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Benzotropolone inhibitors of estradiol methylation: kinetics and in silico modeling studies

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Abstract—Natural and synthetic benzotropolone compounds were assessed in vitro for their ability to inhibit hydroxyestradiol methylation by catechol-*O*-methyltransferase (COMT). The compounds were also modeled in silico with a homology model of human COMT. Purpurogallin (**1**), purpurogallin carboxylic acid (**2**), and theaflavin-3,3′-digallate (**6**) were the most potent inhibitors of 2-hydroxy and 4-hydroxyestradiol methylation (IC_{50} 0.22–0.50 μM). Compounds **1** and **6** decreased the V_{max} and increased the K_m of COMT, indicating a mixed-type inhibition. Compounds **1** and **2** bound to COMT by inserting the six-membered ring of the benzotropolone into the active site. Decreased acidity of the hydroxyl groups on this ring or increased bulkiness reduced potency. Compound **6** bound by inserting the galloyl ester into the active site, which allowed the compound to overcome increased bulkiness and resulted in restored potency. Further studies are needed to determine the impact in vivo of COMT inhibition by these compounds.

1. Introduction

Catechol-*O*-methyltransferase (COMT) is responsible for the *S*-adenosylmethionine (AdoMet)-dependent methylation of a wide variety of endogenous and catechol-containing xenobiotic compounds.[1] For example, methylation of dopamine by COMT in the central nervous system contributes to the process which results in termination of the neurotransmission signal.[2] Methylation of catechols results in a decrease in the polarity and limits the redox activity of these potentially toxic endogenous and exogenous substrates.[1] Both soluble and membrane-bound forms of COMT have been characterized, and COMT has been found in all mammalian tissues studied.[3] COMT activity is highest in the liver followed by the kidney and small intestine. Inhibition of COMT potentially has both beneficial and deleterious effects. For example, entacapone, a selective inhibitor of COMT, is currently included in a regimen to treat Parkinson's disease in humans.[4,5] This compound blocks the methylation, and subsequent inactivation, of 3,4-dihydroxy-L-phenylalanine (L-dopa) in the periphery. This improves the clinical efficacy of L-dopa by increasing the amount of L-dopa that crosses the blood–brain barrier.

The metabolic pathway of estradiol (E_2) includes cytochrome P450-mediated hydroxylation of estradiol, resulting in the formation of the catechol intermediates, 2-hydroxy (2-OH E_2) and 4-hydroxyestradiol (4-OH E_2).[6] These intermediates, which are redox active and have been shown to be carcinogenic (especially 4-OH E_2), are then detoxified by COMT-mediated methylation. Inhibition of this methylation process has been shown to increase the formation of 8-hydroxy-2-deoxyguanosine in MCF-7 human breast cancer cells.[6] Treatment of male Syrian hamsters with 3% dietary quercetin, a flavonoid inhibitor of COMT, resulted in a significant

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increase in E_2-mediated kidney tumorigenesis presumably by increasing the accumulation of these carcinogenic intermediates.[7]

Previously, we have reported the synthesis of a series of benzotropolone-containing polyphenolic compounds ranging in structural complexity from purpurogallin (**1**, mw = 220.2) to theaflavin-3,3′-digallate (**6**, mw = 878.0) (Fig. 1) by the peroxidase-catalyzed reaction of various polyphenolic starting materials.[8]

Purpurogallin is a benzotropolone-containing natural product, which occurs in the nut gall of *Quercus* spp.[9] Human exposure to this compound occurs mainly through consumption of edible oils to which purpurogallin has been added as a stabilizer.[10] Veser has previously reported that purpurogallin competitively inhibits COMT isolated from the yeast, *Candida tropicalis*, but the inhibitory effects of this compound on human COMT and with physiologically-important substrates have not been studied.[11] Theaflavins, which also contain a benzotropolone moiety, are present as 3–6% of the dry weight of black tea (*Camellia sinensis*) water extract. This beverage represents nearly 80% of the world consumption of tea.[12]

In the present study, we report the inhibitory activity of a series of these benzotropolone-containing compounds against COMT-mediated methylation of 2-OH E_2 and 4-OH E_2. The kinetics of inhibition were determined for compounds **1** and **6**, and inhibitory activity was compared with inhibitory activity predicted by molecular modeling with a homology model of human COMT.

2. Results

2.1. HPLC analysis of estradiol metabolites

HPLC analysis of enzyme reactions with 2-OH E_2 as the substrate revealed a clear peak with retention time (t_R) = 10.2 min corresponding to 2-methoxyestradiol (2-OCH_3 E_2) (Fig. 2). A similar retention time was observed for 4-methoxyestradiol when 4-OH E_2 was used as the substrate for the reaction.

Under these HPLC conditions, 2-OH and 4-OH E_2 were eluted in the void volume and could not be quantified. Additionally, two additional peaks ($t_R \approx 12$ min) were observed in chromatograms following incubation with human liver cytosol. We hypothesize that these peaks represent the minor methylation products, 2-hydroxy-3-methoxyestradiol (from 2-OH E_2) and 3-methoxy-4-hydroxyestradiol (from 4-OH E_2), however, we did not have reference standards of these compounds and could not therefore confirm this hypothesis nor quantify these metabolites (Fig. 2). The standard curve was linear over the range of 0.5–5 μg/mL for both 2-OCH_3 ($y = 6.74x$–0.09, $R^2 = 0.998$) and 4-OCH_3 ($y = 18.28$–0.16, $R^2 = 0.998$).

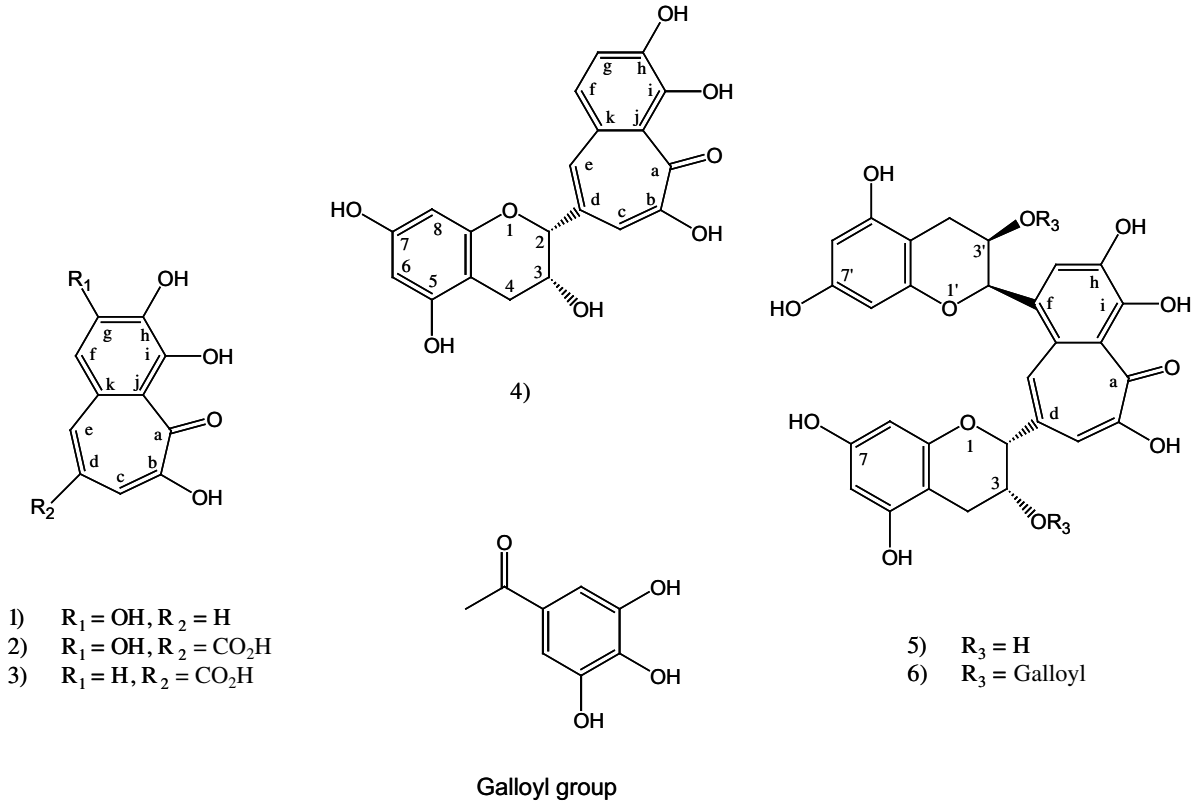


Figure 1. Structures of benzotropolone-containing inhibitors of catechol-*O*-methyltransferase.

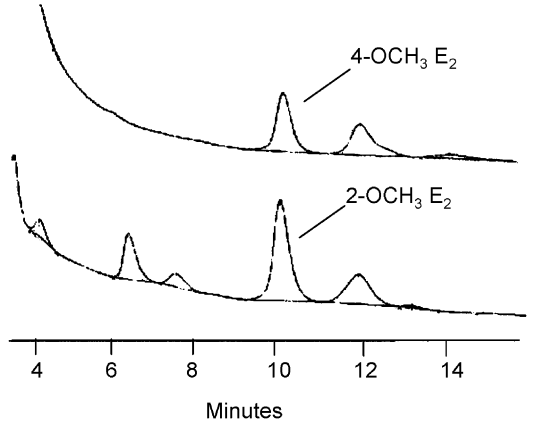


Figure 2. HPLC analysis of 2-OH and 4-OH E_2 methylation by human liver cytosol. Representative chromatograms of reactions containing 50 μM 2-OH E_2 (A) or 4-OH E_2 (B) as the substrate. Identification of the main methylated metabolites were performed by comparison with pure 2-OCH_3 E_2 and 4-OCH_3 E_2 reference standards.

2.2. Inhibition of COMT-mediated methylation of hydroxylated estradiol metabolites

The COMT inhibitory activity of compounds **1**–**6** were determined using 2-OH E_2 (50 μM) as a substrate (Fig. 3). Following a 30 min incubation time, compounds **1** and **2** displayed relatively high potency with IC_{50} values of 0.50 ± 0.04 and 0.30 ± 0.02 μM, respectively. Loss of the hydroxyl groups at the *g*-position (*g*-OH) or increased bulkiness decreased the potency of the molecules as demonstrated by compounds **3**–**5**, which had IC_{50} values of 7–15 μM. Compounds **5** and **6** differ in structure by the presence of two galloyl moieties. This difference significantly enhanced the potency of compound **6**, and resulted in an IC_{50} value of 0.50 ± 0.01 μM: the higher potency was in spite of the higher molecular weight and greater steric bulk.

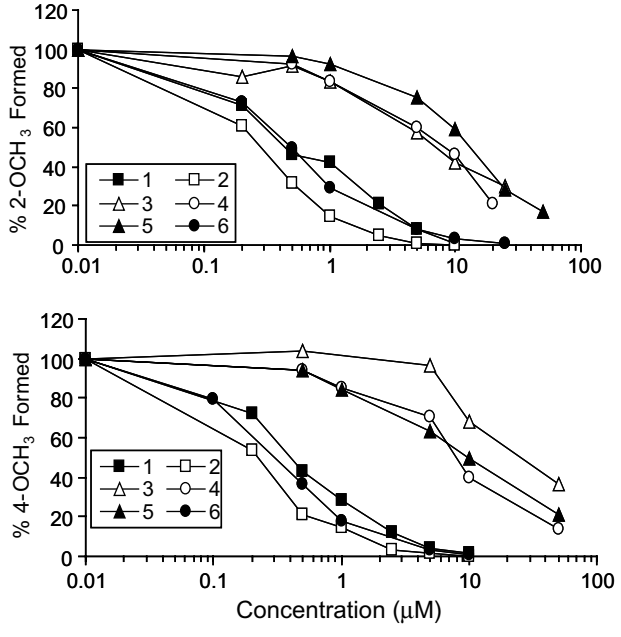


Figure 3. Inhibition of human liver cytosol-mediated methylation of 2-OH and 4-OH E_2 by benzotropolone inhibitors (0–50 μM). Points represent $n = 3$–5. Error bars are omitted for clarity; the standard deviation was 10% or less for each point.

Compounds **1**–**6** were also shown to inhibit methylation 4-OH E_2 in a dose-dependent manner (Fig. 3). The IC_{50} values were very similar to those observed when 2-OH E_2 was used as the substrate. The potency of inhibition of the compounds showed the following rank order: **2** > **6** > **1** ≫ **4** > **5** > **3**.

Kinetic analysis of compounds **1** and **6** was conducted using 2-OH E_2 and 4-OH E_2 as substrates (Fig. 4). Increasing concentrations of compound **1** resulted in decreased V_{max} and increased K_m.

For example, using 2-OH E_2 as the substrate the K_m values after treatment with 0, 1, and 2 μM compound **1** were 17.5 ± 0.7, 39.3 ± 2.6, and 70.0 ± 4.3 μM, respectively: V_{max} was decreased from 424.8 ± 9.1 to 192.2 ± 8.3 pmol/mg/min (Table 1). Such changes indicate a mixed-type inhibition for compound **1** with regard to 2- and 4-OH E_2. Similarly, in the presence of compound **6** (0–2 μM) the K_m was increased from 18.2 ± 0.6 to 73.2 ± 3.5 μM and the V_{max} was decreased from 395.1 ± 11.1 to 180.7 ± 4.9 pmol/mg/min when 2-OH E_2 was used as the substrate. This again indicates a mixed-type inhibition. Similarities of the results between 2-OH E_2 and 4-OH E_2 when compound **1** was tested, we did not determine the inhibitory kinetics of compound **6** with 4-OH E_2 as the substrate.

2.3. Molecular modeling and docking studies of benzotropolone inhibitors

To characterize the inhibitory mechanism of compounds **1**–**6** against COMT, a homology model of human COMT was prepared by comparison with the published crystal structure of rat COMT. Docking of compounds **1**–**5** revealed that the six-membered ring of these compounds occupied the active site of COMT (Figs. 5 and 6).

Calculated values of FLEXX scoring function for compounds **1**–**3** were −13.05 ± 2.54, −14.39 ± 0.26, and −14.13 ± 0.38 kcal/mol, respectively. A more negative value of FLEXX scoring function indicates stronger ligand–COMT binding affinity, and our results suggest that compound **2** binds more tightly to human COMT than compound **1** and **3**, however the difference is not statistically significant. The distance between the hydroxyl groups *g*, *h*, and *i* on the six-membered ring of the compound **1** and the N atom of the terminal NH_2 group on the side chain of Lys144 were calculated. The distance between Lys144 and *g*-OH, *h*-OH, and *i*-OH were 4.79 ± 0.96, 4.00 ± 0.58, and 3.79 ± 0.92 Å, respectively. For compound **2**, the average distance between these groups were 5.63 ± 0.60, 4.09 ± 0.14, and 2.91 ± 0.71 Å for *g*-OH, *h*-OH, and *i*-OH, respectively. Compound **3**, which lacked the *g*-OH, had the distance of 4.58 ± 0.25 and 2.64 ± 0.00 Å between Lys144 and the *h*- and *i*-OH groups, respectively.

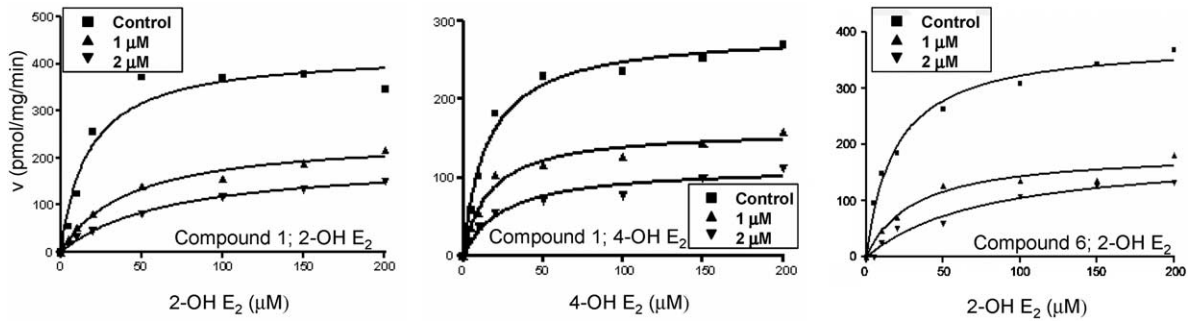


Figure 4. Kinetics of COMT-inhibition by compound **1** and **6** (0–2 μM) with respect to 2-OH E_2 and 4-OH E_2 concentration (5–200 μM). Both compounds exhibited mixed-type inhibition with respect to hydroxyestradiol concentration. Changes in K_m and V_{max} are presented in Table 1. Each point represents $n = 3$. Error bars are omitted for clarity; the standard deviation was 10% or less for each point.

Table 1. Kinetics of inhibition of COMT-mediated methylation of 2-OH and 4-OH E_2 by purpurogallin (**1**) and theaflavin-3,3′-digallate (**6**)[a]

Concentration (μM)	Compound **1**				Compound **6**	
	2-OH E_2		4-OH E_2		2-OH E_2	
	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m
0	424.8	17.5	287.8	16.1	395.1	18.2
1	241.8[b]	39.3[b]	162.6[b]	17.0	194.3[b]	35.3[b]
2	192.2[b]	70.0[b]	113.8[b,c]	24.0[b,c]	180.7[b,c]	73.2[b,c]

[a] Units of V_{max} = pmol/mg/min; units of K_m = μM.
[b] Statistically different from control by one-way ANOVA and Tukey's Multiple Comparison Test, $p < 0.05$.
[c] Statistically different from 1 μM by one-way ANOVA and Tukey's Multiple Comparison Test, $p < 0.05$.

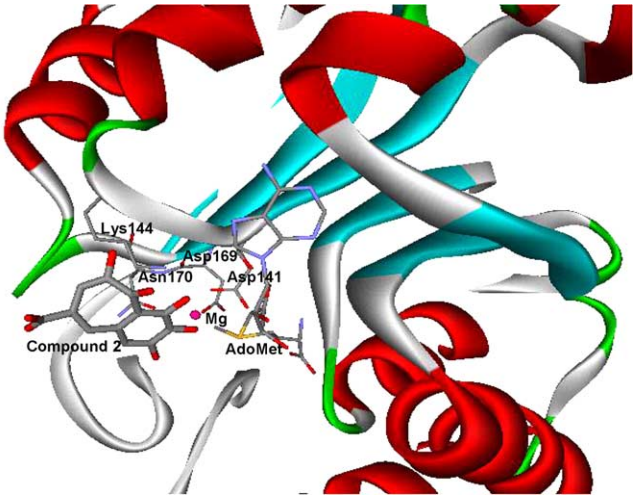


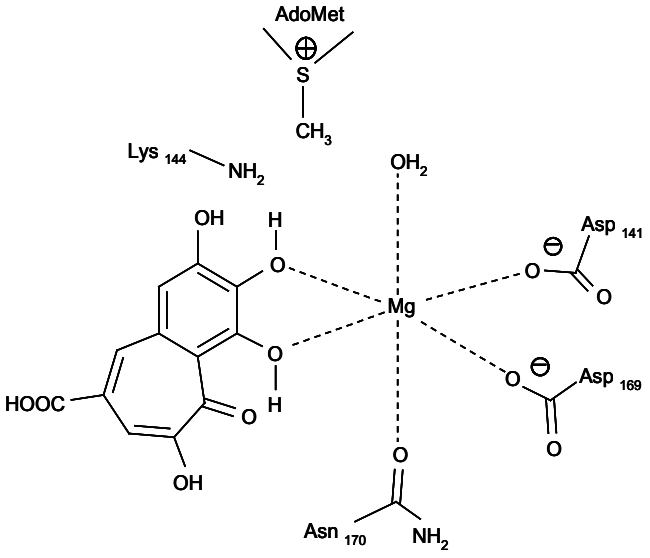
Figure 5. Molecular modeling of the interaction between compound **2** and hCOMT showing the consensus orientation for compound **2**. The protein is depicted in ribbon representation and colored by secondary structures (i.e., helix, strand, and loop). Both compound **2** and ligand contact residues are represented in stick form and colored by atom type with carbon in gray, oxygen in red, sulfur in yellow, and magnesium in magenta.

Compound **6** docked in a different manner from compounds **1**–**5**: in this case, the galloyl moiety occupied the active site of COMT (Fig. 6). The FLEXX scoring function for this compound was −1.02 kcal/mol: the distance between 3″-OH, 4″-OH, and 5″-OH were 3.20 ± 0.57, 3.12 ± 0.76, and 4.72 ± 0.90 Å. Compound **5**, which resembles **6** but lacks the galloyl moieties at R_3, was unable to find a consensus binding mode within human COMT.

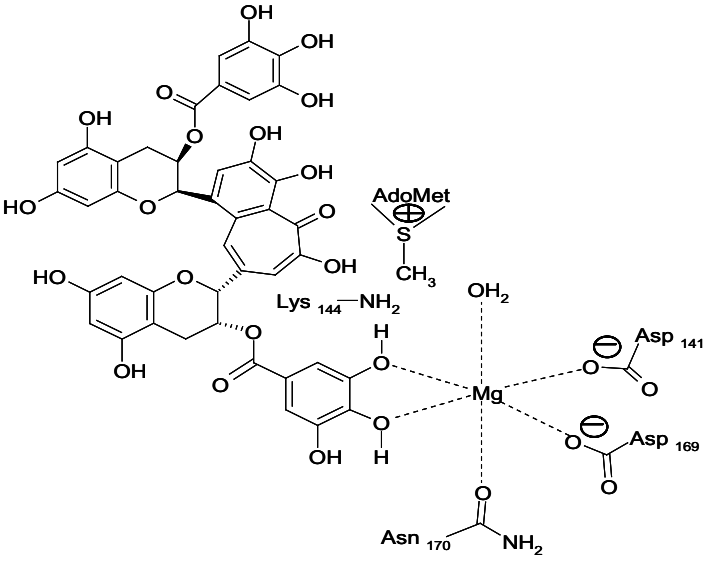
3. Discussion

In the present report, we describe the inhibition of COMT by a series of benzotropolone-containing molecules including purpurogallin (**1**), theaflavin (**5**), and theaflavin-3,3′-digallate (**6**). These compounds displayed IC_{50} values of 0.22–15 μM: potency appeared to depend largely on the presence of a vicinal hydroxyl group and small molecular size. An exception to this observation was theaflavin-3,3′-digallate (**6**), which had the highest molecular weight but was also quite potent with an IC_{50} of 0.30–0.50 μM. The inhibitory kinetics of compounds **1** and **6** were determined using 2-OH E_2 and 4-OH E_2 as substrates. In both cases, these compounds demonstrated a mixed-type inhibition as indicated by the increasing K_m and the decreasing V_{max}. We hypothesize that this mixed-type inhibition is due to competitive inhibition by the test compound and noncompetitive inhibition by the predicted methylated metabolite of the test compound. Previously we have observed such a pattern of inhibition with the tea polyphenol epigallocatechin-3-gallate (EGCG, competitive inhibitor) and its di-methylated metabolite (noncompetitive inhibitor) (unpublished results). We are currently identifying the methylated metabolites of compounds **1** and **6**; these will be tested for inhibitory activity against COMT.

In order to gain a clearer understanding of the differences in potency of compounds **1**–**6**, docking studies



Compound **2**



Compound **6**

Figure 6. Compound **2** forms a hexa-coordination complex with Mg^{2+} and surrounding residues using the six-membered ring moiety. Compound **6** forms a hexa-coordination complex with Mg^{2+} and surrounding residues using the galloyl moiety instead.

were performed using the FLEXX software and a homology model of human COMT. These studies indicated that the six-membered ring of benzotropolone moiety occupied the binding pocket of COMT and formed a stable hexa-coordination complex with Mg^{2+} metal ion through the vicinal hydroxyl groups. As the bulkiness of the molecule increases, this insertion becomes impaired and the potency of the inhibitor is decreased. In the case of compound **6**, which has the highest molecular weight, binding to COMT occurred via the galloyl moiety instead of the six-membered ring of the benzotropolone pharmacophore. The galloyl moiety had greater freedom than the benzotropolone ring to insert into the binding pocket and thus the potency was restored despite the increased bulkiness. The results of the molecular modeling studies correlate quite well with what was observed in the in vitro studies.

The tri-hydroxyl group on the six-membered ring of the benzotropolone moiety is also important for the potency of the inhibitor. Compounds **2** and **3** differ only with respect to the presence of this *g*-hydroxyl group and yet had a 15-fold difference in potency. The loss of potency may result from the lower acidity of the *i*-OH group due to the absence of the *g*-OH group. As an analogy, the overall p*K*a of catechol is 9.5 ± 0.2 whereas that of pyrogallol, which has a tri-hydroxyl structure, is 9.1 ± 0.2 (calculated by SciFinder Scholar, ACS). Modeling

studies suggest that the *i*-OH group interacts with Lys144 in the active site of COMT via a general acid–base reaction, in which Lys144 acts as the general base.[13] A decrease in the acidity of this hydroxyl group would cause compound **3** to interact less strongly within the binding pocket of COMT and would thus result in decreased potency.

Finally, the carboxylic acid moiety on the *d*-carbon of the benzotropolone ring appears to be an important determinant of potency, although less so than the tri-hydroxyl structure. This carboxylic acid group contributes additional resonance stabilization to the deprotonated intermediate of the inhibitor and its importance is demonstrated by comparing the potency of **1** and **2** (IC_{50} = 0.50 ± 0.04 vs 0.30 ± 0.02 μM).

Inhibition of COMT in vivo may be seen either as beneficial or deleterious depending upon the particular situation. For example, current clinical treatment of Parkinson's disease includes COMT inhibitors (e.g., entacapone and tolcapone) as adjunct therapy in combination with the standard regimen of L-dopa and carbidopa.[4] COMT inhibitors prolong the half life of L-dopa and allow that compound to enter the central nervous system. By contrast, chronic inhibition of COMT-mediated methylation of hydroxylated estradiol metabolites may represent a risk factor for mammary carcinogenesis since such metabolites are known to be redox active.[14,15] Methylation of these compounds is generally considered to be a detoxification pathway. For example, Zhu and Liehr have reported that administration of quercetin, a flavonoid inhibitor of COMT, in the estradiol-induced hamster kidney tumor model increased the incidence of large tumors and abdominal metastases relative to animals treated only with estradiol.[7–16] We have found no reports of an increased risk of estrogen-related malignancies in human beings associated with the use of selective COMT inhibitors.

In summary, we have demonstrated the efficacy of a series of benzotropolone-containing molecules as inhibitors of COMT. Using classical enzymology and computer modeling studies, we have characterized these compounds in terms of the mechanism of inhibition using 2- and 4-OH estradiol as substrates. Further studies are needed to determine the potential beneficial or deleterious implications of the observed COMT inhibition in vivo.

4. Experimental

4.1. Chemicals and enzymes

Pooled human liver cytosol (20 mg/mL protein concentration) was purchased from BD-Gentest Co. (Woburn, MA). Purpurogallin and derivatives were prepared as previously reported, dissolved as 50 mM stock solutions in DMSO, and stored at −80 °C (Sang et al., 2004). Estradiol metabolites (2-OCH_3 E_2 and 4-OCH_3 E_2) were purchased from Steraloids Inc. (New Port, RI), prepared as a 5 mM stock solution in DMSO and stored at −80 °C. All other chemicals were of the highest grade available.

4.2. HPLC analysis of methylated estradiol metabolites

The methylation of hydroxylated E_2 was monitored using HPLC with electrochemical detection. The HPLC system consisted of two Waters Model 510 pumps (Waters Corp., Milford, MA) equipped with an ESA Coulochem II electrochemical detector (ESA Inc., Chelmsford, MA). Samples were injected with a Waters 717plus autoinjector equipped with a 200 μL loop. Separation was achieved using a Supelcosil C-18 reversed-phase column (250 mm × 4.6 mm) with a 5 μm pore size (Supelco Inc., Bellefonte, PA) and an isocratic mobile phase of sodium phosphate monobasic buffer (17.1 mM, pH 3.4) containing 33% acetonitrile and 6% tetrahydrofuran. The flow rate was 1 mL/min and eluant was monitored at a potential of 300 mV. Data was processed using MILLENIUM[32] software (Waters Corp., Milford, MA). Identification of 2-OCH_3 E_2 and 4-OCH_3 E_2 was accomplished by comparison of retention times between experimental samples and pure reference standards of those metabolites.

4.3. Inhibition of human liver COMT-mediated methylation of estradiol metabolites

The IC_{50} of the purpurogallin derivatives for the inhibition of human liver cytosol-mediated methylation of 2-OH E_2 (or 4-OH E_2) was determined as follows. Human liver cytosol (2 mg/mL), 2-OH E_2 (50 μM), DTT (1 mM), $MgCl_2$ (1 mM), Tris–HCl (10 mM, pH 7.4), and the compound of interest (0–50 μM) were combined and pre-incubated for 2 min at 37 °C. AdoMet (60 μM) was added and the reaction was incubated at 37 °C for 30 min. The reaction was stopped by the addition of an equal volume of ice-cold methanol containing 1% ascorbic acid. After centrifugation at 10,000*g* for 10 min, 50 μL of the supernatant was analyzed by HPLC with electrochemical detection.

To determine inhibition kinetics, the concentration of substrate (2-OH E_2 or 4-OH E_2) was varied between 5 and 200 μM at saturating AdoMet concentrations (200 μM) in the presence of 0, 1, or 2 μM test compound. After pre-incubation at 37 °C for 3 min, AdoMet was added to start the reaction. The incubation was carried out at 37 °C for 30 min and terminated as above. Aliquots (50 μL) were analyzed by HPLC with electrochemical detection.

4.4. In silico molecular studies of human COMT

The protein sequence of human COMT (accession number BC000419) was retrieved from the NCBI Reference Sequence (RefSeq) Collection. A structural model of human COMT was constructed using the Insight II Homology Module (Accelrys, Inc., San Diego, CA) from the published crystal structure of rat COMT (RCSB Protein Data Bank www.rcsb.org/pdb; PDB ID = 1VID) as the modeling template. The quality of the model was confirmed by the WHATIF-CHECK program (www.cmbi.kun.nl/gv/servers/WIWWWI). All molecule modeling operations for compounds **1**–**6** were performed using SYBYL 6.9 (Tripos, Inc., St. Louis, MO), running on a

Silicon Graphics (SGI) O2 workstation under the IRIX 6.5 operation system. Initial structures of compounds **1–6** were constructed using the Sketch Molecule module in SYBYL and subsequently energy minimized to yield a stable conformation using the MMFF94 molecular force field with a dielectric constant of $\varepsilon = 4.0$.[17–19] Each ligand was docked in the active site using the FLEXX software implemented in SYBYL (Tripos).[20] FLEXX is a fast flexible docking method using an incremental construction algorithm that allows maximum rotations and conformation of ligands in the binding pocket.[21] Distances between the terminal NH_2 group of the Lys144 side chain and the hydroxyl groups were calculated as an indicator of the rate of deprotonation. FLEXX scoring function (kcal/mol), was calculated as an indicator of the strength of the protein–ligand interactions.

4.5. Statistical analysis

Kinetic parameters (V_{max}, K_m) of methylation were calculated using GRAPHPAD PRISM 3.0 (GraphPad Software, San Diego, CA). Statistical differences in V_{max}, K_m, and dose–response curves were assessed using one-way ANOVA followed by a Tukey Multiple Comparison Test. Significance was achieved at $p < 0.05$.

Acknowledgements

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References and notes

1. *Casarett s Toxicology: The Basic Science of Poisons*; Klaassen, C. D., Ed., 5th ed.; McGraw-Hill: New York, NY, 1996; p 1111.
2. Akil, M.; Kolachana, B. S.; Rothmond, D. A.; Hyde, T. M.; Weinberger, D. R.; Kleinman, J. E. *J. Neurosci.* **2003**, *23*, 2008.
3. Lu, H.; Meng, X.; Yang, C. S. *Drug Metab. Dispos.* **2003**, *31*, 572.
4. Stocchi, F.; Barbato, L.; Nordera, G.; Bolner, A.; Caraceni, T. *J. Neural Transm.* **2004**, *111*, 173.
5. Blandini, F.; Nappi, G.; Fancellu, R.; Mangiagalli, A.; Samuele, A.; Riboldazzi, G.; Calandrella, D.; Pacchetti, C.; Bono, G.; Martignoni, E. *J. Neural Transm.* **2003**, *110*, 911.
6. Saintot, M.; Malaveille, C.; Hautefeuille, A.; Gerber, M. *Int. J. Cancer* **2003**, *107*, 652.
7. Zhu, B. T.; Liehr, J. G. *Toxicol. Appl. Pharmacol.* **1994**, *125*, 149.
8. Sang, S.; Lambert, J. D.; Tian, S.; Hong, J.; Hou, Z.; Ryu, J. H.; Stark, R. E.; Rosen, R. T.; Huang, M. T.; Yang, C. S.; Ho, C. T. *Bioorg. Med. Chem.* **2004**, *12*, 459.
9. Abou-Karam, M.; Shier, W. T. *Phytother. Res.* **1999**, *13*, 337.
10. Prasad, K.; Kapoor, R.; Lee, P. *Mol. Cell. Biochem.* **1994**, *139*, 27.
11. Veser, J. *J. Bacteriol.* **1987**, *169*, 3696.
12. Lambert, J. D.; Yang, C. S. *J. Nutr.* **2003**, *133*, 3262S.
13. Zheng, Y. J.; Bruice, T. C. *J. Am. Chem. Soc.* **1997**, *119*, 8137.
14. Yoo, K. Y.; Kang, D. *Breast Cancer* **2003**, *10*, 289.
15. Creveling, C. R. *Neurotox. Res.* **2003**, *5*, 473.
16. Zhu, B. T.; Taneja, N.; Loder, D. P.; Balentine, D. A.; Conney, A. H. *J. Steroid Biochem. Mol. Biol.* **1998**, *64*, 207.
17. Halgren, T. A. *J. Am. Chem. Soc.* **1990**, *112*, 4710.
18. Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490.
19. Halgren, T. A. *J. Comput. Chem.* **1999**, *20*, 720.
20. Rarey, M.; Wefing, S.; Lengauer, T. *J. Comput. Aided Mol. Des.* **1996**, *10*, 41.
21. Rarey, M.; Kramer, B.; Lengauer, T.; Klebe, G. *J. Mol. Biol.* **1996**, *261*, 470.