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Combining cross-metathesis and activity-based protein profiling: New β-lactone motifs for targeting serine hydrolases

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Abstract

β-Lactones are a privileged structural motif as enzyme inhibitors and chemical probes, particularly for the inhibition of enzymes from the serine hydrolase class. Herein, we demonstrate that cross-metathesis (CM) of α-methylene-β-lactones offers rapid access to structurally diverse, previously unexplored β-lactones. Combining this approach with competitive activity-based protein profiling (ABPP) identified lead β-lactone inhibitors/probes for several serine hydrolases, including disease-associated enzymes and enzymes of uncharacterized function. The structural diversity afforded by the α-methylene-β-lactone scaffold thus expands the landscape of serine hydrolases that can be targeted by small-molecule inhibitors and should further the functional characterization of enzymes from this class through the optimization of target-selective probes.

Keywords

β-Lactones; Serine hydrolases; Cross metathesis; α-Methylene-β-lactones; ABPP

β-Lactones are a versatile class of electrophilic small molecules that have found use as biological research probes and therapeutic agents largely due to their capacity to react with and inhibit enzymes from the serine/threonine hydrolase class.[1] More recently, β-lactones have emerged as attractive probes for activity-based protein profiling (ABPP) across the larger serine hydrolase class directly in native biological systems.[2] Many of the β-lactone

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Notes

The authors declare competing financial interests. BFC is a founder and advisor to Abide Therapeutics, a biotechnology company interested in developing serine hydrolase inhibitors and therapeutics

Supplementary Material

Experimental procedures, spectral characterization for all new compounds. This material is free of charge via the Internet at http://bmcl.

inhibitors and probes are 3,4-disubstituted, designed around the natural product-based β-lactone, tetrahydrolipstatin (Orlistat®-Figure 1), a pancreatic lipase inhibitor used to treat obesity.[3] While there are many stereoselective approaches to such β-lactones,[4] we believe that cross-metathesis (CM) of α-methylene-β-lactones **1** offers rapid access to structurally diverse, previously unexplored 3,4-disubstituted-β-lactones.

In investigating strained heterocycles with exocyclic unsaturation, we found that α-methylene-β-lactones **1** participated in CM reactions with Type I[5] alkenes, with couplings proceeding in high yields with excellent *Z*-stereoselectivities (Figure 2).[6] This methodology is attractive for making focused libraries of β-lactones for several reasons. First, the α-alkylidene-β-lactones **4** themselves represent a new class of probes. In addition, metathesis reactions tend to be highly tolerant of a broad range of functionality, thus allowing for the preparation of diverse compounds from a single template. Moreover, it should be possible to selectively access either *cis*- or *trans*-β-lactones **5** from the α-alkylidene-β-lactones. *cis*-Lactones would be the expected products from hydrogenation reactions. Limited literature precedent with exocyclic α,β-unsaturated lactones suggests that 1,4-reductions can provide *trans*-diastereomers under the right conditions (*vide infra*). Most monocyclic β-lactone natural products and close analogs (see examples in Figure 1) that have been explored as drugs or probes are *trans* diastereomers.[1d, 2b, 7] *cis*-β-Lactones, however, have been shown to be as potent as their *trans*-isomers in some cases.[7b, 7c] Overall, then, CM with α-methylene-β-lactones would provide a new class of β-lactone probes (α-alkylidene-β-lactones), while subsequent stereoselective reduction would lead to previously unexplored *cis*- or *trans*-β-lactones. Moreover, an opportunity to examine the potential of 2-methyleneoxetanes (see **11b**/**11d**) in competitive ABPP is offered. We have previously shown that the 2-methyleneoxetane analog **6** (see Figure 1) of the anti-obesity drug, tetrahydrolipstatin (THL), was nearly as potent an inhibitor of porcine pancreatic lipase (PPL) as THL.[8]

Herein, the development of this straightforward CM approach to β-lactone probes, highlighted by a four-step synthesis of (±)-nocardiolactone (Figure 1),[9] is described. Nineteen probes, including nocardiolactone, were prepared from a single α-methylene-β-lactone scaffold **9**. The probes include *E*-and *Z*-α-alkylidene-β-lactones, *cis*- and *trans*-3,4-disubstituted-β-lactones, and 2-methyleneoxetanes. The utility of the probes in competitive ABPP was demonstrated by assaying these compounds in native cell (COLO205 human colon cancer cell line) and tissue (mouse brain) proteomes to assess their *in vitro* inhibitory activity against serine hydrolases. A combination of competitive gel- and MS-based ABPP methods identified novel β-lactone probes that target diverse members of the serine hydrolase family, including uncharacterized enzymes that lack selective inhibitors.

As previously noted, an attractive feature of our planned CM approach to monocyclic β-lactone analogs was that a single template could be used to access a broad range of lactones. The first important choice was the identity of the substituent at C4, since this would be a part of the initial set of analogs. THL has been shown to inhibit the thioesterase domain of fatty acid synthase,[10] forming a covalent bond with an active site serine. Crystallographic studies show the C4 chain to be buried in a hydrophobic channel.[11] While this channel may not be a universal motif, we and others have found that THL interacts with a variety of

serine hydrolases.[12] Böttcher and Sieber also took inspiration from the aliphatic chains of β-lactone natural products in the design of β-lactone ABPPs.[1e] In the initial series, for ease of synthesis we elected to use a simple alkyl chain at C4 and chose the chain length based on nocardiolactone, which has the same number of carbons in its alkyl chain as THL, giving us the opportunity to develop the methodology around a straightforward synthesis of (±)-nocardiolactone. Thus, the key intermediate for its synthesis and for diversification was α-methylene-β-lactone **9**.

We previously reported the synthesis of α-methylene-β-lactones via lactonization of readily accessible, hydrolyzed Morita-Baylis-Hillman (MBH) adducts like **7** (Scheme 1).[13] While α-methylene-β-lactone **9** was readily prepared from tetradecanal by this approach, a more direct sequence involved MBH reaction between tetradecanal and *t*-butyl thioacrylate, followed by mercury-mediated cyclization of **8**[13] to **9**. For nocardiolactone the remaining steps were CM and reduction.

CM between **9** and 1-nonadecene, catalyzed by Grubbs second-generation catalyst **2** under conditions we had previously developed for α-methylene-β-lactones,[6] was straightforward, yielding mainly the *Z*-diastereomer (*Z*-**4a**). The enoate diastereomers were separable, providing two probes to evaluate. While it was anticipated that conversion to the *cis*-isomer of nocardiolactone could be readily achieved using hydrogenation, selective conversion to the *trans*-isomer was more challenging. Romo and coworkers have used epimerization of *cis*-3,4-disubstituted-β-lactones to access the *trans*-isomers,[7c] but the yields were relatively low (35–40%). Our preference was to avoid this extra, material depleting transformation. 1,4-Reduction of enoates **4** represented an alternative pathway that might be manipulated to provide the *trans*-isomers. We found no examples in the literature of 1,4-reduction of α-alkylidene-β-lactones; however, there were a number of examples involving α-alkylidene-γ-lactones.[14] Although simple steric arguments would suggest that *cis*-diastereomers should dominate from protonation from the less hindered face of intermediate enolates, the literature cited showed that other factors play a role. Consequently, a variety of 1,4-reduction conditions were explored.

Iwasaki and coworkers had examined the use of transition metals (Co, Ni, Cu) in conjunction with $NaBH_4$ to effect the reduction of α-alkylidene-γ-lactones with high *trans*-selectivity.[14c] They demonstrated that the protonation of the enoate was not reversible under the reaction conditions, making it the determinant of the relative stereochemistry. The high stereoselectivities were rationalized on the basis of conformational effects from 1,3-allylic strain. While our substituents were different, we reasoned that it might also be possible to use ligands to bias the outcome. Focusing first on cobalt(II) additives with $NaBH_4$ as the reductant, $CoCl_2$ (with MeOH as the proton source) (Table 1, entry 1) gave more *cis*-**5a**.

The stereoselectivity improved with the use of $Co(acac)_2$ (Entry 2), with a further improvement with a slightly more sterically hindered proton source, *i*-PrOH (Entry 4). Using phosphine-containing $CoCl_2(Ph_3P)_2$ provided additional improvement in stereoselectivity (Entry 5). No further enhancement was seen with *i*-PrOH (Entry 7) or by using alternative phosphine ligands (not shown). Separating the product from phosphine related byproducts proved problematic, but this was resolved by cutting back on the additive

(Entry 6). It is noteworthy that running the reaction at higher temperatures resulted in considerable quantities of methyl esters (Entry 3). Although $NiCl_2$ initially looked more promising than the corresponding $CoCl_2$ (Entry 8 vs 1), this was not the case with the optimal phosphine ligand (Entry 9). Copper(II) reagents gave very little conversion to product (not shown). Alternative reductants, such as L-selectride[14a] or Mg,[14b] that had been reported for the stereoselective reduction of α-alkylidene-γ-lactones (Entries 10 and 11) did not provide significant quantities of product. Although other 1,4-reduction conditions, such as those shown in Entries 12[15] and 13,[16] did lead to product, none of these provided higher stereoselectivities than the use of $NaBH_4$ with catalytic $CoCl_2(Ph_3P)_2$ as an additive. While we continue to explore other conditions, the optimized 1,4-reduction conditions identified thus far (Entry 6) do provide access to both *cis*- and *trans*-β-lactones **5**, and these diastereomers are separable by column chromatography.

With α-methylene-β-lactone scaffold **9** in hand, the initial diversification was undertaken. Using four additional alkene partners (**10b–e**) that varied in chain length, with three (**10c–e**) incorporating a terminal aromatic moiety, five α-alkylidene-β-lactones **4** were isolated, and reduced to eight *cis*/*trans*-lactones **5** (Table 2). In addition, compounds *trans*-**5b** and *trans*-**5d** were methylenated to provide probes **11b** and **11d**, respectively, based on our earlier finding of the comparable activity of THL and its 2-methyleneoxetane analog in a PPL assay.[8] Thus, overall, 19 β-lactone-based probes were readily assembled from a single scaffold for evaluation by competitive ABPP.

The β-lactone probe set was screened against the membrane proteomes of mouse brain and the human COLO205 colon cancer cell line by competitive gel-based ABPP. Briefly, proteomes (1mg/mL) were incubated with β-lactone compounds at 25 μM for 30 minutes at 37 °C and then treated with the serine hydrolase-directed activity-based probe fluorophosphonate (FP)-rhodamine[17] (2 μM, 45 min, 37 °C) and analyzed by SDS-PAGE and in-gel fluorescence scanning. Proteomes were also treated with DMSO and THL (25 μM) as controls. The β-lactone probes partially or fully inhibited activities of several serine hydrolase targets in the mouse brain membrane proteome (Figure 3A), including established targets of this inhibitor class, such as fatty acid synthase (FASN)[1a, 18] and lysophospholipase 1 and 2 (LYPLA1, LYPLA2),[2d, 19] and poorly characterized enzymes like ABHD10 and ABHD16A. These enzyme assignments were based on previous studies where gel-based ABPP experiments of mouse membrane proteome were related to mass spectrometry-based identifications of enzymes.[12b, 20] Profiles of the human COLO205 colon cancer membrane proteome also revealed a diverse set of serine hydrolase targets for the β-lactone probes (Figure 3B).

Among the β-lactones, *trans*-**5c** exhibited one of the broadest reactivity profiles across the detected serine hydrolases (Figure 3A, 3B) and was therefore selected for a more in-depth, mass spectrometry (MS)-based analysis of the landscape of serine hydrolases susceptible to inhibition by the β-lactone probes. COLO205 cells were cultured in media with isotopically heavy or light amino acids and their membrane proteomes isolated (1mg/mL) and treated with DMSO or *trans*-**5c** (25 μM, 30 min, 37 °C), respectively. The heavy and light proteomes were then incubated with FP-biotin (10 μM, 45 min, 37 °C),[21] combined, and biotinylated proteins enriched and analyzed using avidin chromatography and

multidimensional liquid chromatography (LC)-MS using an LTQ-Orbitrap instrument, where serine hydrolase activities were identified and quantified based on tryptic peptide MS2 and MS1 data, respectively.[22] Enriched serine hydrolases that showed substantially greater (≥ 3-fold) MS1 signals in DMSO (heavy) versus *trans*-**5c** (light)-treated proteomes were considered targets of the *trans*-**5c** probe (Figure 3C). Eight serine hydrolases satisfied this criterion, with the most potently inhibited targets being ABHD16A, PNPLA4, FAAH, ABHD2, CES2, and ABHD12 (all inhibited > 90%). A second set of enzymes that included FASN and ABHD3 were inhibited from 70–90%. Among these enzymes, only FAAH and FASN, to our knowledge, are well-annotated in terms of biological functions and have selective inhibitors suitable for cellular or *in vivo* pharmacology studies. Our findings thus suggest that α-alkylidene-β-lactones could serve as useful starting points for developing selective inhibitors that target a diverse range of poorly characterized serine hydrolases. We have successfully tested this premise in a separate study, where we identified an α-alkylidene-β-lactone inhibitor and structurally related inactive control probe, that facilitated the functional characterization of the membrane-bound enzyme ABHD16A.[23]

In summary, a diverse set of 19 β-lactones has been readily prepared from a single α-methylene-β-lactone scaffold **9** by a straightforward sequence involving cross-metathesis and subsequent reduction. A combination of competitive gel- and MS-based ABPP experiments identified individual α-alkylidene-β-lactones and their reduced β-lactone counterparts that target a diverse array of serine hydrolases, including disease-relevant and uncharacterized enzymes that lack selective inhibitors. Thus, the utility of CM with α-methylene-β-lactones to access novel biologically active motifs and substitution patterns has been demonstrated. Coupling these tools with ABPP amplifies the significance of this approach for discovering new small-molecule inhibitors as chemical probes of cell biology. We are currently working to identify conditions for 1,4-reductions of the α-alkylidene-β-lactones that provide greater *trans*-selectivity and on the enantioselective synthesis of the α-methylene-β-lactones. In addition, efforts to optimize lead inhibitors discovered in this study are underway, as exemplified in the development of an inhibitor and accompanying inactive control probe of ABHD16A that have been used for functional characterization of this enzyme.[23]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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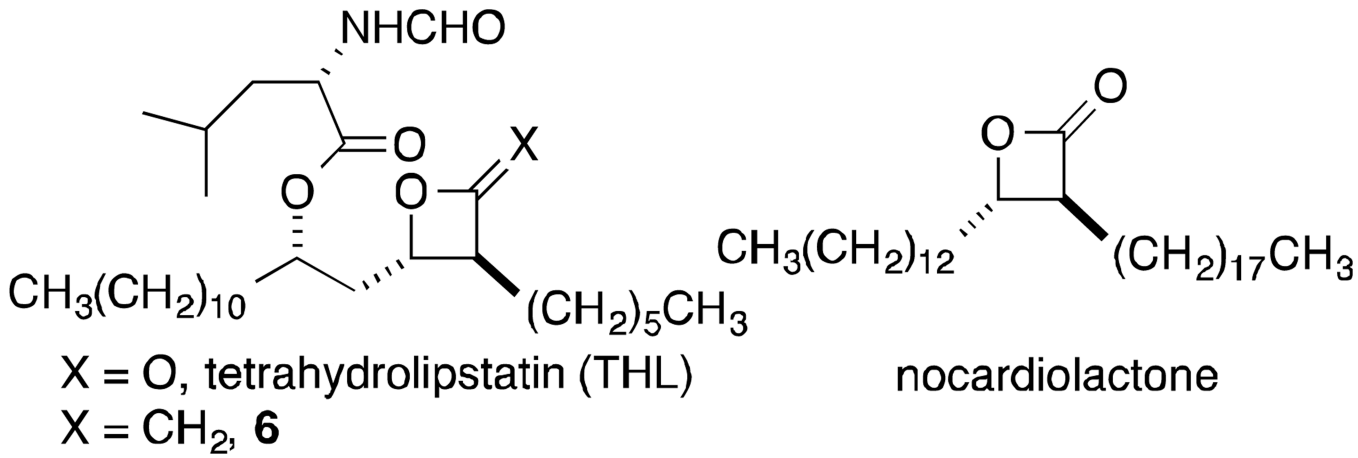


Figure 1.
Examples of biologically active β-lactones.

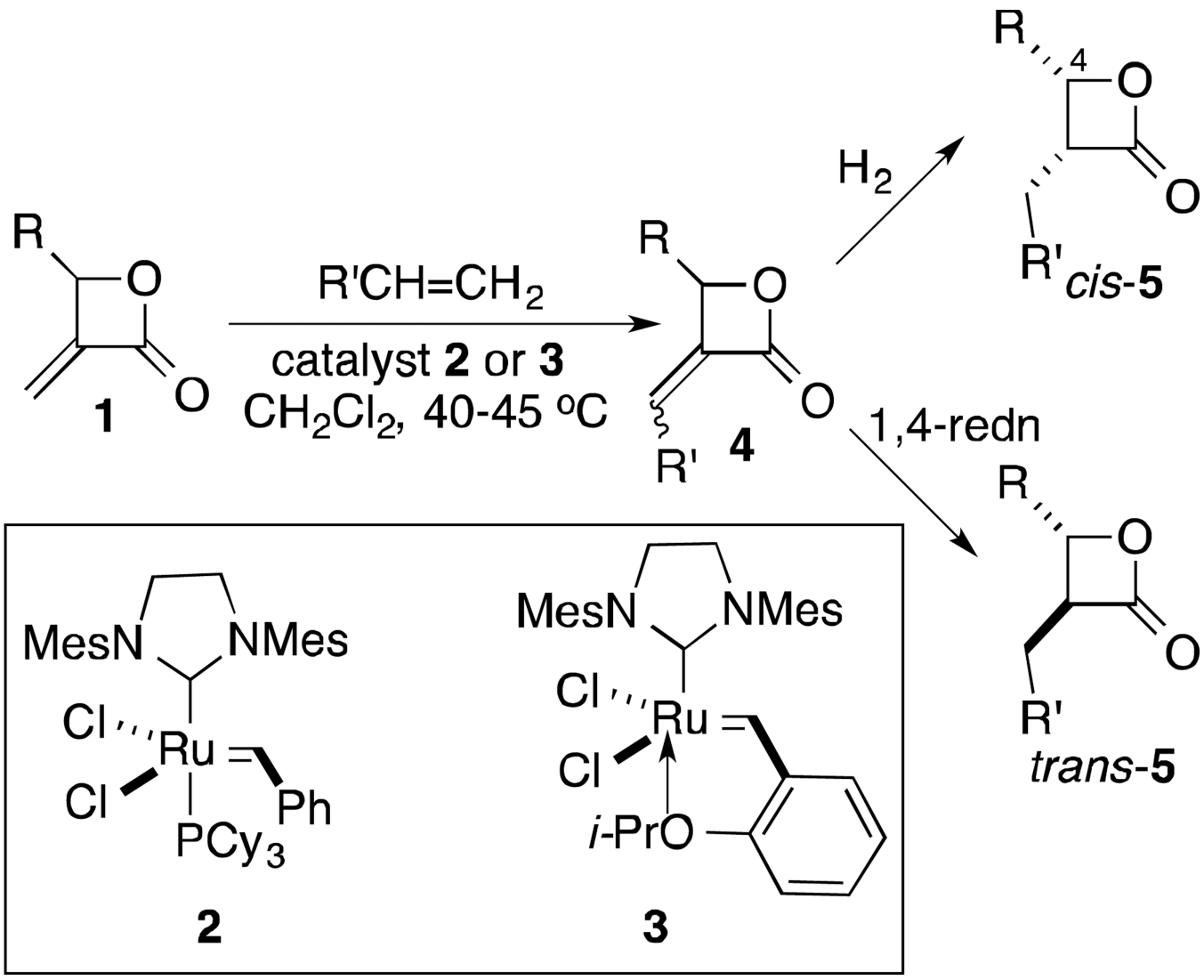


Figure 2.
Cross-metathesis approach to β-lactone libraries.

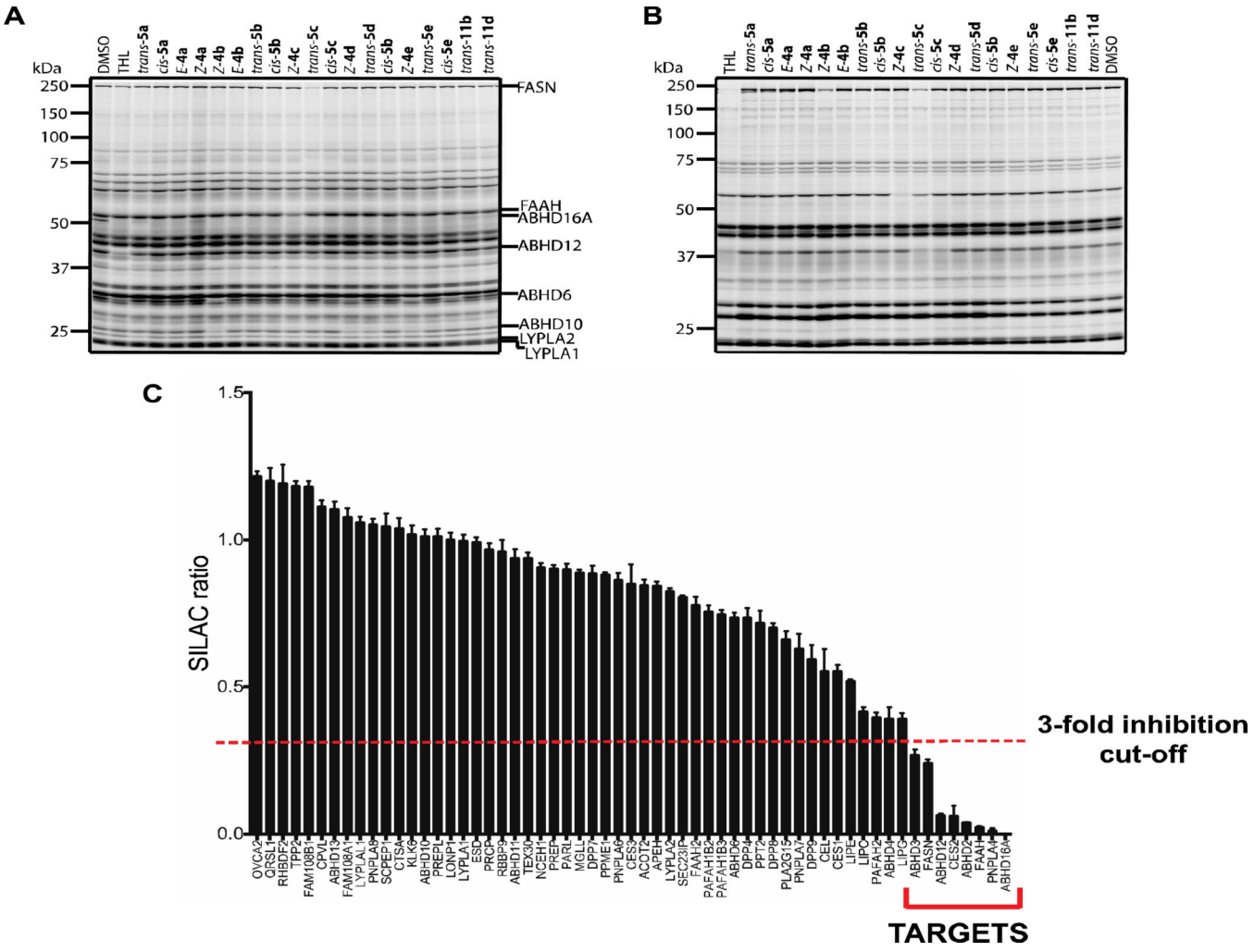
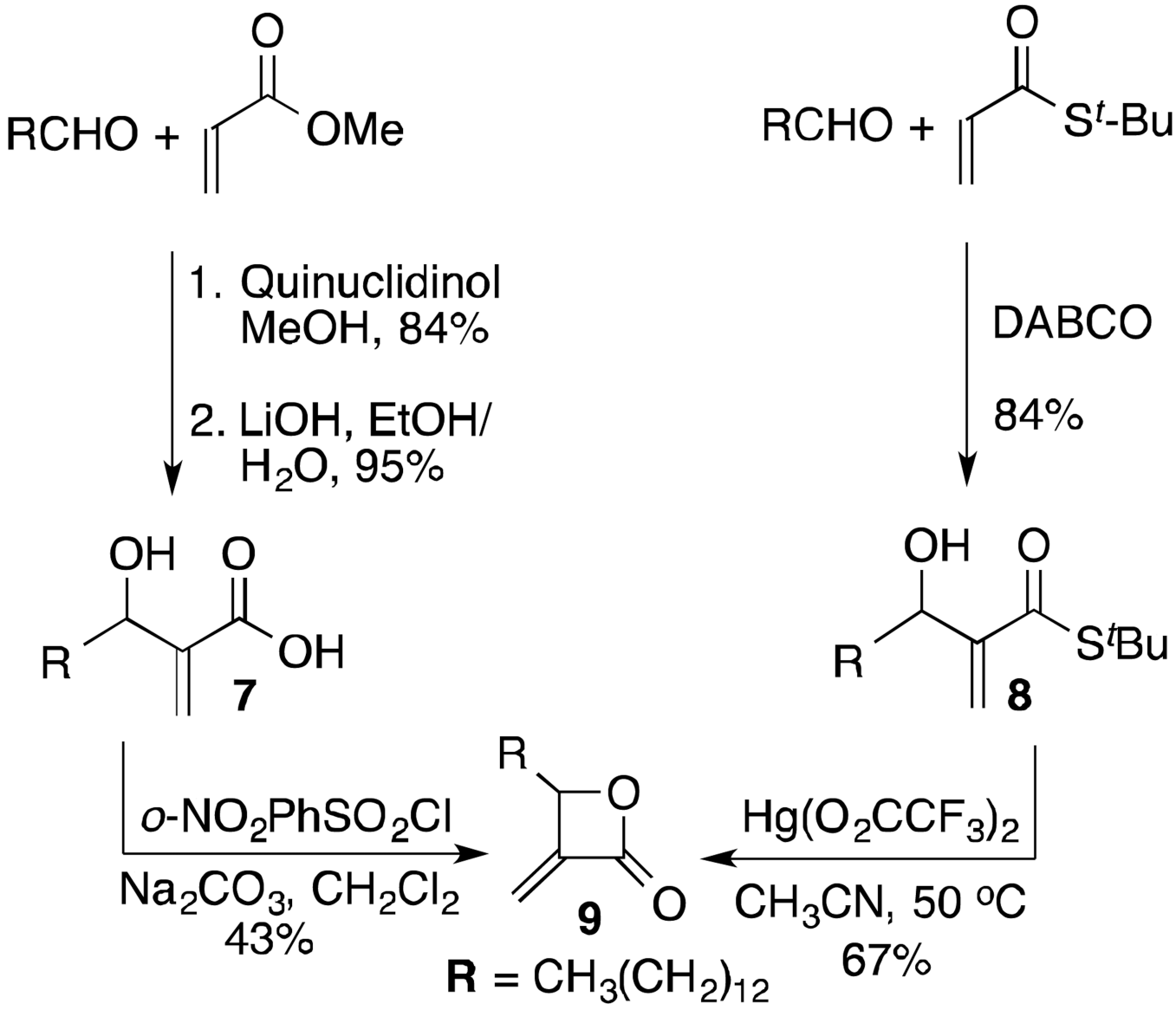
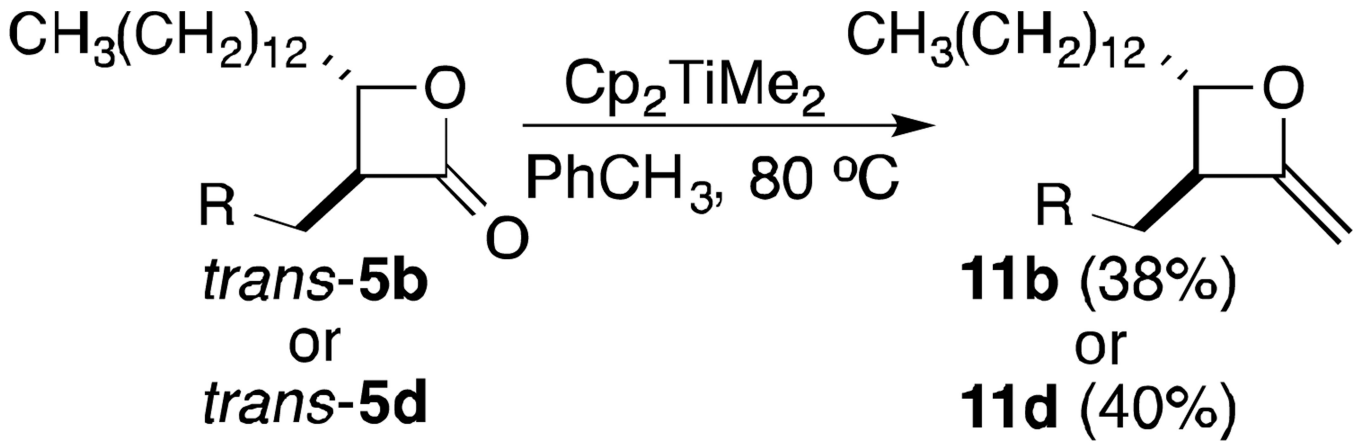


Figure 3.

Competitive ABPP screening and identification of the serine hydrolase targets of β-lactone probe set. Competitive gel-based ABPP in mouse brain membrane proteome (**A**) and COLO205 colon cancer cell membrane proteome (**B**) for the β-lactone probe set. (**C**) ABPP-SILAC analysis of the COLO205 membrane proteome treated with *trans*-**5c**.



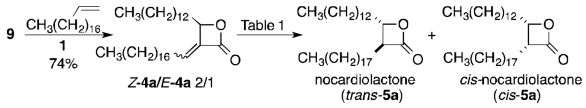
Scheme 1.



Scheme 2.

Table 1

Optimization of stereoselectivity of 1,4-reduction of **4a**.



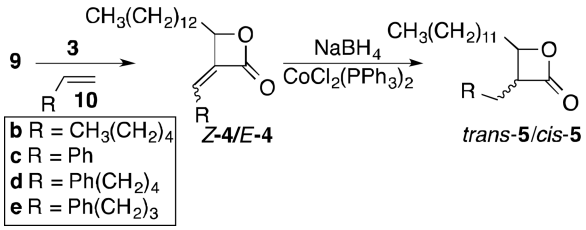
Entry	Conditions	Solvent	Temp (°C)	*trans*-5a/*cis*-5a (yield)
1	$CoCl_2.6H_2O$ (1 equiv), $NaBH_4$	THF:MeOH 5:1	−5	1/2
2	$Co(acac)_2$ (1 equiv), $NaBH_4$	THF:MeOH 5:1	−5	1.5/1
3	$Co(acac)_2$ (1 equiv), $NaBH_4$	THF:MeOH 5:1	5	4/1 (34%) opened ring product (28%)
4	$Co(acac)_2$ (1 equiv), $NaBH_4$	THF:*i*-PrOH 5:1	−5	1.85/1
5	$CoCl_2(Ph_3P)_2$ (1equiv), $NaBH_4$	THF:MeOH 5:1	−5	2/1
6	$CoCl_2(Ph_3P)_2$ (18 mol%), $NaBH_4$	THF:MeOH 5:1	−5	2/1 (61%)
7	$CoCl_2(Ph_3P)_2$ (18 mol%), $NaBH_4$	THF:*i*-PrOH 5:1	−5	1/1
8	$NiCl_2.6H_2O$ (1equiv), $NaBH_4$	THF:MeOH 5:1	−5	1/1
9	$NiCl_2(Ph_3P)_2$ (1 equiv), $NaBH_4$	THF:MeOH 5:1	−5	1.75/1 with sm[b]
10	L-Selectride	THF	−78 to rt	2.5/1 with sm[a,b]
11	Mg^0, ultrasound	MeOH	rt	Reduced opened ring product
12	$Pd(Ph_3P)_4$, $ZnCl_2$, *n*-Bu_3SnH	THF	rt	1/1 with sm[b]
13	$CuCl_2.2H_2O$, PMHS, (*R*)-BINAP, ^{t}BuONa	$PhCH_3$, pentane *i*-PrOH	−20	1/1 sm[b]

[a] Results were inconsistent on repetition, and material recovery was low.

[b] sm = starting material.

Table 2

Preparation of α-alkylidene-β-lactones and their corresponding β-lactones



Entry	R	Alkylidene lactone *Z*-4/*E*-4, (yield)	β-Lactone *trans*-5/*cis*-5, (yield)
1	**10b**; $CH_3(CH_2)_4$	3.3/1 (97%)	1. 3 /1 (44%)
2	**10c**; Ph	*Z*-only (40%)	2/1 (39%)
3	**10d**; $Ph(CH_2)_4$	*Z*-only (68%)	2/1 (32%)
4	**10e**; $Ph(CH_2)_3$	*Z*-only (51%)	1. 4 /1 (46%)