COMPUTER-AIDED DESIGN OF NOVEL TSPO-LIGANDS - POTENTIAL NEUROPSYCHOTROPIC AGENTS

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Problem

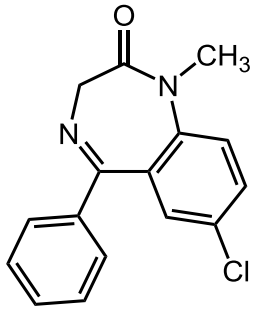
According to WHO data for 2019, the central nervous system diseases were diagnosed in approximately 1 billion people on the planet.

The most common disorders in the population are **anxiety, depressive disorders and neurodegenerative diseases**. The economic losses associated with this type of disease exceed a **trillion dollars** annually.

The **COVID-19** pandemic has made a significant additional contribution to the prevalence and severity of neuropsychiatric diseases. According to preliminary estimates, in **2020** alone, amid the COVID-19 pandemic, the prevalence of anxiety and major depressive disorders increased by **almost 30%.**

Known neuropsychotropic drugs

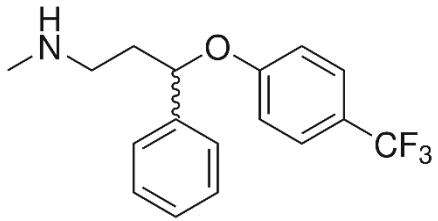
Benzodiazepines – **anxiolytics**



Diazepam

Side effects:
- Sedative
- Muscle relaxant
- Amnestic
- Addictive

Selective serotonin reuptake inhibitors – **antidepressants**



Fluoxetine

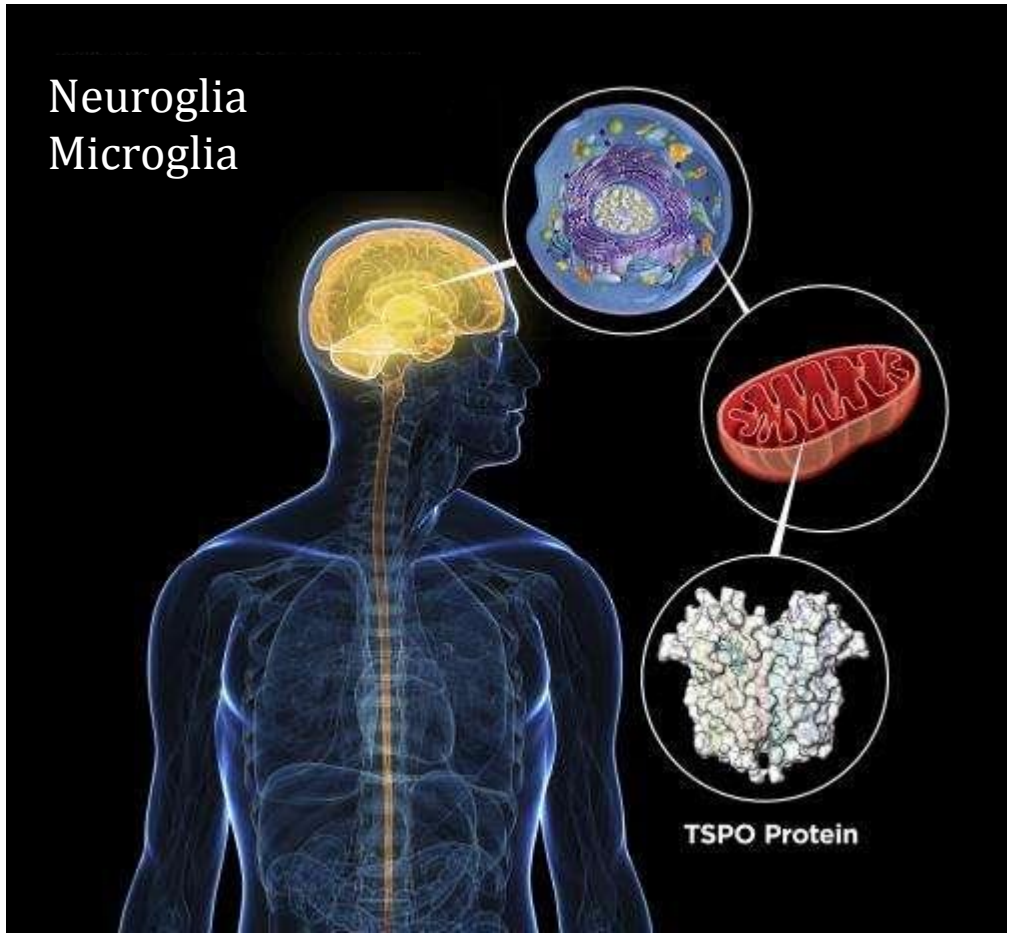
Side effects:
- Convulsions
- Gastrointestinal disorders
- Aggression
- Withdrawal symptoms

The search for neuropsychotropic drugs with new mechanisms of action that meet the following requirements **is actual**:
- Efficiency
- Speed of action
- Safety

Biotarget

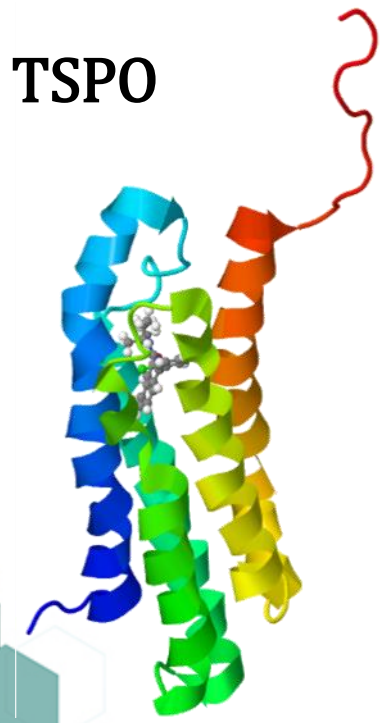
18 kDa translocator protein (TSPO)

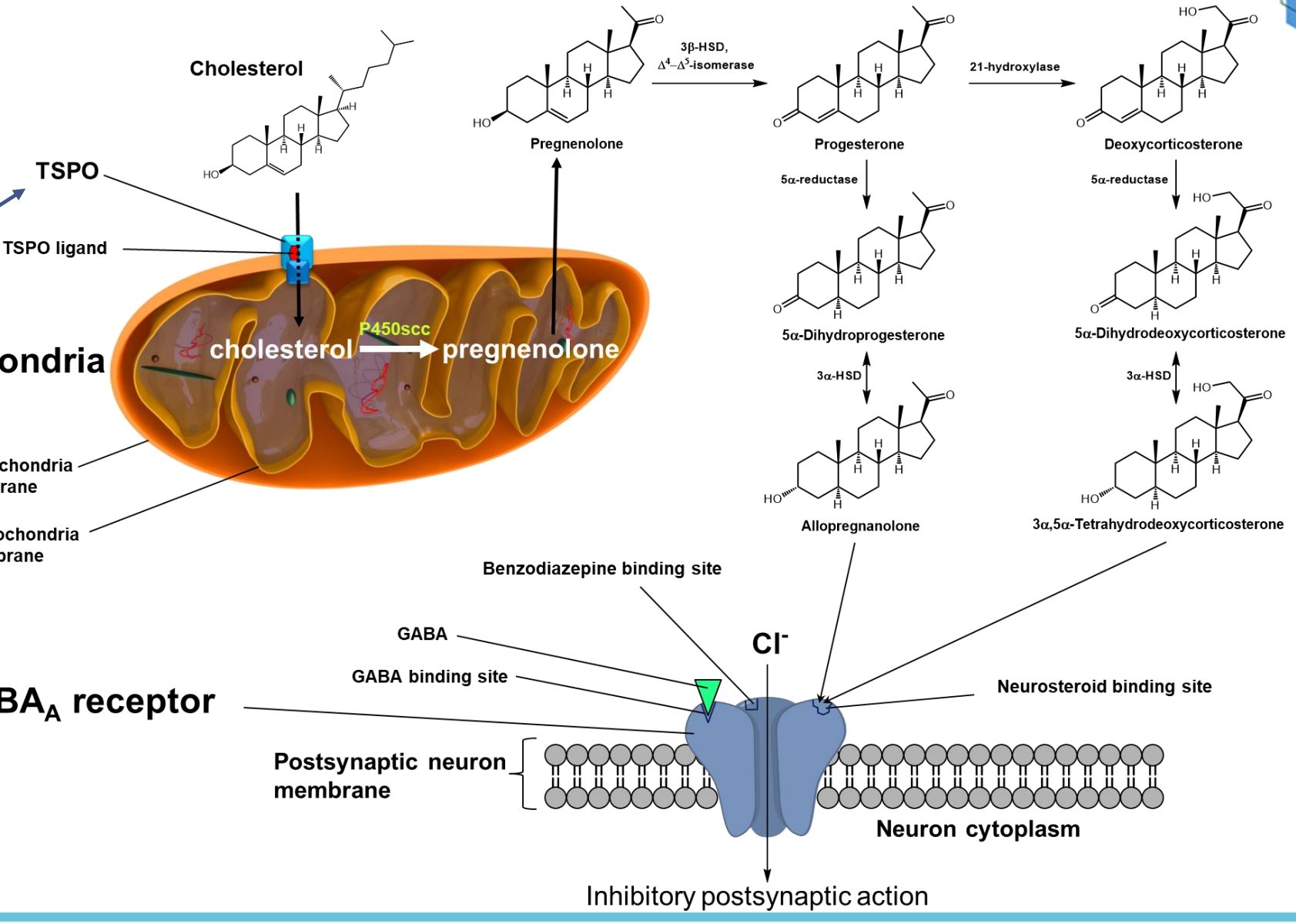
Until 2006, TSPO was known as the peripheral benzodiazepine receptor (PBR), but due to better understanding of its mechanism of action, it was renamed translocator protein. The protein consists of 169 amino acids and 5 helical subunits. Localized on the outer membrane of mitochondria.



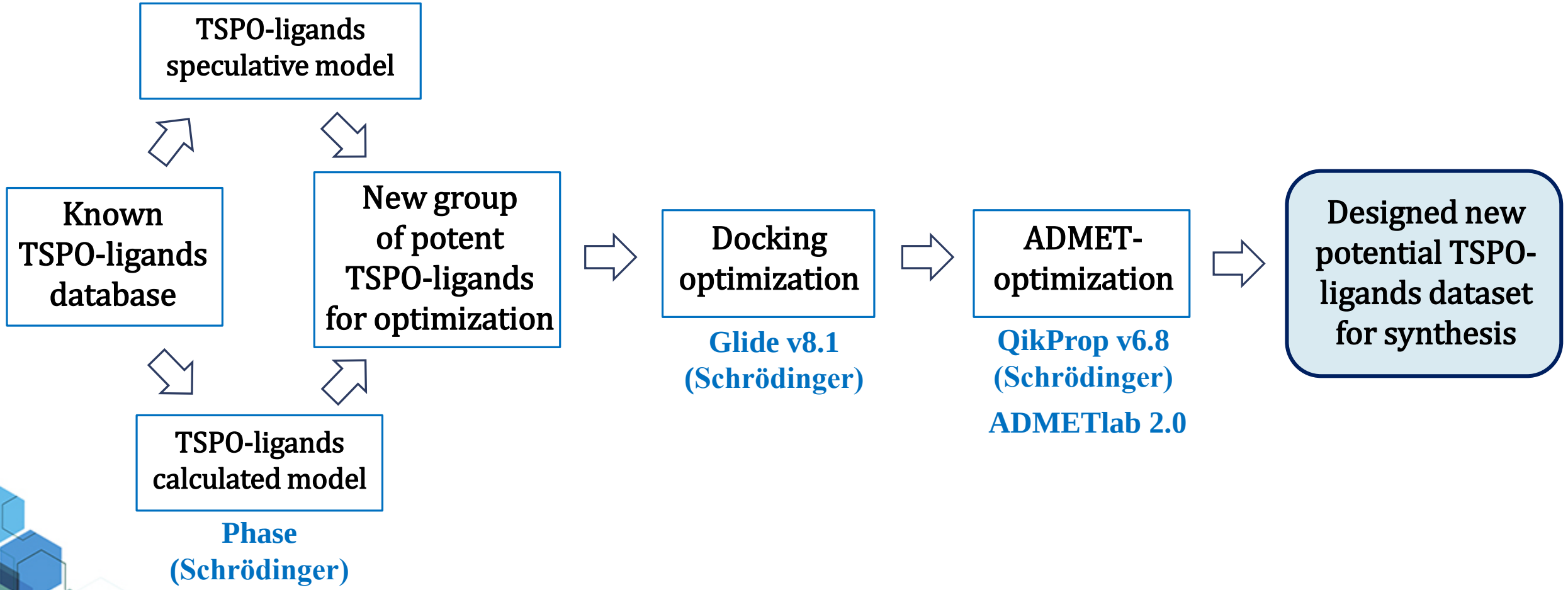
Neurosteroidogenesis

The mechanism of neurosteroidogenesis and the inhibitory effect of neurosteroids upon ligand action on TSPO



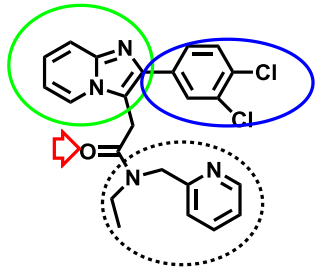


New TSPO ligands design. Strategy

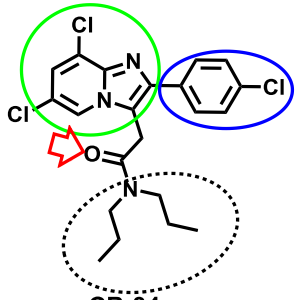


New TSPO ligands design. Stage 1: Pharmacophore design

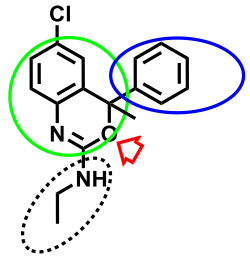
TSPO-ligands database



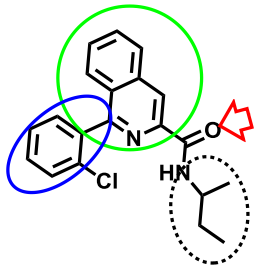
YL-IPA08
IC_{50} = 0.23 nM



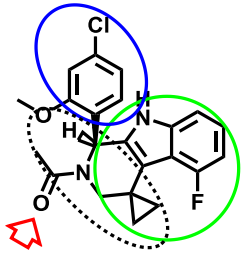
CB-34
IC_{50} = 2.59 nM



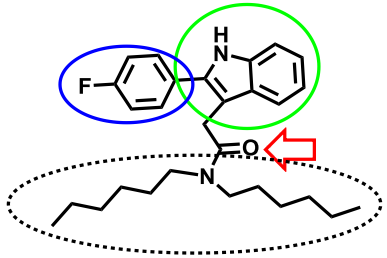
Etifoxine
IC_{50} = 18.3 μM



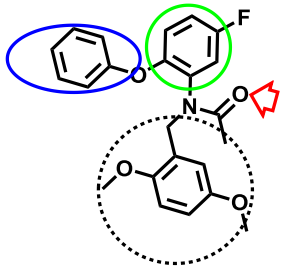
PK-11195
K_i = 9.3 nM



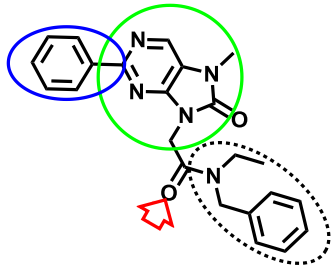
ONO-2952
K_i = 0.33 nM



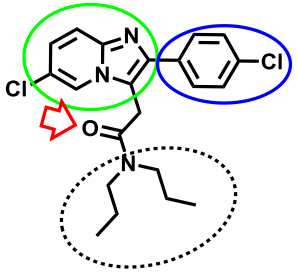
FGIN-1-27
K_i = 7.75 nM



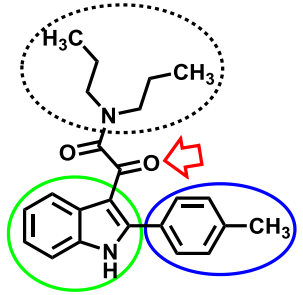
DAA-1106
IC_{50} = 0.28 nM



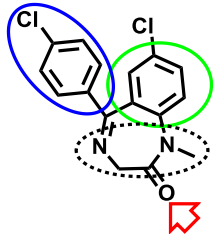
XBD-173
K_i = 0.297 nM



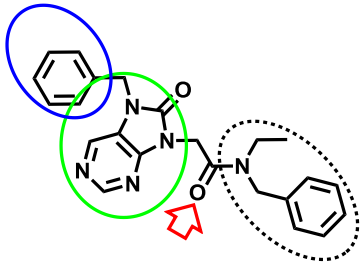
Alpidem
K_i = 0.5-7 nM



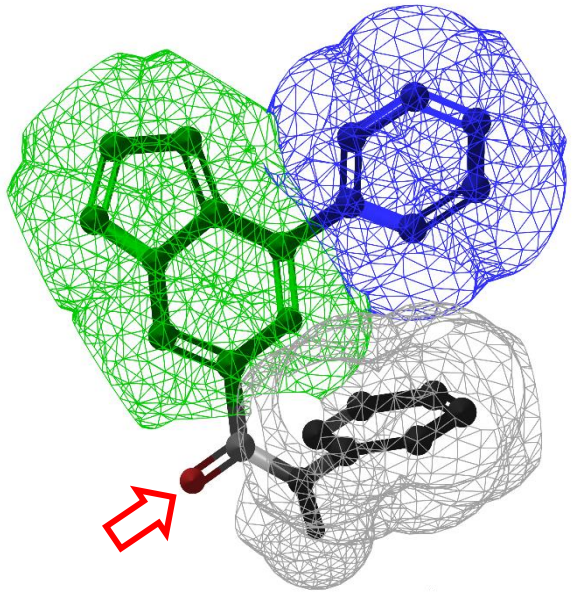
MPIGA
K_i = 5.5 nM



Ro5-4864
K_i = 23 nM



ZBD-2
K_i = 0.463 nM



Flat aromatic heterocycle

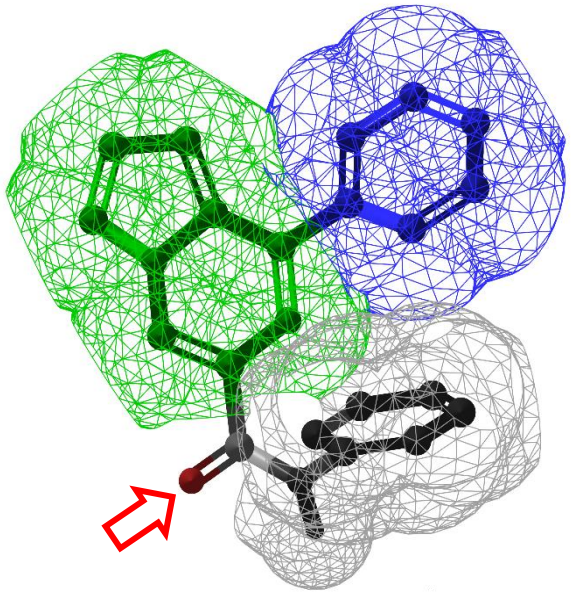
Aromatic substituent

Amide lipophilic substituent

Hydrogen bond acceptor

New TSPO ligands design. Stage 1: Pharmacophore design

TSPO-ligands speculative model



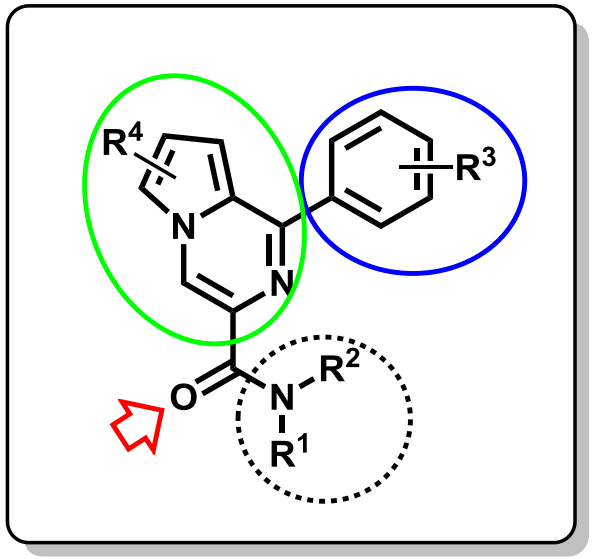
Flat aromatic heterocycle

Aromatic substituent

Amide lipophilic substituent

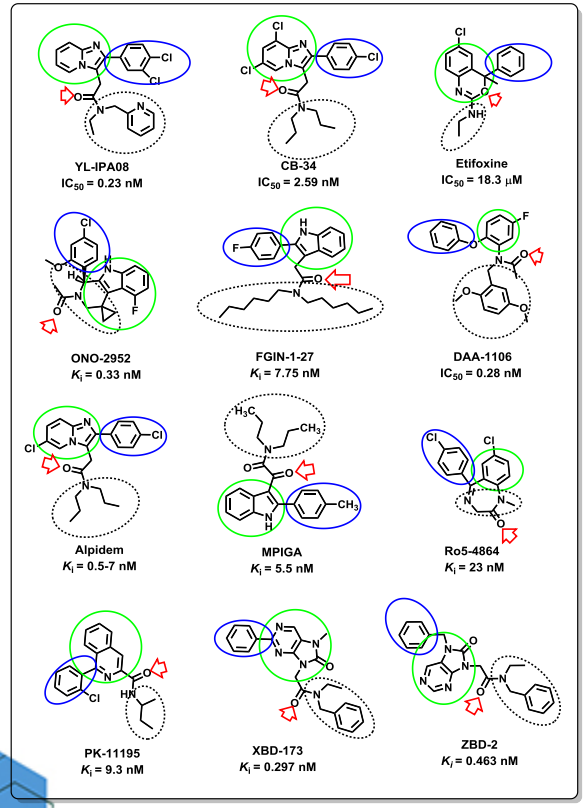
Hydrogen bond acceptor

New pyrrolo[1,2-a]pyrazine group

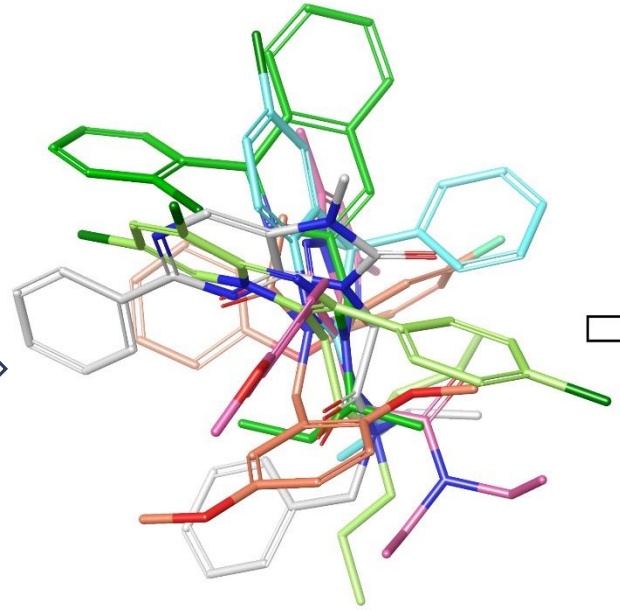


New TSPO ligands design. Stage 2: Pharmacophore calculation

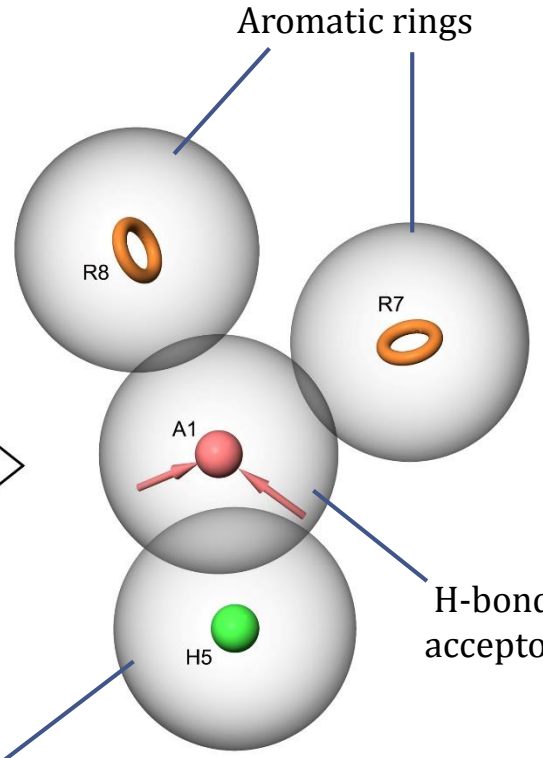
TSPO-ligands Database



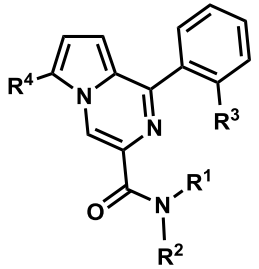
Schrödinger | Phase

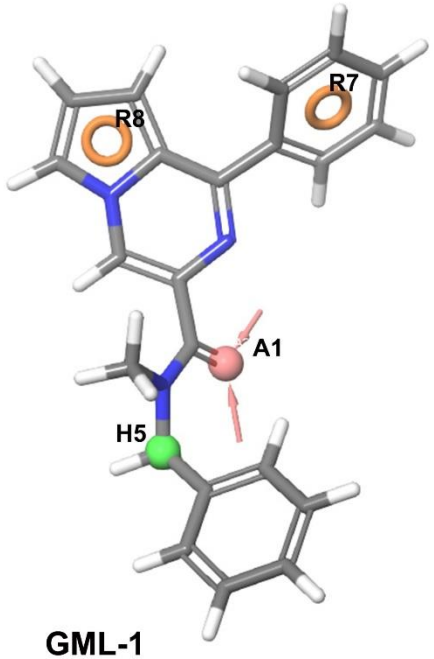


TSPO-ligands



Pharmacophore model

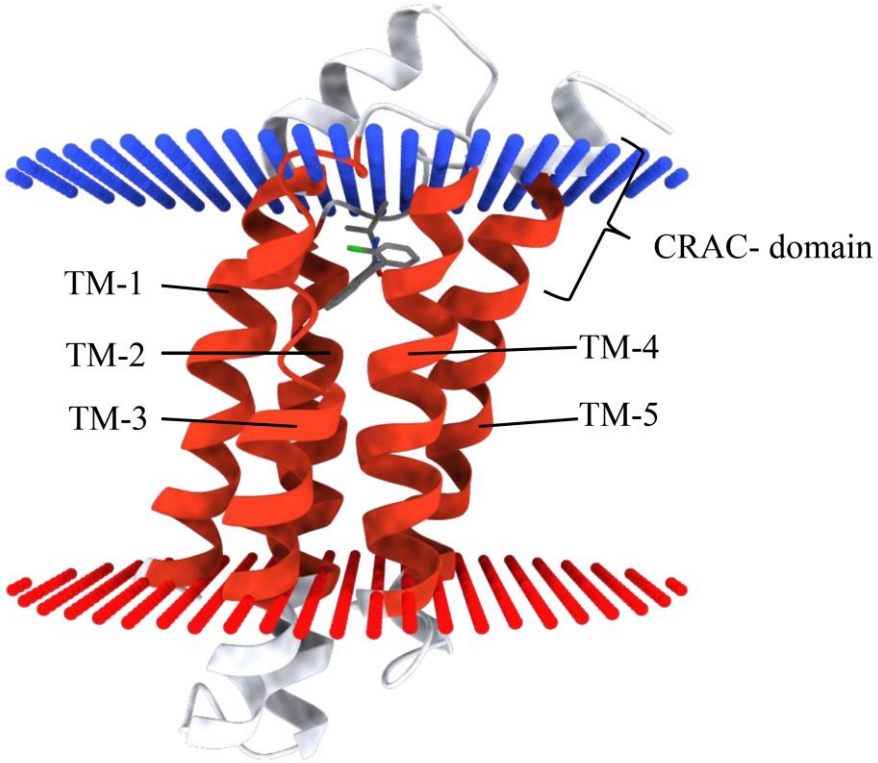




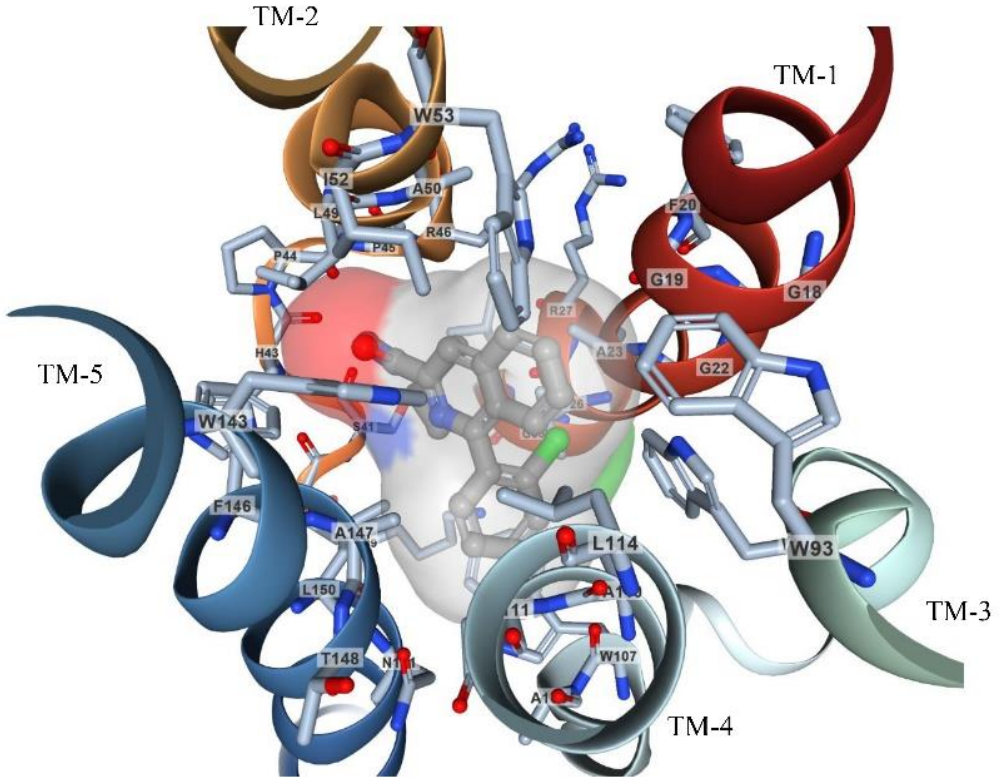
GML-1

Molecules corresponding to the calculated pharmacophore model were selected

New TSPO ligands design. Stage 3: Docking



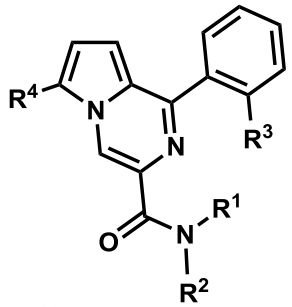
Structure of mTSPO in complex with PK11195 PDB ID: 2MGY



The key amino acid residues of the binding site are:
Ala23, Val26, Leu49, Ala50, Ile52, Trp53 (**TM-2**);
Trp107, Ala110, Leu114 (**TM-4**);
Ala147, Trp143 and Leu150 (**TM-5**)

New TSPO ligands design. Stage 3: Docking

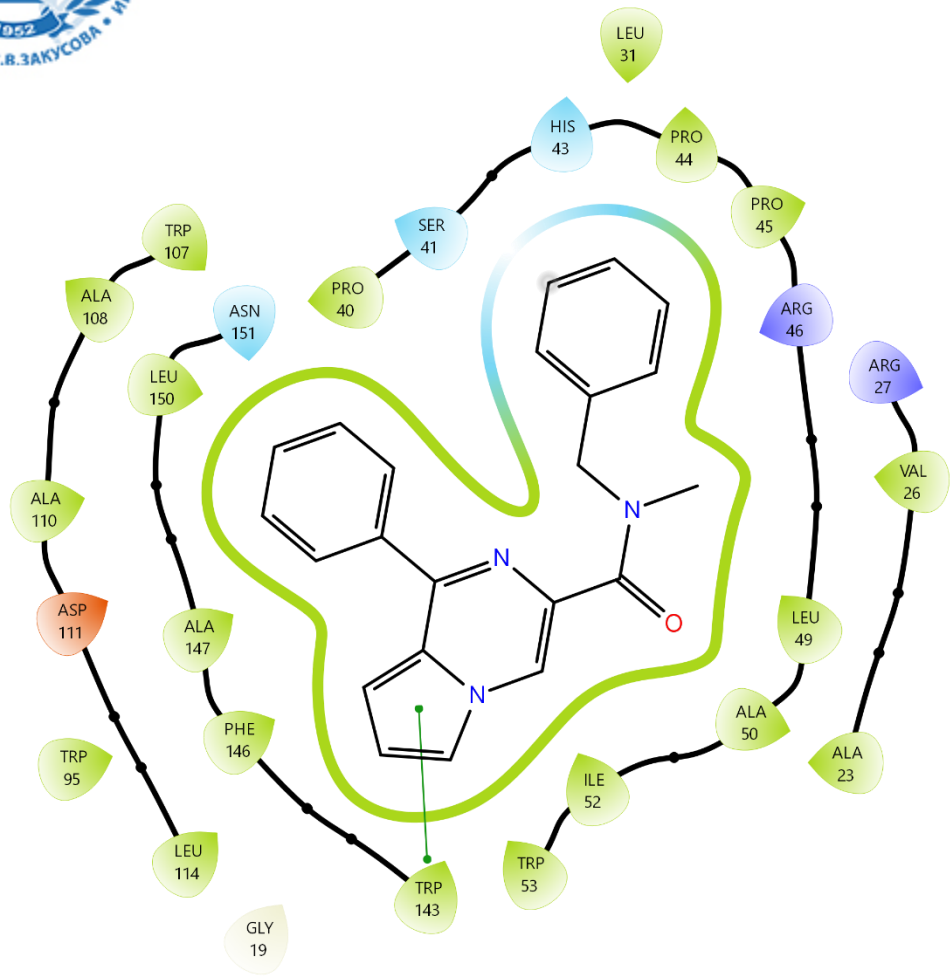
Molecules with a "**Docking Score**" of no more than **-7.0** were selected in docking optimization



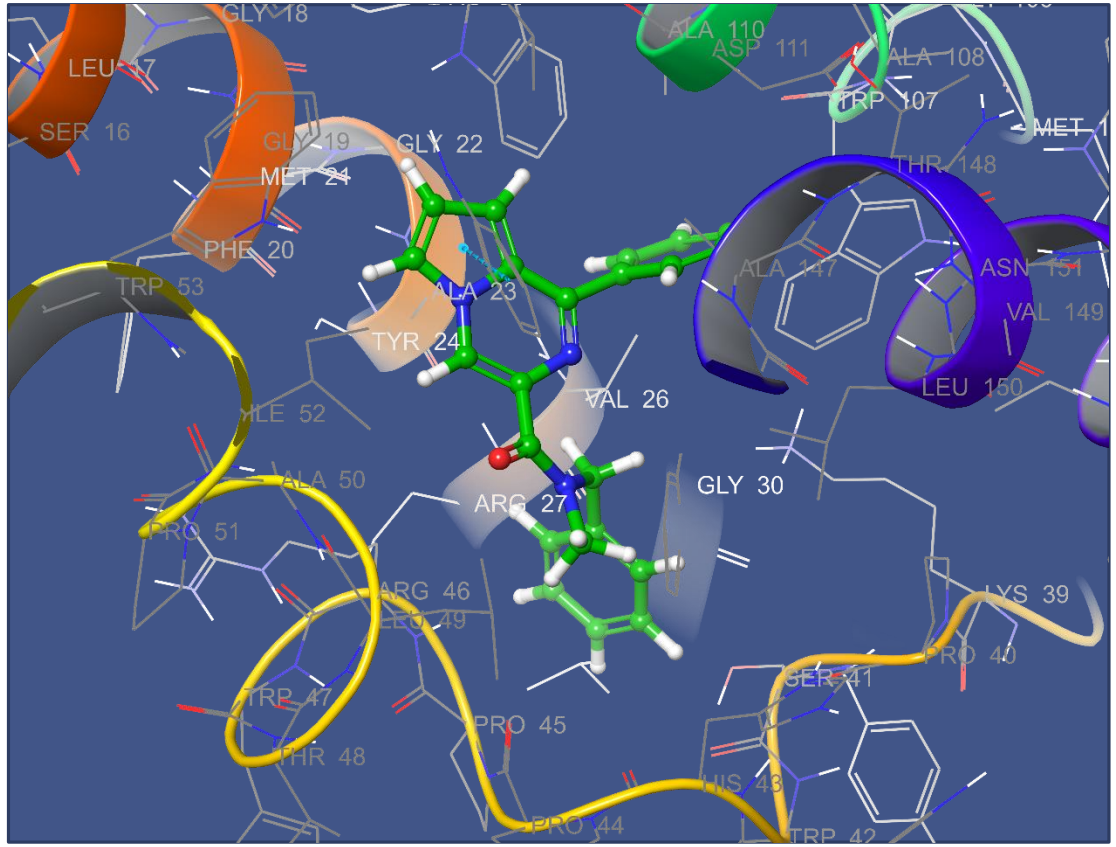
Schrödinger Glide v8.1

Code	R^1	R^2	R^3	R^4	Docking score	π-π stacking with TRP107	π-π stacking with TRP143	Hydrophobic interactions		
								LEY49-TRP53	TRP107-LEY114	SER41-ARG46
GML-1	Bn	Me	H	H	-9.622	-	+	+	+	-
GML-2	n-Bu	Me	Cl	H	-9.016	-	+	+	-	+
GML-3	n-Bu	Me	H	H	-8.282	-	+	+	-	+
GML-4	n-Bu	Me	F	H	-9.057	-	+	+	-	+
GML-5	n-Bu	Me	Br	H	-8.949	-	-	+	+	+
ML-291	i-Bu	Me	H	H	-8.279	+	-	+	+	+
GML-6	sec-Bu	Me	H	H	-8.253	+	-	+	+	+
GML-7	Bn	H	H	H	-9.590	+	+	+	+	-
GML-8	Me	H	H	H	-8.147	-	-	+	+	-
GML-9	Bn	Me	F	H	-10.150	-	+	+	-	-
GML-10	Bn	Me	Cl	H	-10.334	-	+	+	-	-
GML-11	Ph	Bn	H	H	-10.215	+	-	+	+	+
GML-12	n-Pr	n-Pr	H	H	-8.999	+	+	+	+	-
GML-21	Ph	Me	H	H	-7.223	-	+	+	+	-
GML-22	Ph	Et	H	H	-7.105	-	+	+	+	-
GML-23	Ph	n-Bu	H	H	-8.397	-	-	+	-	+
GML-24	Ph	H	H	H	-9.085	+	+	+	-	-
GML-101	Et	Bn	H	H	-10.144	-	-	+	+	+
GML-102	$CHPh_2$	H	H	H	-9.551	-	+	+	+	+
GML-103	Bn	Bn	H	H	-9.278	-	+	+	+	-
GML-104	$(CH_2)_2Ph\ (OMe)_2$-3,4	H	H	H	-9,404	-	+	+	-	+
GML-105	$CH_2Ph\ (OMe)_3$-3,4,5	H	H	H	-9.391	-	+	+	-	+
GML-106	L-Phe-OMe	H	H	H	-10.208	-	+	+	-	-
GML-107	$-(CH_2)_7-$		H	H	-9.193	-	+	+	-	-
GML-108	L-Glu-OEt	H	H	H	-9.125	-	+	+	-	-
GML-109	L-Asp-OEt	H	H	H	-9.603	-	+	+	-	-
GML-110	L-Ala-OMe	H	H	H	-8.726	+	+	+	-	-
GML-111	L-Trp-OEt	H	H	H	-8.083	-	+	+	+	-
GML-112	D-Tyr-OEt	H	H	H	-10.119	-	+	+	-	-
GML-113	L-Phe-OH	H	H	H	-10.095	-	+	+	+	-
GML-114	Me	Bn	H	Br	-8.702	+	+	+	+	-
GML-115	Me	Bn	H	CHO	-9.163	+	+	+	+	+

New TSPO ligands design. Stage 3: Docking

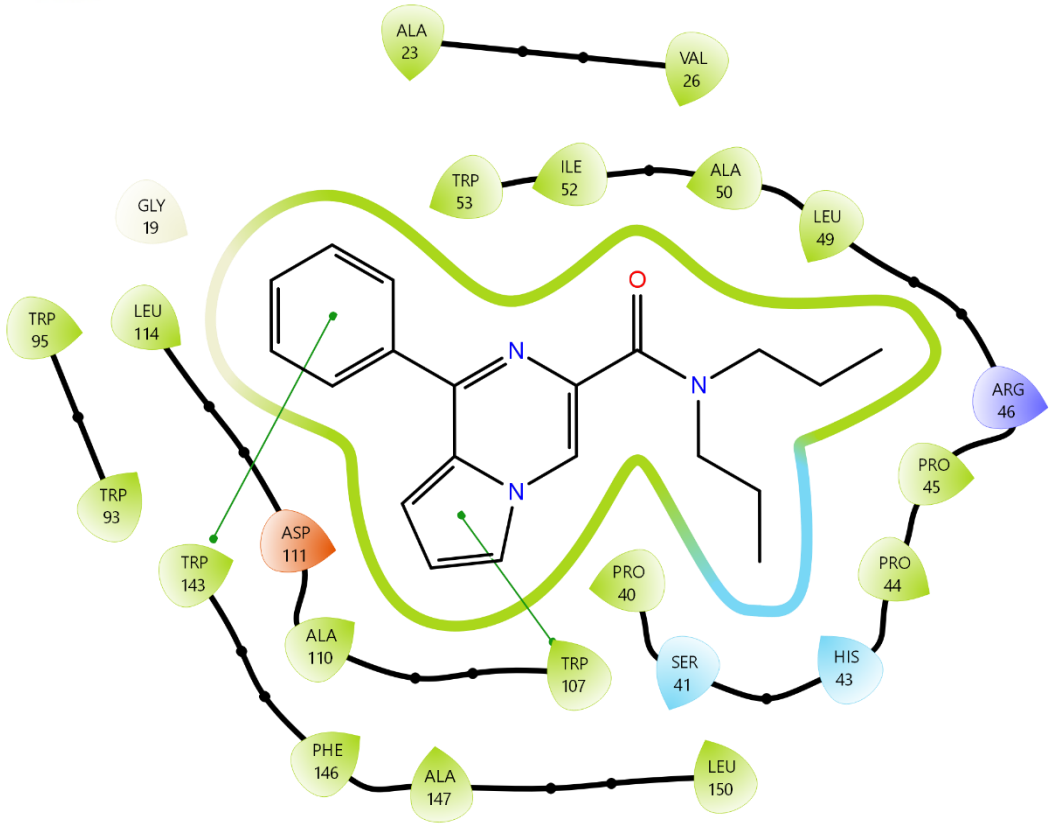


Docking of GML-1 in the TSPO binding site

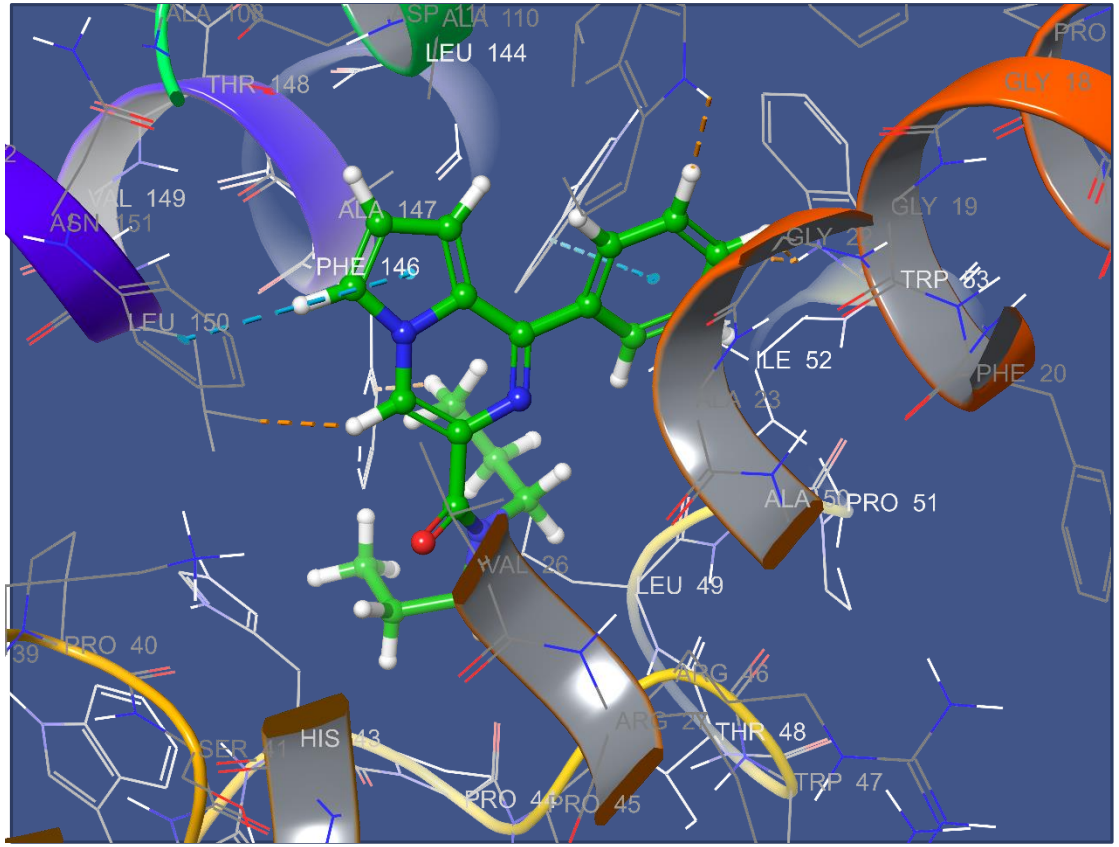


The key ligand-protein interactions:
π-π stacking with TRP143;
hydrophobic interactions with LEY49-TRP53 and TRP143-LEY150

New TSPO ligands design. Stage 3: Docking

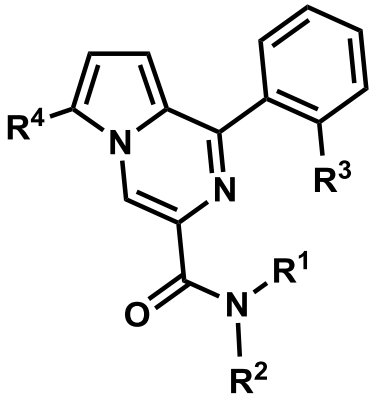


Docking of GML-12 in the TSPO binding site



The key ligand-protein interactions:
π-π stacking with TRP107;
π-π stacking with TRP143;
hydrophobic interactions with TRP107-LEY114 and LEY49-TRP53

New TSPO ligands design.
Stage 4: ADMET-optimization

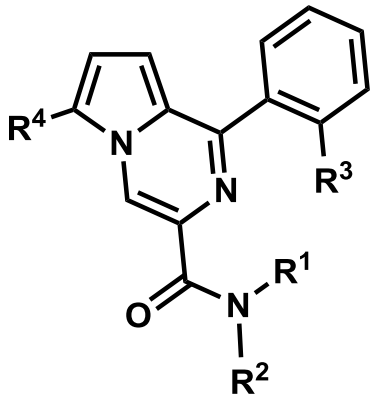


Main analyzed ADMET-parameters:

- Molecular weight
- Dipole moment
- LogP
- LogBB
- Lipinsky "Rule of 5"
- Jorgensen "Rule of 3"
- Oral availability
- Ames test
- Acute toxicity in rats

ADMET: absorption, distribution, metabolism, excretion, toxicity

New TSPO ligands design. Stage 4: ADMET-optimization

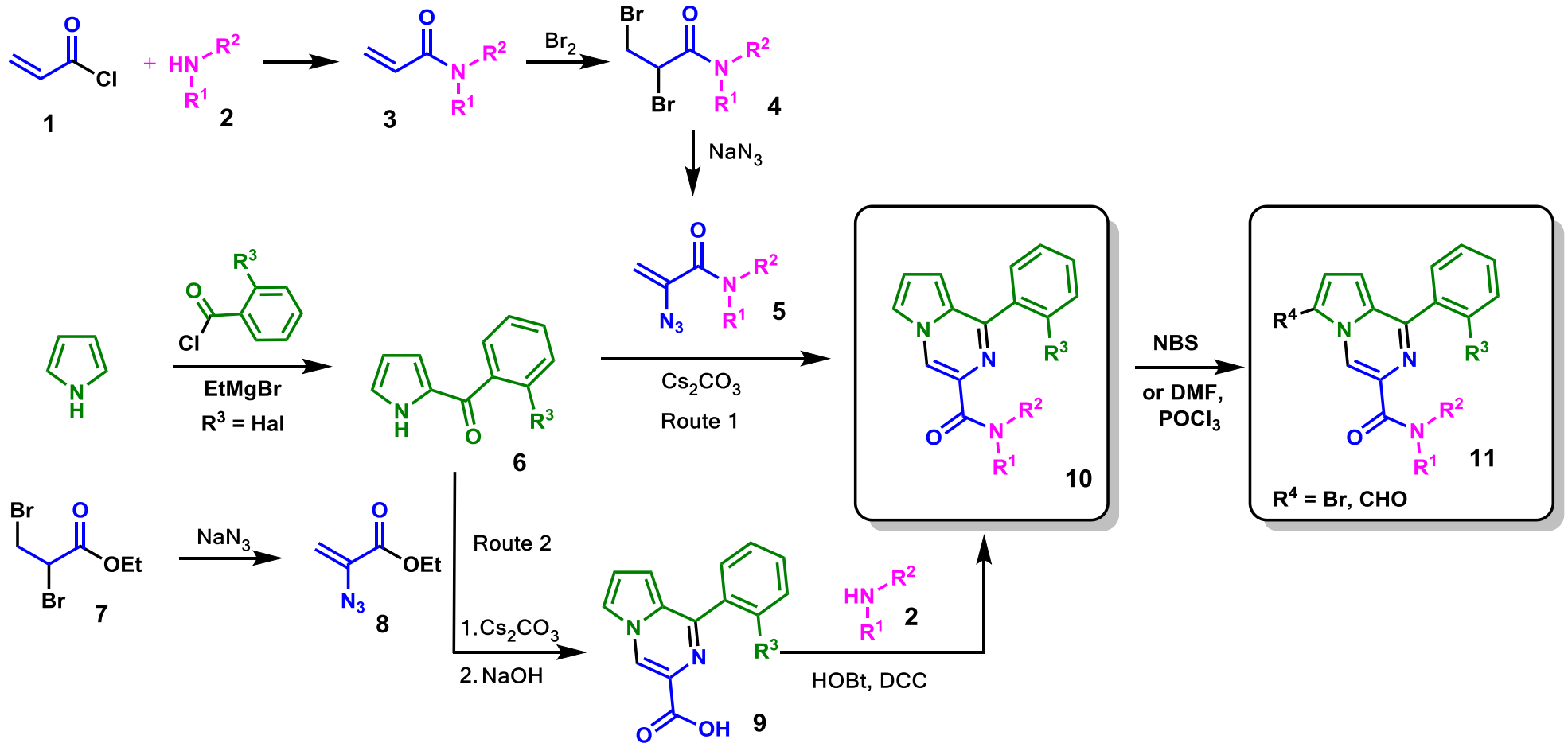


QikProp v6.8 (Schrödinger)

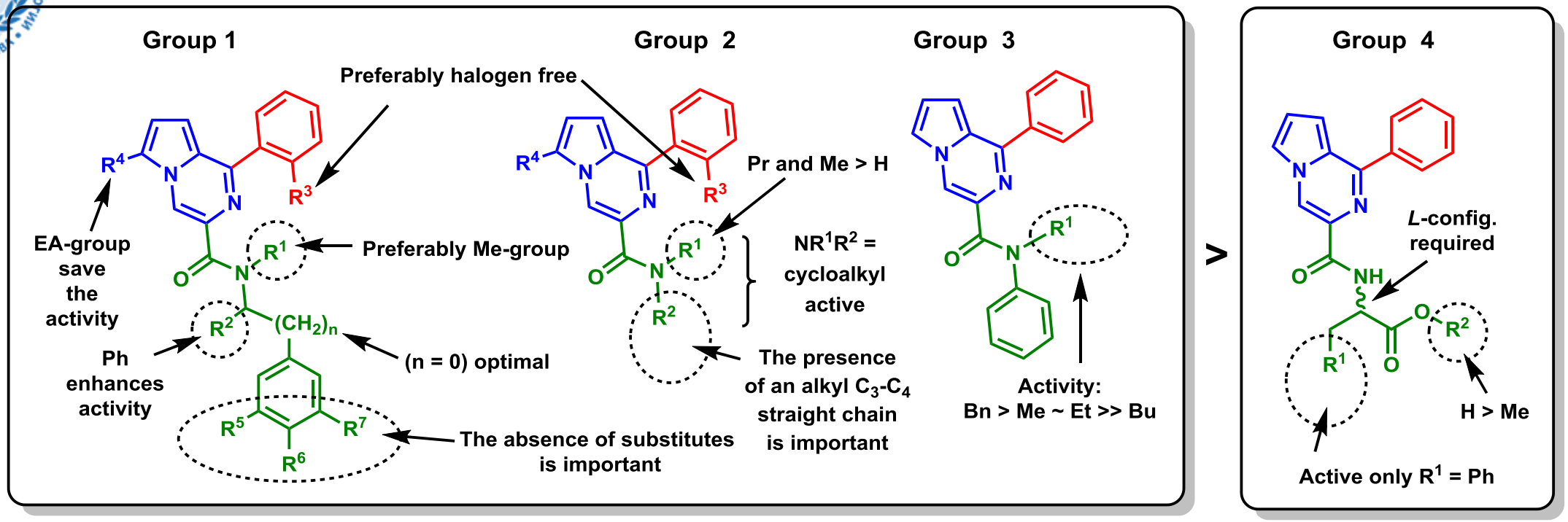
ADMETlab 2.0

Code	R^1	R^2	R^3	R^4	MW	Dipole moment	LogP	LogBB	Lipinsky "Rule of 5"	Jorgensen "Rule of 3"	Oral availability	Ames test	Acute toxicity in rats
GML-1	Bn	Me	H	H	341.412	4.601	5.074	-0.086	1	0	100%	---	---
GML-2	n-Bu	Me	Cl	H	341.839	4.196	4.792	-0.020	0	0	100%	---	---
GML-3	n-Bu	Me	H	H	307.394	4.686	4.508	-0.109	0	0	100%	---	---
GML-4	n-Bu	Me	F	H	325.385	4.053	4.707	-0.062	0	0	100%	---	---
GML-5	n-Bu	Me	Br	H	386.290	3.992	4.939	-0.030	0	0	100%	---	---
ML-291	i-Bu	Me	H	H	307.394	4.650	4.358	-0.043	0	0	100%	---	---
GML-6	sec-Bu	Me	H	H	307.394	4.345	4.345	0.070	0	0	100%	---	---
GML-7	Bn	H	H	H	327.385	5.031	4.877	-0.220	0	1	100%	--	---
GML-8	Me	H	H	H	251.287	4.812	3.189	-0.182	0	0	100%	--	---
GML-9	Bn	Me	F	H	359.402	5.862	5.316	0.044	1	1	100%	---	---
GML-10	Bn	Me	Cl	H	403.482	3.927	6.034	-0.042	1	1	100%	---	---
GML-11	Ph	Bn	H	H	325.385	4.053	4.707	-0.062	0	0	100%	--	---
GML-12	n-Pr	n-Pr	H	H	321.421	5.218	4.826	-0.223	0	0	100%	---	---
GML-21	Ph	Me	H	H	327.385	4.599	4.764	-0.008	0	0	100%	-	---
GML-22	Ph	Et	H	H	341.412	4.275	4.961	-0.021	0	0	100%	--	---
GML-23	Ph	n-Bu	H	H	369.465	4.040	5.391	-0.083	1	0	100%	---	---
GML-24	Ph	H	H	H	313.358	5.108	4.597	-0.153	0	0	100%	-	---
GML-101	Et	Bn	H	H	355.438	4.266	5.433	-0.045	1	1	100%	---	---
GML-102	$CHPh_2$	H	H	H	403.482	5.183	6.506	-0.209	1	1	100%	---	---
GML-103	Bn	Bn	H	H	417.509	4.066	5.993	-0.112	1	0	100%	---	---
GML-104	$(CH_2)_2Ph\ (OMe)_2$-3,4	H	H	H	401.464	6.692	5.032	-0.222	1	0	100%	+	---
GML-105	$CH_2Ph\ (OMe)_3$-3,4,5	H	H	H	417.463	6.112	4.786	-0.335	0	0	100%	--	---
GML-106	L-Phe-OMe	H	H	H	399.448	6.215	5.163	-0.676	1	1	100%	--	---
GML-107	$-(CH_2)_7-$		H	H	319.405	4.690	4.398	0.096	0	0	100%	---	---
GML-108	L-Glu-OEt	H	H	H	423.468	5.230	4.310	-1.027	0	0	100%	---	---
GML-109	L-Asp-OEt	H	H	H	409.441	7.367	4.001	-1.325	0	0	100%	---	---
GML-110	L-Ala-OMe	H	H	H	323.351	4.478	3.689	-0.751	0	0	100%	---	---
GML-111	L-Trp-OEt	H	H	H	452.512	8.433	5.743	-0.954	1	1	100%	--	---
GML-112	D-Tyr-OEt	H	H	H	429.474	4.216	4.874	-1.240	0	1	100%	---	---
GML-113	L-Phe-OH	H	H	H	385.421	6.838	5.126	-1.043	1	1	84%	---	---
GML-114	Me	Bn	H	Br	420.308	4.335	5.636	0.178	1	1	100%	---	---
GML-115	Me	Bn	H	CHO	369.422	5.045	4.012	-0.949	0	0	100%	---	---

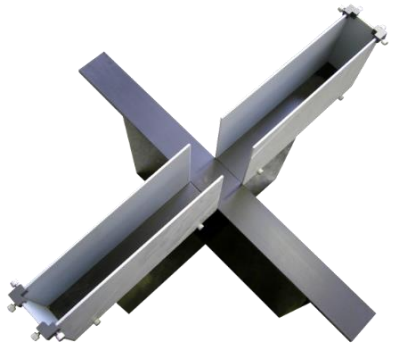
Synthesis of the selected compounds



Anxiolytic activity screening



Open field test
Balb/C mice

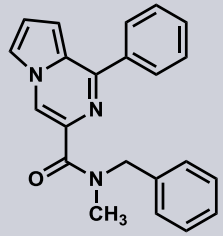
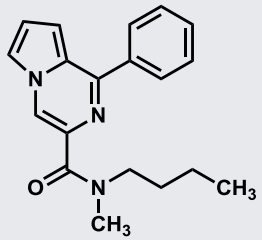
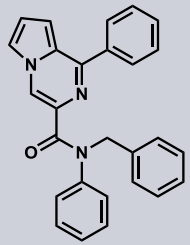
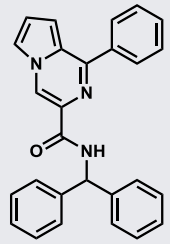


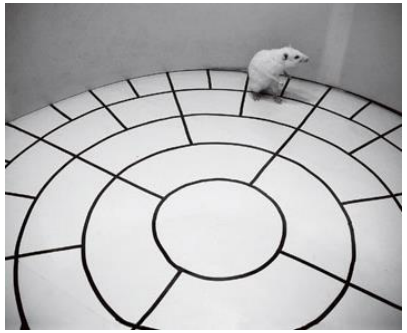
Elevated plus maze test
ICR mice

Compounds were studied in doses of 0.01-5.0 mg/kg (i.p.)

Parameter	Active compounds	Inactive compounds	Difference between active and inactive
Docking Score	-9.16	-8.89	0.28
LogP	4.96	4.64	0.32
LogBB	-0.22	-0.53	0.30

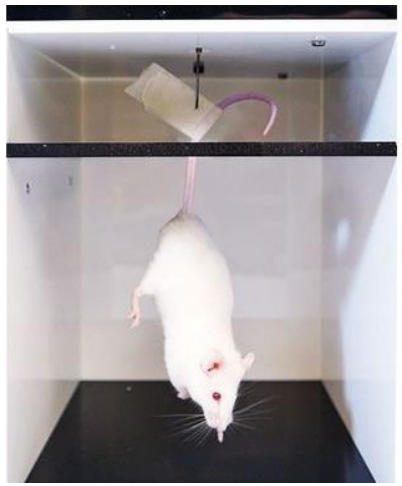
Lead compounds

Code	Structure	Active doses in OF-test (mg/kg)	Active doses in EPM-test (mg/kg)	Active doses in TH-test (mg/kg)
GML-1		0.1-1.0	0.1-1.0	0.5 (sub-chron.)
GML-3		0.1-0.5	0.1-0.5	0.5-5.0
GML-11		0.05-0.1	1.0-5.0	1.0
GML-102		0.1-1.0	0.1-5.0	5.0



Open field test
Balb/C mice

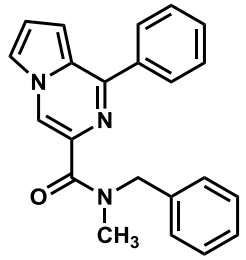
Elevated plus maze test
ICR mice



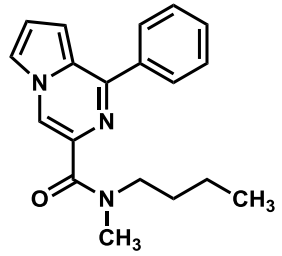
Tail hang test
CD-1 mice

Drug candidates GML-1 and GML-3

GML-1

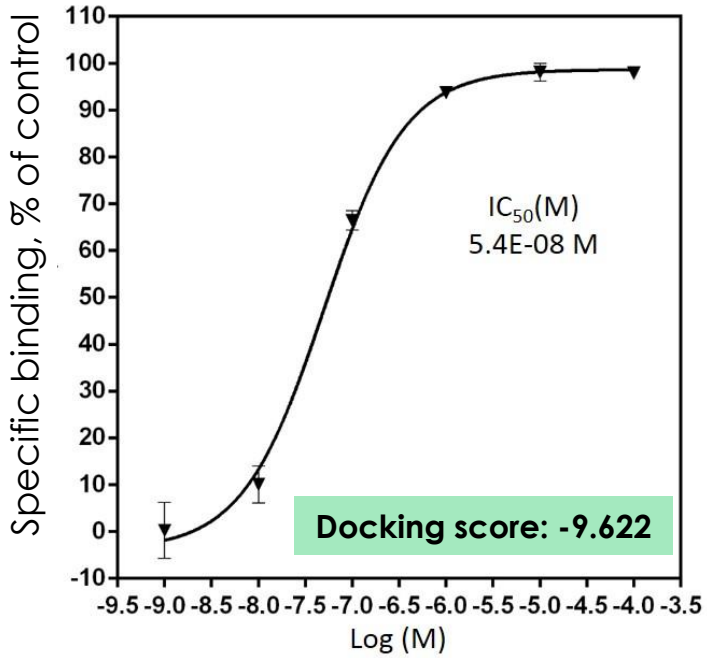


GML-3

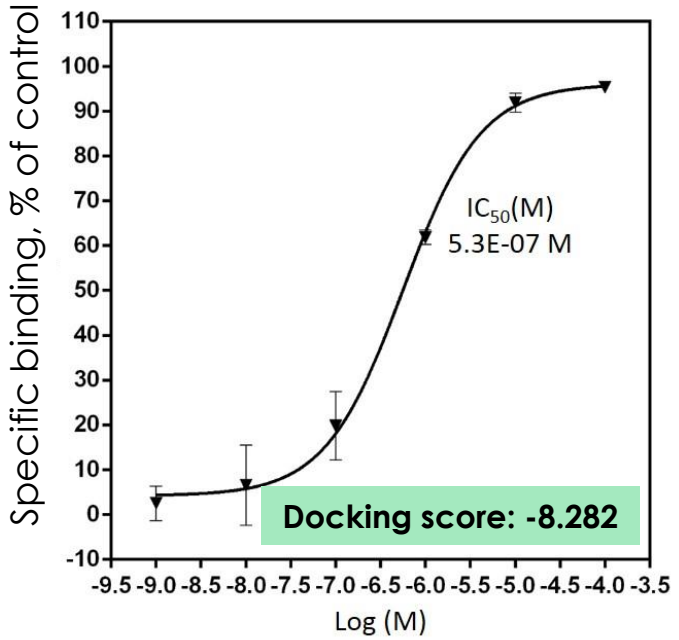


$LD_{50} > 1000$ mg/kg

Potential rapid anxiolytic

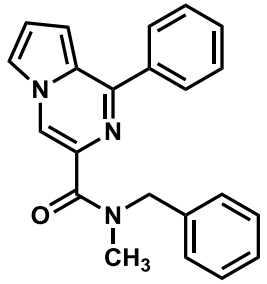


Potential fast antidepressant



Analysis of the affinity of GML-1 and GML-3 for TSPO was carried out by radioligand binding with [^{3}H]PK11195 (Cerep)

GML-1 preclinical study as rapid anxiolytic



Specific activity: **Anxiolytic activity** in open field test, elevated plus maze test, Vogel conflict test (mice, rats; doses 0.01-5.0 mg/kg i.p. and p.o.)

Additional activity: **Antidepressant activity** in tail hang test

Nootropic activity in scopolamine test

Proof of TSPO-mechanism: inhibitory analysis with TSPO-blocker PK11195 and neurosteroids biosynthesis enzymes inhibitors

Dosage form: A tablet dosage form of GML-1 has been developed

Pharmacokinetics: The compound quickly and in sufficient quantities penetrates the target organ, the brain (absolute bioavailability in rats was 21.5%)

Safety: In the maximum doses possible for administration GML-1 (at doses of 1 g/kg (i.p.) and 4 g/kg (p.o.)) **did not have any toxic effect** on mice and rats. In addition, the drug **did not have an immunotoxic effect**, did not cause a systemic anaphylaxis reaction, active cutaneous anaphylaxis, delayed-type hypersensitivity and pseudoallergic reactions. GML-1 has been proven to **lack embryotoxic, fetotoxic, teratogenic, mutagenic and carcinogenic effects**

GML-1 is ready for clinical trials as rapid anxiolytic with procognitive effects

Acknowledgments

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- Department of Pharmacokinetics: prof. V.P. Zherdev and group
- Toxicology Department: corresponding member A.D. Durnev and group
- Department of dosage forms: prof. S.V. Minaev and group

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Thank you for your attention!