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PAPER

Albumin-directed stereoselective reduction of 1,3-diketones and β-hydroxyketones to *anti* diols†‡

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The reduction of 1,3-diketones and β-hydroxyketones with $NaBH_4$ in aqueous acetonitrile is highly stereoselective in the presence of stoichiometric amounts of bovine or human albumin, giving *anti* 1,3-diols with d.e. up to 96%. The same reaction, without albumin, gives *syn* and *anti* 1,3-diols in approximately 1 : 1 ratio. The presence of an aromatic carbonyl group is essential for diastereoselectivity in the $NaBH_4$/albumin reduction of both 1,3-diketones and β-hydroxyketones. Thus, 3-hydroxy-1-(*p*-tolyl)-1-butanone is stereoselectively reduced in the presence of albumin, while reduction of its isomer 4-(*p*-tolyl)-4-hydroxy-2-butanone is not stereoselective. The albumin-controlled reduction is not stereospecific as both enantiomers of 1-aryl-3-hydroxy-1-butanones are reduced to diols with identical stereoselectivities. Circular dichroism of the bound substrates confirms that aromatic ketones are recognized by the protein's IIA binding site. Binding studies also suggest that 1,3-diketones are recognized in their enol form. From the effect of pH on binding of a diketone it is concluded that, in the complex with the substrate, ionizable residues His242 and Lys199 are in the neutral and protonated forms, respectively. A homology model of BSA was obtained and docking of model substrates confirms the preference of the protein for aromatic ketones. Modelling of the complexes with the substrates also allows us to propose a mechanism for the reduction of 1,3-diketones in which the chemoselective reduction of the first (aliphatic) carbonyl is followed by the diastereoselective reduction of the second (aromatic) carbonyl. The role of albumin is thus a combination of chemo- and stereocontrol.

Introduction

Albumin is the most abundant protein in the blood system where it serves several functions, including the control of osmotic blood pressure and the maintenance of blood pH.[1] However, albumin's most striking property is its ability to bind and transport an extraordinary large variety of small molecules. Albumin is the main carrier of unesterified fatty acids and other endogenous ligands such as hemin and bilirubin; it possesses a broad specificity for small aromatic and heteroaromatic compounds, including many drugs, and is able to bind metal cations and several inorganic ions.[1,2] A possible role as carrier of nitric oxide has also been proposed.[3]

Binding[4] and crystallographic[2a,5] studies have disclosed the three-dimensional structure and binding motifs of human serum albumin (HSA). This 585 a.a. protein (MW = 65 kDa) consists of three highly helical domains (Fig. 1) held together by seventeen disulfide bonds. At least five binding sites for long chain fatty acids, with different affinities, are located in domains I and III and at the interfaces between domains I–II and II–III.[5c] Two binding sites for small aromatic and heteroaromatic molecules, with different specificities, are present in subdomains IIA (drug site 1) and IIIA (drug site 2)[2,5a] (Fig. 1). Cystein-34 and the N terminus are the main binding sites for metal ions.[2a]

Primary structures of mammalian albumins are highly conserved. In particular, 76% homology is found between the amino acid sequences of HSA and bovine serum albumin (BSA).[6] Thus, a similar three-dimensional structure and binding site architecture can be expected for the two proteins.

A broad chemical reactivity is associated with the small-molecule binding site located in albumin's IIA subdomain. A lysine residue is present in this site in both HSA (Lys-199) and BSA (Lys-222). In addition to being a site for covalent interactions with many drugs such as, for example, aspirin[2] and benzylpenicillin,[7] this basic residue, surrounded by a hydrophobic environment, is responsible for albumin's ability to behave as

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† Dedicated to Professor Charles J. M. Stirling on his 80th birthday.

‡ Electronic supplementary information (ESI) available: ^{1}H NMR of the Mosher esters of racemic and enantiomerically pure hydroxy ketone **1b**; Scatchard plot of the data from Fig. 4b; superimposition of the IIa binding sites of HSA and BSA. See DOI: 10.1039/c0ob00648c

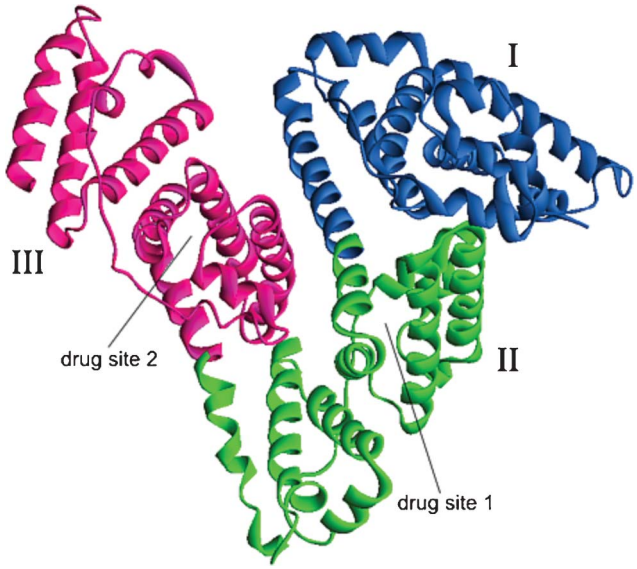


Fig. 1 3-D structure of HSA. The three domains are colour-coded: I, blue; II, green; III, red. The small-molecule binding sites in domains IIa (drug site 1) and IIIa (drug site 2) are shown.

an enzyme-like catalyst in reactions such as β-eliminations,[8] the decomposition of Meisenheimer adducts[9] and the eliminative ring fission of 5-nitrobenzisoxazole to 2-hydroxy-5-nitrobenzonitrile (Kemp elimination).[10] In the latter reaction, in particular, the catalytic activity of BSA and HSA is similar to that of specifically designed catalytic antibodies.[10c]

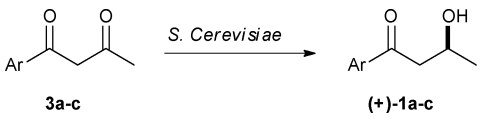
Due to its low cost, large scale availability, and exceptional ability to bind a wide range of substrates in an asymmetric environment, BSA has found many applications as a stationary phase in chiral chromatography,[2c,11] and as a chiral auxiliary and catalyst in organic reactions.[12] Asymmetric oxidations of sulfides,[13] disulfides,[14] thioacetals,[15] and tertiary amines,[16] reduction of aromatic ketones,[17] Diels–Alder cycloadditions,[18] epoxidation[19] and dihydroxylation[20] of alkenes, and the enantioselective hydrolysis of esters[21] have been reported to proceed with varying levels of stereoselectivity in the presence of BSA.

Recently,[22] we have reported on the remarkable ability of albumin to direct the borohydride reduction of β-diketones to the formation of *anti*-1,3-diols. Here we give a full account of this work and present new data on the albumin controlled reduction of β-hydroxyketones; we also describe the results of a study on the binding of diketones and hydroxyketones to BSA that allows us to identify the site of interaction. Finally, we present a model for the BSA-substrate complex that fully accounts for the selectivity observed in the albumin directed borohydride reduction.

Results and discussion

Preliminary results[22] indicated that albumin provides efficient *anti* control in the reduction of aromatic β-diketones, but has no effect in the reduction of aliphatic substrates. Thus, in the present study, we focused on the reduction of aromatic 3-hydroxyketones (**1a–e**) and 1,3-diketones (**3a–j**), to the corresponding *anti* and *syn* 1,3-diols **4** and **5** (Scheme 1). In order to determine whether albumin discriminates between enantiomers, hydroxyketones **1a–c** were studied both in racemic and enantiomerically pure form

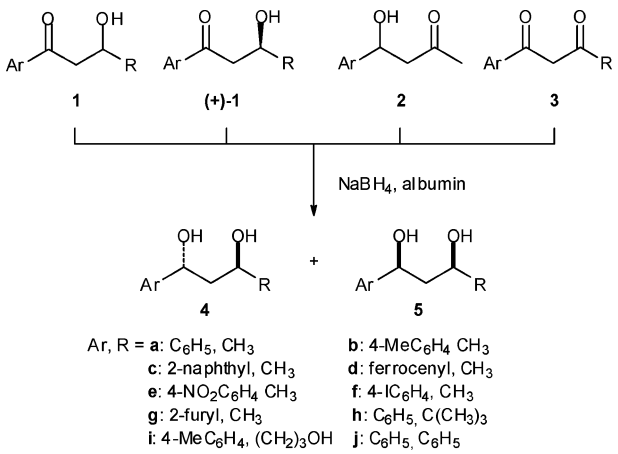
Table 1 Baker's yeast reduction of diketones **3**



3a-c **(+)-1a-c**

Diketone	Ar	Hydroxyketone	Yield [%]	e.e. [%][a]
3a	phenyl	**(*S*)-(+)-1a**	30	96
3b	*p*-tolyl	**(*S*)-(+)-1b**	83	>99
3c	2-naphthyl	**(*S*)-(+)-1c**	35	95

[a] e.e. was determined from the NMR spectra of the corresponding Mosher's esters.



Scheme 1 $NaBH_4$/albumin reduction of β-hydroxyketones and 1,3-diketones.

((+)-**1a–c**). The hydroxyketone **2b**, in which the aromatic group is remote from the carbonyl, was also included in this series for comparison with the corresponding aromatic ketone **1b**.

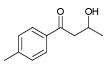
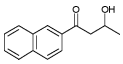
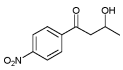
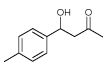
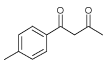
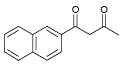
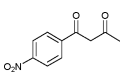
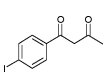
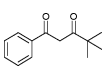
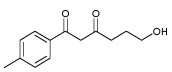
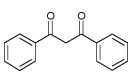
Substrates were obtained by conventional literature methods, as described in the experimental part. The enantiomerically pure hydroxy ketones (+)-**1a–c** were obtained by bakers' yeast reduction of the corresponding diketones **3a–c** (Table 1), as originally described by Chênevert[23] for the phenyl derivative (+)-**1a**. The (*S*) configuration was established for this compound,[23] in agreement with Prelog's rule for the reduction of prochiral ketones.[24] Considering the well known preference of yeast dehydrogenase for Prelog-like reduction of methyl ketones,[23b] it seems safe to assign the same configuration also to hydroxyketones **1b,c**. Enantiomeric excesses have been calculated from the ^{1}H NMR spectra of the corresponding Mosher esters‡ and are reported in Table 1.

Reduction of hydroxyketones

The reduction of hydroxyketones **1a–e** with six equivalents of sodium borohydride in a 9 : 1 water–acetonitrile mixture proceeds with no appreciable stereoselectivity (Table 2). However, when the same reaction is carried out in the presence of BSA, formation of the *anti* isomers **4** is favoured, with diastereoselectivities up to 94 : 6 for **1a**, **1b** and **1d**.[25]§ The BSA-induced *anti* selectivity is

§ The stereochemistry of the novel diols **4/5b,e,f,i** was confirmed by: a) ^{13}C spectral analysis of the corresponding acetonides with characteristic resonances for the geminal methyl groups at around 20 and 30 ppm

Table 2 $NaBH_4$/BSA reduction of hydroxyketones **1**, **2** and diketones **3** to *anti* and *syn* diols **4**, **5**[a]

Substrate		*anti*/*syn* ratios[b,c]		Chemoselectivity[b,d]	
		$NaBH_4$	$NaBH_4$/BSA	$NaBH_4$	$NaBH_4$/BSA
	**1a** **(+)-1a**	47 : 53 45 : 55	93 : 7 94 : 6		
	**1b** **(+)-1b**	45 : 55 44 : 56	94 : 6 94 : 6		
	**1c** **(+)-1c**	44 : 56 40 : 60	80 : 20 80 : 20		
	**1d**	60 : 40	93 : 7		
	**1e**	35 : 65	75 : 25		
	**2b**	50 : 50	50 : 50		
	**3a**	47 : 53	93 : 7	60 : 40	>98 : 2
	**3b**	45 : 55	98 : 2	66 : 33	>98 : 2
	**3c**	45 : 55	80 : 20	65 : 35	90 : 10
	**3d**	60 : 40	94 : 6	60 : 40	90 : 10
	**3e**	35 : 65	75 : 25	n.d.	n.d.
	**3f**	43 : 57	75 : 25	65 : 35	80 : 20
	**3g**	30 : 70	70 : 30	55 : 45	80 : 20
	**3h**	33 : 66	56 : 44	40 : 60	>90 : 10
	**3i**	40 : 60	>90 : 10	60 : 40	>90 : 10
	**3j**	52 : 48	96 : 4		
	**3k**	50 : 50	50 : 50		

[a] All reactions were carried out in 9 : 1 H_2O–CH_3CN with 6 equiv $NaBH_4$ and 1 equiv BSA. [b] By HPLC. [c] Ratios of *anti* and *syn* diols **4** and **5**. [d] Ratios of hydroxyketones **1** and **2** at 50% conversion.

for the *syn* acetonides and 20–22 ppm for the *anti* acetonides;[26] b) comparison with authentic samples of *trans* diols **5b,e** prepared by the stereoselective reduction of aldols **2b,e** with tetramethylammonium triacetoxyborohydride (Evans' reagent).[27]

also good with the 2-naphthyl and *p*-nitrophenyl ketones **1c** and **1e** that are preferentially reduced to *syn* diols in the absence of protein. Thus, the directing effect of BSA is little influenced by the steric and electronic properties of the aromatic group. Clearly, the origin of this directing effect must be a binding interaction

between the protein and the hydroxyketones, resulting in the selective presentation of one diastereotopic face of the carbonyl of the bound substrate to the reducing agent.

Data in Table 2 also show that, in the presence of BSA, the reduction of enantiomerically pure and racemic hydroxyketones **1** proceeds with the same diastereoselectivity. Thus, the protein does not discriminate between enantiomers.

The reduction of hydroxyketone **2b** is not stereoselective in the presence of BSA, indicating that an aromatic carbonyl is essential for stereoselection. This conclusion is confirmed by the lack of selectivity observed in the reduction of 2,4-pentanedione **3k** (Table 2).

Reduction of diketones

The $NaBH_4$ reduction of 1,3-diketones **3** in 9 : 1 H_2O–CH_3CN gives the corresponding *anti* and *syn* diols in ratios close to 1 : 1 (Table 2). The reaction also fails to display any appreciable chemoselectivity: a typical profile for the reduction of diketone **3b** (Fig. 2) shows that the intermediate isomeric hydroxyketones **1b** and **2b** are formed (and react) at comparable rates. The ratios of hydroxyketones **1** and **2** at 50% conversion (Table 2) confirm this behaviour for all the diketones studied.

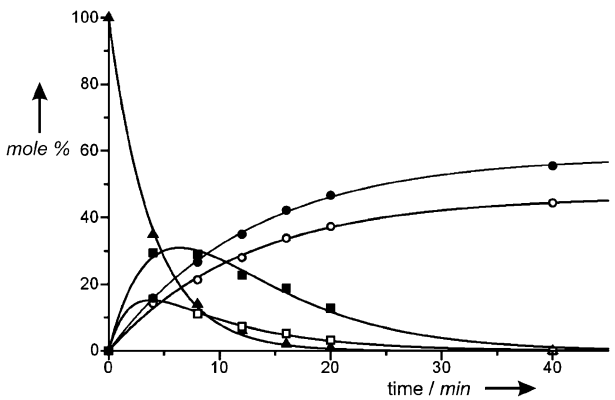


Fig. 2 Time profile for the $NaBH_4$ reduction of diketone **3b** in 9 : 1 H_2O–CH_3CN. Triangles: diketone **3b**; empty squares: hydroxyketone **2b**; filled squares: hydroxyketone **1b**; empty circles: *anti* diol **4b**; filled circles: *syn* diol **5b**.

A completely different pattern emerges when the reduction is carried out in the presence of albumin (Table 2). Again formation of the anti diols **4** is preferred, with stereoselectivities comparable to those already observed in the reduction of hydroxyketones **1**.

The $NaBH_4$/BSA reduction of diketones is not enantioselective and all diols are formed as racemic mixtures. *Anti*/*syn* Ratios higher than 9 : 1 are observed for phenyl (**3a,j**), *p*-tolyl (**3b,i**) and ferrocenyl derivatives (**3d**). Overall stereoselectivities are lower with *para*-substituted compounds **3e,f** or when the aromatic group is 2-naphthyl (**3c**) or 2-furyl (**3g**). Even with these substrates, however, the directing effect of BSA is clearly evident, resulting in an inversion in the stereoselectivity from the *syn* diol, which is favoured without protein, to the *anti* isomer; a similar behaviour was observed in the reduction of hydroxyketones **1c** (*p*-NO_2) and **1e** (2-naphthyl). Replacement of the methyl group of **3a,b** with a longer and functionalized chain, as in **3i**, or with a phenyl group (**3j**) has little effect on the stereoselectivity. On the contrary, introduction of a bulky *t*-butyl group in this position (**3h**) is not tolerated by the protein, resulting in a complete loss of selectivity. The lack of selectivity observed in the reduction of acetylacetone (**3k**) and 1,3-cyclopentanedione (not shown) confirms that the presence of an aromatic carbonyl is necessary for stereoselection control by BSA.

Table 3 Effect of the pH on the stereoselectivity of the $NaBH_4$/BSA reduction of hydroxyketone **1b** and diketone **3b**[a]

	anti/*syn* ratios	
pH	**1b** → **4b,5b**	**3b** → **4b,5b**
5.0[b]	94 : 6	94 : 6
6.0[c]	95 : 5	86 : 14
7.0[c]	90 : 10	85 : 15
8.0[c]	82 : 18	77 : 23
9.0[d]	67 : 33	n.d.

[a] Conditions: 1 μM substrate, 1 μM BSA, 5 μM $NaBH_4$, 1 : 9 acetonitrile/buffer (10 mM, containing 50 mM NaCl). [b] Sodium citrate buffer. [c] Sodium phosphate buffer. [d] Sodium borate buffer.

pH dependence

The reduction of hydroxyketone (±)-**1b** and diketone **3b**, in the presence of BSA, was studied between pH 5 and 9 (Table 3). Data in the table show that the stereoselectivity depends strongly on the pH. In the reduction of hydroxyketone (±)-**1b** the *anti*/*syn* ratio decreases from 95 : 5 at pH 5–6 to 67 : 33 at pH 9 (Table 3). The reduction of diketone **3b** follows a similar trend with *anti*/*syn* ratios dropping from 94 : 6 at pH 5 to 77 : 23 at pH 8, indicating the probable involvement of protonated sites in binding of both hydroxyketones and diketones. In consideration of this pH-dependence all synthetic reactions were generally carried out at pH 6 even if, under these conditions, an excess of sodium borohydride (6 equiv) is required for the reaction to be complete, due to the partial decomposition of borohydride.

Other albumins

To determine whether other albumins possess the same ability to control the selectivity of the conversion of 1,3-diketones into diols, reduction of diketone **3b** was carried out in the presence of Human Serum Albumin (HSA) and Ovalbumin (OVA). HSA has a high (76%) sequence homology with BSA[6] and, not surprisingly, a good *anti*/*syn* stereoselectivity (88 : 12) was observed when the reaction was carried out in the presence of this protein. OVA, belonging to the serpin family, is the main component of chicken egg's albumen: its function is similar to that of BSA and HSA but the sequence and 3-D structure are completely unrelated to those of mammalian albumins.[28] Nevertheless, the stereoselectivity observed in the presence of this protein is comparable to that obtained with HSA and only slightly lower than with BSA. This finding might be interesting for practical applications, as egg albumin is very cheap.

All the reactions described so far were carried out in a water–acetonitrile solution containing equimolar amounts of substrate and BSA. To test experimental conditions more amenable to large scale reactions, reduction of diketone **3b** was also carried out in a heterogeneous system; lyophilized BSA and sodium borohydride were suspended in a toluene solution of the ketone and the

mixture was stirred at room temperature for 24 h. The results were encouraging as, without optimization, the stereoselectivity was still 80% in favour of the *anti* diol **4b**, while the protein could be simply recovered by filtration and recycled.

Binding of diketones and hydroxyketones by albumin

To gain more insight on the mechanism by which albumins control the regio- and stereoselectivity of the reduction of diketones and hydroxyketones, the interaction of substrates **1b**, **2b** and **3b** with BSA was investigated by UV and CD spectroscopy. The analysis was restricted to the region above 300 nm, in which the protein is transparent. The broad band at 316 nm ($\varepsilon = 5000$), present in the UV spectrum of diketone **3b**, recorded in 10% aqueous acetonitrile (Fig. 3a), indicates partial enolization of the aromatic carbonyl in this solvent system.[29] The intensity of the enol band strongly increases upon addition of one equivalent of BSA, indicating that the tautomeric equilibrium is further shifted toward the enol form by the interaction with the protein. The same interaction also causes a shift of the enol band to higher wavelengths (λ_{max} = 324 nm, ε = 13500). Binding of this enol by BSA is confirmed by CD. Being achiral species, neither the diketone **3b**, nor its enol display any CD. In the complex with the protein, however, the enol is surrounded by an asymmetric environment, resulting in the appearance, in the CD spectrum of a mixture of **3b** and BSA, of an induced band[30] at the wavelength characteristic of the enol absorption (Fig. 3b). Titration of the protein with increasing amounts of **3b** clearly shows saturation by the ligand (Fig. 4) and standard treatment of the data‡ gives a value of $5.2 \pm 0.2 \times 10^4$ L mol^{-1} for the association constant between **3b** and BSA. Similar values are found for the binding constants of albumin with other small aromatic molecules.[2]

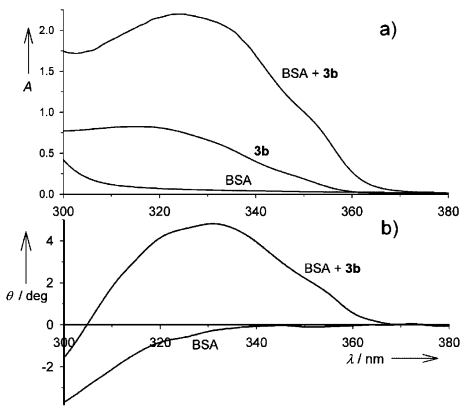


Fig. 3 a) UV spectra of BSA, diketone **3b** and of a 1 : 1 mixture of BSA and **3b**. Spectra were recorded in 9 : 1 H_2O–CH_3CN with all concentrations equal to 1.67×10^{-4} M. b) CD spectra of BSA and of a 1 : 1 mixture of BSA and **3b** in 9 : 1 water–acetonitrile. [BSA] = [**3b**] = 57 μM.

The interaction of hydroxyketones **1b**, **2b** and diols **4b**, **5b** with BSA could not be studied directly, as these species have no UV absorption bands above 300 nm. Nevertheless, their binding could be investigated indirectly by competition experiments. Fig. 4 shows that binding of diketone **3b** to BSA is inhibited in the presence of a ten-fold excess of both racemic hydroxyketone **1b** and enantiomerically pure (+)-**1b**; it can thus be concluded that **1b** and **3b** bind to the same protein site. A comparison of the

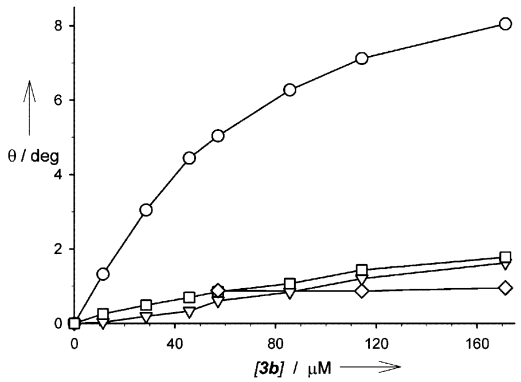


Fig. 4 Ellipticities at 324 nm of albumin solutions in 9 : 1 water–acetonitrile containing diketone **3b**. Circles: BSA (57 μM); squares: BSA (57 μM) and hydroxyketone (±)-**1b** (570 μM); triangles: BSA (57 μM) and (+)-**1b** (570 μM); diamonds: fluoresceinated BSA (57 μM). All values are corrected for the ellipticities at 330 nm of BSA and fluoresceinated BSA, respectively.

effects observed for (+)-**1b** and (±)-**1b** indicates that BSA does not discriminate between enantiomeric hydroxyketones; a similar lack of enantiospecificity has been observed in the binding of warfarin (3-(α-acetonylbenzyl)-4-hydroxycoumarin) by HSA.[5e,31]

The isomeric hydroxyketone **2b**, and the diol products **4b**, **5b**, on the contrary, do not inhibit binding of **3b** to BSA, indicating that these compounds are not recognized by the protein. This may appear surprising considering the structural similarity between molecules of this set and the broad specificity of albumins, but is consistent with the absence of selectivity observed in the reduction of **2b**. Clearly, in these small ligands possessing a limited number of functional groups, the presence of an aromatic carbonyl is essential for efficient recognition by albumin.

In conclusion, data presented in this section strongly suggest that specific binding of **1b** and **3b** to BSA is at the origin of the high selectivities observed in the reduction of these substrates (Table 2), while for **2b** lack of binding results in complete absence of selectivity.

Identification of the binding site

Binding and crystallographic studies indicate that drugs and other small aromatic molecules containing anionic or electronegative groups bind to the IIA site of HSA (drug site 1), a sock shaped hydrophobic cavity lined at its entrance and bottom with positively charged residues.[2a] This site, highly conserved in BSA, is therefore a good candidate for binding of hydroxyketones **1** and diketones **3**.

A single lysine residue is present in this site both in HSA (Lys199) and BSA (Lys222); no lysines are present in the other small molecule binding site (IIIA site; drug site 2) of either albumin. This lysine can be selectively modified with fluorescein isothiocyanate (FITC)[9c] and the modification prevents this residue from participating in binding and catalysis.[9c,10c] Fluoresceinated BSA (FITC-BSA) was thus prepared and its interaction with **3b** was studied by CD (Fig. 4). It can be clearly seen that fluoresceination of Lys199 inhibits over 90% of the binding of the diketone, confirming that recognition takes place in the IIA site. Accordingly, the *anti*/*syn* diastereoselectivity in the borohydride reduction of **3b** drops from 94 : 6 with BSA to 75 : 25 with

FITC-BSA. The residual binding and diastereoselectivity observed in the presence of FITC-BSA can be accounted for by the presence of a small amount of non-fluoresceinated protein in the sample, as indicated by the modification index of 0.9 calculated for FITC-BSA.

Further insight into the interactions of diketone **3b** with BSA is provided by a study of the pH-dependence of the induced band in the CD spectrum of the diketone-protein complex (Fig. 5). A bell-shaped curve is found between pH 3 and 11, with a broad maximum between pH 6 and 8, in general agreement with the variation of selectivity observed in the reduction of **3b** (Table 3). It would seem reasonable to attribute the changes in binding with the pH to the titration of the only ionizable residues present in BSA's IIA site, namely His242 and Lys199. In this hypothesis, pK_a values of around 4.5 and 9.5 for His242 and Lys199, respectively, can be derived from the shape of the curve, in agreement with an estimate of the pK_a's of the protein's protonated residues, carried out with the PROPKA programme,[32] which indicates these two amino acids as the protein's most acidic histidine and lysine, respectively. However, care should be taken with this interpretation as the curve of Fig. 5 might simply reflect the intrinsic pH stability of the protein.[33] In either case, from the location of the maximum we can conclude that in the complex with the bound enol His242 and Lys199 are in the neutral and protonated forms, respectively.

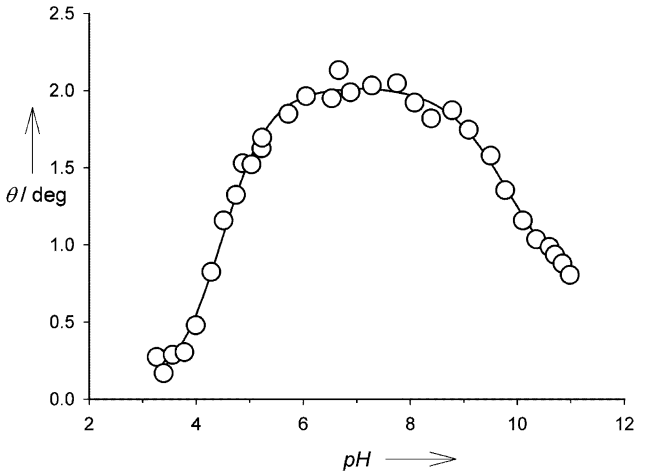


Fig. 5 Ellipticities at 324 nm of a 1 : 1 solution of diketone **3b** and BSA (28.5 μM; water–acetonitrile 9 : 1) as a function of pH. The following buffers were used (40 mM, containing 100 mM NaCl): sodium acetate (pH < 6); sodium phosphate (pH 6–8); sodium borate (pH 8–10); sodium carbonate (pH > 10).

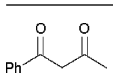
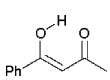
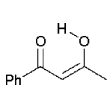
Modelling

A molecular modelling study was carried out to test the mechanistic hypothesis suggested by the experimental data. Diketone **3a** and hydroxyketones **1a** and **2a** were chosen as reference ligands for this study. The geometries of diketone **3a** and of the *s-cis* aryl and alkyl enols **6a** and **7a**, respectively, were initially optimized *in vacuo* at the B3LYP/6.31G(d,p) level of the DF theory,[34] and then re-optimized in water applying the IEF polarizable continuum model for solvation;[35] results are in Table 4. The corresponding *s-trans* enols were also considered initially, but their energies were so high that their contribution to the tautomeric population of **3a** at room temperature can be considered negligible. The electronic spectra of the three isomers were also calculated, at the CI-S/B3LYP/6.31G+(d,p) level and selected absorption wavelengths are reported in Table 4.

The results show that, *in vacuo*, diketone **3a** is present as a mixture of intramolecularly hydrogen bonded enols, while a significant amount of the diketone form is present in water, in agreement with experimental data.[29] A strong UV absorption at 321 nm is calculated for the aryl enol **6a**, shifted to 302.1 nm in the alkyl enol **7a**. This band is absent in the calculated electronic spectrum of **3a**. Breaking the intramolecular hydrogen bond of the enols by rotating the hydrogen away from the carbonyl causes the bands to shift down to 307 and 273 nm, respectively. These results support the hypothesis that diketones **3** are recognized by albumin as enols, the tautomeric equilibrium being shifted to this form by the hydrophobic environment of albumin's binding site. The observed value of 324 nm for the UV maximum of diketone **3a** bound to BSA is in good agreement with the calculated value of 321 nm for **6**; we can thus conclude that the aryl enol is most likely present in the BSA complex, and that a strong hydrogen bond is established by this enol, either intramolecularly or with donor/acceptor groups in the protein binding site.

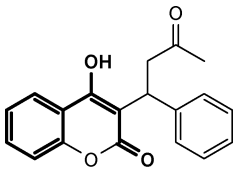
No crystal structure of BSA has ever been reported, to the best of our knowledge, and we were unable to obtain crystals

Table 4 Calculated energies (B3LYP/6.31G(d,p)) and electronic transitions in the 300–330 nm range (CI-S/B3LYP/6.31G+(d,p)) for diketone **3a** and enols **6a**, **7a**

Tautomer		Rel. energy[a] [kcal mol^{-1}] (% population)[b]	Rel. energy[c] [kcal mol^{-1}] (% population)[b]	Wavelength [nm] (energy) [eV] [oscillator strength]
	**3a**	1.78 (2.7)	0.19 (29.3)	—
	**6a**	0.17 (41.6)	0.17 (30.3)	321.0 (3.8685) [0.6158]
	**7a**	0 (55.4)	0 (40.4)	302.1 (4.1036) [0.4913]

[a] Gas phase. [b] At 300 K. [c] In water.

of the protein or its complexes with diketones. BSA, however, is highly homologous with HSA, for which many structures have been described;[5] in particular, in the region corresponding to the IIa domain (residues 190–300) the two proteins differ for only twelve residues. We have thus build an homology model of BSA starting from the crystallographic structure of the complex of HSA with the *R*-(+) enantiomer of warfarin (pdb file 1H9Z).[5e] This structure was chosen for the structural similarity between the 4-hydroxycoumarin ring of warfarin and the aryl enols **6** (highlighted).



Warfarin

The structure of the homology model was obtained by mutating all the necessary amino acid side chains in the HSA structure, and by submitting the mutated structure to a series of molecular dynamics and optimization cycles carried out with the Amber force field as implemented in Sybyl 7.3.[36] The two sites are almost perfectly superimposable, the only exception being HSA's Arg222 and Lys199 whose positions are exchanged in BSA.‡ The diketone **3a** and its tautomers **6a** and **7a** were then docked to the IIA site of the BSA model thus obtained, with the docking suite Grid 22b,[37] and model geometries of the complexes were obtained by submitting again the complexes to molecular dynamics and mechanics optimizations. Very similar docking solutions were obtained for the three tautomers, with the phenyl ring and the polar groups similarly oriented in the three complexes and occupying the same region that hosts the aromatic group of the benzocoumarin system in the warfarin-HSA complex. The structure of the model complex of BSA with the aryl enol **6a** is shown in Fig. 6.

Table 5 Calculated relative complexation energies of BSA with both enantiomers of hydroxyketones **1a** and **2a**

Hydroxyketone	$\Delta\Delta E^a$ [kcal mol^{-1}]
(***R***)-**1**	0
(***S***)-**1**	0.1
(***R***)-**2**	7.2
(***S***)-**2**	10.6

[a] Relative Amber complexation energies $\Delta E = E_{complex} - (E_{ligand} + E_{protein})$.

It can be seen from Fig. 6 that the aromatic ring of the diketone is deeply buried into the hydrophobic cavity lined by Ala291, Ile290, Ile264, Ser287, Leu260, Leu238, Leu219. The polar region of the enol is oriented toward the polar, positively charged rim of the binding site with a water molecule at hydrogen bond distance from the enol oxygen on one side and from the $N_{\varepsilon 2}$ of His242 on the other. The carbonyl oxygen of the substrate is at hydrogen bond distance from Arg199, with the cationic head of Lys222 lying nearby. The model suggests a simple explanation for the regioselectivity observed in the reduction of the diketone (Fig. 6b): while the aromatic carbonyl is inaccessible, completely buried into the site, the alkyl-carbonyl is exposed to the solvent at the top of the site leaving both its faces available to the incoming hydride.

The B3LYP/6.31G(d,p) geometries of hydroxyketones **1a** and **2a** were similarly obtained, following a systematic Amber conformational search,[38] which yielded four conformations for **1a** and two conformations for **2a**. These structures were then docked to the IIa binding site of the BSA homology model and the process was repeated for both enantiomers of each compound. The relative complexation energies for the four complexes obtained after optimization of the best docking solutions are reported in Table 5.

Data in Table 5 show that binding of hydroxyketone **2a** is less favourable than that of **1a** by 7–10 kcal mol^{-1}, matching the experimental observation that **2a** is not recognized by BSA. In agreement with the experiments, comparable affinities are

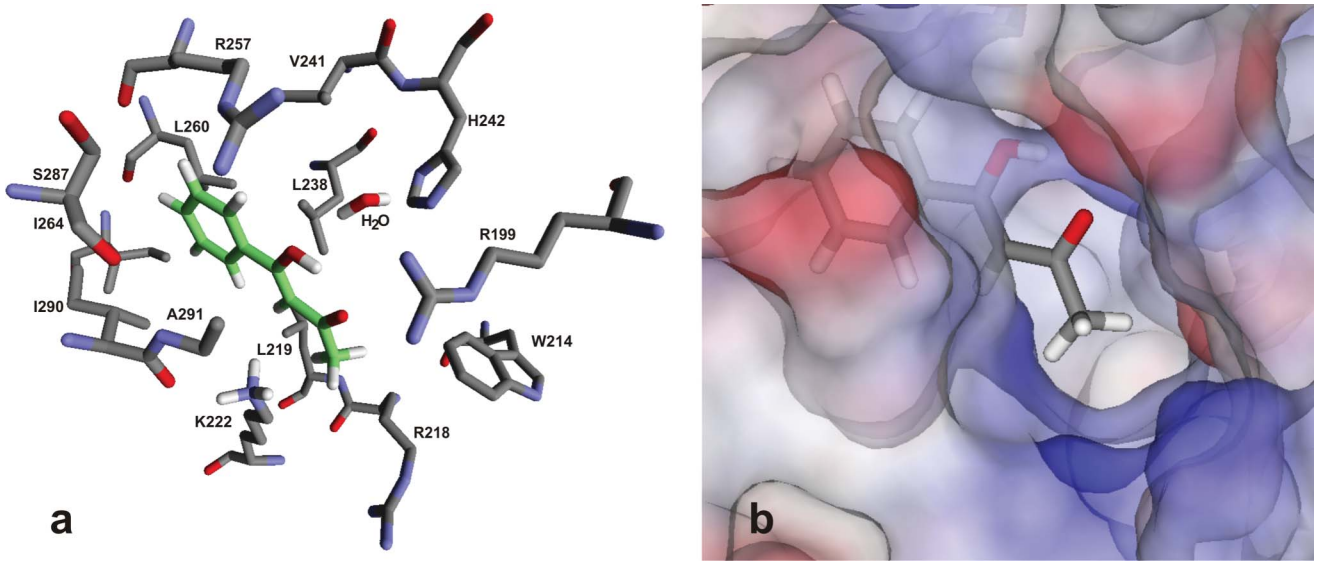


Fig. 6 a) Model of the complex between enol **6a** and BSA's IIa site. b) Connolly surface showing the alkyl carbonyl of **6a** emerging from the hydrophobic cavity of the IIa site; the surface is coloured according to the electrostatic potential.

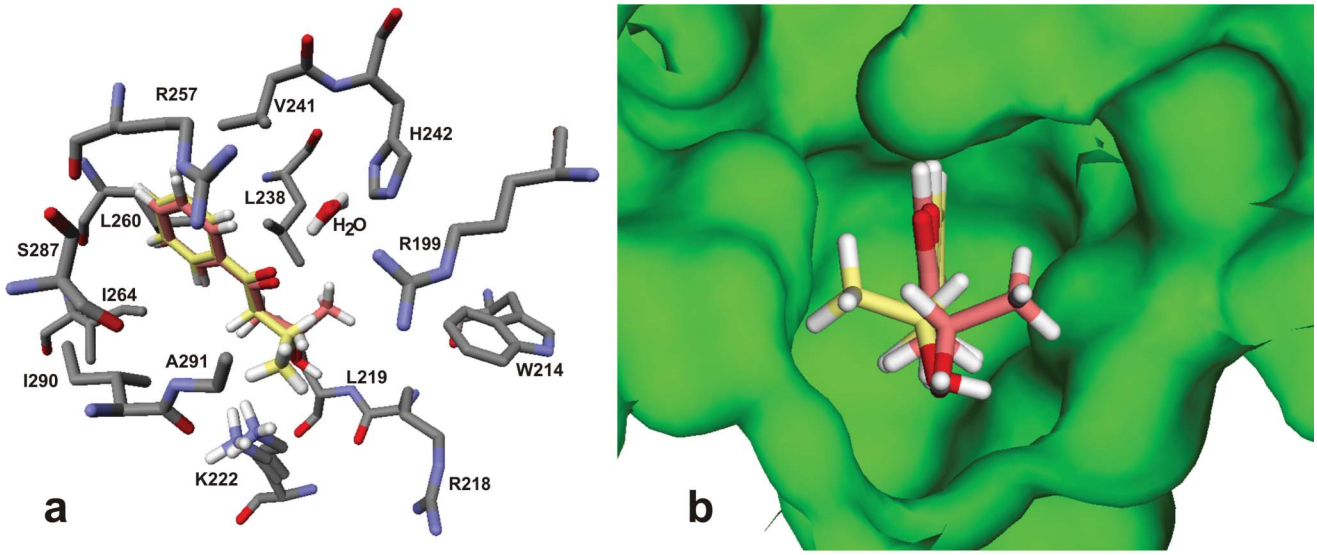
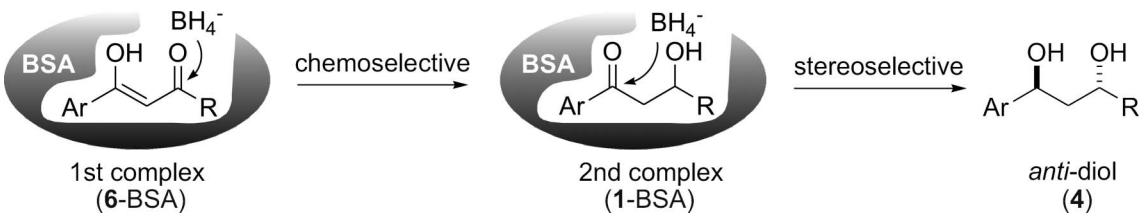


Fig. 7 Superimposition of the calculated structures of the complexes of BSA with hydroxyketones ***R*-1a** (yellow) and ***S*-1a** (pink). a) The side view shows the main interactions with the binding site residues; b) a front view shows that the methyl groups block the access to opposite diastereotopic faces of the carbonyl.



Scheme 2 Mechanism for the BSA-directed reduction of 1,3-diketones and 3-hydroxyketones. The aromatic diketone is bound by BSA to give a ketoenol-BSA complex that is chemoselectively reduced by borohydride in the first step. The resulting second complex (BSA-hydroxyketone) is then stereoselectively reduced to *anti* diol in the second step.

calculated for the enantiomers of hydroxyketone **1a**, which are recognized by the protein with a similar pattern of interactions and in the same *gauche* conformation, with respect to the carbon chain (Fig. 7a). In the complex, the stereogenic carbon is located near the entrance of the binding site, and thus there is enough space to host the methyl group of both enantiomers.

The model clearly shows (Fig. 7b) that, in the best solutions found for both the complexes with ***R*-1a** and ***S*-1a**, the carbonyl is deeply buried inside the inner cavity and access to one face is blocked by the methyl group. Reduction of the hydroxyketone may then take place either in an intermolecular mode, with the hydride approaching the carbonyl from the less hindered side, or by the classical internal delivery mode in which hydride is transferred from a boron species complexed with the substrate's hydroxy group.[39] Inspection of the model indicates that enough space is available in the binding site for 120° rotation around the CH_2–CH(OH) bond of the hydroxyketone (anticlockwise for ***R*-1a** and clockwise for ***S*-1a**) leading to the *gauche* conformation which is necessary for the latter mechanism to operate. Either mechanism would lead to the formation of the *anti* diol.

Conclusions

The data presented show that, in the reduction of diketones **3** in the presence of BSA, there is a strong correlation between the stereoselectivity (*anti*/*syn* ratios) of the overall conversion to diols **4** and **5** and the chemoselectivity observed in the first reduction. This correlation, combined with the observation that only hydroxyketones **1**, and not the isomeric ketones **2**, are stereoselectively reduced in the presence of BSA, suggests a possible model for the stereoselective reduction of hydroxyketones and 1,3 diketones that has been validated by binding studies and by docking simulations. According to this model (Scheme 2) a complex is formed between the diketone, in its enol form **6**, and BSA, in which the aliphatic carbonyl is more available and is reduced first. The model predicts that in the complex both faces of the carbonyl are sufficiently exposed to the incoming nucleophile. Accordingly, no enantioselectivity is observed in this first reduction. The carbonyl group of the hydroxyketone **1** thus formed is then stereoselectively reduced in the second step. Complexation of this substrate by the protein controls the *anti* diastereoselectivity of this step (Scheme 2, Fig. 7), either by directing the path of the incoming external nucleophile or by favouring an alternative intramolecularly assisted mechanism, in which the hydroxy group directs the delivery of hydride to the *syn* face of the carbonyl. The overall diastereoselectivity, in this hypothesis, derives from a combination of chemoselectivity, favouring the formation of hydroxyketones **1** in the first step of the reaction, and albumin-controlled stereoselectivity in the second step.

Unlike other reactions promoted by albumins, in this case there is no catalytic effect associated with the protein. No multiple turnover is observed and a stoichiometric amount of protein is required for efficient control of the regio- and stereoselectivity. While inhibition by the reaction products can prevent multiple turnover in enzyme-catalyzed reactions, this is not the case here, as we have demonstrated that 1,3-diols **4,5** are much poorer binders with respect to substrates **1** and **3**.

In the mechanism of Scheme 2 albumin's function is to present the substrate to an external reducing agent so as to differentiate between the carbonyls in the first step and between the diastereotopic faces of the aromatic ketone in the second step. This role is reminiscent of that of dirigent proteins (DP) that control the enantioselective oxidative coupling of phenols in plant secondary metabolism, but do not possess catalytic activity.[40] Our work suggests that the DP concept is not restricted to biosynthetic pathways, but may also be applied to organic synthesis, offering new possibilities to the control of regio- and stereoselectivity in organic reactions.

Experimental

Optical rotations were measured on a Perkin–Elmer 241 polarimeter fitted with a 10 cm cell. IR spectra were recorded using a Thermo-Nicolet FT-IR Avatar 320. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, unless otherwise stated, on a Jeol EX400 spectrometer operating at 400 MHz and 104 MHz, respectively; chemical shifts (δ) are downfield from TMS. EI-mass spectra were measured at 70 eV on a VG 70/70 spectrometer. ESI-mass spectra were recorded on a Perkin–Elmer API1 spectrometer. Elemental analyses were obtained with a Carlo Erba EA1110 elemental analyzer. UV spectra were obtained on a Perkin–Elmer Lambda 2 or on a Unicam Helios β spectrophotometer. CD spectra were recorded on a Jasco J-710 spectropolarimeter. HPLC analyses were run on a Hewlett–Packard 1100 HPLC system. THF was freshly redistilled from sodium/benzophenone. Flash column chromatography was performed on silica gel 60H (230–400 mesh) Merck 9385; thin layer chromatography was performed on silica-coated Merck kieselgel $60F_{254}$ 0.25 mm plates and visualized by UV irradiation at 254 nm. Diketones **3a** and **3j** were purchased from Sigma–Aldrich. Diketone **3h**[41] was synthesized according to the literature. The hydroxy ketone **2b** was obtained from diketone **3b** by selective hydrogenation of the aromatic carbonyl, as described by Schöpf.[42]

General procedure for the synthesis of hydroxyketones 1a–e

Racemic hydroxyketones **1a–e** (R = CH_3) were obtained from the lithium enolate of the appropriate aromatic ketone and acetaldehyde.[43] A solution of the corresponding ketone (15 mmol) in 15 mL dry THF was added dropwise to a freshly prepared solution of LDA (16.5 mmol) in 15 mL of dry THF at −78 °C. After 30 min the aldehyde (15 mmol) in dry THF (15 mL) was added dropwise and the reaction mixture was stirred for 15 min at −78 °C. The reaction was quenched by addition of ice cold, sat. aqueous $NaHCO_3$ and extracted with $CHCl_3$ (3 × 25 mL). The combined extracts were dried over an. Na_2SO_4 and evaporated under reduced pressure; silica gel chromatography of the residue (CH_2Cl_2) gave the pure products. Aldols **1a**,[43a] **1b**,[43b] and **1d**[44] are known compounds. Spectral data for aldols **1c** and **1e** are given below.

3-Hydroxy-1-(2-naphthalenyl)-1-butanone (1c). (65% from 2-acetyl-naphthalene): m.p. 46–47 °C, from diisopropyl ether; ^{1}H NMR: δ = 1.34 (d, J = 6.1 Hz, 3H), 3.15 (dd, J = 17.5, 3.1 Hz, 1H), 3.25 (dd, J = 17.5, 8.6 Hz, 1H), 3.5 (1H, OH), 4.44 (m, 1H), 7.50–8.41 (m, 7H); ^{13}C NMR: δ = 22.4, 46.5, 64.0, 123.4, 126.7, 127.6, 128.4, 128.5, 129.5, 132.3, 133.9, 135.6, 200.5, one peak is missing due to overlapping; EI-MS: m/z (%): 214 (16) [M^+], 170 (22) [$M^+ - C_2H_4O$], 155 (100) [$C_{10}H_7CO^+$]; elemental analysis calcd (%) for $C_{14}H_{14}O_2$: C 78.5, H 6.59; found C 78.4, H 6.60.

3-Hydroxy-1-(4-nitrophenyl)-1-butanone (1e). (oil, 33% from *p*-nitroacetophenone): ^{1}H NMR: δ = 1.33 (d, J = 6.2 Hz, 3H), 3.05 (1H, OH), 3.15 (m, 2H), 4.46 (m, 1H), 8.11–8.34 (m, 4H); ^{13}C NMR: δ = 22.4, 47.2, 63.8, 123.8, 129.1, 141.0, 150.5, 198.9; EI-MS: m/z (%): 209 (1) [M^+], 165 (28) [$M^+ - C_2H_4O$], 155 (100) [$ArCO^+$].

General procedure for the synthesis of diketones 3 by the Claisen condensation

Diketones **3a–d,f,g,i** were obtained by the Claisen condensation of aromatic ketones with the appropriate ester; the procedure is described for the synthesis of 1-(2-furanyl)butane-1,3-dione (**3g**).

2-Acetylfuran (3.29 g, 30 mmol) in dry ethyl acetate (15 mL) was added dropwise, at 0 °C, to a suspension of NaH (0.79 g of a 60% suspension in mineral oil, 33 mmol) in dry ethyl acetate (15 mL) and the reaction mixture was stirred at 0 °C for 2 h and at 25 °C for further 12 h (TLC). 10% Aqueous NH_4Cl was then carefully added (30 mL) and the mixture was acidified to pH 5 with HCl. The aqueous phase was separated and extracted with ethyl acetate (2 × 15 mL). The combined organic phases were dried over anhydrous sodium sulfate and evaporated under reduced pressure; the crude product was purified by FC, with ethyl acetate/petroleum ether 1 : 1 as eluant, to give the oily diketone **3g** (0.90 g, 20%). ^{1}H NMR: δ = 2.14 (s, 3H), 6.07 (s, 1H, =CH, enol form), 6.54–7.60 (m, 3H), 16.3 (s, 1H, OH, enol form); ^{13}C NMR: δ = 24.4, 96.0 (=CH, enol), 112.4, 115.5, 145.9, 154.7, 176.1, 189.5; EI-MS: m/z (%): 152 (43) [M^+], 137 (28) [$M^+ - CH_3$], 95 (100) [$C_4H_5OCO^+$]. With the same procedure were obtained the known diketones **3b** (65%; m.p. 55 °C, lit.[45] 56 °C), **3c** (54%; m.p. 97.5 °C, lit.[46] 98–98.5 °C), **3d** (58%, m.p. 79 °C, lit.[44] 80 °C), **3f** (33%, m.p. 113–114 °C, lit.[47] 116–117 °C). Compound **3i** was obtained similarly from equimolar amounts of *p*-methyl acetophenone and γ-butyrolactone, in THF (45%; m.p. 38 °C, lit.[48] 38–39 °C).

1-(4-Nitrophenyl)butane-1,3-dione 3e

Pyridinium chlorochromate (160 mg, 0.740 mmol) was added in small portions, at 25 °C, to aldol **1e** (140 mg, 0.670 mmol) in dry dichloromethane (20 mL) and the mixture was stirred for 6 h. Light petroleum was added (100 mL) and the mixture was filtered. Upon concentrating the resulting solution to approximately 10 mL, pure **3e** separated as yellow crystals (105 mg, 75%); m.p. 110 °C (lit.[45] 111 °C).

Bakers' yeast reduction of diketones

A suspension of fresh bakers' yeast (*Saccharomyces cerevisiae*, 50 g) in water (1 L) containing sucrose (30 g) was kept at 37 °C for 1 h. The diketone was added (1 g) and the mixture was stirred at 37 °C for 3 days, adding sucrose (12 g) every 24 h. The mixture was centrifuged and the aqueous phase was extracted with dichloromethane (8 × 100 mL); the combined organic phases were dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude products were purified as described for the racemic hydroxyketones and the enantiomeric excess was established from the NMR analysis of the corresponding Mosher's esters. (***S***)**-1a**: e.e. 96%; $[\alpha]_D^{20} = +64.7$ ($c = 4.4$ in $CHCl_3$) (lit.[49] $[\alpha]_D^{20} = +68.7$ ($c = 1.01$ in $CHCl_3$)); (***S***)**-1b**: e.e. > 99%; $[\alpha]_D^{20} = +60.4$ ($c = 4.1$ in $CHCl_3$); (***S***)**-1c**: e.e. 95%; $[\alpha]_D^{20} = +37.6$ ($c = 2.6$ in $CHCl_3$).

Mosher's esters of hydroxyketones

The hydroxyketone (racemic or single enantiomer, 10 mg) and (*R*)-(−)-α-methoxy-α-trifluoromethyl-phenylacetylchloride (1.3 equiv) in dry pyridine (0.2 mL) were stirred at room temperature for 18 h. Dichloromethane was added (2 mL) and the solution was extracted with 10% aqueous citric acid (1 mL). The aqueous phase was extracted with dichloromethane (1 mL) and the combined organic layers were passed through a short silica column; the column was rinsed with dichloromethane (5 mL) and the flow-through was evaporated under reduced pressure to give the esters; ^{1}H NMR data are listed below.

Mosher's ester of (*S*)-(+)-1a. ^{1}H NMR (C_6D_6) $\delta = 1.39$ (d, J = 4.8 Hz, 3H), 3.05 (dd, J = 17.7, 8.6 Hz, 1H), 3.16 (dd, J = 17.7, 2.9 Hz, 1H), 3.47 (s, 3H), 5.81 (m, 1H), 6.86–7.73 (m, 10H).

Mosher's ester of (*R*)-1a (from racemic 1a). ^{1}H NMR (C_6D_6) $\delta = 1.47$ (d, 3H), 2.80 (m, 1H), 3.12 (m, 1H), 3,41 (s, 3H), 5.81 (m, 1H), 6.86–7.73 (m, 10H).

Mosher's ester of (*S*)-(+)-1b. ^{1}H NMR (C_6D_6) $\delta = 1.07$ (d, J = 6.23 Hz, 3H), 2.00 (s, 3H), 2.29 (dd, J = 17.2, 4.2 Hz, 1H), 2.95 (dd, J = 17.2, 8.3 Hz, 1H), 3.47 (s, 3H), 5.81 (m, 1H), 6.86–7.73 (m, 9H).

Mosher's ester of (*R*)-1b (from racemic 1b). ^{1}H NMR (C_6D_6) $\delta = 1.14$ (d, $J = 6.4$ Hz, 3H), 1.99 (s, 3H), 2.39 (dd, $J = 17.0$, 5.5 Hz, 1H), 2.98 (dd, $J = 17.0$, 7.14 Hz, 1H), 3.42 (s, 3H), 5.78–5.90 (m, 1H), 6.83–7.77 (m, 9H).

Mosher's ester of (*S*)-(+)-1c. ^{1}H NMR (C_6D_6) $\delta = 1.35$ (d, J = 6.22 Hz, 3H), 3.19 (dd, J = 17.6, 8.8 Hz, 1H), 3.32 (dd, J = 17.6, 2.7 Hz, 1H), 3,66 (s, 3H), 4.47 (m, 1H), 7.36–8.05 (m, 12H).

Mosher's ester of (*R*)-1c (from racemic 1c). ^{1}H NMR (C_6D_6) $\delta = 1.44$ (d, $J = 6.4$ Hz, 3H), 2.91 (dd, $J = 17.4$, 3.3 Hz, 1H), 2.96 (dd, $J = 17.6$, 8.9 Hz, 1H), 3.75 (s, 3H), 4.47 (m, 1H), 7.36–8.05 (m, 12H).

$NaBH_4$ reductions

Sodium borohydride (1.14 g, 30 mmol) was added portionwise to a solution of hydroxyketones **1**, **2** or diketones **3** (5 mmol) in 10% aqueous acetonitrile (30 mL) and the mixture was stirred at room temperature for 1–4 h, diluted with water (30 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were extracted with water and sat. brine (30 mL each) and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure gave the diols as mixtures of *anti* (**4**) and *syn* (**5**) isomers which were separated by flash chromatography on silica gel, eluting with 1 : 1 ethyl acetate/petroleum ether. Diols **4a** and **5a**,[50] **4c** and **5c**,[51] **4d** and **5d**,[44] **4g** and **5g**,[52] **4h** and **5h**,[53] **4j** and **5j**[54] are known compounds and were identified from their published spectral data. Spectroscopic data for diols **4b,e,f,i** and **5b,e,f,i** and for their acetonides are given below.

***anti*-1-*p*-Tolylbutane-1,3-diol (4b).** Oil. ^{1}H NMR $\delta = 1.11$ (d, $J = 6.3$ Hz, 3H), 1.65–1.80 (m, 2H), 2.24 (s, 3H), 3.26 (2H, OH), 3.93 (m, 1H), 4.87 (dd, $J = 7.9$, 3.7 Hz, 1H), 7.04 (d, 2H), 7.13 (d, 2H). ^{13}C NMR $\delta = 21.1$, 23.5, 46.4, 65.3, 71.5, 125.8, 129.3, 137.1, 141.8. EI-MS m/z (%): 180 (10) [M$^+$], 162 (10), 147 (20), 121 (100), 120 (36), 119 (54), 91 (33), 77 (18). **Acetonide:** ^{1}H NMR $\delta = 1.27$ (d, $J = 6.2$ Hz, 3H), 1.41 (s, 3H), 1.56 (s, 3H), 1.5–1.7 (m, 2H), 2.33 (s, 3H), 3.88 (m, 1H), 4.62 (dd, $J = 11.3$, 2.5 Hz, 1H), 7.15 (d, 2H), 7.27 (d, 2H). ^{13}C NMR $\delta = 20.1$ (acetonide-CH_3), 21.4 (acetonide-CH_3), 21.5, 21.8, 40.6, 72.8, 78.5, 99.2, 126.2, 129.3, 137.4, 139.7.

***syn*-1-*p*-Tolylbutane-1,3-diol (5b).** Oil. ^{1}H NMR $\delta = 1.10$ (d, $J = 6.2$ Hz, 3H), 1.61 (ddd, $J = 14.5$, 2.8, 2.9 Hz, 1H), 1.73 (dt, J = 14.5, 10.0 Hz, 1H), 2.25 (s, 3H), 3.60 (1H, OH), 3.69 (1H, OH), 4.02 (m, 1H), 4.77 (dd, $J = 10.0$, 2.9 Hz, 1H), 7.06 (d, 2H), 7.15 (d, 2H). ^{13}C NMR $\delta = 21.2$, 24.0, 47.1, 68.8, 75.2, 125.9, 129.4, 137.4, 141.8. EI-MS m/z (%): 180 (11) [M$^+$], 162 (10), 147 (20), 121 (100), 120 (37), 119 (48), 91 (38), 77 (23). **Acetonide:** ^{1}H NMR $\delta = 1.21$ (d, $J = 6.2$ Hz, 3H), 1.4–1.5 (m, 1H), 1.50 (s, 3H), 1.56 (s, 3H), 1.68–1.73 (m, 1H), 2.33 (s, 3H), 4.13 (m, 1H), 4.86 (dd, $J = 11.9$, 2.7 Hz, 1H), 7.15 (d, 2H), 7.26 (d, 2H). ^{13}C NMR δ = 20.1 (acetonide-CH_3), 21.4, 22.4, 30.6 (acetonide-CH_3), 41.2, 65.6, 71.6, 99.2, 126.2, 129.3, 137.4, 139.7.

***anti*-1-(4-Nitrophenyl)butane-1,3-diol (4e).** Oil. ^{1}H NMR δ = 1.28 (d, $J = 6.3$ Hz, 3H), 1.89 (m, 2H), 4.09 (m, 1H), 5.17 (m, 1H), 6.25–6.75 (broad, 2H), 7.54 (d, 2H), 8.19 d (2H). ^{13}C NMR δ = 23.7, 45.7, 65.8, 71.1, 123.9, 126.6, 147.4, 152.4.

***syn*-1-(4-Nitrophenyl)butane-1,3-diol (5e).** Oil. ^{1}H NMR δ = 1.27 (d, $J = 6.3$ Hz, 3H), 1.79 (m, 2H), 2.0–2.5 (broad, 2H), 4.23 (m, 1H), 5.06 (dd, $J = 4.5$, 2.2 Hz, 2H), 7.55 (d, 2H), 8.20 (d, 2H). ^{13}C NMR δ = 24.8, 47.1, 69.5, 74.5, 123.9, 126.6, 137.7, 152.0. **Acetonide:** ^{1}H NMR $\delta = 1.23$ (d, $J = 6.1$ Hz, 3H), 1.36 (m, 1H), 1.53 (s, 3H), 1.57 (s, 3H), 1.79 (dt, $J = 13.0$, 2.6 Hz, 1H), 4.17 (m, 1H), 5.02 (dd, $J = 11.8$, 2.7 Hz, 1H) 7.54 (d, 2H), 8.20 (d, 2H). ^{13}C NMR $\delta = 20.0$ (acetonide-CH_3), 22.3, 30.4 (acetonide-CH_3), 40.9, 65.3, 70.8, 99.4, 123.9, 126.7, 147.4, 150.0.

***anti*-1-(4-Iodophenyl)butane-1,3-diol (4f).** Oil. ^{1}H NMR δ = 1.14 (d, $J = 6.1$ Hz, 3H), 1.75 (m, 2H), 2.48 (1H, OH), 3.42 (1H, OH), 3.96 (m, 1H), 4.91 (dd, $J = 6.08$, 4.86 Hz, 1H), 7.02 (d, 2H), 7.59 (d, 2H). ^{13}C NMR $\delta = 23.8$, 46.0, 65.7, 71.4, 92.8, 127.9, 137.6, 144.3. **Acetonide:** ^{1}H NMR $\delta = 1.22$ (d, $J = 6.5$ Hz, 3H), 1.23 (s, 6H), 1.40 (m, 1H), 1.83 (m, 1H), 4.04 (m, 1H), 4.99 (dd, $J = 6.7$, 4.4 Hz, 1H), 7.09 (d, 2H), 7.65 (d, 2H).

***syn*-1-(4-Iodophenyl)butane-1,3-diol (5f).** Oil. ^{1}H NMR δ = 1.15 (d, $J = 6.2$ Hz, 3H), 1.60–1.78 (m, 2H), 3.04 (1H, OH), 3.74 (1H, OH), 4.05 (m, 1H), 4.79 (dd, $J = 9.91$, 3.06 Hz, 1H) 7.02 (d, 2H), 7.59 (d, 2H). ^{13}C NMR δ = 24.4, 47.1, 69.2, 74.9, 93.1,

127.9, 137.7, 144.4. **Acetonide:** ^{1}H NMR δ = 1.21 (d, J = 6.1 Hz, 3H), 1.39 (m, 1H), 1.50 (s, 3H), 1.55 (s, 3H), 1.68 (dt, J = 13.1, 2.5 Hz 1H), 4.12 (m, 1H), 4.84 (dd, J = 11.7, 2.5 Hz, 1H), 7.12 (d, 2H), 7.64 (d, 2H). ^{13}C NMR δ = 19.8 (acetonide-CH_3), 22.1, 30.3 (acetonide-CH_3), 40.8, 65.24, 71.0, 92.8, 99.1, 127.9, 137.5, 142.2.

***anti*-1-*p*-Tolylhexane-1,3,6-triol (4i).** Oil.^{1}H NMR δ = 1.50 (m, 2H), 1.60 (m, 2H), 1.60–1.85 (m, 2H), 2.30 (s, 3H), 3.53 (m, 2H), 3.83 (m, 1H), 4.0–5.0 (broad, 3H, OH), 4.90 (dd, J = 8.5, 3.1 Hz, 1H), 7.08 (d, 2H), 7.17 (d, 2H). ^{13}C NMR δ = 21.22, 29.0, 34.5, 45.4, 68.6, 71.0, 74.6, 125.7, 129.2, 136.8, 141.9.

***syn*-1-*p*-Tolylhexane-1,3,6-triol (5i).** Oil.^{1}H NMR δ = 1.50 (m, 2H), 1.59 (m, 2H), 1.60–1.85 (m, 2H), 2.30 (s, 3H), 3.53 (m, 2H), 3.83 (m, 1H), 4.0–5.0 (broad, 3H, OH), 4.80 (dd, J = 9.7, 2.9 Hz, 1H), 7.09 (d, 2H), 7.18 (d, 2H). ^{13}C NMR δ = 21.24, 28.7, 35.0, 45.4, 62.6, 72.1, 74.6, 125.8, 129.2, 137.2, 141.6. **Acetonide:** ^{1}H NMR δ = 1.49 (s, 3H), 1.55 (s, 3H), 1.57–1.71 (m, 6H), 2.32 (s, 3H), 3.64 (m, 2H), 4.00 (m, 1H), 4.86 (dd, J = 11.6, 2.7 Hz), 7.14 (d, 2H), 7.25 (d, 2H). ^{13}C NMR δ = 20.0 (acetonide-CH_3), 21.3, 29.0, 30.4 (acetonide-CH_3), 33.4, 39.4, 62.9, 69.6, 71.6, 99.4, 126.0, 129.3, 137.5, 139.4.

Preparation of *anti*-diols 4b, 4e by reduction of aldols 2 with Evans' reagent

A solution of aldol **2b**[42] or **2e**[55] (2 mmol) in dry acetonitrile (3.5 mL) was added by cannula, at –40 °C, to a freshly prepared solution of tetramethylammonium triacetoxyborohydride (15 mmol) in 15 mL of a 1 : 1 mixture of dry acetonitrile and dry acetic acid. The reaction mixture was stirred under argon, at –40 °C for 18 h and then quenched with 20 mL of 0.5 N aqueous sodium potassium tartrate and allowed to reach room temperature. The mixture was partitioned between ethyl acetate (40 mL) and sat. aqueous $NaHCO_3$ and the aqueous phase was extracted with ethyl acetate (4 × 30 mL). The combined organic phases were extracted with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure gave the crude diols (87% and 85% from **2b** and **2e**, respectively) as 4 : 1 mixtures of *anti*/*syn* isomers from which pure anti diols **4b** and **4e** were separated by FC (CH_2Cl_2–EtOAc = 6/4 for **4b**; CH_2Cl_2–CH_3OH = 9/1 for **4e**).

Reductions with $NaBH_4$/albumin

The reduction of diketone **1b** was carried out on the mg scale and is described as a representative example. The diketone (26.4 mg, 150 μmol) in acetonitrile (15 mL) was added to a solution of albumin (10 g, 150 μmol) in water (130 mL) and the mixture was kept at room temperature for 30 min. A solution of $NaBH_4$ (34 mg, 90 μmol) in water (5 mL) was added dropwise, at 20 °C and the resulting mixture was stirred at the same temperature for 2 h. The mixture was acidified with trifluoroacetic acid (5 mL) and ethanol was added (150 mL). The denatured protein was separated by centrifugation at 4200 r.p.m. at 4 °C for 20 min and the solution was concentrated to 5 mL and extracted with dichloromethane (3 × 5 mL). The combined extracts were dried over Na_2SO_4 and evaporated giving a 93 : 7 mixture of diols **4a** and **5a** (20.5 mg, 76%). The other reactions were carried out similarly on the 1.5 μmol scale and analyzed as described in the following section.

HPLC analysis of the $NaBH_4$ and $NaBH_4$/albumin reductions

For the analysis, 200 μL aliquots of the reaction mixtures were taken and acidified with TFA (5 μL). Samples containing albumin were centrifuged at 4200 r.p.m. at 4 °C for 20 min to separate the denaturated protein. The resulting solutions were directly analyzed by HPLC with an Alltech Alltima C18 5 μ column (250 × 4.6 mm). Elution with water–acetonitrile 68 : 32 gave in all cases base-line separated peaks for the diketones, the corresponding hydroxyketones and *anti* and *syn* 1,3-diols.

Fluoresceinated BSA

Fluorescein isothiocyanate (1.3 mg, 3.3 μmol) in DMF (30 μl) was added to a solution of BSA (200 mg, 3 μmol) in 0.02 M phosphate buffer, pH 8.2 (75 mL) and the mixture was stirred at 20 °C for 3 h. The solution was dialyzed against 0.01 M citrate buffer, pH 3, at 4 °C for 3 days and then against water, at 4 °C for 1 day. A modification index of 0.93 was calculated for the conjugate, assuming an extinction coefficient of 15.900 for fluoresceinated BSA.[9c,10b]

Calculations

DFT calculations were carried out with the parallel version of the Gaussian03 suite[56] running on an Intel Core™2 cpu 6600@2.40 GHz, 3.50 GB RAM. All the makeup of the HSA pdb structure, molecular mechanics optimizations, conformational searches and dynamics were carried out with the Sybyl 7.3 suite running on SGI Octane2 MIPS R12000 workstations, or on a RedHat Linux Intel Pentium 4 2.53 GHz machine. Docking calculations were performed with the Grid22b suite on a Kubuntu Linux Intel Core™2 cpu 6600@2.40 GHz, 3.50 GB RAM.

Homology model of BSA

The structure of the warfarin-HSA complex was downloaded from the Protein Data Bank[57] and all the crystallization water molecules were removed before the optimization, with the exception of water 13 and 28. All the hydrogen atoms were then added, and the structure was submitted to the multistage relaxation protocol of Levit and Lifson.[58] The Amber 4.1 force field was used for all the optimizations;[59] Gasteiger–Marsili charges were applied to the protein atoms, while the charges on the ligand molecule were obtained from a B3LYP/6.31G(d,p) calculation. After relaxing the complex, the structure was allowed to reach thermal equilibrium at 300 K by a multistep molecular dynamic run in the NTV ensemble (5000 fs at 100 K and at 200 K and then 405000 fs at 300 K). Finally, the structure was globally reoptimized to give a model of the HSA-warfarin complex. This geometry was used to build the BSA homology model. The protein sequence was mutated at the domain IIa level as follows: K195R, K199R, F211L, A215S, R222K, S232T, T243K, E265D, S270T, E280D, N295K, E297A, M298I, A300E. The mutated model was then submitted again to the relaxation/dynamics/optimization scheme to give a model of the IIa site of BSA. The model was conformationally consistent and passed a Ramachandran analysis.

Docking

The docking analysis was performed with the GRID 22b package.[37] To build the molecular interaction maps, all the residues within 15 Å from the $N_{\varepsilon 2}$ atom of His 242 were considered; the residues were included into a 20 Å cubic grid made of 8000 knots. His 242 was considered to be in its neutral state, while Lys 222 was considered in the protonated cationic state. The grid was mapped with eight different GRID molecular probes (H, OH2, OH, O-, O═, C3, C1═, DRY), and after having obtained the maps, docking of diketone **3a** and hydroxyketones **1a** and **2a** were performed considering all the tautomeric forms of the diketone and all the conformations of the hydroxyketones.

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