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Original scientific paper

Synthesis of novel 2-(piperazino-1-yl-alkyl)-1*H*-benzimidazole derivates and assessment of their interactions with the D_2 dopamine receptor

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Abstract: A total of 14 novel arylpiperazines were synthesized, and pharmacologically evaluated by measuring their affinities towards the D_2 dopamine receptor (DRD2) in a [^{3}H]spiperone competition assay. All the herein described compounds consist of a benzimidazole moiety connected to *N*-(2-methoxyphenyl)piperazine *via* linkers of various lengths. Molecular docking analysis and molecular dynamics simulations were performed on the DRD2–arylpiperazine complexes with the objective of exploring the receptor–ligand interactions and properties of the receptor binding site. The recently published crystal structure of DRD2 was used throughout this study. The major finding is that high affinity arylpiperazines must interact with both the orthosteric binding site and the extended binding pocket of DRD2 and therefore should contain a linker of 5 or 6 methylene groups long.

Keywords: arylpiperazines; molecular dynamics; molecular docking; receptor binding site.

INTRODUCTION

Dopamine receptors belong to the rhodopsin-like, aminergic G protein-coupled receptors (GPCRs) group. They are involved in many physiological processes and play important role in the central nervous system (CNS).[1–4]

Targeting the dopamine D2 receptors (DRD2) is a common strategy for the treatment of neurodegenerative diseases, such as schizophrenia, Parkinson's disease, dementia and depression.[5–8]

It is a well-documented fact that *N*-substituted arylpiperazines are compounds with pronounced DRD2 activity.[9,10] Since arylpiperazines have a wide

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spectrum of therapeutic potentials and the design, synthesis and characterization of new arylpiperazine like drugs is an ever growing field of research.[11–14]

In this paper, the synthesis of 14 new *N*-(2-methoxyphenyl)piperazines of the general structure **5** (Scheme 1) is presented. Their affinities towards DRD2 were evaluated in the [^{3}H]spiperone competition assay.

Recent discovery of DRD2 crystal structure with bound risperidone[15] defined the receptor binding site with greater accuracy than existing homology models. This finding prompted us to investigate DRD2–arylpiperazine binding features, using molecular docking analysis and molecular dynamics simulations in order to define key receptor–ligand interactions and explain the dopaminergic properties of the herein described compounds.

EXPERIMENTAL

The reagents and solvents used in this work were obtained from Alfa–Aesar or Sigma–Aldrich and used without further purification. Solvents were routinely dried over anhydrous Na_2SO_4 prior to evaporation.

General

A Boetius PHMK apparatus (VEB Analytic, Dresden, Germany) was used to determine the melting points, which are here presented uncorrected. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 200 and 50 MHz, respectively, on a Gemini 2000 (Varian, Oxford). The spectra were recorded in deuterochloroform with tetramethylsilane as the internal standard; the chemical shifts (δ) are reported in parts per million (ppm); all coupling constants (J values) are reported in Hz. LC/MS was performed on a 6210 time-of-flight LC–MS system (Agilent Technologies, Germany). For data analysis, MassHunter workstation software was used. The infrared (IR) spectra were obtained on a Thermo Scientific spectrometer. For analytical thin-layer chromatography (TLC), Polygram SIL G/UV$_{254}$ plastic-backed thin layer silica gel plates were used (Macherey–Nagel, Germany). The chromatographic purifications were performed on Merck-60 silica gel columns (230–400 mesh ASTM) under medium pressure (dry column flash chromatography). Analytical and spectral data for the synthesized compounds are given in Supplementary material to this paper. A MicroSYNTH Milestone and a Biotage Initiator 2.5 EXP were used for the microwave experiments.

Chemistry

*General procedure for the synthesis of compounds **3a**–**g***. A suspension of 1-(2-methoxyphenyl)piperazine (**1**, 0.084 mol), triethylamine (0.0874 mol), K_2CO_3 (0.175 mol) and bromoester **2a**–**g** (0.084 mol) in 2-butanone (100 mL) was stirred for 24 h at 80 °C. After cooling, the mixture was poured into cold water and the organic layer was extracted with CH_2Cl_2 and concentrated *in vacuo*. The resulting ester was purified by silica gel column chromatography using a gradient of methanol (0–5 %) in dichloromethane.

*General procedure for the synthesis of compounds **5a**–**n***. Compounds **3a**–**g** (0.0035 mol) and diamines **4a**–**c** (0.0035 mol) were suspended in 8 mL 50 % methanesulfonic acid in water, transferred into a sealed tube, and microwave irradiated at 180 °C for 45 min at 300 W. After cooling to room temperature, the reaction mixture was poured into ice-cold water and neutralized with a saturated solution of NaOH. The product was extracted with CH_2Cl_2 and concentrated *in vacuo.* The resulting 1*H*-benzimidazoles were purified by silica gel column chromatography using a gradient of methanol (0–5 %) in dichloromethane.

Biological assays

Membrane preparation. Rat caudate nuclei synaptosomal membranes for the DRD2 binding experiments were prepared as previously described.[16] Striatal tissue acquired from male Wistar rats (150–200 g) was used as the source of DRD2. The tissue was homogenized in 20 volumes of ice-cold 50 mM Tris-HCl buffer containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 2 mM $CaCl_2$ using a Potter–Elvehjem homogenizer (6×800 rpm). The membrane fraction obtained after centrifugation at 20000 rpm for 15 min was used in the binding experiments.

[^{3}H]Spiperone receptor binding assay. [^{3}H]Spiperone (73.36 Ci mmol^{-1}, Perkin Elmer LAS GmbH, Rodgau, Germany) binding was assayed in 1.0 mM EDTA, 4 mM $MgCl_2$, 1.5 mM $CaCl_2$, 5 mM KCl, 120 mM NaCl, 25 mM Tris-HCl solution, pH 7.4, with rat caudate nuclei synaptosomal membranes (protein concentration 0.6 mg mL^{-1}), at 37 °C for 10 min in a total volume of incubation mixture of 0.4 mL. The binding of the radioligand to 5-HT_2 receptors was prevented by 50 mM ketanserin. The K_i values of the tested compounds were determined by competition binding at 0.2 nM of the radioligand and eight different concentrations of each compound (10^{-4}–10^{-10} M). Nonspecific binding was determined in the presence of 10 μM spiperone. The reaction was terminated by rapid filtration through Whatman GF/C filters, washed three times with 5.0 mL of ice-cold incubation buffer, and the retained radioactivity was measured in a 1219 Rackbeta Wallac scintillation counter (EG&G Wallac, Turku, Finland). Inhibition curve construction and statistical (Student's *t*-test) analysis were performed by Graph-Pad Prism (GraphPad Software Inc). Hill slope coefficients were fixed to unity during the calculations.

Computational study

Docking simulations. The docking procedure was performed using Forecaster software.[17] The receptor model PDB code 6CM4[18] was used together with 2D structures of the ligands, prepared in ChemDraw.[19] All structures were prepared in the software using default procedures. Rigid receptor, flexible ligand docking was carried out. The obtained docking structures were examined and structures with the maximum number of receptor–ligand interactions were selected for further analysis.

Binding poses metadynamics. The docking pose quality was assessed in terms of the fluctuations of the ligand *RMSD* (the root-mean-square deviation of atomic positions), and the persistence of important contacts between the ligand and the receptor (Metadynamics Binding PoseScore and Metadynamics Binding Persistence) using Desmond software and default parameters.[20] One docking pose with the lowest RMSD and best overall score was selected for molecular dynamics (MD) simulations.

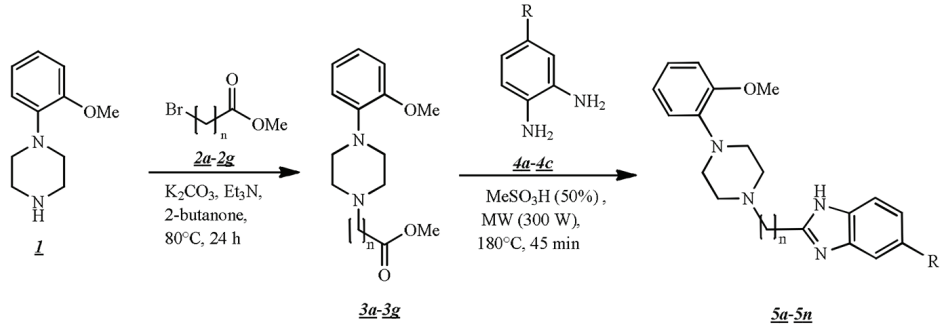
Construction of a protein–membrane system for molecular dynamics. The protein protonation state was adjusted using the Schrodinger Protein Preparation module, at physiological pH (pH 7.4). The prepared protein was embedded into a POPC membrane bilayer using the Desmond system builder module,[20] and oriented according to data from the Orientations of Proteins in Membranes (OPM) server.[21] The embedded protein was solvated with TIP3P explicit water model, and the system was neutralized *via* counter ions and a salt solution of 0.15 M KCl. In this way, systems were obtained that were subjected to membrane relaxation protocol.[20]

MD simulations. Molecular dynamics (MD) simulations of the system were performed using Schrodinger Desmond software packages.[20] OPLS 2003 forcefield[22] was used to calculate the interactions between all the atoms. For the calculation of long-range coulombic interactions, the particle–mesh Ewald (PME) method was used, with a cut-off radius of 9 Å for short-range van der Waals (vdW) and electrostatic interactions.

During the course of the simulation, a constant temperature of 310 K and a pressure of 1.01235 bar were maintained, using a Nose–Hoover thermostat,[23] and the Martyna–Tobias––Klein method.[24] Time increments of 2.0 fs were used in the simulations. Finally, 100 ns MD simulation for the each ligand– DRD2 complex was performed and the collected trajectory frames used in the MD analysis to quantify the protein–ligand interactions.

RESULTS AND DISCUSSION

Compounds **5a**–**n** were synthesized according to Scheme 1. The synthesis started with *N*-(2-methoxyphenyl)piperazine (**1**) that was alkylated with a series of homologous bromo-esters **2a**–**g**, providing *N*-alkylated products **3a**–**g**. Counterpart diamines **4a**–**c** were obtained by reduction of the corresponding 2-nitro precursors, using Raney-Ni and hydrazine hydrate under conditions described in earlier publications.[25,26] Microwave assisted condensation of piperazines **3a**–**g** and diamines **4a**–**c**, under forcing, strongly acidic conditions, secured the desired benzimidazoles **5a**–**n**.



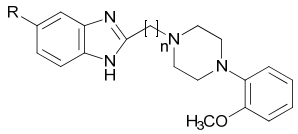
Scheme 1. Synthesis of the compounds **5a**–**n** n = 1–7 for compounds **2a**–**g** and **3a**–**g**; ethyl esters of the general structure **2** were used in the synthesis of **3b**, **3c**, **3e** and **3f**; **4a** (R = H); **4b** (R= OMe); **4c** (R= Cl); structures **5a**–**n** are presented in Table I.

DRD2 binding affinities of compounds **5a–5n** were evaluated *in vitro* using [^{3}H]spiperone as a standard dopaminergic radioactive ligand (Table I).[27]

Molecular docking simulation of the herein described 2-{[4-(2-methoxyphenyl)piperazin-1-yl]alkyl}-1*H*-benzo[d]imidazoles on D2DR was performed on the D2DR crystal structure published recently by Wang *et al.*[15] They reported that the benzisoxazole moiety of risperidone interact with D2DR through Cys118$^{3.36}$, Thr119$^{3.37}$, Ser197$^{5.46}$, Phe198$^{5.47}$, Phe382$^{6.44}$, Phe390$^{6.52}$ and Trp386$^{6.48}$ in the orthosteric binding site (OBS). OBS of D2DR is defined by the amino acid side chains of helices III, V and VI and also harbour Asp114$^{3.32}$. Asp114$^{3.32}$ forms an essential salt-bridge with protonated piperidine nitrogen of risperidone molecule. In addition D2DR has a secondary binding pocket, extended binding pocket (EBP), that encloses the tetrahydropyridopyrimidinone

moiety of risperidone. EBP is bordered by the extracellular part of TM VII consisting of an extracellular loop 1 (EL1) and the junction of helices I, II and VII.[15]

TABLE I. Chemical structures and DRD2 binding constants of 2-{[4-(2-methoxyphenyl)piperazin-1-yl]alkyl}-1*H*-benzimidazoles (**5a**–**n**); DRD2 binding constants (K_i) were determined in a [^{3}H]spiperone displacement assay. The values are the mean of three independent experiments realized in triplicate, performed at eight competing ligand concentrations



Ligand	*n*	R	K_i ± SEM /nM
5a	1	H	>1000
5b	2	H	>1000
5c	3	H	>1000
5d	4	H	>1000
5e	5	H	24±1
5f	6	H	16±2
5g	7	H	>1000
5h	4	OCH_3	124±5
5i	5	OCH_3	12±3
5j	6	OCH_3	76±8
5k	7	OCH_3	>1000
5l	4	Cl	109±9
5m	5	Cl	25±3
5n	6	Cl	102±3

Molecular docking simulations on the binding of 2-{[4-(2-methoxyphenyl)-piperazin-1-yl]alkyl}-1*H*-benzimidazoles into the crystal structure of DRD2 show that the (2-methoxyphenyl)piperazine moiety occupies DRD2 OBS, and interacts with Asp114$^{3.32}$, Cys118$^{3.36}$, Trp386$^{6.48}$ and Phe390$^{6.52}$, while the benzimidazole part interacts with Leu94$^{2.64}$, Ile184^{EL2},Trp100^{EL1}, Phe389$^{6.51}$, Thr412$^{7.39}$ and Tyr408$^{7.35}$ in the EBP (Fig. 1).

Compounds with optimal linker length (five or six methylene groups in the linker) allow the benzimidazole moiety to reach EBP and to interact with Leu94$^{2.64}$, Trp100^{EL1}, Phe389$^{6.51}$, Thr412$^{7.39}$ and Tyr408$^{7.35}$ (Fig. 2). Compounds with shorter linker (**5a**–**d**) do not reach into the EBP, while ligands with seven methylene groups in the linker (**5g** and **5k**) are too long to fit optimally into the D2DR binding cleft and protrude into the extracellular space.

These results are in agreement with experimental data: compound **5d** (with a 4 methylene groups linker) has affinity of over 1000 nM, while compounds **5e** and **5f** (with 5 and 6 methylene groups linker, respectively) have 24 and 16 nM, respectively. Compound **5g** shows a sharp drop in affinity because of the length of the linker, which cannot be accommodated in the DRD2 bind cleft.

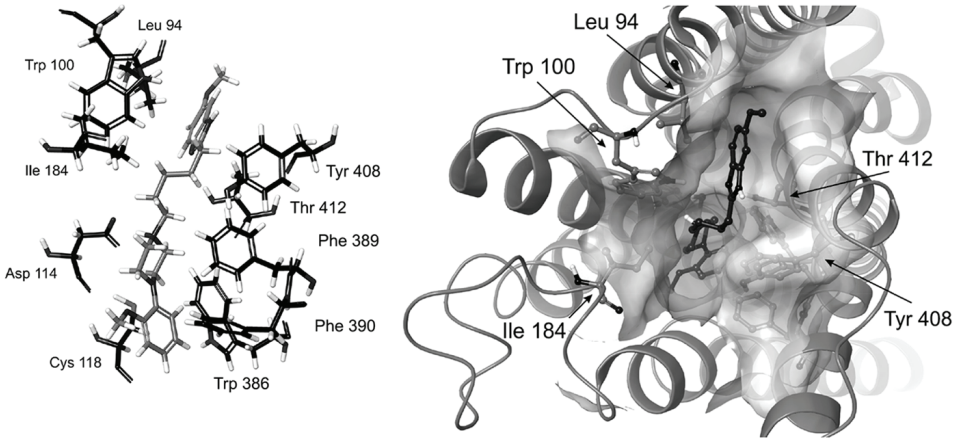


Fig. 1. Docking of ligand **5i** to DRD2 is presented. View of the interactions between the 3D model of the DRD2 binding site and ligand **5i**. The images show only the key amino acid residues of the receptor binding pocket. Figures (side view–left and top view–right) show docking of **5i** viewed from different angles. Binding site ligand accessible surface is shown in the top view.

In series of compounds substituted with methoxy and chloro groups, the highest DRD2 affinity was obtained with compounds **5i** and **5m.** Linker with 5 methylene groups facilitates optimal positioning of substituted benzimidazole part in the receptor EBP (Fig. 1). Shorter linkers, as it is obvious in series **5h**–**k** and **5l**–**n**, lead to decrease in receptor affinity due to sub-optimal placement of benzimidazole part in regard to the interacting residues Trp100^{EL1} and Tyr408$^{7.35}$.

To test the stability of obtained docking poses, MD simulations of the DRD2 and selected ligands were performed on inactive receptor state for 100 ns for each ligand. Obtained trajectories were analyzed with focus on the residues that form OBS and EBP (Table S-I of the Supplementary material).

Most of the receptor–ligand interactions in OBS, observed in molecular docking simulations, persisted for a significant portion of MD run (>20 % total simulation time). Compounds with significant DRD2 affinity (**5e**–**f**, **5h**–**j** and **5l**–**n**) had a salt bridge between the protonated piperazine nitrogen of the ligand and Asp114$^{3.32}$ of DRD2 preserved for more than 79–84 % of the simulation time. Additional interactions in OBS are aromatic interaction with Cys118$^{3.36}$ (32–75 % of the simulation time), and edge-to-face interactions with Trp386$^{6.48}$ (76–98 % of the simulation time) and Phe390$^{6.52}$ (20–49 % of the simulation time). In the EBP, significant interactions are aromatic interactions (edge-to-face type) with Trp100^{EL1}, Phe389$^{6.51}$ and Tyr408$^{7.35}$. Compounds **5e**, **5f**, **5i** and **5m** form an additional hydrogen bond with Thr412$^{7.39}$.

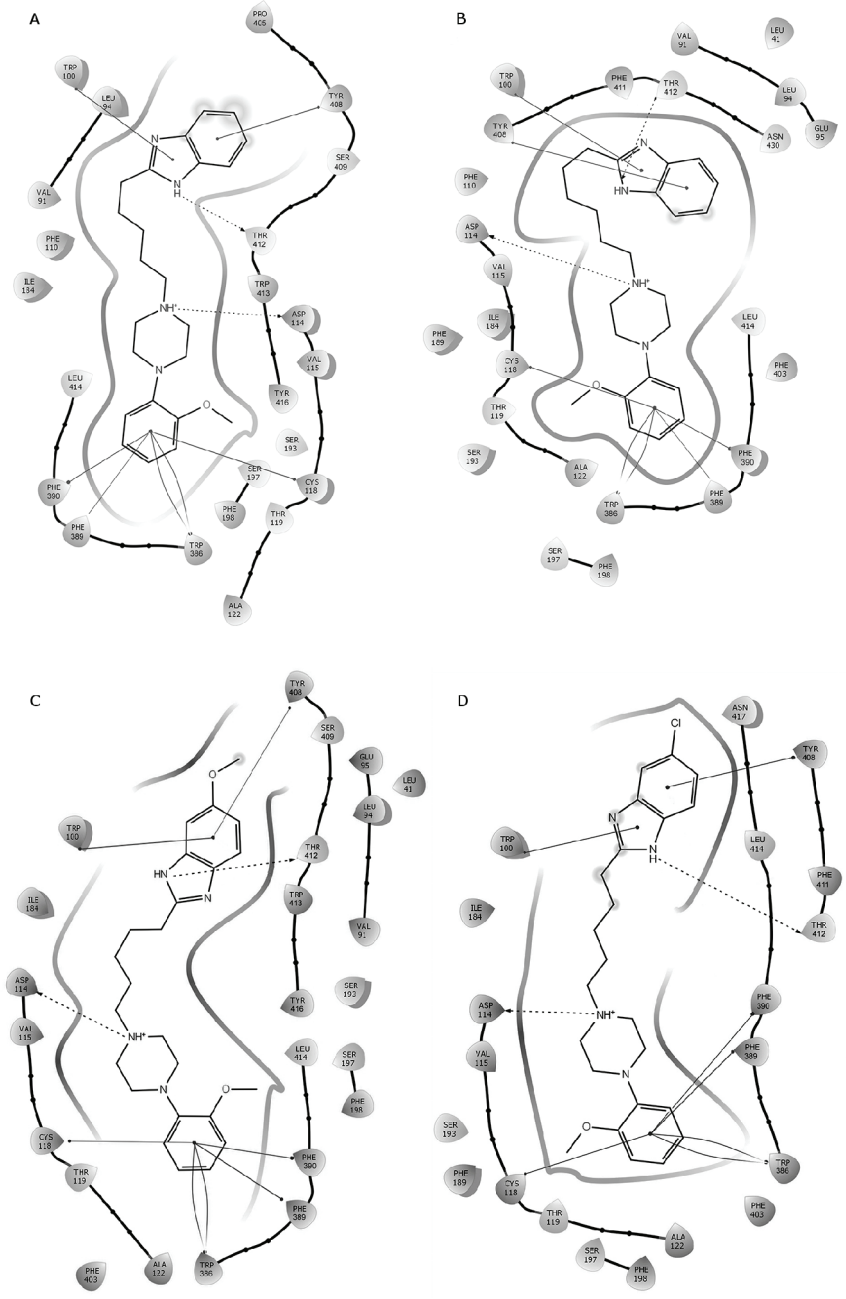


Fig. 2. Results of docking simulations for ligand **5e** (A), **5f** (B), **5i** (C) and **5m** (D) are presented. Schematic representation of the best docking pose for all ligands are provided. For clarity, only amino acid residues in close contact with ligands are shown. Solid lines represent aromatic, while dotted lines represent electrostatic interactions.

CONCLUSIONS

Molecular docking and MD simulation provide important information that explains how the receptor–ligand complexes are formed. High affinity 2-{[4-(2--methoxyphenyl)piperazin-1-yl]alkyl}-1*H*-benzimidazoles must simultaneously occupy both OBS and EBP.

To establish key interactions both in OBS (salt bridge formation and aromatic interactions) and EBP (aromatic interactions and hydrogen bond formation), the ligands should have a linker of five or six methylene groups. Linker flexibility and substituent size in the benzimidazole moiety determine ligand positioning inside the EBP and brings it in close contact with Trp100^{EL1} and Tyr408$^{7.35}$, which are key interacting residues. Additionally, as can be concluded from the results of molecular dynamics, the affinity of the arylpiperazine ligands benefit greatly from possible formation of interactions of the arylpiperazine part of ligands with Thr412$^{7.39}$ in EBP.

It is clear that both Trp100^{EL1} and Tyr408$^{7.35}$ can form aromatic interactions and/or hydrogen bonds. To establish the exact nature of interactions in EBP, modification of presented ligands, in terms of target synthesis of the compounds which can strictly form only one of these interactions, represent a guideline for further investigation.

SUPPLEMENTARY MATERIAL

Analytical and spectral data for the synthesized compounds, as well as additional results, are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА НОВИХ ДЕРИВАТА 2-(ПИПЕРАЗИНО-1-ИЛ-АЛКИЛ)-1*H*-БЕНЗИМИДАЗОЛА И ПРОУЧАВАЊЕ ИНТЕРАКЦИЈА СА Д2 ДОПАМИНСКИМ РЕЦЕПТОРОМ

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У овом раду је презентована синтеза 14 нових арилпиперазина и одређен је њихов афинитет везивања за Д2 допамински рецептор (DRD2) тестовима компетиције са [^{3}H]спипероном. По својој хемијској структури ова једињења представљају супституисане бензимидазоле повезане са *N*-(2-метоксифенил)пиперазинским делом, линкерима различитих дужина. У циљу испитивања лиганд-рецептор интеракција и особина везивног места DRD2, урађена је докинг анализа новосинтетисаних једињења и симулација молекулске динамике, користећи кристалну структуру рецептора. Резултати добијени у овом раду указују да арилпиперазини високог афинитета остварују интеракције у ортостерном везив-

ном месту и у екстензији ортостерног места везивања DRD2 и да стога треба да поседују линкер оптималне дужине, од 5 или 6 метиленских група.

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