# Bisubstrate Inhibitors of the Enzyme Catechol *O*-Methyltransferase (COMT): Efficient Inhibition Despite the Lack of a Nitro Group

Ralph Paulini,[a] Christian Lerner,[a]
Roland Jakob-Roetne,[b] Gerhard Zürcher,[b]
Edilio Borroni,[b] and François Diederich*[a]

Catechol *O*-methyltransferase (COMT) catalyzes the *O*-methylation of catechols by *S*-adenosylmethionine (SAM) in the presence of $Mg^{2+}$ ions.[1] Inhibition of COMT offers a therapeutic handle to reduce catecholamine metabolism, therefore providing a valuable complement for the treatment of CNS (central nervous system) disorders, such as Parkinson's disease[2] and possibly schizophrenia.[3] The most efficacious therapy for Parkinson's disease uses L-Dopa.[4] The introduction of COMT-inhibitors (tolcapone (Tasmar®)[5] and entacapone (Comtan®)[6]) as adjuncts to this treatment has resulted in considerable therapeutic improvement, helping to substantially prolong the efficacy of L-Dopa dosage by preventing its catabolism through *O*-methylation.

On the other hand, in some cases, adverse effects of hepatotoxicity have been associated with the use of tolcapone.[7,8] It has been hypothesized that the hepatotoxic effect may be related to the nitrocatechol core structure of the drug.[9] Therefore, the preparation of COMT inhibitors lacking the nitro group might be of advantage. However, this group is regarded as a key element for tight and reversible binding to the substrate pocket in the active site.[5] Substitution of the nitro group by weaker electron-withdrawing substituents drastically reduces the affinity of catechols to COMT. Furthermore, the electron-withdrawing effect of the nitro group is reflected by a

[a] *R. Paulini, Dr. C. Lerner, Prof. Dr. F. Diederich*
*Laboratorium für Organische Chemie, ETH-Hönggerberg, HCI*
*8093 Zürich (Switzerland)*
*Fax: (+41) 1-632-1109*
*E-mail: diederich@org.chem.ethz.ch*

[b] *Dr. R. Jakob-Roetne, G. Zürcher, Dr. E. Borroni*
*Pharma Division, Präklinische Forschung, F. Hoffmann–La Roche AG*
*4070 Basel (Switzerland)*

  DOI: 10.1002/cbic.200400084 

dramatic fall in the $pK_a$ value, which is paralleled by a strongly reduced nucleophilicity of the corresponding catechol OH group, thereby greatly reducing substrate behavior, that is, *O*-methylation. In contrast, bisubstrate inhibitors[10] that block both the SAM and catechol binding sites of COMT might offer the opportunity to circumvent this prerequisite for a nitro group.

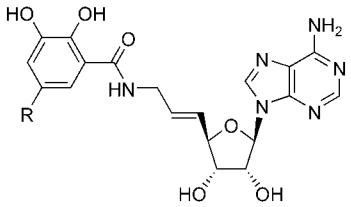
Recently, we described the potent bisubstrate inhibitor **1a** (Table 1; $IC_{50}$ = 9 nM: $IC_{50}$ = concentration of inhibitor at which 50% maximum initial velocity is observed) and demonstrated by crystal-structure analysis and enzyme kinetics that its adenosine and nitrocatechol moieties bind to the SAM and substrate sites of COMT, respectively.[11] Here we report that the bisubstrate inhibition approach eliminates the need for nitro-substituted catechols, and describe the synthesis and in vitro evaluation of a new generation of potent COMT inhibitors that lack the nitro group.

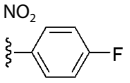
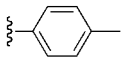
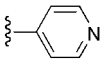
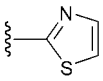
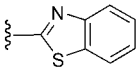
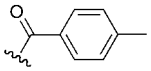
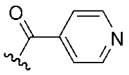
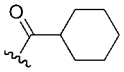
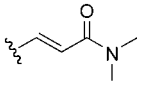
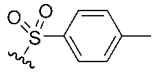
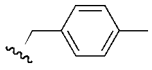
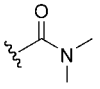
Analysis of the crystal structure of the ternary complex formed between **1a**, COMT, and a $Mg^{2+}$ ion,[11a,b] by using the molecular modeling package MOLOC,[12] suggested that analogues of **1a** could take advantage of a hydrophobic cleft[2e] at the enzyme surface that extends in the direction of the nitro group that we wished to replace. The modeled complex of the 4-methylphenyl derivative **1c** (Figure 1) shows that Trp38, Leu198, Val173, and Pro174 form a pocket[13,14] in which the hydrophobic residue departing from position 5 of the catechol moiety is well accommodated. A series of substituents of suitable size and spanning a wide range of electron-withdrawing capacities, as exemplified by their Hammett substituent constants $\sigma_p$,[15] was selected (Table 1). According to the modeling, all proposed inhibitors **1b**–**t** fully maintain the favorable interactions of the adenosine, linker, and catechol moieties with the protein and the $Mg^{2+}$ ion that had been observed in the crystal structure of the ternary complex formed by **1a** (Figure 1).

The synthesis of the new inhibitors[16] takes advantage of a convergent, two-building-block strategy, in which the allylic amine **2**[11a,b] is coupled with the *N*-hydroxysuccinimide esters **3b**–**t**, featuring diphenylmethylketal or di(4-methoxyphenyl)-methylketal moieties as catechol-protecting groups,[17] to give amides **4b**–**t**. Deprotection of the catechol and ribose residues finally yielded the desired inhibitors **1b**–**t**. This protocol is illustrated in detail in Scheme 1 for the preparation of 4-methylphenyl derivative **1c**.

The key building block for the synthesis of the diphenylmethylketal-protected catechol derivatives is the 5-bromo derivative **7**, obtained from 5-bromo-2,3-dihydroxybenzoic acid (**5**)[18] by esterification to give **6** and protection with dichlorodiphenylmethane. Starting from **7**, the desired substituents at position 5 are readily introduced either by using Pd-catalyzed Suzuki and Heck cross-couplings or Br–Li exchange, followed by treatment with an appropriate electrophile. For the synthesis of **1c**, **7** was subjected to a Suzuki cross-coupling with 4-methylphenylboronic acid to give **8c**. The ester was hydrolyzed (LiOH, THF/$H_2O$), and the resulting acid, **9c**, was transformed into the *N*-hydroxysuccinimide ester **3c**. Coupling of **2** with the activated ester **3c** provided amide **4c**, which was fully deprotected with TFA/$H_2O$ to afford the desired inhibitor **1c**.

***Table 1.*** *Structures, biological activities ($IC_{50}$ [nM], $\Delta G_{inh}$ (310 K) [kJ mol$^{-1}$][21]), Hammett substituent constants $\sigma_p$,[15] and $pK_a$ values[20] of the bisubstrate inhibitors **1a**–**t**.*



| Compound | R | $IC_{50}$ [nM] | $\Delta G_{inh}$ [kJ mol$^{-1}$] | $\sigma_p$ | $pK_a$ |
|---|---|---|---|---|---|
| **1a** | $NO_2$ | 9 | −47.8 | 0.78 | 4.42 |
| **1b** |  | 21 | −45.6 | 0.06 | 6.87 |
| **1c** |  | 23 | −45.3 | −0.03 | 7.06 |
| **1d** |  | 23 | −45.3 | 0.23 | 6.89 |
| **1e** |  | 27 | −44.9 | – | 6.15 |
| **1f** | Br | 28 | −44.8 | 0.23 | 6.54 |
| **1g** |  | 29 | −44.7 | 0.29 | 6.18 |
| **1h** | CN | 29 | −44.7 | 0.66 | 5.18 |
| **1i** |  | 34 | −44.3 | 0.43 | 5.42 |
| **1j** | $CF_3$ | 35 | −44.3 | 0.54 | 6.22 |
| **1k** |  | 39 | −44.0 | 0.80 | 5.74 |
| **1l** |  | 42 | −43.8 | – | 6.95 |
| **1m** | Cl | 44 | −43.7 | 0.23 | 6.65 |
| **1n** |  | 83 | −42.0 | – | 5.46 |
| **1o** |  | 97 | −41.6 | 0.05 | 6.34 |
| **1p** |  | 213 | −39.6 | – | 5.07 |
| **1q** |  | 608 | −36.9 | −0.09 | 7.56 |
| **1r** |  | 1370 | −34.8 | −0.15 | 7.63 |
| **1s** |  | 2000 | −33.8 | 0.36 | 6.25 |
| **1t** | H | 2600 | −33.1 | 0 | 7.37 |

The binding affinity ($IC_{50}$ values) of the new inhibitors towards COMT in the presence of $Mg^{2+}$ ions was determined by using a radiochemical assay that has previously been reported in full detail.[10b,19] Gratifyingly, 14 out of the 19 new inhibitors tested showed $IC_{50}$ values in the double-digit nanomolar

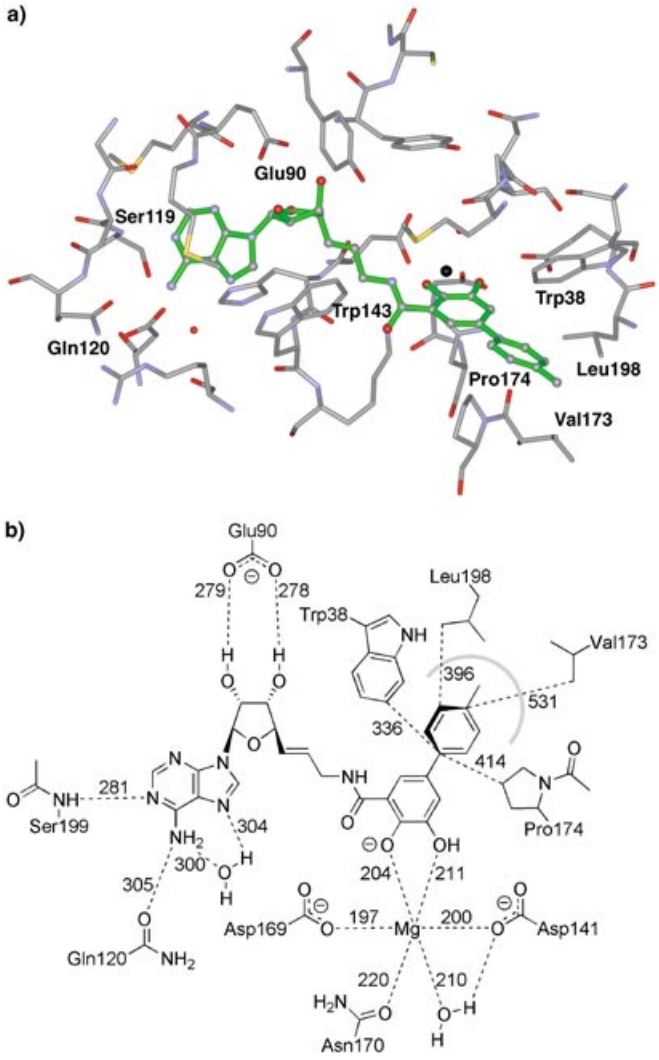



***Figure 1.*** *a) Ball-and-stick and b) schematic representation of* ***1c*** *modeled in the active site of COMT in the presence of a* $Mg^{2+}$ *ion. a) Inhibitor skeleton: green, C atoms of COMT: gray, O atoms: red, N atoms: blue, S atoms: yellow, Mg atom: black; b) distances are given in pm.*

range; this convincingly shows that the nitro group can be successfully substituted in bisubstrate inhibitors. Kinetic studies conducted to investigate the mechanism of enzyme inhibition by ligands **1a**–**t** confirmed that a competitive mechanism with respect to the SAM binding site is operative in all cases.

Table 1 also shows the Hammett substituent constants $\sigma_p$ for residues R introduced at position 5 of the catechol moiety, as well as the p$K_a$ values[20] measured for the most acidic catechol OH group of the inhibitors. The data clearly reveal that no linear free energy relationships (LFERs) exist between the free enthalpy of inhibition ($\Delta G_{inh}$)[21] and the Hammett parameters or the p$K_a$ values. In other words, the electron-withdrawing capacity of the substituent R and the acidity of the catechol OH group *para* to this substituent, are not the only determinants of the relative potencies of inhibition displayed by **1a**–**t**. On the contrary, the 4-methylphenyl-substituted inhibitor **1c** is a $\sigma$ donor ($\sigma_p = -0.03$) and possesses one of the highest p$K_a$ values (6.87) in the series; yet its affinity is one of the highest ($IC_{50} = 23$ nM) and approaches that of the nitro derivative **1a** ($IC_{50} = 9$ nM, $\sigma_p = 0.78$, p$K_a = 4.42$).

The data in Table 1 provide additional useful pieces of information for the design of future generations of COMT inhibitors. The strong inhibition by **1b**–**g** ($IC_{50}$ 21–29 nM) with residues R that are weak $\sigma$ acceptors but feature large hydrophobic surfaces demonstrates the efficiency of favorable apolar interactions of these residues in the hydrophobic cleft formed by Trp38, Leu198, Val173, and Pro174 (see Figure 1).[22] These lipophilic bonding interactions clearly compensate for losses in binding free enthalpy resulting from the increase in p$K_a$ values of the catechol OH groups. In the absence of suitable lipophilic residues, however, as in **1t** (R = H, $IC_{50} = 2600$ nM, p$K_a = 7.37$), binding affinity deteriorates. While lipophilic residues R might simply contribute to a better affinity by ensuring a more favorable partitioning of the inhibitor between water and protein, an enhanced binding strength is only observed in the case of proper molecular recognition of the residue in the hydrophobic cleft; inhibitors **1q** ($IC_{50} = 608$ nM) and **1r** ($IC_{50} = 1370$ nM) possess large hydrophobic residues, yet their activity is low. According to the modeling studies, the fit of the *p*-toluenesulfonyl (in **1p**) and *p*-toluenemethyl (in **1q**) residues to the hydrophobic cleft is less favorable. Both experimental and modeling data suggest that aromatic substituents connected to the catechol through a biaryl-type linkage (**1b**–**e**, **1g**), represent some of the best replacements for the nitro group.

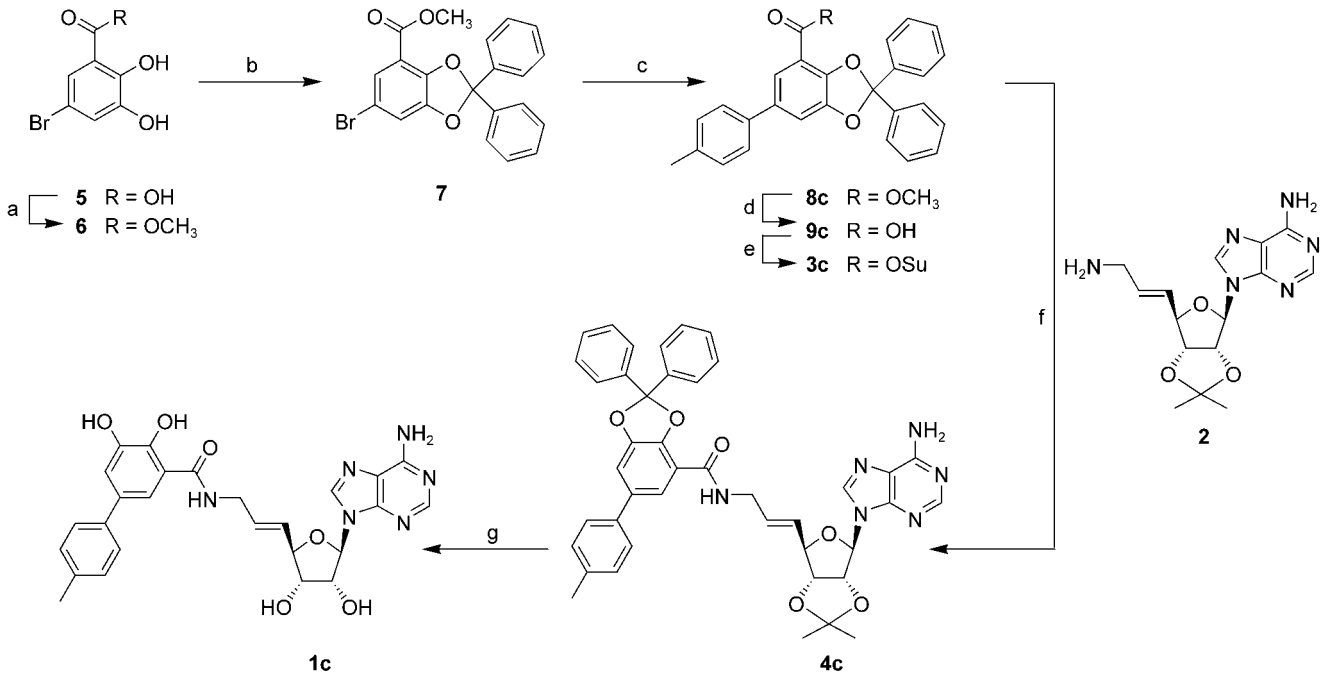
Some of the inhibitors show good activities ($IC_{50}$ between 35 and 42 nM) by benefiting from both the hydrophobic character and the $\sigma$-acceptor capacity of their substituent at position 5 of the catechol moiety (e.g. **1i**, **1j**, **1k**, and **1l**). Remarkably, others with similar acceptor capacity do not (e.g. *N,N*-dimethylacetamido-substituted **1s**; $IC_{50} = 2000$ nM). Good binding is also observed for small electron-withdrawing substituents such as Br (**1f**), CN (**1h**), and Cl (**1m**).

In conclusion, the bisubstrate inhibition approach has, for the first time, provided a family of inhibitors for the enzyme catechol *O*-methyltransferase (COMT) that do not require a nitrocatechol core for high binding activity. Efficient inhibition was achieved through analysis and exploitation of structural information, according to molecular-recognition principles, followed by convenient synthesis. Further in vitro and in vivo studies are underway to evaluate the pharmacokinetic properties of the new inhibitors, the optimization of which could ultimately lead to new therapeutical entities for Parkinson's disease and possibly other CNS disorders, such as schizophrenia. We are at the same time focusing on structural variations of the ribose and nucleobase moieties for a next generation of bisubstrate inhibitors, and are exerting efforts to further validate the proposed binding modes by crystal structure analysis.

## Acknowledgements

*We thank F. Hoffmann–La Roche, Basel, for generously supporting this work. We are grateful to Dr. B. Wagner and Dr. M. Kansy for the measurement of p$K_a$ values.*




***Scheme 1.*** *Synthesis of inhibitor* ***1c.*** *Reagents and conditions: a) $SOCl_2$, MeOH, 12 h, reflux, 91%; b) $Ph_2CCl_2$, 40 min, 160°C, 76%; c) 4-methylphenylboronic acid, $[Pd(PPh_3)_4]$, $Na_2CO_3$, benzene/EtOH/$H_2O$, 12 h, reflux, 78%; d) LiOH, THF/$H_2O$, 4 h, reflux, 89%; e) N-hydroxysuccinimide (HOSu), EDC·HCl, $CH_2Cl_2$, 1.5 h, 20°C; f)* ***2****, $CH_2Cl_2$, 12 h, 20°C, 57% (two steps); g) TFA/$H_2O$ (1:1), 1 h, 20°C, 56%. THF = tetrahydrofuran, EDC·HCl = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, TFA = trifluoroacetic acid.*

**Keywords:** bisubstrate inhibitors · medicinal chemistry · structure–activity relationships · structure-based design · transferases


[1] a) J. Axelrod, R. Tomchick, *J. Biol. Chem.* **1958**, *233*, 702–705; b) H. C. Guldberg, C. A. Marsden, *Pharmacol. Rev.* **1975**, *27*, 135–206; c) P. B. Molinoff, J. Axelrod, *Annu. Rev. Biochem.* **1971**, *40*, 465–500; d) For a crystal structure of COMT, see: J. Vidgren, L. A. Svensson, A. Liljas, *Nature* **1994**, *368*, 354–358.

[2] a) P. T. Männistö, I. Ulmanen, K. Lundström, J. Taskinen, J. Tenhunen, C. Tilgmann, S. Kaakkola, *Prog. Drug Res.* **1992**, *39*, 291–350; b) P. T. Männistö, S. Kaakkola, *Pharmacol. Rev.* **1999**, *51*, 593–628; c) M. J. Bonifácio, M. A. Vieira-Coelho, P. Soares-da-Silva, *Eur. J. Pharmacol.* **2003**, *460*, 163–170; d) P. N. Palma, M. J. Bonifácio, A. I. Loureiro, L. C. Wright, D. A. Learmonth, P. Soares-da-Silva, *Drug Metab. Dispos.* **2003**, *31*, 250–258; e) M. J. Bonifácio, M. Archer, M. L. Rodrigues, P. M. Matias, D. A. Learmonth, M. A. Carrondo, P. Soares-da-Silva, *Mol. Pharmacol.* **2002**, *62*, 795–805.

[3] a) D. R. Weinberger, M. F. Egan, A. Bertolino, J. H. Callicott, V. S. Mattay, B. K. Lipska, K. F. Berman, T. E. Goldberg, *Biol. Psychiatry* **2001**, *50*, 825–844; b) J. J. McCarthy, *Trends Biotechnol.* **2001**, *19*, 283–284; c) S. Shifman, M. Bronstein, M. Sternfeld, A. Pisanté-Shalom, E. Lev-Lehman, A. Weizman, I. Reznik, B. Spivak, N. Grisaru, L. Karp, R. Schiffer, M. Kotler, R. D. Strous, M. Swartz-Vanetik, H. Y. Knobler, E. Shinar, J. S. Beckmann, B. Yakir, N. Risch, N. B. Zak, A. Darvasi, *Am. J. Hum. Gen.* **2002**, *71*, 1296–1302.

[4] a) O. Hornykiewicz, *Wien. Klin. Wochenschr.* **2001**, *133*, 855–862; b) J. G. Nutt, J. H. Fellman, *Clin. Neuropharmacol.* **1984**, *7*, 35–49.

[5] J. Borgulya, H. Bruderer, K. Bernauer, G. Zürcher, M. Da Prada, *Helv. Chim. Acta* **1989**, *72*, 952–968.

[6] E. Nissinen, I.-B. Linden, E. Schultz, P. Pohto, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1992**, *346*, 262–266.

[7] a) C. W. Olanow, *Arch. Neurol.* **2000**, *57*, 263–267; b) P. Watkins, *Neurology* **2000**, *55*, 51–52.

[8] For a controversial discussion on entacapone-induced hepatoxicity, see: a) A. Fisher, J. Croft-Baker, M. Davis, P. Purcell, A. J. McLean, *Mov. Disord.* **2002**, *17*, 1362–1365; b) S. Beck, J. Hubble, K. Reinikainen, *Mov. Disord.* **2002**, *17*, 1397–1399.

[9] a) K. S. Smith, P. L. Smith, T. N. Heady, J. M. Trugman, W. D. Harman, T. L. Macdonald, *Chem. Res. Toxicol.* **2003**, *16*, 123–128. However, hepatotoxicity is believed not to be a class effect of nitrocatechols: b) D. J. Brooks, *Neurology* **2004**, *62*, S39–S46; c) L. V. P. Korlipara, J. M. Cooper, A. H. V. Schapira, *Neuropharmacology* **2004**, *46*, 562–569.

[10] a) D. Broom, *J. Med. Chem.* **1989**, *32*, 2–7; b) B. Masjost, P. Ballmer, E. Borroni, G. Zürcher, F. K. Winkler, R. Jakob-Roetne, F. Diederich, *Chem. Eur. J.* **2000**, *6*, 971–982; c) A. Radzicka, R. Wolfenden, *Methods Enzymol.* **1995**, *249*, 284–312; d) S. Hanessian, P.-P. Lu, J.-Y. Sancéau, P. Chemla, K. Gohda, R. Fonne-Pfister, L. Prade, S. W. Cowan-Jacob, *Angew. Chem.* **1999**, *111*, 3353–3356; *Angew. Chem. Int. Ed.* **1999**, *38*, 3159–3162.

[11] a) C. Lerner, A. Ruf, V. Gramlich, B. Masjost, G. Zürcher, R. Jakob-Roetne, E. Borroni, F. Diederich, *Angew. Chem.* **2001**, *113*, 4164–4166; *Angew. Chem. Int. Ed.* **2001**, *40*, 4040–4042; b) C. Lerner, B. Masjost, A. Ruf, V. Gramlich, R. Jakob-Roetne, G. Zürcher, E. Borroni, F. Diederich, *Org. Biomol. Chem.* **2003**, *1*, 42–49; c) C. Lerner, R. Siegrist, E. Schweizer, F. Diederich, *Helv. Chim. Acta* **2003**, *86*, 1045–1061.

[12] a) P. R. Gerber, K. Müller, *J. Comput.-Aided Mol. Design* **1995**, *9*, 251–268; b) Gerber Molecular Design (http://www.moloc.ch).

[13] J. Vidgren, M. Ovasaka in *Structure-Based Drug Design*, (Ed.: P. Veerapandian), Marcel Dekker, New York, **1997**, p. 343–363.

[14] a) T. Lotta, J. Vidgren, C. Tilgman, I. Ulmanen, K. Melén, I. Julkunen, J. Taskinen, *Biochemistry* **1995**, *34*, 4202–4210; b) E. Y. Lau, T. C. Bruice, *J. Am. Chem. Soc.* **1998**, *120*, 12387–12394; c) A. J. Tervo, T. H. Nyrönen, T. Rönkkö, A. Poso, *J. Comput.-Aided Mol. Des.* **2003**, *17*, 797–810.

[15] C. Hansch, A. Leo, R. W. Taft, *Chem. Rev.* **1991**, *91*, 165–195.

[16] All new compounds were fully characterized by $^1H$ and $^{13}C$ NMR spectroscopy, FTIR spectroscopy, EI- or MALDI-MS, and elemental analysis or high-resolution MS. Further details and synthetic procedures will be published as part of a forthcoming full paper.

[17] For the use of diphenylmethylketal moieties as catechol-protecting groups, see for example: a) M. D. Bachi, J. Klein, *J. Chem. Soc. Perkin Trans. 1*, **1983**, *8*, 1925–1928; b) M.-G. Baudart, L. F. Hennequin, *J. Antibiot.* **1993**, *46*, 1458–1470.

[18] Y. Huang, R. R. Luedtke, R. A. Freeman, L. Wu, R. H. Mach, *Bioorg. Med. Chem.* **2001**, *9*, 3113–3122.

[19] G. Zürcher, M. Da Prada, *J. Neurochem.* **1982**, *38*, 191 – 195. Enzyme preparations were preincubated for 15 min at 37 °C with inhibitor concentrations varying from $10^{-4}$ to $10^{-9}$ mol $L^{-1}$. The reaction was started by addition of substrate (benzene-1,2-diol, 0.025 mol $L^{-1}$), [$^1$H]/[$^3$H]SAM, and deionized $H_2O$, reaching a final substrate concentration of 2.5 mM and a final [$^1$H]/[$^3$H]SAM concentration of 183 μM. After the vials had been incubated in a water-bath at 37 °C for 15 min, the reaction was stopped by the addition of acetic acid (5.7 %) containing guaiacol (0.1 g $L^{-1}$).

[20] For a detailed protocol of the p$K_a$ determination, see: J. Olsen, P. Seiler, B. Wagner, H. Fischer, T. Tschopp, U. Obst-Sander, D. W. Benner, M. Kansy, K. Müller, F. Diederich, *Org. Biomol. Chem.* **2004**, *2*, 1339 – 1352.

[21] $\Delta G_{inh} = -RT \ln(c^\circ/IC_{50})$, where $c^\circ$ = standard concentration, 1 M.

[22] In the crystal, tolcapone exhibits a different orientation of the 4-methylphenyl substituent, as verified by X-ray structure analysis of the quaternary complex formed between COMT, adenosylornithine (sinefungin), tolcapone, and a $Mg^{2+}$ ion; F. K. Winkler, unpublished results.

Received: March 30, 2004