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Isosorbide-Based Aspirin Prodrugs: Integration of Nitric Oxide Releasing Groups

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Aspirin prodrugs and related nitric oxide releasing compounds hold significant therapeutic promise, but they are hard to design because aspirin esterification renders its acetate group very susceptible to plasma esterase mediated hydrolysis. Isosorbide-2-aspirinate-5-salicylate is a true aspirin prodrug in human blood because it can be effectively hydrolyzed to aspirin upon interaction with plasma BuChE. We show that the identity of the remote 5-ester dictates whether aspirin is among the products of plasma-mediated hydrolysis. By observing the requirements for aspirin release from an initial panel of isosorbide-based esters, we were able to introduce nitroxymethyl groups at the 5-position while maintaining ability to release aspirin. Several of these compounds are potent inhibitors of platelet aggregation. The design of these compounds will allow better exploration of cross-talk between COX inhibition and nitric oxide release and potentially lead to the development of selective COX-1 acetylating drugs without gastric toxicity.

Introduction

Aspirin is the world's most widely used medicine. Some of its effects are due to its ability to trans-acetylate platelet cyclooxygenase-1 (COX-1[a]), resulting in effective abolition of platelet derived thromboxane A_2 (TXA_2), leading to cardiovascular and cerebrovascular protection.[1] Other effects can be attributed to COX-2 inhibition and the shunting of arachidonic acid that occurs when COX-2 is acetylated.[2] Long-term aspirin use is associated with reduced lung cancer death as well as decreased incidence of colorectal cancer in patients with colon polyps.[3] The pharmacological target here may be COX-2 or be COX-independent, and the dose requirement has yet to be resolved but it is likely to be higher than in antiplatelet therapy. Aspirin may have a role in attenuating drug-induced liver damage[4] and in preventing Alzheimer's disease progression.[5] COX acetylation is generally beneficial in preventing, curing, and ameliorating human disease.

Aspirin use carries a 2−3 fold increase in risk of a serious gastrointestinal bleed that is of particular importance in the elderly population.[6] Given the widespread use of aspirin, this side effect is a matter of serious public concern. Furthermore, aspirin-induced gastropathy prevents its full clinical exploitation because in an individual instance the risk of ulceration tends to exceed the perceived risk of a disease event.[7] Ulceration is not reduced by pharmaceutical approaches such as enteric coating[8] or buffering,[9] its severity is dose-dependent, and, crucially, it may not be directly attributable to the mechanism of action or pharmacological target of the drug.[10,11] Therefore, there is a need to separate COX-1 acetylation from aspirin's side effects.[12]

Nitric oxide releasing aspirin compounds (termed NO-aspirins) are a type of hybrid intended to be capable of liberating nitric oxide and aspirin.[13] Nitric oxide is gastroprotective through multiple mechanisms.[14] However, the combination of aspirin and nitric oxide release exhibits pharmacological effects in cardiovascular, cancer, and inflammatory models that hold significant therapeutic potential.[15] The NO-aspirin **1** was tested in a number of clinical trials, and it has little or no gastric toxicity either because it releases NO or simply because it is an ester.[13] Aspirin has been linked to a number of other nitric oxide pro-moieties (Figure 1), including furoxan (**2**) and diazeniumdiolate (**3**) groups.[16,17] An interesting group of salicylates were recently reported with incorporation of a nitroxyl group on the acetyl moiety rather than at the carboxylate (**4**).[18]

While the concept holds rich promise, the design of true aspirin nitric oxide hybrids has hitherto proven difficult. Human blood plasma butyrylcholinesterase (BuChE, EC 3.1.1.7) tends to catalyze the hydrolysis of the acetyl group of aspirin esters before the pendant ester whether or not it bears a nitric oxide precursor (this is shown in the special case of isosorbide-based aspirin esters as pathway B in Figure 2).[19,20] The design challenge in nitric oxide releasing aspirins is therefore similar to the design challenge with aspirin prodrugs generally—how to induce release of the group bearing the nitric oxide precursor before deacetylation (pathway A in Figure 2). None of the

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[a] Abbreviations: AChE, acetyl cholinesterase; ADP, adenosine diphosphate; BNPP, bis-*p*-nitrophenylphosphate; (hu)BuChE, (human) butyrylcholinesterase; BW254c51, 1:5-bis(4-allyl-dimethyl)ammonium-phenyl-pentan-3-one; CES, carboxylesterase; COX, cyclooxygenase; DCC, dicyclohexylcarbodiimide; DMAP, dimethylaminopyridine; EDTA, ethylenediaminetetraacetic acid; EtOAc, ethyl acetate; GIT, gastrointestinal tract; GSH; glutathione; Hex, *n*-hexane; ISAS, isosorbide-2-aspirinate-5-salicylate; ISDA, isosorbide-2,5-diaspirinate; ISMN, isosorbide mononitrate; ISMNA, isosorbide mononitrate aspirinate; *iso*-OMPA, tetraisopropylpyrophosphoramide; MeCN, acetonitrile; MeOH, methanol; NO, nitric oxide; ODQ, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one; PMSF, phenylmethylsulfonyl fluoride; PRP, platelet rich plasma; RPHPLC, reverse-phase high performance liquid chromatography; SAR, structure−activity relationship; TXA_2, thromboxane A_2.

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aspirin nitric oxide hybrids reported so far generate aspirin in blood. The overall aim of the work described in the present study was to design an aspirin ester capable of liberating aspirin and a nitric oxide precursor in human plasma.

We have recently reported on isosorbide-2-aspirinate-5-salicylate **5a** (Figure 2) which acts as a true aspirin prodrug because of an unusually productive interaction with BuChE.[21] The 5-salicylate group in **5a** appeared to have a decisive effect on the specificity of processing in this compound because the isosorbide-2-aspirinate **5b** was not an aspirin prodrug [pathway B in Figure 2]. We had earlier reported the aspirin ester (**6**)of the clinically used nitrate isosorbide mononitrate (ISMN), a promising hybrid design as its two components are already approved for human medicine and in similar doses.[22] This paper describes initially an evaluation of **6** as a potential human aspirin-nitrate hybrid prodrug. Following this we investigated the influence of the 5-ester group in **5** on the A/B hydrolysis ratio, leading eventually to successful incorporation of a nitrate group (**7**, Figure 2) while maintaining aspirin release characteristics in human plasma.

Evaluation of Aspirin Ester of Isosorbide-5-mononitrate. Isosorbide-5-mononitrate-2-aspirinate (**6**) was evaluated as an aspirin prodrug by incubating it in human plasma at 37 °C (10 and 50%) and monitoring its decay by RPHPLC. In human plasma solution (10%), compound **6** was hydrolyzed mainly along the salicylate pathway (B in Figure 2), producing < 10% aspirin. The amount of aspirin evident under these conditions can be used to estimate the ratio of pathways A to B because aspirin hydrolysis is slow in dilute plasma solution ($t_{1/2}$ for aspirin in 10% plasma is reported to be 9.8 h).[19] Hydrolysis of **6** in 10% plasma solution was rapid, with an apparent first-order half-life of 52 s. This behavior is not unusual for aspirin esters, especially where deacetylation predominates, but the ISMN−salicylate product of the hydrolysis process was consumed with striking rapidity (Figure 3). Typically, the rate of plasma-mediated hydrolysis of salicylic acid esters is slow. For example, methyl salicylate hydrolyzes in 80% human plasma with a half-life of 17.6 h.[23] It would appear therefore that the ISMN group promotes rapid hydrolysis of the salicylate ester in human plasma solution. Indeed productive hydrolysis of the parent **6** almost competes with the unproductive acetyl group detachment. The isomeric isosorbide-2-nitrate-5-aspirinate[21] (**6a**) was tested under the same conditions. It too disappeared rapidly ($t_{1/2}$ = 1.3 min) but with the exclusive liberation of the isosorbide-2-mononitrate-5-salicylate, which was stable in plasma. Isosorbide-2-mononitrate has been used as a nitrovasodilator but it has significant side effects due to rapid nitric oxide release; the more slowly metabolized 5-mononitrate has a better clinical profile.[24] The progress curve for the hydrolysis of **6a** (Figure 3) illustrates how much faster hydrolysis of the 2-*exo*-ester is and how close **6** is to acting as a true nitro-aspirin prodrug in human plasma. The identity of BuChE in mediating aspirin release from **6** was confirmed by repeating the hydrolysis experiment in the presence of selective esterase inhibitors, eserine (cholinesterases), *iso*OMPA (BuChE), phenylmethylsulfonyl fluoride (PMSF; serine proteases, AChE), and dibucaine (BuChE-subtype) (Table 1).[25] The hydrolysis was also monitored in human plasma solution in the concentration range 2−30% (pH7.4, 37 °C). The extent of aspirin production relative to salicylate was found to be invariant with plasma concentration, although the k_{obs} increased linearly with plasma concentration (Figure 3). Because **6** did not have desirable aspirin release characteristics in human plasma, we were forced to consider how to replace the 5-nitrate with a substituent that could promote hydrolysis to aspirin at position 2 while at the same time being itself amenable to nitrate substitution. To better define the requirements for isosorbide−aspirinate activation to aspirin, we prepared a number of isosorbide−aspirinate 5-esters (**5**). We focused on esters because these would ultimately be hydrolyzed to isosorbide, which is innocuous. This led to the design and synthesis of a second group of

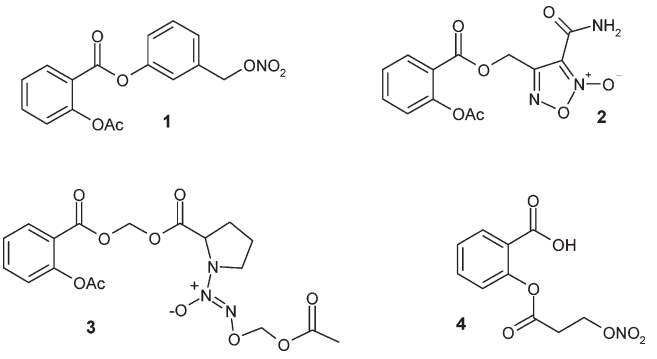


Figure 1. Aspirin-nitrate, **1**, furoxans **2**, diazeniumdiolates (NONOates) **3**, nitroxyacyl salicylates **4**.

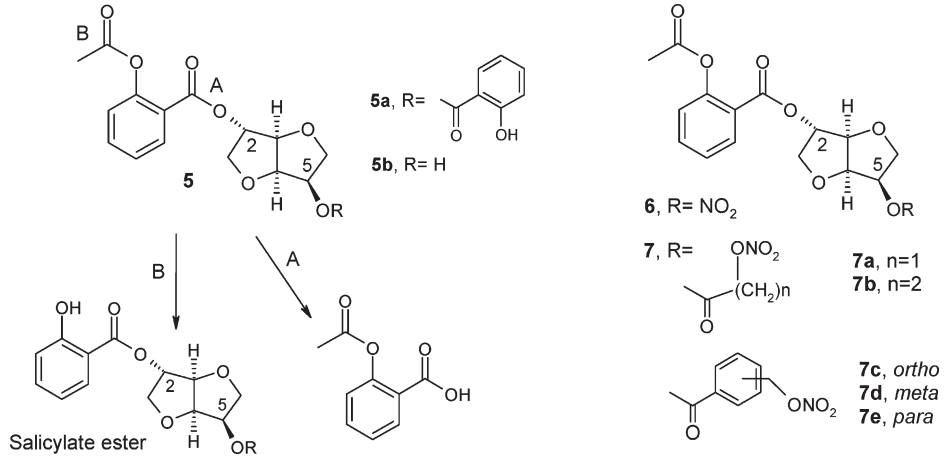
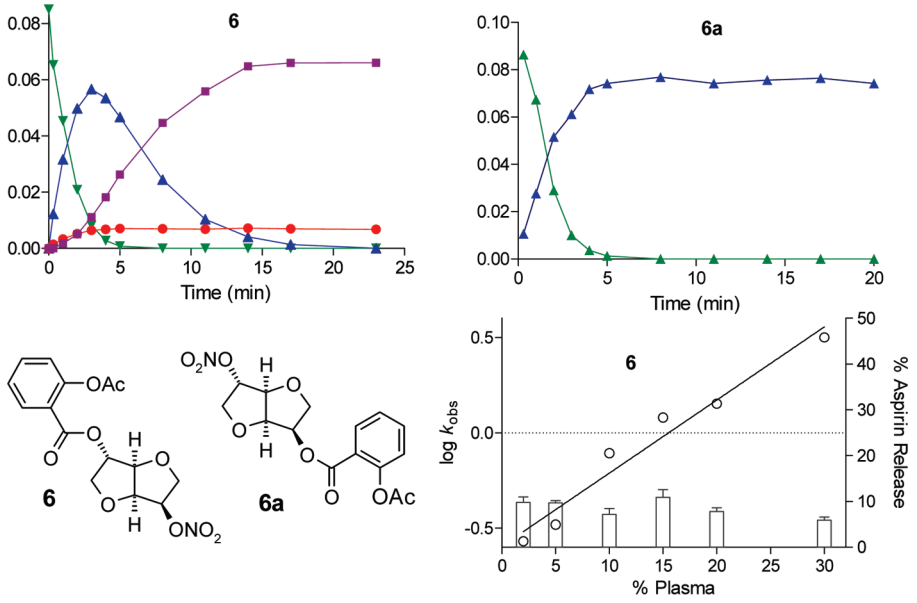


Figure 2. Hydrolysis pathways of aspirin prodrugs.



	Inhibitor	k_{obs}	half-life	% aspirin
6, 10% hp	None	0.86	52 sec	9.1
6, 50% hp	None	3.7	11.3 sec	7.7
6a, 10% hp	None	0.52	1.3 min	nd
6, 50% hp	Dibucaine (1mM)	0.15	4.2 min	6.75
6, 50% hp	PMSF (100μM)	0.25	2.9 min	6.2
6, 50% hp	Eserine (100μM)	0.03	178 min	nd
6, 50% hp	isoOMPA (10μM)	0.02	38.3 min	nd
Whole blood	None	3.12	13.4 sec	<1%

Figure 3. Progress curves for the disappearance of **6** and **6a** in 10% human plasma buffered at pH 7.4 (37 °C): **6** (downward-pointing solid green triangle), aspirin (solid red circle), isosorbide-2/5-salicylate-2/5-nitrate (upward-pointing solid blue triangle), salicylic acid (solid purple square). Plot showing the relationship between rate of hydrolysis and proportion of **7** undergoing hydrolysis to aspirin in the range 2−30% human plasma buffered at pH 7.4 and 37 °C. (n = 3).

compounds in which the 5-ester was elaborated with a nitrate group as a nitric oxide donor (**7**).

Chemistry

Compounds **5** were obtained by esterification of **5b** with the appropriate acid and DCC coupling procedures or by treatment with the corresponding acid chloride in the presence of Et_3N (Scheme 1). We were unable to obtain the 3- and 4-hydroxybenzoates (**5c**, **5d**) by direct coupling because of competition between the isosorbide-5-OH and phenolic −OH groups. In these cases, the benzylprotected hydroxybenzoic acids were coupled first to **5b** by DCC-mediated coupling, followed by reduction in the presence of H_2 over Pd/C. The synthesis of the alkylnitrate esters **7a**,**b** is shown in Scheme 2. The intermediate nitroxyalkyl acids **10** were first prepared by treating the corresponding commercially available bromo-acids **9** with $AgNO_3$ in MeCN. Nitroxyacids **10** were linked by esterification with DCC and DMAP to **5b** to yield the isosorbide-2-aspirinate-nitroxyalkyl esters **7a**,**b**. The nitroxymethylbenzoate compounds **7c**−**e** were prepared by treating **5b** with the appropriate chlorobenzoyl chloride (Scheme 3). The chloromethyl esters were carried through unpurified and the halide−nitrate exchange accomplished using $AgNO_3$ in MeCN. In the case of the ortho compound **7c**, the corresponding *ortho*-chloromethyl benzoic acid was generated from phthalide in the presence of dichlorotriphenylphosphorane and used unpurified.[26] The final compounds were characterized by HRMS, CHN, NMR, and HPLC. Compounds **5a**, **5b**, **6**, and **6a** were obtained as described previously.[21,22]

Evaluation of Isosorbide-Based Aspirinates. The panel of **5** esters was incubated in 10 and 50% plasma solution (pH7.4, 37 °C) and the reaction progress monitored at intervals by RPHPLC. Ester consumption followed first-order kinetics with half-lives of 2−5 min in 10% human plasma and around 1 min in 50% human plasma (Table 1). The salicylates eluted after the parent aspirinates in each case and were quantitated using the response of the parent at 230 nm. The identity of the

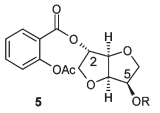
salicylates was confirmed using photodiode array (PDA) (λ_{max}, ~300 nm) and in some cases by LCMS. The extent of aspirin release was estimated from progress curves at maximum aspirin concentration relative to the extrapolated initial parent ester concentration (Table 1). The esters underwent hydrolysis with markedly different outcomes in rate and extent of aspirin release.

The four 5-alkyl esters **5e**−**h** were exclusively hydrolyzed to the corresponding salicylates (pathway B). The benzoate esters tended to be hydrolyzed along pathway A to some extent. The hydroxybenzoate ester compounds **5c**,**d**, which are isomers of **5a**, underwent typically rapid hydrolysis in human plasma. Isomer **5c** produced significant amounts of aspirin (29−44%), but the para isomer **5d** was hydrolyzed along the salicylate pathway (< 1% aspirin). The *ortho*- and *meta*-toluate esters **5j** (46−59%) and **5k** (38−54%) were excellent aspirin prodrugs, but the *para*-toluate (**5l**) was less effective (~15% aspirin release). Of the methoxylbenzoates (**5m**−**o**) the meta isomer was most efficient (17−19%). The 5-biphenyl ester **5t** was not consumed over a period of one hour at either 10% or 50% plasma. Instead, the typical initially rapid hydrolysis tapered quickly and stopped abruptly following around 20% consumption of the parent compound (n = 3). No aspirin was produced. The interaction of the isosorbide-based compounds with the cholinesterases is not straightforward. Whereas isosorbide diesters (including those here) are in general extremely rapidly hydrolyzed by BuChE, some are micromolar inhibitors of the homologous enzyme AChE.[27] Therefore, the failure of **5t** to undergo significant consumption could be due to enzyme inhibition by the intact compound or one of its hydrolysis products. Overall, in the benzoate group, ortho-substitution and to a lesser extent meta-substitution conferred good aspirin release characteristics in human plasma solution. The para-substituted 5-benzoates tended not to act as aspirin prodrugs as evidenced by the behavior of **5d**, **5l**, **5o**, **5s**, and **5t**. Some heterocyclic substituted compounds (**5v**−**5bb**) were also prepared and evaluated, initially with the objective of improving water solubility. The nicotinate (**5v**) is an interesting compound because it produces substantial amounts of aspirin (40%) and is subsequently hydrolyzed to nicotinic acid and isosorbide. The water solubility of the two nicotinates (**5v**,**w**) was significantly better than the alkyl and carbocyclic aryl compounds, but the others, apart from thiadazole (**5aa**), were not sufficiently efficient as aspirin prodrugs to warrant further investigation.

Having established the general requirements for productive hydrolysis in human plasma solution for the 2-aspirinate-5-esters, we next turned to the problem of integrating a potential nitric oxide releasing moiety (**7**, Table 2, Figure 4).

We had chosen to evaluate nitrate esters as potential NO delivery groups because nitrates are synthetically accessible, stable, lipophilic, and a have a long history of clinical use. We were drawn toward nitroxymethylbenzoates because these corresponded most closely to the benzoates that were associated with good aspirin release characteristics in human plasma in series **5**. We evaluated two nitroxyalkyl compounds for comparison. Nitroxy-substitution directly to the benzene ring was rejected because phenylnitrates are reported to spontaneously disproportionate to nitrophenols.[28] The 5-nitroxyalkyl esters **7a**,**b** were hydrolyzed exclusively along the salicylate pathway in human plasma solution (10 and 50%) as observed for the unsubstituted analogs **5e**−**h**. The ortho- and meta-substituted compounds

Table 1. Kinetic Data for the Hydrolysis of Compounds **5** and Extent of Aspirin Released Based on Initial Ester Concentration in Moles Measured at Peak Aspirin Production Following Addition of Candidate Esters to Buffered Human Plasma (10 or 50%) at 37 °C and pH 7.4 by HPLC



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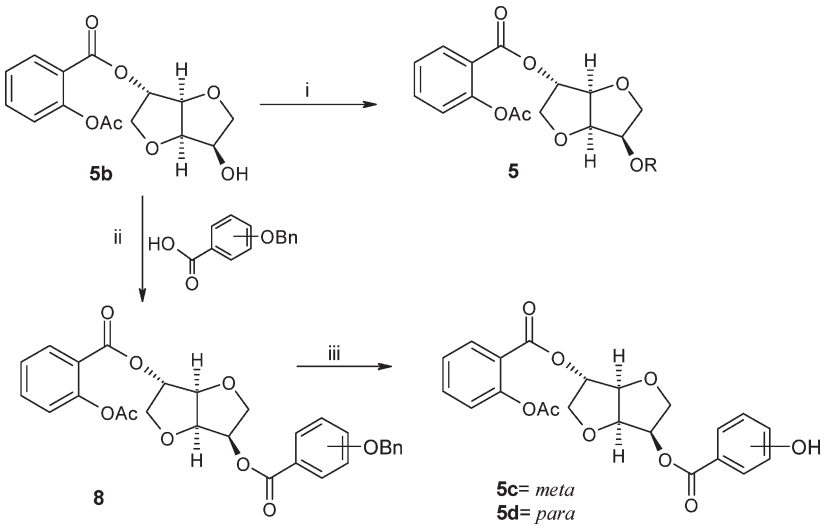
	First-order rate constant (min^{-1})[a], half-life (min)[b] and mole % aspirin[c] released						[†]Solubility in water (μg/mL)
	10% plasma			50 % plasma			
	a	b	c	a	b	c	[†]Sol
R=2-hydroxybenzoate, **5a**	0.14	4.95	72	0.61	1.14	85	11.2
R=3-hydroxybenzoyl, **5c**	0.63	1.10	44	0.77	0.90	29	
R=4-hydroxybenzoyl, **5d**	0.58	1.19	1	0.58	1.19	0.5	
R=acetoyl, **5e**	0.19	3.65	1.2	1.22	0.57	1.3	18.8
R=propanoyl, **5f**	0.19	3.65	6.8	1.05	0.66	7	57
R=pentanoyl, **5g**	0.58	1.19	nd	0.99	0.70	<0.5	
R=cyclopropanoyl, **5h**	0.36	1.93	2	0.77	0.90	<0.5	
R=benzoyl, **5i**	0.21	3.30	19	1.08	0.64	29	1.2
R=2-methylbenzoyl, **5j**	0.33	2.10	46	0.83	0.83	59	5.34
R=3-methylbenzoyl, **5k**	0.07	9.90	38	0.57	1.22	54	0.6
R=4-methylbenzoyl, **5l**	0.11	6.30	16	0.32	2.17	14.7	5.3
R=2-methoxybenzoyl, **5m**	0.2	3.47	5	0.7	0.99	7	9.9
R=3-methoxybenzoyl, **5n**	0.22	3.15	19	0.82	0.85	17	15.6
R=4-methoxybenzoyl, **5o**	0.22	3.15	2.6	0.63	1.10	4	0.31
R=2-benzoyloxybenzoyl, **5p**	0.03	23.10	28	0.16	4.33	19	0.51
R=4-nitrobenzoyl, **5q**	0.25	2.77	13	0.51	1.36	22	1.72
R=4-cyanobenzoyl, **5r**	0.13	5.33	19	0.17	4.08	<0.5	
R=4-phenylbenzoyl, **5s**	na	>20	<0.5	na	>20	<0.5	
R=3,5-diethoxybenzoyl, **5t**	nt	nt	nt	0.41	1.7	<0.5	
R=3-acetamidobenzoyl, **5u**	nt	nt	nt	0.2	3.5	11	
R=nicotinoyl, **5v**	0.5	1.39	41	1.87	0.37	32	87
R=*iso*-nicotinoyl, **5w**	0.18	3.7	19	2.1	0.33	27	207.3
R=6-chloronicotinoyl, **5x**	0.18	3.85	18	0.36	1.93	17	
R=  , **5y**	0.6	1.16	24	0.6	1.16	14	
R=  , **5z**	0.06	11.55	18	12	0.06	14	
R=  , **5aa**	0.18	3.85	32	1.8	0.39	31	
R=  , **5bb**	0.12	5.78	7	0.18	3.85	6	
R=H, **5b**	0.21	3.30	<0.5	0.63	1.10	<0.5	

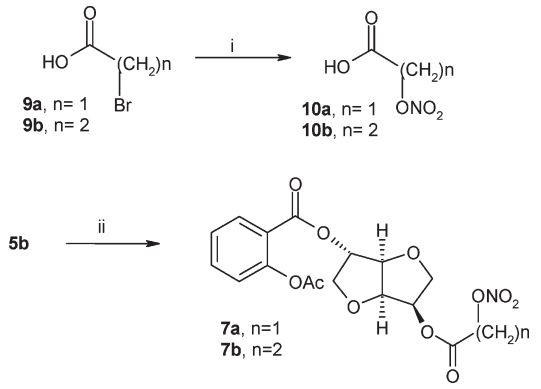
7c and **7d** were effective aspirin prodrugs generating ~30 and ~50% aspirin respectively in human plasma solution. Compounds **7c** and **7d** were incubated in solutions of wild type BuChE purified from human plasma with similar A/B hydrolysis ratio supporting the identity of BuChE in their

Scheme 1. Synthesis of Isosorbide-2-aspirinate-5-esters **5**



Conditions: (i) RCOOH, DCC, DMAP in DCM or RCOCl, Et_3N, DCM; (ii) DCC, DMAP, DCM; (iii) H_2, Pd/C, EtOAc/MeOH.

Scheme 2. Synthesis of Isosorbide Based Alkylnitrate Aspirinates **7a**−**b**

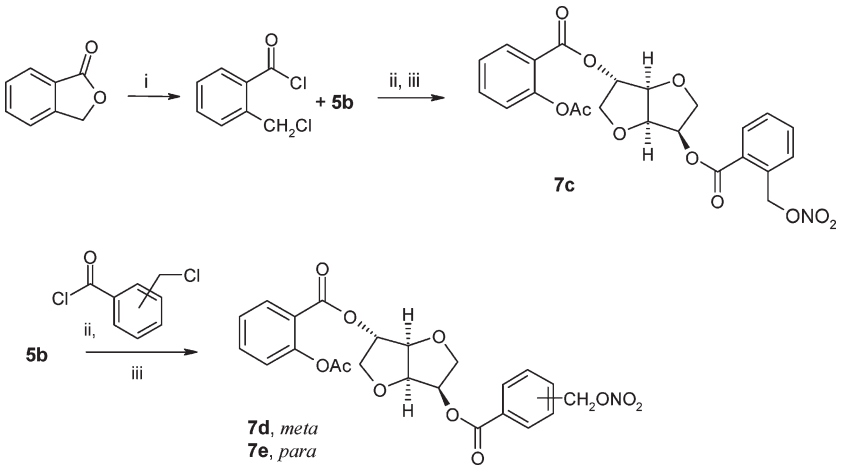


Conditions: (i) $AgNO_3$, MeCN; (ii) RCOOH, DCC, DMAP; (iii) Et_3N, DCM.

hydrolysis in plasma. The *para*-substituted compound **7e** did not act as an aspirin prodrug, consistent with the observations about para-substitution in series **5** (Table 3). The 50% plasma samples following incubation of **7d** were analyzed by LCMS to confirm the hydrolysis pathway evident by HPLC-UV and to look for evidence of nitrate hydrolysis/metabolism. All of the expected plasma esterase products were observed—aspirin, salicylic acid, the salicylate analogue of **7d**, isosorbide-5-*m*-nitroxymethyl benzoate, and *m*-nitroxymethyl benzoic acid. However there was no evidence of the hydroxymethyl products that would be expected following loss of NO/NO_2. Alkyl nitrates, including clinically used nitrates, degrade very slowly in plasma and buffer solution but produce pharmacologically effective amounts of nitric oxide in media containing cell types including smooth muscle cells and, to a lesser extent, platelets.[24,29]

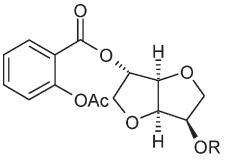
The remote control over the direction of hydrolysis by the 5-ester is peculiar. Isosorbide aspirinate diesters are V-shaped with the 2-substituent directed exo to the fused ring system and the 5-group endo. The exo group is predicted to be more reactive on purely steric grounds, but the high susceptibility of isosorbide-2-esters to BuChE processing is connected to their overall shape and topological complementarity with the BuChE active site. Isosorbide-2,5-diesters are in general rapidly and exclusively hydrolyzed at the 2-ester.[35] The rate of hydrolysis of the isosorbide-2-benzoate ester increases 20-fold across the series −5-OH, −5-nitrate, −5-benzoate with an accompanying depression of K_M values, indicating that the rate enhancement is substrate affinity driven.[30] We also reported recently on isosorbide-2-carbamate esters as low nM and selective inhibitors of BuChE over AChE (6×10^4).[31] These compounds were characterized as time-dependent pseudosubstrate competitive inhibitors. In that class, potency (and presumably affinity) significantly increased in the series −5-nitrate, −5-alkyl ester, −5-aryl ester. The general problem in aspirin prodrug chemistry is that the acetyl group is chemically and enzymatically susceptible to hydrolysis by plasma esterase for electronic, steric, and mechanistic reasons. It seems that in the isosorbide-aspirinate series, 5-aryl substitution can stabilize substrate orientations associated with hydrolysis along pathway A so that it can be competitive with acetate group hydrolysis. We decided to see if a modeling approach could be used to investigate why some benzoates might be better in this regard than others. In ester hydrolysis, we can assume that the transition state resembles the ensuing tetrahedral intermediate and it follows that differences in stability between competing transition states in parallel ester processing will be reflected in the relative energies and geometries of the respective tetrahedral intermediates. We therefore modeled compounds **5a** and **5i** in BuChE using a docking approach in which the carbonyls were rehybridized and linked covalently to the active site serine (198 in HuBuChE: PDB code 1p0i).[32] This approach has been used previously for carbamate interactions with the cholinesterases.[31,33] It finds significant justification in the fact that the X-ray crystal structure of BuChE had a fragment at the active site that could be modeled as covalently bound butyrate with the anionic intermediate directed into the oxyanion hole.[32] One of the virtues of the approach is that it limits the containment volume of the substrate and reduces the problem to a unimolecular one although rehybridization of the carbonyl carbon creates a new sp^3 stereocenter. Docking runs were therefore performed using AUTODOCK 3 with full bond rotation for both stereoisomers of *E*-**5a** and *E*-**5i**.[34,35] It was found that the only realistic poses for processing involving attack on the

Scheme 3. Synthesis of Isosorbide Based Alkylnitrate Aspirinates **7c−e**



Conditions: (i) PPh_3Cl_2, 180 °C, 4 h; (ii) Et_3N, DCM; (iii) $AgNO_3$, MeCN.

Table 2. Kinetic Data for the Hydrolysis of Nitroxy-Substituted Compounds **6**, **7a−e**, and Extent of Aspirin Released Based on Initial Ester Concentration in Moles Measured at Peak Aspirin Production Following Addition of Candidate Esters to Buffered Human Pplasma (10 and 50%) at 37 °C and pH 7.4 by HPLC



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	first-order rate constant (mol^{-1}) (a), half-life (min) (b), and mol % aspirin (c) released					
	10% plasma			50% plasma		
	a	b	c	a	b	c
R = (nitroxy)-acetoyl, **7a**	0.19	3.6	nd	0.77	0.9	nd
R = (nitroxy)-propanoyl, **7b**	0.18	3.9	nd	0.53	1.3	nd
R = (2-nitroxymethyl)-benzoyl, **7c**	0.26	2.7	32	0.21	3.2	34
R = (3-nitroxymethyl)-benzoyl, **7d**	0.26	2.6	51	0.25	2.7	55
R = (4-nitroxymethyl)-benzoyl, **7e**	0.11	6.0	< 5	0.29	2.4	< 1

benzoate ester were those in which the isosorbide-5-ester was orientated toward Trp82 (the cation−π site) and the aspirin phenyl group into the acyl pocket (Leu286/Val288). The 5-salicylate group in **5a** is predicted to interact with Trp82 via $\pi-\pi$ attractions. There were also interactions between the salicylate −OH group and a H-bond network involving Asp70, Tyr332, Ser79, and Trp82 (Supporting Information (SI)). Figure 5 shows the 5-benzoate derivative **5i** in its most favorable pose following attack on the benzoate. The overall conformation is similar to that of **5a** apart from the H-bonding contacts to the salicylate −OH. There is a predicted energy difference between the docked poses of 2.4 kcal·mol^{-1} in favor of **5a**. Compound **5a** was then covalently docked to BChE such that the attack occurred at the acetate ester rather than the benzoic acid ester. While binding was predicted to be good, there were no specific interactions between the macromolecule and the OH group of the salicylate moiety (SI). The binding energy of the substrate in this orientation was predicted to be similar to that of the benzoate in Figure 5 (11.25 kcal·mol^{-1} compared to 12.15 kcal·mol^{-1}). It therefore appears that the −OH group of the salicylate ester in **5a** can form specific interactions with the enzyme when it is being hydrolyzed at the phenyl ester (specifically with the residues of the peripheral site). For compounds lacking the hydroxyl group, there is a smaller energy difference between formation of the intermediate/transition states for hydrolysis at the acetyl and benzoate esters.

Inhibition of Platelet Aggregation in Vitro. Compounds **5a**, **7c−d**, and aspirin were evaluated as inhibitors of platelet aggregation in platelet rich plasma (PRP) in response to collagen (5 μg/mL) and ADP (3 μM). At these concentrations, platelet aggregation induced by collagen is highly dependent on the release of arachidonic acid and TXA_2 generation, while aggregation by ADP is less thromboxane-dependent. As shown in Figure 6, **5a** and the nitroaspirin hybrids **7c** and **7d** and aspirin in the range 10−300 μM caused concentration-dependent inhibition of collagen-induced platelet aggregation. **5a** (20.6 μM) and hybrid **7d** (17.1 μM) were significantly more potent than aspirin (92.7 μM), which was similar in potency to **7c** (90.3 μM), a moderate aspirin releasing compound that also bears a nitroxyl ester (Table 3). Hybrid **7e**, which does not liberate aspirin in human plasma, did not cause inhibition of collagen-induced

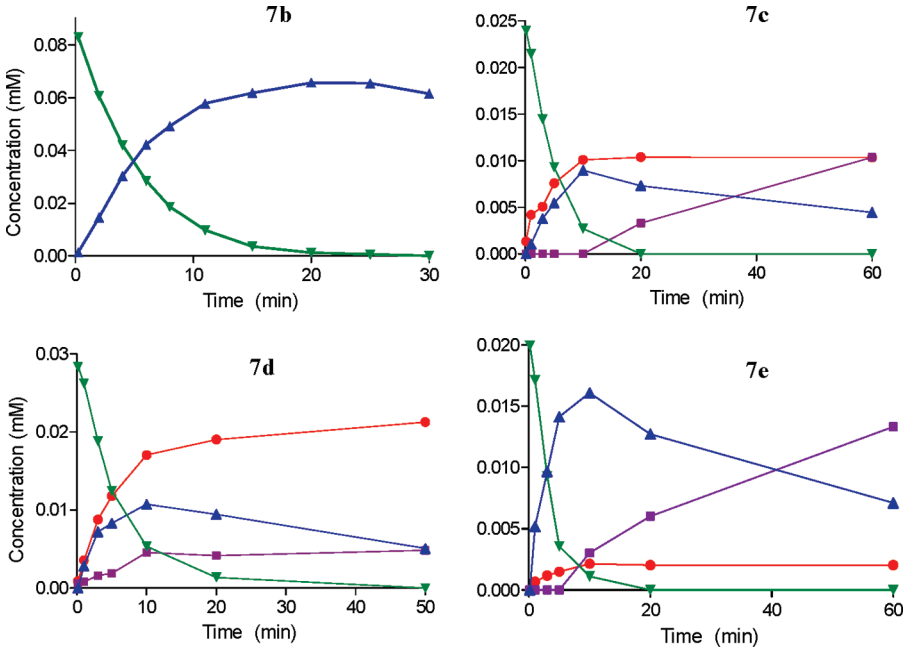

Figure 4. Progress curves for the disappearance of **7b** and the isomeric nitroxymethyl benzoates **7c**−**e** in 50% human plasma buffered at pH 7.4 (37 °C): prodrug (downward-pointing solid green triangle), aspirin (solid red circle), isosorbide-2-salicylate-5-ester (upward-pointing solid blue triangle), salicylic acid (solid purple square).

Table 3. Inhibition Data for Platelet Aggregation in Response to Collagen (5μg/mL) in PRP

compd	*n*	IC_{50} (μM)	SE	CI	% aspirin
5a	3	20.6	1.6	17.2−23.9	80
7c (*ortho*)	5	90.3	5.3	79.5−100.1	34
7d (*meta*)	3	17.1	4.1	8.8−26.4	55
7e (*para*)	3	> 300			1
aspirin	3	92.7	3.6	84.9−100.4	100

aggregation at concentrations < 300 μM. Compounds **5a** and **7d** were also significantly more potent inhibitors of ADP (3 μM)-induced aggregation than aspirin. However, when the aggregation experiments were repeated using collagen or ADP in the presence of the cGMP inhibitor 1*H*-[1,2,4]-oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ), there was no attenuation of platelet inhibition by **7d**, indicating that in PRP in vitro, NO release from **7d** did not make a significant contribution to inhibition. In washed platelets suspension (WP), the addition of 1 mM glutathione (GSH), which promotes NO release from nitrates, enhanced the inhibitory effects of **7d** (Figure 6c). This subtle effect could be abolished by preincubating WP with ODQ. The data indicates that significant inhibition of aggregation by compounds **7** in PRP requires the presence of promoters of NO release. This is consistent with the potency of the non-nitrate **5a**, the lack of effect of the nonaspirin releasing **7e**, and the generally reported failure of platelets to release substantial amounts of nitric oxide from organic nitrates. The unexpected potency of **7d** in this context will be the subject of careful pharmacological studies in vitro, and in vivo where nitric oxide release from other cell types can be expected to contribute to effects.

Conclusion

We have shown that by taking account of the substrate preferences, human BuChE can be used as a vector for the release of aspirin and a nitric oxide precursor from aspirin

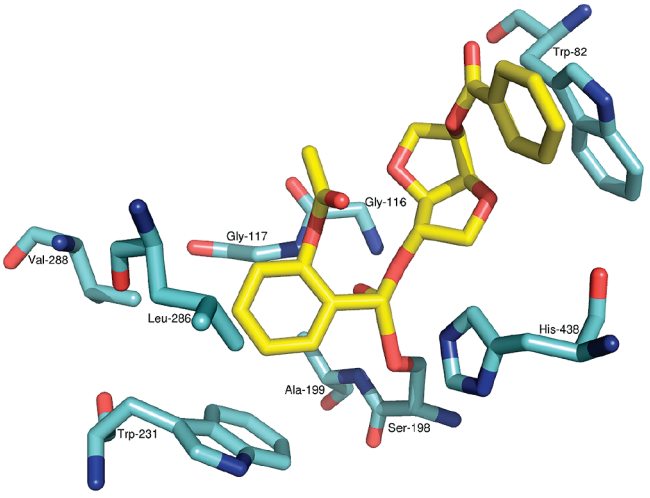

Figure 5. Predicted (and representative) lowest energy conformation for **5i** bound covalently to BChE at the phenyl ester as the tetrahedral intermediate using Autodock 3.

hybrid esters. Compounds **7c** and **7d** are the first compounds reported with the ability to generate aspirin and a potential nitric oxide releasing group under physiologically relevant conditions. They are potent inhibitors of ADP and collagen induced platelet aggregation. They will prove valuable in pharmacological studies on the interactions between nitric oxide and COX inhibition in vivo.

Experimental Section

Chemistry. ^{1}H and ^{13}C spectra were recorded at 27 °C on a Bruker DPX 400 MHz FT NMR spectrometer (400.13 MHz ^{1}H, 100.61 MHz ^{13}C) or a Bruker AV600 (600.13 MHz ^{1}H, 150.6 MHz ^{13}C) in either $CDCl_3$ or $(CD_3)_2CO$ with TMS as internal standard. In $CDCl_3$, ^{1}H spectra were assigned relative to the TMS peak at 0.0 ppm and ^{13}C spectra were assigned relative to the middle $CDCl_3$ triplet at 77.00 ppm. In $(CD_3)_2CO$, ^{1}H spectra

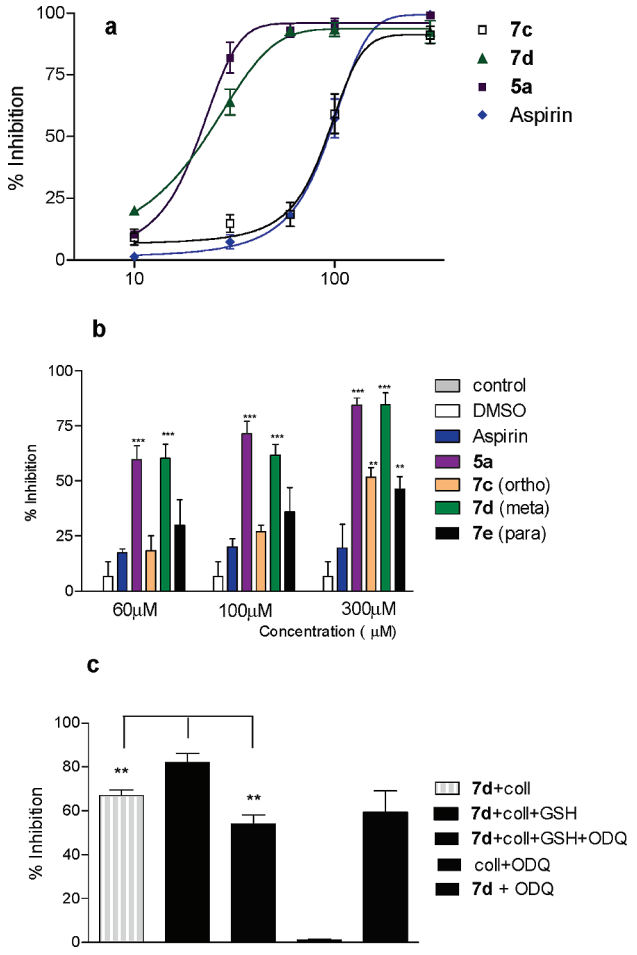

Figure 6. (a) Concentration−response curves showing inhibition of collagen-induced human platelet aggregation in vitro by **5a**, **7c**−**7d**, and aspirin. Data are mean ± SEM, n = 3−7, collagen 5 μg mL^{-1}. (b) Inhibition of platelets aggregation by aspirin, **5a**, and **7c**−**7e** in human rich plasma (PRP), using ADP (3 μM). (c) Inhibition of platelet aggregation in WP using **7d** (200 μM), and 1 mM GSH; ODQ (10 μM) preincubated for 5 min; using collagen 5 μg mL^{-1}. Data are mean ± SEM, n = 3−7, ANOVA.

were assigned relative to the $(CD_3)_2CO$ peak at 2.05 ppm and ^{13}C spectra were assigned relative to the $(CD_3)_2CO$ at 29.5 ppm. Coupling constants were reported in hertz (Hz). HRMS was performed using a Micromass mass spectrophotometer with electrospray ionization at the School of Chemistry, Trinity College, Dublin. Elemental analyses were performed at the Microanalytical Laboratory, Department of Chemistry, University College, Dublin. Flash chromatography was performed on Merck Kieselgel (particle size: 60 mm). Compounds **5a**, **5b**, **6a**, and **6b** were prepared as previously reported. Compounds described were synthesized according to general procedures outlined below.

General Procedure 1 (GP1). To a solution of **5b** (1 equiv) and carboxylic acid (1.1 equiv) in anhydrous dichloromethane was added DMAP (0.1 equiv) and the solution was left stirring at 0 °C for 10 min. DCC (1.1 equiv) was then added and the solution was left stirring for 12 h. The solution was then filtered, and the filtrate washed successively with 1 M HCl, saturated NaH_2CO_3 (aq), water, and brine. The solution was then dried with Na_2SO_4, filtered, and solvent removed under reduced pressure. Compounds were purified by flash chromatography.

General Procedure 2 (GP2). To a solution of **5b** (1 equiv) in anhydrous dichloromethane was added anhydrous triethylamine (1.1 equiv) followed by carboxylic acid chloride (1.1 equiv). The reaction mixture was left stirring for 12 h under a nitrogen atmosphere with the absence of light. The reaction mixture was then washed successively with 1 M HCl, saturated NaH_2CO_3 (aq), water, and brine. The solution was then dried with Na_2SO_4, filtered, and solvent removed under reduced pressure. Compounds were purified by flash chromatography with eluents stated.

Isosorbide-2-aspirinate-5-(-3-hydroxybenzoate) (5c). 5b (0.3 g, 0.98 mmol) was reacted with 3-benzyloxy benzoic acid (1.07 mmol.) according to GP1 to give isosorbide-2-aspirinate-5-(3-benzyloxybenzoate) (0.05 g, 9%) after flash chromatography using EtOAc and hexane (1:4) as eluent. HRMS: $C_{29}H_{26}O_9$ $[M + Na]^+$ requires: 541.1475; found: 541.1474. ^1H NMR δ ($CDCl_3$): 2.37 (3H, s), 4.07 (1H, m), 4.11 (3H, m), 4.65 (1H, d, J = 5.0 Hz), 5.05 (1H, t, J = 5.5 and 5.0 Hz), 5.16 (2H, s), 5.56 (1H, m), 7.13 (1H, d, J = 8.04 Hz), 7.27 (1H, t, J = 8.04 and 6.52 Hz), 7.33−7.42 (5H, m), 7.47 (2H, t, J = 8 Hz), 7.6 (1H, td, J = 8 Hz, 2 Hz), 7.7 (2H, m), 8.05 (1H, dd, J = 8 Hz, 2 Hz). HRMS: $C_{29}H_{26}O_9$, $[M + H]^+$ requires 518.4344, found 518.4357. Anal. $C_{29}H_{26}O_9$ requires C 67.19, H 5.05; found C 67.28, H 5.09. Isosorbide-2-aspirinate-(3-benzyloxybenzoate) (0.2 g, 0.07 mmol) was dissolved in methanol and EtOAc (10 mL, 1:1) and left stirring under an atmosphere of hydrogen for 12 h in the presence of palladium on carbon. After removal of palladium by filtration through celite and removal of solvent under reduced pressure, crude product was recrystallized from diethyl ether to give **5c** as white crystals (30 mg, 91%). HRMS: $C_{22}H_{20}O_9$ $[M + Na]^+$ requires 451.1005; found: 451.0996. Anal. $C_{22}H_{20}O_9$ requires C 61.69, H 4.71; found C 61.72, H 4.75. ^1H NMR δ ($CDCl_3$): 2.37 (3H, s), 4.07 (1H, m), 4.11 (3H, m), 4.65 (1H, d, J = 5.0 Hz), 5.05 (1H, t, J = 5.5 and 5.0 Hz), 5.56 (1H, m), 7.10 (2H, m), 7.33 (2H, m), 7.60 (3H, m), 8.0 (1H, d, J = 8 Hz).

Isosorbide-2-aspirinate-5-(4-hydroxybenzoate) (5d). 5b (0.25 g, 0.8 mmol) and *p*-benzyloxybenzoic acid (0.182 g, 0.88 mmol) were reacted together according to GP1 to give isosorbide-2-aspirinate-5-(4-benzyloxybenzoate) (0.346 g 85%) as a white solid after flash chromatography with EtOAc:Hex (1:2) as eluent. HRMS $C_{29}H_{26}O_9$, $[M + Na]^+$ requires 541.1470, found 541.1475. Anal. $C_{29}H_{26}O_9$ requires C 67.19, H 5.05; found C 67.35, H 5.18. ^1H NMR δ ($CDCl_3$) 2.4 (3H, s), 4.1 (4H, m), 4.65 (1H, d, J = 5.5 Hz), 5.05 (1H, t, J = 5 Hz), 7.1 (1H, d, J = 8.5 Hz), 5.5 (3H, m), 7.25 (5H, m), 7.35 (1H, t, J = 1 Hz), 7.45 (m, 4H), 7.7 (1H, dt, J = 8 Hz, 1.5 Hz), 8.0 (1H, d, J = 8 Hz). Isosorbide-2-aspirinate-(4-benzyloxybenzoate) (0.2 g, 0.35 mmol) was dissolved in methanol and EtOAc (20 mL, 1:1) and left stirring under an atmosphere of hydrogen for 12 h in the presence of palladium on carbon. After removal of palladium by filtration through celite, crude product was recrystallized from diethyl ether to give **5d** as white crystals (0.15 g, 91%). HRMS: $C_{22}H_{20}O_9$ $[M + Na]^+$ requires 451.1005; found 451.1003. Anal. $C_{22}H_{20}O_9$ requires C 61.69, H 4.71; found C 61.65, H 5.16. ^1H NMR δ ($CDCl_3$): 2.37 (3H, s), 4.10 (4H, m), 4.65 (1H, d, J = 5 Hz), 4.95 (1H, t, J = 5 Hz), 5.43 (1H, q, J = 5 Hz), 5.47 (1H, s), 6.87 (2H, d, J = 8.5 Hz), 7.12 (1H, dd, J = 8 and 1 Hz), 7.33 (2H, dt, J = 5.5 and 1.5 Hz), 7.65 (1H, dt, J = 8 and 1.5 Hz), 7.95 (2H, m).

Isosorbide-2-aspirinate-5-acetate (5e). 5b (0.2 g, 0.65 mmol) was reacted with acetic anhydride (0.073 g, 0.72 mmol) according to GP2 to give compound **5e** as white crystalline material (0.1 g, 43.8%) after flash chromatography using EtOAc:hexane (2:3) as eluent; mp 96−98 °C. HRMS: $C_{17}H_{18}O_8$ $[M + H]^+$ requires 351.1080; found 351.1087. Anal. $C_{17}H_{18}O_8$ requires C 58.28, H 5.18; found C 58.20, H 5.20. ^1H NMR δ ($CDCl_3$): 2.13 (3H, s), 2.37 (3H, s), 3.85 (1H, q, J = 5.55, 4.52, 4.96 Hz), 3.99 (1H, q, J = 6.0, 3.52, and 6.04 Hz), 4.10 (2H, t, J = 3.52, 2.0 Hz), 4.59 (1H, d, J = 4.52 Hz), 4.90 (1H, t, J = 5.0, 5.04 Hz), 5.19 (1H, d, J = 5.52 Hz), 5.44 (1H, d, J = 5.52 Hz), 7.12 (1H, d, J = 8.04 Hz), 7.33 (1H, t, J = 7.52, 7.56 Hz), 7.59 (1H, m), 8.01 (1H, dd, J = 6.04, 2.05 Hz).

Isosorbide-2-aspirinate-5-propionate (5f). 5b (0.3 g, 0.98 mmol) was reacted with propionic anhydride (0.14 mL, 1.07 mmol) according to GP2 to yield crude product as a yellow oil (0.19 g).

Purification by flash chromatography using hexane and EtOAc (5:2) as eluent yielded product as white crystals (0.3 g, 84.3%); mp 54−56 °C. HRMS: $C_{18}H_{20}O_8$ $[M + Na]^+$ requires 387.1056; found 387.1069. ^{1}H NMR δ ($CDCl_3$): 1.19 (3H, t, J = 8.04 and 7.52 Hz), 2.37 (3H, s), 2.44 (2H, q, J = 7.52, 8.04, and 7.52 Hz), 3.86 (1H, q, J = 5.52, 4.52, and 5.04 Hz), 3.98 (1H, q, J = 5.52, 4.04, and 6.0 Hz), 4.08 (2H, m), 4.59 (1H, d, J = 4.52 Hz), 4.91 (1H, t, J = 5.0 and 5.04), 5.20 (1H, q, J = 5.04, 6.0, and 5.52 Hz), 5.43 (1H, d, J = 3.0 Hz), 7.12 (1H, dd, J = 1.0 and 1.0 Hz), 7.33 (1H, t, J = 1.0, 6.56, and 8.0 Hz), 7.59 (1H, t, J = 6.0 and 6.52 Hz), 8.01 (1H, dd, J = 1.48 and 2.0 Hz).

Isosorbide-2-aspirinate-5-pentanoate (5g). 5b (0.25 g, 0.82 mmol) was reacted with valeroyl chloride (0.108 g, 0.9 mmol) according to GP2 to give a yellow oil, which was purified by flash chromatography using hexane and EtOAc (4:1) as eluent yielding **5g** as a yellow oil (0.25 g, 65%). HRMS: $C_{20}H_{24}O_8$ $[M + H]^+$ requires 393.1549; found 393.1559. Anal. $C_{20}H_{24}O_8$ requires C 61.22, H 6.16; found C 61.35, H 6.02. ^{1}H NMR δ ($CDCl_3$): 0.97 (3H, t, J = 8 Hz), 1.40 (2H, m), 1.65 (2H, m), 2.37 (3H, s), 2.41 (2H, t, J = 8 Hz), 3.95 (2H, m), 4.1 (2H, m), 4.60 (1H, d, J = 5 Hz), 4.95 (1H, t, J = 5 Hz), 5.20 (1 H, q, J = 4.5 Hz), 5.45 (1 H, d, J = 3 Hz), 7.13 (1H, d, J = 8 Hz), 7.34 (1H, t, J = 8 Hz), 7.60 (1H, dt, J = 8 Hz, 2.5 Hz), 8.05 (1H, dd, J = 8 Hz, 2 Hz).

Isosorbide-2-aspirinate-5-cyclopropanoate (5h). 5b (0.506 g, 1.6 mmol) was reacted with cyclopropane carbonyl chloride (2 mmol) according to GP2 to followed by purification by flash chromatography using hexane and EtOAc (2:1) as eluent to give **5h** as a clear oil (0.396 g, 65%). ^{1}H NMR δ ($CDCl_3$) 0.9−1.18 (m, 4H), 2.32 (3H, s), 3.78 (m, 1H), 3.9 (m, 1H), 4.06 (2H, m), 4.5 (1H, d, J = 5 Hz), 4.83 (1H, t, J = 5 Hz), 5.12 (1H, q, J = 4.5 Hz), 5.38 (1H, s), 7.06 (1H, d, J = 8 Hz), 7.26 (1H, dt, J = 8, 2.5 Hz), 7.52 (1H, t, J = 8 Hz), 7.95 (1H, dd, J = 8, 2 Hz).

Isosorbide-2-aspirinate-5-benzoate (5i). 5b (1.0 g, 3.25 mmol) was reacted with benzoic acid (0.59 g, 4.88 mmol), according to GP1 to give colorless oil, which was recrystallized in ethanol to afford **5i** as white crystals (1.13 g, 84.3%); mp 80−82 °C. HRMS: $C_{22}H_{20}O_8$ $[M + H]^+$ requires 413.1236; found 413.1226. Anal. $C_{22}H_{20}O_8$ requires C 64.07, H 4.89; found C 63.99, H 4.96. ^{1}H NMR δ ($CDCl_3$): 2.37 (3H, s), 4.07 (1H, m), 4.11 (3H, m), 4.65 (1H, d, J = 5.0 Hz), 5.05 (1H, t, J = 5.5 and 5.0 Hz), 5.56 (1H, m), 7.13 (1H, d, J = 8.04 Hz), 7.27 (1H, t, J = 8.04 and 6.52 Hz), 7.33 (1H, d, J = 7.56 Hz), 7.49 (2H, t, J = 7.52 and 7.56 Hz), 8.01 (1H, d, J = 7.56 Hz), 8.12 (2H, d, J = 7.52 Hz).

Isosorbide-2-aspirinate-5-(2-methylbenzoate) (5j). 5b (0.2 g, 0.65 mmol) was reacted with *2*-toluoyl chloride (0.09 mL, 0.72 mmol) according to GP2 to give 0.41 g of crude product as brown oil. Purification by flash chromatography using hexane and EtOAc (2:1) as eluent gave product as yellow oil. This was recrystallized in ethanol to yield **5j** as a white solid (0.11 g, 39.6%); mp 104−106 °C. HRMS: $C_{23}H_{22}O_8$, $[M + Na]^+$ requires 449.1212; found 449.1238. Anal. $C_{23}H_{22}O_8$ requires C 64.78, H 5.20; found C 64.78, H 5.29. ^{1}H NMR δ ($CDCl_3$): 2.38 (3H, s), 2.65 (3H, s,), 4.01 (1H, dd, J = 5.52 and 5.52 Hz), 4.12 (3H, m), 4.66 (1H, d, J = 4.52 Hz), 5.04 (1H, t, J = 5.04 and 5.0 Hz), 5.41 (1H, q, J = 5.52 Hz), 5.47 (1H, d, J = 2.0 Hz), 7.13 (1H, dd, J = 1.0 and 1.0 Hz), 7.33 (1H, t, J = 7.0 and 6.52 Hz), 7.59 (1H, t, J = 6.52 and 6.52 Hz), 8.02 (1H, dd, J = 1.52 and 2.0 Hz).

Isosorbide-2-aspirinate-5-(3-methylbenzoate) (5k). 5b (0.2 g, 0.65 mmol) was reacted with 3-toluic acid (0.09 g, 0.72 mmol) according to GP1 to yield crude product as a clear oil. Purification by flash chromatography using hexane and EtOAc (3:2) as eluent yielded compound **5k** as white crystals (0.12 g, 43.2%); mp 96−98 °C. HRMS: $C_{23}H_{22}O_8$, $[M + Na]^+$ requires 449.1212; found 449.1234. Anal. $C_{23}H_{22}O_8$ requires C 64.78, H 5.20; found C 64.67, H 5.28. ^{1}H NMR δ ($CDCl_3$): 2.36 (3H, s), 2.43 (3H, s), 4.09 (4H, m), 4.65 (1H, d, J = 5.0 Hz), 5.04 (1H, t, J = 5.04 and 5.0 Hz), 5.43 (2H, m), 7.12 (1H, d, J = 8.0 Hz), 7.35 (3H, m), 7.58 (1H, q, J = 1.0, 6.56, and 1.48 Hz), 8.01 (1H, dd, J = 1.0 and 1.52 Hz).

Isosorbide-2aspirnate-5-(4-phenylbenzoate) (5s). Isosrbide-2-aspirinate **5b** (200 mg, 0.6 mmol) and 4-phenylbenzoylchloride (0.156 g, 0.72 mmol) were reacted together to give **5s** (0.185 g, 65%) as a colorless oil after flash chromatography using hexane and EtOAc (4:1) as eluent. HRMS $C_{28}H_{24}O_8$, $[M + H]^+$ requires 489.4933; found 489.5021. Anal. $C_{28}H_{24}O_8$ requires C 68.85, H 4.95; found C 68.88, H 5.08. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.4 (3H, s), 4.1 (4H, m), 4.65 (1H, d, J = 5 Hz), 5.05 (1H, t, J = 5 Hz), 5.45 (2H, m), 7.1 (1H, d, J = 8.5 Hz), 7.35 (1H, dt, J = 6.5 Hz, 1 Hz), 7.45 (1H, t, J = 8 Hz), 7.5 (2H, t, J = 7.5 Hz), 7.6 (1H, dt, J = 8 Hz, 1.5 Hz), 7.65 (2H, d, J = 7 Hz), 7.7 (2H, d, J = 8.5 Hz), 8.0 (1H, dd, J = 8 Hz, 1.5 Hz), 8.2 (2H, d, J = 8.5 Hz).

Isosorbide-2-aspirinate-5-nicotinate (5v). 5b (0.3 g, 0.98 mmol), was reacted with nicotinic acid (0.12 g, 0.98 mmol) according to GP1 to give product as a crude oil (0.95 g). Purification by flash chromatography using DCM and EtOAc (95:5) as eluent yielded compound **5p** as white crystals (0.12 g, 29.7%); mp 94−96 °C. HRMS: $C_{21}H_{19}NO_8$ $[M + Na]^+$ 436.1008; found 436.1011. Anal. $C_{21}H_{19}NO_8$ requires C 61.02, H 4.63, N 3.39; found C 61.13, H 4.28, N 3.45. ^{1}H NMR δ ($CDCl_3$): 2.36 (3H, s), 4.11 (9H, m), 6.64 (1H, d, J = 4.52 Hz), 5.05 (1H, t, J = 5.04 and 5.52 Hz), 5.46 (2H, dd, J = 2.0 and 2.52 Hz), 7.11 (1H, d, J = 8.52 Hz), 7.32 (1H, q, J = 6.52, 8.04, and 8.52 Hz), 7.43 (1H, q, J = 6.53, 8.04, and 8.52 Hz), 7.59 (1H, t, J = 6.04 and 6.52 Hz), 8.00 (1H, dd, J = 1.52 and 2.0 Hz), 8.34 (1H, m), 8.82 (1H, dd, J = 2.0 and 1.48 Hz), 9.28 (1H, d, J = 2.0 Hz).

Isosorbide-2-aspirinate-5-iso-nicotinate (5w). 5b (0.2 g, 0.65 mmol) was reacted with isonicotinic acid (0.08 g, 0.65 mmol) according to GP1 to give compound **5w** (0.17 g, 63.1%) as white crystals following purification by flash chromatography using DCM and EtOAc (95:5) as eluent. mp 86−88 °C. HRMS: $C_{21}H_{19}NO_8$ $[M + Na]^+$ requires 436.1008; found 436.1004. ^{1}H NMR δ ($CDCl_3$): 2.37 (3H, s), 4.09 (5H, m), 4.65 (1H, d, J = 4.52 Hz), 5.05 (1H, t, J = 5.52 and 5.04 Hz), 5.46 (2H, dd, J = 5.52 and 5.04 Hz), 7.12 (1H, d, J = 7.04 Hz), 7.33 (1H, m), 7.59 (2H, t, J = 6.04 and 6.04 Hz), 7.90 (1H, d, J = 5.04 Hz), 8.01 (H, dd, J = 2.0 and 1.52 Hz), 8.84 (1H, s), 8.98 (1H, s).

Isosorbide-2-aspirinate-5-(-4-methyl-1,2,3-thiadiazole-5-oate) (5aa). 5b (0.2 g, 0.65 mmol) and 4-methyl-1, 2, 3-thiadiazole-5-carboxylic acid were reacted together according to GP1 to give **5aa** (0.228 g 83%) as a pale-pink foam after flash chromatography using hexane and EtOAc (3:1) as eluent. HRMS $C_{19}H_{18}N_2O_8S$ $[M + Na]^+$ requires 457.0682; found 457.0679. ^{1}H NMR δ ($CDCl_3$) 2.4 (3H, s), 3.05(3H, s), 4.1 (4H, m), 4.65 (1H, d, J = 5.5 Hz), 5.05 (1H, t, J = 5 Hz), 5.5 (2H, m), 7.15 (1H, d, J = 8.5 Hz), 7.35 (1H, t, J = 1 Hz), 7.7 (1H, dt, J = 8 Hz, 1.5 Hz), 8.1 (1H, d, J = 8 Hz).

Isosorbide-2-aspirinate-5-(1-methyl-(1*H*)-pyrazole-5-carboxylate) (5bb). 5b (0.15 g, 0.48 mmol) and 1-methyl-(1*H*)-pyrazole-5-carboxylic acid (0.055 g, 0.44 mmol) were reacted according to GP1 to give **5bb** (60 mg 30%) as a yellow oil after flash chromatography using hexane and EtOAc (1:1) as eluent. HRMS: $C_{20}H_{20}N_2O_8$ $[M + Na]^+$ requires 439.1117; found 439.1113. ^{1}H NMR δ ($CDCl_3$) 2.37 (3H, s), 3.87 (1H, q, J = 5.55, 4.52 Hz), 4.05 (1H, q, J = 6.05, 3.52 Hz), 4.13 (5H, m), 4.63 (1H, d, J = 4.52 Hz), 4.95 (1H, t, J = 5.00 Hz), 5.30 (1H, m), 5.44 (1H, d, J = 5.52 Hz), 6.95 (1H, d, J = 2.01 Hz), 7.15 (1H, dd, J = 8.04, 1.00 Hz), 7.35 (1H, dt, J = 7.56, 1.00 Hz), 7.55 (1H, d, J = 2.01 Hz), 7.59 (1H, m), 8.05 (1H, dd, J = 6.05, 2.0 Hz).

Isosorbide-2-aspirinate-5-(2-nitroxymethyl)benzoate (7c). Phthalide (37 mmol) and dichlorotriphenylphosphorane (38 mmol) were heated at 180 °C for 4 h with stirring. Color change from green to brown was seen over the course of 4 h. 2-Chloromethylbenzoyl chloride (600 μL) was reacted with compound **5b** (0.52 g, 1.7 mmol) according to GP2, producing 769.5 mg of a brown/green oil. This was purified by flash chromatography using hexane and EtOAc (2:1) as eluent to give (0.419 g, 51%) of a white solid. ^{1}H NMR δ ($CDCl_3$) 400 MHz: 2.38 (3H, s), 4.03 (4H, m), 4.66 (1H, d), 5.04 (2H, m), 5.10 (1H, s), 5.42 (2H, m), 7.12 (1H, d),

7.28 (1H, m), 7.42 (1H, m), 7.6 (3H, m), 8.01 (2H, dd). 400 mg was dissolved in CH_3CN/THF (6 mL, 4/2 v/v) and treated with $AgNO_3$ (1.7 mmol) and refluxed for 4 h before stirring overnight at room temperature while protected from light. The mixture was filtered and concentrated. This was reconstituted in ethyl acetate (10 mL) and water (2 mL). The organic phase was washed with water (3 × 2 mL), brine (2 mL), and dried over sodium sulfate and concentrated, producing an oil that was purified by flash chromatography using hexane/ethyl acetate (2:1) resulting in 95 mg of yellow wax-like material. HRMS: $C_{23}H_{21}NO_{11}$ $[M + Na]^+$ requires 510.1113; found 510.1195. Anal. $C_{23}H_{21}NO_{11}$ requires C 56.68, H 4.34, N 2.87; found C 56.50, H 4.24, N 2.37. ^{1}H NMR δ ($CDCl_3$) 400 MHz: 2.4 (3H, s), 4.01 (4H, m), 4.65 (1H, d), 5.02 (1H, t), 5.41 (2H, m), 5.86 (2H, s), 7.11 (1H, d), 7.28 (1H, t), 7.49 (2H, q), 7.61 (2H, q), 8.01 (1H, d), 8.10 (1H, d).

Isosorbide-2-aspirinate-5-(3-nitrooxymethyl)benzoate (7d). 3-Chloromethylbenzoyl chloride (3.5 mmol) was reacted with compound **5b** (1.6 mmol) according to GP2, producing 1.18 g of an oil. This was chromatographed using hexane/ethyl acetate (3:1), resulting in 903.4 mg of an oil. ^{1}H NMR δ ($CDCl_3$) 400 MHz: 2.37 (3H, s, $OCOCH_3$), 4.06 (4H, m), 4.65 (3H, ds), 5.03 (1H, t), 5.43 (2H, dd), 7.10 (1H, d), 7.32 (1H, t), 7.47 (1H, t), 7.57 (2H, m), 8.00 (2H, m), 8.10 (1H, s). This was dissolved in CH_3CN/THF (6 mL, 4/2 v/v) and treated with $AgNO_3$ (3.9 mmol) and refluxed for 4 h before stirring overnight at room temperature while protected from light. Mixture was filtered and concentrated. This was reconstituted in ethyl acetate (10 mL) and water (2 mL). The organic phase was washed with water (3 × 2 mL), brine (2 mL), and dried over sodium sulfate and concentrated, producing an oil that was chromatographed using hexane/ethyl acetate (1:1), resulting in 184.3 mg of yellow wax-like material. HRMS: $C_{23}H_{21}NO_{11}$ $[M + Na]^+$ requires 510.1113; found 510.1575. Anal. $C_{23}H_{21}NO_{11}$ requires C 56.68, H 4.34, N 2.87; found C 56.41, H 4.46, N 2.75. ^{1}H NMR δ ($CDCl_3$) 400 MHz: 2.38 (3H, s), 4.09 (4H, m), 4.65 (1H, d), 5.05 (1H, t), 5.5 (4H, dd), 7.12 (1H, d), 7.29 (1H, t), 7.50 (3H, m), 7.65 (1H, d), 8.01 (2H, s).

Isosorbide-2-aspirinate-5-(4-nitrooxymethyl)benzoate (7e). 4-Chloromethylbenzoyl chloride (650 μL) was reacted with compound **5b** (1.7 mmol) according to GP2 and chromatographed using hexane/ethyl acetate (2:1), resulting in 100 mg of white solid. ^{1}H NMR δ ($CDCl_3$) 400 MHz: 2.35 (3H, s), 4.04 (4H, m), 4.6 (4H, m), 5.04 (1H, d), 5.42 (2H, t), 7.09 (1H, d), 7.26 (1H, t), 7.47 (2H, m), 7.51 (1H, q), 8.00 (1H, d), 8.06 (2H, m). This was dissolved in CH_3CN/THF (6 mL, 4/2 v/v) and treated with $AgNO_3$ (0.4 mmol) and refluxed for 4 h before stirring overnight at room temperature while protected from light. Mixture was filtered and concentrated. This was reconstituted in ethyl acetate (10 mL) and water (2 mL). The organic phase was washed with water (3 × 2 mL) and brine (2 mL) and dried over sodium sulfate. Concentrated, producing an oil which was chromatographed using hexane/ethyl acetate (2:1) resulting in 28.3 mg of off-white solid. HRMS: $C_{23}H_{21}NO_{11}$ $[M + Na]^+$ requires 510.1113; found 510.1575. Anal. $C_{23}H_{21}NO_{11}$ requires C 56.68, H 4.34, N 2.87; found C 56.58, H 4.43, N 2.67. ^{1}H NMR δ ($CDCl_3$) 400 MHz: 2.35 (3H, s), 4.04 (4H, m), 4.62 (1H, d), 5.01 (1H, t), 5.41 (2H, m), 5.48 (2H, s), 7.09 (1H, d), 7.31 (1H, t), 7.48 (2H, d), 7.55 (1H, t), 8.00 (1H, d), 8.10 (2H, d).

Experimental Method: Hydrolysis Studies Using Plasma/Enzyme Solutions. Pooled plasma/serum solutions (4 mL) were prepared to the correct strength by dilution of plasma with phosphate buffer pH 7.4 (e.g., for a 10% solution, 0.4 mL of plasma/serum was added to 3.6 mL of phosphate buffer pH 7.4). Following equilibration of the plasma/serum sample at 37 ± 0.5 °C, 100 μL of a stock solution of test compound in acetonitrile (1×10^{-4} M) was added and 250 μL aliquots were removed at specified time intervals. Samples were transferred to 1.5 mL Eppendorf tubes containing 500 μL of a 2% w/v solution of $ZnSO_4 \cdot 7H_2O$ (water:acetonitrile, 1:1). Tubes were vortexed for 2 min and then centrifuged at 10000 rpm for 3 min at room temperature. Supernatant was aspirated off and analyzed by HPLC. The concentration of test compound and metabolites were determined with reference to calibration curves run on that day in the same concentration range and under the same experimental conditions. The metabolic fate of the esters under these conditions was also determined by RPHPLC by measuring the concentration of drug and metabolites in the medium as a function of time. The identity of participating enzymes was confirmed by using purified enzyme in the case of plasma (BuChE) and by repeating the hydrolysis experiments in the presence of esterase specific inhibitors: isoOMPA for BuChE and BNPP for carboxylesterase. The BuChE activity of plasma and microsomal samples was determined using the Ellman assay.[35]

Molecular Modeling Studies. The approach we used for investigating covalent docking of the substrate is described fully in ref 31.

HPLC/HPLCMS. High performance liquid chromatography was performed using a system consisting of a Waters 600 pump and controller, Waters 717 autosampler, and a Waters 2996 photodiode array detector controlled by Empower software. A Hichrom Nucleosil C18 column (4.0 mm × 250 mm) was used. For LCMS, measurement were carried out on a Thermo Accela chromatography system fitted with a Waters Xbridge C18 column, 2.1 mm × 50 mm, 2.5 μm at room temperature. Mobile phase A was 0.1% formic acid in water, mobile phase B was acetonitrile. Gradient conditions were 0−4 min, 20−80%B, 4−10 min hold at 80%B, 11 min 80−20%B, equilibrate to 15 min. Flow rate was 100 μL/min. The prodrug and its hydrolysis products were detected using positive and negative electrospray ionization on a Thermo LTQ-XL ion-trap coupled to the Thermo Orbitrap Discovery. Capillary temp (°C), 275.00; sheath gas flow, 30.00; aux gas flow, 10.00; sweep gas flow, 0.00. Positive polarity: source voltage (kV), 3.00; source current (μA), 100.00; capillary voltage (V), 46.00; tube lens (V) 55.00. Negative polarity: source voltage (kV), 3.50; source current (μA), 100.00; capillary voltage (V), −50.00; tube lens (V) −87.29.

Platelet Aggregation. Platelet aggregation was measured by light aggregometry. PRP (2.5×10^8/mL) was placed in a whole blood ionized calcium lumi-aggregometer (Chronolog Corp., Havertown, PA) and (BIO/DATA Corp.) and incubated for 10 min at 37 °C with stirring at 900 rpm prior to the addition of aggregating agents. Aggregation was initiated by the addition of agonists and monitored by Aggro-Link software for at least 6 min. For experiments using inhibitors, aggregation was initiated after 10 min preincubation. To study the aggregatory potency of ADP, the concentration−response (0.3−10 μM) curves were generated. Collagen at different concentrations (0.3−5.0 μg/mL) was also used to induce platelet aggregation. The submaximal concentrations of agonists, i.e., the concentrations that gave approximately 95% of the maximal aggregation, were used to study the effects of inhibitors of aggregation. Results were expressed in % changes in maximal light transmission, with 100% representing light transmission of platelet-poor plasma

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Supporting Information Available: Synthesis of Compounds **5e**, **5g**, **5j**, **5m**−**o**, **5p**, **5r**, **5t**−**u**, **5x**−**z**, **7a**−**b**, **10a**−**b**, modeling and purity data. This material is available free of charge via the Internet at http://pubs.acs.org.

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