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Application of technologies and parallel chemistry for the generation of actives against biological targets

Combinatorial synthesis of anilinoanthraquinone derivatives as non-nucleotide-derived $P2Y_2$ receptor antagonists

$P2Y_2$ receptors belong to the family of G protein-coupled nucleotide (P2) receptors. These receptors are activated by the physiological nucleotides (UTP) and (ATP) and also by dinucleotides. $P2Y_2$ receptors show a wide distribution in the human body, including repositories in the lung, heart, skeletal muscle, spleen, kidney and brain [1]. To date, there is a paucity of compounds acting as pharmacological tools that are potent and selective $P2Y_2$ receptor antagonists. These tools are needed to elucidate the (patho)physiological roles of the receptors [1,2]. Additionally, $P2Y_2$ receptor antagonists have potential as novel therapeutics acting as, for example, anti-inflammatory agents and as neuroprotective drugs. Recent work aimed at identifying new tool compounds [3] has centered around the development of non-nucleotide-derived P2Y2 receptor antagonists using Reactive Blue 2 (i) as a lead structure. This compound is a potent $P2Y_2$ antagonist but the compound also inhibits ectonucleotidases [4] and is non-selective through blocking of other P2 receptor subtypes as well [5]. Additionally, one would not expect the compound to possess good oral bioavailability as it has a high molecular weight (MW = 840 g/mol) and possesses three negatively charged sulfonate groups. The present study investigated whether the preparation of a library of 4-phenylamino-substituted 1-amino-2-sulfoanthraquinones, synthesized through an automated combinatorial synthetic approach using a parallel synthesizer, followed by HPLC purification of final (singleton) compounds, would lead to potent, novel tool compounds as $P2Y_2$ receptor antagonists. For the synthesis of compounds in parallel, polypropylene vials were used in a MiniBlock™ synthesizer (Mettler Toledo, Switzerland). An Ullmann coupling reaction [6] was performed between bromaminic acid salt (ii) and anilines to deliver products of generic structure (iii). After HPLC purification of final products, the compounds underwent pharmacological testing. $P2Y_2$ receptors are coupled to phospholipase $C\beta_1$ via $G\alpha_{q/11}$ protein mediating the production of inositol trisphosphate, which in turn leads to intracellular calcium release [7]. All products were evaluated as antagonists at mouse $P2Y_2$ receptor natively expressed in neuroblastoma × glioma hybrid (NG108-15) cells [8] and at human $P2Y_2$ receptor heterologously expressed in 1321N1 astrocytoma cells [9]. UTP was used for activating the $P2Y_2$ receptor at a concentration where it exhibited approximately 50–80% of the maximal effect (1 μM for 1321N1 astrocytoma cells expressing the human $P2Y_2$ receptor, 3 μM for NG108-15 cells). The starting point for this investigation (i) exhibited an IC_{50} value of 5.0 μM at mouse and 1.85 μM at human $P2Y_2$ receptors respectively, both in agreement with literature data. From this library, several compounds with low micromolar potency against the $P2Y_2$ receptor were obtained. For example, (iv) possessed an IC_{50} of 9 μM. This work is of interest because several novel analogues have been synthesized with similar potency to the known starting point (i). This work lays the foundation for a more exhaustive library synthesis using a diverse set of anilines for example, with a view to both improving activity and establishing more drug like properties (e.g. oral bioavailability) in the series through replacement of the sulfonate group with, for example, a carboxylic acid moiety.

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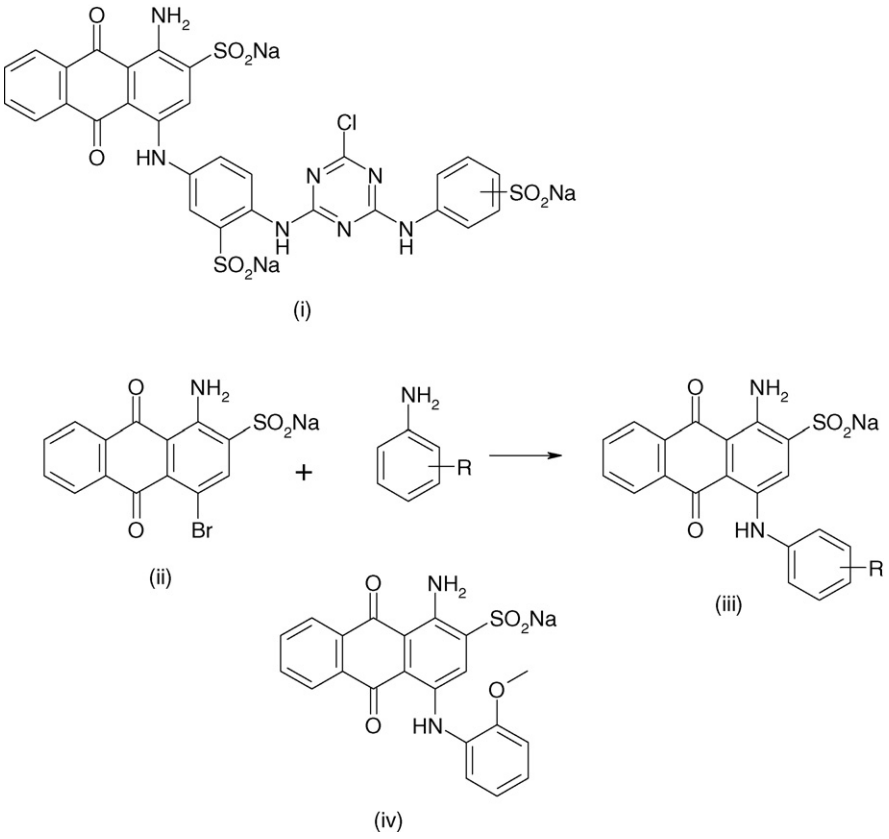
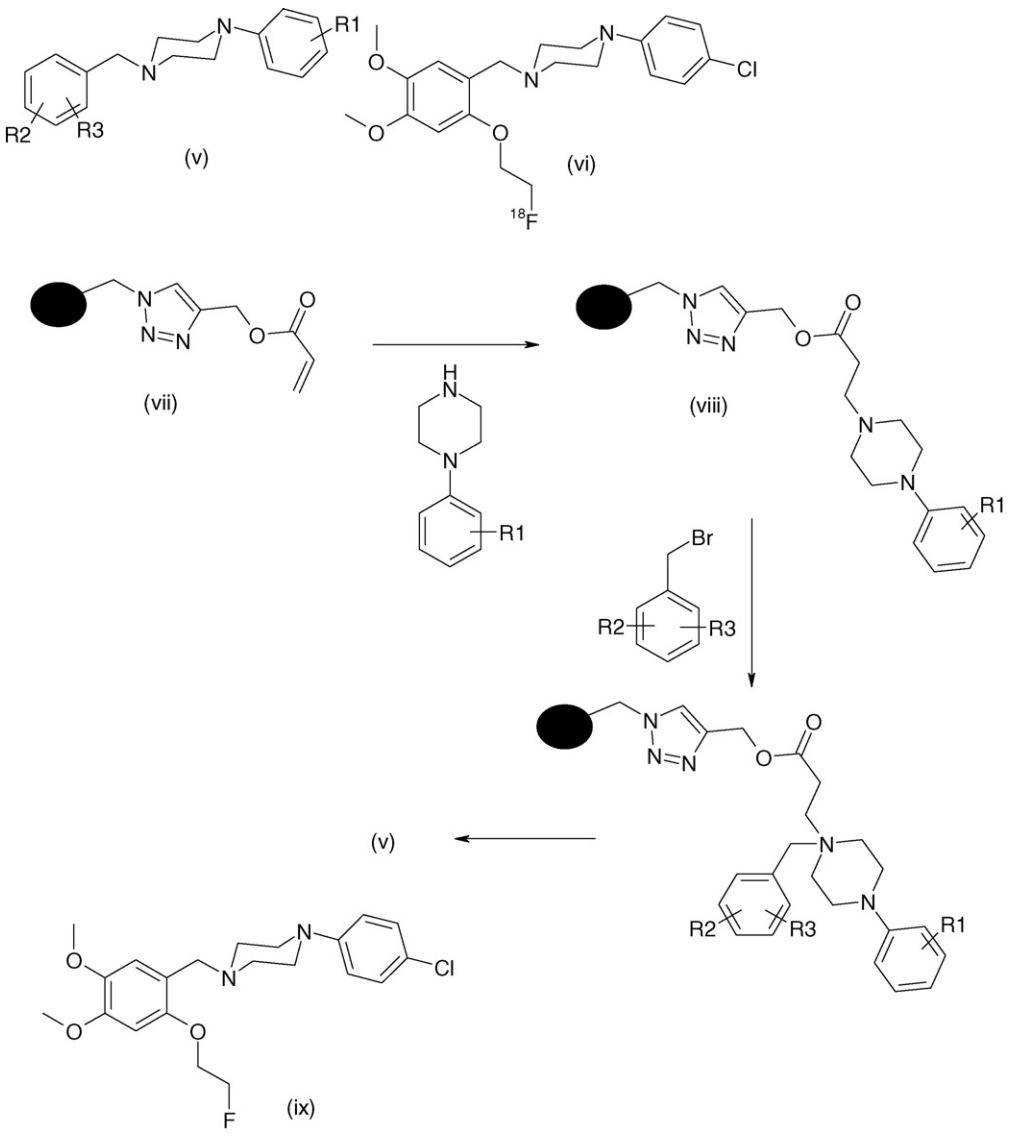


Dopamine D4 selective ligands synthesized from a click chemistry-based regenerative Michael acceptor (REM) linker strategy

The dopamine D4 receptor has attracted attention as a pharmacological target for the treatment of diseases, such as schizophrenia, Parkinson's disease, depression and attention deficit hyperactivity disorder (ADHD) [10]. Within the field of D4 research, the *N*-arylpiperazine framework has proved to be a privileged structural unit when the second nitrogen atom of the piperazine moiety is preferably attached to a benzylic $-CH_2-$ position of a fused heteroarene moiety [11], see general structure (v). Recent work [12] on discovering subtype specific dopaminergic PET candidates has utilized a two-step procedure involving, first, a click chemistry-promoted approach to a focused chemical library, and further lead optimization facilitating a fine tuning of both selectivity profiles and lipophilicity, which resulting in the development of the PET ligand (vi). 'Click resins' enable solid phase supported reactions to work under conditions ideal for this type of chemistry. Utilizing the triazolylmethyl acrylate (TMA) linker [11,13] and an REM strategy [14], a library of tertiary amines were synthesized via parallel chemistry. Specifically, the resin bound Michael acceptor (vii) was treated with phenylpiperazines derivatives to give intermediates (viii). *N*-alkylation with benzyl bromides followed by Hofmann elimination of the resulting ammonium ions gave final products of general structure (v). Once compounds were isolated, dopamine receptor screening of the test compounds was performed, assessing their ability to bind to the cloned human $D2_{long}$, $D2_{short}$, D3 and D4 dopamine receptors, and the porcine D1 subtypes was evaluated *in vitro* [15]. This was performed in a screening system by measuring the displacement of the radioligands [^{3}H]spiperone for D2, D3, D4 and [^{3}H]SCH23390 for D1 receptors using 10 μM, 100 nM and 1 nM concentrations of the test compounds. These sets of experiments indicate a D4 receptor preference for most of the compounds tested. Comparison of non-polar arenes with the derivatives bearing H-bond accepting groups were found to indicate that for successful bioisosteric replacements of the azaindole moiety, an electronegative congener that could be represented by a methoxy or cyano unit was required. From this work, several potent compounds were obtained. One of the most potent was (ix) which possessed a K_i value of 1.7 nM. The use of 'click chemistry' has allowed the rapid generation of a library of compounds and further optimization led to a series of potent compounds with potential for development as D4 PET ligands. Further work in this area is warranted in further improving the properties of this series of inhibitors.



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