Pure Appl. Chem., Vol. 84, No. 7, pp. 1543–1556, 2012.
http://dx.doi.org/10.1351/PAC-CON-11-11-09

Targeting neurodegenerative diseases: Drug discovery in a challenging arena*

Magid Abou-Gharbia‡ and Wayne Childers

Moulder Center for Drug Discovery Research, School of Pharmacy, Temple University, 3307 North Broad Street, Philadelphia, PA 18938, USA

Abstract: Neurodegenerative diseases represent one of the health care community's truly unmet medical needs. They can be loosely classified into two categories, acute and chronic. One of the best known chronic neurodegenerative diseases, Alzheimer's disease, represents a serious health care problem that may well exceed the limits of current fiscal and care giver resources. No disease-modifying therapeutic agents have been identified, and the few available symptomatic treatments possess limitations in their duration of action and side effects. Despite decades of drug discovery research and numerous clinical trials, no truly effective treatment for stroke, the most prevalent acute neurodegenerative disease, has been identified. This article summarizes two recent drug discovery projects, one targeting Alzheimer's disease and the other targeting ischemic stroke. Both projects involved design, synthesis, and biological evaluation of a novel series of heterocyclic derivatives.

Keywords: Alzheimer's; BACE1; bioactive molecules; bioorganic chemistry; computer-aided molecular design; enzyme ILS-920; immunophilin; inhibitors; macromolecular chemistry; medicinal chemistry; stroke; WAY-258,131.

According to the National Institute of Neurological Disorders and Stroke, there are over 600 diseases known to afflict the nervous system. Among these are a large group of afflictions that can be generally classified as neurodegenerative diseases. Neurodegenerative diseases, as the name suggests, are characterized by progressive dysfunction of the nervous system. They can be further subdivided into two general categories. Chronic neurodegenerative diseases are characterized by a slow, progressive loss of neuronal function. The etiologies of chronic neurodegenerative diseases are numerous (see Saxena and Caroni [1] for recent references), and many are still poorly understood. Chronic neurodegenerative diseases were once thought to be exclusively hereditary in nature. However, the discovery of acquired chronic conditions such as Creutzfeld–Jacob disease and bovine spongiform encephalopathy (mad cow disease) has dispelled that generalization. The second category includes acute conditions like stroke, traumatic brain injury, and ischemic hypoxia such as that seen in myocardial infarction and prolonged cardiac surgery. These were once thought to be simply injury-related conditions rather than neurodegenerative disorders, but subsequent research has shown that all of these conditions have components of progressive neurodegeneration that can occur over a period of weeks to months. Taken together, neurodegenerative diseases are one of the major causes of death today.

Pure Appl. Chem.* **84, 1543–1667 (2012). A collection of invited papers based on presentations at the 23rd International Conference on Heterocyclic Chemistry (ICHC-23), Glasgow, UK, 31 July–4 August 2011.
‡Corresponding author

A discussion of the plethora of past and present drug discovery strategies for the various chronic and acute neurodegenerative diseases is beyond the scope of this manuscript [2]. In the following pages, I will focus on two recent neurodegenerative drug discovery projects that were undertaken by scientists from Wyeth Research, Inc. during the time that I headed the Chemical and Screening Sciences Division of that company. The discussion will highlight our work in two neurodegenerative disease areas: Identification of the BACE1 inhibitor WAY-258131 as disease-modifying drugs for Alzheimer's disease, a chronic disorder, and the discovery of novel immunophilin ligand ILS-920 for the treatment of stroke, an acute neurodegenerative disease.

BACE1 INHIBITORS FOR ALZHEIMER'S DISEASE – WAY-258131

Alzheimer's disease is a chronic neurodegenerative disorder characterized by neuronal and synaptic loss, brain atrophy and slow, progressive dementia [3]. Figures from the 2010 World Alzheimer Report suggest that nearly 35 million patients suffer from Alzheimer's disease worldwide [4]. However, that number could grow significantly by the year 2050 and could exceed our ability to properly care for the victims. Currently available treatments (Fig. 1) target only the cognitive symptoms of Alzheimer's disease. These drugs do little to halt the progressive neurodegeneration. In addition, the neurotransmitter systems that these drugs target (acetylcholine and glutamate) are located on neurons in brain regions that are among the first to decline. This unfortunate situation results in a limited time in the disease progression during which the drugs are effective. Drug discovery efforts over the past 10 years have increasingly become focused on identifying what is considered to be the "golden fleece" of Alzheimer's research, a "disease-modifying" drug that will halt or reverse the progression of the disease.

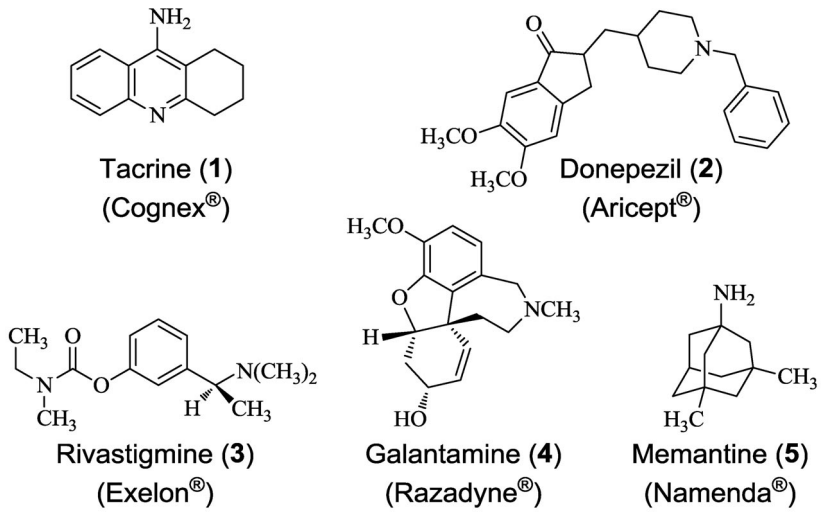


Fig. 1 Approved drugs for Alzheimer's disease.

The pathological hallmarks of Alzheimer's disease, and the means by which definitive diagnosis of the disease is still made, are extracellular plaques and intracellular neurofibrillary tangles. Plaques are composed of β-amyloid peptides, which are formed from the misprocessing of amyloid precursor protein (APP) [5]. Neurofibrillary tangles are derived from over-phosphorylated tau proteins. The causative relationship between these proteins and neurodegeneration has come to be known as the "amyloid cascade hypothesis" of Alzheimer's disease [6]. Recent evidence suggests that these amyloid peptides may not be cleared effectively from the brains of Alzheimer's patients [7]. There is currently

much debate over whether the plaques are beneficial or detrimental, but it is widely accepted that the β-amyloid peptides as well as the soluble oligomers of these peptides are themselves neurotoxic and can induce many of the neurodegenerative and cognitive symptoms of the disease in animal models long before actual plaque density increases [5].

The transmembrane protein APP is processed by a series of proteases (Fig. 2). In non-Alzheimer's individuals, the major pathway involves cleavage of APP by α-secretase to give soluble α-APPs and a smaller transmembrane product, which is further processed by γ-secretase (Fig. 2A). In Alzheimer's disease, the APP is processed at a different cleavage site by β-secretase converting enzyme-1 (BACE1) and the remaining transmembrane product is subsequently cleaved by γ-secretase to give a series of 39–43 amino acid-containing β-amyloid peptides (Fig. 2B). These β-amyloid peptides (Aβs), and in particular the 42 amino acid peptide $A\beta_{1\text{-}42}$, form oligomers that ultimately deposit as amyloid plaques. A significant amount of drug discovery effort has been applied over the past decade to inhibiting the formation of Aβs and their resulting plaques as a means of halting the progression of the disease process.

Inhibition or allosteric modulation of the two enzymes responsible for formation of Aβ peptides (γ-secretase and BACE1) have been pursued as drug targets for several years [8–10]. Efforts targeting γ-secretase ultimately led to the first competitive inhibitor to enter clinical trials, Eli Lilly's semagacestat [11]. However, development of semagacestat was recently discontinued following discouraging results from two Phase III IDENTITY clinical trials, where the compound failed to halt disease progression and, in fact, worsened cognition and the ability to perform activities of daily living [12]. The exact reasons for semagacestat's failure are not known, but the compound suffers from poor selectivity against Notch-1 processing, an off-target activity associated with many competitive γ-secretase inhibitors [13,14]. Clinical trials are currently under way on more selective γ-secretase inhibitors, but semagacestat's disappointing results have led many to question γ-secretase as a viable Alzheimer's drug discovery target.

An alternative target for reducing APP misprocessing, and the subject of this manuscript, is inhibition of the other catabolic enzyme BACE1. Unlike γ-secretase, which is a complex composed of four subunits, BACE1 is a single protein that readily undergoes crystallization and X-ray crystallographic analysis, as do co-crystals with inhibitors. Structural information shows that the active site contains two aspartate residues that can be exploited for drug design (Fig. 3). There is also a FLAP region that offers the potential for additional binding interactions. These structural features, coupled with the ability to use rational design techniques, make BACE1 an attractive drug discovery target.

Most non-peptidic BACE1 inhibitors (Fig. 4) fall into two general classes [15]. The first class is the transition-state inhibitors, which often contain a hydroxyethylamine group that mimics the transition state of the cleavage site on APP. Many of these compounds also contain sulfonamide or sulfonyl groups that interact with the FLAP region and enhance potency. A second class of compounds is the guanidines. One of the first examples of this class (compound **9**) came out of Wyeth [16], and a number of other groups have since disclosed guanidine-based BACE1 inhibitors. In fact, one such compound, LY-2811376 (**11**), advanced to clinical trials where it established proof of concept in humans by lowering β-amyloid levels in cerebral spinal fluid, although it was ultimately dropped from development over toxicology concerns.

Imidazopyrimidine **12** (Fig. 5) was identified through a high-throughput screen of Wyeth's internal compound library using a FRET-based assay (fluorescence resonance energy transfer) [16,17]. The molecule showed relatively weak potency for the BACE family in both biochemical FRET and cellular ELISA (enzyme-linked immunosorbant assay) assays but was reasonably selective for BACE over cathepsin-D, an aspartyl protease that shares similar structural requirements with BACE1 for ligands [18]. The compound displayed relatively low bioavailability and brain partitioning following a 3 mg/kg oral dose. X-ray crystallographic analysis of the compound in complex with BACE1 revealed a number of structural aspects of the compound's binding mode. The guanidinyl moiety formed hydrogen bonds with both active site aspartates. No interactions with the FLAP region of BACE1 were evident, and the 6-membered ring of the imidazopyrimidine did not appear to make any relevant interactions

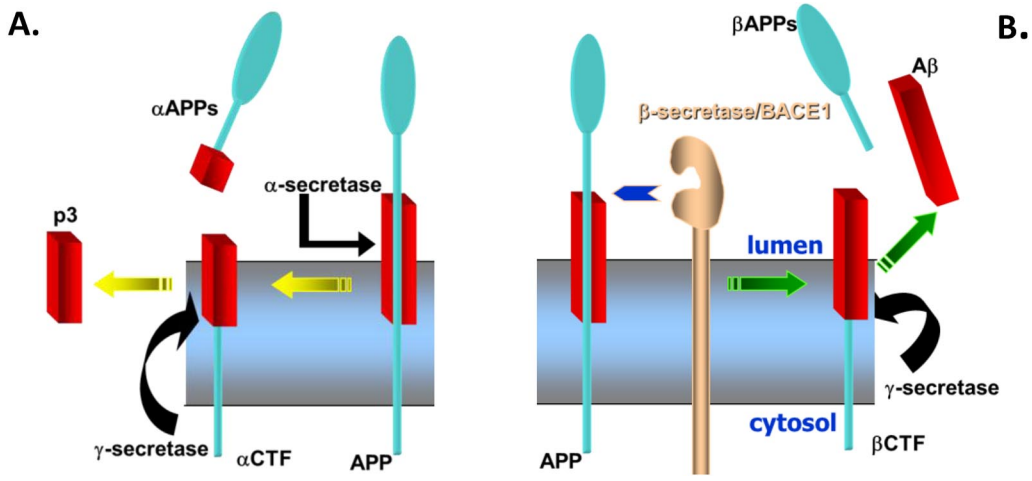


Fig. 2 (A) Processing of APP in non-Alzheimer's individuals; (B) Processing of APP in Alzheimer's individuals.

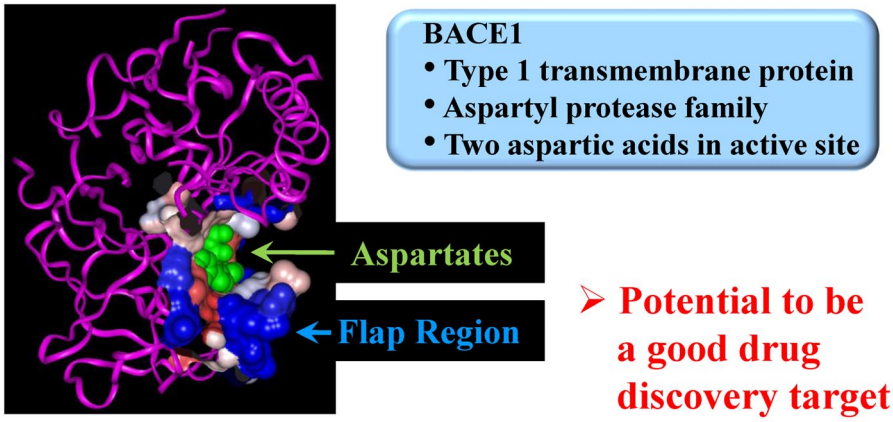


Fig. 3 BACE1 as a potential drug discovery target.

with the binding pocket. It appeared that additional interactions with the S2' and S3 regions of BACE1 might be established through substitution on the two phenyl rings.

One of the primary goals of the project was to enhance potency for BACE1 and selectivity for BACE1 over BACE2 (Fig. 6). Our strategy was to synthesize and test racemates of target compounds and then resolve any promising racemic mixtures into enantiomers. The structure–activity relationship (SAR) of the two phenyl rings initially led to a 10-fold improvement in BACE inhibitory potency by installing the 4-trifluoromethoxy group on the phenyl ring adjacent to the S2' pocket to give compound **13** [16]. Analysis of the X-ray co-crystal structure suggested that placing an aryl group possessing hydrogen bond acceptors in the meta position of the other phenyl ring might establish significant interactions within the S3 pocket. SAR along these lines led to the identification of compound **14**, which was 100-fold more potent than the parent molecule at inhibiting BACE1 and now demonstrated over 30-fold selectivity for BACE1 over BACE2. An X-ray co-crystal structure revealed that the original rational design hypotheses had been correct, with the 4-trifluoromethoxy group on the one phenyl ring interacting with residues in the S2' pocket of BACE1 and the nitrogen atoms of the pyrimidinyl group on the other phenyl ring forming hydrogen bonds with residues in S3.

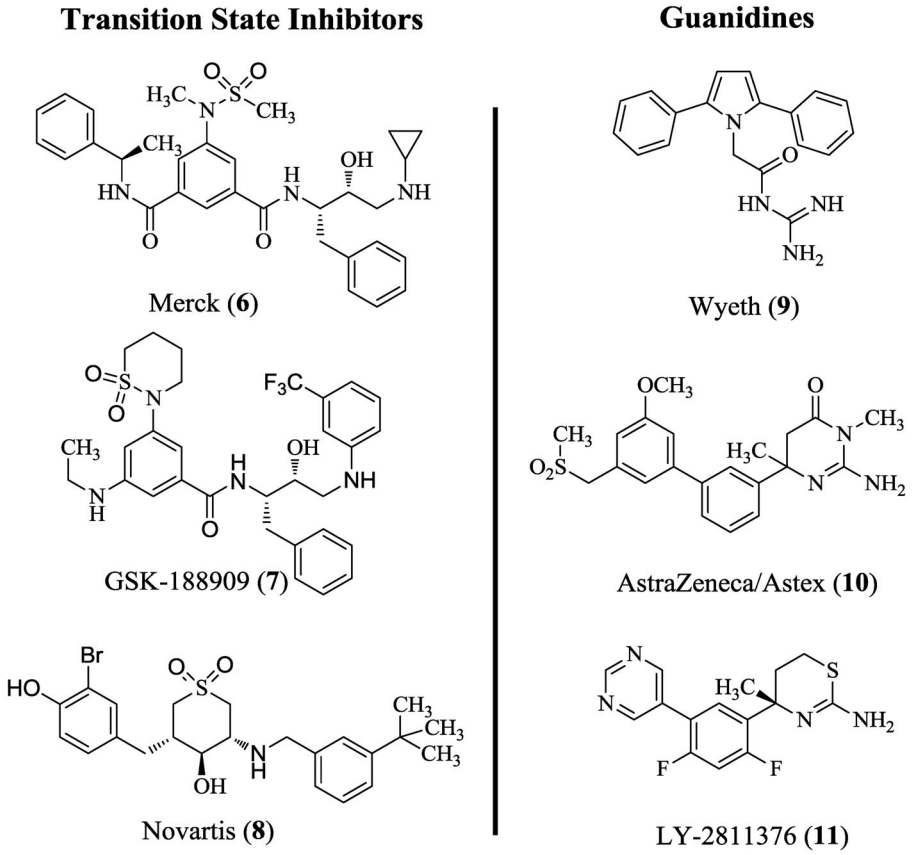
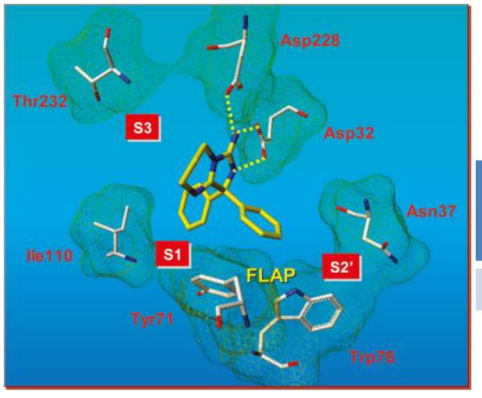
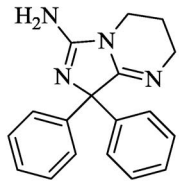


Fig. 4 Examples of two major classes of BACE1 inhibitors.





(12)

BACE1 (IC_{50}, uM)	BACE2 (IC_{50}, uM)	Cath-D (IC_{50}, uM)	ELISA (EC_{50}, uM)
38	38	> 200	27

PK: Cmax = 300 nM (3 mg/kg po)
Brain/Plasma = 0.2

Fig. 5 HTS Hit (**12**): Data and binding interactions with BACE1 (X-ray co-crystal structure reproduced with permission from the *Journal of Medicinal Chemistry* [19]).

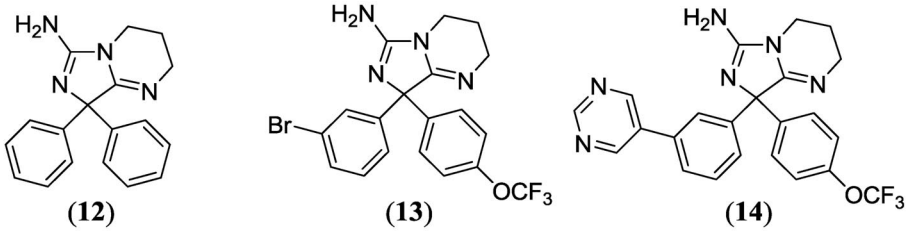


Fig. 6 Interactions with S2' and S3 pockets enhances BACE1 potency and selectivity.

Other weaknesses of the initial hit **12** that required improvement included poor aqueous solubility and a less than optimal pharmacokinetic profile, especially brain penetration. We felt that both of these parameters could be improved by eliminating the lipophilic 6-membered ring of the imidazopyrimidine, which did not contribute significantly to BACE1 binding in the first place. Truncation of **12** gave aminohydantoin analog **15** (Fig. 7), which was 10-fold more potent at inhibiting BACE1 than the parent imidazopyrimidine and retained good selectivity for BACE1 over cathepsin-D [19]. This initial analog was somewhat more selective for BACE2, but previous SAR studies on the imidazopyrimidine homologs suggested that this undesirable selectivity issue might be improved through further structural elaboration. Gratifyingly, aminohydantoin **15** demonstrated better bioavailability and brain penetration than the imidazopyrimidine **12** as well as a 2-fold improvement in aqueous solubility.

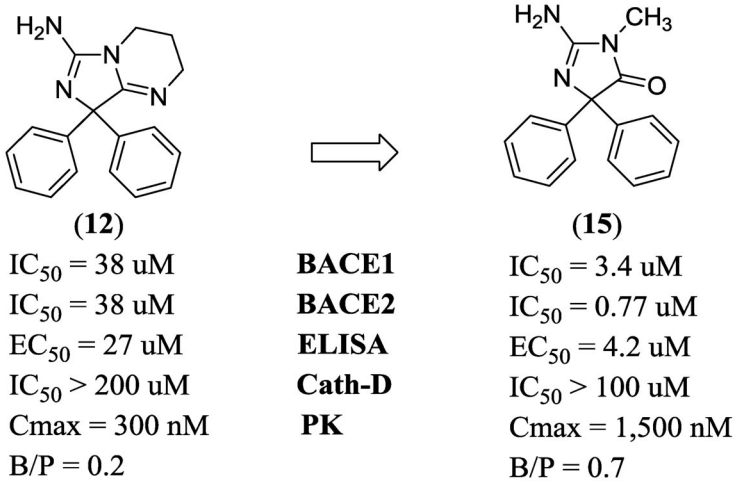
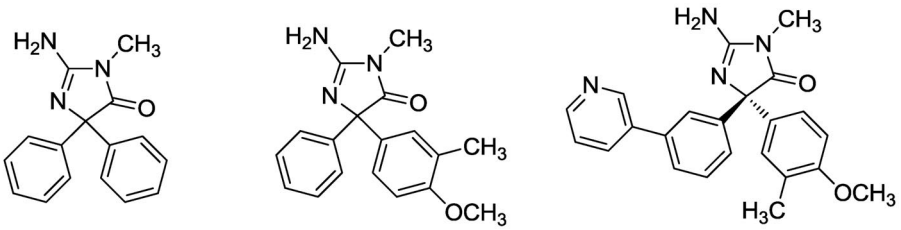


Fig. 7 Data for compounds **12** and **15**.

Efforts within the aminohydantoin series [19] were then focused on the phenyl rings (Fig. 8). SAR of the phenyl proximal to the S2' pocket identified the 3-methyl-4-methoxy substitution pattern as optimal (compound **16**). The 3,4-disubstitution pattern did not increase BACE1 potency relative to substitution in the 4-position only, but did improve BACE1/BACE2 selectivity slightly. Similar to the imidazopyrimidine scaffold, a nitrogen-containing heteroaryl ring in the 3-position of the other phenyl ring (proximal to the S3 pocket) provided the most potent compounds such as compound **17** (Fig. 8),



(15)

BACE1 - IC_{50} = 3.4 uM
BACE2 - IC_{50} = 0.77 uM
Cath-D - EC_{50} > 100 uM

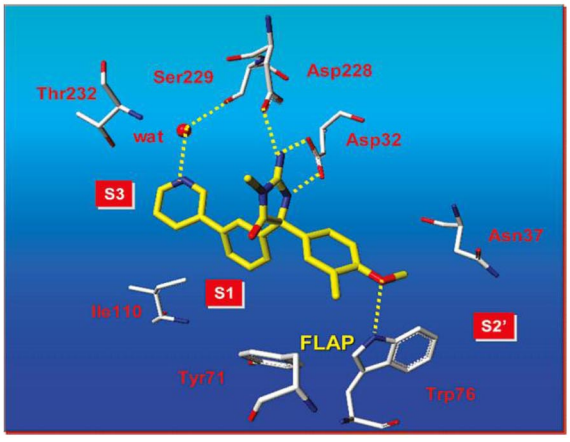
(16)

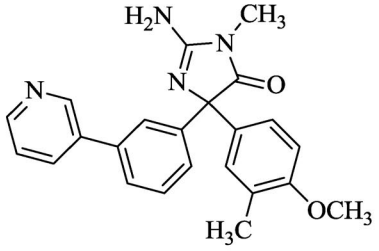
IC_{50} = 3.1 uM
IC_{50} = 1.7 uM
EC_{50} = >100 uM

(17)

IC_{50} = 0.04 uM
IC_{50} = 0.78 uM
EC_{50} = 12.5 uM

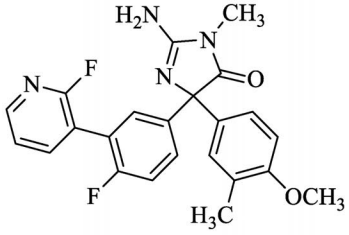
Fig. 8 Evolution of SAR on aminohydantoins leading to WAY-258131.





(17)

Fig. 9 X-ray co-crystal structure of compound **17** and BACE1 (X-ray co-crystal structure reproduced with permission from the *Journal of Medicinal Chemistry* [19]).



Compound **18**

Cd.	BACE1 (IC_{50}, uM)	BACE2 (IC_{50}, uM)	Cath-D (IC_{50}, uM)	ELISA (EC_{50}, uM)
18	0.04	1.84	2.41	0.06
(S)-18	0.01	0.81	0.82	0.02
(R)-18	0.56	1.64	2.71	1.2

Fig. 10 Data for racemic compound **18** and its enantiomers.

although some activity for cathepsin-D was now seen (IC_{50} ~ 2–25 μM). An X-ray co-crystal structure of **17** revealed the expected hydrogen bonding interactions between the 4-methoxy group and the S2' pocket as well as some additional lipophilic interactions with the 3-methyl moiety. Surprisingly, it showed that the binding interactions in the S3 pocket for compound **17** were different from that seen

with compound **14**, with the pyridine forming an interaction with Ser 229 through a bridging water molecule (Fig. 9).

The potency and selectivity of compound **17** were acceptable for advancement to in vivo studies, but the compound suffered from poor metabolic stability. Introduction of fluorine atoms in the ortho-positions of the phenyl and pyridine rings (compound **18**, Fig. 10) did not significantly affect BACE1 potency, but did enhance BACE1/BACE2 selectivity and, more importantly, improved metabolic stability and selectivity for cathepsin-D. The promising data obtained with racemic compound **18** encouraged us to examine the effect of chirality. As expected, the (*S*)-enantiomer of **18**, which was expected to place the two phenyl rings in the appropriate confirmation relative to their respective binding pockets on BACE1, was shown to be significantly more potent than the (*R*)-enantiomer and was the most potent BACE1 inhibitor of the series (Fig. 10). It possessed adequate selectivity for BACE1 vs. BACE2 (81-fold) and cathepsin-D (82-fold). Compound (*S*)-**18** (WAY-258131) was chosen for assessment in vivo.

The synthesis of (*S*)-**18** is shown in Fig. 11 [16]. The required diketone intermediate **19** was prepared in two steps by treatment of the triphenylphosphonium ylide of 3-methyl-4-methoxy benzyl chloride with 3-bromo-4-fluorobenzoyl chloride, followed by conversion of the resulting intermediate to the diketone through treatment with potassium permanganate. Condensation of **19** with methylguanidine under basic conditions yielded the penultimate intermediate **20**, which was converted through a Suzuki coupling reaction to the racemic mixture containing WAY-258131. The desired (*S*)-enantiomer **18** was obtained by reversed-phase HPLC on a Chiralpak® AD column.

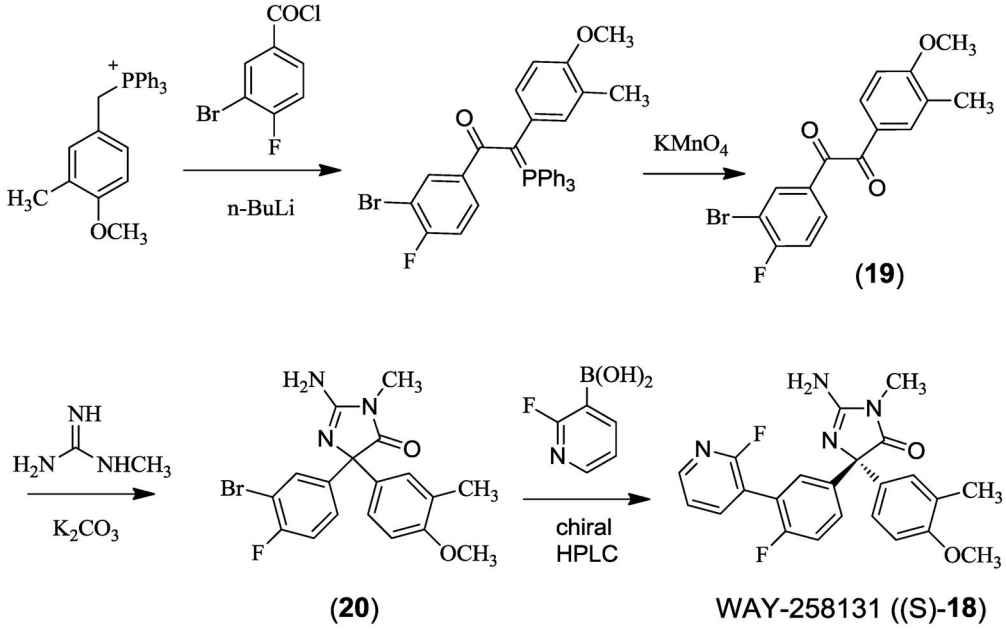


Fig. 11 Synthesis of WAY-258131 ((*S*)-**18**).

The in vivo effects of **18** on amyloid processing were assessed in transgenic Tg2576 mice (Fig. 12) [20]. The Tg2576 mouse expresses APP that contains the human Swedish mutation. The result is that these animals display an amyloidotic phenotype that is similar to that seen in Alzheimer's patients, including increased production of the neurotoxic $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ peptides and increased plaque load. Subchronic oral treatment with **18** twice daily for either 7 or 14 days reduced plasma lev-

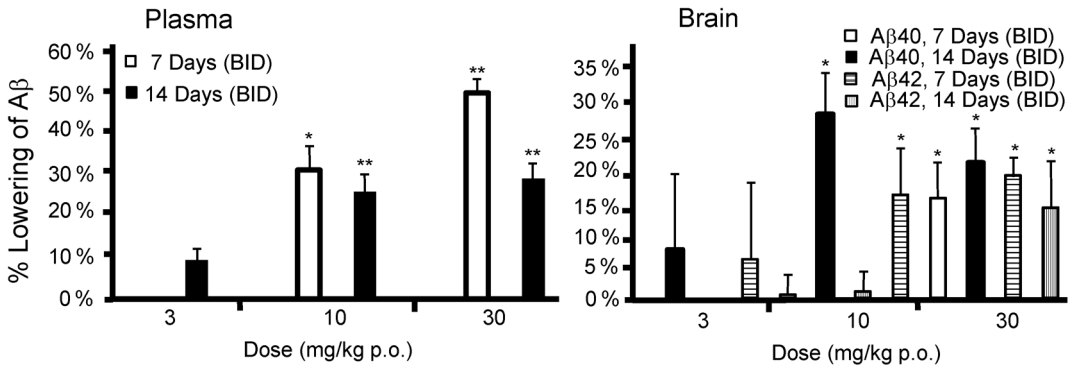


Fig. 12 WAY-258131 ((S)-**18**) reduces amyloidotic phenotype in Tg2576 mice. $*p < 0.01$; $**p < 0.001$ vs. vehicle.

els of β-amyloid peptides by 30–50 %. Changes in brain levels of β-amyloid peptides were variable at the 10 mg/kg dose. However, the 30 mg/kg dose significantly lowered brain levels of both $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ by 15–25 % at both 7 and 14 days. Plaque load in the cortex and hippocampus was reduced by approximately 30 % (data not shown). Additionally, acute administration of WAY-258131 (10–100 mg/kg po) dose-dependently reversed the deficits in contextual memory seen in Tg2576 mice [21] in a contextual fear-conditioning paradigm (data not shown).

IMMUNOPHILINS FOR STROKE – ILS-920

Recent figures suggest that nearly 5.7 million victims world-wide die from stroke or stroke-related conditions annually [22]. Despite these unfortunate figures, many stroke victims survive the acute event only to suffer long-term disabilities. The only registered treatment for stroke is the anti-thrombotic agent human recombinant tissue plasminogen activator. However, the mechanism of action and short window during which it can be administered following a stroke and risks associated with that agent have limited its use [23,24]. The progress in understanding the processes that occur after a stroke has been impressive. Unfortunately, almost no effective drug candidates have been identified in the wake of that understanding. Over 1000 drug candidates, mostly neuroprotective agents, have failed in clinical trials [25]. This disappointing record has led many researchers in the field to question the viability of neuroprotection as a therapeutic target for treating stroke and to explore the possibilities of neuroregeneration, stimulating the establishment of new neuronal connections in place of the ones lost to the stroke.

But can neuroregeneration be stimulated in adult human brains? This question has been explored and debated in the literature for several years [26–28]. Neurogenesis and migration is a routine event in rodents. Caches of neuronal progenitor cells exist in the subgranular zone of the dentate gyrus and in the zones surrounding the ventricles. These progenitors migrate along the rostral migratory stream to the olfactory bulb, where they become incorporated and mature into functional neurons. These progenitor cells have also been shown to migrate to the infarct area in animal stroke models, where they play a role in re-establishing neuronal connections within the lesion. Furthermore, several pharmacological agents have demonstrated an ability to stimulate neuroregenerative activity in animal models of stroke [29]. However, human brains are not rodent brains. Neuronal progenitors are limited to the lateral ventricles, and neurogenesis is more tightly controlled in humans than it is in rodents. Strong evidence for human neuroregeneration is lacking, although at least one clinical study suggests that it may occur following stroke [28].

The story of ILS-920 (**24**) begins with rapamycin (**21**) (Fig. 13), an immunophilin discovered in soil samples from Easter Island [30]. Rapamycin exerts its immunosuppressant effects by forming a tertiary complex with FK-506 binding protein 12 (FKBP12) and mammalian target of rapamycin (mTOR)

[31]. mTOR is a kinase that regulates a number of cellular processes involved with cell growth and survival, protein synthesis, and transcription. mTOR forms at least two complexes (mTORC1 and mTORC2), which have different functions [32,33]. X-ray crystallographic analysis has revealed which portion of rapamycin binds to FKBP12 and which region interacts with mTOR (Fig. 13) [34].

At the time the project began, it was known that certain macrocyclic immunophilins like FK-506, meridamycin, and normeridamycin displayed neuroprotective activity in animal models of stroke [35,36]. More interestingly, the compounds stimulated neuronal outgrowth in culture. The mechanism by which these activities occurred was not known. Wyeth was in the process of exploring the therapeutic potential of rapamycin and related imminophilins by analoging the scaffold. Some changes to the rapamycin scaffold were not tolerated. Modification to the C-42 hydroxyl group (Fig. 13) produced compounds (including the anticancer drug Torisel®) that enhanced neurofilament expression and stimulated neuronal outgrowth in culture [37]. However, these derivatives were still immunosuppressive, an unacceptable off-target effect for a stroke drug. A breakthrough came when it was discovered that the poly-ene portion of rapamycin (part of the region thought to interact with mTOR) would react with dienophiles to give Diels–Alder adducts.

Since the exact mechanism by which these new compounds acted was not known at the time, in vitro functional assays were used as the primary screens. One of the first lead molecules to be examined was WAY-124,466 (**22**) [38]. This compound promoted cortical neuronal survival in culture [39] with moderate potency and was known to be devoid of in vitro immunosuppressive activity at concentrations up to 3 μM [40]. However, the physical and PK properties of WAY-124,466 were not optimal. A breakthrough was realized when the Diels–Alder adducts of rapamycin with nitrosobenzenes were prepared (Table 1). Treatment of rapamycin with nitrosobenzene provided WYE-592 (**23**), which was roughly twice as potent at promoting neuronal survival as rapamycin [38] but showed some immunosuppressive activity in vitro [41]. Substitution on the dienophile-derived phenyl ring did not significantly improve neuroprotective activity, with the exception of the 2,6-dichloro substitution pattern (**25**), which increased neuroprotective potency by 15-fold.

It was later determined that the immunosuppressive activity seen with **23** was the result of the compound partially (3–5 %) undergoing a retro-Diels–Alder reaction to give rapamycin. This retro reaction could be eliminated by reducing the double bond in the oxazenene ring. Interestingly, when the reduction was performed on analogs substituted in the 2- and 6-position of the phenyl ring, the resulting oxazenanes were significantly less potent in the neuronal survival assay (compound **26**). However, when the unsubstituted WAY-592 (**23**) was hydrogenated, the resulting oxazenane (**24**) was more potent than the parent molecule or rapamycin at promoting neuronal survival in vitro. The compound also promoted neurite outgrowth in cultured rat cortical neurons (EC_{50} = 0.54 μM) and showed no immunosuppressive activity at concentrations up to 10 μM. Compound **24** (ILS-920) and was selected for in vivo assessment.

The synthesis of **24** is straightforward and has been presented elsewhere [38,42]. Treatment of **21** with nitrosobenzene in acetone for 24 h followed by catalytic hydrogenation over palladium on carbon afforded **24** in 26 % overall yield.

ILS-920 was examined for its neuroprotective effects in a transient middle cerebral artery occlusion model. When administered as an intravenous bolus starting 4 h post-occlusion, **24** significantly reduced infarct volume by 23–24 % (Fig. 14A) [43]. By contrast, rapamycin was reported to be devoid of efficacy in a similar model [44]. **24** also promoted neuronal survival in culture (Fig. 14B) and stimulated neurite outgrowth (Fig. 14C). In a permanent cerebral artery occlusion model, **24** produced long-lasting improvements in neurological outcome relative to treatment with placebo, as assessed using a sensorimotor-based neurological deficits rating scale (data not shown) [45]. This combination of both neuroprotective and neuroregenerative activities without immunosuppressive effects led to the advancement of **24** to clinical trials for stroke. Studies into the mechanism of action of **24** [43] revealed that the compound inhibits L-type calcium currents, which may contribute to its neuroprotective effects. The ability to stimulate neuronal survival and neurite outgrowth are likely due to the compounds binding,

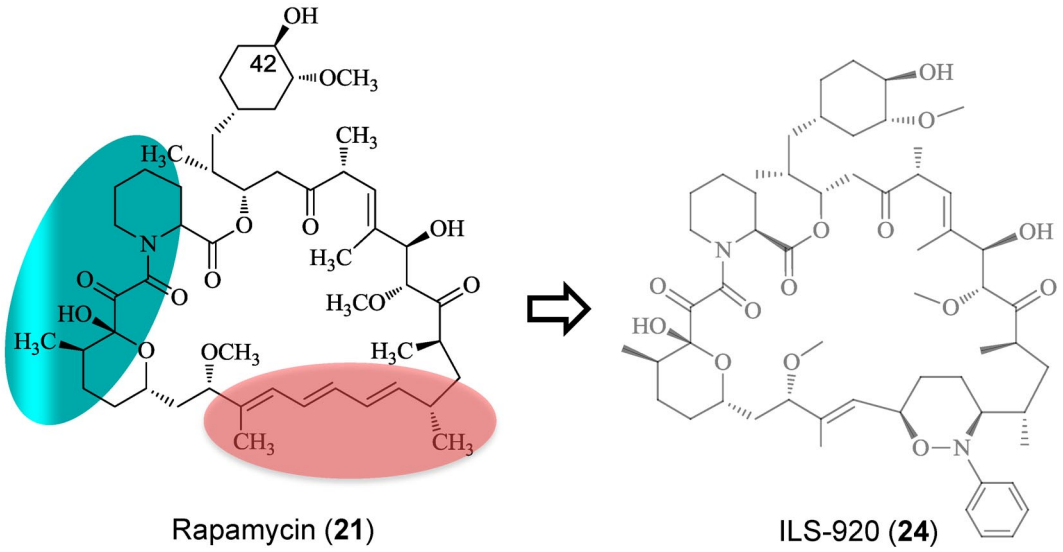
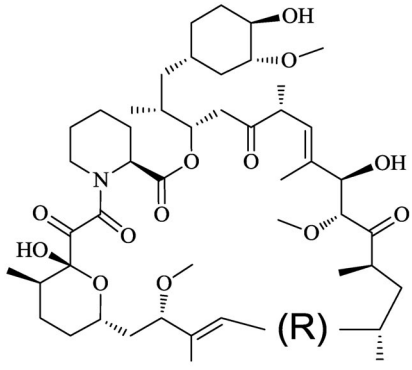


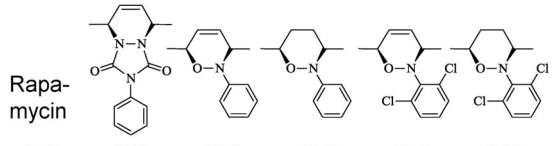
Fig. 13 Structures of rapamycin (**21**) and ILS-920 (**24**). Blue = FKBP12 binding domain; red = mTOR binding domain.

not to FKBP12, but to FKBP52, another immunophilin binding protein that has been associated with neurotrophic activity [46]. This selectivity would also explain the absence of immunosuppressant effects.

Table 1 Data for rapamycin (**21**), ILS-920s (**24**), and selected Diels–Alder adducts. NR = not reported.



(R):



	(21)	(22)	(23)	(24)	(25)	(26)
Cortical Neuron Survival EC_{50}, uM	**1.3**	0.85	0.69	0.15	0.085	3.7
T-Cell Proliferation IC_{50}, uM	0.005	> 3	0.15	> 10	NR	NR

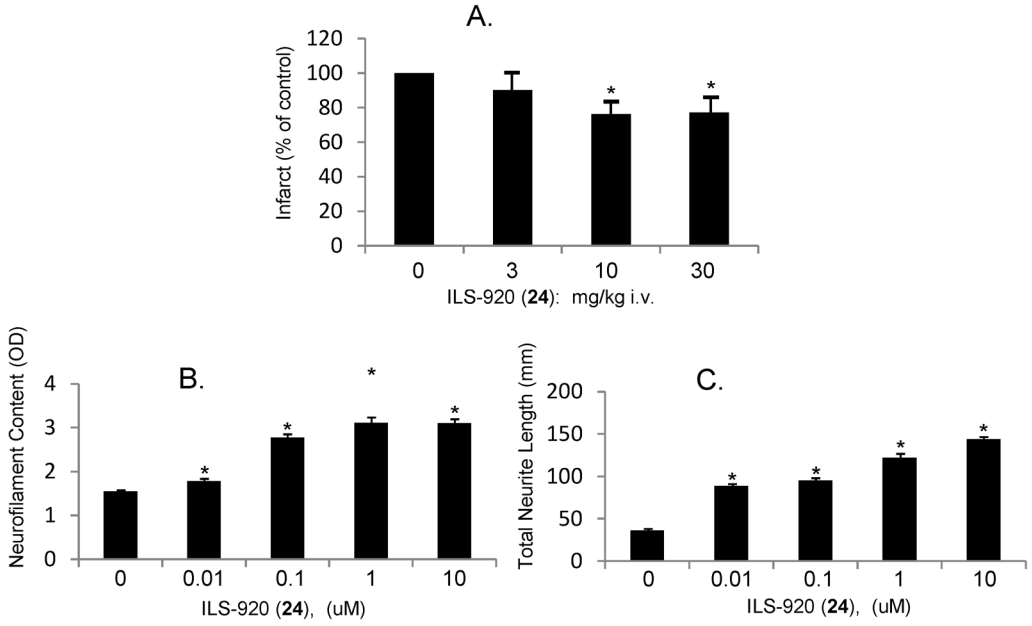


Fig. 14 Data for ILS-920 (**24**): (A) Reduction of infarct volume in transient middle cerebral artery occlusion model; (B) Effect of **24** on neuronal survival as measured by neurofilament content; (C) Effect of **24** on neuronal outgrowth. *$p < 0.05$.

CONCLUSION

Identifying effective drugs for treating neurodegenerative disorders, both chronic and acute, has been an epic struggle for drug discovery scientists and a significant investment for the government, academic, and industrial organizations that have supported this struggle. The stories that were presented above barely scratch the surface of the tremendous amount of data that has been generated in the field. And while the successes have been relatively few, the struggle must and does continue, with new insights and new potential drug discovery targets being disclosed every year. As the average human lifespan continues to increase, so does the incidence of neurodegenerative diseases. We must remain committed to the goal of discovering new drugs to treat neurodegenerative disorders for the sake of patients, care givers, and society in general.

ACKNOWLEDGMENTS

The authors thank the *Journal of Medicinal Chemistry* for their kind permission to reproduce the X-ray co-crystal structure graphics in Figs. 5 and 9.

REFERENCES AND NOTES

1. S. Saxena, P. Caroni. *Neuron* **71**, 35 (2011).
2. For a recent review, see: N. Herrmann, S. A. Chau, I. Kircanski, K. L. Lanctot. *Drugs* **71**, 2031 (2011).
3. X.-P. Wang, H.-L. Ding. *Neurosci. Bull*. **24**, 105 (2008).
4. A. Wimo, M. Prince. *World Alzheimer Report 2010*, Alzheimer's Disease International, available online at: <http://www.alz.co.uk/research/files/WorldAlzheimerReport2010.pdf>.

5. G. M. Shanker, D. M. Walsh. *Mol. Neurodegen*. (2009). Available online at: <http://www.molecularneurodegeneration.com>.
6. For a recent review, see: A. D. Korczyn. *Alzheimer's Dementia* **4**, 176 (2008).
7. K. G. Mawuenyega, W. Sigurnson, V. Ovod, L. Munsell, T. Kasten, J. C. Morris, K. E. Yarasheski, R. J. Bateman. *Science* **330**, 1774 (2010).
8. N. Marks, M. J. Berg. *Neurochem. Res*. **35**, 181 (2010).
9. A. F. Kreft, R. Martone, A. Porte. *J. Med. Chem*. **52**, 6169 (2009).
10. W.-H. Huang, R. Sheng, Y.-A. Hu. *Curr. Med. Chem*. **16**, 1806 (2009).
11. B. P. Imbimbo, I. Peretto. *Curr. Opin. Invest. Drugs* **10**, 721 (2009).
12. Eli Lilly Press Release, 17 August 2010, accessed on 14 October 2011 at: <http://newsroom.lilly.com/releasedetail.cfm?ReleaseID=499794>.
13. S. C. Mayer, A. F. Kreft, B. Harrison, M. Abou-Gharbia, M. Antane, S. Aschmies, K. Atchison, M. Chlenov, D. C. Cole, T. Comery, G. Diamantidis, J. Ellingboe, K. Fan, R. Galante, C. Gonzales, D. M. Ho, M. E. Hoke, Y. Hu, D. Huryn, U. Jain, M. Jin, K. Kremer, D. Kubrak, M. Lin, P. Lu, R. Magolda, R. Martone, W. Moore, A. Oganesian, M. N. Pangalos, A. Porte, P. Reinhart, L. Resnick, D. R. Riddell, J. Sonnenberg-Reines, J. R. Stock, S.-C. Sun, E. Wagner, T. Wang, K. Woller, Z. Xu, M. M. Zaleska, J. Zeldis, M. Zhang, H. Zhou, J. S. Jacobsen. *J. Med. Chem*. **51**, 7348 (2008).
14. A. Hall, R. L. Elliot, G. M. P. Giblin, I. Hussain, J. Musgrave, A. Naylor, R. Sasse, B. Smith. *Bioorg. Med. Chem. Lett*. **20**, 1306 (2010).
15. W.-H. Huang, R. Sheng, H.-A. Hu. *Curr. Med. Chem*. **16**, 1806 (2009).
16. M. S. Malamas, J. Erdei, I. Gunawan, K. Barnes, M. Johnson, Y. Hui, J. Turner, Y. Hu, E. Wagner, K. Fan, A. Olland, J. Bard, A. J. Robichaud. *J. Med. Chem*. **52**, 6314 (2009).
17. P. Wu, L. Brand. *Anal. Biochem*. **218**, 1 (1994).
18. I. Schechter, E. Ziv. *Biol. Chem.* **389**, 313 (2008).
19. M. S. Malamas, J. Erdei, I. Gunawan, J. Turner, Y. Hu, E. Wagner, K. Fan, R. Chopra, A. Olland, J. Bard, S. Jacobsen, R. L. Magolda, M. Pangalos, A. J. Robichaud. *J. Med. Chem*. **53**, 1146 (2010).
20. K. Hsiao, P. Chapman, S. Nilson, C. Eckman, Y. Harigaya, S. Younkin, F. Yang, G. Cole. *Science* **274**, 99 (1996).
21. T. A. Comery, R. L. Martone, S. Aschmies, K. P. Atchison, G. Diamantidis, X. Gong, H. Zhou, A. F. Kreft, M. N. Pangalos, J. Sonnenberg-Reines, J. S. Jacobsen, K. L. Marquis. *J. Neurosci*. **25**, 8898 (2005).
22. World Health Organization. "STEPwise approach to stroke surveillance", accessed online on 14 October 2011 at: <http://www.who.int/chp/steps/stroke/en/index.html>.
23. H. P. Adams Jr., G. del Zoppo, J. J. Alberts, D. L. Bhatt, L. Brass, A. Furlan, R. L. Grubb, R. T. Higashida, E. C. Jauch, C. Kidwell, P. D. Lyden, L. B. Morgenstern, A. I. Qureshi, R. H. Rosenwasser, P. A. Scott, E. F. M. Wijdicks. *Stroke* **38**, 1655 (2007).
24. J. Kaur, Z. Zhao, G. M. Kline, E. H. Lao, A. M. Buchan. *J. Cereb. Blood Flow Metab*. **24**, 945 (2004).
25. V. E. O'Collins, M. R. Macleod, G. A. Donnan, L. L. Horky, B. H. van der Worp, D. W. Howells. *Ann. Neurol*. **59**, 467 (2006).
26. H. T. Ghashghaei, C. Lai, E. S. Anton. *Nat. Rev. Neurosci*. **8**, 141 (2007).
27. A. M. Enciu, M. I. Nicolescu, C. G. Manole, D. F. Muresanu, L. M. Popescu, B. O. Popescu. *BMC Neurol*. **11**, 75 (2011).
28. G. Pyne-Geithman. *Neurology* **74**, 352 (2010).
29. M. M. Zaleska, M. L. T. Mercado, J. Chavez, G. Z. Feuerstein, M. N. Pangalos, A. Wood. *Neuropharmacology* **56**, 329 (2009).

30. C. Vezina, A. Kudelski, S. N. Sehgal. *J. Antibiot.* **28**, 721 (1975).
31. S. N. Sehgal. *Clin. Biochem*. **31**, 335 (1998).
32. N. Hay, N. Sonenberg. *Genes Dev*. **18**, 1926 (2004).
33. S. Wullschleger, R. Loewith, M. N. Hall. *Cell* **124**, 471 (2006).
34. J. Choi, J. Chen, S. L. Schreiber, J. Clardy. *Science* **273**, 239 (1996).
35. W. E. Lyons, E. B. George, T. M. Dawson, J. P. Steiner, S. H. Snyder. *Proc. Nat. Acad. Sci. USA* **94**, 3191 (1994).
36. M. Y. Summers, M. Leighton, D. Liu, K. Pong, E. I. Graziani. *J. Antibiot*. **59**, 184 (2006).
37. D. Liu, B. McIlvain, M. Fennell, J. Dunlop, A. Wood, M. M. Zaleska, E. I. Graziani, K. Pong. *J. Neurosci. Meth.* **163**, 310 (2007).
38. E. Graziani. In *RSC Drug Discovery Series, #4. Accounts in Drug Discovery: Case Studies in Medicinal Chemistry*, J. C. Barrish, P. H. Carter, P. T. W. Cheng, R. Zohler (Eds.), pp. 316–330, RCS Publishing, London (2011).
39. Neuronal survival was determined by measuring neurofilament content in cultured rat cortical neurons using an ELISA assay.
40. T. D. Ocain, K. Longhi, R. J. Steffan, R. G. Caccese, S. N. Sehgal. *Biochem. Biophys. Res. Commun*. **192**, 1340 (1993).
41. In vitro immunosuppression was measured as the inhibition of IL-2-stimulated human CD4+ T cell proliferation.
42. M. Abou-Gharbia. *J. Med. Chem*. **52**, 2 (2009).
43. B. Ruan, K. Pong, F. Jow, M. Bowlby, R. A. Crozier, D. Liu, S. Liang, Y. Chen, M. L. Mercado, X. Feng, F. Bennett, D. von Schack, L. McDonald, M. M. Zaleska, A. Wood, P. H. Reinhart, R. L. Magolda, J. Skotnicki, M. N. Pangalos, F. E. Koehn, G. T. Carter, M. Abou-Gharbia, E. I. Graziani. *Proc. Natl. Acad. Sci. USA* **105**, 33 (2008).
44. J. Sharkey, S. P. Butcher. *Nature* **371**, 336 (1994).
45. Manuscript in preparation.
46. B. G. Gold, V. Densmore, W. Shou, M. M. Matzuk, H. S. Gordon. *J. Pharmacol. Exp. Ther*. **289**, 1202 (1999).

