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Towards the discovery of drug-like epigallocatechin gallate analogs as Hsp90 inhibitors

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ABSTRACT

(−)-Epigallocatechin gallate (EGCG) is the major flavonoid of green tea and has been widely explored for a range of biological activities including anti-infective, anti-inflammatory, anti-cancer, and neuroprotection. Existing structure–activity data for EGCG has been largely limited to exploration of simple ethers and hydroxyl deletion. EGCG has poor drug-like properties because of multiple phenolic hydroxyl moieties and a metabolically labile ester. This work reports a substantial expansion of structure–activity understanding by exploring a range of semi-synthetic and synthetic derivatives with ester replacements and variously substituted aromatic and alicyclic groups containing more drug-like substituents. Structure–activity relationships for these molecules were obtained for Hsp90 inhibition. The results indicate that amide and sulfonamide linkers are suitable ester replacements. Hydroxylated aromatic rings and the *cis*-stereochemistry in EGCG are not essential for Hsp90 inhibition. Selected analogs in this series are more potent than EGCG in a luciferase refolding assay for Hsp90 activity.

(−)-Epigallocatechin gallate (EGCG, **1**, Fig. 1) is the primary flavonoid component of green tea and has been widely studied due to its diversity of biological properties including anti-inflammatory, anti-oxidative, anti-cancer, anti-infective and neuroprotective activity.[1–3] Structure–activity studies on **1** reported to date have focused primarily on a limited spectrum of analogs involving methylation and/or deletion of phenolic hydroxyl moieties in **1**–**3**.[4–6] These compounds have been studied for biological activity in systems related to the therapeutic applications mentioned above. The resulting derivatives retain unfavorable characteristics of EGCG including chemical and metabolic instability. Our approach to modification of EGCG is focused on discovery of more drug-like derivatives in the context of an expanded structure–activity study that includes exploration of the influence of stereochemistry in the natural product on biological/target specific properties.

This Letter details our initial efforts toward the discovery of more drug-like analogs of EGCG using inhibition of Hsp90 as a representative biological target. Hsp90 is an intracellular chaperone protein essential for cell growth and division as well as protein trafficking.[7] It is a target of interest for treatment of cancer and potentially neurodegenerative disorders.[8] We prepared a series of semisynthetic and synthetic EGCG analogs in which the metabolically labile ester is replaced by more stable linkers, aromatic rings

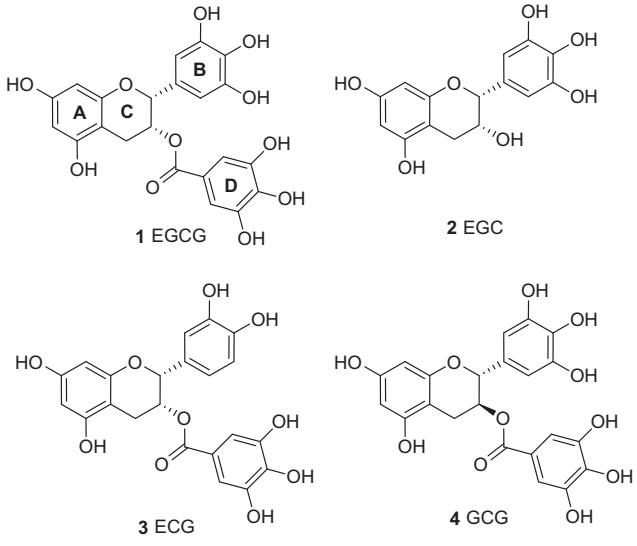

Figure 1.

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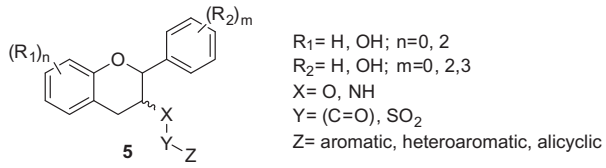
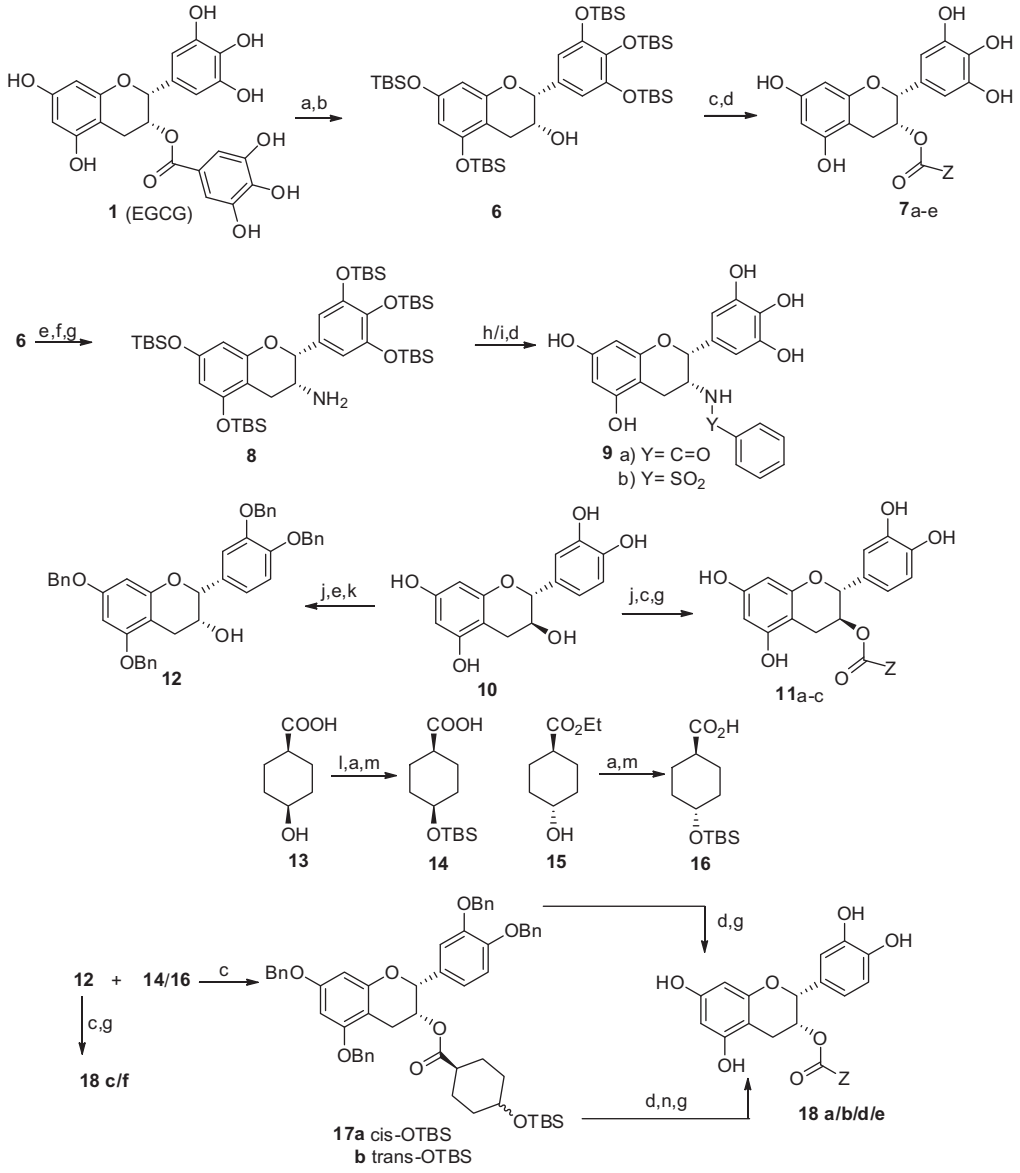


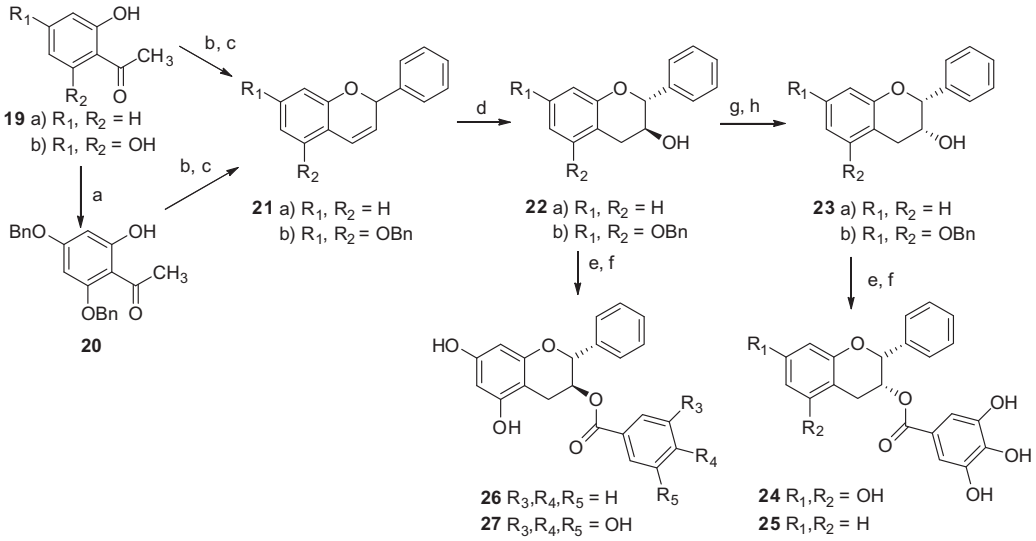
Figure 2.

are replaced by alicyclic systems and phenolic hydroxyl groups are removed and/or replaced with other substituents as outlined in Figure 2. Stereochemical effects on Hsp90 activity are evaluated using a commercially available derivative, gallocatechin gallate (GCG, **4**). EGCG was characterized previously as a C-terminal binding Hsp90 inhibitor.[9] EGCG analogs were evaluated as Hsp90 inhibitors using a luciferase refolding assay because a direct Hsp90 binding assay for C-terminal inhibitors is not yet available. Since the recovery of luciferase enzymatic activity after heat-induced denaturation is dependent on Hsp90 chaperone function, this assay is commonly used to test compounds for their ability to inhibit Hsp90.[9,10]

The semi-synthesis of EGCG analogs is outlined in Scheme 1 below.[11] Silyl protection of the phenolic hydroxyl groups in **1** followed by lithium aluminum hydride reduction furnished protected EGC derivative **6**. Esterification by DCC-mediated coupling provided silyl-protected intermediates in which silyl deprotection was best accomplished with aqueous HF in tetrahydrofuran, leading efficiently to targets **7a**–**e**. Oxidation of **6** with Dess–Martin reagent followed by reductive amination with benzylamine and hydrogenolytic debenzylation provided primary amine **8** that was treated with benzoyl chloride or phenylsulfonyl chloride to give the respective amide and sulfonamide which were de-silylated as above leading to amide **9a** and sulfonamide **9b** in good yield. Beginning with (+)-catechin (**10**), selective phenolic hydroxyl benzylation, esterification and hydrogenolytic debenzylation led to *trans* esters **11a**–**c**. Benzyl protection of **10** followed by



Scheme 1. Reagents and conditions: (a) TBDMS-Cl, Et_3N, DMF, rt 95%; (b) $LiAlH_4$/THF, 0 °C 75%; (c) Z-CO_2H, DCC, DMAP, CH_2Cl_2, rt 70%; (d) 48% aq HF, THF, rt 80%; (e) Dess–Martin, CH_2Cl_2, rt 85%; (f) benzylamine, cat. HOAc, $NaBH_3CN$, THF, rt 75%; (g) H_2 (30 psi), 10% Pd-C, MeOH, rt 95%; (h) benzoyl chloride, Et_3N, CH_2Cl_2, rt 76%; (i) phenyl sulfonyl chloride, Et_3N, CH_2Cl_2, rt 68%; (j) BnBr, K_2CO_3, DMF, rt 30%; (k) L-Selectride, THF, −78 °C to rt, 76%; (l) EtOH/H^+ reflux, 85%; (m) NaOH/EtOH rt, 89%; (n) methyl DAST, CH_2Cl_2 0 °C to rt, 75%.



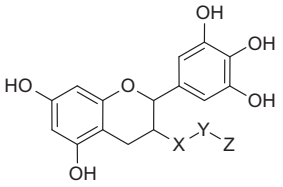
Scheme 2. Reagents and conditions: (a) BnBr, K_2CO_3, DMF, rt, 45%; (b) benzaldehyde, piperidine, ethanol, reflux, 1N HCl, 43%; (c) $NaBH_4$, EtOH/THF, reflux, rt 16 h, 15% HOAc, 40%; (d) BH_3-THF, THF, 0 °C, H_2O_2, NaOH, THF, 59%; (e) Z-CO_2H, DCC, DMAP, DCM, rt, 74%; (f) 30 psi H_2, 10% Pd/C, THF, 90%; (g) Dess–Martin, 0 °C to rt, DCM, 80%; (h) L-Selectride, THF, −78 °C to rt, 63%.

Dess–Martin oxidation and L-Selectride reduction efficiently gave an epimeric alcohol **12** that was esterified and debenzylated as before to give protected *cis* esters **18c** and **18f**. *cis*- and *trans*-hydroxy-substituted cyclohexyl intermediates (**14** and **16**) necessary for esters **18d** and **18e** were prepared by standard methods and coupled with **12** then converted as indicated in Scheme 1. Fluoro derivatives **18a** and **18b** were prepared from their respective hydroxyl intermediates by methyl DAST.

Synthetic EGCG analogs lacking phenolic hydroxyl groups were prepared as outlined in Scheme 2. Chalcone synthesis using **19a** and benzyl protected acetophenone **20** furnished **21a** and **21b**, respectively, which underwent hydroboration/oxidation to give a mixture of alcohols in which the *trans* isomers **22a** and **22b** predominate.[12,13] Esterification of **22b** and deprotection as above provided *trans* esters **26** and **27**. Dess–Martin oxidation of **22a/b** followed by stereoselective reduction by L-Selectride, esterification and debenzylation furnished *cis*-analogs **24** and **25**.

EGCG (**1**), GCG (**4**) and the new analogs were evaluated for functional inhibition of Hsp90 activity using a luciferase refolding assay as described elsewhere.[9,10] The results are shown in Tables 1–3

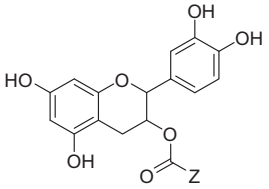
Table 1
Semisynthetic D-ring and linker analogs

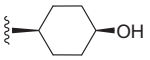
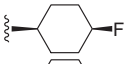
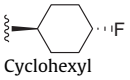
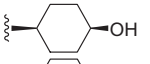
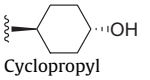


Compd	*cis*/*trans*[a]	X	Y	Z	IC_{50}[b] (μM)
1	*cis*	O	C=O	Gallate	155
4	*trans*	O	C=O	Gallate	34
7a	*cis*	O	C=O	Phenyl	24
7b	*cis*	O	C=O	4-F-Ph	41
7c	*cis*	O	C=O	3,4-Di-F-Ph	58
7d	*cis*	O	C=O	4-Pyridyl	64
7e	*cis*	O	C=O	Cyclohexyl	30
9a	*cis*	NH	C=O	Phenyl	75
9b	*cis*	NH	SO_2	Phenyl	44

[a] Gallate B ring/X (absolute stereochemistry as in EGCG).
[b] Average value based on at least two independent determinations.

Table 2
Semisynthetic catechol D-ring analogs

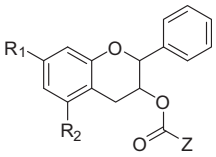


Compd	*cis*/*trans*[a]	Z	IC_{50}[b] (μM)
11a	*trans*	Cyclohexyl	98
11b	*trans*	Phenyl	110
11c	*trans*		280
18a	*cis*		65
18b	*cis*		69
18c	*cis*	Cyclohexyl	81
18d	*cis*		310
18e	*cis*		440
18f	*cis*	Cyclopropyl	410

[a] Catechol B ring/ester (absolute stereochemistry derived from GCG).
[b] Avg based on at least two independent determinations.

below. The following structure–activity observations can be made: (1) The metabolically labile ester can be replaced by an amide (**9a**) or sulfonamide (**9b**) leading to more potent Hsp90 inhibitors. This further suggests that a poly-hydroxy D ring is not necessary for Hsp90 inhibition and the relative geometry of a pyramidal sulfonamide compared to the more planar amide or ester does not disrupt potential binding interactions in this region. The sulfonamide **9b** is approximately 3-fold more potent compared to EGCG. (2) Hsp90 inhibition associated with these and other des-hydroxy derivatives shows that there are a variety of potentially more drug-like options for replacement of the gallate ester including an electron deficient heterocycle (**7d**) and substituted alicyclics (**18a**, **18b**). Fluoro-substituted aromatic groups (**7b**, **7c**) also result in improved Hsp90 inhibition. Selected D-ring analogs

Table 3
Racemic, synthetic analogs



Compd	*cis*/*trans*[a]	R_1	R_2	Z	IC_{50}[b] (μM)
24	*cis*	OH	OH	Phenyl	63
25	*cis*	H	H	Gallate	35
26	*trans*	OH	OH	Phenyl	120
27	*trans*	OH	OH	Gallate	150

[a] Relative phenyl/ester stereochemistry.
[b] Avg based on at least two independent determinations.

are measurably more potent than EGCG, for example cyclohexyl ester **7e** and benzoate ester **7a**. (3) Deletion of both A- and B-ring hydroxyl groups as in **25** (a racemate) notably furnishes a more potent Hsp90 inhibitor (IC_{50} 35 μM) compared to EGCG. (4) B-ring catechol esters are less active compared to analogous gallate analogs, cf. **11a** versus **7e** and **11b** versus **7a**. This observation suggests the possibility of more complex SAR in view of the improved potency of racemic des-hydroxy **25**. In this catechol series, fluoro substitution of the cyclohexane ring is preferable to hydroxyl (cf. **18b** vs **18e**) and the relative stereochemistry of fluorine has little effect on Hsp90 inhibition. It is also interesting to observe that the difference in potency between *cis*- and *trans*-isomers in the racemic, synthetic des-hydroxy analogs in Table 3 differs from that observed with EGCG and GCG. These results collectively suggest a number of avenues for future SAR studies.

This initial SAR survey of synthetic and semi-synthetic analogs was intended to highlight stereochemical and structural variations in rings A, B and D of EGCG that can serve as starting points for preparation and study of more drug like derivatives of this biologically interesting natural product. *trans*-Geometry on the C ring furnishes a more potent Hsp90 inhibitor compared to **1**. Selected compounds were more potent than EGCG in an Hsp90 functional assay. Furthermore our data show that multiple hydroxyl groups can be eliminated and/or replaced with other functional groups. This is especially important given the known tendency of the 4-hydroxy group in the B-ring to play a role in epimerization of EGCG.[14] The metabolically labile ester can be replaced by amide and sulfonamide linkages and the aromatic D ring can be replaced by an alicyclic system. Ongoing work is focused on using this data to identify a combination of sites and analogs where significant improvements in potency of Hsp90 inhibition can be achieved. Other research in this laboratory is exploring connections between increased Hsp90 potency, structural variations and biological outcomes such as neuroprotection and cytotoxicity.

Acknowledgments

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