Novel antagonists for the human adenosine A2A and A3 receptor via purine nitration: synthesis and biological evaluation of C2-substituted 6-trifluoromethylpurines and 1-deazapurines

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Summary

Novel antagonists for the human adenosine $A_{2A}$ and $A_3$ receptor via purine nitration

Synthesis and biological evaluation of C2-substituted 6-trifluoromethylpurines and 1-deazapurines

The development of novel medicines plays a crucial role in the health of people. Over many years, people tried to find bioactive compounds to cure people. Early historical records of the founder of modern medicine, Hippocrates, already describe the use of a bitter powder from the bark of the willow tree to cure headaches or pains. Drug discovery was accelerated in the 19th century by advances in chemistry and laboratory techniques. In these times, the active component of the above natural extract was discovered as salicylic acid and led to development of a more stable acetylated form (aspirin) that is still marketed these days as inhibitor of the enzyme cyclooxygenase. In the early 20th century, the discovery of protein structures (enzymes, ion channels, receptors) could explain the mechanism of action of drug
molecules. Later, the visualization and characterization of binding sites for drugs via advanced techniques and most recently, genome sequencing of the human proteome further boosted drug development.

In the body, proteins are often molecular targets for drug molecules. Proteins can act as a receptor for endogenous ligands (agonists), which upon binding induce a chemical signal and give a physiological effect. Early drug development focused on the development of synthetic analogues of these endogenous ligands. Later, it was found that blocking receptors via (synthetic) ligands (antagonists) could also prevent the release of signalling molecules and sometimes also could have therapeutic value.

The three dimensional and often dynamic protein structure is often visualized as a lock (receptor) that can be opened only with a precisely cut key (endogenous or synthetic agonist). Another key (ligand) may still fit the lock, but is not able to open it (initiate a cell response). By obstructing the lock, this imperfect key prevents the original key to open the lock and causes an antagonist action. In the development of new therapeutics, another factor is of crucial importance: drug receptor (subtype) selectivity. A drug with activity on one receptor and also displaying activity on another receptor could cause unwanted side effects. In modern drug development high receptor selectivity is important in the development of new agonists and antagonists.

In this thesis we focused on the development of selective ligands for the adenosine receptor, of which four subtypes have been characterized: the A₁, A₂A, A₂B and A₃ receptors. In Chapter 1, therapeutic applications for antagonists for the A₂A adenosine receptor are discussed. A₂A receptor antagonists are thought to play a role in several neurotransmitter related disorders in brain. Most intriguing, is the observed effect that antagonizing the A₂A adenosine receptor can increase dopamine release in brain via an alternative pathway. This opens an opportunity for the treatment of patients suffering from Parkinson’s disease, a neurodegenerative process that reduces the amount and efficiency of dopamine producing neurons.
Using a ligand based A$_{2A}$ adenosine receptor model, key structural features for future candidate ligands for the adenosine A$_{2A}$ receptor were selected, leading to two synthetically challenging classes of compounds. One series of compounds focused on highly substituted purines in which a trifluoromethyl group was introduced. In the other series we explored synthetic routes to substituted 1-deazapurines as novel A$_{2A}$ adenosine receptor antagonists.

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\begin{align*}
\text{X} &= \text{NH or O}
\end{align*}
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In Chapter 2, attention was focused on the development of novel trifluoromethyl purines. First, the use of N-9 protective groups was studied on the nitration of the purine ring. This led to the finding of a new purine protective group, Bocom (-CH$_2$-O-Boc), which proved to have excellent properties for purine functionalization. The highly activated difunctional chloro-nitro purine was used for the insertion of a trifluoromethyl group in the purine ring at, not until that time reported, low temperature conditions (0°C and room temperature). The second part of the chapter describes the elucidation of the purine nitration mechanism using extraordinary physical organic techniques: low temperature NMR, $^{15}$N-labeled reagents and CIDNP experiments.

The advantage of increased reactivity of the trifluoromethylated nitro purines towards C-2 substitution is presented in Chapter 3. The effect and elegance of two electronegative substituents, i.e. the trifluoromethyl group in combination with the nitro group was shown via
unprecedented C-2 substitutions at temperatures between -20°C and 0°C. A diverse set of amino substituted and alkoxy substituted 6-trifluoromethylpurines was composed for biological evaluation and further functionalization.

Chapter 4 deals with the introduction of substituents at C-8, making use of lithiation and halogenations reactions at the C-2 substituted trifluoromethyl purines. A set of 8-aminoalkyl substituted analogs was synthesized via direct S_NAr substitution. Using Suzuki, Stille and Sonogashira palladium coupling techniques a complete series of C-8 alkyl substituted 6-trifluoromethyl purines was obtained. The thus developed unique sequence of techniques allowed the development of novel purine structures, with preferred substituents at C-2, C-6, C-8 and N-9.

In Chapter 5, the synthetic efforts towards the synthesis of 2-substituted 1-deazapurines are discussed. Novel efficient routes were presented towards selective C-2 nitration and N-9 alkylation of 1-deazapurines using Boc and azide chemistry. This nitro group was converted to the highly reactive nitroso species, giving access to new series of azo- and hydrazone compounds via amine condensation reactions with the nitroso group. Diels-Alder reactions
and eventual additional ring opening reactions yielded highly attractive compounds for biological study. In analogy of purine nitration, the mechanism of 1-deazapurine nitration was studied and compared with pyridine nitration.

The receptor affinity, functionality and selectivity of the purine analogues that were synthesized as discussed in the preceding chapters are described in **Chapter 6**. Several compounds were identified with very high affinity and antagonistic functionality for the human adenosine receptors. Surprisingly, ligands were highly selective for the adenosine A<sub>3</sub> receptor, instead of the anticipated adenosine A<sub>2A</sub> receptor. The introduction of a single trifluoromethyl group shifted, highly selective, the affinity from the adenosine A<sub>2A</sub> receptor to the adenosine A<sub>3</sub> receptor. This research led to a new class of adenosine receptor A<sub>3</sub> antagonists. The regulation of the adenosine A<sub>3</sub> receptor is currently being evaluated for
several types of diseases like glaucoma, inflammation reactions (asthma and COPD) and regulation of cell growth in some types of cancer (bladder- and thyroid cancer, leukemia).

The biological evaluation of substituted 1-deazapurines revealed that changing substituents from nitro to nitroso groups, can lead to selectivity differences on the $A_1$ and $A_3$ receptors. The C-2 functionalization of the 1-deazapurines led to moderately active antagonists for the adenosine $A_{2A}$ receptor.