Journal Name

ARTICLE

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

DOI: 10.1039/x0xx00000x

www.rsc.org/

Chemo-enzymatic synthesis of (*E*)-2,3-diaryl-5-styryl-*trans*-2,3-dihydrobenzofuran-based scaffolds and their *in vitro* and *in silico* evaluation as a novel sub-family of potential allosteric modulators of the 90 kDa heat shock protein (Hsp90)

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Herein we propose a facile, versatile and selective chemo-enzymatic synthesis of substituted (*E*)-2,3-diaryl-5-styryl-trans-2,3-dihydrobenzofurans based on the exploitation of the laccase-mediated oxidative (homo)coupling of (*E*)-4-styrylphenols. Thanks to this novel synthetic strategy, a library of benzofuran-based potential allosteric activators of the Heat shock protein 90 (Hsp90) was easily prepared. Moreover, considering their structural analogies to previously reported allosteric modulators, the sixteen new compounds synthesized in this work were tested in vitro for their potential stimulatory action on the ATPase activity of the molecular chaperone Hsp90. Combining experimental and computational results, we propose a mechanism of action for these compounds, and expand the structure-activity relationship (SAR) information available for benzofuran-based Hsp90 activators.

Introduction

Heat shock protein 90 (Hsp90) is a 90 kDa molecular chaperone overexpressed by cells in stress conditions. It is known to play a pivotal role in the regulation of a wide-range of 'client proteins' and to modulate different pathways connected with cell growth and maintenance.[1,2] As such, the dysregulation of its natural functions has been proposed as a promising target in the treatment of vascular and neurodegenerative diseases. Moreover, due to its key participation in the control of cell cycle and apoptosis, it is nowadays an established target in the development of novel cancer therapies.[3–7]
Hsp90 functions as a homodimer and its chaperone activity and the consequent downstream signalling pathways are controlled by complex internal dynamics. At the molecular level, the binding and hydrolysis of ATP regulates Hsp90 activity by 'selecting' conformational protein sub-states with distinct functional properties and regulating the transitions between them.[8–10]
Allosteric ligands have been shown to tune the normal functions of the chaperone by perturbing ATPase activity, working either as inhibitors or stimulators. In this context, allosteric modulation may interfere with crucial protein-protein interactions by modifying the timing and kinetics with which suitable chaperone conformations are presented to client proteins for the formation of viable complexes.[11]
In previous work, we demonstrated that rationally designed and differentially decorated allosteric activators based on a 2-(4-hydroxyphenyl)-3-methylbenzofuran scaffold (Figure 1A) may offer the possibility of modulating the activity of the Hsp90 chaperone avoiding the so-called 'heat shock response',[11–14] a prosurvival mechanism affecting the action of drugs acting as pure inhibitors.[15,16] Moreover, a quantitative structure-dynamics-activity relationship model (SDAR) was built, thanks to the integration of computationally evaluated ligand efficiencies and the experimentally measured modulated activities of the Hsp90's ATPase cycle. This allowed to highlight the pivotal role of the substitution pattern of the phenolic moiety at the C_{13} of this family of compounds.[11]

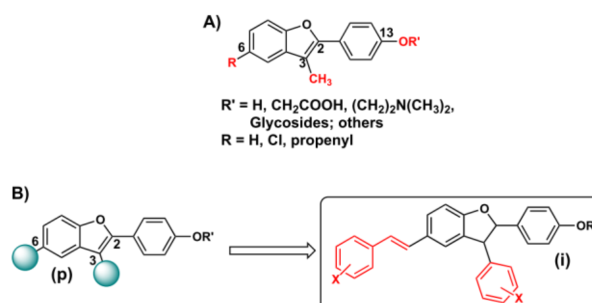

Figure 1. A): previously investigated 2-(4-hydroxyphenyl)-3-methylbenzofuran-based allosteric activators; **B)**: 2,3-

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Electronic Supplementary Information (ESI) available: Metal-mediated oxidation, *in vitro* assay, structures and activity data of Hsp90 modulators, ^{1}H, ^{13}C-NMR and mass spectra. See DOI: 10.1039/x0xx00000x

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dihydrobenzofuran-based potential modulators proposed in this study (**i**).

As the above-mentioned studies were mainly focused on the effects of the phenol in C_{13} and, marginally, of the C_6 substitution(s) on the structure-activity relationship (SAR), in this work the SAR analysis was further amplified by building a library of analogues (**i**, Figure 1B) structurally modified at the C_2, C_3 and C_6 positions of the parental compounds (**p**).

Laccases (EC 1.10.3.2) are copper-containing enzymes known to oxidize a wide range of organic molecules, both directly or in the presence of low-molecular-weight redox mediators,[17] promoting the formation of four highly reactive organic radicals, by reducing molecular oxygen to water.[18–22] We have previously reported several examples dealing with the oxidation of natural compounds.[23–30] Specifically, hydroxystilbenes were successfully oxidized by the action of laccases from *Trametes pubescens* or *T. versicolor* obtaining mixtures of dimers and oligomers (depending on the nature of the substrate used) enriched in the 2,3-dihydrobenzofuran-based dimers, always isolated as a racemic mixture of *trans* stereoisomers.[31–36]

Here we report the convenient exploitation of these 'blue oxidases' for the novel, mild, 'green' and straightforward chemo-enzymatic synthesis of substituted (*E*)-2,3-diaryl-5-styryl-*trans*-2,3-dihydro benzofurans (**i**), structurally-modified analogues of the described parental compounds (**p**), by means of the (homo)coupling of chemically synthesized (*E*)-4-styrylphenols (**ii**, Figure 2). Moreover, to estimate the structure-activity relationship (SAR) of the novel synthesized compounds (**12**-**28**), we evaluated their ligand efficiencies *in silico* using the previously mentioned SDAR model, and combined this to the experimentally measured ability to modulate Hsp90's ATPase activity. The good correlation obtained confirms the validity of the structure-dynamics-activity model and allows to expand its reach to a new set of derivatives.[11,37]

Results and discussion

Chemo-enzymatic synthesis of dimers 12-28

A library of (±)-*trans*-2,3-dihydrobenzofurans bearing two aryl groups at the C_3 and C_6-positions of the parental benzofuran moiety (**p**) was prepared (Figure 1B).

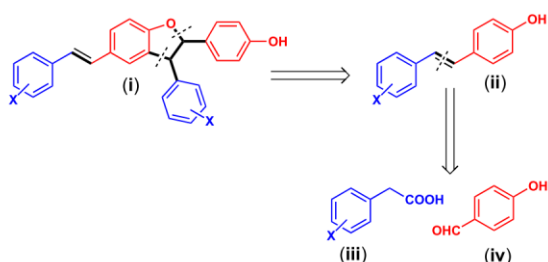


Figure 2. Retrosynthetic analysis for compounds (**i**).

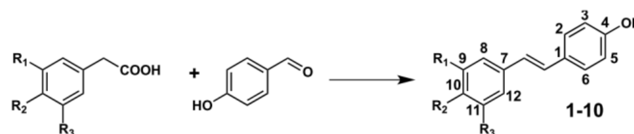
Specifically, as a convenient and easy entry to this family of compounds was needed, the oxidative coupling of (*E*)-stilbenes was considered as a suitable strategy. In fact, it was previously reported that this reaction could be promoted by metal-based catalysts (Fe, Cu, Mn) and, more efficiently, by copper-containing redox enzymes, *i.e.* laccases, under mild reaction conditions.[38, 39]

The retrosynthetic approach that was selected is proposed in Figure 2. (*E*)-4-styrylphenols (**ii**) were prepared from commercially available phenyl acetic acids (**iii**) and *p*-hydroxybenzaldehyde (**iv**) *via* a Perkin-like reaction and transformed into the target oxygenated heterocycles (**i**) exploiting radical oxidative (homo)couplings.

After a set of experiments aimed at the optimization of the reported conditions in terms of isolated yield, the desired (*E*)-4-styrylphenols (**ii**, compounds **1**-**10**) were obtained using *p*-hydroxybenzaldehyde in the presence of a slight excess of substituted phenylacetic acid and piperidine, following the procedure proposed by De Filippis *et al.*[40] The results are summarized in Table 1.

All the synthesized products were analyzed by ^{1}H-NMR and the data were compared with previous literature reports. Evidence on the formation of the desired (*E*)-stilbenes came from the presence of all the expected aromatic and (if any) aliphatic signals as well as from the presence of a (*E*)-olefinic bond (two doublets resonating between 7.20-7.50 ppm with coupling constants of *ca*. 16.5 Hz).

Table 1. Synthesis of substituted (*E*)-4-styrylphenols.[a]



Product	R_1	R_2	R_3	Reaction Time (h)	Isolated Yield %
1	H	H	H	24	52
2	H	CH_3	H	48	44
3	H	CN	H	24	64
4	H	NO_2	H	18	62
5	H	OCH_3	H	28	38
6	CH_3	H	H	24	28
7	CN	H	H	24	60
8	SCH_3	H	H	48	57
9	OCH_3	H	OCH_3	48	28
10	CH_3	H	CH_3	72	39

[a] Reagents and conditions: 4-hydroxy benzaldehyde (1 equiv. 4.0 M), phenylacetic acid (2.4 equiv.), piperidine (2.4 equiv.), DCM, 130 °C, 24-72 h.

Once stilbenes **1**-**10** had been synthesized, their oxidative coupling to give the target 2,3-dihydrobenzofuran-based scaffolds (**i**) was investigated. As previously stated, different metal-based chemical oxidants are available for this transformation as well as biocatalytic entries. Using the unsubstituted compound **1** as a model substrate, MnO_2, $FeCl_3$, $CuCl_2$ and the laccases from *T. versicolor* were tested for their ability of promoting the formation of the oxygenated heterocycles.

Oxidations with iron(III) trichloride, copper(II) chloride or manganese dioxide, using a monophasic mixture of ethanol and water as solvent,[38] gave complex mixtures of oxidized products (for additional details see *Supporting Information*). Therefore, we turned our attention to the oxidative radical couplings mediated by

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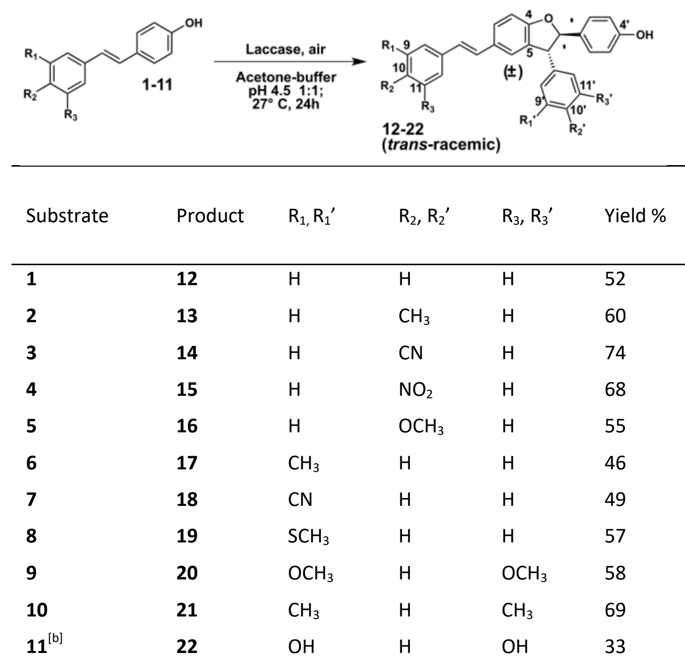
laccases.[39] The (bio)oxidation of the model substrate **1** was conducted using the commercially available laccase from *T. versicolor* at 27 °C in a 1 : 1 mixture of acetate buffer (pH 4.5, 50 mM) and acetone as solvent. The reaction, monitored by TLC and reverse-phase HPLC, proceeded smoothly.

The only detectable product was isolated by silica gel chromatography (76 % yield). It proved to be homogenous also by RP-HPLC and its structure was determined by mass spectroscopy, ^{1}H- and ^{13}C-NMR. The ^{1}H-NMR spectra underlined the formation of the dihydrodimer **12** (Table 2, for additional details see *Supporting Information*). Specifically, in addition to the expected signals of the aromatic protons, the presence of the target 2,3-*trans*-dihydrobenzofuran was confirmed by the two doublets resonating at 5.53 and 4.60 ppm, characterized by the typical value of *trans*-coupling constants in oxygenated 5-membered rings (*J* ca.8.5 Hz). Moreover, two doublets resonating at 7.06 and 6.92 ppm and showing the typical value for a coupling constant of (*E*)-configured C-C double bonds (*J* ca.16.5 Hz) were detected, attesting the presence of unsaturation between C_{α} and C_{β}.

Table 2. Synthesized racemic *trans*-2,3-dihydro benzofurans.[a]



Substrate	Product	R_1, R_1'	R_2, R_2'	R_3, R_3'	Yield %
1	**12**	H	H	H	52
2	**13**	H	CH_3	H	60
3	**14**	H	CN	H	74
4	**15**	H	NO_2	H	68
5	**16**	H	OCH_3	H	55
6	**17**	CH_3	H	H	46
7	**18**	CN	H	H	49
8	**19**	SCH_3	H	H	57
9	**20**	OCH_3	H	OCH_3	58
10	**21**	CH_3	H	CH_3	69
11[b]	**22**	OH	H	OH	33

[a] Reagents and conditions: laccase from *T. versicolor* (42 U $mmol_{substrate}^{-1}$), [substrate] = 78 mM, acetate buffer (pH 4.5, 50 mM) and acetone (1 : 1), 27°C, 160 rpm, 24 h. [b] Commercially available compound.

Once verified that the laccase-catalyzed oxidation could be the best method for the selective preparation of the target (±)-*trans*-dihydrobenzofuran **12** from stilbene **1** in terms of isolated yield, product purity and 'green' reaction conditions, the same protocol was applied to the other (*E*)-4-styrylphenols **2**-**10**. Moreover, the commercially available resveratrol (**11**), whose laccase-catalyzed oxidation was investigated by us years ago,[31] was also subjected to the action of the enzyme from *T. versicolor*, aiming at isolating the corresponding dimer (compound **22**), named ε-viniferin. The results obtained in the (bio)oxidation of stilbenes **1**-**11** are summarized in Table 2. In all cases the oxidations run efficiently and the expected (±)-*trans*-dehydrodimers were isolated in 55-74 % yield after 24 h of reaction. As previously reported, the dimer of resveratrol (**22**) was isolated in lower yield due to presence of more than one oxidizable phenolic OH in **12**, which facilitated the formation of byproducts and undesired over-oxidations. All products were fully characterized by means of mass spectroscopy and ^{1}H- and ^{13}C-NMR. Moreover, thanks to these results, the significant selectivity of these enzymatic transformations along with their functional groups tolerance (sulphides, oxidizable benzylic positions, cyanide or nitro groups) was once again verified.

Previously, Colombo *et al*. found that insertion of a tertiary amine (a basic nitrogen able to promote hydrogen bonding and establish salt-bridges with the chaperone protein) on the benzofuran-based modulators could increase their ability to stimulate the ATPase process. Specifically, the alkylation of the 4-hydroxyl group of the 2-(4-hydroxy-phenyl)-3-methylbenzofuran scaffold with *N,N*-dimethylethanamine side chain gave promising results.[13] Accordingly, it was decided to investigate the introduction of a nitrogen-containing basic functional group on some of the new dimers, following two distinct strategies: the reduction of the nitro groups of compound **15** and the alkylation of the phenolic OH of the dimers **12**, **20** and **21** to introduce the *N,N*-dimethylethanamine moiety.

A potential H-bonding group could be inserted in the *para*-positions of compound **15** exploiting a metal-free method developed by Benaglia and co-workers for the reduction of aromatic nitro compounds, which consisted in the use of trichlorosilane in combination with DIPEA.[41] Accordingly, from the reduction of **15**, the diamine **23** was obtained in 21 % isolated yield and its structure was confirmed both by NMR and mass analysis. Moreover, during the purification process, the two mono-amine derivatives **24** and **25** were also isolated in 3 % and 8 % yield respectively (Figure 3). The structural attribution of compounds **24** and **25** was far from easy, as the two regioisomers share the same molecular weight and show very similar NMR spectra. Their identification was simply based on the different Uv-Vis-responses of their TLC spots. The first one appeared yellow in the Vis-spectrum and responded with a bright spot when irradiated with Uv-light at 254 nm. At variance, the other mono-amino derivative was only Uv-responsive. Since the parental compound **15** showed a behavior similar to the former, the structure of the mono-amine **25**, in which a π-conjugated system still connects the nitro group and the two aromatic rings, was attributed to that yellow product.

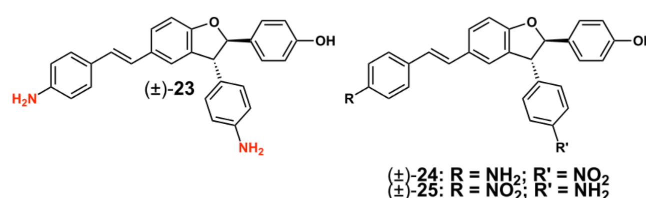


Figure 3. Amine derivatives obtained from dimer **15**.

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The alkylation of the $C_{4'}$-phenolic group of compounds **12**, **20** and **21** was successfully accomplished following published protocols, producing compounds **26**, **27** and **28** (Figure 4).[13] Compound **26** was synthesized to investigate whether the previously mentioned activation effects of tertiary amines could be operating also on *trans*-2,3-dihydrobenzofuran moieties on the 'nude', unsubstituted skeleton of **12**. Moreover, to investigate the possibility of activating potential Hsp90's ATPase modulators sharing structural similarities with compound **22** but lacking its peculiar H-bonding ability, compounds **20** and **21** were derivatized as well to give products **27** and **28**.

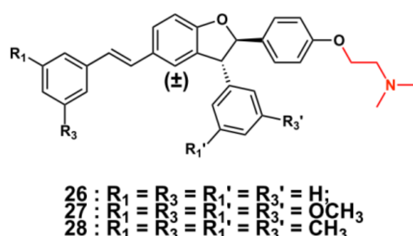

Figure 4. Compounds **26**, **27** and **28**.

In vitro chaperone ATPase stimulation and relationships to structural decoration

In this study, our main goals were to explore the effects of substitutions on positions 2, 3, and 6 of the initial benzofuran scaffold (see Figure 1), and to test the ability of the Dynamic Ligand Efficiency method to correctly predict and/or explain the expected stimulatory activity of newly designed compounds**.** In this context, the chemoenzymatic generation of new benzofuran derivatives with novel substitution patterns combined to the computational study of their potential allosteric binding modes, allows us to expand Structure-Activity Relationship (SAR) investigations. Consequently, compounds **12**-**22** were tested *in vitro* for their potential activity as allosteric modulators of Hsp90 ATPase activity, in comparison to previously reported benzofurans with no sterically hindering substituent on positions 2, 3, and 6 (See *Supporting Information*, for structures and activities of previously published compounds). The yeast isoform of Hsp90 was used here for the measurement of the enzymatic activity.[42] The selected spectrophotometric test, as reported in Sattin *et al.*,[13] consisted in a colorimetric assay in which the chaperone promoted hydrolysis of ATP was coupled with the final oxidation of NADH to NAD^+ and monitored by following the continuous decrease in absorbance at 340 nm in a set of cascade reactions catalyzed by the enzymes pyruvate kinase (PK) and lactate dehydrogenase (LDH) (for supplementary details see Scheme S1).[43]

First, the 'native' ATPase activity of the heat shock protein was tested in the presence of DMSO (Table 3, Entry A) as a control experiment with the cosolvent used to dissolve compounds **12**-**22**, to set a standard value to normalize all the forthcoming experiments.

After that, all the synthesized potential modulators were screened by directly incubating in cuvette the selected compound, a sample of the protein, the reactant and enzymes in HEPES buffer. The enzymatic cascade was initiated by ATP addition.

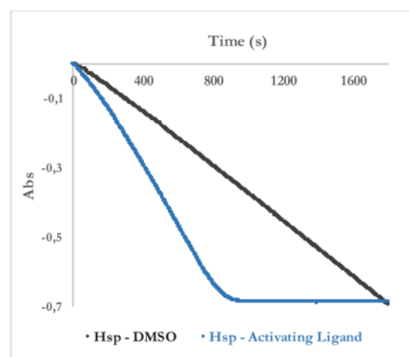

Figure 5: Chaperone ATPase kinetics in the presence of DMSO (black) or compound **22** (blue) (detected at 340 nm).

An example of the ATPase kinetics both in the presence of DMSO (blank control experiment) and of a potential modulator (compound **22**) is reported in Figure 5.

The results obtained from the *in vitro* activity measurements are summarized in Table 3, together with Standard Deviations calculated from three replicates. As shown, compound **22** (Entry N, the dimer of the commercially available resveratrol **11**) was the derivative in this series that could promote the larger increase (2.5 fold) in the normalized ATPase activity. It is important to underline that in the previous series of benzofurans activations up to more than 5-fold could be observed.[13,37] This result indicates that increased steric hindrance on the benzofuran scaffold may still induce activation but to a lower degree than previously observed. Interestingly, computational investigations indicate that these compounds could bind the allosteric site with less favourable ligand efficiencies than the non-hindered series. Nonetheless, it is interesting to observe that some of the newly synthesized compounds are still able to act as stimulators of enzymatic ATPase. On the other hand, we provide access to a diverse series of compounds that permit to modulate Hsp90 activation to variable degrees. Indeed, Hsp90 plays a pivotal role in a large number of different biological pathways. In some cases the full inhibition/activation of its activity may be needed to perturb a specific pathway, while in some other cases a more tuned regulation may be desirable to determine different outcomes. In perspective, we suggest that this battery of compounds can be tested *in vivo* to investigate the potential correlation between the degree of Hsp90 stimulation/activation and observed effects in different cell models.

Since all the tested dimers were obtained as racemic mixtures of *trans*-diastereoisomers, it was of interest to verify whether their single enantiomers could give different results. As the enantiomers of **22**, **(2*R*, 3*R*)-22** and **(2*S*, 3*S*)-22**, were available thanks to previous studies,[33] both these compounds were tested separately (Table 3, entries O and P). The chaperone ATPase modulation efficiency of the two isolated enantiomers turned out to be comparable with the *in vitro* activity of racemic **22**. Docking studies conducted on compounds **(2*S*, 3*S*)-22** and **(2*R*, 3*R*)-22** further confirmed that the two species, possessing docking scores of -11.697 and -10.024 kcal mol^{-1} respectively, interact with the Heat shock protein in a similar fashion (see also Fig. S3 in *Supporting Information*). Therefore,

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while *a priori* it may be expected that binding of different enantiomers to the same protein would result in diastereoisomeric complexes, the properties of such complexes in terms of enzymatic activation need not necessarily be different: Indeed, on the bases of our experimental and computational observations, it was possible to conclude that the absolute configuration of the synthesized racemic dimers **12**-**22** did not have a strong effect on measured Hsp90 stimulation.

Table 3. ATPase assays of compounds **12**-**22**.[a)]

Entry	Compound	Normalized ATPase Activity	STD
A	DMSO	1.00	0.07
B	**12**	1.10	0.07
C	**13**	0.70	0.05
D	**14**	0.80	0.10
E	**15**	1.10	0.10
F	**16**	1.00	0.14
G	**17**	0.90	0.10
H	**18**	1.00	0.05
I	**19**	1.20	0.05
L	**20**	0.90	0.05
M	**21**	1.00	0.09
N	**22**	2.50	0.10
O	**(2*R*, 3*R*)-22**	2.40	0.11
P	**(2*S*, 3*S*)-22**	2.30	0.10

[a)] All compounds were tested three times and standard deviations (STD) were calculated accordingly. Reagents and conditions: HEPES (20 mM, pH 7.5) solution containing KCl (100 mM), $MgCl_2$ (1 mM), PEP (phosphoenolpyruvate, 1 mM), NADH (0.18 mM), PK (pyruvate kinase, 2.5 U ml^{-1}), LDH (L-lactate dehydrogenase, 4 U ml^{-1}), heat shock protein (2 μM) and the desired dihydrodimer (50 μM) and ATP (1 mM); 30 °C, 30 min.

The results of the *in vitro* testing of the reduced and alkylated dimers **23**-**28** are summarized in Table 4. While the reduction of **15** produced compounds with still negligible modulatory ability toward the chaperone ATPase activity (Entries R-T), the alkylation of **12** to give compound **26** demonstrated the ability of the *N*,*N*-dimethylethanamine side chain to promote a modulatory activity in the 2,3-dihydrobenzofuran scaffold. In fact, the incubation of compound **26** with Hsp90 showed a remarkable 2.3 increase over the 'native' ATPase activity (Entry **U**). The same was not true for the alkylation of the more sterically-demanding compounds **20** and **21**, as shown by the data collected for **27** and **28** (Entries V and Z).

Table 4. ATPase assays of dimers **23-28**.[a)]

Entry	Compound	Normalized ATPase Activity	STD
Q	DMSO	1.00	0.07
R	**23**	1.40	0.14
S	**24**	0.90	0.05
T	**25**	0.90	0.02
U	**26**	2.30	0.24
V	**27**	0.90	0.02
Z	**28**	1.00	0.02

[a)] All compounds were tested three times and standard deviations were calculated accordingly.

Docking studies and SAR considerations

To assess at the molecular level the binding properties that guide the efficiency of designed compounds in modulating Hsp90 ATPase activity, protein-ligand docking calculations were performed using the structure of yeast Hsp90 (Figure 6).[42]

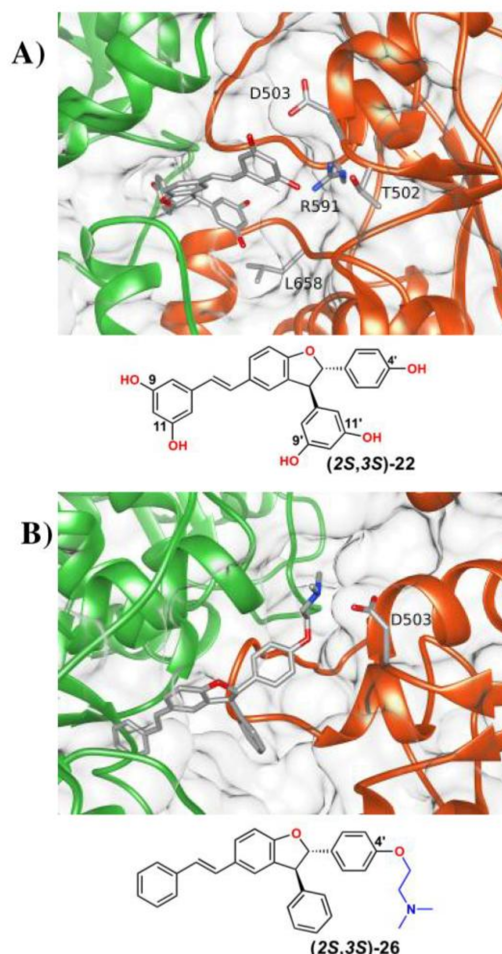

Figure 6. Docking of **2*S*,3*S*-22** (**A**) and **2*S*,3*S*-26** (**B**). Similar interactions are established by the enantiomer of each compound, providing comparable interaction energies.

The results obtained are fully in line with the described experimental assays. Indeed, compound **22** turned out to be the strongest binder with a predicted binding energy of -11.697 kcal mol^{-1} for the best pose (Figure 6A).

The hydroxyl substituents in positions $C_{9/9'}$, $C_{10/10'}$, $C_{11/11'}$ and $C_{4'}$ were found to establish a series of hydrogen bonds with the residues T502, D503, R591 and L658 of protomer B of Hsp90, thus strongly stabilizing its closed and activated conformation and supporting the higher stimulatory activity observed for **22**. Indeed, the elimination of such substituents able to form hydrogen bonds strongly decreased the binding affinity, as in the case of compounds **12**, **20**, **21**, **27** and **28**. This behavior was also in line with the results

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previously obtained for a series of different, yet related, benzofuran-based compounds.[13,37]

To shed light on origin of the observed lower stimulatory activity of the new series of derivatives with respect to the previously presented benzofuran, we compared the best docking poses of **22** and of the original compound presented in Figure 1A, with R=Cl and R'=$(CH_2)_2$-$N(CH_3)_2$. From these models, it is possible to observe that the best stimulator (Figure 7A) is able to establish strong electrostatic interactions with both E477 and D503 from protomer B. The formation of this electrostatic clamp was previously shown (via FRET measurements) to stabilize the closed, catalitically active state of Hsp90. The presence of a large substituent in place of the Cl atom on the benzofuran anchors **22** deep in a hydrophobic pocket on protomer A (Figure 7B), inducing a slightly different binding pose that allows only the di-hydroxylated phenyl ring linked to the furan ring to establish a stable hydrogen-bonding interactions with D503. We hypothesize that the absence of the strong interaction with both charged groups in the allosteric site causes a decreased stabilization of the closed state compared to the best stimulators. In this frame of thought, the structure of the complex resulting from the docking of **22** is less compact than the one observed for the un-substituted compounds (Figure 7).

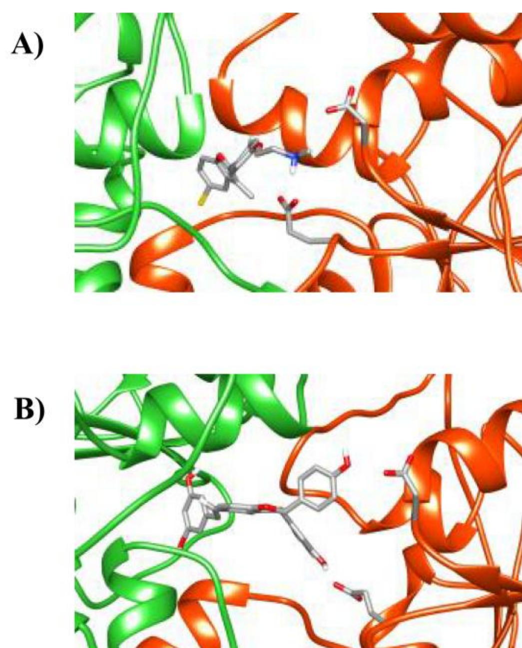

Figure 7. Comparison of the ligand pose in the allosteric pocket between the best stimulator previously identified[13] (**A**) and compound **22** (**B**). The lateral chains of residues E477 and D503 are shown. Protomers A and B are depicted in green and orange, respectively.

Interestingly, while for **27** and **28** the addition of a methylamine substituent in position $OC_{4'}$, to promote the formation of hydrogen bonds or salt bridges did not enhance the binding efficiency of the respective precursors, this was not the case for compound **26**, the $OC_{4'}$-dimethylamine derivative of **12**. While **12** was inactive, a stimulatory effect in promoting ATPase activity by **26** was observed with a similar value to the one measured for compound **22** (2.30 ± 0.14 vs 2.50 ± 0.10). This effect could be rationalized using our multiconformational docking protocol and linked to the formation of a hydrogen bond and a salt bridge with residue D503 from protomer B (Figure 6B).

Combining all above SAR observations, it is possible to argue that the introduction of a dimethylamine substituent in position $OC_{4'}$ is beneficial only when the other phenyl rings are not substituted. Indeed, the presence of large groups in positions $C_{9/9'}$, $C_{10/10'}$, $C_{11/11'}$ increases the steric hindrance of the compounds that adopt a less suitable conformation into the binding pocket, thus potentially affecting the stability of the binding.

To put these observations on a more quantitative ground, we made use of the recently introduced Dynamic Ligand Efficiency (DLE) parameter. DLE associates the average ligand efficiency calculated for each derivative from docking it to a multiconformational ensemble of Hsp90 structures, and experimental ATPase activities. An interesting linear correlation (Figure 8) between DLE and the observed stimulations holds for the new set of chemoenzymatically synthesized compounds.

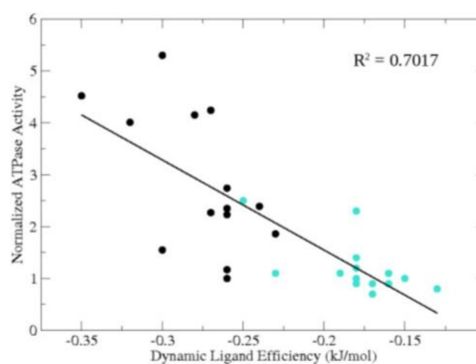

Figure 8. Correlation between the dynamic ligand efficiency (DLE) parameter and the measured ATPase activities. The cyano spots represent the compounds synthesized in this study while the black ones are the previously synthetized ligands.

Importantly, such correlation holds also when combining the data obtained for **12-28** (cyano circles in Figure 8) from this paper together with the ones previously described in literature (See *Supporting Information*, for the structures and activities). This finding further supports the validity of both the Quantitative Structure Dynamics Activity Relationship model previously used for the *de-novo* design of allosteric modulators of Hsp90[11] and the SAR studies conducted on the new sub-family of ligands synthesized in this work.

Overall, our model suggests that a necessary requirement for proficient stimulators is the presence of a flexible amino-group able to engage both E477 and D503. The presence of a bulky group on the lipophilic chain on the aromatic ring of the benzofuran scaffold on the one hand seems to favor anchoring to protomer A, while on the other hand appears to induce a modification in the binding pose

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that does not permit to efficiently reach E477 and D503 with the substituents at the opposite side of the molecule.

Overall, the derivatives prepared in this paper appear to be stimulators of Hsp90 ATPase. Recent experimental and theoretical findings[10,44] indicate that in the chaperone cycle ATP binding by both protomers shifts the protein population to a closed, highly strained asymmetric state. In this complex, one of the two protomers is significantly distorted compared to the symmetric dimer. Hydrolysis happens first in the distorted, asymmetric protomer and then in the other. In our model, allosteric stimulators bind in an asymmetric mode, establishing different sets of interactions with the two protomers. In the absence of a crystal structure for Hsp90-allosteric ligand complexes, it is then possible to speculate that the binding mode we observe could stabilize the asymmetric state necessary to set the stage for the first ATP hydrolysis. This would in general favor activated structures of the chaperone, resulting in the promotion of ATP hydrolysis.

Conclusions

To expand the number of available compounds and further investigate the SAR characteristics of benzofuran-based Hsp90 (potential) stimulators, a novel and straightforward chemo-enzymatic synthesis of (*E*)-2,3-diaryl-5-styryl-*trans*-2,3-dihydro benzofurans has been proposed and successfully validated with the preparation of sixteen derivatives.

The aryl groups installed at the C_3 and C_6 positions of the parental benzofuran platform were diversified using differently substituted phenylacetic acids in the chemical synthesis of the (*E*)-4-styrylphenols soon-to-be oxidized by the action of laccases.

The 2,3-*trans*-dihydrobenzofuran core was successfully and selectively built in a one-step process *via* radical-based oxidative (homo)couplings. At variance to previous reports on the biocatalyzed oxidation of phenolic derivatives,[27–30] these compounds proved to be suitable substrates and their main (and often only) dimeric products were isolated in yield up to 75 %. Moreover, this enzymatic approach was better performing when compared to the metal-catalyzed oxidations in terms of selectivity.

All the synthesized compounds were tested *in vitro* for their ability to modulate the ATPase activity of Hsp90.[14] The polyphenolic compound **22** and the amine containing dimer **26** behaved as activators with interesting degrees of potency. Their mechanism of interaction with the chaperone protein was investigated at a molecular level *via* multiconformational docking studies, highlighting different binding features depending on the chemical structure. Overall, the results support the validity of the Dynamic Ligand Efficiency (DLE) approach for designing and evaluating the potential effects of novel allosteric derivatives on Hsp90 ATPase activities.

Experimental section

Materials, chemicals, and equipment

All reagents were of the highest purity grade from commercial suppliers: Sigma-Aldrich (St Louis, MO, USA) or VWR (Radnor, PA, USA). Laccase from *Trametes versicolor* was from Sigma-Aldrich. The enzymes were used based on their respective activities evaluated according to literature assay based on the ABTS as model substrate. [28] The yeast homologue of Hsp90 was a kind gift from Prof. Johannes Buchner, Technical University of Munich. Biotransformations were performed in a G24 Environmental Incubator New Brunswick Scientific Shaker (Edison, USA) or in a Thermomixer Comfort (Eppendorf, DE). Reactions were monitored by thin-layer chromatography (TLC) [precoated silica gel 60 F254 plates (Merck, DE)]; development with UV lamp, Komarovsky reagent (1 mL 50% ethanolic H_2SO_4 with 10 mL 2% methanolic 4-hydroxybenzaldehyde), a 20% solution of H_2SO_4 in ethanol or a molybdate reagent ($(NH_4)_6Mo_7O_{24}$·4 H2O, 42 g; $Ce(SO_4)_2$, 2 g; H_2SO_4 conc., 62 mL; made up to 1 L of deionized water). Flash chromatography: silica gel 60 (70–230 mesh, Merck, DE). NMR spectra were recorded with a Bruker AC spectrometer (400 or 500 MHz) in [D_4]MeOH, [D_6]DMSO or [D_1]$CHCl_3$. Mass spectra were recorded with a Bruker Esquire 3000 Plus spectrometer. Uv-kinetics were recorded on a nitrogen flushed Jasco J-1100 spectrophotometer (Easton, MD, USA) interfaced with a thermostatically controlled cell holder.

General procedure A: chemical synthesis of (*E*)-stilbenes

A solution of *p*-hydroxybenzaldehyde (0.5 M, 1 eq) and of the desired substituted phenylacetic acid (1.2-2.4 eq) was prepared in CH_2Cl_2 at rt. Piperidine (2.5 eq) was added and the resulting mixture was gradually heated to 130 °C, distilling the solvent. The resulting neat mixture was left reacting at 130 °C for 24-48 h. After that, the crude residue was dissolved in MeOH, analyzed by TLC and purified by flash chromatography (ETP :AcOEt = 7 : 3), obtaining the target (*E*)-stilbene.

Compound 1. According to General Procedure **A**, the desired stilbene **1** (250 mg, 1.27 mmol, white solid, isolated yield: 56 %) was obtained after 24 h from phenylacetic acid (803 mg, 5.90 mmol), *p*-hydroxybenzaldehyde (300 mg, 2.46 mmol) and piperidine (608 µl, 6.15 mmol). **^{1}H-NMR** (400 MHz; [D_1]$CDCl_3$): δ 7.52-7.50 (m, 2H), 7.45-7.42 (AA'BB' system, 2H), 7.39-7.35 (m, 2H), 7.28-7.24 (m, 1H), 7.07 (d, J = 16.3 Hz, 1H), 6.99 (d, J = 16.3 Hz, 1H), 6.87-6.84 (AA'BB' system, 2H), 4.81 (s, 1H); **^{13}C-NMR** (101 MHz; [D_1]$CDCl_3$): 155.2, 137.6, 130.4, 128.6, 128.1, 127.9, 127.2, 126.7, 126.3, 115.6; **MS**, *m/z* ESI =194.9 $[M-H]^-$. Data in agreements with those reported in literature.[45]

Compound 2. According to General Procedure **A**, the desired stilbene **2** (113 mg, 0.54 mmol, white solid, isolated yield: 44 %) was obtained after 48 h from 4-methyl phenylacetic acid (443 mg, 2.95 mmol), *p*-hydroxybenzaldehyde (150 mg, 1.23 mmol) and piperidine (304 µl, 3.07 mmol). **^{1}H-NMR** (400 MHz; [D_4]MeOH, r.t.): δ 7.40-7.37 (m, 4H), 7.18-7.12 (AA'BB' system, 2H), 7.03 (d, J = 16.4 Hz, 1H), 6.94 (d, J = 16.4 Hz, 1H), 6.80-6.77 (AA'BB' system, 2H), 2.34 (s, 3H). **^{13}C-NMR** (101 MHz; [D_4]MeOH, r.t.): δ 156.8, 136.5, 135.1, 129.3, 128.8, 127.27, 127.19, 125.7, 125.3, 115.1, 19.8; **MS**, *m/z* ESI

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=225.0 [M-H]$^-$. Data in agreements with those reported in literature.[45]

Compound 3. According to General Procedure **A**, the desired stilbene **3** (58 mg, 0.26 mmol, white solid, isolated yield: 64 %) was obtained after 44 h from 4-cyano phenylacetic acid (148 mg, 0.98 mmol), *p*-hydroxybenzaldehyde (50 mg, 0.41 mmol) and piperidine (101 μl, 1.02 mmol). **^{1}H-NMR** (400 MHz; [D$_4$]MeOH, r.t.): δ 7.71-7.65 (m, 4H), 7.48-7.45 (AA'BB' system, 2H), 7.29 (d, J = 16.4 Hz, 1H), 7.04 (d, J = 16.3 Hz, 1H), 6.83-6.81 (AA'BB' system, 2H). **^{13}C-NMR** (101 MHz; [D$_6$]DMSO, r.t.): δ 158.6, 143.0, 132.98, 132.82, 129.0, 127.9, 127.1, 123.9, 119.6, 116.1, 109.1; **MS**, *m/z* ESI =244.0 [M+Na]$^+$. Data in agreements with those reported in literature.[46]

Compound 4. According to General Procedure **A**, the desired stilbene **4** (0.63 g, 2.61 mmol, yellow solid, isolated yield: 64 %) was obtained after 18 h from 4-nitro phenylacetic acid (2.27 g, 12.52 mmol), *p*-hydroxybenzaldehyde (0.50 g, 4.09 mmol) and piperidine (1.01 ml, 10.24 mmol). **^{1}H-NMR** (400 MHz; [D$_4$]MeOH, r.t.): δ 8.22-8.19 (AA'BB' system, 2H), 7.74-7.71 (AA'BB' system, 2H), 7.49-7.47 (AA'BB' system, 2H), 7.34 (d, J = 16.4 Hz, 1H), 7.10 (d, J = 16.4 Hz, 1H), 6.84-6.81 (AA'BB' system, 2H); **^{13}C-NMR** (101 MHz; [D$_4$]MeOH, r.t.): δ 158.1, 146.2, 144.9, 133.2, 128.28, 128.10, 126.2, 123.6, 122.90, 122.88, 122.86, 115.3; **MS**, *m/z* ESI =264.0 [M+Na]$^+$. Data in agreements with the one reported in literature.[47]

Compound 5. According to General Procedure **A**, the desired stilbene **5** (140 mg, 0.62 mmol, oil, isolated yield: 38 %) was obtained after 48 h from 4-hydroxy phenylacetic acid (654 mg, 3.93 mmol), *p*-hydroxybenzaldehyde (200 mg, 1.64 mmol) and piperidine (406 μl, 4.10 mmol). **^{1}H-NMR** (500 MHz; [D$_6$]DMSO, r.t.): δ 7.48 (AA'BB' system, 2H), 7.38 (AA'BB' system, 2H), 7.01-6.92 (m, 4H), 6.76 (AA'BB' system, 2H), 3.77 (s, 3H); **^{13}C-NMR** (101 MHz; [D$_6$]DMSO, r.t.): 159.0, 157.4, 130.7, 128.9, 127.9, 127.7, 126.7, 125.3, 116.0, 114.6, 55.6; **MS**, *m/z* ESI =225.0 [M-H]$^-$. Data in agreements with those reported in literature.[48]

Compound 6. According to General Procedure **A**, the desired stilbene **6** (147 mg, 0.7 mmol, white solid, isolated yield: 28 %) was obtained after 24 h from 3-methyl phenylacetic acid (886 mg, 5.90 mmol), *p*-hydroxybenzaldehyde (300 mg, 2.46 mmol) and piperidine (608 μl, 6.15 mmol). **^{1}H-NMR** (400 MHz; [D$_4$]MeOH, r.t.): δ 7.40-7.38 (AA'BB' system, 2H), 7.32-7.29 (m, 2H), 7.20 (t, J = 7.6 Hz, 1H), 7.07 (d, J = 16.4 Hz, 1H), 7.04-7.02 (m, 1H), 6.94 (d, J = 16.4 Hz, 1H), 6.80-6.78 (m, 2H), 2.36 (s, 3H); **^{13}C-NMR** (101 MHz; [D$_4$]MeOH, r.t.): δ 156.9, 137.8, 129.1, 128.08, 127.94, 127.38, 127.32, 127.29, 126.4, 125.5, 122.9, 115.1, 20.0; **MS**, *m/z* ESI =208.9 [M-H]$^-$. Data in agreements with those reported in literature.[49]

Compound 7. According to General Procedure **A**, the desired stilbene **7** (52 mg, 0.49 mmol, white solid, isolated yield: 60 %) was obtained after 24 h from 3-cyano phenylacetic acid (317 mg, 1.97 mmol), *p*-hydroxybenzaldehyde (100 mg, 0.82 mmol) and piperidine (204 μl, 2.05 mmol). **^{1}H-NMR** (400 MHz; [D$_6$]DMSO, r.t.): δ 9.66 (s, 1H), 8.01 (s, 1H), 7.85 (d, J = 8.0 Hz, 1H), 7.64 (d, J = 7.7 Hz, 1H), 7.54 (t, J = 7.8 Hz, 1H), 7.45 (AA'BB' system, J = 8.6 Hz, 2H), 7.32 (d, J = 16.5 Hz, 1H), 7.05 (d, J = 16.5 Hz, 1H), 6.80 (AA'BB' system, J = 8.6 Hz, 2H); **^{13}C-NMR** (101 MHz; [D$_6$]DMSO, r.t.): δ 158.3, 139.5, 131.5, 131.0, 130.5, 130.3, 129.7, 128.7, 128.0, 123.3, 119.3, 116.1, 112.3; **MS**, *m/z* ESI = 220.0[M-H]$^-$. Data in agreements with those reported in literature.[50]

Compound 8. According to General Procedure **A**, the desired stilbene **8** (113 mg, 0.26 mmol, white solid, isolated yield: 57 %) was obtained after 48 h from 3-methylthio phenylacetic acid (358 mg, 1.97 mmol), *p*-hydroxybenzaldehyde (100 mg, 0.82 mmol) and piperidine (204 μl, 2.05 mmol). **^{1}H-NMR** (400 MHz; [D$_1$]CHCl$_3$, r.t.): δ 7.43-7.39 (m, 3H), 7.31 (dt, J = 7.6 Hz, 1.6 1H), 7.25 (t, J = 7.2, 1H), 7.13 (dt, J = 7.6, 1.6 Hz, 1H), 7.10 (d, J = 16.0 Hz, 1H), 6.95 (d, J = 16.3 Hz, 1H), 6.81-6.78 (AA'BB' system, 2H), 2.51 (s, 3H); **^{13}C-NMR** (101 MHz; [D$_1$]CHCl$_3$, r.t.): δ 156.8, 136.5, 135.1, 129.3, 128.8, 127.27, 127.19, 125.7, 125.3, 115.1, 19.8; **HRMS (EI)**, calcd for C15H15OS(M$^-$): 241.0687, found: 241.0684; **MS***m/z* ESI **=** 240.9 Da [M-H]$^-$.

Compound 9. According to General Procedure **A**, the desired stilbene **9** (55 mg, 0.22 mmol, oil, isolated yield: 28 %) was obtained after 48 h from 3,5-dimethoxy phenylacetic acid (386 mg, 1.97 mmol), *p*-hydroxybenzaldehyde (100 mg, 0.82 mmol) and piperidine (204 μl, 2.05 mmol). **^{1}H-NMR** (400 MHz; [D$_1$]CHCl$_3$, r.t.): δ 7.44-7.40 (AA'BB' system, 2H), 7.05 (d, J = 16.2 Hz, 1H), 6.92 (d, J = 16.2 Hz, 1H), 6.87-6.84 (AA'BB' system, 2H), 6.68-6.67 (m, J = 2.2 Hz, 2H), 6.42 (t, J = 2.2 Hz, 1H), 3.86 (s, 6H); **^{13}C-NMR** (101 MHz; [D$_1$]CHCl$_3$, r.t.): 160.9, 155.4, 139.7, 130.1, 128.8, 128.0, 126.6, 115.7, 104.5, 99.7, 77.4, 77.0, 76.7, 55.4; **MS**, *m/z* ESI = **255.0**[M-H]$^-$. Data in agreements with those reported in literature. [51]

Compound 10. According to General Procedure **A**, the desired stilbene **10** (72 mg, 0.32 mmol, white solid, isolated yield: 39 %) was obtained after 48 h from 3,5-dimethyl phenylacetic acid (323 mg, 1.97 mmol), *p*-hydroxybenzaldehyde (100 mg, 0.82 mmol) and piperidine (204 μl, 2.05 mmol). **^{1}H-NMR** (400 MHz; [D$_1$]CHCl$_3$, r.t.): δ 7.39-7.37 (AA'BB' system, 2H), 7.12 (bs, 2H), 7.04 (d, J = 16.3 Hz, 1H), 6.91 (d, J = 16.4 Hz, 1H), 6.87 (bs, 1H), 6.80-6.77 (AA'BB' system, 2H), 2.17 (s, 6H). **^{13}C NMR** (101 MHz; [D$_4$]-MeOH, r.t.), δ 156.9, 137.76, 137.63, 129.2, 128.2, 127.7, 127.3, 125.6, 123.6, 115.1, 20.0; **HRMS (EI)**, calcd for C16H15O(M$^-$): 223.1123, found: 223.1125; **MS**, *m/z* ESI = 222.9 [M-H]$^-$.

General Procedure B: Laccase-mediated oxidation of substituted stilbenes

A solution of laccase (*ca* 40 U mmol$_{subtrate}^{-1}$) from *T. versicolor* (1 mg mL^{-1} in 50 mM acetate buffer pH 4.5, average activity 4 U mL^{-1}) was added to a solution of (*E*)-stilbenes (78 mM) dissolved in a 1:1 mixture of acetone and acetate buffer (pH 4.5, 50 mM). The resulting mixture was shaken (160 rpm) at 27 °C and monitored by TLC (CHCl$_3$: acetone, 9 : 1) till the complete disappearance of the starting stilbene substrate. After 24 h, the reaction was quenched by extraction with AcOEt, the organic solvent evaporated *in vacuo* and the crude residue purified by flash chromatography (ETP :AcOEt, 7 : 3) to give a racemic mixture of 2,3-*trans*-dihydrobenzofurans.

Compound 12. According to General Procedure **B**, the desired (±)-2,3-*trans*-dihybenzofuran **12** (62 mg, 0.16 mmol, white solid, isolated yield: 76 %) was obtained from compound **1** (83 mg, 0.41 mmol) and laccase (17.7 U). **^{1}H-NMR** (400 MHz; [D$_1$]CHCl$_3$, r.t.): δ 7.47-7.45 (m, 2H), 7.40-7.32 (m, 6H), 7.26-7.21 (m, 6H), 7.06 (d, *J* = 16.3 Hz, 1H), 6.97-6.94 (d, *J* = 8.0 Hz, 1H), 6.93 (d, *J* = 16.3 Hz, 1H), 6.86-6.83 (m, 2H), 5.53 (d, *J* = 8.5 Hz, 1H), 4.87 (s, 1H), 4.59 (d, *J* = 8.4 Hz, 1H).**^{13}C-NMR** (101 MHz; [D$_1$]CHCl$_3$, r.t.): δ 159.6, 155.6,

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141.5, 137.7, 132.7, 131.0, 128.9, 128.6, 128.5, 128.4, 127.9, 127.6, 127.3, 127.1, 126.3, 126.2, 122.9, 115.5, 109.7, 93.3, 57.6. **MS**, *m/z* ESI = 389.0 [M-H]$^-$. Data in agreements with those reported in literature.[52]

Compound 13. According to General Procedure **B**, the desired (±)-2,3-*trans*-dihybenzofuran **13** (60 mg, 0.14 mmol, white solid, isolated yield: 60 %) was obtained from compound **2** (95 mg, 0.45 mmol) and laccase (19.0 U). **^{1}H-NMR** (400 MHz; [D_1]$CHCl_3$, r.t.): δ 7.39-7.34 (m, 2H), 7.26-7.22 (AA'BB' system, 2H), 7.19-7.10 (m, 6H), 7.00 (d, J = 16.3 Hz, 1H), 6.94 (d, J = 8.3 Hz, 1H), 6.89 (d, J = 16.3 Hz, 1H), 6.86-6.82 (AA'BB' system, 2H), 5.49 (d, J = 8.6 Hz, 1H), 4.85 (s, 1H), 4.55 (d, J = 8.5 Hz, 1H), 2.39 (s, 3H), 2.36 (s, 3H). **^{13}C-NMR** (101 MHz; [D_1]$CHCl_3$, r.t.): δ 159.5, 155.5, 138.5, 136.9, 136.9, 134.9, 132.8, 131.2, 129.6, 129.3, 128.3, 127.6, 127.6, 127.5, 126.2, 126.1, 122.8, 115.5, 109.6, 93.4, 57.3, 21.2, 21.1; **HRMS (EI)**, calcd for C30H25O2(M^-): 417.1855, found: 417.1858; **MS**, *m/z* ESI =417.0 [M-H]$^-$.

Compound 14. According to General Procedure **B**, the desired (±)-2,3-*trans*-dihybenzofuran **14** (37 mg, 0.08 mmol, white solid, isolated yield: 74 %) was obtained from compound **3** (50 mg, 0.27 mmol) and laccase (9.5 U). **^{1}H-NMR** (400 MHz; [D_1]$CHCl_3$, r.t.): δ 7.69-7.67 (AA'BB' system, 2H), 7.63-7.61 (AA'BB' system, 2H), 7.53-7.51 (AA'BB' system, 2H), 7.46 (dd, J = 8.4, 1.5 Hz, 1H), 7.35-7.33 (AA'BB' system, 2H), 7.22-7.20 (AA'BB' system, 2H), 7.17-7.12 (m, 2H), 7.00 (d, J = 8.3 Hz, 1H), 6.92-6.86 (m, 3H), 5.48 (d, J = 8.3 Hz, 1H), 5.17 (s, 1H), 4.66 (d, J = 8.3 Hz, 1H). **^{13}C-NMR** (101 MHz; [D_1]$CHCl_3$, r.t.): ▯ 160.4, 156.2, 156.1, 146.7, 142.0, 132.8, 132.5, 131.8, 131.6, 130.4, 130.0, 129.1, 129.1, 127.6, 126.5, 124.7, 124.7, 123.2, 119.0, 118.5, 115.7, 115.7, 111.5, 110.3, 110.3, 110.1, 93.1, 57.7; **HRMS (EI)**, calcd for C30H19N2O2 (M^-): 439.1446, found: 439.1444; **MS**, *m/z* ESI = 463.2 [M+Na]$^+$.

Compound 15. According to General Procedure **B**, the desired (±)-2,3-*trans*-dihybenzofuran **15** (68 mg, 0.14 mmol, white solid, isolated yield: 68 %) was obtained from compound **4** (100 mg, 0.45 mmol) and laccase (17.4 U). **^{1}H-NMR** (400 MHz; [D_4]MeOH, r.t.): δ 8.24-8.22 (AA'BB' system, 2H), 8.17-8.15 (AA'BB' system, 2H), 7.68-7.66 (AA'BB' system, 2H), 7.54 (dd, J = 8.3, 1.8 Hz, 1H), 7.46-7.44 (AA'BB' system, 2H), 7.32 (d, J = 16.3 Hz, 1H), 7.27 (bs, 1H), 7.19-7.17 (AA'BB' system, 2H), 7.06 (d, J = 16.4 Hz, 1H), 6.96 (d, J = 8.3 Hz, 1H), 6.81-6.79 (AA'BB' system, 2H), 5.52 (d, J = 8.4 Hz, 1H), 4.79 (d, J = 8.3 Hz, 1H). **^{13}C-NMR** (101 MHz; [D_4]MeOH, r.t.): δ 208.6, 160.5, 157.7, 149.1, 147.3, 146.3, 144.5, 132.7, 130.6, 130.5, 130.3, 129.1, 128.9, 127.3, 126.4, 123.8, 123.6, 123.5, 123.2, 115.1, 109.6, 99.9, 93.3, 56.9; **HRMS (EI)**, calcd for C28H19N2O6 (M^-): 479.1243, found: 479.1244; **MS**, *m/z* ESI = 479.0 [M-H]$^-$.

Compound 16. According to General Procedure **B**, the desired (±)-2,3-*trans*-dihybenzofuran **16** (33 mg, 0.07 mmol, white solid, isolated yield: 55 %) was obtained from compound **5** (60 mg, 0.27 mmol) and laccase (9.3 U). **^{1}H-NMR** (400 MHz; [D_1]$CHCl_3$, r.t.): δ 7.41-7.39 (AA'BB' system, 2H), 7.36 (dd, J = 8.3, 1.0 Hz, 1H), 7.29-7.26 (m, 3H), 7.18-7.17 (m, 3H), 6.94-6.88 (m, 7H), 6.69-6.20 (AA'BB' system, J = 6.9 Hz, 2H), 5.46 (d, J = 8.7 Hz, 1H), 4.54 (d, J = 8.7 Hz, 1H), 3.84 (s, 3H), 3.83 (s, 3H). **^{13}C-NMR** (101 MHz; [D_1]$CHCl_3$, r.t.): δ 159.3, 159.9, 158.8, 155.6, 133.4, 132.6, 131.3, 131.6, 130.5, 129.4, 127.6, 127.5, 127.3, 126.5, 125.9, 122.6, 115.5, 114.3, 114.1, 109.6, 93.5, 56.9, 55.3, 55.3. **MS**, *m/z* ESI = 449.0 [M-H]$^-$. Data in agreements with those reported in literature. [52]

Compound 17. According to General Procedure **B**, the desired (±)-2,3-*trans*-dihybenzofuran 1**7** (66 mg, 0.16 mmol, white solid, isolated yield: 66 %) was obtained from compound **6** (100 mg, 0.45 mmol) and laccase (20.0 U). **^{1}H-NMR** (400 MHz; [D_1]$CHCl_3$, r.t.): δ 7.40 (dd, J = 8.3, 1.8 Hz, 1H), 7.26-7.19 (m, J = 4.2 Hz, 6H), 7.14 (bd, J = 7.6 Hz, 1H), 7.06-7.00 (m, 4H), 6.95 (d, J = 8.3 Hz, 1H), 6.90 (d, J = 16.3 Hz, 1H), 6.87-6.82 (AA'BB' system, 2H), 5.53 (d, J = 8.4 Hz, 1H), 4.82 (s, 1H), 4.54 (d, J = 8.4 Hz, 1H), 2.37 (s, 3H), 2.36 (s, 3H). **^{13}C-NMR** (400 MHz; [D_1]$CHCl_3$, r.t.): ▯ 159.6, 155.6, 141.5, 138.6, 138.1, 137.8, 132.9, 131.2, 131.1, 128.9, 128.7, 128.5, 128.3, 128.1, 127.9, 127.7, 127.6, 126.8, 126.4, 125.5, 123.4, 122.9, 115.6, 109.6, 97.3, 57.6, 30.9, 214.5, 21.4. **MS**, *m/z* ESI = 417.0 [M-H]$^-$. Data in agreements with those reported in literature.[53]

Compound 18. According to General Procedure **B**, the desired (±)-2,3-*trans*-dihybenzofuran **18** (23 mg, 0.06 mmol, white solid, isolated yield: 49 %) was obtained from compound **7** (50 mg, 0.23 mmol) and laccase (9.5 U). **^{1}H-NMR** (400 MHz; [D_1]$CHCl_3$, r.t.): δ 7.73-7.70 (bt, J = 1 Hz, 1H), 7.67 (dt, J = 8.0, 1.2 Hz, 1H); 7.64 (dt, J = 7.2, 1.2 Hz, 1H) 7.52-7.42 (m, 6H), 7.23-7.21 (AA'BB' system, 2H), 7.16 (s, 1H). 7.10 (d, J = 16.3 Hz, 1H), 7.00 (d, J = 8.3 Hz, 1H), 6.90-6.86 (m, 3H), 5.47 (d, J = 8.2 Hz, 1H), 4.64 (d, J = 8.2 Hz, 1H). **^{13}C-NMR** (101 MHz; [D_1]$CHCl_3$, r.t.): δ 160.2, 156.1, 143.0, 138.8, 132.8, 131.8, 131.6, 131.2, 130.8, 130.5, 130.3, 130.2, 129.8, 129.6, 129.4, 128.8, 127.5, 124.2, 123.1, 118.8, 118.5, 115.8, 113.2, 112.9, 110.3, 93.1, 57.2; **HRMS (EI)**, calcd for C30H19N2O2 (M^-): 439.1447, found: 439.1445; **MS**, *m/z* ESI = 463.2 [M+Na]$^+$.

Compound 19. According to General Procedure **B**, the desired (±)-2,3-*trans*-dihybenzofuran **19** (23 mg, 0.05 mmol, white solid, isolated yield: 46 %) was obtained from compound **8** (50 mg, 0.21 mmol) and laccase (8.7 U). **^{1}H-NMR** (400 MHz; [D_1]$CHCl_3$, r.t.): δ 7.40 (dd, J = 8.3, 1.7 Hz, 1H), 7.35 (bs, 1H), 7.32-7.19 (m, 7H), 7.14 (dt, J = 6.7, 2.0 Hz, 1H), 7.10 (t, J = 1.7 Hz, 1H), 7.05 (d, J = 16.3 Hz, 1H), 6.99 (dt, J = 7.6, 1.3 Hz, 1H), 6.96 (d, J = 8.3 Hz, 1H), 6.88 (d, J = 16.3 Hz, 1H), 6.86-6.83 (AA'BB' system, 2H), 5.52 (d, J = 8.4 Hz, 1H), 4.54 (d, J = 8.4 Hz, 1H), 2.52 (s, 3H), 2.48 (s, 3H). **^{13}C-NMR** (101 MHz; [D_1]$CHCl_3$, r.t.): δ 159.7, 155.7, 142.2, 139.3, 138.7, 138.3, 132.6, 130.90, 130.74, 129.4, 129.06, 128.99, 128.1, 127.6, 126.3, 125.8, 125.39, 125.24, 125.04, 124.4, 123.06, 123.02, 115.5, 109.8, 93.1, 57.6, 15.9, 15.7; **HRMS (EI)**, calcd for C30H25O2S2 (M^-): 481.1296, found: 481.1298; **MS**, *m/z* ESI = 481.1 [M-H]$^-$.

Compound 20. According to General Procedure **B**, the desired (±)-2,3-*trans*-dihybenzofuran **20** (29 mg, 0.06 mmol, white solid, isolated yield: 58 %) was obtained from compound **9** (50 mg, 0.20 mmol) and laccase (8.2 U). **^{1}H-NMR** (400 MHz; [D_1]$CHCl_3$, r.t.): δ 7.39 (dd, J = 8.3, 1.7 Hz, 1H), 7.26-7.22 (m, 3H), 7.03 (d, J = 16.2 Hz, 1H), 6.94 (d, J = 8.3 Hz, 1H), 6.86 (d, J = 16.2 Hz, 1H), 6.86-6.82 (AA'BB' system, 2H), 6.64 (d, J = 2.2 Hz, 2H), 6.43 (t, J = 2.3 Hz, 1H), 6.39 (t, J = 2.2 Hz, 1H), 6.37 (d, J = 2.3 Hz, 2H), 5.54 (d, J = 8.3 Hz, 1H), 4.50 (d, J = 8.3 Hz, 1H), 3.83 (s, 6H), 3.78 (s, 6H). **^{13}C-NMR** (101 MHz; [D_1]$CHCl_3$, r.t.): δ 161.1, 160.9, 159.7, 155.8, 143.9, 139.7, 132.7, 130.8, 130.7, 129.0, 128.0, 127.6, 126.3, 123.1, 115.5, 109.7, 106.5, 104.3, 99.7, 99.1, 93.0, 57.8, 55.3, 55.3. **MS**, *m/z* ESI = 509.3 [M-H]$^-$. Data in agreements with those reported in literature.[52]

Compound 21. According to General Procedure **B**, the desired (±)-2,3-*trans*-dihybenzofuran **21** (34 mg, 0.08 mmol, white solid, isolated yield: 58 %) was obtained from compound **10** (50 mg, 0.22 mmol), and laccases (9.4 U). **^{1}H-NMR** (400 MHz; [D_1]$CHCl_3$, r.t.): δ

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7.39 (dd, J = 8.3, 1.7 Hz, 1H), 7.27-7.23 (AA'BB' system, 2H), 7.18 (bs, 1H), 7.09 (bs, 2H), 7.03 (d, J = 16.3 Hz, 1H), 6.96-6.89 (m, 4H), 6.85-6.83 (m, 4H), 5.54 (d, J = 8.2 Hz, 1H), 4.50 (d, J = 8.2 Hz, 1H), 2.33 (s, 6H), 2.32 (s, 6H). **^{13}C-NMR** (101 MHz; [D$_1$]CHCl$_3$, r.t.): δ 159.8, 158.5, 142.6, 139.4, 138.5, 137.9, 133.8, 132.3, 132.1, 129.9, 128.7, 128.3, 127.6, 127.5, 126.6, 126.2, 124.4, 123.5, 115.7, 109.7, 94.2, 58.5, 45.7, 21.4, 21.2; **HRMS (EI)**, calcd for C32H29O2 (M$^-$): 445.2167, found: 445.2169; **MS**, *m/z* ESI =445.0 [M-H]$^-$.

Compound 22. According to General Procedure **B**, the desired (±)-2,3-*trans*-dihybenzofuran **22** (17 mg, 0.04 mmol, white solid, isolated yield: 33 %) was obtained from compound **11** (50 mg, 0.22 mmol), and laccases (9.2 U). **^{1}H-NMR** (400 MHz; [D$_4$]MeOH, r.t.): δ 7.39 (dd, J = 8.4, 1.4 Hz, 1H), 7.20-7.18 (m, 3H), 6.99 (d, J = 16.3 Hz, 1H), 6.87 (d, J = 8.3 Hz, 1H), 6.82-6.79 (m, 3H), 6.46 (bd, J = 2.2 Hz, 2H), 6.22 (td, J = 2.2, 1.0 Hz, 1H), 6.18 (td, J = 2.1, 1.0 Hz, 1H), 6.16 (d, J = 2.2 Hz, 2H), 5.42 (d, J = 8.3 Hz, 1H), 4.42 (d, J = 8.4 Hz, 1H). **^{13}C-NMR** (101 MHz; [D$_4$]MeOH, r.t.): δ 161.30, 160.44, 160.22, 159.10, 145.91, 141.46, 133.28, 132.81, 132.43, 129.80, 129.29, 129.23, 127.92, 124.62, 116.85, 110.81, 108.11, 106.42, 103.43, 103.08, 94.72, 58.52. **MS**, *m/z* ESI = 453.5 [M-H]$^-$. Data in agreements with those reported in literature.[33]

Structural Modification of the synthesized dimers

Reduction of compound 15

Compound **15** (0.14 M) and DIPEA (20 equiv.) were dissolved in CH_2Cl_2 under magnetic stirring and nitrogen atmosphere. A solution of $HSiCl_3$ (7.1 equiv.) in dry CH_2Cl_2 (1.25 M) was prepared apart and added drop-wise to the first solution over 10 minutes at 0 °C, following the reaction with TLC analysis. As the reduction was not completed after 22 h, 10 equivalents of DIPEA and 3.5 equivalents of $HSiCl_3$ (1.25 M in dry CH_2Cl_2) were added to the reacting mixture, which was stirred for additional 24 h. After that, a saturated solution of $NaHCO_3$ was added drop-wise and the biphasic mixture allowed to stir for 30 min. The crude mixture was extracted with ethyl acetate, dried over Na_2SO_4, filtered and then the solvent was removed under reduced pressure to afford the crude product. The crude amine was then purified by flash chromatography ($CHCl_3$: acetone, 9.75 : 0.25) to give the main di-amine product **23** and the two reaction intermediates **24** and **25**.

Compound 23. 13.7 mg, 0.033 mmol, yield: 21%; **^{1}H-NMR** (400 MHz; [D$_4$]MeOH, r.t.): δ 7.47-7.24 (m, 3H), 7.16-6.94 (m, 6H), 6.84-6.70 (m, 8H), 5.36-5.34 (m, 1H), 4.43 (d, J = 8.9 Hz, 1H); **^{13}C-NMR** (101 MHz; [D$_4$]MeOH, r.t.): δ 159.0, 157.2, 145.9, 145.6, 131.68, 131.63, 131.44, 131.32, 128.8, 128.5, 127.3, 126.9, 126.7, 126.0, 124.6, 122.0, 116.0, 115.5, 114.9, 108.9, 93.8, 78.2, 77.8, 77.5, 56.9, 29.3; **HRMS (EI)**, calcd for C28H23N2O2 (M$^-$): 419.1759, found: 419.1760; **MS**, *m/z* ESI = 419.0 [M-H]$^-$.

Compound 24. 1.8 mg, 0.004 mmol, yield: 3%; **^{1}H-NMR** (400 MHz; [D$_1$]CHCl$_3$, r.t.): δ 8.25-8.23 (AA'BB' system, 2H), 7.41-7.38 (m, 3H), 7.30-7.26 (AA'BB' system, J = 8.5 Hz, 2H), 7.24-7.20 (AA'BB' system, J = 8.5 Hz, 2H), 7.10 (bs, 1H), 6.96 (d, J = 8.4 Hz, 1H), 6.87-6.84 (m, 4H), 6.68-6.65 (AA'BB' system, 2H), 5.46 (d, J = 8.4 Hz, 1H), 4.70 (d, J = 8.4 Hz, 1H); **^{13}C-NMR** (101 MHz; [D$_1$]CHCl$_3$, r.t.): δ 1159.0, 156.0, 149.0, 145.9, 132.1, 131.9, 129.5, 129.2, 127.9, 127.61, 127.44, 126.9, 124.6, 124.2, 122.3, 115.7, 115.2, 110.1, 92.8, 57.7, 29.7; **HRMS (EI)**, calcd for C28H21N2O4 (M$^-$): 449.1502, found: 449.1504; **MS**, *m/z* ESI = 449.0 [M-H]$^-$.

Compound 25. 5.6 mg, 0.012 mmol, yield: 8 %; **^{1}H-NMR** (400 MHz; [D$_4$]MeOH, r.t.): δ 8.25-8.19 (AA'BB' system, 2H), 7.57-7.55 (AA'BB' system, 2H), 7.43-7.40 (m, 1H), 7.25-7.19 (m, 4H), 7.01-6.93 (m, 4H), 6.85-6.83 (AA'BB' system, 2H), 6.71-6.69 (AA'BB' system, 2H), 5.48 (d, J = 8.8 Hz, 1H), 4.49 (d, J = 8.8 Hz, 1H); **^{13}C-NMR** (101 MHz; [D$_4$]MeOH, r.t.), δ 160.6, 155.7, 146.4, 145.7, 144.4, 133.2, 130.9, 129.8, 129.3, 128.7, 127.6, 126.4, 124.1, 123.7, 123.4, 115.52, 115.47, 109.9, 93.8, 56.8, 29.7; **HRMS (EI)**, calcd for C28H21N2O4 (M$^-$): 449.1502, found: 449.1501; **MS**, *m/z* ESI = 449.0 [M-H]$^-$.

General procedure C: Alkylation of phenols

Phenolic compound (0.05 M, 1 equiv.) and 2-chloro-*N,N*-dimethylamine hydrochloride (3 equiv.) were suspended in chloroform. Tetrabutylammoniumhydrogensulfate (0.05 equiv.), potassium carbonate (8 equiv.) and water (H_2O : $CHCl_3$ = 2: 100) were then added and the reaction stirred at 50 °C. After that, the mixture was diluted with chloroform, the organic phase washed with water and dried over sodium sulfate. The solvent was evaporated at reduced pressure and the crude residue purified by flash chromatography ($CHCl_3$:MeOH = 9 : 1) to give desired alkylated product.

Compound 26. According to General Procedure **C**, the desired alkylated (±)-2,3-*trans*-dihybenzofuran **26** (12 mg, 0.03 mmol, oil, isolated yield: 35 %) was obtained from compound **11** (21 mg, 0.05 mmol), 2-chloro-*N,N*-dimethylamine hydrochloride (35 mg, 0.16 mmol), TBAHSO$_4$ (9 mg, 0.03 mmol). **^{1}H-NMR** (400 MHz; [D$_1$]CHCl$_3$, r.t.): δ 7.47-7.45 (m, 2H), 7.42-7.32 (m, 6H), 7.30-7.26 (m, 3H), 7.25-7.20 (m, 4H), 7.05 (d, J = 16.3 Hz, 1H), 6.97-6.90 (m, 4H), 5.53 (d, J = 8.4 Hz, 1H), 4.60 (d, J = 8.4 Hz, 1H), 4.14 (t, J = 5.7 Hz, 2H), 2.83 (t, J = 5.6 Hz, 2H), 2.42 (s, 7H). **^{13}C-NMR** (101 MHz; [D$_1$]CHCl$_3$, r.t.): δ 160.3, 159.4, 142.1, 138.3, 133.3, 131.6, 129.5, 129.21, 129.10, 129.00, 128.5, 127.99, 127.93, 127.7, 126.94, 126.78, 123.6, 115.3, 110.3, 94.0, 66.3, 58.6, 58.2, 46.2, 30.30, 30.2; **HRMS (EI)**, calcd for C32H32NO2 (M$^+$): 462.2433, found: 462.2435; **MS**, *m/z* ESI =462.3 [M+H]$^+$.

Compound 27. According to General Procedure **C**, the desired alkylated (±)-2,3-*trans*-dihybenzofuran **27** (19 mg, 0.03 mmol, oil, isolated yield: 60 %) was obtained from compound **21** (27 mg, 0.05 mmol), 2-chloro-*N,N*-dimethylamine hydrochloride (35 mg, 0.24 mmol), TBAHSO$_4$ (9 mg, 0.03). **^{1}H-NMR** (400 MHz; [D$_1$]CHCl$_3$, r.t.): δ 7.39 (dd, J = 8.3, 1.7 Hz, 1H), 7.27-7.23 (AA'BB' system, 2H), 7.18 (bs, 1H), 7.09 (bs, 2H), 7.03 (d, J = 16.3 Hz, 1H), 6.96-6.89 (m, 4H), 68.30-6.86 (m, 4H), 5.54 (d, J = 8.2 Hz, 1H), 4.50 (d, J = 8.2 Hz, 1H), 2.33 (s, 6H), 2.32 (s, 6H). **^{13}C-NMR** (101 MHz; [D$_4$]MeOH, r.t.): δ 161.1, 160.9, 159.7, 158.5, 158.2, 143.9, 139.7, 133.0, 130.81, 130.71, 130.3, 129.0, 128.0, 127.55, 127.36, 126.3, 123.1, 115.7, 114.7, 114.1, 113.8, 109.7, 106.5, 104.3, 99.7, 99.0, 92.9, 65.15, 65.10, 57.8, 57.4, 55.4, 44.96, 44.91, 44.2; **HRMS (EI)**, calcd for C36H40NO6 (M$^+$): 582.2856, found: 582.2858; **MS**, *m/z* ESI = 582.4 [M+H]$^+$.

Compound 28. According to General Procedure **C**, the desired alkylated (±)-2,3-*trans*-dihybenzofuran **28** (22 mg, 0.03 mmol, oil, isolated yield: 28 %) was obtained from compound **22** (30 mg, 0.07 mmol), 2-chloro-*N,N*-dimethylamine hydrochloride (44 mg, 0.30

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mmol), $TBAHSO_4$ (12 mg, 0.04). **^{1}H-NMR** (400 MHz; [D_1]$CHCl_3$, r.t.): δ 7.40-38 (m, 1H), 7.28-7.20 (m, 3H), 7.01 (d, J = 16.5 Hz, 1H), 6.93-6.83 (m, 5H), 6.62 (s, 2H), 6.41-6.35 (m, 3H), 5.53 (d, J = 8.3 Hz, 1H), 4.48 (d, J = 8.5 Hz, 1H), 4.14 (s, 2H), 3.81 (s, 6H), 3.76 (s, 6H), 2.97 (s, 3H), 2.46 (s, 6H). **^{13}C-NMR** (101 MHz; [D_1]$CHCl_3$, r.t.): δ 159.5, 158.7, 141.6, 138.4, 138.0, 137.6, 133.1, 131.3, 131.1, 128.98, 128.92, 128.2, 127.6, 127.3, 126.4, 126.1, 124.1, 123.0, 114.7, 109.6, 93.2, 65.7, 58.0, 57.5, 45.6, 29.7, 21.3, 21.3; **HRMS (EI)**, calcd for C36H40NO2 (M+): 518.3059, found: 518.3057; **MS**, *m/z* ESI **=** 518.2 $[M+H]^+$.

ATPase assay. The heat shock protein (2 μM) was added to a solution prepared in HEPES buffer (20 mM, pH 7.5) containing KCl (100 mM), $MgCl_2$ (1 mM), NADH (0.18 mM), L-lactate dehydrogenase (4 U ml^{-1}), phosphoenolpyruvate (1 mM), pyruvate kinase (2.5 U ml^{-1}) and the desired compound (dissolved in DMSO) to a final concentration of 50 μM). The reaction was initiated by the addition of ATP (1 mM) and absorbance changes at 360 nm, 30 °C, were monitored for 30 min.

HPLC methods: Oxidations of **1** to **11**
- Column = Kinetex C18 HPLC 5µm EVO C18 100Å, 150x4.6 mm
- Flow = 0.3 mL/min; - Loop = 20 μL;
- Detection Lambda = 270 nm;
- Injection = 10 μL
- Gradient of CH_3CN and H_2O (0.1 ppm TFA, pH 3): 0 min, 80% H_2O, 20% CH_3CN; 0–60 min, 15% H_2O, 85% CH_3CN; 61-70 min, 80% H_2O, 20% CH_3CN.

Docking Methods. The MAESTRO suite (www.Schrodinger.com) was used to obtain the three-dimensional structures of compounds **12**, **20**, **21**, **22**, **26**, **27** and **28** to be used for protein-ligand docking calculations. The compounds' structures were first refined by assigning charges and atomic types with the LigPrep module of Maestro using the OPLS2005 force field.[54–56] The Hsp90 homologue from yeast set of conformations to be used as target receptors were extracted from molecular dynamics simulations as described previously.[11,37] Briefly, the trajectory was clustered and the centroids of the ten most representative clusters, which together represent the 90% of all conformations sampled along the simulation, were selected and used as receptor representatives. Thus, for each compound 10 docking experiments were performed, for a total of 70, using the Glide module of MAESTRO in the extended precision (XP) mode.[54–58] The docking grid, which defines the binding pocket, was built and centered around residues 474-487, 502, 503, 591-599, 602, 603, 652-657 of protomer A and residues 502-504, 591-595, 656-662 of protomer B. This pocket has been previously identified and validated as the allosteric binding site by combined experimental and computational approaches.[11,14,37] For each compound, the Dynamic Ligand Efficiency (DLE) was calculated by averaging the LE values calculated for the aforementioned 10 best poses in each of the ten docking runs.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors acknowledge for financial support from AIRC (Grants IG15420 and IG20019), from italian MAE (PERTNET project) and Fondazione Cariplo, grant n. 2014-0568 (INBOX project). We thank Prof. Johannes Buchner, Technical University of Munich, for generously providing us the yeast homologue of Hsp90.

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