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Magnetic beads method for determination of binding of drugs to melanin

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ABSTRACT

Binding to melanin is considered to be a reason for several adverse effects of drugs and should be known to reduce the failure rate due to inappropriate pharmacokinetics in search for better pharmaceuticals. A new, reliable and convenient method of determination of affinity of drugs and drug candidates to melanin has been proposed employing magnetic beads. For that aim the reaction conditions to effectively covalently immobilize melanin on surface of superparamagnetic beads have been determined. Binding efficiency of melanin towards antipsychotic and other basic drugs has been determined and compared to that obtained in the affinity HPLC systems employing aminopropylsilica stationary phases with immobilized melanin. The magnetic beads method provided melanin binding data correlating well with the ability of agents to evoke extrapyramidal symptoms. Quantitative structure–property relationships have been derived describing the melanin binding efficiency in terms of structural descriptors of drugs from calculation chemistry. Thus, an approach has been proposed to evaluate *a priori* melanin binding potency of drug candidates based solely on their chemical formula.

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1. Introduction

For years antipsychotic drugs almost invariably caused extrapyramidal symptoms (EPS) at clinically effective doses. This led to the false impression that EPS were an unavoidable consequence of antipsychotic therapy. With introduction of "atypical" antipsychotic agents it appeared that a great degree of separation is possible between respective dose–response curves for antipsychotic and EPS effects [1].

In search for new drugs important is to evaluate the risk of adverse effects of a drug candidate at as early stage of research and development process as possible to avoid failure in the phase of extremely costly clinical tests. There are hypotheses that EPS associated with chronic administration of antipsychotic drugs is correlated to the neuromelanin affinity of these drugs [2–5]. Binding to melanin has also been reported to correlate with drugs' occular toxicity, ototoxicity, pigment disturbances of skin and hair and carcinogenicity [6–10].

Determination of binding of drugs and drug candidates to protein of interest by standard slow equilibrium methods is a time and reagents consuming procedure. Hence it is unsuitable for high-throughput screening (HTS), employed in the present day approaches to drug candidate identification and preselection [11,12]. Therefore, above cited affinity HPLC methods called a wider interest of medicinal chemists. However, these methods can only be treated as indirectly reflecting the binding of drug analytes to melanin, which forms one of the components of the dynamic, fast equilibrium chromatographic system. A more direct drug–melanin binding measurement system is requested.

The micro- and nano-sized magnetic supports provide an original modern technology for bioseparations, especially for ligand "fishing", protein, enzyme, DNA, RNA and cell isolation or purification [13]. Because of the superparamagnetic properties, magnetic beads (MB) can be used to simply isolate any target and can be linked with diverse manual and automated applications [14,15]. An innovative biomedical research has produced new applications of magnetic nanoparticles that have exciting clinical potential, e.g. can be used for gene therapy and magnetic-force-based tissue engineering [16]. Most recently, these particles were used for a drug delivery, reducing its toxicity and enhancing efficacy [17]. As a means of drug targeting, they can be accumulated at desired locations within the body with the help of a magnet.

Furthermore, the recent clinical research showed that exposure to iron nanoparticles increased the endothelial cell permeability caused by oxidative stress coming from reactive oxygen material [18]. This phenomenon has opened a new opportunity for application of magnetic beads in cancer therapies [19].

The wide application of the MB is due to their "flexible" and multifunctional surface [20,21]. Modifications of that surface depend on

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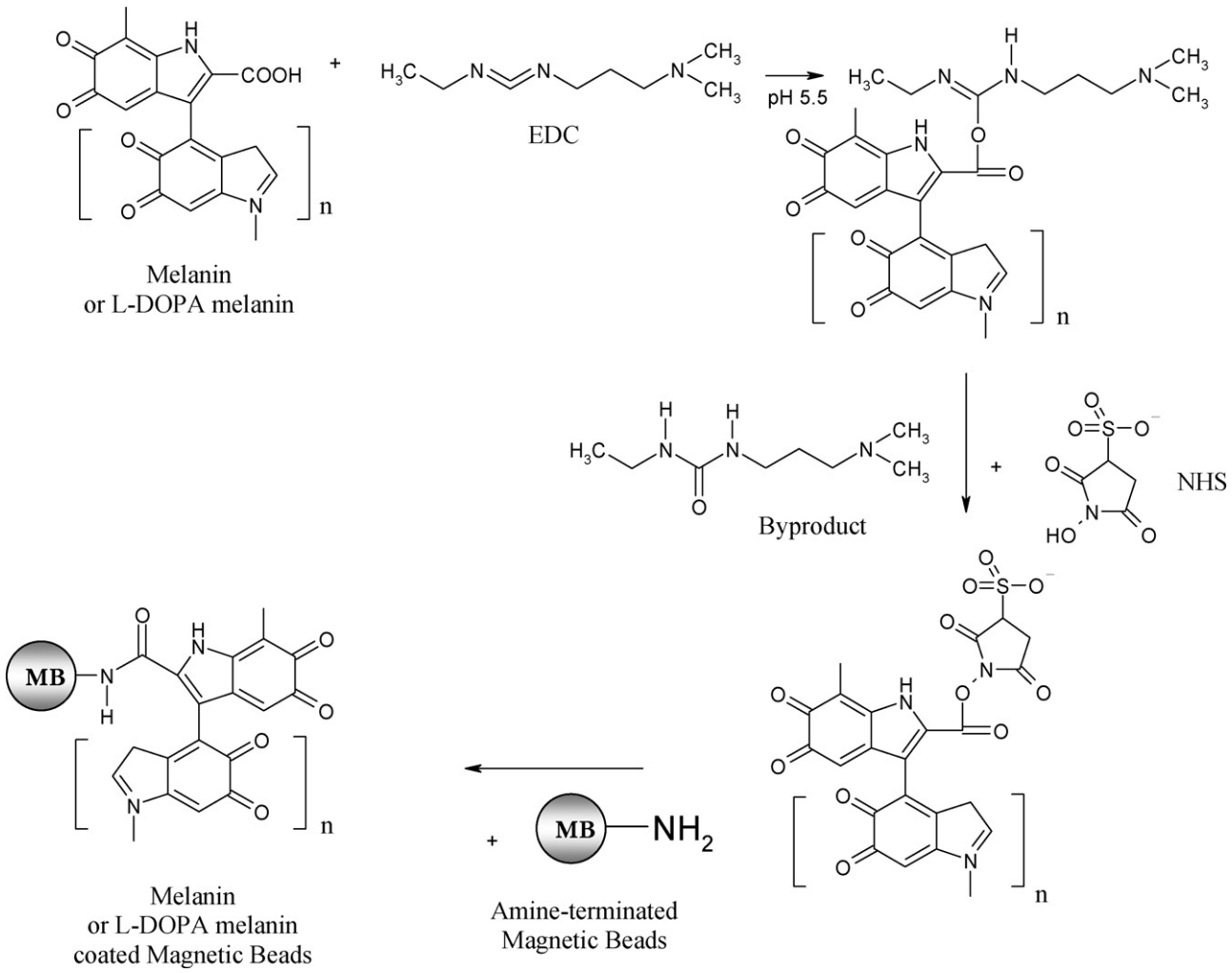

Fig. 1. Covalent immobilization of melanin or L-DOPA melanin on an amine-terminated beads surface.

the nature of target, sample and reaction medium applied [22,23]. In the present study melanin-coated magnetic beads were adapted to measure the binding data of a series of antipsychotics and other selected basic drugs to melanin. We also intended to compare the performance of the method employing MB with a corresponding affinity HPLC procedure. Both kinds of melanin-binding efficiency parameters were to be related to EPS measures, which have been reported for several antipsychotics in literature. Finally, we planned to quantitatively relate the determined melanin-binding data to the molecular structure of the drugs aiming at a convenient means of predicting affinity to melanin of any structurally defined compound based solely on its structural formula.

2. Experimental

2.1. Chemicals

Bovine serum albumin (BSA), clozapine, 3,4-dihydroxy-L-phenyl-alanine (L-DOPA), dimethyl sulfoxide (DMSO), diphenhydramine, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC), fluphenazine dihydrochloride, glutaric acid, glycine, haloperidol, imipramine hydrochloride, melanin, melatonin, nicotine hydrogen tartrate, (N-hydroxy-succinimidyl)-ester (NHS), quinine, sodium azide and ziprasidone hydrochloride monohydrate were purchased from Sigma–Aldrich (Stainhaim, Germany). Chloropromazine hydrochloride, ethopropazine hydrochloride, promazine hydrochloride, thioridazine hydrochloride, trifluoperazine, dihydrochloride, triflupromazine hydrochloride, selegiline and venlafaxine hydrochloride were from a reference drug substance collection of the Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk. Acetonitrile (ACN) and methanol (MeOH) of chromatographic quality were from Merck (Darmstadt, Germany). Ammonia (NH_4OH) was from POCh (Gliwice, Poland).

Amine terminated magnetic beads as an aqueous suspension (50 mg/mL, 1 μm diameter) of "sophistically coated iron oxide particles to provide primary amino groups", were purchased from Bioclone Inc. (San Diego, CA, USA). The surface area of the beads was determined as a 100 m^2/g of MB with functional group density of ~250 μmol/g of MB. All supernatants were separated from MB using a magnetic separator Dynal MPC-S (Invitrogen Corporation, Carlsbad, CA, USA). Water used in the study was prepared using a Milli-Q Water Purification System (Millipore, Bedford, MA, USA). The aminopropyl silica (APS) stationary phase with the pore size 300 Å and particle size 7 μm (Nucleosil 300-7 NH_2) was purchased from Macherey-Nagel (Düren, Germany).

2.2. Preparation of melanin-coated magnetic beads

2.2.1. Covalent coupling of melanin

The immobilization of melanin onto the amine-terminated magnetic beads (MB) was performed by the formation of an amide

bound between carboxyl group of melanin and the primary amino groups of the MB (Fig. 1).

Melanin was immobilized onto the MB following the protocol provided by Bioclone Inc. with slight modifications. A 9 mg sample of MB was placed in a 1.5 mL centrifuge tube and rinsed with 25 mM potassium phosphate buffer (pH 6). Next, 6 mg of melanin was dissolved in 100 μL of DMSO. 10 μL of melanin solution in DMSO was diluted 100-fold in 25 mM potassium phosphate buffer (pH 6). Then, 800 μL of this solution was placed in a centrifuge tube along with the previously rinsed beads. The mixture was mixed for 1 min, followed by the addition of 200 μL of a 10 mg/mL solution of EDC and NHS. The mixture was then rotated at 250 rpm in an orbital shaker for 24 h at ambient temperature. After an overnight incubation the resulted melanin-coated magnetic beads were divided into three equal parts in 1.5 mL centrifuge tubes. Next, 50 μL of aqueous solution of glycine (1 M, pH 8) or hydroxylamine (1 M, pH 8) or 0.5% (w/v) of BSA were added to one set of beads (3 mg) to quench the reaction. The resulting mixtures were rotated at 250 rpm in an orbital shaker for 15 min and then the supernatant was discarded. Finally, three sets of melanin-coated magnetic beads were prepared: 3 mg of Mel (1)-MB, Mel (2)-MB or Mel (3)-MB, with glycine, hydroxylamine and BSA as reaction quenchers, respectively. The melanin-coated beads were rinsed three times with 1 mL of 20% (v/v) DMSO-water solution and 1 mM NH_4OH. The control magnetic beads: control-Mel (1)-MB, control-Mel (2)-MB and control-Mel (3)-MB, were made in the same manner but without the addition of melanin to the reaction mixture.

2.2.2. Covalent coupling of polymerization product of L-DOPA

A 15 mg sample of L-DOPA was dissolved in 6 mL of 25 mM potassium phosphate buffer (pH 8) and left to stand at ambient temperature for 7 days. A 400 μL sample of the resulting solution (2.5 mg/mL of L-DOPA/melanin) of polymerized L-DOPA/melanin was diluted with 400 μL of 25 mM potassium phosphate buffer (pH 6). Then, 800 μL of this solution was added to a centrifuge tube along with 9 mg of the previously rinsed beads. Next, 200 μL of a 10 mg/mL solution of EDC and NHS was added to the tube and further steps were repeated from the above described procedure (Section 2.2.1). Finally, three 3 mg-portions of L-DOPA melanin-coated magnetic beads were prepared: L-D (1)-MB, L-D (2)-MB and L-D (3)-MB, with glycine, hydroxylamine and BSA as reaction quenchers, respectively. The control magnetic beads: control-L-D (1)-MB, control-L-D (2)-MB and control-L-D (3)-MB, were made in the same manner but without the addition of L-DOPA melanin to the reaction mixture.

2.3. Preparation of melanin-based chromatographic stationary phase Mel-APS

Synthesis of melanin-based stationary phase was performed using the published method with slight modifications [24]. A 150-mg portion of APS was rinsed with 10 mL of 25 mM potassium phosphate buffer (pH 6). Next, 5 mL of the melanin solution (2.5 mg/mL of L-DOPA/melanin), prepared as described in Section 2.2.1, was added to a centrifuge tube with the previously rinsed APS. The mixture was mixed for 1 min, followed by the addition of 200 μL of a 10 mg/mL solution of EDC and NHS. The mixture was then rotated at 250 rpm in an orbital shaker for 24 h at ambient temperature. Next, the mixture was centrifuged at 1500 rpm for 5 min and the supernatant was discarded. Then, 20 μL of 1 M hydroxylamine was added, the mixture shaken for 30 min at 4 °C and the supernatant discarded. The resulted end-capped melanin-coated silica was rinsed three times with 5 mL of 20% (v/v) DMSO–water solution and 1 mM NH_4OH. Then, the suspension of melanin-APS was placed in a glass column with inner diameter of 5 mm and length of 20 mm (5/20 Tricorn, GE Healthcare Bio-Science AB/Amersham Biosciences, Freiburg, Germany) and allowed to settle. The fittings on the Mel-APS column were tightened and the column was washed with methanol–water (15:85, v/v) for 2 h using a chromatographic pump providing solvent flow rate of 0.2 mL/min.

2.4. Preparation of polymerization product of L-DOPA-based chromatographic stationary phase L-D-APS

A 150 mg portion of APS was placed in a 10 mL plastic tube and rinsed with 10 mL of 25 mM potassium phosphate buffer (pH 6). A 2.5 mL sample of polymerized L-DOPA/melanin solution (2.5 mg/mL of L-DOPA/melanin) from Section 2.2.2 was diluted with 2.5 mL of 25 mM potassium phosphate buffer (pH 6). Then, 5 mL of this solution was added to a 10 mL tube along with 150 mg of the previously rinsed APS. The mixture was mixed for 1 min, followed by the addition of 200 μL of a 10 mg/mL solution of EDC and NHS. The further immobilization procedure was continued with the above described method I (Section 2.3) resulting in an L-D-APS column.

2.5. Compound binding studies to melanin- and L-DOPA melanin-based magnetic beads

The following compounds were subjected to the binding studies to melanin: chloropromazine, clozapine, diphenhydramine, ethopropazine, fluphenazine, haloperidol, imipramine, melatonin, nicotine, promazine, quinine, selegiline, thioridazine, trifluoperazine, triflupromazine, venlafaxine, ziprasidone (Fig. 2). 3 mg of Mel (2)-MB, L-D (2)-MB, control-Mel (2)-MB and control-L-D (2)-MB were incubated with serial concentrations for each ligand: 135, 225, 375, 500, 750, 1000, 1500 and 2000 nM. The same concentrations were used for determination of standard curves for each ligand. 350 μL solution of each concentration in 10 mM ammonium acetate buffer of pH 7.4, were placed into 1 mL centrifuge tubes along with 3 mg of the studied magnetic beads and incubated (vortexed) for 10 min. After 1 min, the supernatant was removed using the magnetic separator and analyzed by LC–MS-ESI. The magnetic beads washing procedure consisted of a triple wash with 500 μL of 15% MeOH in 10 mM ammonium acetate buffer (pH 7.4) and a single wash with 10 mM ammonium acetate buffer of pH 7.4 for 2 min. The scheme of ligands incubation with the coated magnetic beads and washing procedure is presented in Fig. 3.

The optimization of the washing procedure of the Mel (2)-MB and L-D (2)-MB consists of the different concentration of MeOH (elution step) and wash time. With respect to that, the elution step consisting of the triple wash with 15% MeOH for 15 min each appeared to be most sufficient to remove ligand unbound and, at the same time, harmless for melanin activity. Finally, after the washing procedure Mel (2)-MB and L-D (2)-MB could be reused for further study.

The measured free ligand concentration ([D]) in the first supernatant was used to calculate the fraction of compound that is unbound. The amount of ligand bound ($[D_B]$) was obtained from:

$$[D_B] = [D_{TOT}] - [D] \quad (1)$$

where $[D]_{TOT}$ is total ligand concentration.

The melanin- and the L-DOPA-derived melanin–drug binding efficiency (E_B) was determined as the slope of a plot representing the relationship between the amount of drug bound ($[D_B]$) vs. amount added ($[D_{TOT}]$). The above indicated serial concentrations of ligands were used to get the analyzed linear relationships (Fig. 4).

In order to test the performance of Mel (2)-MB and L-D (2)-MB, the E_B values were determined for fluphenazine after 60 and 120 incubations The attained values were 0.91 (±0.04) and 0.93 (±0.02), in comparison to the starting value of 0.94 (±0.03). When using the L-D (2)-MB, the corresponding values were 0.85 (±0.02) and 0.85

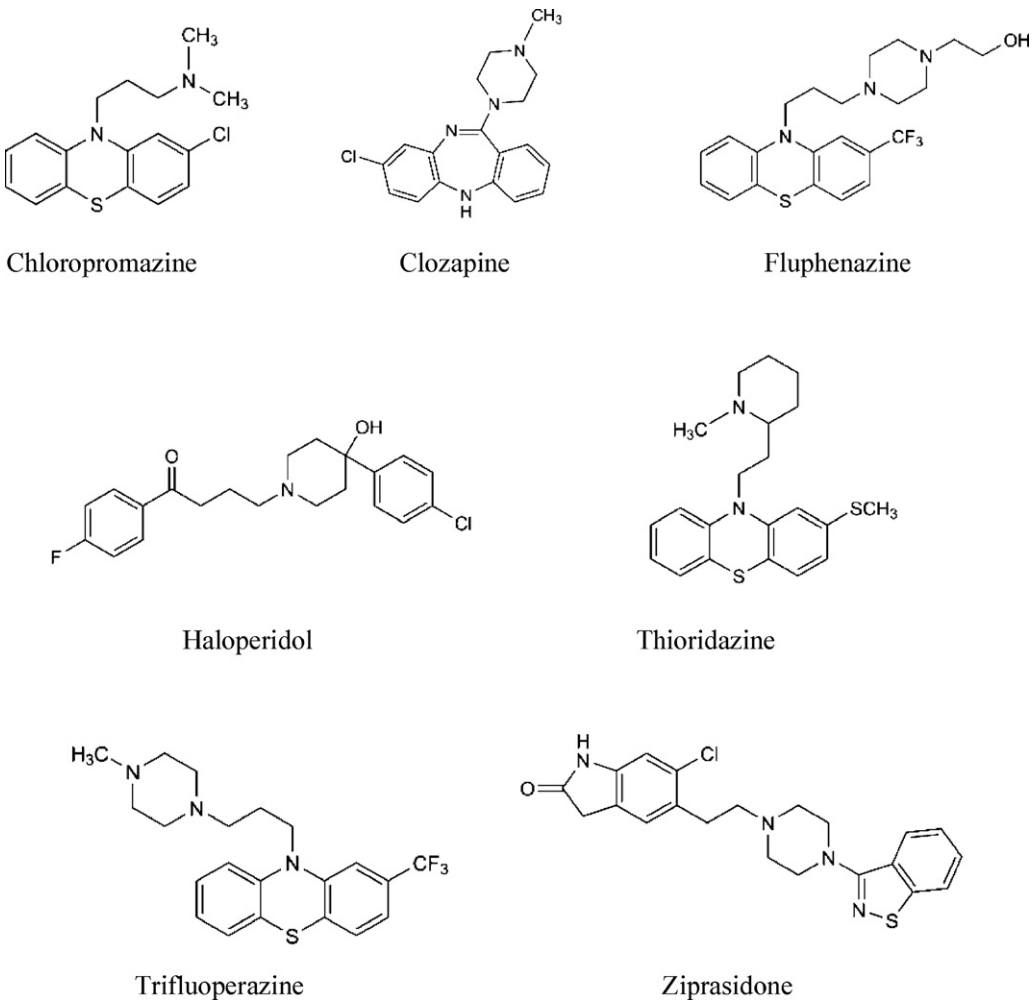

Fig. 2. Structural formulas of drug studied.

(±0.04) vs. 0.87 (±0.01) (Table 1). The slight change in the E_B proves that the beads remain active.

All the analyses of supernatants were done after a 10 min incubation (vortexing) at 18 °C in triplicate. The supernatant was assayed with a Shimadzu LC–MS 2010 eV system (Kyoto, Japan) composed of a vacuum degasser (DGU-2A3), two solvent pumps (LC-20AD), an autosampler (SIL-20AC) with temperature adjusted at 18 °C, a diode array detector (SPD-M20A) and a column oven

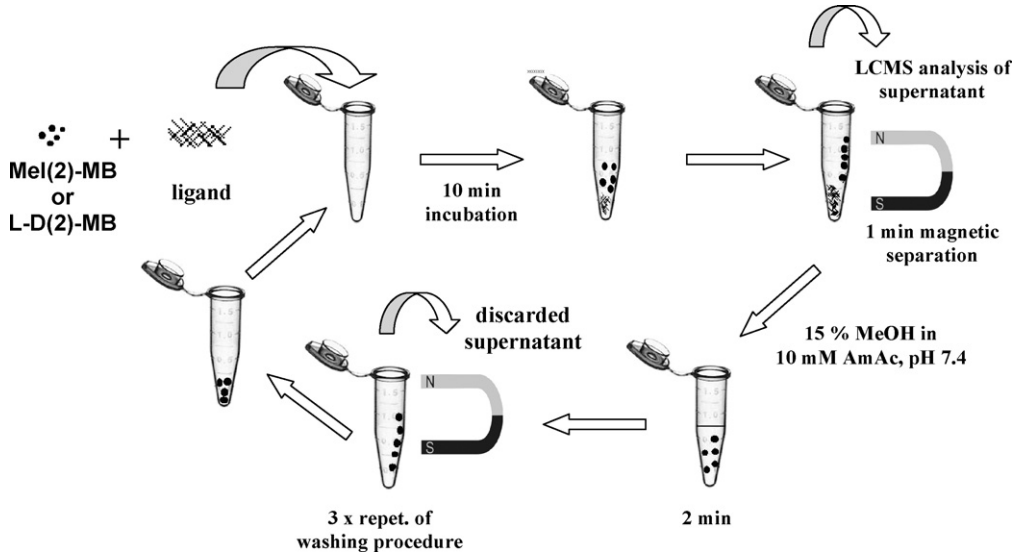

Fig. 3. Scheme of incubation studied ligands with the L-DOPA (L-D (2)-MB) – and melanin (Me l (2)-MB) based magnetic beads and washing procedure.

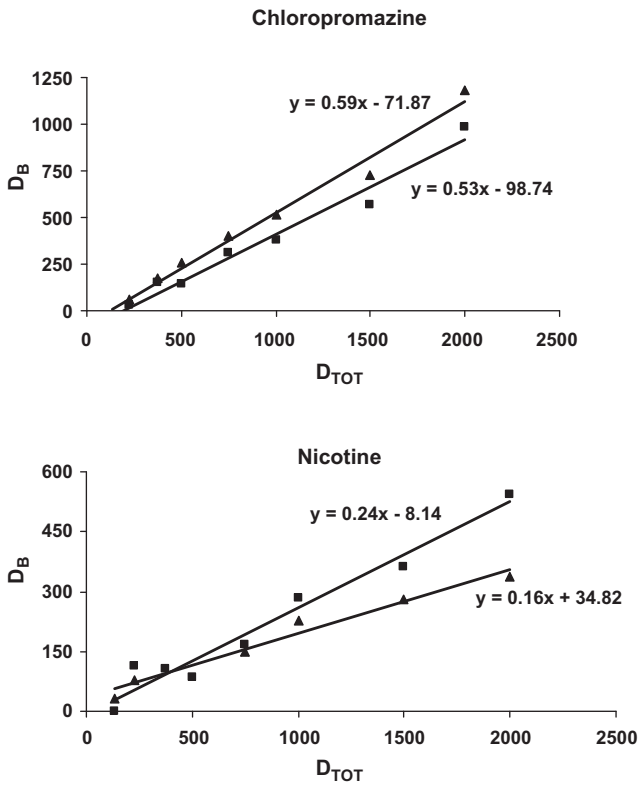

Fig. 4. The exemplary linear analysis of data obtained from incubation of chlorpromazine and nicotine using the Mel (2)-MB (▲) and L-D (2)-MB (■).

(CTO-20AC). The experiments were carried out using positive capillary voltage (1.5 kV), drying gas flow rate of 1.5/min, temperature 250 °C and the mobile phase composed of 10 mM ammonium acetate buffer (pH 7.4)/acetonitrile (20/80, v/v) with the flow rate of 0.4 mL/min. Each collected supernatant was injected in sample volume of 25 μL. The studied compounds were quantified in the positive-ion mode using the single ion monitoring mode (SIM) at *m*/*z* 319 (chloropromazine), *m*/*z* 327 (clozapine), *m*/*z* 256 (diphenhydramine), *m*/*z* 313 (ethopropazine), *m*/*z* 438 (fluphenazine), *m*/*z* 376 (haloperidol), *m*/*z* 281 (imipramine), *m*/*z* 233 (melatonin), *m*/*z* 163 (nicotine), *m*/*z* 286 (promazine), *m*/*z* 325 (quinine), *m*/*z* 188 (selegiline), *m*/*z* 371 (thioridazine), *m*/*z* 409 (trifluoperazine), *m*/*z* 353 (triflupromazine), *m*/*z* 278 (venlafaxine), *m*/*z* 413 (ziprasidone). The calculated standard curves for all the analytes were linear with correlation coefficient ranging from 0.991 to 0.999.

Table 1
Binding efficiency, E_B, of melanin for a set of antipsychotics and other basic drugs as determined by the magnetic beads method using beads covered with melanin or polymerized L-DOPA melanin.

Compounds	Mel (2)-MB		L-D (2)-MB	
	E_B	SD	E_B	SD
Chloropromazine	0.60	0.00	0.55	0.05
Clozapine	0.24	0.07	0.21	0.09
Diphenhydramine	0.03	0.00	0.06	0.00
Ethopropazine	0.25	0.03	0.22	0.02
Fluphenazine	0.94	0.03	0.87	0.01
Haloperidol	0.72	0.03	0.59	0.04
Imipramine	0.18	0.04	0.26	0.02
Melatonine	0.11	0.03	0.05	0.03
Nicotine	0.15	0.05	0.23	0.04
Promazine	0.08	0.02	0.14	0.01
Quinine	0.27	0.05	0.49	0.09
Selegiline	0.12	0.04	0.06	0.02
Thioridazine	0.71	0.02	0.76	0.00
Trifluoperazine	0.35	0.09	0.54	0.10
Triflupromazine	0.39	0.03	0.50	0.01
Venlafaxin	0.14	0.04	0.12	0.03
Ziprasidone	0.39	0.00	0.38	0.06

2.6. Chromatographic binding studies to Mel-APS and L-D-APS

The retention coefficient, *k*, of the studied drugs on the Mel-APS and L-D-APS columns was determined using the isocratic mobile phase methanol: 25 mM Tris–HCl buffer pH 7.4 (12:88, v/v) with flow rate of 0.4 mL/min. A signal of sodium nitrate was a dead-time marker. All the measurements were done in triplicate with UV detection at 254 nm wavelength and sample injection volume of 5 μL. The Shimadzu (Kyoto, Japan) chromatographic system was composed of two solvent pumps (LC-20AD), autosampler (SIL-20A), diode array detector (SPD-M20A), column oven (CTO-20AC) and degasser DGU-20A3.

3. Results and discussion

Covalent immobilization on magnetic beads of both the commercially available melanin and the product of L-DOPA polymerization was successful. The immobilization of both melanins was performed by the formation of an amide bound between the amine group of magnetic beads or APS between carboxyl group of melanin. An advantage of the product of L-DOPA polymerization with respect to melanin is its solubility [24]. The main problem of natural melanin is its insolubility in water and many organic solvents, except for DMSO. However, this reagent, as a strong polar aprotic solvent, can cause unstable condition during the activation with the cross-linker. Thus, the efficiency of final product of immobilization of melanin is not equal or even lower in comparison with the use of product of L-DOPA polymerization as a substrate. Perhaps that is the reason for a better consistency of melanin–drug binding data derived with use of the L-DOPA polymer, as will be described further.

The immobilization of melanin- and the L-DOPA-derived melanin was carried out on to the surface of the amine terminated magnetic beads. In order to address the issue of nonspecific binding, the magnetic beads were endcapped with glycine (as suggested in the manufacturer's protocol), hydroxylamine and BSA. The 350 μL solution of 1 μM of fluphenazine, imipramine or triflupromazine in 10 mM ammonium acetate buffer of pH 7.4, was placed into a 1 mL centrifuge tube with 3 mg of Mel (1)-MB, Mel (2)-MB, Mel (3)-MB, L-D (1)-MB, L-D (2)-MB or L-D (3)-MB and their controls and incubated (vortexed) for 10 min. Next, the supernatant was removed using the magnetic separator (1 min) and immediately analyzed by LC–MS-ESI. The amount of the ligand bound and unbound was determined. For triflupromazine, the less nonspecific binding of control beads (0.6% for control-Mel (1)-MB and 2.1% for control-L-D (1)-MB) was achieved using the glycine endcapped beads (Fig. 5). However, the best resolution between control and the melanin- as well as L-DOPA melanin-coated magnetic beads was observed for hydroxylamine quencher (9.2% binding for control vs. 48.4% for melanin and 5.3% binding for control vs. 58.2% for L-DOPA melanin-coated magnetic beads). Similar results were observed for fluphenazine and imipramine. Finally, for further study on the binding to melanin of a set of antipsychotics and other basic drugs, the hydroxylamine-quenched magnetic beads: Mel (2)-MB, L-D (2)-MB and their controls were used.

The quantitative measure of binding efficiency, E_B, with a good reliability, was derived by linear relationships involving D_B and D_{TOT} (Table 1). The similar bindings (E_B) were observed for all the tested drugs, either to Mel (2)-MB or to L-D (2)-MB. For the melanin-based magnetic beads, Mel (2)-MB, a better resolution was achieved, with the strongest binding fluphenazine (0.94)

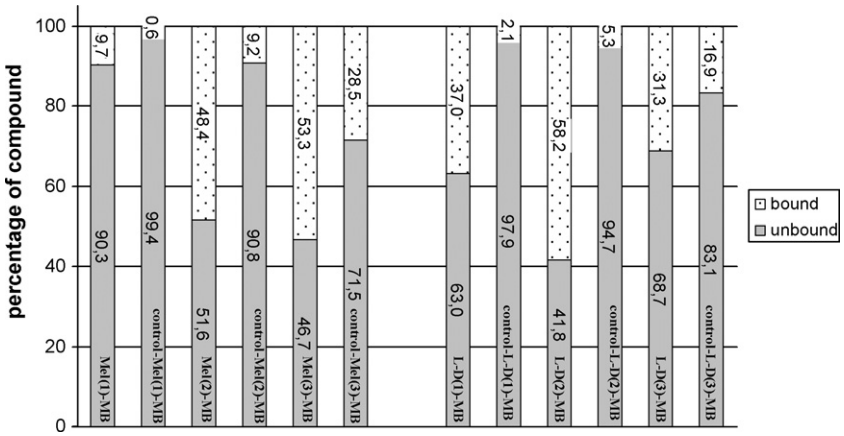

Fig. 5. Comparison of triflupromazine binding to magnetic beads with immobilized melanin, Mel (x) MB, L-DOPA melanin, L-D (x)-MB and control magnetic beads obtained in reactions quenched with glycine ($x = 1$), hydroxylamine ($x = 2$) and BSA ($x = 3$).

and the least binding diphenhydramine (0.03). For the product of polymerization of L-DOPA-based magnetic beads, L-D (2)-MB, the corresponding E_B values were 0.87 and 0.06, respectively.

There is a significant correlation between melanin-binding efficiency determined by the magnetic beads method, E_B, and retention coefficient of individual agents determined on HPLC columns packed with stationary phases comprising immobilized melanin (Fig. 6). That concerns both the commercially available melanin and the product of polymerization of L-DOPA, although in the latter case the correlation of E_B with retention coefficient, k, is slightly higher (R are 0.7903 and 0.8464, respectively). Therefore, the two procedures of melanin–drug binding may be treated as alternative. The more so that both are of a similar complexity. Still, the melanin binding parameters provided by the two methods are not identical. Hence, a question appeared which method produces the more pharmacologically relevant data.

To test the possible relationships between the determined melanin binding efficiency data and the risk of extrapyramidal side effects (EPS) of the studied drugs, we related our E_B data (as well as retention coefficients, k, from the corresponding affinity HPLC systems) to the available semiquantitative data on EPS from literature [25,26]. To evaluate the presence of EPS a severity scale is used. The scale is based on a subjective questionnaire in which the clinician performs a detailed evaluation of EPS and rates two global impressions of the severity of parkinsonism and dyskinesia [27,28].

Correlation of chromatographic k (or $\log k$) parameters with EPS was rather low ($R < 0.7$), whereas E_B determined on both the commercial melanin- and the L-DOPA melanin-covered beads correlated significantly with EPS (Fig. 7). It must be noted here that thioridazine appeared to be an outlier in the correlation analysis. That can be rationally explained as Baldessarini and Tarazi noted that thioridazine has "a somewhat lower incidence of adverse

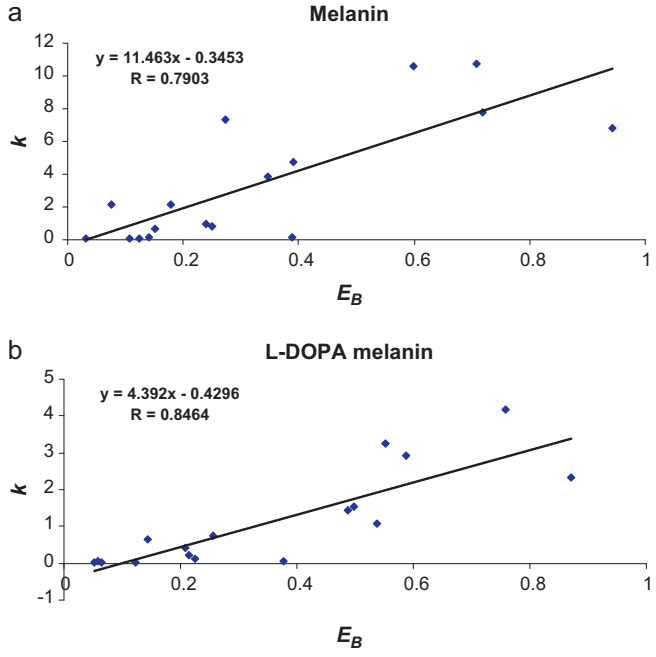

Fig. 6. Comparison of the relationships between the melanin binding efficiency from magnetic beads method, E_B, and the chromatographic retention determined on the melanin-based column, k: (a) Mel (2)-MB; (b) L-D (2)-MB.

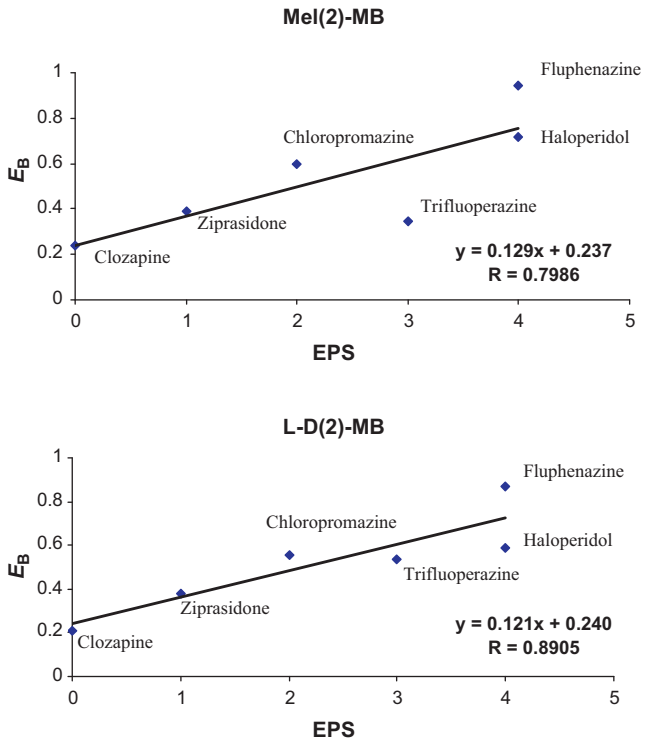

Fig. 7. Relationships between the melanin binding efficiency, E_B, determined on Mel (2)-MB and on L-D (2)-MB, and the risk of extrapyramidal symptoms (EPS) estimated in a 0–4 degree scale [25].

Table 2
Structural descriptors of studied drugs used in regression analysis with binding efficiency, E_B.

Compounds	$\log E_{B\ L\text{-}D\ (2)\text{-}MB}$	Q_{index}	T(N. . .N)	MWC04
Chloropromazine	−0.0603	23.0	14.0	6.8
Clozapine	−0.2573	16.0	4.0	6.4
Diphenhydramine	−0.5901	15.0	4.0	6.4
Ethopropazine	−0.3034	19.0	4.0	6.6
Fluphenazine	−1.2333	10.0	0.0	6.1
Haloperidol	−0.2316	18.0	0.0	6.6
Imipramine	−0.3116	21.0	6.0	6.7
Melatonine	−0.6459	9.0	4.0	5.8
Nicotine	−1.1938	6.0	0.0	5.7
Promazine	−0.8416	15.0	4.0	6.4
Quinine	−0.2700	23.0	14.0	6.8
Selegiline	−0.6669	16.0	3.0	6.5
Thioridazine	−0.9077	13.0	0.0	6.3
Trifluoperazine	−0.1196	20.0	4.0	6.7
Triflupromazine	−1.2896	11.0	5.0	6.1
Venlafaxine	−0.6826	20.0	22.0	6.6
Ziprasidone	−0.4229	25.0	37.0	6.8

extrapyramidal effects, possibly due to increased central antimuscarinic activity" [26].

The melanin binding efficiency data, after a standard logarithmic conversion, log $E_{B\ L\text{-}D\ (2)\text{-}MB}$, were correlated with molecular descriptors calculated by the HyperChem and Dragon softwares. Statistical methodology, based on the multiple linear regression analysis, produced the following quantitative structure–property relationship (QSPR) equation:

$$\log E_{B\,L\text{-}DOPA} = 12.74(\pm 3.77) + 0.27\, Q_{index}(\pm 0.05) - 0.04T(\mathrm{N}\ldots\mathrm{N})\,(\pm 0.01) - 2.72\mathrm{MWC04}(\pm 0.71) \quad (2)$$

$n = 17$; $R = 0.94$; $F = 30.65$; $p < 0.00$; $s = 0\ .15$

where the independent variables are the calculated theoretically structural descriptors of the agents studied: Q_{index} is a topological index – the so-called Zagreb quadratic index, T(N. . .N) is a sum of topological distances between the nitrogen atoms, and MWC04 is a molecular walk count of order 04 (Table 2). Other symbols at Eq. (2) refer to: n – number of compound studied, R – multiple correlation coefficient, F – Fischer coefficient of significance, p – equation significance level, s – standard error of estimate, numbers in parenthesis – standard errors of individual regression coefficients.

All the molecular graph-derived indices, employed in Eq. (2), were provided and selected by the Dragon software. Definitions and calculation rules for these indices can be found in a book of Todeschini and Cansonni [29]. Selection of these specific indices from the multitude of the existing descriptors for the QSPR equation (Eq. (2)) was done according to the rules of meaningful statistics.

It is difficult to assign unequivocally a concrete physical sense to the individual descriptors used in Eq. (2). Nonetheless, the derived equation offers an opportunity that the binding efficiency of antipsychotic and other basic drugs to melanin can be estimated *a priori* for any structurally defined agent, providing that the QSPR equation is not a fortuitous one, obtained by chance. The proof of relevance of any of the linear free-energy relationship (LFER)-based models can only be statistical in nature.

The good statistical quality of Eq. (2) is demonstrated by a high correlation between the experimentally determined and the calculated melanin binding efficiency data (Fig. 8). Its reliability is confirmed by the regular distribution of the prediction errors (Fig. 9).

Above derived Eq. (2) must be treated with extreme care because the analyte descriptors employed in it are calculated from molecular formula or molecular graph and as such have no definite physical meaning. Actually, as discussed in Ref. [30], the problem is if they bear on property of a compound or only its symbolic representation. The fact is that they often happen to provide reliable predictions of properties of individual members of a series of compounds, e.g., chromatographic retention parameters [31].

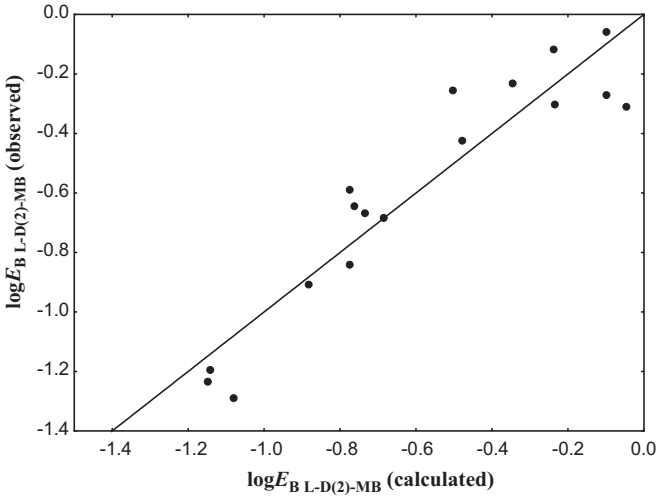

Fig. 8. Plot of melanin binding efficiency determined on L-D (2)-MB vs. data calculated by Eq. (2).

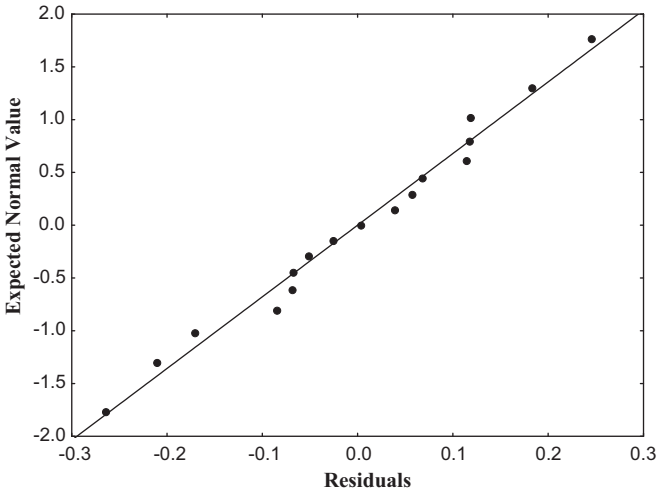

Fig. 9. Normal probability plot of residuals for Eq. (2).

4. Conclusions

Magnetic beads with covalently immobilized melanin offer a reliable, fast, inexpensive and convenient system for high throughput screening of affinity of antipsychotic and other basic drugs and drug candidates to melanin. Hence, a new method can be proposed to estimate potential of bioactive agents to evoke adverse effects related to melanin binding, like ocular toxicity, ototoxicity, extrapyramidal syndrome and carcinogenicity.

Melanin can be immobilized effectively on magnetic beads as well as on aminopropylsilica stationary phase for HPLC by the proposed chemical procedures using either a commercially available reagent or a product of polymerization of L-DOPA. The melanin efficiency parameters determined by the magnetic beads method seem to be more biorelevant than the corresponding parameters (retention coefficients) from the affinity chromatography, as the former correlate significantly with the scaled potential of antipsychotic drugs to evoke extrapyramidal syndrome.

Measures of affinity of agents to melanin, determined for a series of 17 compounds, have been described in terms of molecular descriptors provided by the standard calculation chemistry software. The resulting quantitative structure–property relationships (QSPR) allow an *a priori* evaluation of affinity to melanin of drug candidates, thus helping to eliminate agents of inappropriate ADMETox properties at the early stages of the drug development process.

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