UDC 54.057:547.7/.8:615.22
DOI: 10.15587/2519-4852.2024.291626

COMPUTER-AIDED RATIONAL DESIGN AND SYNTHESIS OF NEW POTENTIAL ANTIHYPERTENSIVE AGENTS AMONG 1,2,3-TRIAZOLE-CONTAINING NIFEDIPINE ANALOGS

Alexander Kyrychenko, Igor Bylov, Anna Geleverya, Sergiy M. Kovalenko, Iryna Zhuravel, Volodymyr Fetyukhin, Thierry Langer

1,2,3-Triazole-containing Nifedipine analogues offer the opportunity to increase biostability, bioavailability, efficacy and binding selectivity to target receptors. Here, we applied a computer-aided rational design for identifying new Nifedipine analogues containing a 1,2,3-triazole moiety. First, a new chemical library of 796 derivatives combining the 1,4-dihydropyridine (DHP) fragment and 1,2,3-triazole moiety was generated. Second, to reduce the library size, the library was pre-filtered using two 3D-pharmacophore models with different complexity, which allowed us to gradually reduce the chemical space, ending up with 26 hit candidates. Molecular docking calculations against the rCav1.1 receptor allowed the identification of eight derivatives 5a-h, characterized by the binding affinity towards the rCav1.1 receptor of the same level as approved Nifedipine-like drugs. Next, our molecular docking results were used to guide and optimize the retrosynthetic approaches for new analogues of Nifedipine as promising antihypertensive agents. So, a retrosynthetic approach for Nifedipine analogues with a 1,2,3-triazole ring in position 4 was proposed. Finally, eight analogues 5a-h determined by molecular docking calculations were synthesized using the suggested retrosynthetic approach.

***The aim of this study** is to identify new Nifedipine analogues using a computer-aided drug design and a retrosynthetic approach.*

***Materials and methods.** The organic synthesis of new Nifedipine analogues containing a 1,2,3-triazole moiety. Computer-aided drug design of new DHP derivatives using pharmacophore screening and molecular docking calculations.*

***Results**. Molecular docking of new Nifedipine analogues made it possible to estimate the binding affinity of new Nifedipine derivatives to the rCav1.1 receptor. Pharmacophore screening of a chemical library of analogues, consisting of 796 derivatives, allowed gradually reducing the chemical space and obtaining 26 candidates with high affinity to the rCav1.1 receptor. Using the method of molecular docking, eight hits 5a-h were identified, and the synthesis of the recommended compounds was proposed and performed.*

***Conclusions**. The results of molecular docking showed that Nifedipine analogues 5a-h are characterized by binding affinity to the rCav1.1 receptor at the same level as approved Nifedipine-like drugs. Pharmacophore screening and molecular docking calculations indicate key features of the ligand-receptor interaction that can guide and optimize the synthesis of new Nifedipine analogues as promising new antihypertensive agents. A retrosynthetic approach was proposed, and the recommended compounds were synthesized*

***Keywords**: 1,4-dihydropyridine, 1,2,3-triazole, calcium channel blockers, antihypertensive agents, molecular docking, synthesis*

How to cite:
Kyrychenko, A., Bylov, I., Geleverya, A., Kovalenko, S. M., Zhuravel, I., Fetyukhin, V., Langer, T. (2024). Computer-aided rational design and synthesis of new potential antihypertensive agents among 1,2,3-triazole-containing nifedipine analogs. ScienceRise: Pharmaceutical Science, 3 (49), 4–12. doi: http://doi.org/10.15587/2519-4852.2024.291626

1. Introduction

Nowadays, calcium ion channel blockers play a prominent role in treating hypertension [1]. Within this drug family, the special attention of medical practitioners and research teams is attracted to drugs containing 1,4-dihydropyridine (DHP) core [2, 3]. This is because of their high selectivity towards blood vessels compared to phenylalkylamine and benzothiazepine derivatives [4].

A classic drug that founded the family of DHP-blockers is Nifedipine. Later on, other members, such as Isradipine, Nicardipine, Felodipine, Nimodipine (2nd generation), Amlodipine, Lacidipine and Lercanidipine (3rd generation), were also implemented into medical practice (Fig. 1).

In most DHP blockers, a phenyl radical containing a nitro group or chlorine atom is located in position 4 of the dihydropyridine system; only Isradipine has a fused benzoxadiazole system (Fig. 1). Therefore, one of the promising strategies for the structural modification of DHP derivatives is the introduction of various heterocyclic systems into position 4. The synthesis of DHP derivatives, containing fragments of furan [5], thiophene [6], indole [7], imidazole [8, 9], pyridine [7, 10], pyrazine [11] and others have been reported. Some DHP derivatives containing 1,2,3-triazole fragments

have also been synthesized [12]; however, in this case, the two heterocycles are connected to each other through a methoxyphenyl fragment [13]. An example of a 1,2,3-triazole-containing DHP derivative is shown in Fig. 2, *a*.

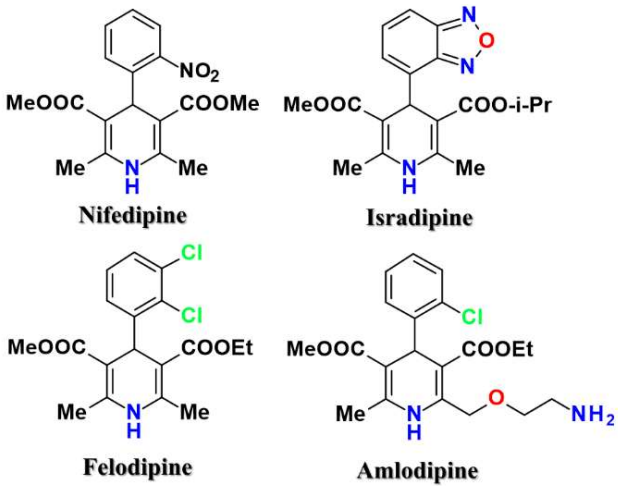
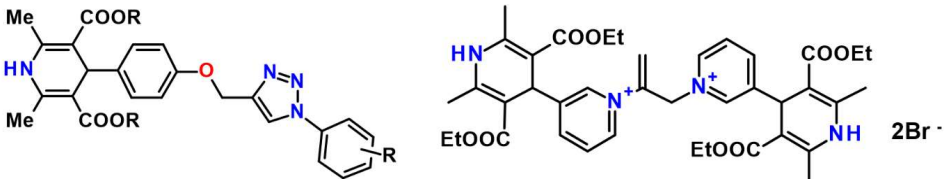


Fig. 1. The drug family of DHP-blockers



a *b*

Fig. 2. Example of a DHP-blocker containing heterocyclic moieties: *a* – 1,2,3-triazole; *b* – bispyridinium

Meanwhile, DHP derivatives have recently attracted attention in addition to their classical role as potential Calcium ion channel blockers. For example, 1,2,3-triazole-derivatives, mentioned above and shown in Fig. 2, *b*, is considered a promising antidiabetic agent. Compounds containing a thiophene fragment in position 4 affect not only Calcium ion channels but also the transport of chloride ions, which opens up opportunities for treating cystic fibrosis [14].

Some studies have demonstrated that DHP derivatives have an almost classical structure that reveals antimicrobial [15, 16] and antifungal effects. In addition, DHP derivatives, in which 3,5-carbo-ester groups are symmetrically amidated with thiosemicarbazide, showed anticoagulant activity [17]. Finally, symmetrical amidation of 3,5-carbo-ester groups with deacylated melatonin led to the appearance of antioxidant properties against the background of blocking activity concerning Calcium ion channels [18]. Bispyridinium dibromide DHP analogues, shown in Fig. 2, *b*, were synthesized by quaternization of 4-pyridyl derivative DHP with propargyl bromide and showed antiradical activity [19].

The goal of our study is synthesis and a computer-aided rational design of new, more effective analogs of Nifedipine.

2. Planning (methodology) of research

The methodology of our study was as follows: new derivatives combine the DHP fragment and a variable 1,2,3-triazole moiety, which promise to increase biostability, bioavailability, efficacy and binding selectivity to target receptors [2, 12]. First, 45 FDA-approved Nifedipine analogues were docked against the rCav1.1 receptor to estimate the best analogues, which were further used for estimating the binding energy threshold. Second, we generated a virtual library of DHP analogues composed of 796 molecules, all containing 1,2,3-triazole moieties, using evolution/combinatorial principles. Third, to reduce chemical space, the generated library was pre-filtered against the Nifedipine pharmacophore model. Fourth, selected candidates were docked against the rCav1.1 receptor. Finally, eight suggested derivatives were synthesized using the retrosynthetic approaches. The results of our molecular docking calculations can guide and optimize the practical synthesis of new analogues of Nifedipine.

3. Materials and Methods

Chemistry. Starting materials and reagents were commercially available and used as obtained from Sigma-Aldrich or Fischer Scientific without further purification. NMR spectra were recorded on Bruker AV 400 in DMSO-d6 using TMS as an internal standard. LC/MS spectra were recorded with a Waters UPLC Acquity equipped with a Waters LCT Premier XE Mass Detector for UPLC-HRMS and with Waters Alliance systems. Control of the reactions was carried out using thin-layer chromatography (eluent – ethyl acetate-hexane 1:2) on Fluka silica gel (60 F 254) plates (0.25 mm), and compounds were visualized with UV light (λ=254 nm) or $KMnO_4$ stain.

Molecular Docking Setup. Molecular docking was performed for selected DHP derivatives against the Cryo-EM structure of the rabbit Cav1.1 channel (PDB ID: 6JP5). The co-crystalized ligand and water molecules were removed. All semi-flexible molecular docking calculations were performed using LigandScout software (version 4.4.9) [20] with the built-in AutoDock Vina 1.1 [21]. Docking was performed for rigid receptors and conformationally flexible ligand molecules. For each ligand, three independent runs were performed. The best docking mode corresponds to the highest ligand binding affinity. Molecular graphics and visualization were performed using LigandScout 4.4.9 and VMD 1.9.3 [22].

4. Results

The Structure of Calcium Ion Channel Receptor. Recently, the high-resolution Cryo-EM structure of the rabbit Cav1.1 channel, also known as the L-type Cav or dihydropyridine receptors (DHPRs), has been resolved [23]. The rCav1.1 is a hetero-multimeric complex containing the pore-forming α1 core subunit and some other auxiliary subunits, such as α2γ-1, β1a, and γ, as shown in Fig. 3.

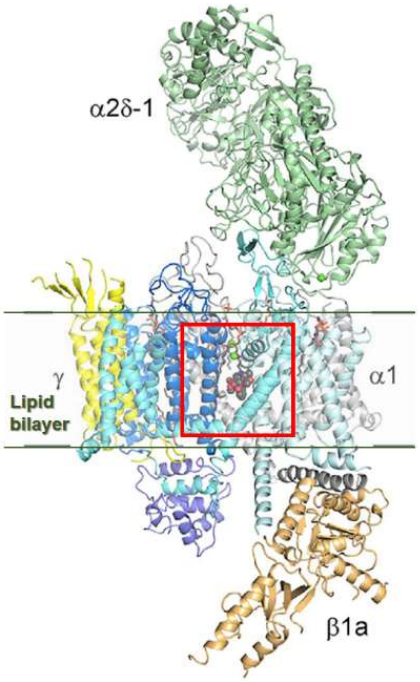


Fig. 3. Cryo-EM structure of the rCav1.1-Nifedipine complex. The bound Ca^{2+} ion and Nifedipine are shown as green spheres and as ball-and-sticks. The sugar moieties are omitted for clarity (PDB 6JP5) [23]. The active binding site and the docking cell are shown in red

The DHP derivatives have been widely used for the treatment of hypertension, angina pectoris, and Raynaud's phenomenon [24]. Therefore, the rCav1.1-Nifedipine complex is a good atomic structural model for *in silico* screening of novel DHP antagonists.

The CryoEM structure of α1-subunit of the rCav1.1 after removing the co-crystalized water and Nifedipine was used as a target receptor for *in silico* screening of the DPH library and the molecular docking calculation, as shown in Fig. 3.

Molecular Docking Calculations.

To benchmark our docking procedure and used force-field parameters, we first re-docked Nifedipine against the rCav1.1 receptor using LigandScout software. Fig. 4 compares the experimental X-ray binding mode of Nifedipine in its co-crystalized complex with the rCav1.1 receptor and its best binding mode obtained by molecular docking calculation. Our docking results demonstrate a good overlap of the X-ray experimental data and the predicted bound conformation of Nifedipine with the root-mean-square deviation of less than 0.15 nm, as estimated by LigandScout. This agreement allowed us to use this docking procedure further for the large-scale screening of a broad family of DHP analogues.

A series of 45 FDA-approved DHP drugs and existing Calcium ion channel blockers, available in PubChem database (https://pubchem.ncbi.nlm.nih.gov), were docked against the rCav1.1 receptor to identify the hit candidates. The docking was performed against the rCav1.1 receptor using the identical docking

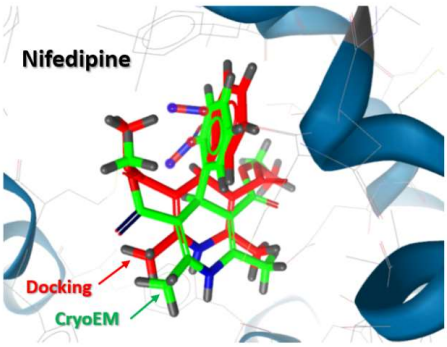


Fig. 4. The comparison of the experimental CryoEM structure (green) at the active site of the rCav1.1 (PDB ID: 6JP5) and the best binding mode (red) of Nifedipine estimated by molecular docking calculations

procedure. Table 1 summarizes the molecular docking results for the eight hits characterized by their LigandScout binding affinity score, which is higher than the reference ligand Nifedipine.

Table 1 and Fig. 5, *a*, *b*, *d* demonstrate that Elgodipine, Dexniguldipine and Vatanidipine revealed the highest affinity towards the rCav1.1 receptor in terms of AutoDock Vina Binding Affinity and LigandScout Binding Affinity Score. Therefore, the results of Table 1 and Fig. 5, *a*–*h* form the basis for further *in silico* screening of new 1,2,3-triazole-containing DHP analogs.

Nifedipine-Based Pharmacophore Screening. Next, we carried out *in silico* screening of our local library composed of 796 Nifedipine analogues containing a 1,2,3-triazole moiety. This library is too big for direct molecular docking. Therefore, the library was pre-filtered by using two different pharmacophore models created based on the cryo-EM structure of the rCav1.1-nifedipine complex (Fig. 6, *a*, *b*). First, using the original CryoEM structure of crystal-bound Nifedipine, a 3-point pharmacophore model was built using LigandScout software (Fig. 6, *a*).

Table 1

Summary of the molecular docking calculation and physic-chemical parameters for the eight best Calcium channel blockers.

No.	Ligand Name	AutoDock Vina Binding Affinity (kcal/mol)	LigandScout Binding Affinity Score	M_W (g/mol)	cLogP	PSA[a] (Å^2)
1	Elgodipine	–8.1	–38.5	524.6	5.07	94.2
2	Dexniguldipine	–9.7	–35.4	609.7	5.84	119.7
3	Teludipine	–6.7	–34.8	498.6	3.94	94.1
4	Vatanidipine	–9.0	–34.6	686.8	6.45	122.9
5	Pranidipine	–7.9	–31.6	448.5	4.26	116.4
6	Cilnidipine	–8.4	–30.6	492.5	4.28	125.7
7	Lercanidipine	–8.7	–30.4	611.7	6.52	119.7
8	Manidipine	–8.6	–25.0	610.7	4.70	122.9
9	Nifedipine	–7.3	–24.9	346.3	2.18	116.5

Note: [a] – polar surface area (PSA) is the surface sum over all polar atoms, such as oxygen, nitrogen, sulfur and phosphorus, including also attached hydrogens

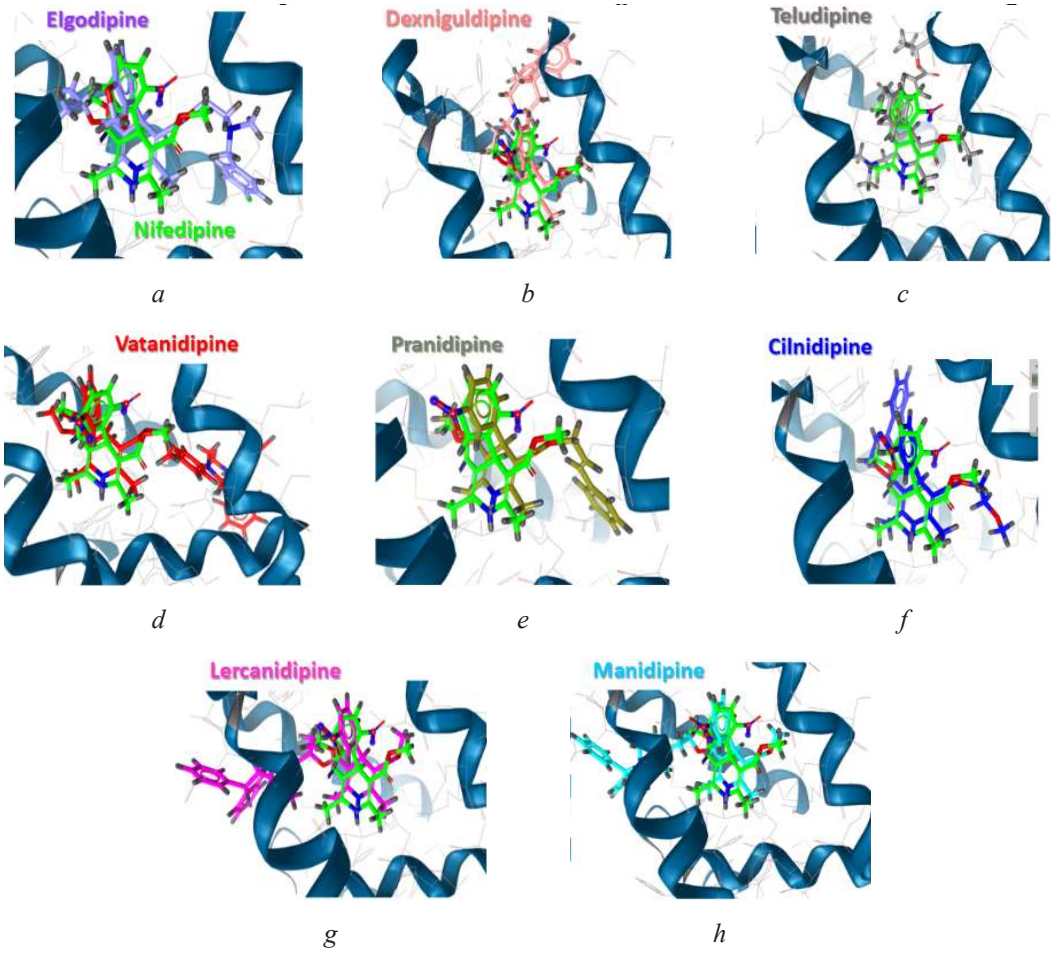


Fig. 5. The best docking poses of eight hit channel blockers at the active site of the rCav1.1 receptor: *a* – elgodipine; *b* – dexniguldipine; *c* – teludipine; *d* – vatanidipine; *e* – pranidipine; *f* – cilnidipine; *g* – lercanidipine; *h* – manidipine. The ligand binding pose is colour-coded and overlapped with the experimental binding mode of Nifedipine (green) (PDB ID: 6JP5) versus the molecular docking calculations (colour-coded)

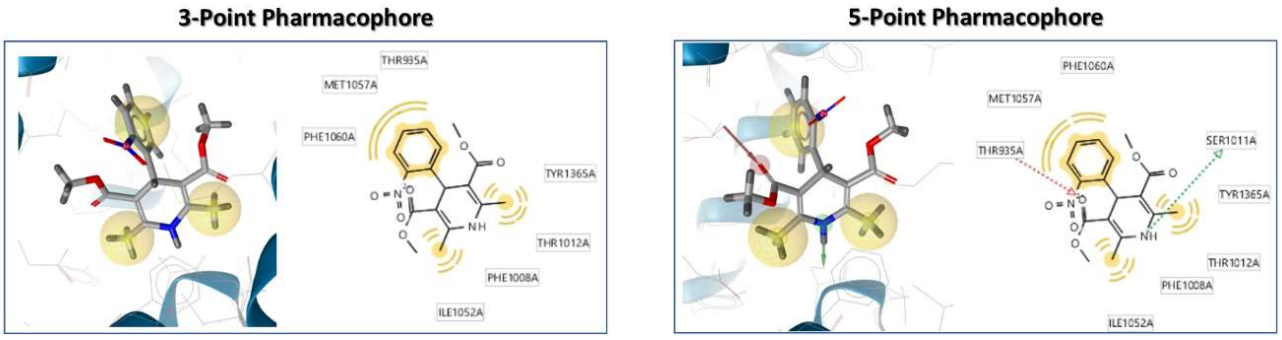


Fig. 6. Two pharmacophore models of Nifedipine bound to the rCav1.1 receptor (PDB ID: 6JP5) in 3D-representation (*left*) and a 2D cartoon view (*right*): *a* – 3-point model; *b* – 5-point model

Second, the structure of the rCav1.1-Nifedipine complex was energy-minimized by the empirical MMFF94 force field, following the pharmacophore building. This procedure allowed us to identify additional ligand-receptor interactions, such as H-bonding with Thr935 and Ser1011, resulting in a five-point pharmacophore model, as shown schematically in Fig. 6, *b* for 3D and 2D representations.

Next, the pharmacophore filtering of our Nifedipine library using 3- and 5-point models allowed gradual narrowing the library chemical space from 796 to up to 124 (using 3-point model) and 26 (using 5-point model) analogs, characterized by a pharmacophore fit score of above Nifedipine reference value of 53.5.

To evaluate the binding affinity of 26 selected candidates, we carried out their molecular docking against the rCav1.1 structure. The docking results for 8 best-binding ligands are summarized in Table 2. The docking poses of the first four best-binding derivatives are shown in Fig. 7, *a–d*. The best docking pose was selected by two criteria:

1) first, the bound conformation of a DHP ring of a studied ligand should match the position of this ring in the co-crystalized rCav1.1-Nifedipine complex, as shown in Fig. 7;

2) second, the best binding pose corresponds to the structure with the most negative LigandScout binding affinity score.

Table 2 shows that among eight 1,2,3-triazole-containing DHP analogues, compounds **5a** and **5b** revealed the same or higher affinity towards the rCav1.1 receptor than parent Nifedipine in terms of LigandScout Binding Affinity Score. These parameters for **5a** and **5b** were found to be –25.2 and –24.8, respectively, compared to –24.9 for Nifedipine (Table 1). In terms of AutoDock Vina Binding Affinity, all identified analogues except **5e**, are characterized by a higher affinity from –7.5 up to –7.9 kcal/mol, as compared to –7.3 kcal/mol for Nifedipine (Table 1). In addition, most of the selected analogues are characterized by low PSA values below 100 Å^2. PSA is a commonly used medicinal chemistry criterion for estimating cell permeability, so drug-like molecules with PSA>140 Å^2 are typically poorly permeable into cell membranes [25, 26]. Finally, our molecular docking results suggest that introducing the 1,2,3-triazole moiety to position 4 of a 1,4-dihydropyridine core offers a promising scaffold for developing novel DHP blockers.

Fig. 7 shows molecular aspects of ligand-receptor interactions and crucial amino acid residues of the receptor active site. It can be noted that the binding interactions of a DHP moiety in Nefidipine and ligands **5a-d** with the rCav1.1 receptor are governed by residues T935, Q939, F1008 and M1366, respectively. The further binding preference of ligands **5a-d** are driven by steric interactions of their bulky aryl-1,2,3-triazole moiety with the receptor residues of F1060 and F1370, respectively.

Table 2

Summary of the molecular docking calculation and physico-chemical parameters for derivatives **5a-h**

No.	Ligand	AutoDock Vina Binding Affinity (kcal/mol)	LigandScout Binding Affinity Score	M_W (g/mol)	cLogP	PSA (Å^2)
1	**5a**	–7.7	–25.2	448.9	3.42	95.4
2	**5b**	–7.7	–24.8	464.4	3.69	95.3
3	**5c**	–7.3	–23.4	441.4	1.92	141.2
4	**5d**	–7.9	–22.9	459.4	2.68	147.2
5	**5e**	–7.1	–22.5	440.1	2.30	95.3
6	**5f**	–7.8	–22.4	432.4	2.91	95.3
7	**5g**	–7.6	–20.8	414.4	2.77	95.4
8	**5h**	–7.7	–20.4	420.8	2.64	95.3

Synthesis of Nifedipine Analogs containing a 1,2,3-Triazole Moiety.

Based on the docking results, we designed a retrosynthetic plan for the synthesis of new DHP derivatives and synthesized a series of derivatives **5a-h**.

The Huesgen dipolar cycloaddition reaction (CuAAC) was used to construct the 1,2,3-triazole scaffold (Fig. 8) [27–29]. Propargyl alcohol was introduced into a click reaction with arylazides (step *b*), which, in turn, were synthesized by the diazotization reaction of the corresponding arylamines (step *a*). Next, alcohols **3a-h** were subjected to standard oxidation by Pyridinium chlorochromate (PCC) and aldehydes **4a-h** were obtained in a yield of 68–79 % [30, 31].

The synthesis of the target 1-N-aryl-substituted 1*H*-1,2,3-triazolyl-1,4-dihydropyridines **5a–h** was carried out according to the Hanch reaction by condensation of two equivalents of 1,3-dicarbonyl compound **6**, one equivalent of aldehyde **4a–h** and ammonium acetate (Fig. 9). The reaction was carried out in ethanol at a temperature of 80–85 °C for 2 hours yielding derivatives **5a–h** with yields of 80–96 %.

General method of 2,6-dimethyl-4-(1H-1,2,3-triazol-4-yl)-1,4-dihydropyridine-3,5-dicarboxylate synthesis (5a–h). The mixture of 1H-1,2,3-triazole-4-carbaldehyde ***4a–h*** (1 mmol), methyl/ethyl acetoacetate (2.1 mmol), ammonium acetate (1.5 mmol, 258 mg) was dissolved in ethanol (10 mL). The solution was refluxed for 2 h at 80 °C. After completion of the re-

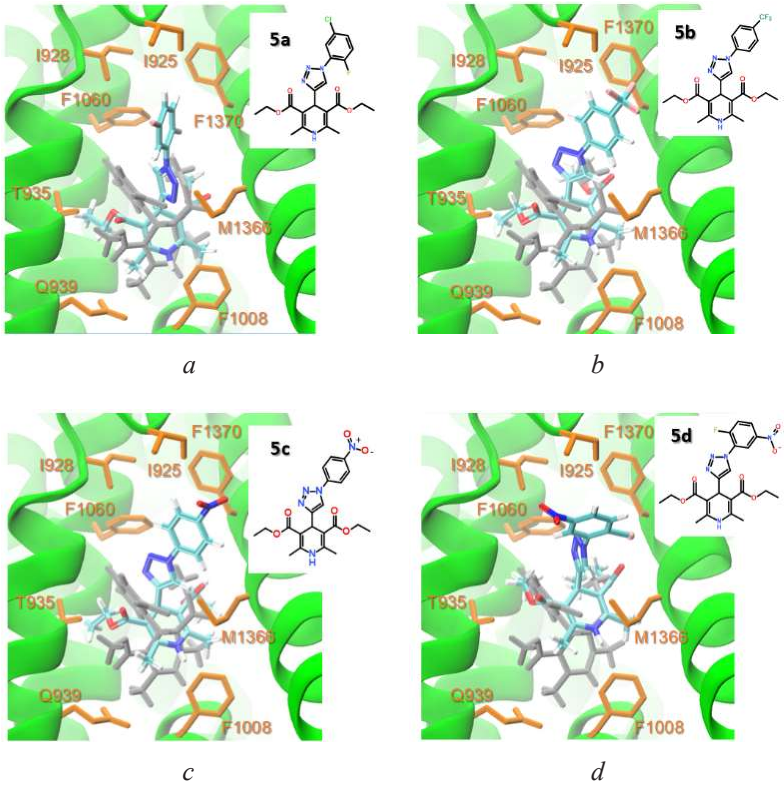


Fig. 7. The docking poses for the four 1,2,3-triazole-containing Nifedipine analogues **5a-d** with the best-binding scores located at the active site of the rCav1.1 receptor: *a* – **5a**; *b* – **5b**; *c* – **5c**; *d* – **5d**. For comparison, the crystallographic pose of Nefidipine (PDB ID: 6JP5) is shown in grey

action, according to TLC data, the reaction mixture was cooled to room temperature. The precipitate was filtered off, washed with ethanol, and crystallized from ethanol. A pure product was obtained with a yield of 80–96 % based on the starting aldehyde.

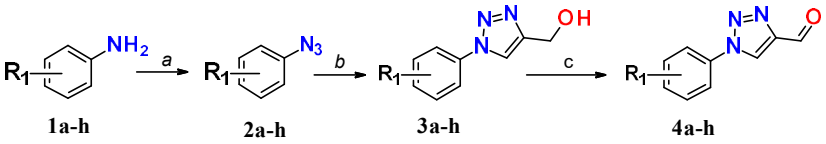


Fig. 8. Synthetic way for 1H-1,2,3-triazole-4-carbaldehydes. Reagents and conditions: a – $NaNO_2$, HCl, 0 °C; NaN_3, 2–4 h, rt; b – propargyl alcohol, $CuSO_4$, sodium ascorbate, THF:H_2O (1:1), rt; c – PCC, CH_2Cl_2, 2 h, rt

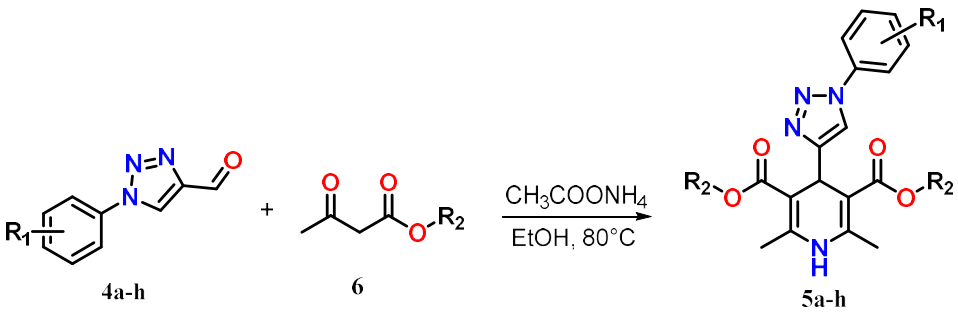


Fig. 9. Synthesis of derivatives 2,6-dimethyl-4-(1H-1,2,3-triazol-4-yl)-1,4-dihydropyridine-3,5-dicarboxylates **5a–h**

3,5-Diethyl 4-(1-(5-chloro-2-fluorophenyl)-1H-1,2,3-triazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***5a***). White solid, yield 80 % (290 mg) ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.95 (*s*, 1H), 8.42 (*s*, 1H), 7.69 (*ddd*, *J*=6.0, 3.7, 2.2 Hz, 1H), 7.28 (*dd*, *J*=10.1, 6.3 Hz, 1H), 5.16 (*s*, 1H), 4.43 (*dq*, *J*=1.9, 1.0 Hz, 1H), 3.98 (*q*, *J*=7.1 Hz, 4H), 2.23 (*s*, 6H), 1.12 (*t*, *J*=7.1 Hz, 6H). MS (ESI+) m/z calculated for $C_{21}H_{22}ClFN_4O_4$ $[M+H]^+$ 449.88, found 449.13.

3,5-Diethyl 2,6-dimethyl-4-(1-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)-1,4-dihydropyridine-3,5-dicarboxylate (***5b***). White solid, yield 82 % (395 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.94 (*s*, 1H), 8.45 (*d*, *J*=0.5 Hz, 1H), 8.19–8.09 (*m*, 2H), 7.98–7.88 (*m*, 2H), 5.14 (*s*, 1H), 4.11–4.03 (*m*, 4H), 2.27 (*s*, 6H), 1.16 (*t*, *J*=7.1 Hz, 6H). MS (ESI+) m/z calculated for $C_{22}H_{23}F_3N_4O_4$ $[M+H]^+$ 465.4, found 465.2.

3,5-Diethyl 2,6-dimethyl-4-(1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)-1,4-dihydropyridine-3,5-dicarboxylate (***5c***). Yellow solid, yield 94 % (950 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.94 (*s*, 1H), 8.51 (*s*, 1H), 8.42–8.37 (*m*, 2H), 8.22–8.18 (*m*, 2H), 5.15 (*s*, 1H), 4.06 (*p*, *J*=7.2 Hz, 4H), 2.27 (s, 6H), 1.16 (*t*, *J*=7.1 Hz, 6H). MS (ESI+) m/z calculated for $C_{21}H_{23}N_5O_6$ $[M+H]^+$ 442.1, found 442.0.

3,5-Diethyl 4-(1-(2-fluoro-5-nitrophenyl)-1H-1,2,3-triazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***5d***). Yellow solid, yield 91 % (245 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (*s*, 1H), 8.16 (*s*, 1H), 7.79 (*d*, *J*=0.6 Hz, 1H), 7.46 (*dd*, *J*=10.2, 7.6 Hz, 1H), 5.16 (*s*, 1H), 4.43 (*dq*, *J*=1.9, 1.0 Hz, 1H), 3.98 (*q*, *J*=7.1 Hz, 4H), 2.23 (*s*, 6H), 1.12 (*t*, *J*=7.1 Hz, 6H). MS (ESI+) m/z calculated for $C_{21}H_{22}FN_5O_6$ $[M+H]^+$ 460.4, found 460.1.

3,5-Dimethyl 4-(1-(2-fluoro-4-methylphenyl)-1H-1,2,3-triazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***5e***). White solid, yield 89 % (420 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (*s*, 1H), 7.98 (*s*, 1H), 7.60 (*ddd*, *J*=8.0, 1.9, 0.9 Hz, 1H), 7.03–6.95 (*m*, 1H), 5.16 (*s*, 1H), 4.43 (*dq*, *J*=1.9, 0.9 Hz, 1H), 2.33–2.26 (*m*, 3H), 2.23 (*d*, *J*=1.0 Hz, 6H). MS (ESI+) m/z calculated for $C_{20}H_{21}FN_4O_4$ $[M+H]^+$ 401.4, found 401.1.

3,5-Diethyl 4-(1-(2,3-difluorophenyl)-1H-1,2,3-triazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***5f***). White solid, yield 93 % (340 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (*s*, 1H), 8.29 (*s*, 1H), 6.93–6.76 (*m*, 2H), 5.16 (*s*, 1H), 4.43 (*dq*, *J*=1.9, 1.0 Hz, 1H), 3.98 (*q*, *J*=7.1 Hz, 4H), 2.23 (*s*, 6H), 1.12 (*t*, *J*=7.1 Hz, 6H). MS (ESI+) m/z calculated for $C_{21}H_{22}F_2N_4O_4$ $[M+H]^+$ 433.4, found 433.2.

3,5-Diethyl 4-[1-(2-fluorophenyl)-1H-1,2,3-triazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***5g***). White solid, yield 96 % (910 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (*s*, 1H), 8.02 (*d*, *J*=2.4 Hz, 1H), 7.77 (*td*, *J*=7.9, 1.7 Hz, 1H), 7.61–7.47 (*m*, 2H), 7.39 (*ddd*, *J*=8.1, 6.9, 1.8 Hz, 1H), 5.12 (*s*, 1H), 4.15–3.96 (*m*, 4H), 2.26 (*s*, 6H), 1.16 (*t*, *J*=7.1 Hz, 6H). MS (ESI+) m/z calculated for $C_{21}H_{23}FN_4O_4$ $[M+H]^+$ 415.1, found 415.0.

3,5-Dimethyl 4-[1-(3-chloro-2-fluorophenyl)-1,2,3-triazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***5h***). White solid, yield 81 % (270 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (*s*, 1H), 7.98 (*s*, 1H), 7.43 (*ddd*, *J*=8.5, 3.7, 1.2 Hz, 1H), 6.97 (*t*, *J*=8.6 Hz, 1H), 5.16 (*s*, 1H), 4.43 (*dq*, *J*=1.9, 0.9 Hz, 1H), 3.44 (*s*, 6H), 2.23 (*s*, 6H). MS (ESI+) m/z calculated for $C_{19}H_{18}ClFN_4O_4$ $[M+H]^+$ 421.8, found 421.2.

5. Discussion

Our computer-aided rational design demonstrated that adding a 1,2,3-triazole ring to existing DHP and Nifedipine scaffolds has a strong potential for developing novel rCav1.1 receptor selective ligands. To follow this strategy, we generated a new chemical library of 796 derivatives combining the DHP fragment and a 1,2,3-triazole moiety using structural modifications of the parent Nefidipine scaffold by evolution/combinatorial principles [26]. The generated library contained new promising virtual molecules; however, it was too large for direct molecular docking calculations. Therefore, we used a multi-step procedure for its gradual reduction. The li-

brary size was filtered using two 3D-pharmacophore models of different complexity. The 5-point 3D-pharmacophore screening decreased the chemical space up to 26 hit candidates. The selected ligands were further subjected to molecular docking calculations against the rCav1.1 receptor, which allowed us to reduce the chemical space, ending up with eight hit derivatives **5a-h** (Fig. 7, Table 2). The identified hit molecules **5a-h** were characterized by the high selectivity and strong binding affinity towards the rCav1.1 receptor. We found that the DHP moiety of Nefidipine and ligands **5a-h** interacted mostly with residues T935, Q939, F1008 and M1366 of the rCav1.1 receptor, while the bound conformation of bulky aryl-1,2,3-triazole moieties of ligands **5a-h** is stabilized by steric interactions with the receptor residues of F1060 and F1370, respectively.

We used the results of our computer-aided rational design of novel promising antihypertensive agents to set up the retrosynthetic approach for the experimental synthesis of new Nifedipine analogues (Fig. 8, 9). Finally, we performed the retrosynthetic procedure based on the Hanch reaction to obtain new Nifedipine analogues **5a-h** bearing a 1,2,3-triazole moiety in position 4.

Study limitations. The high-throughput screening of the antihypertensive activity for derivatives **5a-h** was not available in our lab. Therefore, direct comparison of the molecular docking and the activity assays is not possible, so the potential therapeutic effect of the selected derivatives is only based on a theoretical predictions.

The prospects for further research. The suggested computer-aided screening protocol followed by the retrosynthetic analysis of organic synthesis have a strong potential for developing novel promising antihypertensive agents by varying sizes and nature of the substituents in the position 4.

6. Conclusion

Introducing a 1,2,3-triazole ring to available DHP blockers and Nifedipine analogues holds promise for increasing biostability, bioavailability, efficacy, and binding selectivity to target receptors [2, 12]. To identify new Nifedipine analogues, a computer-aided rational design of Nifedipine analogues containing a 1,2,3-triazole moiety was performed. Our design procedure is based on generating a virtual chemical library of Nefidipine analogues, followed by gradual filtering and decreasing its size by pharmacophore screening and direct molecular docking calculations. Our generated chemical library of 796 derivatives combined the DHP fragment and 1,2,3-triazole moiety. Reducing the library size was carried out by using two 3D-pharmacophore models with different complexity, ending up with 26 hit candidates. The molecular docking calculations against the rCav1.1 receptor allowed the identification of eight hit derivatives **5a-h**, characterized by the binding affinity towards the rCav1.1 receptor of the same level as approved Nifedipine-like drugs. Our molecular docking results were used to guide and optimize the experimental synthesis of new analogues of Nifedipine as novel promising antihypertensive agents. The retrosynthetic approach for new analogues of Nifedipine with a 1,2,3-triazole ring in position 4 was proposed. The eight hit analogues **5a-h** determined by the molecular docking calculations were synthesized using the suggested retrosynthetic approach.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

Funding

This study was funded by the Ministry of Health of Ukraine from the state budget according to the topic 'Hybrid pharmacophore approach to innovative pharmacologically active compounds of hypotensive action – molecular design, synthesis and screening of the hybrid molecules containing 1,4-dihydropyridine and 1,2,3-triazole frameworks' (state registration number: 0123U101752).

Data availability

The manuscript has no associated data.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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Received date 15.02.2024
Accepted date 10.05.2024
Published date 30.06.2024

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