

Scientific reseARCh article / <http://dx.doi.org/10.15446/rcciquifa.v51n1.102679>

Tachykinin/neurokinin 3 receptor antagonists: *In silico* ADME/T analysis of novel compounds for menopause hot flashes therapy

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Received: March 31, 2021

Corrected: May 28, 2021

Accepted: June 4, 2021

SUMMARY

Introduction: Neurokinin-B receptor (NK3R) activation is tightly involved in the onset of vasomotor symptoms during menopause, yet there are still no NK3R antagonistic drugs approved for hot flashes therapy. Determining the pharmacokinetic properties of current drug candidates is crucial for scaffold identification and prediction of feasible outcomes in future clinical trials. **Aim:** To develop a pharmacokinetic profile of new NK3R blockers with hot flashes reducing activity and by comparing them with trial-suspended NK3R antagonists (Osanetant & Talnetant), it is expected to identify enhanced properties in novel compounds. **Methodology:** For *in silico* evaluation, Smiles were retrieved from PubChem and DrugBank, and further analysis was carried out through ADMETlab and SwissADME to calculate compounds drug-likeness and pharmacokinetics. **Results:** Pavinetant & Fezolinetant and SB-222.200 & SB-218.795 exhibited higher compliance with drug-likeness rules and more suitable physicochemical properties when compared to Osanetant & Talnetant. ADME/T evaluation showed considerable disparities between groups, yet no significant difference was reported. Pharmacokinetic properties varied irregularly among studied compounds. **Conclusion:** Novel NK3R antagonists exhibit enhanced properties when compared to formerly suspended ones. Fezolinetant is predicted to have more favorable outcomes based on *in silico* evaluation.

Keywords: Hot flashes, menopause, tachykinins, NK3 receptor antagonists, ADMET.

RESUMEN

Antagonistas del receptor taquiquinina/neuroquinina 3: Análisis *in silico* ADME/T de nuevos compuestos para la terapia de los sofocos en la menopausia

Introducción: la activación del receptor de neuroquinina-B (NK3R) está estrechamente relacionada con la aparición de síntomas vasomotores durante la menopausia, no obstante, a la fecha no se reportan fármacos antagonistas de NK3R aprobados para el manejo de sofocos. La evaluación de las propiedades farmacocinéticas de los compuestos inhibidores de NK3R resulta indispensable para la identificación de potenciales farmacóforos y para la estimación de posibles resultados en ensayos clínicos. **Objetivo:** determinar las características farmacocinéticas de los nuevos compuestos inhibidores de NK3R con propiedades reductoras de los fogajes asociados a la menopausia, y por medio de un análisis comparativo con los antagonistas de NK3R cuyo ensayo clínico fue suspendido (Osanetant & Talnetant), se espera identificar propiedades superiores en los nuevos compuestos desarrollados. **Metodología:** se obtuvieron los códigos Smiles a partir de PubChem y DrugBank, posteriormente, el análisis se basó en el cálculo de las propiedades farmacocinéticas y *drug-like* mediante las plataformas ADMETlab y SwissADME. **Resultados:** Pavinetant & Fezolinetant y SB-222.200 & SB-218.795 exhiben mejores propiedades fisicoquímicas y cumplen a mayor cabalidad las reglas *drug-likeness* al compararse con Osanetant & Talnetant. La evaluación ADMET reveló variaciones entre los grupos, pero ninguna fue significativa. Las propiedades farmacocinéticas varían de forma irregular entre los distintos compuestos. **Conclusiones:** los antagonistas de NK3R recientemente desarrollados exhibieron propiedades superiores frente a los compuestos de ensayos suspendidos. Los resultados del estudio *in silico* permiten deducir que el Fezolinetant podría tener mejores resultados en futuros ensayos clínicos.

Palabras clave: Sofocos, menopausia, taquiquininas, antagonistas del receptor NK3, ADMET.

RESUMO

Antagonistas do receptor de taquicinina/neurocinina 3: análise ADME/T *in silico* de novos compostos para a terapia de ondas de calor na menopausa

Introdução: a ativação do receptor de neuroquinina-B (NK3R) está intimamente relacionada ao aparecimento de sintomas vasomotores durante a menopausa, entretanto, até o momento não há relatos de drogas antagonistas de NK3R aprovadas para o manejo das ondas de calor. A avaliação das propriedades farmacocinéticas de compostos inibidores de NK3R é essencial para a identificação de potenciais farmacóforos e para a estimativa de possíveis resultados em ensaios clínicos. **Objetivo:** determinar as características farmacocinéticas dos novos compostos inibidores de NK3R com propriedades redutoras de ondas de calor associadas à menopausa, e através de uma análise comparativa com os antagonistas de NK3R cujo ensaio clínico foi suspenso (Osanetant & Talnetant), espera-se identificar propriedades superiores nos compostos recém-desenvolvidos. **Metodologia:** os códigos Smiles foram obtidos do PubChem e DrugBank, posteriormente, a análise foi baseada no cálculo das propriedades farmacocinéticas e farmacocinéticas utilizando as plataformas ADMETlab e SwissADME. **Resultados:** Pavinetant & Fezolinetant e SB-222.200 & SB-218.795 apresentam melhores propriedades físico-químicas e atendem mais plenamente às regras de “*drug-likeness*” quando comparados ao Osanetant & Talnetant. A avaliação ADMET revelou variações entre os grupos, mas nenhuma foi significativa. As propriedades farmacocinéticas variam irregularmente entre os diferentes compostos. **Conclusões:** os antagonistas de NK3R recém-desenvolvidos exibiram propriedades superiores em relação aos compostos de teste suspensos. Os resultados do estudo *in silico* permitem-nos deduzir que o Fezolinetant poderá ter melhores resultados em futuros ensaios clínicos.

Palavras-chave: Ondas de calor, menopausa, taquicininas, antagonistas do receptor NK3, ADMET.

INTRODUCTION

Tachykinins are a diverse family of pleiotropic neuropeptides which exhibit significant neuromodulating properties. Several tachykinins have been identified in humans, some of which include substance P, hemokinin-1, endokinin-A, endokinin-B, neurokinin-A,

and neurokinin-B [1]. These neuroactive compounds act through an array of receptors encoded in the human genome and which are actively involved in numerous neuroendocrine circuits [2, 3].

Tachykinin/neurokinin 3 receptor (NK3R) exhibits a high affinity for neurokinin-B [1] and when activated, it displays cholinergic modulation and endocrine reproductive regulation mediated by neurohormonal action on the hypothalamus [4]. In the arcuate/infundibular nucleus (ARC), neurokinin-B is co-expressed with kisspeptin and dynorphin by KNDy neurons which project to GnRH-releasing cells and thermoregulatory centers. During menopause, KNDy neurons hypertrophy in response to the absent feedback of estrogen, therefore there is an augmented firing rate towards NK3R-expressing hypothalamic neurons either within the ARC nucleus (GnRH neurons) or towards the preoptic area (thermoregulatory center and GnRH neurons) [5].

Soon after menopause onset, induced-hypertrophy stimulates KNDy neurons to release kisspeptin leading to GnRH increased secretion and further FSH and LH serum elevation which is commonly seen in the initial phases of menopause. Simultaneously, greater stimulation of the preoptic area via neurokinin-B signaling results in autonomic activation causing the frequent menopause-associated vasomotor symptoms commonly known as hot flashes [5] (figure 1).

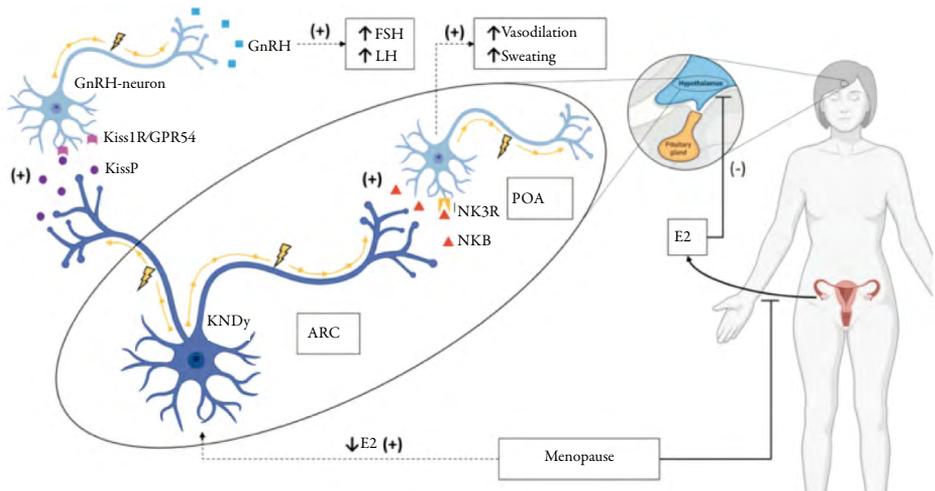


Figure 1. Neuroendocrine circuits involved in hot flashes onset. Postmenopausal hormonal decrease results in the blockage of E2 hypothalamic downregulation of ARC neurons, therefore, inducing KNDy hypertrophy with increased excitability. Augmented release of KissP stimulates GnRH secretion towards the median eminence and results in FSH and LH raised levels. POA neurons are

stimulated via NKB-NK3R leading to vasodilation and sweating mediated by autonomic outputs. E2: Estradiol; ARC: arcuate nucleus; POA: Preoptic area; KNDy: Kisspeptin-NeurokininB-Dynorphin neuron; NKB: Neurokinin-B; NK3R: Neurokinin 3 receptor; KissP: Kisspeptin; Kiss1R/GPR54: Kisspeptin receptor.
Source: Own elaboration.

As a result, NK3R has become a suitable target for drug development in disorders such as schizophrenia, and non-hormonal therapy during menopause [6, 7]. Around 2005, the first non-peptidic NK3R antagonists were reported, Osanetant (SR142801) and Talnetant (SB223412), both developed as antipsychotics [8]. More recently, a reduction of hot flashes was identified when inhibiting NK3R, giving rise to compounds for vasomotor symptoms therapy which are currently under study in humans and animal models [9]. NK3R antagonists are shown in figure 2.

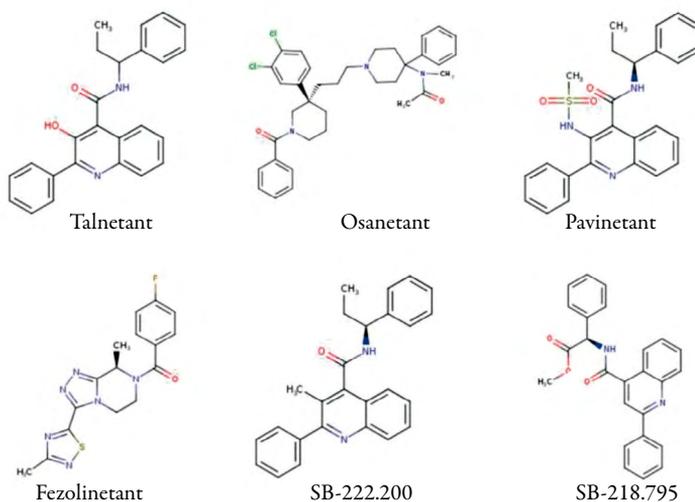


Figure 2. Molecular structure of NK3R antagonists. Compounds advised for schizophrenia (Talnetant, Osanetant) and hot flashes therapy (Pavinetant, Fezolinetant, SB-222.200, SB-218.795).
Source: Structures taken from [10].

Osanetant and Talnetant were tested for schizophrenia treatment and showed promising results in hospitalized patients, however, despite their efficacy, both drugs' clinical trials were finally suspended due to their poor pharmacokinetic profile [8]. On the other hand, several NK3R antagonists, yet not intended for schizophrenia but hot flashes therapy, have recently been raised as potential therapeutic agents. Pavinetant and

Fezolinetant are both currently under clinical trials and have shown a significant reduction of hot flashes [11], with Fezolinetant presently undergoing Phase IIb [12]. Reduction of hot flashes has also been reported for compounds tested in animals, among them, SB-222.200 and SB-218.795 show high affinity for hypothalamic NK3R and exhibited promising results in preclinical trials [9].

NK3R antagonists have shown encouraging outcomes when studying their efficacy whether it is for schizophrenia or hot flashes reduction. Regardless of their distinct therapeutic effect, these compounds are orally-delivered drug candidates that target structures within the nervous system, therefore, assessing their pharmacokinetics is of great importance when evaluating their potential as drugs. Determining the pharmacokinetic properties of novel compounds requires the design of wasteful *in vivo* assays, as a result, *in silico* experimentation emerges as a promising strategy to accelerate drug filtering by providing several compound screening methods ranging from structure-activity analysis to toxicology and pharmacokinetic studies [13]. Physicochemical and ADME/T parameters (absorption, distribution, metabolism, excretion, and toxicity) can be predicted based on topological and molecular descriptors determined in bioinformatic platforms such as ADME/Tlab and SwissADME [10, 14].

As previously mentioned, Osanetant and Talnetant were discontinued as a result of their poor pharmacokinetics, consequently, novel NK3R antagonists are expected to exhibit superior drug-likeness in order to achieve better outcomes in future trials. This study aims to develop a first-time reported pharmacokinetic profiling of NK3R antagonists. By comparing the pharmacokinetic profile of Osanetant & Talnetant and novel NK3R antagonists, we intend to identify enhanced drug-like properties for Fezolinetant, Pavinetant, SB-222.200, and SB-218.795. Results derived from this investigation could help to elucidate optimal physicochemical and pharmacokinetic properties in current drug candidates for hot flashes reduction as well as to point out important features in possible scaffolds for future lead-compounds synthesis of potential drugs with NK3R antagonistic activity.

METHODOLOGY

NK3R antagonists, selection and structure retrieval

Compounds with hot flashes reducing activity were retrieved from Muñoz et al. [9]. NK3R antagonists under study were divided into three groups; schizophrenia drugs for which clinical trials were suspended [8], drugs under current testing in humans which have shown to ameliorate menopause vasomotor symptoms [12, 15], and animal-tested

NK3R antagonists with a reported lessening of hot flashes [16, 17]. Carried out *in silico* analysis required the SMILES chemical identifiers which were taken from DrugBank (<https://www.drugbank.com>) and PubChem (<https://pubchem.ncbi.nlm.nih.gov>).

Physicochemical properties prediction

The assessment of physicochemical properties was based on predicted values calculated in the SwissADME platform (<http://www.swissadme.ch>). Analyzed parameters were those stated as crucial for suitable drug-like compounds [18]; lipophilicity (Log P), solubility (Log S), size (molecular weight), polarity (topological polar surface area), unsaturation (fraction of sp³ carbons) and flexibility (number of rotatable bonds). Retrieved values were plotted on a bioavailability radar chart [10].

Drug-likeness rules compliance

Drug-like properties for all NK3R antagonists were evaluated through their compliance with four drug-likeness rules: Lipinski, Ghose, Veber, and Varma [19-21]. Results were obtained from ADMETlab and further on analyzed as percentage values according to the number of violations for each assessed rule.

***In silico* ADME/T evaluation**

The pharmacokinetic profile of each NK3R antagonist was determined through the calculation of absorption, distribution, metabolism, excretion and toxicity (ADMET) performed in the ADMETlab platform (<http://ADMET.scbdd.com>) [22]. Values retrieved from ADMETlab were based on Smiles inputs and were predicted with a 67% to 93% accuracy, depending on the estimated parameter.

Absorption & Distribution

Human gastrointestinal absorption (HIA), membrane permeability (Caco-2 permeability) and P-glycoprotein substrate likeliness were determined to evaluate absorption properties. In addition, distribution assessment was carried out by blood-brain barrier (BBB) diffusion and bioavailability prediction. Distribution results were reported as probability values for BBB diffusion and the achievement of a systemic circulating fraction of 20% and 30% when orally consumed.

Metabolism & Excretion

Interactions with cytochrome P450 enzymes, both as substrates and inhibitors, were predicted for the NK3R antagonists. Studied CYP450 included: CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4. Results were reported as probability values for inhibition and substrate interactions.

Toxicity

Toxicity evaluation was based on three estimated parameters: long-term human hepatotoxicity (H-HT), drug-induced liver injury (DILI) and probability of hERG blockage.

Data analysis

The aforementioned groups were compared in terms of physicochemical properties, drug-likeness compliance, and ADME/T predicted values. Statistical analysis was carried out by a one-way Anova test and taking into consideration significant differences with a probability of less than 0.05 ($\alpha=0.05$).

RESULTS AND DISCUSSION

Physicochemical evaluation

Predicted values for lipophilicity, solubility, polarity, flexibility, size and unsaturation are presented in table 1. The overall results from physicochemical assessment did not report significantly high or low values for most of the studied compounds, still, some exhibited a greater number of non-suitable features. Statistical analysis revealed no significant difference ($p>0.05$) between the three groups in terms of physicochemical properties.

Table 1. Physicochemical properties of NK3R antagonists. Lipophilicity (Log P), Solubility (Log S), Polarity (TPSA), Flexibility (N.° RB), Size (MW) and Unsaturation (Fraction C sp³).

Property	Suspended trial		Human tested		Animal tested	
	Talne-tant	Osane-tant	Pavine-tant	Fezolinetant	SB-222.200	SB-218.795
Log P (XLOGP3)	5.75*	6.97*	5.07*	1.56	5.92*	4.75
Log S (ESOL)	-6.00	-7.65*	-5.85	-3.32	-6.09*	-5.37
TPSA (Å ²)	62.22	43.86	96.54	105.04	41.99	68.29
N.° RB	6.00	10.00*	8.00	3.00	6.00	7.00
MW (g.mol ⁻¹)	382.45	606.62*	459.56	358.39	380.48	396.44
Fraction C sp ³	0.12*	0.43	0.15*	0.31	0.15*	0.08*

*Exceeding values. MW: Molecular weight; TPSA: Topological polar surface area; RB: Rotatable bonds.

Results from the physicochemical evaluation suggest that there are slight variations among the studied compounds which, in turn, affect their drug-like properties. In the first place, schizophrenia (suspended) drugs exhibited exceedingly high values of lipophilicity, insolubility, flexibility, and molecular weight. Human-tested drugs, on the other hand, showed only two violations concerning unsaturation and lipophilicity. Similar results were found in animal-tested compounds, which exhibited three violations for lipophilicity, solubility and unsaturation. For easier visual analysis, assessed properties for each compound are displayed as bioavailability radar charts (figure 3).

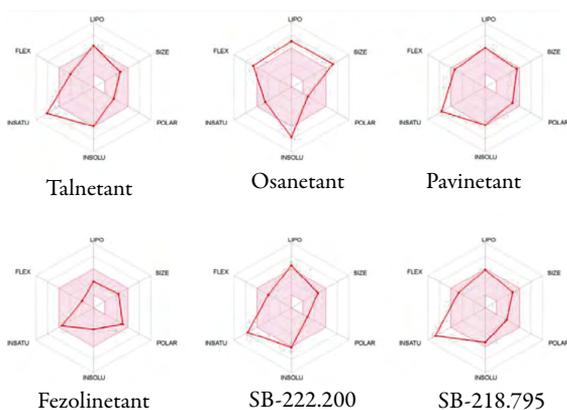


Figure 3. Bioavailability radar chart. Red-colored area: suitable drug-like properties values. Lipo: $\log P$; Size: Molecular weight; Polar: Topological polar surface area; Insolu: $\log S$; Insat: Fraction of sp^3 carbons; Flex: N° of rotatable bonds. Plotted data within the red area represent suitable properties for drug-like compounds. Limit values are: $-0.7 < \log P < 5$, $150 \text{ g}\cdot\text{mol}^{-1} < \text{MW} < 500 \text{ g}\cdot\text{mol}^{-1}$, $20 \text{ \AA}^2 < \text{TPSA} < 130 \text{ \AA}^2$, $-6 < \log S < 0$, $0.25 < \text{Fraction C } sp^3 < 1$ and $0 < N^\circ \text{ RB} < 9$. Source: [10].

All six compounds under study exhibited adequate polarity (TPSA), yet several deviations were found in terms of the five sparing properties. Obtained results show that Talnetant has undesirable lipophilicity and unsaturation. Lipophilicity is a crucial parameter in drug-likeness evaluation; while low values indicate poor lipid bilayer permeability, exceeding values suggest, as in the case of Talnetant, that this compound would have poor blood transport and would tend to deposit in lipid-abundant tissues [23]. In terms of unsaturation, the fraction of sp^3 carbons alludes to the spatial complexity and is tightly connected to solubility [24], hence Talnetant is predicted to exhibit properties that compromise its solvation, blood transport, and plasma distribution. Osanetant, on the other hand, was reported to be the least suitable drug-like compound, with violations for flexibility, size, lipophilicity, and solubility. Absorption and

distribution are compromised by inappropriate values of solubility and lipophilicity [25], additionally, unsuitable size also conveys poor permeability and absorption, and indicates low synthetic accessibility [26]. Optimal flexibility, on the other hand, has recently shown to be crucial in drugs that target proteins (such as NK3R) given their continuous conformational changes [27], hence inadequate flexibility might compromise ligand-target affinity.

In regards to human-tested drugs, only Pavinetant reported slight violations for lipophilicity and unsaturation, whereas Fezolinetant showed no violations at all. Compounds with satisfactory values for all physicochemical parameters are predicted to exhibit superior pharmacological qualities [28]. On the other hand, animal-tested compounds displayed better properties than suspended drugs, yet not better than human-tested. Reported violations included lipophilicity, solubility, and unsaturation for SB-222.200, and unsaturation for SB-218.795.

When comparing novel NK3R antagonists undergoing clinical trials for hot flashes reduction and NK3R antagonists for which trials were suspended, results show that human-tested compounds exhibit enhanced drug-like properties. Fezolinetant showed more satisfactory results when compared to Talnetant and Osanetant, indicating that, in terms of physicochemical descriptors, Fezolinetant displays advantages that might play an important role when evaluating bioavailability and effectiveness following oral administration [29, 30].

Drug-likeness compliance

Based on predicted physicochemical properties, several guidelines have been developed in order to assess compounds in terms of drug-likeness. These are known as drug-likeness rules, in which different criteria are evaluated for each filter. In this study, we analyzed the compliance with Lipinski's rule of 5, Ghose filter, Veber's rules, and Varma's rules. An array of chemical descriptors are assessed by these guidelines, including molecular weight (MW), lipophilicity (Log P), hydrogen-bond acceptors and donors, molar refractivity (MR), number of atoms, number of rotatable bonds, polar surface area (TPSA), and solubility at pH=7.4 (Log D) [31, 32].

Results obtained for the three groups under study were reported as a mean percentage of compliance with each set of rules, hence a higher value represented a lower number of violations. The statistical analysis showed no significant difference ($p > 0.05$) among the three groups in terms of drug-likeness, yet Talnetant and Osanetant show less compliance with Lipinski, Ghose, and Varma rules when compared to Fezolinetant, Pavinetant, SB-222.200, and SB-218.795. Mean compliance percentage values are shown in figure 4.

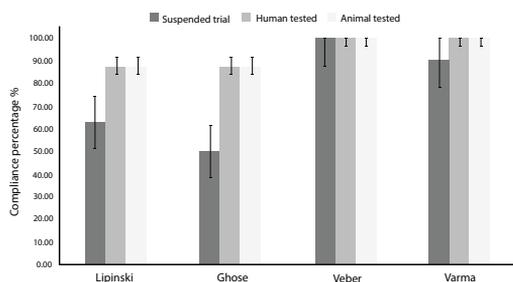


Figure 4. Drug-likeness rules mean compliance percentage for each of the four assessed guidelines \pm SEM (standard error of the mean).

Although an *in silico* drug-likeness assessment is limited when classifying a molecule as a suitable drug [33], these guidelines provide valid information of proper pharmacokinetic behavior when administered orally [14, 34]. In concordance with our results, up to date carried out clinical trials showed slightly better outcomes for Fezolinetant when compared to Talnetant and Osanetant [9] and given the data obtained from this investigation, our findings suggest superior outcomes in more advanced clinical trials based on *in silico* pharmacokinetics prediction.

In silico ADME/T analysis

The assessment of the pharmacokinetic parameters for the NK3R antagonists was carried out in the ADMETlab platform. Initial analysis was based on BBB and GI absorption (figure 5), membrane permeability, and P-glycoprotein substrate properties (table 2).

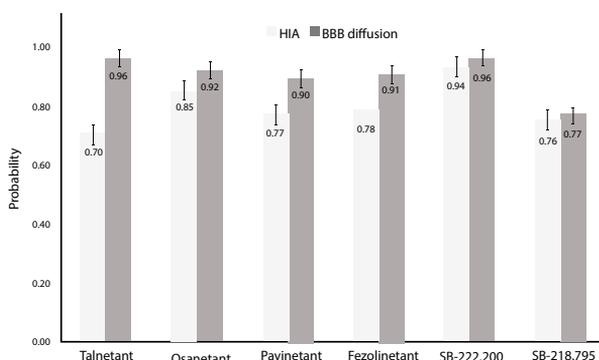


Figure 5. Human gastrointestinal absorption and blood-brain barrier diffusion probability \pm SE. HIA: Human intestinal absorption; BBB: blood-brain barrier; SE: standard error.

Human intestinal absorption was predicted as stated in [35] with an accuracy of 78%. ADMETlab threshold for absorbable compounds states a cut-off point of 50%, meaning that compounds with probability values higher than 0.50 are classified as GI absorbable. All six compounds exhibited a human intestinal absorption value $\geq 70\%$, with SB-222.200 showing the highest probability. Smith et al. [36] determined that SB-222.200 was highly absorbable when orally administered to male rats, and complemented with this study, results suggest that SB-222.200 could exhibit high human GI absorption. Despite lower values for Fezolinetant absorption when compared to Talnetant and Osanetant, previous studies have shown that oral administration results in successful reduction of hot flashes [37], thus this degree of difference in GI absorption may not be significant enough to argue lower effectiveness of novel Fezolinetant.

Blood-brain barrier (BBB) diffusion evaluation show a 92% accuracy based on calculations proposed in [38]. Five compounds reported values $\geq 90\%$, except for SB-218.795. However, all six molecules were above the 0.50 threshold, suggesting suitable BBB diffusion. Compounds targeting structures in nervous tissue are required to have an equilibrium between solubility and lipophilicity balanced enough to cross the blood-brain barrier [39], our study shows that all six compounds exhibit appropriate BBB diffusion properties. Nevertheless, it should be noted that ionization states and stereochemical modifications are not considered when predicting this parameter. Further on, Caco-2 permeability [40] was evaluated parallel to P-glycoprotein substrate probability [41] (table 2).

Table 2. Caco-2 permeability and P-glycoprotein (Pgp) substrate probability of NK3R antagonists.

Parameter	Suspended trial		Human tested		Animal tested	
	Talnetant	Osanetant	Pavinetant	Fezolinetant	SB-222.200	SB-218.795
Caco-2 permeability (cm·s ⁻¹)	-5.12	-4.98	-5.16	-4.55	-4.74	-4.95
Pgp substrate (probability)	0.06	0.40	0.09	0.13	0.10	0.06

Caco-2 permeability values of all six compounds were reported to be > -5.15 cm·s⁻¹ suggesting optimal absorption and membrane permeability [40]. Assessing Caco-2 permeability has been widely used to predict drug absorption with considerable accuracy [42]. *In silico* analysis carried out in the present study indicated that all six NK3R antagonists are likely GI absorbable.

P-glycoprotein acts by extruding absorbed xenobiotics from cells [43], hence assessing substrate likeliness is of great importance. No compound exhibited a probability >50%, yet Osanetant showed a considerable 40% probability of being a P-glycoprotein substrate.

Bioavailability estimation was carried out as stated in [44] with an accuracy of 70%. Cut-off points of 20% and 30% when orally administered were selected for this study. Results are reported as probability values of achieving a bioavailability (F) greater than 20% or 30% (table 3).

Table 3. Bioavailability prediction for NK3R antagonists.

Parameter	Predicted probability values					
	Suspended trial		Human tested		Animal tested	
	Talnetant	Osanetant	Pavinetant	Fezolinetant	SB-222.200	SB-218.795
F >20%	0.73	0.73	0.73	0.73	0.80	0.71
F >30%	0.57	0.48	0.55	0.59	0.57	0.48

F >20%: Bioavailability>20%; F >30%: Bioavailability>30%.

When analyzing the probability of reaching a bioavailability greater than 20%, suspended trial drugs and human-tested drugs exhibited an oddly same value of 0.73, still, the reported probability was over the 0.50 threshold of ADMETlab. While SB-218.795 scored the lowest, SB-222.200 showed the highest probability when compared to all previously mentioned groups.

Regarding >30% bioavailability, the highest value was exhibited by Fezolinetant, whereas the lowest was for Osanetant. Besides Osanetant, all the compounds were above the 0.50 threshold. In concordance with our results, suspended trials for Osanetant reported a slight low bioavailability when compared to novel drugs under current testing [8].

Excretion evaluation was carried out by assessing half-life and clearance [45] with an accuracy of 85% and 88%, respectively. Results of the evaluated parameters for each compound are shown in table 4.

Table 4. Elimination, Clearance and half-life of NK3R antagonists.

Parameter	Suspended trial		Human tested		Animal tested	
	Talnetant	Osanetant	Pavinetant	Fezolinetant	SB-222.200	SB-218.795
Half-life (h)	2.18	1.91	1.81	1.41	2.10	2.07
Clearance (cm ³ ·min ⁻¹ ·kg ⁻¹)	2.14	1.02	1.37	1.54	1.92	1.67

The predicted half-life for NK3R antagonists was reported to be within a range of 1.41 to 2.18 hours. Obtained values were over 0.50 and below 3.00 hours suggesting drug-like features, however, they still exhibited a moderately low half-life [22]. Reported low half-life values indicate that an optimal therapeutic effect is achieved only with an increased dosing regimen [46], hence these results show that it is advisable to carefully evaluate dosing when analyzing these compounds as therapeutic agents. Interestingly, animal-tested drugs (SB-222.200 and SB-218.795) were reported to have higher half-life when compared to the other two groups, and on the contrary, human-tested drugs exhibited the lowest half-life values of all six compounds.

Clearance results were all <5 cm³·min⁻¹·kg⁻¹, suggesting that the clearance rate for these compounds is rather low [22]. Talnetant exhibited the highest clearance (2.14 cm³·min⁻¹·kg⁻¹), being the only compound that reported >2 cm³·min⁻¹·kg⁻¹. These results support Talnetant clinical trial suspension based on the poor pharmacokinetic profile as proposed by Malherbe et al. [8]. In contrast, Pavinetant and Fezolinetant seem to exhibit lower clearance and a considerably different pharmacokinetic profile in terms of excretion. Animal-tested drugs, on the other hand, reported intriguingly high values of both clearance and half-life.

Metabolism evaluation was carried out as detailed in [47]. Results are reported as the probability of inhibition or substrate interaction with CYP450 enzymes (table 5).

Table 5. CYP450 predicted inhibition and substrate-like properties.

Parameter	Predicted probability of CYP450 interactions					
	Suspended trial		Human tested		Animal tested	
	Talnetant	Osanetant	Pavinetant	Fezolinetant	SB-222.200	SB-218.795
CYP1A2 inhibitor	0.28	0.06	0.19	0.43	0.39	0.97*
CYP1A2 substrate	0.39	0.60	0.36	0.52*	0.40	0.47

Parameter	Predicted probability of CYP450 interactions					
	Suspended trial		Human tested		Animal tested	
	Talnetant	Osanetant	Pavinetant	Fezolinetant	SB-222.200	SB-218.795
CYP3A4 inhibitor	0.78*	0.85*	0.90*	0.57*	0.93*	0.93*
CYP3A4 substrate	0.49	0.70*	0.50*	0.45	0.43	0.50*
CYP2C9 inhibitor	0.30	0.27	0.24	0.48	0.05	0.05
CYP2C9 substrate	0.57*	0.38	0.56*	0.42	0.60*	0.43
CYP2C19 inhibitor	0.50*	0.19	0.32	0.68*	0.51*	0.55*
CYP2C19 substrate	0.51*	0.65*	0.47	0.38	0.49	0.46
CYP2D6 inhibitor	0.29	0.72*	0.34	0.47	0.28	0.27
CYP2D6 substrate	0.33	0.57*	0.26	0.46	0.31	0.34

*Values that label the compound as a feasible inhibitor/substrate.

Given ADMETlab categorization, feasible interactions are likely to occur when the probability is higher than 0.50, hence every compound showed at least one interaction with a CYP450 enzyme. Most importantly, all six NK3R antagonists were reported to have induced toxicity based on their predicted CYP3A4 inhibition, still, Fezolinetant exhibited a much lower probability for the occurrence of this particular interaction. Osanetant showed the highest number of interactions with cytochromes, for which two out of five were inhibitions. On the other hand, Talnetant and SB-218.795 reported four interactions with two and three possible inhibitions, respectively. Pavinetant, Fezolinetant and SB-222.200 showed three interactions including both inhibitions and substrate-enzyme. For all three groups, the mean probability values of CYP450 interactions are shown in figure 6.

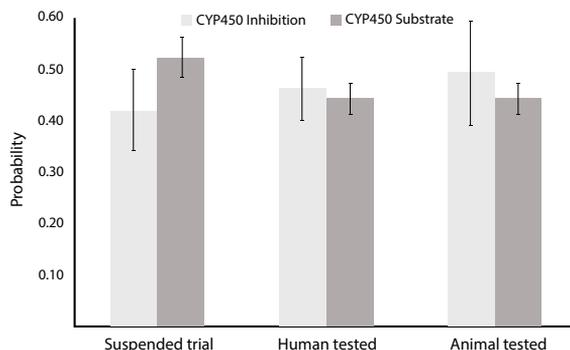


Figure 6. Probability of CYP450 interactions. Mean probability values for inhibition and substrate properties \pm SEM. SEM: standard error of the mean; CYP450: cytochrome P450.

The statistical analysis reported no significant difference ($p > 0.05$) in terms of probability, yet clear differences were evidenced. Talnetant and Osanetant exhibited a higher number of CYP450 substrate interactions, while animal-tested drugs (SB-222.200 and SB-218.795) showed greater CYP450 inhibitions. Human-tested drugs mean interacting probability lies between both aforementioned groups.

The evaluation of cytochrome interactions with drug-like compounds is crucial. As CYP450 enzymes are tightly involved in xenobiotic metabolism, the interacting probability of Talnetant and Osanetant with CYP450 indicates a higher catabolic rate leading to poor efficacy as therapeutic agents [48], most importantly, lower substrate-enzyme interactions were reported for novel NK3R antagonists. Inhibition, on the other hand, was greater for animal-tested drugs, thus these compounds are more likely to produce drug-induced hepatotoxicity when compared to human-tested drugs [49].

Toxicity profiles for NK3R antagonists were determined by the estimated probability of hERG blockage [50], human hepatotoxicity (H-HT) [51], and drug-induced liver injury (DILI) [52] with an accuracy of 84%, 69% and 84%, respectively. Results are shown in table 6.

Table 6. Toxicity profile of NK3R antagonists.

Parameter	Predicted probability values					
	Suspended trial		Human tested		Animal tested	
	Talnetant	Osanetant	Pavinetant	Fezolinetant	SB-222.200	SB-218.795
hERG blockers	0.90	0.93	0.96	0.73	0.91	0.92
H-HT	0.86	0.62	0.90	0.79	0.82	0.76
DILI	0.73	0.34	0.90	0.72	0.68	0.90

hERG blockage was found to be considerably elevated, with five compounds reporting a probability $\geq 90\%$, nonetheless, Fezolinetant hERG blockage probability was significantly low in comparison. H-HT probability was found to be within a range of 0.62-0.90, with Pavinetant scoring the highest and, oddly, Osanetant the lowest. Assessment of DILI probability was also within a range of 0.34-0.90, reporting the lowest for Osanetant and the highest for Pavinetant and SB-218.795. Data show that Pavinetant exhibits the greatest toxicity profile based on *in silico* evaluation, and in concordance with these results, clinical trial outcomes showed higher toxicity for Pavinetant as a result of an increase in subjects serum transaminases, which was not evidenced with Fezolinetant administration [12].

CONCLUSION

In silico analysis revealed a physicochemical and pharmacokinetic profile that enabled a non-previously carried out comparison between the novel and suspended NK3R antagonists. Given Talnetant and Osanetant poor outcomes in clinical trials, and as expected in this study, results show that drugs currently undergoing clinical trials (Pavinetant & Fezolinetant) and animal-tested drugs (SB-222.200 & SB-218.795) exhibited higher compliance with drug-likeness rules. When compared to trial-suspended drugs, human-tested compounds reported better physicochemical features based on molecular descriptors calculation. Because of these facts, results suggest more suitable drug-like properties for novel Fezolinetant and Pavinetant, and thus, a likely enhanced activity as NK3R antagonists. ADME/T evaluation showed considerable disparities between groups, yet no significant difference was reported in terms of absorption, distribution, metabolism, excretion, and toxicity. Results derived from this study served to elucidate crucial pharmacological and chemical traits of novel drug-like compounds currently under study for hot flashes therapy.

ACKNOWLEDGMENTS

We thank Professor Claudia M. Cruz (Gimnasio Vermont) for her advice and assistance regarding experimental design and the chemoinformatic approach.

DISCLOSURE STATEMENT

The authors report no conflict of interest.

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HOW TO CITE THIS ARTICLE

J.A. Barrero, A.M. González-Clavijo, Tachykinin/neurokinin 3 receptor antagonists: In silico ADME/T analysis of novel compounds for menopause hot flashes therapy, *Rev. Colomb. Cienc. Quim. Farm.*, *51*(1), 100-122 (2022).