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# Novel and potent NPY5 receptor antagonists derived from virtual screening and iterative parallel chemistry design

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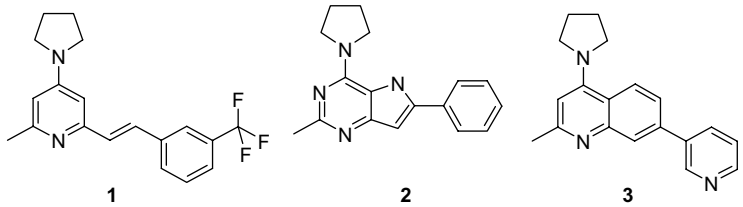
**Abstract**—In the quest for NPY5 receptor antagonists a virtual screening approach yielded a novel and potent hit class from a limited compound selection. The tight and seamless integration between virtual screening and rapid parallel chemistry within the framework of the Roche Lead Generation unit led in only two rounds of iterative chemistry optimisation to a much broader understanding of the factors which influence the potency of the thiazole hit class.


Neuropeptide Y (NPY) is a 36 amino acid neuropeptide which was discovered in 1982 as a member of the pancreatic polypeptide family.[1] At least 6 G-protein coupled receptors (GPCR) are known which bind with high affinity to members of this family such as NPY, peptide YY and pancreatic polypeptide.[2] Since it was demonstrated that antagonists of the NPY5 receptor (NPY5R) cause the reduction of food intake in animal feeding models,[3] NPY5R antagonists have been targeted by many pharmaceutical companies as potential anti-obesity drugs.

In the quest for novel NPY5R antagonists a virtual screening campaign was initiated to select a small set of approximately 600 molecules suitable for lab-scale testing. Virtual screening based on topological similarity (e.g., Daylight fingerprints[4]) tends to retrieve molecules which are structurally similar to the original query molecules. Pure 3D pharmacophore queries have the potential to yield structurally diverse scaffolds, but there is a higher risk of losing binding affinity as compared to topologically similar hits. Therefore, a hybrid approach was chosen to translate three reference compounds from Banyu **1**,[5] Amgen **2**[6] and Roche **3**[7] (Scheme 1) into a Catalyst™[8] virtual screening query containing 3D pharmacophore information and topological elements.

From the comparison of the three seed structures it was concluded that they had two hydrophobic moieties in common. In Catalyst™, hydrophobic features are defined as a set of chemical functions consisting of aliphatic and aromatic substructures. The pharmacophore hypothesis also contained topological elements such as an aromatic nitrogen ($N_{ar}$) and a central aromatic carbon atom ($C_{ar}$) which was attached to an aliphatic nitrogen ($N_{al}$) and two Markush-type atoms




**Scheme 1.** Seed compounds for deriving 3D pharmacophore hypothesis.

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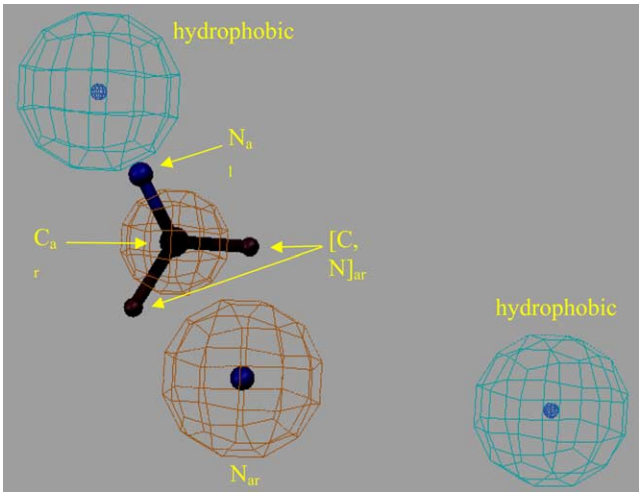


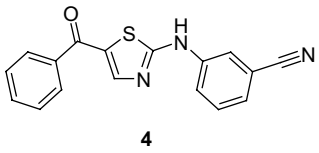

**Scheme 2.** 3D pharmacophore hypothesis with location constraints.

([C, N]$_{ar}$) representing aromatic carbon or nitrogen atoms (Scheme 2).

Location constraints were added to both the hydrophobic moieties, the aromatic nitrogen and the central aromatic carbon atom. The substituents of the aromatic carbon were not constrained in order to make the query less restrictive. Since all three reference compounds had a similar size, a molecular shape filter was defined to eliminate hits which did not resemble the overall shape of the seed molecules.

The virtual screening of the Roche compound depository yielded 632 molecules from which 31 compounds had an $IC_{50} < 10\ \mu M$. The most interesting compound class was represented by **4** which showed an $IC_{50}$ of 40 nM at the mouse Y5 receptor and was active at 10 mg/kg ip in a mice feeding model. Furthermore, the 4-aminothiazole hit class was free of patent claims (Scheme 3).

Compound **4** is structurally quite diverse from the seeds (Tanimoto coefficient Daylight fingerprint = 0.23 with




**4**

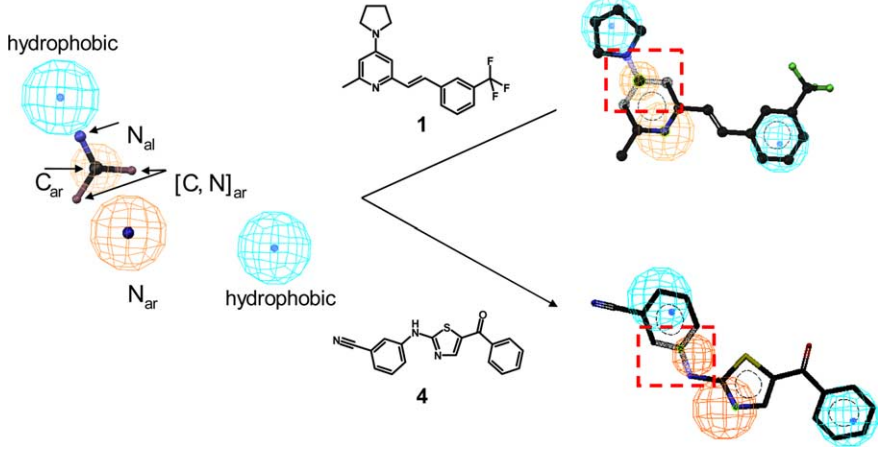
**Scheme 3.** Aminothiazole **4**.

respect to **1**), and it would not have been discovered by a topological similarity search. The critical success factors of this mixed 3D/topological query were the spatial conservation of the hydrophobic moieties and the aromatic nitrogen atom, whereas the unconstrained substituents of the aromatic carbon atom allowed for a novel connectivity pattern of the aliphatic nitrogen. In the seed compounds the aliphatic nitrogen is bound in an exocyclic position, in **4**, however, this nitrogen is part of the central scaffold motif. As it is illustrated in Scheme 4, the 3D coordinates of the central aromatic carbon atom are maintained both in the reference structure and in **4**. Since no rotational constraint was imposed on the substituents around $C_{ar}$, a structurally novel scaffold matching the pharmacophoric pattern of the seeds **1**, **2** and **3** could be identified via 3D virtual screening.

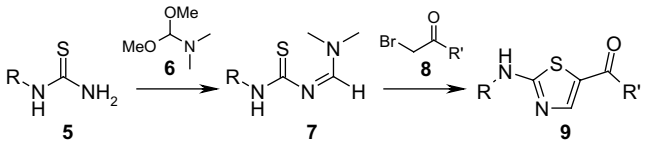
Due to the promising binding affinity, in vivo activity and secure intellectual property position, the potential of this chemical class was further explored. Therefore, this hit class underwent two chemistry optimisation cycles for establishing a conclusive structure activity relationship which finally yielded novel, potent NPY5R antagonists.

Since the access to multiple members of this thiazole class deemed straightforward based on literature precedent[9] an efficient iterative chemistry design was set in place (Scheme 5).

Thioureas **5** are conveniently condensed with *N*,*N*′-dimethylformamide dimethyl acetal **6** under reflux conditions without employing a solvent. In most of the




**Scheme 4.** Mapping of the 3D pharmacophore hypothesis onto **1** and **4**. The trigonal substitution pattern around $C_{ar}$ is allowed to rotate yielding a novel scaffold in the 3D virtual screening campaign (the match is highlighted by the dashed red rectangle).



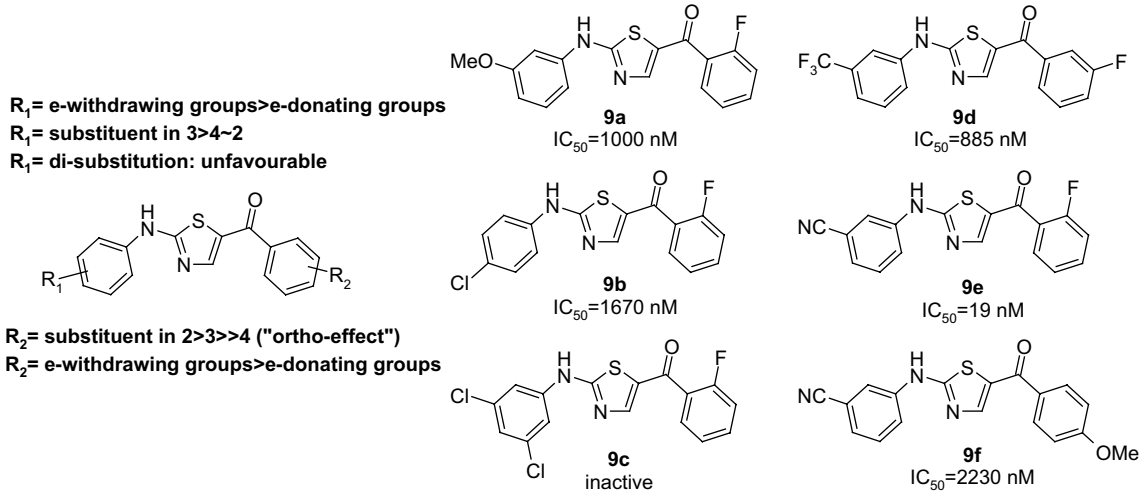
**Scheme 5.** Synthetic access to aminothiazole derivatives.

cases investigated the dimethylaminomethylene-aryl-thiourea **7** precipitated from the reaction mixture which made the isolation of pure material a quick process. The introduction of the second vector of diversity (R′) was achieved by reaction of **7** with various α-bromoketones **8** (either commercially available or synthesised according to procedures described in literature[10]) in a solvent like DMF.[11] The proceeding purification via preparative HPLC on reversed phase eluting with an acetonitrile/water gradient was straightforward since no work-up of the reaction mixtures was required. All water miscible components like DMF, base or other polar side products were eluted first. A time and UV directed fraction collection protocol ensured fast and straightforward access to the desired aminothiazoles **9**. However, only in cases where the product precipitated from the reaction mixture filtration, washing of the compound was the most appropriate method to access pure material.

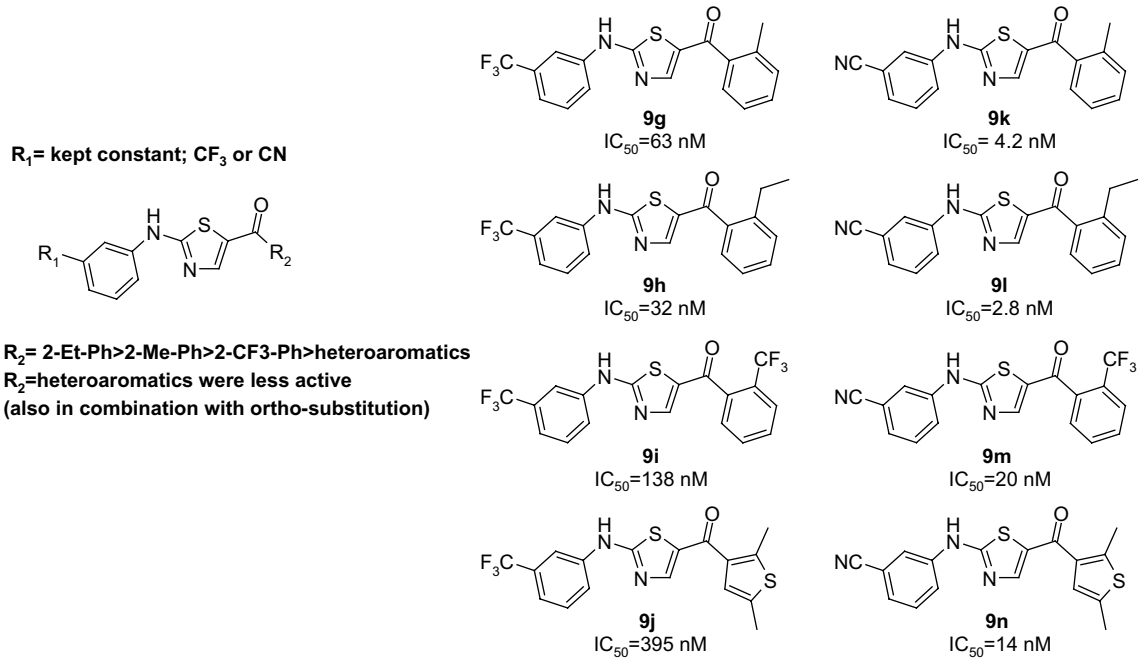
In a first synthesis round the influence and role of various combinations of substitution patterns on the dimethylaminomethylene-aryl-thiourea **7** were explored in combination with various substitution patterns on the α-bromoketone **8**. All compounds were tested at the mouse NPY 5 receptor. The preliminary SAR from this compound array is shown and some representative examples are also presented in Scheme 6.

From the first array of ca. 100 compounds several effects could be observed and the following conclusions were drawn: Electron-donating groups were disfavoured in $R_1$ (**9a**; $mIC_{50}$ = 1000 nM), however, the position of the substituent was important. The 3 position proved to be the most important since substitution at 2 or 4 position lowered the activity of the thiazoles considerably (**9b**; $mIC_{50}$ = 1670 nM). In addition, a disubstitution did not lead to active compounds (**9c**, inactive). However, the combination of $R_1$ being an electron withdrawing group in the 3 position with a substituent ($R_2$) on the other aromatic ring in meta position (**9d**; $mIC_{50}$ = 885 nM) yielded compounds which were only slightly more active. Shifting $R_2$ into the *ortho* position changed the picture considerably, since compounds active in the low nanomolar range were obtained (**9e**; $mIC_{50}$ = 19 nM). The para position seemed to be somehow forbidden not only for electron donating groups (**9f**; $mIC_{50}$ = 19 nM) but in general proved to be less favourable for any substitution. With this first array of compounds we identified the importance of substitution of the *ortho* position which we further pursued with a second array of compounds. These should clarify the influence of the nature of this substituent on the activity of the compounds. In this second array ca. 40 compounds were synthesised with $R_1$ being only allowed 3-$CF_3$ and 3-CN thereby broadly varying the α-bromoketone portion. The SAR which was deduced from this array and some representative compounds are shown in Scheme 7.

In general the 3-$CF_3$ series (exemplified by **9g–j**) was less potent than the 3-CN series (exemplified by **9k–n**). However, different substituents in the respective *ortho* position had a great influence on the potency of the respective compounds. Methyl substitution yielded compounds with acceptable (**9g**; $mIC_{50}$ = 63 nM) and excellent (**9k**; $mIC_{50}$ = 4.2 nM) potencies. An ethyl substituent made the thiazole derivatives (**9h**; $mIC_{50}$ = 32 nM, **9l**; $mIC_{50}$ = 2.8 nM) even more potent and seemed to be the optimal substituent amongst the investigated alkyl variations. As a variation of the alkyl group a $CF_3$ group was introduced, however, this rendered the compounds (**9i**; $mIC_{50}$ = 138 nM, **9m**; $mIC_{50}$ = 20 nM) less potent indicating a steric restriction in this position. Various heteroaromatic substitutions combined with the *ortho* effect (i.e., $R_2$ = 2-Me-3-pyridine, 2-Me-6-pyridine, 2-Me-3,6-pyrazine, etc.) lowered




**Scheme 6.** SAR deduced from first optimisation round.




**Scheme 7.** SAR deduced from second optimisation round.

the potency of the compounds into the high nanomolar range. Nevertheless, 2,4-dimethyl-3-thiophene (**9j**; $mIC_{50}$ = 395 nM, **9n**; $mIC_{50}$ = 14 nM) proved to be an adequate surrogate for 2-Me-phenyl. This indicates that there is still room for improvement and manipulation of compound profiles.

In conclusion, a successful virtual screening strategy is not aiming towards high hit rates but the goal is to yield the right balance between maintaining the pharmacophoric pattern and shape of the seed compounds and at the same time allowing for topological variability of the scaffold. A hybrid approach applying pharmacophoric features, 3D constraints and topological elements delivered a novel and potent hit class from a limited compound selection. The tight and seamless integration between virtual screening and rapid parallel chemistry within the framework of the Roche Lead Generation unit led in only two rounds of iterative optimisation to a much broader understanding of the factors which influence the potency of the thiazole hit class. Electron-withdrawing substituents, most preferably in the meta position of one aromatic moiety combined with an electronically and sterically suitable *ortho*-substituent on the other aromatic moiety, provided access to several compounds with potencies in the low nanomolar range. The established SAR of this compound class forms the basis for further optimisation cycles in the quest for novel and potent NPY5R antagonists with a balanced pharmacodynamic and pharmacokinetic profile.

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10. General experimental procedure for the synthesis of **9**: A mixture of aryl thiourea (1 mmol) and *N*,*N*-dimethylformamide dimethyl acetal (1 ml) was heated to 100 °C for 1 h. The precipitate was filtered off, washed with THF and dried to obtain the respective 1-dimethylaminomethylene-aryl-thiourea. When no precipitation occurred the mixture was evaporated to dryness and the residue was suspended

in DCM, filtered and dried to yield the title compound. Then a solution of 1-dimethylaminomethylene-aryl-thiourea (0.13 mmol) in DMF (0.33 ml) was added α-bromoketone (0.13 mmol) and the mixture was allowed to stir at room temperature for 16 h. Then *N*,*N*-diisopropylethylamine (0.13 mmol) was added and the mixture was subjected to preparative HPLC separation on reversed phase eluting with an acetonitrile/water gradient to yield the title compound after evaporation of the product fractions. The structural identity of the compounds was corroborated by LC–MS and NMR.

11. General experimental procedure for the synthesis of α-bromoketones: To a solution of aryl-ethanone (40 mmol) in HBr (33%, 21 ml) and methanol (7 ml) was added bromine (40 mmol) and the mixture was heated to 60 °C for 3 h. After removal of the volatiles under reduced pressure the residue was washed with diethyl ether and ethyl acetate to obtain the title compound which could be optionally further purified. For a more detailed description of workflow procedures utilised in the preparation of compound arrays please refer to: Nettekoven, M.; Thomas, A. W. *Curr. Med. Chem.* **2002**, *9*, 2179–2190.