

Article

AI-Assisted Prioritisation of Novel SSTR2 Ligands for High-Risk Stage 4 Neuroblastoma: Integrating Generative Analogue Design, Deep-Learning ADMET Prediction and Retrosynthetic Planning

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Abstract

Somatostatin receptor subtype 2 (SSTR2), encoded at chromosome 17q25.1, is overexpressed in high-risk stage 4 neuroblastoma with a 17q gain, yet no non-peptide small-molecule ligand exists for this indication. From the agonist **L-054,522**, BROOD generated 5766 analogues, of which 292 were docked to SSTR2 (PDB 7XN9) with FRED, HYBRID, and POSIT; no physicochemical filter was applied. The query achieved an ROC AUC of 0.798 against known ligands and decoys. Eight compounds reaching GREAT pose confidence were re-docked with AutoDock Vina Extended, FITTED, Flare, and DiffDock and then assessed across five ADMET and two retrosynthesis platforms. Redocking the crystallographic ligand recovered the experimental pose with four site-directed programs (RMSD 0.85–1.79 Å), but none run blind or as ensembles, indicating that pose recovery depends on the search protocol rather than the scoring function. Docking scores were not reproducible across repeat runs, so candidates are not ranked. Compound **8** reproduced the Asp122, Gln126, and Thr194 contacts, alone received a Spaya RScore of 1.0 indicating a fully precedented route, and it held its pose over three 100 ns molecular dynamic simulations in a POPC bilayer (ligand RMSD 1.70 ± 0.12 Å). Because docking cannot distinguish agonism from antagonism, this work delivers a synthesisable hypothesis set, not a characterised pharmacological agent.

Keywords: artificial intelligence; machine learning; deep learning; virtual screening; retrosynthesis prediction; ADMET prediction; somatostatin receptor 2; neuroblastoma; molecular docking; structure–property relationship



Academic Editor: Irini Doytchinova

Received: 9 September 2026

Revised: 1 October 2026

Accepted: 8 October 2026

Published: 10 October 2026

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1. Introduction

Neuroblastoma (NB) is the third most common solid tumour in infants and children [1], accounting for approximately 15% of all paediatric cancer deaths globally [2,3]. The disease originates from embryonic sympathoadrenal cells of the neural crest and is closely linked to genetic and molecular characteristics rather than behavioural or environmental factors [4,5]. Clinical outcomes vary dramatically: some tumours undergo spontaneous regression, while others progress aggressively despite intensive therapy [6]. For patients diagnosed with stage 4 disease (NBS4), survival rates remain between 40 and 50%, underscoring the urgent need for novel therapeutic strategies [2].

A hallmark genetic abnormality in high-risk NBS4 is the segmental gain of chromosome 17q, detected in over 80% of tumours [2]. The region 17q23.1 to 17qter is most

frequently amplified, with gains at 17q25 strongly associated with an unfavourable prognosis. Notably, somatostatin receptor subtype 2 (SSTR2), located at 17q25.1, is overexpressed in high-risk NBS4 [2,7–12]. Previous studies have demonstrated that somatostatin receptors, particularly SSTR2, are expressed in many neuroblastoma tumours and cell lines and that the activation of these receptors can inhibit neuroblastoma cell proliferation and tumour growth [13–15].

Somatostatin receptors belong to the G protein-coupled receptor (GPCR) superfamily and mediate diverse physiological effects [16], including the inhibition of growth hormone, regulation of neurotransmission, and suppression of cell proliferation [14]. SSTR2 is the most extensively studied mediator of somatostatin's antiproliferative action and the primary pharmacological target of clinically prescribed somatostatin analogues [17,18]. Its activation inhibits adenylyl cyclase, blocks voltage-dependent calcium channels, and upregulates cell-cycle inhibitors such as CDKN1B [19].

The nonpeptide compound **L-054,522** was selected as the structural template for this work. **L-054,522** binds SSTR2 with a dissociation constant of 0.01 nM and exhibits greater than 3000-fold selectivity over other somatostatin receptor subtypes [20,21]. Using the co-crystal structure of **L-054,522** with SSTR2 (PDB: 7XN9) [22] as a reference, this study employed a multi-platform computational workflow to design and evaluate potential SSTR2 ligands.

Computational approaches offer significant value at early stages of drug discovery by enabling the rapid screening of chemical space, predicting binding modes, and prioritising candidates for synthesis and testing [23]. However, docking studies alone cannot determine whether a compound acts as an agonist, antagonist, partial agonist, or inactive binder—such functional characterisation requires experimental validation [24]. The present study therefore aims to identify computationally promising SSTR2 ligand candidates and provide a foundation for subsequent experimental investigation.

Two additional GPCRs on chromosome 17 are also expressed in NB cell lines: corticotropin-releasing hormone receptor 1 (CRHR1) at 17q21.31 and galanin receptor type 2 (GALR2) at 17q25.3 [25–29]. As an exploratory analysis, we assessed whether top-ranked SSTR2 ligand candidates might also bind these receptors, though the therapeutic implications of such multi-target binding remain speculative pending experimental studies.

2. Materials and Methods

2.1. Lead Compound and Receptor Selection

Receptor preparation, docking and pose selection were performed with MakeReceptor, FRED, HYBRID and POSIT from the OEDocking suite (OpenEye Scientific, Cadence Molecular Sciences, Santa Fe, NM, USA), OEDocking 4.3.5.0, applications release 2026.1.0.

L-054,522 (C₃₅H₄₇N₇O₅; MW 645.80) was identified as a structural template based on its high affinity and selectivity for SSTR2 [20,21]. The crystal structure of SSTR2 in complex with **L-054,522** (PDB: 7XN9) [22] was retrieved from the Protein Data Bank and used as the primary docking target. The receptor was prepared for docking using the MakeReceptor application (OpenEye, Santa Fe, NM, USA), which defines the binding site based on the crystallographic ligand position and generates a shape-based receptor grid [30].

A second SSTR2 structure (PDB: 7WIG) [31] served as a confirmatory target to assess binding site consistency. For exploratory multi-target analysis, CRHR1 (PDB: 8GTI) [32] and GALR2 (PDB: 7WQ4, 7XBD, 7XJK, 7XJL) [31,33,34] structures were also obtained.

During validation, **L-054,522** failed to re-dock successfully using FRED and HYBRID [35,36] (“Undocked”) and received a POOR confidence score in POSIT [30]. This likely reflects the conformational constraints of the ligand and the sensitivity of pose prediction to the starting geometry. The crystallographic pose was used directly as the reference for

evaluating analogue binding modes, and the MakeReceptor-defined binding site faithfully represents the experimental ligand position.

2.2. Analogue Generation Using BROOD

The BROOD (version 4.2.1.3) application (OpenEye Scientific, Santa Fe, NM, USA) was used to generate structural analogues of **L-054,522** by replacing molecular fragments with bioisosteres of similar shapes and electrostatics but different chemical properties [37,38]. BROOD identifies fragments from known chemical space (medicinal chemistry databases) and substitutes them into the lead structure while preserving the overall molecular shape and pharmacophore features. Ten rounds of fragment searching were performed:

- Shape and Electrostatics mode: 4 rounds (1552 analogues)
- Shape and Colour mode: 6 rounds (4214 analogues)

Druglike filters were applied within BROOD to the replacement fragments rather than to the assembled analogues: molecular weight ≤ 500 Da, LogP ≤ 5.0 , topological polar surface area ≤ 150 Å², Lipinski donor count ≤ 5 , Lipinski acceptor count ≤ 10 , rotatable bonds ≤ 10 , heavy atom count ≤ 30 , and ≤ 1 Lipinski violation. Because BROOD evaluates these limits based on the fragment being substituted, with the contribution of the query excluded, the assembled analogues are not themselves constrained by them: those returned here have a median molecular weight of 669 Da and a median topological polar surface area of 169 Å², and only 26% fall below 500 Da. From each round, the top 22–30 analogues were selected based on Tanimoto combo scores (combined shape and electrostatic/colour similarity), giving 289 cluster representatives. Three reference compounds—**octreotide**, **lanreotide**, and **L-054,522**—were added, yielding 292 structures for docking.

2.3. Benchmark Construction and Query Validation

The performance of the shape-based query was assessed prospectively against a purpose-built benchmark. Known SSTR2 ligands were retrieved from ChEMBL (target ChEMBL1804, Homo sapiens, single protein), yielding 3804 bioactivity records. Records were filtered to Ki or IC50 determinations reported with an exact relation in human assays and with an associated pChEMBL value, and compounds with pChEMBL ≥ 7 (100 nM or better) were retained, giving 227 unique molecules. Because shape-based similarity to a 646 Da non-peptide template is not a meaningful test for macrocyclic peptides, the set was restricted to non-peptidic and peptidomimetic ligands of molecular weight ≤ 900 Da, giving 81 compounds. **L-054,522** was excluded, as it constitutes the query. To limit analogue bias arising from congeneric series, at most five compounds were retained per source publication, selected by potency, giving 35 actives spanning ten publications.

Property-matched decoys were generated for these actives using the DUDE-Z service (tldr.docking.org, accessed on 1 September 2026). Decoys were matched based on the molecular weight (± 125 Da), calculated logP (± 3.6), rotatable bonds (± 5), hydrogen-bond acceptors (± 4), hydrogen-bond donors (± 3), and net formal charge (exact match), with topological similarity to any active constrained below a Tanimoto coefficient of 0.35 and mutual decoy similarity below 0.8. Fifty decoys were requested per active, with a minimum of 20 required for an active to be retained. Thirteen actives met this criterion, yielding 650 decoys at a uniform ratio of 50 decoys per active.

Conformers for actives and decoys were generated together in a single OMEGA run (OMEGA 6.1.1.3, classic mode; maximum 200 conformers per molecule, 10 kcal mol^{−1} energy window, 0.5 Å RMSD duplicate threshold, MMFF94s_NoEstat force field) to ensure identical treatment of both sets. Two macrocyclic actives and 58 decoys were not processed in classic mode and were excluded, leaving 11 actives and 592 decoys. The query was the crystallographic conformation of **L-054,522** extracted from PDB 7XN9, expanded to 200 con-

formers under the same settings. Screening was performed with ROCS 3.9.0.3 (OEChem 4.3.0.3) using the Implicit Mills–Dean colour force field, ranking by TanimotoCombo with no hit-list limit so that all molecules were ranked. Performance was quantified by the area under the receiver operating characteristic curve, by enrichment factors at the top 1% and 5% of the ranked database, and by BEDROC ($\alpha = 20$).

Validation against the DUD and DUD-E decoy collections was considered and rejected. Decoys in these collections are property-matched to the actives of their respective targets: across the 40 DUD sets (124,413 molecules), the median decoy molecular weight ranged from 229 to 518 Da, and across five class A GPCR DUD-E sets (approximately 99,800 molecules), the median was 429 Da, against a query molecular weight of 645.8 Da; fewer than 0.05% of DUD molecules exceeded 600 Da. Enrichment measured against such a background would reflect the molecular size rather than shape complementarity. In addition, SSTR2 is represented in neither collection, so neither can supply target-relevant actives. A property-matched benchmark was therefore constructed specifically for this work, in which 33.7% of decoys exceed 600 Da, and the median decoy molecular weight (546 Da) approximates that of the actives (582 Da).

2.4. Molecular Docking

Primary docking (OpenEye suite): all 292 structures were docked to 7XN9 using FRED (fast exhaustive docking) and HYBRID (ligand-guided docking) [35,36]. FRED performs the systematic, exhaustive sampling of ligand poses within the binding site, while HYBRID uses the co-crystallised ligand to guide pose generation.

Final pose selection and ranking were performed using POSIT [30], which automatically selects the optimal docking algorithm based on 2D (graph) and 3D (structural) similarity between the docked ligand and the bound reference ligand. POSIT assigns pose confidence categories based on the probability that the predicted pose lies within 2.0 Å of the true binding mode:

- GREAT: 75–100% probability
- GOOD: 50–75%
- MEDIOCRE: 33–50%
- POOR: <33%

POSIT probabilities were validated against X-ray crystallography data in the original method development [30], providing a rigorous basis for the pose confidence assessment. No physicochemical or property-based filter was applied at the docking stage: all 292 structures were docked, and the reduction to the final set was affected solely by the pose-prediction confidence. POSIT returned poses for nine structures at its confidence threshold, of which eight were returned at GREAT confidence and constitute the final candidate set.

2.4.1. Re-Docking Across Additional Platforms

Compounds achieving GREAT confidence were re-docked using AutoDock Vina (Samson 2026 R1; version 11.0.1) Extended [39–41] and FITTED (version 1.8.0) (Molecular Forecaster, Montréal, QC, Canada) [42] within the Samson Suite (Version 2026 R1; version 11.0.1), followed by the Flare Suite (version 12.0.0) [43–45]. Docking was also performed against 7WIG to confirm binding site consistency.

2.4.2. Interpretation of Cross-Platform Results

Different docking programs use different scoring functions with different scales and physical meanings. FRED scores reflect shape complementarity and chemical matching; AutoDock Vina (Samson 2026 R1; version 11.0.1) uses an empirical free energy function;

FITTED (version 1.8.0) incorporates induced-fit effects. Raw scores are not directly comparable across platforms [46]. Scores are therefore used only to identify gross incompatibility with the binding site and are not used to rank the candidates; the results are interpreted in Section 3.4.

2.4.3. Exploratory Multi-Target Docking

The eight top-ranked compounds were docked to CRHR1 (8GTI) and GALR2 (7WQ4) using AutoDock Vina Extended. Binding site similarity across receptors was assessed using Protein Aligner (version 7.2.0) [47]. These results are presented as hypothesis-generating rather than confirmatory.

2.4.4. Docking Parameters

For the OpenEye suite (FRED, HYBRID, POSIT) and FITTED, the binding site was defined from the co-crystallised coordinates of **L-054,522** using MakeReceptor, with an expanded search volume of approximately 14,000 Å³. AutoDock Vina Extended, as implemented in Samson 2026 R1 (version 11.0.1), used a grid box of approximately 27,000 Å³ centred on the same site, with exhaustiveness set to 8 and up to 20 binding modes retained per ligand. FITTED does not employ a Vina-style exhaustiveness parameter; search depth is governed by its genetic algorithm settings, and 10 non-redundant poses were retained per ligand. Flare 12.0.0 [43–45] was run with default accuracy settings, with the binding site defined from the co-crystallised ligand. In each case, the largest available search volume was used; this increases the computational cost but reduces the risk of excluding valid poses.

2.4.5. Re-Docking Validation

The crystallographic ligand **L-054,522** (residue GI9) was extracted from PDB 7XN9 and re-docked into the prepared receptor with each platform used in this study, applying the parameters given above. Pose accuracy was assessed based on heavy-atom root-mean-square deviation from the crystallographic coordinates, calculated in place without superposition, with atoms matched within element classes via global optimal assignment (Hungarian algorithm), so that no crystallographic atom can be claimed by more than one atom of the docked pose. This symmetry-corrected treatment replaces the nearest-neighbour matching used previously, which permits many-to-one mapping and systematically flatters poorly reproduced poses. A deviation below 2 Å was taken to indicate successful pose recovery. For AutoDock Vina (Samson 2026 R1; version 11.0.1), two search boxes were compared: a blind box generated automatically from the receptor (69.6 × 75.6 × 89.6 Å), and a site-directed box of 24 × 26 × 28 Å centred on the centroid of the crystallographic ligand at (18.52, 46.04, 54.56), with exhaustiveness of 32. Closest-approach distances to residues implicated in SSTR2 ligand recognition were computed for each re-docked pose and compared with the crystallographic values.

2.4.6. Ligand and Receptor Preparation

Compound **8** was modelled with the primary amine protonated, giving a net charge of +1, consistent with the dominant microspecies expected at physiological pH; this assignment was confirmed during force field parameterisation with CGenFF. Receptor protonation states were assigned by each docking program's default preparation routine at pH 7.4 (MakeReceptor for the OpenEye suite; the built-in protein preparation routines of Samson 2026 R1 and Flare 12.0.0 for the remaining platforms). No manual adjustment of ionisable residues or histidine tautomers was performed.

2.4.7. Independent Blind Docking with DiffDock

DiffDock [48], a diffusion-based generative docking model that employs no scoring function, was applied as an independent blind test. The unmodified 7XN9 structure was used with the crystallographic ligand and all waters removed, and the same prepared receptor file was used for every compound. DiffDock searches the whole receptor rather than a defined site and returns poses ranked by a learned confidence estimate. All eight candidates and **L-054,522** as the reference were submitted under identical settings. Rank-1 poses were assessed by distance from the centroid of the crystallographic ligand and by SMINA rescoring. This test establishes whether the binding site is recovered without site information; it is not presented as supporting evidence for the site-directed results.

2.5. Molecular Dynamic Simulations

The complex of compound **8** with SSTR2 was examined via all-atom molecular dynamic simulation in an explicit lipid bilayer to establish whether the docked pose is physically stable rather than a static optimum of a scoring function.

2.5.1. System Preparation

The receptor–ligand complex was prepared from the docked pose and built into a membrane system with the CHARMM-GUI Membrane Builder (v3.7) [49]. The receptor model spans residues 37–331; residues 240–242 of the third intracellular loop were not modelled, and the disulfide bond between Cys115 and Cys193 was retained. The complex was oriented in the membrane using the PPM server as implemented in CHARMM-GUI and embedded in a bilayer of 203 POPC molecules. The system was solvated with TIP3P water and neutralised with K^+ and Cl^- ions, giving a final system of 94,151 atoms in a box of $9.02 \times 9.02 \times 12.47$ nm. Ligand parameters were generated with the CHARMM General Force Field (CGenFF v4.0), with the primary amine protonated, consistent with the form used for docking.

2.5.2. Simulation Protocol

Simulations were run with GROMACS 2026.3 [50] using the CHARMM36m force field for protein and lipids. Van der Waals interactions were truncated at 1.2 nm with a force-switch function from 1.0 nm, and long-range electrostatics were treated using the particle-mesh Ewald method with a 1.2 nm real-space cut-off. Bonds involving hydrogen were constrained with LINCS. Temperature was maintained at 303.15 K with the velocity-rescale thermostat ($\tau_T = 1.0$ ps), coupling the protein–ligand, membrane, and solvent separately, and pressure was at 1 bar with the stochastic cell-rescaling barostat under semi-isotropic coupling ($\tau_P = 5.0$ ps). The system was energy-minimised based on the steepest descent and equilibrated in six stages following the CHARMM-GUI protocol, with positional restraints on protein and lipids released progressively. Three independent unrestrained production runs of 100 ns were then performed from different random initial velocities, giving 300 ns in total, with a 2 fs time step and coordinates saved every 100 ps.

2.5.3. Trajectory Analysis

Analysis covered the protein backbone RMSD, ligand RMSD after fitting on the protein backbone, $C\alpha$ RMSF, the number of ligand–protein hydrogen bonds, heavy-atom contacts within 4 Å, and the minimum ligand–protein distance. Averages are reported over 10–100 ns, excluding the initial equilibration drift, and the combined figures are the mean $\pm$ SD of the three run means.

2.6. ADMET Evaluation

Toxicity profiles were assessed using Toxicity Estimation Software Tools (T.E.S.T.; version 5.1.2) [51], including the following:

- Bioconcentration factor;
- Mutagenicity (Ames test prediction);
- Oral rat LD₅₀;
- 48 h IGC₅₀ in *Tetrahymena pyriformis*;
- Developmental toxicity.

Pharmacokinetic properties were predicted using DEEP-PK [52]:

- Human oral bioavailability (50% threshold);
- Blood–brain barrier penetration;
- CYP1A2 substrate status;
- Predicted half-life;
- Carcinogenicity.

Additional ADME parameters (TPSA, LogP, water solubility, GI absorption, skin permeability) were calculated using SwissADME (version 2.0-accessed 28 November 2025) [53].

Two further platforms were added during revision. ADMETlab 3.0 was used because it reports applicability–domain information explicitly, and ADMET-AI [54] was used as an independent check on the endpoints where the other platforms disagreed; both were run on all nine structures, the eight candidates and **L-054,522**. Predictions from all five platforms are reported as the numerical value returned by the platform, with its unit where it has one, and the classification cut-off applied is stated in the corresponding table. Each platform's own classification label is retained alongside its probability so that the underlying value remains visible, and no endpoint is reported as a classification without that value. Where a platform returned no value, or placed a structure outside its applicability domain, the tables record that explicitly; no such case is reported as a favourable outcome.

2.7. Retrosynthetic Analysis

Synthetic feasibility was evaluated using two AI-driven platforms: ChemAIRS (version 3.9.0) [55] and Spaya (version 1.13.10) [56]. Spaya assigns an RScore (0–1), where values above 0.5 indicate well-precedented synthetic routes; lower scores suggest novel or higher-risk transformations. ChemAIRS was run in standard mode where possible; compounds requiring “High Risk” settings were flagged. Retrosynthetic analysis was performed using ChemAIRS (software release 3.9.0, database version 3.9.0) and Spaya (version 1.13.10), both neural retrosynthesis platforms.

2.8. Structure Visualisation and Analysis

Molecular structures and binding interactions were visualised using Discovery Studio Visualizer (version 24.1.0.23298) [57] and VIDA (OpenEye, version 5.0.7.1) [58]. Chemical structures were drawn using PICTO (OpenEye, version 5.1.4.3) [59] and ChemDraw (version 25.0.2.14.) [60]. Predicted ¹H and ¹³C NMR spectra were generated using MestReNova [61] to support future synthetic characterisation.

2.9. Machine-Learning Components of the Workflow

For machine-learning components of the workflow, four elements of the pipeline rest on trained models rather than physics-based calculations. POSIT assigns pose confidence from a probabilistic model calibrated against crystallographic poses. T.E.S.T. applies QSAR models built on curated experimental toxicity endpoints. DEEP-PK applies deep neural networks trained on curated pharmacokinetic datasets. ChemAIRS (3.9.0) and Spaya (1.13.10) apply neural retrosynthesis models trained on reaction corpora, with the Spaya RScore

reflecting the density of the literature precedent for the proposed disconnections. Predictions from each are interpreted as prioritisation signals rather than measurements. The compounds prioritised here have molecular weights of approximately 620.81–691.84 Da and topological polar surface areas above 160 Å², placing them beyond the chemical space on which most ADMET models have been trained and validated; predictions for this series should therefore be regarded as indicative rather than quantitative.

2.10. Use of Generative Artificial Intelligence

During the preparation of this manuscript, the authors used Claude (version Opus 5) (Anthropic, San Francisco, CA, USA) for the purposes of manuscript drafting and editing, computation of virtual screening validation metrics, and responsibility for the content of this publication.

3. Results

3.1. Analogue Generation

Ten rounds of BROOD searching generated 5766 candidate structures (Table 1). The highest-ranked analogues of each round were then advanced to docking—between 22 and 30 per round, giving 289 cluster representatives—together with three peptide references, for a total of 292 structures (Figure 1). No physicochemical or property-based filter was applied at this stage.

Table 1. BROOD screening summary.

Search Mode	Rounds	Compounds Generated
Shape and Electrostatics	4	1552
Shape and Colour	6	4214
Total	10	5766

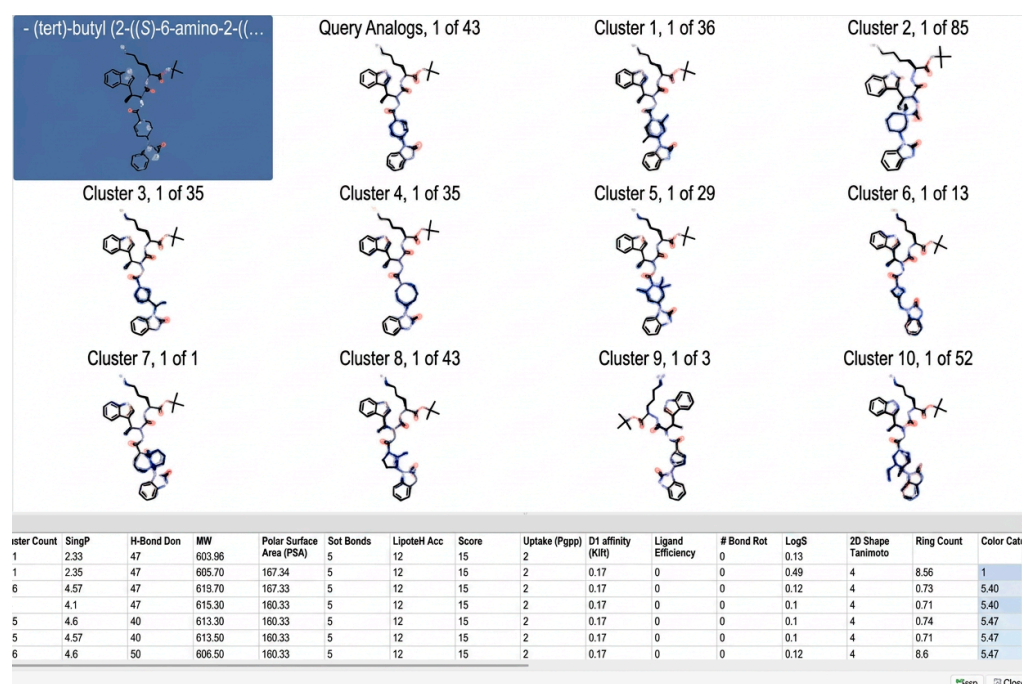


Figure 1. Representative of a section of BROOD output from round 6, showing analogues and associated Tanimoto scores.

3.2. Query Validation

The **L-054,522**-derived query was screened against a benchmark of 11 known SSTR2 ligands and 592 property-matched decoys (Table 2, Figure 2). The query achieved an area under the receiver operating characteristic curve of 0.798, with enrichment factors of 45.7 at the top 1% and 11.0 at the top 5% of the ranked database, and a BEDROC ($\alpha = 20$) of 0.600. Five of the eleven known actives ranked within the top six molecules of 603, and the highest-scoring molecule in the database was a known SSTR2 ligand. Active TanimotoCombo scores spanned 0.484 to 1.188 (median 0.734) against a decoy distribution of 0.373 to 0.746 (median 0.571). The two lowest-ranked actives are structurally unrelated to the **L-054,522** scaffold and are not retrieved by shape-based similarity, which is expected for a single-query method.

Table 2. Validation of the **L-054,522** query against property-matched decoys.

Metric	Value
Known actives	11
Property-matched decoys	592
Decoys per active (as generated)	50
ROC AUC	0.798
Enrichment factor, top 1%	45.7
Enrichment factor, top 5%	11.0
BEDROC ($\alpha = 20$)	0.600
Active ranks (of 603)	1, 2, 3, 4, 5, 9, 66, 89, 169, 474, 559
Active TanimotoCombo, median (range)	0.734 (0.484–1.188)
Decoy TanimotoCombo, median (range)	0.571 (0.373–0.746)

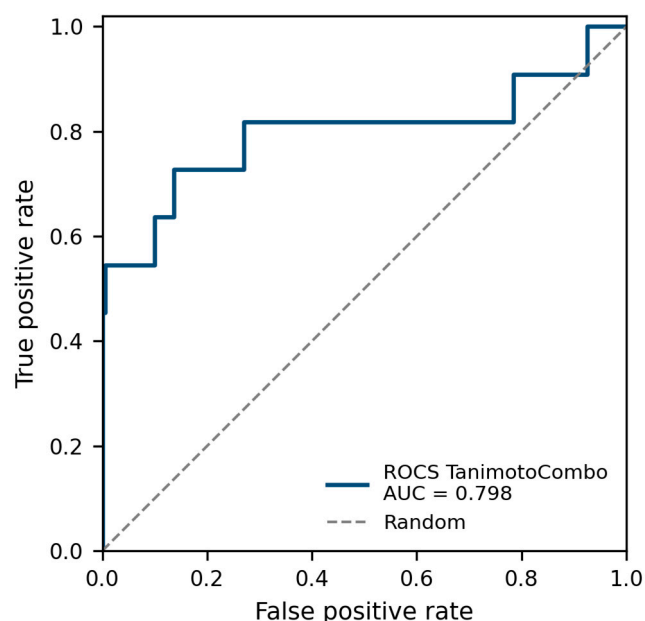


Figure 2. Receiver operating characteristic curve for the **L-054,522**-derived shape query screened against 11 known SSTR2 ligands and 592 property-matched decoys generated with DUDE-Z. Molecules were ranked by TanimotoCombo using ROCS with the Implicit Mills–Dean colour force field. The area under the curve is 0.798; the dashed diagonal indicates random selection. Enrichment factors were 45.7 and 11.0 at the top 1% and 5% of the ranked database, respectively.

All eight prioritised compounds were subsequently scored against the same validated query (Table 3). Every candidate exceeded the highest-scoring decoy in the benchmark (0.746), and seven of the eight ranked within the top 0.5% of the combined database. Compound **30** achieved the highest similarity of any molecule examined (TanimotoCombo 1.294), exceeding all eleven known actives, while compound **8** scored 1.074, exceeding nine of eleven. Because these compounds were generated via shape-based analogue design from **L-054,522**, high similarity to that template is an expected consequence of the design method and does not constitute independent evidence of receptor binding. The result establishes that the prioritised set falls within the similarity range occupied by experimentally confirmed SSTR2 ligands under a query of demonstrated retrieval performance and outside the range occupied by property-matched decoys.

Table 3. Shape and electrostatic similarity of the prioritised compounds to **L-054,522**. Ranks are positions within the combined 604-molecule set.

Compound	TanimotoCombo	ShapeTanimoto	ColorTanimoto	Rank	Actives Exceeded
30	1.294	0.790	0.504	1	11 of 11
12	1.128	0.609	0.518	3	9 of 11
17	1.105	0.633	0.471	3	9 of 11
13	1.104	0.637	0.468	3	9 of 11
8	1.074	0.762	0.312	3	9 of 11
27	1.070	0.596	0.474	3	9 of 11
22	1.058	0.642	0.415	3	9 of 11
14	0.930	0.521	0.409	5	7 of 11
Highest-scoring decoy	0.746	—	—	—	—
Median decoy	0.571	—	—	—	—

3.3. Primary Docking and Compound Selection

All 292 structures were docked to SSTR2 (7XN9). POSIT analysis identified eight compounds (**8**, **12**, **13**, **14**, **17**, **22**, **27**, and **30**) with GREAT pose confidence, indicating a 75–100% probability of the predicted pose lying within 2.0 Å of the true binding mode (Table 4).

Table 4. Primary docking scores (OpenEye suite) and POSIT confidence.

Compound	FRED (7XN9)	FRED (7WIG)	HYBRID (7XN9)	HYBRID (7WIG)	POSIT Confidence
8	−47.67	−49.69	−26.50	−20.23	GREAT
12	−49.59	−52.53	−24.36	−20.04	GREAT
13	−49.48	−51.38	−24.40	−20.28	GREAT
14	−48.92	−52.22	−25.00	−21.32	GREAT
17	−49.51	−53.92	−20.36	−19.07	GREAT
22	−49.87	−52.72	−23.62	−20.70	GREAT
27	−46.47	−48.40	−18.12	−17.01	GREAT
30	−45.95	−49.10	−26.00	−18.13	GREAT
L-054,522	Undocked	Undocked	Undocked	Undocked	Undocked

L-054,522 itself did not dock successfully (“Undocked” in FRED/HYBRID; “POOR” in POSIT), reflecting the conformational constraints discussed in Section 2.1. The crystallographic pose served as the reference for all evaluations.

More negative scores indicate stronger predicted binding. Scores are not comparable across different docking programs. The docking of 292 structures to 7XN9 in FRED is shown in Figure 3, and POSIT results in VIDA are in Figure 4.

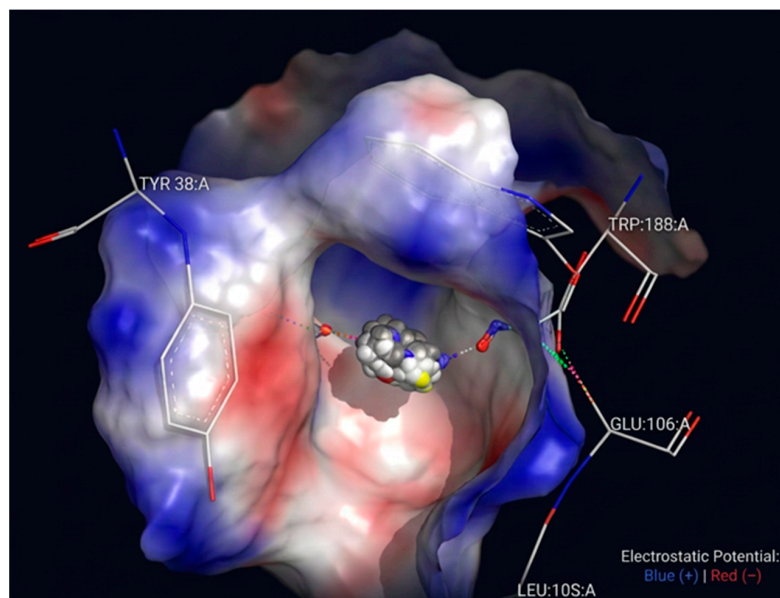


Figure 3. Docking of 292 structures to 7XN9 in FRED, showing binding site occupancy. The receptor is shown as a grey molecular surface clipped at the orthosteric site; docked poses are displayed as sticks, with oxygen in red, nitrogen in blue and polar hydrogen in white.

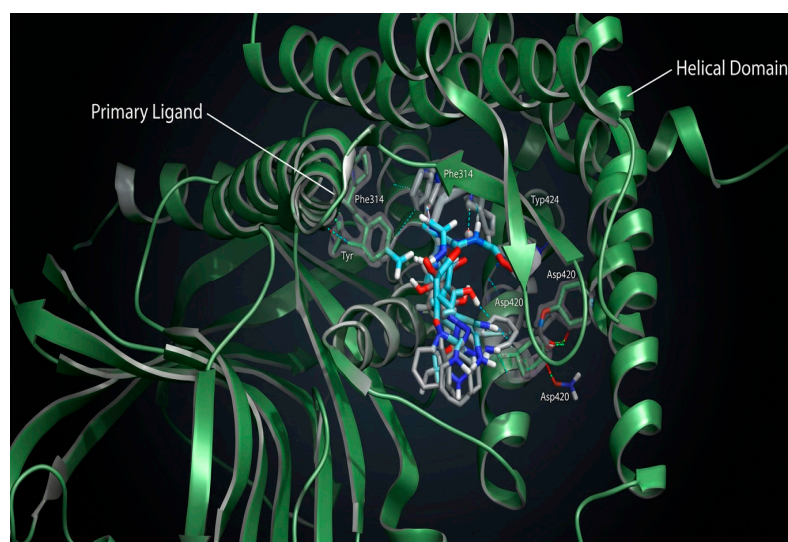


Figure 4. POSIT results in VIDA, highlighting the eight GREAT-confidence compounds. The receptor is shown as a grey molecular surface clipped at the orthosteric site of 7XN9. The eight compounds are displayed as sticks with carbon atoms coloured individually per compound to distinguish the superposed poses; oxygen is red, nitrogen blue and polar hydrogen white in all structures. Surrounding residues are shown as thin lines.

3.4. Redocking Validation

The results of the re-docking validation are shown in Table 5.

Table 5. Re-docking of the crystallographic ligand **L-054,522** into SSTR2 (PDB 7XN9).

Platform	Heavy-Atom RMSD (Å)	Median Deviation (Å)	Atoms Within 1 Å	Outcome
Flare 12.0.0 (normal docking)	0.85	0.77	40/47 (85%)	Pose recovered
AutoDock Vina Extended (Samson 2026 R1; version 11.0.1) (site-directed box)	1.38	0.86	30/47 (64%)	Pose recovered
FITTED (version 1.8.0)	1.54	1.11	20/47 (42%)	Pose recovered
POSIT	1.79	0.52	32/47 (68%)	Pose recovered, then rejected on steric grounds
HYBRID	4.18	2.07	8/47 (17%)	Pose not recovered
FRED	5.28	3.77	7/47 (15%)	Pose not recovered
AutoDock Vina Extended (blind box)	15.66	15.8	0/47 (0%)	Pose not recovered
DiffDock (version 1.1.3) (blind)	67.90	68.18	0/47 (0%)	Pose not recovered
Flare 12.0.0 (ensemble docking)	73.70	73.83	0/47 (0%)	Pose not recovered

RMSD calculated over all 47 heavy atoms without superposition, against the crystallographic coordinates of residue G19, with atoms matched within element classes via global optimal assignment (symmetry-corrected). A deviation below 2 Å was taken to indicate successful pose recovery. For AutoDock Vina Extended in the blind box, the value is for the top-ranked pose.

The closest approach (Å) between the re-docked ligand and residues implicated in SSTR2 ligand binding is shown in Table 6.

Table 6. Closest approach (Å) between the re-docked ligand and residues implicated in SSTR2 ligand binding.

Residue	Crystallographic	Flare	POSIT	HYBRID	FRED	Vina Ext. (Blind Box)
Asp122	2.65	2.72	2.56	3.70	5.53	7.64
Gln126	2.93	2.89	3.60	2.30	3.45	9.98
Thr194	2.53	2.78	2.46	3.85	1.04	2.83
Phe208	4.24	3.54	4.02	3.40	3.69	7.66
Phe272	3.76	2.17	3.53	2.01	3.71	7.17
Phe294	3.14	2.75	2.24	3.25	2.73	3.28

The Asp122 salt bridge is reproduced by Flare and POSIT, degraded by HYBRID and FRED, and lost entirely by AutoDock Vina Extended in the blind box.

The crystallographic ligand **L-054,522** was extracted from PDB 7XN9 and re-docked into the prepared receptor under each protocol used in this study. Four protocols recovered the experimental binding mode within 2 Å: Flare in normal docking at 0.85 Å, with 85% of heavy atoms within 1.0 Å of their crystallographic positions; AutoDock Vina Extended in a site-directed box at 1.38 Å; FITTED at 1.54 Å; and POSIT at 1.79 Å, with a median atom deviation of 0.52 Å. POSIT rejected its pose on steric grounds; it is included in the comparison because it demonstrates that the correct binding mode was identified, with its rejection reflecting the clash criterion applied to the prepared receptor rather than a failure of pose prediction. The remaining protocols did not recover the experimental pose: HYBRID placed the ligand at 4.18 Å, FRED at 5.28 Å, AutoDock Vina Extended in the blind box at 15.66 Å, DiffDock at 67.90 Å, and Flare in ensemble mode at 73.70 Å.

HYBRID and FRED placed the ligand within the orthosteric pocket but in an incorrect orientation, inverted end to end relative to the crystallographic pose. The blind-box AutoDock Vina run placed it outside the site altogether, with the ligand centroid 14.9 Å from its crystallographic position, and none of the nine returned poses came within 6 Å of the crystallographic pose—indicating that the correct binding mode was not sampled rather than merely mis-ranked. Reproduction of individual binding-site contacts followed the same pattern (Table 6). The Asp122 salt bridge, at 2.65 Å in the crystal structure, was reproduced by Flare (2.72 Å) and POSIT (2.56 Å), degraded by HYBRID (3.70 Å) and FRED (5.53 Å), and lost entirely by AutoDock Vina Extended in the blind box (7.64 Å). Contacts with the aromatic residues Phe208, Phe272, and Phe294 were more consistently reproduced across platforms, indicating that the aromatic pocket constrains ligand placement more strongly than the polar contacts do.

A related observation concerns the scoring rather than the poses. Three platforms assigned **L-054,522** the least favourable score of the nine molecules examined: FRED −10.76 against −48.79 to −53.60 for the eight candidates, HYBRID −10.10 against −29.07 to −32.92, and AutoDock Vina (Samson 2026 R1; version 11.0.1) Extended −9.65 against −21.39 to −23.07. Since **L-054,522** has a reported K_i of 0.01 nM, while none of the eight candidates has been tested experimentally, this inverts the ranking that the available experimental data would predict.

These results show that pose recovery on this target depends on the search protocol rather than on the scoring function: all four protocols that recovered the crystallographic pose used a focused, site-directed search, while every blind, ensemble and batch-screening protocol failed, as did FRED and HYBRID. A site-directed search was therefore necessary but not sufficient for pose recovery. No platform is given greater weight on this basis, and the repeat-run analysis reported in Section 4.2 shows that the docking scores themselves are not reproducible between runs.

3.5. Confirmatory Docking

Scores for the eight candidates are given in Table 7. No compound scored most favourably under every method, and the spread between candidates is small relative to the variation between repeat runs of the same program (Section 4.3; Supplementary Table S1). The scores are reported for completeness and are not used to rank the candidates. The AutoDock Vina Extended scores span only 1.7 kcal mol^{−1} across the eight candidates, with the four highest-ranked separated by less than 0.1 kcal mol^{−1}, offering little discrimination; the Flare scores span 2.1 kcal mol^{−1}. No weighting between platforms is applied, because the repeat-run analysis reported in Section 4.3 shows these scores to be unstable between runs.

Table 7. Confirmatory docking scores.

Compound	FITTED (7XN9)	FITTED (7WIG)	AutoDock Vina Ext. (7XN9)	Flare LF dG (7XN9)	Flare LF dG (7WIG)
8	−20.91	−13.78	−22.42	−12.311	−11.303
12	−19.31	−16.04	−22.97	−11.117	−10.852
13	−15.78	−16.06	−22.99	−11.021	−10.765
14	−19.59	−15.83	−22.26	−11.823	−9.817
17	−13.85	−12.33	−22.97	−12.309	−9.655
22	−17.05	−13.90	−23.07	−12.256	−11.168

Table 7. Cont.

Compound	FITTED (7XN9)	FITTED (7WIG)	AutoDock Vina Ext. (7XN9)	Flare LF dG (7XN9)	Flare LF dG (7WIG)
27	−15.77	−13.13	−21.74	−13.080	−10.304
30	−19.74	−15.41	−21.39	−13.083	−10.976
L-054,522	−16.60	−14.54	−9.65	−12.453	Dock unsuccessful

AutoDock Vina Extended scores were obtained with a search box of $28 \times 28 \times 28$ Å centred on the crystallographic ligand at (19.1, 44.7, 54.5), grid spacing of 0.375 Å, and exhaustiveness of 32, and receptor side-chain flexibility enabled. Flare scores are LF dG values from Flare 12.0.0 at default accuracy. Bold indicates the most favourable score in each column.

No compound ranked first under every method. Compound 8 scored within the range of the other candidates across the seven receptor–program combinations examined, with no consistent position; the repeat-run analysis in Section 4.3 shows that this ordering is not reproducible. Figure 5 shows the Flare docking poses with compound 8.

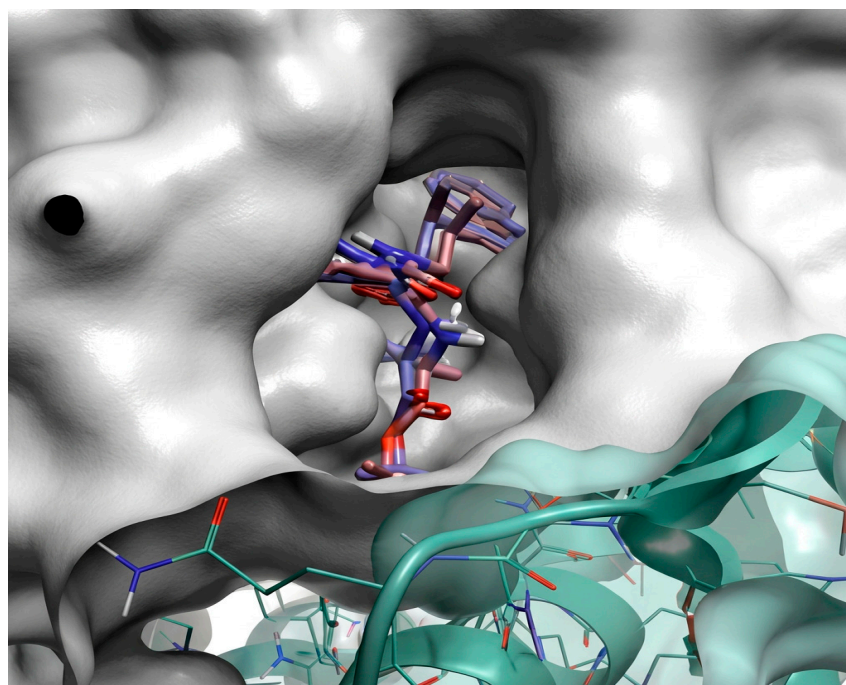


Figure 5. Flare docking results showing the binding pose of compound 8. Ligand poses are shown as sticks with carbon atoms coloured individually per pose; oxygen is red, nitrogen blue and polar hydrogen white throughout. The receptor is rendered as a grey molecular surface clipped at the binding site, with the adjacent helix shown as a pale green cartoon.

3.6. Binding Interactions

An analysis of compound 8's predicted binding pose revealed the following interactions:
Hydrogen bonds:

- Asp122 (salt bridge);
- Gln126;
- Thr194;
- Phe208 (backbone);
- Ala283.

Hydrophobic contacts:

- Val103;
- Phe272;
- Phe294;
- Leu290;
- Val298.

Aromatic interactions:

- π -stacking with Phe208, Phe272, Phe275, Phe294.

These interactions closely (Figure 6) mirror those observed in the **L-054,522** crystal structure [22] and align with residues identified in published molecular dynamic studies as critical for SSTR2 ligand binding [62]. Gervasoni et al. demonstrated, through multi-microsecond MD simulations, that ligands stabilising the active conformation of SSTR2 engage Asp122, Gln126, and the aromatic residues lining the binding pocket [62]. The interaction pattern of compound **8** is consistent with these binding determinants.

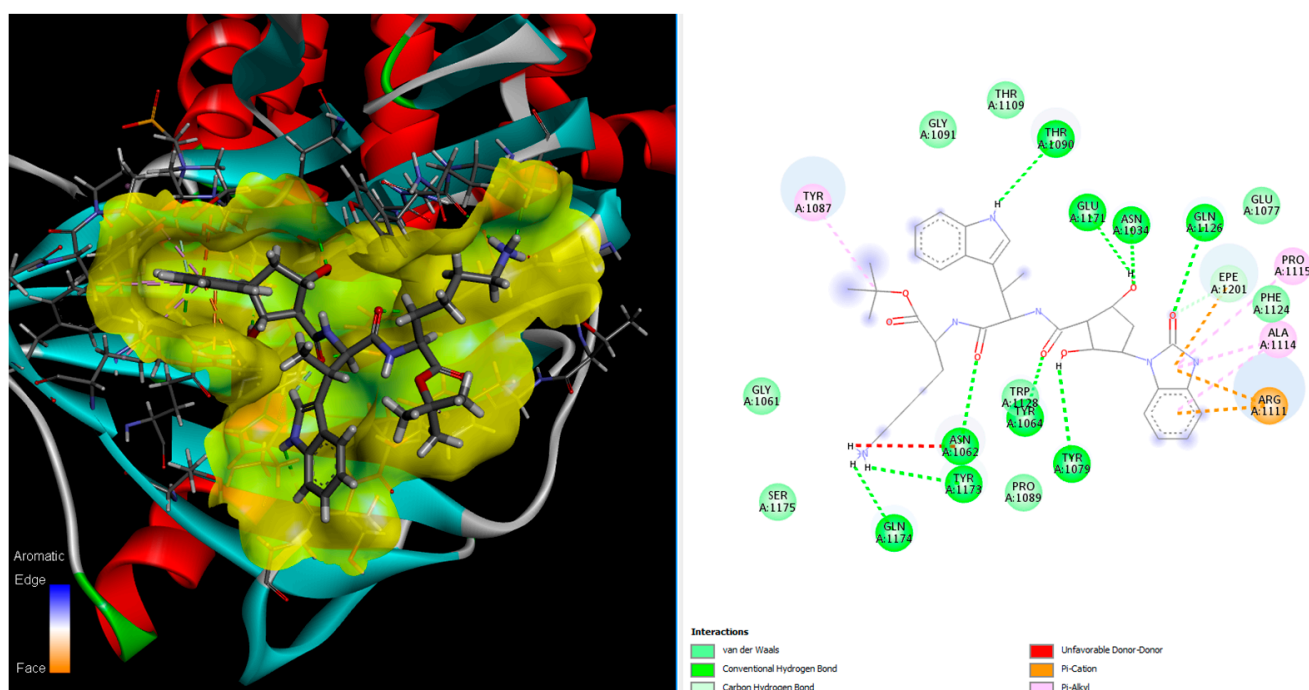


Figure 6. Compound **8** interaction map with 7XN9, showing hydrogen bonds, hydrophobic contacts, and aromatic interactions.

While these interactions are consistent with ligand binding, docking cannot determine functional activity. Whether compound **8** acts as an agonist, antagonist, partial agonist, or inactive binder requires experimental determination through binding assays and functional studies.

DiffDock, a diffusion-based generative docking method employing no scoring function, was applied as an independent blind test to all eight compounds and to **L-054,522** as the reference, with the receptor prepared identically in every case. Under this protocol DiffDock did not reproduce the binding site: rank-1 poses for six of the nine structures, including the reference ligand at 67.74 Å, fell outside the pocket entirely, and all nine rank-1 poses gave positive SMINA affinities, indicating steric clash rather than binding. Only compounds **12**, **17**, and **14** placed rank-1 poses within the site (0.78, 2.38, and 3.73 Å from the crystallographic centroid). A previously reported placement of compound **8** at 1.48 Å is withdrawn: the receptor file used for that run still contained the crystallographic

ligand and buffer molecule as HETATM records, so the result was not a blind prediction. DiffDock performs blind docking across the entire receptor rather than a defined site, and its confidence estimates did not separate the successful placements from the failures (−1.35 to −3.22 across the nine structures), so the confidence score was not informative based on this target. These results are reported as a negative control and are not used to support any claim about the compounds.

Molecular formulae, masses, and elemental compositions were computed directly from the three-dimensional structures used for docking, so that formula and composition are derived from the same source and cannot disagree (Table 8). Percentages are by mass, from IUPAC standard atomic weights. Compositions are given for the protonated species predominant at pH 7.4, consistent with the forms used throughout.

Table 8. Molecular formulae and elemental composition of the eight candidates.

Compound	Molecular Formula	MW (g mol ^{−1})	C (%)	H (%)	N (%)	O (%)
8	C35H47N6O7	663.80	63.33	7.14	12.66	16.87
12	C37H50N7O5	672.85	66.05	7.49	14.57	11.89
13	C37H51N6O7	691.85	64.23	7.43	12.15	16.19
14	C36H51N7O5	661.85	65.33	7.77	14.81	12.09
17	C37H52N7O5	674.87	65.85	7.77	14.53	11.85
22	C37H47N6O7	687.82	64.61	6.89	12.22	16.28
27	C34H46N7O5	632.79	64.54	7.33	15.49	12.64
30	C34H50N7O4	620.82	65.78	8.12	15.79	10.31

The chemical structures, molecular weights, and names of all eight compounds are presented in Table 9. All retain key structural features: an aromatic moiety capable of π -stacking, a basic centre capable of interacting with Asp122/Gln126, and overall shape similarity to L-054,522.

Table 9. Structures and names of top-ranked compounds.

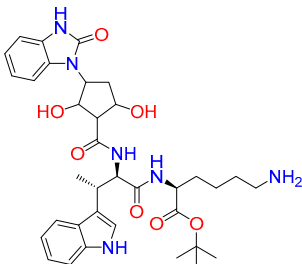
Compound	Chemical Structure and Molecular Weight (MW)	Name
8	 Chemical Formula: C₃₅H₄₆N₆O₇ Exact Mass: 662.34 Molecular Weight: 662.79 m/z: 662.34 (100.0%), 663.35 (38.7%), 664.35 (8.7%), 663.34 (2.2%), 665.35 (1.6%) Elemental Analysis: C, 63.43; H, 7.00; N, 12.68; O, 16.90	[(5S)-5-[[[(2R,3S)-2-[[2,5-dihydroxy-3-(2-oxo-3H-benzimidazol-1-yl)cyclopentanecarbonyl]amino]-3-(1H-indol-3-yl)butanoyl]amino]-6-[(2-methylpropan-2-yl)oxy]-6-oxohexyl]azanium.

Table 9. Cont.

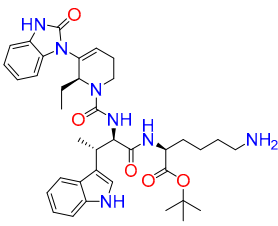
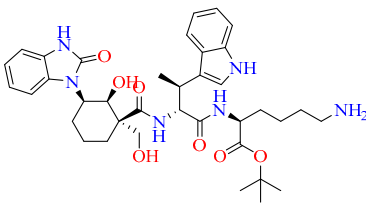
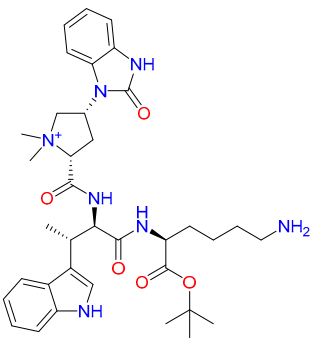
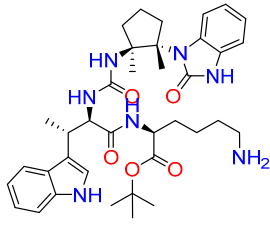
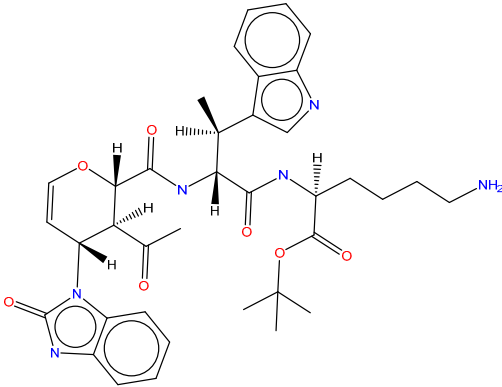
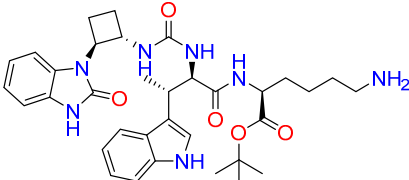
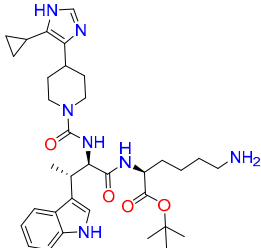
Compound	Chemical Structure and Molecular Weight (MW)	Name
12	 Chemical Formula: C₃₇H₄₉N₇O₅ Exact Mass: 671.38 Molecular Weight: 671.84 m/z: 671.38 (100.0%), 672.38 (42.8%), 673.39 (8.1%), 673.38 (2.1%), 674.39 (1.5%) Elemental Analysis: C, 66.15; H, 7.35; N, 14.59; O, 11.91	[(5S)-5-[[[(2R,3S)-2-[[[(6S)-6-ethyl-5-(2-oxo-3H-benzimidazol-1-yl)-3,6-dihydro-2H-pyridine-1-carbonyl]amino]-3-(1H-indol-3-yl)butanoyl]amino]-6-[(2-methylpropan-2-yl)oxy]-6-oxohexyl]azanium.
13	 Chemical Formula: C₃₇H₅₀N₆O₇ Exact Mass: 690.37 Molecular Weight: 690.84 m/z: 690.37 (100.0%), 691.38 (40.9%), 692.38 (9.6%), 691.37 (2.2%), 693.38 (1.8%) Elemental Analysis: C, 64.33; H, 7.30; N, 12.17; O, 16.21	[(5S)-5-[[[(2R,3S)-2-[[[(1R,2S,3R)-2-hydroxy-1-(hydroxymethyl)-3-(2-oxo-3H-benzimidazol-1-yl)cyclohexanecarbonyl]amino]-3-(1H-indol-3-yl)butanoyl]amino]-6-[(2-methylpropan-2-yl)oxy]-6-oxohexyl]azanium.
14	 Chemical Formula: C₃₆H₅₀N₇O₅⁺ Exact Mass: 660.39 Molecular Weight: 660.84 m/z: 660.39 (100.0%), 661.39 (39.7%), 662.39 (9.5%), 661.38 (2.6%), 663.40 (1.0%) Elemental Analysis: C, 65.43; H, 7.63; N, 14.84; O, 12.11	[(5S)-5-[[[(2R,3S)-2-[[[(2R,4R)-1,1-dimethyl-4-(2-oxo-3H-benzimidazol-1-yl)pyrrolidin-1-ium-2-carbonyl]amino]-3-(1H-indol-3-yl)butanoyl]amino]-6-[(2-methylpropan-2-yl)oxy]-6-oxohexyl]azanium.
17	 Chemical Formula: C₃₇H₅₁N₇O₅ Exact Mass: 673.40 Molecular Weight: 673.86 m/z: 673.40 (100.0%), 674.40 (40.8%), 675.40 (10.2%), 674.39 (2.6%), 676.41 (1.1%) Elemental Analysis: C, 65.95; H, 7.63; N, 14.55; O, 11.87	[(5S)-5-[[[(2R,3S)-2-[[[(1S,2S)-1,2-dimethyl-2-(2-oxo-3H-benzimidazol-1-yl)cyclopentyl]carbamoylamino]-3-(1H-indol-3-yl)butanoyl]amino]-6-[(2-methylpropan-2-yl)oxy]-6-oxohexyl]azanium.

Table 9. Cont.

Compound	Chemical Structure and Molecular Weight (MW)	Name
22	 Chemical Formula: C₃₇H₄₄N₆O₇ Exact Mass: 684.33 Molecular Weight: 684.79 m/z: 684.33 (100.0%), 685.33 (40.8%), 686.33 (10.2%), 685.32 (2.2%), 687.34 (1.1%) Elemental Analysis: C, 64.90; H, 6.48; N, 12.27; O, 16.35	[(5S)-5-[[[(2R,3S)-2-[[[(2S,3S,4R)-3-acetyl-4-(2-oxo-3H-benzimidazol-1-yl)-3,4-dihydro-2H-pyran-2-carbonyl]amino]-3-(1H-indol-3-yl)butanoyl]amino]-6-[(2-methylpropan-2-yl)oxy]-6-oxohexyl]azanium.
27	 Chemical Formula: C₃₄H₄₅N₇O₅ Exact Mass: 631.35 Molecular Weight: 631.78 m/z: 631.35 (100.0%), 632.35 (40.0%), 633.35 (8.5%), 634.36 (1.2%) Elemental Analysis: C, 64.64; H, 7.18; N, 15.52; O, 12.66	[(5S)-5-[[[(2R,3S)-3-(1H-indol-3-yl)-2-[[[(1S,2S)-2-(2-oxo-3H-benzimidazol-1-yl)cyclobutyl]carbamoylamino]butanoyl]amino]-6-[(2-methylpropan-2-yl)oxy]-6-oxohexyl]azanium.
30	 Chemical Formula: C₃₄H₄₉N₇O₄ Exact Mass: 619.38 Molecular Weight: 619.81 m/z: 619.38 (100.0%), 620.39 (37.5%), 621.39 (7.7%), 620.38 (2.6%), 622.39 (1.3%), 621.38 (1.0%) Elemental Analysis: C, 65.89; H, 7.97; N, 15.82; O, 10.33	[(5S)-5-[[[(2R,3S)-2-[[4-(5-cyclopropyl-1H-imidazol-4-yl)piperidine-1-carbonyl]amino]-3-(1H-indol-3-yl)butanoyl]amino]-6-[(2-methylpropan-2-yl)oxy]-6-oxohexyl]azanium.

3.7. Comparative Structural Analysis

All eight candidates retain three features of the parent scaffold: the 2-oxo-benzimidazole head group, the indole side chain, and the tert-butyl ester terminus, with the protonated primary amine positioned to engage Asp122. Structural variation between them is confined to the fragment replaced by BROOD.

Predicted properties vary systematically with the substituted fragment. TPSA spans 159.85 Å² (compound **30**) to 206.18 Å² (compounds **8** and **13**), while XLOGP3 varies from 3.40 to 5.64. Predicted skin permeability tracks lipophilicity closely ($r = 0.954$ with XLOGP3)

rather than the polar surface area ($r = -0.33$), with the most lipophilic candidates (12 and 17, XLOGP3 5.41–5.64) showing the least restricted permeability (-6.40 and -6.58) and the least lipophilic (30, XLOGP3 3.40) showing the most restricted (-7.67). Compounds **8** and **13** share an identical TPSA of $206.18 \text{ \AA}^2$, as do compounds **17** and **27** at $177.75 \text{ \AA}^2$, indicating that several BROOD substitutions are isosteric with respect to the polar surface while differing in lipophilicity. Predicted aqueous solubility discriminates within the set: compounds **8**, **13**, **22**, **27**, and **30** are classified as moderately soluble, while compounds **12**, **14**, and **17** and the lead compound **L-054,522** are poorly soluble. Gastrointestinal absorption was predicted as low for all nine.

The only predicted pharmacokinetic parameter that distinguishes within the set is half-life: compounds **8**, **13**, **14**, **22**, and **27** are predicted to exceed three hours, while compounds **12**, **17**, and **30** fall below, as does **L-054,522**. All other predicted toxicity and pharmacokinetic parameters are uniform across the eight candidates.

The clearest structure–property relationship in the set concerns synthetic accessibility rather than predicted binding: the only candidate with an unmodified cyclopentane-derived fragment (compound **8**) received a Spaya RScore of 1.0, while candidates incorporating strained, spiro, or quaternary-nitrogen fragments (14, 17, 22, 27, 30) scored 0.2–0.4 or required high-risk settings in ChemAIRS. Because no experimental activity data are available for any member of the series, these are structure–property rather than structure–activity relationships, and no inference about potency should be drawn from them.

3.8. ADMET Properties

3.8.1. Toxicity

T.E.S.T. returned a consensus prediction for developmental toxicity for every structure, and all nine—the eight compounds and **L-054,522**—were classified as developmental toxicants, with consensus values between 0.82 and 1.08 (Table 10). The eight compounds therefore show no predicted developmental toxicity advantage over the parent ligand. For the remaining T.E.S.T. endpoints, including mutagenicity, the oral rat LD50, and three of the four ecotoxicity measures, the consensus models returned no prediction for most or all structures; these are reported as N/A, which indicates the absence of a prediction rather than a negative result. Across platforms, the toxicity calls were not concordant. DEEP-PK classified hERG blockade and micronucleus induction as toxic at maximum confidence ($p = 1$) for all nine structures and liver injury II as toxic ($p = 0.75$ – 0.90) for all nine, whereas ADMETlab 3.0 returned low probabilities for the same endpoints (hERG 0.10–0.34). Because these DEEP-PK flags apply equally to **L-054,522**, a compound carried through medicinal chemistry optimisation, they are more consistent with a systematic response of the models to large, highly polar peptidomimetics than with compound-specific risk. Based on AMES mutagenicity, the platforms also diverged: DEEP-PK classified compounds **8**, **13**, and **27** as positive (compound **8**, $p = 0.847$), whereas ADMETlab (0.03–0.21) and ADMET-AI (0.060–0.180) returned low probabilities for every structure, placing compound **8** among the least mutagenic of the set and below **L-054,522** on both. Two of the three platforms therefore do not support a mutagenicity flag for compound **8**. The hERG signal is more consistent: ADMET-AI returned 0.855–0.943 across the series, including 0.942 for **L-054,522**, in agreement with DEEP-PK and against ADMETlab. Predicted hERG liability therefore tracks the scaffold rather than the substitution pattern and applies equally to the parent ligand; it is reported here as a property of the chemotype requiring experimental resolution, not as a basis for discriminating between the candidates.

Table 10. T.E.S.T. toxicity predictions.

Compound	Developmental Toxicity (Value)	Classification	Mutagenicity	Oral rat LD50 (mg/kg)	BCF	Daphnia Magna LC50 (mg/L)	Water Solubility (mg/L)
8	0.82	Toxicant	N/A	N/A	N/A	24.08	1.63
12	1.08	Toxicant	N/A	N/A	N/A	8.81	0.31
13	0.96	Toxicant	N/A	N/A	N/A	15.47	1.20
14	0.90	Toxicant	N/A	N/A	4.91	24.96	2.22
17	0.82	Toxicant	N/A	N/A	N/A	7.04	0.30
22	0.99	Toxicant	N/A	N/A	N/A	20.27	1.42
27	0.92	Toxicant	N/A	N/A	5.21	13.73	0.55
30	1.04	Toxicant	N/A	884.64	19.04	6.36	0.86
L-054,522	1.00	Toxicant	−0.04 (negative)	N/A	4.29	5.51	0.79

3.8.2. Pharmacokinetics

DEEP-PK predictions indicated that all compounds, including L-054,522, exhibit low oral bioavailability, do not penetrate the blood–brain barrier, are not CYP1A2 substrates, and have safe carcinogenicity profiles (Table 11). Compounds 8, 13, 14, 22, and 27 showed predicted half-lives ≥ 3 h, while compounds 12, 17, 30, and L-054,522 showed shorter half-lives.

Table 11. DEEP-PK pharmacokinetic predictions.

Compound	Intestinal Absorption	Half-Life	AMES	hERG	Micronucleus	Liver Injury II	Respiratory
8	Non-absorbed	≥ 3 h	Toxic (0.85)	Toxic (1.00)	Toxic (1.00)	Toxic (0.88)	Toxic (0.79)
12	Absorbed	< 3 h	Safe (0.00)	Toxic (1.00)	Toxic (1.00)	Toxic (0.82)	Toxic (0.98)
13	Non-absorbed	≥ 3 h	Toxic (0.65)	Toxic (1.00)	Toxic (1.00)	Toxic (0.88)	Toxic (0.77)
14	Non-absorbed	≥ 3 h	Safe (0.00)	Toxic (1.00)	Toxic (1.00)	Toxic (0.75)	Toxic (0.58)
17	Non-absorbed	< 3 h	Safe (0.32)	Toxic (1.00)	Toxic (1.00)	Toxic (0.80)	Toxic (0.81)
22	Absorbed	≥ 3 h	Safe (0.42)	Toxic (1.00)	Toxic (1.00)	Toxic (0.90)	Safe (0.01)
27	Non-absorbed	≥ 3 h	Toxic (0.64)	Toxic (1.00)	Toxic (1.00)	Toxic (0.84)	Toxic (0.60)
30	Non-absorbed	< 3 h	Safe (0.00)	Toxic (1.00)	Toxic (1.00)	Toxic (0.78)	Toxic (1.00)
L-054,522	Absorbed	< 3 h	Safe (0.33)	Toxic (1.00)	Toxic (1.00)	Toxic (0.90)	Toxic (0.89)

3.8.3. Physicochemical Properties

The results from SwissADME are shown in Table 12.

Table 12. SWISSADME predictions.

Compound	Formula	MW Da	TPSA (Å ²)	XLOGP3	Consensus LogP	Log Kp (cm/s)	Solubility	GI Absorption	Lipinski Violations
8	C ₃₅ H ₄₇ N ₆ O ₇ ⁺	663.78	206.18	4.26	0.42	−7.32	Moderate	Low	3
12	C ₃₇ H ₅₀ N ₇ O ₅ ⁺	672.84	168.96	5.64	2.06	−6.40	Poor	Low	2
13	C ₃₇ H ₅₁ N ₆ O ₇ ⁺	691.84	206.18	4.18	0.87	−7.55	Moderate	Low	3
14	C ₃₆ H ₅₁ N ₇ O ₅ ²⁺	661.83	165.72	4.54	−0.41	−7.11	Poor	Low	2
17	C ₃₇ H ₅₂ N ₇ O ₅ ⁺	674.85	177.75	5.41	2.34	−6.58	Poor	Low	3
22	C ₃₇ H ₄₇ N ₆ O ₇ ⁺	687.81	192.02	4.33	1.10	−7.42	Moderate	Low	2

Table 12. Cont.

Compound	Formula	MW Da	TPSA (Å ²)	XLOGP3	Consensus LogP	Log Kp (cm/s)	Solubility	GI Absorption	Lipinski Violations
27	C ₃₄ H ₄₆ N ₇ O ₅ ⁺	632.77	177.75	4.68	1.45	−6.84	Moderate	Low	3
30	C ₃₄ H ₅₀ N ₇ O ₄ ⁺	620.81	159.85	3.40	2.41	−7.67	Moderate	Low	2
L-054,522	C ₃₅ H ₄₇ N ₇ O ₅	645.79	167.34	4.79	2.62	−6.84	Poor	Low	2

Descriptors were computed for the protonated species predominant at pH 7.4, consistent with the forms used for docking. Compound **14** carries an additional permanent positive charge from its quaternary ammonium. iLOGP could not be determined for any compound in this series, and XLOGP3 is reported in its place.

A fifth platform, ADMET-AI, was applied to all nine structures as an independent check on the toxicity endpoints where DEEP-PK and ADMETlab disagreed (Table 13). Values are probabilities, with higher values indicating greater predicted liability. ADMET-AI returns low mutagenicity probabilities for every structure (0.060–0.180), placing compound **8** among the least mutagenic of the set, and high hERG probabilities for every structure (0.855–0.943), including L-054,522—in agreement with DEEP-PK on hERG and ADMETlab based on mutagenicity.

Table 13. ADMET-AI predictions for the eight candidates and L-054,522.

Compound	AMES	hERG	DILI	Carcinogenicity	ClinTox	P-gp Substrate	Bioavailability
8	0.084	0.902	0.395	0.081	0.018	0.603	0.183
12	0.076	0.943	0.623	0.090	0.013	0.867	0.201
13	0.099	0.931	0.419	0.088	0.018	0.724	0.222
14	0.114	0.917	0.349	0.117	0.025	0.641	0.145
17	0.089	0.929	0.550	0.109	0.017	0.784	0.278
22	0.131	0.855	0.592	0.172	0.015	0.956	0.289
27	0.180	0.916	0.521	0.104	0.024	0.697	0.229
30	0.060	0.912	0.344	0.026	0.018	0.686	0.141
L-054,522	0.096	0.942	0.641	0.057	0.424	0.834	0.436

3.9. Synthetic Accessibility

A retrosynthetic analysis confirmed that all eight compounds have identifiable synthetic routes (Table 14). Critically, compound **8** achieved an RScore of 1.0 in Spaya, indicating a fully precedented pathway with established reaction types. Other compounds required “High Risk” settings in ChemAIRS or achieved lower RScores (0.2–0.4), indicating less established or more challenging synthetic routes.

Table 14. Retrosynthetic accessibility summary.

Compound	ChemAIRS	Spaya RScore	Interpretation
8	Direct route	1.0	Fully precedented; straightforward synthesis
12	High Risk required	Not scored	Challenging synthesis
13	High Risk required	Not scored	Challenging synthesis
14	High Risk required	Not scored	Challenging synthesis
17	High Risk required	0.4	Higher-risk transformations
22	High Risk required	0.3	Higher-risk transformations

Table 14. Cont.

Compound	ChemAIRS	Spaya RScore	Interpretation
27	High Risk required	0.4	Higher-risk transformations
30	High Risk required	0.2	Novel/challenging route

RScore > 0.5 indicates well-precedented routes; lower scores suggest novel transformations with less literature precedent.

Given its superior synthetic accessibility, compound **8** is prioritised as the lead candidate. Detailed retrosynthetic schemes for all compounds are provided in Supplementary Materials (Scheme S1–S9).

3.10. Exploratory Multi-Target Analysis

As exploratory analysis, the eight compounds were docked to CRHR1 (8GTI) and GALR2 (7WQ4)—two additional GPCRs on chromosome 17q that are expressed in neuroblastoma cell lines [25–29]. A Protein Aligner [47] comparison indicated structural similarity between the SSTR2 binding pocket and those of CRHR1/GALR2 (Figure 7).



Figure 7. Protein Aligner comparison of 7XN9, 8GTI, and 7WQ4 binding sites.

Compound **8** returned the least favourable CRHR1 score of the nine molecules examined (−6.46, against −7.32 for **L-054,522**) and a less favourable GALR2 score than the lead compound (−7.66 against −8.16). Its predicted engagement with both off-target receptors is therefore weaker than that of **L-054,522** and of most of its own analogues. Consistent with this, the SSTR2-specific Asp122 salt bridge is absent from the predicted CRHR1 and GALR2 poses. Taken together these observations suggest a degree of predicted selectivity for SSTR2 over the two other chromosome 17 GPCRs examined, although docking scores alone cannot establish selectivity, and receptor-specific binding assays would be required to test this (Table 15).

Table 15. Exploratory multi-target docking scores (AutoDock Vina).

Compound	CRHR1 (8GTI)	GALR2 (7WQ4)
8	−6.46	−7.66
12	−6.94	−7.41
13	−7.64	−7.41
14	−6.88	−7.12
17	−7.07	−7.69
22	−7.63	−7.43

Table 15. Cont.

Compound	CRHR1 (8GTI)	GALR2 (7WQ4)
27	−7.71	−6.85
30	−8.92	−7.94
L-054,522	−7.32	−8.16

These findings do not imply functional activity but highlight the possibility of polypharmacology that may influence GPCR signalling networks in neuroblastoma. Experimental receptor-specific binding assays will be required to determine whether compound **8** exhibits measurable affinity or functional effects at CRHR1 or GALR2.

3.11. Predicted NMR Spectra

To support future synthetic characterisation, predicted ^1H (Figure 8) and ^{13}C NMR (Figure 9) spectra were generated for compound **8** and L-054,522 using MestReNova (version 17.0.01).

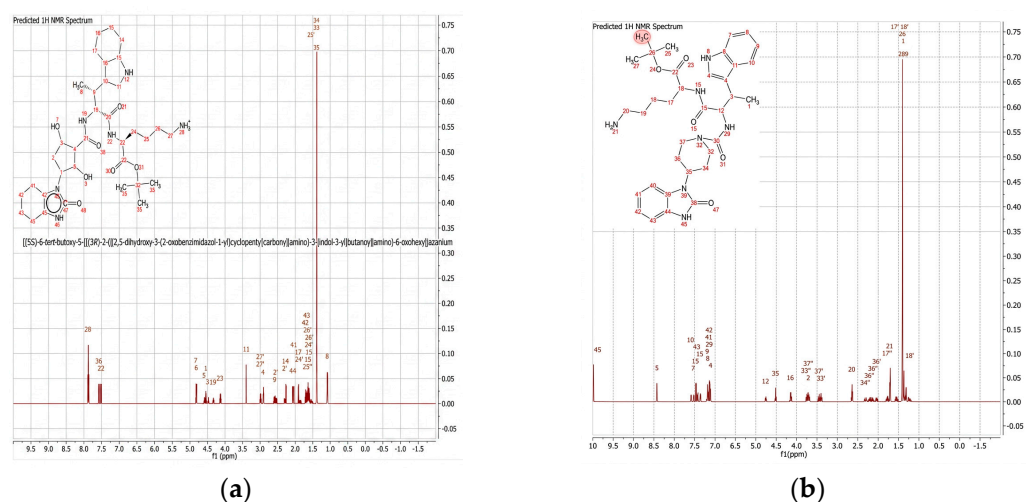


Figure 8. Predicted ^1H NMR spectra. (a) Compound **8**; (b) L-054,522.

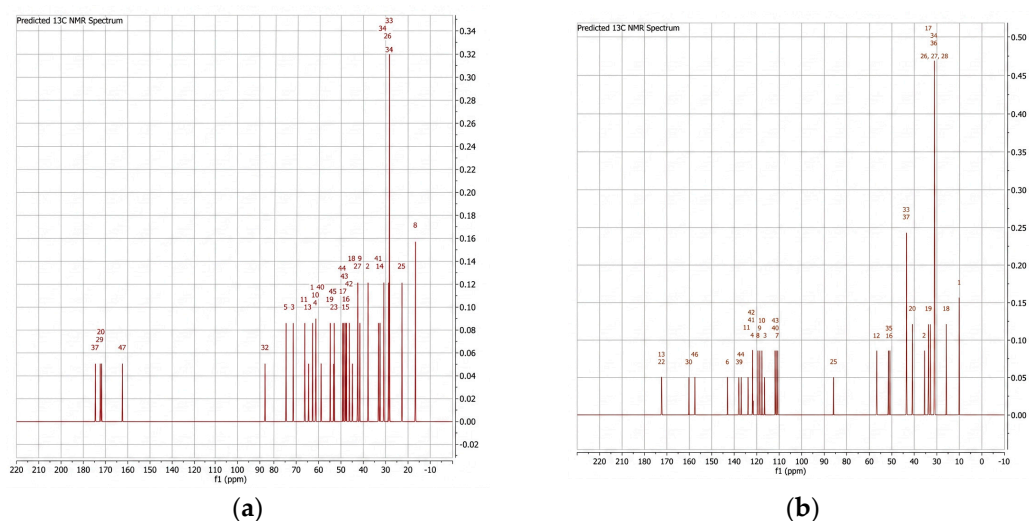


Figure 9. Predicted ^{13}C NMR spectra. (a) Compound **8**; (b) L-054,522.

3.12. Molecular Dynamics of the Compound 8 Complex

The docked pose of compound **8** was tested via three independent 100 ns simulations in an explicit POPC bilayer, 300 ns in total. The receptor was stable in all three runs, with a backbone RMSD of 1.85 ± 0.04 Å, and the C α RMSF profiles almost overlap, with the same flexible regions in each run: the termini, the residues adjacent to the unmodelled ICL3 segment, and the loops near residues 110, 187, and 285. The ligand remained within the binding pocket in every frame of every run. Ligand RMSD was 1.70 ± 0.12 Å and never exceeded 2.8 Å, the minimum ligand–protein distance never exceeded approximately 2.0 Å, and at least one ligand–protein hydrogen bond was present in every frame (Table 16, Figure 10).

Table 16. Comparison of the three independent 100 ns replicas. Run values are means over 10–100 ns; the combined column is the mean $\pm$ SD of the three run means with its 95% confidence interval.

Property (10–100 ns)	Run 1	Run 2	Run 3	Mean $\pm$ SD ($n = 3$)	95% CI
Backbone RMSD (Å)	1.84	1.82	1.89	1.85 ± 0.04	1.75–1.95
Ligand RMSD (Å)	1.68	1.58	1.83	1.70 ± 0.12	1.39–2.01
Maximum ligand RMSD (Å)	2.54	2.77	2.71	–	–
Ligand–protein H-bonds	5.5	5.3	5.7	5.5 ± 0.2	4.9–6.1
Atom contacts < 4 Å	568	565	643	592 ± 44	483–701
Minimum distance, max (Å)	1.93	2.02	1.98	–	–
C α RMSF median (Å)	0.75	0.66	0.74	0.71 ± 0.05	0.59–0.83

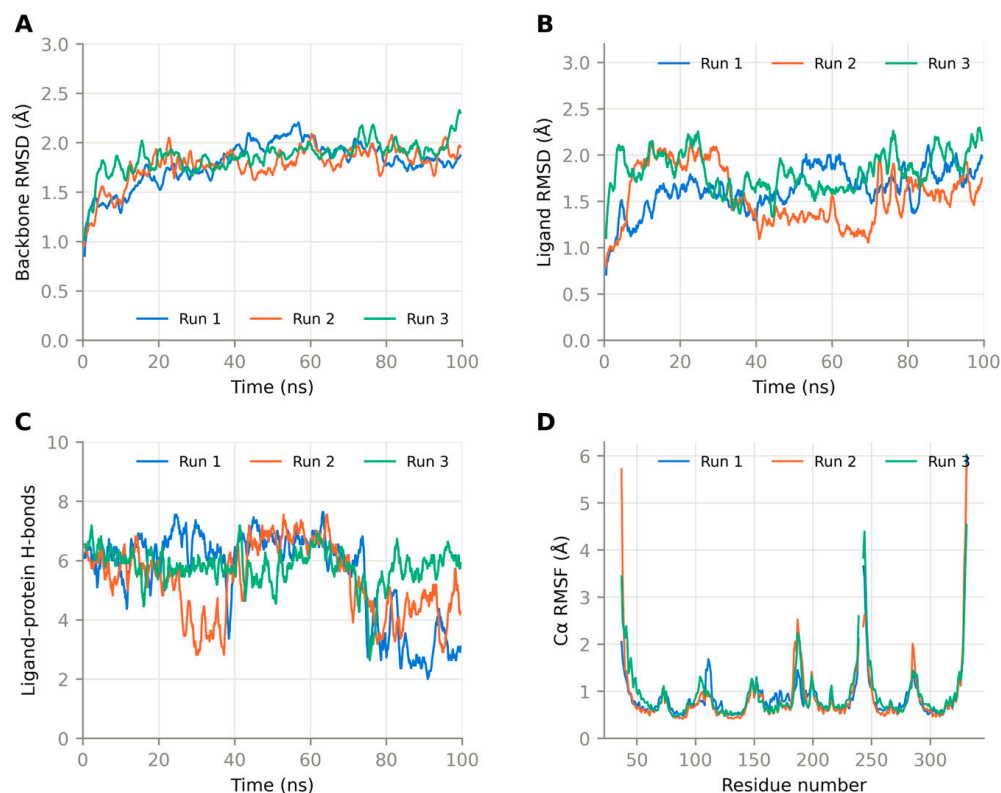


Figure 10. Stability of the compound **8**–SSTR2 complex over three independent 100 ns replicas. (A) Protein backbone RMSD; (B) ligand RMSD after fitting on the protein backbone; (C) number of ligand–protein hydrogen bonds; (D) C α RMSF by residue.

Residue-level analysis identified a consistent anchor. Asp122 and Phe294 were in contact with the ligand in 100% of frames in all three runs, with Phe272, Tyr205, and Ser279 in at least 89%. The protonated amine of compound **8** formed a hydrogen bond or salt bridge with Asp122 in 64–77% of frames in every run, making Asp122 the consistent anchor; Ser279 (57–76%) and Tyr205 (45–71%) were also reproducible hydrogen-bond partners (Figure 11). Interactions with Gln126, Asn276, and Arg184 varied between runs.

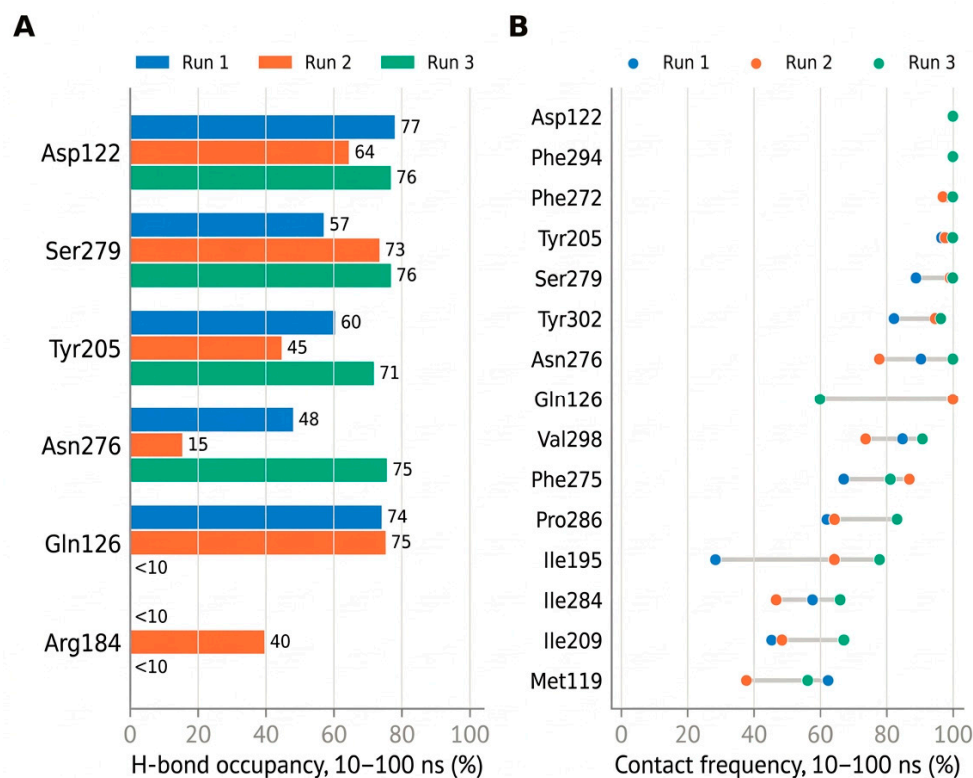


Figure 11. Ligand–receptor interactions over 10–100 ns in each replica. (A) Hydrogen-bond occupancy for the six most frequent partners; (B) heavy-atom contact frequency within 4 Å for the fifteen most frequent residues.

All three runs showed a fall in the number of ligand–protein hydrogen bonds between approximately 70 and 80 ns, but its extent and persistence differed: in run 1, about half the hydrogen bonds were lost for the remainder of the simulation (6.4 → 3.2), in run 2, the loss was partial (5.7 → 4.3) and followed an earlier, fully reversed dip at 25–38 ns, and in run 3, the dip lasted about 5 ns before the count returned to its earlier level. The ligand therefore switches reversibly between tighter and looser hydrogen-bonded arrangements within the same site, rather than drifting systematically out of the pose. The similar timing across runs may reflect a slow relaxation common to all three, which start from the same structure, or may be coincidental; longer simulations would be required to distinguish these.

These contacts correspond closely to those determined experimentally for the crystallographic ligand. In the SSTR2–L-054,522 structure, L-054,522 forms a salt bridge with Asp122 and hydrophobic contacts with Phe272 and Phe294, and alanine substitution at each of these positions reduces the L-054,522 potency in functional assays. The simulation therefore reproduces, for compound **8** and without reference to the experimental data, the residues shown via mutagenesis to be required for binding of the parent ligand. Ser279, among the more persistent hydrogen-bond partners of compound **8**, is not a reported contact of L-054,522 and may reflect the hydroxyl substitution distinguishing this analogue.

Two limitations should be stated. Simulations were performed for the lead compound only; equivalent sampling for all eight candidates was not computationally feasible, so these

results characterise the compound **8** pose rather than discriminate between candidates. And stability of a pose on a 100 ns timescale is not evidence of binding affinity, nor does it distinguish agonism from antagonism; it establishes only that the pose predicted via site-directed docking is physically persistent in a membrane environment.

4. Discussion

4.1. Therapeutic Rationale and Nature of the Present Contribution

The therapeutic rationale for targeting SSTR2 in high-risk stage 4 neuroblastoma rests on three established observations. First, the segmental gain of chromosome 17q occurs in over 80% of high-risk tumours, and SSTR2 lies at 17q25.1 within the most frequently amplified interval. Second, SSTR2 is overexpressed in these tumours, providing a target that is enriched in malignant relative to normal tissue. Third, SSTR2 engagement is coupled to antiproliferative signalling through the inhibition of adenylyl cyclase, blockade of voltage-dependent calcium channels, and upregulation of the cell-cycle inhibitor CDKN1B, and somatostatin analogues have been shown to inhibit neuroblastoma cell proliferation through this axis.

The mechanistic gap this work addresses is the absence of small-molecule chemical matter directed at SSTR2 in this specific disease context. The clinically available somatostatin analogues are peptidic, with the pharmacokinetic constraints that follow, and the one well-characterised non-peptide ligand, **L-054,522**, carries a predicted developmental toxicity liability that is particularly unwelcome in a paediatric indication.

It is important to state precisely what the present study does and does not deliver. It delivers a synthetically accessible set of candidate SSTR2 ligands, obtained through a workflow whose query has been validated against property-matched decoys and whose pose predictions are reproducible across repeat runs. Predicted toxicity liabilities were identified for every structure in the set, with the lead compound included. It does not deliver a characterised pharmacological agent. Because docking cannot distinguish agonism from antagonism, whether compound **8** would activate the antiproliferative signalling described above, block it, or neither, is undetermined and cannot be determined computationally. The translational value of this work is therefore that of hypothesis generation: it converts an untargeted receptor into a testable, synthesisable set of candidates, and the experimental programme required to resolve the functional question is set out in Section 4.6.

4.2. Convergent Evidence for Compound **8**

This computational study employed a multi-platform workflow to identify potential SSTR2 ligands for high-risk NBS4, a paediatric cancer characterised by frequent 17q gain and SSTR2 overexpression. From 5766 BROOD-generated analogues of **L-054,522**, eight compounds achieved GREAT pose confidence in POSIT, and confirmatory docking across three additional platforms (AutoDock Vina, FITTED, and Flare) gave results consistent with these, though consistency between docking programs is not independent evidence of binding. Compound **8** was prioritised on the following grounds:

- The docking scores do not support a ranking of the eight candidates, for the reasons set out below. The prioritisation of compound **8** therefore does not rest on the docking rank, but on acceptable predicted binding across platforms combined with the only fully precedented synthetic route, moderate rather than poor predicted aqueous solubility, and placement within the similarity range of experimentally confirmed SSTR2 ligands under a validated query. Compound **30**, while scoring more favourably in several docking and similarity measures and returning the most favourable predicted toxicity profile of the set based on ADMET-AI (the lowest predicted mutagenicity, hepatotoxicity, and carcinogenicity of the nine structures), requires high-risk synthetic

transformations and shows stronger predicted engagement with both off-target receptors examined. Based on predicted properties alone, compound **30** is therefore the stronger candidate, and it is deprioritised here based on synthetic risk and off-target engagement rather than on any predicted advantage of compound **8**. It is retained in the candidate set for that reason.

The docking scores reported above were each obtained from a single run. To test whether they support a ranking, the eight candidates were re-docked into the same prepared 7XN9 receptor on separate occasions: three times with FTTED under identical parameters, and twice each with FRED, HYBRID, and POSIT. The programs behaved differently. POSIT returned identical pose-confidence probabilities on both occasions, to six decimal places, with identical confidence classes. FRED, HYBRID, and FTTED did not reproduce their own ordering: Spearman rank correlations between repeat runs were +0.19, +0.00, and, across the three FTTED runs, between −0.36 and +0.19—none distinguishable from chance for eight items (Supplementary Table S1). Compound **13** ranked eighth, seventh, and second across the FTTED runs; compound **22** ranked seventh, second, and first.

What separates POSIT from the other three is the quantity reported. POSIT estimates the probability that the predicted pose lies within 2 Å of the true binding mode, a property of the pose itself; FRED, HYBRID, and FTTED report energy-like scores, which are sensitive to small differences in the atomic position. On this series the pose-confidence estimate is stable and the energy-like scores are not. Because POSIT was reproducible across the same two occasions on which the other three varied, the variation cannot be attributed simply to a different preparation pipeline having been used on the two dates.

This distinction is material to the workflow reported here. The reduction from 292 docked structures to the final eight candidates was made on POSIT pose confidence, which is reproducible; that step of the funnel is therefore stable. The energy-like scores are used only to identify gross incompatibility with the binding site and are not used to rank the candidates. The ranking statements in the previous version of this manuscript have been withdrawn accordingly, and the prioritisation of compound **8** rests on synthetic accessibility, predicted solubility, and similarity to experimentally confirmed SSTR2 ligands, none of which depends on a docking score. We report the observation because a reader repeating this work would otherwise have to rediscover it.

- Interaction pattern. The predicted binding pose engages Asp122, Gln126, Thr194, and aromatic residues (Phe208, Phe272, Phe294) identified in crystallographic and simulation studies as key determinants of SSTR2 ligand binding.
- Shape and electrostatic similarity. Under a query of validated retrieval performance, compound **8** scored above nine of eleven experimentally confirmed SSTR2 ligands and well above the entire decoy distribution, placing it within the similarity range occupied by known binders. Compound **30** scored higher based on this measure, as it did on predicted binding at the two off-target receptors examined.
- Predicted safety. Compound **8** offers no predicted safety advantage over **L-054,522**: both, and all the other candidates, are classified as developmental toxicants by T.E.S.T., and predicted hERG liability is returned for every structure examined, **L-054,522** included, by two of the three toxicity platforms. Compound **8** is therefore not prioritised on predicted safety; it is prioritised based on synthetic accessibility, predicted solubility, and similarity to confirmed SSTR2 ligands, with hERG resolution identified as a prerequisite for experimental follow-up of the series as a whole.
- Synthetic accessibility. Compound **8** alone achieved a Spaya RScore of 1.0, indicating a fully precededented synthetic route. Other candidates required high-risk transformations, making compound **8** the most practical choice for experimental follow-up.

4.3. Limitations

Several important limitations must be acknowledged:

- Functional activity cannot be inferred from docking or global MD stability alone. Throughout this manuscript, compound **8** is described as a “ligand” rather than an “agonist” because docking scores and pose geometries cannot distinguish agonists from antagonists, partial agonists, or inactive binders. Functional characterisation requires experimental binding assays (e.g., radioligand displacement) and functional assays (e.g., cAMP inhibition, calcium flux, or β -arrestin recruitment).
- Protocol dependence of pose recovery. The lead compound re-docked successfully under four site-directed protocols but was not recovered by FRED or HYBRID in focused redocking, nor by any blind, ensemble or batch-screening protocol. This does not invalidate the approach—the crystallographic pose defined the binding site accurately—but it shows that pose recovery on this target depends on how the search is configured, and it limits the weight that can be placed on any single docking result.
- ADMET predictions require experimental validation. Computational ADMET tools provide useful filters but have known accuracy limitations. The predicted low GI absorption and poor water solubility of all compounds represent genuine developability concerns.
- Multi-target claims are speculative. The exploratory docking to CRHR1 and GALR2 demonstrates only that compound **8** can occupy these binding sites computationally. No evidence supports the therapeutic benefit from the simultaneous modulation of these receptors in NBS4.
- Scoring-function reliability outside conventional small-molecule space. The compounds prioritised here have molecular weights of approximately 620–692 Da and topological polar surface areas above 160 Å², placing them beyond the chemical space on which most docking scoring functions were parameterised and validated. Empirical and knowledge-based scoring functions are known to lose discriminatory power for large, flexible and highly polar ligands, where desolvation and conformational entropy contributions are poorly captured. The failure of **L-054,522** itself to re-dock under FRED and HYBRID illustrates this directly. The docking results are therefore treated as a filter against gross incompatibility with the binding site rather than as an estimate of binding affinity.
- Differential platform reliability. The re-docking results reported in Section 3.4 provide direct evidence for these limitations within the present system. Of the eight protocols examined, four reproduced the crystallographic pose of **L-054,522** within the conventional 2 Å threshold, and only under a focused, site-directed search. POSIT generated a pose at 1.79 Å but rejected it on steric grounds, indicating that the clash criterion applied to the prepared receptor was more stringent than the experimental structure warrants; the GREAT confidence classifications reported for the eight candidates should therefore be understood as a conservative filter based on shape and chemical-feature complementarity rather than as independent evidence of pose accuracy. HYBRID, FRED, and AutoDock Vina Extended in the blind box placed the ligand within the orthosteric pocket but in an inverted orientation, losing the Asp122 salt bridge.

The scoring behaviour is equally informative. Three platforms assigned the experimentally validated lead compound the least favourable score of the nine molecules examined, inverting the ranking implied by the available affinity data. Taken together with the pose results, this indicates that both pose prediction and score-based ranking degrade substantially for ligands of this size, flexibility, and polarity and

supports the approach taken here of resting compound prioritisation on evidence beyond docking alone.

- Static binding-mode prediction. All poses reported here are derived from docking to fixed receptor conformations. Receptor flexibility, ligand-induced conformational change, and the influence of the lipid environment on the transmembrane bundle were not modelled. This is addressed in part in Section 3.12, where three independent 100 ns replicas of the prioritised complex in an explicit POPC bilayer are reported; longer timescales and equivalent sampling for the remaining candidates would be required before the predicted binding mode is treated as established.
- Scale of the validation benchmark. The benchmark comprises 11 known SSTR2 ligands, a modest number reflecting the limited non-peptidic chemical matter reported for this receptor and the requirement that each active be matched to at least 20 property-matched decoys. Confidence intervals based on the reported area under the curve are correspondingly wide, and the enrichment factors are sensitive to the rank of individual compounds. The reported performance should be regarded as an indication that the query discriminates known ligands from property-matched decoys rather than as a precise estimate of retrieval efficiency.

4.4. Developability Considerations

The predicted low oral bioavailability and poor water solubility of compound **8** (and all analogues) represent significant hurdles for drug development. For a paediatric neuroblastoma population, practical considerations include the following:

- Intravenous administration: May be clinically acceptable in patients with high-risk neuroblastoma, as treatment commonly involves intensive multimodal regimens incorporating repeated cycles of intravenous chemotherapy, surgery, radiotherapy, stem-cell transplantation, and immunotherapy.
- Formulation strategies: Nanoparticle encapsulation, cyclodextrin complexation, or lipid-based formulations could improve solubility and bioavailability.
- Prodrug approaches: Chemical modification to improve absorption followed by metabolic conversion to the active compound.

These strategies should be explored in parallel with experimental validation of binding and functional activity.

The physicochemical profile of this series—molecular weight above 600 Da, TPSA above 160 Å², poor predicted aqueous solubility, and low predicted oral bioavailability—places it in the “beyond Rule of 5” region of chemical space. This region is not a barrier to clinical use in itself: several approved agents occupy it, including venetoclax (868 Da), which requires food co-administration to achieve adequate exposure; elbasvir and velpatasvir (both ~880 Da), which are co-formulated to ensure delivery; naloxegol (652 Da), a PEGylated derivative designed with a high polar surface area to restrict central nervous system entry; and vancomycin (1449 Da), administered parenterally because oral absorption is negligible. Fostamatinib (581 Da) illustrates the prodrug route to addressing exactly this combination of properties. The precedent therefore exists for developing agents in this space, but it is uniformly conditional on a deliberate delivery strategy, which, for the present series, would need to be established alongside the experimental confirmation of target engagement.

4.5. Comparison with Previous Work

Previous studies established the therapeutic relevance of somatostatin receptors in neuroblastoma. Work in the 1990s identified SSTR2 expression across NB cell lines and tumours and demonstrated antiproliferative effects mediated through somatostatin re-

ceptors [14,15,63]. The present study extends this foundation by applying contemporary computational methods to design novel non-peptide ligand candidates, one of which has a fully precedented synthetic route.

Combination approaches are a plausible direction for this target: multi-target inhibitors against HDAC, BRD, Hedgehog, and TRK pathways have been described in high-risk NBS4 [64], and an SSTR2 ligand could, in principle, be paired with one. The present work provides no evidence on that question, and it would require experimental testing.

4.6. Future Directions

The following experimental studies are required to validate the computational findings:

Synthesis of compound **8**: The RScore of 1.0 indicates straightforward chemistry; synthesis should be prioritised.

- Binding assays: Radioligand displacement or fluorescence polarisation assays to confirm SSTR2 binding and determine affinity (K_i or K_d).
- Functional assays: cAMP inhibition assays to determine whether compound **8** acts as an agonist (inhibits adenylyl cyclase) or antagonist. Additional assays (β -arrestin recruitment, calcium flux) could further characterise the pharmacological profile.
- Selectivity profiling: Assessment of binding to SSTR1, SSTR3, SSTR4, and SSTR5 to determine subtype selectivity.
- Cell-based studies: Evaluation of antiproliferative activity in NB cell lines (e.g., SH-SY5Y, IMR-32, SK-N-BE(2)) expressing SSTR2.
- Free-energy and enhanced-sampling studies: Binding free-energy calculations or enhanced-sampling approaches could help refine prioritisation and distinguish stable binding from activation-relevant conformational effects.
- In vivo studies: If in vitro results are promising, xenograft models of NBS4 could evaluate therapeutic efficacy and safety.

5. Conclusions

This study identified compound **8** as a computationally prioritised candidate SSTR2 ligand for high-risk neuroblastoma stage 4, through an AI-assisted workflow integrating generative analogue design, validated shape-based screening, multi-platform docking, deep-learning ADMET prediction, and neural retrosynthetic analysis. The shape query underpinning the workflow was validated against known SSTR2 ligands and property-matched decoys, achieving an ROC AUC of 0.798 with enrichment factors of 45.7 and 11.0 at the top 1% and 5% of the ranked database.

Compound **8** returned acceptable predicted binding across every platform examined, was predicted to be moderately soluble where the lead compound was not, and was the only candidate with a fully precedented synthetic route. Its predicted interaction pattern involving Asp122, Gln126, and neighbouring aromatic residues aligns with established structural determinants of SSTR2 ligand recognition, and its similarity to the validated query places it within the range occupied by experimentally confirmed SSTR2 binders. Re-docking validation established that four programs reproduce the crystallographic pose when run as a site-directed redocking and that pose recovery based on this target depends on the search protocol rather than on the scoring function.

These results establish a hypothesis rather than a pharmacological finding. Docking and similarity calculations cannot distinguish agonists from antagonists or inactive binders, and no experimental data are reported here. The next essential steps are synthesis, receptor-binding studies, functional assays, and membrane-embedded molecular dynamic simulations. If these confirm SSTR2 engagement and favourable biological activity, com-

pound **8** could represent a useful lead structure for targeted therapeutic development in high-risk NBS4.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/aichem1040016/s1>. All data supporting this study are available at <https://doi.org/10.5281/zenodo.22648179>. Detailed retrosynthetic schemes for compounds **8**, **12**, **13**, **14**, **17**, **22**, **27**, **30**, and **L-054,522** are provided in Scheme S1–S9.

Author Contributions: Conceptualisation, A.G.; methodology, A.G.; software, A.G.; validation, A.G. and U.C.; formal analysis, A.G.; investigation, A.G.; resources, A.G.; data curation, A.G.; writing—original draft preparation, A.G.; writing—review and editing, A.G. and U.C.; visualisation, A.G.; supervision, U.C.; project administration, A.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable for studies not involving humans or animals.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data supporting this study are available at <https://doi.org/10.5281/zenodo.22648179>.

Acknowledgments: The authors thank Corinne Kay for her advice and support over the years and Colin Gaudion for testing some of the programs used in this work. This work is dedicated to the memory of Isabella Gerges (1998–2005), who was diagnosed with neuroblastoma stage 4 in January 2003. Isabella relapsed in March 2005 and passed away in July 2005, one week after her seventh birthday. Her memory continues to inspire this research.

Conflicts of Interest: The authors declare no conflicts of interest. The personal connection to this disease (see Dedication) motivated this research but did not influence the design, execution, or interpretation of the study.

Abbreviations

The following abbreviations are used in this manuscript:

SSTR2	Somatostatin receptor subtype 2
NBS4	Neuroblastoma stage 4
GPCR	G protein-coupled receptor
CRHR1	Corticotropin-releasing hormone receptor 1
GALR2	Galanin receptor type 2
ADMET	Absorption, distribution, metabolism, excretion, and toxicity
MW	Molecular weight
TPSA	Topological polar surface area
BBB	Blood–brain barrier
ECL2	Extracellular loop 2
PDB	Protein Data Bank
FRED	Fast Rigid Exhaustive Docking
POSIT	Pose prediction tool (OpenEye)
T.E.S.T.	Toxicity Estimation Software Tools

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