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# Discovery of a Long-Acting, Peripherally Selective Inhibitor of Catechol-*O*-methyltransferase

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Novel nitrocatechol-substituted heterocycles were designed and evaluated for their ability to inhibit catechol-*O*-methyltransferase (COMT). Replacement of the pyrazole core of the initial hit **4** with a 1,2,4-oxadiazole ring resulted in a series of compounds endowed with longer duration of COMT inhibition. Incorporation of a pyridine *N*-oxide residue at position 3 of the 1,2,4-oxadiazole ring led to analogue **37f**, which was found to possess activity comparable to entacapone and lower toxicity in comparison to tolcapone. Lead structure **37f** was systematically modified in order to improve selectivity and duration of COMT inhibition as well as to minimize toxicity. Oxadiazole **37d** (2,5-dichloro-3-(5-(3,4-dihydroxy-5-nitrophenyl)-1,2,4-oxadiazol-3-yl)-4,6-dimethylpyridine 1-oxide (BIA 9-1067)) was identified as a long-acting, purely peripheral inhibitor, which is currently under clinical evaluation as an adjunct to L-Dopa therapy of Parkinson's disease.

## Introduction

Catechol-*O*-methyltransferase (COMT[a])[1] catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine to catecholic substrates such as endogenous catechol neurotransmitters[2] and xenobiotics[3,4] including (*S*)-2-amino-3-(3,4-dihydroxyphenyl)propanoic acid (L-Dopa), the gold standard[5] drug for treatment of Parkinson's disease (PD).[6,7] Coadministration of a peripheral amino acid decarboxylase (AADC) inhibitor prevents breakdown of L-Dopa in the periphery by blocking enzymatic decarboxylation,[8] and inhibition of COMT further improves its bioavailability by reducing the formation of 3-*O*-methyl-L-Dopa (3-OMD).

"First-generation" COMT inhibitors[3,9] such as pyrogallol, tropolone, and gallic acid are poorly selective and have poor efficacy *in vivo*. "Second-generation" inhibitors[10,11] are exemplified by tolcapone **1**,[12] entacapone **2**,[13] and nebicapone (BIA 3-202) **3**[14] (Figure 1). Structure−activity (SAR) studies exploring the position of the nitro group[15] and various side-chain substituents[16] have been reported. Subtle differences in the mode of COMT inhibition by **1**−**3** are thought to be relevant in terms of efficacy. Entacapone **2** is a short-acting,[17] peripherally selective inhibitor which is taken concomitantly with every dose of L-Dopa. Albeit the most widely marketed COMT inhibitor, the clinical efficacy of **2** has been questioned.[18] Tolcapone **1** is a more potent, longer acting but nonselective inhibitor of both cerebral and peripheral COMT. Unlike **2**, clinical use of **1** is severely restricted due to its elevated hepatotoxicity risk,[19] postulated to occur through uncoupling of oxidative phosphorylation.[20,21] Nebicapone **3** possesses a longer duration of peripheral COMT inhibition than **2** and more limited access to the brain than **1**, but due to limited clinical experience, firm conclusions concerning safety have not yet been established. Undoubtedly therefore, a requirement exists for improved COMT inhibitors to address the unmet medical needs of many PD patients.

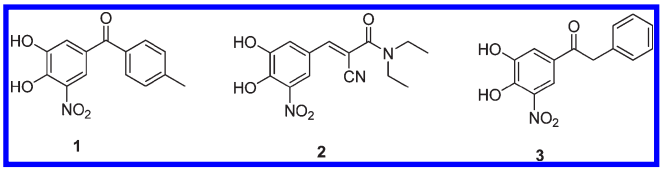


**Figure 1.** Chemical structures of tolcapone **1**, entacapone **2**, and nebicapone **3**.

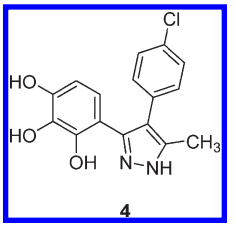


**Figure 2.** Chemical structure of screening hit **4**.

Our group has recently focused on the design of longer acting COMT inhibitors, and a cell viability assay was developed in order to assess the safety of the compounds. Pyrazole **4** (Figure 2) was found to possess activity in a preliminary *in vitro* screen. Although **4** has an atypical structure compared to classical COMT inhibitors, it offered scope for an optimization program, since several fragments of the molecule were amenable to structural modification. Introducing electron-withdrawing groups onto the catechol ring would be expected to increase COMT inhibition. The pyrazole could be replaced

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[a] Abbreviations: COMT, catechol-*O*-methyltransferase; PD, Parkinson's disease; AADC, amino acid decarboxylase; SAR, structure−activity relationship; ADMET, absorption, distribution, metabolism, excretion, toxicity; L-Dopa, (*S*)-2-amino-3-(3,4-dihydroxyphenyl)propanoic acid; 3-OMD, 3-*O*-methyl-L-Dopa.

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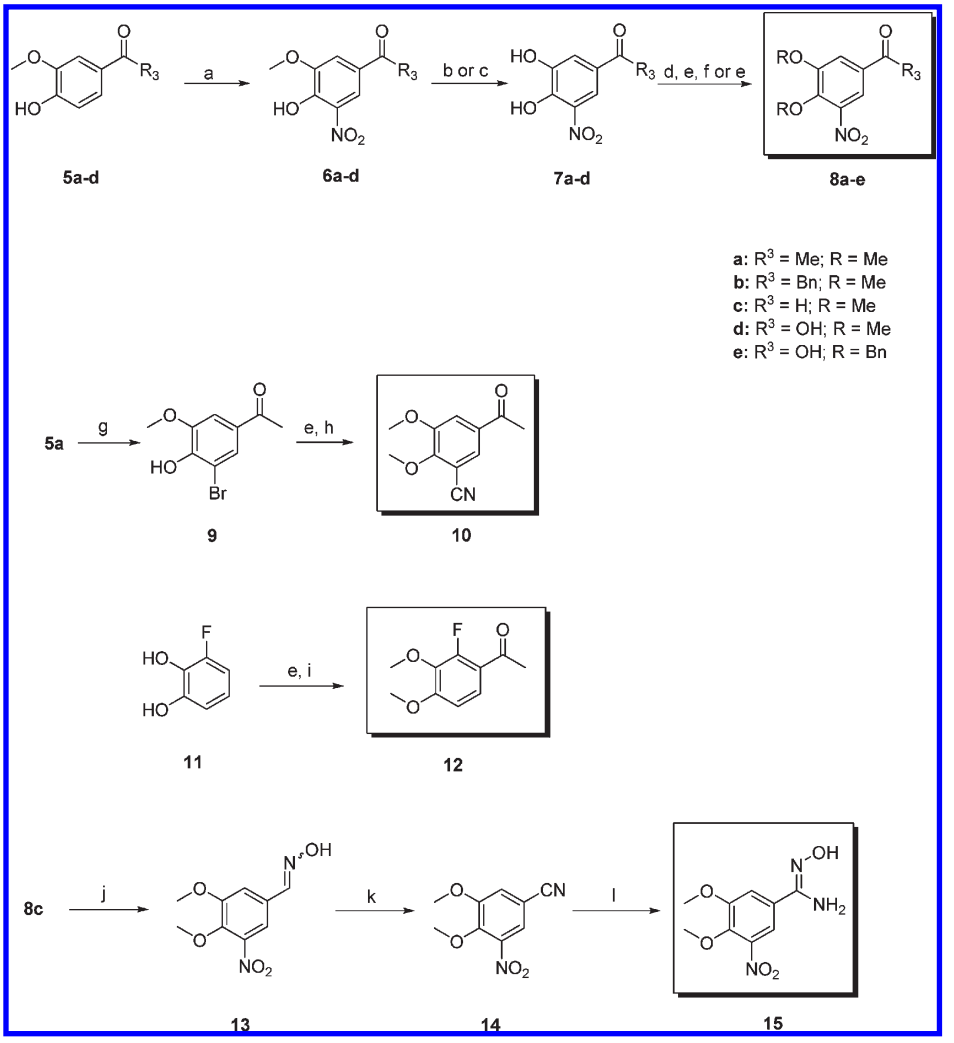
**Scheme 1**[a]




[a] Reagents and conditions: (a) 60% $HNO_3$, AcOH, room temperature; (b) 48% HBr, 120−140 °C; (c) $AlCl_3$, pyridine, DCE, reflux; (d) MeOH, HCl(g); (e) $Me_2SO_4$ or BnBr, acetone, reflux; (f) NaOH, MeOH; (g) $PhN(Me)_3^+Br_3^-$, THF, 0 °C; (h) CuCN, NMP, 170 °C; (i) $CH_3COCl$, $AlCl_3$, DCE, room temperature; (j) $NH_2OH \cdot HCl$, NaOAc, water, 60 °C; (k) $Ac_2O$, 140 °C; (l) $NH_2OH \cdot HCl$, KOH, ethanol, 70 °C.

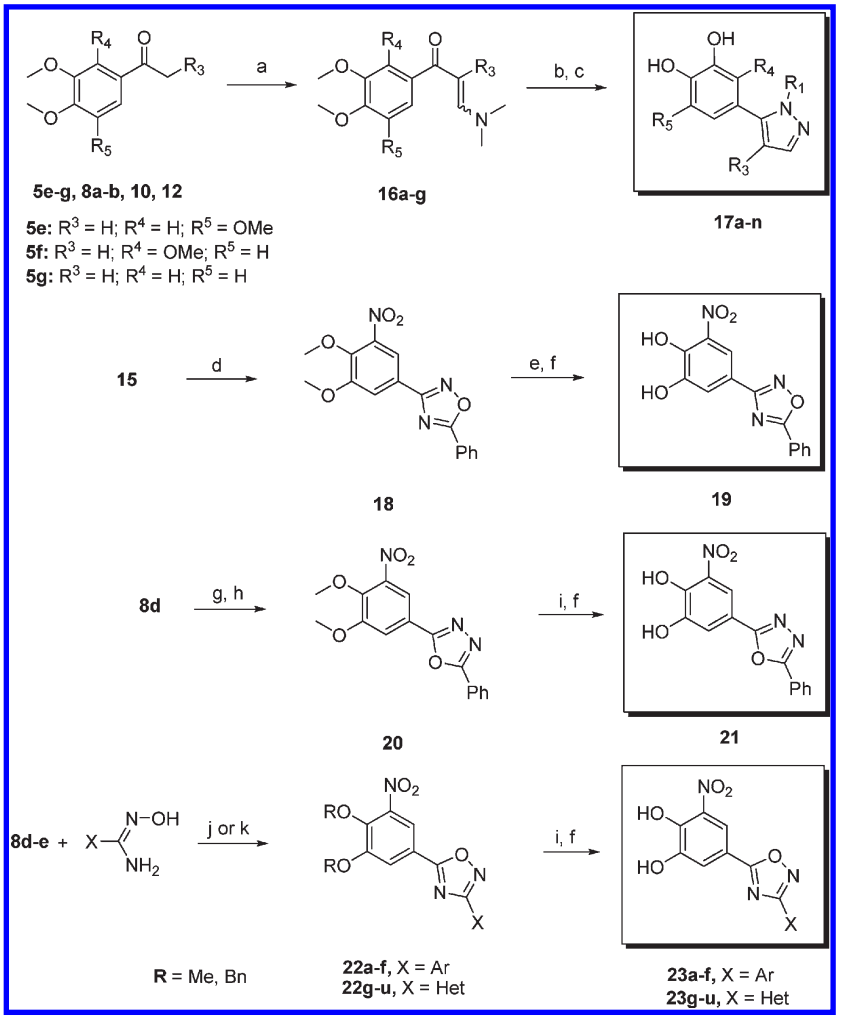
with other heterocycles of different size or containing other heteroatoms in alternative spatial arrangements within the ring. Finally, variation of the substituents on the central heterocyclic ring could allow manipulation of the physicochemical properties of the compounds, important for influencing ADMET properties.

## Chemistry

The catechol intermediates **8a**−**e**, **10**, **12**, and **15** containing various electron-withdrawing groups ($NO_2$, CN, F) were prepared as summarized in Scheme 1. Vanillin derivatives **5a**−**d** underwent regioselective nitration to the corresponding 5-nitro derivatives **6a**−**d** in moderate to good yields (55−95%). Attempts to directly protect the phenolic hydroxyl group through alkylation of **6a**−**c** to obtain **8a**−**c** failed. Thus, the monohydroxyl compounds **6b**−**d** were demethylated in boiling 48% aqueous hydrobromic acid (HBr). A milder procedure was applied for **6a**, wherein cleavage was effected with aluminum chloride ($AlCl_3$) and pyridine in refluxing 1,2-dichloroethane (1,2-DCE).[22] The nitrocatechols **7a**−**c** thus obtained were treated with dimethyl sulfate ($Me_2SO_4$) and potassium carbonate ($K_2CO_3$) in warm acetone to provide the fully protected intermediates **8a**−**c**. 3,4-Dihydroxy-5-nitrobenzoic acid (**7d**) was converted to the corresponding methyl ester and then etherified by reaction with either $Me_2SO_4$ or benzyl bromide (BnBr). Hydrolysis of the esters to carboxylic acid derivatives **8d** and **8e** was accomplished using aqueous sodium hydroxide in methanol at 60 °C. 5-Acetyl-2,3-dimethoxybenzonitrile (**10**) was prepared via the 5-bromo intermediate **9**. Regioselective bromination of acetovanillone **5a** was followed by *O*-methylation of the phenolic group (acetone, $Me_2SO_4$, $K_2CO_3$). The resulting intermediate **9** was heated with copper(I) cyanide in *N*-methyl-2-pyrrolidinone (NMP) to yield **10** in 40% yield. 1-Fluoro-2,3-dimethoxybenzene, which was prepared by exhaustive methylation of 3-fluorocatechol **11**, underwent Friedel−Crafts acylation with acetyl chloride to furnish acetophenone **12** in 58% yield. Synthesis of the amidoxime **15** began with 3,4-dimethyl-5-nitrobenzaldehyde (**8c**), which reacted with hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) to give the oxime **13** in 93% yield. Dehydration of **13** occurred in hot acetic anhydride, and the resulting nitrile **14** was converted to **15** on reaction with $NH_2OH \cdot HCl$.

Pyrazoles were prepared by the general method depicted in Scheme 2. Condensation of appropriately substituted acetophenones (**5e**−**g**, **8a**,**b**, **10**, **12**) with dimethylformamide

**Scheme 2**[a]



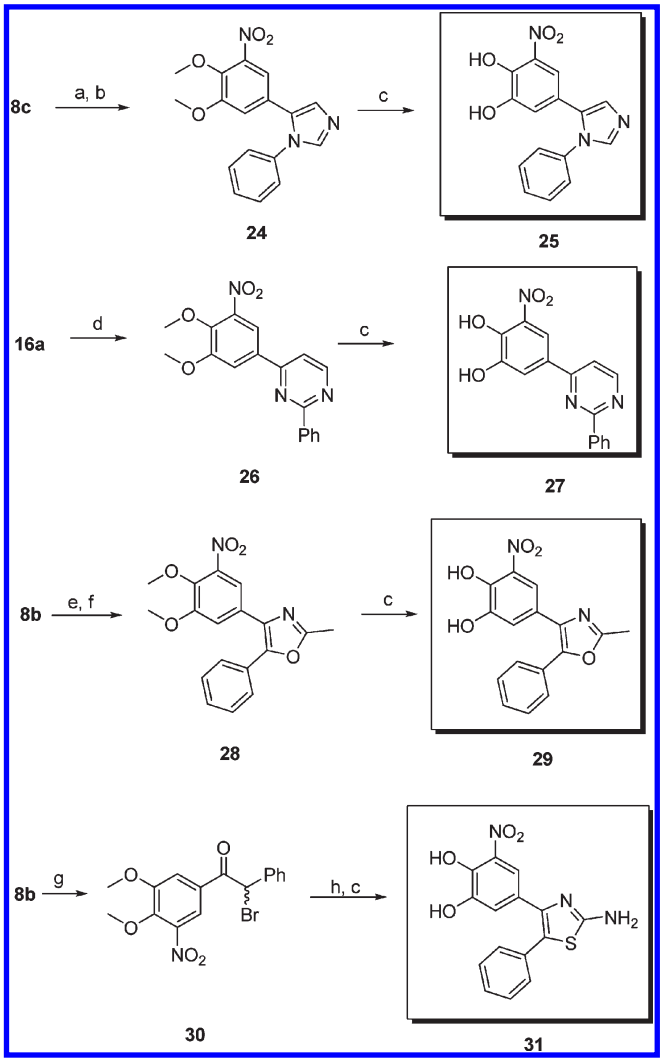

[a] Reagents and conditions: (a) DMF-dimethylacetal, neat, 140 °C; (b) $R_1$-NH-$NH_2$, EtOH, reflux; (c) 48% HBr, 140 °C; (d) $PhCO_2H$, CDI, DMF, room temperature, then 120 °C; (e) 48% aqueous HBr, 30% HBr in AcOH, 120 °C; (f) $AlCl_3$, pyridine, DCE, reflux; (g) Ph-(CO)-NH-$NH_2$, CDI, THF, reflux; (h) $POCl_3$, neat, 120 °C; (i) $BBr_3$, DCM, −78 °C, then room temperature; (j) CDI, DMF, room temperature, then 100−157 °C; (k) CDI, DMF, room temperature, then TBAF, THF, room temperature.

dimethylacetal (DMF-DMA) provided enaminones **16a**−**g** in good to excellent yield (67−96%), which were subsequently reacted with selected hydrazines to provide the dimethoxypyrazole derivatives.[23] Removal of both *O*-methyl protecting groups was achieved with aqueous 48% aqueous HBr to furnish the final catechol products **17a**−**k** and **17m**. In the case of the cyano-substituted pyrazole derived from the acetophenone **10**, deprotection of the methyl protecting groups under the strongly acidic conditions caused concomitant hydrolysis of the nitrile group, thereby forming the 5-carboxyl-substituted catechol derivative **17l**.

The synthetic routes employed to obtain the three possible oxadiazole regioisomers are briefly outlined in Scheme 2. Benzoic acid was condensed with amidoxime **15** using *N*,*N*′-carbonyldiimidazole (CDI) as the coupling agent, followed by thermal cyclization of the O-acylated intermediate to form the oxadiazole ring. Intermediate **18** was monodemethylated in a mixture of a 30% solution of HBr in AcOH and aqueous 48% HBr. Compound **19** was then obtained by heating the above mono-*O*-methyl intermediate (2-methoxy-6-nitro-4-(5-phenyl-1,2,4-oxadiazol-3-yl)phenol) with $AlCl_3$ and pyridine. In a similar fashion, O-protected 5-nitrovanillic acids **8d**,**e** were activated by CDI and then allowed to react with the corresponding amidoximes. The O-acylated amidoxime intermediates underwent thermal cyclization to provide oxadiazoles **22a**−**u**.[24] Alternatively, cyclization of certain isolated O-acylated amidoximes was promoted by tetrabutylammonium fluoride (TBAF) in THF.[25] Cleavage of the protecting groups of **22a**−**u** involved a two-step procedure. First, **22a**−**u** were treated with boron tribromide ($BBr_3$) in dichloromethane at −78 °C to furnish the intermediate monomethoxy intermediates, which were then fully demethylated on treatment with $AlCl_3$ and pyridine to access the desired 1,2,4-oxadiazolylnitrocatechols **23a**−**u**. The regioisomeric 1,3,4-oxadiazoles were prepared according to the following procedure. Benzohydrazide was acylated with 3,4-dimethoxy-5-nitrobenzoic acid (**8d**) using CDI in refluxing THF. Without purification, the intermediate thus obtained was dehydrated with phosphorus oxychloride to give the 1,3,4-oxadiazole **20**.[26] Heating **20** in aqueous 48% HBr led to destruction of the heterocyclic ring, while reaction of **20** with $BBr_3$ stalled after removal of only one methyl group. The second methyl group could however be removed on further treatment of this intermediate with $AlCl_3$ and pyridine.

Representative syntheses of various heterocyclic analogues are depicted in Scheme 3. Imidazole derivatives were prepared

**Scheme 3**[a]




[a] Reagents and conditions: (a) Ph-$NH_2$, ethanol reflux; (b) TOSMIC, DME−MeOH, $K_2CO_3$, reflux; (c) 48% HBr, 140 °C; (d) R-C(=NH)-$NH_2$·HCl, KO$^t$Bu, ethanol, reflux; (e) $Pb(OAc)_4$, AcOH, 110 °C; (f) $NH_4OAc$, AcOH, 120 °C; (g) $PhN(Me)_3{}^+Br_3{}^-$, THF, 0 °C; (h) $NH_2$-(C=S)$NH_2$, ethanol, reflux.

by a known method.[27] Thus aldehyde **8c** was condensed with aniline, and the resulting Schiff base was cyclocondensed with tosylmethyl isocyanide (TOSMIC) under basic conditions to provide the protected imidazole **24**, which underwent cleavage on treatment with 48% aqueous HBr to afford hydrobromide salt **25**. The pyrimidine **26** was prepared by cyclization of benzamidine hydrochloride with enaminone **16a** and potassium *tert*-butoxide in ethanol.[28] Demethylation of **26** in aqueous 48% HBr furnished catechol **27** in 67% yield. The oxazole analogue **29** was synthesized starting from ketone **8b**. Reaction of **8b** with lead(IV) tetraacetate in acetic acid and treatment of the intermediate with ammonium acetate in AcOH[29] gave oxazole **28**. Finally, cleavage of both methyl protecting groups occurred smoothly on reaction with 48% aqueous HBr. Aminothiazole **31** was also prepared by a literature method.[30] Bromination of ketone **8b** and treatment of phenacyl bromide **30** with thiourea in ethanol led to the cyclic thiazole intermediate, which underwent methyl ether cleavage on reaction with 48% aqueous HBr.

The general synthetic routes to support SAR exploration within the series of 1,2,4-oxadiazoles substituted with various pyridine *N*-oxide residues are outlined in Scheme 4 and are similar to the methods employed for preparation of 1,2,4-oxadiazoles (**23a**−**u**). Due to the difficulties encountered in the demethylation of intermediates **22a**−**u**, the more labile *O*-benzyl group was chosen for protection of the catechol. Pyridines containing electron-withdrawing groups (halogen, trifluoromethyl) did not readily undergo N-oxidation with either peroxyacetic acid or *m*-chloroperoxybenzoic acid (MCPBA), and only trace amounts of the desired *N*-oxides were detected. Therefore, a considerably stronger oxidant was required to obtain compounds **36a**−**d** and **32e**−**m**. The pyridine intermediates were oxidized using a mixture of urea−hydrogen peroxide addition complex (UHP) and trifluoroacetic anhydride (TFAA) in dichloromethane.[31] In most cases, conversions around 90% were achieved, even for those pyridines containing electron-withdrawing groups. Either the pyridyl *N*-oxide group could be introduced on a downstream building block (**32n**−**u**) or, alternatively, the oxidation step could be performed on the *O*-benzyl protected oxadiazole derivatives **35a**−**d**. The 2-trifluoromethylnicotinonitrile **32t** was prepared via a novel route[32] from a readily available fluorinated precursor. Accordingly, substituted nicotinonitriles **32a**−**m** were converted to the corresponding amidoximes **33a**−**m** on reaction with aqueous $H_2NOH$, and these were allowed to react with di-*O*-benzylnitrovanillic acid (**8e**) activated by CDI. After isolation of the O-acylated amidoxime intermediates **34a**−**m**, cyclization was performed either thermally in DMF or by treatment with TBAF in THF. Conversion of the protected pyridine *N*-oxides **36a**−**m** to the desired nitrocatechols **37a**−**m** was readily achieved on exposure to $BBr_3$.

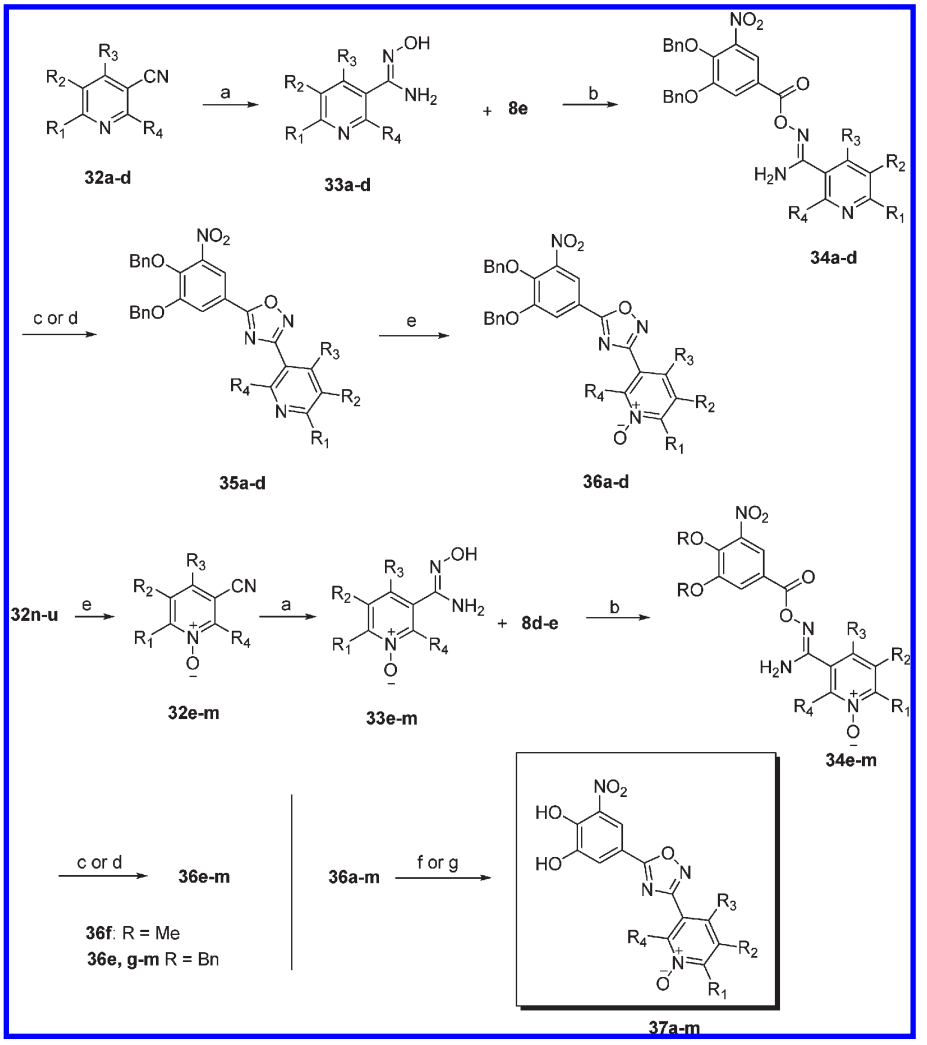
## Results and Discussion

*In vitro* COMT inhibition by new compounds was evaluated in rat liver homogenates by measuring the formation of metanephrine (*O*-methylated adrenaline) as previously described.[33] All nonnitrated catechols were evaluated under competitive conditions at a concentration of 30 $\mu$M, whereas nitrocatechols were tested under tight-binding conditions at a 10-fold lower concentration of 3 $\mu$M. The results from a representative selection of compounds (**17a**−**m**) are shown in Table 1. The 3,4,5-trihydroxypyrogallol (**17i**) was more active than the 2,3,4-trihydroxy isomers (**4**, **17j**) and the catechol **17m**, as well as compounds incorporating weakly electron-withdrawing groups (**17k**, fluorine, and **17l**, carboxyl). As expected, pyrazoles **17a**−**g** containing a nitro group exhibited the strongest inhibition.

Focus then shifted to the central pyrazole ring, and we looked first at the effect of substitution of the nitrogen atom, while maintaining a hydrogen atom at position $R_2$. The parent compound **17g** abolished COMT activity, as did compounds containing various substituents on the phenyl group (**17a**−**c** and **17e**). Residual COMT activity was observed when the phenyl group was replaced by smaller alkyl groups, such as methyl (**17d**). The introduction of a second aryl group at position $R_3$ however led to a drastic reduction in COMT inhibition (**17f**), which could be partially restored by insertion of a carbonyl group between the nitrogen of the pyrazole and the aryl ring (**17h**).

Thereafter, the pyrazole ring itself was modified. Several five- and six-membered heterocyclic analogues were prepared in which the number and arrangement of the heteroatoms varied. The majority of these compounds, as well as some of

**Scheme 4**[a]



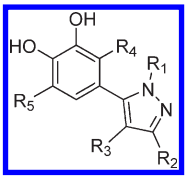

[a] Reagents and conditions: (a) $NH_2OH$, MeOH−$H_2O$, 80 °C; (b) CDI, DMF, room temperature; (c) DMF, 110−155 °C; (d) TBAF, THF, room temperature; (e) UHP, TFAA, DCM, room temperature; (f) $BBr_3$, DCM, −78 °C; (g) $BBr_3$, DCM, −78 °C, then $AlCl_3$, pyridine, DCE, reflux.

the most active pyrazole derivatives from the initial *in vitro* screen (**17c**, **17e**, and **17h**) were administrated orally (30 mg/kg) to mice. Thereafter, at 6 h postadministration, the animals were killed by decapitation, and the livers were removed and used to determine COMT activity. Results are shown in Table 2, which includes comparative data for reference compounds **1**−**3**. *N*-Phenyl-substituted pyrazole compounds (**17c**, **17e**) showed slightly improved *in vivo* inhibition over both **2** and **3**, but they were significantly less potent than **1**, which shows sustained COMT inhibition. Compound **17h**, which had performed reasonably well *in vitro*, did not display promising COMT inhibition *in vivo*. Surprisingly, it was discovered that replacement of pyrazole (**17c**) with an imidazole ring (**25**) led to an abrupt diminishment of COMT inhibition. On the contrary, pyrimidine **27** performed better than the pyrazoles and the reference standards **2** and **3**. Replacement of a nitrogen atom either with oxygen, as in oxazole **29**, or with sulfur, as in thiazole **31**, provided compounds with poor *in vivo* efficacy. Heterocyclic rings containing three heteroatoms were considered at this point, and oxadiazoles represented an attractive option, as one could envisage the synthesis of two 3,5-disubstituted 1,2,4-oxadiazolyl isomers as well as a third 1,3,4-oxadiazole isomer. While all three prototype oxadiazoles **19**, **21**, and **23a** showed greater COMT inhibition than pyrazoles **17c** and **17e**, the 1,3,4-isomer 21 was clearly the least active of this family. The 1,2,4-isomer 19, which incorporates the nitrocatechol fragment at position 3 of the heterocyclic ring, was significantly more active and reduced COMT activity by approximately 75%. However, the "reverse" 1,2,4-oxadiazole **23a**, in which the nitrocatechol group is located at position 5 of the heterocyclic ring, was found to the most active of the trio and essentially equipotent to **1**. This hierarchy of activity, 5-(3-nitrocatechol-5-yl)-1,2,4-oxadiazole > 3-(3-nitrocatechol-5-yl)-1,2,4-oxadiazole > 2-(3-nitrocatechol-5-yl)-1,3,4-oxadiazole, was observed consistently in each family of three regioisomeric oxadiazoles.

The selectivity of COMT inhibition by **19**, **21**, and **23a** was determined in rats at a lower dose of 3 mg/kg and at 3 h postadministration (Table 3). As expected, **21** was poorly active and uninteresting in terms of selectivity. Isomer **19** was found to be essentially equipotent in both the liver and the brain, causing an approximately 50% reduction of COMT activity. However, **23a** displayed a clear, 3-fold preference for peripheral COMT inhibition and was thus selected as a lead for further optimization.

The next round of SAR concentrated on substitution at position 3 of the oxadiazole ring. Table 4 details results (mice, 3 mg/kg, 3 h postdose) for new compounds and includes comparative data for **1** and **2**. First, with regard to

**Table 1.** *In Vitro* COMT Inhibition by **4** and Pyrazoles **17a**−**m** in Rat Liver Homogenates



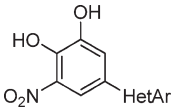

| no. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | % of control[c] |
|---|---|---|---|---|---|---|
| **4** | | | | | | $15.6 \pm 0.2^a$ |
| **17a** | *p*-tolyl | H | H | H | $NO_2$ | $0 \pm 0^a$ |
| **17b** | 3-chlorophenyl | H | H | H | $NO_2$ | $0 \pm 0^a$ |
| **17c** | phenyl | H | H | H | $NO_2$ | $0 \pm 0^a$ |
| **17d** | methyl | H | H | H | $NO_2$ | $1.1 \pm 0.1^a$ |
| **17e** | 4-cyanophenyl | H | H | H | $NO_2$ | $0.3 \pm 0.1^a$ |
| **17f** | 3-chlorophenyl | H | phenyl | H | $NO_2$ | $68.6 \pm 2.3^a$ |
| **17g** | H | H | H | H | $NO_2$ | $0 \pm 0^a$ |
| **17h** | 4-methylbenzoyl | H | phenyl | H | $NO_2$ | $2.8 \pm 0.4^a$ |
| **17i** | phenyl | H | H | H | OH | $0.8 \pm 0.2^b$ |
| **17j** | phenyl | H | H | OH | H | $26.8 \pm 0.6^b$ |
| **17k** | 4-(trifluoromethyl)phenyl | H | H | F | H | $32.3 \pm 1.8^b$ |
| **17l** | 3-chlorophenyl | H | H | H | COOH | $24.1 \pm 0.3^b$ |
| **17m** | *p*-tolyl | H | H | H | H | $41.2 \pm 1.2^b$ |

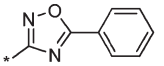
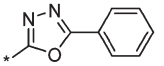
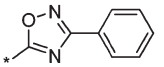
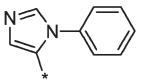
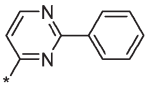
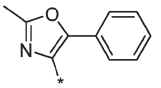
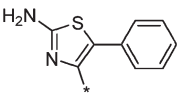
[a] Tight binding conditions, $C_{inhib} = 3\ \mu M$. [b] Competitive conditions, $C_{inhib} = 30\ \mu M$. [c] Results are given as percentage (%) of metanephrine formed relative to the control measured in the absence of inhibitor and are expressed as the mean ± SEM of four experiments per group.

phenyl rings, lipophilic substituents, in particular halogens, were beneficial in terms of potency (**23a**−**d**) whereas more polar piperazine or carboxamide residues were clearly detrimental (**23e**,**f**). Calculated log *P* values (see Experimental Section for details) for the most active oxadiazole compounds (**23a**−**d**) (log *P* 4.03−5.11) are considerably higher than for **1** (log *P* 3.30) and **2** (log *P* 2.00). Thus, despite their promising activity, **23a**−**d** might raise toxicity concerns. To this end, compounds **23a**−**f** were subjected to an *in vitro* toxicity test in a mouse neuroblastoma cell line as previously described.[34] This assay was validated by the finding that over 80% of cells remained viable after exposure to **2**, whereas **1** markedly reduced the viable cell count to less than 30%. Unfortunately, the viable cell count was reduced to between 12% and 40% on exposure to compounds **23a**−**d**. Paradoxically, cell viability was not significantly reduced on exposure to less lipophilic analogues **23e**,**f**, the least effective COMT inhibitors.

Incorporating lipophilicity index as a filter in the design of further compounds, several more hydrophilic compounds containing heterocyclic or heteroaromatic rings at position 3 of the oxadiazole ring were prepared. The morpholine **23g** and piperazine analogues **23i**,**j**, each having log *P* below 2, displayed higher inhibition than **2** and **23e**,**f** and showed borderline (**23g**) or low toxicity risk (**23i**,**j**). The more lipophilic 2-pyridyl-substituted piperazine **23h** showed interesting inhibition (32% of control) but proved to be as toxic as **1**. The phenyl ring was then replaced with various heterocyclic residues giving compounds covering a range of log *P* values. Bioisosteres of **23a** such as thiophene **23l** and isoxazole **23m** showed comparable activity and toxicity. Thiazole **23k** and pyrimidine **23q**, which contain weakly basic nitrogen atoms, both showed respectable COMT inhibition but borderline toxicity. The three pyridyl-substituted regioisomers **23n**−**p** were similar in terms of inhibition (approximately 30% of control), but only the 2-pyridyl analogue was relatively nontoxic. The more lipophilic pyrimidyl analogue **23q** showed no improvement. Substitution of the pyridyl ring was then

**Table 2.** *In Vivo* COMT Inhibition by Selected Heterocyclic Analogues in Mouse Liver Homogenates




| No. | HetAr | % of control[a,b] |
|---|---|---|
| **1** | | $6.8 \pm 3.0$ |
| **2** | | $93.6 \pm 29.1$ |
| **3** | | $85.7 \pm 10.2$ |
| **17c** | | $60.8 \pm 22.2$ |
| **17e** | | $61.1 \pm 20.9$ |
| **17h** | | $89.3 \pm 6.6$ |
| **19** |  | $24.4 \pm 17.5$ |
| **21** |  | $47.3 \pm 11.2$ |
| **23a** |  | $7.0 \pm 2.6$ |
| **25** |  | $109.0 \pm 16.8$ |
| **27** |  | 44.8±9.6 |
| **29** |  | $84.1 \pm 30.3$ |
| **31** |  | $77.4 \pm 14.3$ |

[a] All at 30 mg/kg, *po*, mice; COMT activity was determined 6 h after administration. [b] Results are given as percentage (%) of metanephrine formed relative to the control measured in the absence of inhibitor and are expressed as the mean ± SEM of four experiments per group. [c] An asterisk represents point of attachment.

**Table 3.** *In Vivo* COMT Inhibition of Oxadiazoles **19**, **21**, and **23a** in Homogenates of Rat Liver and Brain[a]

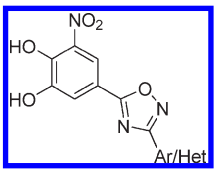
| no. | brain[a,b] | liver[a,b] |
|---|---|---|
| **19** | $55.6 \pm 2.4$ | $51.9 \pm 4.8$ |
| **21** | $87.9 \pm 21.2$ | $67.8 \pm 10.2$ |
| **23a** | $71.7 \pm 11.6$ | $20.6 \pm 4.4$ |

[a] Results are given as percentage (%) of metanephrine formed relative to the control measured in the absence of inhibitor and are expressed as the mean ± SEM of four experiments per group. [b] 3 mg/kg, *po*; COMT activity was determined 3 h after administration.

examined. Compounds (**23r**−**u**) presented similar inhibitory activity to that of **23a**, yet despite having lower log *P* also showed a relatively high toxicity risk.

The pyridines were further functionalized through oxidation of the nitrogen atom. The resulting *N*-oxides could be expected to have significantly lower lipophilicity. Although the very hydrophilic (log *P* 1.05) nicotinic *N*-oxide derivative **37f** (Table 5) displayed slightly lower activity than **2** (3 mg/kg *po*, rat, 3 h), encouragingly it was devoid of toxicity. It was hypothesized that the low activity of **37f** could be due to poor

**Table 4.** *In Vivo* COMT Inhibition in Mice Liver Homogenates and Cell Viability Count for Selected Oxadiazoles



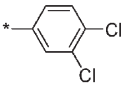
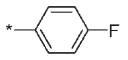
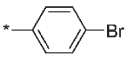
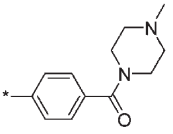
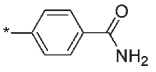
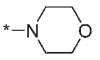
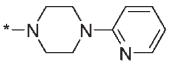
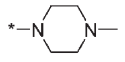
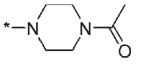
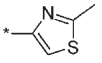
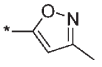
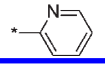
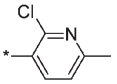
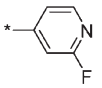
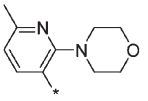
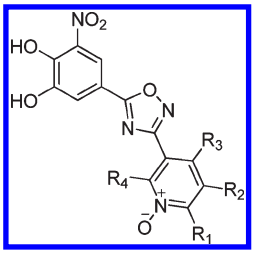

| No. | Ar/Het | Cyclisation[a]-Cleavage[b] method | % of Control[c,d] | Log*P* | Cell Viability[d,e] |
|---|---|---|---|---|---|
| **1** | | | 15.1 ± 4.1 | 3.30 | 27.2 ± 5.6 |
| **2** | | | 79.8 ± 17.1 | 2.00 | 81.4 ± 2.0 |
| **23a** |  | A/C, D | 20.6 ± 4.4 | 4.10 | 43.1 ± 3.5 |
| **23b** |  | A/C, D | 8.8 ± 2.3 | 5.11 | 37.7 ± 1.8 |
| **23c** |  | A/C, D | 12.7 ± 3.8 | 4.03 | 29.5 ± 1.8 |
| **23d** |  | A/D | 12.7 ± 5.8 | 5.00 | 12.2 ± 0.8 |
| **23e** |  | A/C | 74.0 ± 27.6 | 2.66 | 90.7 ± 2.2 |
| **23f** |  | A/C | 79.2 ± 21.2 | 3.05 | 91.2 ± 2.4 |
| **23g** |  | B/C | 45.8 ± 13.8 | 1.48 | 67.5 ± 4.3 |
| **23h** |  | B/C | 32.2 ± 8.2 | 2.64 | 24.6 ± 1.5 |
| **23i** |  | B/C | 56.8 ± 9.8 | 1.93 | 90.2 ± 2.9 |
| **23j** |  | B/C | 68.2 ± 11.3 | 1.80 | 103.7 ± 3.9 |
| **23k** |  | A/C | 22.9 ± 6.8 | 2.98 | 58.7 ± 2.1 |
| **23l** |  | A/C, D | 29.6 ± 15.3 | 3.90 | 19.3 ± 0.9 |
| **23m** |  | A/C | 32.4 ± 2.9 | 3.02 | 27.6 ± 1.7 |
| **23n** |  | A/C, D | 33.6 ± 8.0 | 2.78 | 26.7 ± 0.4 |
| **23o** |  | A/C | 32.1 ± 5.3 | 2.71 | 27.3 ± 1.3 |
| **23p** |  | B/C | 27.5 ± 13.6 | 2.75 | 70.5 ± 2.3 |

**Table 4.** Continued

| No. | Ar/Het | Cyclisation[a]-Cleavage[b] method | % of Control[c,d] | Log*P* | Cell Viability[d,e] |
|---|---|---|---|---|---|
| **23q** |  | A/C | 44.7 ± 5.3 | 1.93 | 60.5 ± 1.8 |
| **23r** |  | A/C, D | 21.6 ± 5.2 | 3.80 | 53.1 ± 4.5 |
| **23s** |  | A/C | 18.7 ± 14.3 | 2.80 | 25.0 ± 1.1 |
| **23t** |  | B/C | 19.3 ± 6.1 | 3.80 | 14.7 ± 0.6 |
| **23u** |  | B/C | 23.5 ± 5.8 | 2.96 | 43.8 ± 2.1 |

[a] A, CDI/DMF, room temperature, then Δ; B, TBAF, THF, room temperature. [b] C, $BBr_3$, DCM, −78 °C; D, $AlCl_3$, pyridine, 1,2-DCE or NMP, Δ. [c] All at 3 mg/kg, *po*; COMT activity was determined 3 h after administration; results are given as percentage (%) of metanephrine formed relative to the control measured in the absence of inhibitor. [d] Results are mean ± SEMs of four experiments. [e] % of remaining viable cells. [f] An asterisk represents point of attachment.

**Table 5.** *In Vivo* COMT Inhibition in Rat Liver Homogenates and Cell Viability Count for Selected Oxadiazolylpyridine *N*-Oxides



| no. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | cyclization method | % of control[a,b] | log *P* | cell viability[b,c] |
|---|---|---|---|---|---|---|---|---|
| **1** | | | | | | 23.8 ± 14.2 | 3.30 | 27.2 ± 5.6 |
| **2** | | | | | | 66.6 ± 25.6 | 2.00 | 81.4 ± 2.0 |
| **37a** | Me | H | Me | Cl | TBAF/THF | 13.9 ± 10.9 | 2.45 | 92.3 ± 2.8 |
| **37b** | Me | H | H | Br | CDI/DMF | 33.7 ± 7.7 | 2.08 | 76.3 ± 3.1 |
| **37c** | Me | Me | Me | Cl | TBAF/THF | 7.9 ± 3.8 | 2.91 | 95.1 ± 6.2 |
| **37d** | Me | Cl | Me | Cl | CDI/DMF | 0.7 ± 1.1 | 2.95 | 86.6 ± 2.3 |
| **37e** | H | H | $CF_3$ | H | TBAF/THF | 23.6 ± 13.3 | 2.03 | 93.4 ± 3.0 |
| **37f** | H | H | H | H | CDI/DMF | 77.0 ± 5.7 | 1.05 | 93.8 ± 2.7 |
| **37g** | Me | H | H | Cl | TBAF/THF | 31.6 ± 10.9 | 1.99 | 100.2 ± 3.2 |
| **37h** | H | H | H | Cl | TBAF/THF | 71.6 ± 8.2 | 1.53 | 93.7 ± 2.0 |
| **37i** | $CF_3$ | H | H | Me | TBAF/THF | 13.9 ± 7.2 | 2.48 | 62.2 ± 3.9 |
| **37j** | Me | H | $CF_3$ | Me | CDI/DMF | 1.3 ± 0.3 | 2.95 | 37.2 ± 4.0 |
| **37k** | Ph | H | $CF_3$ | Me | CDI/DMF | 24.3 ± 30.9 | 4.53 | 26.6 ± 2.8 |
| **37l** | H | H | H | $CF_3$ | TBAF/THF | 5.5 ± 1.8 | 2.03 | 96.4 ± 3.5 |
| **37m** | $CF_3$ | H | H | H | TBAF/THF | 34.4 ± 13.2 | 2.02 | 87.0 ± 3.1 |

[a] All at 3 mg/kg, *po*; COMT activity was determined at 3 h after administration; results are given as percentage (%) of metanephrine formed relative to the control measured in the absence of inhibitor. [b] Results are mean ± SEMs of four experiments. [c] % of remaining viable cells.

absorption caused by the very polar nature of the *N*-oxide group and that oral bioavailability might be improved by substituents on the pyridine *N*-oxide residue. Introduction of a trifluoromethyl group (**37e** and **37 m**) revealed that interesting levels inhibition could be achieved, without toxicity risk. Shifting the trifluoromethyl group to the 6-position gave **37l**, which was exceptionally active and devoid of toxicity. Further substitution (**37i**, methyl; **37j**, dimethyl; **37k**, phenyl) was investigated, and although **37j** was found to be even more potent than **37l**, these more lipophilic compounds significantly reduced the cell viability count.

Replacing the trifluoromethyl group chlorine as in **37h** failed to provide improvement over the parent 37**f**. However, the introduction of one, two, and three methyl groups to **37h**

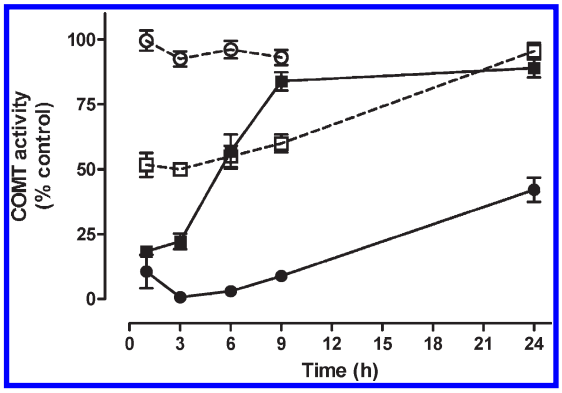



**Figure 3.** COMT activity in rat liver (closed symbols) and brain (open symbols) homogenates, determined at 1, 3, 6, 9, and 24 h after oral administration of 3 mg/kg **1** (squares) or **37d** (circles). Each point represents mean values ± SEM of $n = 4-8$.

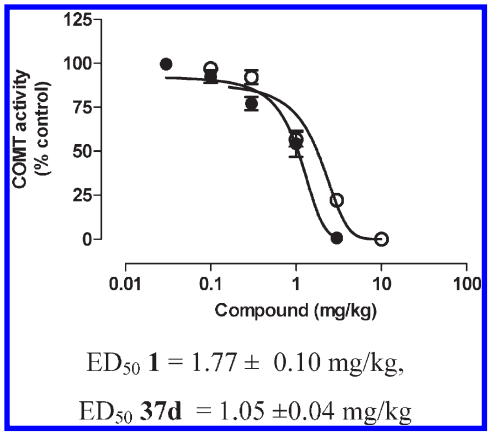



**Figure 4.** Dose-dependent inhibition of rat liver COMT determined 3 h after oral administration of 0.03, 0.1, 0.3, 1, 3, and 10 mg/kg **1** (open circles) or **37d** (closed circles). Each point represents mean values ± SEM of $n = 4-10$.

resulted in consecutive increases in COMT inhibition (**37g**, methyl; **37a**, dimethyl; **37c**, trimethyl) with no reduction in cell viability. Replacing the 3-methyl substituent of compound **37c** with a second chlorine atom gave **37d**, which abolished COMT activity while cell viability was not compromised. On the basis of these findings, **37d** was selected for further pharmacological studies.

Figure 3 compares the COMT inhibition profiles of **37d** and **1** in rat liver and brain homogenates. Compounds **1** and **37d** achieve maximal inhibitory effect at 1 and 3 h, respectively, and **1** is essentially inactive against peripheral COMT at 9 h while central inhibition remains around 50%. Oxadiazole **37d** has no measurable effect on central COMT, but significant peripheral inhibition (50%) is maintained up to 24 h. The concentration-dependent peripheral inhibitory potency of **1** and **37d** was then evaluated in rats given increasing doses of each compound (0.03−3 mg/kg). Animals were killed 3 h after administration, and COMT activity was then determined. The results are shown in Figure 4. Compound **37d** was found to be more potent than **1**, with $ED_{50}$'s of 1.05 ± 0.04 and 1.77 ± 0.10 mg/kg, respectively.

Finally, the pharmacodynamic interaction of **37d** with L-Dopa was assessed[35] and compared to **1**. Both compounds were administered to overnight fasted rats (3 mg/kg each); thereafter, at defined intervals (1, 6, and 23 h) L-Dopa (12 mg/kg) was coadministered with benserazide (3 mg/kg). One hour

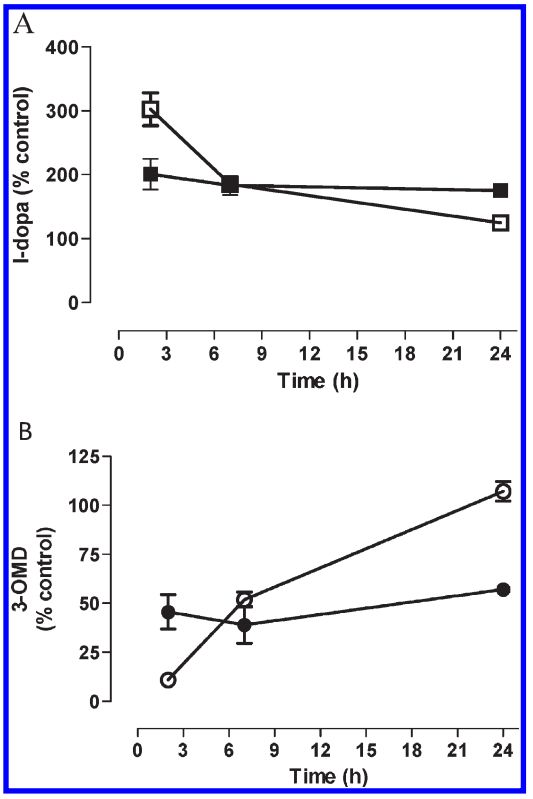



**Figure 5.** Plasma levels of L-Dopa (A) and 3-OMD (B) determined 1 h after oral administration of L-Dopa/benserazide (12/3 mg/kg) and 2, 7, or 24 h after oral administration of 3 mg/kg **1** (open circles) or **37d** (closed circles). Each point represents mean values ± SEM of $n = 4-16$.

later (i.e., 2, 7, and 24 h after administration of **1** and **37d**), L-Dopa and 3-OMD levels were assayed by HPLC. As shown in Figure 5A, **1** caused an abrupt, 3-fold increase in L-Dopa levels after 2 h, which fell by one-third at 7 h and was followed by a gradual return to baseline at 24 h. This rapid fluctuation in L-Dopa levels could potentially cause undesired CNS-related side effects. Conversely, **37d** conferred a more conservative 2-fold increase in L-Dopa levels at 2 h, but importantly this increase was sustained virtually over the entire 24 h period. The ability of **37d** to deliver a consistent increase in L-Dopa levels over time as a result of prolonged peripheral COMT inhibition could potentially make it suitable for once daily administration. A complementary trend could be observed for 3-OMD levels in plasma (Figure 5B). Two hours after administration of **1**, significantly reduced 3-OMD levels were observed, which then rose sharply up to 7 h followed by a gradual return to baseline over 24 h. With **37d**, however, 3-OMD levels were initially reduced to just below 50% of control and sustained essentially over the entire 24 h period.

## Conclusion

Novel heterocyclic COMT inhibitors were derived from the initial *in vitro* screening hit **4**. 1,2,4-Oxadiazoles substituted with a pyridine *N*-oxide motif (**37a**−**m**) were found to have reduced toxicity risk and were endowed with longer duration of inhibition than reference compounds **1** and **2**. Oxadiazole **37d** (2,5-dichloro-3-(5-(3,4-dihydroxy-5-nitrophenyl)-1,2,4-oxadiazol-3-yl)-4,6-dimethylpyridine 1-oxide, BIA 9-1067) was selected for further pharmacological studies and was found to be a purely peripheral inhibitor of COMT with a unprecedented duration of action. In addition, **37d** presents

favorable pharmacodynamics with L-Dopa, which results in stable and sustained plasma L-Dopa levels over prolonged periods, such that a single daily dose of **37d** may be effective in the clinical setting of PD.

## Experimental Section

**Chemistry.** 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), number of protons, multiplicity (s, singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quartet; m, multiplet; br, broad), approximate coupling constant (*J*) in hertz, and assignment of a signal. Elemental analyses were performed on a CE Instruments, EA 1110 CHNS analyzer. The purity of the compounds in all cases was higher than 95%. Analytical TLC was performed on precoated silica gel plates (Merck 60 Kieselgel F 254) and visualized with UV light. Solvents and reagents were purchased from Aldrich, Merck, and Fluka and were used without further purification unless otherwise noted. Compounds **32b**,[36] **32c**,[37] **32r**,[38] and **32s**[39] were synthesized by known methods.

**1-(4-Hydroxy-3-methoxy-5-nitrophenyl)ethanone (6a).** To a solution of acetovanillone **5a** (20 g, 120.35 mmol) in acetic acid (200 mL) was added 60% nitric acid (9.7 mL, 126.4 mmol) dropwise. The reaction was allowed to stir at room temperature for 0.5 h and then poured into ice−water (1 L). The resulting precipitate was filtered off, washed with water, and dried under vacuum to give the product as a yellow powder (20.37 g, 80%). $^1$H NMR ($CDCl_3$): $\delta$ 11.14 (1H, s), 8.31 (1H, d, $J$ = 2 Hz), 7.76 (1H, d, $J$ = 2 Hz), 4.00 (3H, s), 2.63 (3H, s). $^{13}$C NMR ($CDCl_3$): $\delta$ 194.9, 150.3, 150, 132.9, 128.3, 117.7, 115.1, 56.8, 26.

**1-(3,4-Dihydroxy-5-nitrophenyl)ethanone (7a).** Compound **6a** (20.3 g, 96.13 mmol) was suspended in 1,2-dichloroethane (200 mL) at 0 °C and treated with $AlCl_3$ (14.10 g, 105.74 mmol) followed by addition of pyridine (30.41 g, 384.52 mmol) dropwise under argon. The red reaction mixture was heated at reflux for 2 h, then was cooled to room temperature, and poured onto a mixture of ice and 2 N HCl. The resulting yellow precipitate was filtered off, washed with water, and dried under vacuum to give a yellow solid (17.3 g, 91%). $^1$H NMR (DMSO-$d_6$): $\delta$ 10.86 (2H, br), 7.94 (1H, d, $J$ = 2 Hz), 7.56 (1H, d, $J$ = 2 Hz), 2.52 (3H, s). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 195.4, 147.6, 145.8, 137.1, 127.3, 117.1, 116.5, 26.3.

**3,4-Dihydroxy-5-nitrobenzaldehyde (7c).** A suspension of **6c** (19 g, 96.4 mmol) in 48% aqueous HBr (165 mL) was heated at 140 °C for 4 h; then the reaction was cooled to room temperature and poured onto ice−water. The resulting dark yellow precipitate was filtered off, washed with water, then dissolved in ethyl acetate, and dried over $MgSO_4$. The solution was filtered and evaporated under vacuum. The residue was stirred with diethyl ether, filtered, and dried in air to leave a yellow powder (10.65 g, 60%). $^1$H NMR (DMSO-$d_6$): $\delta$ 11.0 (2H, br), 9.80 (1H, s), 7.98 (1H, d, $J$ = 2 Hz), 7.46 (1H, d, $J$ = 2 Hz). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 190.2, 148, 147, 137, 126.7, 119.6, 115.5.

**3,4-Dihydroxy-5-nitrobenzoic Acid (7d). 6d** (18.5 g, 86.8 mmol) was placed in a mixture of aqueous 48% HBr (120 mL) and 30% HBr in acetic acid (120 mL). The reaction mixture was heated at 120 °C for 3.5 h, then cooled to room temperature, and poured onto ice−water. The precipitated solid was filtered off and washed with water. The filtrate was extracted by ethyl acetate, and the extracts were washed with brine, then dried over $MgSO_4$, filtered, and evaporated leaving a yellow solid. Both solids were combined and dried by azeotropic distillation with toluene twice to leave a yellow solid (16.42 g, 95%). $^1$H NMR (DMSO-$d_6$): $\delta$ 10.7 (1H, br), 7.85 (1H, s br), 7.57 (1H, s br). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 166.1, 147.9, 145.8, 137.3, 121.2, 119.3, 117.1.

**1-(3,4-Dimethoxy-5-nitrophenyl)ethanone (8a).** A mixture of **6a** (17.26 g, 87.55 mmol), $K_2CO_3$ (42.3 g, 306.52 mmol), and dimethyl sulfate (55.21 g, 437.76 mmol) in acetone (350 mL) was stirred at vigorous reflux for 1.5 h. The reaction mixture was then cooled to room temperature and filtered through Celite, and the filter cake was washed with acetone. The combined filtrate was evaporated to leave an oil that crystallized from 2-propanol to leave a pale yellow solid (12.29 g, 62%). $^1$H NMR ($CDCl_3$): $\delta$ 7.90 (1H, d, $J$ = 2 Hz), 7.72 (1H, d, $J$ = 2 Hz), 4.05 (3H, s), 3.98 (3H, s), 2.62 (3H, s). $^{13}$C NMR ($CDCl_3$): $\delta$ 195.1, 154.2, 146.8, 144.2, 132.1, 117.1, 114.2, 62.1, 56.6, 26.3.

**3,4-Dimethoxy-5-nitrobenzoic Acid (8d).** Acid **7d** (16.4 g, 82.36 mmol) was dissolved in MeOH (300 mL), and dry HCl gas was bubbled through the mixture. After 30 min, the HCl stream was shut off, and the mixture was stirred at room temperature for 1 h. The methanol was removed by evaporation, and the residue was dissolved in a mixture of 2-propanol/dichloromethane (1:9). After being washed with water, the organic phase was dried over $MgSO_4$ and filtered. Evaporation of the solvent afforded 16.68 g of a yellow solid. The thus obtained methyl 3,4-dihydroxy-5-nitrobenzoate (16.65 g, 78.1 mmol) was taken up in acetonitrile (250 mL), and freshly powdered $K_2CO_3$ (54.0 g, 391 mmol) was added, followed by dimethyl sulfate (49.31 g, 391 mmol). The resulting mixture was heated at 100 °C for 1.5 h and then allowed to cool to room temperature. The insoluble material was filtered off, and the filter cake was washed with acetonitrile. The combined filtrate was evaporated to leave an orange oil that solidified on standing overnight. Recrystallization from 2-propanol gave a white solid (9.5 g, 50%). The thus obtained methyl 3,4-dimethoxy-5-nitrobenzoate (9.5 g, 39.39 mmol) was suspended in a mixture of methanol (150 mL) and water (30 mL). Aqueous sodium hydroxide solution (3 N) (39 mL, 117 mmol) was added in one portion, and the mixture was stirred at 60 °C for 1 h. The resulting deep red solution was cooled to room temperature. The methanol was removed by evaporation, and water (200 mL) was added to the residue. The mixture was cooled in ice−water, and 37% aqueous HCl was added until pH 2 was reached. The copious white precipitate was filtered off, washed with water, and dried under vacuum (8.2 g, 92%). $^1$H NMR ($CDCl_3$): $\delta$ 8.11 (1H, s), 7.81 (1H, s), 4.08 (3H, s), 4.02 (3H, s). $^{13}$C NMR ($CDCl_3$): $\delta$ 169.0, 153.7, 146.9, 144.3, 124.2, 118.4, 116.4, 62.3, 56.7.

**1-(3-Bromo-4-hydroxy-5-methoxyphenyl)ethanone (9).** To an ice-cooled solution of acetovanillone **5a** (5.0 g, 30.12 mmol) in THF (50 mL) was added phenyltrimethylammonium tribromide (12.49 g, 33.23 mmol), allowing each portion to dissolve before adding the next. After being stirred for 15 min in the cold, the reaction was poured onto cold water (400 mL). The resulting precipitate was filtered off, washed with water, and dried in air. Recrystallization from 2-propanol afforded beige crystals (3.98 g, 54%). $^1$H NMR ($CDCl_3$): $\delta$ 7.75 (1H, d, $J$ = 1.8 Hz), 7.48 (1H, d, $J$ = 1.8 Hz), 6.45 (1H, s, exch), 3.98 (3H, s), 2.57 (3H, s).

**5-Acetyl-2,3-dimethoxybenzonitrile (10).** Compound **9** (3.92 g, 16 mmol) was converted to 1-(3-bromo-4,5-dimethoxyphenyl)ethanone by a similar procedure described for **8a**. Recrystallization from dichloromethane−petroleum ether afforded a white solid (3.15 g, 76%). A mixture of the thus obtained 1-(3-bromo-4,5-dimethoxyphenyl)ethanone (1.69 g, 6.52 mmol) and Cu(I)CN (642 mg, 7.18 mmol) was heated in NMP (10 mL) at 170 °C for 5 h. The reaction mixture was cooled to room temperature and then poured onto water. The brown precipitate was filtered off and washed with water. The solid was stirred in a dichloromethane−2-propanol mixture (9:1) and left in an ultrasound bath at room temperature for 10 min. After the suspended material was allowed to sediment, the mixture was then filtered through Celite. The filtrate was washed with water and brine and then dried over $MgSO_4$. Evaporation of the solvent left a brown solid, which was recrystallized from dichloromethane−2-propanol to leave a pale brown solid (512 mg, 40%). $^1$H NMR ($CDCl_3$): $\delta$ 7.74 (1H, d, $J$ = 1.8 Hz), 7.73 (1H, d, $J$ = 1.8 Hz), 4.16 (3H, s), 3.96 (3H, s), 2.60 (3H, s). $^{13}$C NMR ($CDCl_3$): $\delta$

194.8, 155.1, 152.1, 132.6, 125.8, 115.6, 115.2, 105.6, 61.8, 56.3, 26.4.

**1-(2-Fluoro-3,4-dimethoxyphenyl)ethanone (12).** 3-Fluorocatechol **11** (5.0 g, 39.06 mmol) was converted to 1-fluoro-2,3-dimethoxybenzene by a similar procedure as described for **8a**, and the product was then taken up in 1,2-dichloroethane (50 mL), whereupon acetyl chloride (5.685 g, 72.42 mmol) was added. After the mixture was cooled in an ice−water bath, $AlCl_3$ (12.37 g, 92.77 mmol) was added in portions. The mixture was stirred in the cold for 1 h and then at room temperature for 0.5 h. The reaction was then cooled again and carefully quenched with a mixture of ice and 2 N HCl. The phases were separated, and the organic phase was washed with water and brine, then dried over $Na_2SO_4$, filtered, and evaporated. The residue was chromatographed (petroleum ether−ethyl acetate, 4:1) to give 4.45 g of a mobile oil that solidified on standing (58%). $^1$H NMR ($CDCl_3$): $\delta$ 7.66 (1H, dd, $J$ = 8.2 and 9 Hz), 6.77 (1H, dd, $J$ = 1.6 and 9 Hz), 3.95 (3H, s), 3.94 (3H, d, $J$ = 1 Hz), 2.62 (3H, d, $J$ = 5.5 Hz). $^{13}$C NMR ($CDCl_3$): $\delta$ 194.3 (d, $J$ = 3.5 Hz), 157.6 (d, $J$ = 5 Hz), 156.4 (d, $J$ = 254 Hz), 136.8 (d, $J$ = 15 Hz), 125.1 (d, $J$ = 4.5 Hz), 119.6 (d, $J$ = 12.5 Hz), 107.1 (d, $J$ = 3 Hz), 61.7 (d, $J$ = 3.3 Hz), 56.4, 31.7 (d, $J$ = 7 Hz).

**3,4-Dimethoxy-5-nitrobenzaldehyde Oxime (13).** A mixture of aldehyde **8c** (1.5 g, 7.11 mmol), hydroxylamine hydrochloride (2.07 g, 29.79 mmol), and sodium acetate (5.21 g, 63.53 mmol) was heated in water (55 mL) at 60 °C for 1 h and then cooled to room temperature. The resulting precipitate was filtered off, washed with water, and dried under vacuum to give the product as off-white crystals (1.5 g, 93%). $^1$H NMR (DMSO-$d_6$): $\delta$ 11.53 (1H, s), 8.17 (1H, s), 7.61 (1H, s, br), 7.57 (1H, s, br), 3.92 (3H, s), 3.88 (3H, s). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 153.3, 146, 144.3, 141.7, 129.2, 113.3, 113.3, 61.7, 56.5.

**3,4-Dimethoxy-5-nitrobenzonitrile (14).** Oxime **13** (1.45 g, 6.41 mmol) was suspended in acetic anhydride (10 mL) and the mixture then heated at 140 °C for 24 h. After being cooled to room temperature, the mixture was poured onto ice−water. The resulting precipitate was filtered off, washed with water, and dried under vacuum. Trituration in a mixture of petroleum ether and diethyl ether followed by filtration and drying gave the title compound as an off-white powder (1.15 g, 86%). $^1$H NMR ($CDCl_3$): $\delta$ 7.66 (1H, d, $J$ = 2 Hz), 7.31 (1H, d, $J$ = 2 Hz), 4.06 (3H, s), 3.98 (3H, s). $^{13}$C NMR ($CDCl_3$): $\delta$ 154.4, 146.5, 144.6, 120.3, 118.1, 116.6, 107.2, 62.5, 56.9.

**3,4-Dimethoxy-5-nitrobenzamidoxime (15).** To a stirred mixture of nitrile **14** (1.10 g, 5.29 mmol) and hydroxylamine hydrochloride (1.481 g, 21.30 mmol) in ethanol (35 mL) was added 12 mL of 10% aqueous KOH solution. The reaction was heated 70 °C for 1 h and then allowed to cool to room temperature. The ethanol was removed by vacuum, the residue was dissolved in ethyl acetate, dried over $MgSO_4$, and filtered, and the solvent was evaporated. Trituration in a mixture of petroleum ether and diethyl ether followed by filtration and drying gave the title compound as an off-white powder (1,15 g, 90%). $^1$H NMR (DMSO-$d_6$): $\delta$ 9.88 (1H, s), 7.69 (1H, s), 7.62 (1H, s), 6.05 (2H, s), 3.93 (3H, s), 3.38 (3H, s). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 152.8, 148.6, 143.9, 141.4, 129.2, 113.3, 112, 61.6, 56.6.

**1-(3,4-Dimethoxy-5-nitrophenyl)-3-(dimethylamino)prop-2-en-1-one (16a).** A suspension of acetophenone **8a** (12.22 g, 54.26 mmol) in dimethylformamide dimethylacetal (80 mL) was stirred at 140 °C for 5 h and then allowed to cool to room temperature. The resulting orange precipitate was filtered off, washed with diethyl ether, and dried in air to give the product (13.12 g, 86%). $^1$H NMR ($CDCl_3$): $\delta$ 7.84 (1H, d, $J$ = 12.3 Hz), 7.81 (1H, d, $J$ = 2 Hz), 7.72 (1H, d, $J$ = 2 Hz), 5.64 (1H, d, $J$ = 12.3 Hz), 4.00 (3H, s), 3.98 (3H, s), 3.19 (3H, s), 2.96 (3H, s). $^{13}$C NMR ($CDCl_3$): $\delta$ 184.7, 155, 153.9, 144.8, 144, 135.9, 115.1, 114.9, 90.7, 62, 56.5, 45.3, 37.5.

**3-Nitro-5-(1- *p*-tolyl-1*H*-pyrazol-5-yl)benzene-1,2-diol (17a).** A mixture of enaminone **16a** (522 mg, 1.86 mmol) and *p*-tolylhydrazine hydrochloride (310 mg, 1.95 mmol) in ethanol (10 mL) was heated at reflux for 1.5 h. The reaction mixture was cooled to room temperature, then poured onto water (100 mL), and extracted with dichloromethane. The organic phase was washed with water and brine, then dried over $MgSO_4$, filtered, and evaporated. Chromatography (petroleum ether−ethyl acetate, 4:1) gave 5-(3,4-dimethoxy-5-nitrophenyl)-1-*p*-tolyl-1*H*-pyrazole as a thick yellow oil (510 mg, 68%). A solution of this intermediate (490 mg, 1.44 mmol) in 48% aqueous HBr (16 mL) was heated at 130 °C for 3.5 h. The reaction mixture was then allowed to cool to room temperature and poured onto ice−-water (150 mL). The resulting orange precipitate was filtered off, washed with water, and dried under vacuum to give the product (312 mg, 69%). $^1$H NMR (DMSO-$d_6$): $\delta$ 10.45 (2H, s, br), 7.71 (1H, s), 7.24 (2H, d, $J$ = 8 Hz), 7.23 (1H, s), 7.17 (2H, d, $J$ = 8 Hz), 6.82 (1H, s), 6.64 (1H, s), 2.33 (3H, s). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 147.4, 141.5, 140.4, 139.8, 137.0, 136.9, 136.9, 129.4, 124.8, 120.4, 119.0, 114.4, 107.6, 20.8.

**4-(5-(3,4-Dihydroxy-5-nitrophenyl)-1*H*-pyrazol-1-yl)benzonitrile (17e).** Compound **16a** (400 mg, 1.43 mmol) was cyclized with 4-cyanophenylhydrazine hydrochloride (267 mg, 1.57 mmol) in ethanol (5 mL) as described for **17a**. A solution of the obtained pyrazole derivative (340 mg, 0.97 mmol) in dichloromethane (5 mL) was cooled to −78 °C and treated with $BBr_3$ (2.43 g, 9.70 mmol). The resulting deep purple mixture was allowed to stir at room temperature for 5 h, then cooled to −20 °C, and carefully quenched with ice−water. The mixture was then extracted with a dichloromethane−2-propanol mixture (7:3), and the extracts were washed with water, then dried over $MgSO_4$, filtered, and evaporated to leave an orange solid. The thus obtained 4-(5-(4-hydroxy-3-methoxy-5-nitrophenyl)-1*H*-pyrazol-1-yl)benzonitrile (330 mg, 0.98 mmol) was suspended in 1,2-dichloroethane (4 mL) and treated with $AlCl_3$ (142 mg, 1.07 mmol), followed by addition of pyridine (307 mg, 3.88 mmol). The reaction was stirred at reflux for 1.5 h and then allowed to cool to room temperature. After being poured onto a mixture of 2 N HCl and ice, the resulting precipitate was filtered off, washed with water, and dried under vacuum. The product was obtained as a yellow solid (284 mg, 91%). $^1$H NMR (DMSO-$d_6$): $\delta$ 10.42 (2H, s), 7.93 (2H, d, $J$ = 8.0 Hz), 7.84 (1H, s), 7.51 (2H, d, $J$ = 8.0 Hz), 7.29 (1H, s), 6.82 (1H, s), 6.72 (1H, s). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 147.5, 142.5, 141.7, 141.1, 140.9, 137.1, 133.2, 125.0, 119.7, 118.9, 118.1, 114.6, 109.7, 109.1.

**3-(3,4-Dimethoxy-5-nitrophenyl)-5-phenyl-1,2,4-oxadiazole (18).** CDI (294 mg, 1.81 mmol) was added to a stirred solution of benzoic acid (201 mg, 1.65 mmol) in DMF (8 mL). The colorless reaction mixture was stirred at room temperature for 1 h under argon, whereupon the amidoxime **15** (361 mg, 1.5 mmol) was added, and the reaction was stirred for further 2 h at room temperature. After addition of a second aliquot of CDI (294 mg, 1.81 mmol), the reaction was then heated at 120 °C for 2 h. After being cooled to room temperature, the mixture was slowly poured onto water, and the resulting precipitate was filtered off, washed with water, and dried under vacuum. Chromatography using neat dichloromethane gave the title compound as a white powder (244 mg, 50%). $^1$H NMR ($CDCl_3$): $\delta$ 8.23 (2H, d, $J$ = 8.3 Hz), 8.19 (1H, d, $J$ = 1.8 Hz), 7.89 (1H, d, $J$ = 1.8 Hz), 7.65 (1H, t, $J$ = 7.2 Hz), 7.58 (2H, t, $J$ = 7.5 Hz), 4.07 (3H, s), 4.05 (3H, s). $^{13}$C NMR ($CDCl_3$): $\delta$ 175.9, 166.9, 154.2, 144.9, 144.7, 132.9, 129, 128.1, 123.7, 122.6, 115.4, 113.9, 62.2, 56.8.

**3-Nitro-5-(5-phenyl-1,2,4-oxadiazol-3-yl)benzene-1,2-diol (19).** Compound **18** (197 mg, 0.60 mmol) was heated at 120 °C in a mixture of aqueous 48% HBr (3 mL) and 30% HBr in acetic acid (2 mL) for 30 min. The reaction was then allowed to cool to room temperature and poured onto ice−water. The resulting yellow precipitate was filtered off, washed with water, and dried under vacuum. The thus obtained 2-methoxy-6-nitro-4-(5-phenyl-1,2,4-oxadiazol-3-yl)phenol (156 mg, 0.50 mmol) was suspended in 1,2-dichloroethane (8 mL) and treated with $AlCl_3$ (73 mg, 0.55 mmol), followed by addition of pyridine (291 mg, 3.68 mmol). The red reaction mixture was heated at reflux for 2 h under argon, then cooled to room temperature, and poured onto

a mixture of ice and 1 N HCl solution. The resulting yellow precipitate was filtered off, washed with water, and dried under vacuum (72 mg, 40%). $^1$H NMR (DMSO-$d_6$): $\delta$ 10.94 (2H, br), 8.17 (2H, d, $J$ = 7.5 Hz), 8.0 (1H, d, $J$ = 2 Hz), 7.74 (2H, m), 7.65 (2H, t, $J$ = 7.8 Hz). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 175.1, 166.6, 148.2, 144.4, 137.2, 133.3, 129.4, 127.8, 123.0, 116.3, 116.1, 114.0.

**2-(3,4-Dimethoxy-5-nitrophenyl)-5-phenyl-1,3,4-oxadiazole (20).** A mixture of acid **15** (532 mg, 2.34 mmol) and CDI (418 mg, 2.57 mmol) was heated in THF (10 mL) at reflux for 3 h under argon and then cooled to room temperature. Benzohydrazide (351 mg, 2.57 mmol) was added in one portion, and the yellowish mixture was stirred at reflux overnight. After being cooled to room temperature, the mixture was poured onto water (100 mL), and the copious precipitate was filtered off, washed water, and dried under vacuum (630 mg, 78%). The thus obtained *N*′-benzoyl-3,4-dimethoxy-5-nitrobenzohydrazide (359 mg, 1.04 mmol) was heated in phosphorus oxychloride (10 mL) at 120 °C for 3 h. The pale yellow solution was then allowed to cool to room temperature and carefully poured onto ice−water (200 mL). After being stirred for 1 h at room temperature, the resulting precipitate was filtered off and washed with plenty of water. Drying under vacuum afforded the title compound as a white solid (309 mg, 91%). $^1$H NMR ($CDCl_3$): $\delta$ 8.16 (2H, d, $J$ = 7.6 Hz), 8.04 (1H, d, $J$ = 2 Hz), 7.93 (1H, d, $J$ = 2 Hz), 7.58 (3H, m), 4.09 (3H, s), 4.07 (3H, s).

**3-Nitro-5-(5-phenyl-1,3,4-oxadiazol-2-yl)benzene-1,2-diol (21).** A solution of compound **20** (200 mg, 0.61 mmol) in dichloromethane (8 mL) was cooled to −78 °C and treated with $BBr_3$ (766 mg, 3.06 mmol) under argon. The resulting deep purple mixture was allowed to reach room temperature for 1 h, then cooled again to −78 °C, and carefully quenched by the addition of methanol. After removal of the solvents by evaporation, the residual yellow solid was triturated with diethyl ether, then filtered, and dried under vacuum. The thus obtained monomethyl ether (200 mg, 0.69 mmol) was suspended in 1,2-dichloroethane (5 mL) and treated with $AlCl_3$ (180 mg, 1.355 mmol) followed by pyridine (385 mg, 4.88 mmol). The reaction was stirred at reflux for 4 h under argon. The reddish suspension was then cooled to room temperature and poured onto a mixture of 2 N HCl and ice. After being stirred for 30 min at room temperature, the resulting precipitate was filtered off, washed with water, and dried under vacuum to give the product as a yellow powder (112 mg, 59%). $^1$H NMR (DMSO-$d_6$): $\delta$ 11.0 (2H, s, br), 8.11 (2H, d, $J$ = 7.3 Hz), 8.05 (1H, s, br), 7.76 (1H, s, br), 7.63 (3H, m). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 163.6, 162.5, 148.2, 144.4, 137.5, 131.8, 129.2, 126.4, 123.0, 115.8, 113.5, 113.4.

**4-(5-(3,4-Bis(benzyloxy)-5-nitrophenyl)-1,2,4-oxadiazol-3-yl)-morpholine (22g).** A stirred solution of acid **8e** (727 mg, 1.92 mmol) in DMF (5 mL) was stirred with CDI (358 mg, 2.21 mmol) for 1.5 h under argon. Thereupon, 4-morpholinoamidoxime (320 mg, 2.21 mmol) was added, and the mixture was stirred for additional 3 h and then poured onto water. The resulting precipitate was filtered off, washed with water, and dried under vacuum. The thus obtained O-acylated amidoxime intermediate (858 mg, 1.69 mmol) was dissolved in THF (10 mL) and treated with a 1 N solution of TBAF in THF (1.7 mL). The reaction was allowed to stir at room temperature for 3 h under argon and then poured onto water. The resulting precipitate was filtered off, washed with water, and dried under vacuum. Recrystallization from ethanol gave **22g** as a white powder (527 mg, 56%). $^1$H NMR ($CDCl_3$): $\delta$ 8.09 (1H, d, $J$ = 2 Hz), 7.86 (1H, d, $J$ = 2 Hz), 7.52−7.29 (10H, m), 5.27 (4H, s), 3.84 (4H, m), 3.54 (4H, m). $^{13}$C NMR ($CDCl_3$): $\delta$ 172.0, 170.4, 153.4, 145.4, 144.7, 135.4, 134.8, 128.7 (2 signals), 128.6, 128.5, 128.3, 127.7, 120.0, 116.3, 115.6, 76.4, 71.9, 66.2, 46.3.

**3-Nitro-5-(3-phenyl-1,2,4-oxadiazol-5-yl)benzene-1,2-diol (23a).** A solution of compound **22a** (283 mg, 0.86 mmol) in DCM (12 mL) was cooled to −78 °C and treated with $BBr_3$ (1.65 g, 6.58 mmol). The resulting dark mixture was allowed to reach room temperature over 0.5 h under argon, then carefully poured onto ice−water, and extracted with ethyl acetate. The organic phase was dried over $MgSO_4$, filtered, and evaporated to leave a yellow solid (259 mg, 95%). The thus obtained 2-methoxy-6-nitro-4-(3-phenyl-1,2,4-oxadiazol-5-yl)phenol intermediate was taken up in 1,2-dichloroethane (15 mL) and treated with $AlCl_3$ (117 mg, 0.88 mmol) followed by pyridine (300 mg, 3.80 mmol). The red reaction mixture was heated at reflux for 2 h under argon, then cooled to room temperature, poured onto a mixture of ice and 37% aqueous HCl, and extracted with ethyl acetate. The organic phase was dried over $MgSO_4$, filtered, and evaporated to leave a colorless oil, which crystallized as a white powder on trituration with diethyl ether (157 mg, 60%). $^1$H NMR (DMSO-$d_6$): $\delta$ 11.10 (2H, br), 8.09 (1H, s), 8.07 (2H, d, $J$ = 7 Hz), 7.76 (1H, s), 7.60 (3H, m). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 173.7, 167.8, 148.2, 145.5, 137.4, 131.4, 129.0, 126.8, 125.8, 116.5, 115.0, 113.1.

**5-(3-(4-Bromophenyl)-1,2,4-oxadiazol-5-yl)-3-nitrobenzene-1,2-diol (23d).** The monomethyl ether **22d** (500 mg, 1.27 mmol) was placed in NMP (2 mL) and treated with $AlCl_3$ (211 mg, 1.58 mmol), followed by the addition of pyridine (403 mg, 5.10 mmol). The red reaction mixture was heated at reflux for 0.5 h under argon, then cooled to room temperature, and poured onto a mixture of ice and 37% aqueous HCl. The resulting yellow precipitate was filtered off, washed with water, and dried under vacuum (460 mg, 96%). $^1$H NMR (DMSO-$d_6$): $\delta$ 11.17 (2H, br), 8.09 (1H, d, $J$ = 2 Hz), 7.99 (2H, d, $J$ = 8.5 Hz), 7.79 (2H, d, $J$ = 8.5 Hz), 7.73 (1H, d, $J$ = 2 Hz). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 174.3, 167.5, 148.6, 146.0, 137.7, 132.4, 129.1, 125.3, 116.7, 115.4, 113.1.

**(4-(5-(3,4-Dihydroxy-5-nitrophenyl)-1,2,4-oxadiazol-3-yl)phenyl)-(4-methylpiperazin-1-yl)methanone Hydrobromide (23e).** A solution of compound **22e** (269 mg, 0.59 mmol) in dichloromethane (10 mL) was cooled to −78 °C and treated with $BBr_3$ (7.08 g, 28.25 mmol). The resulting purple mixture was allowed to reach room temperature overnight under argon, then cooled again to −78 °C, and carefully quenched with methanol. The mixture was evaporated to dryness and azeotroped twice with ethanol−toluene to leave a yellowish orange solid (112 mg, 37%). $^1$H NMR (DMSO-$d_6$): $\delta$ 11.10 (2H, br), 9.94 (1H, br), 8.17 (2H, d, $J$ = 8.4 Hz), 8.12 (1H, d, $J$ = 2 Hz), 7.78 (1H, d, $J$ = 2 Hz), 7.68 (1H, d, $J$ = 8.4 Hz), 3.47−3.16 (8H, br), 2.84 (3H, s). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 174.0, 167.9, 167.3, 148.3, 145.7, 137.4, 127.9, 127.1, 116.5, 115.2, 113.0, 52.1, 44.1, 42.3.

**5-(3,4-Dimethoxy-5-nitrophenyl)-1-phenyl-1*H*-imidazole (24).** A mixture of aniline (232 mg, 2.49 mmol), aldehyde **8c** (527 mg, 2.50 mmol), and acetic acid (0.25 mL) was stirred in refluxing ethanol (12.5 mL) for 2 h. The reaction was then cooled to room temperature, and the ethanol was removed by vacuum distillation. The residue was dissolved in a mixture of methanol (17.5 mL) and 1,2-dimethoxyethane (7.5 mL). This solution was treated with $K_2CO_3$ (690 mg, 5 mmol) followed by TOSMIC (731 mg, 3.74 mmol). The mixture was stirred at reflux for 3 h under argon and then allowed to cool to room temperature, and the solvents were evaporated off. The residue was dissolved in dichloromethane, and the organic phase was washed with water, then dried over $MgSO_4$, filtered, and evaporated. The crude product was purified by chromatography (dichloromethane−methanol, 99:1). Trituration of the crude product with diethyl ether and filtration provided **24** as a white powder (363 mg, 45%). $^1$H NMR ($CDCl_3$): $\delta$ 7.74 (1H, s), 7.49 (3H, m), 7.34 (1H, s), 7.24 (2H, m), 7.18 (1H, s), 6.70 (1H, s), 3.95 (3H, s), 3.60 (3H, s). $^{13}$C NMR ($CDCl_3$): $\delta$ 153.5, 144.6, 141.6, 139.3, 135.8, 130.6, 129.6, 129.3, 128.6, 125.7, 125.2, 114.9, 114.9, 62.1, 56.1.

**3-Nitro-5-(1-phenyl-1*H*-imidazol-5-yl)benzene-1,2-diol Hydrobromide (25).** Compound **24** (289 mg, 0.89 mmol) was heated in 48% aqueous HBr (5 mL) at 140 °C for 2.5 h, whereupon the reaction mixture was cooled to room temperature, and the volatiles were removed by distillation under vacuum. The resulting yellow crystalline mass was dried under high vacuum. Trituration of the crude product with diethyl ether and filtration provided **25** as a yellow powder (285 mg, 85%). $^1$H NMR (DMSO-$d_6$): $\delta$ 10.60 (2H, br), 9.54 (1H, s), 8.12 (1H, d, $J$ = 1.5 Hz), 7.58

(2H, m), 7.57 (1H, m), 7.51 (2H, m), 7.25 (1H, d, $J$ = 2 Hz), 6.83 (1H, d, $J$ = 2 Hz). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 147.6, 142.4, 136.9, 136.8, 133.4, 132.1, 130.2, 129.6, 126.2, 119.1, 118.3, 115.7, 115.5.

**4-(3,4-Dimethoxy-5-nitrophenyl)-2-phenylpyrimidine (26).** A suspension of the enaminone **16a** (300 mg, 1.07 mmol), benzamidine hydrochloride (186 mg, 1.18 mmol), and potassium *tert*-butoxide (264 mg, 1.47 mmol) in ethanol (6 mL) was heated at 100 °C in a sealed tube for 1 h. The reaction mixture was then allowed to cool to room temperature, and water was added. The resulting yellow precipitate was filtered off, washed with water, and dried under vacuum (195 mg, 58%). $^1$H NMR ($CDCl_3$): $\delta$ 8.89 (1H, d, $J$ = 5.2 Hz), 8.55 (2H, m), 8.10 (2H, m), 7.58 (1H, d, $J$ = 5.2 Hz), 7.54 (3H, m), 4.09 (3H, s), 4.08 (3H, s). $^{13}$C NMR ($CDCl_3$): $\delta$ 164.4, 160.9, 158.1, 154.2, 144.8, 144.7, 137.1, 132.4, 130.9, 128.5, 128.1, 114.7, 114.1, 114.0, 62.2, 56.7.

**3-Nitro-5-(2-phenylpyrimidin-4-yl)benzene-1,2-diol Hydrobromide (27).** Compound **26** was synthesized by a similar procedure as described for **7c**. Treatment of compound **26** (182 mg, 0.54 mmol) in a mixture of 48% aqueous HBr (8 mL) and 30% HBr in acetic acid (8 mL) at 150 °C for 6 h afforded **27** (142 mg, 67%) as a yellow solid. $^1$H NMR (DMSO-$d_6$): $\delta$ 10.80 (2H, br), 8.92 (1H, d, $J$ = 5.4 Hz), 8.49 (2H, m), 8.29 (1H, d, $J$ = 2 Hz), 8.14 (1H, d, $J$ = 2 Hz), 7.97 (1H, d, $J$ = 5.4 Hz), 7.57 (3H, m). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 162.8, 160.8, 158.3, 147.8, 143.8, 137.8, 136.9, 130.9, 128.6, 127.6, 126.2, 116.8, 114.3, 113.8.

**4-(3,4-Dimethoxy-5-nitrophenyl)-2-methyl-5-phenyloxazole (28).** A suspension of **8b** (1.00 g, 3.32 mmol) and $Pb(OAc)_4$ (1.68 g, 3.80 mmol) in acetic acid (10 mL) was heated at 110 °C for 2 h. The reaction was then allowed to cool to room temperature and poured onto water. The mixture was extracted with ethyl acetate, and the organic extracts were washed by water, saturated aqueous $NaHCO_3$ solution, and brine, then dried over $MgSO_4$, filtered, and evaporated to leave a pale yellow oil. Chromatography (petroleum ether−ethyl acetate, 4:1) gave 2-(3,4-dimethoxy-5-nitrophenyl)-2-oxo-1-phenylethyl acetate (917 mg, 77%) as a pale yellow oil that solidified on standing. A mixture of this intermediate (860 mg, 2.34 mmol) and ammonium acetate (554 mg, 7.19 mmol) in acetic acid (10 mL) was heated at 120 °C overnight. The reaction was allowed to cool to room temperature and then poured onto water. The mixture was extracted with ethyl acetate, and the organic extracts were washed by water, saturated aqueous $NaHCO_3$ solution, and brine, then dried over $MgSO_4$, filtered, and evaporated to leave an orange oil. Chromatography (petroleum ether−ethyl acetate, 4:1) gave **28** (633 mg, 78%) as a pale yellow oil that slowly solidified on standing. $^1$H NMR ($CDCl_3$): $\delta$ 7.63 (1H, d, $J$ = 2 Hz), 7.60 (2H, m), 7.45−7.35 (4H, m), 4.01 (3H, s), 3.84 (3H, s), 2.57 (3H, s). $^{13}$C NMR ($CDCl_3$): $\delta$ 160.3, 153.7, 145.9, 144.8, 141.9, 132.6, 129.0, 128.7, 128.6, 127.9, 126.7, 114.7 (2 signals), 62.1, 55.4, 14.1.

**5-(2-Methyl-5-phenyloxazol-4-yl)-3-nitrobenzene-1,2-diol Hydrobromide (29).** Compound **29** was synthesized by a similar procedure as described for **7c**. Treatment of compound **28** (551 mg, 1.62 mmol) with 48% aqueous HBr (13 mL) at 140 °C for 3 h afforded **29** (220 mg, 34%) as an orange solid. $^1$H NMR (DMSO-$d_6$): $\delta$ 10.40 (2H, br), 7.55 (1H, d, $J$ = 2 Hz), 7.55 (m, 2H), 7.47 (2H, m), 7.42 (1H, m), 2.49 (3H, s). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 160.4, 147.8, 144.6, 141.8, 137.2, 132.6, 129.1, 129.1, 128.2, 126.7, 122.4, 117.9, 113.3, 13.6.

**2-Bromo-1-(3,4-dimethoxy-5-nitrophenyl)-2-phenylethanone (30).** To an ice-cooled solution of **8b** (2.0 g, 6.64 mmol) in THF (68 mL) was added a solution of phenyltrimethylammonium tribromide (2.87 g, 7.63 mmol) in THF (80 mL) dropwise. After being stirred for 15 min in the cold, the reaction was allowed to stir at room temperature for 1 h. The resulting white precipitate was filtered off and washed with diethyl ether. The combined filtrate was evaporated under vacuum, and the residue was dissolved in dichloromethane. The organic layer was washed with water and brine, then dried over $MgSO_4$, filtered, and evaporated. Chromatography (petroleum ether−ethyl acetate, 2:1) afforded **30** (1.75 g, 69%) as a pale yellow oil. $^1$H NMR ($CDCl_3$): $\delta$ 7.95 (1H, d, $J$ = 2 Hz), 7.75 (1H, d, $J$ = 2 Hz), 7.53 (2H, d, $J$ = 8.5 Hz), 7.44−7.34 (3H, m), 6.29 (1H, s), 4.05 (3H, s), 3.96 (3H, s). $^{13}$C NMR ($CDCl_3$): $\delta$ 188.1, 154.0, 147.0, 143.9, 134.9, 129.3, 129.0, 128.9, 128.7, 117.3, 115.6, 62.3, 56.7, 50.2.

**5-(2-Amino-5-phenylthiazol-4-yl)-3-nitrobenzene-1,2-diol Hydrobromide (31).** A mixture of compound **30** (500 mg, 1.32 mmol) and thiourea (110 mg, 1.44 mmol) was heated in ethanol (5 mL) at reflux for 15 min. The reaction was allowed to cool to room temperature, and the solvent was removed by distillation under vacuum. The residue was added to 48% aqueous HBr (11 mL) and heated at 140 °C for 2.5 h. After being cooled to room temperature, the mixture was poured onto water, and the resulting yellow precipitate was filtered off, washed with water, and dried under vacuum (450 mg, 83%). $^1$H NMR (DMSO-$d_6$): $\delta$ 10.50 (1H, br), 8.69 (1H, br), 7.43 (1H, d, $J$ = 2.2 Hz), 7.41−7.32 (3H, m), 7.28 (2H, dd, $J$ = 2.0 and 8.0 Hz), 7.02 (1H, d, $J$ = 2.2 Hz). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 166.9, 147.4, 142.1, 136.8, 134.7, 129.6, 129.0, 128.6, 128.4, 120.5, 119.3, 118.2, 115.2.

**3-Cyano-4-(trifluoromethyl)pyridine 1-Oxide (32e).** To a stirred mixture of 4-(trifluoromethyl)nicotinonitrile **32n** (343 mg, 1.99 mmol) and urea−hydrogen peroxide addition complex (UHP) (564 mg, 6.00 mmol) in dichloromethane (30 mL) was added trifluoroacetic anhydride (TFAA) (1.26 g, 6.0 mmol) at 0 °C under argon. The reaction was allowed to stir at room temperature for 0.5 h. Excess peroxide was destroyed by the addition of 10% aqueous potassium iodide solution (30 mL). The organic phase was washed with saturated 10% aqueous $Na_2S_2O_3$ solution and water, then dried over $MgSO_4$, and filtered. The solvent was removed by evaporation, and the residue was triturated by diethyl ether to give **32e** (156 mg, 42%) as a white solid. $^1$H NMR ($CDCl_3$): $\delta$ 8.49 (1H, m), 8.39 (1H, m), 7.66 (1H, d, $J$ = 7 Hz). $^{13}$C NMR ($CDCl_3$): $\delta$ 143.1, 142.5, 126.9 (q, $J$ = 35.5 Hz), 124.0 (q, $J$ = 4.5 Hz), 120.8 (q, $J$ = 273 Hz), 110.7, 110.5 (br).

**2-Chloro-4,6-dimethylnicotinamidoxime (33a).** To a stirred suspension of 2-chloro-4,6-dimethylnicotinonitrile **32a** (623 mg, 3.74 mmol) in ethanol (15 mL) was added a 50% aqueous solution of hydroxylamine (1.23 mL, 20.07 mmol). The reaction was allowed to stir at reflux overnight and then cooled to room temperature, whereupon the solvent was evaporated off. The residue was triturated with diethyl ether. The resulting white precipitate was filtered off, washed with diethyl ether, and dried under vacuum (574 mg, 77%). $^1$H NMR (DMSO-$d_6$): $\delta$ 9.39 (1H, s), 7.17 (1H, s), 5.88 (2H, s), 2.41 (3H, s), 2.26 (3H, s). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 157.7, 150.1, 148.7, 147.8, 126.8, 123.3, 23.3, 18.9.

**Nicotinamidoxime 1-Oxide (33f).** To a stirred solution of 3-cyanopyridine 1-oxide **32g** (600 mg, 5.0 mmol) in a mixture of ethanol (50 mL) and water (10 mL) was added hydroxylamine hydrochloride (1.564 g, 22.5 mmol) followed by sodium acetate (2.05 g, 25 mmol). The reaction was heated at 70 °C for 1 h and then cooled to room temperature, and the solvent was evaporated off. The resulting white precipitate was filtered off, washed with water, and dried under vacuum (395 mg, 52%). $^1$H NMR (DMSO-$d_6$): $\delta$ 10.16 (1H, s), 8.47 (1H, s), 8.23 (1H, d, $J$ = 6.3 Hz), 7.61 (1H, d, $J$ = 8.0 Hz), 7.43 (1H, dd, $J$ = 6.3 and 8.0 Hz), 6.09 (2H, s). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 146.9, 138.4, 135.3, 132.4, 126.0, 122.1.

**5-(3,4-Bis(benzyloxy)-5-nitrophenyl)-3-(2-chloro-4,6-dimethylpyridin-3-yl)-1,2,4-oxadiazole (35a).** A stirred solution of acid **8e** (758 mg, 2.0 mmol) in DMF (10 mL) was stirred with CDI (340 mg, 2.1 mmol) for 1.5 h under argon. The amidoxime **33a** (399 mg, 2.0 mmol) was then added, and the mixture was stirred for an additional 2 h, whereupon it was poured onto water. The resulting precipitate was filtered off, washed with water, and dried under vacuum. Recrystallization from dichloromethane−2-propanol gave **34a** (632 mg, 56%) as an off-white solid. This intermediate **34a** (614 mg, 1.09 mmol) was dissolved in THF (40 mL) and treated with a 1 N solution of TBAF (2 mL) in

THF. The reaction was allowed to stir at room temperature for 3 h under argon, then poured onto water, and extracted with dichloromethane. The organic phase was dried over $MgSO_4$ and filtered, and the solvent was removed by evaporation. The residue was chromatographed (dichloromethane−methanol, 99:1), and the crude product was recrystallized from 2-propanol−diethyl ether to give **35a** as a white powder (288 mg, 48%). $^1$H NMR ($CDCl_3$): $\delta$ 8.22 (1H, m), 8.00 (1H, d, br, $J$ = 1.8 Hz), 7.50 (2H, d, $J$ = 7.8 Hz), 7.47−7.41 (3H, m), 7.39−7.30 (5H, m), 7.11 (1H, br), 5.30 (4H, s), 2.59 (3H, s), 2.28 (3H, s). $^{13}$C NMR ($CDCl_3$): $\delta$ 173.8, 166.1, 160.5, 153.7, 150.6, 149.8, 145.5, 145.2, 135.3, 134.6, 128.7, 128.7, 128.7, 128.6, 128.4, 127.7, 123.6, 119.9, 119.2, 116.5, 115.8, 76.4, 72, 24.3, 20.1.

**5-(3,4-Bis(benzyloxy)-5-nitrophenyl)-3-(2-bromo-6-methylpyridin-3-yl)-1,2,4-oxadiazole (35b).** To a stirred solution of acid **8e** (500 mg, 1.32 mmol) in DMF (5 mL) was added CDI (246 mg, 1.52 mmol) in one portion. The resulting mixture was stirred at room temperature for 1 h under argon; then the amidoxime **33b** (357 mg, 1.52 mmol) was added in one portion. The mixture was allowed to stir at room temperature overnight and then poured onto water (100 mL). Brine (10 mL) was added, and a white precipitate formed that was filtered off and washed with water. The solid was dissolved in dichloromethane and washed with water. The organic phase was dried over $MgSO_4$, filtered, and evaporated. Recrystallization from dichloromethane−2-propanol afforded **34b** (601 mg, 76%) as a white powder. This intermediate **34b** (590 mg, 0.99 mmol) was treated with CDI (178 mg, 1.097 mmol) in DMF (10 mL) at 120 °C for 3 h. The reaction was cooled to room temperature and poured onto water (100 mL). The resulting orange precipitate was filtered off, washed with water, and dried in air. Recrystallization from dichloromethane−ethanol mixture gave **35b** (261 mg, 38%) as a pale yellow solid. $^1$H NMR ($CDCl_3$): $\delta$ 8.21 (1H, d, $J$ = 2 Hz), 8.12 (1H, d, $J$ = 7.8 Hz), 7.99 (1H, d, $J$ = 2 Hz), 7.51 (2H, d, $J$ = 7.9 Hz), 7.45 (3H, m), 7.40−7.29 (6H, m), 5.31 (2H, s), 5.30 (2H, s), 2.66 (3H, s). $^{13}$C NMR ($CDCl_3$): $\delta$ 173.2, 167.3, 161.8, 153.6, 145.5, 145.2, 140.0, 139.9, 135.3, 134.7, 128.8, 128.7 (2 signals), 128.5, 128.4, 127.7, 122.5, 122.1, 119.1, 116.5, 115.8, 76.4, 72.0, 24.5.

**2-Chloro-3-(5-(3,4-dihydroxy-5-nitrophenyl)-1,2,4-oxadiazol-3-yl)-4,6-dimethylpyridine 1-Oxide (37a).** A solution of compound **36a** (175 mg, 0.31 mmol) in dichloromethane (8 mL) was cooled to −78 °C and treated with $BBr_3$ (314 mg, 1.25 mmol) under argon. The resulting dark mixture was allowed to reach room temperature for 0.5 h and then cooled again to −20 °C, whereupon water was carefully added to quench the reaction mixture. After being stirred for 1 h at room temperature, the resulting yellow precipitate was filtered off, washed with water, and dried under vacuum. Recrystallization from a dichloromethane−2-propanol mixture gave compound **37a** (57 mg, 48%) as a yellow solid. $^1$H NMR (DMSO-$d_6$): $\delta$ 11.15 (2H, br), 8.11 (1H, s), 7.74 (1H, s), 7.61 (1H, s), 2.48 (3H, s), 2.19 (3H, s). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 174.5, 164.2, 150.7, 148.3, 145.8, 139.8, 137.5, 135.2, 125.8, 123.0, 116.4, 115.3, 112.6, 18.7, 18.3.

## Pharmacology

**Animals.** Adult male Wistar rats and NMRI mice, supplied by Harlan (Barcelona, Spain), were kept five per cage under controlled environmental conditions (12 h light/dark cycle, room temperature 22 ± 1 °C and humidity 50 ± 5%, food and tap water ad libitum). Rats weighed 250−300 g and mice weighed 27−40 g. All animal procedures were conducted in the strict adherence to the European Directive for Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609CEE), Portuguese legislation, and the rules of the "Guide for the Care and Use of Laboratory Animals", 7th ed., 1996, Institute for Laboratory Animal Research, Washington, DC.

**Animal Treatments.** Animals were kept without bedding (gridded cages) for 48 h before the experiments. Animals fasted overnight were administered orally (rat 4 mL/kg; mice 8 mL/kg) with the test compounds (3 or 30 mg/kg) suspended in carboxymethylcelulose (0.5% w/v). Then at the required time the animals were anesthetized by ip administration of sodium pentobarbital (60 mg/kg) and then were perfused with NaCl (0.9%) by cardiac route. Liver and brain samples were collected and stored at −80 °C in 5 mM sodium phosphate buffer.

**Homogenate Preparation.** Tissues were thawed at room temperature and homogenized using a Diax homogenizer (Heidolph, 2 cycles of 20 s in position 5, on ice). Total protein content in homogenates was measured with Bio-Rad standard protein assay using a standard curve of bovine serum albumin (50−250 $\mu$g/mL). Samples were then diluted to obtain a concentration of 4 mg/mL.

**COMT Activity Determination.** The reaction mixture (total volume of 1 mL) contained homogenate (2 mg/mL), NCE (3 $\mu$M for nitrocatechols or 30 $\mu$M for non-nitrocatechols), 100 $\mu$M $MgCl_2$, 1 mM EGTA, 100 $\mu$M pargyline, and *S*-adenosyl-L-methionine (500 $\mu$M) in phosphate buffer (5 mM, pH 7.8). After a 20 min preincubation period at 37 °C, the reaction was initiated with adrenaline (1000 $\mu$M when testing nitrocatechols or 25 $\mu$M when testing non-nitrocatechols) and proceeded for 5 min at 37 °C. Reactions were terminated with the addition of $^1/_{10}$ volume of 2 N perchloric acid. Samples were centrifuged (16000*g* for 3 min at 4 °C) and filtered through 0.22 $\mu$m pore size Spin-X 200 filters. Metanephrine was then quantified by HPLC with electrochemical detection as previously detailed.[32] Evaluation of COMT activity in tissues of animals administered with the NCEs was performed as described above with changes in the *S*-adenosyl-L-methionine concentration (500 $\mu$M for rat liver, 100 $\mu$M for rat brain, and 250 $\mu$M for mice tissues), adrenaline concentration (1000 $\mu$M for rat liver, 100 $\mu$M for rat brain, and 50 $\mu$M for mice tissues), and incubation times (5 min for rat liver, 15 min for rat brain, and 10 min for mice tissues).

**Toxicity Studies.** Neuro-2A cells seeded in 96-well plastic culture clusters (Costar) were grown in minimum essential medium (Sigma-Aldrich, St. Louis, MO) supplemented with 1.5 g $L^{-1}$ sodium bicarbonate, 1.0 mM sodium pyruvate, 10% fetal bovine serum, 100 units $mL^{-1}$ penicillin G, 0.25 $\mu$g $mL^{-1}$ amphotericin B, 100 $\mu$g $mL^{-1}$ streptomycin, and 25 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES) under a $CO_2$/air (5%/95%) humidified atmosphere at 37 °C. Five days after seeding, cell cultures were incubated with NCEs (30 $\mu$M) for 24 h. Negative controls (no NCE) and positive controls (cells incubated with ethanol) were run in parallel. After treatment, cells were washed twice with Hanks' medium (medium composition, in mM: NaCl, 137; KCl, 5; $MgSO_4$, 0.8; $Na_2HPO_4$, 0.33; $KH_2PO_4$, 0.44; $CaCl_2$, 0.25; $MgCl_2$, 1.0; Tris-HCl, 0.15; sodium butyrate, 1.0; pH 7.4) and loaded with 2 $\mu$M calcein−AM in Hanks medium at room temperature for 30 min. Fluorescence was measured at 485 nm excitation and 530 nm emission wavelengths in a multiplate reader (Spectromax Gemini; Molecular Devices). Minimun staining for calcein−AM ($calcein_{min}$) was determined by treating six wells with ethanol 15 min before calcein−AM addition. The viability (percent) was then calculated as $[(calcein_{sample} - calcein_{min})/(calcein_{control} - calcein_{min})] \times 100$.[35]

**Assay of L-Dopa and 3-OMD in Plasma.** Plasma samples were deproteinized by adding to 600 $\mu$L of plasma, 300 $\mu$L of perchloric acid (1 N), and 100 $\mu$L of 0.1 N HCl. After an incubation of 10 min at 4 °C, samples were centrifuged at 16000*g* for 5 min at 4 °C, and supernatants were filtered through 0.22 $\mu$m filters (Costar Spin-X). L-Dopa and 3-OMD were quantified on the filtrate samples (50 $\mu$L) by HPLC with electrochemical detection as previously described.[40]

**Log *P* Calculation.** Octanol/water partition coefficients were calculated using the log *P* fragmentation algorithm implemented in ACD Laboratories v.10 software (Advanced Chemistry Development, Inc.). The standard log *P* model was corrected with ACD/PhysChem Accuracy Extender, by supplementing the internal database of fragment contributions with experimentally determined log *D* (pH = 7.4) values of selected oxadiazolylnitrocatechol compounds.

**Supporting Information Available:** Further experimental details, NMR, and elemental analyses data. This material is available free of charge via the Internet at http://pubs.acs.org.

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