Synthesis of 1-(3,4-Dihydroxy-5-nitrophenyl)-2-phenyl-ethanone and Derivatives as Potent and Long-Acting Peripheral Inhibitors of Catechol-*O*-methyltransferase

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A homologous series of novel nitro-catechol structures (**7a**−**7e**) were synthesized and tested as inhibitors of the enzyme catechol-*O*-methyltransferase (COMT). Increasing chain length was found to have significant impact on both brain penetration and duration of COMT inhibition in the rat. Of this series, compound **7b** (1-(3,4-dihydroxy-5-nitrophenyl)-2-phenyl-ethanone) was found to exhibit the most potent and selective inhibition of peripheral COMT, with an inhibition profile more similar to entacapone **2** than tolcapone **1** (an equipotent peripheral and central inhibitor) but with much improved duration of action (**7b**, 70% inhibition and **2**, 25% inhibition at 9 h after administration). The effects of structural modifications to **7b** on COMT inhibitory profile were investigated, and it is concluded that the carbonyl group and preferably unsubstituted aromatic ring are essential features to maintain prolonged peripheral COMT inhibition. The introduction of the α-methylene group, the major structural difference between **7b** and **1**, would appear responsible for the observed enhancement in selectivity of peripheral COMT inhibition of **7b**, which has more limited access to the brain than **1**.

Introduction

The enzyme-catalyzed *O*-methylation of catechol-based neurotransmitters was reported as early as 1958[1] and attributed to catechol-*O*-methyltransferase (COMT),[2] a magnesium-dependent enzyme found in both cerebral tissues and the central nervous system (CNS). COMT catalyzes the transfer of a methyl group from its cofactor *S*-adenosyl-L-methionine (AdoMet) to one hydroxyl group of a catecholic substrate via an S_N2-type reaction, giving rise to *O*-methylated products and *S*-adenosylhomocysteine (SAH). COMT is now known to play a key role in the inactivation of endogenous catecholamines,[3] catechol estrogens,[4] and the detoxification of several xenobiotic catechols.[5−7] Accordingly, medicinal chemists over the last 20 years have been greatly interested in exploring the design and clinical potential of inhibitors of the COMT enzyme.[8,9]

Development of such inhibitors has been accelerated mainly on the premise that inhibition of COMT may provide therapeutic benefit to patients afflicted by Parkinson's disease (PD), a dopamine deficiency disorder resulting from the degeneration of striatal dopaminergic neurons. Orally administered L-Dopa (3-(3,4-dihydroxyphenyl)-L-alanine), conceived as a dopamine precursor able to cross the blood-brain barrier (BBB), continues as standard therapy for PD,[10,11] in conjunction with a peripherally acting aromatic amino acid decarboxylase (AADC) inhibitor such as carbidopa or benserazide[12,13] (also inaccessible to the brain) to prevent breakdown of L-Dopa in the periphery. Enzymatic decarboxylation of L-Dopa successfully reaching the brain (AADC) permits an 'artificial' increase of dopamine levels. The rationale for the use of COMT inhibitors is based on their capacity to inhibit the *O*-methylation of L-Dopa to 3-*O*-methyl-L-Dopa (3-OMD), the major metabolic deactivation pathway of L-Dopa in the periphery when administered with an AADC inhibitor.[14−16] 3-OMD, which offers no therapeutic effect and a long elimination half-life compared to L-Dopa, accumulates in plasma and tissues and competes with L-Dopa for transport across the BBB into the brain. In fact, a close relationship between accumulation of 3-OMD and the 'end-of-dose' or 'wearing-off' syndrome has been described.[17] In principle therefore, COMT inhibition should improve L-Dopa/AADC inhibitor therapy namely by reducing metabolic formation of 3-OMD—increasing the bioavailability of L-Dopa to enter the brain would extend the duration of antiparkinsonian action and permit dosage lowering and/or increasing the time interval between doses.

The so-called 'first-generation' inhibitors[3,18] such as pyrogallol, tropolone, and gallic acid, which besides being toxic and lacking potency with inhibition constants (K_i) in the micromolar range, are themselves competitive substrates for COMT. It was subsequently discovered that incorporation of the strongly electronegative nitro group to catechol-containing molecules gave rise to highly potent and tight-binding inhibitors of COMT, which were in addition very poor substrates for the enzyme.[19−21] The electron-withdrawing nitro group could be envisaged to both stabilize the ionized catechol−COMT complex and to reduce the nucleophilicity of the hydroxyl oxygen normally prone to undergo methylation. Typical second-generation inhibitors con-

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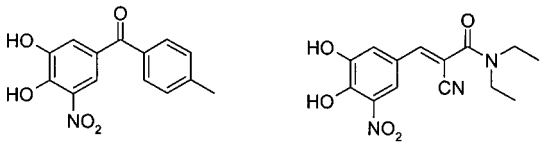


1 **2**

Figure 1. Chemical structures of tolcapone (**1**) and entacapone (**2**).

taining the 3-nitrocatechol motif such as tolcapone[22] **1** and entacapone[23] **2** (Figure 1) are very potent with inhibition constants in the low nanomolar range and were introduced into clinical practice as adjuncts to L-Dopa therapy for PD. Due to liver toxicity concerns however (several cases of fatal fulminant hepatitis were reported[24−26]), **1** was recently withdrawn from the market.

The subject of selectivity of COMT inhibition remains controversial, selectivity being defined in terms of the predominance of peripheral over central COMT inhibition. Compound **1**, a more potent inhibitor of peripheral COMT, also penetrates into the brain to inhibit cerebral COMT. Conversely, **2** is considered a purely peripheral COMT inhibitor. This distinction is of key importance − while COMT inhibitors penetrating the brain may theoretically have the benefits of decreasing dopamine methylation, indeed central inhibition may be unimportant if the more significant action is to protect L-Dopa from breakdown in the periphery. Furthermore, COMT inhibitors with limited access to the brain may avoid undesired side-effects of these agents. It is interesting to highlight both the lack of antiparkinsonian action of tolcapone when administered alone,[27] and the relatively frequent observations of increased dopaminergic stimulation expressed as dyskinesia and confusion in patients taking both L-Dopa and tolcapone.[28] Such observations lead to the conclusion that COMT inhibitors entering the brain have minimal beneficial effect alone and, when administered together with L-Dopa, may provoke serious symptoms requiring cessation of therapy.

A further concern with established COMT inhibitors concerns their relatively short half-life[29,30] (**1**, 2 h and **2**, 0.3 h) such that recommended doses of **2** are twice that of **1** and in either case are administered up to thrice daily. To date, the development of a potent, long-acting, and selective inhibitor of peripheral COMT as an adjunct to L-Dopa/AADC inhibitor therapy for the treatment of PD remains an unfulfilled objective.

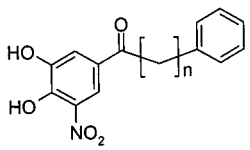
Our initial strategy therefore was to synthesize a preliminary restricted series of homologous nitro-catechol compounds in order to ascertain the effect of chain length (increasing lipophilicity) on potency, duration, and selectivity of COMT inhibition. One compound from this series uniquely displayed potent, selective, and long-acting inhibition of peripheral COMT. This lead structure was modified, and the effects on COMT inhibition in terms of peripheral selectivity and duration of action were determined.

Chemistry

The initial nitro-catechol homologues (**7a**−**7e**, Table 1) were prepared according to Scheme 1. Protection of the hydroxyl group of commercially available vanillin (**3**, R = H) as the benzyl ether (**3**, R = Bn; BnBr, K_2CO_3, acetone, Δ, 96%) and subsequent reaction with the appropriate preformed arylalkyl Grignard reagents ($Ar(CH_2)_nMgX$, n = 0−4) furnished the alcohols (**4a**−**4e**, R = Bn, n = 0−4, 87−94%). Oppenauer oxidation using sodium *tert*-butoxide as base and cyclohexanone as hydrogen acceptor in warm toluene proved quicker and more expedient than Jones oxidation under PTC conditions ($Na_2Cr_2O_7$, H_2SO_4, CH_2Cl_2, H_2O, $Bu_4N^+Br^-$) both in terms of ease of product isolation and yields (ketones **5a**−**5e**, R = Bn, 87−94%). Selective deprotection of the benzyl-protecting groups proceeded smoothly under acidic conditions (excess 30% HBr in acetic acid, CH_2Cl_2, room temperature, 2 h (75−87%)) or more conveniently by catalytic hydrogen transfer using ammonium formate as hydrogen donor and palladium catalysis to provide the phenols (**5a**−**5e**, R = H, 84−92%), which underwent regioselective nitration in moderate to good yields under mild conditions (70% HNO_3, AcOH) to **6a**−**6e** (67−77%). Although methyl ether cleavage proceeded under standard forcing conditions (48% HBr, AcOH, Δ), these rather harsh conditions and only moderate yields prompted us to develop an improved method for the demethylation of nitro-catechol methyl ethers using aluminum chloride and pyridine in warm ethyl acetate,[31] which furnished the target compounds (**7a**−**7e**, Table 1) in excellent yields (90−99%) and purity (>99%, HPLC).

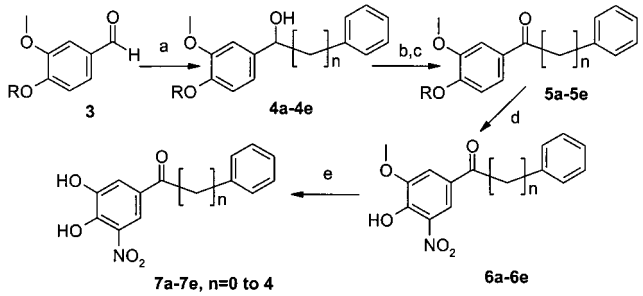
Structural modifications considered for the ethanone **7b**, identified from the preliminary series, are outlined in Figure 2. The 3,4-dihydroxy-5-nitrophenyl group as mentioned earlier is essential for high activity, so our initial focus centered on the carbonyl group. Catalytic

Table 1. Physical Constants for Homologous Nitro-catechols **7a**−**7e**



compd	n	mp (°C)	recryst. solvent	formula	anal.
7a	0	134−136	EtOH	$C_{13}H_9NO_5$	C, H, N
7b	1	178−179	EtOH	$C_{14}H_{11}NO_5$	C, H, N
7c	2	131−132	Et_2O/PE	$C_{15}H_{13}NO_5$	C, H, N
7d	3	123−125	CH_2Cl_2/heptane	$C_{16}H_{15}NO_5$	C, H, N
7e	4	109−111	CH_2Cl_2/heptane	$C_{17}H_{17}NO_5$	C, H, N

Scheme 1. Synthesis of Homologous Nitro-catechol Series[a]



[a] Reagents: (a) i. $Ar(CH_2)nMgX$, ii. H_3O^+, (87−94%); (b) NaO^t-Bu, cyclohexanone, $PhCH_3$, Δ, (87−94%); (c) 10% Pd/C, $NH_4^+HCO_2^-$, MeOH, Δ, (84−94%); (d) 70% HNO_3, AcOH, (67−77%); (e) $AlCl_3$, pyridine, EtOAc, Δ, (90−99%).

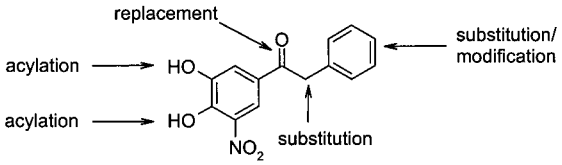


Figure 2. Structural modifications to lead structure **7b**.

hydrogenation of benzyl protected alcohol **4b** (R = Bn, n = 1) under acidic conditions allowed concomitant removal of both benzyl ether and benzylic hydroxyl groups, and subsequent nitration (100% HNO_3/Et_2O) and demethylation step e outlined in Scheme 1 afforded deoxy analogue **8**. Direct oximation of **7b** with hydroxylamine hydrochloride gave **9** while standard procedures afforded aza-derivatives **10**−**14** (Table 2) in generally moderate yields.

Modification of the hitherto unsubstitued aryl ring was achieved via Friedel−Crafts acylation of guaiacol (2-methoxyphenol) with an appropriate arylacetic acid in a zinc chloride−phosphorus oxychloride mixture[32] (Scheme 2), which gave access to the intermediate ketones of general structure **15** albeit in generally low yields (25−38%). Only marginally better yields were obtained with the same reagents using boron trifluoride as both solvent and catalyst for the Friedel−Crafts reaction.[33] Subsequent nitration and demethylation steps afforded the target compounds **16**−**23** (Table 3).

Introduction of strongly electronegative substituents to this aryl ring was achieved by direct nitration of the diacetate **24** derived from **7b** (Ac_2O, H_2SO_4, 77%) which afforded a separable mixture of 4′- and 2′-nitro isomers **25** and **26** (ratio ∼ 1:1, 45% combined yield). Construction of the skeleton of the 2′-carboxyl derivative **27** was achieved by lateral lithiation of *o*-toluic acid and reaction with methyl vanillate.[34] Exposure of **27** to warm acid effected cyclization to **28** (both Scheme 3).

Finally, attention was turned to the free α-methylene group of ethanone **7b**, which was changed by the introduction of alkyl substituents via either mono- or dialkylation of the benzyl protected intermediate ketone **5b** (n = 1) using sodium hydride and an alkyl halide (Scheme 4). Subsequent elaboration afforded the α-alkylated nitro-catechol derivatives, which were more conveniently isolated and purified as their diacetates **29**−**32** (Table 4). Direct bromination of **7b** using phenyltrimethylammonium tribromide in THF afforded bromo-derivative **33** while the α-phenyl analogue **34** was conveniently obtained by reaction of guaiacol with diphenylacetic acid under conditions described in Scheme 2.

Results and Discussion

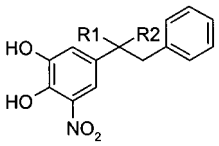
Initial experiments were designed to evaluate the effect of increasing chain length of new compounds **7a**−**7e** on both duration of inhibition and brain access and comparison with standards **1** and **2**. Test compounds were administered by gastric tube to overnight fasted rats. Thereafter at defined intervals, the animals were killed by decapitation, and the livers and brains were removed and used to determine COMT activity (see Experimental Section). Compounds **7a**−**7e** were found to rapidly achieve maximal inhibitory effect (within 30 min) after oral administration. The time course inhibition profiles differed significantly, however (Table 5). Compound **7a** presented an almost identical inhibitory profile in brain and liver COMT (very similar to that of **1**), whereas longer chained **7c**−**7e** were noticeably more potent inhibitors of liver rather than brain COMT after only 3 h. Similarly at 6 and 9 h, compound **7b** was distinctly more potent as a peripheral COMT inhibitor, and notably endowed with a particularly long duration of action, such that 70% inhibition of liver COMT was still attained even at 9 h. These results demonstrate that increasing chain length has a definite effect on the selectivity of COMT inhibition and imply that the introduction of the α-methylene group, the major structural difference between **7b** and **1**, is most probably responsible for the selectivity enhancement of peripheral COMT inhibition observed for **7b**.

Figure 3 clearly highlights differences in inhibitory profile between **7b** and standards **1** and **2**. As indicated earlier, **1** produces almost equipotent inhibition of both liver and brain COMT at 6 and 9 h after administration. Although both **2** and **7b** possess similar central COMT inhibition profiles, **2** is characterized by markedly lower peripheral COMT inhibitory activity at 9 h (only 25%) compared to **7b**.

In vitro COMT inhibition studies were then carried out to evaluate and compare the potency of compounds **1**, **2**, and **7b**. Incubation of liver and whole brain homogenates in the presence of increasing concentrations of adrenaline resulted in a concentration-dependent formation of metanephrine, yielding K_m (in μM) and V_{max} (in nmol mg protein^{-1} h^{-1}) values of 0.7 (0.5, 0.9; 95% confidence intervals) and 1.31 ± 0.02 for brain and 238.5 (128.5; 348.5) and 61.6 ± 3.8 for liver, respectively. From these kinetic parameters, a saturating concentration of adrenaline was chosen to use in inhibition studies (liver, adrenaline = 1000 μM; brain, adrenaline = 100 μM). The protein content was similar in all samples (approximately 5 mg/500 μL homogenate). Compounds **1**, **2**, and **7b** produced a concentration-dependent decrease in the *O*-methylation of adrenaline with IC_{50} values in the low nanomolar range for the brain and in the micromolar range for the liver (Table 6). Interestingly, **7b** displayed, along with **1**, potent inhibition of both cerebral and peripheral COMT (thereby implying that **7b** is a potent inhibitor of both forms of COMT), whereas **2** was endowed with noticeably lower potency. Confirmation of the reduced potency of **2** compared to **1** and **7b** came from further in vitro testing (SK-N-SH cell monolayers, Table 7).

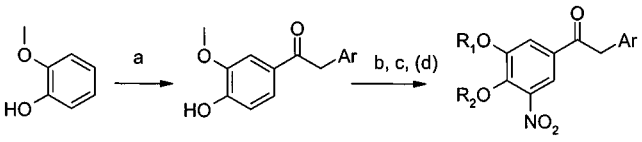
The in vivo inhibitory potency of **1**, **2**, and **7b** was evaluated in experiments in which rats were given increasing doses of the compounds (0.3 to 30 mg/kg). Animals were killed 1 h after administration, and COMT activity was determined. The results (Figure 4) show that **1** and **7b** are essentially equipotent inhibitors of peripheral COMT with ED_{50}'s of 0.7 ± 1.1 and 0.7 ± 0.1 mg/kg, respectively; **2** again was less potent with an ED_{50} value of 1.9 ± 0.2 mg/kg. Significantly, however, **7b** was less potent than **1** at inhibiting cerebral COMT with ED_{50}'s of 5.3 ± 1.1 and 1.6 ± 0.1 mg/kg, respectively (**2** failed to reach the 50% inhibition level). Taken together, these results indicate that the observed selectivity of peripheral COMT inhibition may be attributed to the fact that **7b** (unlike **1**) has limited access to the brain.

Table 2. Modified Carbonyl Derivatives of **7b**



compd	R1, R2	mp (°C)	cryst. solvent	formula	anal.
8	H, H	108−110	Et_2O/PE	$C_{14}H_{13}NO_4$	C, H, N
9	=N-OH	135−139	CH_2Cl_2/PE	$C_{14}H_{12}N_2O_5 \cdot 0.2H_2O$	C, H, N
10	=N-NH-Ph	198−200	AcOH	$C_{20}H_{17}N_3O_4$	C, H, N
11	=N-NH-Ph-(4-CF_3)	173−175	AcOH	$C_{21}H_{16}N_3F_3O_4$	C, H, N
12	=N-NH-Ph-(4-NO_2)	216−217	AcOH	$C_{20}H_{16}N_4O_6$	C, H, N
13	=N-NH-CO-Ph	238−240	AcOH	$C_{21}H_{17}N_3O_5$	C, H, N
14	=N-NH-CO-Ph-(4-Cl)	255−257	AcOH	$C_{21}H_{16}N_3ClO_5$	C, H, N

Scheme 2. Modification of Unsubstituted Aryl Ring of **7b**[a]



15 **16-23**

[a] Reagents: (a) $ArCH_2CO_2H$, $ZnCl_2$, $POCl_3$, 80 °C, (25−38%); (b) 70% HNO_3, AcOH (65−74%); (c) $AlCl_3$, pyridine, EtOAc, Δ, (90−96%); (d) Ac_2O, H_2SO_4 (cat.), (73%).

That even simple alterations of the carbonyl group of **7b** caused marked reduction in COMT inhibition was quickly determined during in vitro screening (SK-N-SH cell monolayers, Table 7). Of the aza-compounds **10**−**14** (Table 2), only the phenyl derivative **10** retained reasonable inhibition (77%, identical to **2**) compared to **7b** (90%). Carbonyl derivatives of **10** such as **13** and **14** displayed only approximately half the activity associated with **7b**, while substitution of the aryl ring of **10** (**11**, 4-CF_3; **12**, 4-NO_2) led to a substantial two to 3-fold decrease in activity. In tandem, time course experiments confirmed that deoxy compound **8** and oxime **9** were essentially devoid of inhibition of liver COMT at only 6 and 9 h, respectively (Table 8). These results prove that additional conjugation of the 3,4-dihydroxy-5-nitrophenyl pharmacophore with the electron-withdrawing carbonyl group is absolutely necessary for high inhibition. Attempts toward crystallization and crystallographic characterization of the COMT complex with **7b** are in progress and could serve to clarify in more detail the role of the carbonyl group in enhancing COMT inhibitory activity.

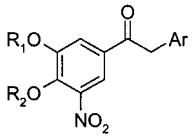
In contrast, replacement of the hitherto unsubstituted aromatic ring of **7b** with larger, more lipophilic aromatic groups was reasonably well tolerated (1- or 2-naphthyl, 4′-biphenyl), compounds **20**−**22** having very good selectivity at 6 h and moderate to high inhibition of liver COMT at 9 h (Table 8). The saturated cyclic hydrocarbon derivative **23**, however, exhibited notably higher inhibition (∼40%) of brain COMT at 6 h. Introduction of the *p*-methyl substituent to the aryl ring (as **1** possesses) gave a highly potent and selective inhibitor **17**, with an almost identical inhibition profile as **7b**. This would suggest that enzymatic hydroxylation of the aromatic ring, which normally occurs at the para position, may not be a major metabolic deactivation pathway for **7b**. The *o*-methyl analogue **16** also retained almost 50% liver inhibition at 9 h but was slightly less selective than **17** at the time points studied. Other para substituents as in the *p*-methoxy (**18**) and *p*-chloro (**19**) derivatives also gave long-lasting liver COMT inhibition (50−60%) until 9 h. At this junction, having established that potent and selective liver COMT inhibition could be achieved until at least 9 h, this time-point became our main focus of interest. Interestingly, electron-withdrawing groups in particular were very poorly tolerated (NO_2, **25** and **26**) in terms of duration of inhibition, and only carboxyl derivative **27** managed moderate, though erratic inhibition at 6 and 9 h; even this effect was virtually lost on cyclization to **28**.

Substituents at the α-methylene carbon to the carbonyl group were then investigated and also found to be poorly tolerated. Alkyl derivatives **29**−**32** were more conveniently isolated and purified as their diacetates; rapid hydrolysis of the acetate residues in vivo was expected to give origin to the corresponding nitrocatechols. Alkylation with small residues such as the α-methyl and α-dimethyl derivatives **29** and **30** was sufficient to noticeably diminish liver COMT inhibition at 9 h, an effect also seen for α-aryl and α-bromo analogues **34** and **33**, respectively, both equipotent with **29** at 9 h. The α-spiro compound **32** was thereafter unsurprisingly found as inactive as **30**. Interestingly, the bulkier benzyl derivative **31** did exhibit respectable liver COMT inhibition at 9 h (41%), but should perhaps be more correctly regarded as a propan-1-one derivative more closely related to **7c** rather than the ethanone **7b**.

Conclusions

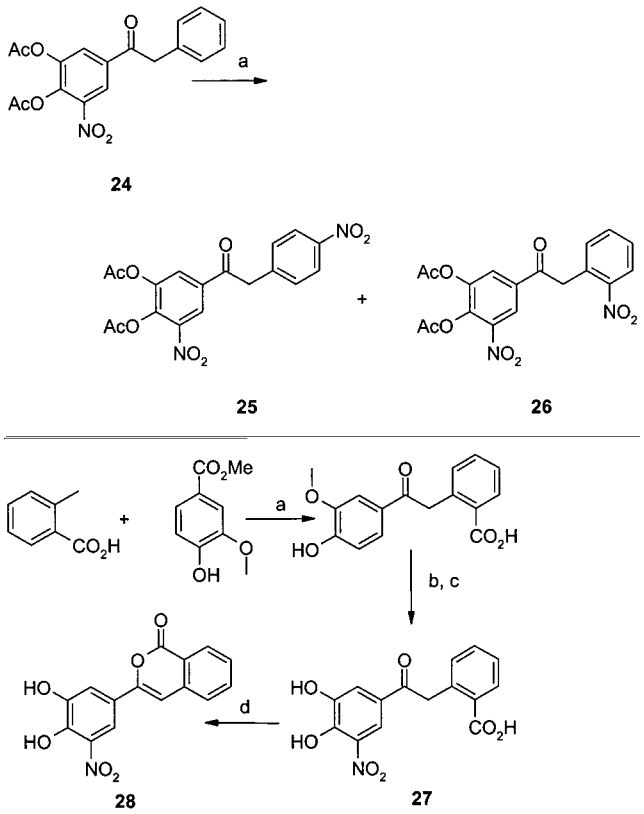
From the initial series of homologous nitro-catechol structures, increasing chain length was found to have a profound effect on the selectivity and duration of COMT inhibition. 1-(3,4-Dihydroxy-5-nitrophenyl)-2-phenyl-ethanone **7b** was found to possess the optimum combined properties of potent and selective inhibition of peripheral COMT with outstanding duration of action. A structure−activity relationship study revealed that conjugation of an unmodified carbonyl moiety with the already established 3,4-dihydroxy-5-nitrophenyl pharmacophore is absolutely essential for maintaining high and prolonged inhibitory activity. Likewise, the unsubstituted methylene carbon atom α to this carbonyl group, the major structural difference between **7b** and **1**, is strictly required to achieve selectively peripheral COMT inhibition. Prolonged and selective peripheral COMT inhibition is also dependent upon the nature of the aromatic ring of **7b**. While electron-donating sub-

Table 3. Modifications of Unsubstituted Aryl Ring of **7b**



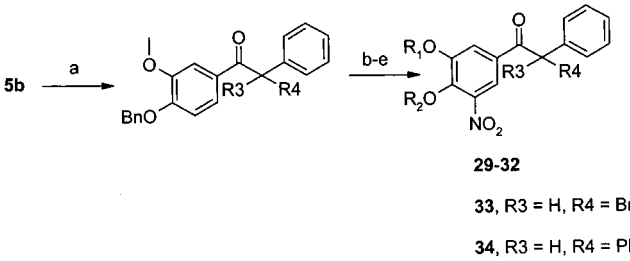
compd	R_1	R_2	Ar	mp (°C)	recryst. solvent	formula	anal.
16	H	H	Ph-(2-CH_3)	163−165	AcOH	$C_{15}H_{13}NO_5$	C, H, N
17	H	H	Ph-(4-CH_3)	189−190	AcOH	$C_{15}H_{13}NO_5$	C, H, N
18	Ac	Ac	Ph-(4-OCH_3)	88−89	EtOH	$C_{19}H_{17}NO_8$	C, H, N
19	H	H	Ph-(4-Cl)	162−164	AcOH	$C_{14}H_{10}NClO_5$	C, H, N
20	H	H	1-naphthyl	206−208	AcOH	$C_{18}H_{13}NO_5$	C, H, N
21	H	H	2-naphthyl	190−192	AcOH	$C_{18}H_{13}NO_5 \cdot 0.3H_2O$	C, H, N
22	H	H	4′-biphenyl	182−184	AcOH	$C_{20}H_{15}NO_5$	C, H, N
23	H	H	cyclohexyl	113−114	AcOH	$C_{14}H_{17}NO_5$	C, H, N
25	Ac	Ac	Ph-(4-NO_2)	140−145	CH_2Cl_2/PE	$C_{18}H_{14}N_2O_9$	C, H, N
26	Ac	Ac	Ph-(2-NO_2)	127−130	CH_2Cl_2/PE	$C_{18}H_{14}N_2O_9$	C, H, N
27	H	H	Ph-(2-CO_2H)	244−247	EtOH	$C_{15}H_{11}NO_7$	C, H, N

Scheme 3. Introduction of Electronegative Substituents to **7b**[a,b]



[a] Reagents: (a) i. 100% HNO_3, 0 °C; ii. chromatographic separation. [b]Reagents: (a) LDA, THF, −78 °C then RT, (66%); (b) 70% HNO_3, AcOH, (62%); (c) $AlCl_3$, pyridine, EtOAc, Δ, (72%); (d) AcOH, H_2SO_4 (cat.), Δ, (61%).

Scheme 4. Substitution at α-Methylene Group of **7b**[a−c]



[a] Reagents: (a) NaH, alkyl halide, DMF, (65−73%); (b) 10% Pd/C, $NH_4^+HCO_2^-$, MeOH, Δ, (82−93%); (c) 70% HNO_3, AcOH, (65−73%); (d) $AlCl_3$, pyridine, EtOAc, Δ, (90−99%); (e) Ac_2O, H_2SO_4 (cat.), (90−95%). [b]Compound **24**, $PhMe_3N^+Br_3^-$,THF, (67%). [c]As in Scheme 2 from guaiacol and Ph_2CO_2H.

stituents and *p*-alkyl substitution in particular are well tolerated, electron-withdrawing substituents lead to a striking reduction in activity and/or selectivity at 6 and 9 h after administration. However, replacement of the same ring with larger, more lipophilic aromatic rings such as naphthyl and biphenyl rings is relatively well tolerated, suggesting possibilities for further investigation.

Ethanone **7b** (BIA 3-202) presents a COMT inhibition profile more similar to entacapone **2** than tolcapone **1**, but it possesses the additional benefit of markedly prolonged duration of peripheral COMT inhibition. The peripheral selectivity of COMT inhibition is attributed to the limited access of **7b** to the brain. This compound is presently under clinical evaluation as an adjunct to L-Dopa/AADC therapy for the treatment of Parkinson's disease.

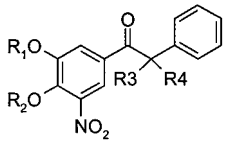
Experimental Section

Chemistry. Melting points were measured in open capillary tubes on an Electrothermal model 9100 hot stage apparatus and are uncorrected. NMR spectra were recorded on a Bruker Avance DPX (400 MHz) spectrometer with solvent used as internal standard, and data are reported in the following order: chemical shift (ppm), multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad), number of protons, approximate coupling constant in hertz, and assignment of a signal. IR spectra were measured with a Bomem Hartmann & Braun MB Series FTIR spectrometer using KBr tablets. Analytical HPLC was performed on a Gilson System equipped with a model 305 pump and 117 UV detector, LiChrospher 100 RP-18 EcoCART 125-3 Cartridges (Merck), in combination with acetonitrile/water mixtures. Analytical TLC was performed on precoated silica gel plates (Merck 60 Kieselgel F 254) and visualized with UV light. Preparative chromatography was done on Merck 60 Kieselgel (0.063−0.2 mm). Elemental analyses were performed on a Fisons EA 1110 CHNS instrument, and all analyses are consistent with theoretical values to within ±0.4% unless otherwise indicated (Tables 1−4). Solvents and reagents were purchased from Aldrich, E. Merck, and Fluka.

The following details are representative procedures for synthesis of compounds **7a**−**7e**.

4-Benzyloxy-3-methoxybenzaldehyde (3, R = Bn). To a vigorously stirred solution of vanillin (**3**, R = H, 60 g, 395 mmol) in acetone (600 mL) at room temperature was added finely powdered potassium carbonate (148 g, 1072 mmol). The

Table 4. Modifications of α-Methylene Group of **7b**



compd	R_1	R_2	R3	R4	mp (°C)	recryst. solvent	formula	anal.
29	Ac	Ac	H	CH_3	110−111	EtOH	$C_{19}H_{17}NO_7$	C, H, N
30	Ac	Ac	CH_3	CH_3	116−118	EtOH	$C_{20}H_{19}NO_7$	C, H, N
31	Ac	Ac	H	CH_2Ph	126−128	EtOH	$C_{25}H_{21}NO_7$	C, H, N
32	Ac	Ac	$-(CH_2)_5-$		166−168	EtOH	$C_{23}H_{23}NO_7$	C, H, N
33	Ac	Ac	H	Br	124−126	Et_2O	$C_{18}H_{14}NBrO_7$	C, H, N
34	H	H	H	Ph	204−205	AcOH	$C_{20}H_{15}NO_5$	C, H, N

Table 5. Percent Inhibition of COMT Activity by Compounds **7a**−**7e**, Tolcapone **1**, and Entacapone **2** in Homogenates of Rat Brain and Liver, Determined at 0.5, 1, 3, 6, and 9 h after Their Administration (all at 30 mg/kg) by Gastric Tube[a]

Brain Time Course % Inhibition					
no.	0.5 h	1 h	3 h	6 h	9 h
7a	96 ± 1	96 ± 1	97 ± 1	86 ± 7	35 ± 6
7b	84 ± 1	81 ± 3	65 ± 4	32 ± 3	22 ± 3
7c	90 ± 1	86 ± 1	60 ± 6	33 ± 7	1 ± 5
7d	85 ± 2	69 ± 5	33 ± 4	27 ± 4	12 ± 6
7e	87 ± 1	74 ± 4	25 ± 3	−6 ± 7	−7 ± 5
2	72 ± 7	45 ± 7	30 ± 6	20 ± 7	23 ± 3
1	99 ± 1	99 ± 1	97 ± 1	86 ± 8	78 ± 2
Liver Time Course (% Inhibition)					
no.	0.5 h	1 h	3 h	6 h	9 h
7a	99 ± 1	98 ± 1	97 ± 3	81 ± 7	32 ± 6
7b	99 ± 1	97 ± 2	96 ± 1	76 ± 4	70 ± 4
7c	98 ± 1	98 ± 1	95 ± 1	71 ± 13	40 ± 11
7d	97 ± 1	95 ± 1	67 ± 8	52 ± 10	39 ± 13
7e	99 ± 1	99 ± 1	88 ± 4	36 ± 6	−4 ± 8
2	98 ± 1	96 ± 1	86 ± 2	74 ± 5	25 ± 8
1	100 ± 1	99 ± 1	98 ± 1	94 ± 1	67 ± 4

[a] Results are means ± SEM of four experiments per group.

resulting suspension was stirred at steady reflux for 2 h and then allowed to cool to room temperature. The inorganic material was removed by filtration, and the filter cake was washed by acetone (100 mL). Evaporation of the solvent (40 °C, water aspirator pressure) left an oily residue, which was crystallized from a diethyl ether/petroleum ether mixture (500 mL, 1:2) to afford glistening white crystals (88.4 g, 93%) of mp 61−63 °C. ^{1}H NMR ($CDCl_3$) δ 9.86 (s, 1H, CHO), 7.5−7.3 (m, 7H, H-2, H-6, Ph), 7.01 (d, 1H, J = 8.2 Hz, H-5), 5.27 (s, 2H, CH_2), 3.97 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$) δ 191.5, 154.2, 150.6, 136.6, 130.8, 129.3, 128.8, 127.8, 127.2, 112.9, 109.8, 71.4, 56.6.

1-(4-Benzyloxy-3-methoxyphenyl)-2-phenyl-ethanol (4b, R = Bn, *n* = 1). In a flame-dried three-necked (500 mL) round-bottomed flask flushed by a stream of nitrogen was placed oven-dried magnesium turnings (8.3 g, 341 mmol) and a single crystal of iodine. Anhydrous diethyl ether (Aldrich, 75 mL) was added, followed by a few drops of neat benzyl chloride. On discoloration of the yellow color, stirring was started and a solution of benzyl chloride (35.7 g, 282 mmol) in anhydrous diethyl ether (150 mL) was added so as to maintain steady reflux. When the exothermic reaction subsided, the mixture was cooled in an ice−water bath and a solution of the aldehyde **3** (54.5 g, 225 mmol) in anhydrous tetrahydrofuran (Aldrich, 200 mL) was added dropwise. The resulting mixture was allowed to stir at room temperature for 1 h, then cooled again and quenched by careful addition of excess 2 N hydrochloric acid. Unreacted magnesium was removed by filtration, and the organic phase of the filtrate separated. The aqueous phase was extracted by diethyl ether (50 mL), and the combined organic layers were washed by water (100 mL) and brine (100 mL), then dried over anhydrous sodium sulfate, filtered, and

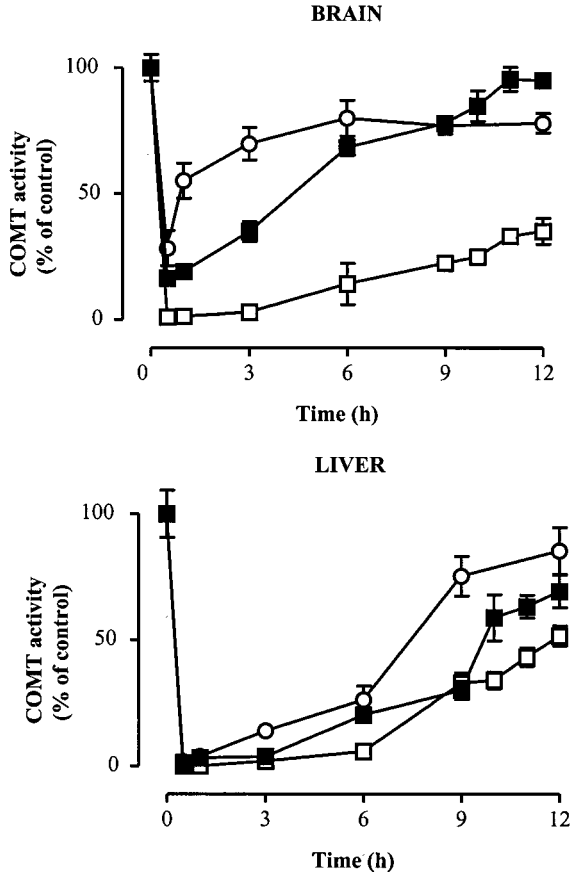


Figure 3. Brain and liver COMT activity after oral administration of **1** (open squares), **2** (open circles), and **7b** (closed squares) (all at 30 mg/kg).

Table 6. IC_{50} Values (in nM) for Inhibition of Rat Brain and Liver COMT

no.	brain	liver
1	2.2 (0.8, 6.4)	927 (551, 1561)
2	12.8 (4.0, 41.3)	2320 (741, 7263)
7b	3.7 (1.7, 8.1)	696 (356, 1360)

evaporated (40 °C, water aspirator pressure). The residue was recrystallized from a dichloromethane/petroleum ether mixture (200 mL, 1:2) to afford white crystals (70.1 g, 93%) of mp 96−98 °C. ^{1}H NMR ($CDCl_3$) δ 7.5−7.15 (m, 10H, 2 × Ph), 6.92 (d, 1H, J = 1.9 Hz, H-2), 6.87 (d, 1H, J = 8.2 Hz, H-5), 6.82 (dd, 1H, J = 1.9, 8.2 Hz, H-6), 5.17 (s, 2H, OCH_2), 4.86 (t, 1H), 3.02 (m, 2H, CH_2), 3.89 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$) δ 150.2, 148.1, 138.6, 137.7, 137.6, 130.1, 129.1, 129.1, 128.4, 127.8, 127.2, 118.6, 114.4, 110.2, 75.8, 71.7, 56.6, 46.6.

1-(4-Benzyloxy-3-methoxyphenyl)-2-phenyl-ethanone (5b, R = Bn, *n* = 1). To a stirred suspension of the

Table 7. Percent Inhibition of COMT Activity in SK-N-SH Cells by Compounds **1**, **2**, **7b,** and **10**−**14** (100 nM)[a]

compound	% inhibition
1	97 ± 1
2	77 ± 3
7b	90 ± 1
10	77 ± 3
11	38 ± 4
12	28 ± 4
13	45 ± 7
14	50 ± 2

[a] Results are means ± SEM of four experiments per group.

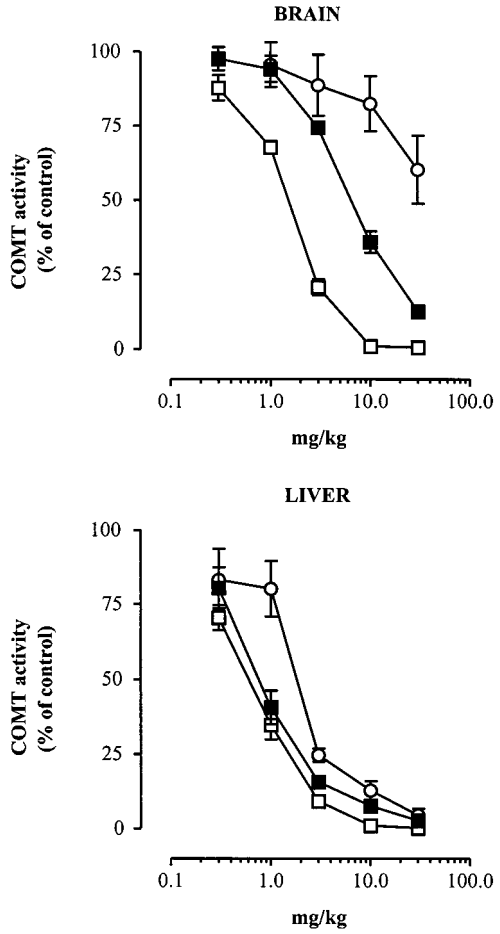


Figure 4. Concentration dependent inhibition of brain and liver COMT activity by **1** (open squares), **2** (open circles), and **7b** (closed squares) at 1 h after administration.

foregoing ethanol **4b** (70 g, 210 mmol) in toluene (600 mL) at room temperature was added sodium *tert*-butoxide (25.2 g, 262 mmol) followed by cyclohexanone (102.6 g, 1046 mmol). The resulting mixture was warmed until gentle reflux and after 30 min left to cool to 50 °C. Water (150 mL) was added slowly with stirring, and the phases were separated. The aqueous phase was extracted by ethyl acetate (100 mL), and the combined organic layers were washed by brine (100 mL) and then evaporated (60 °C, water aspirator pressure). The residue was recrystallized from 96% ethanol (200 mL) to afford off-white crystals (63.5 g, 91%) of mp 136−138 °C. ^{1}H NMR ($CDCl_3$) δ 7.61 (m, 2H, H-2, H-6), 7.5−7.2 (m, 10H, 2 x Ph), 6.91 (d, 1H, J = 8.9 Hz, H-5), 5.24 (s, 2H, OCH_2), 4.25 (s, 2H, CH_2), 3.94 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$) δ 196.7, 152.9, 149.9, 136.6, 135.4, 130.4, 129.7, 129.1, 129.1, 128.6, 127.6, 127.2, 123.7, 112.5, 111.5, 71.2, 56.4, 45.6.

1-(4-Hydroxy-3-methoxyphenyl)-2-phenyl-ethanone (5b, R = H, *n* = 1). To a stirred suspension of the benzyl ketone **5b** (60.7 g, 183 mmol) from above and ammonium formate (46.1 g, 732 mmol) in methanol (550 mL) at room temperature was added carefully a slurry of 10 % palladium on charcoal (2.5 g) in methanol (50 mL). The resulting dark suspension was gradually warmed to gentle reflux (vigorous gas evolution) and after 30 min allowed to cool to room temperature. After cooling in an ice−water bath, water (200 mL) and concentrated hydrochloric acid (60 mL) was slowly added. Further water (500 mL), followed by dichloromethane (500 mL), was added, and the mixture was filtered through a Celite pad. The filter cake was washed by dichloromethane (100 mL), and the organic phase of the filtrate was separated. The aqueous phase was extracted by dichloromethane (100 mL), and the combined organic layers were washed by water (100 mL) and brine (100 mL), then dried over anhydrous sodium sulfate, filtered, and evaporated (40 °C, water aspirator pressure). The residue was recrystallized from a dichloromethane/petroleum ether mixture (300 mL, 1:2) to give white crystals (41.6 g, 94%) of mp 106−108 °C. ^{1}H NMR ($CDCl_3$) δ 7.65 (dd, 1H, J = 2.1, 8.2 Hz, H-6), 7.59 (d, 1H, J = 2.1 Hz, H-2), 7.4−7.2 (m, 5H, Ph), 6.96 (d, 1H, J = 8.2 Hz, H-5), 6.16 (br, 1H, 4-OH), 4.26 (s, 2H, CH_2), 3.94 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$) δ 196.8, 150.9, 147.1, 135.5, 129.9, 129.7, 129.1, 127.2, 124.6, 114.2, 110.7, 56.4, 45.5.

Table 8. Percent Inhibition of COMT Activity by Derivatives of **7b** in Homogenates of Rat Brain and Liver, Determined between 6 and 9 h after Administration (all at 30 mg/kg) by Gastric Tube[a]

	liver		brain	
compd	6 h	9 h	6 h	9 h
8	0 ± 13	12 ± 3	10 ± 3	16 ± 2
9	48 ± 9	18 ± 10	13 ± 8	15 ± 2
16	74 ± 5	46 ± 11	33 ± 2	25 ± 7
17	83 ± 7	70 ± 8	23 ± 6	21 ± 9
18	72 ± 5	53 ± 3	25 ± 2	9 ± 8
19	69 ± 4	57 ± 8	12 ± 3	26 ± 2
20	70 ± 3	38 ± 4	10 ± 5	1 ± 7
21	83 ± 2	46 ± 10	17 ± 4	7 ± 3
22	78 ± 5	51 ± 4	28 ± 5	27 ± 5
23	72 ± 12	36 ± 3	39 ± 7	6 ± 3
25	49 ± 7	11 ± 10	0 ± 3	1 ± 4
26	28 ± 2	20 ± 13	20 ± 3	7 ± 3
27	39 ± 9	16 ± 5	34 ± 5	42 ± 3
28	15 ± 12	14 ± 14	29 ± 8	11 ± 2
29	42 ± 3	24 ± 8	44 ± 2	0 ± 7
30	19 ± 3	9 ± 5	23 ± 4	15 ± 1
31	63 ± 6	41 ± 2	44 ± 2	0 ± 8
32	14 ± 2	9 ± 8	19 ± 2	7 ± 2
33	48 ± 2	36 ± 8	20 ± 2	13 ± 5
34	13 ± 1	20 ± 7	7 ± 5	0 ± 1

[a] Results are means ± SEM of four experiments per group.

1-(4-Hydroxy-3-methoxy-5-nitrophenyl)-2-phenyl-ethanone (6b, *n* = 1). To a stirred semi-solution of the phenol **5b** (40 g, 165 mmol) obtained above in acetic acid (400 mL) at room temperature was added dropwise 70% nitric acid (88 mL, 177 mmol), causing formation of a deep red solution followed by a copious orange precipitate. After stirring for 30 min, the mixture was poured slowly onto stirred ice−water (1000 mL). The resulting precipitate was filtered and washed by water (200 mL). Recrystallization from ethanol (150 mL) afforded bright yellow crystals (33.6 g, 71%) of mp 129−130 °C. ^{1}H NMR ($CDCl_3$) δ 11.14 (br, 1H, 4-OH), 8.43 (d, 1H, J = 2.1 Hz, H-6), 7.79 (d, 1H, J = 2.1 Hz, H-2), 7.4−7.2 (m, 5H, Ph), 4.30 (s, 2H, CH_2), 4.00 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$) δ 195.1, 150.9, 150.5, 134.3, 133.3, 129.7, 129.3, 127.9, 127.7, 118.3, 116.3, 57.3, 45.6.

1-(3, 4-Dihydroxy-5-nitrophenyl)-2-phenyl-ethanone (7b, *n* = 1). To a stirred yellow suspension of the methyl ether **6b** (30 g, 104 mmol) obtained above in ethyl acetate (300 mL) at room temperature was added aluminum chloride (16.7 g, 125 mmol) in one portion. To the resulting orange/red suspension was added dropwise pyridine (33 g 418 mmol), causing the internal temperature to rise to 45 °C. The orange solution was then heated at reflux for 2 h and then allowed to cool to 60 °C whereupon the reaction mixture was carefully added to

a mixture of ice/concentrated hydrochloric acid (120 mL). After being stirred at 50 °C for 1 h, the mixture was cooled in an ice/water bath for 1 h and then filtered. The filter cake was washed by water (100 mL), and the product then dried (70 °C, 0.02 mmHg, 5 h) to afford the title product as a yellow solid (28.3 g, 99%). IR(KBr) 3352 (OH), 1669 (C═O), 1539 (NO_2). ^{1}H NMR (DMSO-d_6) δ 10.9 (br, 2H, 3-OH, 4-OH), 8.09 (d, 1H, J = 2.1 Hz, H-6), 7.63 (d, 1H, J = 2.1 Hz, H-2), 7.35−7.20 (m, 5H, Ph), 4.33 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ 196.0, 148.5, 146.6, 138.0, 135.9, 130.6, 129.2, 127.5, 127.4, 118.2, 117.4, 45.0.

By application of similar procedures, the following were also obtained:

1-(3, 4-Dihydroxy-5-nitrophenyl)-1-phenyl-methanone (7a, *n* = 0). IR(KBr) 3353 (OH), 1640 (C═O), 1539 (NO_2). ^{1}H NMR (DMSO-d_6) δ 10.94 (br, 2H, 3-OH, 4-OH), 7.77 (d, 2H, *o*-Ph), 7.72 (t, 1H, *p*-Ph), 7.72 (d, 1H, J = 2.1 Hz, H-6), 7.57 (t, 2H, *m*-Ph), 7.49 (d, 1H, J = 2.1 Hz, H-2). ^{13}C NMR (DMSO-d_6) δ 194.2, 148.8, 147.0, 138.0, 137.7, 133.5, 130.3, 129.6, 127.7, 120.0, 119.0.

1-(3, 4-Dihydroxy-5-nitrophenyl)-3-phenyl-propan-1-one (7c, *n* = 2). IR(KBr) 3373 (OH), 1664 (C═O), 1549 (NO_2). ^{1}H NMR ($CDCl_3$) δ 11.0 (br, 1H, 4-OH), 8.29 (d, 1H, J = 2.1 Hz, H-6), 7.86 (d, 1H, J = 2.1 Hz, H-2), 7.35−7.20 (m, 5H, Ph), 6.1 (br, 1H, 3-OH), 3.30 (t, 2H, $COCH_2$), 3.10 (t, 2H, CH_2-Ph). ^{13}C NMR ($CDCl_3$) δ 196.5, 147.2, 146.7, 141.1, 133.5, 129.3, 129.0, 128.8, 126.7, 120.6, 117.0, 40.5, 30.4.

1-(3,4-Dihydroxy-5-nitrophenyl)-4-phenyl-butan-1-one (7d, *n* = 3). IR(KBr) 3381, 3335 (OH), 1663 (C═O), 1548 (NO_2). ^{1}H NMR (DMSO-d_6) δ 10.8 (br, 2H, 3-OH, 4-OH), 7.93 (d, 1H, J = 2.1 Hz, H-6), 7.57 (d, 1H, J = 2.1 Hz, H-2), 7.35−7.10 (m, 5H, Ph), 2.97 (t, 2H, $COCH_2$), 2.63 (t, 2H, CH_2Ph), 1.89 (qui, 2H, $CH_2CH_2CH_2$). ^{13}C NMR (DMSO-d_6) δ 198.2, 148.6, 146.6, 142.8, 138.1, 129.35, 129.3, 128.0, 126.8, 118.0, 116.9, 37.8, 35.4, 26.6.

1-(3, 4-Dihydroxy-5-nitrophenyl)-5-phenyl-pentan-1-one (7e, *n* = 4). IR(KBr) 3368 (OH), 1675 (C═O), 1544 (NO_2). ^{1}H NMR ($CDCl_3$) δ 11.0 (br, 1H, 4-OH), 8.29 (d, 1H, J = 2.1 Hz, H-6), 7.85 (d, 1H, J = 2.1 Hz, H-2), 7.40−7.10 (m, 5H, Ph), 6.0 (br, 1H, 3-OH), 2.97 (t, 2H, $COCH_2$), 2.69 (t, 2H, CH_2-Ph), 1.85−1.70 (m, 4H, $CH_2CH_2CH_2CH_2$). ^{13}C NMR ($CDCl_3$) δ 197.5, 147.3, 146.7, 142.6, 133.7, 129.6, 129.0, 128.9, 126.4, 120.8, 117.1, 38.6, 36.3, 31.5, 24.3.

1-(3,4-Dihydroxy-5-nitrophenyl)-2-phenyl-ethane (8).

a. To a stirred solution of the benzyl alcohol **4b** (R = Bn, n = 1) (1 g, 3 mmol) in methanol (40 mL) at room temperature was added concentrated hydrochloric acid (1 mL) followed by 10% palladium on charcoal (0.35 g). Hydrogen gas was bubbled through the dark suspension for 2 h, and then the catalyst was removed by filtration through Celite. The filter cake was washed by methanol (10 mL), and the combined filtrate was evaporated (40 °C, water aspirator pressure) to leave a pale orange oil (0.67 g, 98%), which was used without further purification. ^{1}H NMR ($CDCl_3$) δ 7.35−7.15 (m, 5H, Ph), 6.86 (d, 1H, J = 8.2 Hz, H-5), 6.71 (dd, 1H, J = 2.1, 8.2 Hz, H-6), 6.64 (d, 1H, J = 2.1 Hz, H-2), 5.50 (br, 1H, 4-OH), 3.86 (s, 3H, OCH_3), 2.90 (m, 4H, CH_2CH_2). ^{13}C NMR ($CDCl_3$) δ 146.6, 144.1, 142.2, 134.1, 129.0, 128.7, 126.3, 121.4, 114.6, 111.5, 56.2, 38.7, 38.0.

b. A stirred and cooled (ice bath) solution of the oil from above (0.57 g, 2.51 mmol) in diethyl ether (15 mL) was treated dropwise with 100% nitric acid (0.11 mL, 2.76 mmol). The resulting deep red solution was stirred at room temperature for 1 h and then poured onto ice−water (20 mL). The mixture was extracted by diethyl ether (20 mL) and the organic phase washed by water (20 mL) and brine (20 mL), then dried over anhydrous sodium sulfate, filtered, and evaporated (40 °C, water aspirator pressure). The residue was chromatographed over silica gel (petroleum ether/ethyl acetate, 4:1) to give an orange oil (0.3 g, 44%). ^{1}H NMR ($CDCl_3$) δ 10.68 (br, 1H, 4-OH), 7.52 (d, 1H, J = 2.1 Hz, H-6), 7.4−7.1 (m, 5H, Ph), 6.80 (d, 1H, J = 2.1 Hz, H-2), 3.85 (s, 3H, OCH_3), 2.93 (m, 4H, CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 149.9, 145.1, 141.0, 134.0, 133.2, 129.0, 128.9, 126.7, 119.2, 115.4, 57.0, 37.9, 37.6.

c. A stirred solution of the orange oil (0.28 g, 1 mmol) in ethyl acetate (15 mL) was treated with aluminum chloride (0.24 g, 1.83 mmol) and pyridine (0.35 g, 4.48 mmol) as described for **7b** to give **8** as yellow crystals (0.1 g, 38%). IR-(KBr) 3385 (OH), 1551 (NO_2). ^{1}H NMR ($CDCl_3$) δ 10.51 (br, 1H, 4-OH), 7.46 (d, 1H, J = 2.1 Hz, H-6), 7.09 (d, 1H, J = 2.1 Hz, H-2), 7.35−7.15 (m, 5H, Ph), 5.80 (br, 1H, 3-OH), 2.9 (m, 4H, CH_2CH_2). ^{13}C NMR ($CDCl_3$) δ 146.6, 141.5, 141.0, 134.2, 133.7, 128.9, 128.8, 126.7, 122.7, 115.3, 37.7, 37.3.

1-(3,4-Dihydroxy-5-nitrophenyl)-1-hydroxyimino-2-phenyl-ethane (9). A stirred suspension of **7b** (0.33 g, 1.2 mmol) in absolute ethanol (6 mL) was treated with hydroxylamine hydrochloride (0.29 g, 4.2 mmol) and pyridine (0.28 g, 3.6 mmol), and the mixture was heated at reflux for 1 h. The mixture was evaporated (40 °C, water aspirator pressure), and the residue treated with water (5 mL). The precipitate was collected by filtration and recrystallized from a dichloromethane/toluene mixture to give orange crystals (0.12 g, 34%). IR(KBr) 3385 (OH), 3254 (OH, oxime), 1548 (NO_2). ^{1}H NMR ($CDCl_3$) δ 8.6 (vbr, 2H, 3-OH, 4-OH), 7.92 (d, 1H, J = 2.1 Hz, H-6), 7.62 (d, 1H, J = 2.1 Hz, H-2), 7.35−7.15 (m, 5H, Ph), 4.20 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ 156.0, 147.1, 144.3, 136.3, 134.0, 129.4, 129.1, 128.2, 127.3, 120.0, 114.7, 32.1.

1-(3,4-Dihydroxy-5-nitrophenyl)-2-phenyl-1-phenylhydrazono-ethane (10). A stirred solution of **7b** (0.2 g, 0.73 mmol) and phenylhydrazine (0.28 g, 2.6 mmol) in 96% ethanol (5 mL) and acetic acid (3 drops) was stirred at reflux for 3 h. On cooling, the orange precipitate was filtered off and recrystallized from acetic acid to give orange crystals (0.14 g, 53%). IR(KBr) 3536 (OH), 3332 (NH), 1535 (NO_2). ^{1}H NMR (DMSO-d_6) δ 10.3 (br, 2H, 3-OH, 4-OH), 9.84 (br, 1H, NH), 7.71 (d, 1H, J = 2.1 Hz, H-2), 7.58 (d, 1H, J = 2.1 Hz, H-6), 7.40−7.10 (m, 9H, 2 × Ph), 6.79 (t, 1H, NH*Ph*), 4.21 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ 148.4, 146.7, 142.7, 140.3, 137.9, 137.7, 130.3, 130.0, 129.8, 129.1, 127.3, 120.2, 117.1, 113.8, 112.5, 31.7.

By application of similar procedures, the following were also obtained:

1-(3,4-Dihydroxy-5-nitrophenyl)-2-phenyl-1-(4-trifluoromethyl)phenylhydrazono-ethane (11). Yield, 42%. IR-(KBr) 3496 (OH), 3330 (NH), 1532 (NO_2). ^{1}H NMR ($CDCl_3$) δ 10.8 (br, 1H, 4-OH), 7.98 (d, 1H, J = 2.1 Hz, H-6), 7.95 (d, 1H, J = 2.1 Hz, H-2), 7.75 (br, 1H, NH), 7.55−710 (m, 9H, 2 x Ph), 5.9 (br, 1H, 3-OH), 4.14 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ 147.4, 147.1, 143.8, 142.0, 134.5, 134.0, 131.5, 130.2, 128.3, 128.2, 127.2, 127.2, 119.3, 113.5, 113.3, 32.6.

1-(3,4-Dihydroxy-5-nitrophenyl)-1-(4-nitrophenyl)hydrazono-2-phenyl-ethane (12). Yield, 45%. IR(KBr) 3493 (OH), 3305 (NH), 1541 (broad, NO_2). ^{1}H NMR (DMSO-d_6) δ 10.7 (br, 1H, NH), 10.4 (br, 2H, 3-OH, 4-OH), 8.17 (d, 2H, NH*Ph*), 7.69 (d, 1H, J = 2.1 Hz, H-2), 7.66 (d, 1H, J = 2.1 Hz, H-6), 7.40−7.10 (m, 7H, Ph, Ph), 4.29 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ 152.1, 148.5, 146.0, 143.4, 139.8, 138.1, 137.2, 129.9, 129.1, 129.1, 127.5, 127.0, 117.5, 113.6, 113.2, 32.3.

1-(3,4-Dihydroxy-5-nitrophenyl)-1-benzoylhydrazido-2-phenyl-ethane (13) Yield, 56%. IR(KBr) 3362 (OH), 3200 (NH), 1668 (C═O), 1541 (NO_2). ^{1}H NMR (DMSO-d_6) δ 11.0 (br, 1H, NH), 10.5 (br, 2H, 3-OH, 4-OH), 7.80−7.10 (m, 12H, H-2, H-6, Ph, Ph), 4.38 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ 165.4, 153.5, 148.5, 144.0, 138.0, 137.2, 135.0, 132.6, 129.8, 129.3, 129.1, 128.9, 127.5, 118.0, 114.7, 32.9.

1-(3,4-Dihydroxy-5-nitrophenyl)-1-(4-chlorobenzoyl)-hydrazido-2-phenyl-ethane (14). Yield, 49%. IR(KBr) 3304 (OH), 1647 (C═O), 1532 (NO_2). ^{1}H NMR (DMSO-d_6) δ 11.0 (br, 1H, NH), 10.5 (br, 2H, 3-OH, 4-OH), 7.80−7.20 (m, 11H, Ph), 4.36 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ 164.4, 154.0, 144.1, 138.0, 137.2, 133.6, 130.9, 129.8, 129.3, 129.1, 128.7, 127.5, 118.0, 114.8, 33.1.

The following details are representative procedures for synthesis of compounds **16**−**23**.

1-(3,4-Dihydroxy-5-nitrophenyl)-2-(1′-naphthyl)-ethanone (20). A stirred suspension of guaiacol (1.24 g, 10 mmol), 1-naphthylacetic acid (1.86 g, 10 mmol), and zinc chloride (5 g, 36.7 mmol) in phosphorus oxychloride (24.7 g, 160 mmol)

was heated at 80 °C for 2 h and then allowed to cool. The purple mixture was carefully added to ice−water (200 mL) (CAUTION: exothermic) and then extracted by dichloromethane (2 × 50 mL). The extracts were washed by water (50 mL) and brine (50 mL), then dried over anhydrous sodium sulfate, filtered, and evaporated (40 °C, water aspirator pressure). The residue was chromatographed over silica gel (petroleum ether/ethyl acetate, 2:1) to give the intermediate ketone **15** (Ar = 1-naphthyl) as beige crystals, (1.08 g, 37%) of mp 121−122 °C. ^{1}H NMR ($CDCl_3$) δ 8.0−7.8 (m, 3H, Ar), 7.74 (dd, 1H, J = 1.8, 8.2 Hz, H-6), 7.61 (d, 1H, J = 1.8 Hz, H-2), 7.55−7.35 (m, 4H, Ph), 6.98 (d, 1H, J = 8.2 Hz, H-5), 6.11 (br, 1H, 4-OH), 4.71 (s, 2H, CH_2), 3.92 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$) δ 196.8, 151.1, 147.3, 134.5, 132.8, 132.4, 130.2, 129.4, 128.5, 128.3, 126.9, 126.3, 126.1, 124.5, 124.4, 114.4, 110.9, 56.6, 43.3.

Nitration (as described for **6b**, n = 1, 78%) and demethylation (as described for **7b**, 93%) gave the title compound **20** as yellow crystals. IR(KBr) 3376 (OH), 1669 (C═O), 1546 (NO_2). ^{1}H NMR (DMSO-d_6) δ 10.9 (vbr, 2H, 3-OH, 4-OH), 8.22 (d, 1H, J = 1.8 Hz, H-6), 8.0−7.8 (m, 3H, Ar), 7.66 (d, 1H, J = 1.8 Hz, H-2), 7.6−7.3 (m, 4H, Ar), 4.85 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ 196.2, 148.7, 146.9, 138.3, 134.4, 133.2, 133.1, 129.4, 129.3, 128.3, 127.8, 127.0, 126.6, 126.5, 125.5, 118.3, 117.5, 43.0.

By application of similar procedures the following were obtained:

1-(3,4-Dihydroxy-5-nitrophenyl)-2-(2′-methylphenyl)-ethanone (16). IR(KBr) 3365 (OH), 1669 (C═O), 1544 (NO_2). ^{1}H NMR (DMSO-d_6) δ 10.9 (vbr, 2H, 3-OH, 4-OH), 8.11 (d, 1H, J = 2.1 Hz, H-6), 7.62 (d, 1H, J = 2.1 Hz, H-2), 7.20−7.10 (m, 4H, Ph), 4.37 (s, 2H, CH_2), 2.14 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 196.0, 148.7, 146.8, 138.2, 137.9, 135.1, 131.5, 130.9, 127.9, 127.8, 126.7, 118.2, 117.3, 43.5, 20.3.

1-(3,4-Dihydroxy-5-nitrophenyl)-2-(4′-methylphenyl)-ethanone (17). IR(KBr) 3357 (OH), 1670 (C═O), 1540 (NO_2). ^{1}H NMR (DMSO-d_6) δ 10.9 (vbr, 2H, 3-OH, 4-OH), 8.07 (d, 1H, J = 2.1 Hz, H-6), 7.61 (d, 1H, J = 2.1 Hz, H-2), 7.20−7.00 (m, 4H, Ph), 4.26 (s, 2H, CH_2), 2.26 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 196.2, 148.7, 146.8, 138.2, 136.5, 132.9, 130.5, 130.0, 127.6, 118.4, 117.5, 44.8, 21.7.

1-(3,4-Diacetoxy-5-nitrophenyl)-2-(4′-methoxyphenyl)-ethanone (18). IR(KBr) 1779 (C═O, ester), 1687 (C═O, ketone), 1543 (NO_2). ^{1}H NMR ($CDCl_3$) δ 8.60 (d, 1H, J = 2.1 Hz, H-6), 8.12 (d, 1H, J = 2.1 Hz, H-2), 7.18 (d, 2H, J = 8.6 Hz, Ph), 6.9 (d, 2H, J = 8.6 Hz, Ph), 4.25 (s, 2H, CH_2), 3.81 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 194.4, 168.1, 167.4, 159.5, 145.3, 141.0, 134.8, 131.0, 129.0, 125.3, 123.6, 115.1, 55.8, 45.3, 21.1, 21.0.

2-(4′-Chlorophenyl)-1-(3,4-dihydroxy-5-nitrophenyl)-ethanone (19). IR(KBr) 3374 (OH), 1671 (C═O), 1540 (NO_2). ^{1}H NMR (DMSO-d_6) δ 10.9 (vbr, 2H, 3-OH, 4-OH), 8.09 (d, 1H, J = 2.1 Hz, H-6), 7.61 (d, 1H, J = 2.1 Hz, H-2), 7.38 (d, 2H, J = 8.3 Hz, Ph), 7.26 (d, 2H, J = 8.3 Hz, Ph), 4.37 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ 195.8, 148.7, 146.8, 138.2, 135.1, 132.7, 132.3, 129.2, 127.6, 118.2, 117.5, 44.3.

1-(3,4-Dihydroxy-5-nitrophenyl)-2-(2′-naphthyl)-ethanone (21). IR(KBr) 3383, 3260 (OH), 1684 (C═O), 1548 (NO_2). ^{1}H NMR (DMSO-d_6) δ 10.8 (vbr, 2H, 3-OH, 4-OH), 8.14 (d, 1H, J = 1.9 Hz, H-6), 8.0−7.7 (m, 4H, Ar), 7.65 (d, 1H, J = 1.9 Hz, H-2), 7.55−7.3 (m, 3H, Ar), 4.51 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ 196.2, 148.7, 146.9, 138.2, 134.0, 133.9, 132.8, 129.3, 129.1, 128.7, 128.5, 128.4, 127.7, 127.1, 126.7, 118.3, 117.6, 45.3.

2-(4′-Biphenyl)-1-(3,4-dihydroxy-5-nitrophenyl)-ethanone (22). IR(KBr) 3380 (OH), 1674 (C═O), 1554 (NO_2). ^{1}H NMR (DMSO-d_6) δ 10.9 (vbr, 2H, 3-OH, 4-OH), 8.12 (d, 1H, J = 2.1 Hz, H-6), 8.0−7.3 (m, 10H, H-2, Ar), 4.39 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ 196.1, 148.7, 146.9, 141.0, 139.5, 138.2, 135.3, 131.3, 129.9, 128.4, 127.6, 127.6, 118.4, 117.6, 44.8.

2-Cylohexyl-1-(3,4-dihydroxy-5-nitrophenyl)-ethanone (23). IR(KBr) 3361 (OH), 1669 (C═O), 1540 (NO_2). ^{1}H NMR ($CDCl_3$) δ 10.98 (br, 1H, 4-OH), 8.29 (d, 1H, J = 2.1 Hz, H-6), 7.85 (d, 1H, J = 2.1 Hz, H-2), 6.02 (br, 1H, 3-OH), 2.81 (d, 2H, J = 6.8 Hz, CH_2), 1.97 (m, 1H, CH), 1.8−1.0 (m, 10H, *c*-hexyl). ^{13}C NMR ($CDCl_3$) δ 197.5, 147.3, 146.7, 133.7, 130.1, 120.9, 117.2, 46.3, 35.1, 33.9, 26.7, 26.6.

1-(3,4-Dihydroxy-5-nitrophenyl)-2,2-diphenyl-ethanone (34). IR(KBr) 3400 (OH), 1669 (C═O), 1547 (NO_2). ^{1}H NMR (DMSO-d_6) δ 10.9 (vbr, 2H, 3-OH, 4-OH), 8.10 (d, 1H, J = 2.1 Hz, H-6), 7.64 (d, 1H, J = 2.1 Hz, H-2), 7.40−7.10 (m, 10H, 2 x Ph), 6.33 (s, 1H, CH). ^{13}C NMR (DMSO-d_6) δ 196.4, 148.7, 147.0, 140.4, 138.1, 130.0, 129.6, 127.9, 127.4, 118.9, 118.0, 58.2.

3,4-Diacetoxy-5-nitrophenyl-2-phenyl-ethanone (24). To a stirred suspension of **7b** (4.1 g, 15 mmol) in acetic anhydride (48 g, 470 mmol) was added two drops of concentrated sulfuric acid. After being stirred for 30 min, the mixture was poured onto ice−water (500 mL) and the precipitate was filtered off, washed by water (50 mL), and dried to give the product as an off-white solid, (41.5 g, 77%) of mp 92−93 °C. (C, H, N). IR(KBr) 1778 (C═O, ester), 1699 (C═O, ketone), 1539 (NO_2). ^{1}H NMR ($CDCl_3$) δ 8.61 (d, 1H, J = 2.1 Hz, H-6), 8.59 (d, 1H, J = 2.1 Hz, H-2), 7.45−7.15 (m, 5H, Ph), 4.3 (s, 2H, CH_2), 2.42 (s, 3H, $COCH_3$), 2.38 (s, 3H, $COCH_3$). ^{13}C NMR ($CDCl_3$) δ 193.9, 168.0, 145.1, 143.1, 140.9, 134.6, 133.3, 129.8, 129.5, 129.1, 128.8, 128.6, 127.9, 125.7, 123.4, 45.9, 20.9, 20.8.

3,4-Diacetoxy-5-nitrophenyl-2-(4′-nitrophenyl)-ethanone (25) and 3,4-Diacetoxy-5-nitrophenyl-2-(2′-nitrophenyl)-ethanone (26). To 100% nitric acid (3 mL) at 0 °C (ice−salt bath) was added **24** (1.07 g, 3 mmol) in portions with stirring over 15 min. When addition was complete, the mixture was poured onto ice−water (100 mL). The precipitate was filtered off and washed with water (20 mL). Chromatography on silica gel (petroleum ether/ethyl acetate, 2:1) allowed separation of the faster-running product which, after recrystallization was identified as the 4′-nitro isomer 25 (0.29 g, 24%). IR(KBr) 1779 (C═O, ester), 1696 (C═O, ketone), 1535 (very strong, NO_2). ^{1}H NMR ($CDCl_3$) δ 8.61 (d, 1H, J = 2.1 Hz, H-6), 8.10 (d, 1H, J = 2.1 Hz, H-2), 8.30−7.4 (m, 4H, Ph), 4.45 (s, 2H, CH_2), 2.42 (s, 3H, $COCH_3$), 2.39 (s, 3H, $COCH_3$). ^{13}C NMR ($CDCl_3$) δ 192.7, 168.1, 167.3, 148.0, 145.6, 143.5, 141.5, 140.8, 131.3, 128.8, 124.6, 123.2, 45.4, 21.1, 21.0.

The slower running product obtained from the column was recrystallized and identified as the 2′-nitro isomer **26** (0.25 g, 21%). IR(KBr) 1779 (C═O, ester), 1696 (C═O, ketone), 1529 (very strong, NO_2). ^{1}H NMR ($CDCl_3$) δ 8.64 (d, 1H, J = 2.1 Hz, H-6), 8.10 (d, 1H, J = 2.1 Hz, H-2), 8.20−7.45 (m, 4H, Ph), 4.7 (s, 2H, CH_2), 2.43 (s, 3H, $COCH_3$), 2.41 (s, 3H, $COCH_3$). ^{13}C NMR ($CDCl_3$) δ 192.3, 168.0, 167.2, 148.9, 145.2, 143.3, 141.3, 134.4, 134.1, 129.4, 129.8, 128.7, 126.0, 123.0, 44.7, 20.9, 20.8.

2-(2′-Carboxylphenyl)-1-(3,4-dihydroxy-5-nitrophenyl)-ethanone (27). To a stirred and cooled (−78 °C) solution of diisopropylamine (1.8 g, 17.65 mmol) in anhydrous THF (10 mL) under nitrogen was added *n*-butyllithium (8.8 mL, 2 M solution in hexanes, 17.6 mmol). After the mixture was stirred for 30 min, *o*-toluic acid (0.6 g, 4.41 mmol) in THF (5 mL) was added dropwise, and the resulting deep red solution was stirred at −78 °C for 1 h. Thereupon, a solution of methyl vanillate (0.84 g, 4.63 mmol) in THF (10 mL) was added dropwise, and the temperature was gradually allowed to reach room temperature. The mixture was then poured onto ice/2 N HCl (350 mL) and extracted by diethyl ether (3 × 50 mL). The organic extracts were washed by water (50 mL) and brine (50 mL), then dried over anhydrous sodium sulfate, filtered, and evaporated (40 °C, water aspirator pressure). The residue was recrystallized from 50% aqueous ethanol to afford white crystals (0.83 g, 66%) of mp 166−168 °C.

Nitration (as described for **6b**, n = 1, 62%) and demethylation (as described for **7b**, 72%,) gave the title compound **27** as yellow crystals. IR(KBr) 3374 (OH), 2903, 2652 (CO_2H, acid), 1686 (broad, C═O), 1543 (NO_2). ^{1}H NMR (DMSO-d_6) δ 12.8 (vbr, 1H, COOH), 10.8 (vbr, 2H, 3-OH, 4-OH), 8.07 (d, 1H, J = 2.1 Hz, H-6), 7.95 (d, 1H, Ar), 7.63 (d, 1H, J = 2.1 Hz, H-2), 7.6−7.3 (m, 3H, Ar), 4.68 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ 195.7, 169.2, 148.6, 146.5, 138.2, 138.1, 133.8, 133.0, 131.5, 131.4, 128.3, 128.0, 118.2, 116.8, 45.0.

3-(3,4-Dihydroxy-5-nitrophenyl)-isochromen-1-one (28). A stirred suspension of **27** (0.3 g, 0.94 mmol) in acetic acid (12 mL) was treated with concentrated sulfuric acid (3 drops) and heated at 95 °C for 10 min and then allowed to cool to room temperature. The mixture was poured onto ice−water (30 mL), and the orange precipitate was filtered off and washed by water (5 mL). Recrystallization from ethanol afforded orange crystals (0.17 g, 61%) of mp 285−288 °C. (Anal. C, H, N). IR(KBr) 3226 (OH), 1701 (C═O, lactone), 1533 (NO_2). ^{1}H NMR (DMSO-d_6) δ 10.7 (vbr, 2H, 3-OH, 4-OH), 8.16 (d, 1H, Ar), 7.9−7.8 (m, 2H, H-6, Ar), 7.71 (d, 1H, Ar), 7.65−7.5 (m, 2H, H-2, Ar), 7.46 (s, 1H, CH). ^{13}C NMR (DMSO-d_6) δ 162.1, 151.8, 149.2, 144.2, 138.7, 138.3, 136.5, 129.9, 129.6, 127.6, 123.1, 120.7, 116.1, 112.7, 102.7.

The following details are representative procedures for synthesis of compounds **29**−**32**.

1-(3,4-Diacetoxy-5-nitrophenyl)-2-methyl-2-phenyl-propan-1-one (30). Sodium hydride (0.92 g, 55−65% mineral oil dispersion) was washed with dry diethyl ether (6 mL) under nitrogen and then covered by dry THF (10 mL). After cooling to 0 °C, a solution of the ketone **5b** (R = Bn, n = 1) (2 g, 6.02 mmol) in dry THF (50 mL) was added dropwise, and the resulting suspension was allowed to stir for 45 min at room temperature. The suspension was thereafter cooled again (0 °C), and a solution of methyl iodide (4.27 g, 30.12 mmol) in dry THF (5 mL) was added dropwise. The resulting mixture was stirred at 55 °C for 3 h and then quenched by careful addition of cold water (10 mL). The solvent was removed (40 °C, water aspirator pressure) and the residue partitioned between dichloromethane (50 mL) and water (50 mL). The organic phase was separated, washed by water (10 mL) and brine (10 mL), then dried over anhydrous sodium sulfate, filtered, and dried. Evaporation (40 °C, water aspirator pressure) afforded a pale yellow oil which was chromatographed over silica gel (petroleum ether/ethyl acetate, 95:5) to afford the dimethylated product as a clear oil (1.55 g, 71%). ^{1}H NMR ($CDCl_3$) δ 7.4−7.25 (m, 10H, 2 x Ph), 7.21 (d, 1H, J = 2.1 Hz, H-2), 7.07(dd, 1H, J = 2.1, 8.5 Hz, H-6), 6.67 (d, 1H, J = 8.5 Hz, H-5), 5.13 (s, 2H, OCH_2), 3.71 (s, 3H, OCH_3), 1.62 (s, 6H, 2 × CH_3). ^{13}C NMR ($CDCl_3$) δ 202.6, 151.7, 149.1, 146.7, 136.9, 129.5, 129.3, 129.2, 128.6, 127.7, 127.2, 126.2, 124.8, 113.6, 112.2, 71.2, 56.3, 51.8, 28.7.

Subsequent debenzylation (91%), nitration (69%), demethylation (93%), and acetylation (95%) using procedures described above gave **30** as off-white crystals. IR(KBr) 1775 (C═O, ester), 1684 (C═O, ketone), 1545 (NO_2). ^{1}H NMR ($CDCl_3$) δ 8.04 (d, 1H, J = 2.1 Hz, H-6), 7.70 (d, 1H, J = 2.1 Hz, H-2), 7.50−7.30 (m, 5H, Ph), 2.35 (s, 3H, $COCH_3$), 2.30 (s, 3H, $COCH_3$), 1.65 (s, 6H, 2 × CH_3). ^{13}C NMR ($CDCl_3$) δ 199.7, 168.0, 167.5, 144.5, 144.2, 142.6, 139.8, 134.1, 130.5, 130.1, 128.2, 126.1, 124.9, 52.2, 28.0, 21.0, 21.0.

By application of similar procedures the following were obtained:

1-(3,4-Diacetoxy-5-nitrophenyl)-2-phenyl-propan-1-one (29). IR(KBr) 1780 (C═O, ester), 1690 (C═O, ketone), 1548 (NO_2). ^{1}H NMR ($CDCl_3$) δ 8.54 (d, 1H, J = 2.1 Hz, H-6), 8.08 (d, 1H, J = 2.1 Hz, H-2), 7.40−7.20 (m, 5H, Ph), 4.61 (q, 1H, J = 6.8 Hz, CH), 2.38 (s, 3H, $COCH_3$), 2.36 (s, 3H, $COCH_3$), 1.57 (d, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 196.5, 167.9, 167.2, 144.9, 142.9, 140.5, 140.3, 134.5, 129.9, 129.1, 128.1, 128.0, 123.7, 48.8, 20.9, 20.8, 19.7.

1-(3,4-Diacetoxy-5-nitrophenyl)-2,3-diphenyl-propan-1-one (31). IR(KBr) 1782 (C═O, ester), 1686 (C═O, ketone), 1544 (NO_2). ^{1}H NMR ($CDCl_3$) δ 8.47 (d, 1H, J = 2.1 Hz, H-6), 8.03 (d, 1H, J = 2.1 Hz, H-2), 7.40−7.00 (m, 10H, 2 × Ph), 4.72 (t, 1H, J = 7.2 Hz, CH), 3.56 (dd, 1H, J = 7.5, 13.7 Hz, CH_2), 3.09 (dd, 1H, J = 6.8, 13.7 Hz, CH_2), 2.36 (s, 3H, $COCH_3$), 2.34 (s, 3H, $COCH_3$). ^{13}C NMR ($CDCl_3$) δ 195.6, 168.0, 167.3, 145.1, 143.1, 140.7, 139.5, 138.1, 134.8, 130.0, 129.7, 129.1, 129.0, 128.7, 128.4, 127.0, 123.7, 57.0, 40.5, 21.1, 21.0.

(3,4-Diacetoxy-5-nitrophenyl)-1-(phenyl-cyclohexyl)-methanone (32). IR(KBr) 2943 (aliphatic CH), 1780 (C═O, ester), 1679 (C═O, ketone), 1540 (NO_2). ^{1}H NMR ($CDCl_3$) δ 7.93 (d, 1H, J = 2.1 Hz, H-6), 7.54 (d, 1H, J = 2.1 Hz, H-2), 7.50−7.30 (m, 5H, Ph), 2.5 (m, 2H, c-hexyl), 2.35 (s, 3H, $COCH_3$), 2.30 (s, 3H, $COCH_3$), 1.8 (m, 2H, c-hexyl), 1.7 (m, 3H, c-hexyl), 1.4 (m, 3H, c-hexyl). ^{13}C NMR ($CDCl_3$) δ 200.8, 168.0, 167.5, 144.3, 143.3, 142.5, 139.4, 136.0, 130.1, 129.7, 128.3, 126.7, 124.0, 56.2, 36.2, 26.2, 23.7, 21.0, 21.0.

2-Bromo-1-(3,4-diacetoxy-5-nitrophenyl)-2-phenyl-ethanone (33). To a stirred solution of **24** (0.36 g, 1 mmol) in dry THF (5 mL) was added phenyltrimethylammonium tribromide (0.45 g, 1.2 mmol) in one portion. After stirring at room temperature for 45 min, the mixture was poured onto ice−water (20 mL) and extracted by dichloromethane (3 × 30 mL). The organic extracts were washed by water (30 mL) and brine (30 mL), then dried over anhydrous sodium sulfate, filtered, and evaporated (40 °C, water aspirator pressure). The residue was chromatographed over silica gel (dichloromethane/petroleum ether, 2:1) and the major product obtained recrystallized (diethyl ether) to give **33** as off-white crystals (0.67 g, 51%). IR(KBr) 1786 (C═O, ester), 1703 (C═O, ketone), 1546 (NO_2). ^{1}H NMR ($CDCl_3$) δ 8.58 (d, 1H, J = 2.1 Hz, H-6), 8.15 (d, 1H, J = 2.1 Hz, H-2), 7.60−7.30 (m, 5H, Ph), 6.25 (s, 1H, CH), 2.41 (s, 3H, $COCH_3$), 2.38 (s, 3H, $COCH_3$). ^{13}C NMR ($CDCl_3$) δ 187.9, 168.0, 167.3, 145.4, 143.3, 141.4, 134.9, 132.3, 130.3, 129.9, 129.7, 129.7, 124.1, 50.3, 21.1, 21.0.

Pharmacology. Male Wistar rats (Harlan-Interfauna Ibérica, Barcelona, Spain) aged 60 days and weighing 240−260 g were used in all experiments. Rats were kept under controlled environmental conditions (12 h light/dark cycle and room temperature 22 ± 1 °C) with food and tap water allowed ad libitum. All test compounds were given by gastric tube (30 mg/kg) in 0.5% carboxymethylcellulose to overnight fasted rats. Thereafter, at defined intervals (0.5, 1, 3, 6, and 9 h), animals were sacrificed and tissues removed to determine COMT activity. Rats were anaesthetised with sodium pentobarbital (60 mg/kg) and perfused through the left ventricle with 0.9% (w/v) NaCl. Livers and brains were immediately removed and homogenized in 5 mM sodium phosphate buffer, pH 7.8 at 4 °C with a Teflon homogenizer (Heidolph). The crude homogenates were directly used for the COMT assay. The protein content in the homogenates was determined by the method of Bradford[35] with human serum albumin as standard.

COMT activity was determined by the ability of enzyme preparations to methylate adrenaline to metanephrine as previously described.[36] Briefly, aliquots of 0.5 mL of liver or brain homogenates were preincubated for 20 min with 0.4 mL of phosphate buffer in the presence of a saturating concentration of *S*-adenosyl-L-methionine, the methyl donor (500 μM and 100 μM for liver and brain, respectively). The incubation medium contained also pargyline (100 μM), $MgCl_2$ (100 μM), and EGTA (1 mM). The reaction mixture was incubated with a saturating concentration of adrenaline (1000 μM and 100 μM for liver and brain, respectively). The preincubation and incubation were carried out at 37 °C under conditions of light protection with continuous shaking. At the end of the incubation period (5 and 15 min for liver and brain, respectively), the tubes were transferred to ice, and the reaction was stopped by the addition of 200 μL of 2 M perchloric acid. The samples were then centrifuged (200 g, 4 min, 4 °C), and 500 μL aliquots of the supernatant, filtered on 0.22 μm pore size Spin-X filter tubes (Costar), were used for the assay of metanephrine by means of HPLC-ED, as previously described.[36] In brief, aliquots of 50 μL of the filtered supernatant were injected into the chromatograph. The chromatography system consisted of a pump (Gilson model 302; Gilson Medical Electronics, Villiers le Bel, France) connected to a manometric module (Gilson model 802 C) and a stainless steel 5 μm ODS column (Biophase; Bioanalytical Systems, West Lafayette, IN) of 25 cm length. Samples were injected by means of an automatic sample injector (Gilson model 231) connected to a Gilson dilutor (model 401). The mobile phase was a degassed solution of citric acid (0.1 mM), sodium octylsulfate (0.5 mM), sodium acetate (0.1 M), EDTA (0.17 mM), dibutylamine (1 mM), and methanol (10% v/v), adjusted to pH 3.5 with perchloric acid (2 M) and pumped at a rate of 1.0 mL/min. The detection was carried out electrochemically with a glassy carbon electrode,

an Ag/AgCl reference electrode, and an amperometric detector (Gilson model 141); the detector cell was operated at 0.75 V. The current produced was monitored using the Gilson 712 HPLC software. The lower limits for detection of metanephrine ranged from 350 to 500 fmol.

SK-N-SH cells, a human neuroblastome derived cell line, were obtained from the American Type Culture Collection (ATTC HTB-11). The SK-N-SH cell line expresses high levels of COMT activity with similar kinetics parameters to rat brain COMT and is considered to be an appropriate in vitro model for the assay of human COMT.[37] SK-N-SH cells were maintained in a humidified atmosphere of 5% CO_2-95% air at 37 °C. The cells were grown in Minimal Essential Medium supplemented with 10% foetal bovine serum, 100 U/mL penicillin G, 0.25 μg/mL amphotericin B, 100 μg/mL streptomycin, and 25 mM HEPES. For the assays, the cells were plated in 24-well plates, and 24 h prior to each experiment the medium was changed to medium free of foetal bovine serum. Experiments were generally performed after cells reached confluence (5−7 days), and each cm^2 contained about 100 μg of cell protein. COMT activity was evaluated in cell monolayers by the ability to methylate adrenaline to metanephrine in the presence of saturating concentration (100 μM) of the methyl donor (*S*-adenosyl-L-methionine). The incubation medium also contained 100 μM pargyline, 100 μM $MgCl_2$, and 1 mM EGTA. The preincubation (20 min) and incubation (15 min) were carried out at 37 °C, in the dark, with continuous shaking and without oxygenation. At the end of the incubation period, the plates were transferred to ice and the reaction was stopped by the addition of 2 M perchloric acid. The assay of metanephrine was performed by means of HPLC-ED, as described above.

References

(1) Axelrod, J.; Senoh, S.; Witkop, B. *O*-Methylation of catechol amines in vivo. *J. Biol. Chem*. **1958**, *233*, 697−701.

(2) Axelrod, J.; Tomchick, R. Enzymatic *O*-methylation of epinephrine and other catechols. *J. Biol. Chem*. **1958**, *233*, 702−705.

(3) Guldberg, H.; Marsden, C. Catechol-*O*-methyltransferase: pharmacological aspects and physiological role. *Pharmacol. Rev*. **1975**, *27*, 135−206.

(4) Creveling, C.; Dalgard, N.; Shimizu, H.; Daly, J. Catechol-*O*-methyltransferase III. M- and p-*O*-methylation of catecholamines and their metabolites. *Mol. Pharmacol*. **1970**, *6*, 691−696.

(5) Zhu, B. T.; Ezell, E. L.; Liehr, J. G. Catechol-*O*-methyltransferase-catalysed rapid *O*-methylation of mutagenic flavanoids. Metabolic inactivation as a possible reason for their lack of carcinogenicity *in vitro*. *J. Biol. Chem*. **1994**, *269*, 292−299.

(6) Borcharrdt, R. T.; Thakker, D. R. Affinity labeling of catechol-*O*-methyltransferase by *N*-haloacetyl derivatives of 3,5-dimethoxy-4-hydroxyphenylethylamine and 3,4-dimethoxy-5-hydroxyphenylethylamine. Kinetics of inactivation. *Biochemistry* **1975**, *14*, 4542−4551.

(7) Burba, J. V.; Becking, G. C. Effect of the antioxidant norhydroguaiaretic acid on the in vitro activity of catechol-*O*-methyltransferase. *Arch. Int. Pharmacodyn. Ther*. **1969**, *180*, 323−329.

(8) Mannisto, P. T.; Kaakkola, S. New selective COMT inhibitors: Useful adjuncts for Parkinson's disease? *Trends Pharmacol. Sci*. **1989**, *10*, 54−56.

(9) Mannisto P. T.; Kaakkola, S. Rationale for selective COMT inhibitors as adjuncts in the drug treatment of Parkinson's disease. *Pharmacol. Toxicol*. **1990**, *66*, 317−323.

(10) Cotzias, G. C.; Papavasiliou, P. S.; Ginos, J.; Steck, A.; Duby, S. Aromatic amino acids and modification of parkinsonism. *N. Engl. J. Med*. **1967**, *276*, 374−379.

(11) Calne, D. B. Treatment of Parkinson's disease. *N. Engl. J. Med*. **1993**, *329*, 1021−1027.

(12) Diamond, S. G.; Markham, C. H.; Treciokas, L. J. A double-blind comparison of levodopa, Madopar and Sinemet in Parkinson's disease. *Ann. Neurol*. **1978**, *3*, 267−272.

(13) Bartholini, G.; Pletscher, A. Decarboxylase inhibitors. *Pharmacol. Ther*. **1975**, *1*, 407−421.

(14) Nutt, J. G.; Woodward, W. R.; Anderson, J. L. The effect of carbidopa on the pharmacokinetics of intavenously administered levodopa: the mechanism of action in the treatment of parkinsonism. *Ann. Neurol*. **1985**, *18*, 537−543.

(15) Messiha, F.; Hsu, T.; Bianchine, J. Peripheral aromatic L-amino acid decarboxylase inhibitor in parkinsonism. I. Effect on *O*-methylated metabolites of L-Dopa-2-^{14}C. *J. Clin. Invest*. **1972**, *51*, 452−455.

(16) Mannisto, P. T.; Ulmanen, I.; Lundstrom, K.; Taskinen, J.; Tenhunen, J.; Tilgmann, C., Kaakkola, S. Characteristics of catechol-*O*-methyltransferase (COMT) and properties of selective COMT inhibitors. *Prog. Drug Res*. **1992**, *39*, 291−350.

(17) Tohgi, H.; Abe, T.; Kikuchi, T.; Takahashi, S.; Nozaki, Y. The significance of 3-*O*-methyldopa concentrations in the cerebrospinal fluid in the pathogenesis of wearing-off phenomenon in Parkinson's disease. *Neurosci. Lett*. **1991**, *132*, 19−22.

(18) Ericsson A. D. Potentiation of the L-Dopa effect in man by the use of catechol-*O*-methyltransferase inhibitors. *J. Neurol. Sci*. **1971**, *14*, 193−197.

(19) Borgulya, J.; Bruderer, H.; Bernauer, K.; Zurcher, G.; Da Prada, M. Catechol-*O*-methyltransferase-inhibiting pyrocatechol derivatives: synthesis and structure−activity studies. *Helv. Chim. Acta*. **1989**, *72*, 952−968.

(20) Backstrom, R.; Honkanen, E.; Pippuri, A.; Kairisalo, P.; Pystynen, J.; Heinola, K.; Nissinen, E.; Lindén, I.-B.; Mannisto, P. T.; Kaakkola, S.; Pohto, P. Synthesis of some novel potent and selective catechol-*O*-methyltransferase inhibitors. *J. Med. Chem*. **1989**, *32*, 841−846.

(21) Perez, R. A.; Fernandez-Alvarez, E.; Nieto, O.; Piedrafita, F. J. Dihydroxynitrobenzaldehydes and hydroxymethoxynitrobenzaldehydes: synthesis and biological activity as catechol-*O*-methyltransferase inhibitors. *J. Med. Chem*. **1992**, *35*, 4584−4588.

(22) Borgulya, J.; Da Prada, M.; Dingemanse, J.; Scherschlicht, R.; Schlappi, B.; Zurcher, G. Ro 40−7592. Catechol-*O*-methyltransferase (COMT) inhibitor. *Drugs Future* **1991**, *16*, 719−721.

(23) Nissinen, E.; Lindén, I.-B.; Schultz, E.; Pohto, P. Biochemical and pharmacological properties of a peripherally acting catechol-*O*-methyltransferase inhibitor Entacapone. *Nauyn Schmiedebergs Arch. Pharmacol*. **1992**, *346*, 262−266.

(24) Assal, F.; Spahr, L.; Hadengue, A.; Rubbici-Brandt, L.; Burkhard, P. R. Tolcapone and fulminant hepaptitis. *Lancet* **1998**, *352*, 958.

(25) Bonifati, V.; Meco, G. New, selective catechol-*O*-methyltransferase inhibitors as therapeutic agents in Parkinson's disease. *Pharmacol. Ther*. **1999**, *81*, 1−36.

(26) Colissimo, C. The rise and fall of tolcapone. *J. Neurol*. **1999**, *246*, 880−882.

(27) Hauser, R. A.; Molho, E.; Shale, H.; Pedder, S.; Dorflinger, E. E. A pilot evaluation of the tolerability, safety and efficacy of tolcapone alone and in combination with oral selegiline in untreated Parkinson's disease patients. *Mov. Disord*. **1998**, *13*, 643−647.

(28) Nutt, J. G. Catechol-*O*-methyltransferase inhibitors for treatment of Parkinson's disease. *Lancet* **1998**, *351*, 1221−1222.

(29) Dingemanse, J.; Jorga, K.; Schmitt, M.; Gieschke, R.; Fotteler, B.; Zurcher, G.; Da Prada, M.; van Brummelen, P. Integrated pharmacokinetics and pharmacodynamics of the novel catechol-*O*-methyltransferase inhibitor tolcapone during first administration to humans. *Clin. Pharmacol. Ther*. **1995**, *57*, 508−517.

(30) Keranen, T.; Gordin, A.; Harjola, V.-P.; Karlsson, M.; Korpela, K.; Pentikainen, P. J.; Rita, H.; Seppala, L.; Wikberg, T. Inhibition of soluble catechol-*O*-methyltransferase and single-dose pharmacokinetics after oral and intravenous administration of entacapone. *Eur. J. Clin. Pharmacol*. **1994**, *46*, 151−157.

(31) Learmonth, D. A.; Alves, P. C. Improved method for the demethylation of nitro-catechol methyl ethers. *Synth. Commun*. In press.

(32) Devi, N.; Jain, N.; Krishnamurty, H. G. Synthesis of deoxybenzoins. *Indian J. Chem. Sect. B*. **1993**, *32B* (8), 874−875.

(33) Wahala, K.; Hase, T. A. Expedient synthesis of polyhydroxyisoflavones. *J. Chem. Soc., Perkin Trans. 1* **1991**, 3005−3008.

(34) Guion, T. S.; Koller, M. U.; Lachicotte, R. J.; Rutledge, R. N.; Hildebran, K. C.; Le, P. H.; Beam, C. F. The preparation of 2-(2-oxo-2-phenylethyl)benzoic acids from dilithiated ortho-toluic acid. *Synth. Commun*. **1996**, *26* (9), 1753−1762.

(35) Bradford, M. M. A rapid method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*. **1976**, *72*, 248−254.

(36) Borges, N.; Vieira-Coelho, M. A.; Parada, A.; Soares-da-Silva, P. Studies on the tight-binding nature of tolcapone inhibition of soluble and membrane-bound rat brain catechol-*O*-methyltransferase. *J. Pharmacol. Exp. Ther*. **1997**, *282*, 812−817.

(37) Costa, J. L.; Vieira-Coelho, M. A.; Soares-da-Silva, P. Catechol-*O*-methyltransferase activity in SK−N−SH cells. *Eur. J. Neurosci*. **2000**, *12* (suppl. 11), 280.

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