence of the test drug and EC_{50-1} is the value in the absence of the uptake inhibitor.

Results

Initial screen of compounds. As noted in Methods, compounds were first evaluated in the [3 H]DA uptake inhibition assay. Some agents acted as full efficacy [3 H]DA uptake inhibitors (for example, see SRI-31335 (Fig. 2A)). A large set of agents also acted as partial inhibitors when the dose-response curves were fit to the one-component equation (for example, see SRI-29986 and SRI-30835 in Fig 2A). However, upon visual inspection, it is clear that whereas the SRI-29986 dose-response curve is well described by a one-component equation, the SRI-30835 dose-response curve was not. Fitting the same three dose-response curves to a two-component equation led to a highly significant improvement in the goodness-of-fit for SRI-30835, but not the other two agents (see Fig. 2B and Table 1). These data illustrate that the initial set of compounds binned into three groups of [3 H]DA uptake inhibitors: 1) apparent full efficacy one-component agents, 2) apparent full efficacy two-component agents and 3) partial efficacy agents. We focused further studies on the initial set of 36 partial efficacy agents. Of interest, preliminary experiments suggest that some of the apparent full efficacy one-component agents are also allosteric modulators (data not shown).

Evaluation of test agents for inhibition of DAT, SERT and NET uptake and DAT binding. Fig 3 illustrates that SRI-29574 was not only a partial inhibitor of DAT uptake, but also

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of SERT and NET uptake. All agents tested (Table 2) were partial inhibitors of DAT, SERT, and NET uptake, though in general the efficacy was lower at SERT than at NET and DAT. While many of the test agents had similar IC_{50} values for BAT uptake inhibition, in general the order of potency was DAT>SERT>NET. Another striking aspect of the data set was that most compounds were about three orders of magnitude less potent in inhibiting [3 H]WIN35428 binding to DAT, than in blocking uptake of [3 H]DA. This is illustrated in Fig. 4A for two compounds. SRI-29574 partially inhibited DAT uptake ($IC_{50} = 2.3 \pm 0.4$ nM) while being inactive in inhibiting DAT binding. In contrast, SRI-29786 partially inhibited DAT uptake ($IC_{50} = 7.1 \pm 2.2$ nM), but also inhibited DAT binding, with full efficacy and an IC_{50} value (1100 ± 10 nM) 155-fold weaker than the IC_{50} for inhibition of DAT uptake. Overall, only 5 of the 36 compounds were full efficacy inhibitors of DAT binding, and in most cases the agents were much less potent at DAT binding inhibition than at DAT uptake inhibition. In contrast, the prototypical DAT blockers GBR12935 and cocaine displayed similar potency and efficacy in both assays. There was no significant correlation between the E_{max} values observed in the DAT uptake and binding assays (Fig. 4B).

Effect of test agents on DAT-mediated [3 H]MPP⁺ release. The first set of release experiments determined the effect of test agents on DAT-mediated [3 H]MPP⁺ release in the absence and presence of 100 nM *d*-amphetamine. Overall, at concentrations less than 1 μ M, none of the agents altered DAT-mediated [3 H]MPP⁺ release in the absence or presence of 100 nM *d*-amphetamine (data not shown). The ability of these agents to shift *d*-amphetamine-induced DAT-mediated [3 H]MPP⁺ release, using blocking concentrations about 25-times greater than the corresponding IC_{50} for DAT uptake inhibition, were then determined. Fig. 5A reports representative results. SRI-29574 had no significant effect on the *d*-amphetamine EC_{50} or E_{max} value. SRI-29213, in contrast, significantly increased the EC_{50} value and also decreased the E_{max} value. Of the 23 agents tested in this manner (see Table 3), only SRI-29213 increased EC_{50} and decreased E_{max} . GBR12935, a competitive DAT uptake inhibitor, shifted the *d*-

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amphetamine release curve to the right in a parallel fashion without changing the E_{\max} value. Similar results were obtained when [^3H]DA was used instead of [^3H]MPP $^+$ (Fig. 5B).

Effect of SRI-29574 and cocaine on [^3H]DA uptake/accumulation. We next assessed the effect of SRI-29574, a potent partial [^3H]DA uptake inhibitor, on the time course of [^3H]DA uptake, in comparison with cocaine. We predicted that SRI-29574 would reduce the maximum level of [^3H]DA accumulation, consistent with noncompetitive inhibition. As reported in Fig. 6A and Table 4A, SRI-29574 had no significant effect on the $T_{1/2}$ (time to half-maximal accumulation), but decreased the E_{\max} in a dose-dependent manner (Fig. 6B) ($EC_{50} = 5.4 \pm 0.2$ nM, $E_{\max} = 69 \pm 1$ %). In contrast, the most striking effect of cocaine (Fig. 7A and 7B) was to increase the $T_{1/2}$ in a dose-dependent linear manner. The effect of cocaine on the E_{\max} was more complex. Post-hoc student's t-test showed that two of the four E_{\max} values were not significantly different from control, indicating that cocaine did not have a consistent effect on the E_{\max} . Viewed collectively, these results are consistent with SRI-29574 being a noncompetitive inhibitor of [^3H]DA uptake.

Discussion

Our previously published papers identified three quinazolinamine DAT allosteric modulators: SRI-20040, SRI-20041 and SRI-9804 (see Fig 1A, Fig 1B and Supplemental Fig. 1 for structures). While two of the quinazolinamine modulators (SRI-9804 and SRI-20040) partially inhibit both uptake of [^3H]DA (forward transport) and DAT-mediated release of preloaded [^3H]DA (reverse transport), the third compound (SRI-20041) inhibits substrate uptake, but has no appreciable effect on efflux (Rothman et al., 2009). This latter compound appeared to be the first DAT ligand to differentially affect substrate uptake versus release, suggesting that the two functional modes of substrate translocation are unique, and that it may be possible to design compounds

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selectively affecting a single part of the NSS translocation cycle. The experiments reported here significantly extend these findings.

The first generation DAT allosteric modulators partially inhibited DAT uptake and DAT binding (measured using [125 I]RTI-55) with micromolar potency. A major advance made in this study is the development of second generation compounds with nanomolar potency for partial inhibition of DAT uptake. Unlike the first generation compounds, the second generation compounds were generally 100- to 1000-fold less potent inhibitors of DAT binding when compared to DAT uptake. Some agents, such as SRI-29574 and SRI-30522, were inactive as inhibitors of DAT binding. To our knowledge, these compounds are the first compounds that discriminate inhibition of DAT uptake from inhibition of DAT binding, providing strong support for the hypothesis that these second generation DAT allosteric modulators bind to a site on the DAT distinct from the cocaine binding site. Interestingly, the second generation DAT allosteric modulators also partially inhibited SERT and NET uptake. This observation suggests that further research should be aimed at developing allosteric modulators selective for DAT, SERT and NET.

The data reported here clearly demonstrate that the second generation DAT allosteric modulators and standard DAT inhibitors, such as cocaine and GBR12935, interact differently with the DAT. A defining difference is that the second generation DAT allosteric modulators partially inhibit DAT uptake with nanomolar potency. Moreover, as noted above, the second generation DAT allosteric modulators are much less potent in inhibiting DAT binding, unlike standard DAT uptake inhibitors (Table 2). Perhaps most interesting, with the exception of one agent (SRI-29213), the second generation DAT allosteric modulators fail to significantly alter *d*-amphetamine-induced DAT-mediated release of [3 H]MPP⁺ or [3 H]DA (Table 3) when tested using concentrations of “blockers” 20-25 times higher than the corresponding IC₅₀ value for inhibiting DAT uptake (Table 3). SRI-29213 reduced the E_{max} value for *d*-amphetamine-induced release, similar to what had been observed with the first generation agents, SRI-9804 and SRI-20040. These results suggest that the second generation SRI compounds affect forward

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transport (i.e., uptake), but not reverse transport (i.e., release), indicating that these two processes are separable and independently regulated, as has previously been suggested (Cao and Reith, 2002). This possibility is supported by the finding that the phosphorylation of Thr⁵³ of DAT can partially reduce forward transport by DAT, but completely eliminate reverse transport produced by amphetamine (Foster et al., 2012). Similar findings were reported for site-directed mutagenesis of Thr⁶² (Fraser et al., 2014), and by Khoshbouei (Khoshbouei et al., 2004), who reported that N-terminal phosphorylation shifts DAT from a “reluctant” state to a “willing” state for *d*-amphetamine induced DA efflux, without affecting inward transport. Moreover, a recent study showed that impairing the interaction of PIP-2 with DAT impairs amphetamine-induced DA efflux without affecting DA uptake (Hamilton et al., 2014).

In summary, this paper reports a new generation of potent DAT allosteric modulators that partially inhibit DAT uptake without altering DAT-mediated reverse transport and with minimal inhibition of DAT binding. The molecular mechanism by which these second generation allosteric modulators alter DAT function remains to be determined. As reviewed elsewhere (Schmitt et al., 2013), the current understanding of this complex mechanism is still evolving. It is possible that some of the compounds reported here will help elucidate these mechanisms. Some possibilities for future research include radiolabeling the most potent ligands, such as SRI-31040, to allow the direct study of the hypothesized allosteric binding site, which would be aid future structure-activity studies and in vivo investigations with selected compounds to explore their behavioral/therapeutic effects. Finally, in-silico molecular modeling experiments could be utilized to identify the putative allosteric binding site on DAT, as was recently accomplished for SERT (Kortagere et al., 2013).

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Authorship Contributions

Participated in research design: Rothman, Ananthan, Partilla, Baumann

Conducted experiments: Partilla

Contributed new reagents or analytic tools: Ananthan, Saini, Moukha-Chafiq, Pathak

Performed data analysis: Rothman, Ananthan, Partilla,

Wrote or contributed to the writing of the manuscript: Rothman, Ananthan, Partilla, Baumann, Saini, Moukha-Chafiq, Pathak

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Legends to Figures.

Figure 1A and 1B. Structure of test compounds. Note that SRI-20040, SRI-20041 and SRI-9804 were formerly designated SoRI-20040, SoRI-20041, SoRI-9804, respectively.

Figure 2. Initial screening of compounds for inhibition of [³H]DA uptake using a one-component (Panel 2A) or two-component (Panel 2B) fit. Panel 2A shows that some agents acted as full efficacy [³H]DA uptake inhibitors (e.g., SRI-31335), whereas others acted as partial inhibitors (e.g., SRI-29986 and SRI-30835), when the dose-response curves were fit to the one-component equation. Panel 2B shows that the SRI-30835 dose-response curve was better described by a two-site fit (also see Table 1) and was a full efficacy agent when fit to a two-component model. We focused further studies on the initial set of 36 partial efficacy agents well described by a one component model.

Figure 3. Inhibition of [³H]DA, [³H]5-HT and [³H]NE uptake by SRI-29574 in rat brain synaptosomes. SRI-29574 was not only a partial inhibitor of DAT uptake, but also of SERT and NET uptake. All agents tested (see Table 2) were partial inhibitors of DAT, SERT, and NET uptake, though in general the efficacy was lower at SERT than at NET and DAT. The data were fit to a one-component dose-response curve equation for the best-fit estimates of the IC₅₀ (±SD) and E_{max} (±SD): [³H]DA uptake (2.3 ± 0.4 nM, 68 ± 2%), [³H]5-HT uptake (23 ± 5 nM, 52 ± 2%), [³H]NE uptake (52 ± 15 nM, 72 ± 4%). Each data point is the mean±SD of three separate experiments.

Figure 4. Comparison of the inhibition of [³H]WIN35428 binding versus [³H]DA uptake by test agents. The test drugs shown here (Panel 4A), and most of the drugs examined (see Table 2), were about three orders of magnitude less potent in inhibiting [³H]WIN35428 binding to DAT, than in blocking uptake of [³H]DA. For example, SRI-29574 partially inhibited DAT uptake (IC₅₀ = 2.3±0.4 nM) while being inactive in inhibiting DAT binding. In contrast, SRI-29786 partially inhibited DAT uptake (IC₅₀ =

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7.1±2.2 nM), but also inhibited DAT binding, with full efficacy and an IC₅₀ value (1100±10 nM) 155-fold weaker than the IC₅₀ for inhibition of DAT uptake. Panel 4B shows the correlation plot of DAT uptake E_{max} versus DAT binding E_{max} for 36 DAT partial inhibitors shows that there was no significant correlation between the E_{max} values observed in the DAT uptake and binding assays for 36 DAT partial inhibitors. Each data point is the mean±SD of three separate experiments. The data were fit to a one-component dose-response curve equation for the best-fit estimates of the IC₅₀ (±SD) and E_{max} (±SD), which are reported in Table 1.

Figure 5. Effect of test agents on DAT-mediated release of [³H]MPP⁺ (Panel 5A) or [³H]DA (Panel 5B). Panel 5A ([³H]MPP⁺ release): SRI-29574 had no significant effect on the *d*-amphetamine EC₅₀ or E_{max} value. SRI-29213, in contrast, significantly increased the EC₅₀ value and also decreased the E_{max} value. GBR12935, a competitive DAT uptake inhibitor, shifted the *d*-amphetamine release curve to the right in a parallel fashion without changing the E_{max} value. Panel B ([³H]DA release): Similar results were observed for [³H]DA release. Each data point is the mean±SD of three separate experiments. The data were fit to a one-component dose-response curve equation for the best-fit estimates of the EC₅₀ (± SD) and E_{max} (± SD), which are reported in Table 2.

Figure 6. Effect of SRI-29574 on [³H]DA uptake/accumulation. As shown in Panel 6A and Table 4A: SRI-29574 had no significant effect on the T_{1/2} (time to half-maximal accumulation), but decreased the E_{max} in a dose-dependent manner (Panel 6B) (IC₅₀ = 5.4±0.2 nM, E_{max} = 69±1 %). Each data point is the mean±SD of three separate experiments. The data were fit to a one-component time-response curve equation for the best-fit estimates of the time to half-maximal accumulation (T_{1/2}) (min ± SD) and E_{max} (% ± SD), which are reported in Table 4A.

Figure 7. Effect of cocaine on [³H]DA uptake/accumulation. As shown in Panel 7A and Table 4B, cocaine increased the T_{1/2} for [³H]DA uptake/accumulation in a dose-

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dependent linear manner. The effect of cocaine on the E_{\max} was more complex. Post-hoc student's t-test showed that two of the four E_{\max} values were not significantly different from control, indicating that cocaine did not have a consistent effect on the E_{\max} . Each data point is the mean \pm SD of three separate experiments. The data were fit to a one-component time-response curve equation for the best-fit estimates of the time to half-maximal accumulation ($T_{1/2}$) (min \pm SD) and E_{\max} (% \pm S D), which are reported in Table 4B.

Table 1

[³H]DA Uptake Inhibition By Select SRI-Compounds: One-Component vs Two-Component Fits

DRUG	One-site	One-site	Two-site	Two-site	Two-site	Two-site	SS	SS	F-test
SRI-	IC50 (nM ± SD)	Emax (%) ± SD)	IC50 ₁ (nM ± SD)	Emax ₁ (%) ± SD)	IC50 ₂ nM ± SD)	Emax ₂ (%) ± SD)	One Site Fit	Two Site Fit	
29986	18 ± 4	75 ± 3	18 ± 1.7E5	37 ± 2.7E8	18 ± 1E5	37 ± 0.7E8	175	175	0
31335	156 ± 12	100 ± 1.5	99 ± 63	76 ± 54	564 ± 1177	27 ± 53	28.9	19.0	1.56
30835	15 ± 7	75 ± 5	6 ± 0.9	57 ± 2	4000 ± 1330	42 ± 4	677	14.2	140*

Dose-response curves (8 points) reported in Fig. 1 were fit to one- and two-component equations for the best-fit estimates of the IC50 and Emax values (±SD). *p<0.001 when compared to the one-component fit (F-test).

Table 2

Summary of Results Obtained for the 36 Partial Efficacy DAT Uptake Blockers

SRI-	DAT	DAT	NET	NET UPTAKE	SERT	SERT	DAT	DAT	5HT/DA	NE/DA
	UPTAKE IC50 (nM ±S D)	UPTAKE Emax (% ± SD)	UPTAKE IC50 (nM ± SD)	Emax (% ± SD)	UPTAKE IC50 (nM ±S D)	UPTAKE Emax (% ± SD)	BINDING IC50 (uM ± SD)	BINDING Emax (% ± SD)	IC50 (UPTAKE)	IC50 (UPTAKE)
29070	174 ± 58	66 ± 4	1740 ±1150	64 ± 10	699 ±164	48 ± 3	1.8 ± 0.4	71 ± 4	4	10
29072	212 ± 49	71 ± 3	5850±1746	62 ± 5	6382 ± 2636	56 ± 9	2.7 ± 0.3	77 ± 2	30	28
29153	20 ± 1	73 ± 1	181 ± 46	73 ± 4	37 ± 18	55 ± 5	1.7 ± 0.2	76 ± 2	1.9	9
29155	10 ± 1.0	74 ± 1	290 ± 52	70 ± 3	68 ± 13	54 ± 2	0.9 ± 0.2	87 ± 4	6.8	29
29212	672 ± 204	67 ± 4	8126±2273	64 ± 5	3805±1645	50 ± 7	2.2 ± 0.5	72 ± 4	5.7	12
29213	16 ± 4	81 ± 3	346 ± 59	77 ± 3	89 ± 51	43 ± 4	0.2 ± 0.0	94 ± 2	5.6	22
29338	9.0 ± 1.5	71 ± 2	204 ± 47	62 ± 3	56 ± 21	52 ± 3	1.18 ± 0.33	63 ± 4	6	23
29554	11 ± 1	71 ± 1	179 ± 27	78 ± 2	57 ± 18	60 ± 3	0.98 ± 0.46	59 ± 6	5	16
29574	2.3 ± 0.4	68 ± 2	52 ± 15	72 ± 4	23 ± 5	52 ± 2	inactive	inactive	10	23
29577	4.4 ± 0.8	70 ± 2	90 ± 15	71 ± 2	20 ± 5	56 ± 2	3.4 ± 1.2	65 ± 6	4.6	20

29776	19 ± 4	69 ± 2	229 ± 43	71 ± 3	106 ± 19	61 ± 2	6.09 ± 0.97	58 ± 3	6	12
29779	7.3 ± 2.2	63 ± 3	147 ± 63	71 ± 6	42 ± 12	51 ± 2	1.2 ± 0.2	83 ± 3	5.8	20
29786	7.1 ± 2.2	70 ± 3	143 ± 61	68 ± 7	49 ± 27	44 ± 4	1.1 ± 0.1	100 ± 3	6.9	20
29982	13 ± 2	73 ± 2	259 ± 41	71 ± 2	54 ± 7	51 ± 1	2.46 ± 1.21	47 ± 6	4	20
29983	11 ± 2	70 ± 2	203 ± 79	73 ± 6	35 ± 9	57 ± 2	1.29 ± 0.34	55 ± 3	3	19
29991	2.1 ± 0.3	68 ± 1	63 ± 9	69 ± 2	4.7 ± 0.6	51 ± 1	1.22 ± 1.12	18 ± 4	2	30
30503	9.2 ± 1.2	70 ± 1	98 ± 23	65 ± 3	16 ± 5	55 ± 2	0.67 ± 0.37	34 ± 4	2	11
30504	11 ± 2	70 ± 2	426 ± 49	70 ± 2	50 ± 14	55 ± 3	0.97 ± 0.65	47 ± 7	5	39
30507	18 ± 3	71 ± 2	132 ± 16	77 ± 2	28 ± 6	54 ± 2	4.80 ± 1.81	62 ± 7	2	7
30508	9.3 ± 1.1	65 ± 1	69 ± 18	76 ± 3	3.9 ± 0.4	56 ± 1	0.15 ± 0.09	34 ± 3	0.4	8
30513	12 ± 2	72 ± 2	153 ± 55	65 ± 4	83 ± 28	59 ± 4	2.97 ± 1.03	50 ± 4	7	13
30517	6.0 ± 0.7	70 ± 1	95 ± 12	70 ± 2	23 ± 3	54 ± 1	0.16 ± 0.08	43 ± 4	4	16
30522	8.8 ± 1.1	63 ± 1	86 ± 55	40 ± 5	13 ± 6	35 ± 3	inactive	inactive	1	10
30524	4.8 ± 0.6	71 ± 1	44 ± 6	76 ± 2	8.7 ± 0.9	56 ± 1	2.36 ± 1.17	52 ± 6	2	9
30810	5.6 ± 0.8	64 ± 1	60 ± 10	69 ± 2	11 ± 3	51 ± 2	0.82 ± 0.36	25 ± 2	2	11
30826	6.1 ± 1	64 ± 2	88 ± 15	81 ± 2	18 ± 4	56 ± 2	1.28 ± 0.40	42 ± 3	3	14
30827	0.5 ± 0.1	63 ± 2	21 ± 7	67 ± 3	3.2 ± 0.9	58 ± 3	1.99 ± 0.33	79 ± 3	6	42
30828	8.9 ± 1.6	60 ± 2	144 ± 30	65 ± 3	20 ± 5	50 ± 2	2.61 ± 1.14	60 ± 7	2	16
30837	11 ± 1	61 ± 1	300 ± 56	75 ± 3	67 ± 8	55 ± 1	1.70 ± 0.62	44 ± 4	6	27

30946	21 ± 3	70 ± 2	78 ± 17	75 ± 3	35 ± 11	59 ± 3	1.17 ± 0.32	44 ± 3	2	4
31034	7.4 ± 1.1	69 ± 1	94 ± 22	63 ± 3	25 ± 7	49 ± 2	inactive	inactive	3	13
31039	7.4 ± 2	74 ± 4	31 ± 7	71 ± 3	8.0 ± 2.8	58 ± 3	3.15 ± 1.11	72 ± 7	1	4
31040	1.2 ± 0.1	69 ± 1	11 ± 4	70 ± 4	3.1 ± 0.7	54 ± 2	3.74 ± 1.10	91 ± 7	3	9
31043	11 ± 1	67 ± 1	47 ± 10	67 ± 2	22 ± 5	51 ± 2	2.16 ± 0.97	30 ± 3	2	4
31142	1.9 ± 0.3	72 ± 2	17 ± 4	61 ± 2	2.4 ± 0.4	48 ± 1	2.34 ± 0.45	92 ± 4	1.3	9
31143	1.7 ± 0.1	69 ± 1	16 ± 5	69 ± 4	3.0 ± 0.6	51 ± 2	3.39 ± 1.99	52 ± 8	1.8	9
cocaine	200 ± 19	100 ± 2	329 ± 22	102 ± 2	273 ± 24	98 ± 2	0.28 ± 0.03	97 ± 3	1.4	1.7
GBR12935	1.1 ± 0.1	104 ± 3	nd	nd	nd	nd	2.0e-3 ± 0.08e-3	101 ± 0.9		

Dose response curves for each indicated agent were generated as described in Methods for DAT, NET and SERT uptake inhibition, and DAT binding. Each value is the mean±SD, n=3.

Table 3

Effect of Test Agents on D-Amphetamine DAT-Mediated [³H]MPP⁺ or [³H]DA Release

Blocker	IC50 for DAT Uptake Inhibition (nM)	E _{max} for DAT Uptake Inhibition (%)	Blocker Concentration nM	D-Amphetamine EC50 (nM ± SD)	D-Amphetamine E _{max} (% ± SD)	K _e App nM
[³ H]MPP ⁺ Release						
none	----	----	----	6.4 ± 1.2	104 ± 4	---
29574	2	68	50	5.4 ± 0.6	103 ± 3	-388
29577	4	70	125	4.8 ± 0.4	102 ± 2	-553
29786	7	70	250	7.0 ± 0.5	101 ± 2	1940
29779	7	63	250	7.9 ± 0.6	99 ± 2	912
29155	10	74	250	9.6 ± 1.0	94 ± 2	456
29213	16	81	500	9.7 ± 1.0	78 ± 2	886

29153	20	73	500	7.9 ± 0.9	101 ± 3	1820
29070	174	66	5000	10.6 ± 0.7	98 ± 1	7050
29072	212	71	5000	7.4 ± 1.0	96 ± 3	25830
29212	672	67	12500	7.4 ± 0.8	103 ± 2	64580
29991	2	68	50	6.1 ± 0.7	102 ± 2	-1070
30517	6	70	150	7.0 ± 1.3	104 ± 4	1600
30522	9	63	250	6.4 ± 1.1	105 ± 4	N/A
30524	5	71	125	7.1 ± 0.9	103 ± 3	1140
30810	6	64	150	7.2 ± 1.5	104 ± 5	1200
30826	6	64	150	7.0 ± 1.3	105 ± 4	1600
30827	0.5	63	12.5	6.7 ± 0.9	104 ± 3	267
31034	7	69	200	6.8 ± 1.5	104 ± 5	3200
31040	1	69	25	9.3 ± 1.3	105 ± 3	55
31142	2	72	50	7.2 ± 1.0	103 ± 3	400
31143	2	67	50	6.9 ± 1.3	104 ± 4	46
GBR12935	2	100	5	150 ± 25	122 ± 8	0.22
[³ H]DA Release						

none	----	----	----	67 ± 10	97 ± 3	
GBR12935	2	100	5	519 ± 95	91 ± 6	0.74
29574	2	68	50	53 ± 6	95 ± 2	-239
29213	16	81	500	72 ± 12	71 ± 3	6700

D-amphetamine dose-response curves were generated in the absence and presence of each test agent as described in Methods and illustrated in Fig. 5A. Each value is the mean±SD, n=3. The apparent Ke was calculated according to the following equation: $\text{Apparent Ke} = [\text{Blocker}] / ((\text{EC50}_{.2} / \text{EC50}_{.1}) - 1)$ where EC50_{.1} is the EC50 in the absence of blocker and EC50_{.2} is the EC50 in the presence of blocker. *p<0.05 when compared to control (Students t-test).

Table 4

Effect of SRI-29574 and Cocaine on [³H]DA accumulationA. Effect of SRI-29574 on [³H]DA accumulation

[SRI-29574] nM	T1/2 (MIN±SD)	E _{max} (%±SD)	% Inhibition of the E _{max} value
0	32 ± 4	98 ± 4	0
1	28 ± 3	89 ± 4	11
4	27 ± 3	71 ± 2*	29
8	34 ± 3	58 ± 2*	42
25	31 ± 3	43 ± 3*	57

[³H]DA uptake was assessed at various time points in the absence and presence of the indicated concentrations of SRI-29574. The combined data of all three experiments (Fig. 6A) (180 data points) were fit to the equation: $B = E_{max} \times (T / (T + T_{1/2}))$ where “B” is the observed level of uptake, “T” is the time in minutes and T1/2 is the time to half-maximal accumulation. The time course data of each drug condition were separately applied to the above equation, each of which differed only in the names of the corresponding parameters: E_{max}₁, E_{max}₂, E_{max}₃, E_{max}₄ and E_{max}₅ and T1/2₁, T1/2₂, T1/2₃, T1/2₄ and T1/2₅. Thus, the unconstrained fit led to the above reported parameter values. The data were then fit with the constraint that the E_{max} values were all equal. This resulted in a highly significant increase in the sum-of-squares with an (F=18.9, p=0). In

contrast, fitting the data with the constraint that the T1/2 values were equal did not significantly increase the sum-of-squares (F=0.46, p=0.76). *p<0.05 when compared to control (unpaired students t-test)

B. Effect of Cocaine on [³H]DA accumulation

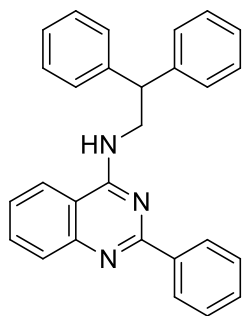
[Cocaine] nM	T1/2 (MIN±SD)	Emax (%±SD)	FOLD Increase in T1/2
0	40 ± 2	100 ± 2	0
100	63 ± 5*	112 ± 4*	1.57
300	121 ± 15*	121 ± 15	3.0
600	266 ± 24*	132 ± 9*	6.65
1200	440 ± 165*	140 ± 43	11.0

[³H]DA uptake was assessed at various time points in the absence and presence of the indicated concentrations of cocaine. The combined data of all three experiments (Fig. 7A) were (180 data points) and fit to the equation described in Table 4A. The time course data of each drug condition were separately applied to the above equation, each of which differed only in the names of the corresponding parameters: Emax₁, Emax₂, Emax₃, Emax₄ and Emax₅ and T1/2₋₁, T1/2₋₂, T1/2₋₃, T1/2₋₄ and T1/2₋₅. Thus, the unconstrained fit led to the above reported parameter values. The data were then fit with the constraint that the Emax values were all equal. This resulted in a significant increase in the sum-of-squares with an (F=6.34, p<0.001). Fitting the data with the constraint that the T1/2 values were equal led to a highly significant increased sum-of-squares (F=89, p=0).

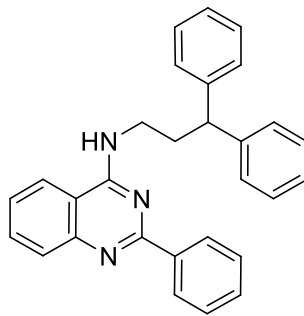
Constraining the Emax values to equal 100% also led to a modestly significant increase in sum-of-squares ($F=4.19$, $p=0.003$).

* $p<0.05$ when compared to control (unpaired students t-test).

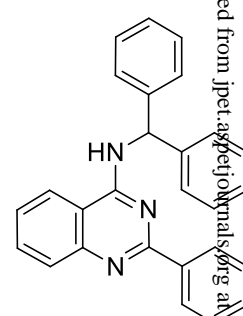
Figure 1A



SRI-20040

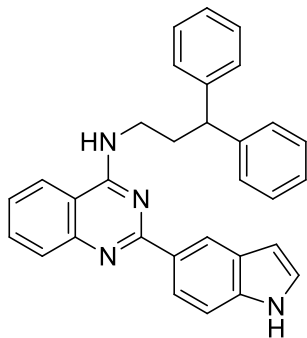


SRI-20041

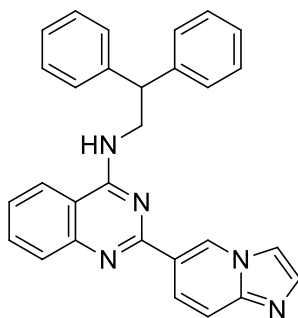


SRI-9804

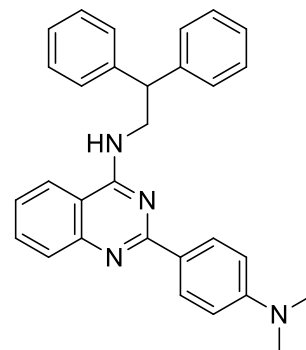
Figure 1B



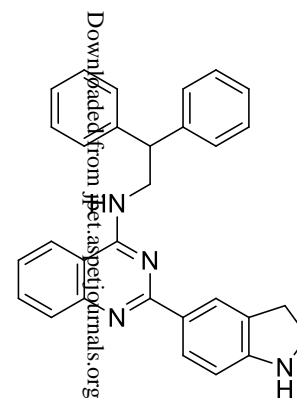
SRI-29213



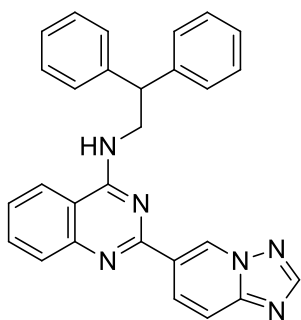
SRI-29574



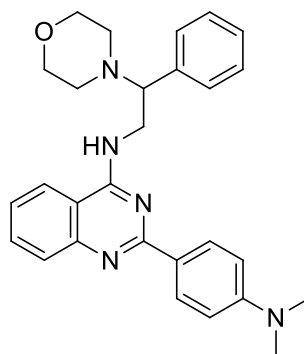
SRI-29786



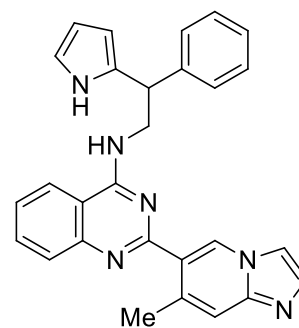
SRI-29986



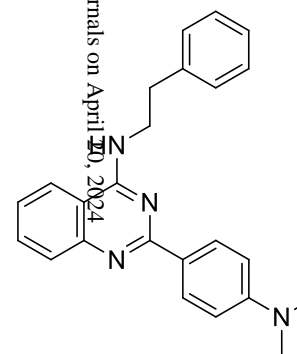
SRI-30522



SRI-30835



SRI-31040



SRI-31335

FIGURE 2A

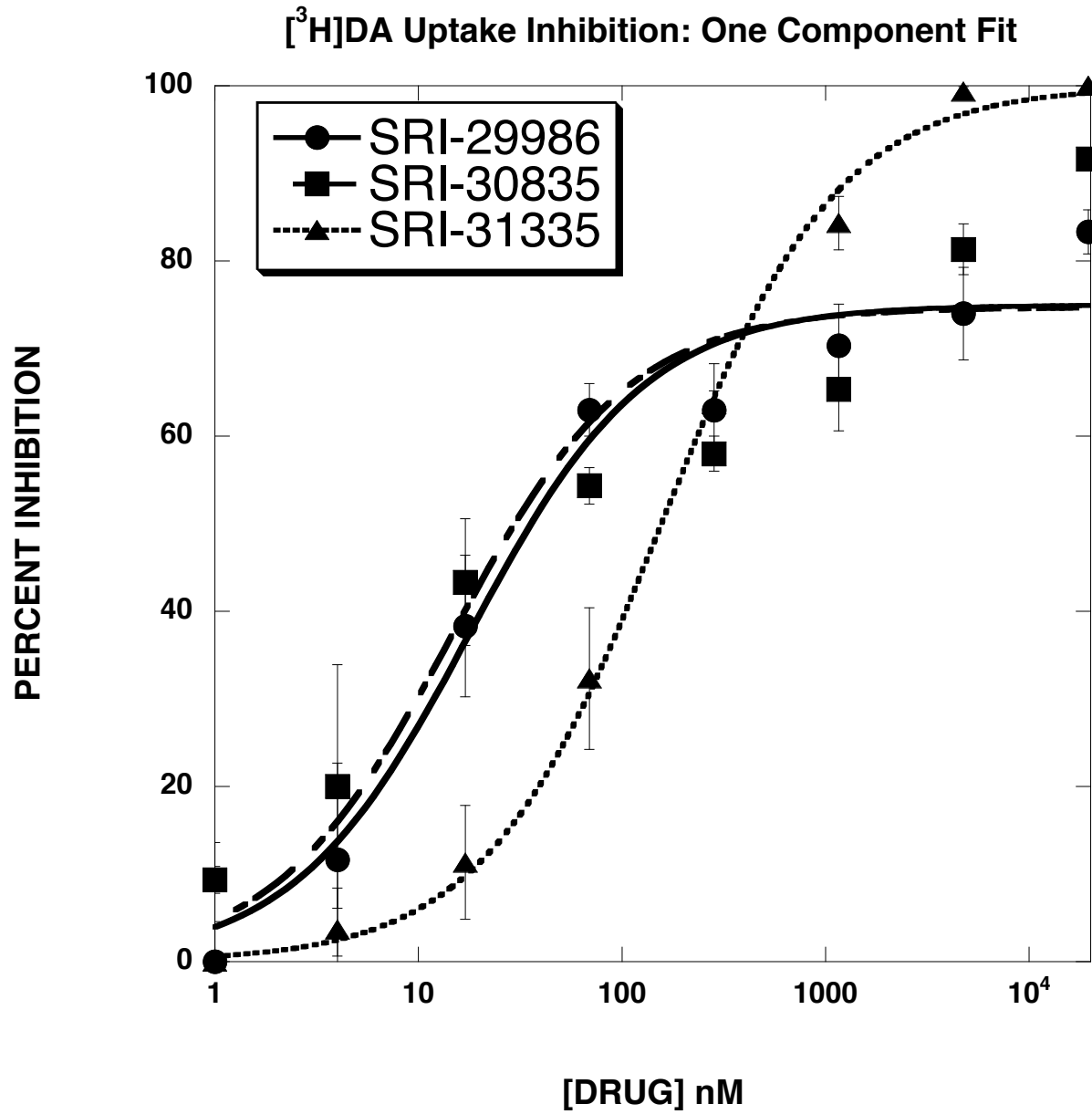


FIGURE 2B

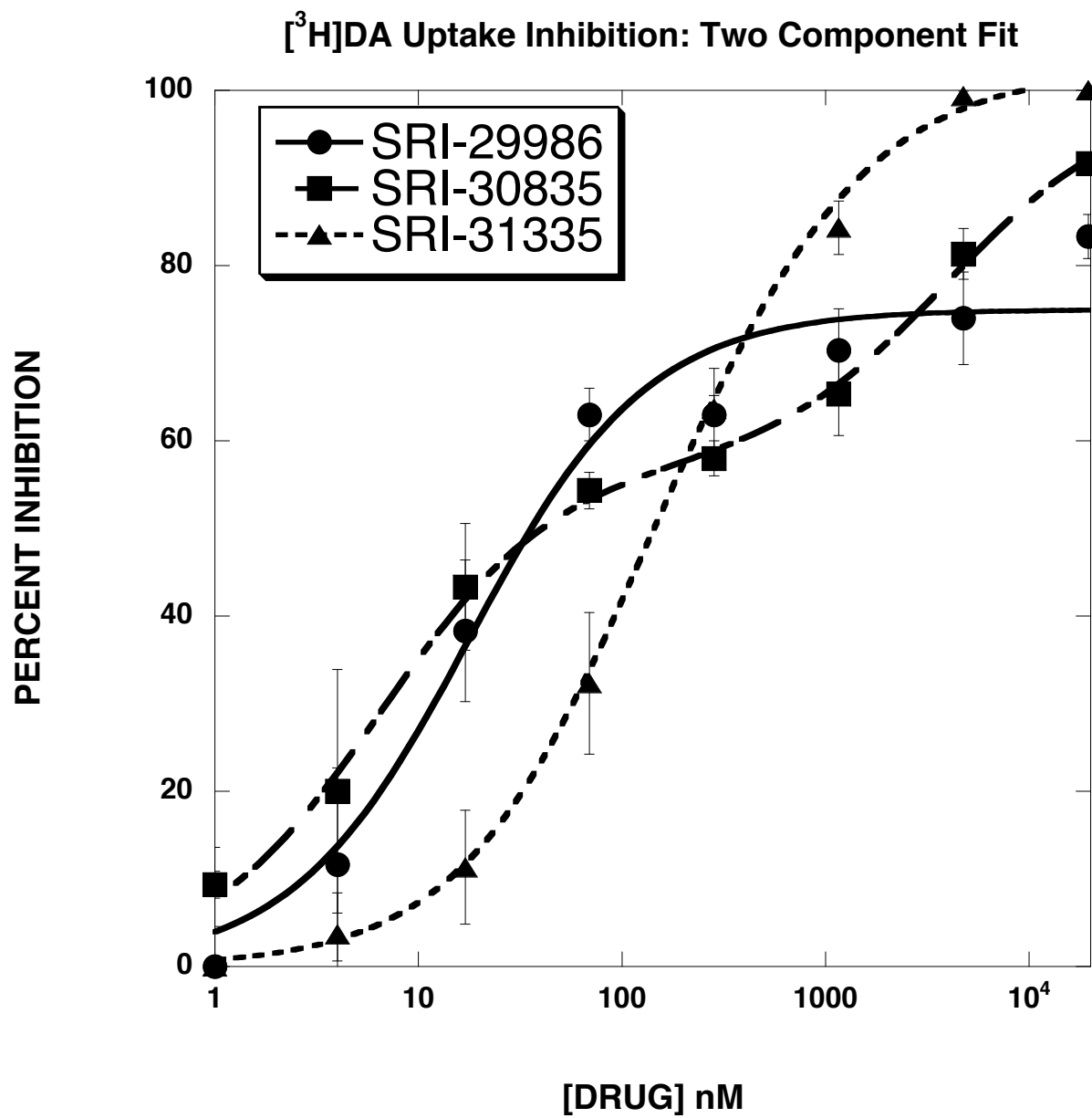
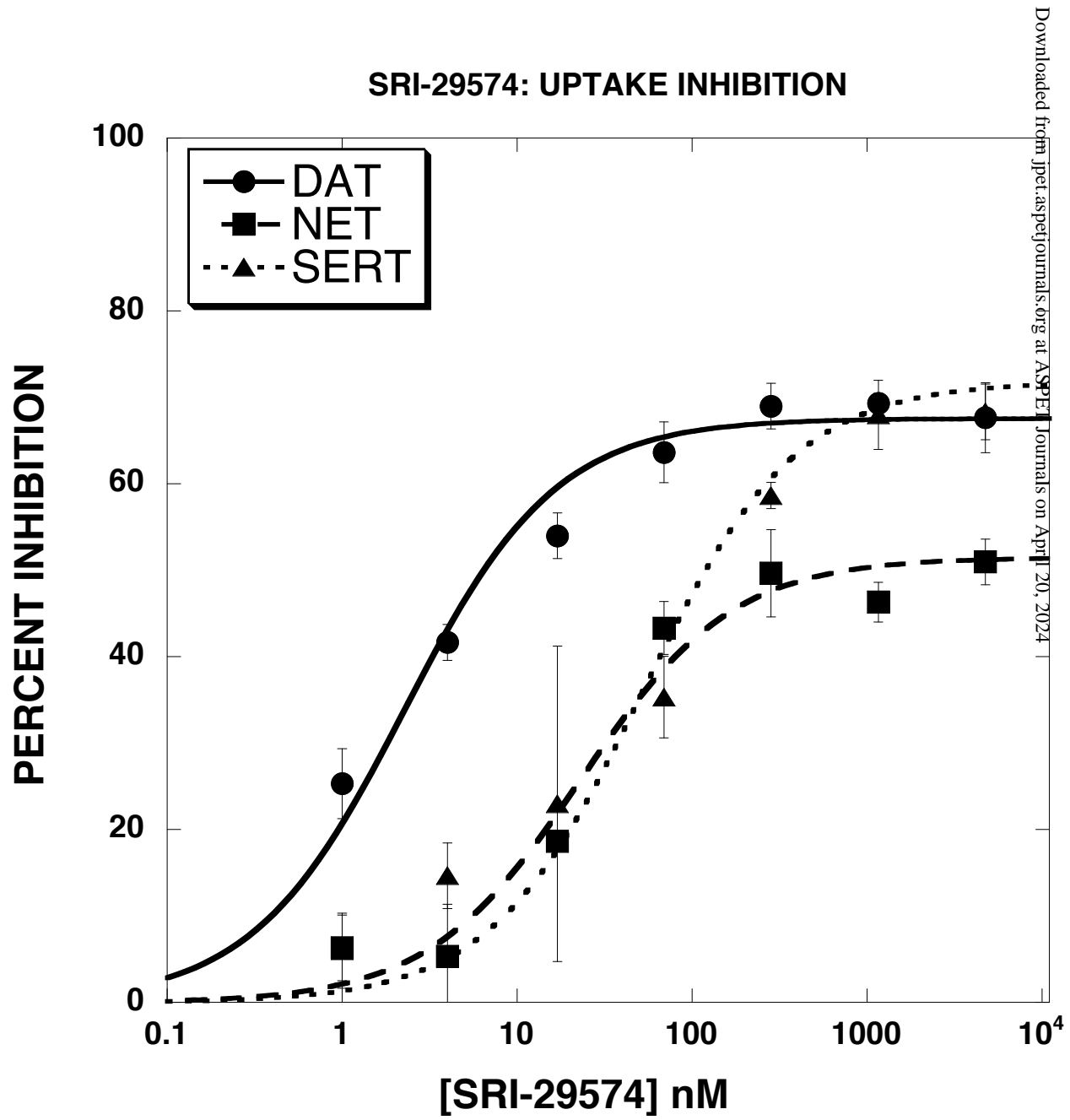


Figure 3



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Figure 4A

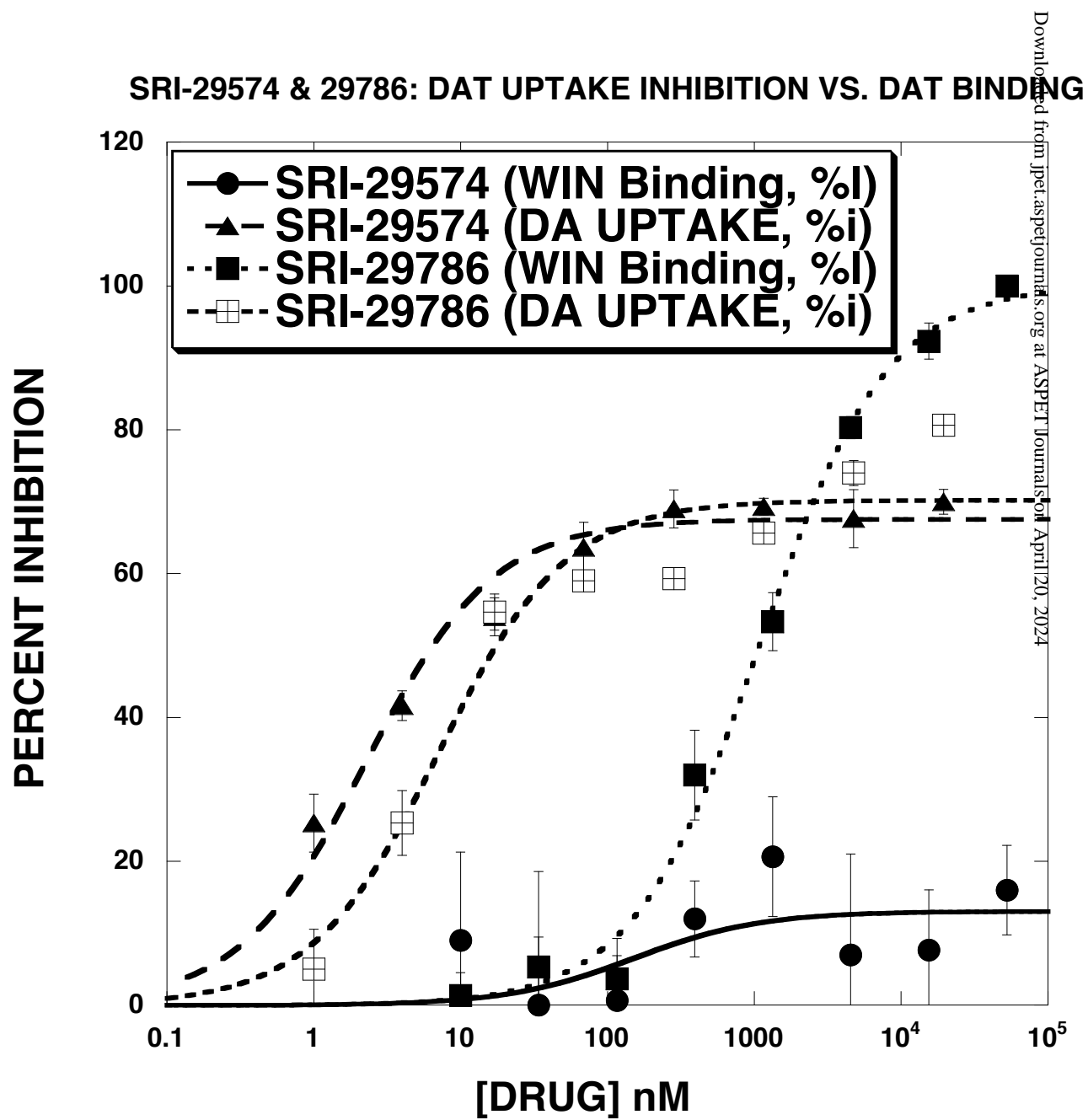


Figure 4B

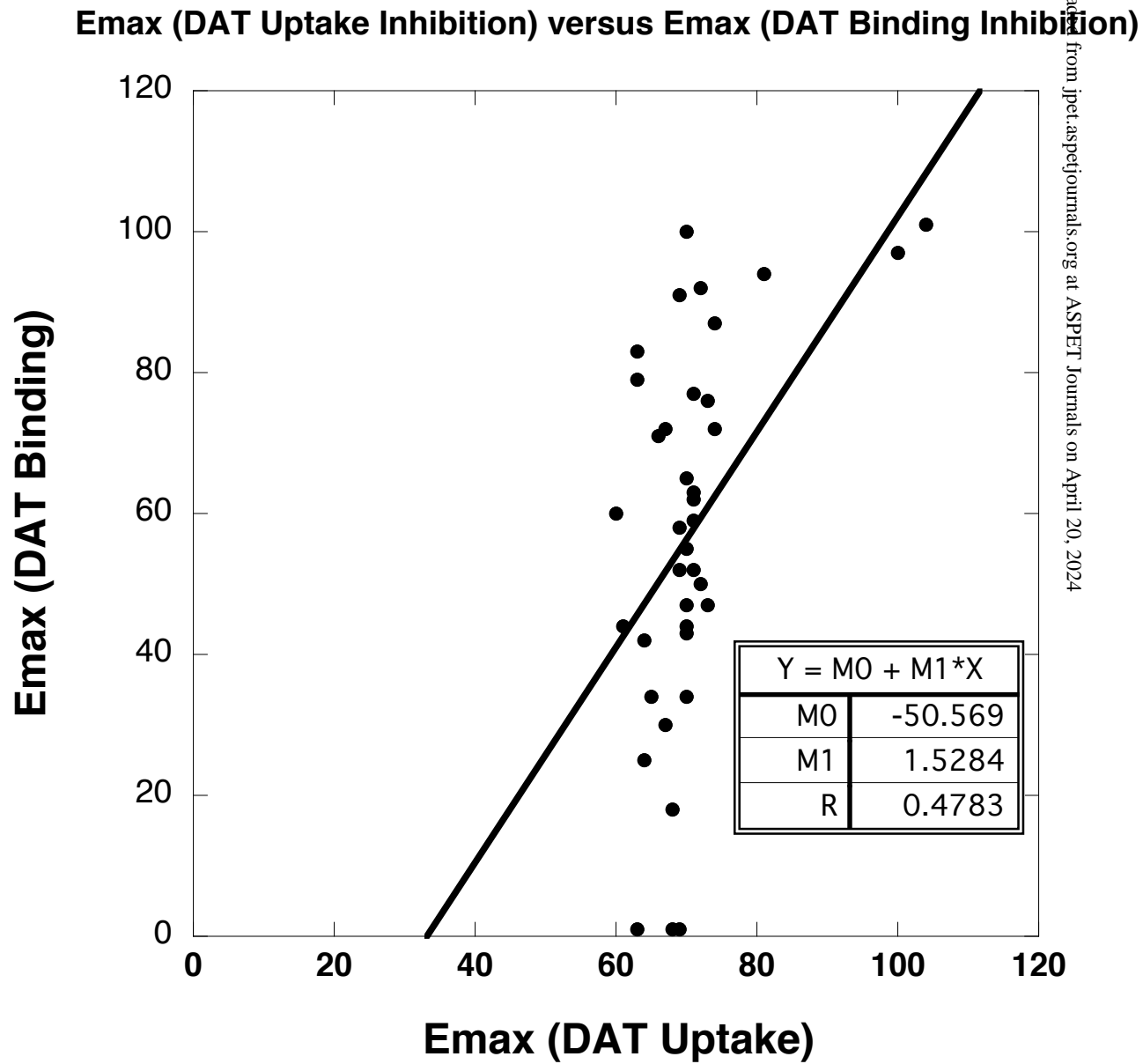
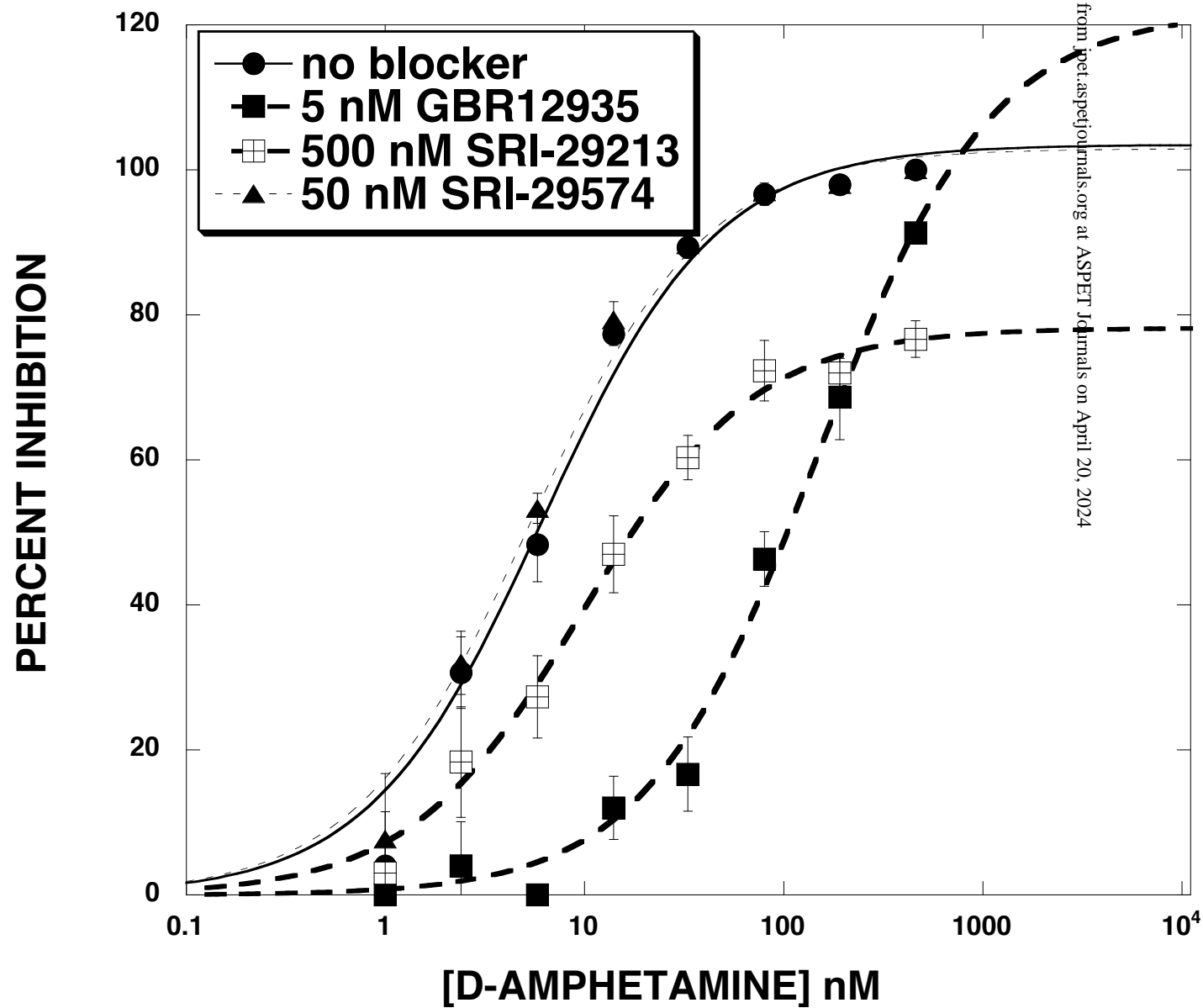


FIGURE 5A

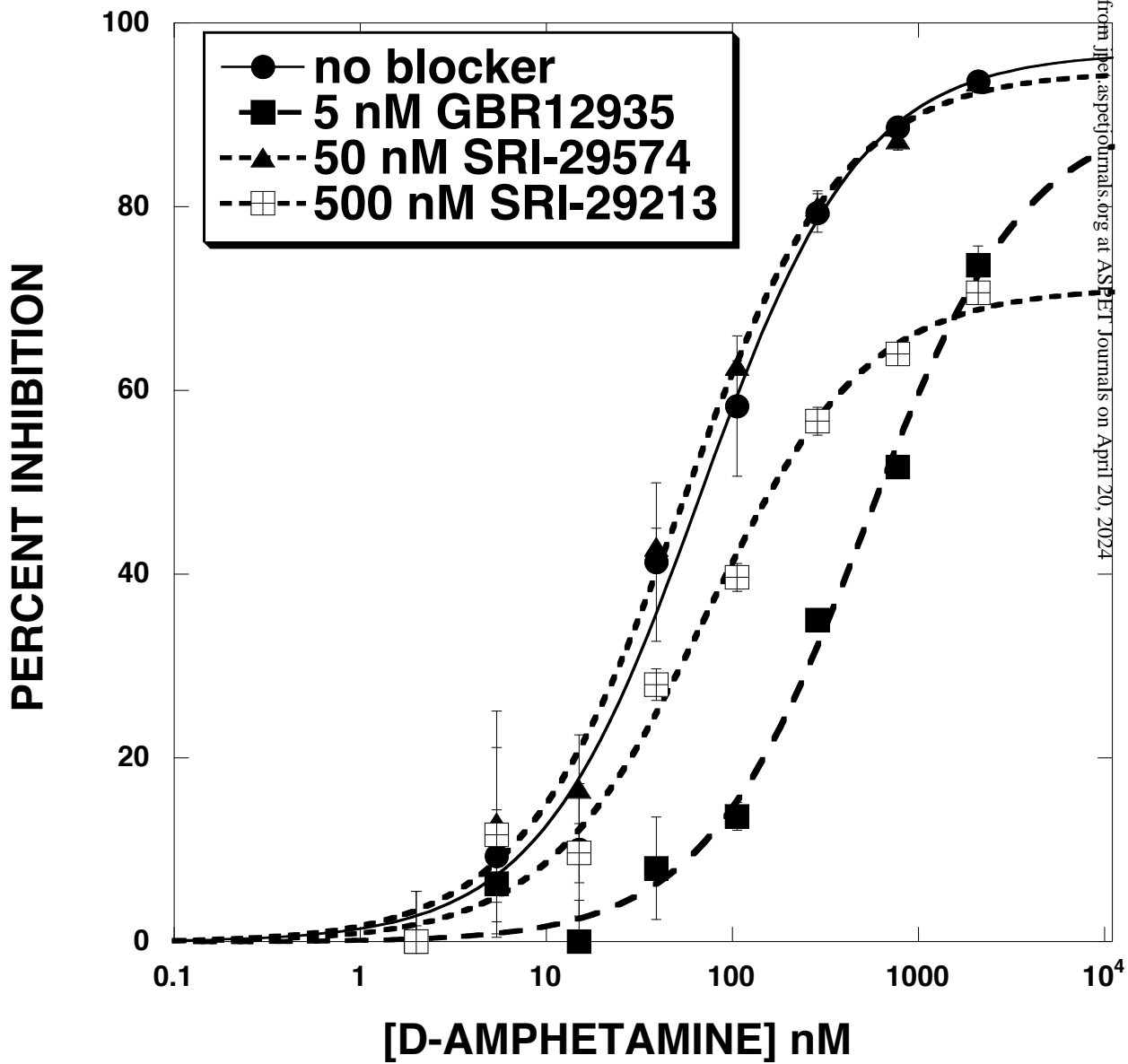
EFFECT OF TEST DRUGS ON D-AMPH INDUCED DAT-MEDIATED [³H]MPP⁺ RELEASE



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FIGURE 5B

EFFECT OF TEST DRUGS ON D-AMPH INDUCED [³H]DOPAMINE RELEASE



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FIGURE 6A

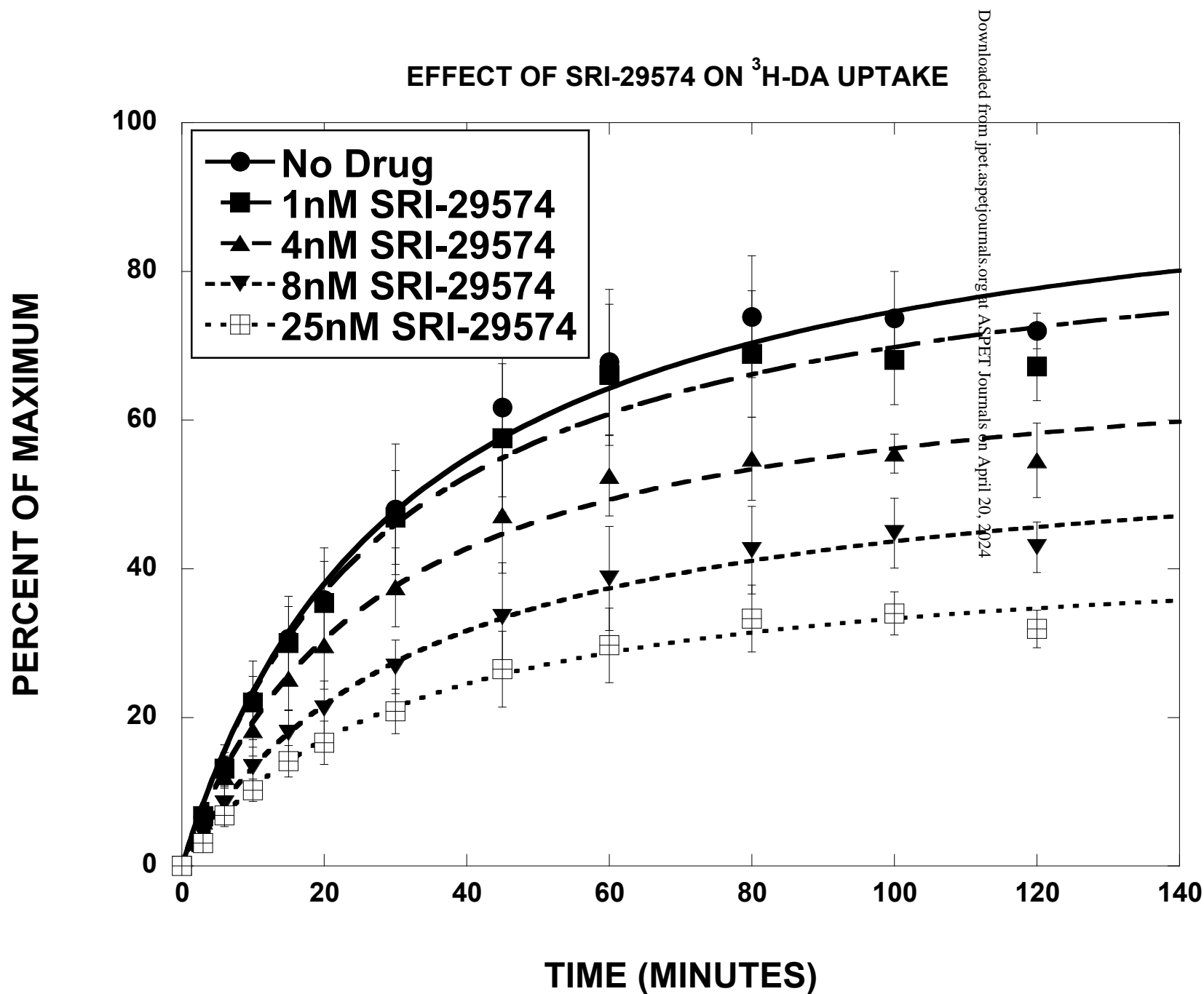


FIGURE 6B

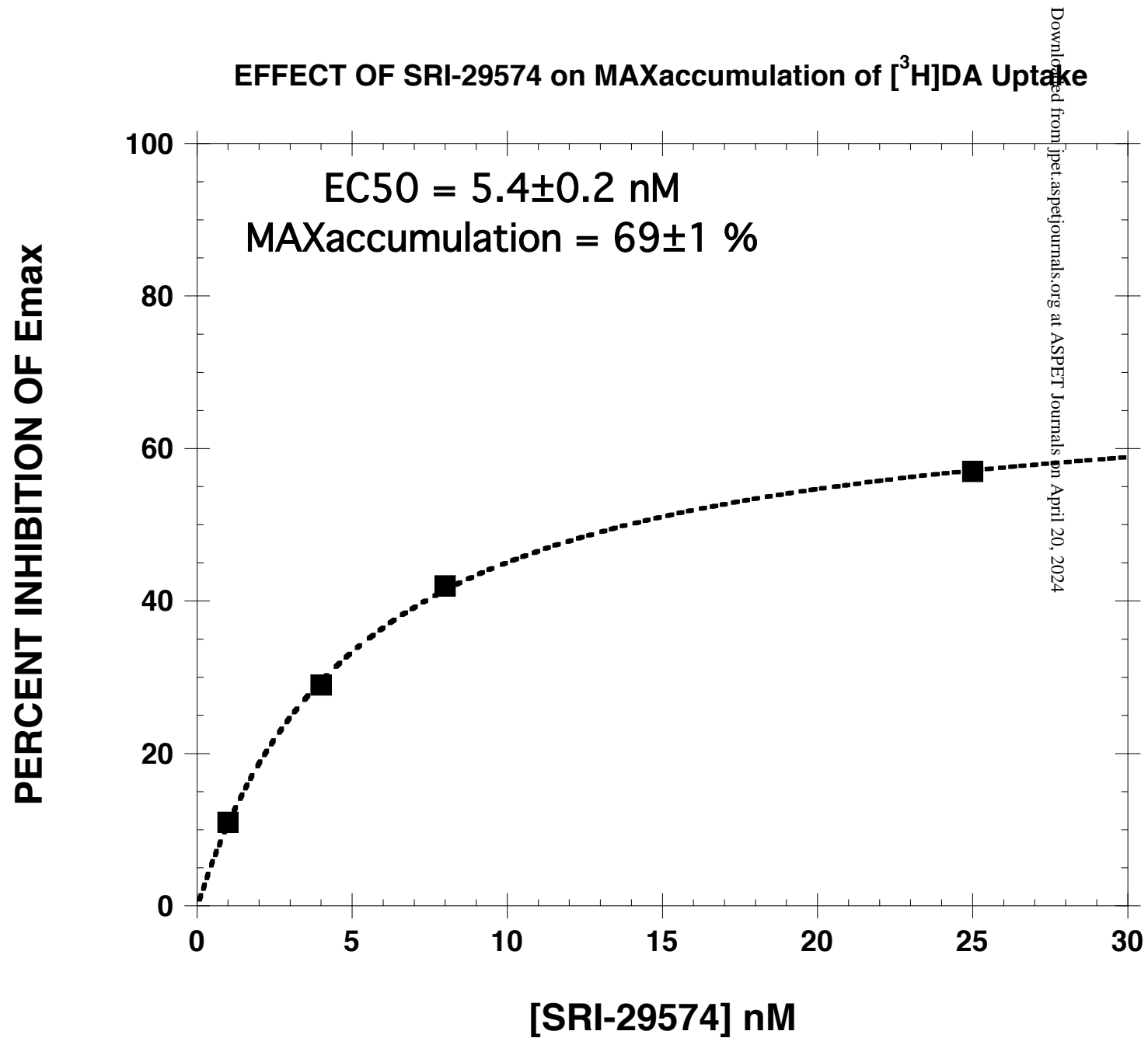
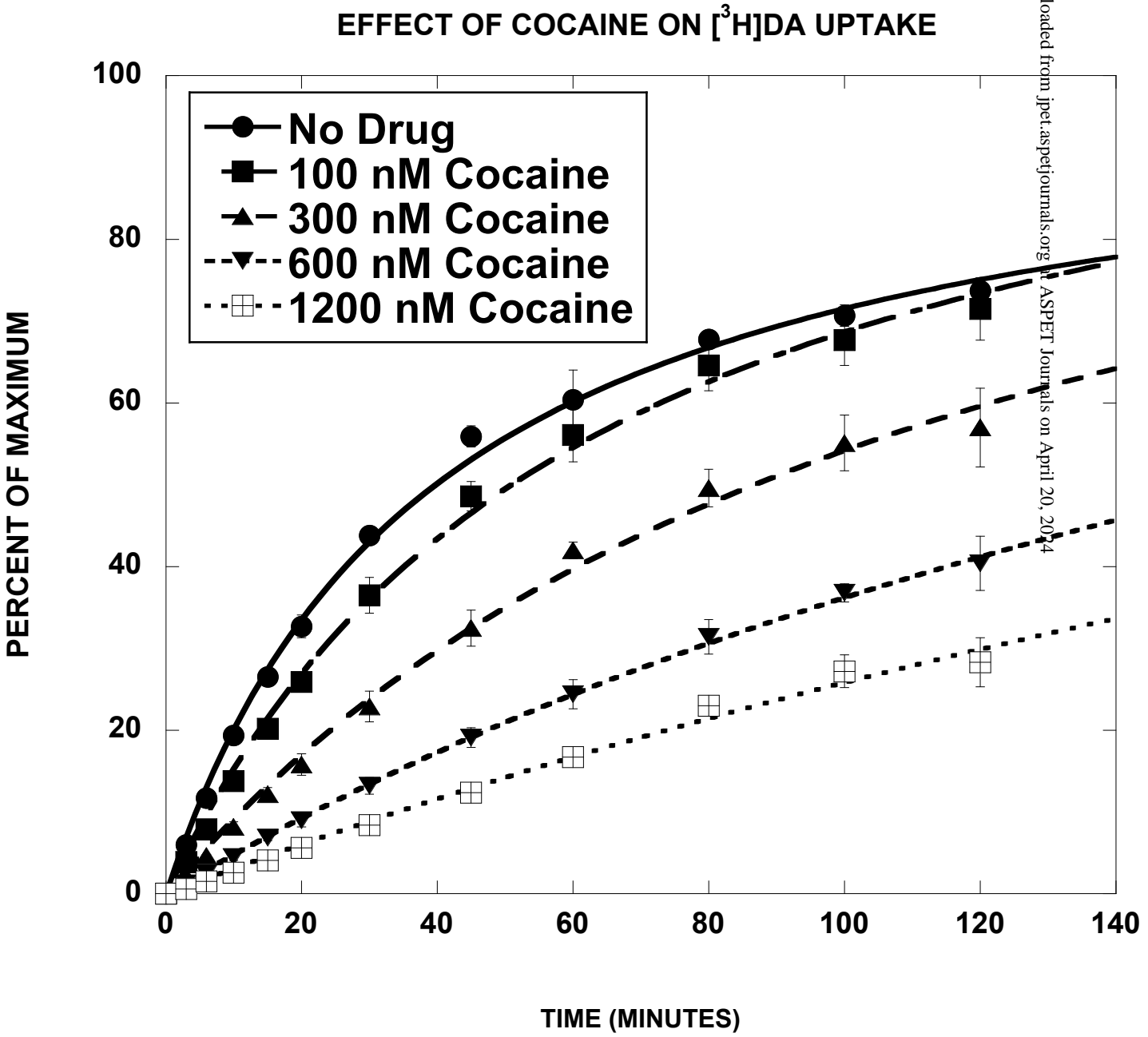
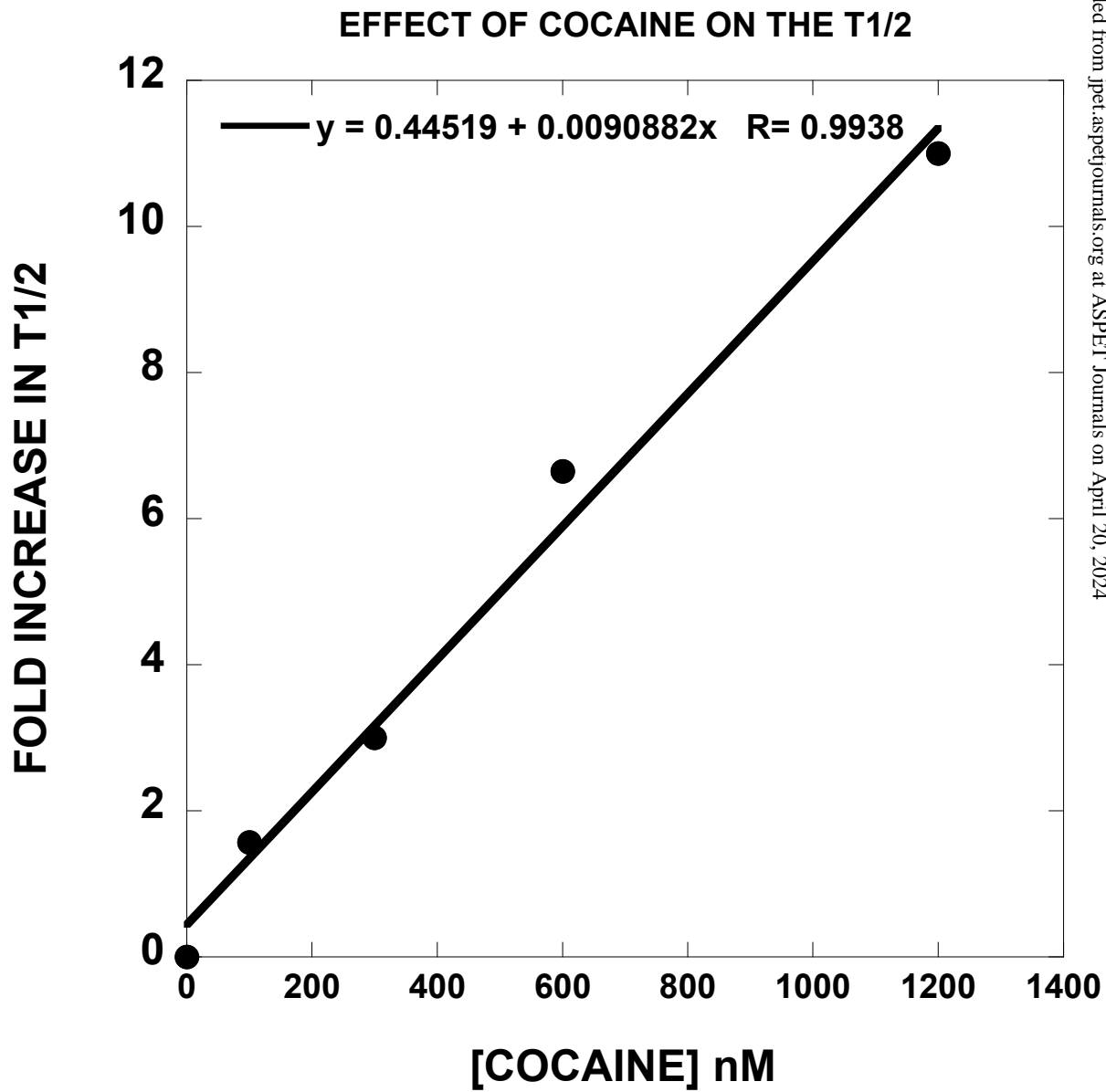


FIGURE 7A



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Fig. 7B

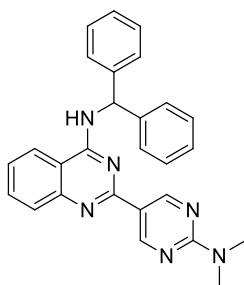


Studies of the Biogenic Amine Transporters. 15. Identification of Novel Allosteric Dopamine Transporter Ligands with Nanomolar Potency

Richard B. Rothman, Subramaniam Ananthan, John S. Partilla, Surendra K. Saini, Omar Moukha-Chafiq, Vibha Pathak, and Michael H. Baumann

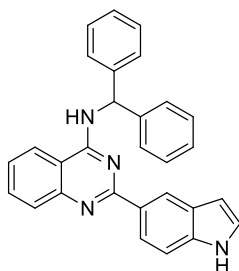
SUPPLEMENTAL FIGURE 1

Structures of Dopamine Transporter Ligands



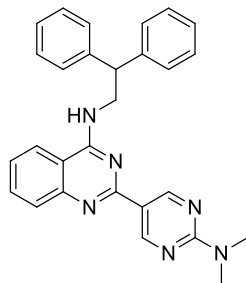
SRI-29070

N-Benzhydryl-2-(2-(dimethylamino)pyrimidin-5-yl)quinazolin-4-amine



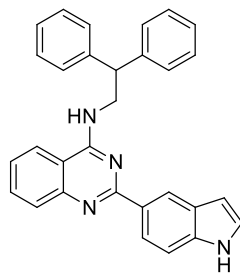
SRI-29072

N-Benzhydryl-2-(1H-indol-5-yl)quinazolin-4-amine



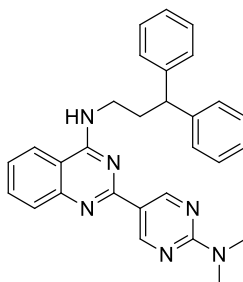
SRI-29153

2-(2-(Dimethylamino)pyrimidin-5-yl)-N-(2,2-diphenylethyl)quinazolin-4-amine



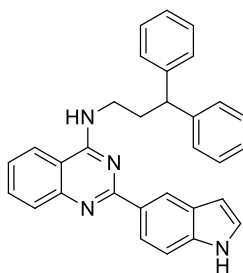
SRI-29155

N-(2,2-Diphenylethyl)-2-(1H-indol-5-yl)quinazolin-4-amine



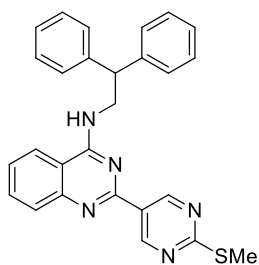
SRI-29212

2-(2-(Dimethylamino)pyrimidin-5-yl)-N-(3,3-diphenylpropyl)quinazolin-4-amine



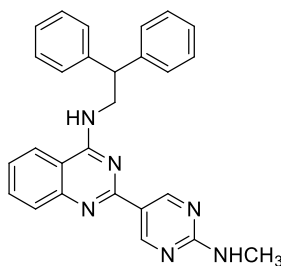
SRI-29213

N-(3,3-Diphenylpropyl)-2-(1H-indol-5-yl)quinazolin-4-amine



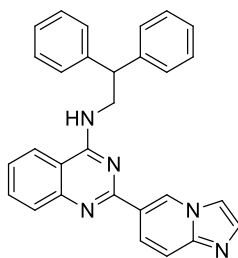
SRI-29338

N-(2,2-Diphenylethyl)-2-(2-(methylthio)pyrimidin-5-yl)quinazolin-4-amine



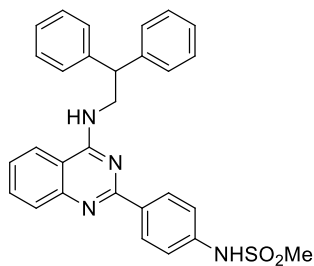
SRI-29554

N-(2,2-Diphenylethyl)-2-(2-(methylamino)pyrimidin-5-yl)quinazolin-4-amine



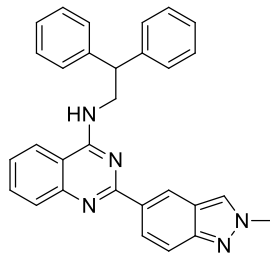
SRI-29574

N-(2,2-Diphenylethyl)-2-(imidazo[1,2-a]pyridin-6-yl)quinazolin-4-amine



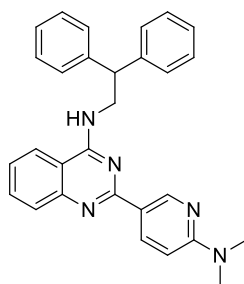
SRI-29577

N-(4-(4-((2,2-Diphenylethyl)amino)quinazolin-2-yl)phenyl)methanesulfonamide



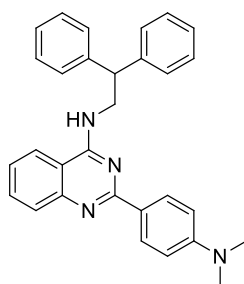
SRI-29776

N-(2,2-Diphenylethyl)-2-(2-methyl-2H-indazol-5-yl)quinazolin-4-amine



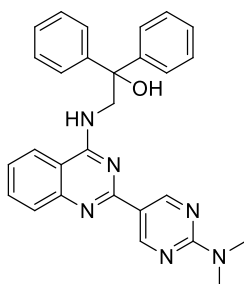
SRI-29779

2-(6-(Dimethylamino)pyridin-3-yl)-N-(2,2-diphenylethyl)quinazolin-4-amine



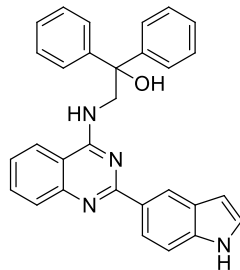
SRI-29786

2-(4-(Dimethylamino)phenyl)-N-(2,2-diphenylethyl)quinazolin-4-amine



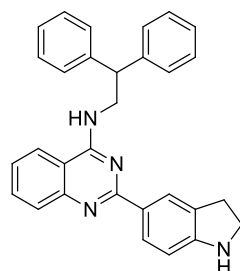
SRI-29982

2-((2-(2-(Dimethylamino)pyrimidin-5-yl)quinazolin-4-yl)amino)-1,1-diphenylethanol



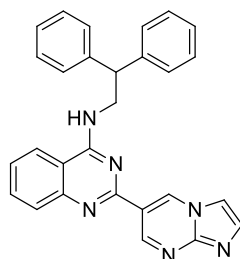
SRI-29983

2-((2-(1H-Indol-5-yl)quinazolin-4-yl)amino)-1,1-diphenylethanol



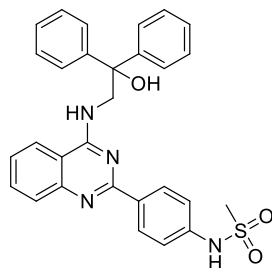
SRI-29986

N-(2,2-Diphenylethyl)-2-(indolin-5-yl)quinazolin-4-amine



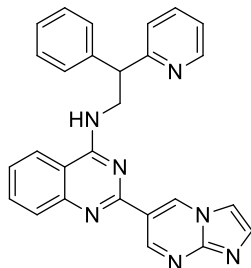
SRI-29991

N-(2,2-Diphenylethyl)-2-(imidazo[1,2-a]pyrimidin-6-yl)quinazolin-4-amine



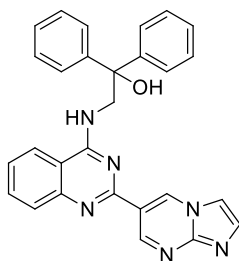
SRI-30504

N-(4-(4-((2-Hydroxy-2,2-diphenylethyl)amino)quinazolin-2-yl)phenyl)methanesulfonamide



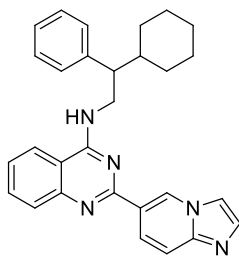
SRI-30507

2-(Imidazo[1,2-a]pyrimidin-6-yl)-N-(2-phenyl-2-(pyridin-2-yl)ethyl)quinazolin-4-amine



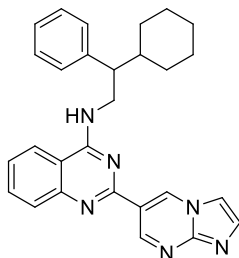
SRI-30508

2-((2-(Imidazo[1,2-a]pyrimidin-6-yl)quinazolin-4-yl)amino)-1,1-diphenylethanol



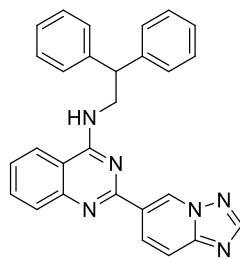
SRI-30513

N-(2-Cyclohexyl-2-phenylethyl)-2-(imidazo[1,2-a]pyrimidin-6-yl)quinazolin-4-amine



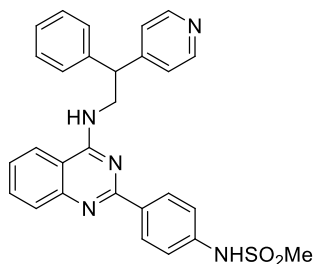
SRI-30517

N-(2-Cyclohexyl-2-phenylethyl)-2-(imidazo[1,2-a]pyrimidin-6-yl)quinazolin-4-amine



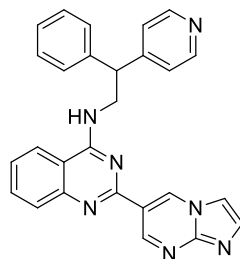
SRI-30522

2-([1,2,4]Triazolo[1,5-a]pyridin-6-yl)-N-(2,2-diphenylethyl)quinazolin-4-amine



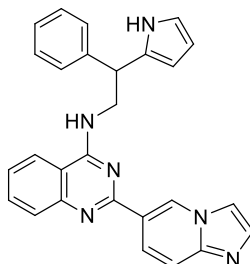
SRI-30524

N-(4-(4-((2-Phenyl-2-(pyridin-4-yl)ethyl)amino)quinazolin-2-yl)phenyl)methanesulfonamide



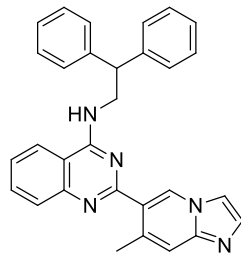
SRI-30810

2-(Imidazo[1,2-a]pyrimidin-6-yl)-N-(2-phenyl-2-(pyridin-4-yl)ethyl)quinazolin-4-amine



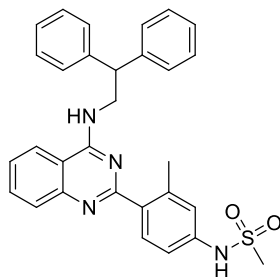
SRI-30826

2-(Imidazo[1,2-a]pyridin-6-yl)-N-(2-phenyl-2-(1H-pyrrol-2-yl)ethyl)quinazolin-4-amine



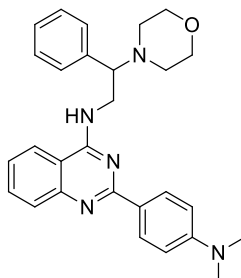
SRI-30827

N-(2,2-Diphenylethyl)-2-(7-methylimidazo[1,2-a]pyridin-6-yl)quinazolin-4-amine



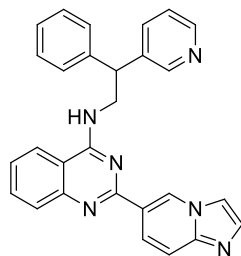
SRI-30828

N-(4-(4-((2,2-Diphenylethyl)amino)quinazolin-2-yl)-3-methylphenyl)methanesulfonamide



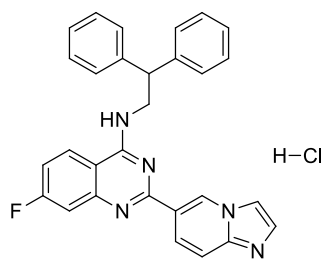
SRI-30835

2-(4-(Dimethylamino)phenyl)-N-(2-morpholino-2-phenylethyl)quinazolin-4-amine



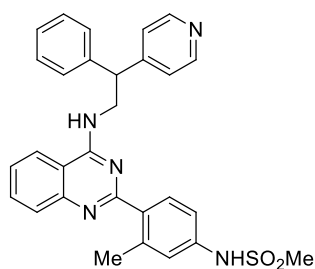
SRI-30946

2-(Imidazo[1,2-a]pyridin-6-yl)-N-(2-phenyl-2-(pyridin-3-yl)ethyl)quinazolin-4-amine



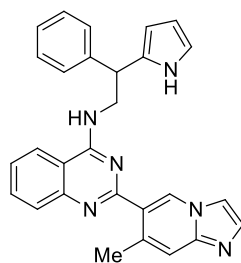
SRI-31034

N-(2,2-Diphenylethyl)-7-fluoro-2-(imidazo[1,2-a]pyridin-6-yl)quinazolin-4-amine hydrochloride



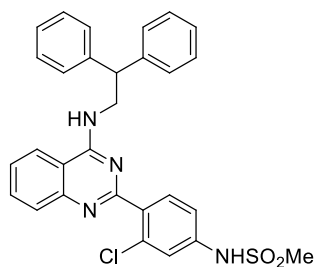
SRI-31039

N-(3-Methyl-4-(4-((2-phenyl-2-(pyridin-4-yl)ethyl)amino)quinazolin-2-yl)phenyl)methanesulfonamide



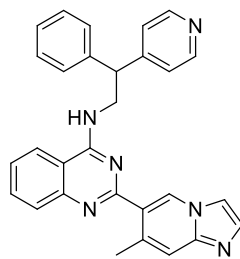
SRI-31040

2-(7-Methylimidazo[1,2-a]pyridin-6-yl)-N-(2-phenyl-2-(1H-pyrrol-2-yl)ethyl)quinazolin-4-amine



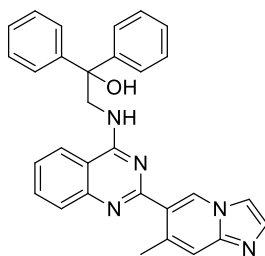
SRI-31043

N-(3-Chloro-4-(4-((2,2-diphenylethyl)amino)quinazolin-2-yl)phenyl)methanesulfonamide



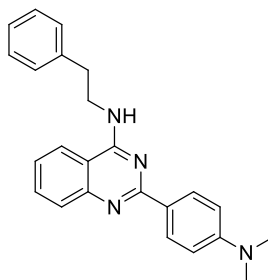
SRI-31142

2-((2-(7-Methylimidazo[1,2-a]pyridin-6-yl)-N-(2-phenyl-2-(pyridin-4-yl)ethyl)quinazolin-4-amine



SRI-31143

2-(((2-(7-Methylimidazo[1,2-a]pyridin-6-yl)quinazolin-4-yl)amino)-1,1-diphenylethanol



SRI-31335

2-(4-(Dimethylamino)phenyl)-N-phenethylquinazolin-4-amine

The structures and chemical names of compounds reported in the paper are reported here.