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Synthesis and Evaluation of Bicyclic Hydroxypyridones as Inhibitors of Catechol *O*-Methyltransferase

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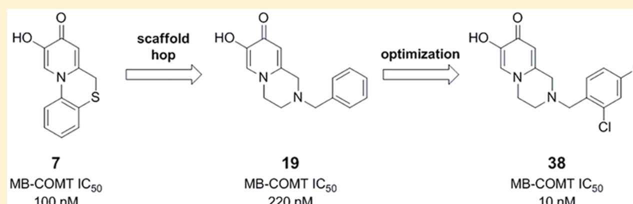
Supporting Information

ABSTRACT: A series of bicyclic pyridones were identified as potent inhibitors of catechol *O*-methyltransferase (COMT). Substituted benzyl groups attached to the basic nitrogen of the core scaffold gave the most potent inhibitors within this series. Rat pharmacokinetic studies showed medium to high levels of clearance for this series, but with high free fraction due to remarkably low levels of protein and tissue binding. In rat biomarker studies, levels of unbound drug exposure are seen in the brain, which exceed their respective IC_{50}s, leading to changes in the levels of dopamine metabolites in a manner consistent with COMT inhibition.



KEYWORDS: *Catechol O-methyl transferase, catechol mimic, enzyme inhibitors, dopamine metabolism*

Impaired dopamine activity in the prefrontal cortex (PFC) is thought to contribute to the cognitive deficits in several conditions such as obsessive-compulsive disorder, traumatic brain injury, attention deficit hyperactivity disorder (ADHD), and schizophrenia.[1] An approach toward selectively modulating dopamine signaling in the PFC is by inhibiting the activity of catechol *O*-methyltransferase (COMT). COMT is a magnesium-containing metalloenzyme that transfers a methyl group from the cofactor *S*-adenosylmethionine (SAM) to dopamine, as well as a number of endogenous and exogenous catechols. Due to the lack of expression of synaptic dopamine transporters in the PFC as compared to the striatum,[2,3] COMT is the predominant means of dopamine processing in the PFC.

COMT exists in two isoforms, designated MB-COMT for the membrane-bound form and S-COMT for the soluble form.[4] The isoforms have identical enzymatic domains, with MB-COMT having an extra 50 amino acids at the N-terminus for membrane anchoring that also influences enzymatic activity.[5] The two COMT isoforms also differ in their expression pattern, with MB-COMT being more prevalent in the brain, while S-COMT is predominant in the periphery, although this differential expression is more significant in humans as compared to rodents.[6,7] Selectivity for MB-COMT over S-COMT may then be theoretically desirable to achieve the goal of central COMT inhibition.

COMT inhibitors have demonstrated utility and are widely prescribed for treatment of Parkinson's disease to increase levels of exogenously administered L-DOPA through their ability to inhibit peripheral metabolism.[8] Among known COMT inhibitor scaffolds, nitrocatechols have distinguished themselves by providing clinically used drugs tolcapone **1** and entacapone **2**, as well as the more recently approved opicapone **3** (Figure 1).[9] Although the nitrocatechols show efficacy for inhibition of COMT, entacapone and opicapone have negligible CNS penetration, and tolcapone gives low but measurable brain exposure.[10] Parkinson's nonmotor symptoms are more effectively treated by COMT inhibitors with greater brain penetration,[11] and these compounds are useful for

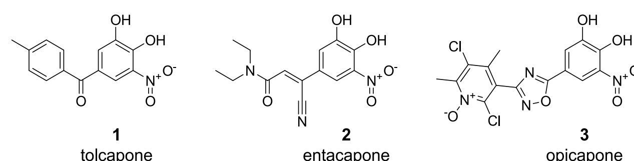


Figure 1. COMT inhibitors used clinically.

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additional neurological and psychiatric conditions such as cognitive impairment associated with schizophrenia.[12] Tolcapone has achieved some early clinical success, but its impact is limited by hepatic toxicity associated with the drug.[13] The risk-benefit profile of tolcapone has severely limited its more general use. As a result, a need for new COMT inhibitors exists, particularly those that are CNS penetrant as well as those arising from alternative pharmacophores to help mitigate the possible toxicity risk related to the nitrocatechol moiety.

The nitrocatechols are among COMT inhibitors that have been shown to bind in the catechol binding site of the enzyme, rather than the site to which the cofactor *S*-adenosylmethionine (SAM) occupies. The catechols and their mimetics are typically bidentate chelators to the magnesium in the catalytic domain, providing two of the six coordinates in the octahedral geometry of the dication. Besides nitrocatechols, other scaffolds that have given rise to COMT inhibitors are generically shown in Figure 2 including 8-hydroxyquinazolinones **4**,[14] 8-hydroxyquinolines **5**,[15] and 3-hydroxy-4-pyrimidinones **6**.[16]

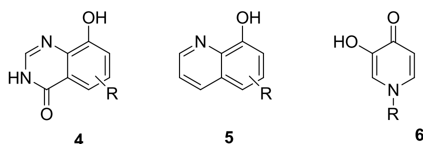


Figure 2. Scaffolds of additional known COMT inhibitors.

As reported by Harrison et. al,[16] a particular series of *N*-aryl 4-pyridones arose from a formal removal of the thiomorpholine ring from the initial screening hit **7**. Potency of these analogues was further enhanced by building off the *N*-aryl ring with additional hydrophobic substituents as shown with **8**, which is 10-fold more potent than the HTS hit. We began by examining the same tricyclic scaffold of **7** and envisioned removal of the N-aryl ring and replacement of the sulfur atom with nitrogen to give a bicyclic hydroxypyridone **9** (formally 1,2,3,4-tetrahydro-8*H*-pyrido[1,2-*a*]pyrazin-8-one). The nitrogen would then serve as a useful position on which substituents could be introduced, enabling rapid synthesis of analogues (Figure 3).

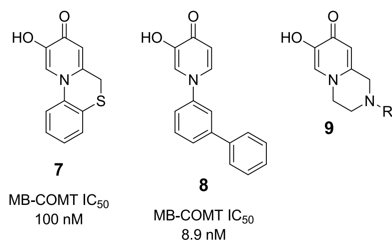
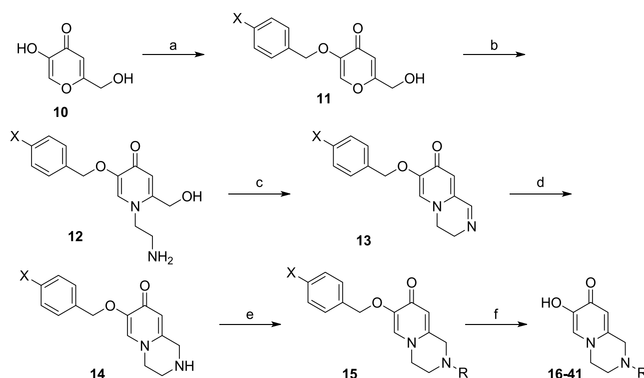


Figure 3. Origins of existing and inspiration for new hydroxypyridone COMT inhibitors.

Compounds were prepared using the synthetic sequence shown in Scheme 1.[17] Kojic acid, **10**, was *O*-alkylated with either benzyl chloride or 4-methoxybenzyl chloride to give the benzyl- or PMB-protected alcohol **11**. This material was reacted with ethylenediamine to give the protected alcohol/amine **12**. A one-pot oxidation/cyclocondensation was achieved by reaction with MnO_2 to give the cyclic imine **13**. The imine was then reduced with sodium borohydride to give the *O*-protected parent **14**. Addition of various N-substituents was achieved through reductive amination, alkylation, acylation, or sulfonylation to enable rapid analogue synthesis and give penultimate material with the general structure **15**. Deprotection under acidic conditions then afforded the final hydroxypyridone products **16−41**.

Scheme 1. Synthesis of 1,2,3,4-Tetrahydro-8*H*-pyrido[1,2-*a*]pyrazin-8-ones 16−41[a]



[a]Reagents and conditions: (a) BnCl, NaOH, EtOH/H_2O, 60−75%; or PMB-Cl, K_2CO_3, DMF, 80 °C, 90%; (b) ethylenediamine, EtOH, 90 °C; (c) MnO_2, $CHCl_3$, 60 °C; (d) $NaBH_4$, MeOH, rt, 48 h, 50%, 3 steps; (e) RX, DIPEA, $CHCl_3$, or where R = R′CH_2, R′CHO, $Na(OAc)_3BH$, $CHCl_3$; (f) 6 N HCl, EtOH, reflux. Experimental details and characterization data may be found in the supplementary material and in ref 17.

MB-COMT inhibition data[21] for an initial set of *N*-substituted hydroxypyridones are shown in Table 1. The parent unsubstituted compound **16** shows no measurable

Table 1. SAR of 2-Substitution[a]

#	R_2	Human MB-COMT IC_{50} (nM)
16	H	>10000
17		1600
18		630
19		220
20		2000
21		6300
22		>10000
23		>10000

[a]For assay protocol, see ref 21. All data are the mean values of at least three independent measurements.

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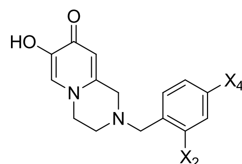
inhibition of the enzyme under assay conditions. Introduction of aliphatic *N*-substituents, as with **17** and **18**, shows incremental improvements in potency, giving a submicromolar IC_{50} in the case of **18**. This could be viewed as due to nonspecific binding from the addition of lipophilicity, but an analysis of subsequent compounds show this to be more nuanced. The *N*-benzyl analogue **19** displays an additional potency improvement with an IC_{50} of 220 nM. Incorporation of an additional methylene in the *N*-phenethyl compound **20** shows a 10-fold loss in potency compared to **19**, suggesting a limit to potency increases that can be attributed merely to lipophilic bulk. Modification of the phenyl of **19** to the 3-pyridyl of **21** results in a greater than 20-fold loss in potency. A change from the methylene linker to a carbonyl, **22**, or sulfonyl, **23**, completely eroded MB-COMT inhibition, highlighting the importance of maintaining the basicity of that nitrogen for inhibitory activity.

An exploration of substitution in the 2- and 4-positions of the phenyl ring of **19** is shown in Table 2. An overall

Table 2. SAR of Monosubstitution on Phenyl of 2-Benzyl Derivatives[a]



Cmpd	X_2	X_4	Human MB-COMT IC_{50} (nM)
19	H	H	220
24	CN	H	100
25	H	CN	2000
26	OCH_3	H	250
27	H	OCH_3	400
28	CF_3	H	60
29	H	CF_3	450
30	CH_3	H	50
31	Cl	H	40

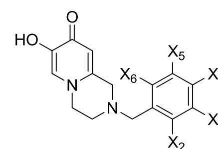
[a]All data are the mean values of at least three independent measurements.

preference for 2- versus 4-substitution is seen for some examples (cyano, **24** and **25**; and trifluoromethyl, **28** and **29**) within a limited set of matched pairs across both electron withdrawing and donating substituents, while no clear difference is seen for the methoxy-substituted pair, **26** and **27**. A pair of compounds with mono 2-substituents but no corresponding 4-substituted matched pair, 2-methyl **30** and 2-chloro **31**, each serve as potent and intriguing starting points for the effect of disubstitution on the pendant phenyl ring.

First, a more focused look at the effect of dimethyl substitution while maintaining a 2-methyl substituent is shown in Table 3. A preference for 2,4- and 2,6- disubstitution (**33** and **35**, respectively) is exhibited over 2,3- and 2,5-disubstitution (**32** and **34**). This trend, while modest in magnitude, was used to guide subsequent probes for the effect of pendant phenyl ring substituent patterns on MB-COMT inhibitory activity in this series.

The effect of 2,4- and 2,6-dihalo substitution is explored in Table 4. The 2-fluoro analogue, **36**, shows a similar inhibitory potency as seen with the 2-chloro, **31**. Addition of a second chloro or fluoro substituent to the 4- or 6-positions gives

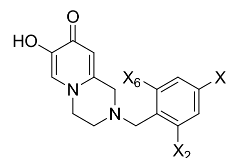
Table 3. SAR of Dimethyl Substitution on Phenyl of 2-Benzyl Derivatives[a]



Cmpd	X_2	X_3	X_4	X_5	X_6	Human MB-COMT IC_{50} (nM)
30	CH_3	H	H	H	H	50
32	CH_3	CH_3	H	H	H	40
33	CH_3	H	CH_3	H	H	13
34	CH_3	H	H	CH_3	H	63
35	CH_3	H	H	H	CH_3	6.3

[a]All data are the mean values of at least three independent measurements.

Table 4. SAR of Dihalo Substitution on Phenyl of 2-Benzyl Derivatives[a]



Cmpd	X_2	X_4	X_6	Human MB-COMT IC_{50} (nM)
19	H	H	H	220
36	F	H	H	50
31	Cl	H	H	40
37	Cl	F	H	40
38	Cl	Cl	H	10
39	F	H	F	38
40	Cl	H	F	16
41	Cl	H	Cl	10

[a]All data are the mean values of at least three independent measurements.

compounds with either a modest improvement in potency (as with **38**, **40**, and **41**) or ones that are equipotent with their respective 2-halo parents (**37** and **39**), but no loss in potency was observed.

Having several compounds that exhibited potent MB-COMT inhibition in the enzyme assay, we wished to measure the effects of COMT activity in vivo in the rat. Representative compounds in this series displayed similar potencies using rat MB-COMT as with human enzyme (data not shown). As a result, to simplify the testing funnel, inhibitory activity against human MB-COMT alone was used as the primary screening tool.

To determine whether compounds in this series possessed properties suitable for in vivo studies, we first measured a set of physicochemical and DMPK properties, both in vitro and in vivo, which are shown in Table 5 for five compounds that span a range of potencies. Most of the selected compounds have reasonable aqueous solubilities, but **35** is less soluble. In rat hepatocytes, the five compounds tested show moderate to good stability. In MDCK-MDR1 cells, the compounds display good permeability, and none are P-gp substrates, giving these compounds a good chance of being CNS-penetrant. In rat PK studies, clearance was quite high, suggesting that rat hepatocyte stability is not predictive of in vivo clearance for this scaffold, although reasonable oral bioavailability was

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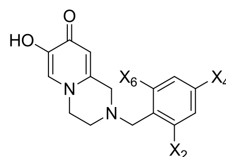
Table 5. Potency and ADME Properties of 7-Hydroxy-3,4-dihydro-1*H*-pyrido[1,2-*a*]pyrazin-8-ones



#	X_2	X_4	X_6	Human MB-COMT IC_{50} (nM)	Kinetic solubility in water (μg/mL)	Hepatocyte Stability (Rat) (μL/min/10^6 cells)	P_{app} ($\times 10^{-6}$ cm/s)	Efflux ratio[a] A→B/B→A	Rat PK[b] %F	Rat PK[b] T1/2 (h) - iv	Rat PK[b] Clp (mL/min/kg)	Rat Plasma Protein Binding[c]	Rat Brain Tissue Binding[d]
19	H	H	H	220	60.3	<4	25.5	1.0	51	0.38	32	61.4	12.9
31	Cl	H	H	40	58.0	11	26.1	1.0	57	0.33	49	27.5	5.9
40	Cl	H	F	16	115	11	25.1	1.1	64	0.80	87	52.8	25.8
38	Cl	Cl	H	10	34.9	17	11.5	1.8	68	0.42	138	28.0	11
35	CH_3	H	CH_3	6.3	9.9	<4	21.0	1.3	19	0.42	145	31.1	2.8

[a]A:B/B:A efflux ratio from P-glycoprotein expressing MDCK cells. [b]Rat pharmacokinetics after a 1 mpk iv dose and 10 mpk oral dose. [c]Percent free from rat plasma as measured by equilibrium dialysis. [d]Percent free from rat brain homogenate.

Table 6. In Vivo Exposures of COMT Inhibitors and Their Effects on Biomarkers of Dopamine Metabolism

#	Dose (mpk)	Time (h)	Total Plasma (nM)	Total Brain (nM)	CSF (nM)	Total B/P	Free Plasma (nM)[a]	Free Brain (nM)[a]	$K_{p,uu}$	Human MB-COMT IC_{50} (nM)	Free Brain/IC_{50}	HVA[c]	DOPAC[c]
1	15	4	3805	<732	<183	<0.19	35	BLQ	N/A	<1	N/A	28%	304%
19	100	4	8884	4373	4902	0.49	5454	564	0.10	220	2.6	13%	126%
31	100	4	2732	4795	989	1.75	751	285	0.38	40	7.1	29%	197%
40	100	4	3579	1912	355	0.53	1890	493	0.26	16	31	25%	217%
38	100	4	1037	3911	78	3.77	290	430	1.48	10	43	22%	209%
35	100	4	801	564	214	0.70	249	16	0.06	6.3	2.5	29%	203%

[a]Free plasma and brain levels calculated using ratios listed in Table 5 on plasma and brain samples taken immediately after CSF sampling. [b]$K_{p,uu}$ is the ratio of free brain to free plasma. [c]CSF HVA and DOPAC changes (% of vehicle) 4 h after 100 mpk oral dose compared to vehicle (n = 7−8 rats per group), except **1** (15 mpk ip). BLQ = below the limit of quantitation.

observed. Rat plasma protein binding and rat brain tissue binding assays indicated that this scaffold has strikingly low levels of plasma protein binding, with the amount of free drug exceeding 25% for all five compounds tested (Table 5). The percent free in the brain tissue binding studies was also fairly high, but not to the degree seen with plasma protein binding. While having a large free fraction is a boon with regard to target engagement, it may leave more unbound drug exposed to metabolism and other clearance mechanisms, which could partly explain the high clearance of these compounds in vivo.[18]

We then proceeded to test the in vivo efficacy of these compounds in rats while mindful of the pharmacokinetic data. Inhibition of COMT in the CNS should produce measurable changes in dopamine metabolites in the central nervous system: an increase in the concentration of dihydroxyphenyl acetic acid (DOPAC) and a decrease in that of homovanillic acid (HVA). These dopamine metabolites were measured in rat CSF, while concentrations of COMT inhibitors were assessed in CSF, brain homogenate, and plasma concurrently. Taken as a whole, these measurements provide an assessment of both CNS penetration and activity for the dosed compounds. It has been previously shown that CSF levels of HVA and DOPAC are responsive to brain-penetrant COMT inhibitors[19] and that these levels mirror those seen in total brain.[20] All compounds in Table 5 have IC_{50}s for S-COMT that are greater than 5 μM (Supplementary Table S1),[15] giving confidence that any changes in biomarker level would be driven by MB-COMT inhibition.

Table 6 shows the results from a single oral administration (100 mpk) of COMT inhibitors to rats (n = 7−8). The animals were sacrificed 4 h postdose, then total drug was measured in plasma, brain, and CSF, while dopamine metabolite levels were determined in CSF. The free plasma and brain concentrations were calculated using the plasma protein and brain tissue binding data (Table 5). LC−MS was used to measure the levels of HVA and DOPAC in CSF; the percent change in these levels versus vehicle-treated control animals is shown in Table 6. Tolcapone, **1**, was used as a positive control (15 mpk, ip injection) and gave expected movement of biomarkers, where HVA was decreased and DOPAC increased relative to control. All five compounds tested decreased HVA levels to the same degree as tolcapone. Increases in DOPAC were below the levels seen with tolcapone, and the change seen with **19** was only slightly increased versus control. The other four compounds roughly doubled the levels of DOPAC as compared to control but below that seen with tolcapone.

All five hydroxypyridone analogues exhibited good peripheral exposure and brain levels. Free drug concentrations in brain homogenate for all compounds exceeded their respective MB-COMT IC_{50}s by at least 2-fold, with **38** displaying a 40-fold level of coverage over its IC_{50}. However, the change in the biomarker levels is roughly the same for all compounds except **19** and does not reflect the range of free compound concentration coverage above the IC_{50}s. While these compounds clearly inhibit COMT in vivo, they do not increase the levels of DOPAC as robustly as tolcapone. A

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possible explanation for this is off-target activity at other enzymes of dopamine processing; however, compounds **31** and **38** were tested for, and had no activity against, monoamine oxidases A and B (IC_{50}> 10 μM) and very weak inhibition of tyrosine hydroxylase ($IC_{50} \approx 10$ μM).

In summary, a series of bicyclic pyridones were prepared and evaluated for their ability to inhibit COMT, and several potent inhibitors of MB-COMT were identified. Incorporation of a halo or methyl 2-substituent on the benzyl group attached to the basic nitrogen of the core scaffold gave compounds with increased potency; further methyl or halo substitution at the 4- or 6-position gave the most potent inhibitors within this series. Although rat pharmacokinetic studies showed medium to high levels of clearance for this series, they also have remarkably low levels of protein and tissue binding, increasing the availability of circulating compounds to interact with the target of interest. In rat biomarker studies, levels of unbound drug exposure are seen in the brain, which exceed their respective IC_{50}s. This target inhibitory coverage manifests itself in altering the levels of dopamine metabolites in a manner consistent with COMT inhibition, although not reflecting the magnitude of change in DOPAC concentration as seen with tolcapone.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.9b00345.

Procedures for synthesis and characterization of compounds, details of COMT enzymatic assays, and a table of S-COMT activity for key compounds (PDF)

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I.B., S.D., P.d.L., T.D., V.D., G.E., Y.H., M.P., D.S., F.M., M.-C.V., N.V., and J.C.B. designed and synthesized compounds. M.K., H.W., M.W., E.J., and J.C.B. developed the in vitro assays. D.A., V.A., S.B., G.C., M.D., A.K., E.J., D.S., N.W., and J.C.B. developed and analyzed the in vivo assays. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

%F, bioavailability; ADHD, attention deficit hyperactivity disorder; ADME, absorption, distribution, metabolism, and excretion; BLQ, below limit of quantitation; BnCl, benzyl chloride; B/P, ratio of concentration of drug in brain to concentration of drug in plasma; $CHCl_3$, chloroform; Cl_p, plasma clearance; COMT, catechol-*O*-methyltransferase; Cmpd, compound; CNS, central nervous system; CSF, cerebrospinal fluid; DIPEA, *N*,*N*-diisopropylethylamine; DMF, dimethylformamide; DMPK, drug metabolism and pharmacokinetics; DOPAC, dihydroxylphenyl acetic acid; EtOH, ethanol; F_u, fraction unbound; H_2O, water; HCl, hydrochloric acid; HTS, high-throughput screening; HVA, homovanillic acid; IC_{50}, compound/substance concentration required for 50% inhibition; K_2CO_3, potassium carbonate; $K_{p,uu}$, ratio of free concentration of drug in brain to free concentration of drug in plasma; LC–MS, liquid chromatography–mass spectrometry; L-DOPA, L-3,4-dihydroxyphenylalanine; MB-COMT, membrane bound catechol-*O*-methyltransferase; MDCK, Madin–Darby canine kidney cells; MDR1, multidrug resistance protein 1; MeOH, methanol; MnO_2, magnesium(IV) oxide; $NaBH_4$, sodium borohydride; nM, nanomoles per liter of solution; $Na(OAc)_3BH$, sodium triacetoxyborohydride; NaOH, sodium hydroxide; P_{app}, apparent intrinsic permeability; PFC, prefrontal cortex; P-gp, P-glycoprotein 1; PK, pharmacokinetics; PMB, *p*-methoxybenzyl; PMB-Cl, *p*-methoxybenzyl chloride; PPB, plasma protein binding; SAM, *S*-adenosyl methionine; SAR, structure–activity relationship; S-COMT, soluble catechol-*O*-methyltransferase; $T_{1/2}$, half-life of the product

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