Discovery of Novel TRPV1-Targeting Analogs for Safer Pain Management

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Acetaminophen is widely recognized as a first-line analgesic in clinical practice and is recommended by numerous treatment guidelines, particularly for vulnerable populations such as older adults, children, pregnant women, and patients with peptic ulcer disease, owing to its favorable safety profile. Its pharmacokinetic characteristics also allow for flexible and practical dosing regimens. However, the risk of hepatotoxicity limits its use in some patients, especially those with contraindications to alternative analgesics. The aim of this study was to facilitate the identification and development of novel compounds with comparable or improved efficacy and safety profiles, thereby expanding therapeutic options for patients requiring pain management. To achieve this, we employed a computational workflow that integrates structure-based and ligand-based drug design approaches with a transformer-based framework to accelerate the drug discovery process. This integrated strategy yielded three promising candidate compounds, two of which share notable structural similarity and exhibit strong predicted activity alongside favorable safety-relevant properties. These findings demonstrate the potential of modern AI-enabled drug design pipelines to uncover next-generation analgesics and lay the groundwork for subsequent experimental validation and optimization.

1 Introduction

Acetaminophen remains the most commonly used analgesic for managing acute and chronic pain [9]. Several factors contribute to its widespread use, including its over-the-counter availability, favorable safety profile at therapeutic doses, and lower risk of gastrointestinal adverse effects compared to non-steroidal anti-inflammatory drugs (NSAIDs) [6, 10, 1]. Consequently, acetaminophen is widely recommended as a first-line analgesic in both outpatient and clinical care settings across diverse patient populations [5, 10]. Although acetaminophen is generally safe at recommended doses [9, 6], exceeding this range can be harmful due to accumulation of the toxic metabolite N-acetyl-p-benzoquinoneimine (NAPQI), which can lead to acute liver failure[7, 12]. These limitations of acetaminophen, particularly its risk of hepatotoxicity at higher doses, limit its use

in certain patient populations, including those with pre-existing liver disease, chronic alcohol use, multiple comorbidities, and the elderly. It remains unclear whether simple dose reduction is sufficient to mitigate these risks in certain cases. These safety concerns emphasize the need for alternative analgesics that are both safer and more effective[6, 12, 14]. Acetaminophen is first deacetylated in the liver to produce p-aminophenol, which crosses the blood–brain barrier (BBB) and is converted in the CNS to N-arachidonoylphenolamine (AM404) [8]. This metabolite is believed to play a central role in the drug's analgesic action [11]. AM404 engages several molecular targets involved in pain signaling, including TRPV1 receptors, and these interactions are thought to contribute to acetaminophen's overall analgesic effect [4, 13]. To circumvent the formation of the hepatotoxic metabolite N-acetyl-p-benzoquinone imine (NAPQI) associated with acetaminophen metabolism, our objective is to design novel compounds with enhanced affinity and specificity for TRPV1, while demonstrating minimal or no hepatotoxic potential. Developing TRPV1-targeted analgesics with improved safety profiles would address the dose-limiting liver toxicity of acetaminophen and expand therapeutic options for patients in whom its use is currently restricted. Currently, no FDA-approved oral medication directly targets the TRPV1 receptor for pain management. Although several TRPV1 modulators have been investigated clinically, none have progressed to regulatory approval due to challenges such as off-target effects and thermoregulatory disturbances. Capsaicin, a well-known TRPV1 agonist, remains the only approved therapeutic targeting this pathway; however, its use is limited to topical administration. Clinically, capsaicin is formulated as an 8% dermal patch and is indicated for the treatment of neuropathic pain through localized activation and subsequent desensitization of TRPV1-expressing sensory neurons [2, 3]. Building upon conventional in silico strategies, this work represents, to our knowledge, one of the earliest applications of generative artificial intelligence (AI) for the design of next-generation TRPV1 modulators.

2 Results and Discussion

2.1 Molecular Generation

We generated novel molecules by performing conditional sequence generation using a generalized pre-trained transformer (GPT) architecture, seeding the model with known molecular structures. In total, 24 unique and chemically valid molecules were obtained. Then, we applied PCA (principal component analysis) to map the high dimensional features space to two-dimensional (2D) space, represented by scatterplot in Figure 2. In the scatter plot, the blue dots correspond to the original data, while the red dots represent the generated molecules. As shown in Figure 2, the generated data occupy a broader region of chemical space compared to the original dataset. In some cases, multiple distinct molecules were generated from the same input, yet they remained structurally similar and closely positioned in the continuous representation due to inherent

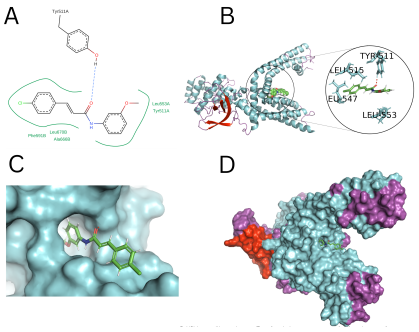

Figure 1: Structural visualization of ligand-binding interactions within the TRPV1 pocket. The reference ligand forms key polar interactions with tyrosine 511, a critical residue implicated in vanilloid binding. The remaining portion of the ligand is embedded within a hydrophobic pocket formed by leucine 553 (chain A), tyrosine 511 (chain A), leucine 670 (chain B), alanine 666 (chain B), and phenylalanine 591 (chain B) (panels A and B). Panels C and D show the molecular docking analysis of the reference compound within the TRPV1 active site. The close-up (panel C) highlights the fit of the ligand within the binding cavity, while the full surface view (panel D) depicts its overall orientation.

randomness.

For subsequent analysis, 7 out of the 24 compounds failed during ligand preparation process. During the docking process, 6 additional compounds failed to dock within the defined binding site. A summary of the results from the docking of the newly generated TRPV1 analogues is shown in Table 1. Overall, the compounds exhibited reproducibly strong binding affinities, as reflected by their favorable docking scores across repeated docking simulations. The docking scores for these novel TRPV1 inhibitors ranged from -9.74 kcal/mol to -8.03 kcal/mol. These results suggest that the newly generated analogues possess substantial potential as promising leads for the design and optimization of potent TRPV1 inhibitors with improved binding characteristics. Among the screened seed molecules, AM404 (N-Arachidonoylphenolamine) exhibited a docking score of –8.72 kcal/mol.

2.2 ADMET and Molecular Interaction Analysis of Novel TRPV1 Compounds

To complement the docking analyses, a comprehensive ADMET evaluation was conducted on compounds 1-11 to predict the bioavailability, pharmacokinetic behavior, and toxicity risks of the novel TRPV1 analogues. The results from the analysis are presented in Table 2. Despite compounds 1, 2, and 3 exhibiting the highest docking scores and demonstrating relatively favorable synthetic accessibility based on the Graph Attention–based assessment of Synthetic Accessi-

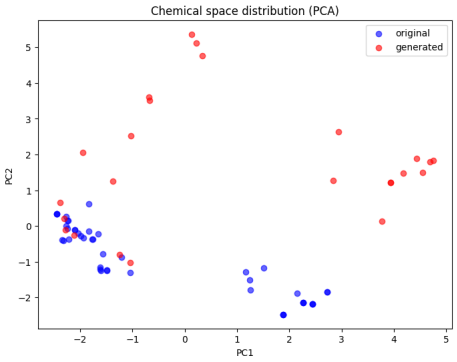

Figure 2: Chemical space analysis of novel TRPV1 analogues

Table 1: Table showing the binding energies of newly generated TRPV1 analogues alongside their corresponding seed molecules.

Generated Molecule ID	**Binding Affinity (kcal/mol)**
compound 1	-9.73904
compound 2	-9.55459
compound 3	-9.34425
compound 4	-8.71824
compound 5	-8.67747
compound 6	-8.54432
compound 7	-8.45130
compound 8	-8.44623
compound 9	-8.42479
compound 10	-8.15210
compound 11	-8.02511

bility (GASA), all three compounds were predicted to have undesirable toxicity profiles, including drug-induced liver injury and nephrotoxicity. In addition, predictions indicated that these compounds contain substructures commonly associated with pan-assay interference compounds (PAINS), which are chemical motifs known to produce false-positive results in bioassays due to nonspecific interactions rather than true biological activity. This suggests that, although these compounds show strong predicted binding affinities, their potential as genuine drug candidates may be limited. Moreover, all compounds were predicted to be P-glycoprotein (P-gp) inhibitors except compound 4, which displayed a lower probability of P-gp inhibition compared to the others. Notably, strong P-gp inhibitors can increase intracellular drug concentrations but also carry a higher risk of drug–drug interactions and may alter the pharmacokinetics of co-administered therapeutics.

None of the screened compounds were predicted to be promiscuous or carcinogenic, indicating a low likelihood of off-target binding and mutagenic po-

tential. Furthermore, all compounds demonstrated high human intestinal absorption and the ability to permeate the blood–brain barrier (BBB), suggesting favorable pharmacokinetic characteristics for oral bioavailability and central nervous system accessibility. The BBB is a highly selective physiological barrier that restricts the passage of most molecules into the central nervous system. The ability to cross this barrier is particularly advantageous for compounds targeting neurological or pain-related pathways, such as TRPV1 analogues. However, compounds 6, 7, 8, 9, and 11 were also predicted to induce nephrotoxicity, highlighting potential safety concerns.

Overall, compounds 4, 5, and 10 exhibited the most favorable ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiles, with compound 5 emerging as the most promising candidate due to its balanced combination of good bioavailability, low toxicity risk, and optimal pharmacokinetic properties. When benchmarked against acetaminophen, particularly with respect to drug-induced liver toxicity, these lead compounds displayed superior safety profiles. Taken together, these findings highlight the potential of compounds 4, 5, and 10 as safer and more effective alternatives to standard analgesics such as acetaminophen, warranting further experimental validation and optimization for clinical development.

Parameters	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C11**	**Acet.**
GASA	Easy	Easy	Easy	Hard	Hard	Hard	Hard	Hard	Hard	Hard	Hard	Easy
Promiscuous compounds	No	No	No	No	No	No	No	No	No	No	No	No
PAINS	Yes	Yes	Yes	No	No	No	No	No	No	No	No	No
Human Intestinal Absorption	High	High	High	High	High	High	High	Low	High	High	High	High
BBB permeant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Probable
P-gp inhibitor	Yes	Yes	Yes	Probable	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Carcinogenicity	No	No	No	No	No	No	No	No	No	No	No	Probable
Drug-Induced Liver Injury	Yes	Yes	Yes	No	No	Yes	No	Probable	Yes	No	Probable	Probable
Drug-Induced Neurotoxicity	No	No	Probable	No	No	No	No	No	No	No	No	Probable
Drug-Induced Nephrotoxicity	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Probable	Yes	Probable

Table 2: Predicted bioavailability, pharmacokinetic parameters and toxicity profiles of the novel TRPV1 analogues and standard drug(Acetaminophen).

Table 3: Predicted binding affinities and interacting residues for selected lead compounds.

Compound name	**Binding Affinity (kcal/mol)**	**H-Bond Interactions**	**Hydrophobic Interacting Amino Acids**
Compound 4	−8.718	SER512, ARG557, GLN701	PHE543, LEU547, THR550, LEU553, ILE569, GLU570, ILE573, PHE587, PHE591, LEU670
Compound 5	−8.677	TYR511, THR550, TYR554	PHE522, PHE543, ALA546, LEU547, THR550, LEU553, PHE587, PHE591, LEU670
Compound 10	−8.152	THR550, TYR554	PHE522, PHE543, ALA546, LEU547, THR550, ILE573, PHE587, PHE591, LEU670

The key hydrogen bond and hydrophobic interactions formed by the top TRPV1 lead compounds are summarized in Table 3. Regarding hydrogen bonding, only two of the three interactions observed for compound 5 were retained in compound 10, involving Thr550 and Tyr554, while the interaction with Tyr511 was absent. The loss of this polar contact likely reduced the overall binding

affinity of compound 10 relative to compound 5. Nevertheless, the retention of key hydrogen bonds, together with extensive hydrophobic interactions, ensured favorable ligand–receptor contacts, indicating that compound 10 remained effectively stabilized within the TRPV1 binding pocket despite minor differences in its interaction profile.

3 Conclusion

In this study, we applied an integrated computational workflow combining ligand-based screening, structure-based docking, and transformer-based molecular generation to identify novel TRPV1-targeting analogues. Among the evaluated compounds, compounds 4, 5, and 10 demonstrated the most favorable combination of binding affinity and ADMET profiles. Detailed protein–ligand interaction analyses revealed that these compounds achieved stabilization within the TRPV1 binding pocket through a combination of specific hydrogen bonds, π–π stacking, and extensive hydrophobic contacts with key residues. Notably, compound 5 emerged as the most promising candidate, exhibiting optimal hydrogen bonding and hydrophobic interactions alongside low predicted toxicity and favorable pharmacokinetic properties. When benchmarked against acetaminophen, particularly with respect to drug-induced liver toxicity and overall ADMET performance, these lead compounds demonstrated superior safety profiles. Collectively, these findings suggest that rational design of TRPV1 modulators guided by computational modeling and generative artificial intelligence can generate novel ligands with enhanced binding characteristics and improved safety profiles. These findings provide a foundation for further experimental validation and optimization, supporting the development of safer, more effective analgesics that target TRPV1.

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