Bioorganic & Medicinal Chemistry Letters 18 (2008) 5789–5791

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The identification of neurotensin NTS1 receptor partial agonists through a ligand-based virtual screening approach

Yi Fan [a,*], Margaret H. Lai [b], Kelly Sullivan [b], Michael Popiolek [b], Terrance H. Andree [b], Paul Dollings [a], Mark H. Pausch [b]

[a] Chemical & Screening Sciences, Wyeth Research, Princeton, NJ 08543, USA
[b] Discovery Neuroscience, Wyeth Research, Princeton, NJ 08543, USA

ARTICLE INFO

Article history:
Received 2 July 2008
Revised 16 September 2008
Accepted 17 September 2008
Available online 24 September 2008

Keywords:
Neurotensin
ROCS
Virtual screening
Agonist
Antagonist

ABSTRACT

We identified small molecule NTS1R agonist compounds through virtual screening of the corporate database using a ROCS approach that searches multi-conformer representations efficiently. As a starting point for the ROCS search, we used the known NTS1R selective antagonist, SR-48527, based on the hypothesis that NT agonists and antagonists might share similar binding regions. Conformations were expanded and selected as database search queries based on a cluster analysis. The search provided us with virtual hits that were tested in intracellular calcium mobilization assays of NTS1R agonist and antagonist activities measured in FLIPR format as well as in [^{3}H]NT competition binding studies. The results indicated that two initial hits produced partial agonist activity with potency in the moderate micromolar range.
© 2008 Elsevier Ltd. All rights reserved.

Neurotensin (NT, pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu) is a tridecapeptide that acts as a neuromodulator in the CNS.[1] Several lines of evidence have shown that NT modulates dopamine (DA) neurotransmission, predominantly in the mesolimbic pathway.[2] Due to its effects on DA and the activity in a range of animal behavioral models, NT has been implicated in the pathophysiology of schizophrenia and has been proposed as an endogenous neuroleptic.[3] NT mediates its effects through three receptors, NTS1R, NTS2R, and NTS3R. NTS1R and NTS2R are members of the seven-transmembrane domain family of G protein-coupled receptors (GPCRs), with NTS1R believed to be principally responsible for transducing the DA modulatory effects of NT.[4] Although considerable effort has been devoted towards identifying suitable NT agonists, the most advanced compounds remain peptidic in nature.[3] Small molecule, non-peptide NTS1 agonists would be of considerable value in elucidating the role of NT in regulation of DA neurotransmission and could serve as leads for development of a novel class of anti-psychotic compounds.

To date, direct structural information about the agonist binding site in NTS1R is not available. Pang and his coworkers first reported the three-dimensional structure of the extracellular loops, in particular E3, and the helical bundle of NTS1R in complex with neurotensin (8–13) through a heuristic approach in conjunction with site-directed mutagenesis studies.[5] The proposed ligand binding site remains one of many possible models. In addition, a tentative molecular model of the NTS1R antagonist, SR-48692, complexed with NTS1R indicated important residues involved in the antagonist binding site for rat NTS1R.[6] A similar approach was later applied to build a molecular model between agonist (NT8–13) and NTS1R.[7] Taken together, these studies suggest that agonist and antagonist sites might share common points of anchor in the receptor although their binding sites could be distinct.

With little information available for the receptor structure, we decided to identify small molecule NTS1R agonist compounds through virtual screening of the corporate database using a ligand-based approach called ROCS (*R*apid *O*verlay of *C*hemical *S*tructures).[8,9] ROCS is a shape-based superimposition method, based on the idea that molecules have similar shape if their volumes overlay well and any volume mismatch is a measure of dissimilarity. Due to Gaussian-based function to represent the molecular volume, ROCS allows for searching multi-conformer representations of corporate collections efficiently by first comparison of shape followed by simple matching of chemical functionalities.

As a starting point for the ROCS search, we chose SR-48527, the NTS1R selective antagonist, as queries based on the experimental observations that NT agonist and antagonist might share similar binding regions.[5–7] The structure of SR-48527 is shown in Figure 1.

The structure was first minimized using MMFF94 force field.[10–14] and then expanded using OMEGA[15] with the energy cut-off of 5 kcal/mol. OMEGA is believed to be one of the most reliable conformational expansion tools in terms of speed and

* Corresponding author. Tel.: +1 609 2080309; fax: +1 732 2744390.
E-mail address: fank@wyeth.com (Y. Fan).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.09.075

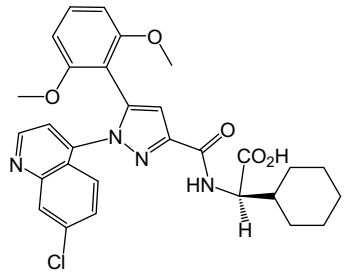


Figure 1. Chemical structure of SR-48527.

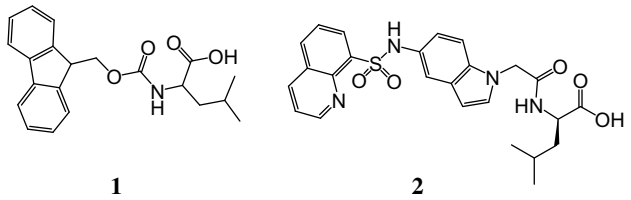
performance. Five representative conformers were retained as search queries as a result of clustering analysis of torsional RMS using the XCluster utility implemented in the Schroedinger Maestro interface.[16]

To identify a novel NTS1 small molecule agonist, ROCS shape-based searches were performed on Wyeth corporate collections. The chemical/or color force field (CFF), Mills Dean, was added to the shape matching procedure during the searches. In other words, after finding the best alignment based on the shape, the program calculates the color force field score (color) to measure chemical complementarities, and to refine shape-based superimpositions based on chemical similarity. A scaled color value is calculated by taking a hit's actual score value and dividing it by the color score of the query molecule against itself. The score used for ranking the hit list in this experiment is combo score that is the sum of the shape Tanimoto coefficient and the scaled color value. Since both shape Tanimoto coefficient and the scaled color are in the range of 0 and 1, the combo score has a value from 0 to 2.

The library used in the ligand-based virtual screening is the lead-like subset of Wyeth corporate collections (LEADCORP). The LEADCORP contains about 200,000 compounds with molecular weights between 180 and 400, less than 9 rotatable bonds, no more than one chiral center, a $\log D_{7.4}$ of less than 4.0, fewer than 4 ionizable groups, and applied filter of non-reactive functionalities. The 'lead-like' molecules were expanded into a set of 3D conformations using the program OMEGA[11] with the energy cut-off of 5 kcal/mol. The torsional sampling ranges from 5° to 60° depending on bond type. For chiral molecules lack of a stereospecific designation, both enantiomers were generated and conformationally expanded.

The effects of agonists were measured using CHOK1 cells stably expressing the human NTS1R and were monitored with intracellular Ca^{2+} measurements (fluorometric imaging plate reader, FLIPR). Cells were plated 24 h before assay at a density of 40,000 cells/well in 96-well plates. FLIPR Calcium 3 dye (Molecular Probes, Eugene, OR) was prepared in Hank's balanced salt supplemented with 20 μM Hepes and 2.5 μM probenecid. Dye loads were for 50 min at 37 °C. The intracellular calcium increases were detected by measuring increases in fluorescence with FLIPR after compound's addition. Data were analyzed with PRISM 4.0 (GraphPAD Software Inc.).

The NTS1R antagonist experiments were also performed using FLIPR. The assay conditions were the same as the ones described above. Compounds (3 nM NT or 50 μM of compounds **1** and **2**) were added after 30 min. pretreatment with SR-48692 (10^{-5}–10^{-11} M). Data were analyzed with PRISM 4.0 (GraphPAD Software Inc.).



1 **2**

Figure 2. Two confirmed hits identified from virtual screening.

Table 1
NTS1R agonist activity of compounds **1** and **2**

Compound	EC_{50}[a]	E_{max}[b] (%)
NT	0.31 (±0.26)	100
1	215.50 (±84.15)	60
2	178.01 (±77.58)	17

[a] EC_{50} of NT is reported in nM, while others are expressed in μM. Values are means in triplicates of at least two independent experiments, and the standard deviation is given in parentheses.
[b] Agonist efficacy is expressed as a percentage of the maximal value produced by NT. No detectable agonist activities were observed when the compounds were tested on non-transfected CHOK1 cells.

[^{3}H]NT competition binding analysis conducted using crude cell membranes were prepared from CHOK1 cells stably expressing the human NTS1R as described.[17] Membranes were suspended in 50 mM Tris–HCl, pH 7.4, and protein concentration determined. Compounds were serially diluted in 50 mM Tris–HCl, pH 7.4, in 96-well plates. [^{3}H] neurotensin (5 nM) and 25 μg of protein were added to each well for the competition binding assay. Non-specific binding was determined with 10 μM SR-48692. After incubation at 27 °C for 60 min, the reaction was stopped by rapid filtration (Tomtek, Hamden, CT) and radioactivity was counted using a Betaplate liquid scintillation counter (PerkinElmer Life, Shelton, CT). Data were analyzed with PRISM 4.0 (GraphPAD Software Inc.).

Virtual screening hits were selected based on the minimum combo score of 1.2 in the ROCS searches. After removing the unavailable compounds, 170 were finally submitted for testing with the consideration of chemical diversity. Primary FLIPR screening identified two initial hits having NTS1R partial agonist activity

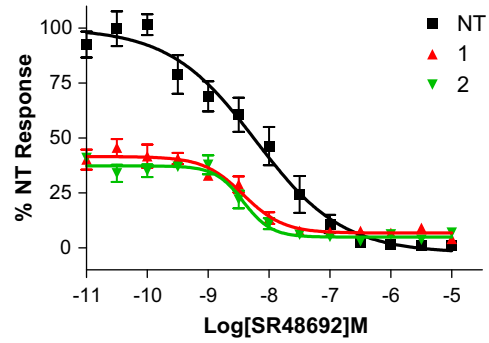


Figure 3. Antagonist FLIPR functional assay. Demonstrations of blockade by SR-48692 of NTS1R agonist activity were conducted in FLIPR as described in Experimental methods.

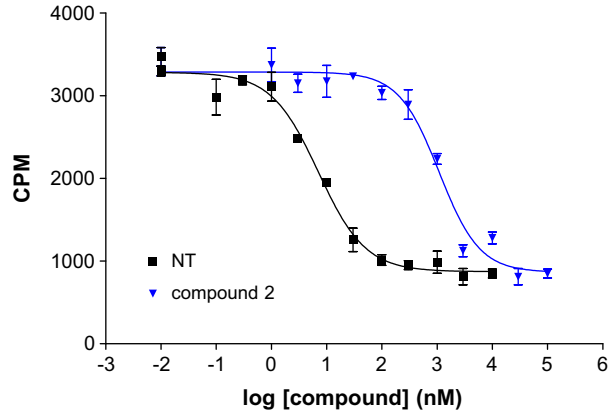


Figure 4. Competition binding assay. Competition binding of NT and compound **2** with [3H]NT studies were conducted as described in Experimental methods. Binding for compound **1** was undeterminable.

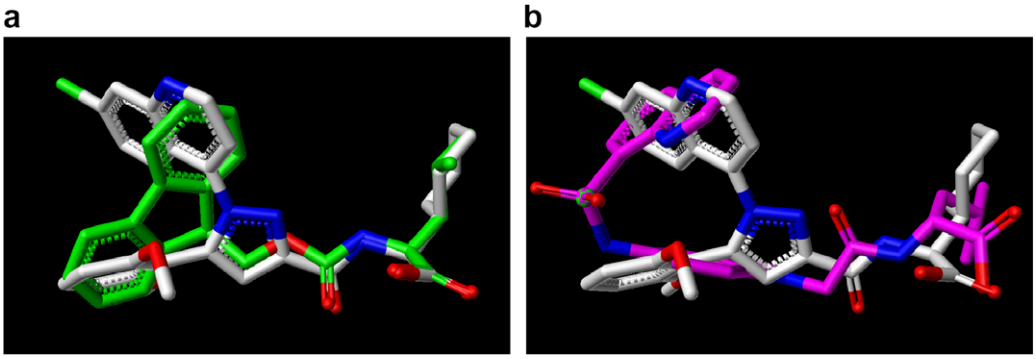


Figure 5. (a) The ROCS alignment of compound **1** with the NTS1 antagonist, SR-48527. All carbons were shown in white for SR-48527 and in green for compound **1**. Oxygen and nitrogen atoms were highlighted in red and blue, respectively. Hydrogen atoms were not explicitly displayed. (b) The ROCS alignment of compound **2** with the NTS1 antagonist, SR-48527. All carbons were shown in white for SR-48527 and in magenta for compound **2**. Oxygen and nitrogen atoms were highlighted in red and blue, respectively. Hydrogen atoms were not explicitly displayed.

with potency in the moderate micromolar range. To follow-up the most potent compound **1** (Fig. 2), we have also performed 2D similarity searches using topological torsion and atom pair based descriptors.[18,19] The subsequent similarity searches led two additional potent compounds with compound **2** as a representative shown in Figure 2.

Compounds **1** and **2** were tested for NTS1R agonist activity. Table 1 showed the EC_{50} values of the tested compounds in the NTS1R FLIPR functional assay. The results indicated the production of the agonist activity by both compounds.

In order to confirm the effect of the compounds on the NTS1R, antagonist assays using the selective NTS1R antagonist, SR-48692, were conducted in FLIPR format. SR-48692 was added for a 30 min pretreatment prior to addition of compounds (3 nM NT or 50 μM of other compounds). The results were shown in Figure 3. The antagonist effects of SR-48692 in CHOK1 stably expressing NTS1 cells can be observed by inhibition of the stimulatory effects of the agonist compounds [SR-48692 IC_{50} (NT) = 6.03 nM; IC_{50} (compound **1**) = 3.79 nM; IC_{50} (compound **2**) = 3.76 nM]. In addition, the [^{3}H]NT competition binding assay indicated that the compound **2**, in particular, showed competition binding (K_i = 545 nM, Fig. 4), however, there was no binding observed for compound **1**.

The virtual screening hits, compounds **1** and **2**, were overlaid with NTS1 antagonist, SR-48527, in Figure 5 using the ROCS shape-based method. The initial ROCS hit compound **1** and the query molecule SR-48527 have substantially different chemistry but reasonably high shape similarity. Compound **2** identified by subsequent 2D similarity searches also showed reasonable degrees of shape similarity with NTS1 antagonist, SR-48527. In addition, the three molecules shared common pharmacophoric features according to the alignment shown in Figure 5a and b.

Early studies[4] based on the model of the SR-48692-rNTS1 complex suggested that the carboxylic group of NTS1 antagonist might make an ionic interaction with Arg^{327} from helix 6. As shown in the ROCS alignment, the carboxylic group in the structures of compounds **1** and **2** is oriented toward the same pharmacophore space as that with SR-48527. The Leu side chains of compounds **1** and **2** share the same space as cyclohexyl of SR-48527, the group hypothesized to interact with the hydrophobic pocket contributed by the residues such as Met^{228} of H4 and Phe^{331} of H6.[6] Although compounds **1** and **2** show reasonable degree of similarity with the NTS1 antagonist, SR-48527, in terms of shape and pharmacophore, they also showed structural differences. The observed structural difference might be responsible for the identified agonist activity observed for compound **2**, in particular.

In conclusion, virtual screening using SR-48527, the NTS1R selective antagonist as a query, successfully identified two hits that produced partial agonist activity that was blocked by the NTS1R antagonist SR-48692 in FLIPR assays. Among those, compound **2** showed competition binding with [^{3}H]NT in NTS1R binding assays. It was noted that none of these compounds were identified from an earlier high throughput screen that generated no leads from our standard screening collections.

Acknowledgments

The authors thank Christine Humblet for her guidance and thoughtful discussions in the preparation of the manuscript, and Yongbo Hu who generated 'drug-like' corporate database that makes this work possible.

References and notes

1. Carraway, R.; Leeman, S. E. *J. Biol. Chem.* **1973**, *248*, 6854.
2. Binder, E. B.; Kinkead, B.; Owens, M. J.; Nemeroff, C. B. *Pharmacol. Rev.* **2001**, *53*, 453.
3. Boules, M.; Shaw, A.; Fredrickson, P.; Richelson, E. *CNS Drugs* **2007**, *21*, 13.
4. Vincent, J. P.; Mazella, J.; Kitabgi, P. *Trends Pharmacol. Sci.* **1999**, *20*, 302. 1999.
5. Pang, Y.-P.; Cusack, B.; Groshan, K.; Richelson, E. *J. Biol. Chem.* **1996**, *271*, 15060.
6. Labbe-Jullie, C.; Barroso, S.; Nicolas-Eteve, D.; Reversat, J. L.; Botto, J. M.; Mazella, J.; Bernassau, J. M.; Kitabgi, P. *J. Biol. Chem.* **1998**, *273*, 16351.
7. Barroso, S.; Richard, F.; Nicolas-Etheve, D.; Reversat, J.-L.; Bernassau, J.-M.; Kitabgi, P.; Labbe-Jullie, C. *J. Biol. Chem.* **2000**, *275*, 328.
8. Grant, J. A.; Gallard, M. A.; Pickup, B. T. *J. Comp. Chem.* **1996**, *17*, 1653.
9. ROCS; OpenEye Scientific Software, Santa Fe, NM, 2004. Available from: http://www.eyesopen.com.
10. Halgren, T. A. *J. Comp. Chem.* **1996**, *17*, 616.
11. Halgren, T. A. *J. Comp. Chem.* **1996**, *17*, 553.
12. Halgren, T. A. *J. Comp. Chem.* **1996**, *17*, 520.
13. Halgren, T. A. *J. Comp. Chem.* **1996**, *17*, 490.
14. Halgren, T. A.; Nachbar, R. B. *J. Comp. Chem.* **1996**, *17*, 587.
15. Omega; OpenEye Scientific Software, Santa Fe, NM, 2004. Available from: http://www.eyesopen.com.
16. Maestro 6.0, Schrodinger, New York, NY, 2004. Available from: http://www.schrodinger.com.
17. Heinrich, J. N.; Brennan, J.; Lai, M. H.; Sullivan, K.; Hornby, G.; Popiolek, M.; Jiang, L. X.; Pausch, M. H.; Stack, G.; Marquis, K. L.; Andree, T. H. *Eur. J. Pharmacol.* **2006**, *552*, 36.
18. Nilakantan, R.; Bauman, N.; Dixon, J. S.; Venkataraghavan, R. *J. Chem. Inf. Comput. Sci.* **1987**, *27*, 82.
19. Carhart, R. E.; Smith, D. H.; Venkataraghavan, R. *J. Chem. Inf. Comput. Sci.* **1985**, *25*, 64.