Synthesis and Biological Evaluation of a Novel Series of "Ortho-Nitrated" Inhibitors of Catechol-*O*-methyltransferase

David A. Learmonth,[†] Maria João Bonifácio,[‡] and Patrício Soares-da-Silva*,[‡]

Laboratory of Chemistry and Laboratory of Pharmacology, Department of Research & Development, BIAL, 4745-457 S. Mamede do Coronado, Portugal

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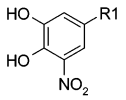
Novel regioisomeric "ortho-nitrated" catechols related to the catechol-*O*-methyltransferase (COMT) inhibitors BIA 3-202 **3** and BIA 3-335 **4** were synthesized and biologically evaluated. Changing the position of the nitro group from the "classical" meta- to the ortho-position relative to the side-chain substituent of the nitrocatechol pharmacophore exerted profound effects on selectivity and duration of COMT inhibition. Alkylaryl compounds **7a**−**d** possessed shorter duration of action than their regioisomers, but **7b** displayed reversed selectivity over **3** at 3 and 6 h, exhibiting preferential central inhibition. In the amino-substituted series, ortho-nitrated regioisomer **14k** was less peripherally selective than **4** and short-acting, whereas decahydroquinoline **14g** displayed an unprecedented combination of long-acting and selective peripheral inhibition. **7b** could provide a useful tool to probe the pharmacological utility of short-acting, centrally selective COMT inhibitors in the treatment of depression in Parkinsonian patients, and **14g** represents a promising candidate for clinical evaluation as an adjunct to L-Dopa therapy.

Introduction

Despite the fact that levodopa (3-(3,4-dihydroxyphenyl)-L-alanine, L-Dopa) was introduced into clinical practice as long ago as the early 1960s, it still continues to be the gold standard drug for the symptomatic treatment of Parkinson's disease (PD).[1−3] PD is a dopamine-deficiency disorder resulting from the degeneration of striatal dopaminergic neurons. Because dopamine itself is unable to permeate across the blood−brain barrier (BBB), Parkinsonian patients are treated with the dopamine precursor L-Dopa in combination with an aromatic amino acid decarboxylase (AADC) inhibitor such as carbidopa or benserazide,[4,5] which prevent premature decarboxylation of L-Dopa in the periphery. However, when this decarboxylation degradation pathway is blocked, catechol-*O*-methyltransferase (COMT), catalyzed *O*-methylation of L-Dopa to give 3-*O*-methyl-L-Dopa (3-OMD), becomes the predominant degradation pathway in the periphery[6−8] such that only a very limited percentage of the orally administered L-Dopa dose actually reaches the site of action intact.

Accordingly, keen interest has been maintained in the development of novel, more clinically effective inhibitors of COMT.[9,10] COMT[11] is a magnesium-dependent enzyme which catalyses the transfer of a methyl group from its cofactor *S*-adenosyl-L-methionine (SAM) to substrates containing a catechol motif. COMT is now known to play a key role in the inactivation of endogenous catecholamines,[12] catechol estrogens,[13] and the detoxification of several xenobiotic catechols.[14,15]

It has been postulated that it should be possible to improve the bioavailability of L-Dopa by reducing metabolic *O*-methylation in the periphery. On one hand, 3-OMD is not known to provide any beneficial therapeutic effect and has a long elimination half-life compared to L-Dopa, which means that it accumulates in plasma and tissues.[16] On the other hand, 3-OMD may compete with L-Dopa for the same active transport system[17,18] that permits permeation across the BBB, and a close relationship between the accumulation of 3-OMD and the "end-of-dose" or 'wearing-off' syndrome has been described.[16] In principle therefore, COMT inhibition should protect L-Dopa from undesirable metabolic degradation in the periphery and facilitate passage into the brain, thereby extending the duration of antiparkinsonian action and permitting a reduction in the dose and/or number of daily doses of L-Dopa.

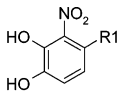


1, R_1 = C(O)-Ph-(4'-CH_3)

2, R_1 = (*E*)-C=C(CN)-$CONEt_2$

3, R_1 = C(O)-CH_2-Ph

4, R_1 = C(O)-$(CH_2)_2$-piperazine-Ph-(3'-CF_3).HCl



5, R_1 = CH=CH-C(O)-Ph

6, R_1 = CH=C(C(O)CH_3)$_2$

Figure 1. Chemical structures of tolcapone (**1**), entacapone (**2**), BIA 3-202 (**3**), BIA 3-335 (**4**), [2-(3,4-dihydroxy-2-nitrophenyl)vinyl]phenyl ketone (**5**), and nitecapone (**6**).

Of the second-generation COMT inhibitors, the most commonly known are tolcapone (**1**)[19]and entacapone (**2**)[20] (Figure 1). Both of these molecules contain a catecholic pharmacophore to which the highly electronegative nitro group has been added to occupy the meta position relative to the side-chain substituent. Both of these molecules are potent, tight-binding inhibitors of

* Author to whom correspondence should be addressed. Tel: 351-22-9866100. Fax: 351-22-9866192. E-mail: psoares.silva@bial.com.
† Laboratory of Chemistry.
‡ Laboratory of Pharmacology.

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COMT, which themselves are very poor substrates for the enzyme[21,22] unlike earlier, nonfunctionalized catecholic inhibitors.[23] Pharmacologically, **1** can be distinguished from **2** in that it is an entirely indiscriminate inhibitor of both central and peripheral COMT, whereas **2** is a peripherally selective inhibitor. The nitro group is thought to have the twofold function of stabilizing the ionized inhibitor−enzyme complex and to reduce the nucleophilicity of the hydroxyl group of the catechol usually susceptible to methylation. Although both of these molecules were approved and reached the marketplace as adjuncts to L-Dopa therapy, **1** was subsequently withdrawn due to hepatotoxicity concerns.[24,25] Presently, **1** can only be administered to Parkinsonian patients unresponsive to other PD treatments and strictly only with a regular liver function testing regime, which is both expensive and inconvenient for the patient. The actual mechanism(s) by which **1** induces liver toxicity remain controversial, but hepatotoxicity has not thus far been associated with **2**, which is generally regarded as a safe drug. This may be significant, given that it shares with **1** the same nitrocatecholic pharmacophore. Studies into the metabolism of **1** in humans[26] have shown that among extensive phase I transformations (methylation, oxidation, and reduction), significant reduction of the nitro group occurs to form the corresponding amino metabolite that then undergoes a number of phase II conjugation reactions including acetylation of the amino group. On the other hand, analogous metabolic reduction of the nitro group of **2** is only observed in rats, not humans.[27] Recent in vitro studies[28] showed that these metabolites of **1** could be further oxidized to reactive intermediates capable of forming covalent adducts with hepatic proteins, thereby explaining the potential of **1**, but not **2**, to cause hepatocellular injury. Other workers have found that **1** but not **2** is a potent uncoupler of oxidative phosphorylation in mitochondria[29,30] and is thereby able to reduce the cell's capacity to generate ATP, and they have proposed this aspect as the source of toxicity for **1**. Furthermore, it was recently reported[31] that tolcapone has a significantly higher lipophilicity compared to that of **2** at physiological pH (**1**, log P_{app} 1.03 vs **2**, log P_{app} 0.174); thus, it would be expected that **1** would be able to permeate across cell membranes more efficiently than **2** and, once therein, could exert the above-mentioned uncoupling effects.

Notwithstanding the reduced toxicity risk, however, **2** is endowed with a very short in vivo half-life[32] which means that the dosages are high and must be repeatedly administered throughout the day. Indeed, the clinical efficacy of **2** has been recently questioned,[33] and this aspect is often cited by patients as the principal motive for cessation of treatment.

Recent efforts from this group sought to address these problems, and this work led to the discovery of BIA 3-202 (**3**)[34] and BIA 3-335 (**4**)[35] (Figure 1). Immediately, one can see that both of these structures also display what can now be regarded as the "classical" 3,4-dihydroxy-5-nitrophenyl pharmacophore, wherein the nitro group is located at the *meta* position relative to the carbonyl substituent. Both **3** and **4** are potent, tight-binding, long-acting, and highly selective inhibitors of peripheral COMT. In either case, it was clearly demonstrated through structure−activity relationships and molecular modeling studies that variation of the side-chain substituent was found to exert profound influence on both the peripheral selectivity and duration of COMT inhibition. Thus, although the nitrocatechol moiety may be regarded as responsible for "anchoring" the inhibitor molecule to the magnesium ion at the active site of the enzyme, due consideration must be given to appropriate side-chain substitution of the pharmacophore, because this side chain can modulate the overall physicochemical properties and, thus, performance of the compounds. Animal and human studies indicate that **3** and **4** are endowed with significantly improved pharmacokinetic and pharmacodynamic properties over both **1** and **2** and are currently in advanced clinical investigation as potential adjuncts to L-Dopa/AADC therapy in human studies.[36,37]

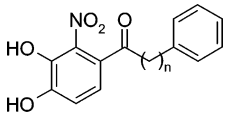
During this work, we became increasingly curious as to what pharmacological effects might be observed by changing the position of the nitro group from the *meta* position (i.e., 3,4-dihydroxy-5-nitro substitution pattern as in **3**, **4**, and their analogues) to the *ortho* position (i.e., 3,4-dihydroxy-2-nitrophenyl substitution pattern) in relation to the side-chain substituent. To the very best of our knowledge, no systematic comparative study of the pharmacology of regioisomerically nitrated derivatives of COMT inhibitors currently in clinical use or development has thus far been published. However, over a decade ago, Perez et al.[38] reported the synthesis and inhibitory activity of a series of regioisomeric dihydroxy nitrobenzaldehydes against COMT isolated from pig liver. These authors concluded that the substitution of the catechol with a nitro group ortho to both one hydroxyl group and the carbonyl group (3,4-dihydroxy-2-nitro) gave the most potent COMT inhibitor in this series, on the basis of the evidence of a lower IC_{50} than that of the corresponding 3,4-dihydroxy-5-nitro regioisomer. The same authors went on to synthesize and evaluate the kinetics of COMT inhibition in vivo by a series of ortho- and meta-nitrated vinyl-substituted catechols[39−41] derived from these benzaldehydes and concluded that the ortho-nitrated vinylphenyl ketone (**5**) (Figure 1) was a particularly potent and selective COMT inhibitor, with an apparent K_i (K_{app}) 3.5-fold lower than nitecapone (**6**) (Figure 1). Despite this promise, it appears that **5** did not progress further in terms of clinical development, and indeed we are unaware of any ortho-nitrated COMT inhibitor currently in development.

Accordingly, we proceeded to synthesize a restricted series of ortho-nitrated catechols including several analogues of **3** and **4** to ascertain the effect of changing the position of the nitro substituent on pharmacological parameters such as potency, duration, and selectivity of COMT inhibition.

Chemistry

The ortho-nitrated analogues of the previously reported[34] BIA 3-202 homologous series (**7a**−**d**, Table 1) were prepared according to Scheme 1. The hydroxyl group of vanillin (**8**, R = H) was temporarily protected as the benzyl ether (**8**, R = Bn) by the Williamson reaction and was reacted with homologous arylalkyl Grignard reagents ($Ar(CH_2)_nMgX$, $n = 0-3$) to provide

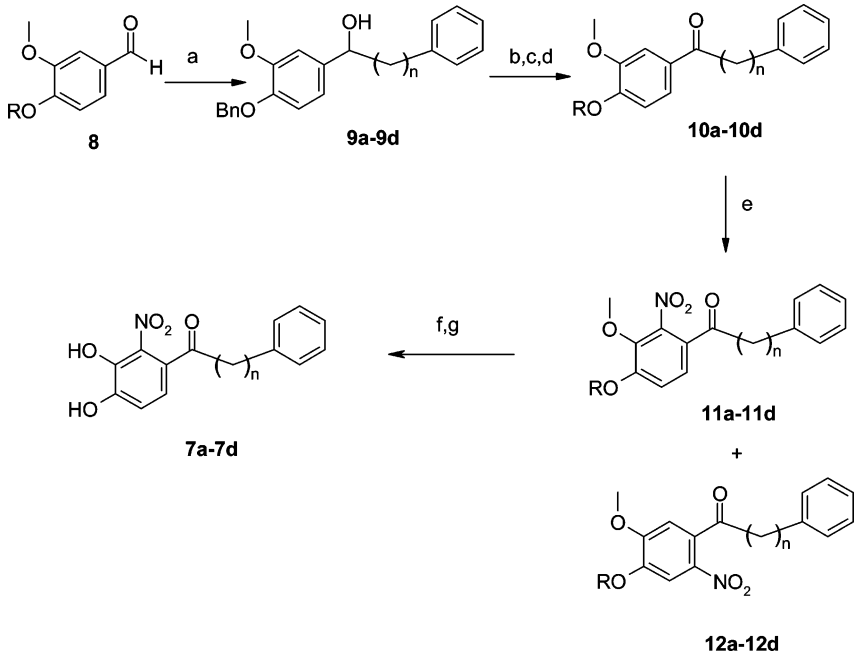
Table 1. Physical Constants, Percent Inhibition of COMT Activity in SK-N-SH Cells by Compounds **1**−**3** and **7a**−**d** (100 nM),[a] and IC_{50} Values for Inhibition of Rat Brain and Liver COMT[b]



No.	n	mp (°C)	Formula	Anal.	% Inhibition (SK−N−SH)	IC_{50} Liver (μM)	IC_{50} Brain (nM)
1					97 ± 1	0.93 (0.55, 1.56)	2.2 (0.8, 6.4)
2					77 ± 1	2.32 (0.74, 7.26)	12.8 (4.0, 41.3)
3					90 ± 1	0.69 (0.36, 1.36)	3.7 (1.7, 8.1)
7a	1	155−157	$C_{13}H_9NO_5$	C,H,N	98 ± 0	0.13 (0.08, 0.2)	3 (2,4)
7b	2	153−154	$C_{14}H_{11}NO_5$	C,H,N	76 ± 1	2.0 (1.4, 2.8)	4 (3,5)
7c	3	108−109	$C_{15}H_{13}NO_5$	C,H,N	95 ± 2	0.13 (0.08, 0.2)	4 (3,5)
7d	4	142−143	$C_{16}H_{15}NO_5$	C,H,N	87 ± 4	0.11 (0.08, 0.17)	4 (3,5)

[a] Results are mean SEMs of four experiments per group. [b] 95% confidence intervals in brackets.

Scheme 1. Synthesis of Homologous Ortho-Nitrated BIA 3-202 Analogues[a]

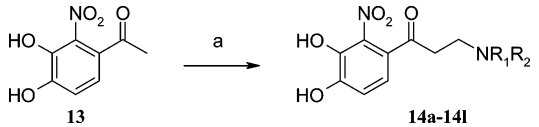


[a] Reagents: (a) i. $Ar(CH_2)_nMgX$, ii. H_3O^+; (b) NaO^tBu, cyclohexanone, $PhCH_3$, Δ; (c) 30% HBr−AcOH, CH_2Cl_2; (d) Ac_2O, pyridine, CH_2Cl_2; (e) $Cu(NO_3)_2$, Ac_2O; (f) 3 N NaOH, MeOH; (g) $AlCl_3$, pyridine, $Cl(CH_2)_2Cl$, Δ.

the alcohols **9a**−**d** (n = 0−3) in good yields. Oppenauer oxidation furnished the ketones **10a**−**d** (R = Bn) in moderate to excellent yields. Deprotection of the *O*-benzyl protecting groups proceeded smoothly on contact with excess hydrogen bromide in acetic acid to provide the phenols **10a**−**d** (R = H) which were then converted to the *O*-acetates **10a**−**d** (R = $COCH_3$) in quantitative yields. Regioselective introduction of the nitro group at C-2 proved elusive and could not be satisfactorily achieved in acceptable yields using nitric acid under a variety of conditions, principally due to predominant formation of the undesired 6-nitro isomers **12a**−**d** (R = $COCH_3$) or nitration occurring on the nonfunctionalized aromatic ring. Fortunately, however, reaction of the acetates **10a**−**d** (R = $COCH_3$) with copper (II) nitrate trihydrate in acetic anhydride gave better results, and the 2-nitro compounds **11a**−**d** (R = $COCH_3$) could be obtained in moderate yields after separation from the 6-nitro regioisomers by chromatography or crystallization. The acetates were then hydrolyzed to the corresponding phenols **11a**−**d** (R = H) in aqueous methanolic sodium hydroxide. Finally, scission of the methyl groups was achieved using aluminum chloride and pyridine in warm 1,2-dichloroethane,[42] which furnished the target compounds **7a**−**d** (Table 1) in excellent yields.

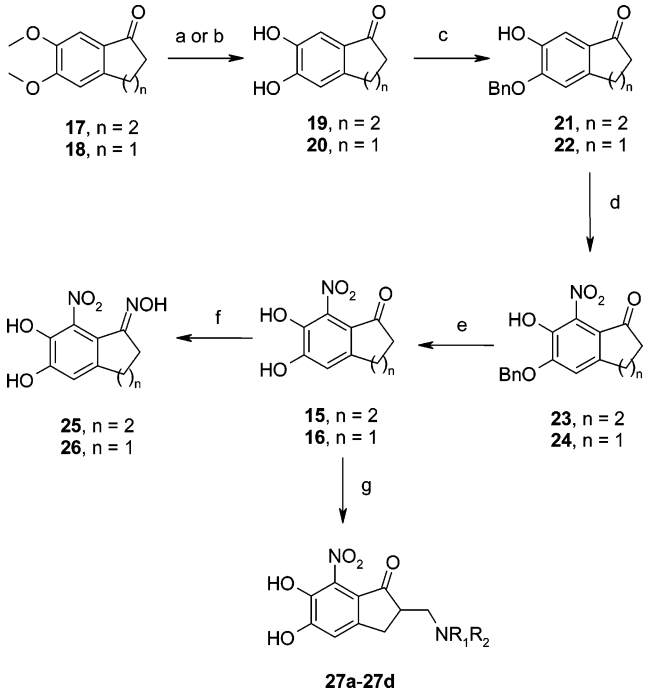
Selected ortho-nitrated analogues (**14a**−**l**) of the previously reported[35] piperidines and piperazines from the BIA 3-335 series were prepared by the Mannich reaction of the nitrocatecholic acetophenone building block **13** with appropriate cyclic secondary amines (Scheme 2). Acetophenone (**13**) was prepared from acetovanillone by acetylation, nitration, hydrolysis, and methyl ether cleavage as in steps d−g of Scheme 1.

Scheme 2. Synthesis of Ortho-Nitrated BIA 3-335 Analogues[a]



[a] Reagents: (a) HNR_1R_2, H_2CO, c·HCl, ^{i}PrOH, 80 °C.

Scheme 3. Synthesis of Ortho-Nitrated Ring-Constrained BIA 3-335 Analogues[a]



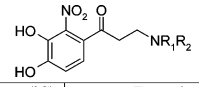
[a] Reagents: (a) 48% HBr (aq), reflux; (b) BBr_3, CH_2Cl_2, −78 °C to room temperature; (c) BnBr, K_2CO_3, DMF, 80 °C; (d) 65% HNO_3, AcOH; (e) 30% HBr−AcOH, 48% HBr, 110 °C; (f) H_2NOH·HCl, pyridine, EtOH, reflux; (g) HNR_1R_2, H_2CO, c·HCl, ^{i}PrOH, 80 °C.

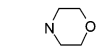
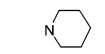
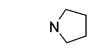
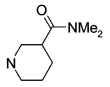
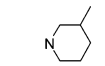
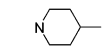
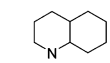
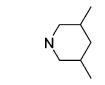
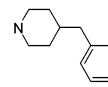
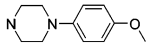
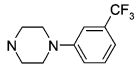
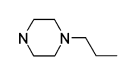
Constrained carbocyclic analogues **15** and **16** were synthesized from 6,7-dimethoxy-1-tetralone (**17**) and 5,6-dimethoxy-1-indanone (**18**), respectively (Scheme 3). Exhaustive demethylation of **17** was readily achieved in boiling 48% aqueous hydrobromic acid, whereas treatment of **18** with boron tribromide gave superior yields of the corresponding catechol **20**. The para hydroxyl group of catechols **19** and **20** were regioselectively benzylated via the Williamson reaction to provide the para-*O*-benzyl ethers **21** and **22** in moderate yields, each of which permitted a regioselective introduction of the nitro substituent ortho to the carbonyl group upon reaction with dilute nitric acid in acetic acid to give exclusively **23** and **24**. The benzyl groups of each were then cleaved on brief contact with warm hydrobromic acid to furnish the nitrocatechols **15** and **16**. These were subsequently further elaborated by the Mannich reaction with selected secondary amines giving access to **27a**−**d**, and the carbonyl group of the parent ketones **15** and **16** were modified by reaction with hydroxylamine to give the oximes **25** and **26**.

Results and Discussion

In vitro screening in human neuroblastoma SK-N-SH cells, as previously described,[43] was employed to make a preliminary assessment of the ability of the newly synthesized ortho-nitrated catechols to inhibit COMT. Homologous alkylaryl compounds **7a**−**d** were further tested for their ability to decrease *O*-methylation of adrenaline to metanephrine in rat liver and whole brain homogenates, as previously described,[44] and were compared with the standards **1**−**3** (Table 1). Compounds **7a**, **c**, and **d** exerted significant inhibition of COMT in SK-N-SH cells (87−98%) and were found to be unexpectedly potent inhibitors of both peripheral and cerebral COMT, having noticeably lower IC_{50}'s for inhibition of peripheral COMT (0.11−0.13 M) than **1** and **3** (0.93 and 0.69 M, respectively). Rather surprisingly however, the ethanone **7b**, which structurally represents the ortho-nitrated analogue of BIA 3-202 **3**, was found to be a significantly less potent inhibitor in the SK-N-SH assay (76%). The lower potency of **7b**, compared to those of **7a**, **c**, and **d**, was confirmed by its IC_{50} value for liver COMT (2 μM), which was closest to that of the weakest inhibitor **2** tested.

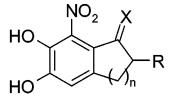
Table 2. Physical Constants and Percent Inhibition of COMT Activity in SK−N−SH Cells by Compounds **4** and **14a**−**l** (100 nM)[a]

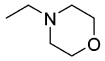
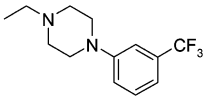
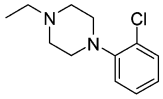


No.	NR_1R_2	mp (°C)	Formula	Anal.	% Inhibition
4					96 ± 1
14a		153-155	$C_{13}H_{16}N_2O_6$.HCl.1.3H_2O	C,H,N	88 ± 1
14b		197-198	$C_{14}H_{18}N_2O_5$.HCl.1.5H_2O	C,H,N	95 ± 1
14c		187-188	$C_{13}H_{16}N_2O_5$.HCl.1.25H_2O	C,H,N	93 ± 1
14d		141-143	$C_{19}H_{27}N_3O_6$.HCl.1.5H_2O	C,H,N	87 ± 2
14e		143-45	$C_{12}H_{20}N_2O_5$.HCl	C,H,N	89 ± 2
14f		183-184	$C_{15}H_{20}N_2O_5$.HCl1.5H_2O	C,H,N	86 ± 0
14g		179-180	$C_{18}H_{25}N_2O_5$.HCl	C,H,N	89 ± 2
14h		193-194	$C_{16}H_{22}N_2O_5$.HCl.H_2O	C,H,N	87 ± 1
14i		184-185	$C_{21}H_{24}N_2O_5$.HCl.H_2O	C,H,N	91 ± 2
14j		200-201	$C_{20}H_{23}N_3O_6$.HCl.H_2O	C,H,N	94 ± 2
14k		199-200	$C_{20}H_{20}F_3N_3O_5$.HCl	C,H,N	94 ± 1
14l		228-229	$C_{16}H_{23}N_3O_5$.2HCl	C,H,N	89 ± 0

[a] Results are mean SEMs of four experiments per group.

Table 3. Physical Constants and Percent Inhibition of COMT Activity in SK−N−SH Cells by Compounds **15**, **16**, **25**, **26** and **27a**−**d** (100 nM)[a]



No.	n	x	R	mp (°C)	Formula	Anal.	% Inhibition
15	2	O	H	224-6	$C_{10}H_9NO_5$	C,H,N	54 ± 1
16	1	O	H	223-5	$C_9H_7NO_5$	C,H,N	76 ± 1
25	2	NOH	H	196-8	$C_{10}H_{10}N_2O_5$	C,H,N	37 ± 2
26	1	NOH	H	200-1	$C_9H_8N_2O_5$	C,H,N	61 ± 3
27a	1	O		193-5	$C_{14}H_{16}N_2O_6$.HCl	C,H,N	79 ± 1
27b	1	O		177-9	$C_{15}H_{18}N_2O_5$.HCl	C,H,N	83 ± 1
27c	1	O		198-9	$C_{21}H_{21}F_3N_3O_5$.HCl	C,H,N	83 ± 1
27d	1	O		225-7	$C_{20}H_{21}ClN_3O_5$.HCl	C,H,N	91 ± 2

[a] Results are mean SEMs of four experiments per group.

On the other hand, all of the open-chain amino analogues **14a**−**l** maintained good inhibition of COMT in SK-N-SH cells (86−94%) irrespective of the side-chain substituent, and indeed, **14k** was essentially equipotent to its nitro-regioisomer **4** (Table 2). We then turned our attention to five- and six-membered ring-constrained carbocyclic analogues, and it was interesting to observe that the unsubstituted tetralone **15** was significantly less active than the lower indanoyl homologue **16** (Table 3). Modification of the carbonyl group, as in the corresponding oximes **25** and **26**, led to a further significant decrease in inhibition, confirming previous findings[34] in another series. Substitution to the carbonyl group of indanone **16** led to generally slight improvements in activity, with restoration of respectable inhibition observed only for the 2-(chlorophenyl)piperazine **27d** from this series. The 3-(trifluoromethylphenyl)piperazinyl indanone **27c** was found to be approximately 10% less active than both the open-chain analogue **14k** and the nitro regioisomer **4**. Of the three series tested, the cyclic derivatives listed in Table 3 appeared least interesting and were thus not studied further.

Attention was then turned to evaluating the effects of incorporation of the nitro group at the ortho position on in vivo parameters such as the duration of COMT inhibition and its ability to access the brain. Test compounds were administered by gastric tube to overnight-fasted rats (**1**−**3** and **7a**−**d**) or mice (**4**, **14a**−**g**, **14k**). Thereafter, at defined intervals, the animals were sacrificed and the liver and brains removed and used to assess COMT activity. Compounds **7a**−**d** were all found to have a rapid onset of action, with a maximum inhibitory effect observed at 30 min after administration, and general trends could be observed from the time course profiles (Table 4). In the brain, compounds **7b** and particularly **7c** presented an inhibition pattern over time similar to that of **3**, which is itself placed between **1** and **2**, and both retained still significant inhibition at 6 h post dose. Benzophenone **7a**, on the other hand, was essentially inactive at this latter time point. In this sense, **7a** has a much shorter duration of inhibition in the brain than its previously reported meta-nitrated analogue (97% at 3 h and 86% at 6 h), whereas **7b**, as mentioned above, is very similar in performance to its regioisomer **3**. However, more significant differences were observed in the liver. All compounds **7a**−**d** were relatively short-acting (surprisingly even more so than **2**), and **7b**−**d** in particular were practically inactive against liver COMT at 6 h post dose. The differences in inhibition over time between the regioisomers **3** and **7b** are particularly striking. In practical terms, it can be seen that **7a** and, to a slightly lesser extent, **d** have very similar inhibition patterns in both the brain and liver. Although **7b** and **c** are both highly active against both liver and brain COMT at shorter time points (0.5 and 1 h), the rapid reduction in peripheral inhibition at 3−6 h post dose means that, in effect, they are more active against brain COMT at these intermediate time points. Accordingly, these compounds represent the first COMT

Table 4. Percent Inhibition of COMT Activity by Compounds **1**−**3** and **7a**−**d** in Homogenates of Rat Brain and Liver after Administration by Gastric Tube (all at 30 mg/kg)[a]

	Time Course % Inhibition				
No.	0.5 h	1 h	3 h	6 h	9 h
			Brain		
1	99 ± 1	99 ± 1	97 ± 1	86 ± 8	78 ± 2
2	72 ± 7	45 ± 7	30 ± 6	20 ± 7	23 ± 3
3	84 ± 1	81 ± 3	65 ± 4	32 ± 3	22 ± 3
7a	88 ± 0.3	75 ± 2	48 ± 2	7 ± 8	−2.3 ± 1
7b	81 ± 2	81 ± 1	63 ± 6	35 ± 4	1.6 ± 3
7c	87 ± 1	82 ± 1	63 ± 1	39 ± 10	13 ± 9
7d	87 ± 1	65 ± 2	48 ± 2	33 ± 3	5 ± 10
			Liver		
1	100 ± 1	99 ± 1	98 ± 1	94 ± 1	67 ± 4
2	98 ± 1	96 ± 1	86 ± 2	74 ± 5	25 ± 8
3	99 ± 1	97 ± 2	96 ± 1	76 ± 4	70 ± 4
7a	98 ± 0.5	73 ± 1	48 ± 3	21 ± 15	−9 ± 7
7b	94 ± 1	92 ± 2	38 ± 11	8 ± 11	−26 ± 9
7c	99 ± 0.5	95 ± 1	34 ± 12	5 ± 14	4 ± 7
7d	99 ± 0.1	70 ± 8	45 ± 4	8 ± 1	18 ± 4

[a] Results are mean SEMs of four experiments per group.

Table 5. Percent Inhibition of COMT Activity by **4** and Compounds **14a**−**k** in Homogenates of Mouse Brain and Liver after Administration by Gastric Tube (All at 30 mg/kg)[a]

	% Inhibition			
	COMT Activity at 1 h		COMT Activity at 6 h	
No.	Liver	Brain	Liver	Brain
4	82 ± 7	14 ± 14	74 ± 4	14 ± 11
14a	58 ± 6	25 ± 3	7 ± 17	8 ± 4
14b	85 ± 3	8 ± 14	35 ± 7	−24 ± 12
14c	87 ± 2	18 ± 4	21 ± 14	−10 ± 8
14d	69 ± 5	−27 ± 14	10 ± 15	−72 ± 8
14e	76 ± 6	−11 ± 15	−10 ± 10	−45 ± 18
14f	88 ± 3	26 ± 18	80 ± 2	−12 ± 9
14g	91 ± 3	7 ± 8	87 ± 3	−19 ± 10
14k	91 ± 3	78 ± 5	36 ± 4	17 ± 16

[a] Results are mean SEMs of four experiments per group.

inhibitors presenting some degree of selectivity for central rather than peripheral COMT.

The in vivo time course results for **4**, **14a**−**g**, and **14k** in the mouse were equally revealing (Table 5). Whereas **4** has been pharmacologically characterized as a potent, long-acting, and peripherally selective inhibitor of COMT, the ortho-nitrated regioisomer **14k** was endowed with a much shorter duration of action, exhibiting only 50% of the peripheral activity of **4** at the 6 h time point. In contrast to **4**, **14k** was also a potent inhibitor of cerebral COMT at 1 h post dose. In analogy with **7b**, **14k** seems to be able to penetrate the BBB more effectively than its meta-nitro regioisomer **4** but is less able to sustain inhibition of COMT. Morpholine **14a** was poorly selective at the shorter time point and short acting, whereas piperidine **14b** and pyrrolidine **14c** were peripherally selective but still appreciably short acting compared to **4**. Adding substituents to the piperidine ring, such as 3-diethylamide **14d** and 3-methyl **14e**, abolished central activity at the cost of a further reduction in the duration of action. However, shifting the methyl group from C-3 to C-4 of the piperidine ring, as in **14f**, effectively restored both peripheral selectivity and duration of action, such that 80% inhibition was maintained at 6 h after administration. Annelation of a lipophilic ring to the piperidine substituent, as in the decahydroquinoline **14g**, resulted in a COMT inhibitor possessing thus far unprecedented properties, in that **14g** is virtually devoid of central action even shortly after administration (1 h), yet maintains almost 90% inhibition of peripheral COMT at 6 h post dose.

Previously, we reported details[45,46] on the structure of the ternary complex between recombinant rat liver S-COMT, the cosubstrate SAM, and compound **4**, as determined by X-ray crystallography. More recently, we disclosed preliminary details on the crystallization and crystallographic data of the ternary complex between S-COMT, SAM, and **7a**.[47] The crystals of this complex are not isomorphous to those of **4**, which may reflect different crystal contacts due to the nature of the side-chain substituent or position of the nitro group. Further studies are currently in progress to further characterize this and other S-COMT−SAM−ortho-nitrated inhibitor complexes, which are expected to reveal the effect on binding conformations of the inhibitors described herein caused by altering the position of the nitro group from the meta- to the ortho-position. Furthermore, following previous molecular modeling studies[48] on the interaction of **3** with COMT, molecular modeling efforts are currently being focused on analyzing the effects of the binding conformations of ortho-nitrated catechols, such as **7a**, on their metabolism. The results of crystallographic and molecular modeling studies will be reported in due course.

Conclusions

Several novel regioisomeric ortho-nitrated catechols structurally related to BIA 3-202 (**3**) and BIA 3-335 (**4**) have been synthesized and evaluated for their ability to inhibit COMT. Altering the position of the nitro group from the meta- to the ortho-position relative to the side-chain substituent of the nitrocatechol pharmacophore was found to have a profound effect on the in vitro potency and in vivo selectivity/duration of COMT inhibition. Compounds **7a**, **c**, and **d** of the alkylaryl series were found to be extremely potent inhibitors in vitro with significantly lower IC_{50}'s than their meta-nitrated regioisomers and had a rapid onset but shorter duration of action in vivo than their previously reported regioisomers. This may reflect subtle differences in the metabolic profiles of ortho- and meta-nitrated catechols, and this aspect is currently under further investigation. On the other hand, ethanone **7b** displayed reversed selectivity over **3** at 3 and 6 h post dose, thus exhibiting a degree of preferential inhibition of central COMT. In the amino-substituted series, regioisomer **14k** was found to be much less peripherally selective than **4** and endowed with a shorter duration of action, whereas the decahydroquinoline **14g** displayed an unprecedented combination of almost totally selective peripheral inhibition and excellent duration of action. Ethanone **7b** could be a useful tool to probe the pharmacological utility of short-acting but centrally selective COMT inhibitors in the treatment of depression in Parkinsonian patients. **14g** is also presented as a promising candidate for clinical evaluation as an adjunct to L-Dopa therapy for the treatment of the symptoms 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 order: chemical shift (ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), number of protons, approximate coupling constant (*J*) in hertz, and assignment of a signal. IR spectra were measured with a Bomem Hartmann & Braun MB Series FTIR spectrometer using KBr disks. Analytical TLC was performed on precoated silica gel plates (Merck 60 Kieselgel F_{254}) and visualized with UV light. 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. Solvents and reagents were purchased from Aldrich, Merck, and Fluka and used as received unless otherwise noted.

The following details are representative procedures for the synthesis of alkylaryl compounds **7a**−**d**. Compounds **10a**−**d** (R = H) were prepared as previously described.[34]

1-(4-Acetyloxy-3-methoxyphenyl)-2-phenyl-methanone (10a, *n* = 0, R = Ac). To a stirred solution of the phenol **10a** (*n* = 0, R = H) (14.9 g, 65 mmol) in dichloromethane (150 mL) at room temperature was added pyridine (9.17 g, 117 mmol) and DMAP (spatula tip) followed by acetic anhydride (10.2 g,

117 mmol) dropwise. The resulting solution was allowed to stir for 15 min and then washed by cold 2 N hydrochloric acid, saturated aqueous sodium bicarbonate solution, water, and brine and then dried over anhydrous sodium sulfate, filtered, and evaporated to leave a pale yellow solid. Recrystallization from dichloromethane−diethyl ether−petroleum ether gave colorless crystals (16.57 g, 94%) of mp 103−104 °C. ^{1}H NMR ($CDCl_3$) δ 7.62 (d, 1H, J = 2.0 Hz, H-2), 7.60 (dd, 1H, J = 8.0, 2.0 Hz, H-6), 7.40−7.21 (5H, m, Ar-H), 7.10 (d, 1H, J = 8.0 Hz, H-5), 3.9 (s, 3H, OCH_3), and 2.40 (s, 3H, $COCH_3$). ^{13}C NMR ($CDCl_3$) δ 198.4, 169.0, 151.8, 144.1, 141.6, 136.0, 128.9, 128.8, 126.6, 123.2, 121.8, 111.9, 56.5, and 21.1.

1-(4-Acetyloxy-3-methoxy-2-nitro-phenyl)-2-phenyl-methanone (11a, *n* = 0, R = Ac) and 1-(4-Acetyloxy-3-methoxy-6-nitro-phenyl)-2-phenyl-methanone (12a, *n* = 0, R = Ac). To a stirred solution of the *O*-acetate obtained above (16.4 g, 60.7 mmol) in acetic anhydride (170 mL) at room temperature was added copper (II) nitrate trihydrate (18.3 g, 75.87 mmol) in one portion. After approximately 10 min, an exothermic reaction began, which was controlled by occasionally cooling the reaction mixture in an ice−water bath over 30 min. Once the exotherm had subsided, the reaction mixture was poured onto water (1.2 L), and the resulting precipitate was filtered off, washed by water, and dried in air. Recrystallization from dichloromethane−petroleum ether gave **12a** (n = 0, R = Ac) as a pale-yellow solid (3.75 g, 20%) of mp 210.2−211.1 °C. ^{1}H NMR ($CDCl_3$) δ 7.85−7.75 (m, 5H, Ar-H), 7.71 (s, 1H, H-5), 7.20 (s, 1H, H-2), 3.91 (s, 3H, OCH_3), and 2.42 (s, 3H, $COCH_3$). ^{13}C NMR ($CDCl_3$) δ 193.8, 169.0, 154.0, 148.3, 144.8, 134.7, 129.7, 129.6, 129.2, 111.8, 111.4, 57.5, and 21.1.

The mother liquors were evaporated, and the residue was chromatographed over silica gel (dichloromethane−petroleum ether, 1:1). Homogeneous fractions were pooled and evaporated to leave an oil that solidified on standing. Recrystallization from dichloromethane−diethyl ether−petroleum ether gave **11a** (n = 0, R = Ac) as a very pale-yellow solid (9.89 g, 52%) of mp 84.5−86 °C. ^{1}H NMR ($CDCl_3$) δ 7.80−7.54 (m, 5H, Ar-H), 7.33 (d, 1H, J = 8.5 Hz, H-6), 7.22 (d, 1H, J = 8.5 Hz, H-5), 4.02 (s, 3H, OCH_3), and 2.41 (s, 3H, $COCH_3$). ^{13}C NMR ($CDCl_3$) δ 192.6, 169.0, 144.7, 144.1, 140.6, 134.0, 130.5, 129.1, 127.4, 125.8, 117.8, 56.5, and 21.1.

1-(4-Hydroxy-3-methoxy-2-nitro-phenyl)-2-phenyl-methanone (11a, *n* = 0, R = H). A stirred suspension of **11a** (n = 0, R = Ac) obtained above (11.3 g, 35.8 mmol) in methanol (120 mL) at room temperature was treated with aqueous 3 N sodium hydroxide solution (36 mL, 108 mmol) dropwise. The resulting red solution was stirred for 15 min and then cooled in an ice−water bath and acidified to pH 2 by the addition of concentrated hydrochloric acid. The resulting yellow precipitate was filtered off, washed with water, and dried to give a yellow solid (9.12 g, 93%) of mp 168.9−170 °C. ^{1}H NMR ($CDCl_3$) δ 7.80 (dt, 2H, J = 7.5, 1.7 Hz, Ar-H), 7.71 (tt, 1H, J = 7.5, 1.7 Hz, Ar-H), 7.55 (td, 2H, J = 7.5, 1.7 Hz, Ar-H), 7.32 (d, 1H, J = 8.5 Hz, H-6), 7.21 (d, 1H, J = 8.5 Hz, H-5), 6.35 (br, 1H, OH), and 4.02 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$) δ 192.5, 153.1, 144.7, 140.5, 136.5, 134.0, 130.4, 129.0, 127.3, 125.9, 117.6, and 63.4.

1-(3,4-Dihydroxy-2-nitro-phenyl)-2-phenyl-methanone (7a, *n* = 0). To a stirred solution of the methyl ether **11a** (n = 0, R = H) obtained above (0.27 g, 1 mmol) in 1,2-dichloroethane (3 mL) at room temperature was added aluminum chloride (0.15 g, 1.1 mmol) in one portion followed by pyridine (0.33 g, 4 mmol) dropwise. The resulting deep-red suspension was stirred at 100 °C for 30 min and then allowed to cool to room temperature. The mixture was poured onto ice−water (20 mL) and acidified by the addition of 2 N hydrochloric acid (4 mL). The phases were separated, and the aqueous phase was extracted with 10% 2-propanol−dichloromethane. The combined organic phases were washed with water and brine and then dried over anhydrous sodium sulfate, filtered, and evaporated to leave a yellow-orange solid. Recrystallization from dichloromethane−petroleum ether gave orange crystals (0.77 g, 81%). ν_{max} (KBr disk)/cm^{-1} 3246 (OH), 1656 (CO), 1550 (NO_2). ^{1}H NMR ($CDCl_3$) δ 10.41 (1H, br, OH), 7.83 (dt, 2H, J = 7.5, 1.4 Hz, Ar-H), 7.60 (tt, 1H, J = 7.5, 1.4 Hz, Ar-H), 7.52 (td, 2H, J = 7.5, 1.4 Hz, Ar-H), 7.32 (d, 1H, J = 8.2 Hz, H-6), 6.92 (d, 1H, J = 8.2 Hz, H-5) and 6.41 (br, 1H, OH). ^{13}C NMR ($CDCl_3$) δ 193.2, 148.1, 143.2, 134.1, 132.8, 129.1, 129.6, 129.2, 120.8, and 120.7.

1-(3,4-Dihydroxy-2-nitrophenyl)-3-[4-[3-(trifluoromethyl)-phenyl]-1-piperazinyl]-1-propanone Hydrochloride (14k). A mixture of 3,4-dihydroxy-2-nitroacetophenone (**13**) (0.099 g, 0.5 mmol), 35% aqueous formaldehyde solution (0.2 mL, 2.5 mmol), and concentrated hydrochloric acid (0.25 mL, 3 mmol) in 2-propanol (2.5 mL) was heated at reflux for 24 h. The reaction mixture was allowed to cool to room temperature, and the resulting precipitate was filtered off and dried in air. Recrystallization from acetic acid gave yellow crystals (0.19 g, 80%). ν_{max} (KBr disk)/cm^{-1} 3471 (OH), 1687 (CO), 1543 (NO_2). ^{1}H NMR (DMSO-d_6) δ 9.49 (2H, br, OH), 7.61 (1H, d, J = 8.5 Hz, H-6), 7.51−7.23 (m, 4H, Ar-H), 7.11 (1H, d, J = 8.5 Hz, H-5), 3.65 (t, 2H, $COCH_2$), 3.42 (t, 2H, CH_2), and 3.39−3.2 (m, 8H, 4 × CH_2). ^{13}C NMR (DMSO-d_6) δ 194.1, 153.7, 150.9, 140.0, 139.7, 131.2, 130.9, 125.4 (J = 272.3 Hz), 123.6, 120.3, 119.8, 116.8, 115.9, 112.8, 51.8, 51.5, 45.9, and 34.0.

6-Benzyloxy-7-hydroxy-1-tetralone (21, *n* = 2). To a stirred solution of 6,7-dihydroxy-1-tetralone (**19**, n = 2) (1.0 g, 5.62 mmol) in dimethylformamide (25 mL) at room temperature was added anhydrous potassium carbonate (0.78 g, 5.62 mmol) followed by benzyl bromide (0.96 g, 5.62 mmol) dropwise. The resulting suspension was stirred at 90 °C for 3 h and then allowed to cool to room temperature. Inorganic material was filtered off, and the filter cake was washed with dimethylformamide (5 mL). The solvent was then evaporated off and the residue partitioned between 2 N aqueous sodium hydroxide solution (25 mL) and diethyl ether (25 mL). The aqueous phase was separated and carefully acidified to pH 2 by the addition of 2 N hydrochloric acid. After extraction with ethyl acetate, the organic phase was washed with water and brine and then dried over anhydrous sodium sulfate, filtered, and evaporated to leave a brownish solid. Recrystallization from 2-propanol gave long beige needles (0.86 g, 57%) of mp 135−137 °C. ^{1}H NMR ($CDCl_3$) δ 7.61 (s, 1H, H-8), 7.41−7.28 (m, 5H, Ar-H), 6.75 (s, 1H, H-5), 5.6 (br, 1H, OH), 5.25 (s, 2H, CH_2Ph), 2.92 (t, 2H, J = 6.3 Hz, CH_2), 2.55 (t, 2H, J = 6.3 Hz, $COCH_2$), and 1.91 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ 202.2, 150.4, 145.0, 137.4, 135.9, 129.3, 129.1, 128.3, 125.9, 113.6, 111.1, 71.5, 39.3, 37.4, and 26.0.

6-Benzyloxy-7-hydroxy-8-nitro-1-tetralone (23, *n* = 2). To a stirred suspension of the *O*-benzyl-1-tetralone (**21**, n = 2) obtained above (0.81 g, 3.02 mmol) in glacial acetic acid (8 mL) in a water cooling bath was added 65% nitric acid dropwise (0.32 mL, 4.54 mmol), during which time the initial colorless mixture rapidly became deep-brown followed by the formation of a copious reddish precipitate. After stirring at room temperature for 40 min, the mixture was poured onto ice−water (50 mL), and the dark orange-red precipitate was filtered off, washed with water, and dried in air. Recrystallization from dichloromethane−ethyl acetate afforded yellow crystals (0.60 g, 63%) of mp 232−233 °C. ^{1}H NMR ($CDCl_3$) δ 7.48−7.27 (m, 5H, Ar-H), 6.81 (s, 1H, H-6), 6.06 (br, 1H, OH), 5.22 (s, 2H, CH_2Ph), 2.95−2.91 (t, 2H, J = 6.2 Hz, CH_2), 2.52−2.28 (t, 2H, J = 6.2 Hz, CH_2), and 1.98−1.95 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ 199.3, 150.8, 138.2, 138.1, 135.0, 129.7, 129.6, 128.5, 117.6, 112.3, 72.5, 39.5, 36.8, and 26.4.

6,7-Dihydroxy-8-nitro-1-tetralone (15, *n* = 2). A suspension of the nitro-phenol (**23**, n = 2) obtained from above (0.56 g, 1.79 mmol) in 30% solution of hydrogen bromide in acetic acid (5 mL) and 48% hydrobromic acid (5 mL) was heated at 110 °C for 30 min. The dark solution was then allowed to cool to room temperature and poured onto ice−water (100 mL). The resulting precipitate was filtered off, washed with water, and dried in air. Recrystallization from ethyl acetate gave yellow crystals (0.23 g, 58%). ν_{max} (KBr disk)/cm^{-1} 3477 (OH), 1663 (CO), 1543 (NO_2). ^{1}H NMR (DMSO-d_6) δ 10.61 (br, 2H 2 × OH), 6.81 (s, 1H, H-5), 2.85 (t, 2H, J = 5.8 Hz, CH_2), 2.53 (t, 2H, J = 6.2 Hz, $COCH_2$), and 2.02 (t, 2H, J = 6.1 Hz, CH_2).

^{13}C NMR (DMSO-d_6) δ 194.6, 153.3, 139.7, 139.4, 138.0, 115.9, 115.7, 39.3, 29.5, and 23.6.

6,7-Dihydroxy-8-nitro-1-hydroxyiminotetralone (25, *n* = 2). A suspension of the tetralone (**15**, $n = 2$) obtained from above (0.2 g, 0.89 mmol), hydroxylamine hydrochloride (0.2 g, 2.87 mmol), and pyridine (0.25 g, 3.14 mmol) in ethanol (10 mL) was stirred at 85 °C for 4 h and then allowed to cool to room temperature. The solvent was evaporated and the residue partitioned between ethyl acetate and water. The phases were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with 1 N hydrochloric acid, water, and brine and then dried over anhydrous sodium sulfate, filtered, and evaporated to leave a red-orange solid. Recrystallization from toluene−ethanol gave orange crystals (0.16 g, 75%). ν_{max} (KBr disk)/cm^{-1} 3507(NOH), 3412 (OH), 1531 (NO_2). ^{1}H NMR (DMSO-d_6) δ 10.92 (br, 1H, NOH), 9.85 (br, 2H, 2 × OH), 6.51 (s, 1H, H-5), 2.35−2.20 (m, 4H, 2 × CH_2), and 1.4 (q, 2H, J = 5.8 Hz, CH_2). ^{13}C NMR (DMSO-d_6) δ 150.8, 148.0, 139.1, 138.0, 132.9, 116.4, 114.1, 30.1, 24.4, and 21.8.

Pharmacology. Complete experimental protocols for the evaluation of in vitro and in vivo COMT inhibitory activity of new compounds have been previously reported.[34, 43−44] All animal interventions were performed in accordance with European Directive number 86/609, and the rules of the National Institute of Health's *Guide for the Care and Use of Laboratory Animals* (http://oacu.od.nih.gov/regs/guide/guidex.htm).

Supporting Information Available: Spectroscopic data for compounds **7b**−**d**, **14a**−**j**, **14l**, **15**, **16**, **25**, **26**, and **27a**−**d** and microanalytical data for all test compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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