


Spirostans Design as Novel Ligands for CB1 and CB2 Cannabinoid Receptors

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Abstract: Spirostans (Sp) are useful in diverse therapeutical treatments, e.g., in cancer, inflammation, neurodegenerative diseases, pain, and obesity. This study aimed to design Sp as ligands for CB1 and CB2 cannabinoid receptors, which are involved in several physiological functions and diseases. SwissTargetPrediction platform was used to predict the selective biological potential of some Sp for CB1 and CB2 receptors; then, adducts were evaluated by molecular docking. The energy coupling of 155 Sp was calculated and compared to that of reference drugs. Tridimensional maps of 5TGZ (CB1) and 5ZTY (CB2) proteins were prepared and optimized from the ProteinDataBank in AutodockTools. The molecular docking was performed using Autodock Vina and redocking by means of RMSD (<1.0 Å). The Kruskal-Wallis test analyzed data to determine the influence of SP functionality on selectivity toward cannabinoid receptors. Sp interactions were compared versus reference drugs. Results provide insight into the role of specific and differential interactions of Sp and CB1/CB2 receptors. Remarkably, Sp-48 resulted positive for CB1 and Sp-114, and Sp-115 and Sp-126 for CB2 receptors. The *in silico* selectivity was 4.3 CB1/CB2 and 3.1 CB2/CB1, respectively. Data support the development of new CB1 and CB2 cannabinoid receptor agonists useful for preclinical studies.

Keywords: spirostans; CB1 and CB2 cannabinoid receptors; molecular docking.

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1. Introduction

The endocannabinoid system (ECS) comprises endocannabinoids, CB1 and CB2 cannabinoid receptors, and proteins involved in the transport, synthesis, and catabolism of endocannabinoids [1,2]. ECS is responsible for the regulation of vital functions such as learning, memory, mood, anxiety, pain perception, and nutritional behavior, among others [3-4]. CB1 and CB2 receptors are members of the G protein-coupled receptor (GPCR) family [5-7], particularly present in the brain. The CB1 receptors are expressed in the hippocampus, basal ganglia, cerebellum, and cerebral cortex [6, 8]. Considering those areas, it is not surprising that CB1 modulates psychotropic and behavioral effects. CB1 receptors are not only present in the CNS; they are also related to insulin resistance and cardiac regulation processes [7]. In contrast, CB2 receptors also have an expression on peripheral cells, predominantly cells of the immune system. CB2 is involved in cardiac protection and the regulation of bone resistance and is also

present in the brain, where they have been implicated in nociception and neuroinflammation processes [6,7]. Relevant point: cannabinoid receptors have been related to pain, e.g., pain in breast cancer, bone cancer, terminal cancer, and pain in multiple sclerosis [9-11]. Some commercial and natural drugs (Figure 1) have been studied for the CB receptors modulations on the functions.

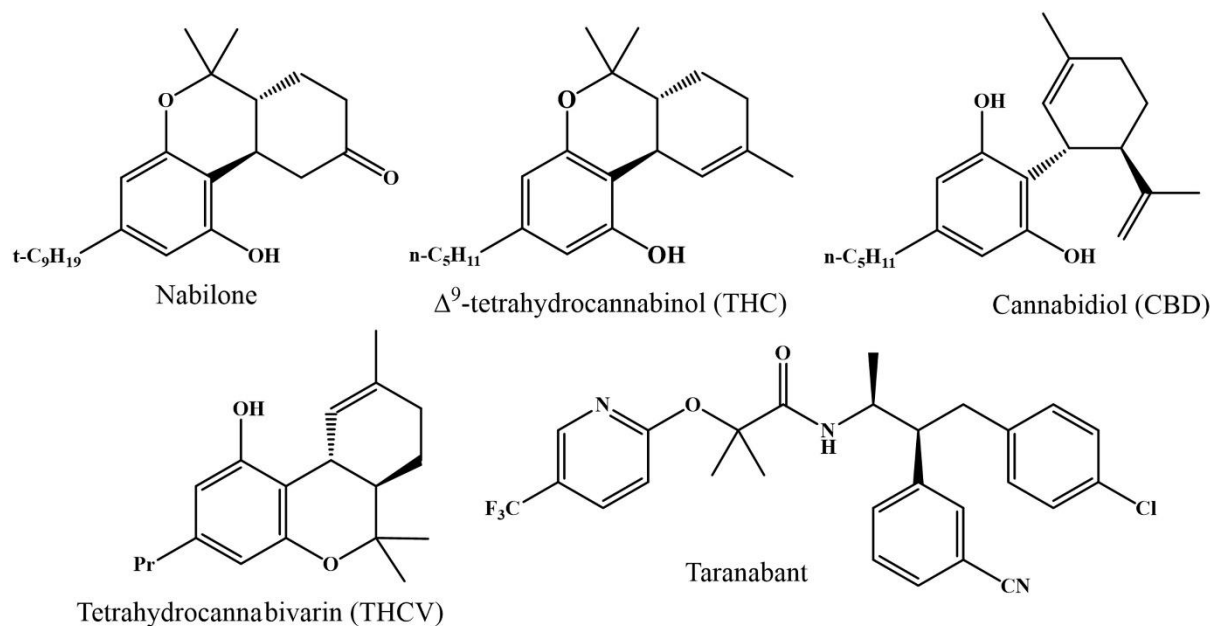


Figure 1. Structure of natural and commercial drugs used for disorders related to cannabinoid receptors.

The most prominent cannabinoid ligands are Δ^9 -tetrahydrocannabinol (THC) and cannabidiol (CBD) (Figure 1), psychoactive compounds relatively abundant in cannabis [12-14]. Endocannabinoids are other classifications of ligands that the body naturally produces: anandamide (AEA) [15], 2-arachidonoylglycerol (2-AG), virodhamine (OAE), 2-arachidonoyl glyceryl ether, N-arachidonoyl-dopamine (NADA) as well as lysophosphatidylinositol (LPI)[16] and synthetic cannabinoids; tetrahydrocannabivarin (THCV) [17], taranabant [18], and nabilone [19,20] (Figure 1).

Several studies present that the pharmacologic regulation of CB1 receptor is a therapeutic strategy for a wide range of central nervous system disorders [21-23]. Meanwhile, CB2 is an interesting therapeutic target for pain management, immunomodulators, treatment of liver diseases, osteoporosis, and others [24-28]. CB1 and CB2 cannabinoid receptors have been analyzed as targets for exhaustive drug development. The studies recommend that the pharmacologic regulation of CB1 receptors could be proposed as a therapeutic strategy in many human disorders [29,30]. Therefore, elucidating the detailed structure of interactions between ligands and CB1 and CB2cannabinoid receptors will facilitate the understanding of how antagonists or agonists participated in modulating the cannabinoid system downstream signaling. Cannabinoid system becomes an attractive area of research for the design of new drugs, spirostans an important kind of compound to be surveyed. Spirostans are natural compounds found as aglycones in plants and have been studied for medical purposes [31]. In this way, we performed an *in silico* study of 155 spirostans (Sp) to obtain specific candidates as novel and direct agonists of the cannabinoid receptors CB1 and CB2. The results open new doors for further investigations for the development of new drugs and pharmacological treatments for human diseases.

2. Materials and Methods

2.1. Ligands.

The 3D structures of cannabinoid references (CR) were obtained from the DrugBank database [32] under codes: cannabidiol (DB09061), THC (DB00470), THCV (DB11755), nabilone (DB00486) and taranabant (DB06624). The proposed 155 Sp were drawn by means of ChemDraw. All structures (CR and Sp) were prepared as with previously reported protocols [31] using Chem Professional 17.1, Chem3D 17.1, MM2 [33], and MOPAC (<http://openmopac.net/>).

2.2. Receptors.

The structure of crystallized proteins was obtained from the Protein Data Bank (<https://pubchem.ncbi.nlm.nih.gov/>) for CB₁ (PDB ID: 5TGZ) [34] and CB₂ (PDB ID: 5ZTY [35]). Proteins were built by adding polar hydrogens and optimized with Autodock Tools [36].

2.3. Docking protocol.

Docking studies were performed considering the previously reported protocols [31], Autodock Vina [37] for reference drugs, and Sp. For the re-docking of ZDG [34] in CB₁ RMSD of 0.69 Å, and 9JU [35] in CB₂ RMSD 0.98 Å.

2.4. Assessment.

Employing SwissTargetPrediction [38], the selective biological potential of Sp for cannabinoid receptors CB₁ and CB₂ was determined. Subsequently, molecular docking evaluated results *in silico*, comparing the coupling energy of each Sp with the reference cannabinoid drug. Data were analyzed statistically by the Kruskal-Wallis test [31, 39] ($p < 0.05$) to determine a probable significant difference between Sp functionality and receptor selectivity. To determine the activated or blocked potential, the interactions of the Sp were analyzed according to the interactions observed for the reference cannabinoid drugs and reported as a percentage of similarity.

3. Results and Discussion

Molecular docking was performed for the 155 Sp from the combination of the properties of the chemical groups and stereochemistry in all stereocenters (see Figure 2). The energetic comparison was evaluated using 2 natural cannabinoids: THC and CBD, and 3 commercial drugs: taranabant, tetrahydrocannabivarin (THCV), and nabilone.

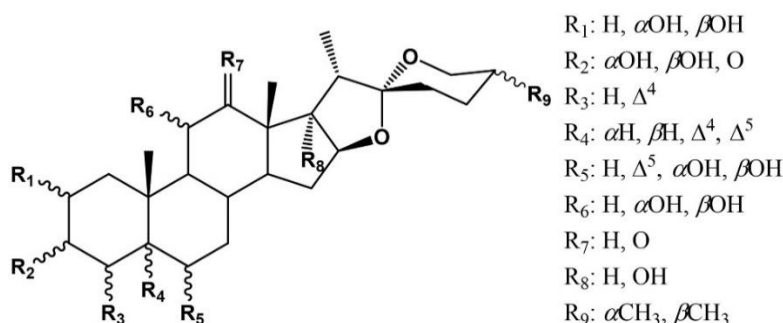


Figure 2. Combinations of functionalization and stereochemistry at the 155 designed spirostans (Sp). For a detailed description of the 155 structures, see Table 5, at the end of this article.

The interest in searching for new compounds is due to the low selectivity of cannabinoids used in several related treatments because they interact with both CB1 and CB2 cannabinoid receptors simultaneously. That is the case of the CR used in this study: CBD, THC, THCV, nabilone, and taranabant, which all have a similar active site (Figure 3). The binding coupling energy (BCE, kcal/mol) for CR is similar in both receptors but in different quantities between them. Starting on the need for selective compounds with CB2 for better anti-inflammatory and analgesic activity and decreased or null psychotropic effect, a study was carried out with SwissTargetPrediction of the 155 Sp, and the probability of interaction for the cannabinoid receptors CB1 and CB2 was obtained using the 50+1 criteria. The probability of interaction was 57 and 53% for CB1 and CB2, respectively. Another important value for the analysis was the selectivity ratio between CB1 and CB2 receptors (k_{CB1}/k_{CB2}); from this ratio, we can compare if a molecule could exhibit specific activity in a single receptor. All CR did not present selectivity; the ratios were close to 1.0-0.65 and 1.0-0.62 for THC and nabilone, respectively. Both drugs showed a slight selectivity for CB2 receptors. CBD and taranabant had 1.0-1.38 and 1.0-1.52 selectivity for CB1; THCV had the same selectivity for both CB1 and CB2 receptors (Figure 3).

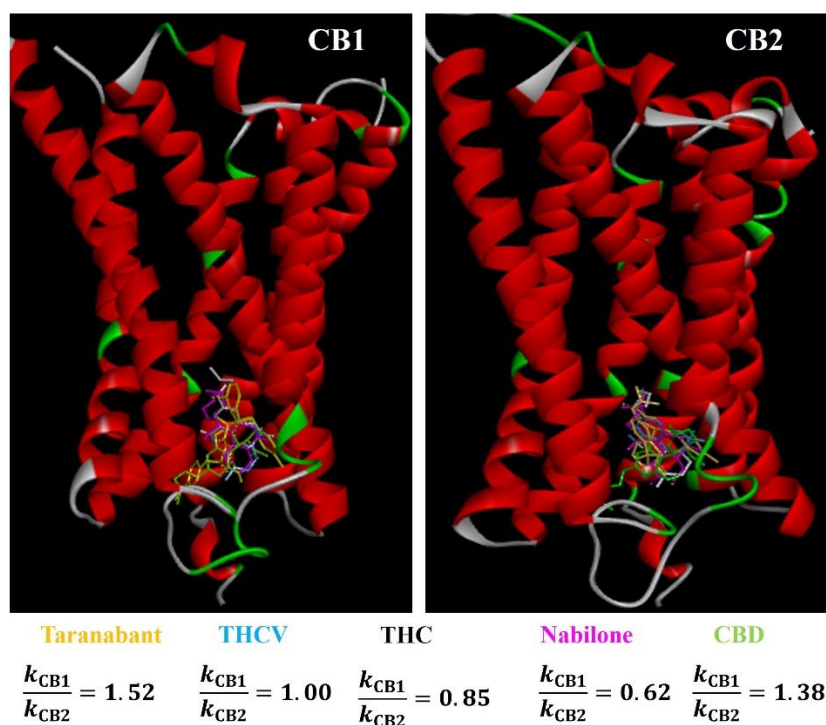


Figure 3. Selective ratio of cannabinoid references towards the CB1 and CB2 active sites.

3.1. Energetic analysis.

3.1.1. Sp energy trend.

For reference cannabinoids drugs, no significant difference was found between binding coupling energies (BCE) for CB1 and CB2 receptors. Whereas, for the Sp, the BCE at both receptors were scattered values; the trend of these energies is shown in Figure 4.

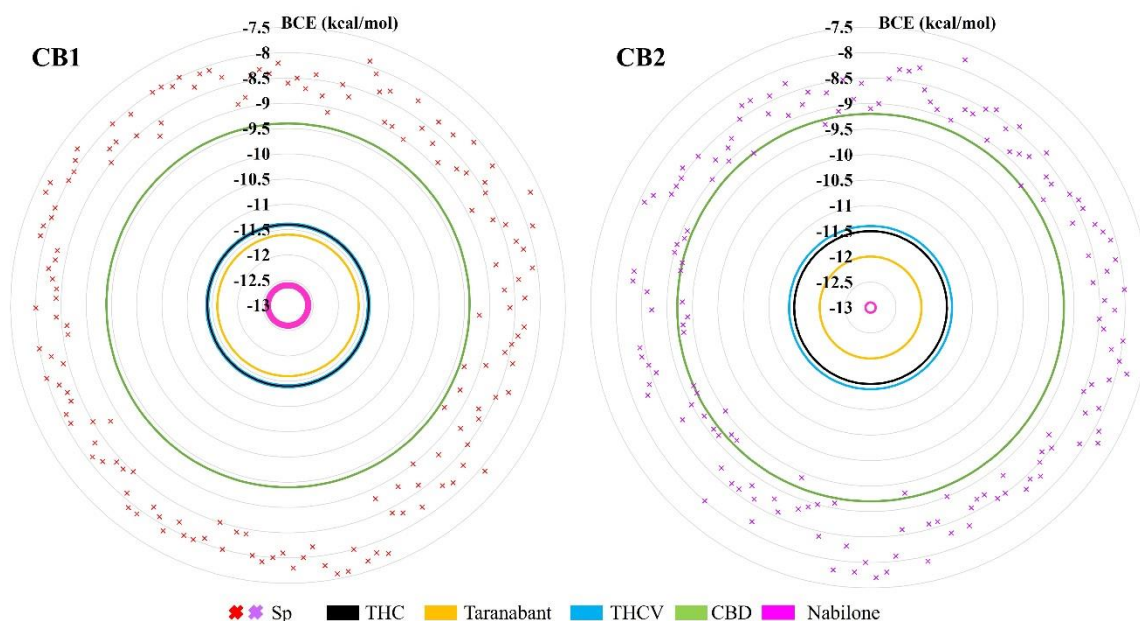


Figure 4. Comparative BCE (kcal/mol) between CB1, CB2 receptors, and Sp, or reference compounds.

In both illustrations, the BCE energies for Sp are not as good as for the reference compounds; generally, the values are close to cannabidiol. Specifically, for CB1 there are 4 Sp with similar activity to CBD; 2 of them were statistically the same, but none were better. For CB2 receptors, a greater number of Sp compounds resulted in positive, close to CBD activity; 3 of them were better, 6 had the same BCE value, and 15 more were statistically the same. Taking into account only the energy values of specific interaction of Sp with CB2 but not with CB1, a useful guide was revealed. The latter could be interesting for new drugs to treat chronic and cancer pain, eliminating psychotropic effects. In the design of molecules, various functions and stereochemical descriptors were considered (see Table S1 at the end of the article). Knowing that Sp has a possible high activity to CB2, statistical analysis was launched to determine the effect of each descriptor.

3.1.2. Statistical analysis.

The BCE values have a non-normal distribution, so a Kruskal-Wallis test was performed to determine the statistically significant differences ($p < 0.05$), which are summarized in Table 1. There was no significant difference between the stereogenic center descriptors at C-3, C-4, C-12, and C-25 and both receptors (CB1 and CB2); for C-17 in CB1, there were no changes, and neither for CB2 at C-5.

Table 1. Data of BCE for SP and CB1, CB2 receptors interactions.

C-#	CB1		CB2	
	Statistical Difference	Better BCE to CB1	Statistical Difference	Better BCE to CB2
C-2	* +	H, α OH β OH	*	H α OH, β OH
C-3		No		No
C-4		No		No
C-5	* +	Δ^5 , α H Δ^4 β H, α OH		No
C-6	*	Δ^5 , O, H α OH, β OH	*	O, α OH α OH, Δ^5 , β OH, H
C-11	*	H, O, α OH O, α OH, β OH	*	O, H α OH, β OH
C-12		No		No
C-17		No	*	H OH
C-25		No		No

*Functionalization difference, +difference due to stereochemistry. Kruskal-Wallis test, $p < 0.05$.

When there was a statistical difference from Sp, the stereochemistry of the hydroxyl groups did not affect the BCE value, but the functional group did. An example of this phenomenon is shown in Figure 5: for the substituents in C-2 with CB1, a better BCE occurred from the presence of a hydrogen atom or an α -hydroxyl group, having both substituents more interactions with the aliphatic zone of the molecule due to van der Waals and alkyl type interactions. Differently, worse BCE occurred when a β -hydroxyl group was present at C-2; the interactions in the same area decreased, and all interactions with the amino acids changed. This situation suggests that the binding site changed, although the type of interactions was maintained. Consequently, the BCE decreased considerably. In other cases, with the statistical difference, such as substitutions at C-5, C-6, or C-11, the interaction site did not change. Still, the distance between the interaction helices was bigger, which generated the binding force decrease.

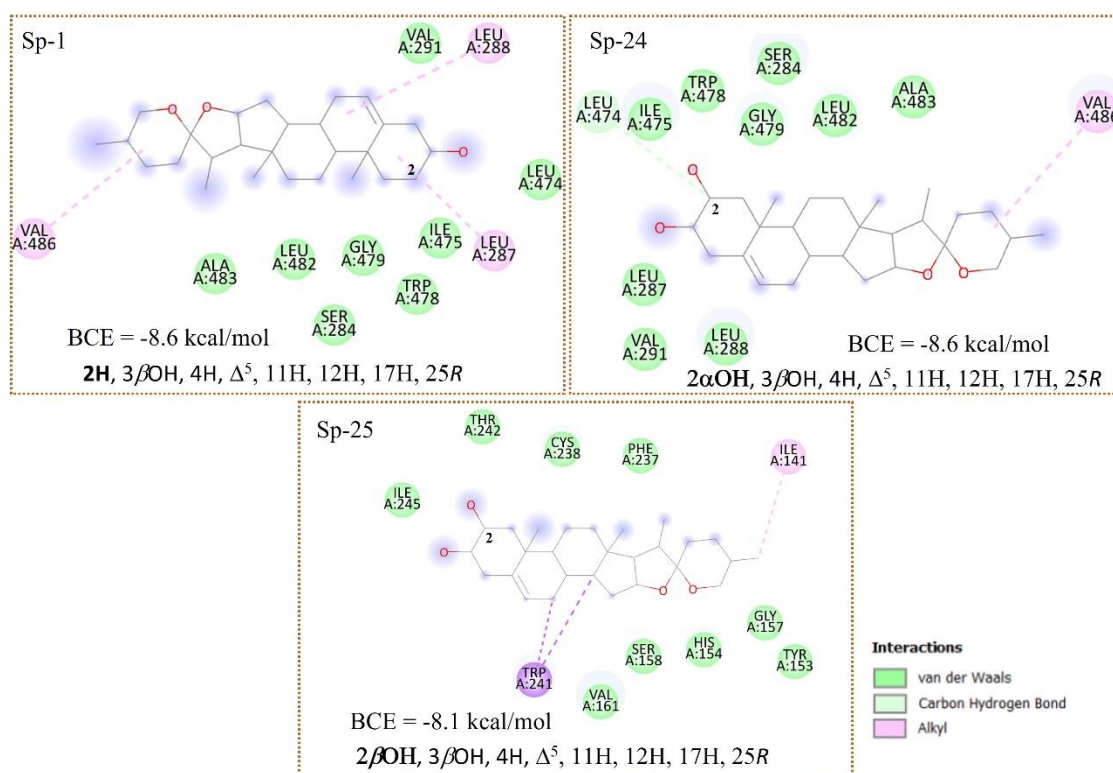


Figure 5. Sp with the statistical difference due to substitution at C-2 at the CB1 receptor site. Kruskal-Wallis test, $p < 0.05$.

An example without statistical difference was obtained from compounds having a hydrogen atom or a hydroxyl group at C-17 (Figure 6) in an α orientation. That did not generate a considerable modification in the BCE value; the change occurred in the interaction with the amino acids. From the 2D diagram, a slight displacement can be appreciated; therefore, a slight movement favors more interactions of the aliphatic area of the molecule. This effect was produced similarly in other centers such as C-3, C-4, or C-12; the functional groups do not generate a great change in the orientation or the type of interactions. Therefore, the energies do not either.

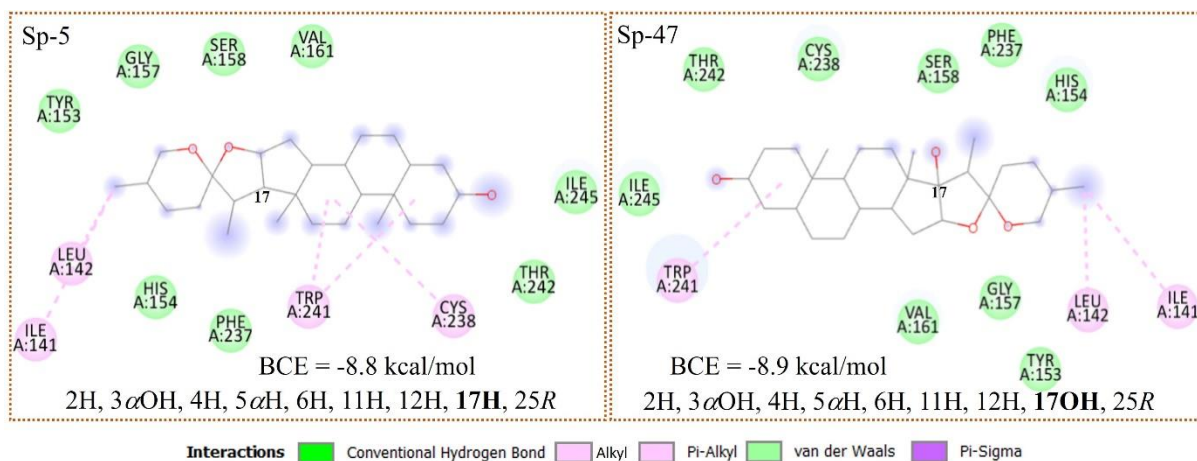


Figure 6. Sp without statistical difference in C-17 at CB1 receptor. Kruskal-Wallis test, $p < 0.05$.

Unlike the case for C-17 with CB1, for CB2, the presence of the substituents in this center does generate a statistically significant change (Figure 7). In this case, both molecules presented the greatest interaction in the aliphatic zone: when the hydrogen is present, it generates a slight repulsion, thus moving the Sp away from the receptor amino acids, and the interaction with Ile A:256 is lost. Even more, when C-17 supports a hydrogen atom, the molecule approaches the amino acids, and this generates the formation of a hydrogen bond with Leu A:255, which generates a better BCE.

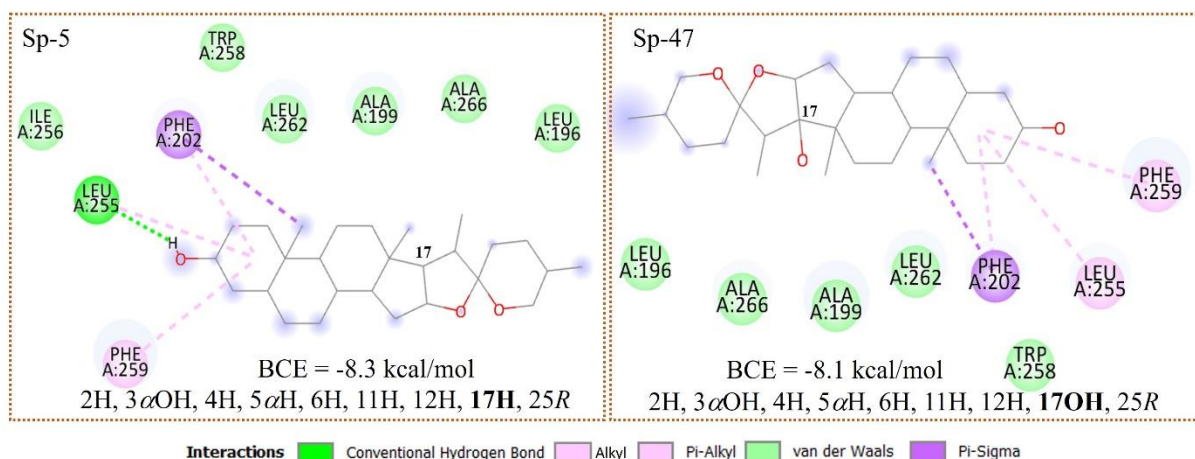


Figure 7. Sp with the statistical difference in C-17 at the CB2 receptor. Kruskal-Wallis test, $p < 0.05$.

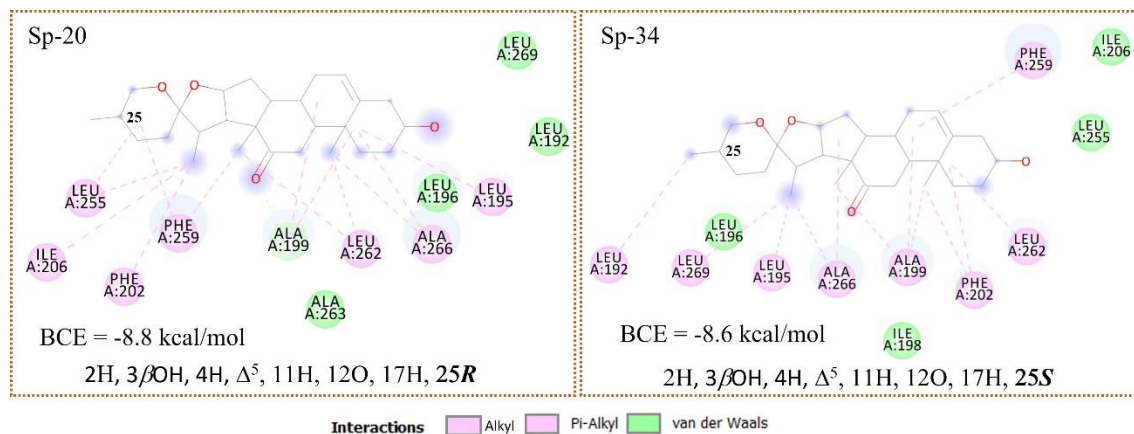


Figure 8. Sp without statistical difference in C-25 at the CB2 receptor. Kruskal-Wallis test, $p < 0.05$.

For the case of C-25, the statistical analysis showed no effect due to the orientation of the methyl group at C-25: axial (ax) or equatorial (eq). Figure 8 shows that both molecules are partially out of phase; the center of both is between the binding site helices. This fact is due to some interacting amino acids present on both sides of the same side, but some higher than others; that is also the case of the alkyl interaction with ax-CH₃ and eq-CH₃, with Leu A:192 and Leu A:255, which produce interaction with Sp-34 and Sp-20, respectively.

From the above observations, 2 Sp (Figure 9a) displays good interaction with CB1 and 24 Sp (Figure 9b) with CB2, selecting those from the statistical point of view as similar, or better BCE, confirming the greater interaction of these derivatives with CB2. To select the best Sp for each receptor, it was necessary to analyze each molecule's binding site and compare it to that of the reference drugs.

3.1.3. Interactional analysis.

From the analysis of 3D structures (Figure 9), the CBD binding site can be established between all the lower parts of the helices and CB1 and CB2 receptors, being like all CR. It is important to note that Sp naturally trends to be bound at a different site producing a competitive activity, as can be seen with respect to CBD, which is the energetically analogous cannabinoid. However, to determine a similarity between these Sp vs. reference drugs, it was necessary to analyze the molecular docking at the active site.

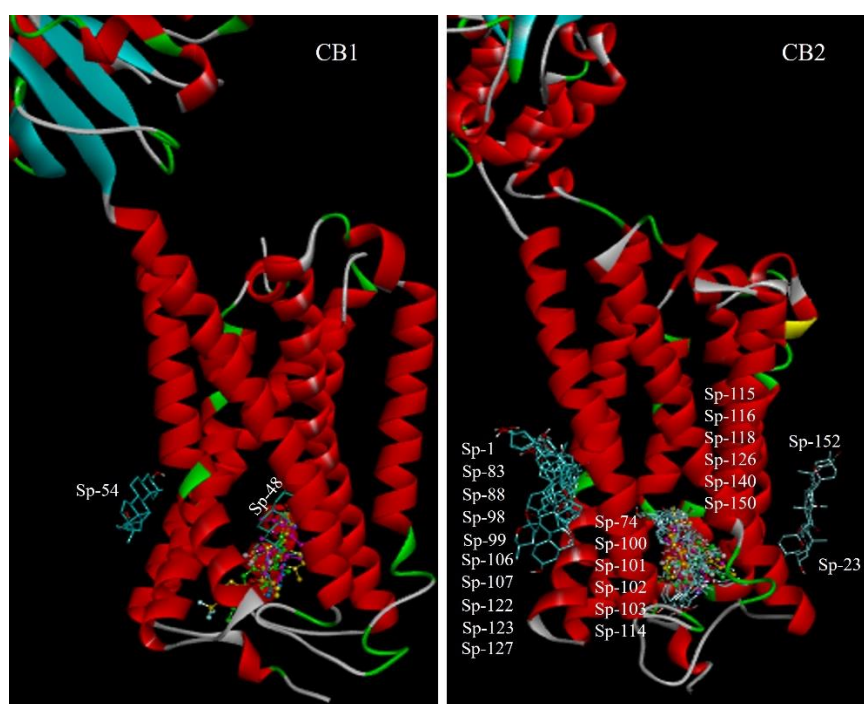


Figure 9. 3D model of CB1 and CB2 receptors interacting with Sp molecules.

For CB1, only Sp-48 binds at the active site, and comparing the interacting amino acid residues with those binded at reference molecules shows a similarity greater than 60% (Table 2). Only half of the interactions with CB2 receptors at the 24 chosen Sp molecules could bind the active site, and the similarity of amino acids was determined. For CBD, the interactions were poor because all Sp were vertically oriented at the receptor, and interactions were favored with the aromatic rings and not with oxygenated substituents. For taranabant, Sp-102 residues reached 83%, attributed to interactions with oxygenated substituents.

The Sp do not have better binding coupling energies to the reference's drugs except CBD. Despite that, when the interaction site is reduced, the similarity is better with tetrahydrocannabivarin: 75% as a minimum. With Sp-114, Sp-115, and Sp-126, the observed value was 90%, which is important to the interaction of these compounds' residues. The greater interaction with the A ring and the spiroketal of structures stands out. For THC residues, Sp-126 presented 95% of similarity. For commercial drug nabilone residues, the Sp presented low identity with a maximum of 87% for Sp-115, as can be appreciated in the table of percentages (Table 2); the same Sp had better interactions at the different CR.

Table 2. Aminoacid similarity (%) at the CB1 and CB2 receptor binding sites.

CR	CB1		CB2				
	Sp-48	Sp-74	Sp-100	Sp-101	Sp-102	Sp-103	
CBD	76.5	57.9	84.2	78.9	68.4	68.4	
Taranabant	68.0	75.0	66.7	70.8	83.3	66.7	
THCV	82.4	80.0	80.0	85.0	75.0	75.0	
THC	81.0	81.0	85.7	90.5	81.0	81.0	
Nabilone	81.0	78.3	69.6	73.9	78.3	69.6	

CR	CB2						
	Sp-114	Sp-115	Sp-116	Sp-118	Sp-126	Sp-140	Sp-150
CBD	78.9	73.7	78.9	57.9	84.2	63.2	78.9
Taranabant	75.0	79.2	75.0	79.2	75.0	75.0	75.0
THCV	90.0	90.0	85.0	70.0	90.0	80.0	85.0
THC	85.7	90.5	90.5	76.2	95.2	81.0	90.5
Nabilone	82.6	87.0	78.3	73.9	78.3	78.3	78.3

CR, cannabinoid reference

3.1.4. Sp as agonist for CB1 and CB2 receptors.

For CB1 receptors, only the spirostan Sp-48 could regulate it; this molecule is classified as statistically equal to CBD: interacts at the active site and exhibits more than 70% similarity to key aminoacids (Table 3), compared to CBD and THC (Figure 10). Of these interactions, we can highlight Ser A:505 with a hydrogen bond and Phe A:268 with alkyl interactions. Sp-48 presented unfavorable van der Waals interactions. The site interaction probability is higher for Sp-48 on CB1. Still, the most important analysis is to compare the interaction rate constants, e.g., Sp-48 has a bigger selectivity 4.3 times for CB1 than for CB2 receptors, favoring interaction with this receptor more than to CR.

Table 3. Molecular docking between CB1 amino acids and reference cannabinoids or Sp.

CR	BCE (kcal/mol) to CB1	van der Waals and alkyl interactions	Hydrogen bond interaction	Unfavorable bump and halogen bond interactions
CBD	-9.4	Pro A:269; Phe A:177; Phe A:189; Phe A:268; Leu A:193; Val A:196; Phe A:170; Ser A:173; Phe A:174; Lys A:498; Phe A:108; Asp A:266; Phe A:501; Ala A:502; Ile A:267; His A:178	Ser A:505	
Taranabant	-11.6	Ser A:173; Lys A:192; Leu A:193; Phe A:170; Val A:196; Trp A:279; Thr A:197; Met A:485; Phe A:200; Phe A:268; Ile A:267; Thr A:499; Phe A:108; Leu A:111; Met A:109; Phe A:501; Ala A:502; Phe A:174; Pro A:269; Phe A:177; Ser A:505; Phe A:189	Val A:110	Lys A:498; His A:178
THCV	-11.4	Thr A:197; Leu A:193; Ser A:173; Phe A:189; Lys A:192; Phe A:177; Pro A:269; Phe A:108; Ile A:267; His A:178; Phe A:174; Phe A:501; Phe A:268; Phe A:170; Val A:196; Phe A:200	Ser A:505	
THC	-11.4	Phe A:108; Ile A:267; Pro A:269; His A:178; Phe A:174; Phe A:501; Thr A:197; Leu A:481; Trp A:279; Trp A:478; Phe A:200; Cys A:508; Phe	Ser A:505	

CR	BCE (kcal/mol) to CB1	van der Waals and alkyl interactions	Hydrogen bond interaction	Unfavorable bump and halogen bond interactions
		A:268; Val A:196; Phe A:170; Cys A:508; Leu A:193; Ser A:173; Lys A:192; Phe A:189; Phe A:177		
Nabilone	-12.6	Pro A:269; His A:178; Phe A:174; Ile A:267; Phe A:501; Leu A:481; Met A:485; Thr A:197; Tyr A:275; Ile A:271; Leu A:276; Trp A:279; Phe A:200; Phe A:170; Phe A:268; Leu A:193; Val A:196; Ser A:173; Phe A:189; Phe A:177	Ser A:505	
Sp-48	-9.3	Pro A:269; Phe A:177; Phe A:189; Leu A:193; Val A:196; Phe A:170; Ser A:505; Phe A:174; Phe A:108; Phe A:501; Ile A:267; His A:178; Thr A:197; Phe A:200; Thr A:201; Trp A:279; Trp A:478; Leu A:481; Met A:485		Phe A:268

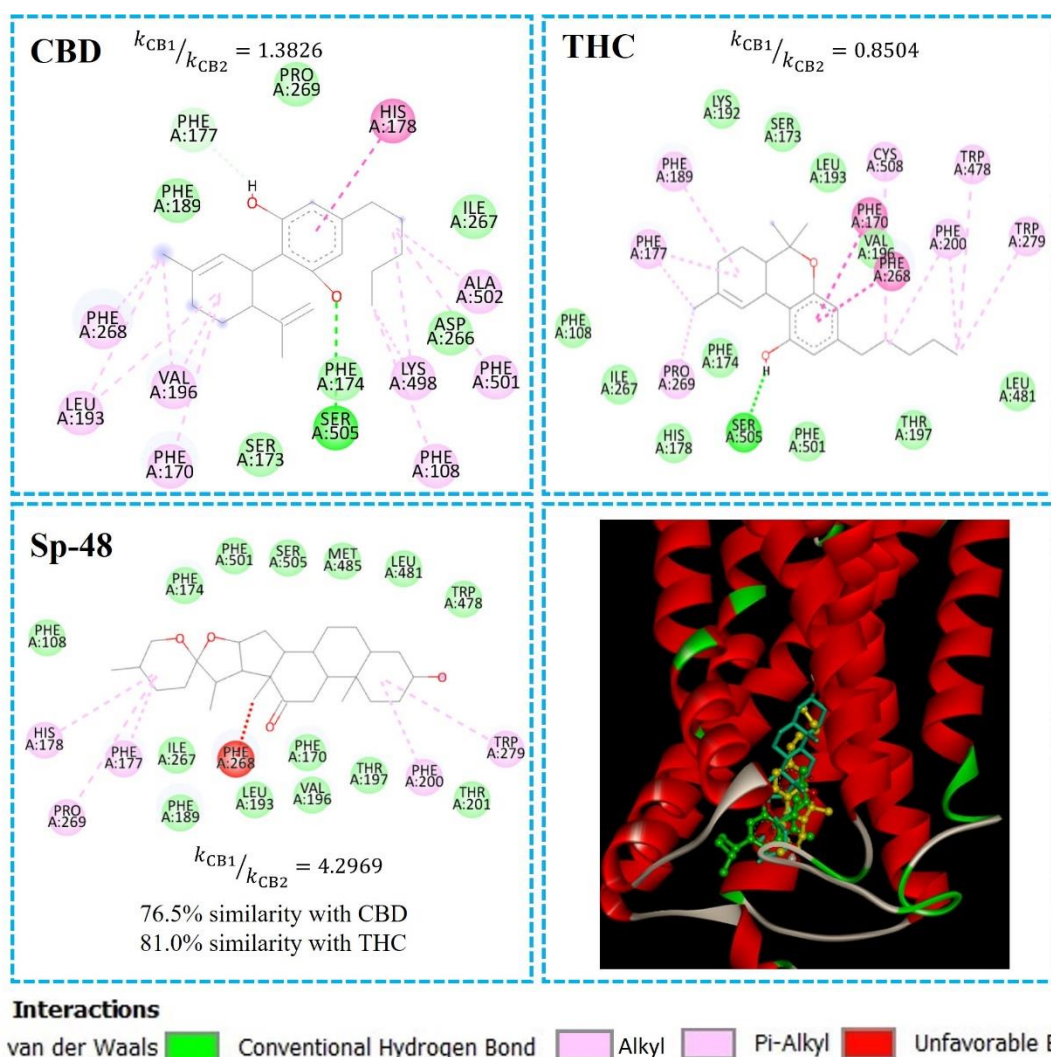


Figure 10. Sp shows the best interactions with CB1 receptors.

Figure 11 shows 3 Sp molecules (Sp-114, Sp-115, and Sp-126) that were the best for selective interaction with the CB2 receptor; these molecules were classified as statistically equal to CBD by their BCE, and all of them can interact in the active site of CBD and THC, and by the percentage of similarity with the amino acids, the minimum was 73.7 and 85.7%, respectively. This high interaction similarity towards the residues (Table 4) can be highlighted: a hydrogen bond is observed for Ser A:505 to THC, which for Sp is presented as a van der Waals interaction, as for CBD. Leu A:182 presents a hydrogen bond and an unfavorable bump

with Sp-116 and Sp-126, respectively, changing the intensity of van der Waals-type and alkyl-type interactions with the CR. In summary, the compounds Sp-114, Sp-115, and Sp-126 would be good candidates for drugs due to their high interaction strength and because they can interact in the CB2 active site. However, the most relevant and novel thing is their selectivity towards only one of the CR; in this balance, CB2 has 3 and 5 times higher interactions than CB1.

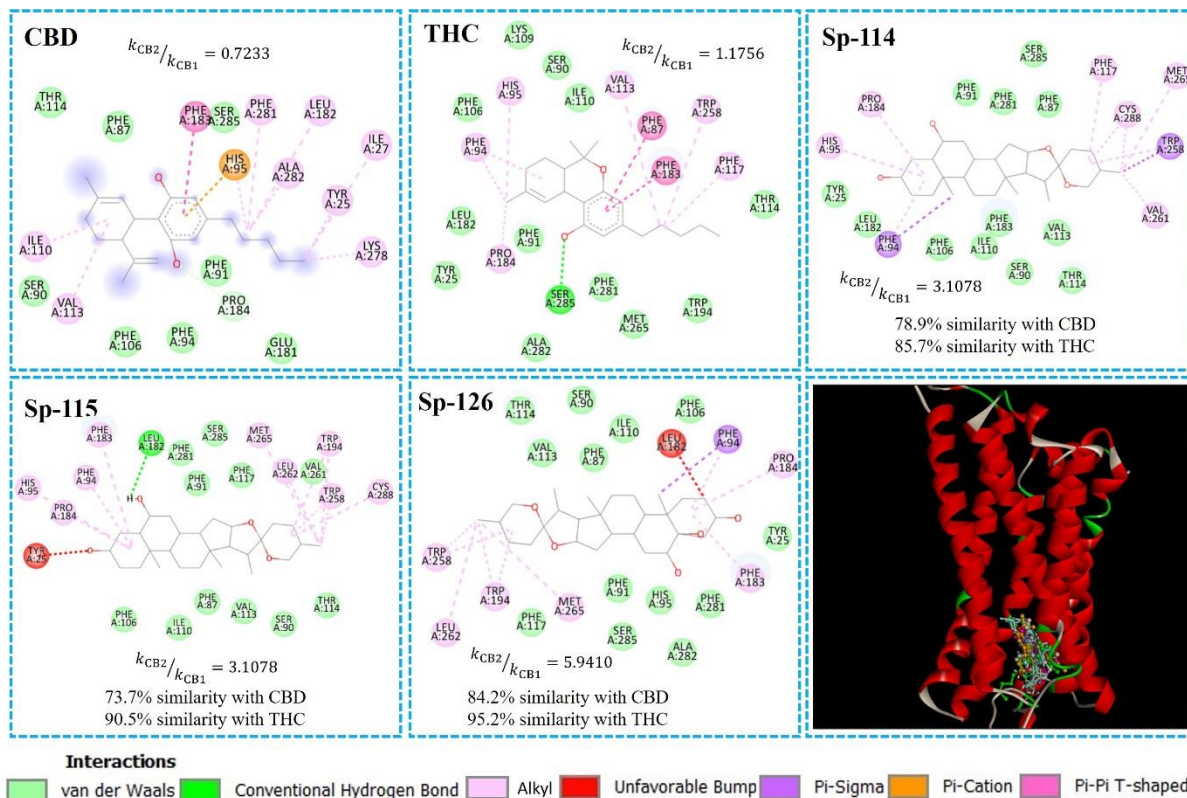


Figure 11. The best Sp selected for their CB2 receptor interaction.

Table 4. Results of molecular docking between CB2 aminoacids and CR or Sp.

CR	BCE (kcal/mol) to CB1	van der Waals and alkyl interactions	Hydrogen bond interaction	Unfavorable bump and halogen bond interactions
CBD	-9.2	Thr A: 114; Phe A:87; Phe A:183; Ser A:285; His A:95; Phe A:281; Leu A:182; Ala A:282; Ile A:27; Tyr A:25; Lys A:278; Phe A:91; Pro A:184; Glu A:181; Phe A:94; Phe A:106; Val A:113; Ser A:90; Ile A:110		
Taranabant	-12.0	Leu A:182; His A:95; Pro A:184; Ser A:90; Lys A:109; Phe A:106; Phe A:94; Val A:113; Trp A:258; Ile A:110; Thr A:114; Trp A:194; Ile A:186; Leu A:191; Tyr A:190; Pro A:168; Met A:265; Phe A:183; Val A:261; Ser A:285; Phe A:87; Phe A:281; Phe A:91; Phe A:117		
THCV	-11.4	Trp A:258; Cys A:288; Phe A:87; Phe A:117; Phe A:281; Phe A:183; Met A:265; His A:95; Ala A:282; Phe A:91; Phe A:94; Pro A:184; Leu A:182; Tyr A:25; Phe A:106; Ser A:90; Lys A:109; Ile A:110; Val A:113		Ser A:285
THC	-11.5	Lys A:109; Ser A:90; His A:95; Phe A:106; Phe A:94; Leu A:182; Tyr A:25; Pro A:184; Phe A:91; Ala A:282; Phe A:281; Met A:265; Trp A:194; Thr A:114; Phe A:117; Trp A:258; Phe A:183; Phe A:87; Val A:113; Ile A:110	Ser A:285	
Nabilone	-12.9	Phe A:94; Leu A:182; Tyr A:25; Phe A:91; Phe A:281; Val A:261; Met A:265; Thr A:114; Ile A:186; Phe A:117; Ile A:110; Leu A:191; Cys A:288; Phe A:183; Val A:113; Phe A:87; Trp A:194; Lys A:109; Ser A:90; Phe A:106; Pro A:184	His A:95; Ser A:285	

CR	BCE (kcal/mol) to CB1	van der Waals and alkyl interactions	Hydrogen bond interaction	Unfavorable bump and halogen bond interactions
Sp-114	-9.3	Pro A:184; His A:95; Tyr A:25; Leu A:182; Phe A:94; Phe A:106; Ile A:110; Phe A:183; Ser A:90; Val A:113; Thr A:114; Val A:261; Trp A:258; Cys A:288; Met A:265; Phe A:117; Phe A:87; Ser A:285; Phe A:281; Phe A:91		
Sp-116	-9.3	Phe A:106; Ile A:110; Phe A:87; Val A:113; Ser A:90; Thr A:114; Cys A:288; Trp A:258; Val A:261; Trp A:194; Leu A:262; Met A:265; Phe A:117; Ser A:285; Phe A:91; -Phe A:281; Phe A:183; Phe A:94; Pro A:184; His A:95	Leu A:182	Tyr A:25
Sp-126	-9.1	Trp A:258; Trp A:194; Leu A:262; Phe A:117; Met A:265; Phe A:91; Ser A:285; His A:95; Ala A:282; Phe A:281; Phe A:183; Tyr A:25; Pro A:184; Phe A:94; Phe A:106; Ile A:110; Phe A:87; Ser A:90; Val A:113; Thr A:114		Leu A:182

4. Conclusions

Spirostanic compounds have a hydrophobic tetracyclic skeleton and could have hydrophilic sites at C-2, C-3, C-5, C-6, C-11, C-12, and C-17, that result in possible interactions with CB1 and CB2 receptors (SwissTargetPrediction). When performing the molecular docking from 155 Sp, the interactions with the active site of these receptors were obtained. Compared to CR, Sp behaves at the same level as CBD; these results suggest agonist activity at the orientation level, different action for THC. Sp supporting polar groups at C-6 and C-11 showed an increased BCE with respect to CB1 and CB2. At the rest of the structure, non-polar groups predominate or do not have a significant influence. At the level of interaction with respect to amino acid residues and type of interaction, the structures with the highest molecular coupling presented percentages greater than 80%, which indicates a high probability of acting as agonists, particularly due to their hydrophobic and van der Waals interactions. Considering these filters and observing the difference between BCE for CB1, only Sp-48 can be an agonist agent, exhibiting 4.3 times greater selectivity than CB2. CB2, Sp-114, Sp-115, and Sp-126 were the compounds that presented greater activity as agonists. Furthermore, the selectivity was higher than 3.1; in particular, for Sp-126-CB2, it was 5.9 vs. CB1.

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Conflicts of Interest

The authors declare no conflict of interest.

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Supplementary materials

Table S1. List of designed spirostans.

Sp	C-2 (R1)	C-3 (R2)	C4 (R3)	C-5 (R4)	C-6 (R5)	C-11 (R6)	C-12 (R7)	C-17 (R8)	C-25 (R9)	CB1 BCE (kcal/mol)	CB2 BCE (kcal/mol)
1	H	β OH	H	Δ		H	H	H	R	-8.6	-9.1
2	H	α OH	H	Δ		H	H	H	R	-8.5	-9.0
3	H	β OH	H	Δ		H	H	H	S	-8.7	-8.5
4	H	α OH	H	Δ		H	H	H	S	-8.4	-8.3
5	H	α OH	H	α H	H	H	H	H	R	-8.8	-8.3
6	H	β OH	H	α H	H	H	H	H	R	-9.1	-8.2
7	H	α OH	H	α H	H	H	H	H	S	-8.5	-8.6
8	H	β OH	H	α H	H	H	H	H	S	-8.7	-8.8
9	H	α OH	H	β H	H	H	H	H	R	-7.9	-8.9
10	H	β OH	H	β H	H	H	H	H	R	-8.1	-7.8
11	H	α OH	H	β H	H	H	H	H	S	-8.4	-9.0
12	H	β OH	H	Δ		H	H	H	S	-8.3	-8.7
13	H	α OH	H	Δ		H	O	H	R	-8.9	-8.7
14	H	α OH	H	Δ		α OH	H	H	R	-8.9	-8.5
15	H	α OH	H	Δ		β OH	H	H	R	-8.2	-8.4
16	H	α OH	H	Δ		O	H	H	R	-9.0	-9.0
17	α OH	α OH	H	Δ		H	H	H	R	-8.6	-8.6
18	β OH	α OH	H	Δ		H	H	H	R	-8.3	-8.4
19	H	α OH	H	Δ		H	H	OH	R	-8.7	-8.9
20	H	β OH	H	Δ		H	O	H	R	-8.3	-8.8
21	H	β OH	H	Δ		α OH	H	H	R	-8.9	-8.7
22	H	β OH	H	Δ		β OH	H	H	R	-8.1	-8.4
23	H	β OH	H	Δ		O	H	H	R	-8.7	-9.2
24	α OH	β OH	H	Δ		H	H	H	R	-8.6	-8.7
25	β OH	β OH	H	Δ		H	H	H	R	-8.1	-8.7
26	H	β OH	H	Δ		H	H	OH	R	-8.4	-9.0
27	H	α OH	H	Δ		H	O	H	S	-8.5	-8.5
28	H	α OH	H	Δ		α OH	H	H	S	-8.9	-8.4
29	H	α OH	H	Δ		β OH	H	H	S	-7.7	-8.4
30	H	α OH	H	Δ		O	H	H	S	-8.6	-8.7
31	α OH	α OH	H	Δ		H	H	H	S	-8.4	-8.3
32	β OH	α OH	H	Δ		H	H	H	S	-7.9	-8.2
33	H	α OH	H	Δ		H	H	OH	S	-8.3	-8.1
34	H	β OH	H	Δ		H	O	H	S	-8.2	-8.6
35	H	β OH	H	Δ		α OH	H	H	S	-8.8	-8.1
36	H	β OH	H	Δ		β OH	H	H	S	-8.1	-8.3
37	H	β OH	H	Δ		O	H	H	S	-8.4	-8.6
38	α OH	β OH	H	Δ		H	H	H	S	-8.7	-8.0
39	β OH	β OH	H	Δ		H	H	H	S	-8.3	-8.4
40	H	β OH	H	Δ		H	H	OH	S	-8.6	-8.2
41	H	α OH	H	α H	H	H	O	H	R	-9.2	-8.5
42	H	α OH	H	α H	H	α OH	H	H	R	-8.3	-8.4
43	H	α OH	H	α H	H	β OH	H	H	R	-8.4	-8.0
44	H	α OH	H	α H	H	O	H	H	R	-8.5	-8.2
45	α OH	α OH	H	α H	H	H	H	H	R	-8.6	-8.0
46	β OH	α OH	H	α H	H	H	H	H	R	-8.4	-7.8
47	H	α OH	H	α H	H	H	H	OH	R	-8.9	-8.1
48	H	β OH	H	α H	H	H	O	H	R	-9.3	-8.4
49	H	β OH	H	α H	H	α OH	H	H	R	-8.3	-8.5
50	H	β OH	H	α H	H	β OH	H	H	R	-8.5	-8.1
51	H	β OH	H	α H	H	O	H	H	R	-9.2	-8.3
52	α OH	β OH	H	α H	H	H	H	H	R	-8.7	-7.9
53	β OH	β OH	H	α H	H	H	H	H	R	-8.6	-7.8
54	H	β OH	H	α H	H	H	H	OH	R	-9.3	-8.1
55	H	α OH	H	α H	H	H	O	H	S	-8.8	-8.7
56	H	α OH	H	α H	H	α OH	H	H	S	-8.5	-8.5
57	H	α OH	H	α H	H	β OH	H	H	S	-7.9	-8.6
58	H	α OH	H	α H	H	O	H	H	S	-8.3	-8.5
59	α OH	α OH	H	α H	H	H	H	H	S	-8.8	-8.3
60	β OH	α OH	H	α H	H	H	H	H	S	-8.2	-8.4
61	H	α OH	H	α H	H	H	H	OH	S	-8.3	-8.3
62	H	β OH	H	α H	H	H	O	H	S	-9.0	-8.8

Sp	C-2 (R1)	C-3 (R2)	C4 (R3)	C-5 (R4)	C-6 (R5)	C-11 (R6)	C-12 (R7)	C-17 (R8)	C-25 (R9)	CB1 BCE (kcal/mol)	CB2 BCE (kcal/mol)
63	H	β OH	H	α H	H	α OH	H	H	S	-8.6	-8.5
64	H	β OH	H	α H	H	β OH	H	H	S	-8.2	-8.5
65	H	β OH	H	α H	H	O	H	H	S	-8.8	-8.7
66	α OH	β OH	H	α H	H	H	H	H	S	-8.3	-8.7
67	β OH	β OH	H	α H	H	H	H	H	S	-8.4	-8.6
68	H	β OH	H	α H	H	H	H	OH	S	-8.8	-8.3
69	H	α OH	H	β H	H	H	O	H	R	-7.7	-9.0
70	H	α OH	H	β H	H	α OH	H	H	R	-7.8	-8.3
71	H	α OH	H	β H	H	β OH	H	H	R	-7.7	-8.6
72	H	α OH	H	β H	H	O	H	H	R	-8.0	-8.6
73	α OH	α OH	H	β H	H	H	H	H	R	-7.6	-7.9
74	β OH	α OH	H	β H	H	H	H	H	R	-7.6	-9.3
75	H	α OH	H	β H	H	H	H	OH	R	-7.8	-8.6
76	H	β OH	H	β H	H	H	O	H	R	-8.2	-8.1
77	H	β OH	H	β H	H	α OH	H	H	R	-8.0	-7.8
78	H	β OH	H	β H	H	β OH	H	H	R	-7.8	-7.7
79	H	β OH	H	β H	H	O	H	H	R	-8.1	-8.0
80	α OH	β OH	H	β H	H	H	H	H	R	-8.0	-7.8
81	β OH	β OH	H	β H	H	H	H	H	R	-7.9	-8.3
82	H	β OH	H	β H	H	H	H	OH	R	-8.0	-7.8
83	H	α OH	H	β H	H	H	O	H	S	-8.4	-9.1
84	H	α OH	H	β H	H	α OH	H	H	S	-8.4	-8.4
85	H	α OH	H	β H	H	β OH	H	H	S	-8.0	-9.0
86	H	α OH	H	β H	H	O	H	H	S	-8.5	-8.9
87	α OH	α OH	H	β H	H	H	H	H	S	-8.1	-8.8
88	β OH	α OH	H	β H	H	H	H	H	S	-8.0	-9.4
89	H	α OH	H	β H	H	H	H	OH	S	-8.0	-8.6
90	H	β OH	H	β H	H	H	O	H	S	-8.2	-8.8
91	H	β OH	H	β H	H	α OH	H	H	S	-7.9	-8.2
92	H	β OH	H	β H	H	β OH	H	H	S	-8.1	-8.6
93	H	β OH	H	β H	H	O	H	H	S	-8.2	-8.8
94	α OH	β OH	H	β H	H	H	H	H	S	-7.9	-8.6
95	β OH	β OH	H	β H	H	H	H	H	S	-7.9	-8.6
96	H	β OH	H	β H	H	H	H	OH	S	-8.1	-8.0
97	α OH	β OH	H	Δ		H	O	H	R	-8.5	-8.7
98	H	β OH	H	H	α H	H	H	H	R	-8.4	-9.3
99	H	α OH	H	H	α H	H	H	H	R	-8.4	-9.3
100	H	α OH	H	H	α H	H	H	H	S	-8.2	-9.1
101	H	β OH	H	H	α H	H	H	H	S	-8.1	-9.1
102	H	β OH	H	H	β H	H	H	H	R	-8.3	-9.5
103	H	α OH	H	H	β H	H	H	H	R	-8.8	-9.1
104	H	α OH	H	H	β H	H	H	H	S	-8.5	-8.7
105	H	β OH	H	H	β H	H	H	H	S	-8.1	-9.0
106	H	β OH	H	H	α H	H	H	H	R	-8.1	-9.2
107	H	α OH	H	H	α H	H	H	H	R	-8.3	-9.2
108	H	α OH	H	H	α H	H	H	H	S	-8.3	-8.3
109	H	β OH	H	H	α H	H	H	H	S	-8.2	-8.4
110	H	β OH	H	H	α H	H	H	H	R	-8.0	-8.6
111	H	α OH	H	H	α H	H	H	H	R	-8.4	-8.5
112	H	α OH	H	H	α H	H	H	H	S	-8.2	-8.4
113	H	β OH	H	H	α H	H	H	H	S	-8.0	-8.4
114	H	β OH	H	H	β H	H	H	H	R	-8.6	-9.3
115	H	α OH	H	H	β H	H	H	H	R	-8.6	-9.3
116	H	α OH	H	H	β H	H	H	H	S	-8.4	-9.4
117	H	β OH	H	H	β H	H	H	H	S	-8.0	-8.7
118	H	β OH	H	H	β H	H	H	H	R	-8.4	-9.3
119	H	α OH	H	H	β H	H	H	H	R	-8.4	-8.6
120	H	α OH	H	H	β H	H	H	H	S	-8.3	-8.3
121	H	β OH	H	H	β H	H	H	H	S	-8.2	-8.3
122	H	β OH	H	H	α OH	H	H	H	R	-8.3	-9.2
123	H	α OH	H	H	α OH	H	H	H	R	-8.3	-9.2
124	H	α OH	H	H	α OH	H	H	H	S	-7.9	-9.0
125	H	β OH	H	H	α OH	H	H	H	S	-7.9	-9.0
126	H	β OH	H	H	α OH	H	H	H	R	-8.0	-9.1
127	H	α OH	H	H	α OH	H	H	H	R	-8.0	-9.1

Sp	C-2 (R1)	C-3 (R2)	C4 (R3)	C-5 (R4)	C-6 (R5)	C-11 (R6)	C-12 (R7)	C-17 (R8)	C-25 (R9)	CB1 BCE (kcal/mol)	CB2 BCE (kcal/mol)
128	H	α OH	H	H	α OH	H	H	H	S	-7.7	-8.1
129	H	β OH	H	H	α OH	H	H	H	S	-7.7	-8.2
130	H	β OH	H	H	α OH	H	H	H	R	-8.0	-8.5
131	H	α OH	H	H	α OH	H	H	H	R	-8.0	-8.5
132	H	α OH	H	H	α OH	H	H	H	S	-7.9	-8.5
133	H	β OH	H	H	α OH	H	H	H	S	-7.8	-8.4
134	H	O		Δ	H	H	H	H	R	-8.5	-9
135	H	O		Δ	H	H	H	H	S	-8.3	-8.5
136	H	O		Δ	α OH	H	H	H	R	-8.3	-8.8
137	H	O		Δ	α OH	H	H	H	S	-8.0	-9
138	H	O		Δ	β OH	H	H	H	R	-8.5	-8.8
139	H	O		Δ	β OH	H	H	H	S	-8.1	-8.7
140	H	O		Δ	O	H	H	H	R	-8.8	-9.2
141	H	O		Δ	O	H	H	H	S	-8.6	-8.5
142	α OH	O		Δ	H	H	H	H	R	-8.0	-8.3
143	α OH	O		Δ	H	H	H	H	S	-8.0	-8.3
144	β OH	O		Δ	H	H	H	H	R	-8.1	-8.7
145	β OH	O		Δ	H	H	H	H	S	-8.0	-8.4
146	H	O		Δ	H	α OH	H	H	R	-8.3	-8.9
147	H	O		Δ	H	α OH	H	H	S	-8.1	-8.3
148	H	O		Δ	H	β OH	H	H	R	-8.1	-8.8
149	H	O		Δ	H	β OH	H	H	S	-8.3	-8.6
150	H	O		Δ	H	O	H	H	R	-8.9	-9.3
151	H	O		Δ	H	O	H	H	S	-8.8	-8.7
152	H	O		Δ	H	H	O	H	R	-8.4	-9.1
153	H	O		Δ	H	H	O	H	S	-8.3	-8.5
154	H	O		Δ	H	H	H	OH	R	-8.4	-8.9
155	H	O		Δ	H	H	H	OH	S	-8.2	-8.6