Design and synthesis of benzothiophene and benzothienopyrimidine derivatives with kinase inhibitory activity

_Doctoral (Ph.D.) Thesis_

_Péter G. Bánhegyi_

Semmelweis University
Doctoral School of Pharmaceutical and Pharmacological Sciences

Supervisor: Dr. László Örfi, Ph.D.
Opponents: Dr. Gábor Dibó, C.Sc.
Dr. Péter Tétényi, Ph.D.
Head of Examination Committee: Dr. Éva Lemberkovics Éva, Ph.D.
Members of Examination Committee:
Dr. Éva Szőke, D.Sc.
Dr. Péter Mátyus, D.Sc.

Budapest
2008
Design and synthesis of benzothiophene and benzothienopyrimidine derivatives with kinase inhibitory activity

Péter G. Bánhegyi, supervisor: Dr. László Örфи

During my Ph.D. research I participated in the development of novel potential pharmaceutical agents against human and bacterial kinase enzymes. In this process I synthesized pyrrolo-pirimidine leads with highly active Epidermal Growth Factor Receptor Protein Tyrosine Kinase (EGFR-PTK) inhibitory activity, as well as the 1-benzothiophene lead with mycobacterial Protein Kinase G (PknG) inhibitory activity, that are acknowledged in the literature.

I have synthesized focused molecule libraries with new patentable structures around these core structures and our research group examined the biological activity profile of these derivatives. By changing the substituents, introducing new molecule fragments, or by changing their positions, more efficient, patentable structures can be created.

During the biological test of the focused benzothiophene and the benzothienopyrimidine molecule libraries we found that several analogues exhibited increased inhibitory activity compared to the parent lead molecules.

Moreover we discovered an unusual chemical method for the amidation of the slightly reactive benzothiophene carboxylate esters by reaction with lithium amide in THF.

The detailed exploration of the structure-activity relationships of these derivatives and the optimization of their biological activity requires further research.

1. Introduction

In recent years, dramatic developments in molecular biology and protein chemistry have greatly enhanced our understanding of the molecular pathomechanisms of various diseases. The aim of the new conception of research was the inhibition of the false and enhanced signals created by the oncogenes and growth factors. The reversible protein phosphorylation, which is generated by protein kinases and ceased by phosphoprotein-phosphatases, plays an essential role in the molecular mechanism of cellular answers to extracellular signals.

The epidermal growth factor receptor protein tyrosine kinase (EGFR-PTK) is the archetypal member of a receptor tyrosine kinase family comprised of four closely related receptors called EGFR, HER2, HER3, and HER4. The kinase activity is stimulated when members of the EGF family of growth factors bind to the receptor. Ligand-induced EGFR activation initiates a signaling cascade that activates gene expression and induces cellular responses such as cell-cycle progression or differentiation. Aberrant activation of this highly regulated signaling pathway is believed to contribute to many tumorigenic processes, including enhanced cellular proliferation, protection from apoptosis, tumor cell invasion and metastasis. Inhibitors of the EGFR-PTK could therefore have great therapeutic potential in the treatment of malignant and nonmalignant epithelial diseases. Due to the involvement of tyrosine kinases in many signal transduction pathways, it can be important to develop inhibitors with high selectivity at the enzyme level.

*Mycobacterium tuberculosis* continues to be one of the world's deadliest pathogens, causing a prospective burden of one billion newly infected individuals. Despite the existence of effective chemotherapies, no new drugs have come to the market over 40 years. Virulence of pathogenic mycobacteria is related to their capacity to survive for prolonged times within macrophage phagosomes. Whereas normally internalized and phagocytosed bacteria are rapidly degraded within phagolysosomes, pathogenic mycobacteria have evolved to block lysosomal delivery. A crucial virulence factor for intracellular mycobacterial survival is protein kinase G (PknG), an eukaryotic-like serine/threonine protein kinase expressed by pathogenic mycobacteria that blocks the intracellular degradation of mycobacteria in lysosomes. Importantly, mycobacteria overexpressing a kinase-dead mutant of PknG are rapidly transferred to lysosomes and killed, demonstrating that PknG kinase activity is crucial for mycobacterial survival. Inhibition of PknG with a highly selective low-molecular-weight inhibitor (such as AX20017) results in mycobacterial transfer to lysosomes and killing the mycobacteria.

2. Targets

During my Ph.D. research I have participated in the development of novel potential pharmaceutical agents against human and bacterial kinase enzymes. In this process first I explored a synthetic route for the preparation of the AX20017 lead including a new amidation method with lithium-amide.

I have synthesised a focused compound library of patentable structures around this lead molecule, and around the QSAR-selected potential EGFR-PTK inhibitor thieno-pyrimidine core structure.
In my Ph. D. work we set the following aims:

1. To work out a synthetic route for the preparation of the most active PknG inhibitor lead AX20017 found during the HTS of a 50,000-membered compound library;
2. The design and preparation of a focused compound library with patentable members around this lead molecule;
3. The biological study of the focused compound library on PknG;
4. The design and synthesis of a focused compound library with patentable members around the QSAR-selected potential EGFR -PTK inhibitor, thieno-pyrimidine core structure;
5. The biological study of this focused compound library on EGFR-PTK, and a panel of other kinases for selectivity profiling.

3. Results

3.1. The preparation of AX20017 and a focused compound library around it

The AX20017 (18) was found by Axxima Pharmaceuticals AG in a high throughput screening of a 55000-membered compound library. The synthesis of the AX20017 had not been described in the literature.

![Figure 1. The AX20017 lead molecule. (PknG IC\textsubscript{50} = 0.5 µM)](image)

The synthetic route was started from the commercially accessible 19 amine, what was acylated in pyridine resulting the 20 ester.

![Figure 2. The acylation of the 19 amine.](image)

The compound 20 could not be converted to amide by the application of conventional methods like treatment with aqueous or alcoholic ammonia solution or ammonia gas. Under mild reaction conditions we have recovered the unconverted starting material, higher temperature and pressure resulted the formation of a ring-closed product (21). The successful amidation reaction was carried out with lithium amide in tetrahydrofurane resulting 18 in good yield.
After acylations of 20 with diverse acid-chlorides followed by the amidation with lithium-amide we prepared the following 2-acyl analogues.

The alkaline hydrolisis of the ester group yielded the 22 acid-analogue, what which was reacted with CDI and hydrazine to obtain the 27 acid-hydrazid compound. The reaction of the 20 ester with aliphatic amines gave the 28, 29 N-alkyl derivatives.

We started the modification of the cycloalkene ring with different ring size homologues. We prepared the 32a-c amines via Gewald-reaction, followed by the acylation and amidation yielding the cyclopentyl (34a), cycloheptyl (34b), and cyclooctyl (34c) derivatives.
We started from heteroatom-containing cyclic ketones in the Gewald reaction followed by acylation and amidation to prepare the 37a pyrane, 37b N-methyl-piperidine, and 37c thiopyrane analogues. The oxidation of the 37c with potassium-persulphate yielded the 37d sulfoxide derivative.

In a similar manner we prepared the alkyl- and arylsubstitued AX20017 derivatives from the alkyl- and arylsubstitued cyclic ketones.

We did experiments for the aromatisation of the cycloalkene ring of AX20017 with actived MnO_2, chinon, DDQ, Pd/C + air, SeO_2, but these attempts were unsuccesful. The solution was the oxydation of the 20 ester with activated MnO_2, followed by amidation at low temperature yielding the 41 aromatic compound.
Figure 10. The preparation of aromatic bromo-, nitro-, amino-, and carbamide derivatives.

Cyclohexanedion-monoethylene-ketal, cyanacetamide and sulphur were used in the Gewald-reaction, yielding the 46 ketal derivative, which was acylated to yield the 47,48 ketal analogues.

Figure 11. The synthesis of ketal derivatives.

The ketal protecting group was removed by trifluor-acetic acid to yield the 49,50 oxo-analogues. These compounds were dehydrogenated with Pd/C and air to yield the 51,52 aromatic phenols. The reduction of 49, 50 with sodium borohydride led to the 53,54 saturated hydroxy derivatives.

Figure 12. The synthetic route for the preparation of aromatic and saturated 6-hydroxy AX20017 analogues.
3.2. Design and synthesis of a focused compound library around the QSAR-selected potential EGFR inhibitor thieno-pyrimidine core structure

The QSAR study showed that the tricyclic 1-benzothieno[2,3-\(d\)]pyrimidin-4-amine core may have very good EGFR-PTK inhibitory activity. We have marked the potential diversity points of this structure in Figure 13.

![Figure 13](image)

**Figure 13.** The 1-benzothieno[2,3-\(d\)]pyrimidin-4-amine core structure and its diversity points.

The key intermediate compound of the tricyclic structure is the early described 19 ester, which was prepared by Gewald-reaction. The ring-closure was carried out with formamidin-acetate in formamide at 180 °C yielding the 70 phenol derivative. After it's chlorination with phosphorus oxychloride we reacted the obtained 71 reactive compound with substituted anilines to get the 72a-x molecules.

![Synthetic route](image)

**Figure 14.** Synthetic route for the preparation of 1-benzothieno[2,3-\(d\)]pyrimidin-4-amine analogues.

For the preparation of the aromatic 1-benzothieno[2,3-\(d\)]pyrimidin-4-amine derivatives we started from the 42 aromatic ester. The cyclopropyl-carbonyl group was removed by methanesulphonic acid, the ring-closure was carried out with formamide. After the chlorination of product 74 we reacted the 75 imidoyl-chloride intermediate with substituted anilines to obtain the 76a-c aromatic derivatives.
Figure 15. The preparation of the aromatic benzothieno[2,3-d]pyrimidine derivatives.

For the preparation of derivatives containing cyclopropyl group in position 2, we started from the 20 and 42 compounds. The reaction of these molecules with formamidin-acetate in formamide yielded the 77a,b derivatives. After the reaction with phosphorus oxychloride the 78a,b chlorinated derivatives were reacted with the corresponding anilines to obtain the 79a-g saturated, and 79h-l aromatic derivatives.

![Reaction Scheme](image)

**Figure 16.** The preparation of 2-cyclopropyl derivatives of benzothieno[2,3-d]pyrimidine.

For the preparation of 4-hydrazone derivatives we started from the 71 imidoyl chloride derivative. The reaction of 71 with hydrazine and a set of aromatic aldehydes led to the 81a-m hydrazone derivatives.

![Reaction Scheme](image)

**Figure 17.** Synthetic route of 4-hydrazone derivatives.
4. Summary of results, conclusions

The aim of my Ph.D. research was to synthesize the highly active PknG kinase inhibitor AX20017 lead molecule. I have synthesized a focused compound libraries around AX20017 and the QSAR predicted potential EGFR-PTK inhibitory benzothienopyrimidine lead and our research group examined the biological profile of these new patentable derivatives. Moreover we have discovered an unusual chemical method for the amidation of the slightly reactive benzothiophene carboxylate esters by reaction with lithium amide in THF.

We could make the following conclusions based on the biological data presented in this dissertation:

1. Testing results of the synthesised benzothiophene derivatives showed that several analogues exhibited increased inhibitory activity compared to the parent AX20017 lead molecule.

<table>
<thead>
<tr>
<th>compound</th>
<th>PknG IC$_{50}$</th>
<th>compound</th>
<th>PknG IC$_{50}$</th>
<th>compound</th>
<th>PknG IC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>0.5 μM</td>
<td>27</td>
<td>&gt;100 μM</td>
<td>41</td>
<td>0.085 μM</td>
</tr>
<tr>
<td>20</td>
<td>&gt;100 μM</td>
<td>28</td>
<td>22 μM</td>
<td>42</td>
<td>58 μM</td>
</tr>
<tr>
<td>22</td>
<td>&gt;100 μM</td>
<td>29</td>
<td>&gt;100 μM</td>
<td>43</td>
<td>0.095 μM</td>
</tr>
<tr>
<td>26a</td>
<td>31 μM</td>
<td>34a</td>
<td>0.35 μM</td>
<td>44a</td>
<td>0.093 μM</td>
</tr>
<tr>
<td>26b</td>
<td>23 μM</td>
<td>34b</td>
<td>68 μM</td>
<td>44b</td>
<td>0.17 μM</td>
</tr>
<tr>
<td>26c</td>
<td>17 μM</td>
<td>34c</td>
<td>27 μM</td>
<td>45a</td>
<td>0.85 μM</td>
</tr>
<tr>
<td>26d</td>
<td>4.79 μM</td>
<td>37a</td>
<td>0.63 μM</td>
<td>45b</td>
<td>0.95 μM</td>
</tr>
<tr>
<td>26e</td>
<td><strong>0.38 μM</strong></td>
<td>37b</td>
<td>68 μM</td>
<td>45c</td>
<td>1.29 μM</td>
</tr>
<tr>
<td>26f</td>
<td>1.66 μM</td>
<td>37c</td>
<td>0.74 μM</td>
<td>47</td>
<td>3.16 μM</td>
</tr>
<tr>
<td>26g</td>
<td>56 μM</td>
<td>37d</td>
<td>28 μM</td>
<td>48</td>
<td>13 μM</td>
</tr>
<tr>
<td>26h</td>
<td>62 μM</td>
<td>40a</td>
<td>16 μM</td>
<td>49</td>
<td><strong>0.49 μM</strong></td>
</tr>
<tr>
<td><strong>26i</strong></td>
<td><strong>0.41 μM</strong></td>
<td><strong>40b</strong></td>
<td><strong>0.29 μM</strong></td>
<td>50</td>
<td>0.63 μM</td>
</tr>
<tr>
<td>26j</td>
<td>&gt;100 μM</td>
<td>40c</td>
<td>0.79 μM</td>
<td>51</td>
<td><strong>0.047 μM</strong></td>
</tr>
<tr>
<td>26k</td>
<td>26 μM</td>
<td>40d</td>
<td>1.17 μM</td>
<td>52</td>
<td><strong>0.058 μM</strong></td>
</tr>
<tr>
<td>26l</td>
<td>&gt;100 μM</td>
<td>40e</td>
<td>74 μM</td>
<td>53</td>
<td><strong>0.31 μM</strong></td>
</tr>
<tr>
<td>26m</td>
<td>&gt;100 μM</td>
<td>40f</td>
<td>1.17 μM</td>
<td>54</td>
<td><strong>0.37 μM</strong></td>
</tr>
</tbody>
</table>

*Table 1.* The PknG inhibitory activity of the prepared AX20017 analogues. (in bold: have higher PknG inhibitory activity than the parent AX20017)

Thirteen compounds showed higher PknG inhibitory activity than the original lead molecule. The most actives were the **51** and **52** aromatic 6-phenol derivatives of AX20017.

2. Testing the focused compound libraries synthesized around the QSAR selected benzothienopyrimidine structure on various targets we found that several members of the compound library showed excellent inhibitory activity against EGFR-PTK as well as extreme high selectivity.
Table 2. The EGFR-PTK inhibitory activity of the prepared benzothienopyrimidine derivatives.

<table>
<thead>
<tr>
<th>compound</th>
<th>EGFR IC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>compound</th>
<th>EGFR IC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>compound</th>
<th>EGFR IC&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>72a</td>
<td>32 nM</td>
<td>72r</td>
<td>27 nM</td>
<td>79h</td>
<td>not active</td>
</tr>
<tr>
<td>72b</td>
<td>not active</td>
<td>72s</td>
<td>not active</td>
<td>79i</td>
<td>not active</td>
</tr>
<tr>
<td>72c</td>
<td>not active</td>
<td>72t</td>
<td>not active</td>
<td>79j</td>
<td>not active</td>
</tr>
<tr>
<td>72d</td>
<td>not active</td>
<td>72u</td>
<td>not active</td>
<td>79k</td>
<td>not active</td>
</tr>
<tr>
<td>72e</td>
<td>not active</td>
<td>72v</td>
<td>not active</td>
<td>79l</td>
<td>not active</td>
</tr>
<tr>
<td>72f</td>
<td>12 nM</td>
<td>72w</td>
<td>not active</td>
<td>81a</td>
<td>not active</td>
</tr>
<tr>
<td>72g</td>
<td>2 nM</td>
<td>72x</td>
<td>not active</td>
<td>81b</td>
<td>not active</td>
</tr>
<tr>
<td>72h</td>
<td>3 nM</td>
<td>76a</td>
<td>13 nM</td>
<td>81c</td>
<td>not active</td>
</tr>
<tr>
<td>72i</td>
<td>8 nM</td>
<td>76b</td>
<td>7 nM</td>
<td>81d</td>
<td>not active</td>
</tr>
<tr>
<td>72j</td>
<td>34 nM</td>
<td>76c</td>
<td>8 nM</td>
<td>81e</td>
<td>not active</td>
</tr>
<tr>
<td>72k</td>
<td>35 nM</td>
<td>79a</td>
<td>not active</td>
<td>81f</td>
<td>not active</td>
</tr>
<tr>
<td>72l</td>
<td>17 nM</td>
<td>79b</td>
<td>not active</td>
<td>81g</td>
<td>not active</td>
</tr>
<tr>
<td>72m</td>
<td>8 nM</td>
<td>79c</td>
<td>not active</td>
<td>81h</td>
<td>not active</td>
</tr>
<tr>
<td>72n</td>
<td>not active</td>
<td>79d</td>
<td>not active</td>
<td>81i</td>
<td>not active</td>
</tr>
<tr>
<td>72o</td>
<td>11 nM</td>
<td>79e</td>
<td>not active</td>
<td>81j</td>
<td>not active</td>
</tr>
<tr>
<td>72p</td>
<td>27 nM</td>
<td>79f</td>
<td>not active</td>
<td>81k</td>
<td>not active</td>
</tr>
<tr>
<td>72q</td>
<td>50 nM</td>
<td>79g</td>
<td>not active</td>
<td>81l</td>
<td>not active</td>
</tr>
</tbody>
</table>

(in bold: the highest EGFR-PTK inhibitory activities; 'not active': the EGFR-PTK inhibition level was lower than 90% at 10 μM)

The most active compound was the 72g with 2 nM IC<sub>50</sub> value on EGFR-PTK and showed extreme high selectivity (did not effect the 19 other kinases).

The detailed exploration of the structure-activity relationships of these derivatives and the optimization of their biological activity is in progress.

5. Publications

Papers of the thesis work:


9. György Kéri, Zsolt Székelyhidi, **Péter Bánhegyi**, Zoltán Varga, Bálint-Hegymegi Barakonyi, Csaba Szántai-Kis, Doris Hafenbradl, Bert Klebl, Gerhard Muller, Axel...


Patents:


8. Missio Andrea; Bacher Gerald; Koul Anil; Choidas Axel; Banhegyi Peter; Greff Zoltan; Keri Gyoergy; Marko Peter; Orﬁ Laszlo; Waczek Frigyes; Pato Janos: 4,5,6,7-Tetrahydrobenzo[b]thiophene derivatives and methods for medical intervention against mycobacterial infections. WO 03084947 A1 PCT patent application.

Oral presentations:


Posters


