Organic & Biomolecular Chemistry

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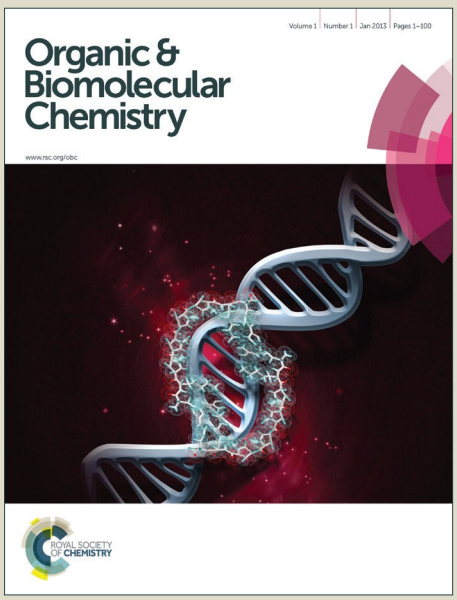

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REVIEW

Synthesis of amino heterocycle aspartyl protease inhibitors

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Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Aspartyl proteases are important pharmacological targets. Historically aspartyl proteases have been commonly targeted with transition state derived peptidomimetics. The strategy to develop aspartyl protease inhibitors has undertaken a dramatic paradigm shift in the last 10 years. The pharmaceutical industry in 2005 disclosed several scaffolds or "head groups" that prompted the field to move beyond peptidomimetic derived inhibitors. Since the discovery of the first amino heterocycle aspartyl protease inhibitor, the amino hydantoin, industry and academia have positioned themselves for a foothold on the new molecular space, designing a variety of related "head groups". Both the design and synthetic efforts involved in constructing these scaffolds are varied and complex. Here we highlight the synthetic strategies used to access these amino heterocycle scaffolds.

1. Introduction

Aspartyl proteases are important pharmacological targets. There are several aspartyl protease inhibitors that have entered the market place or have entered the clinical setting, treating diseases such as hypertension, Alzheimer's disease, and HIV. Aliskiren (Tekturna©) an inhibitor of the aspartyl protease renin, was a first in class drug that entered the market in 2007 for treatment of hypertension.[1] Aliskiren was developed from a transition state peptidomimetic strategy, and although the journey of drug to the marketplace was successful, it was a long exhaustive pathway to the clinic. The issues with undertaking a peptidomimetic approach to develop an inhibitor suitable for *in vivo* dosing, have been well documented,[2] and further exemplified by the low oral bioavailability of Aliskiren. Lessons learnt from the peptidomimetic approach to develop Aliskiren were implemented in the development of HIV aspartyl protease inhibitors. Ten of these inhibitors, such as Lopinavir and Ritonavir, are all in the marketplace. Although these drugs underpin the success of the mimetic approach, the development of drugs treating HIV and hypertension was long and exhaustive, due to difficulties with complex syntheses and oral bioavailability. In addition, some of these drugs have issues with cytochrome P450 inhibition, P-gp efflux and possessed poor blood brain membrane permeability, which was a major issue in developing a beta-secretase inhibitor for the treatment of Alzheimer's disease. This was well documented by Zhu.[3]

The strategy to develop aspartyl protease inhibitors has undertaken a dramatic paradigm shift in the last 10 years. The pharmaceutical industry identified several scaffolds or "head groups" that laid the foundation for the field to move beyond using peptidomimetic derived scaffolds. Key examples that initiated the new approach to develop non-peptidomimetic aspartyl protease inhibitors were disclosure of cyclic acyl guanidines by Schering-Plough,[4] Wyeth,[5] and AstraZeneca[6] as well as an acyclic acyl guanidine by Wyeth.[7] These examples ignited the pharmaceutical industry in the aspartyl protease inhibitor field into a flurry of activity. Each company positioned themselves for a foothold in the new molecular space. This activity resulted in the design of a plethora of novel amino

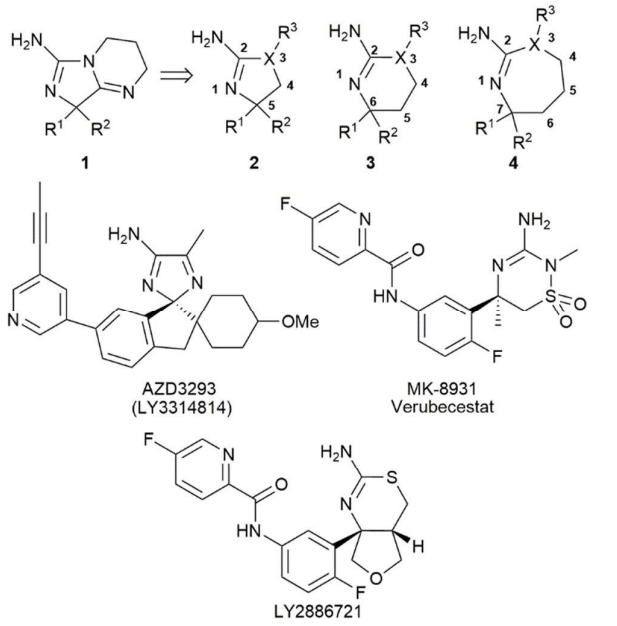

Figure 1. Nomenclature of the 5-, 6- and 7-membered 2-amino heterocycles presented in this review, and examples of this class that have entered into clinic trials.

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heterocycle scaffolds (Figure 1) targeting aspartyl proteases, particularly beta-secretase (BACE-1). Several of these amino heterocycle BACE-1 inhibitors, such as AZD-3292 (also known as LY3314814),[8] MK-8931 (or Verubecestat)[9] and LY2886721[10] (Figure 1) have now entered clinical trials for treatment of Alzheimer's disease.[11]

An overview of the development these amino heterocycle scaffolds targeting beta-secretase was undertaken by both Oehlrich *et al.*[12] and Ghosh *et al.*,[13] and demonstrates the immense effort and resources by both industry and academia to design novel scaffolds. The design of the new scaffolds by industry was not only to capture new molecular space, but as the research in this area evolved, it was clear that some of the scaffolds did not possess ideal physicochemical properties. One particular issue was the pKa of the basic amidine functionality that varied greatly and was dependent on atoms and functionality incorporated into the head group scaffold. The pKa of the head group was not only important for binding to the catalytic dyad, but for optimal pharmacokinetics and mediating off target activity, such as P-gp mediated efflux. The search for optimal physicochemical properties underpins the design of a variety of head groups described here and elsewhere.[12-14]

Several X-ray structures have provided insight into the binding mode of the amino heterocycle scaffold to aspartyl proteases. Figure 2 shows an example of the 2-amino heterocycle class (**21** in Scheme 5), bound to the open flap form of BACE-1.[5c] Note that the flap is in a relatively open conformation similar to that of published apo structures, which is consistent with a number of amino heterocycle aspartyl protease inhibitors and is a contrast to the more closed flap confirmation that is seen with substrate based peptidomimetics. The X-ray structure (Figure 2) shows that the amino functionality interacts with the acid groups of the two catalytic aspartates in the catalytic dyad, while the 2-N forms a hydrogen bond with the protonated carboxylic acid aspartate. The

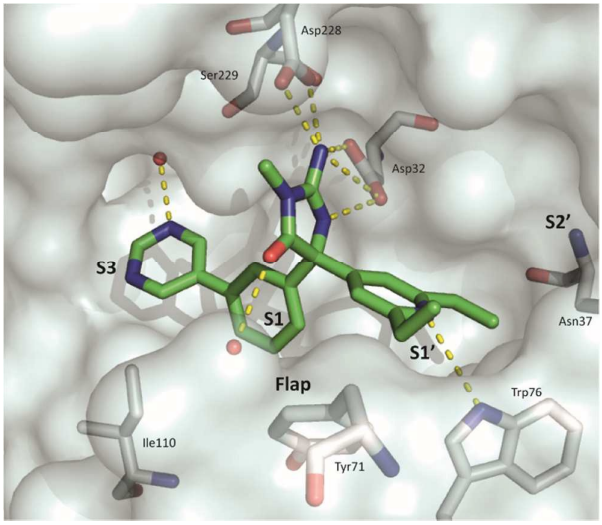


Figure 2. X-ray crystal structure (3IN4) showing the orientation of the 2-amino 1H-imidazol-5(4H)-one (green) (**21** pictured in Scheme 5) bound to the aspartyl protease, BACE-1 (grey).[5c]

3-N that is generally alkylated on all amino heterocycle inhibitors, faces out of the binding cleft, and takes no part in binding affinity. This has allowed the replacement of the 3-N with other atoms such as O, S, and C. The 3-C has been exploited to provide a site of differentiation compared to the 3-N, for example dialkyl substituents. Although the 3-position does not take part in any direct binding, attaching the appropriate substituents to this position has allowed an indirect route to access other pockets in the binding cleft of aspartyl proteases. The 3-position is also critically important in mediating the pKa of the amidine functionality that interacts with the aspartyl residues in the catalytic dyad.

Both the S1 and S1' pockets of the aspartyl proteases can be accessed from the 5-position of the 5-membered heterocycle **2**. This carbon is usually differentially disubstituted which allows one substituent to protrude in the S1 pocket and another into the S1' pocket. Given that this carbon is differentially disubstituted it creates a chiral centre, which adds to the synthetic complexity of this class of inhibitor. The group that is appended to the 5-C and enters the S1 pocket, also permits entry to the S3 pocket.

6- and 7- membered amino heterocycles, **3** and **4** respectively, bind in the same orientation to the 5- membered orthologues **2**. The open flap binding confirmation of this class is conducive to accommodating larger heterocycles, given the additional endocyclic atoms face solvent space. The additional endocyclic atoms in the 6- and 7- membered systems enables extra chemical diversity to be installed in these positions. Although installing functionality in these positions is directed into solvent space, these positions are used to mediate pKa of the amidine group that interacts with the catalytic aspartyl amino acids. Additionally these positions are also used to install chiral cyclic ring systems that conformationally constrain and rigidify the 6- and 7- membered systems. Given the flexibility of these scaffolds, the structural diversity within this class of inhibitor is large and varied.

One aspect that underpins this research are the synthetic strategies that were undertaken to construct these head groups, some with highly functionalised architecture. Here we describe a summary of the synthetic strategies undertaken to access the 2-amino heterocycle head groups that were used as scaffolds to target specific aspartyl proteases.

This review will focus on the synthesis of the 5-, 6- and 7-membered scaffolds or head groups (**2**, **3**, and **4** respectively) broadly depicted in Figure 1. In the literature this class of aspartyl protease inhibitor is referred to as the amino (or imino) hydantoin in the case of the 5-membered lactam system or the amino pyrimidone in the instance of the 6-membered lactam system. In this review, the IUPAC nomenclature convention will be used. For example, the amino hydantoin head group **2** will be referred to as the 2-amino 1H-imidazol-5(4H)-one.

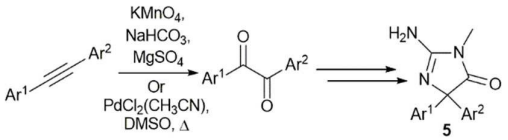
2. 5-Membered heterocycles

One of the first amino heterocycles, the 6-amino 2,3,4,8-tetrahydroimidazo[1,5-a]pyrimidine **1** (Figure 1), was identified from a high throughput screen of BACE-1.[5d, 15] Chemists quickly realised that truncating the tetrahydropyrimidine ring to produce the 2-amino 1H-imidazol-5(4H)-one **5**,[5b] resulted in improved BACE-

1 potency across a range of analogues.[5a, 5c, 16] The discovery of the 2-amino imidazol-5-(4H)-one head group **5** by Malamas *et al.* initiated the search for greater diversity among the amino heterocycle class.

2.1 2-Amino 1H-imidazol-5(4H)-one and surrogates

The pathway to access the imidazolone head group **5** has been predominantly through a benzilic acid rearrangement in the presence of a guanidine equivalent (Scheme 1). Entry to the diketone intermediate building block is depicted in Scheme 1 and 2. The most common method is starting from the alkyne using either $KMnO_4$[5c] or Wacker type conditions, employing catalytic palladium in the presence of DMSO or oxygen at high temperature (Scheme 1).[5a] High yields are achieved in both cases if a diaryl alkyne is used. The alkyne is easily accessed via the Sonogashira reaction. This route is compatible with a diverse array of functional handles that allow late stage diversification, for entry to analogues shown in Scheme 5.

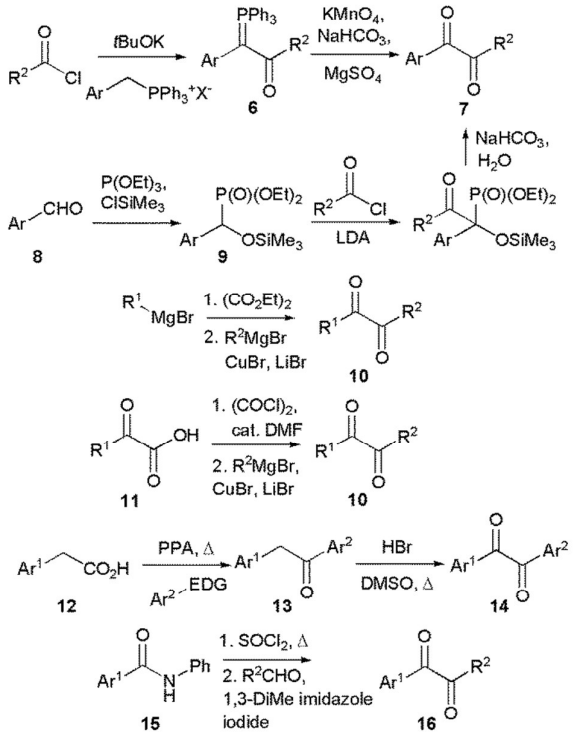


Scheme 1. Access to the 2-amino 1H-imidazol-5(4H)-one **5** via the diketone.

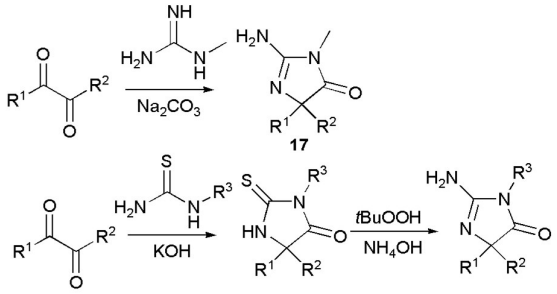
Alternative pathways to access the diketone are shown in Scheme 2. In one synthesis, Malamas *et al.* reacted an acid chloride with a benzyl phosphonium salt, to produce the ylide **6**. This was followed by oxidation of the ylide **6** to yield the diketone **7**.[5b] This route appeared more amenable to producing a diketone where one of the substituents is an alkyl group. Malamas *et al.* also employed a silyloxy ether **9**, which was formed from the aldehyde **8**. The anion of **9** was formed and reacted with an acid chloride and upon hydrolysis gave the diketone **7**.[5b] Other methods include reacting an activated dicarbonyl species, **11** or ethyl malonyl chloride with the appropriate equivalents of a carbon nucleophile, to yield the unsymmetrical diketones **10**.[5b] Starting from aryl carboxylic acids is an attractive option given the number that are commercially available. Zhou *et al.* used phenylacetic acid derivatives **12** in a Friedel-Crafts like alkylation, and the methylene product **13** subsequently oxidised to give the diaryl diketone **14**.[17] While, Cumming *et al.* used benzoic acids to form the phenyl amide **15**, which was activated to the chloro imidate, and on-reacted in an *N*-heterocyclic carbene catalyzed benzoin transformation to give the unsymmetrical diketone **16**. All these methods to access the diketone offer different advantages depending on the functionality on the diketone substituents.[18] These synthetic routes to synthesise the diketone are all geared towards ensuring diversity can be housed in the final amino imidazolone product.

The diketone is most commonly converted to the amino imidazolone **17** via reaction with the N-substituted guanidine in one step or with the N-substituted thiourea in two steps. The two step reaction is generally higher yielding than the one step procedure (Scheme 3). The N-3 substituent is then incorporated into the guanidine or thiourea. Installing the N-3 substituent post 2-amino imidazolone formation can be problematic with respect to regioselectivity.

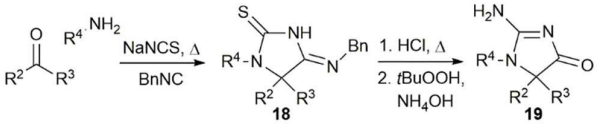
Substitution can be installed on the N-1 substituent regioselectively following the route depicted in Scheme 4. Zhou *et al.* used a Ugi multicomponent reaction starting from simple building blocks to synthesise the benzyl protected intermediate **18**. Hydrolysis of the benzyl imine and oxidative displacement of the thione gave the N-1 substituted product **19**.[4a] Analogues with the N-1 substitution were found to be less active against BACE-1 than the N-3 substituted



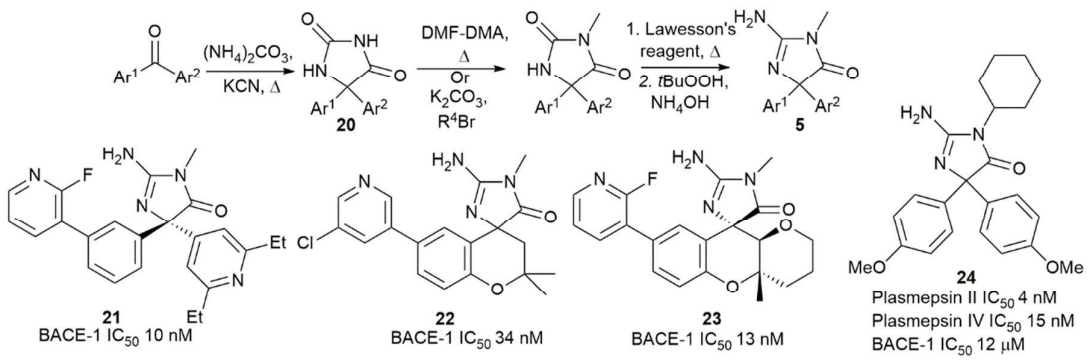
Scheme 2. Synthesis of the diketone precursor.



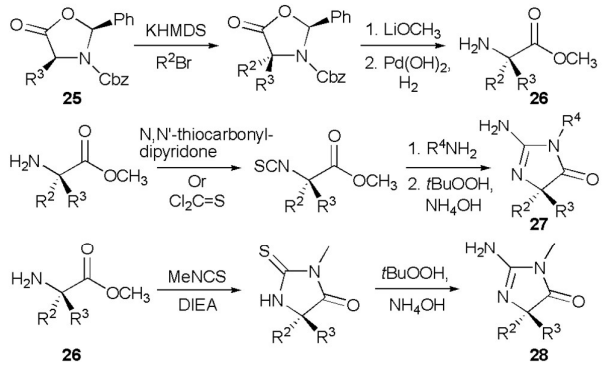
Scheme 3. Access to the amino imidazolone via the diketone.



Scheme 4. Synthesis of the N-1 substituted 2-amino imidazolone **19**.



Scheme 5. Alternate synthesis of the 2-amino imidazolone head group, and examples supporting the imidazolone head group.



Scheme 6. Synthesis of the chiral α,α-disubstituted amino ester **26**, en-route to the chiral 2-amino imidazolone.

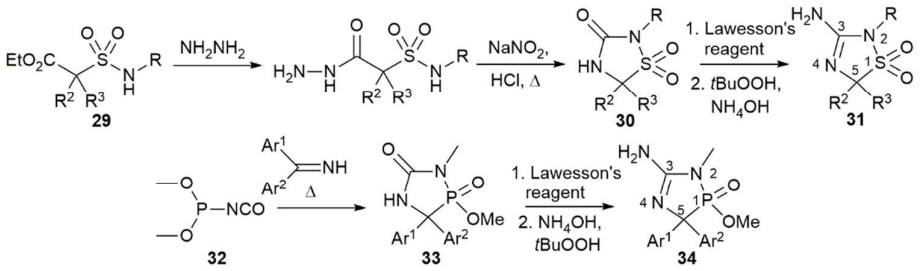
imidazolone analogues.[4a] This was due to the incorrect orientation of N-1 substitution for binding with the catalytic aspartates. The Bucherer–Bergs reaction can also be used to gain entry to the imidazolone scaffold **5** (Scheme 5).[18-19] Here, Cumming *et al*. used DMF-DMA to methylate the N-3 position of the hydantoin **20**, then selectively converted to the 2-thioamide, before conversion to the imidazolone **5**.[18] Alternatively, Hunt *et al.* was able to selectively alkylate the hydantoin at the N-3 position under basic conditions, before conversion to the imidazolone.[18]

These methods all give access to the 5-C disubstituted imidazolone, but only as a racemate. Chromatography with a chiral stationary phase has been used to separate enantiomers, but is inefficient due to recovery yields. Chiral synthesis of α,α-disubstituted amino acids enabled access to enantioselective synthesis of the 5,5-disubstituted imidazolone. The α,α-disubstituted amino acid can be prepared using "self-regeneration of stereocenters" methodology.[20] Here, the chiral oxazolidinone **25** is used to install the substitution to the opposite face of the hindered chirality directing substituent, usually with excellent dr. The chiral α,α-disubstituted amino acids **26** are then converted into the isothiocyanate, and subsequent ring closure is effected by addition of a substituted amine, to gain access to the amino imidazolone **27** post oxidative displacement of the thione.[4a] Alternatively, the chiral α,α-disubstituted amino acid **26** was reacted with isothiocyanate directly and subsequent oxidative displacement with ammonium hydroxide gives the imidazolone **28** (Scheme 6).[21] These procedures enabled the synthesis of potent inhibitors targeting BACE-1 **21**,[5c] **22**,[19] **23**,[22] and plasmepsin II and IV **24**[23] (Scheme 5).

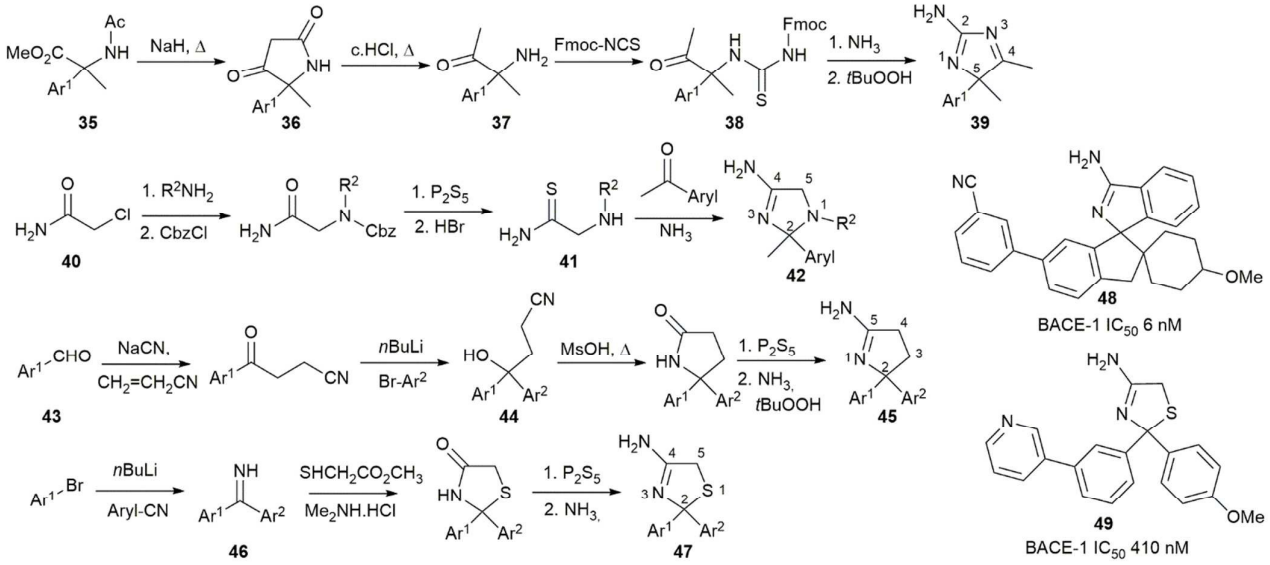
The pKa of the 2-amino-imidazolone was important for its interaction with the aspartates in the catalytic dyad and pharmacokinetics. To modulate the pKa, numerous other scaffolds were designed around the 2-amino-imidazolone scaffold. An obvious change was to install a sulfonyl or phosphonate group in place of the 4-carbonyl functionality on the imidazolone (Scheme 7). The synthesis of the 4-sulfonyl analogues started from the 2,2-substituted malonyl sulfonyl amide **29**. After formation of the hydrazide, a concomitant diazotisation and cyclisation yielded the 3-amino-2,5-dihydro-1,2,4-thiadiazole 1,1-dioxide **30**. This was then converted to the 2-amino imidazol-4-sulfone **31** via the 2-thione.[24] The 4-phosphonate analogue **34**, was generated by first using the disubstituted imine with isocyanate phosphane **32** in a cyclo addition reaction to form the cyclic diazaphospholidinone **33**, and in turn converted to the amino dihydro diazaphosphole **34** (Scheme 7).[24]

2.2 Amino hydro-imidazoles

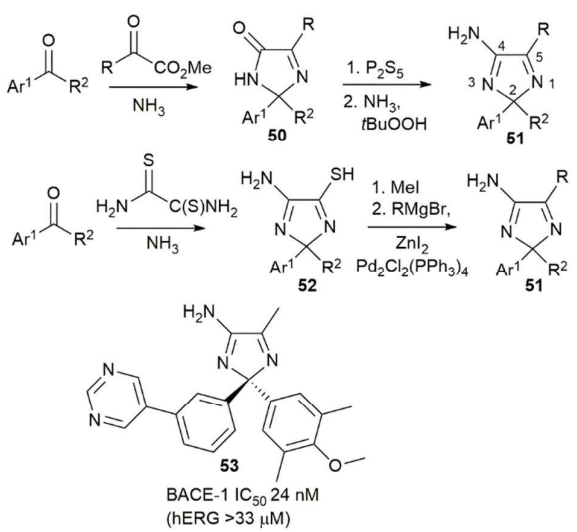
The pKa of the 2-amidine moiety on the imidazolone class of inhibitor was also altered by Ginman *et al.*, with the view to improve permeability and mitigate P-gp efflux and hERG inhibition. The research centered around exploring the removal of the 4-carbonyl group in the imidazolone scaffold in the heterocycles **39**, **42**, **45** and **47** (Scheme 8).[25] The 4-methyl-4H-imidazol-2-amine **39** was accessed from the *N*-acylated α,α-disubstituted amino ester **35**. This synthon **35** is cyclised to **36** using NaH and then the amide hydrolysed and decarboxylated to give the β-amino ketone **37**. Reaction of this intermediate with the protected isocyanate formed the thiourea **38** that was deprotected and cyclised in the one step to give the 2-thione dihydroimidazole, on route to the desired imidazole **39** (Scheme 8). The 2,5-dihydro-1H-imidazol-4-amine **42** was synthesised from the halo acetamide **40**, which was converted to the 2-amino thioacetamide **41**. This intermediate was then condensed with the ketone in the presence of ammonia to give the dihydroimidazole **42** (Scheme 8).[25]



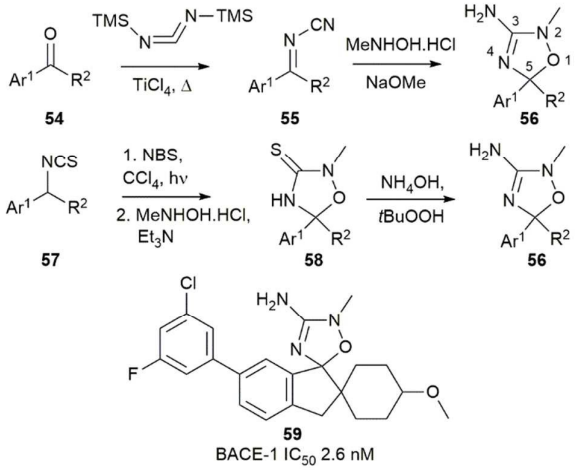
Scheme 7. Synthetic pathways to access the 3-amino-2,5-dihydro-1,2,4-thiadiazole 1,1-dioxide **31** and 3-amino-2,5-dihydro-2,4-diazaphospholidinone **34** scaffolds.



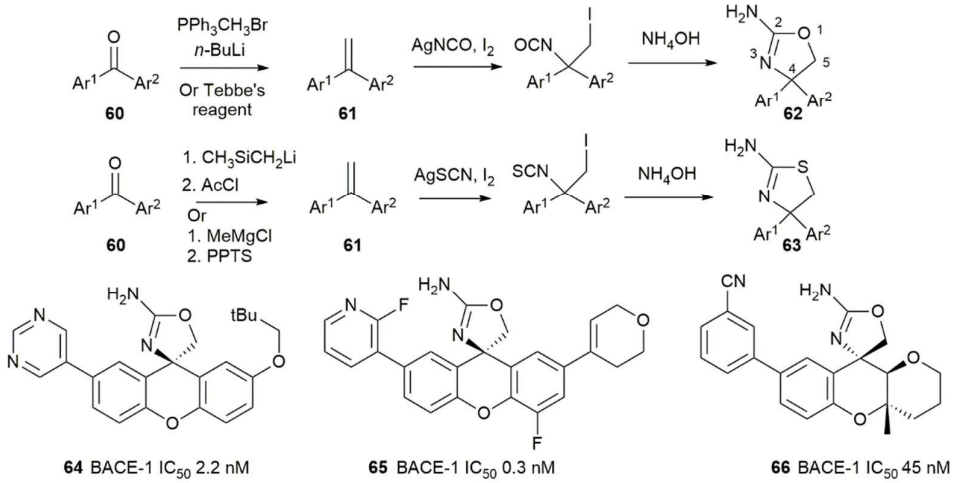
Scheme 8. Synthetic pathways to generate the 2-amino-4-methyl-4H-imidazole **39**, 4-amino-2,5-dihydro-1H-imidazole **42**, 5-amino-3,4-dihydro-2H-pyrrole **45**, and 4-amino-2,5-dihydrothiazole **47** head groups.



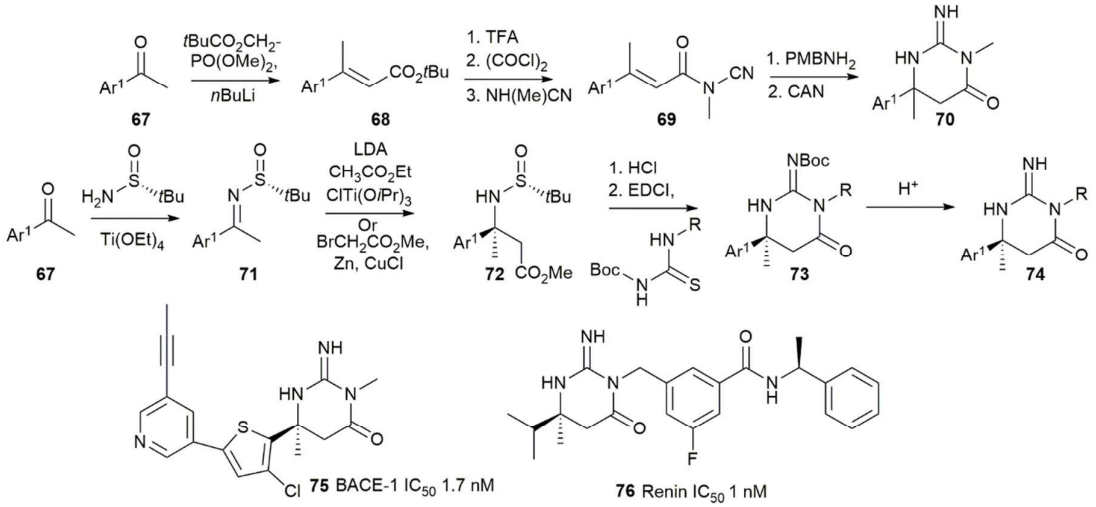
Scheme 9. Syntheses of the 4-amino-2H-imidazole **51** head group.



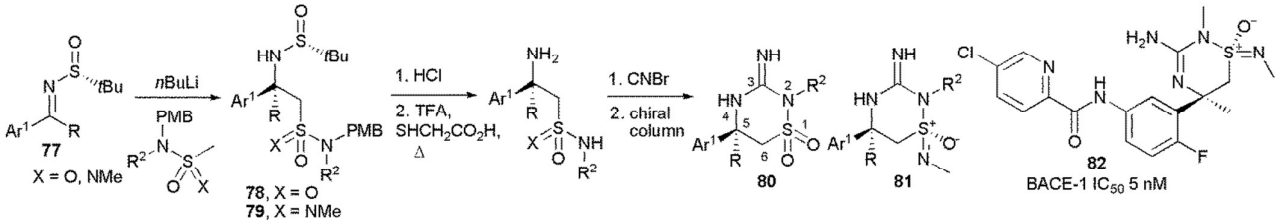
Scheme 10. Synthesis of the 3-amino-2,5-dihydro-1,2,4-oxadiazole **56** scaffold.



Scheme 11. Synthesis of 2-amino 4,5-dihydrooxazole **62** and 2-amino 4,5-dihydrothiazole scaffolds **63**.



Scheme 12. Synthetic pathways to access the 2-amino-5,6-dihydropyrimidin-4-one head groups **70** and **74**.



Scheme 13. Synthetic route to access 3-amino 5,6-dihydro-2H-1,2,4-thiadiazine 1,1-dioxide **80** and 3-amino 1-(methylimino)-5,6-dihydro-2H-1,2,4-thiadiazine 1-oxide **81**.

Ginman *et al.* also demonstrated that the 3-nitrogen can be removed completely to give the 5-amino 3,4-dihydro-2H-pyrrole **45**.[25] The 3,4-dihydro-2H-pyrrole **45** was generated starting from aryl aldehyde **43** that was subjected to a Stetter reaction. Next a lithiated arene attacks the ketone to form the intermediate **44** that is subsequently ring closed under strong acidic conditions. Finally conversion to the 3,4-dihydro-2H-pyrrol-5-amine **45** was undertaken via the thioamide.[25] Entry to the 2,5-dihydrothiazol-4-amine **47** was via a lithiated arene attack of an aryl nitrile. The resulting imine **46** was reacted with the 2-mercaptoacetate to give the cyclic amide en-route to the amino dihydrothiazole **47**.[25] These procedures enabled the synthesis of the potent inhibitors **48**,[26] and **49**[25] that target BACE-1 (Scheme 8).

In a successful attempt to prevent off target hERG activity the 4-amino-2H-imidazole scaffold **51** was designed (Scheme 9).[27] One synthesis started from the multicomponent reaction of a ketone with an α-oxo acetate in the presence of ammonia to give the intermediate **50**, which was converted to the amino imidazole **51** in two steps. The second pathway, started by reacting a ketone with a dithiooxamide to give the 2-amino-3-thioimidazole **52**. This scaffold was well positioned to install diversity at the 3-position via a Kumada reaction,[27] and led to the structural diversity seen in the potent BACE-1 inhibitor **53**.

2.3 3-Amino 2,5-dihydro-1,2,4-oxadiazole

The N-substitution on the guanidine moiety was also maintained in the 2,5-dihydro-1,2,4-oxadiazol-3-amine head group (Scheme 10). To synthesise this scaffold, Dillard *et al.* started from the reaction of ketone **54** with bis TMS carbodiimide to give the N-cyano imine **55**. This intermediate was reacted with the N-substituted hydroxylamine to give the oxadiazole **56**.[28] Zhu *et al.* used a different approach starting from the α-substituted benzyl isothiocyanate **57**. The α-centre of this intermediate was brominated and then on-reacted with N-methyl hydroxylamine to give the 3-thio oxadiazole **58** and subsequently the 3-amino 1,2,4-oxadiazole **56**.[24] These reaction sequences were used to produce the potent BACE-1 inhibitor **59** (Scheme 10).[29]

2.4 2-Amino 4,5-dihydrooxazole and thiazole

In ongoing efforts to generate BACE-1 inhibitors with improved CNS penetration, P-gp efflux and hERG, the head group was altered by several groups to a 2-amino 4,5-dihydrooxazole or 2-amino 4,5-dihydrothiazole scaffold (**62** and **63**).[19, 30] This change altered the pKa of the oxazine head group, in examples **64**,[30b] **65**[30a] and **66**,[30d] which was sufficient to ameliorate all of the aforementioned properties, while retaining potent BACE-1 activity. The general scheme to synthesise the 4,5-dihydrooxazole **62** and 4,5-dihydrothiazole **63** class of inhibitor started with a ketone **60** that was converted to the methylene **61** using a Wittig[30f] or a Tebbe reaction.[19, 30c, 30d] This transformation to the methylene **61** can also be performed by Grignard addition[30b, 30e] or lithiated agent addition[30e] to the ketone **60** followed by dehydration. The methylene **61** is then treated with *in situ* generated iodo isocyanate followed by ammonium hydroxide to give the 4,5-dihydrooxazole **62** or iodo thioisocyanate in the case of the 4,5-dihydrothiazole **63** (Scheme 11).

3. 6-Membered heterocycles

The 5-membered amino imidazolone was the initial scaffold that was discovered to interact and stabilize the catalytic aspartates within the catalytic dyad of BACE-1. Soon after it was discovered that expansion of the ring system or insertion of a carbon adjacent to the carbonyl group of the imidazolone retained binding affinity to BACE-1. Although, the 6-membered head group did not impact on binding to the catalytic aspartates, the 6-membered head group was seen as an opportunity to modulate the pKa of the amidine aspartate binding moiety to fine tune the physicochemical parameters. The extra carbon also allowed for the possibility of extra structural diversity, and resulted in a large number of iterations that are found in literature. Only a few of these modifications could be covered here due to space limitations, therefore the primary focus is on the synthesis of the head group.

3.1 2-Amino-5,6-dihydropyrimidin-4-one and orthologues

The most obvious 6-membered system was the addition of an extra carbon adjacent to the carbonyl in the 2-amino imidazolone **2** to give the 2-amino-5,6-dihydropyrimidin-4-one **70**. The synthesis of **70** by Berg *et al* started from a ketone **67**.[31] Under Wittig conditions the ketone **67** was transformed to the *tert*-butyl acrylate **68**, which was subsequently converted to the *N*-methyl *N*-cyano amide **69**. PMB amine was then added to induce cyclisation and the PMB group removed under oxidative conditions to give **70**. Although the synthesis is not enantioselective it uses inexpensive reagents and is reasonably modular with respect to integration of N-substituents.

The most common synthesis of the 2-amino dihydropyrimidin-4-one scaffold utilises a *tert*-butyl sulfinamide precursor and chemistry championed by the Ellman lab.[32] This chemistry prolifically exploited and allows installation of the P1 and P'1 (or 6, 6-) substituents onto the dihydropyrimidin-4-one scaffold in an enantioselective manner.[24, 29, 33] Using the *tert*-butyl sulfinamide, the imine was formed with a ketone **67** in the presence of a Lewis acid to afford the imine **71**. Then a metalated acetate equivalent was added to the imine **71** to afford the chiral acetate **72**. This intermediate **72** was treated with HCl to remove the sulfinamide group. Addition of *N*-Boc-*N*'-substituted thiourea to the resulting β-amino acid in the presence of EDCI, gave the Boc protected product **73**. The *N*-Boc group at this stage provides adequate protection for further functionalisation reactions that may not be compatible with unprotected guanidine functionality. Facile removal of the Boc group under acidic conditions ultimately provides the desired 2-amino 5,6-dihydropyrimidin-4-one **74**, similar to scaffolds **75** and **76** that are potent inhibitors of BACE-1[34] and renin[33a, 33b] respectively (Scheme 12). Usually, the 6-position is used to gain access to the S3 pocket via the S1 pocket of the aspartyl protease, similar to that shown in Figure 2. Interestingly, McKittrick *et al* used a novel approach to enter the S3 sub-pocket of renin exploiting the N3-substituent, shown in compound **76**.[33a] Here, the *N*-substitution is readily installed with the *N*-Boc-*N*'-substituted thiourea reagent, but can be installed post dihydropyrimidin-4-one formation using a base and an aliphatic halide or via a Mitsunobu reaction using an alcohol (not shown).[33b]

The direct sulfonamide and imino sulfonamide analogues of the 2-amino dihydro pyrimdin-4-one, **70** and **74** respectively, were also produced utilising the sulfinamide chemistry.[35] Here, the sulfinamide imine **77** was reacted with either deprotonated PMB protected methyl sulfonamide or sulfinamide to afford **78** and **79** respectively. The sulfinamide and PMB groups were removed and then cyanogen bromide used to commit cyclization, to give either the sulfone and sulfinamide systems, **80** and **81** respectively. This synthesis was used to produce the sulfinamide analogue **82** which possessed potent BACE-1 activity[35b] (Scheme 13). The phosphonate analogue of **80** was also generated by Zhu *et al.*[24] The synthesis of the 4, 4-disubstituted analogue started from the β-amino

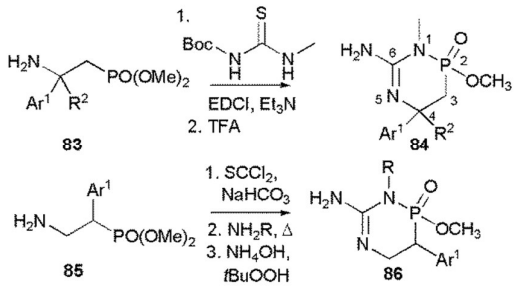
phosphate **83** that was coupled to the *N*-Boc *N*-methyl thiourea followed by Boc deprotection to afford **84**. Similarly the 5 substituted analogue **86** was prepared from the β-amino phosphate **85**. Here, **85** was reacted with thiophosgene, followed by an amine. The resulting cyclised thiourea was converted to the 1,2,3,4-tetrahydro-1,5,2-diazaphosphinine 2-oxide **86** with use of ammonium hydroxide under oxidative conditions (Scheme 14). All 4 scaffolds (**80**, **81**, **84** and **86**) were found to exhibit similar activity against BACE-1 compared to direct analogues of the progenitor **74**.[24, 35b]

3.2. 2-Amino 3,4,5,6-tetrahydropyridines

In several scaffolds the nitrogen adjacent to the amino group was replaced with a disubstituted carbon (Scheme 15). The aim of these head groups was to modulate the pKa of the amidine functionality to ablate P-gp activity, and to assist with BBB permeability to target BACE-1. Although, P-gp activity was abrogated and the potency against BACE-1 *in vitro* was comparable to **75**, no pharmacological response *in vivo* was observed.[36] The synthesis of these scaffolds both utilised the sulfinamide chemistry. For **88**, this started with nucleophilic addition of a metalated functionalised methyl sulfone to the sulfinamide imine **77**. The sulfinamide group was removed from the resulting product **87**, and enabled an $AlMe_3$ mediated cyclization of the amine to the nitrile, to afford **88**. The synthesis of the tetrahydro pyridine **92**, started with a Reformatsky like addition of a zinc species to the sulfinamide imine **89** to give **90**. The sulfinamide group was removed and the amine protected with a DMB group, via reductive amination and subsequently reacted with a functionalised acryloyl chloride. This intermediate **91** was positioned for metathesis reaction using the second generation Grubb's catalyst to afford the cyclised tetrahydropyridine **92**. The 3-cyclopropyl group was next installed over 4 steps, and the 2-hydroxy converted to the 2-amine over these steps to give the tetrahydropyridine **93**. These synthetic pathways led to the potent inhibitors of BACE-1, **94**[35a] and **95**[36] (Scheme 15).

3.3 Amino 5,6-dihydro-oxazine, thiazine and orthologues

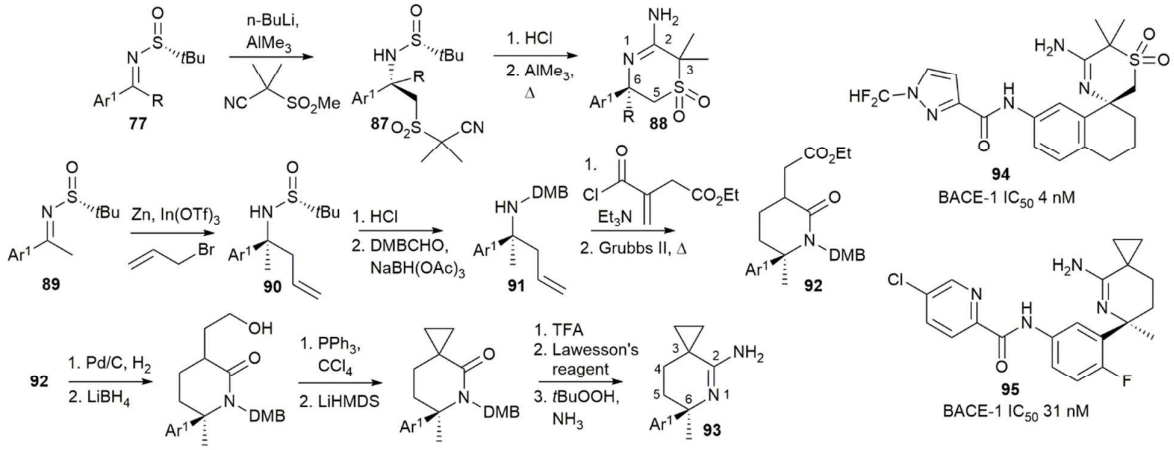
Similar to the five membered dihydrooxazole and thiazole head groups (**62** and **63**), the 6-membered oxazine and thiazines were constructed to improve PK and CNS penetration while maintaining BACE-1 potency. A plethora of analogues harbouring these head groups were created,[37] many of them possessing improved CNS penetration,[37a, 37c, 37o] leading to the potent BACE-1 inhibitors **96**[37o] and **97**[37k] (Scheme 16).



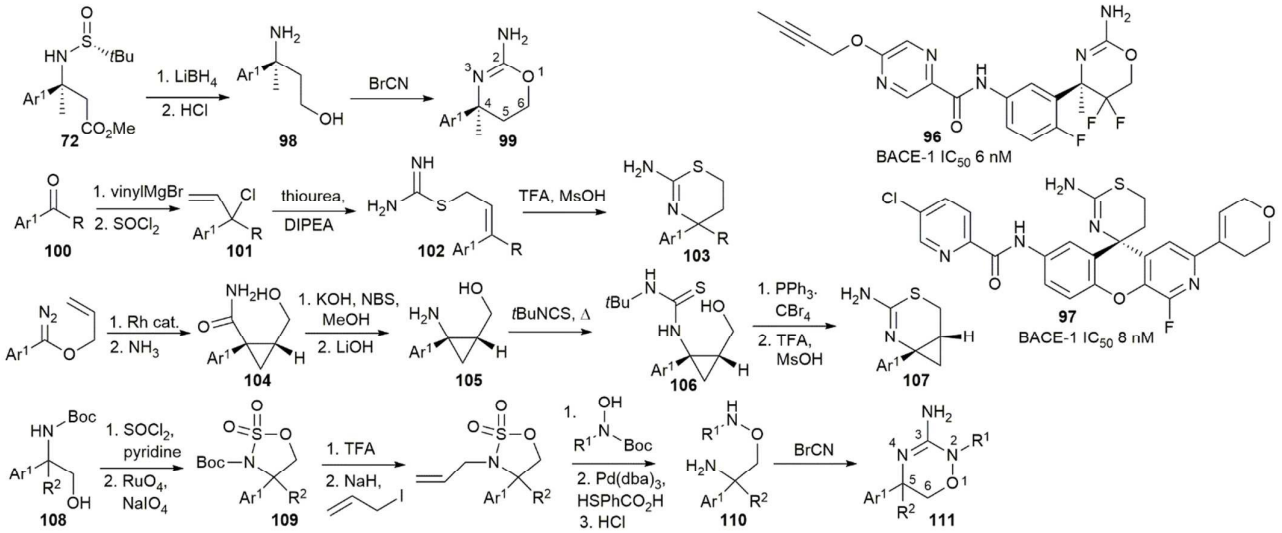
Scheme 14. Synthesis of the 6-amino-2-methoxy-1,2,3,4-tetrahydro-1,5,2-diazaphosphinine 2-oxide head group.

A representative synthesis of the 2-amino 5,6-dihydro-oxazine head group **99** is shown in Scheme 16. In this synthesis, **99** was accessed stereoselectively starting from the chiral sulfinamide **72** building block (its synthesis highlighted earlier in Scheme 12). Here, the chiral sulfinamide ester **72** was reduced, followed by removal of the sulfinamide group to give the amino alcohol **98**. The amino alcohol **98** was then reacted with cyanogen bromide providing the enantiopure 2-amino 5,6-dihydro-oxazine **99**.[37u]

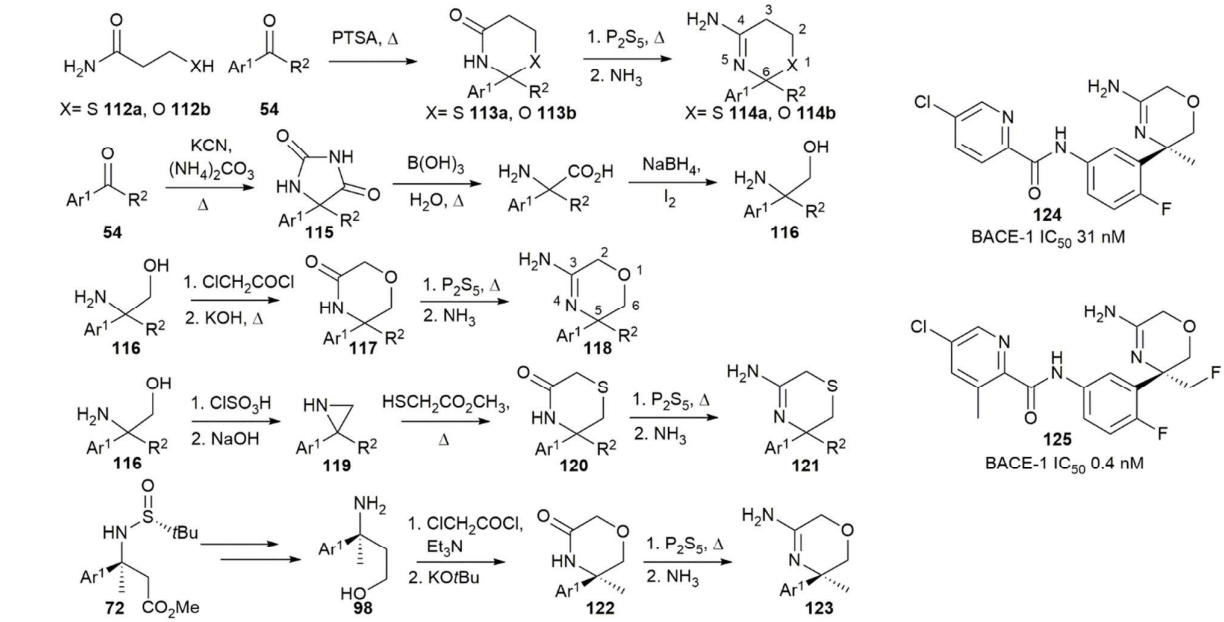
Efficient access to racemic 2-amino 5,6-dihydro-thiazine **103** was performed by starting from readily available ketones **100**. In this synthesis the ketone was treated with the vinyl Grignard, followed by treatment with thionyl chloride to give the chloro species **101**. Addition of thiourea and elimination of chlorine positioned **102** for cyclisation to the amino dihydro-thiazine **103** under acidic conditions[37v] (Scheme 16). This method although not stereoselective was used to generate amino dihydro-thiazine analogues quickly. To access the 2-amino 5,6-dihydro-thiazine head group in an enantiopure form, a representative example in Scheme



Scheme 15. Synthesis of 2-amino 3,4,5,6-tetrahydropyridine-like scaffolds.



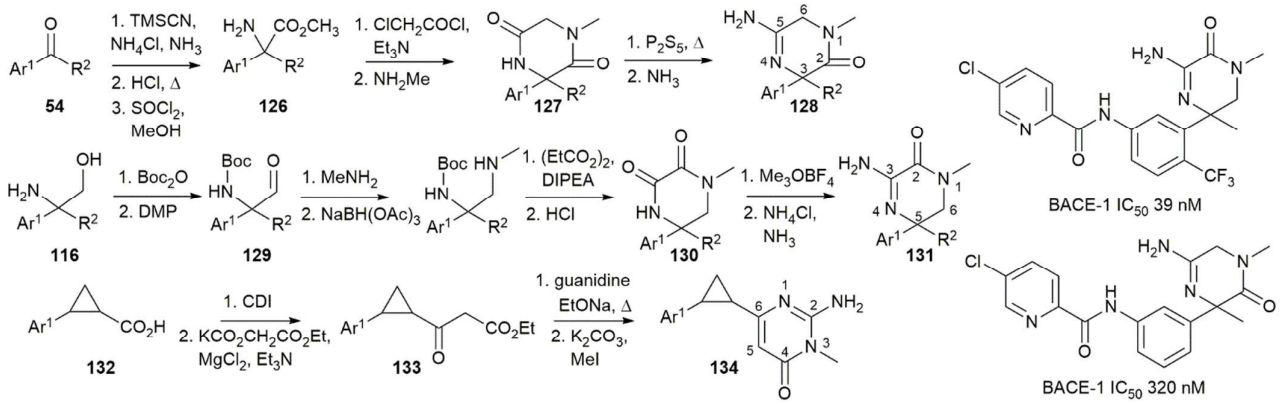
Scheme 16. Synthetic routes to produce the amino dihydro-oxazine, thiazine and oxadiazine head groups.



Scheme 17. Synthetic routes to produce the amino dihydro-oxazine and thiazine scaffolds.

16 is shown that possesses a cyclopropyl group at the 5,6 position of the thiazine. Here, Woltering *et al*[36] produced the starting chiral cyclopropyl amide **104** from an intramolecular carbenoid cyclopropanation induced by a chiral ligated Rh catalyst. **104** was then subjected to a Hoffmann rearrangement followed by basic hydrolysis to generate the amino alcohol **105**, which was treated with *tert*-butylisothiocyanate to yield the thiourea **106**. This intermediate was cyclised under Appel conditions followed by deprotection under strongly acidic conditions to generate the chiral 2-amino 5,6-dihydro-thiazine **107** (Scheme 16). Another iteration on the dihydro-oxazine head group, was the 3-amino 5,6-dihydro-1,2,4-oxadiazine **111**. To synthesise this scaffold, Zhu *et al.* produced the oxathiazolidine **109** from cyclisation of the β-amino alcohol **108**. After replacing the Boc protection with an allyl, addition of a substituted hydroxylamine gave the ring opened product **110**. After protecting group removal, addition of cyanogen bromide yielded the 5,6-dihydro-1,2,4-oxadiazine **111** (Scheme 16).[38]

Ginman *et al.* focused on refining the dihydro-oxazine/thiazine core to address permeability and hERG activity, while maintaining BACE-1 potency. To do this, a series of head groups were generated where the sulfur or oxygen heteroatom were placed in different positions around the 6-membered head group,[25] shown in Scheme 17. The 4-amino 5,6-dihydro- oxazine and thiazine variant **114** can be accessed via condensation of a ketone **54** with either a thiol or alcohol amide **112** under acidic conditions to form the lactam **113**.



Scheme 18. Synthesis of the 5-amino-1,6-dihydropyrazin-2(3H)-one **128**, 3-amino-piperazin-2-one **131** and 2-amino-pyrimidin-4(3H)-one **134** head groups.

This intermediate was converted to the 4-amino 5,6-dihydro-oxazine/thiazine **114** in two steps (Scheme 17).[25]

The synthesis of the 3-amino 2,6-dihydro-oxazine **118** or thiazine **121** head groups started from the amino alcohol **116**. To access the amino alcohol **116**, a ketone **54** was reacted with KCN and $(NH_4)_2CO_3$ in Bucherer-Bergs reaction to form the hydantoin **115**, which was hydrolysed, followed by reduction to afford the amino alcohol **116**.[25] The amino alcohol **116** was then employed in their synthesis of the 2,6-dihydrooxazine head group **118**. Here the amino alcohol **116** was reacted with chloroacetyl chloride and cyclised on addition with base to form the morpholinone **117**. Conversion of the amide to the amidine in two steps gave the 3-amino 2,6-dihydro **118** (Scheme 17).[39]

To access the 3-amino 2,6-dihydro-thiazine **121**, the amino alcohol **116** was converted to the alkyl sulphate by treatment with chlorosulfonic acid followed by a Wenker reaction to afford the aziridine **119**. Aziridine ring opening and expansion with methyl 2-mercaptoacetate gave the 6-membered lactam **120**, which was followed by a 2 step conversion to the 3-amino 2,6-dihydro-thiazine **121** (Scheme 17).[25] These methods only produced the racemic 3-amino 2,6-dihydro-thiazine, and chiral chromatography was required to produce the enantiopure product.

To produce the 2,6-dihydro-oxazine head group **118** in a stereoselective manner, the sulfinamide methodology was again employed.[33e] Starting from the earlier described β-amino alcohol **98**, that was accessed from the sulfinamide **72**, **98** was treated with 2-chloroacetyl chloride, followed by addition of potassium *tert*-butoxide, yielding the cyclic intermediate **122**. The amide functionality was then transformed into the amidine to give **123** in two steps via the corresponding thioamide. This stereoselective synthesis allowed entry to the potent BACE-1 and BACE-2 inhibitors, **124**[36] and **125**[40] respectively (Scheme 17).

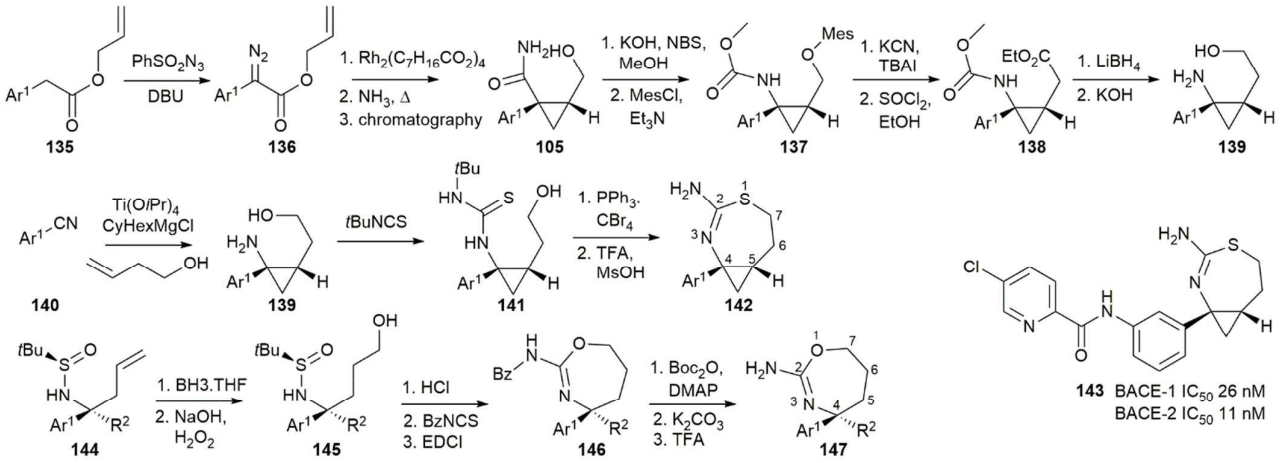
3.4 5-Amino-1,6-dihydropyrazin-2(3H)-one and 3-amino-piperazin-2-one

Another variation on the dihydro oxazine theme to mediate the pKa of the amidine functionality of the head group, was to insert an amide at various positions, giving rise to either a 5-amino-1,6-dihydropyrazin-2(3H)-one **128** or a 3-amino-piperazin-2-one **131**. It was found that the 5-amino dihydropyrazin-2(3H)-one (**128**) compared to other head groups (for example **95** and **124**) possess similar potent BACE-1 inhibitory activity, however the 3-amino-piperazin-2-one scaffold (**131**) was 10 fold less potent (Scheme 18).[41] To form the 1,6-dihydropyrazinone **128**, Trabanco-Suarez *et al.* started by reacting the ketone **54** with trimethylsilyl cyanide in the presence of ammonia to form the intermediate nitrile. The nitrile was hydrolysed under acidic conditions, followed by treatment with thionyl chloride to form the amino ester **126**. The reaction of **126** with chloroacetyl chloride followed by methylamine induced cyclisation to the piperazine-dione **127**. Conversion of the amide functionality to the amidine in two steps gave the desired compound **128**[42] (Scheme 18).

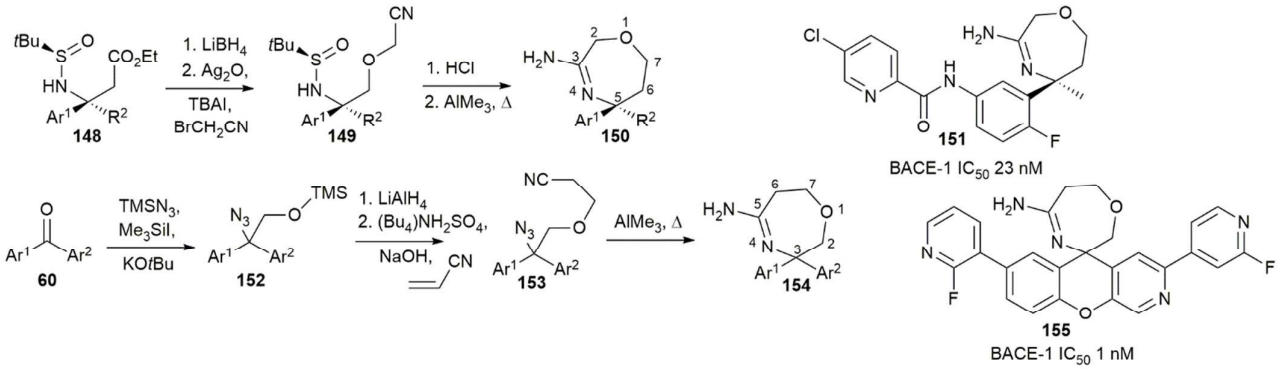
The synthesis of the 3-amino-piperazin-2-one head group **131** was initiated from the amino alcohol **116** which was converted to the protected amino aldehyde **129** employing Dess-Martin periodinane. The *N*-methyl group was introduced by reductive amination followed by acylation and *in situ* cyclisation with ethyl oxalate to the piperazine-2,3-dione **130**. Finally, the piperazinedione was *O*-methylated using Me_3OBF_4 followed by conversion to the amidine after treatment with NH_3 in the presence of NH_4Cl to give **131**[43] (Scheme 18).

3.5 2-Amino-pyrimidin-4(3H)-one

Yonezawa *et al.* generated the pyrimidin-4-one **134**, a sp^2 hybridised form of the 5,6-dihydropyrimidin-4-one **70** in an attempt to improve the binding affinity to BACE-1. It was found the sp^2 character was not well tolerated, resulting in low μM inhibition of BACE-1.[44] To generate such a scaffold, the cyclopropanoic acid **132** was converted to the β-ketoester **133** under Masamune conditions. The ketoester **133** was treated with guanidine carbonate in the presence of sodium ethoxide followed by selective methylation of the lactam nitrogen affording the 2-amino-pyrimidin-4(3H)-one **134** (Scheme 18).



Scheme 19. Synthesis of the 2-amino-4,5,6,7-tetrahydro-1,3-thiazepine and the 2-amino-4,5,6,7-tetrahydro-1,3-oxazepine scaffolds.



Scheme 20. Synthesis of the 3-amino-2,5,6,7-tetrahydro-1,4-oxazepine and the 5-amino-2,3,6,7-tetrahydro-1,4-oxazepine head groups.

4. 7-Membered heterocycles

The binding orientation of the amino heterocycle class in the substrate binding cleft of aspartyl protease inhibitors is not only conducive to 5- and 6-membered systems, but can also accommodate 7-membered systems, as seen by the BACE-1 activity comparison between **124** (Scheme 17) and **151** (Scheme 20), and the head group comparison study performed by Woltering *et al*.[36]

7.1 2-Amino-4,5,6,7-tetrahydro-1,3-thiazepine

Woltering *et al.* generated a 4,5-cyclopropyl fused 4,5,6,7-tetrahydro-1,3-thiazepine **142** to introduce a degree of conformational restriction to improve BACE-1 affinity. To access this scaffold, the initial steps involved the formation of intermediate amino alcohol **139**. This began with a Regitz diazo transfer reaction of allyl ester **135** to afford the allyl α–diazoester **136**, which was cyclised to the lactone intermediate using a intramolecular carbenoid cyclopropanation induced by a chiral ligated Rh catalyst, followed by ring opening to form the amide **105**. Hoffmann rearrangement of **105** to a carbamate **137** followed by homologation of the alcohol over several steps gave the ester **138**. Reduction and saponification produced the amino alcohol **139** (Scheme 19).[45] Alternatively, the amino alcohol **139** could be generated by a cyclopropanation reaction of buten-3-ol in the presence of an aromatic nitrile **140** using Ti(O*i*Pr)$_4$ and a hindered Grignard. With the amino alcohol **139** now in hand, the amine was converted to a thiourea **141** using *t*BuNCS, and cyclisation was performed using Appel reaction conditions. Finally, the *t*Bu group was removed under acidic conditions to give the 4,5,6,7-tetrahydro-thiazepine **142**. Using this synthesis, compound **143** was generated and was shown to have an IC$_{50}$ of 26 nM against BACE-1 and 11 nM against BACE-2 (Scheme 19). It was noted that the addition of the cyclopropyl group had no effect on BACE-1 activity.[36]

7.2 2-Amino-4,5,6,7-tetrahydro-1,3-oxazepine

The synthesis of the 4,5,6,7-tetrahydro-1,3-oxazepine head group **147** by Kusakabe *et al*. began with the hydroboration of the chiral butenyl sulfinamide **144** to form the amino alcohol **145**. The sulfinamide was then removed and the benzoyl thiourea formed. This allowed for an EDCI mediated cyclisation to the 7-membered oxazepine **146**. Deprotection of the benzoyl group over three steps yielded the 2-amino-4,5,6,7-tetrahydro-1,3-oxazepine **147** (Scheme 19).[46]

7.2 3-Amino-2,5,6,7-tetrahydro-1,4-oxazepine and the 5-amino 2,3,6,7-tetrahydro-1,4-oxazepine

The pathway to access 2,5,6,7-tetrahydro-1,4-oxazepine involved the reduction of a sulfinamide ester **148** to form a sulfinamide alcohol, which was selectively alkylated with $BrCH_2CN$ to form the sulfinamide nitrile **149**. The sulfinamide **149** was deprotected, followed by $AlMe_3$ mediated cyclisation to yield the oxazepine **150** (Scheme 20).[37r] Following this method, Woltering *et al*. generated the potent BACE-1 inhibitor **151**[36] (Scheme 20). Dineen *et al*. formed the 2,3,6,7-tetrahydro-1,4-oxazepine head group **154** starting from ketone **60**. The ketone **60** was treated with a MeS^+I^- and a strong base followed by $TMSN_3$ to form the azide **152**. The TMS group was then removed and the azide reduced to the β-amino alcohol. The alcohol was when selectively alkylated via a conjugation reaction with acrylonitrile to the cyano ether **153**. The cyano ether **153** was then cyclised using $AlMe_3$ to the 5-amino 2,3,6,7-tetrahydro-1,4-oxazepine **154**.[47] By this method, Dineen *et al*. synthesised the BACE-1 inhibitor **155** which was shown to have an IC_{50} of 1 nM.[47] In general the 7-membered systems exhibited similar biochemical BACE-1 activity to their direct 6-membered counterparts.

5. Conclusions

Here we have provided a snapshot of the immense effort that has been invested in the synthesis of the amino heterocycle class of aspartyl protease inhibitor. Some 300 publications and patents have been published or filed respectively since the initial disclosure of this inhibitor class in 2005, demonstrating the evolving diversity in the head group and functionality that has been incorporated into this scaffold.

A large majority of literature surrounding the amino heterocycle class is geared toward targeting BACE-1 and BACE-2, indicating that these scaffolds are under utilised in targeting other pharmacologically relevant aspartyl proteases, such as HIV protease for treating AIDS, renin for hypertension,[48] certain plasmepsins for treating malaria,[49] ASP5 for treating Toxoplasmosis,[50] and cathepsin D as an emerging cancer target.[51] Several of the amino heterocycles shown in this review that target BACE-1, are progressing through clinical trials for treating Alzheimer's disease. It is foreseen that the amino heterocycle class will be heavily utilised in aspartyl protease drug discovery programs well into the future.

Acknowledgements

This work was supported by the Wellcome Trust Pathfinder Award (WT109662MA), Australian Cancer Research Foundation, the Victorian State Government Operational Infrastructure Support and Australian Government NHMRC IRIISS. We also thank Jared Cumming, Brian McKittrick, Jack Scott and Christoph Grohmann for their useful advice.

Notes and references

1. a) C. Jensen, P. Herold and H. R. Brunner, *Nat. Rev. Drug Discovery*, 2008, **7**, 399-410; b) N. C. Cohen, *Chem. Biol. Drug. Des.*, 2007, **70**, 557-565.
2. a) A. Dahan, Y. Tsume, J. Sun, J. M. Miller and G. L. Amidon, in *Amino Acids, Peptides and Proteins in Organic Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA, 2011, DOI: 10.1002/9783527631827.ch8, pp. 277-292; b) V. J. Wacher, J. A. Silverman, Y. Zhang and L. Z. Benet, *J. Pharm. Sci.*, 1998, **87**, 1322-1330; c) G. M. Pauletti, S. Gangwar, T. J. Siahaan, A. Jeffrey and R. T. Borchardt, *Adv. Drug Deliv. Rev.*, 1997, **27**, 235-256.
3. Z. Zhu, *Trends Pharmacol. Sci.*, 2012, **33**, 233-240.
4. a) Z. Zhu, Z.-Y. Sun, Y. Ye, J. Voigt, C. Strickland, E. M. Smith, J. Cumming, L. Wang, J. Wong, Y.-S. Wang, D. F. Wyss, X. Chen, R. Kuvelkar, M. E. Kennedy, L. Favreau, E. Parker, B. A. McKittrick, A. Stamford, M. Czarniecki, W. Greenlee and J. C. Hunter, *J. Med. Chem.*, 2010, **53**, 951-965; b) U. Iserloh, Z. Zhu, A. Stamford and J. H. Voigt, US20060281729A1, 2006 CAN 2006:1309978; c) Z. Zhu, B. McKittrick, Z.-Y. Sun, Y. C. Ye, J. H. Voigt, C. Strickland, E. M. Smith, A. Stamford, W. J. Greenlee, Y. Wu, U. Iserloh, R. Mazzola, J. Caldwell, J. Cumming, L. Wang, T. Guo, T. X. H. Le, K. W. Saionz, S. D. Babu and R. C. Hunter, WO2005058311A1, 2005 CAN 2005:570806.
5. a) M. S. Malamas, A. Robichaud, J. Erdei, D. Quagliato, W. Solvibile, P. Zhou, K. Morris, J. Turner, E. Wagner, K. Fan, A. Olland, S. Jacobsen, P. Reinhart, D. Riddell and M. Pangalos, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 6597-6605; b) 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 and A. J. Robichaud, *J. Med. Chem.*, 2010, **53**, 1146-1158; c) M. S. Malamas, K. Barnes, M. Johnson, Y. Hui, P. Zhou, J. Turner, Y. Hu, E. Wagner, K. Fan, R. Chopra, A. Olland, J. Bard, M. Pangalos, P. Reinhart and A. J. Robichaud, *Bioorg. Med. Chem.*, 2010, **18**, 630-639; d) 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 and A. J. Robichaud, *J. Med. Chem.*, 2009, **52**, 6314-6323; e) P. Zhou, M. S. Malamas, Y. Li, A. J. Robichaud and D. A. Quagliato, US20070004730A1, 2007 CAN 2007:17729; f) M. S. Malamas, P. Zhou, W. F. Fobare, W. R. Solvibile, I. S. Gunawan, J. J. Erdei, Y. Yan, P. M. Andrae and D. A. Quagliato, US20070004786A1, 2007 CAN 2007:17778; g) M. S. Malamas, I. S. Gunawan, J. J. Erdei, P. W. Nowak, J. R. Stock and Y. Yan, US20070027199A1, 2007 CAN 2007:119525; h) M. S. Malamas, J. J. Erdei, W. F. Fobare, D. A. Quagliato, S. A. Antane and A. J. Robichaud, US20070072925A1, 2007 CAN 2007:359158; i) M. S. Malamas, J. J. Erdei, I. S. Gunawan, P. Zhou, Y. Yan and D. A. Quagliato, US20050282825A1, 2005 CAN 2005:1335094.
6. a) S. Geschwindner, L.-L. Olsson, J. S. Albert, J. Deinum, P. D. Edwards, T. de Beer and R. H. A. Folmer, *J. Med. Chem.*, 2007, **50**, 5903-5911; b) P. D. Edwards, J. S. Albert, M. Sylvester, D. Aharony, D. Andisik, O. Callaghan, J. B. Campbell, R. A. Carr, G. Chessari, M. Congreve, M. Frederickson, R. H. A. Folmer, S. Geschwindner, G. Koether, K. Kolmodin, J. Krumrine, R. C. Mauger, C. W. Murray, L.-L. Olsson, S. Patel, N. Spear and G. Tian, *J. Med. Chem.*, 2007, **50**, 5912-5925; c) M. Congreve, D. Aharony, J. Albert, O. Callaghan, J. Campbell, R. A. E. Carr, G. Chessari, S. Cowan, P. D. Edwards, M. Frederickson, R. McMenamin, C. W. Murray, S. Patel and N. Wallis, *J. Med. Chem.*, 2007, **50**, 1124-1132; d) J. Albert, G. Chessari and P.

Edwards, WO2007058581A1, 2007 CAN 2007:563315; e) J. Albert, G. Chessari, M. S. Congreve, P. Edwards, C. Murray, S. Patel and M. Sylvester, WO2007058583A2, 2007 CAN 2007:565115; f) J. Albert, J. Arnold, G. Chessari, M. S. Congreve, P. Edwards, C. Murray and S. Patel, WO2007058601A1, 2007 CAN 2007:564530; g) J. Albert, D. Andisik, P. Edwards and M. Sylvester, WO2007058580A1, 2007 CAN 2007:564495; h) J. S. Albert, D. Andisik, J. Arnold, D. Brown, O. Callaghan, J. Campbell, R. A. E. Carr, G. Chessari, M. S. Congreve, P. Edwards, J. R. Empfield, M. Frederickson, G. M. Koether, J. Krumrine, R. Mauger, C. W. Murray, S. Patel, M. Sylvester and S. Throner, WO2006041404A1, 2006 CAN 2006:366969.

7. a) L. D. Jennings, D. C. Cole, J. R. Stock, M. N. Sukhdeo, J. W. Ellingboe, R. Cowling, G. Jin, E. S. Manas, K. Y. Fan, M. S. Malamas, B. L. Harrison, S. Jacobsen, R. Chopra, P. A. Lohse, W. J. Moore, M.-M. O'Donnell, Y. Hu, A. J. Robichaud, M. J. Turner, E. Wagner and J. Bard, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 767-771; b) D. C. Cole, J. R. Stock, R. Chopra, R. Cowling, J. W. Ellingboe, K. Y. Fan, B. L. Harrison, Y. Hu, S. Jacobsen, L. D. Jennings, G. Jin, P. A. Lohse, M. S. Malamas, E. S. Manas, W. J. Moore, M.-M. O'Donnell, A. M. Olland, A. J. Robichaud, K. Svenson, J. Wu, E. Wagner and J. Bard, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 1063-1066; c) B. Hu, US20060183943A1, 2006 CAN 2006:821171; d) W. F. Fobare and W. R. Solvibile, US20060183792A1, 2006 ; e) D. C. Cole, E. S. Manas, L. D. Jennings, F. E. Lovering, J. R. Stock, W. J. Moore, J. W. Ellingboe, J. S. Condon, M. N. Sukhdeo, P. Zhou, J. Wu and K. M. Morris, US20060183790A1, 2006 CAN 2006:821376.
8. S. Eketjall, J. Janson, K. Kaspersson, A. Bogstedt, F. Jeppsson, J. Falting, S. B. Haeberlein, A. R. Kugler, R. C. Alexander and G. Cebers, *J Alzheimers Dis*, 2016, **50**, 1109-1123.
9. D. Wyss, presented in part at the Pacifichem, Honolulu, Hawaii, USA, 2015.
10. P. C. May, B. A. Willis, S. L. Lowe, R. A. Dean, S. A. Monk, P. J. Cocke, J. E. Audia, L. N. Boggs, A. R. Borders, R. A. Brier, D. O. Calligaro, T. A. Day, L. Ereshefsky, J. A. Erickson, H. Gevorkyan, C. R. Gonzales, D. E. James, S. S. Jhee, S. F. Komjathy, L. Li, T. D. Lindstrom, B. M. Mathes, F. Martenyi, S. M. Sheehan, S. L. Stout, D. E. Timm, G. M. Vaught, B. M. Watson, L. L. Winneroski, Z. Yang and D. J. Mergott, *J Neurosci*, 2015, **35**, 1199-1210.
11. R. Vassar, *Alzheimers Res Ther*, 2014, **6**, 89.
12. D. Oehlrich, H. Prokopcova and H. J. M. Gijsen, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 2033-2045.
13. A. K. Ghosh and H. L. Osswald, *Chem. Soc. Rev.*, 2014, **43**, 6765-6813.
14. a) J. Yuan, S. Venkatraman, Y. Zheng, B. M. McKeever, L. W. Dillard and S. B. Singh, *J. Med. Chem.*, 2013, **56**, 4156-4180; b) H. J. M. Gijsen and F. P. Bischoff, in *Annual Reports in Medicinal Chemistry*, ed. C. D. Manoj, Academic Press, 2012, vol. Volume 47, pp. 55-69; c) S. J. Stachel, *Drug Dev. Res.*, 2009, **70**, 101-110; d) R. Silvestri, *Med. Res. Rev.*, 2009, **29**, 295-338; e) W. H. Huang, R. Sheng and Y. Z. Hu, *Curr. Med. Chem.*, 2009, **16**, 1806-1820; f) R. E. Olson and L. R. Marcin, in *Annual Reports in Medicinal Chemistry*, ed. E. M. John, Academic Press, 2007, vol. Volume 42, pp. 27-47.
15. M. S. Malamas, J. J. Erdei, I. S. Gunawan, P. Nowak and B. L. Harrison, WO2006076284A2, 2006 CAN 2006:710863.
16. P. Nowak, D. C. Cole, A. Aulabaugh, J. Bard, R. Chopra, R. Cowling, K. Y. Fan, B. Hu, S. Jacobsen, M. Jani, G. Jin, M.-C. Lo, M. S. Malamas, E. S. Manas, R. Narasimhan, P. Reinhart, A. J. Robichaud, J. R. Stock, J. Subrath, K. Svenson, J. Turner, E. Wagner, P. Zhou and J. W. Ellingboe, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 632-635.
17. P. Zhou, M. Malamas and A. J. Robichaud, *ARKIVOC*, 2010, 84-88.
18. J. N. Cumming, E. M. Smith, L. Wang, J. Misiaszek, J. Durkin, J. Pan, U. Iserloh, Y. Wu, Z. Zhu, C. Strickland, J. Voigt, X. Chen, M. E. Kennedy, R. Kuvelkar, L. A. Hyde, K. Cox, L. Favreau, M. F. Czarniecki, W. J. Greenlee, B. A. McKittrick, E. M. Parker and A. W. Stamford, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 2444-2449.
19. K. W. Hunt, A. W. Cook, R. J. Watts, C. T. Clark, G. Vigers, D. Smith, A. T. Metcalf, I. W. Gunawardana, M. Burkard, A. A. Cox, M. K. Geck Do, D. Dutcher, A. A. Thomas, S. Rana, N. C. Kallan, R. K. DeLisle, J. P. Rizzi, K. Regal, D. Sammond, R. Groneberg, M. Siu, H. Purkey, J. P. Lyssikatos, A. Marlow, X. Liu and T. P. Tang, *J. Med. Chem.*, 2013, **56**, 3379-3403.
20. C. Cativiela and M. D. Díaz-de-Villegas, *Tetrahedron: Asymmetry*, 2007, **18**, 569-623.
21. J. P. Caldwell, R. D. Mazzola, J. Durkin, J. Chen, X. Chen, L. Favreau, M. Kennedy, R. Kuvelkar, J. Lee, N. McHugh, B. McKittrick, P. Orth, A. Stamford, C. Strickland, J. Voigt, L. Wang, L. Zhang, Q. Zhang and Z. Zhu, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 5455-5459.
22. A. A. Thomas, K. W. Hunt, M. Volgraf, R. J. Watts, X. Liu, G. Vigers, D. Smith, D. Sammond, T. P. Tang, S. P. Rhodes, A. T. Metcalf, K. D. Brown, J. N. Otten, M. Burkard, A. A. Cox, M. K. G. Do, D. Dutcher, S. Rana, R. K. DeLisle, K. Regal, A. D. Wright, R. Groneberg, K. Scearce-Levie, M. Siu, H. E. Purkey, J. P. Lyssikatos and I. W. Gunawardana, *J. Med. Chem.*, 2014, **57**, 878-902.
23. M. J. Meyers, M. D. Tortorella, J. Xu, L. Qin, Z. He, X. Lang, W. Zeng, W. Xu, L. Qin, M. J. Prinsen, F. M. Sverdrup, C. S. Eickhoff, D. W. Griggs, J. Oliva, P. G. Ruminski, E. J. Jacobsen, M. A. Campbell, D. C. Wood, D. E. Goldberg, X. Liu, Y. Lu, X. Lu, Z. Tu, X. Lu, K. Ding and X. Chen, *ACS Med. Chem. Lett.*, 2014, **5**, 89-93.
24. Z. Zhu, B. McKittrick, Z.-Y. Sun, Y. C. Ye, J. H. Voigt, C. Strickland, E. M. Smith, A. Stamford, W. J. Greenlee, R. D. Mazzola, Jr., J. Caldwell, J. N. Cumming, L. Wang, Y. Wu, U. Iserloh, X. Liu, Y. Huang, G. Li, J. Pan, J. A. Misiaszek, T. Guo, T. X. H. Le, K. W. Saionz, S. D. Babu, R. C. Hunter, M. L. Morris, H. Gu, G. Qian, D. Tadesse, G. Lai, J. Duo, C. Qu and Y. Shao, WO2008103351A2, 2008 CAN 2008:1106595.
25. T. Ginman, J. Viklund, J. Malmstroem, J. Blid, R. Emond, R. Forsblom, A. Johansson, A. Kers, F. Lake, F. Sehgelmeble, K. J. Sterky, M. Bergh, A. Lindgren, P. Johansson, F. Jeppsson, J. Faelting, Y. Gravenfors and F. Rahm, *J. Med. Chem.*, 2013, **56**, 4181-4205.
26. L. W. Dillard, J. Yuan, K. Leftheris, S. Venkatraman, G. Wu, L. Jia, Z. Xu, S. Cacatian, A. Morales-Ramos, S. Singh and Y. Zheng, WO2011106414A1, 2011 CAN 2011:1104180.
27. Y. Gravenfors, J. Viklund, J. Blid, T. Ginman, S. Karlstroem, J. Kihlstroem, K. Kolmodin, J. Lindstroem, S. von Berg, F. von Kieseritzky, K. Bogar, C. Slivo, B.-M. Swahn, L.-L. Olsson, P. Johansson, S. Eketjaell, J. Faelting, F. Jeppsson, K. Stroemberg, J. Janson and F. Rahm, *J. Med. Chem.*, 2012, **55**, 9297-9311.
28. L. W. Dillard, J. Yuan, L. Jia and Y. Zheng, WO2010021680A2, 2010 CAN 2010:243486.
29. S. Cacatian, D. A. Claremon, L. W. Dillard, K. Fuchs, N. Heine, L. Jia, K. Leftheris, B. McKeever, A. Morales-Ramos, S. Singh, S. Venkatraman, G. Wu, Z. Wu, Z. Xu, J. Yuan and Y. Zheng, WO2010105179A2, 2010 CAN 2010:1161614.

30. a) Y. Cheng, J. Brown, T. C. Judd, P. Lopez, W. Qian, T. S. Powers, J. J. Chen, M. D. Bartberger, K. Chen, R. T. Dunn, 2nd, O. Epstein, R. T. Fremeau, Jr., S. Harried, D. Hickman, S. A. Hitchcock, Y. Luo, A. E. Minatti, V. F. Patel, H. M. Vargas, R. C. Wahl, M. M. Weiss, P. H. Wen, R. D. White, D. A. Whittington, X. M. Zheng and S. Wood, *ACS Med. Chem. Lett.*, 2015, **6**, 210-215; b) J. J. Chen, Q. Liu, C. Yuan, V. Gore, P. Lopez, V. Ma, A. Amegadzie, W. Qian, T. C. Judd, A. E. Minatti, J. Brown, Y. Cheng, M. Xue, W. Zhong, T. A. Dineen, O. Epstein, J. Human, C. Kreiman, I. Marx, M. M. Weiss, S. A. Hitchcock, T. S. Powers, K. Chen, P. H. Wen, D. A. Whittington, A. C. Cheng, M. D. Bartberger, D. Hickman, J. A. Werner, H. M. Vargas, N. E. Everds, S. L. Vonderfecht, R. T. Dunn, II, S. Wood, R. T. Fremeau, Jr., R. D. White and V. F. Patel, *Bioorg. Med. Chem. Lett.*, 2015, **25**, 767-774; c) M. Volgraf, L. Chan, M. P. Huestis, H. E. Purkey, M. Burkard, M. Geck Do, J. Harris, K. W. Hunt, X. Liu, J. P. Lyssikatos, S. Rana, A. A. Thomas, G. P. Vigers and M. Siu, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 2477-2480; d) A. A. Thomas, K. W. Hunt, B. Newhouse, R. J. Watts, X. Liu, G. Vigers, D. Smith, S. P. Rhodes, K. D. Brown, J. N. Otten, M. Burkard, A. A. Cox, M. K. Geck Do, D. Dutcher, S. Rana, R. K. DeLisle, K. Regal, A. D. Wright, R. Groneberg, J. Liao, K. Scearce-Levie, M. Siu, H. E. Purkey and J. P. Lyssikatos, *J. Med. Chem.*, 2014, **57**, 10112-10129; e) O. Epstein, M. C. Bryan, A. C. Cheng, K. Derakhchan, T. A. Dineen, D. Hickman, Z. Hua, J. B. Human, C. Kreiman, I. E. Marx, M. M. Weiss, R. C. Wahl, P. H. Wen, D. A. Whittington, S. Wood, X. M. Zheng, R. T. Fremeau, Jr., R. D. White and V. F. Patel, *J. Med. Chem.*, 2014, **57**, 9796-9810; f) H. Huang, D. S. La, A. C. Cheng, D. A. Whittington, V. F. Patel, K. Chen, T. A. Dineen, O. Epstein, R. Graceffa, D. Hickman, Y. H. Kiang, S. Louie, Y. Luo, R. C. Wahl, P. H. Wen, S. Wood and R. T. Fremeau, *J. Med. Chem.*, 2012, **55**, 9156-9169.
31. S. Berg, J. Hedstroem, S. Hellberg and J. Malmberg, WO2007114771A1, 2007 CAN 2007:1142800.
32. M. T. Robak, M. A. Herbage and J. A. Ellman, *Chemical Reviews*, 2010, **110**, 3600-3740.
33. a) B. A. McKittrick, J. P. Caldwell, T. Bara, G. Boykow, M. Chintala, J. Clader, M. Czarniecki, B. Courneya, R. Duffy, L. Fleming, R. Giessert, W. J. Greenlee, C. Heap, L. Hong, Y. Huang, U. Iserloh, H. Josien, T. Khan, W. Korfmacher, X. Liang, R. Mazzola, S. Mitra, K. Moore, P. Orth, M. Rajagopalan, S. Roy, S. Sakwa, C. Strickland, H. Vaccaro, J. Voigt, H. Wang, J. Wong, R. Zhang and A. Zych, *Bioorg. Med. Chem. Lett.*, 2015, **25**, 1592-1596; b) T. Khan, H. Josien, B. McKittrick, H. Vaccaro, T. Bara, J. Caldwell, C. R. Heap, W. B. Geiss, S. Mitra and A. J. Zych, WO2013142396A1, 2013 CAN 2013:1504710; c) A. W. Stamford, E. J. Gilbert and J. N. Cumming, WO2012138590A1, 2012 CAN 2012:1481946; d) A. E. Minatti, O. Epstein, R. White, M. Weiss, W. Zhong and J. Low, WO2012071279A1, 2012 CAN 2012:767960; e) O. Delgado, A. Monteagudo, M. Van Gool, A. A. Trabanco and S. Fustero, *Org. Biomol. Chem.*, 2012, **10**, 6758-6766; f) Y. Wu, U. Iserloh, J. N. Cumming, X. Liu, R. D. Mazzola, Z.-Y. Sun, Y. Huang, A. Stamford, B. McKittrick and Z. Zhu, US20070287692A1, 2007 CAN 2007:1419743; g) Z. Zhu, B. McKittrick, A. Stamford, Y. Huang and E. M. Smith, US20060281730A1, 2006 CAN 2006:1309980; h) A. Stamford, G. Li, W. J. Greenlee, Z. Zhu, B. McKittrick, R. Mazzola, Y. Huang, T. Guo, T. X. H. Le, G. Qian and Y. Shao, WO2006138265A2, 2006 CAN 2006:1357210.
34. A. W. Stamford, J. D. Scott, S. W. Li, S. Babu, D. Tadesse, R. Hunter, Y. Wu, J. Misiaszek, J. N. Cumming, E. J. Gilbert, C. Huang, B. A. McKittrick, L. Hong, T. Guo, Z. Zhu, C. Strickland, P. Orth, J. H. Voigt, M. E. Kennedy, X. Chen, R. Kuvelkar, R. Hodgson, L. A. Hyde, K. Cox, L. Favreau, E. M. Parker and W. J. Greenlee, *ACS Med. Chem. Lett.*, 2012, **3**, 897-902.
35. a) J. D. Scott, W.-L. Wu and J. N. Cumming, WO2015038446A1, 2015 CAN 2015:457849; b) T. A. Khan, J. D. Scott and J. N. Cumming, WO2014150340A1, 2014 CAN 2014:1600157; c) T. A. Khan, J. D. Scott and J. N. Cumming, WO2014150331A1, 2014 CAN 2014:1605754; d) E. J. Gilbert, J. N. Cumming, A. W. Stamford, Y. Yu, J. D. Scott and U. Iserloh, WO2014062549A1, 2014 CAN 2014:663465.
36. T. J. Woltering, W. Wostl, H. Hilpert, M. Rogers-Evans, E. Pinard, A. Mayweg, M. Gobel, D. W. Banner, J. Benz, M. Travagli, M. Pollastrini, G. Marconi, E. Gabellieri, W. Guba, H. Mauser, M. Andreini, H. Jacobsen, E. Power and R. Narquizian, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 4239-4243.
37. a) Y.-J. Wu, J. Guernon, F. Yang, L. Snyder, J. Shi, A. McClure, R. Rajamani, H. Park, A. Ng, H. Lewis, C. Chang, D. Camac, J. H. Toyn, M. K. Ahlijanian, C. F. Albright, J. E. Macor and L. A. Thompson, *ACS Medicinal Chemistry Letters*, 2016, DOI: 10.1021/acsmedchemlett.5b00432; b) L. L. Winneroski, M. A. Schiffler, J. A. Erickson, P. C. May, S. A. Monk, D. E. Timm, J. E. Audia, J. P. Beck, L. N. Boggs, A. R. Borders, R. D. Boyer, R. A. Brier, K. J. Hudziak, V. J. Klimkowski, P. Garcia Losada, B. M. Mathes, S. L. Stout, B. M. Watson and D. J. Mergott, *Bioorg. Med. Chem.*, 2015, DOI: 10.1016/j.bmc.2015.04.062, Ahead of Print; c) F. J. Rombouts, G. Tresadern, O. Delgado, C. Martinez Lamenca, M. Van Gool, A. Garcia-Molina, S. A. Alonso De Diego, D. Oehlrich, H. Prokopcova, J. M. Alonso, N. Austin, H. Borghys, S. Van Brandt, M. Surkyn, M. De Cleyn, A. Vos, R. Alexander, G. J. Macdonald, D. Moechars, H. J. Gijsen and A. A. Trabanco, *J Med Chem*, 2015, DOI: 10.1021/acs.jmedchem.5b01101; d) C. R. Butler, M. A. Brodney, E. M. Beck, G. Barreiro, C. E. Nolan, F. Pan, F. Vajdos, K. Parris, A. H. Varghese, C. J. Helal, R. Lira, S. D. Doran, D. R. Riddell, L. M. Buzon, J. K. Dutra, L. A. Martinez-Alsina, K. Ogilvie, J. C. Murray, J. M. Young, K. Atchison, A. Robshaw, C. Gonzales, J. Wang, Y. Zhang and B. T. O'Neill, *J. Med. Chem.*, 2015, **58**, 2678-2702; e) M. A. Brodney, E. M. Beck, C. R. Butler, G. Barreiro, E. F. Johnson, D. Riddell, K. Parris, C. E. Nolan, Y. Fan, K. Atchison, C. Gonzales, A. E. Robshaw, S. D. Doran, M. W. Bundesmann, L. Buzon, J. Dutra, K. Henegar, E. LaChapelle, X. Hou, B. N. Rogers, J. Pandit, R. Lira, L. Martinez-Alsina, P. Mikochik, J. C. Murray, K. Ogilvie, L. Price, S. M. Sakya, A. Yu, Y. Zhang and B. T. O'Neill, *J. Med. Chem.*, 2015, **58**, 3223-3252; f) A. E. Minatti, J. D. Low, J. R. Allen, J. Chen, N. Chen, Y. Cheng, T. Judd, Q. Liu, P. Lopez, W. Qian, S. Rumfelt, R. M. Rzasa, N. A. Tamayo, Q. Xue, B. Yang and W. Zhong, WO2014134341A1, 2014 CAN 2014:1474314; g) A. E. Minatti, J. D. Low, J. R. Allen, A. Amegadzie, J. Brown, M. J. Frohn, A. Guzman-Perez, P. E. Harrington, P. Lopez, V. V. Ma, N. Nishimura, W. Qian, S. Rumfelt, R. M. Rzasa, K. Sham, A. L. Smith, R. White and Q. Xue, WO2014138484A1, 2014 CAN 2014:1520687; h) F. M. Martin, D. J. Mergott and W. M. Owton, WO2014066132A1, 2014 CAN 2014:717282; i) M. A. Brodney, C. R. Butler, E. A. Lachapelle and B. T. O'Neill, WO2014097038A1, 2014 CAN 2014:1041505; j) E. M. Beck, M. A. Brodney, C. R. Butler and B. T. O'Neill, WO2014125397A1, 2014 CAN 2014:1389914; k) R. White, Y. Cheng, A. E. Minatti, B. Yang, X. M. Zheng, P. Lopez, J. B. Human, O. Epstein, T. Judd, K. Sham and Q. Xue, WO2013044092A1, 2013 CAN 2013:464395; l) A. Quattropani and D. Swinnen, EP2669286A1, 2013 CAN 2013:1873480; m) R. M. Lueoend, R. Machauer, H. Rueeger and S. J. Veenstra,

WO2013027188A1, 2013 CAN 2013:318778; n) K. Hurth, S. Jacquier, R. Machauer, H. Rueeger, M. Tintelnot-Blomley, S. J. Veenstra and M. Voegtle, WO2013054291A1, 2013 CAN 2013:600740; o) H. Hilpert, W. Guba, T. J. Woltering, W. Wostl, E. Pinard, H. Mauser, A. V. Mayweg, M. Rogers-Evans, R. Humm, D. Krummenacher, T. Muser, C. Schnider, H. Jacobsen, L. Ozmen, A. Bergadano, D. W. Banner, R. Hochstrasser, A. Kuglstatter, P. David-Pierson, H. Fischer, A. Polara and R. Narquizian, *J. Med. Chem.*, 2013, **56**, 3980-3995; p) H. J. M. Gijsen and S. F. A. Van Brandt, WO2013083556A1, 2013 CAN 2013:932308; q) T. Woltering, WO2012168164A1, 2012 CAN 2012:1819565; r) R. Narquizian, E. Pinard and W. Wostl, US20120302549A1, 2012 CAN 2012:1743240; s) E. Borroni, L. Gobbi, H. Hilpert, M. Honer, D. Muri, R. Narquizian and A. Polara, WO2012168175A1, 2012 CAN 2012:1819575; t) C. T. Clark, A. Cook, I. W. Gunawardana, K. W. Hunt, N. C. Kallan, M. Siu, A. A. Thomas and M. Volgraf, WO2011123674A1, 2011 CAN 2011:1269689; u) D. Banner, W. Guba, H. Hilpert, H. Mauser, A. V. Mayweg, R. Narquizian, E. Pinard, E. Power, M. Rogers-Evans, T. Woltering and W. Wostl, WO2011069934A1, 2011 CAN 2011:755654; v) J. E. Audia, D. J. Mergott, S. M. Sheehan and B. M. Watson, WO2009134617A1, 2009 CAN 2009:1367388.

38. Z. Zhu, A. Stamford, M. Mandal and X. Liu, WO2008073365A1, 2008 CAN 2008:735774.
39. S. Badiger, M. Chebrolu, K. Hurth, S. Jacquier, R. M. Lueoend, R. Machauer, H. Rueeger, M. Tintelnotblomley and M. Voegtle, IN2011DE00077A, 2013 CAN 2013:1408303.
40. H. Rueeger, WO2012095521A1, 2012 CAN 2012:1043760.
41. a) A. A. Trabanco-Suarez, H. J. M. Gijsen, M. L. M. Van Gool, J. A. Vega Ramiro and F. Delgado-Jimenez, WO2012117027A1, 2012 CAN 2012:1301381; b) A. A. Trabanco-Suarez, F. Delgado-Jimenez and G. J. Tresadern, WO2012000933A1, 2012 CAN 2012:27902.
42. A. A. Trabanco-Suarez, G. J. Tresadern, G. J. MacDonald and J. A. Vega Ramiro, WO2011154374A1, 2011 CAN 2011:1612989.
43. G. Tresadern, F. Delgado, O. Delgado, H. Gijsen, G. J. Macdonald, D. Moechars, F. Rombouts, R. Alexander, J. Spurlino, M. Van Gool, J. A. Vega and A. A. Trabanco, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 7255-7260.
44. S. Yonezawa, T. Yamamoto, H. Yamakawa, C. Muto, M. Hosono, K. Hattori, K. Higashino, T. Yutsudo, H. Iwamoto, Y. Kondo, M. Sakagami, H. Togame, Y. Tanaka, T. Nakano, H. Takemoto, M. Arisawa and S. Shuto, *J. Med. Chem.*, 2012, **55**, 8838-8858.
45. T. Woltering, WO2013004676A1, 2013 CAN 2013:55151.
46. K.-I. Kusakabe, S. Yoshida, K. Nakahara, T. Hasegawa, G. Tadano and K. Fuchino, WO2014065434A1, 2014 CAN 2014:713484.
47. T. Dineen, M. Weiss, V. F. Patel, X. M. Zheng and R. White, WO2012109165A1, 2012 CAN 2012:1201541.
48. M. Drag and G. S. Salvesen, *Nat. Rev. Drug Discov.*, 2010, **9**, 690-701.
49. a) A. N. Hodder, B. E. Sleebs, P. E. Czabotar, M. Gazdik, Y. Xu, M. T. O'Neill, S. Lopaticki, T. Nebl, T. Triglia, B. J. Smith, K. Lowes, J. A. Boddey and A. F. Cowman, *Nature structural & molecular biology*, 2015, **22**, 590-596; b) B. E. Sleebs, S. Lopaticki, D. S. Marapana, M. T. O'Neill, P. Rajasekaran, M. Gazdik, S. Gunther, L. W. Whitehead, K. N. Lowes, L. Barfod, L. Hviid, P. J. Shaw, A. N. Hodder, B. J. Smith, A. F. Cowman and J. A. Boddey, *PLoS Biol.*, 2014, **12**, e1001897.
50. M. J. Coffey, B. E. Sleebs, A. D. Uboldi, A. L. Garnham, M. Franco, N. D. Marino, M. W. Panas, D. J. P. Ferguson, M. Enciso, M. T. O'Neill, S. Lopaticki, R. J. Stewart, G. Dewson, G. K. Smyth, B. J. Smith, S. L. Masters, J. C. Boothroyd, J. A. Boddey and C. J. Tonkin, *eLife*, 2015, 10.7554/eLife.10809.
51. G. Berchem, M. Glondu, M. Gleizes, J.-P. Brouillet, F. Vignon, M. Garcia and E. Liaudet-Coopman, *Oncogene*, 2002, **21**, 5951-5965.