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Identification of COMT and ErmC inhibitors by using a microplate assay in combination with library focusing by virtual screening

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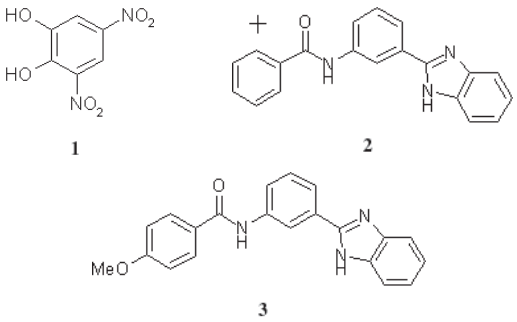
The paper describes a process of facilitated screening by using a combination of molecular modelling and a 96-well microplate assay for the identification of novel inhibitors of catechol-*O*-methyltransferase (COMT) and bacteria expressing ErmC. With the help of virtual screening the number of compounds processed in the *in vitro* screening assay was reduced from over 200 000 to 49. Out of the 49, two structurally very similar compounds were identified as confirmed hits with reasonable activity (IC_{50} values of 26 and 73 μM) and thus as potential core structures for further drug design and development.

Catechol-*O*-methyltransferase (COMT) is an enzyme capable of *O*-methylating catecholamines and other compounds with catechol structure. Inactivation of catecholamine neurotransmitters and elimination of toxic catechols and some other hydroxylated metabolites is the general function of COMT. Catechol-*O*-methyltransferase inhibitors are used for blocking the metabolism of levodopa in the periphery resulting in increased availability in the brain. The main clinical application of COMT inhibitors is as adjunct in the levodopa therapy of Parkinson's disease (Männistö and Kaakkola 1999). Another methyltransferase, ErmC, mediates antibiotic resistance in bacteria (Khan et al. 1999). Recently, we have described the development of a screening assay in 96-well plate format for the identification of COMT inhibitors (Kurkela et al. 2004). Here we describe an idea how the screening of novel antimicrobials, and at the same time COMT inhibitors, can be enhanced by focusing the compound library *in silico* prior to *in vitro* screening and by employing a simple enzyme-based assay before proceeding to cell-based screening.

In the COMT-catalyzed *O*-methylation *S*-(5′-adenocyl)-L-methionine (AdoMet) is used as the universal methyl donor. The crystal structures of several characterized methyltransferases are strikingly similar in the AdoMet-binding regions (Männistö and Kaakkola 1999), including that of ErmC', an ErmC variant differing from ErmC in only five amino acids (Su and Dubnau 1990; Bussiere et al. 1998). The overall aim of our project is to search for novel antimicrobials against erythromycin-resistant bacteria that produce ErmC. However, the assay used for screening bacterial susceptibility is cell-based and more tedious than the enzyme-based COMT assay. Therefore, on the basis of the structural similarity of the AdoMet binding site, we decided to use the COMT assay as a primary screen prior to examining the antimicrobial activity of the hits. First, the 3D structure of ErmC' (Bussiere et al. 1998; Schluckebier et al. 1999) was used as a model for assessing the interactions of library compounds with the binding site. Databases of commercial libraries containing in total over 200 000 compounds [ChemBridge Corp. (San Diego, CA), Maybridge (Cornwall, England), Specs (Delft, Netherlands)] were screened *in silico* using the FlexX program (Rarey et al. 1996) for docking each compound to the AdoMet binding site of ErmC'. The top-scoring compounds were further visually inspected to eliminate hits with unrealistic conformation or binding orientation. 49 drug-like compounds with best binding properties were selected and purchased for *in vitro* screening. The potency of the chosen compounds in inhibiting S-COMT, the water-soluble form of COMT, was determined at a concentration of 100 μM according to Kurkela et al. (2004) in 96-well microplate format. A known COMT inhibitor 3,5-dinitrocatechol (**1**) was used as a positive control.



1 **2** **3**

Four actives (>30% inhibition of S-COMT) were identified from the primary screening and two of these were confirmed to be potential hits in the follow-up experiments. The hits, named as MB5 (= HTS 01780 313.3600, Maybridge, **2**) and MB6 (= HTS 01781 343.3860, Maybridges, **3**), were further evaluated by determining their dose-response profiles. The concentrations causing inhibition of 50% (IC_{50}) were 26 and 73 μM for MB5 and MB6, respectively. For the positive control, 3,5-dinitrocatechol, IC_{50} of 35 nM has been reported (Kurkela et al. 2004). Although the hits MB5 and MB6 possess only modest activity in inhibiting S-COMT, they share a common parent structure which could serve as a relatively simple and low molecular weight template for further drug design and optimization. The COMT inhibitors currently in clinical use, namely entacapone and tolcapone, are based on 3-nitrocatechol, and suffer from several problems such as liver toxicity, poor blood-brain barrier penetration, and poor oral bioavailability. It has therefore been recognized that it would be advantageous to develop new COMT inhibitors not containing the nitrocatechol structure (Männistö and Kaakkola 1999).

Noteworthy, the COMT assay was found to possess two features that should be considered when library compounds are screened. First of all, with some of the compounds the inhibition % in the primary screen was markedly negative as a result of the native fluorescence of the

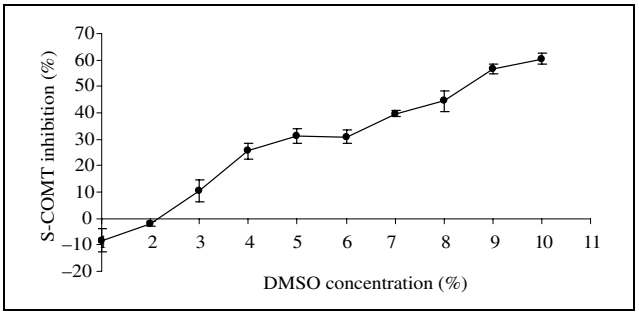


Fig.: DMSO tolerance of the S-COMT assay. The columns represent % inhibitions observed with increasing DMSO concentrations (mean ± SD, n = 3)

compounds. This seemed to interfere with the assay detection and is in fact seen as one of the limiting factor in high throughput screening concerning fluorescence-based assays (Comley 2003). Secondly, the assay was noted to tolerate a limited concentration of DMSO which is the universal solvent in dissolving library compounds. For example, the DMSO concentration of 5% resulted in 31% inhibition of S-COMT (Fig.). With very poorly soluble library compounds the limited DMSO tolerance of the assay may complicate the screening at high concentrations. In this study, the final DMSO concentration used was therefore kept at 1%.

In conclusion, this study shortly describes an approach by which the time and cost-efficiency of an *in vitro* screening process can be improved by a preceding *in silico* screening procedure. Here, the number of compounds screened *in vitro* was reduced from thousands to 49 using virtual screening, resulting in notable savings in time and money. After *in vitro* screening, two inhibitors were identified as reasonable drug candidates for further evaluation in cell-based assays and for chemical optimisation.

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LE300 – New results on its ability to antagonize the discriminative stimulus effects of cocaine

Dopamine/serotonin receptor antagonists, part XI

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LE300, i.e. 7-methyl-6,7,8,9,14,15-hexahydro-5*H*-benz[*d*]indolo[2,3-*g*]azecine, with nanomolar affinities to the hD_1 receptor family, suppresses *in vivo* spontanous locomotor activity and attenuates locomotor activity induced by cocaine. Therefore in this study, LE300 was investigated for its ability to antagonize cocaine's discriminative effects. LE300 was tested in doses from 0.5 to 10.0 mg/kg and partially antagonized the discriminative stimulus effects produced by 10 mg/kg of cocaine in rats. The partial antagonism (39% drug-appropriate responding) occurred following 5 mg/kg LE300. Response rate was decreased following 5 and 10 mg/kg, with the maximum effect (27% of cocaine control) following 10 mg/kg LE300.

The compound LE300 (Witt et al. 2000), which is 7-methyl-6,7,8,9,14,15-hexahydro-5*H*-benz[*d*]indolo[2,3-*g*]-azecine (Fig. 1), has been characterized both by radioligand binding assays and functional testings using cAMP and $[Ca^{2+}]$ as a very potent antagonist for the human dopamine receptors (D_1, D_{2L}, $D_{4.4}$ and D_5) with nanomolar affinities and a 10- to 20-fold selectivity for D_1 over D_{2L} (Kassack et al. 2002): $K_i(D_1) = 1.9$ nM, $K_i(D_{2L}) = 44.7$ nM, $K_i(D_{4.4}) = 109$ nM, $K_i(D_5) = 7.5$ nM. LE300 served as a lead for numerous analogs which are highly potent and show selectivity towards the dopamine receptor subtypes, some of them show selectivity even within the D_1-subtype family (Wittig et al. 2004; Decker and Lehmann 2003). Moderate affinity at the D_3 receptor was determined ($K_i(D_3) = 86.9$ nM), nanomolar affinities were found at the 5-HT_{2A} and 5-HT_{2C} receptors (K_i(5-HT_{2A}) = 11.9 nM; K_i(5-HT_{2C}) = 36.1 nM), micromolar affinity at the 5-HT_{1A} receptor (K_i(5-HT_{1A}) = 1.2 μM) using binding assays (Decker et al. 2004). Functional studies indicate moderate antagonist activity at the 5-HT_{2A} site ($K_e = 86.9$ nM; $pA_2 = 8.35$ nM). No activity was found at dopamine, serotonin and norepinephrine transporters. These results suggested the use of LE300 for cocaine addiction treatment (Piercy et al. 1992; Ciccocioppo et al. 2001). High activities were found *in vivo*: LE300 suppressed spontaneous locomotor activity with an ID_{50} of 1.24 mg/kg and attenuated locomotor activity induced by 20 mg/kg cocaine with AD_{50} of 1.50 mg/kg (Decker et al. 2004). LE300 was also tested for substitution for the dis-