Bachelor's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Chemistry

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Is Thienopyrimidine a Suitable Scaffold for HER2 Tyrosine Kinase Inhibitors?

Bachelor's thesis in MLREAL
Supervisor: Bård Helge Hoff
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Abstract

HER2 positive breast cancer is a form of cancer where the HER2 receptor is overexpressed, leading to uncontrolled cell proliferation. One approach to treat this type of cancer is by inhibiting the intracellular kinase domain of the receptor. Today, three tyrosine kinase inhibitors are approved for treatment of HER2 positive breast cancer, but acquired resistance to these drugs is a widespread challenge. Therefore, the search for new inhibitors continues. Thienopyrimidine derivatives have been proposed as possible inhibitors of HER2. Potent inhibitors have been developed by utilizing substituents from the approved drugs on the thienopyrimidine scaffold, and additionally, potent alkynyl thienopyrimidines have been improved over the course of several studies. In this thesis, studies published on thienopyrimidine derivatives as HER2 inhibitors will be discussed, focusing particularly on whether these compounds provide any improvements over the already existing inhibitors, and the role of these compounds in the battle against drug resistance.

Sammendrag

HER2-positiv brystkreft er en kreftform med økt uttrykk av HER2-reseptoren, som fører til ukontrollert cellevekst og celledeling. En behandlingsmetode for denne typen kreft er hemming av det intracellulære kinasedomenet til reseptoren. I dag finnes det tre godkjente tyrosinkinasehemmere som er godkjent for behandling av HER2-positiv brystkreft, men utvikling av resistens mot disse er et utbredt problem. Derfor fortsetter søket etter nye hemmere. Derivater av tienopyrimidiner har blitt foreslått som mulige hemmere av HER2. Potente hemmere har blitt utviklet ved å benytte seg av substituenter fra godkjente medisiner på et tienopyrimidinskjelett, og tienopyrimidiner med alkynylsubstituenter som har vist god effekt har blitt forbedret over flere studier. I denne oppgaven vil studier på derivater av tienopyrimidiner som HER2-hemmere diskuteres, med et særlig fokus på om disse forbindelsene har noen fordeler over de hemmerne som allerede eksisterer og rollen til disse forbindelsene i kampen mot resistens.

Abbreviation list

Abbreviation	Meaning
ATP	adenosine triphosphate
EGFR	epidermal growth factor receptor
EMA	European Medicines Agency
FDA	United States Federal Drug Administration
HER	human epidermal growth factor receptor
IC_{50}	half-maximal inhibitory concentration
RTK	receptor tyrosine kinase
TKI	tyrosine kinase inhibitor

Contents

1 Introduction

One of the main subtypes of breast cancer is called human epidermal growth factor receptor 2 (HER2) positive breast cancer, and it makes up between 20-30% of breast cancer cases.[1] Patients that are diagnosed with this type of cancer are found to have an overexpression of the HER2 gene, which causes uncontrolled cell growth and cell division. HER2 positive breast cancer is often associated with more aggressive cancer and poorer outcomes.[2] Additionally, HER2 overexpression has also been observed in a series of other cancer types, such as bladder, colorectal, non-small cell lung, esophageal and gastric cancers.[3]

A possible treatment for this type of cancer is inhibition of HER2. There are two main ways of inhibiting the activity of HER2: monoclonal antibodies and tyrosine kinase inhibitors (TKIs).[4] In this thesis, monoclonal antibodies will only be mentioned briefly, while the main focus will lie on TKIs. TKIs are molecules with chemical structures such that they can bind to the adenosine triphosphate (ATP) binding domain of the receptor.[5]

The structure and functions of the HER2 receptor will first be described, followed by a presentation of some approved TKIs for treatment of HER2 positive breast cancer, with emphasis on the chemical properties that causes their function, in addition to their ability to inhibit kinase activity. A common problem with the currently approved drugs is drug resistance.[6] Therefore, the search for new inhibitors continues.

This thesis will attempt to answer the following research question:

"Is thienopyrimidine a suitable scaffold for HER2 tyrosine kinase inhibitors?"

The thienopyrimidine scaffold is one of several alternative TKI scaffolds under investigation.[4] Results from studies on molecules derived from thienopyrimidine scaffolds will be presented, and the potential for these molecules as TKIs will be discussed in comparison to the TKIs currently in use for treating HER2 positive breast cancer, focusing on whether these compound provide potential improvements compared to the approved inhibitors, and possibilities for inhibition of drug resistant cells.

2 Theory

2.1 HER2: A Receptor Tyrosine Kinase

HER2 is a receptor tyrosine kinase (RTK), which is a part of the epidermal growth factor receptor (EGFR) family.[7] This family consist of four RTKs: EGFR (HER1, ErbB1), HER2 (ErbB2), HER3 (ErbB3) and HER4 (ErbB4). These have similar structures, being transmembrane proteins with an intracellular tyrosine kinase domain and an extracellular ligand binding domain, except for HER2, for which there exists no known ligands, and HER3, which does not have tyrosine kinase activity.[8] Instead, HER2 is activated by dimerization, either with itself (homodimerization) or with one of the other family members (heterodimerization).[9] In fact, HER2 seems to be the preferred dimerization partner for the other HERs.[10] In figure 1, the dimerization of HER2 is illustrated.

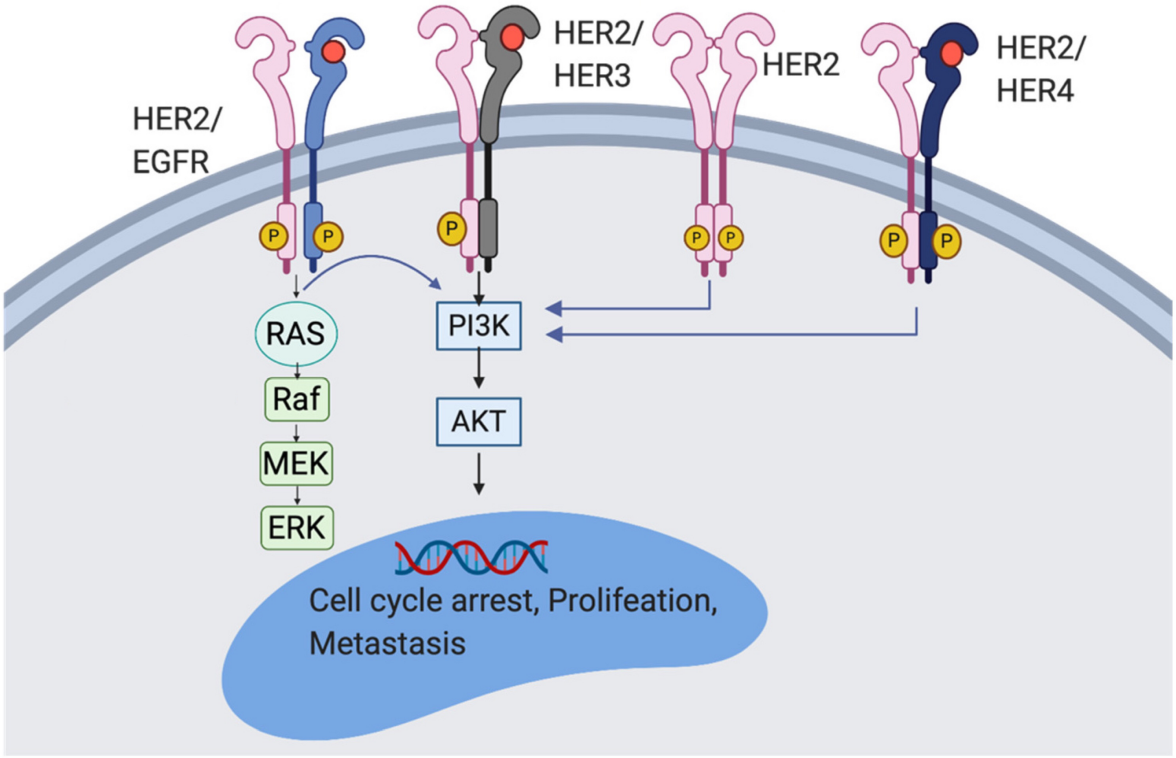


Figure 1: Illustration of the EGFR family. The figure shows homo and heterodimerization of HER2 with consequential cell proliferation as a result of tyrosine residue phosphorylation in the kinase domain. The figure is adapted from Garcia-Sampedro *et al.*.[11]

Upon activation, the tyrosine kinase domain of the receptor becomes catalytically active, and binds ATP, whose chemical structure is shown in figure 2, resulting in phosphorylation of tyrosine residues in the kinase domain.[12] This further initiates signalling pathways in the cell, which causes cell division and cell growth (cell proliferation).[13] An overexpression of HER2 will therefore cause uncontrolled cell division and cell growth, which can lead to cancer.[14]

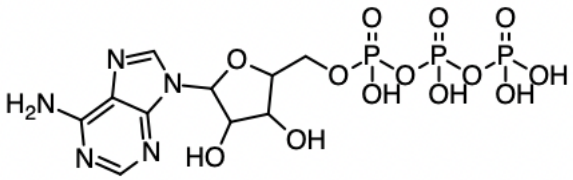


Figure 2: Structure of ATP.

A potential treatment of cancer types with an overexpression of HER2, would therefore be inhibition of the HER2 receptor. Drugs that are commonly used for HER2 inhibition today are monoclonal antibodies, including the approved drugs trastuzumab and pertuzumab.[10] These bind to the extracellular domain of HER2, preventing dimerization, thus hindering activation of the intracellular domain, ultimately leading to a downregulation of the related signalling pathways.[15] However, drug resistance is a common problem, and development of alternative drugs are therefore of interest.[16]

An alternative target for HER2 inhibition, is the intracellular domain. Tyrosine kinase inhibitors (TKIs) are molecules that can bind competitively to the ATP-binding site of the receptor.[4] This inhibits phosphorylation, and downregulates signalling pathways leading to cell proliferation. Many protein kinases in the body share similar structures in their ATP-binding domain, which requires high specificity in the TKI to specifically target HER2.[15] It is therefore necessary to examine the exact structure of the ATP-binding domain of HER2.

2.2 Structure of Kinase Domain of HER2

Aertgeerts *et al.* has performed a structural analysis of the kinase domain of the HER2 receptor.[17] The kinase consist of two lobes, the N-terminal lobe and the C-terminal lobe. Between the two lobes, which are connected in the hinge region, there is a cleft, where the ATP binding

site lies. Depending on whether the kinase is in its active conformation or not, the size of this cleft will be larger or smaller, respectively.

When binding to the enzyme, ATP exists in a complex with magnesium, and the main contributions to the binding of ATP to the catalytic domain, comes from electrostatic interactions caused by the magnesium ions.[18] Additionally, there are several minor contributions to the binding of ATP. The hinge region, although a mostly hydrophobic region, contains amino acid residues that makes hydrogen bonds to the nitrogens of the adenine ring in ATP. The ribose part of ATP is located in a region consisting of mainly hydrophilic amino acid residues, while the phosphate group is located in the phosphate binding region, where it is subject to hydrogen bonding.[19] Additionally, the pocket consists of two hydrophobic regions, which are not utilized by ATP.

Figure 3 shows an illustration of ATP bound to the ATP-binding site of EGFR. The ATP-binding site of HER2 is assumed to have the same general structure, since the catalytic domain of HER2 is 77.7% identical to the catalytic domain of EGFR.[20]

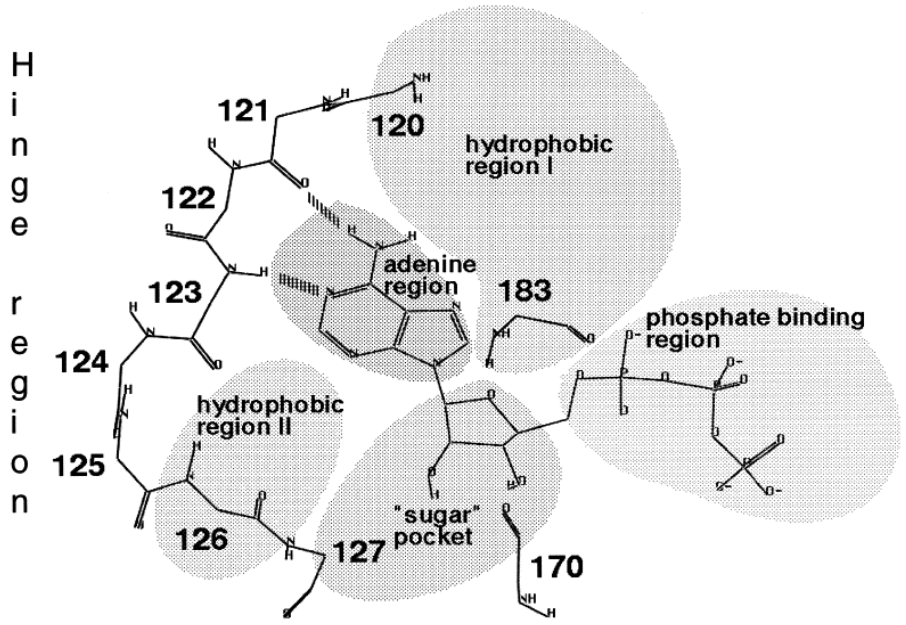


Figure 3: Illustration of an ATP molecule in the ATP-binding site of a member of the EGFR family. Reprinted from Traxler and Furet with permission.[19]

2.3 Approved Drugs Targeting HER2 Inhibition

Figure 4 shows the chemical structure of the three TKI's that are currently approved by the United States Federal Food and Drug Administration (FDA) and the European Medicines Agency (EMA): lapatinib, neratinib and tucatinib.[21–23]

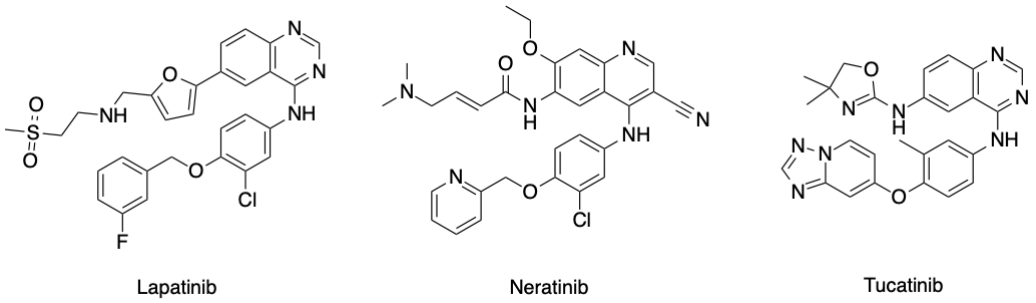


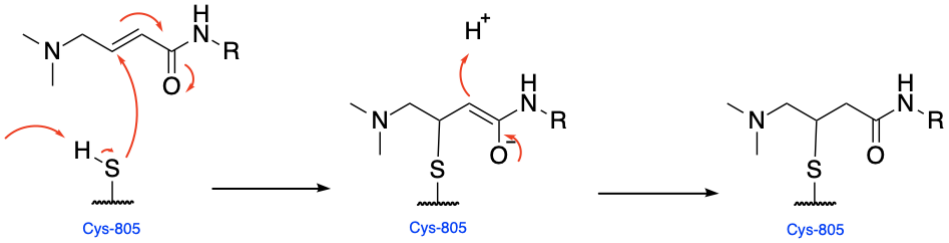
Figure 4: Structures of the three TKIs that are approved for treatment of HER2 positive breast cancer: lapatinib, neratinib and tucatinib.

Lapatinib was the first TKI that was approved for treatment of breast cancer, in combination with other treatments.[24] Lapatinib is a dual EGFR/HER2 inhibitor, which competes with ATP for binding in both the HER2 and EGFR receptor.[20] Its scaffold is a 4-anilinoquinazoline, which is the same as several other approved TKI drugs, such as gefitinib, erlotinib and afatinib.[25] These inhibitors are used in treatment of EGFR-driven non-small celled lung cancer.

As opposed to the binding of ATP, where electrostatic and hydrophilic forces account for the majority of the binding, the binding mode of lapatinib relies mainly on hydrophobic and van der Waals interactions.[18] The scaffold of lapatinib undergoes hydrogen bonding in the hinge region, similarly to ATP, but one of the hydrogen bonds is a water-mediated bond. The substituent groups on the scaffold are located in two separate hydrophobic regions, and the methylsulfonylethylamino moiety is hydrogen bonded to the residues binding phosphate in ATP.

A second HER2 TKI, called neratinib, was approved by the FDA in 2017. As opposed to lapatinib, neratinib is an irreversible inhibitor, binding covalently to the kinase domain.[26] This is possible due to the 4-(dimethylamino)crotonamide substituent, which acts as a Michael acceptor, and undergoes Michael addition with a cystein residue in the ATP binding site.[27] Even though many protein kinases share similar structures, this cystein residue is unique to the EGFR family, leading to neratinib being more selective for EGFR, HER2 and HER4 than other RTKs.

The dimethylamino group at the end of the Michael acceptor is suggested to act as a base catalyst for the reaction.[28] A proposed mechanism for the reaction is shown in scheme 1.



Scheme 1: Proposed mechanism for the covalent bonding of neratinib to the ATP binding domain of HER2. The mechanism is adapted from Piesche *et al.*.[29]

In addition to the covalent bond, the hinge region hydrogen bonds to the nitrogen atom in the cyanoquinoline scaffold and to the nitrogen on the cyano group.[28] The hydrophobic aniline portion of the molecule is bonded in a long hydrophobic pocket.[30]

The most recent addition to the approved HER2 TKIs is tucatinib, which is a molecule with the same scaffold as lapatinib. Tucatinib is highly selective for HER2 over EGFR, as opposed to lapatinib and neratinib, which are dual EGFR/HER2 inhibitors.[3] Tucatinib is a reversible inhibitor, but the details of the binding of tucatinib are yet to be reported.

Table 1 shows half-maximal inhibitory concentrations (IC_{50}) values, both for EGFR and HER2, for the approved TKIs that have been described above. IC_{50} is a commonly used parameter that measures the effect of an inhibitor.[31] An IC_{50} value indicates how much of an inhibitor is needed to inhibit half of the biological activity of a protein, meaning that the lower the IC_{50} value is, the more potent the inhibitor is.[32]

Table 1: IC_{50} values for FDA and EMA approved HER2 tyrosine kinase inhibitors.[21]

Compound	EGFR IC_{50} (nM)	HER2 IC_{50} (nM)
Lapatinib	11	9
Neratinib	92	59
Tucatinib	449	6.9

2.4 Drug resistance

Unfortunately, despite tumors being initially responsive to lapatinib treatment in many cases, resistance develops in advanced diseases.[20] Neratinib and tucatinib resistance has also been observed.[33] When HER2 is inhibited, alternative pathways can be activated, compensating for the downregulated activity of HER2.[34] This can for instance be other RTKs or intracellular kinases.

Another common cause of drug resistance is mutations in the kinase domain, where amino acid residues are exchanged, leading to poorer affinity for the inhibitor.[35] In EGFR, a common mutation is the T790M mutation, where a threonine residue is substituted with a methionine residue.[36] This causes increased affinity for ATP, and the bulky methionine interfers sterically with binding of lapatinib, making it less effective.[37] Irreversible inhibitors, such as neratinib, has been found to be potent for the T790M mutation.[36] However, over time, new mutations arise.[38] The T798I mutation of HER2, where a threonine residue is substituted with a isoleucine residue, corresponds to the T790M mutation of EGFR.[39] Substitution of the same residue with methionine, T798M, has also been observed.[40]

2.5 Thienopyrimidines: An Alternative Scaffold for HER2 Inhibition

In the search for new TKIs, thienopyrimidine scaffolds have been investigated. These compounds are molecules derived from either the thieno[3,2-*d*]pyrimidine (**A**) or thieno[2,3-*d*]-pyrimidine scaffold (**B**), shown in figure 5.[41] When used in HER2 inhibition, these compounds usually have two substituents, an R group in position 6 and an R' group in position 4.

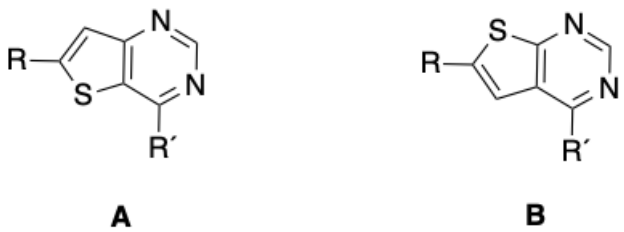


Figure 5: Structures of a thieno[3,2-*d*] and a thieno[2,3-*d*]pyrimidin, **A** and **B** respectively.

Rheault *et al.* has studied thienopyrimidines containing the lapatinib aniline substituent as the R' group, in addition to a furan, thiophene or pyrrole substituent as the R group, as dual EGFR/HER2 inhibitors.[41] In this study, compounds with scaffold **A** were generally found to be more potent than equivalent compounds with scaffold **B**. The study revealed several compounds with high activity. For instance, the furan substituent of lapatinib was tested, resulting in potent dual EGFR/HER2 inhibitors for both scaffold **A** (**1**) and **B** (**2**), both in enzymatic assays and in cells with overexpression of the enzymes. These compounds are also highly selective for EGFR and HER2 over other kinases. The structures of the compounds are shown in figure 6, and the IC_{50} values are given in table 2. In addition to displaying enzymatic inhibition of the EGFR and HER2 receptors, the cellular inhibition of HN5 and BT474 cancer cells are also given. HN5 and BT474 are cell lines with overexpression of EGFR and HER2, respectively.[42,43]

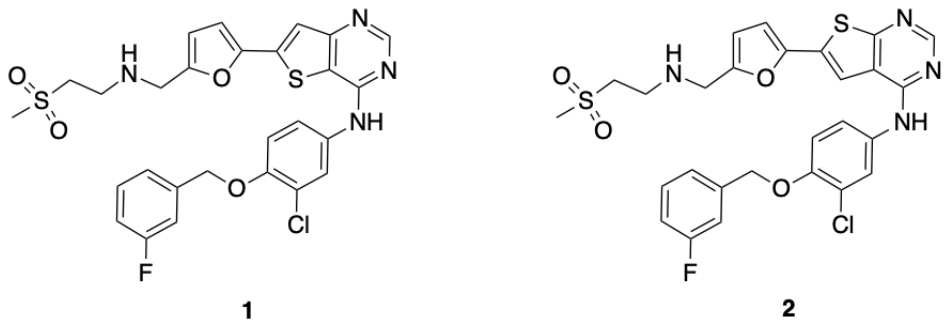


Figure 6: Structures of compounds **1** and **2**.

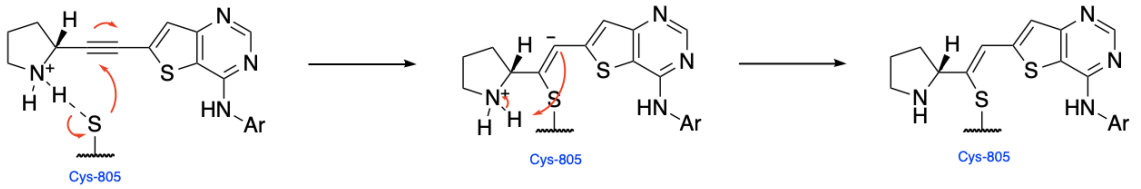
Table 2: IC_{50} values for compounds **1** and **2**.[41]

Compound	EGFR IC_{50} (nM)	HER2 IC_{50} (nM)	HN5 IC_{50} (nM)	BT474 IC_{50} (nM)
1	1	71	410	420
2	12	6	420	420

Wood *et al.* has also investigated thienopyrimidine compounds as TKIs.[44] Here, the lapatinib aniline unit was kept in the structure, while alkynyls were used as the other substituent. Also in this case, structures derived from thienopyrimidine **A** were generally found to have higher inhibitory activity than structures of type **B**.

The alkynyl thienopyrimidines were found to bind covalently to the inhibitors, with R groups containing a basic propargylamine R group, such as pyrrolidine (**3a-b**) (see figure 8), having

particularly high activity. These compounds lack an obvious Michael acceptor, as seen in neratinib, but Wood *et al.* proposes a mechanism for the reaction, where pyrrolidine deprotonates the Cys residue. The suggested mechanism is shown in scheme 2.



Scheme 2: Proposed mechanism for the covalent bonding of alkynyl thienopyrimidines to the ATP binding domain of HER2. The mechanism is adapted from a mechanism proposed by Wood *et al.*.[44]

Hubbard *et al.* has done further studies on derivatives of **3**.[45] A comparison of the two stereoisomers **3a** and **3b** reveals that **3a** is both a more potent inhibitor and more reactive than **3b**. The addition of substituents on the pyrrolidine nitrogen atom, resulted in inhibitors which were less potent than the unsubstituted compound, with poor ability to covalently bond to the receptors.

Instead, a series of different substituents on C4 in the pyrrolidine ring were examined, after comparing models of lapatinib and **3a** in HER2, which indicated that the location of substituents on C4 of the pyrrolidine would be approximately the same as that of the sulfone tail in lapatinib, as seen in figure 7.

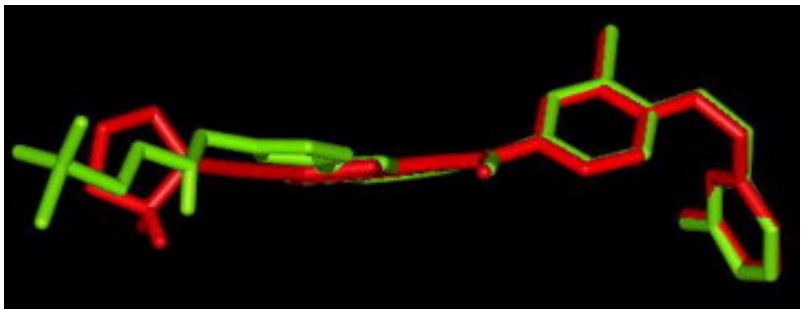


Figure 7: 3a (in red) superimposed on lapatinib (in green). Reprinted from Hubbard *et al.* with permission.[45]

Several potent inhibitors were revealed, including those with methylcarbamate (**4**), ethylcarbamate (**5**), dimethylcarbamate (**6**) and morpholine (**7**) as C4 substituents. Structures of these inhibitors are shown in figure 8 and IC_{50} values are shown in table 3.

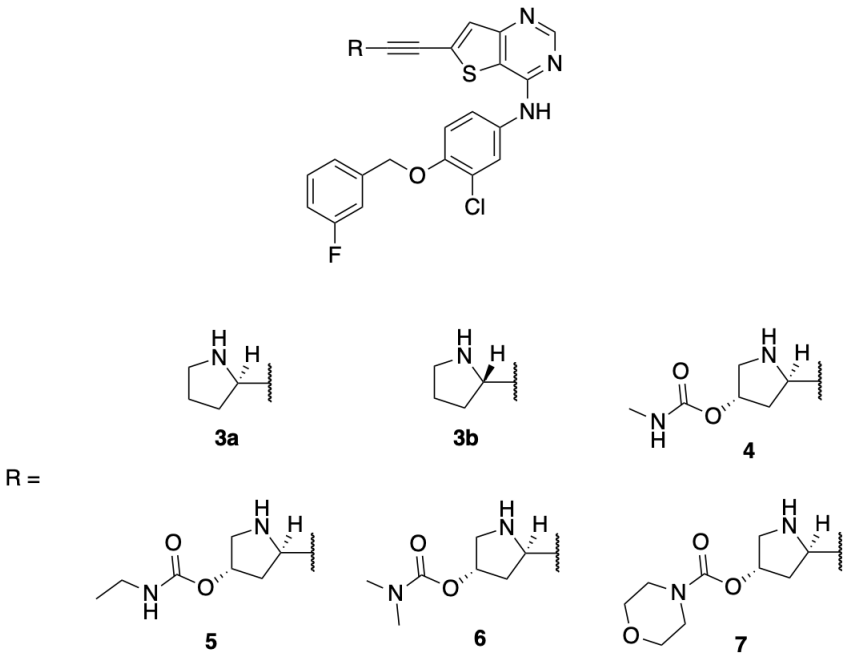


Figure 8: Structures of compounds **3**-**7**.[45]

Table 3: IC_{50} values for compounds **1**-**7**.[45]

Compound	EGFR IC_{50} (nM)	HER2 IC_{50} (nM)	HN5 IC_{50} (nM)	BT474 IC_{50} (nM)
3a	7	13	238	94
3b	109	54	536	205
4	50	20	58	28
5	32	43	95	30
6	28	68	100	30
7	65	84	160	30

Waterson *et al.* has further studied the effect of various aniline substituents in alkynyl thienopyrimidines. In this study, the R group of compound **7** is used as the standard. The structures of a selection of the evaluated substituents are shown in figure 9.

Among the most potent derivatives that were tested, was compound **8** (which has the same aniline substituent as the one in neratinib), **9** and **10**. These compounds display slightly more potent inhibition of HER2 compared to EGFR, which was also the general trend for the substituents tested in this study. However, there were also substituents that gave approximately equipotent EGFR and HER2 inhibitors, such as **11**, while **12** was quite selective for HER2. IC_{50} values for these compounds are given in table 4.

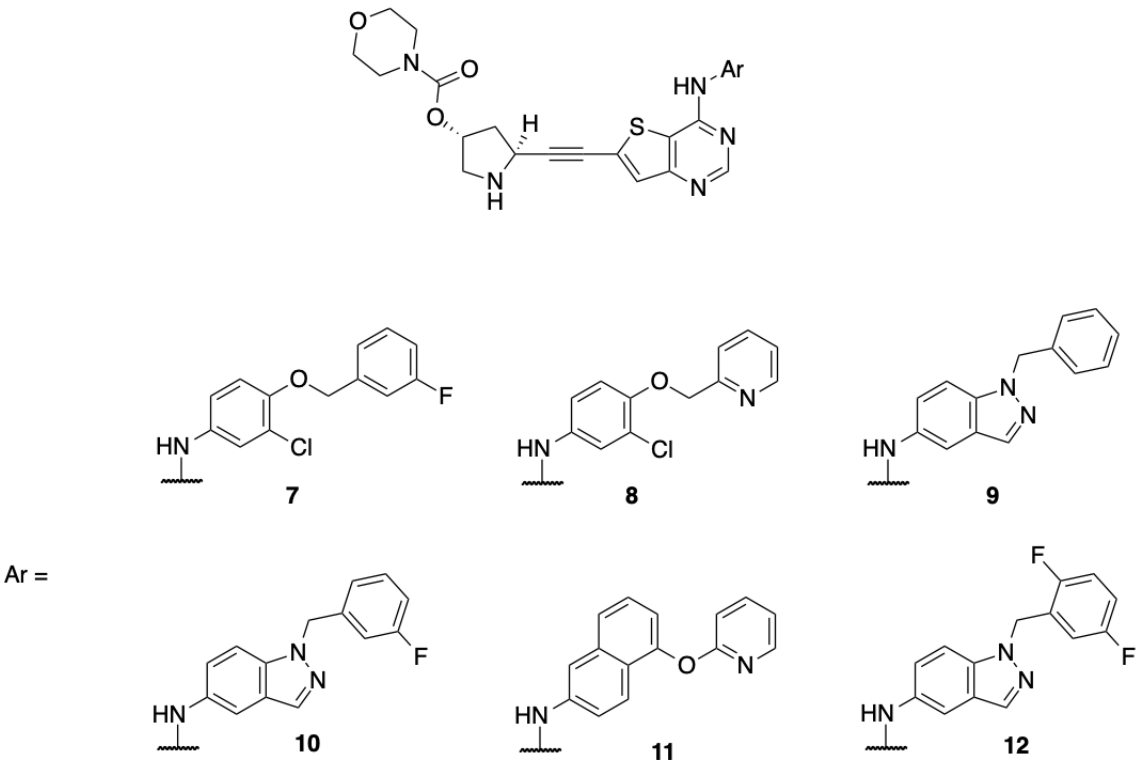


Figure 9: Structures of compounds **7**-**12**.[46]

Table 4: IC_{50} values for compounds **7**-**12**.[46]

Compound	EGFR IC_{50} (nM)	HER2 IC_{50} (nM)	HN5 IC_{50} (nM)	BT474 IC_{50} (nM)
7	80	80	260	90
8	50	30	240	60
9	80	50	70	20
10	30	30	70	30
11	1050	260	120	100
12	33	35	250	10

Yang *et al.* has developed another series of irreversible thienopyrimidine HER2 inhibitors.[48] Here, the α,β-unsaturated amide side chain, which acts as the Michael acceptor in neratinib, is used as the substituent on position 6 in the thienopyrimidine scaffold, while various alternative substituents were investigated in position 4. In this case, compounds with scaffold **B** resulted in vastly more potent inhibitors than compounds with scaffold **A**. Four derivatives of **B** showed potent inhibition of HER2, **13**, **14**, **15** and **16**, whose structures are shown in figure 10, and IC_{50} values are given in table 5. Of these structures, compound **16** seems to be particularly potent. In addition to HER2 inhibition, values for inhibition of the cell line SK-BR-3 is given, which is a cell line with overexpression of HER2.[49]

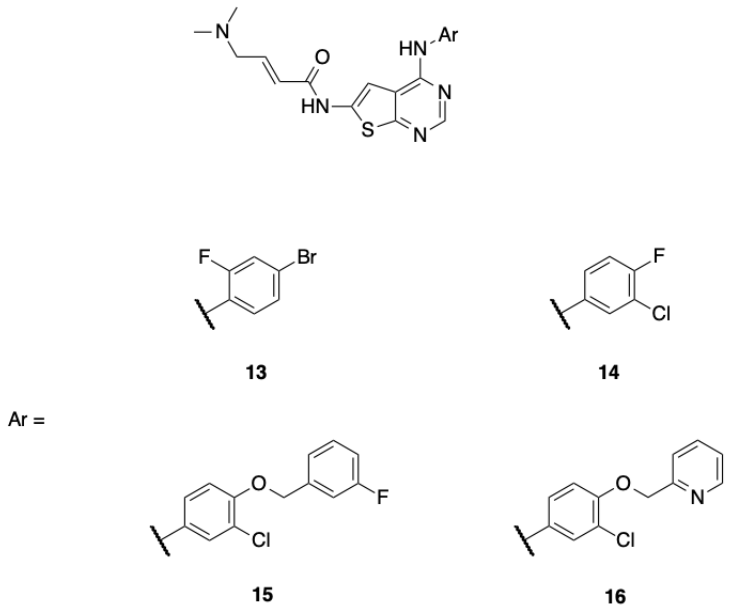


Figure 10: Structures of compounds **13**-**16**.[47]

Table 5: IC_{50} values for compounds **13**-**16** and lapatinib.[47]

Compound	HER2 IC_{50} (nM)	SK-BR-3 IC_{50} (nM)
13	227	450
14	29	200
15	24	450
16	7	130
Lapatinib	17	490

Milik *et al.* focuses on developing dual EGFR/HER2 thienopyrimidine inhibitors that can be effective against kinases that has undergone mutations.[6] For this they use a 6-phenylthieno[2,3-*d*]pyrimidine scaffold. The phenylthiophene unit is suggested to have a higher affinity in the hydrophobic pocket than the phenylfuran in lapatinib. After a screening of different substituents, particularly compound **17** (see figure 11) was found to have good potential.

Table 6 shows EGFR and HER2 IC_{50} values for compound **17**, while table 7 shows IC_{50} values for **17** and lapatinib in the cell lines SK-BR-3 and NCI-H1975. The NCI-H1975 is a cell line with the T790M EGFR mutation.[6]

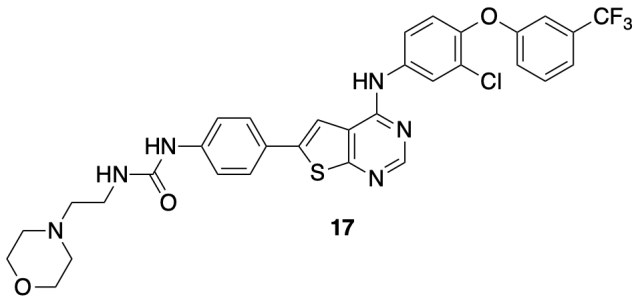


Figure 11: Structure of compound **17**.[6]

Table 6: IC_{50} values for **17** in EGFR and HER2.[6]

Compound	EGFR IC_{50} (nM)	HER2 IC_{50} (nM)
17	91.7	1200

Table 7: IC_{50} values for **17** and lapatinib in the cell lines SK-BR-3 and NCI-H1975.[6]

Compound	SK-BR-3 IC_{50} (nM)	NCI-H1975 IC_{50} (nM)
17	4830	4200
Lapatinib	170	11460

3 Discussion

In early investigations of thienopyrimidine derivatives as HER2 scaffolds, one approach was reusing substituents that were already proven to have good effect in approved drugs, such as lapatinib. This was successful, and both compounds **1** and **2**, which have the same substituents as lapatinib, are potent EGFR and HER2 inhibitors. Rheault *et al.* provides a binding model of inhibitors **1** and **2** in EGFR, and compares it to lapatinib in EGFR.[41] These models indicate that **2** almost overlaps entirely with lapatinib, while the R substituent of **1** has a different spatial orientation, as shown in figure 12. Rheault *et al.* suggests that this may be due to dipole-dipole interactions that forces the sulfur atom in the scaffold and the oxygen atom in the furan in opposites directions, minimizing repulsion.[41]

By comparing IC_{50} values of these two compounds (see table 2) to the values of lapatinib (see table 1), it is also clear that **2** shares similar potency to lapatinib, with slightly higher

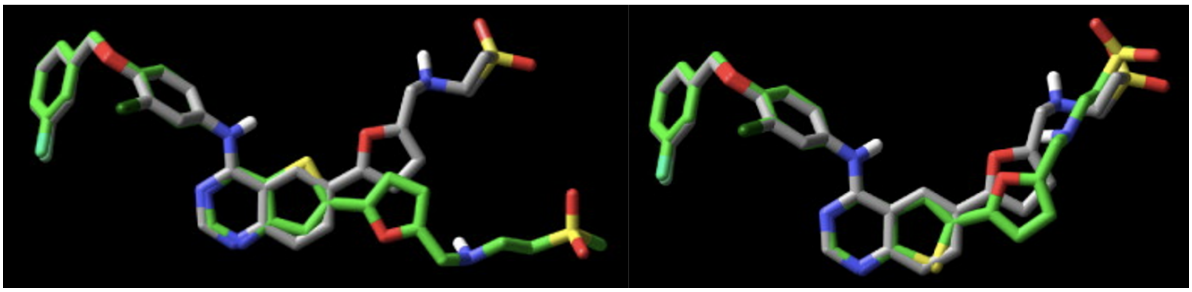


Figure 12: Lapatinib superimposed on compound **1** on the left, and compound **2** on the right. Reprinted from Rheault *et al.* with permission.[41]

selectivity for HER2 than EGFR, while **1** is somewhat more selective for EGFR, and has a bigger difference in potency.

Since binding modes of particularly **1** seems to be highly consistent with that of lapatinib, based on the overlapping three dimensional structures, and similar potencies, there is no reason to believe that these compounds would provide improved inhibition of lapatinib resistant cells compared to lapatinib itself. In fact, resistance is not mentioned at all in this study. Regardless, the results from this study do show that the thienopyrimidine scaffold can indeed be a suitable scaffold for potent HER2 inhibitors.

Around the same time, studies regarding the irreversible alkynyl thienopyrimidines were published. Lapatinib resistance is not mentioned in these articles either, and does not seem to be the motivation for developing these alternative inhibitors. At this point, no irreversible inhibitors were yet approved for treatment of HER2 positive breast cancer. Waterson *et al.* points to benefits of irreversible inhibitors such as prolonged inhibition, and better ability to compete with the high cellular concentrations of ATP.[46]

However, Waterson *et al.* also mentions the risk of higher toxicity.[46] Neratinib, which is also an irreversible inhibitor, has been found to be less selective towards the EGFR family than lapatinib and tucatinib, causing higher toxicity.[3] This is suggested to be a result of the covalent binding mechanism. Since the alkynyl thienopyrimidines also bind covalently, the same could be the case for these compounds.

Irreversible inhibitors that have been approved, such as neratinib, have been effective inhibitors for the T790M mutation in EGFR, but due to toxicity considerations, large enough doses can

not be given.[50] Since the alkynyl thienopyrimidines also binds irreversibly, with a covalent bond to the same threonine residues as neratinib, it is possible that the alkynyl thienopyrimidines also could inhibit resistant cells. However, no data confirming this has been reported, and since the mutations causes steric changes in the binding site, binding may be unfavorable.

The substituents of neratinib have also been attempted placed on a thienopyrimidine scaffold. While keeping the Michael acceptor substituent constant, some alternative aniline subtituents were tested. All of the compounds **13**-**16** are more potent than lapatinib in cellular inhibition of SK-BR-3 (see table 5). The most potent inhibitor is **16**, which contains the same aniline substituent as neratinib. Yang *et al.* provides data only for HER2 inhibition, and not EGFR, and it is therefore not certain whether these inhibitors are dual EGFR/HER2 inhibitors, but since neratinib is not particularly selective for HER2 over EGFR, it is plausible to assume that compound **16** also inhibits EGFR. The longer aniline substituents are more potent than the shorter ones, and since all the approved inhibitors also have longer aniline substituents, this may be generally favorable for HER2 inhibitors.

Whether **A** or **B** is the most suitable scaffold for HER2 inhibition seems to depend on the substituents that are present. For the alkynyl thienopyrimidines, scaffold **A** generally gave the best results, while for the series of compounds with an α,β-unsaturated substituent presented by Yang *et al.*, compounds of scaffold **B** were the most potent. The identity of the R substituent in position 6 seems to be of particular importance in this regard, since the use of the same R' group in position 4, for instance the aniline group of lapatinib, has resulted in potent inhibitors for both scaffold **A** and **B**, such as for compounds **1**-**7** and **15**. This is consistent with the models that Rheault *et al.* provides of **1** and **2**, where the R' substituents are unaffected by the position of the sulfur atom, while the R substituents in the two possible cases have different spatial orientations.[41]

Milik *et al.* focuses on developing dual EGFR/HER2 thienopyrimidine inhibitors that can inhibit resistant cells. This study does not focus on finding inhibitors that can treat HER2 positive breast cancer, but are rather looking for EGFR inhibitors. The reason that dual EGFR/HER2 is desired in this case, is due to the upregulation of HER2 that occurs when EGFR is inhibited.

The HER2 IC_{50} value is significantly higher for compound **17** than the other inhibitors, and the inhibitor is much more potent for EGFR than for HER2.

Compound **17** shows improved inhibition of the cell line where the mutation T790M is present, with an IC_{50} value that is approximately 1/3 of the IC_{50} value of lapatinib. There is given no information about whether the inhibitor is also potent to any cells with HER2 mutations, and it is therefore unclear whether this inhibitor would have any effect in HER2 positive cancer where resistance has been developed.

So far, the potential inhibitors have been discussed primarily based on their measured IC_{50} values. A few comments need to be made about the use of IC_{50} values.

First of all, a good HER2 inhibitor has a relatively low IC_{50} value. A low IC_{50} value indicates a potent inhibitor with lower systemic toxicity.[51] One important thing to keep in mind when discussing IC_{50} values across different studies, is that the obtained values can vary significantly depending on the conditions used when performing the experiments. Hubbard *et al.* points out that when dealing with irreversible inhibitors, the IC_{50} value will depend on the time they have been allowed to react.[45] An example of this can be seen when comparing the obtained IC_{50} values for compound **7** measured by Hubbard *et al.* and Waterson *et al.* (see table 3 and 4), where somewhat different values have been measured.[45,46]

Despite IC_{50} values across different studies not always being entirely comparable, they can still give a general indication of whether a molecule is suitable as an inhibitor or not, and in particular, it is a useful tool to determine the most potent inhibitor of potential compounds within a study, given that equal conditions are applied within the study.

However, a low IC_{50} value alone is not enough to conclude that an inhibitor is satisfactory. In order for an inhibitor to be suitable as a drug, it also needs to have appreciable oral exposure. If solely looking at IC_{50} values, compound **4** seems to be one of the best inhibitors developed in the study by Hubbard *et al.*, with lower IC_{50} values than compound **5**-**7** for all but EGFR (see table 9).[45] Yet, **4** has a relatively low oral exposure in mice compared to compounds **5** and **6**, making these compounds better suited than **4** as potential drugs.

Another important quality for a good HER2 inhibitor, is that it is selective. Selectivity for HER2 versus the other members of the EGFR family has been mentioned earlier, and both approved inhibitors and inhibitors that are currently being researched are in many cases not particularly selective for HER2 over the other members of the EGFR family. However, one essential quality of a HER2 inhibitor, is that it is selective to the EGFR family, in comparison to the other RTKs in the body. Otherwise, the inhibitor can bind to other receptors in the body, leading to adverse effects.

From the preceding discussion, it is clear that potent HER2 inhibitors have been derived from the thienopyrimidine scaffold. But do these compounds provide any improvements compared to the already approved drugs? With regard to resistance, it has already been argued that it is unlikely that the presented compounds are considerably more effective against resistant cells than the approved drugs. The fact that Milik *et al.* has been able to find thienopyrimidine inhibitors that are effective towards resistant EGFR cells, may indicate that it is also possible to develop inhibitors that are potent against resistant HER2 cells. However, even if such compounds were to be developed successfully, the effect of treatment with these compounds would be uncertain, due to the fact that resistant cells have been found to survive without depending on HER2.[52] In this case, HER2 inhibition of resistant cells would not be enough to stop cell proliferation.

In general, most studies that have been presented have had a goal to develop dual EGFR/HER2 inhibitors, rather than inhibitors with high selectivity for HER2 over the other EGFR family members. In the study conducted by Waterson *et al.*, the selectivity towards EGFR/HER2 varies depending on the aniline substituent, indicating that it may be possible to develop inhibitors that are more selective towards HER2 if desirable. Compound **12** is for instance far more selective towards HER2 than the other compounds in the same study. Kulukian *et al.* claims there is an unmet need for inhibitors that target HER2 selectively over the other members of the EGFR family, due to observations of more severe side effects with dual EGFR/HER2 inhibitors.[3] There is uncertainty around the effect of inhibiting EGFR in HER2 positive breast cancers, even though EGFR is also expressed in these cancer types.

Another possible future use for thienopyrimidines could therefore be using this scaffold to develop inhibitors that are selective for HER2 over EGFR. As seen in the study by Waterson *et al.*, the nature of the aniline substituent may effect the selectivity of the inhibitor,[46] and since previous attempts at using substituents from the approved inhibitors on the thienopyrimidine scaffold has been successful, the same may be possible with the aniline substituent of tucatinib. Since tucatinib is highly selective for HER2, a thienopyrimidine with the same substituent may share the same quality. More selective HER2 inhibitors will hopefully reduce severe adverse effects, but again, possible improvements offered by such compounds compared to tucatinib are uncertain.

Research on thienopyrimidines as HER2 inhibitors is currently sparse, and from the studies that have been discussed in this thesis, there are no indications that the thienopyrimidine scaffold will provide the solution to the biggest problem in treatment of HER2 positive breast cancer, which is drug resistance.

4 Conclusion

The thienopyrimidine scaffold has been confirmed as a suitable scaffold for HER2 inhibitors, with several potent compounds being reported in literature. There has been developed both reversible and irreversible inhibitors, and several studies have focused on reusing substituents from the already approved drugs lapatinib and neratinib. This has resulted in inhibitors with potencies comparable to those of the corresponding approved inhibitors, as seen for compound **1**, **2** and **16**.

The use of either scaffold **A** or **B** determines the orientation of the substituent at position 6. Whether a derivative of scaffold **A** or **B** is most potent is therefore dependent on the particular substituent in use. The spatial orientations of the potential compounds have in some cases been compared with that of lapatinib, and the results from these studies indicate that aiming for similar spacial orientation as lapatinib leads to the most potent HER2 inhibitors.

Despite being a suitable scaffold for potent inhibitors, the potential for thienopyrimidine derivat-

ices to provide improvements of significance is uncertain. Today, the main challenge in HER2 inhibition is the development of drug resistance. While thienopyrimidine derivatives have been found to be effective towards the T790M mutation in EGFR, and it has been discussed that this scaffold may also be used to develop inhibitors that can inhibit corresponding mutations in HER2, the effect of such compounds may not be of importance in a realistic setting, due to other resistance mechanisms that allows the cell to survive without HER2.

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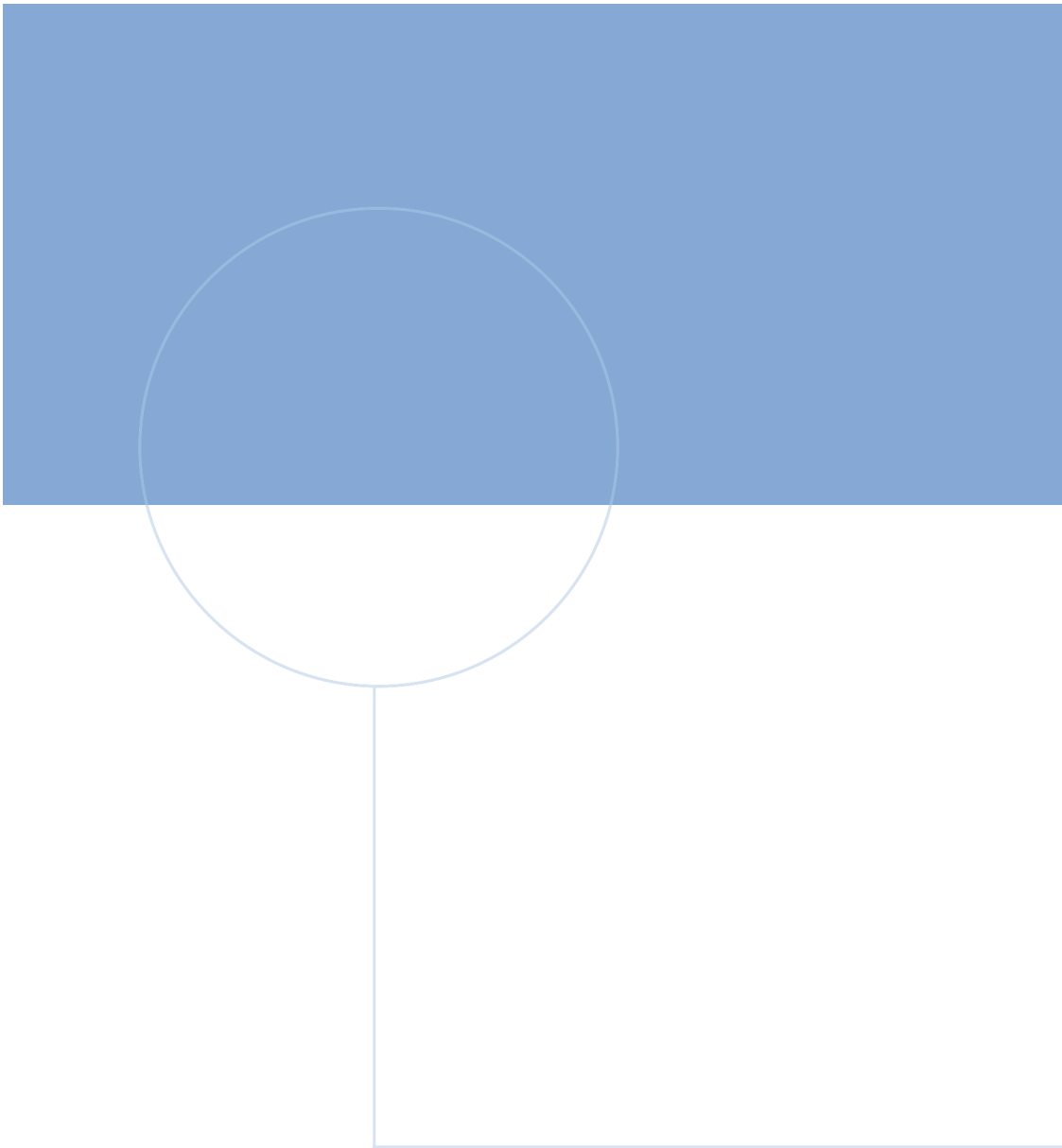

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