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Full Paper

Prediction of biological activity and pharmacokinetic properties of novel nitronorbornene analogs

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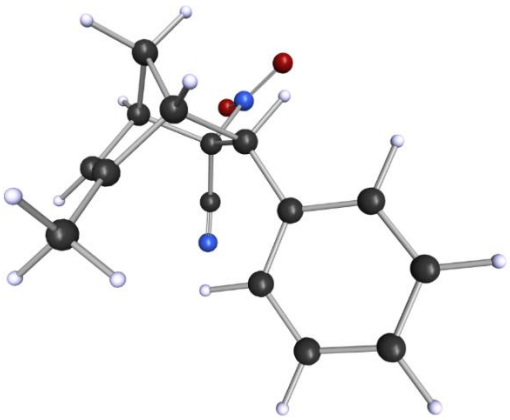

Abstract: Selected nitronorbornene analogs bearing different substituents were analyzed using in silico methods to predict their biological and pharmacokinetic properties. The compounds demonstrated potential biological activity, as indicated by PASS predictions, and exhibited favorable ADME profiles based on SWISS ADME analysis. These findings highlight their promise as candidates for further experimental evaluation and potential pharmacological applications.

Keywords: nitronorbornenes, in silico analysis, PASS prediction, ADME, pharmacokinetics, biological activity

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Introduction

Nitronorbornenes (nitrobicyclo[2.2.1]hept-2-en) represent a particularly intriguing class of compounds due to their unique structural features and potential reactivity. Despite their promising chemical and biological properties, they remain relatively underexplored, with only limited studies addressing their applications or activity profiles.

One of the most straightforward approaches to obtaining compounds with a nitronorbornene framework is the cycloaddition reaction of a cyclopentadiene analogue with an alkene [1,2]. Studies have shown that conjugated nitroalkenes are the most reactive partners in this transformation (Fig. 1).

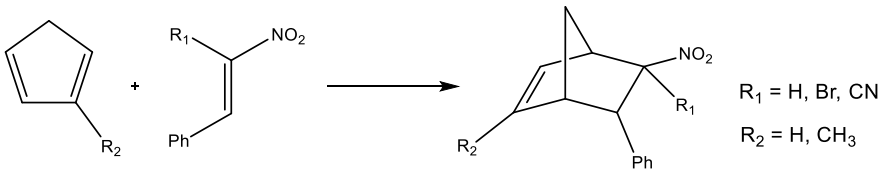

Fig. 1. Diels–Alder reaction of cyclopentadienes analogus with a nitroalkene to afford the corresponding nitronorbornene adducts.

Results and discussion

Thermodynamics

We began our study with a thermodynamic analysis of the investigated compounds **A–E** (Fig. 2) The quantum-chemical calculations were performed using the ωB97X-D functional together with the def2-TZVP basis set in the Turbomole package. As a first step, we examined the HOMO and LUMO energies, which provides insight into the stability and redox properties of a given compound (Fig. 3 and 4). It can be observed that this value is highest for nitronorbornenes A and decreases with the electrophilicity of the substituents. HOMO-LUMO gap is significantly higher for compound A, B and C in contrast to compounds D and E where the energy of LUMO becomes negative. Introduction of an electron-withdrawing group (CN, Br) results in decreasing of the HOMO-LUMO gap and increasing electrophilic (oxidizing) properties of nitronorbornene. Among the isomers of the individual compounds, the most stable isomers are **d** (B2d, D2d and E2d), in which the nitro group occupies the *endo* position relative to the bond formed between the C1 atom of the cyclopentadiene unit and C_{Me}. This

finding is consistent with the CDFT (Constrained Density Functional Theory) analysis of the preferred reaction pathways, which has already been reported in earlier studies [2].

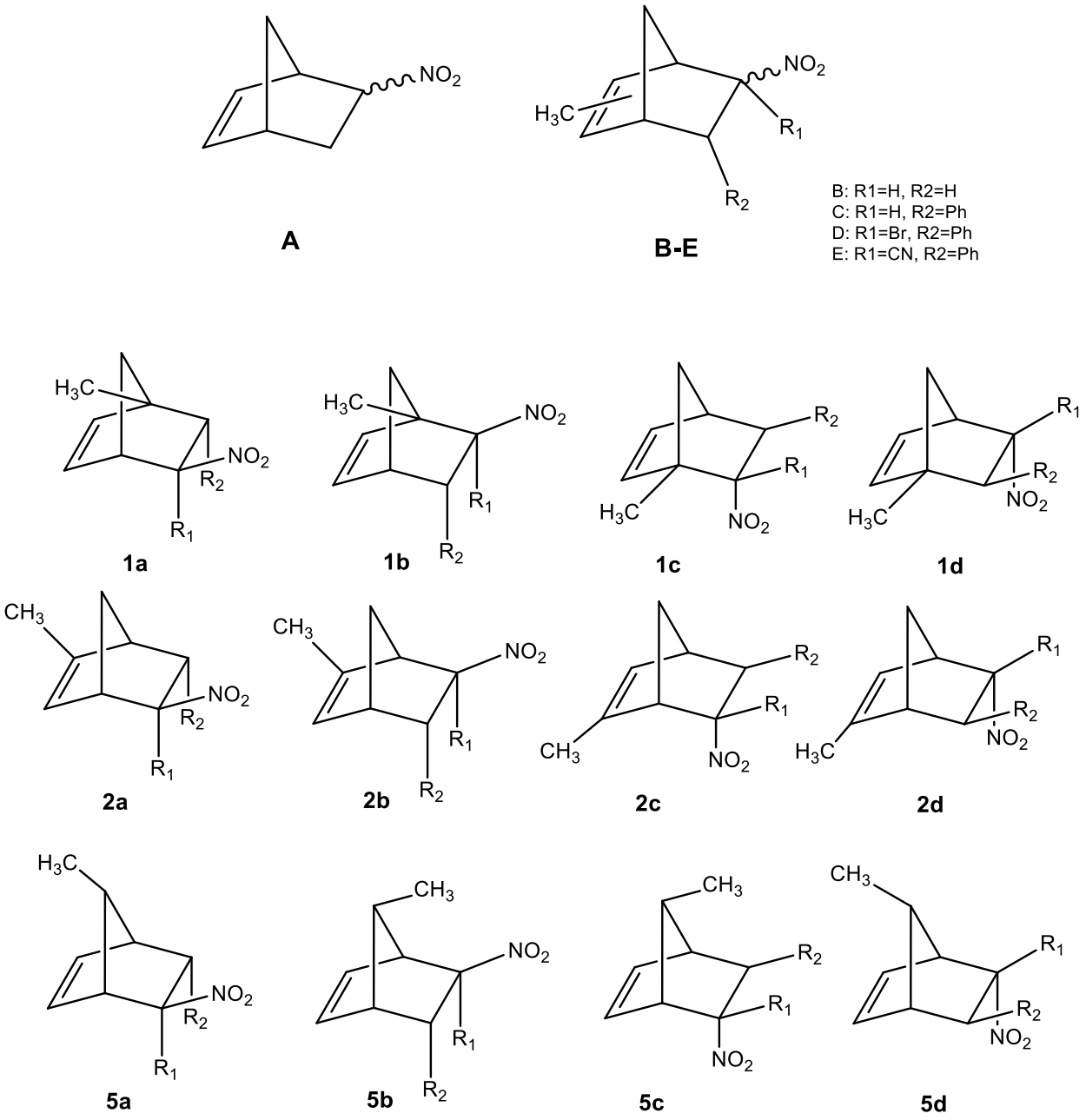

Fig. 2 Chemical structures of the investigated compounds A–E(1,3,5)(a-d).

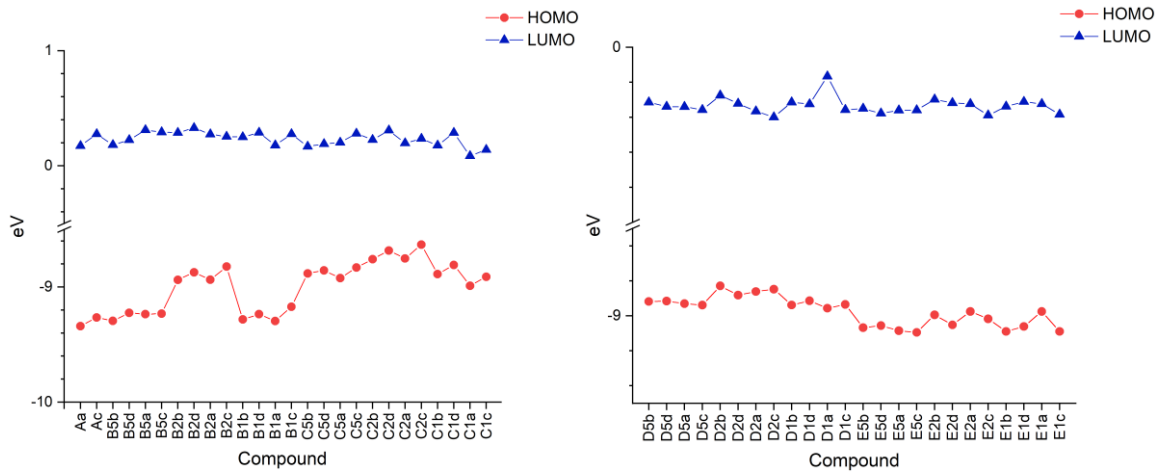

Fig. 3 HOMO and LUMO energies of A–E calculated at the ωB97X-D/def2-TZVP level of theory.

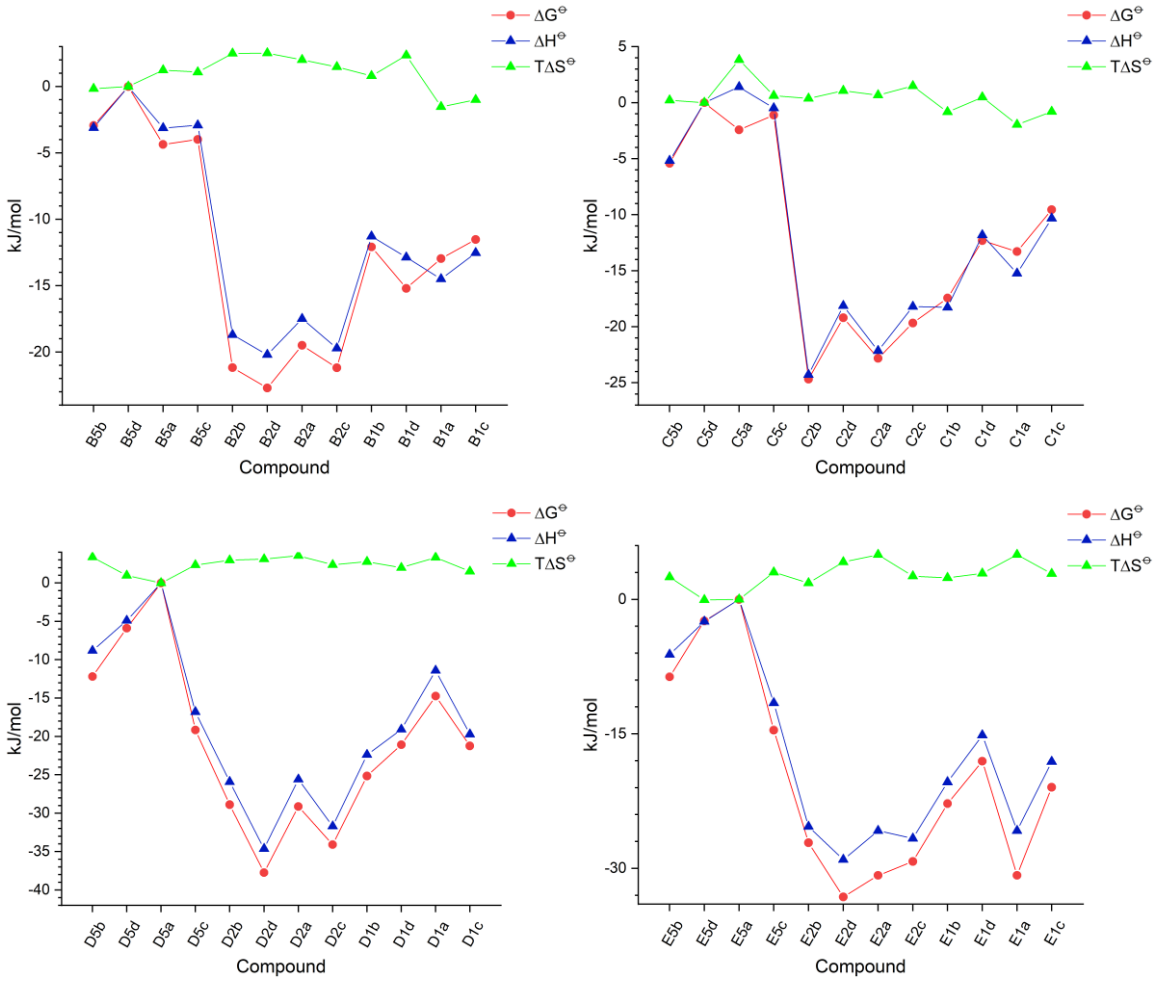

Fig. 4 – Relative stability of the DFT calculated isomers of B-E.

In case of compounds A-E the isomers obtained in the reaction with 2-methylcyclopentadiene are the most thermodynamically stable. The nitro substituent preferably occupies the *endo* position (B2d, D2d and E2d).

Biological activity

We investigated five nitronorbornenes **A-E** bearing different substituents. Our objective was to predict the potential biological properties of these compounds, for which we employed the PASS and SwissADME tools. We employed *in silico* studies because they are faster and more cost-effective than experimental approaches, while still providing a high-probability prediction of the biological properties of the investigated compounds. Such computational methods allow for rapid screening of multiple molecular candidates, guiding experimental work by prioritizing the most promising structures and reducing the need for extensive laboratory testing. It should be noted that most predictive tools, both for biological activity (e.g., PASS) and pharmacokinetic properties (ADME)(Table 1), usually ignore stereoisomerism. This is because the underlying algorithms are primarily based on topological descriptors, which capture the general molecular framework, rather than stereochemical descriptors that reflect the spatial configuration. As a result, enantiomers or diastereomers of a given compound may be treated identically, even though they often differ in biological activity or pharmacokinetic behavior.

Drug-Likeness Evaluation and ADME Analysis

Table 1 Physicochemical properties of A-E

Compound	Formula	MW	#Heavy atoms	#Aromatic heavy atoms	Fraction Csp3	#Rotatable bonds	#H-bond acceptors	#H-bond donors
	[-]	[g/mol]	[-]	[-]	[-]	[-]	[-]	[-]
A	$C_7H_9NO_2$	139.15	10	0	0.71	1	2	0
B	$C_8H_{11}NO_2$	153.18	11	0	0.75	1	2	0
C	$C_{14}H_{15}NO_2$	229.27	17	6	0.43	2	2	0
D	$C_{14}H_{14}BrNO_2$	308.17	18	6	0.43	2	2	0
E	$C_{15}H_{14}N_2O_2$	254.28	19	6	0.40	2	3	0

All the investigated compounds exhibit moderate lipophilicity (Table 2), with LogP values ranging between 0.93 and 2.72. This indicates that the molecules possess a balanced affinity for both aqueous and lipid environments, which is generally considered favorable for oral bioavailability. Such a range of lipophilicity suggests that the compounds are sufficiently soluble in water to ensure absorption, while at the same time lipophilic enough to permeate biological membranes. All the investigated compounds exhibit aqueous solubility, as predicted by different computational models. This indicates that their dissolution in biological fluids should not represent a limiting factor for absorption, which is an important prerequisite for further pharmacokinetic considerations. The BOILED-Egg predictive model (Fig. 5) indicated

that all investigated nitronorbornene analogs are located within the yolk region. This suggests that the compounds are not only predicted to have good intestinal absorption but also a high probability of crossing the blood–brain barrier, which may highlight their potential for applications targeting the central nervous system. The pharmacokinetic predictions (Table 3) indicate that the compound is characterized by high gastrointestinal absorption, is not a substrate of P-glycoprotein, and does not inhibit the main cytochrome P450 isoenzymes, which suggests a low risk of drug–drug interactions. Additionally, its low skin permeation coefficient implies that transdermal administration would not be effective.

Table 2. Lipophilicity of A-E

Compound	iLOGP	XLOGP3	WLOGP	MLOGP	Silicos-IT Log P	Consensus Log P
A	1.36	1.29	1.23	1.53	-0.77	0.93
B	1.60	1.20	1.62	1.05	-0.35	1.02
C	1.88	2.59	3.01	2.51	0.89	2.18
D	2.17	3.27	3.73	2.89	1.53	2.72
E	1.67	2.37	2.91	1.83	0.87	1.93

Table 3. Pharmacokinetics of A-E

Compound	GI absorption	BBB permeant	Pgp substrate	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	log Kp
	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[cm/s]
A	High	Yes	No	No	No	No	No	No	-6.23
B	High	Yes	No	No	No	No	No	No	-6.38
C	High	Yes	No	No	No	No	No	No	-5.86
D	High	Yes	No	No	Yes	No	No	No	-5.86
E	High	Yes	No	No	No	No	No	No	-6.17

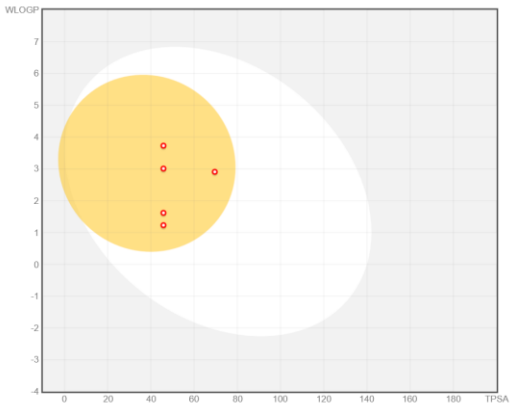

Fig. 5. The BOILED-Egg predictive model

The evaluation of drug-likeness according to commonly applied filters (Lipinski[3], Ghose[4], Veber[5], Egan[6], and Muegge[7]) revealed that the majority of compounds, except A and B, fully comply with the established criteria (Table 4). The single deviation observed was related to low molecular weight, which may slightly reduce the likelihood of drug-likeness but does not exclude potential bioactivity. Overall, the investigated molecules demonstrate favorable drug-like profiles, supporting their suitability for further pharmacokinetic and biological studies.

Table 4 Summary of druglikeness evaluation based on Lipinski's rule of five, Veber's rule, and other relevant molecular descriptors.

Compound	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability Score
A	yes	no (3 violations)	yes	yes	no (1 violation)	0.55
B	yes	no (1 violation)	yes	yes	no (1 violation)	0.55
C	yes	yes	yes	yes	yes	0.55
D	yes	yes	yes	yes	yes	0.55
E	yes	yes	yes	yes	yes	0.55

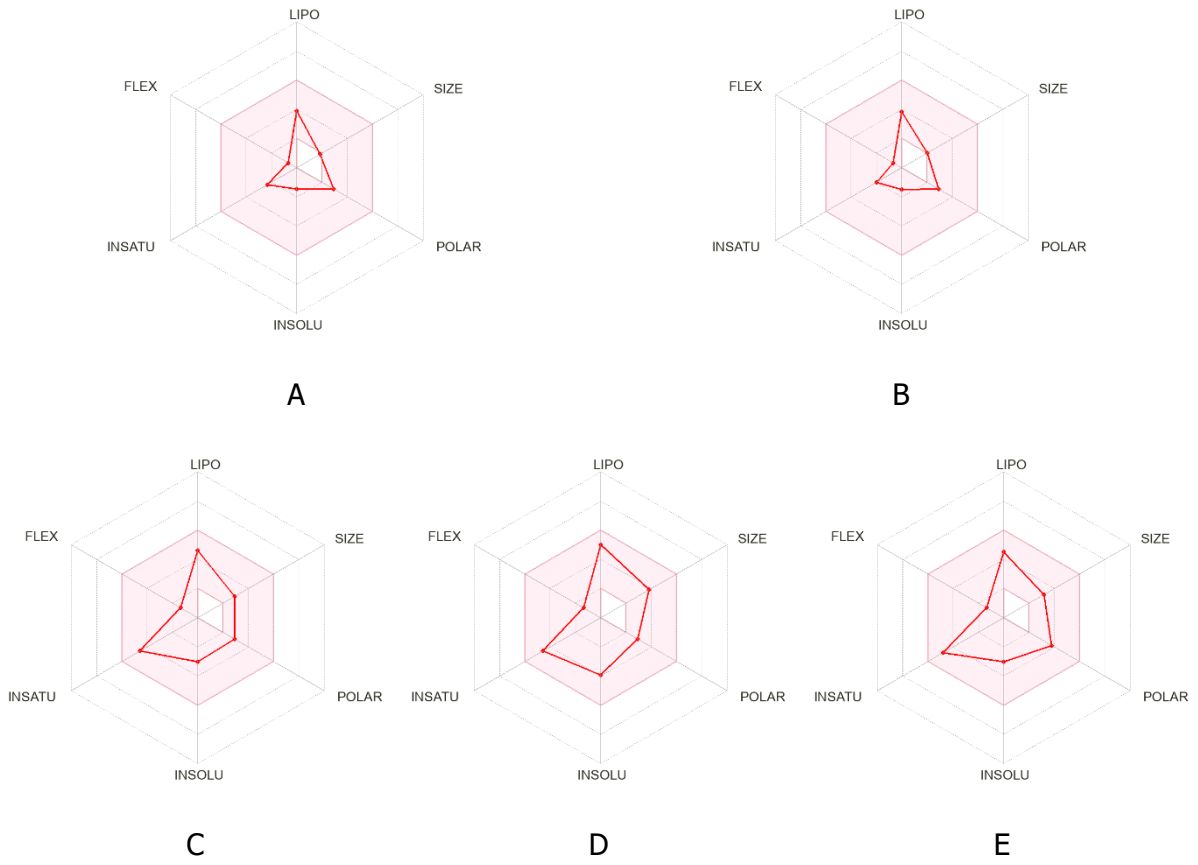

Fig. 6. The bioavailability radar for A-E. The pink area represents the optimal range for each property, including lipophilicity, size, polarity, insolubility, insaturation and flexibility.

Bioavailability radars were also generated for compounds **A–E**, providing a graphical representation of their key physicochemical properties (fig.6). The plot clearly demonstrates that most parameters fall within the optimal range (highlighted in pink). Only for compounds A and B does the molecular size exceed this range. This suggests that, while the overall bioavailability profile of the series is favorable, molecular size may limit the oral drug-likeness of the larger analogues.

PASS-Based Biological Activity Prediction

Table 5. PASS prediction results presenting the most probable biological activities of the studied compounds, expressed as probability values Pa (to be active) and Pi (to be inactive).

Compound	**A**		**B**		**C**		**D**		**E**	
	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi
Ubiquinol-cytochrome-c reductase inhibitor	0.842	0.019	0.838	0.020	0.838	0.020	0.763	0.046	0.649	0.088
Phobic disorders treatment	0.810	0.030	0.718	0.068	0.718	0.068	0.710	0.072	0.572	0.137
Testosterone 17β-dehydrogenase ($NADP^+$) inhibitor	0.771	0.033	0.736	0.043	0.736	0.043	0.706	0.053	0.596	0.092
Acrocylindropepsin inhibitor	0.825	0.015	0.730	0.036	0.730	0.036	0.647	0.060	0.524	0.102
Polyporopepsin inhibitor	0.734	0.032	0.702	0.039	0.702	0.039	0.642	0.055	0.536	0.095
Chymosin inhibitor	0.825	0.015	0.730	0.036	0.730	0.036	0.647	0.060	0.524	0.102
Saccharopepsin inhibitor	0.825	0.015	0.730	0.036	0.730	0.036	0.647	0.060	0.524	0.102
Glucan endo-1.6-β-glucosidase inhibitor	0.807	0.007	0.704	0.019	0.704	0.019	0.654	0.025	0.412	0.075
5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase inhibitor	0.794	0.008	0.725	0.018	0.725	0.018	0.656	0.032	0.556	0.062
CYP2C12 substrate	0.404	0.109	0.621	0.070	0.646	0.066	0.650	0.066	0.769	0.043
Nicotinic α2β2 receptor antagonist	0.793	0.011								
Nicotinic α6β3β4α5 receptor antagonist	0.769	0.017								
L-glutamate oxidase inhibitor	0.748	0.007								
Fusarinine-C ornithinesterase inhibitor	0.743	0.015								
Albendazole monooxygenase inhibitor	0.732	0.004								
β glucuronidase inhibitor	0.729	0.004								

The in silico Prediction of Activity Spectra for Substances PASS [8] analysis (Table 5) of the studied nitronorbornene derivatives indicated a broad spectrum of potential biological activities. The compounds were predicted as inhibitors of various enzymes, including ubiquinol-cytochrome-c reductase, testosterone 17β-dehydrogenase, and several proteases (acrocylindropepsin, polyporopepsin, saccharopepsin), as well as glucan endo-1,6-beta-glucosidase and 5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase. The enzyme prediction in PASS provides only an indication that the compound's structure may interact with fungal proteins, which might, but does not necessarily, result in a fungistatic or fungicidal effect. Additionally, the compounds **A-D** showed potential activity in the treatment of phobic

disorders. For all listed activities, the probability of being active (Pa) exceeded the probability of inactivity (Pi), suggesting a high likelihood of confirming these effects experimentally. The compound **E** was predicted to be a substrate of CYP2C12, indicating that it may undergo metabolism by this hepatic enzyme, which could influence its pharmacokinetic profile and potential drug–drug interactions. The simplest nitronorbornene **A** was predicted to interact with several biologically relevant targets. It showed potential antagonistic activity towards nicotinic acetylcholine receptors (α2β2 and α6β3β4α5 subtypes), as well as inhibitory effects on enzymes such as L-glutamate oxidase, fusarinine-C ornithinesterase, albendazole monooxygenase, and β-glucuronidase. These predicted activities suggest that even the structurally simplest analog may possess a diverse pharmacological profile and warrants further investigation.

Material and methods

The biological activity profiles of the compounds were predicted using the PASS Online platform (https://www.way2drug.com/passonline/)[8]. Pharmacokinetic properties, including absorption, distribution, metabolism, and excretion (ADME[9]), were assessed employing the SWISS ADME tool.

The quantum-chemical calculations were performed using TURBOMOLE rev. V7-9 software package[10]. The DFT (density functional theory) calculation were carried out using full parallelization[11] applying resolution of identity [12,13] as well as multipole accelerated resolution of identity approximation[14]. The def2-TZVP[15] basis sets and auxiliary basis sets[13] were employed across all atoms. In order to correctly treat electron exchange and non-covalent interactions ωB97X-D [16] functional was used. Initial geometries were optimized to explore the potential energy surface. The ground-state molecular structures presented in the text correspond to their respective energy minima. The analysis of vibrational frequencies was performed using Aoforce[17]. The examination of the Hessian matrix revealed no negative eigenvalues for all presented compounds. The standard thermodynamic data (T = 298.15 K, p = 0.1 MPa) were calculated based on vibrational frequencies with zero-point vibrational energy correction (0.9546)[18] using Freeh module.

Conclusions

In the analyzed group of compounds, the ones obtained in the reaction with 2-methylcyclopentadiene (**2**) are thermodynamically the most stable. In the subpopulation of isomers differing in the position of the nitro group relative to the cyclopentadiene ring, compounds with a **d** configuration proved to be the most stable in most cases.

An analysis was conducted on five selected nitronorbornene analogs with various substituents. The results suggest that these compounds may exhibit notable biological activities, highlighting their potential as candidates for further pharmacological evaluation.

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