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Identification and initial SAR of silybin: An Hsp90 inhibitor

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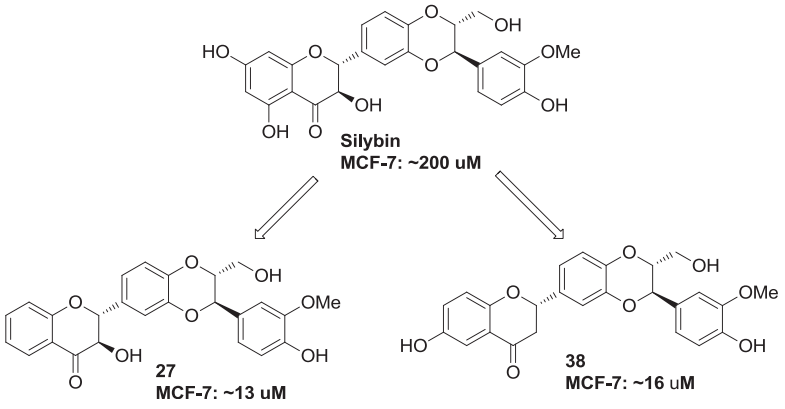
ABSTRACT

Through Hsp90-dependent firefly luciferase refolding and Hsp90-dependent heme-regulated eIF2α kinase (HRI) activation assays, silybin was identified as a novel Hsp90 inhibitor. Subsequently, a library of silybin analogues was designed, synthesized and evaluated. Initial SAR studies identified the essential, non-essential and detrimental functionalities on silybin that contribute to Hsp90 inhibition.



The 90 kDa family of heat shock proteins (Hsp90) is responsible for the conformational maturation of newly synthesized polypeptides and the refolding of denatured proteins into biologically active, three-dimensional structures.[1,2] To date, more than 200 Hsp90-dependent client proteins have been discovered, of which Her2, Src family kinases, Raf, PLK, RIP, Akt, telomerase and Met are directly associated with the six hallmarks of cancer.[3–5] Consequently, inhibition of the Hsp90 folding machinery provides a combinatorial approach towards the disruption of multiple signaling nodes that are critical for malignant cell growth and proliferation. Since the first-in-class drug, 17-AAG (a synthetic derivative of geldanamycin), demonstrated therapeutic benefit at tolerable doses, more than 30 clinical trials have commenced for the treatment of various cancers.[6,7] In contrast, recent studies have demonstrated that Hsp90 is a potential therapeutic target for neurodegenerative diseases, including Alzheimer's, Parkinson's, Prion and Hodgkin's diseases.[8] The potential therapeutic benefits associated with Hsp90 modulation highlight the importance of identifying and optimizing novel Hsp90 inhibitors for the treatment of these diseases.

Silymarin, a flavonolignan extract from the seed of milk thistle (*Silybum marianum*), is native to the Mediterranean regions of Europe, North Africa and the Middle East.[9] It has been used since ancient time for the treatment of liver and gallbladder disorders. In the past three decades, silymarin has been used clinically as an anti-hepatotoxic agent as well as a nutritional supplement to protect the liver from diseases associated with alcohol consumption and exposure to chemical and environmental toxins.[10] Silybin is a mixture of two diastereomers, A and B, in nearly 1:1 ratio, and is the major component of silymarin complex, along with its

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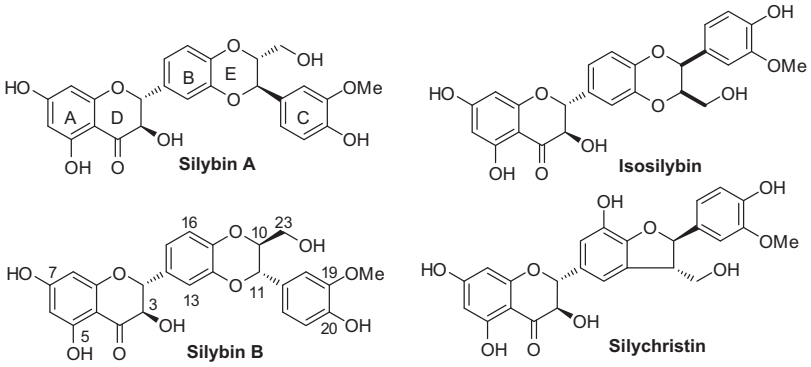


Figure 1. Major components of silymarin.

regio-isomers isosilybin A and B, silydianin, silychristin and isosilychristin[10] (Fig. 1).

Along with the beneficial activities exhibited by silymarin such as hepato-, cardio-, and neuro-protective activities resulting from the anti-oxidant and radical scavenging properties,[10,11] recent studies have demonstrated that silybin exerts cytotoxic activity against cancer cell lines and enhances the efficacy of other chemotherapeutic agents.[10,12] The mechanism for these activities has been investigated at the cellular and molecular levels. For example, in prostate cancer, both silymarin and silybin (50–100 μg/ml) have been shown to inhibit human PC3 cell proliferation, induce cell death, and cause G1 and G2-M cell cycle arrest in a dose-dependent manner.[13,14] G1 arrest is associated with a decrease in cyclin-dependent kinases CDK4, CDK6 and CDK2 protein levels, and CDK2 and CDK4 kinase activity. In addition, silybin inhibits metastatic PC3 cell mobility and adhesion,[15] suggesting that it serves as the active component in silymarin. However, it has also been suggested that other silybin stereoisomers present in silymarin may also contribute to its efficacy.[10] Furthermore, silybin inhibits cell proliferation of colon cancer cell lines by causing cell cycle arrest due to depletion of CDK2.[16] Silybin also inhibits cell growth and induces apoptosis in both small cell and non-small cell human lung carcinoma cells.[17] CDK4, CDK6, and CDK2 are well-studied Hsp90-dependent clients, suggesting that silybin may exhibit Hsp90 inhibitory activity, which may be responsible for the observed anti-cancer activity.

To test this hypothesis, the Hsp90-dependent refolding assay utilizing the rematuration of firefly luciferase was investigated.[18] Analysis showed that silybin inhibited Hsp90-dependent refolding of luciferase in reticulocyte lysate by 50% at a concentration of

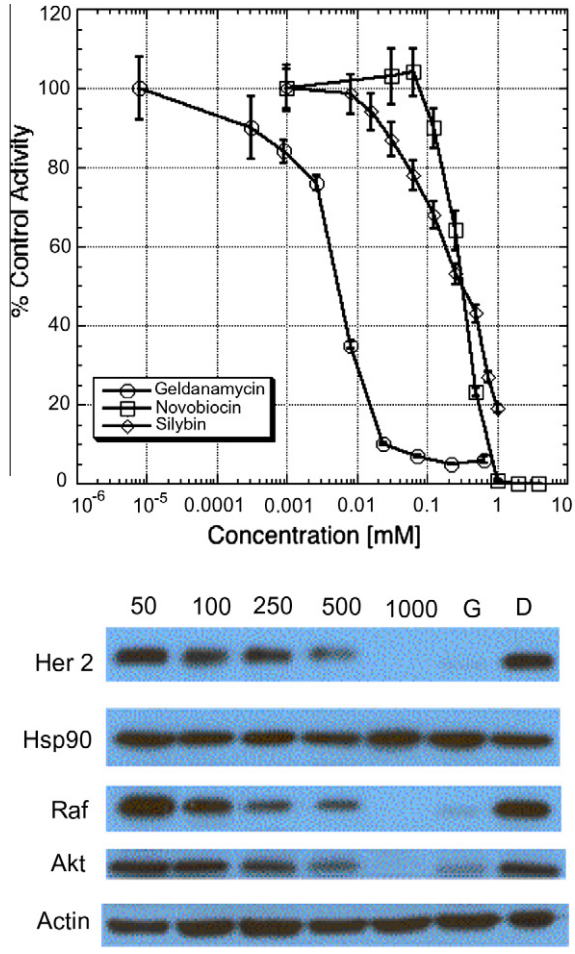


Figure 2. (Top) Effect of geldanamycin, novobiocin and silybin on Hsp90-dependent refolding of heat denatured luciferase in rabbit reticulocyte lysate. Denatured luciferase was incubated in the presence of DMSO (vehicle control) or increasing concentrations (mM) of geldanamycin (open circles), novobiocin (open squares) or silybin (open diamonds) for 30 min at 30 °C. Aliqouts (10 μL) were added to assay buffer and relative light units produced by the refolded luciferase was measured as previously described.[18] Activity is expressed as percent of the DMSO control. Data points correspond to one representative experiment performed in triplicate. (Bottom) Western blot analyses of Hsp90-dependent client proteins from MCF-7 breast cancer cell lysate upon treatment with silybin. Concentrations (in μM) were indicated above each line, and geldanamycin (G, 0.5 μM) and dimethylsulfoxide (D) were employed as positive and negative controls.

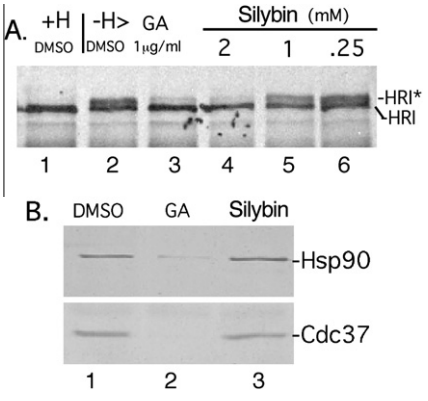


Figure 3. The effect of silybin on HRI maturation/activation, and the interaction of Hsp90 and Cdc37 with HRI in rabbit reticulocyte lysate. (A) Activation of newly synthesized [^{35}S]-labeled His-tagged HRI matured in heme-replete (+H, lane 1) or heme-deficient reticulocyte lysate (−H, lanes 2–6) in the presence of DMSO (lane 2), 1 μg/ml geldanamycin (GA, lane 3) or the indicated concentrations of silybin (lanes 4–6) [−HRI, inactive HRI; −HRI* mature/active HRI]. (B) Effect of DMSO (lane 1), 1 μg/ml geldanamycin (GA, lane 2) or 2 mM silybin (lane 3) on the interaction of Hsp90 and Cdc37 with newly synthesized His-tagged HRI in reticulocyte lysate assayed via anti-His-tag pull down assays and Western blotting.

250 μM, compared to an IC_{50} of 2 and 350 μM for the Hsp90 inhibitors, geldanamycin and novobiocin, respectively. Subsequent evaluation in MCF-7 cells confirmed selective degradation of Hsp90-dependent clients at a concentration that paralleled anti-proliferative activity, linking Hsp90 inhibition to cell viability (Fig. 2).

To further characterize the effect of silybin on the activity of Hsp90 in vitro, we examined the ability of silybin to inhibit Hsp90-dependent activation of heme-regulated eIF2α kinase (HRI).[19–21] When newly synthesized HRI was 'matured' in heme-deficient lysate, silybin inhibited the activation of HRI in a concentration-dependent manner. Silybin at a concentration of 2 mM, inhibited the Hsp90-dependent maturation and activation of HRI to the same extent as 1 μg/mL geldanamycin (Fig. 3A, absence of -HRI*). Western blotting indicated that silybin did not disrupt the interactions between Hsp90 and Cdc37 with HRI (Fig. 3B), suggesting that it inhibits Hsp90 similar to the Hsp90 inhibitor, derrubone.[22] Thus, silybin exhibits properties that are expected for Hsp90-inhibitors, suggesting that other members of this family may also exhibit Hsp90 inhibitory activity.

Although silybin was identified as an Hsp90 inhibitor, it manifests poor anti-proliferative activity against the MCF-7 cell line. Its low bioavailability and poor water solubility may contribute to this low efficacy.[10] Attempts to circumvent these issues have been previously reported, including the esterification,[23] phosphorylation,[24] glycosidation,[25] and oxidation of the C-23 alcohol.[26] Other structural modifications to the phenol and alcohol have also been disclosed to improve efficacy, focusing primarily on the anti-oxidant and radical-scavenging potential.[11,27] Herein, we provide an alternative approach towards improving the anti-proliferative efficacy of silybin through the synthesis of analogues that manifest Hsp90 inhibitory activity. Initial SAR studies focused on the role of individual functionalities on silybin in an effort to identify essential moieties responsible for anti-proliferative activity.

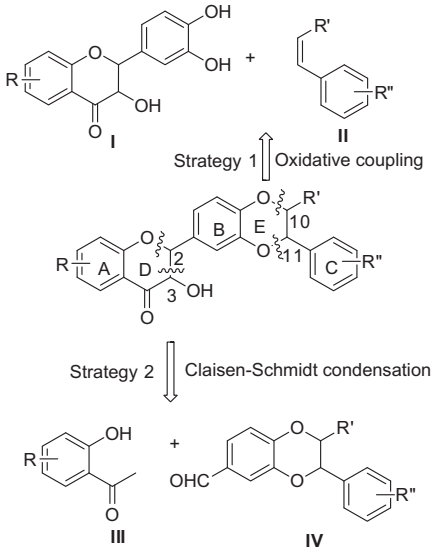
The synthesis of silybin analogues followed two strategies; (1) the biomimetic synthesis of natural silybin through a silver oxide promoted oxidative coupling of a taxifolin analogue (**I**) and a benzylallylic alcohol (**II**) (Scheme 1, Strategy 1);[28] however, this oxidative coupling is not regio-selective, as nearly an equal amount of isosilybin analogues were obtained. The other strategy was to regio-specifically construct the substituted 1,4-benzodioxane (**IV**) (Scheme 1, Strategy 2) as the key intermediate, followed by a Claisen–Schmidt condensation with **III**, and subsequent epoxidation and acid promoted cyclization.[29]

A late stage common intermediate is highly favored for the construction of analogues to generate a diverse library of compounds. Therefore, the latter strategy was employed to investigate substitutions on the A- and D-rings, both of which originate from intermediate **6**. Synthesis of this key intermediate is described in Scheme 2. Demethylation of eugenol (1) with lithium chloride in DMF afforded catechol **2** in modest yield. Likewise, esterification of ferulic acid (**3**) with methanol and thionyl chloride followed by subsequent reduction with diisobutylaluminium hydride in tetrahydrofuran gave allylic alcohol, **4**. Following the procedure of Merlini,[28] the hetero-Diels–Alder reaction between **2** and **4** gave the cyclo-adduct **5**, which upon isomerization and oxidative cleavage afforded key intermediate **6**.

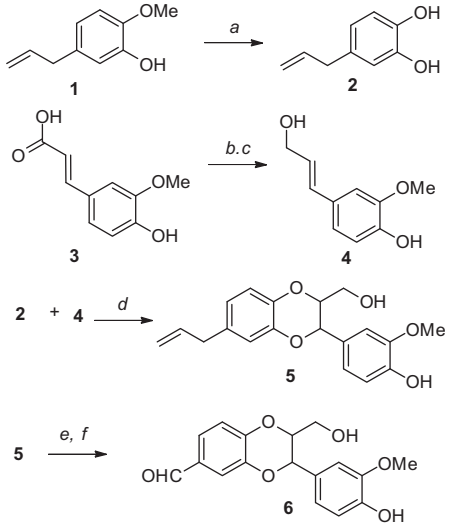
1,4-Benzodioxan **6** and various hydroxy-acetophenones (**8**–**13**) were easily converted to the methoxymethyl ethers (**7** and **14**–**18**) in good yield. Claisen–Schmidt condensation of **7** and acetophenones **14**–**18** were performed in ethanol in the presence of potassium hydroxide to provide chalcones **19**–**23** in good yield. Compounds produced from this reaction gave the *trans* products, as established by ^1H NMR. Alkaline hydrogen peroxide oxidation of compounds **19**–**23** gave the corresponding epoxides, which underwent acid promoted deprotection of the MOM-ether, followed by in situ cyclization to afford silybin analogues **24–28** in modest yields.

To evaluate the effect of the C-3 hydroxyl group on anti-proliferative activity, compound **34,** in which the C-3 hydroxyl group was removed, was synthesized via basic cyclization of chalcone **29**, which was obtained upon acidic deprotection of **19** (Scheme 4). Evaluation of this compound against two breast cancer cell lines indicated that the C-3 hydroxyl group is detrimental to anti-proliferative activity. To validate this observation, compounds **35**–**38**, containing various phenol substitutions on the A-ring, were prepared via the same synthetic sequence.

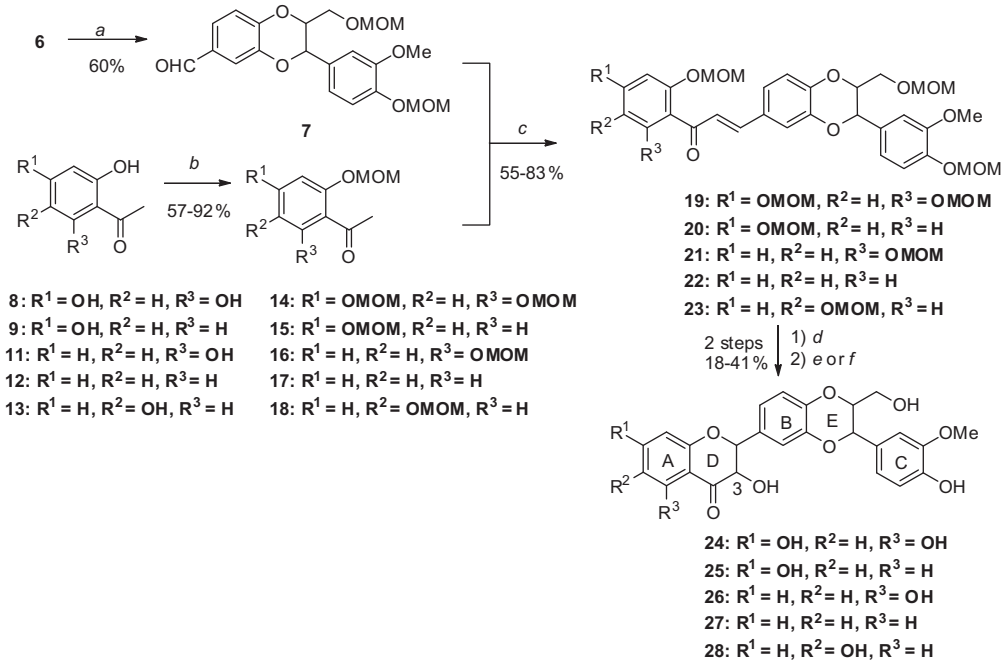
To assess the role of substitutions on the C- and E-rings, Strategy 1 was applied toward the preparation of these analogues. As described in Scheme 5, synthesis of isoeugenol (**39**) was accomplished by catalytic isomerization of eugenol (**1**) with platinum dichloride. Wittig reaction of 3-methoxybenzaldehyde (**40**) with (carbomethoxymethyl)-triphenylphosphonium bromide afforded



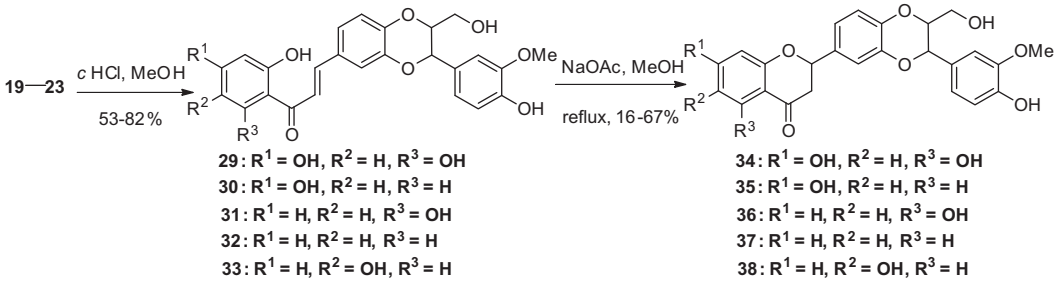
Scheme 1. Retro-synthesis of silybin analogues.



Scheme 2. Synthesis of compound **6**. Reagents and conditions: (a) LiCl, DMF, reflux, 48 h, 43%; (b) $SOCl_2$, MeOH, 0 °C, 12 h, 92%; (c) DIBAL-H, THF, 0 °C, 4 h, 74%; (d) Ag_2O, benzene/acetone, 24 h, 73%; (e) $PtCl_2$, MeOH, 12 h, 94%; (f) OsO_4, $NaIO_4$, dioxane, 8 h, 64%.



Scheme 3. Synthesis of silybin analogues with a modified A ring. Reagents and conditions: (a) NaH, MOMCl, DMF; (b) NaH, MOMCl, THF; (c) KOH, EtOH; (d) 5% NaOH, 50% H_2O_2, MeOH; (e) concd HCl, MeOH, 55 °C; (f) TFA, DCM, 48 h.

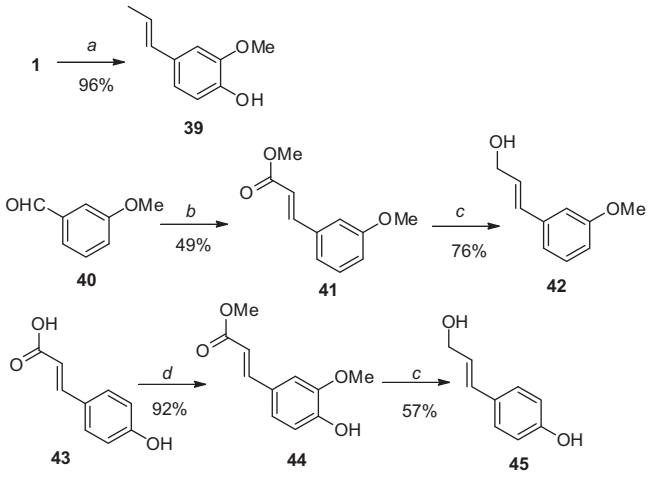


Scheme 4. Synthesis of silybin analogues without the C-3 hydroxy group.

the α,β-unsaturated ester **41**, which was reduced with diisobutylaluminium hydride in tetrahydrofuran to give alcohol **42**. Similarly, compound **45** was obtained from (*E*)-3-(4-hydroxyphenyl)-acrylic acid (**43**) in two steps.

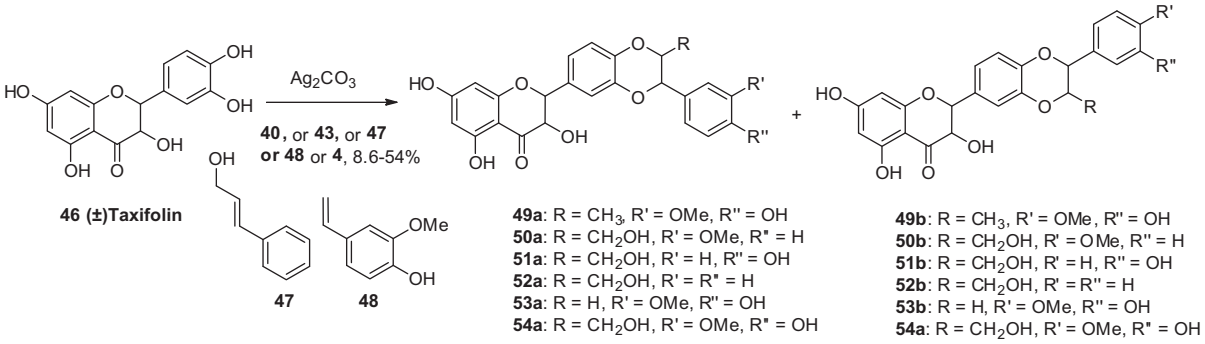
(±)-Taxifolin **46** was synthesized in four steps following the reported procedure.[11] Subsequent coupling of (±)-taxifolin **46** and coniferyl alcohol analogues (**39**, **42, 45, 47**, **48**) afforded silybin analogues **49a–53a**, along with their regio-isomers, **49b**–**53b,** in a 1:1 ratio. Repeated chromatography of the mixture gave **51a**, which was also independently prepared via the procedure described in Scheme 3 to confirm its structure. Separation of other mixtures was not pursued. To compare the anti-proliferative activity between silybin analogues and their corresponding regio-isomers, a mixture of silybin and isosilybin (**54a** and **54b**) was also synthesized, as described in Scheme 6.

Upon construction of the silybin analogues, the compounds were evaluated for anti-proliferative activity against SKBr3 (estrogen receptor negative, HER2 over-expressing breast cancer cells) and MCF-7 (estrogen receptor positive breast cancer cells) cell lines.[30,31] As shown in Table 1, silybin analogues containing modifications to the A- and C-rings exhibited improved anti-proliferative activity compared to silybin. These results indicate that the phenol on the A-ring is not necessary, since compound **27** shows



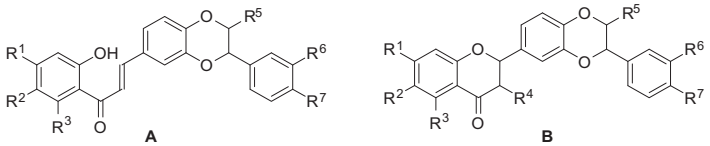
Scheme 5. Synthesis of coniferyl alcohol analogues. Reagents and conditions: (a) $PtCl_2$, MeOH; (b) NaH, (carbomethoxymethyl)-triphenylphosphonium bromide, DCM; (c) DIBAL-H, THF, 0 °C; (d) $SOCl_2$, MeOH.

comparable activity to **25**, **26** and **28**. However, one phenol is tolerated on the A-ring; and the pattern of substitution is not



Scheme 6. Synthesis of silybin analogues with modified C- and E- rings.

Table 1
Anti-proliferative activity of various silybin analogues



		R^1	R^2	R^3	R^4	R^5	R^6	R^7	SKBR3 (μM)	MCF-7 (μM)
Silybin*		OH	H	OH	OH	CH_2OH	OMe	OH	197.0 ± 45.3[a]	222.8 ± 3.6
24	**B**	OH	H	OH	OH	CH_2OH	OMe	OH	196.3 ± 26.0	196.4 ± 28.8
25	**B**	OH	H	H	OH	CH_2OH	OMe	OH	16.04 ± 2.23	11.92 ± 0.64
26	**B**	H	H	OH	OH	CH_2OH	OMe	OH	11.90 ± 2.43	17.66 ± 6.55
27	**B**	H	H	H	OH	CH_2OH	OMe	OH	11.12 ± 1.42	13.42 ± 2.56
28	**B**	H	OH	H	OH	CH_2OH	OMe	OH	13.41 ± 1.32	17.80 ± 7.38
29	**A**	OH	H	OH	OH	CH_2OH	OMe	OH	111.40 ± 10.47	109.3 ± 6.22
30	**A**	OH	H	H	—	CH_2OH	OMe	OH	37.14 ± 0.62	16.91 ± 4.72
31	**A**	H	H	OH	—	CH_2OH	OMe	OH	25.03 ± 1.86	22.58 ± 2.64
32	**A**	H	H	H	—	CH_2OH	OMe	OH	19.87 ± 3.27	12.07 ± 0.21
33	**A**	H	OH	H	—	CH_2OH	OMe	OH	35.84 ± 0.83	12.17 ± 0.38
34	**B**	OH	H	OH	H	CH_2OH	OMe	OH	101.2 ± 2.50	104.2 ± 5.44
35	**B**	OH	H	H	H	CH_2OH	OMe	OH	41.91 ± 2.96	41.58 ± 3.68
36	**B**	OH	H	OH	H	CH_2OH	OMe	OH	47.73 ± 1.51	50.32 ± 2.83
37	**B**	H	H	H	H	CH_2OH	OMe	OH	16.25 ± 0.63	13.66 ± 2.58
38	**B**	H	OH	H	H	CH_2OH	OMe	OH	15.63 ± 4.35	15.62 ± 0.50
49a + 49b	—	OH	H	OH	OH	CH_3	OMe	OH	60.9 ± 2.66	69.8 ± 7.13
50a + 50b	—	OH	H	OH	OH	CH_2OH	OMe	H	>500	140.4 ± 14.4
51a + 51b	—	OH	H	OH	OH	CH_2OH	H	OH	172.0 ± 13.4	151.4 ± 26.0
51a	—	OH	H	OH	OH	CH_2OH	H	OH	210.6 ± 7.5	138.7 ± 33.0
52a + 52b	—	OH	H	OH	OH	CH_2OH	H	H	>500	>500
53a + 53b	—	OH	H	OH	OH	H	OMe	OH	103.3 ± 2.62	101.3 ± 0.92
54a + 54b	—	OH	H	OH	OH	CH_2OH	OMe	OH	233.5 ± 6.15	222.7 ± 2.76

* Purchased from Sigma–Aldrich.
[a] Values represent mean ± standard deviation for at least two separate experiments performed in triplicate.

important. Removal of the C-3 hydroxyl group resulted in an increase in anti-proliferative activity ~2-fold, compared to silybin, suggesting that the C-3 hydroxyl group is not essential. In support of this, removal of C-3 hydroxyl group on compounds **27** and **28** resulted in similar activity (compound **37** and **38**). However, removal of the C-3 hydroxyl group on compounds **25** and **26** resulted in decreased activity (**35** and **36**). Interestingly, the acyclic chalcone intermediates **29**–**33** exhibited comparable anti-proliferative activity to their cyclized counterparts. Although modifications to the C- and E-rings resulted in two inseparable regio-isomers, evaluation of the mixture of **51a** and **51b** gave approximately the same result as that obtained from pure **51a**. This observation was also seen in the case of silybin (**24**) and silybin mixtures (**54a** + **54b**). Consequently, it appears as though the C-23 hydroxyl group is detrimental to anti-proliferative activity (**24** vs **49**), however, removal of the methylene group decreases activity (**53** vs **49**). Only one substitution on the C-ring is required for anti-proliferative activity and the 4'-hydroxyl moiety (**51**) is favored over the 3'-methoxy group (**50**). However, deleting both functional groups significantly diminishes activity (**52**).

Structure–activity relationships produced from this first generation of silybin analogues provide substantial information with regards to structural features necessary for silybin and its exhibition of anti-proliferative activity: the resorcinol structure is detrimental, and any phenol is tolerated on the A-ring. The C-3 hydroxyl group is not essential and the C-23 hydroxyl group is detrimental. Finally, an H-bond donor on the C-ring is more favored than a H-bond acceptor. A summary of these observations is detailed in Figure 4.

To confirm that anti-proliferative activities exhibited by silybin analogues result from Hsp90 inhibition, Western blot analyses of the MCF-7 cell lysate following administration of **27** and **38** were performed.[30,31] Figure 5 shows that the Hsp90-dependent client proteins Her2, Raf, and Akt were degraded in a concentration-dependent manner upon treatment with compound **27** or **38** at concentrations that mirror its anti-proliferative activity, clearly

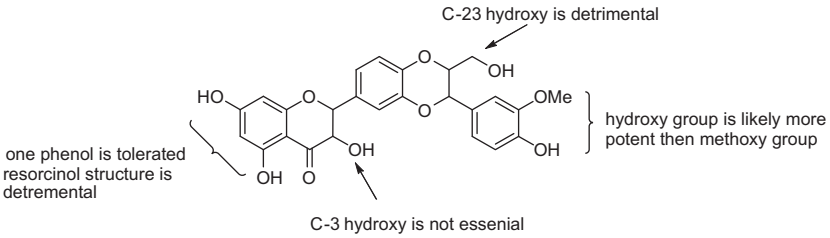


Figure 4. SAR summary of silybin analogues.

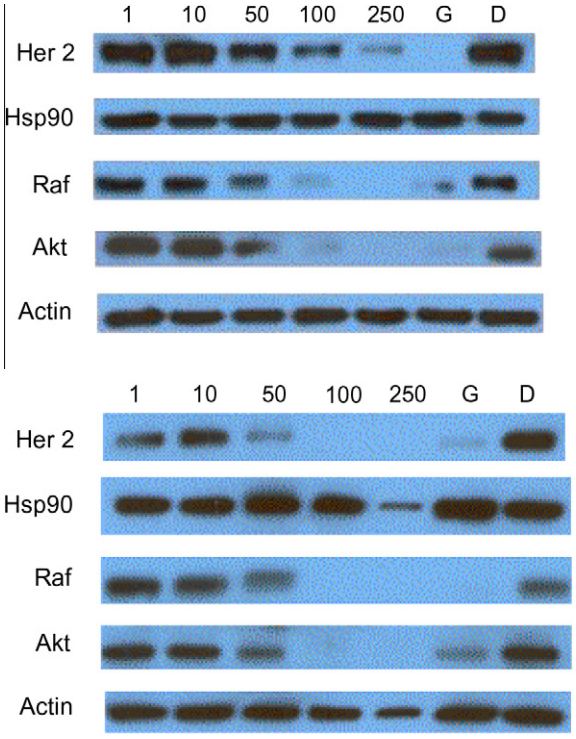


Figure 5. Western blot analyses of MCF-7 cell lysates for Hsp90 client protein degradation after 24 h incubation. Concentrations (in μM) of **27** (Top) and **38** (Bottom) are indicated above each lane. Geldanamycin (G, 500 nM) and DMSO (D) were employed, respectively as positive and negative controls.

linking client protein degradation to cell viability. The non-Hsp90-dependent protein, actin, was not affected upon administration of compound **27** or **38**, indicating that selective degradation of Hsp90-dependent proteins occurs. In addition, Hsp90 levels remained constant at all concentrations tested, which is characteristic of C-terminal Hsp90 inhibition, suggesting these compounds may bind the Hsp90 C-terminus.

In conclusion, silybin was identified as a novel inhibitor of the Hsp90 protein folding machinery and a library of silybin analogues was designed and synthesized to explore the structure–activity relationships for this natural product. Upon biological evaluation, initial SAR was produced to determine the essential, non-essential and detrimental functionalities present on the silybin scaffold that result in anti-proliferative activity. Western blot analyses of silybin and silybin analogues support these compounds bind to the Hsp90 C-terminus, which validates silybin as a novel scaffold for Hsp90 inhibition and analogue development.

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Reference and notes

1. Pearl, L. H.; Prodromou, C.; Workman, P. *Biochem. J.* **2008**, *410*, 439.
2. Blagg, B. S. J.; Kerr, T. D. *Med. Res. Rev.* **2006**, *26*, 310.
3. Xu, W.; Neckers, L. *Clin. Cancer Res.* **2007**, *13*, 1625.
4. Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57.
5. Zhang, H.; Burrows, F. *J. Mol. Med.* **2004**, *82*, 488.
6. Kim, Y. S.; Alarcon, S. V.; Lee, S.; Lee, M. J.; Giaccone, G.; Neckers, L.; Trepel, J. B. *Curr. Top. Med. Chem.* **2009**, *9*, 1479.
7. Biamonte, M. A.; Water, R. V.; Arndt, J. W.; Scannevin, R. H.; Perret, D.; Lee, W. C. *J. Med. Chem.* **2010**, *53*, 3.
8. Peterson, L. B.; Blagg, B. S. J. *Future Med. Chem.* **2009**, *1*, 267.
9. Abenavoli, L.; Capasso, R.; Milic, N.; Capasso, F. *Phytother. Res.* **2010**, *24*, 1423.
10. Gazak, R.; Walterova, D.; Kren, V. *Curr. Med. Chem.* **2007**, *14*, 315.
11. Yang, L. X.; Huang, K. X.; Li, H. B.; Gong, J. X.; Wang, F.; Feng, Y. B.; Tao, Q. F.; Wu, Y. H.; Li, X. K.; Wu, X. M.; Zeng, S.; Spencer, S.; Zhao, Y.; Qu, J. *J. Med. Chem.* **2009**, *52*, 7732.
12. Lu, P.; Mamiya, T.; Lu, L. L.; Mouri, A.; Niwa, M.; Hiramatsu, M.; Zou, L. B.; Nagai, T.; Ikejima, T.; Nabeshima, T. *J. Pharmacol. Exp. Ther.* **2009**, *331*, 319.
13. Deep, G.; Singh, R. P.; Agarwal, C.; Kroll, D. J.; Agarwal, R. *Oncogene* **2006**, *25*, 1053.
14. Davis-Searles, P. R.; Nakanishi, Y.; Kim, N.-C.; Graf, T. N.; Oberlies, N. H.; Wani, M. C.; Wall, M. E.; Agarwal, R.; Kroll, D. J. *Can. Res.* **2005**, *65*, 4448.
15. Mokhtari, M. J.; Motamed, N.; Shokrgozar, M. A. *Cell Biol. Int.* **2008**, *32*, 888.
16. Hogan, F. S.; Krishnegowda, N. K.; Mikhailova, M.; Kahlenberg, M. S. *J. Surg. Res.* **2007**, *143*, 58.
17. Sharma, G.; Singh, R. P.; Chan, D. C.; Agarwal, R. *Anticancer Res.* **2003**, *23*, 2649.
18. Thulasiraman, V.; Matts, R. L. *Biochemistry* **1996**, *35*, 13443.
19. Shao, J.; Grammatikakis, N.; Scroggins, B. T.; Uma, S.; Huang, W.; Chen, J. J.; Hartson, S. D.; Matts, R. L. *J. Biol. Chem.* **2001**, *276*, 206.
20. Shao, J.; Irwin, A.; Hartson, S. D.; Matts, R. L. *Biochemistry* **2003**, *42*, 12577.
21. Yun, B. G.; Huang, W.; Leach, N.; Hartson, S. D.; Matts, R. L. *Biochemistry* **2004**, *43*, 8217.
22. Hadden, M. K.; Galam, L.; Gestwicki, J. E.; Matts, R. L.; Blagg, B. S. J. *J. Nat. Prod.* **2007**, *70*, 2014.
23. Wang, F.; Huang, K.; Yang, L.; Gong, J.; Tao, Q.; Li, H.; Zhao, Y.; Zeng, S.; Wu, X.; Stoeckigt, J.; Li, X.; Qu, J. *Bioorg. Med. Chem.* **2009**, *17*, 6380.
24. Kidd, P.; Head, K. *Altern. Med. Rev. J. Clin. Ther.* **2005**, *10*, 193.
25. Kren, V.; Kubisch, J.; Sedmera, P.; Halada, P.; Prikrylova, V.; Jegorov, A.; Cvak, L.; Gebhardt, R.; Ulrichova, J.; Simanek, V. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2467.
26. Gazak, R.; Svobodova, A.; Psotova, J.; Sedmera, P.; Prikrylova, V.; Walterova, D.; Kren, V. *Bioorg. Med. Chem.* **2004**, *12*, 5677.
27. Gong, J. X.; Weng, L. J.; Wang, F.; Feng, Y. B.; Zhou, C. X.; Li, H. B.; Wu, Y. H.; Hao, X. J.; Wu, X. M.; Bai, H.; Stockigt, J.; Zhao, Y. *Chin. Chem. Lett.* **2006**, *17*, 465.
28. Merlini, L.; Zanarotti, A.; Pelter, A.; Rochefort, M. P. *J. Chem. Soc., Perkin Trans. 1* **1980**, 775.
29. Tanaka, H.; Shibata, M.; Ohira, K.; Ito, K. *Chem. Pharm. Bull.* **1985**, *33*, 1419.
30. Donnelly, A. C.; Zhao, H.; Kusuma, B. R.; Blagg, B. S. J. *Med. Chem. Commun.* **2010**, *1*, 165.
31. Zhao, H.; Kusuma, B. R.; Blagg, B. S. J. *ACS Med. Chem. Lett.* **2010**, *1*, 311.