HHS Public Access

Author manuscript

Bioorg Med Chem. Author manuscript; available in PMC 2017 September 01.

Published in final edited form as:

Bioorg Med Chem. 2016 September 1; 24(17): 3953–3963. doi:10.1016/j.bmc.2016.04.047.

Identification of Selective Covalent Inhibitors of Platelet Activating Factor Acetylhydrolase 1B2 from the Screening of an Oxadiazolone-Capped Peptoid-azapeptoid Hybrid Library

Bani Kanta Sarma[#1], **Xiaodan Liu**[#2], and **Thomas Kodadek**[2,*]

[1]Department of Chemistry, School of Natural Sciences, Shiv Nadar University, Dadri, Uttar Pradesh-201314, India

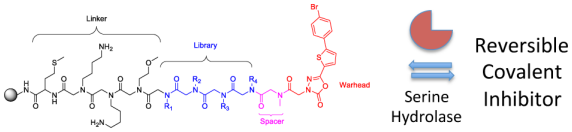
[2]Departments of Chemistry, Scripps Research Institute, Scripps Florida, 130 Scripps Way, #3A2, Jupiter, Fl 33458, USA

[#] These authors contributed equally to this work.

Abstract

A potent and selective inhibitor of platelet-activating factor acetylhydrolase 1B2 (PAFAH1B2) is described. The compound was derived by improvement of a modest affinity primary hit isolated from the screening of a bead-displayed peptoid-azapeptoid hybrid library tethered to an oxadiazolone “warhead”. The oxadiazolone moiety of the inhibitors was found to react covalently with the active site serine residue of PAFAH1B2. This screening strategy may be useful for the identification of many selective, covalent inhibitors of serine hydrolases.

Graphical Abstract



1. INTRODUCTION

In recent years, high-throughput screening (HTS) of large compound collections has become the most common way to identify bioactive small molecules. Most HTS campaigns are conducted using functional assays in which the ability of a small molecule to stimulate or inhibit some process is monitored. An alternative strategy is to screen for binding to a target

[*]Corresponding authors To whom correspondence should be addressed. kodadek@scripps.edu.

Supporting Information

Experimental details and characterization of compounds are provided in the supporting information.

Notes

The authors declare no competing financial interest.

of interest. One way to do this is to incubate a labeled target with a large number of bead-displayed molecules, then somehow pick the beads that retain the labeled target. Vast one bead one compound (OBOC) libraries can be synthesized using the split and pool strategy.[1,2] This approach is particularly suitable for oligomeric compounds such as peptides[3,4] and peptoids[5,6] constructed from diverse building blocks. TentaGel beads have emerged as the screening platform of choice due to their unique combination of mechanical stability, swell ability in both aqueous and organic solvents and low non-specific protein binding.[6] Of course, primary screening hits tend to be of modest affinity or potency, requiring optimization. This can also be done using OBOC screening, for example, by tethering the primary hit to a new combinatorial library[7,8] or through the construction of a "derivative library" that contains thousands of compounds that explore the "chemical space" around the primary screening hit.[9] In both cases, re-screening against the same target but under more demanding conditions has been shown to result in improved ligands. Many bioactive compounds have been identified using this approach.

The discovery of covalent inhibitors via OBOC library screening has attracted much less attention, despite the fact that this is an attractive strategy for the development of potent compounds.[10,11] The idea is that a "warhead" that recognizes most or all of the members of a class of target proteins ("pan-specific") will provide each molecule in the screening collection with a "toehold" on the target. The hope is that the additional residues tethered to the pan-specific ligand will not only provide additional potency through secondary contacts with the target, but that these contacts will also result in selectivity for a particular member of the target class. An attractive feature of protein binding via formation of a covalent adduct is that long-lived ligand-protein complexes are formed, which tends to endow compounds with a favorable pharmacological profile in vivo.[12-15] There are two types of covalent warheads that can be employed in this approach, reversible and irreversible modifiers. A potential advantage of reversible covalent modifiers is that off-target binding is not permanent as is the case for irreversible inhibitors and this may provide a better safety profile for drug candidates.[15] Thus we were interested in exploring the use of OBOC library screening to identify novel covalent, reversible inhibitors.

In considering where to begin with such an effort, a focus on serine hydrolases (SHs) seemed reasonable. SHs are one of the most diverse enzyme classes. They comprise approximately 1% of the genes in the human proteome and many enzymes in the SH superfamily are targets of approved drugs.[16] Various scaffolds having electrophilic groups able to modify the active site serine in SHs covalently have been developed[17-25], including many that are reversible[26-40]. Nonetheless, even with the existence of such diverse scaffolds, it is rather challenging to develop selective inhibitors against individual members of the SH family. A case in point is the interesting SH called platelet-activating factor acetylhydrolase 1B2 (PAFAH1B2), which is involved in various aspects of development and has been implicated in cancer progression.[41] Previous efforts using collections of serine hydrolase-directed inhibitors such as lactones[19], carbamates[22], heterocyclic ureas[24], etc. failed to produce any PAFAH1B2 inhibitors. Indeed, only very recently has a selective PAFAH1B2 inhibitor been reported by the Cravatt laboratory using an elegant activity-based protein profiling (ABPP)-directed strategy.[42] However, this is a noncovalent reversible inhibitor. Thus, we considered PAFAH1B2 as a challenging, worthwhile target with which to develop

bead-based screening for reversible covalent inhibitors. As a first step, we recently developed a method to synthesize 1,3,4-oxadiazol-2-one-terminated compounds on the solid phase.[43] This moiety has been shown previously to be capable of effecting reversible inhibition of SHs. Examples include compound 7600 and its derivatives[32] and CAY10499[33], which are potent human hormone-sensitive lipase (HSL) inhibitors (Figure 1). Recently, potent and selective 1,3,4-oxadiazol-2-ones-based FAAH inhibitors have also been reported.[35,44-46] Of the many 1,3,4-oxadiazol-2-ones we synthesized,[43] one (compound **1**, Figure 2) proved to be a moderately potent, pan-selective inhibitor of SHs as determined by ABPP.[47] Herein, we describe the construction of a OBOC peptoid-azapeptoid hybrid library capped with **1**. Screening of this OBOC library against His_6-tagged PAFAH1B2 led to the discovery of a few modestly potent and moderately selective inhibitors. The optimization of one such screening hit led to the discovery of an inhibitor with good selectivity for PAFAH1B2.

2. RESULTS AND DISCUSSION

2.1. Design and synthesis of OBOC library capped with "warhead" 1

In a previous report,[43] we demonstrated that 1,3,4-oxadiazol-2-one moieties could be constructed efficiently at the end of a peptoid chain using solid-phase synthesis as shown in Figure 2. In this study, we also wished to incorporate aza-peptoid units since these provide additional opportunities for hydrogen bonding to target proteins and also have unique conformational properties. To test the compatibility of solid-phase incorporation of 1,3,4-oxadiazol-2-one moieties with azapeptoids derived from different hydrazide-based submonomers, we synthesized compound **2** as shown in Figure 3. To synthesize **2**, the peptoid and azapeptoid submonomers were incorporated as described previously[48,49] and finally the 1,3,4-oxadiazol-2-one ring was installed using our reported protocol.[43] After trifluoroacetic acid (TFA) cleavage and diethyl ether precipitation, the crude reaction mixture was subjected to an analytical HPLC to obtain **2** in more than 95% purity (Figure S1). This shows that peptoid-azapeptoid hybrid oligomers capped with a 1,3,4-oxadiazol-2-one moiety can be synthesized with high purity on solid phase. With these results in hand, we proceeded to incorporate **1** into an OBOC peptoid-azapeptoid hybrid library as a warhead.

A tetrameric peptoid-azapeptoid hybrid library with a theoretical diversity of 14,641 compounds was constructed by split and pool submonomer synthesis (Figure 4) on TentaGel HL NH_2 beads with a diameter of ~75 μm (4×10^6 beads per g, ~100 pmol compounds per bead). To allow for selective cleavage of the library compounds from the resin-support post screening, we first incorporated Fmoc-L-Met-OH using standard HBTU/HOBt chemistry. Next, a tripeptoid sequence (Nlys-Nlys-Nmea) was introduced as a spacer prior to the variable residues. The combinatorial library was comprised of four variable peptoid-azapeptoid residues (positions R_1-R_4). 11 different submonomers were used at each position. To avoid side reactions[48,49] primary amines and aryl acyl hydrazides were employed at positions R_1 and R_3, whereas positions R_2 and R_4 contain various alkyl/benzyl acyl hydrazides, carbazates and semicarbazides (Figure 4). After the fourth variable position, a peptoid unit containing methylamine was incorporated to provide some space between the

variable positions and the oxadiazolone warhead. We observed that without this spacer, unequivocal sequence determination of the compounds in the OBOC library is difficult and in some cases impossible by using tandem MALDI TOF mass spectrometry. Finally, 5-(4-bromophenyl)thiophene-2-carbohydrazide was incorporated and the beads were treated with 4-nitrophenyl chloroformate. Subsequent treatment with DIEA led to an efficient intramolecular ring closure to form the oxadiazolone warhead (see Figure 2).

After deprotection of the side chain protecting groups with 95% TFA, the library beads were washed thoroughly with DMF followed by DCM, and dried to remove residual DCM. To assess the quality of the library, 20 beads were picked at random and subjected to MALDI MS analysis. In each mass spectrum, a single predominant peak was observed. Tandem MALDI TOF/TOF mass spectrometry allowed each of the peptoids to be sequenced, and all submonomers in the library construction were represented in at least one of the sequences (data not shown). We concluded that the library was of high quality and suitable for screening.

2.2. creening of the OBOC library

We planned to screen this library by incubating it with His_6-tagged PAFAH1B2 followed by anti-His magnetic beads (Talon beads) and pulling out beads that become magnetized.[50] Therefore, it was useful to first clear the library of any compounds that might bind to the magnetic beads directly. Approximately 50 mg of resin (200,000 beads) was incubated with a blocking buffer (1% BSA in 1:1 TBST:TBS Starting Block) for 1h. The beads were then washed with Tris-buffered saline containing 0.05% Tween-20 (TBST) and mixed with 120 μL of Talon beads in blocking buffer and incubated for 1h. The conical tube containing the beads was then placed on a magnetic separator, which attracted the library beads that had bound to magnetic beads, while the remainder of the beads settled to the bottom of the conical tube. 29 beads bound to the magnetic Talon beads, which were discarded. This prescreening process was repeated, and 7 beads were removed during this second round, at which point the library was deemed ready for screening.

The library beads were then incubated with 2 μM His_6-tagged PAFAH1B2 in the blocking buffer for 1h. The beads were washed with TBST to remove unbound proteins and then incubated with 120 μL Talon beads in blocking buffer for 1h. The bead suspension was then exposed to a strong magnet. After the non-magnetized beads that had settled to the bottom of the tube were removed, 16 positive beads ("hits") were retrieved by removing the magnet separator. These hit beads were treated with trypsin and then acetonitrile-water (50:50) containing 0.1% TFA to strip the bound proteins. The hit beads were washed extensively with DMF followed by DCM and then subjected to cyanogen bromide (CNBr) cleavage and tandem MALDI TOF mass spectral analysis. The structure of 15 of the 16 hits obtained from the screen could be identified unequivocally from these data (Figure S2).

2.3. Validation

The hits obtained from the screen were resynthesized on Knorr amide MBHA resin without the methionine residue (hit1-hit15 correspond to compounds **3**-**17**) (Figure S3) and tested for their ability to prevent reaction of PAFAH1B2 with a fluorophosphonate-rhodamine

conjugate.[51] Out of these 15 compounds (**3**-**17**), seven (**3**, **6**, **8**, **11**, **13**, **15** and **17**) inhibited PAFAH1B2 more than 50% at a concentration of 50 μM under these conditions (Figure S4A-B). Compounds **3**, **13**, **15** and **17** (Figure 5A) inhibited PAFAH1B2 significantly at 25 μM (Figure 5B), so further attention was focused on these molecules. To test their selectivity, we carried out ABPP studies with a HeLa cell lysate doped with recombinant PAFAH1B2. Compounds **3**, **13**, **15** and **17** showed moderate selectivity against PAFAH1B2 with some off target inhibition of other SHs (Figure 5C). In the ABPP assay with cell lysates, compounds **3** and **13** showed better potency than **15** and **17**. Therefore, we chose **3** and **13** for further studies.

2.4. Identification of the minimal pharmacophore of inhibitors 3 and 13

To identify the minimal pharmacophores of compounds **3** and **13**, we first deleted the methylamine spacer between the variable region and the warhead in **3** and **13**, creating **18** and **19**, respectively (Figure 6). Removal of this residue did not compromise the activity of the compounds in the ABPP assay (Figure 9 and Figure S5). Compounds **18** and **19** have considerable sequence similarity. Variable residues 2 and 3 (counting from C-terminus) of the variable region are identical in **18** and **19**. Therefore, we chose to synthesize compound **20,** which includes only these residues connected to the warhead along with the linker. Somewhat to our surprise, since this alters the spacing between the conserved residues and the warhead, compound **20** showed better potency against PAFAH1B2 than the parent compounds **18** and **19** (Figure 9). However, compound **20** showed only modest selectivity for PAFAH1B2 in an ABPP assay using the protein doped into HeLa cell extract (Figure 9). Note that due to our choice of submonomers at positions R_1-R_4 (Figure 3), our library did not have any compound with a sequence similar to that present in **20**. We also observed that removal of the linker (Nlys-Nlys-Nmea) from compound **20** does not affect its inhibition potency (Figure S6A). However, removal of the linker decreases the solubility of the compound in buffer and also considerably reduces its peak intensity in MALDI TOF MS spectra (Figure S6B). Therefore, during the optimization process of **20**, we kept the linker part (Nlys-Nlys-Nmea) as well.

2.5. Improvement of the minimal pharmacophore 20

The minimal pharmacophore **20** consists of two azapeptoid residues, excluding the warhead. The warhead is the result of exploring many similar structures,[43] so we focused on improving binding by manipulating the nature of the azapeptoid side chains. We chose to do so initially by varying the nature of the semicarbazide, since only a single semicarbazide (4-phenyl semicarbazide) was included in the original library. Compounds **21-37** were set as the initial targets (Figure 7).

As not many semicarbazides are commercially available, a solid-phase synthetic route to incorporate different semicarbazide residues was developed (Figure 7A). We first incorporated the linker region (Nlys-Nlys-Nmea) using a standard sub-monomer peptoid synthesis protocol.[51] The beads were then bromoacylated using bromoacetic acid and DIC and then treated with NH_2-NHDdz at 37 °C overnight (~15h). The N-terminus was then alloc-protected using allyl chloroformate in DCM in presence of DIEA. Next, the side chain Ddz group was removed using 1% TFA in DCM. The beads were neutralized by washing

with 1% DIEA in DCM and the free hydrazide NH_2 group was reacted with 4-nitrophenyl chloroformate in presence of DIEA for 3h at 37 °C (twice). The beads were washed with DCM and DMF and then reacted with different primary amines for 24h at 60 °C to displace the 4-nitrophenol group. The N-terminal alloc group was then removed using $Pd(PPh_3)_4$ in the presence of phenylsilane. The chain extension from the N-terminus was then achieved by standard sub-monomer synthesis. Finally, the warhead was installed as discussed previously.[42]

The potency and selectivity of compounds **21**-**37** against different SHs were tested in a HeLa cell lysate doped with recombinant PAFAH1B2 using the single dose ABPP assay, as discussed above. All of the compounds (**21**-**37**) inhibited most of the SHs in a HeLa cell lysate detectable by ABPP (Figure S7). Many of them had a potency comparable to that of **20**, but compounds **23**, **28**, **35** and **37** appeared somewhat more active (Figure S7) and thus were analyzed more carefully in a dose-dependence experiment (Figure 8). We observed that compound **35** is more selective than parent compound **20** with less off-target effects at approximate IC_{50} 3 μM. **35** also shows better dose dependent behavior. As can be seen from its chemical structure, the semicarbazide motif of **20** is derived from aniline (Figure 7). Among the derivatives (**21**-**37**) of **20** that we synthesized, **35** is the only compound with a semicarbazide motif derived from aniline (4-Br-aniline). This indicates that anilines may be preferred at this position over other aliphatic and benzylic amines. Therefore, we synthesized compounds **38**-**46** (Figure 7), having different substituted anilines at the semicarbazide position. We carried out the ABPP inhibition assay with compounds **38**-**46** as discussed above and observed that compound **45** exhibited improved selectivity towards PAFAH1B2 compared to **20** and **35** (Figure 9 and Figure S8) The PAFAH1B2 activity was completely abolished by **45** at 100 μM, with little off-target effects (Figure 9). Taken together, as shown in Figure 9, throughout the optimization process, we obtained the final derivative with better potency and selectivity than the initial hits from library screening. The known inhibitor methyl-arachidonyl fluorophosphonate (MAFP) was also tested as a positive control in the ABPP assay. As shown in Figure 9, at concentration of 685 nM, MAFP inhibited more than 50% of PAFAH1B2 activity in the cell lysate; however, the inhibition is totally non-specific with significant off-target effects.

The substrate assay was also performed to evaluate the potency of the primary hits and the derivatives. 2-thiol PAF was used as the substrate. Upon hydrolysis by PAFAH1B2 the product with free thiol can be detected by Ellman's reagent. The IC_{50} values are presented in Figure 10. MAFP inhibited PAFAH1B2 with an IC_{50} value of 70 nM, which is consistent with the literature report.[52] The derivatives have lower IC_{50} values than the primary hits, suggesting that the optimization yields more potent inhibitors. Interestingly, compound **47** (Figure 7) that contains all the residues of **20** except the carbonyl group that is involved in the ester bond formation to generate the oxadiazolone ring, inhibited the enzyme with an IC_{50} value 34 μM, which is seven fold higher than **20**, indicating that the 1,3,4-oxadiazol-2-one moiety contributes significantly to the potency of the compound. This is consistent with the results of the ABPP assay wherein compound **47** exhibited weaker inhibition potency than compound **20** as well (Figure 9). This result also supports our hypothesis that the

peptoid moiety provides secondary contact with the enzyme active site pocket to further increase the reactivity of the tethered warhead.

2.6. Covalent Nature of Inhibition

The design of the warhead **1** was based on the assumption that SHs will react covalently with the oxadiazolone moiety as was the case with oxadiazolone containing HSL inhibitors such as CAY10499[33] and compound 7600[32]. To determine if covalent protein-inhibitor adducts had formed, whole protein MALDI-TOF mass spectroscopic analysis was employed. We used diluted sinapinic acid matrix (saturated sinapinic acid matrix solution was diluted ten-fold) to stabilize the protein-inhibitor adduct while acquiring MALDI TOF mass spectra. The peaks corresponding to the protein-inhibitor adducts with most of our inhibitors were observed after 5 hour incubation (Figure 11 and Figure S10). The protein-inhibitor adduct peaks of PAFAH1B2 with compounds **20** and **35** became more significant after 24 hour incubation (Figure 11), indicating that the inhibitors are slow covalent binders. There is an additional peak observed on the whole protein mass spectrometric analysis of PAFAH1B2-inhibitor incubations, suggesting the possibility of more than one site for reaction (Figure 11 and Figure S10). Interestingly, no such protein-inhibitor complex peak was observed with compound **47** where the oxadiazolone ring is absent indicating that the oxadiazolone ring is necessary for covalent reaction between PAFAH1B2 and inhibitors (Figure 11). We failed to detect protein-inhibitor complex by MALDI TOF MS in saturated sinapinic acid matrix even after 24 h incubation of 2 μM PAFAH1B2 with 100 μM of the inhibitors suggesting that the protein-inhibitor complexes are unstable in the acidic condition. This is not surprising considering that these inhibitors might be reversible covalent inhibitors.[53] It is difficult to detect protein-inhibitor complexes for covalent reversible inhibitors by mass spectrometry and a recent inhibition study of lipolytic proteins with compound 7600 also supports our observation.[39] We tried to identify the protein residues that react with the inhibitors. We could see the disappearance of the peak corresponding to native active site peptide fragment of PAFAH1B2 after incubation with compound **20**, suggesting that modification occurred at this fragment (Figure S11). However, we were not able to detect the modified fragment by MALDI and HPLC/MS/MS.

We also tested the time-dependent inhibition of PAFAH1B2 by our compounds using both ABPP assay (Figure 12) and substrate assay (Figure 13). Both assays indicated that the inhibition potency significantly increased for the primary hits with increase in incubation time, but not for the derivatives. These results suggest that the derivatives may have higher affinity with PAFAH1B2, therefore, the inhibition can quickly reach the equilibrium. In contrast, the primary hits need longer incubation time to display better potency, which is also reflected in their speed of protein-inhibitor adduct formation.

Unlike, HSL inhibitors CAY10499[33] and compound 7600[32] (Figure 14A), the reaction mixture of PAFAH1B2 and our inhibitors (**20** or **35**), did not show any product corresponding to the hydrolysis of **20** or **35**. Therefore, we believe that our compounds are slow covalent inhibitors that work via a different mechanism than HSL inhibitors CAY10499 and compound 7600. Inhibitors CAY10499 and compound 7600 both have a methoxy substituent on the oxadiazolone ring. Once the active site serine residue of HSL attacks at

the carbonyl carbon of the oxadiazolone ring and opens it up, hydrolysis occurs to regenerate the active site of the protein and the compound gets deactivated (Figure 14A).[37] In our case, the inhibitors contain an aromatic substituent on the oxadiazolone ring in place of the methoxy group. We propose that the active site serine residue first opens up the 5-membered oxadiazolone ring. The side chain –NH-hydrogen of the ring opened protein-inhibitor complex may be reactive enough to get deprotonated at physiological pH or by basic amino acid residues close to the active site of PAFAH1B2 to reversibly undergo ring closure to regenerate the oxadiazolone ring and the active site of the protein as shown in Figure 14B. The difference in the mechanism may arise due to the difference in substituents present in inhibitors CAY10499 and compound 7600 (methoxy) and our inhibitors (aromatic ring). We observed that intramolecular cyclization to oxadiazolone works very efficiently when the substituent on the starting compounds are derived from aromatic acyl hydrazides whereas cyclization is very difficult when the starting compounds are derived from alkyl/benzylic hydrazides, carbazates or semicarbazides.[43] In fact, we observed that a methyl hydrazinocarboxylate-derived compound did not undergo cyclization even at 60 °C using the strategy shown at Figure 2.[43]

3. CONCLUSION

In summary, we have developed a solid-phase synthetic method to construct OBOC peptoid-azapeptoid hybrid libraries capped with 1,3,4-oxadiazol-2-ones. We synthesized a OBOC library capped with a 1,3,4-oxadiazol-2-one “warhead” that was previously shown to be a moderate pan-selective SH inhibitor. On bead screening of the library against PAFAH1B2 produced several hits that were also pan-selective inhibitors of SHs but more potent than the “warhead”. Identification of the minimal pharmacophore of the most potent initial hit and further optimization led to the discovery of a selective covalent reversible inhibitor of PAFAH1B2 with low μM inhibition potency in cell lysates. We observed that the oxadiazolone moiety of our inhibitors reacted covalently with the active site serine residue of PAFAH1B2. We believe that such a screening strategy of combinatorial libraries tethered to moderate affinity warheads against specific targets may be used on a general basis to identify more potent and selective compounds.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Institutes of Health (GM090294).

References

1. Lam KS, Salmon SE, Hersh EM, Hruby VJ, Kazmierski WM, Knapp RJ. Nature. 1991; 354:82. [PubMed: 1944576]
2. Houghten RA, Pinilla C, Blondelle SE, Appel JR, Dooley CT, Cuervo JH. Nature. 1991; 354:84. [PubMed: 1719428]
3. Lian W, Upadhyaya P, Rhodes CA, Liu Y, Pei D. J. Am. Chem. Soc. 2013; 135:11990. [PubMed: 23865589]

4. Liu T, Qian Z, Xiao Q, Pei D. ACS Comb. Sci. 2011; 13:537. [PubMed: 21848276]
5. Figliozzi GM, Goldsmith R, Ng SC, Banville SC, Zuckermann RN. Methods Enzymol. 1996; 267:437. [PubMed: 8743331]
6. Alluri PG, Reddy MM, Bacchawat-Sikder K, Olivos HJ, Kodadek T. J. Am. Chem. Soc. 2003; 125:13995. [PubMed: 14611236]
7. Agnew HD, Rohde RD, Millward SW, Nag A, Yeo W–S, Hein JE, Pitram SM, Tariq AA, Burns VM, Krom RJ, Fokin VV, Sharpless BK, Heath JR. Angew. Chem., Int. Ed. Engl. 2009; 48:4944. [PubMed: 19301344]
8. Lim H–S, Reddy MM, Xiao X, Wilson J, Wilson R, Connell S, Kodadek T. Bioorg. Med. Chem. Lett. 2009; 19:3866. [PubMed: 19380225]
9. Gao Y, Amar S, Pahwa S, Fields G, Kodadek T. ACS Comb. Sci. 2015; 17:49. [PubMed: 25434974]
10. Shannon DA, Weerapana E. Curr. Opin. Chem. Biol. 2015; 24:18. [PubMed: 25461720]
11. Potashman MH, Duggan ME. J. Med. Chem. 2009; 52:1231. [PubMed: 19203292]
12. Copeland RA, Pompliano DL, Meek TD. Nat. Rev. Drug. Discov. 2006; 5:730. [PubMed: 16888652]
13. Tummino PJ, Copeland RA. Biochemistry. 2008; 47:5481. [PubMed: 18412369]
14. Lu H, Tonge PJ. Curr. Opin. Chem. Biol. 2010; 14:467. [PubMed: 20663707]
15. Bradshaw JM, McFarland JM, Paavilainen VO, Bisconte A, Tam D, Phan VT, Romanov S, Finkle D, Shu J, Patel V, Ton T, Li X, Loughhead DG, Nunn PA, Karr DE, Gerritsen ME, Funk JO, Owens TD, Verner E, Brameld KA, Hill RJ, Goldstein DM, Taunton J. Nat. Chem. Biol. 2015; 11:525. [PubMed: 26006010]
16. Ross MK, Wang R. Chem. Biol. 2015; 22:808. [PubMed: 26207294]
17. Tew DG, Boyd HF, Ashman S, Theobald C, Leach CA. Biochemistry. 1998; 37:10087. [PubMed: 9665713]
18. Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, Tontini A, Mor M, Tarzia G, La Rana G, Calignano A, Giustino A, Tattoli M, Palmery M, Cuomo V, Piomelli D. Nat. Med. 2003; 9:76. [PubMed: 12461523]
19. Hoover HS, Blankman JL, Niessen S, Cravatt BF. Bioorg. Med. Chem. Lett. 2008; 18:5838. [PubMed: 18657971]
20. Bottcher T, Sieber SA. Angew. Chem. Int. Ed. Engl. 2008; 47:4600. [PubMed: 18383487]
21. Ahn K, Johnson DS, Mileni M, Beidler D, Long JZ, McKinney MK, Weerapana E, Sadagopan N, Liimatta M, Smith SE, Lazerwith S, Stiff C, Kamtekar S, Bhattacharya K, Zhang Y, Swaney S, Van Becelaere K, Stevens RC, Cravatt BF. Chem. Biol. 2009; 16:411. [PubMed: 19389627]
22. Bachovchin DA, Ji T, Li W, Simon GM, Blankman JL, Adibekian A, Hoover H, Niessen S, Cravatt BF. Proc. Natl. Acad. Sci. U.S.A. 2010; 107:20941. [PubMed: 21084632]
23. Bachovchin DA, Mohr JT, Speers AE, Wang C, Berlin JM, Spicer TP, Fernandez-Vega V, Chase P, Hodder PS, Schurer SC, Nomura DK, Rosen H, Fu GC, Cravatt BF. Proc. Natl. Acad. Sci. U.S.A. 2011; 108:6811. [PubMed: 21398589]
24. Adibekian A, Martin BR, Wang C, Hsu K-L, Bachovchin DA, Niessen S, Hoover H, Cravatt BF. Nat. Chem. Biol. 2011; 7:469. [PubMed: 21572424]
25. Bachovchin DA, Cravatt BF. Nat. Rev. Drug Discov. 2012; 11:52. [PubMed: 22212679]
26. Steitz TA, Shulman RG. Annu. Rev. Biophys. Bioeng. 1982; 11:419. [PubMed: 7049067]
27. Imperiali B, Abeles RH. Biochemistry. 1986; 25:3760. [PubMed: 3527255]
28. Liang T-C, Ringe D, Abeles RH. Arch. Biochem. Biophys. 1987; 252:626. [PubMed: 3813553]
29. Brady K, Wei A, Ringe D, Abeles RH. Biochemistry. 1990; 29:7600. [PubMed: 2271520]
30. Zhong S, Haghjoo K, Kettner C, Jordan F. J. Am. Chem. Soc. 1995; 117:7048.
31. Jiang F, Tsai S-W, Chen S, Makinen MW. J. Phys. Chem. B. 1998; 102:4619.
32. Ben Ali Y, Chahinian H, Petry S, Muller G, Lebrun R, Verger R, Carrière F, Mandrich L, Rossi M, Manco G, Sarda L, Abousalham A. Biochemistry. 2006; 45:14183. [PubMed: 17115713]
33. Muccioli GG, Labar G, Lambert DM. ChemBioChem. 2008; 9:2704. [PubMed: 18855964]
34. Tamada T, Kinoshita T, Kurihara K, Adachi M, Ohhara T, Imai K, Kuroki R, Tada T. J. Am. Chem. Soc. 2009; 131:11033. [PubMed: 19603802]

35. Minkkila A, Savinainen JR, Käsnänen H, Xhaard H, Nevalainen T, Laitinen JT, Poso A, Leppanen J, Saario SM. ChemMedChem. 2009; 4:1253. [PubMed: 19472270]
36. Minkkilä A, Savinainen JR, Käsnänen H, Xhaard H, Nevalainen T, Laitinen JT, Poso A, Leppänen J, Saario SM. ChemMedChem. 2009; 4:1253. [PubMed: 19472270]
37. Ben Ali Y, Verger R, Carrière F, Petry S, Muller G, Abousalham A. Biochimie. 2012; 94:137. [PubMed: 22008857]
38. Point V, Kumar PKVP, Marc S, Delorme V, Parsiegla G, Amara S, Carrière F, Buono G, Fotiadu F, Canaan S, Leclaire J, Cavalier J-F. Eur. J. Med. Chem. 2012; 58:452. [PubMed: 23153815]
39. Delorme V, Diomandé SV, Dedieu L, Cavalier J–F, Carrière F, Kremer L, Leclaire J, Fotiadu F, Canaan S. Plos One. 2012; 7:e46493. [PubMed: 23029536]
40. Ehmann DE, Jahić H, Ross PL, Gu R-F, Hu J, Kern G, Walkup GK, Fisher SL. Proc. Natl. Acad. Sci. U.S.A. 2012; 109:11663. [PubMed: 22753474]
41. Arai H, Koizumi H, Aoki J, Inoue K. J. Biochem. 2002; 131:635. [PubMed: 11983068]
42. Chang JW, Zuhl AM, Speers AE, Niessen S, Brown SJ, Mulvihill MM, Fan YC, Spicer TP, Southern M, Scampavia L, Vega VF, Dix MM, Cameron MD, Hodder PS, Rosen H, Nomura DK, Kwon O, Hsu K-L, Cravatt BF. ACS Chem Biol. 2015; 10:925. [PubMed: 25602368]
43. Sarma BK, Liu X, Wu H, Gao Y, Kodadek T. Org. Biomol. Chem. 2015; 13:59. [PubMed: 25354697]
44. Kiss LE, Ferreira HS, Beliaev A, Torrao L, Bonifáciob MJ, Learmonth DA. Med. Chem. Commun. 2011; 2:889.
45. Käsnänen H, Minkkilä A, Taupila S, Patel JZ, Parkkari T, Lahtela-Kakkonen M, Saario SM, Nevalainen T, Poso A. Eur. J. Pharm. Sci. 2013; 49:423. [PubMed: 23557840]
46. Patel JZ, Parkkari T, Laitinen T, Kaczor AA, Saario SM, Savinainen JR, Navia-Paldanius D, Cipriano M, Leppänen J, Koshevoy IO, Poso A, Fowler CJ, Laitinen JT, Nevalainen T. J. Med. Chem. 2013; 56:8484. [PubMed: 24083878]
47. Alexander JP, Cravatt BF. J. Am. Chem. Soc. 2006; 128:9699. [PubMed: 16866524]
48. Sarma BK, Kodadek T. Chem. Comm. 2011; 47:10590. [PubMed: 21892506]
49. Sarma BK, Kodadek T. ACS Comb. Sci. 2012; 14:558. [PubMed: 22958123]
50. Astle JM, Simpson LS, Huang Y, Reddy MM, Wilson R, Connell S, Wilson J, Kodadek T. Chem. Biol. 2010; 17:38. [PubMed: 20142039]
51. Zuckermann RN, Kerr JM, Kent SBH, Moos WH. J. Am. Chem. Soc. 1992; 114:10646.
52. Simon GM, Cravatt BF. J. Biol. Chem. 2010; 285:11051. [PubMed: 20147750]
53. ABPP assay of reaction mixtures of PAFAH1B2 with compounds 3 and 13 before and after filtration through biospin column shows that compounds 3 and 13 are reversible inhibitors of PAFAH1B2 (see Supporting Information Figure S9)]

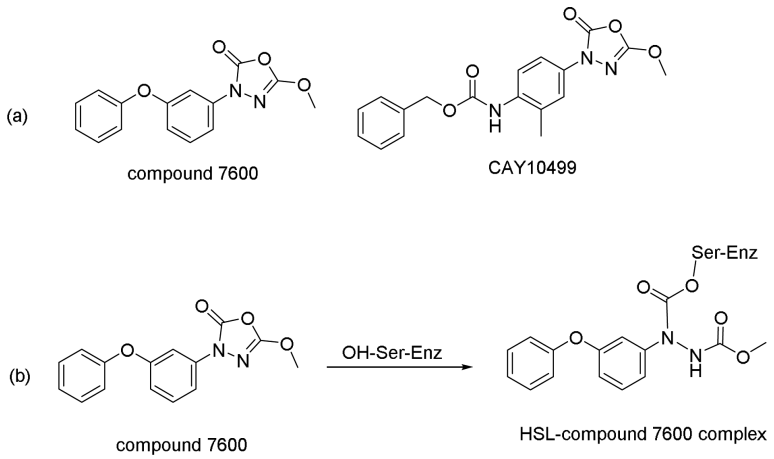


Figure 1.

(a) Chemical structures of hormone-sensitive lipase (HSL) inhibitors compound 7600 and CAY10499 having a 1,3,4-oxadiazol-2-one scaffold. (b) Covalent protein-inhibitor complex formation between HSLs and their inhibitor compound 7600.

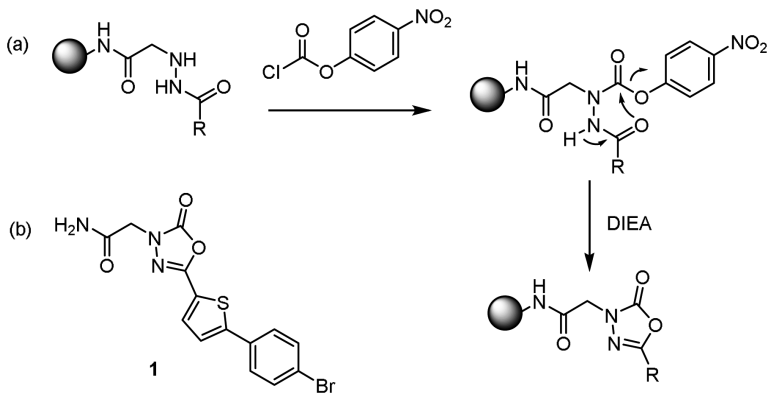


Figure 2.

(a) Synthetic route for the incorporation of 1,3,4-oxadiazol-2-one scaffolds on solid-phase.

(b) Chemical structure of pan-selective SH inhibitor **1**.

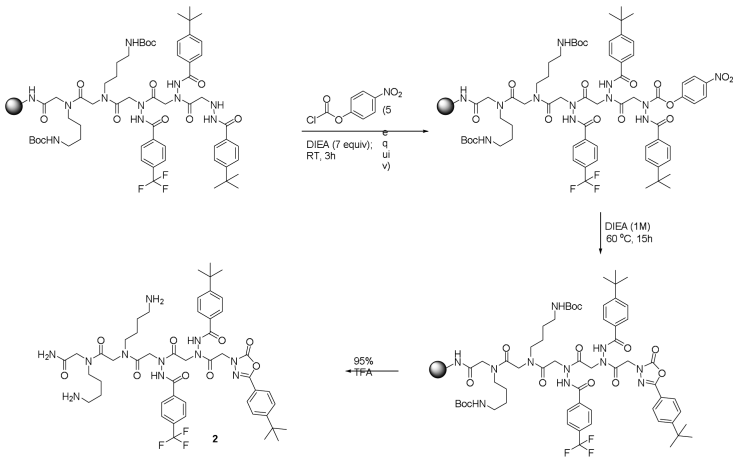


Figure 3.

Synthesis of compound **2**. An azapeptoid synthesized by submonomer method is capped with an oxadiazolone moiety. Incorporation of the oxadiazolone moiety on the solid phase is compatible with the hydrazide-based submonomers present in the azapeptoid.

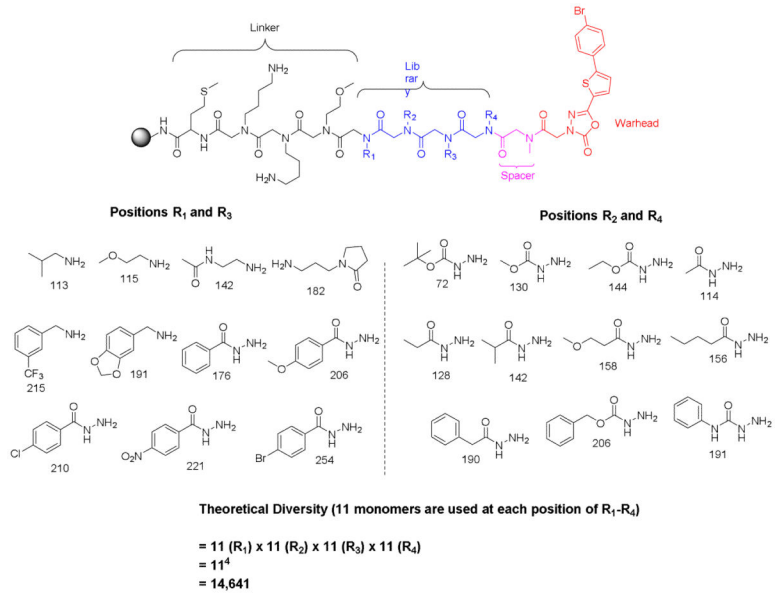


Figure 4.

General structure of the OBOC peptoid-azapeptoid hybrid library tethered with the SH inhibitor **1**. A tetrameric peptoid-azapeptoid hybrid library with a theoretical diversity of 14,641 compounds was constructed by split and pool submonomer synthesis on TentaGel HL NH_2 beads. After incorporating methionine, a constant tripeptoid sequences (Nlys-Nlys-Nmea) was introduced as a spacer prior to library sequence (in black). The variable azapeptoid part (positions R_1-R_4 in blue) of the library has four residues and 11 different submonomers were used in each position. Positions R_1 and R_3 comprise of primary amines and aryl acyl hydrazides whereas positions R_2 and R_4 contain various alkyl/benzyl acyl hydrazides, carbazates and semicarbazides (in blue). After the fourth variable position N-methylglycine (in pink) was incorporated to provide some space between the variable position and the warhead. The warhead is shown in red.

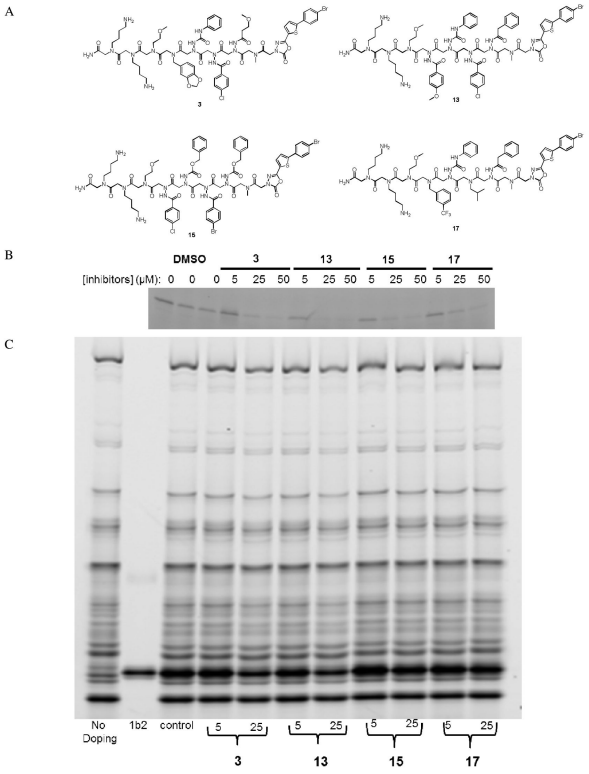


Figure 5.

(A) Chemical structures of most potent initial hits **3**, **13**, **15** and **17**. (B) Dose dependent inhibition on PAFAH1B2 by compounds **3**, **13**, **15** and **17** (5, 25 and 50 μM compounds were used) against PAFAH1B2 (2 μM). (C) Selectivity evaluation of compounds **3**, **13**, **15** and **17** (5 and 25 μM compounds were used) against PAFAH1B2 (100 nM) doped in a HeLa cell lysate (1 mg/mL) by ABPP assay. Compounds **3**, **13**, **15** and **17** show pan-selective inhibition against serine hydrolases (SHs) as suggested by ABPP assay. However, compounds **3** and **13** show better inhibitory potency than **15** and **17**.

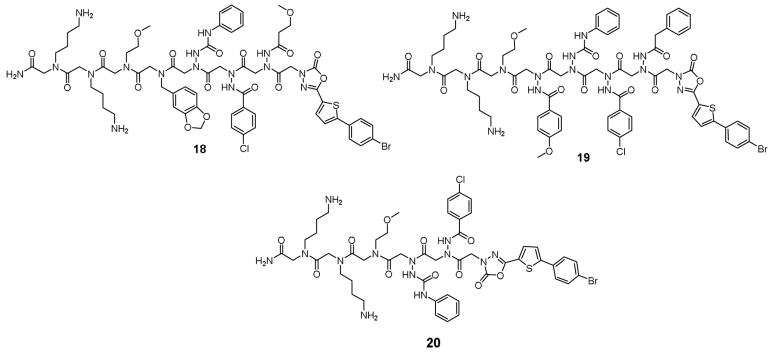


Figure 6.
Chemical structures of compound **18**, **19** and **20**.

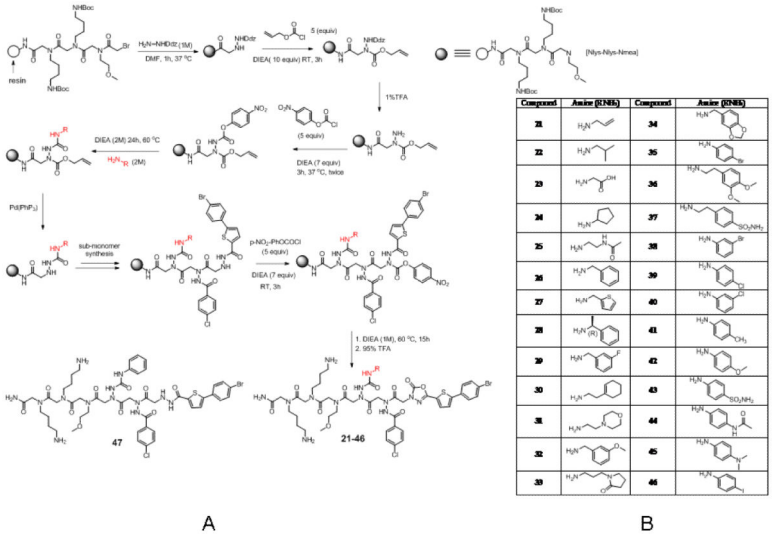


Figure 7.

(A) General route for the synthesis of minimal pharmacophore derivatives **21-46.** (B) Different substituents at the variable position of compounds **21-46**.

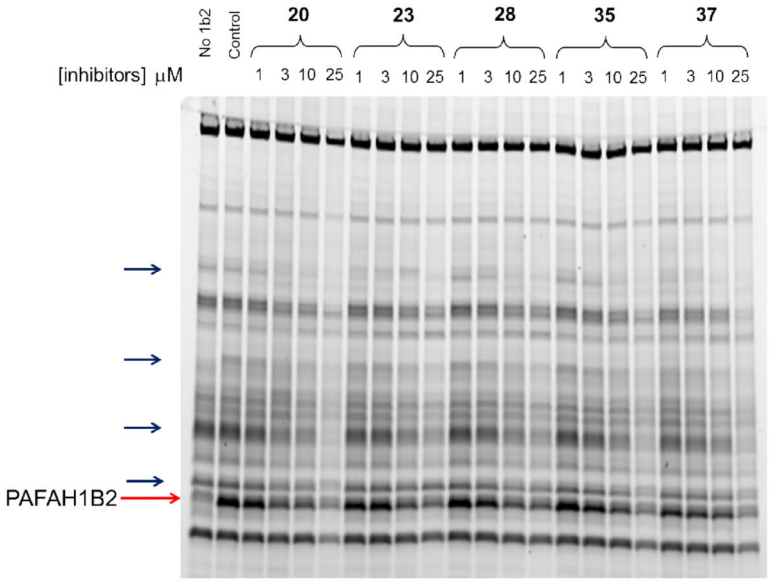


Figure 8.

Dose dependent inhibition of doped PAFAH1B2 (100 nM) by compounds **20**, **23**, **28**, **35** and **37** in a HeLa cell lysate (1mg/mL). Compound concentrations used are 1μM, 3μM, 10μM and 25μM). Compound **35** shows much better dose dependence and better selectivity for PAFAH1B2.

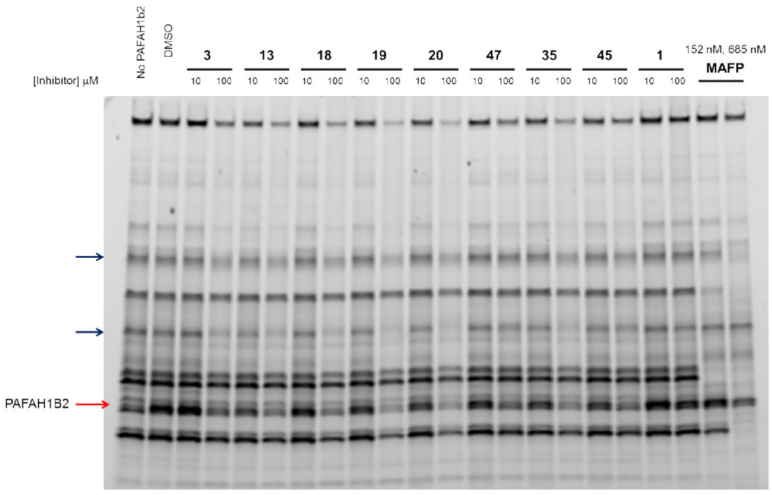


Figure 9.

Selectivity evaluation of the initial hits and the derivatives. Two concentrations of the hits and derivatives (10 and 100 μM) were evaluated. Warhead compound **1** didn't show any significant inhibition at 100 μM. Initial hits (**3**, **13**) displayed significant inhibition at 100 μM, but they were non-selective. After optimization, compound **35** and **45** showed much improved potency and selectivity. Interestingly, both **20** and **47** inhibited PAFAH1B2 but inhibition potency of the compound **47** without the oxadiazolone ring much less than **20**, which has the ring. This means regardless of whether the oxadiazolone ring is present or not the azapeptoid may bind at the active site and compete with FP-Rh for binding with the protein. MAFP, the known inhibitor was used as a positive control. At 150 nM, it inhibited most of the cellular serine hydrolases but PAFAH1B2.

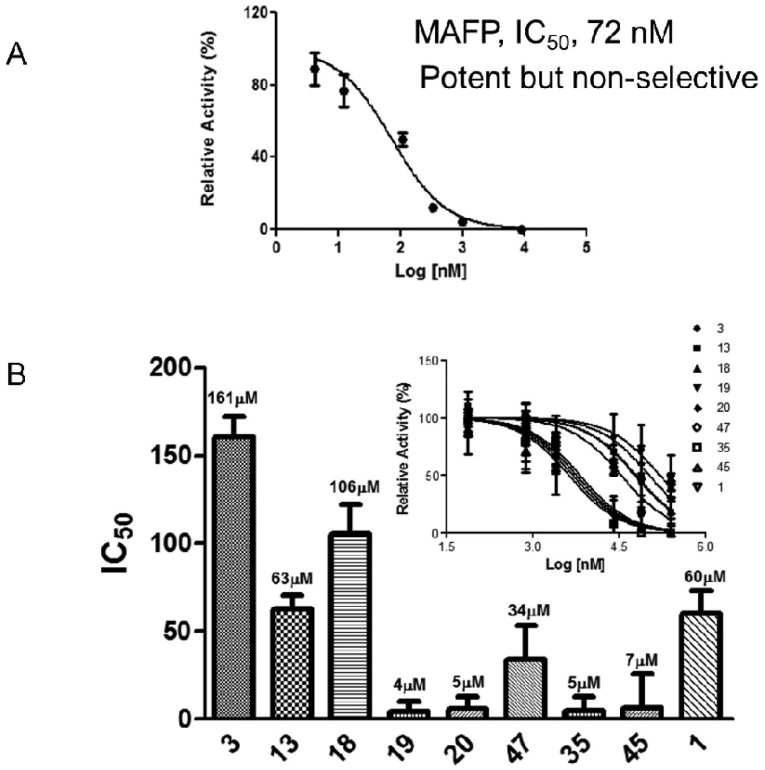


Figure 10.

(A) Determination of IC_{50} value of inhibition of PAFAH1B2 by MAFP by substrate assay. (B) Evaluation of inhibition potency of different inhibitors by substrate assay. The substrate analog 2-thio-PAF was used in the assay. The free thiol generated from the hydrolysis of thio-PAF by PAFAH1B2 was detected by Ellman's reagents, which provide UV absorbance at 412 nm. Inhibiting the enzyme leads to the reduction of DTNB thiolate. IC_{50} values were calculated based on the decrease in DTNB thiolate absorbance by using UV-Visible spectroscopy. Some IC_{50} values are higher than that observed in the ABPP assay. This may be because higher concentration of enzyme is required in this assay and the substrate analog has much higher affinity than FP-Rh for the enzyme, and, therefore, higher concentration of inhibitors is required to achieve same level of inhibition.

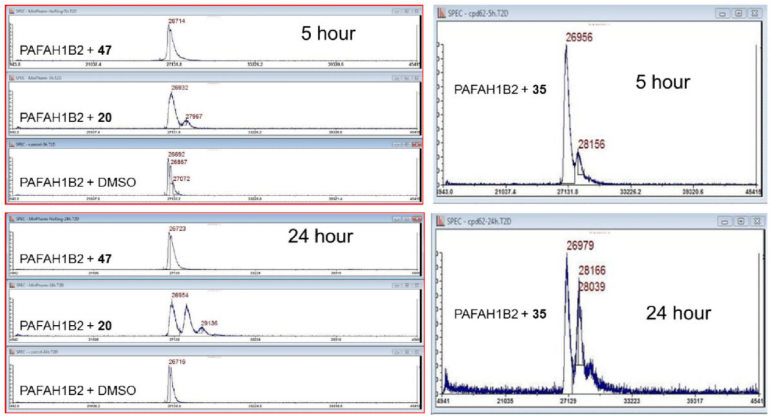


Figure 11.

Representative MALDI TOF mass spectra of control protein [PAFAH1B2 (2 μM) with 2% DMSO] and PAFAH1B2 (2 μM) incubated with compounds **20**, **35** and **47** (100 μM) after 5h or 24h of incubation. Protein-inhibitor adduct formation is clearly visible by MALDI mass spectroscopy for compounds **20** and **35**. No protein-inhibitor adduct was seen with compound **47** indicating that the oxadiazolone ring is important for covalent adduct formation.

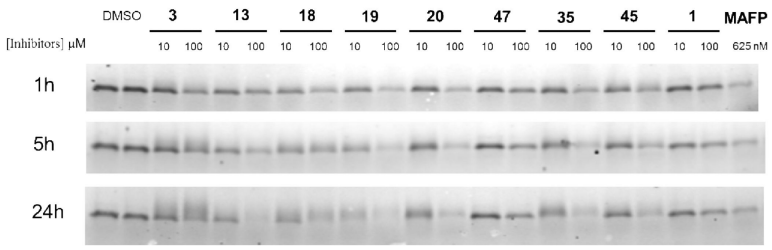


Figure 12.

Time-dependent Inhibition of PAFAH1B2 by ABPP assay. Maximal inhibition was observed after 24 h of incubation of inhibitor with PAFAH1B2, indicating it's a slow reaction between the enzyme and inhibitor. Inhibition potency significantly increased for the primary hits with increase in incubation time, but it's not as prominent for the derivatives. These results suggest that the derivatives may have higher affinity for PAFAH1B2, therefore, the inhibition can quickly reach the equilibrium. In contrast, the primary hits need longer incubation time to display better potency, which is also reflected in the rate of protein-inhibitor adduct formation.

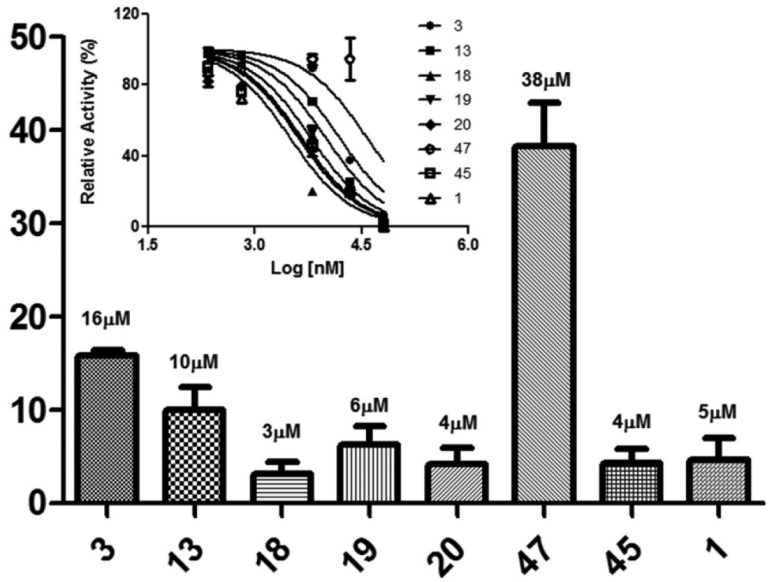


Figure 13.

Substrate assay showing the time-dependent Inhibition. Inhibition potency significantly increased for the primary hits with increase in incubation time to 24h, but not for the derivatives. These results suggest that the derivatives may have higher affinity for PAFAH1B2, therefore, the inhibition can quickly reach the equilibrium. In contrast, the primary hits need longer incubation time to display better potency, which is also reflected in the rate of protein-inhibitor adduct formation. For, compound **47**, there is no difference in IC_{50} value even after increasing the incubation time suggesting noncovalent inhibition by **47**.

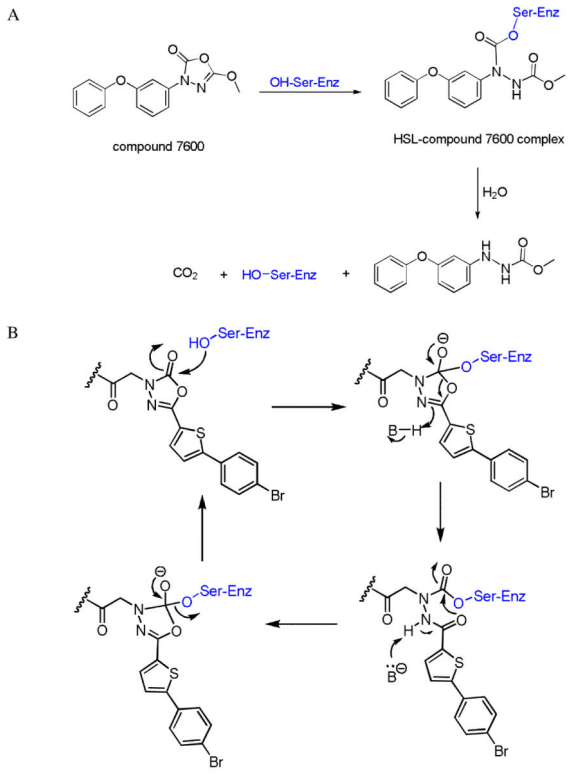


Figure 14.

(a) Mechanism of inhibition of compound 7600.[37] (b) Proposed mechanism of covalent inhibition of our acyl hydrazide-derived oxadiazolone inhibitors.