The Lipophilicity Behavior of Three Catechol-*O*-methyltransferase (COMT) Inhibitors and Simple Analogues

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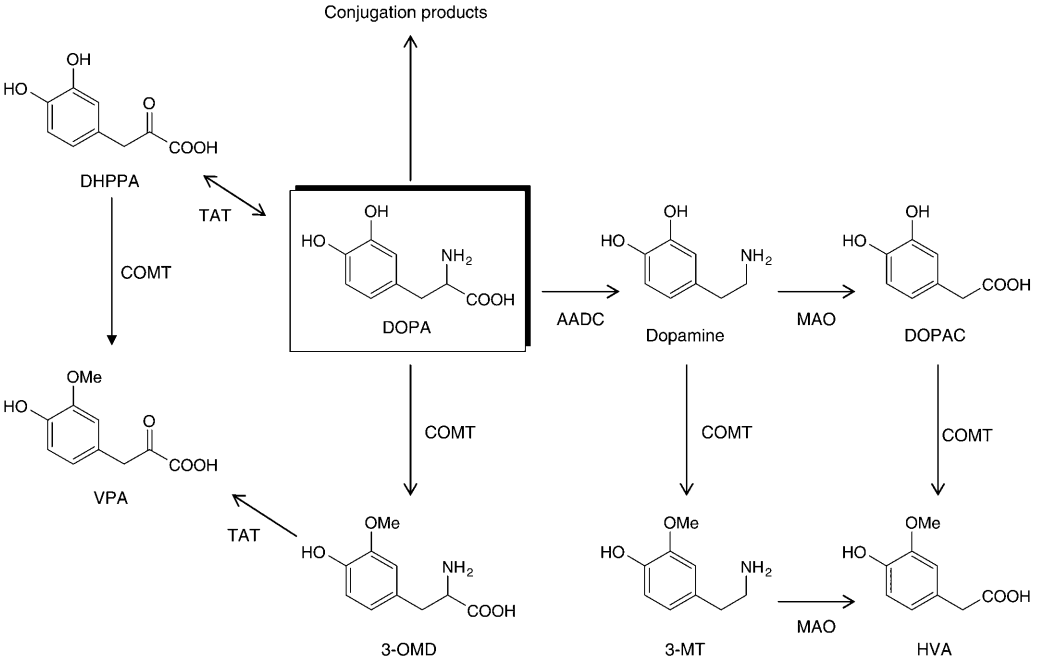
The ionization and lipophilicity properties of the second-generation catechol-*O*-methyltransferase (COMT) inhibitors entacapone (**1**), nitecapone (**2**), and tolcapone (**3**) which share the same nitrocatechol structure but have remarkably different pharmacokinetic profiles are investigated to identify relationships between some of these physicochemical parameters and the blood-brain-barrier (BBB) passage. In addition, the lipophilicity behavior of the simpler, structurally related analogues **4**–**11** is studied. Combined descriptors such as $\Delta\log P$ (difference between log P in two different solvent systems) and *diff*(log P^{N-I}) (difference between log P of two different electrical forms of a given solute in the same system) provide insight into inter- and intramolecular interactions characteristic of the analyzed compounds.

1. Introduction. – *Parkinson*'s disease (PD) is a neurodegenerative disease characterized by the depletion of dopaminergic neurons in the substantia nigra pars compacta (SNc). The loss of striatal dopamine and the resulting disruption of neurotransmitter signaling are responsible for most symptoms of PD [1][2].

Since its introduction in the late 1960s, levodopa (L-DOPA), the natural dopamine precursor, has remained the most efficient treatment for *Parkinson*'s disease. From the 1970s onwards, co-administration of levodopa and a peripheral aromatic amino acid decarboxylase (AADC) inhibitor has become routinely used in PD therapy. AADC inhibitors block the peripheral formation of dopamine and thus result in an approximately tenfold increase in brain levodopa availability, allowing significant levodopa dose reduction [3]. However, the chronic use of levodopa/AADC inhibitor results in a decrease of the clinical response as the disease progresses. After some years of stable benefits, many patients suffer from fluctuations in motor disability (phenomenon referred to as the 'on' and 'off' state) since the effect of a single levodopa dose becomes progressively shorter. Indeed, the main metabolic effect of the combined therapy levodopa/AADC inhibitor is an increase of 3-*O*-methyldopa (3-OMD), the final product of the catechol-*O*-methyltransferase (COMT, E.C. 2.1.1.6) pathway (*Scheme*). During chronic levodopa therapy, 3-OMD accumulates as its elimination half-life is long (about 15 h). Since this metabolite competes with levodopa for transport across the blood–brain barrier (BBB), it has been associated with the fluctuations in motor disability observed during levodopa/AADC inhibitor treatment [4]. Indeed, it has been observed that 3-*O*-methyldopa shows a higher affinity for the LNAA (large neutral

Scheme. *Schematic Illustration of the Levodopa Metabolism*



AADC: aromatic amino acid decarboxylase
COMT: catechol-*O*-methyltransferase
MAO: monoamine oxidase
TAT: tyrosine aminotransferase
DOPA: levodopa
DOPAC: dihydroxyphenylacetic acid
HVA: homovanillic acid
3-MT: 3-methoxy-tyramine
3-OMD: 3-*O*-methyldopa
VPA: vanilpyruvic acid
DHPPA: 3,4-dihydroxyphenylpyruvic acid

amino acid) carrier than levodopa itself. Thus, the association of a COMT inhibitor to the levodopa/AADC inhibitor therapy is a possible approach to provide a prolonged maintenance of serum levodopa levels and hence a longer clinical response. Indeed, COMT inhibition increases the percentage of levodopa that passes the BBB [2][5].

Recently, potent and selective new COMT inhibitors bearing a nitrocatechol (=3-nitrobenzene-1,2-diol) subunit, entacapone (**1**), nitecapone (**2**), and tolcapone (**3**), have become available (*Fig. 1*). Two of them, entacapone (=*Comtan*®; **1**) and tolcapone (=*Tasmar*®; **3**), are used in clinical practice in association with the classical therapy (levodopa/AADC inhibitor).

Although entacapone (**1**), nitecapone (**2**), and tolcapone (**3**) share the same nitrocatechol structure, their pharmacokinetic profiles are remarkably different. Indeed, several studies have shown that after systemic administration, **3** penetrates better into the brain than **1** and **2** [6][7]. The brain COMT inhibition observed with **1** is lower and obtained at higher doses, while **2** is only peripherally active [5]. As shown by *Forsberg et al.*, the rat striatum/serum ratios 60 min after intravenous administration of a single dose of 10 mg/kg was 0.27% for **1** and 1% for **3**, respectively [6].

The aim of this work is to investigate the ionization and lipophilicity properties of entacapone (**1**), nitecapone (**2**), and tolcapone (**3**) to identify relationships between

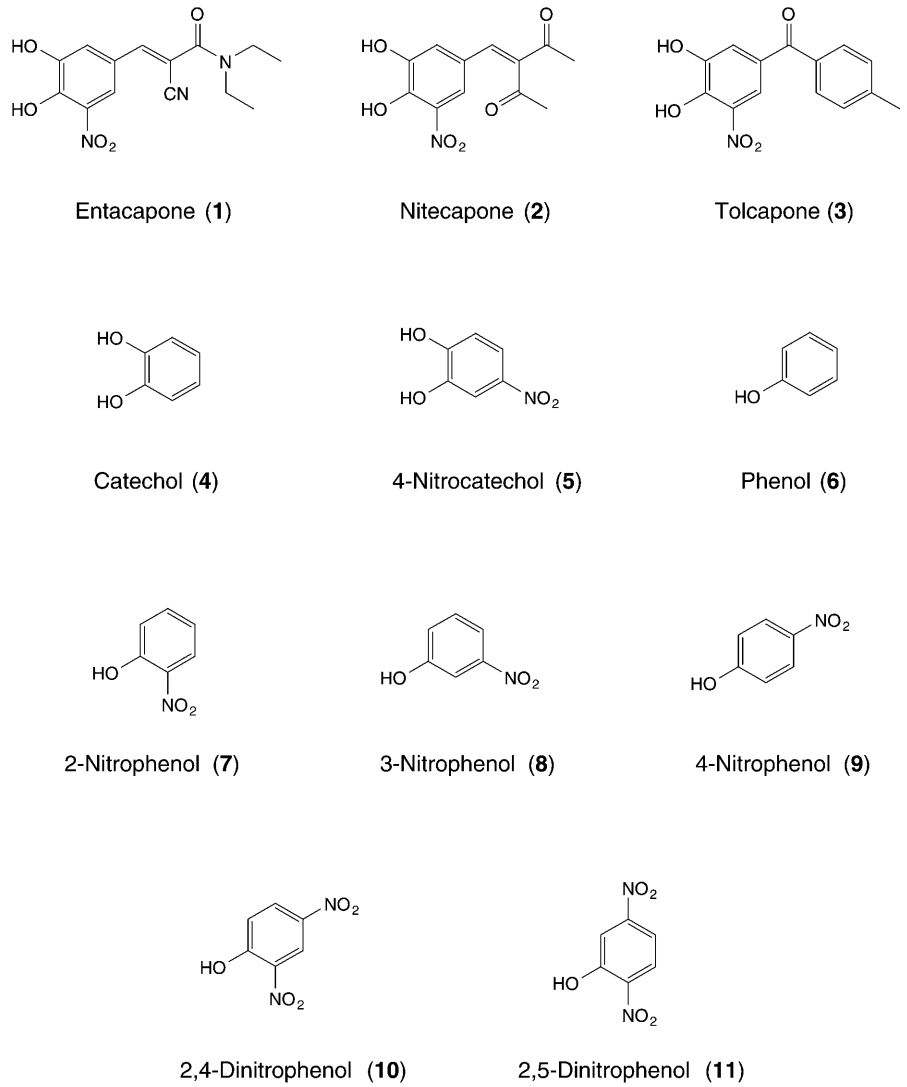


Fig. 1. *Chemical structures of the COMT inhibitors* **1**–**3** *and of the structurally related compounds* **4**–**11**

some of these physicochemical parameters and the BBB passage. It is well-known that the ability to passively cross the BBB can be related to lipophilicity expressed by a number of simple (*i.e.*, log *P* and log *D*) or combined descriptors (Δlog *P* and *diff* (log P_{dce}^{N-I}) [8–10]. The study is extended to the analysis of the behavior of the simpler, structurally related analogues **4**–**11** (*Fig. 1*).

2. Results and Discussion. – 2.1. *Ionization Constants.* The pK_a values of entacapone (**1**), nitecapone (**2**), tolcapone (**3**), catechol (= benzene-1,2-diol; **4**), and 4-nitrocatechol

(**5**) were measured by potentiometry and compared with the known pK_a values [11] of phenol (**6**), nitrophenols **7**–**9**, and dinitrophenols **10** and **11** (*Table 1*). In case of the simple phenols **6**–**9**, the presence of the NO_2 group increases the acidity of the OH group due to its strong and topology-related electron-withdrawing effect. A further decrease of the pK_a values is observed for the dinitrophenol derivatives **10** and **11** in comparison to the monosubstituted phenols **7**–**9**.

The electronic effect of the NO_2 group also lowers the pK_a of 4-nitrocatechol (**5**) as compared to catechol (**4**). Moreover, the three COMT inhibitors **1**–**3** show similar pK_a values around 4.5 for pK_{a1} and 10 for pK_{a2}. The low first-ionization constant reflects the substituent effects of both the NO_2 group and the electron-withdrawing additional substituent. As a result of this ionization scheme, at physiological pH (7.4), the monoanionic form of entacapone (**1**), nitecapone (**2**), and tolcapone (**3**) is largely predominant (*ca.* 99%).

Table 1. *Ionization Constants of the COMT Inhibitors* **1**–**3** *and the Related Analogues* **4**–**11** (see *Fig. 1*)

	pK_{a1}[a])	pK_{a2}[a])		pK_{a1}[a])	pK_{a2}[a])
1	4.59	10.02	**7**[b])	6.92	–
2	4.69	10.16	**8**[b])	8.10	–
3	4.64	10.20	**9**[b])	6.90	–
4	9.20	–[c])	**10**[b])	3.96	–
5	6.61	10.57	**11**[b])	4.97	–
6[b])	9.99	–			

[a]) Measured by the *Sirius* pH-metric method. [b]) Ionization constants taken from [11]. [c]) Not determined.

2.2. *Lipophilicity Behavior*. 2.2.1. *Lipophilicity of Neutral Forms.* The partition coefficients (log *P*) of the neutral forms of entacapone (**1**), nitecapone (**2**), tolcapone (**3**), catechol (**4**), and 4-nitrocatechol (**5**) in octanol/H_2O (log P^N_{oct}) and 1,2-dichloroethane/H_2O (log P^N_{dce}) as well as the difference between these two values ($\Delta\log P^N_{oct-dce}$) were then determined and compared with the literature values [11] for phenol (**6**) nitrophenols **7**–**9**, and dinitrophenols **10** and **11** (*Table 2*). The results clearly indicate that the lipophilicity decreases in the order **3** > **1** > **2**, independently of the investigated solvent system.

2.2.2. *Effect of Intramolecular H-Bonding on Lipophilicity of Neutral Species.* The difference between the partition coefficients of the neutral species obtained in octanol/H_2O and 1,2-dichloroethane/H_2O ($\Delta\log P^N_{oct-dce}$) is an expression of H-bonding interactions as demonstrated by solvatochromic equations and, more specifically, an expression of the α property of solutes [10][12]. By using an optimized training set of compounds not affected by intramolecular effects [13], three regression lines were obtained, as shown in the graph representing log P^N_{oct} *vs.* log P^N_{dce} (*Fig. 2*): one line for two-H-bond-donor solutes ($\alpha = 2$; *Line c*), a second line for one-H-bond-donor solutes ($\alpha = 1$; *Line b*), and a third line for purely H-bond-acceptor solutes ($\alpha = 0$; *Line a*). This clear separation of H-bond donors from non-H-bond donors in the log P^N_{oct} *vs.* log P^N_{dce} plot confirms that the major difference between the log *P* values in octanol/H_2O and 1,2-dichloroethane/H_2O is due to the α property of solutes. Thus, the position of a specific compound on this graph can quantify its H-bond potency which can be modulated

Table 2. *Physicochemical Parameters of the COMT Inhibitors* **1**–**3** *and the Related Analogues* **4**–**11** (see *Fig. 1*)

	$\log P^{N}_{oct}$ [a])	$\log P^{N}_{dce}$ [a])	$\Delta\log P^{N}_{oct-dce}$	$\log P^{I}_{dce}$ [b])	*diff* ($\log P^{N-I}_{dce}$)
1	2.16	2.07	0.09	−0.4	2.5
2	1.17	1.36	−0.19	−1.4	2.8
3	3.17	3.09	0.08	0.2	2.9
4	0.85	−0.53	1.38	−2.6	2.1
5	1.59	−0.14	1.73	−2.8	2.7
6[c])	1.46	0.61	0.85	−2.3	2.9
7[c])	1.77	2.81	−1.04	−2.0	4.8
8[c])	2.00	0.92	1.08	−2.4	3.3
9[c])	1.96	0.72	1.24	−2.5	3.2
10[c])	1.52	2.46	−0.94	−1.7	4.2
11[c])	1.75	2.49	−0.74	−2.3	4.8

[a]) Measured by the *Sirius* pH-metric method. [b]) Measured by cyclic voltammetry. [c]) Partition coefficients of neutral and charged species taken from [11].

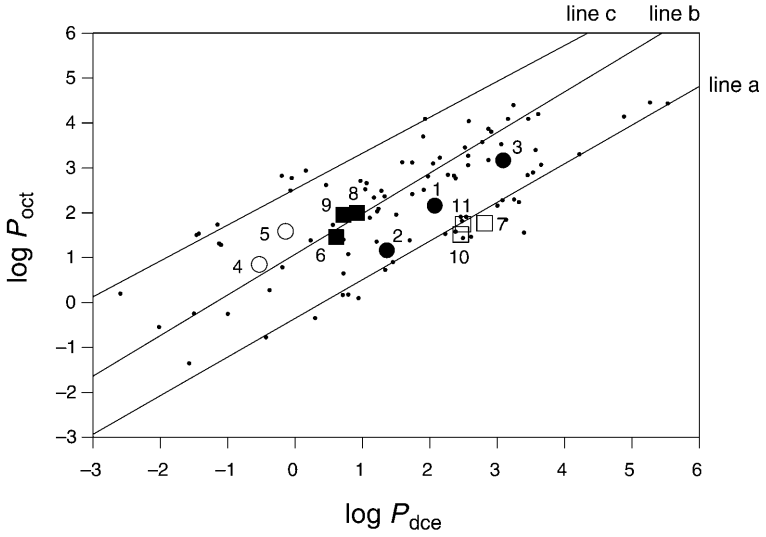


Fig. 2. *Relationships between log* P^{N}_{oct} *and log* P^{N}_{dce} *values for COMT inhibitors* **1**–**3** (•)*, Compounds* **4** *and* **5** (○)*, Compounds* **6**, **8**, *and* **9** (■)*, and Compounds* **7**, **10**, *and* **11** (□). *Line a* describes purely H-bond-acceptor compounds with $\alpha = 0$; *Line b* one H-bond-donor compounds with $\alpha = 1$; *Line c* two H-bond-donor compounds with $\alpha = 2$.

by the presence or absence of intramolecular effects [12]. This approach is illustrated by the effect of intramolecular H-bonds in substituted phenols. Indeed, simple as well as *meta*- and *para*-NO_2-substituted phenols **6**, **8**, and **9** are on the regression line of compounds fully expressing the potency of the one-H-bond-donor group, while phenols **7**, **10**, or **11** with at least one *ortho*-NO_2 substituent belong to the pure H-bond-acceptor regression line. In *ortho*-NO_2-substituted phenols, the OH group cannot be

an intermolecular H-bond donor due to its strong intramolecular H-bond in a nonpolar solvent such as 1,2-dichloroethane [11].

Moreover, the simple catechol compounds **4** and **5** belong to the regression line for compounds having a one-H-bond-donor group despite the presence of the two OH groups suggesting a strong intramolecular H-bond between these two adjacent substituents in nonpolar media. Interestingly, entacapone (**1**), nitecapone (**2**), and tolcapone (**3**) fall between the regression line for solutes with $\alpha = 0$ (*Line a*) and that for solutes with $\alpha = 1$ (*Line b*) (*Fig. 2*). The formation of H-bonds accounts for this behavior, but the different position on the graph of compounds **1**–**3** in comparison to the catechols is due to a complex balance between several intramolecular H-bonds as schematically shown in *Fig. 3*. The position of compounds **1**–**3** on the graph (*Fig. 2*) clearly suggests an equilibrium between different species with one or two intramolecular H-bonds.

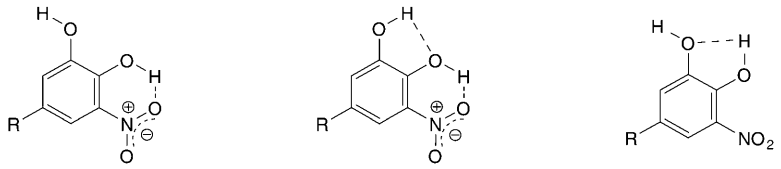
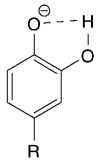


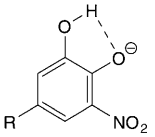
Fig. 3. *Different possible H-bond patterns for the neutral form of the COMT inhibitors* **1**–**3**

2.2.3. *Lipophilicity of Ionized Forms.* The partition coefficients log P^{I}_{dce} of the ionized forms of entacapone (**1**), nitecapone (**2**), tolcapone (**3**), catechol (**4**), and 4-nitrocatechol (**5**), measured in 1,2-dichloroethane/H_2O by cyclic voltammetry, as well as the literature values for phenol and the nitrophenol derivatives, *i.e.*, for compounds **6**–**11**, are given in *Table 2* [11]. As deduced from their pK_a values, compounds **1**–**3** are over 99% mono-ionized at physiological pH. The predominance of monoanions at pH 7.4 suggests that a detailed analysis of their lipophilic behavior in 1,2-dichloroethane/H_2O is mandatory to identify potential intramolecular effects affecting the partition behavior of charged species [12]. The values of the difference between the log P_{dce} values obtained for the neutral and ionized species of each compound, **1**–**11**, *diff* (log P^{N-I}_{dce}) (see *Table 2*), reveal important structural information as widely described in the literature [12][14][15]. In particular, *diff* (log P^{N-I}_{dce}) variations allow to identify charge-delocalization effects, intramolecular interactions, and conformational equilibria in both neutral and ionized species. The *diff* (log P^{N-I}_{dce}) values of *ca.* 3 for phenols **6**, **8**, and **9** are markedly lower than the values of 5–8 found for carboxylate or enolate anions reflecting the strong charge delocalization in the phenolate anions [15]. The large increase measured for the *ortho*-substituted compounds **7**, **10**, and **11** reflects the effect of the intramolecular H-bond which largely increases the partition coefficient of neutral species [15]. For the simple catechols **4** and **5**, the measured *diff* (log P^{N-I}_{dce}) is lower (2.1 and 2.7, resp.) despite the intramolecular H-bonds already identified for the neutral species (see above). The low *diff* (log P^{N-I}_{dce}) for **4** and **5** clearly indicates that an intramolecular H-bond is also operative for the partitioning of the anionic species (*Fig. 4*). The value of *diff* (log P^{N-I}_{dce}) is *ca.* 3 for compounds **1**–**3** suggesting also an enhanced

lipophilicity of the ionized forms since the lipophilicity of the neutral forms is influenced by the presence of intramolecular H-bonds (see above). Due to the withdrawing effect of the *para* substituent, it can be postulated that OH–C(2) is the site of ionization, suggesting only one type of intramolecular H-bond for the ionized species of **1**–**3** (*Fig. 4*).



4 R = H
5 R = NO_2



1–**3**

Fig. 4. *Intramolecular H-bond for the ionized form of catechol* (**4**) *and 4-nitrocatechol* (**5**) *as well as of entacapone* (**1**)*, nitecapone* (**2**)*, and tolcapone* (**3**)

3. Conclusions. – Neutral species are generally assumed to be responsible for the BBB passage by passive mechanisms. The most suitable lipophilic predictor of BBB passage was shown to be the difference between the log *P* of the neutral form in octanol/H_2O and alkane/H_2O systems, *i.e.*, the $\Delta\log P^{N}_{oct-alk}$ parameter was mostly related to the solute H-bonding ability (mainly α) [12][16]. In particular, an inverse linear relation was found by *Young et al.*, between the logarithm of the brain/blood concentration ratios and $\Delta\log P^{N}_{oct-alk}$ for a series of H_2 receptor histamine antagonists [8]. $\Delta\log P^{N}_{oct-dce}$ can replace $\Delta\log P^{N}_{oct-alk}$ in SAR studies of permeation [13]. For entacapone (**1**), nitecapone (**2**), and tolcapone (**3**), $\Delta\log P^{N}_{oct-dce}$ values are very close (*ca.* 0) suggesting a similar and moderate ability to cross the BBB. This result is not surprising considering that the three COMT inhibitors studied share the same H-bond-donor substructure. Thus, the larger BBB passage of **3** compared to **1** and **2** can be related to the higher lipophilicity of the R substituent.

Moreover, for the three COMT inhibitors, the amount of the neutral form present at physiological pH is even lower than expected from their ionization profiles (1%), because they are involved in other equilibria (*e.g.*, HSA binding). However, the lipophilic behavior of the anionic species of **1**–**3** is similar to the partition order of the neutral species with a H-bond-donor potency largely lowered due to the formation of intramolecular H-bonds. These molecular properties could support enhanced brain permeation for the ionized form of **3** even if, at the moment, no evidence has been found in the literature for the passage of monoanions by passive mechanisms.

Experimental Part

1. *Materials.* Entacapone (**1**), nitecapone (**2**), and tolcapone (**3**) were kindly donated by the Dipartimento di Scienza e Tecnologia del Farmaco, University of Turin, Italy. The 4-nitrocatechol (**5**) and pyrocatechol (=catechol=benzene-1,2-diol; **4**) were obtained from *Sigma* (U.K.) and from *Fluka AG* (Buchs, Switzerland), respectively. Octanol was purchased from *Lancaster* (U.K.), and 1,2-dichloroethane (*SDS*, Valdonne-Peypin, France) was used without further purification and handled with all necessary precautions [17]. The organic supporting electrolyte, BTPPATPBCl (bis(triphenylphosphoranylidene)ammonium tetrakis(4-chlorophenyl)borate) was prepared by metathesis of KTPBCl (potassium tetrakis-(4-chlorophenyl)borate) (*Fluka AG*, Buchs, Switzerland) and of BTPPACl (bis(triphenylphosphoranyl-

idene)ammonium chloride) (*Aldrich*, Milwaukee, USA) [18]. The aqueous phase was deionized water (*Milli-QSP* reagent water system, *Millipore*, Switzerland). All other chemicals were of anal. grade and were supplied by *Fluka*.

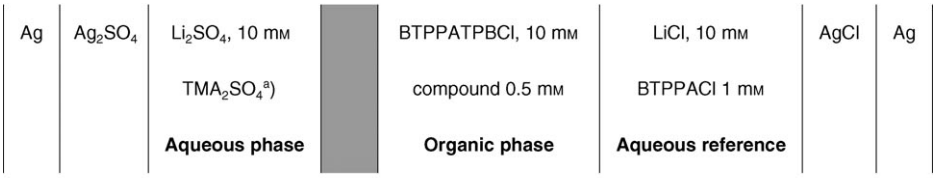
2. *Potentiometric Determination of Ionization Constants and Partition Coefficients.* The ionization constants and the partition coefficients of neutral forms were determined by potentiometric titration with the GlpK_a apparatus of *Sirius Analytical Instruments Ltd.* (Forest Row, East Sussex, UK) [19]. All titrations were conducted under Ar at $25 \pm 0.1°$.

The ionization constants of all tested compounds were determined in H_2O. All compounds were acid; thus to improve their solubility, they were initially alkalinized to an appropriately high pH with standardized KOH. Titrations started immediately to avoid mostly oxidative degradation of catechols at strong basic pHs. The solns. were then titrated with 0.5M HCl to low pH (minimum 2.0).

The neutral partition coefficients in octanol/H_2O and 1,2-dichloroethane/H_2O were measured by titrating drug solns. in presence of different volumes of org. phase (volume ratio oil/H_2O between 0.02 and 1.5). The log *P* values were estimated from difference *Bjerrum* [20] plots and refined by a nonlinear least-squares procedure [21] by including previously determined pK_a values as unrefined contributions. The detailed experimental procedures of the potentiometric method can be found elsewhere [22][23].

3. *Cyclic Voltammetry Measurements.* The partition coefficient of ionic forms in 1,2-dichloroethane/H_2O was studied by cyclic voltammetry. The experimental setup was a home-made four-electrode potentiostat, as described in [24], with ohmic drop compensation [25]. The scanning of the applied potentials was performed by a waveform generator (VA scanner *E 612*, *Metrohm*, Herisau, Switzerland), coupled to an *X-Y* recorder (*Bausch & Lomb*, NY, USA). Both, the cell and the four-electrode potentiostat were housed in a *Faraday* cage. All experiments were carried out at r.t. (25°).

The 1,2-dichloroethane and H_2O were mutually saturated. The compounds were dissolved in the org. phase. The pH of the aq. soln. was adjusted to the desired value with H_2SO_4 and LiOH. The electrochemical chain shown in *Fig. 5* was used.



a) TMA_2SO_4 = trimethylammonium sulfate: reference ion.

Fig. 5. *Schematic illustration of the electrochemical chain used*

All measured half-wave potentials $\Delta_o^w\phi_i^{1/2}$ of the ionized forms were referred to the half-wave potential $\Delta_o^w\phi^0_{TMA^+}$ of the tetramethylammonium cation. Thus, the standard transfer potential $\Delta_o^w\phi_i^0$ of an ion X_i can be calculated with *Eqn. 1*. Since the value of $\Delta_o^w\phi^0_{TMA^+}$ is known (160 mV on the tetraphenylarsonium tetraphenylborate scale), the standard *Gibbs* energy of transfer of the ion, $\Delta G^{0,w\to o}_{tr,i}$, and its standard partition coefficient, log $P^{0,i}_{dce}$, can be calculated with *Eqns. 2* and *3*.

$$\Delta_o^w\phi_i^{1/2} - \Delta_o^w\phi^{1/2}_{TMA^+} = \Delta_o^w\phi_i^0 - \Delta_o^w\phi^0_{TMA^+} \tag{1}$$

$$\Delta_o^w\phi_i^0 = \frac{\Delta G^{0,w\to o}_{tr,i}}{z_i F} \tag{2}$$

$$\log P^{0,i}_{dce} = -\frac{\Delta G^{0,w\to o}_{tr,i}}{RT\ln 10} \tag{3}$$

The details on the electrochemical measurements and the theoretical background have been reported in [24][26][27].

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