STRUCTURAL AND BIOLOGICAL EVALUATION OF NOVEL MULTI-TARGET COMPOUND WITH POTENTIAL APPLICATION IN THE TREATMENT OF SCHIZOPHRENIA

Piotr Stępnicki[1], Katarzyna Targowska-Duda[2], Andrea G. Silva[3], Oliwia Koszła[1], Ewa Kędzierska[4], Angelika Grudzińska[1], Marta Kruk-Słomka[4], Grażyna Biała[4], Marián Castro[3], Agnieszka A. Kaczor[1,5]

[1]Department of Synthesis and Chemical Technology of Pharmaceutical Substances with Computer Modeling Laboratory and [2]Department of Biopharmacy and [4]Department of Pharmacology and Pharmacodynamics, Faculty of Pharmacy, Medical University of Lublin, 4A Chodźki St., PL-20093 Lublin, Poland

[3]Department of Pharmacology, Universidade de Santiago de Compostela, Center for Research in Molecular Medicine and Chronic Diseases (CIMUS), Avda de Barcelona, E-15782 Santiago de Compostela, Spain

*[5]School of Pharmacy, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland; *E-mail piotrstepnicki93@gmail.com*

INTRODUCTION

Due to the fact that currently available antipsychotics are often not sufficiently effective against symptoms of schizophrenia, there is an urgent need to develop new medications. In search for novel potential antipsychotics, structure-based virtual screening was performed in order to identify new antagonists of dopamine D_2 receptor [1]. The compound D2AAK3 (Fig. 1) with affinity to dopamine D_2 receptor was found and then it was subjected to *in vitro* and *in vivo* studies to evaluate it as a potential antipsychotic. D2AAK3 may be considered as an ideal candidate for a multi-target lead structure to develop new drugs, since it exhibits nanomolar or low micromolar affinity also to D_1, D_3, 5-HT_{1A}, 5-HT_{2A} and 5-HT_7 receptors. The compound possesses also some affinity to M_1 and H_1 receptors. Here we present molecular modeling, *in vitro* and *in vivo* studies of D2AAK3 [2].

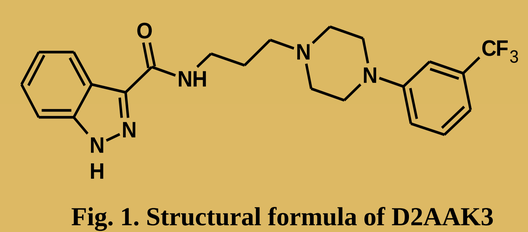


Fig. 1. Structural formula of D2AAK3

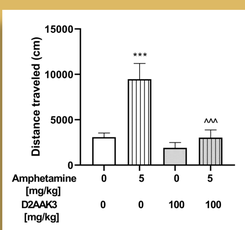


Fig. 2. The influence of an acute injection of D2AAK3 on the amphetamineinduced hyperactivity in mice. Appropriate groups of mice received D2AAK3 [(100 mg/kg; i.p. (n = 7)], amphetamine [5 mg/kg, (n = 8); s.c.], 100 mg/kg D2AAK3 co-injected with 5 mg/kg amphetamine (n = 8), and vehicle (n = 8, indicated as 0). Data are shown as the distance traveled (cm) by mouse recorded for 30 min (mean ± SEM). The results from the Tukey's test analyses presented: ***p < 0.01 amphetamine vs. the vehicle-treated group and ^^^p < 0.001 100 mg/kg D2AAK3 vs. amphetamine-treated group.

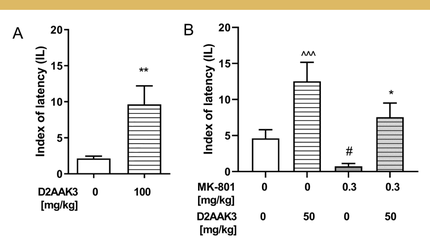


Fig. 3. Acute effect of D2AAK3 on memory consolidation (A) and D2AAK3 effect on MK-801-induced memory impairment (B) in mice assessed in passive avoidance (PA) test.Appropriate mice groups received acute injections of D2AAK3 [50 and 100 mg/kg (n = 8–13), vehicle (n = 9–13, indicated as 0), D2AAK3 (50 mg/kg) + MK-801 (n = 13) and MK-801 alone (n = 12); i.p.] on Day 1 immediately after the PA test. Then, these rodents were retested on Day 2 (i.e., 24 h later) as reported previously [3]. Data are presented as mean ± SEM. For the panel A: the results from the *t*-test analyses indicate: **p = 0.0075 when compared with the control group. For the panel B: the results from the post hoc Newman-Keuls's test indicated: *p < 0.05 for D2AAK3+MK-801 when compared to MK-801-treated group; ^^^p < 0.001 D2AAK3 vs control group and #p < 0.05 for MK-801 vs control group.

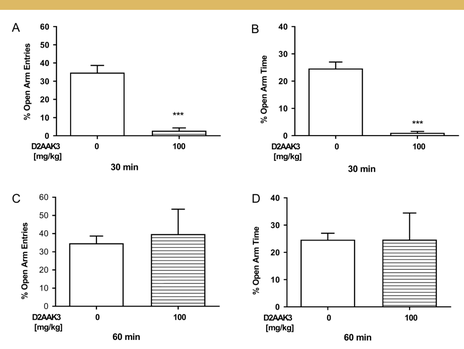
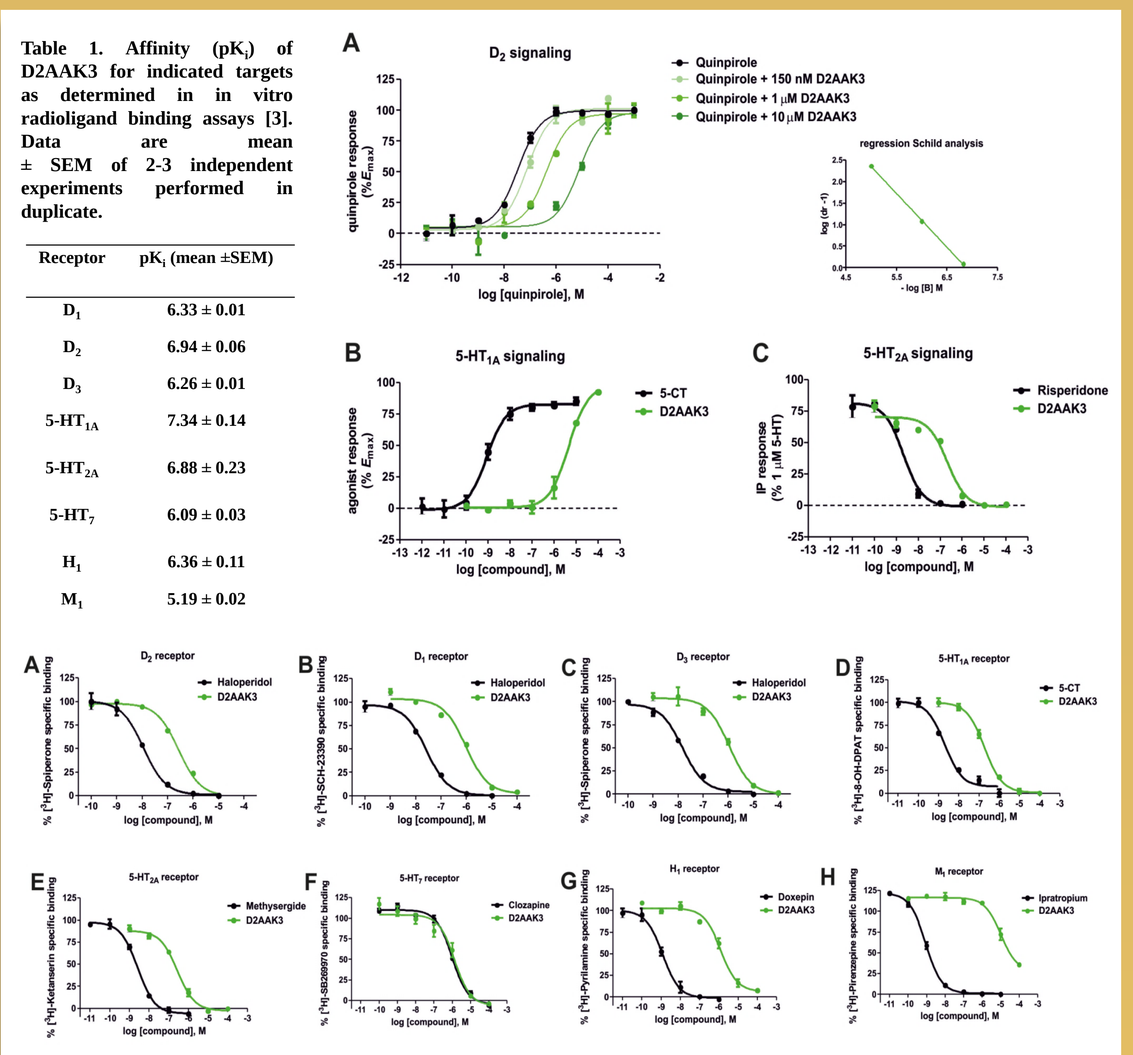


Fig. 4. Acute effect of D2AAK3 on anxiety-like responses in mice evaluated 30 and 60 min after treatment using elevated plus maze (EPM) test. Percentage (%) of open arm entries (A and C) and % of time spent in the open arms (B and D) recorded 30 (A and B) and 60 (C and D) minutes after 100 mg/kg D2AAK3 injection [(n = 8) and vehicle (n = 8, indicated as 0); i.p). Data are presented as mean ± SEM. The *t*-test analyses indicated the anxiogenic activity elicited by D2AAK3 30 min after treatment ***p < 0.001 for time spent in the open arms and open arm entries but not statistically significant after 60 min.

Table 1. Affinity (pK_i) of D2AAK3 for indicated targets as determined in in vitro radioligand binding assays [3]. Data are mean ± SEM of 2-3 independent experiments performed in duplicate.

Receptor	pK_i (mean ±SEM)
D_1	6.33 ± 0.01
D_2	6.94 ± 0.06
D_3	6.26 ± 0.01
5-HT_{1A}	7.34 ± 0.14
5-HT_{2A}	6.88 ± 0.23
5-HT_7	6.09 ± 0.03
H_1	6.36 ± 0.11
M_1	5.19 ± 0.02



METHODS

***In vitro* receptor binding assays. Competition binding experiments were performed in membranes from cells stably expressing the cloned human receptors following previously described procedures. 0.2 nM [^{3}H]-Spiperone (D_2), 1 nM [^{3}H]-Ketanserine (5-HT_{2A}), 2 nM [^{3}H]-8-OH-DPAT (5-HT_{1A}), 0.7 nM [^{3}H]-SCH23390 (D_1) and 1 nM [^{3}H]-Spiperone (D_3), 2 nM [^{3}H]-Pirenzepine (M_1), 2nM [^{3}H]-Pyrilamine (H_1) and 2nM [^{3}H]-SB269970 (5-HT_7) were used as radioligands. Non-specific binding was assessed in the presence of 10 μM sulpiride (D_2), 1 μM methysergide (5-HT_{2A}), 10 μM serotonin (5-HT_{1A}), 1 μM butaclamol (D_1) and 1 μM haloperidol (D_3), 200 μM pirenzepine (M_1), 10 μM triprolidine (H_1) and 25 μM clozapine (5-HT_7).**

***In vitro* functional assays. The efficacy of D2AAK3 at D_2 and 5-HT_{1A} receptors was evaluated in assays of forskolin-stimulated cAMP production in cells stably expressing the receptors, using the homogeneous time-resolved fluorescence (HTRF)-based cAMP dynamic kit (Cisbio, Bioassays, France).**

Molecular modeling studies. Available X-ray structures of studied receptors were downloaded from PDBdb. In case of dopamine D_1 and serotonin 5-HT_{1A} and 5-HT_7 receptor the homology models were used. Homology modeling of the receptors was performed using Modeller v.9.10. D2AAK3 was modeled using LigPrep module of Schrödinger software v. 2019-4. To determine the protonation state, Epik module of Schrödinger software v. 2019-4 was used. For molecular docking of D2AAK3 to the studied receptor models Standard Precision (SP) approach of Glide from Schrödinger v. 2019-4 was applied.

***In vivo* studies. Motor coordination and chimney tests, spontaneous locomotor activity, amphetamine-induced hyperactivity, elevated plus maze (EPM) procedure and passive avoidance test were carried out on naive male Swiss mice (Farm of Laboratory Animals, Warszawa, Poland), 2 months old, weighing 20-30 g.**

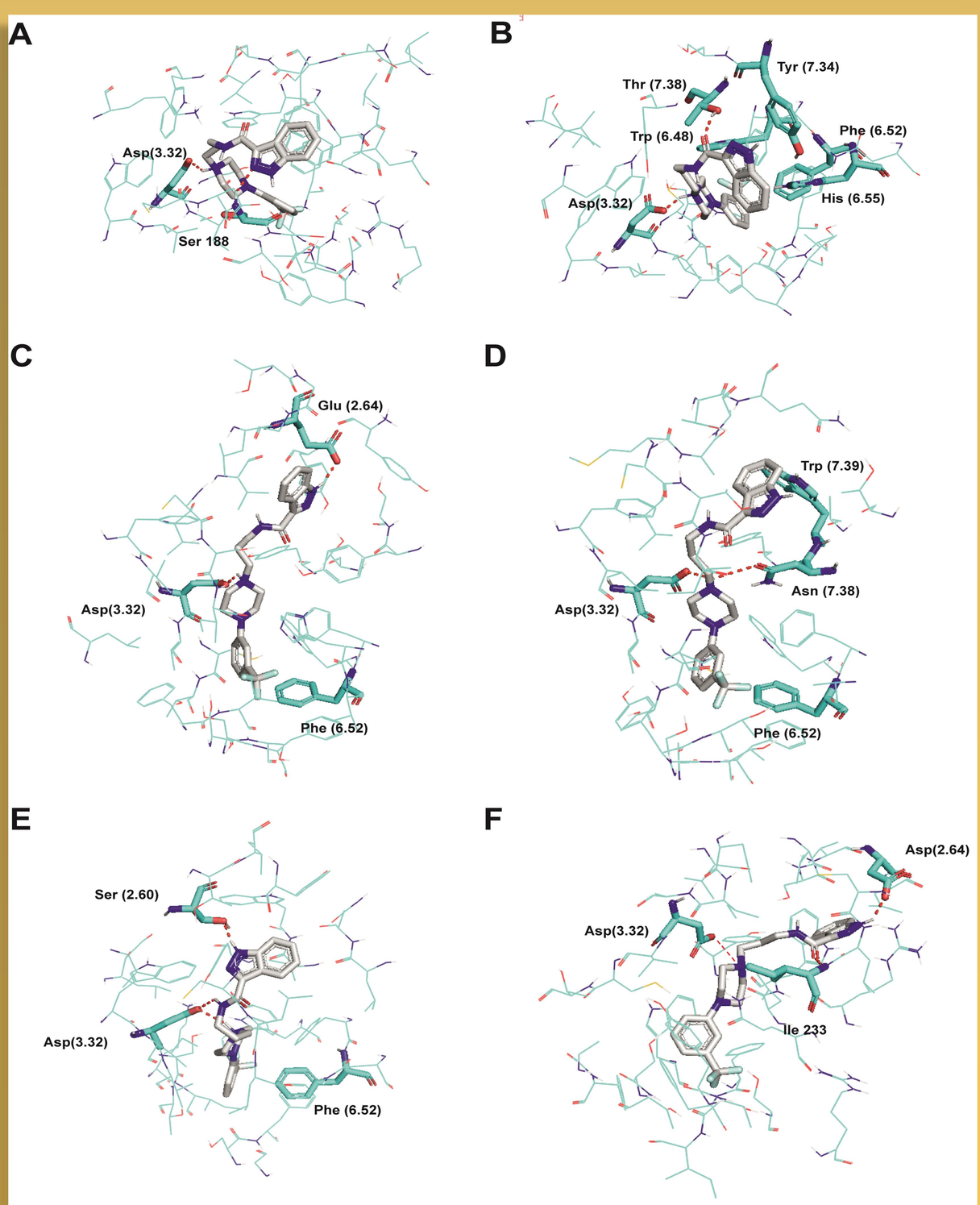


Fig. 5. D2AAK3 in complex with dopamine D_1 (A), D_2 (B), D_3 (C) and serotonin 5-HT_{1A} (D), 5-HT_{2A} (E) and 5-HT_7 receptors (F). Proteins shown in wire representation with cyan carbon atoms. The most important residues shown as sticks. Ligand shown as sticks with grey carbon atoms. Polar interactions represented as red dashed lines. Non-polar hydrogen atoms omitted for clarity.

CONCLUSIONS

Presented *in silico*, *in vitro* and *in vivo* studies of D2AAK3 confirm its multi-receptor profile and good properties for a starting structure for optimization toward developing novel potential antipsychotics.

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