UNIVERSITY OF PARDUBICE
FACULTY OF CHEMICAL TECHNOLOGY
Institute of Organic Chemistry and Technology

Application of *Eschenmoser* coupling reaction in the synthesis of biologically active compounds

Annotation of the Ph.D. Degree thesis

Author: Ing. Lukáš Marek
Supervisor: prof. Ing. Jiří Hanusek, Ph.D.

Pardubice 2024

Author:

Ing. Lukáš Marek

Supervisor:

prof. Ing. Jiří Hanusek, Ph.D.

Year of the defence:

2024

Study program:

Organic Chemistry

Study field:

Organic Chemistry

Reviewers:

Ing. Petr Beier, Ph.D., Ústav organické chemie a biochemie AV ČR

doc. PharmDr. Mgr. Martin Krátký, Ph.D., Farmaceutická fakulta v Hradci Králové, Univerzita Karlova

Reference

Marek, Lukáš. Application of *Eschenmoser* coupling reaction in the synthesis of biologically active compounds. Pardubice, 2024. 280 pages. Dissertation thesis (Ph.D.). University of Pardubice, Faculty of Chemical Technology, Institute of Organic Chemistry and Technology. Supervisor prof. Ing. Jiří Hanusek, Ph.D.

Summary

This dissertation thesis explores the use of α-brominated lactams as novel substrates for the *Eschenmoser* coupling reaction (ECR). These substrates exhibit unique reactivity when combined with primary or secondary thioamides, often eliminating use of a thiophilic agent or a catalytic base. This makes the method highly suitable for the synthesis of a broad spectrum of 3-aminoacrylamide derivatives.

The first section focuses on exploring the structure-reactivity relationships, examining how modifications of both electrophilic and nucleophilic components influences the reaction. Additionally, the effects of the reaction environment, particularly solvent polarity, were analyzed, leading to important insights, which were further supported by DFT calculations.

Given the promising results obtained from the reactions of 3-bromooxindole and 4-bromoisoquinoline-1,3-dione with simple thioamides, the potential application of ECR for the synthesis of known kinase inhibitors was further explored. This approach was successfully employed in the synthesis of both experimental drugs and clinically established compounds, including *Nintedanib* and *Hesperadin*, as well as several cyclin-dependent kinase inhibitors. The overall yields of the mentioned synthetic approach are comparable to the best methods published to date.

The final section delves into the mechanism of ECR between 3-bromooxindole and thiobenzamide, utilizing advanced mass spectrometry techniques such as HRMS combined with ion mobility and IRPD ion spectroscopy. The key finding is the experimental detection of polysulfide intermediates and the elucidation of their autocatalytic role in ECR, which proceeds without the assistance of a thiophilic agent.

KEYWORDS:

Eschenmoser reaction, *Eschenmoser* sulfide contraction, α-bromoamide, α-bromolactam, thioamide, thiophile

Souhrn

Ústředním motivem této disertační práce je využití α-bromovaných laktamů coby nových substrátů pro provedení *Eschenmoserovy* reakce (ECR). Při reakcích s primárními a sekundárními thioamidy vykazuje transformace mechanismem ECR unikátní chování, ve většině případů není nutná přítomnost thiofilního činidla, ani přídavek katalyzující báze. To z ní činí metodu vhodnou pro syntézu celé řady deriátů 3-aminoakrylamidového typu.

V první části práce byly zkoumány vztahy struktura-reaktivita, přičemž byly prověřeny vlivy strukturních modifikací elektrofilní i nukleofilní komponenty. Důležité závěry byly vyvozeny i z vlivů použitého reakčního prostředí, zejm. polarity použitého rozpouštědla. Získané poznatky jsou podpořeny kvantově-chemickými výpočty.

Vzhledem k výborným výsledkům, získaných při reakcích 3-bromoxindolu a 4-bromisochinolin-1,3-dionu s jednoduchými thioamidy, byly prověřeny možnosti aplikace ECR v tomto uspořádání v syntéze známých inhibitorů kináz, a to jak experimentálních léčiv, tak látek zavedených do klinické praxe. To vedlo k úspěšnému zvládnutí syntézy *Nintedanibu* a *Hesperadinu*, dále bylo připraveno několik zástupců inhibitorů cyklin-dependentních kináz. Celkové výtěžky tohoto syntetického přísupu konkurují nejlepším z dosud publikovaných metod.

V poslední části práce byl zkoumán mechanismus ECR 3-bromoxindolu s thiobenzamidem s využitím moderních hmotnostně spektrometrických metod, zejm. HRMS ve spojení s iontovou mobilitou a iontovou spektrometrií IRPD. Klíčovým výstupem této části práce je především experimentální detekce polysirných intermediátů a objasnění jejich autokatalytické role v ECR probíhající bez přítomnosti thiofilního činidla.

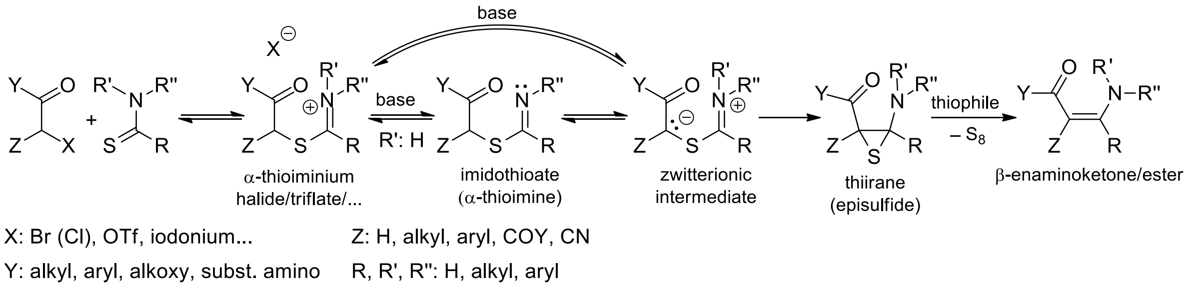
KLÍČOVÁ SLOVA

Eschenmoserova reakce, *Eschenmoserova* sulfidová kontrakce, α-bromamid, α-bromlaktam, thioamid, thiofil

Table of Contents

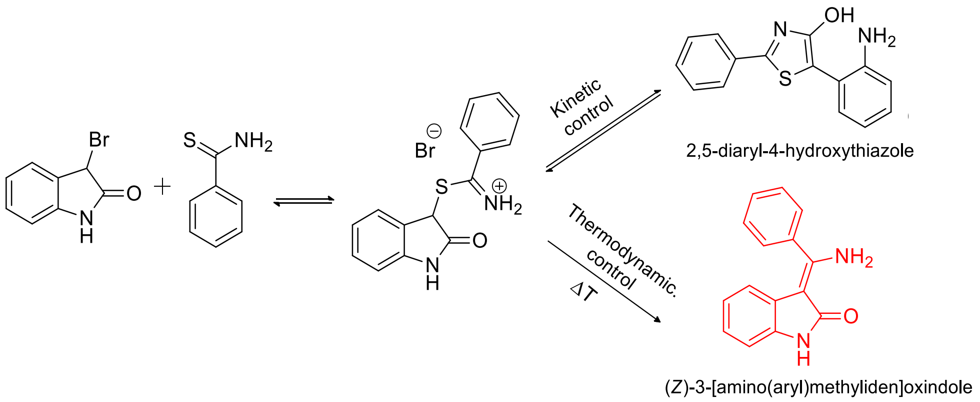
1. Introduction

Eschenmoser coupling reaction (ECR), in literature also referred to as *Eschenmoser* sulfide contraction, represents a versatile synthetic methodology used in the construction of activated double bonds. The typical setup (**Scheme 1**) consists of a carbonyl compound, bearing a leaving group in the α-position, and a thioamide.[1,2] There is an equilibrium between the initially formed α-thioiminium salt and its free base, imidothioate. This electroneutral species undergoes deprotonation at the α-position, which leads to a zwitterionic C-anion. The lone pair of electrons exerts towards the reactive iminium group, and the key intermediate, thiirane, is formed. The subsequent loss of a sulfur atom from the three-membered cycle, called extrusion, leads to the final reaction product. The final step of ECR usually requires a thiophile to promote thiirane ring opening. The reaction gained general popularity during the 1980s after successful attempts[3] in the construction of the corrin scaffold, a critical part of the total synthesis of vitamin B_{12}.



Scheme 1 *– Generally accepted mechanism of ECR.*

Previous studies conducted by our research group[4–6] revealed that the chemical transformation of α-thioiminium salt, initially formed by the reaction of 3-bromooxindole with thiobenzamide in acetonitrile, primarily produces 2,5-diaryl-4-hydroxythiazoles (kinetic product), which then transform into the thermodynamic product of the ECR upon heating (**Scheme 2**).



Scheme 2 *– Possible reaction pathways leading to thiazole or desulfurization product.*

1.1. The current state of the problem

The structural transformation leading to β-enamine derivatives described in **Scheme 2** is unique for several reasons. No previous reports, except by our group, have used α-bromoamides as substrates for ECR. Literature typically cites α-halogenated esters/nitriles and α-haloketones as suitable electrophiles. Additionally, primary thioamides are generally deemed unsuitable for ECR due to the decomposition of the α-thioiminium salt, leading to unwanted nitrile/mercaptan byproducts. Finally, the reaction occurs smoothly without a thiophilic reagent, a rare phenomenon in ECR that is not well understood.

1.2. Objectives

Although ECR has been known for decades, the concept of utilizing α-bromoamides in ECR is novel, and its reactivity is unexplored. To gain a comprehensive understanding of this topic, the main objectives were defined as follows:

- The first aim was to explore the reactivity of substituted 3-bromooxindoles with various thioamides and evaluate the influence of substitution concerning both the thiocarbonyl and nitrogen parts of the thioamide.
- Potential extension of the reaction scope by using analogous α-brominated lactams (e.g., lactam ring expansion) or even acyclic α-bromoamides as electrophiles could be beneficial in understanding structure-reactivity relationships and in future synthetic applications.
- The final objective was to investigate the reaction mechanism, as the reaction proceeds atypically without the assistance of a thiophile.

Additionally, the versatility and robustness that the reaction demonstrated while exploring the first two objectives encouraged me to attempt the reaction in synthesizing known tyrosine and cyclin-dependent kinase inhibitors involving the 3-aminoacrylamide moiety. There is a plethora of compounds containing this structural unit, which are of significant pharmaceutical interest. In particular, compounds with oxindole and isoquinoline-1,3-dione pharmacophore are either already used in clinical practice (e.g., *Nintedanib*) or in various stages of clinical trials (e.g., *Hesperadin*).

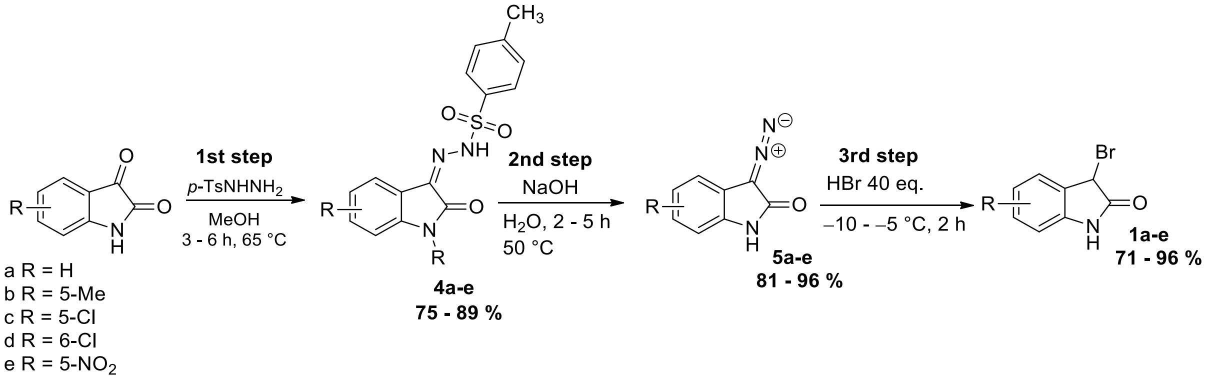
2. Results and discussion

2.1. Scope and limitations of ECR with 3-bromooxindole as electrophile

In the first part of the thesis, the versatility and robustness of ECR using various substituted 3-bromooxindoles (**1a-e**) and primary and secondary thioamides (**2a-d, 3a-j**) were probed (**Table 1, Scheme 4**). Substituents were deliberately chosen to range from strongly electron-donating (MeO) to strongly electron-withdrawing (NO_2, CF_3) to evaluate the influence on reaction yields.

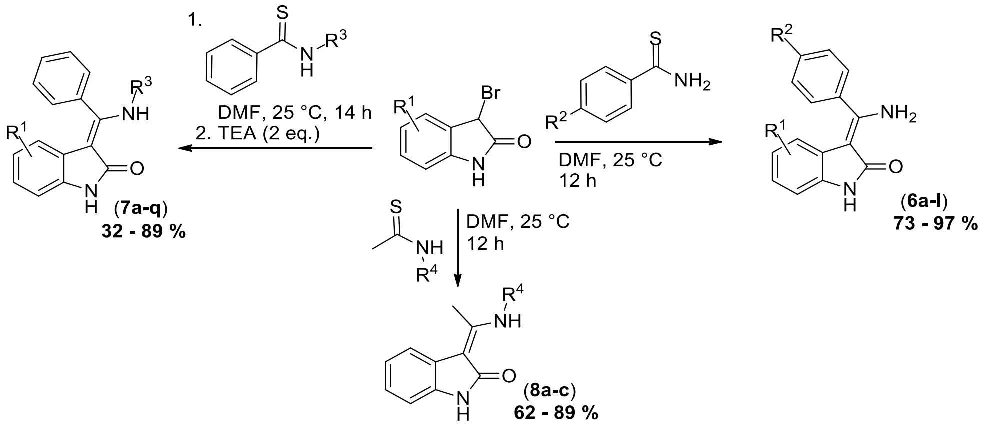
2.1.1. Synthesis of Starting Materials

Primary thioamides (**2a-d**) were synthesized by magnesium chloride-catalyzed thiolysis[7] of nitriles. Secondary thioamides (**3a-j**) were obtained via thionation of amides, which were prepared by reacting benzoyl chloride with aniline, using $Py_2P_2S_5$ as the thionating[8] agent. The method was optimized for higher yields, especially for derivatives with electron-withdrawing groups (e.g., CF_3, NO_2). 3-Bromooxindoles (**1a-e**) were synthesized[9] from isatins using a three-step process: conversion to *p*-toluenesulfonylhydrazones (**4a-e**), elimination to form 3-diazo derivatives (**5a-e**), and substitution with hydrobromic acid (**Scheme 3**).



Scheme 3 – Synthetic route leading to 3-bromoxindoles (**1a-e**).

2.1.2. Reactions with thioamides



Scheme 4 – Reaction of 3-bromoxindoles (**1a-e**) with various thioamides (**2a-d, 3a-j**).

Primary thioamide series				**Secondary thioamide series**				**Thioacetanilide series**			
Compd.	**R^1**	**R^2**	**Yield [%]**	**Compd.**	**R^1**	**R^3**	**Yield [%]**	**Compd.**	**R^1**	**R^4**	**Yield [%]**
6a	H	H	88	**7a**	H	Ph	77	**8a**	H	H	89
6b	H	OMe	82	**7b**	H	4-MeOPh	88	**8b**	H	Me	85
6c	H	Cl	93	**7c**	H	4-ClPh	48	**8c**	H	Ph	62
6d	H	CF$_3$	97	**7d**	H	4-CF$_3$Ph	43				
6e	5-Me	CF$_3$	93	**7e**	H	4-NO$_2$Ph	32				
6f	5-Cl	OMe	73	**7f**	H	Me	76				
6g	5-Cl	Cl	90	**7g**	H	CH$_3$(CH$_2$)$_4$	71				
6h	5-Cl	CF$_3$	76	**7h**	H	Bn	71				
6i	6-Cl	Cl	94	**7i**	H	cHex	64				
6j	6-Cl	CF$_3$	78	**7j**	5-Me	Ph	69				
6k	5-NO$_2$	H	79	**7k**	5-Cl	Ph	89				
6l	5-NO$_2$	Cl	78	**7l**	6-Cl	Ph	72				
				7m	5-NO$_2$	Ph	74				
				7n	5-Me	Me	66				
				7o	5-Cl	Me	71				
				7p	6-Cl	Me	84				
				7q	5-NO$_2$	Me	74				

Table 1 *– Summary of reactions of* **1a-e** *with primary (**2a-d**) and secondary (**3a-j**) thiobenzamides and thioacetanilides.*

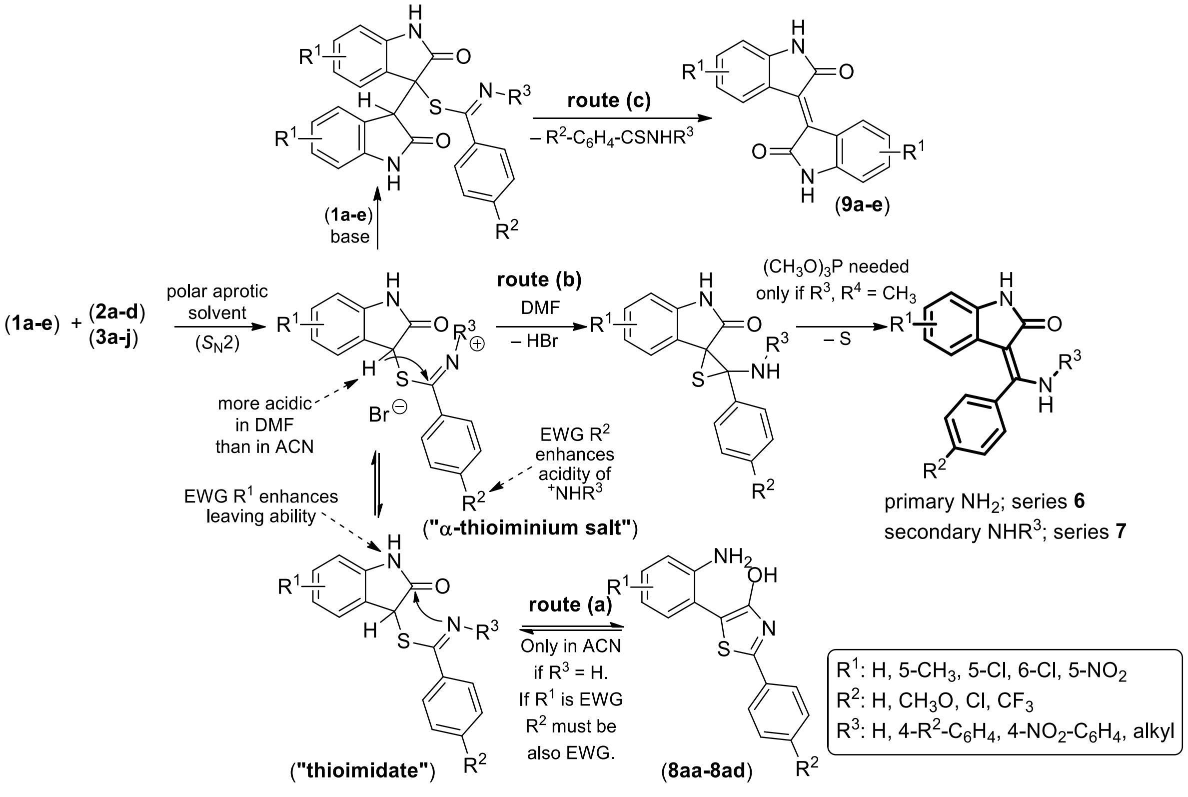
The first series examined the reaction of substituted 3-bromooxindoles (**1a-e**) with primary thiobenzamides (**2a-d**). The reaction proceeded smoothly in dry DMF at room temperature without the need for an additional base or thiophilic agent. The isolated yields of ECR products **6a-l** ranged from 73 % to 97 %. The yield was slightly better with thiobenzamides containing electron-withdrawing groups (e.g., 4-CF$_3$, 4-Cl). The presence of base (TEA) and thiophile (Ph$_3$P) or a combination of both led to a significant decrease in the reaction yield or even complete decomposition.

Reactions with secondary thioamides (**3a-j**) showed a significant influence of electronic effects from the thioamidic nitrogen substituents. For example, the highest yield (88 %) was obtained with 4'-methoxythiobenzanilide (**3b**). Conversely, reactions with electron-withdrawing substituents showed reduced yields, correlating with the decreasing electron density on the thiocarbonyl function. As demonstrated in products **7j-q**, the influence of substituents on the oxindole moiety was insignificant, and other factors, such as the solubility of the products, contributed to the ECR yield.

The reaction of 3-bromooxindole (**1a-e**) was also successfully carried out with thioacetamides as reaction partners, having both primary and secondary nitrogen atom. The reaction gave moderate to good yields of ECR products (64 – 76 %), the lowest yield in this series was obtained with thioacetanilide, which is consistent with the electron-withdrawing nature of the benzene ring.

Summary

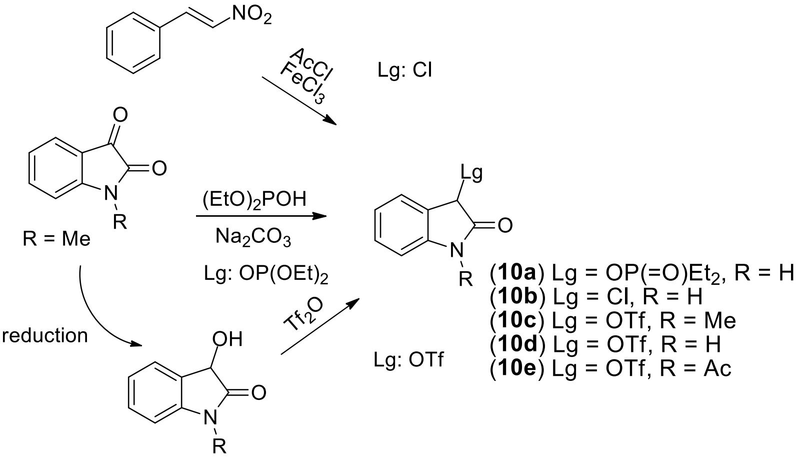
DMF was determined as the solvent of choice for the ECR of 3-bromoxindole (**1a-e**) with both primary (**2a-d**) and secondary thioamides (**3a-j**). In this solvent, the reaction proceeded easily at room temperature; no thiophilic reagent was required for the successful progress of the reaction. Considering ECR, *Hantzsch* thiazole synthesis, and isoindigo formation as main reaction pathways, the observed reactivity was explained by the influence of solvent polarity and electronic effects of the substituents on the equilibrium concentrations of key reaction intermediates, particularly the α-thioiminium salt and thioimidate (**Scheme 5**).



***Scheme 5** – Possible transformations of α-thioiminium salt derived from **1a-e**.*

2.1.3. Reactivity of oxindoles bearing alternative Lg in 3- position

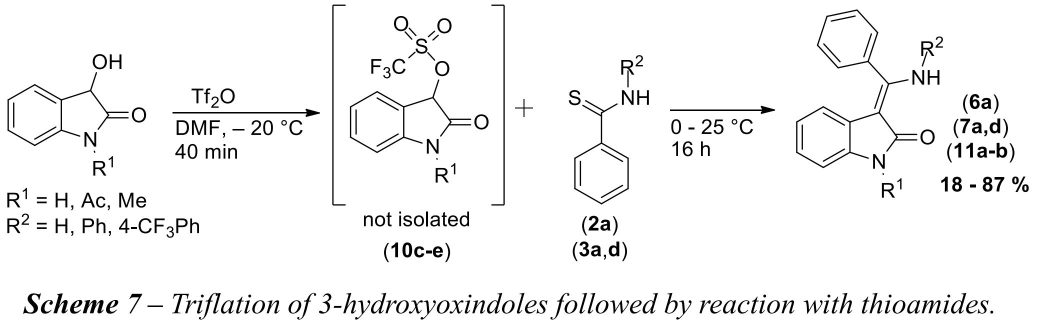
The reactivity discussed so far involves reactions where 3-bromooxindole (**1a**) acts as the electrophilic component. However, bromide is not the only suitable nucleofuge for this purpose. The α-position of the electrophilic component can be substituted with any leaving group (Lg) that has sufficient nucleofugality to allow the rapid formation of the α-thioiminium salt. At the same time, this Lg should be as non-nucleophilic as possible to avoid equilibria shift back to starting compounds. Literature sources[10] indicate that these criteria are typically met by bromide and iodide anions. Recently, however, several cases of ECR reactions using alternative leaving groups have appeared in the literature.[2,11] Therefore, I decided to investigate the reactivity of oxindoles with different leaving groups at position 3 (see **Scheme 6** below). The potential use of these alternative oxindole derivatives **10a-e** is also interesting due to their easy synthetic accessibility.



***Scheme 6** – Synthesis of oxindoles **10a-e** bearing Lg in position 3.*

The substitution of 3-bromoxindole by diethyl oxindol-3ylphosphate (**10a**) and 3-chlorooxindole (**10b**) in reactions with thiobenzamide exhibited low chemoselectivity, predominantly due to the formation of undesired isoindigo by-products. Optimization of the reaction conditions, including the use of excess electrophile and increased reaction temperature, significantly improved the yield of the ECR product, achieving 38 % for **10a** and 62 % for **10b**.

The activation of 3-hydroxyoxindoles by triflation (**Scheme 7**) encountered issues with poor selectivity due to the high reactivity of Tf_2O, leading to undesirable *N*- and *O*, *N*-triflation. Optimization of the reaction conditions resulted in only moderate yields of ECR products (18 – 59 %, **Table 2**). The use of *N*-protected starting materials **10c** and **10e** revealed that this activation method is exclusively suitable for *N*-alkylated derivatives, where yields comparable to those of **1a** were obtained.



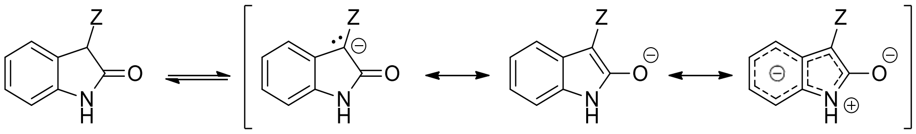
***Scheme 7** – Triflation of 3-hydroxyoxindoles followed by reaction with thioamides.*

Entry	Product	R^1	R^2	Yield [%]
1	**6a**	H	H	59
2	**7a**	H	Ph	36
3	**7d**	H	4-CF_3Ph	18
4	**11a**	Ac	Ph	23
5	**11b**	Me	H	87

Table 2 *– Summarization of experiments using O-triflated hydroxyoxindoles* ***10c-e****.*

2.2. Broadening ECR scope with α-halogenated amides

3-Bromooxindole (**1a**) represents a unique structure - its α-carbon is adjacent to both the carbonyl group of the lactam and the benzene ring, both of which contribute to the stabilization of the carbanion (**Scheme 8**), the formation of which is essential for the successful course of the ECR (see **Scheme 5**).



Scheme 8 *– Possible resonance structures of the anion derived from 3-substituted oxindole.*

To ensure the necessary acidity of the α-hydrogen, a total of seven analogous structures (**Figure 1**) were proposed to verify the general reactivity of α-bromoamides with thioamides in the context of the ECR. These structures share a common feature: retention of the phenylacetyl arrangement with bromine adjacent to the α-carbon. Additionally, attention was given to the potential for carbanion aromatization *via* the involvement of a lone pair on a different heteroatom (N → O) and the prevention of this aromatization by removing the heteroatom (N → CH_2), expanding the ring (N → N-CH_2, N-C=O), or disrupting the bond between the heteroatom and the benzene ring. In this way, six structurally close α-bromolactams/amides, one α-bromolactone and one α-bromoketone were proposed.

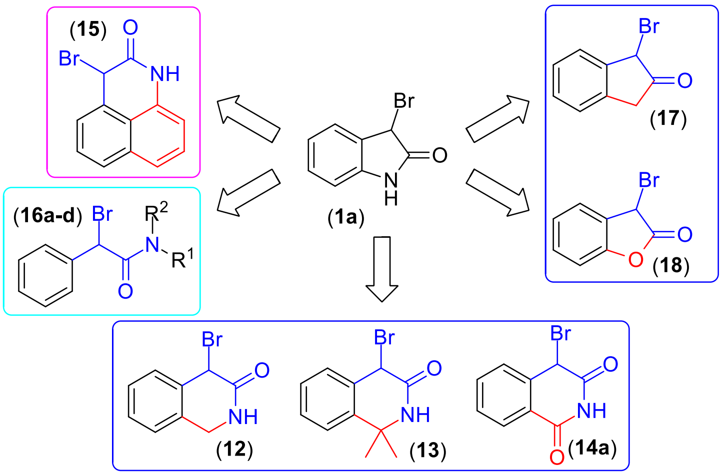
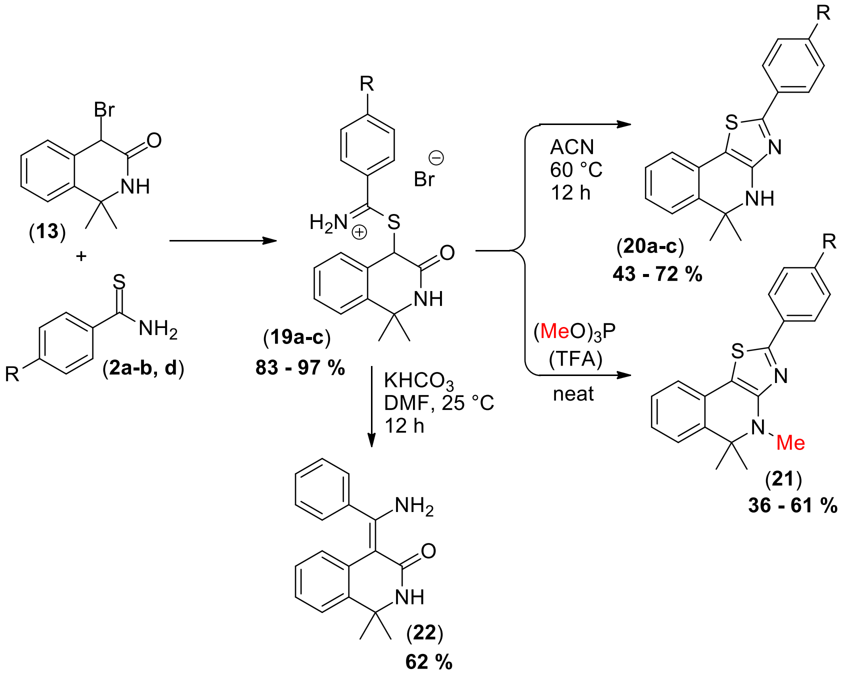


Figure 1 *– α-Bromocarbonyl compounds used in ECR scope study.*

A well-known characteristic of α-brominated amides is their high reactivity and closely associated limited stability. Attempts to synthesize compound **12** were unsuccessful. Efforts to brominate 4-dihydroisoquinolin-3(2*H*)-one failed due to preferential oxidative aromatization. An alternative approach led to the preparation of the 7-chlorinated analogue of compound **12**; however, even this compound underwent aromatization of the lactam ring.

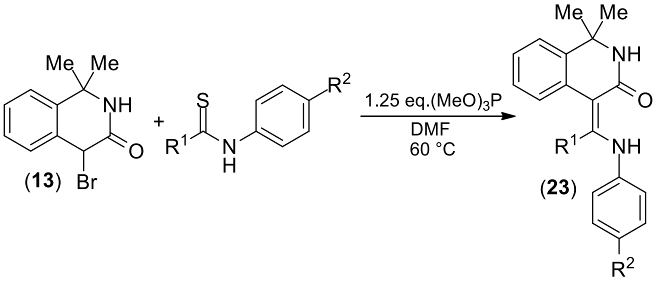
In a related 1,1-dimethyl derivative **13**, the methyl groups (R: Me) prevent such aromatization. Starting lactam was prepared by condensation of benzylcyanide with acetone in PPA at 140 °C, followed by α-bromination using NBS/*m*-PCBA system in $CHCl_3$.

This derivative (**13**) gives isolable and stable α-thioiminium salts **19a-c** by reaction with thiobenzamides (**2a**) in hot ACN. This α-thioiminium salts exhibited impressive condition-dependent reactivity (**Scheme 9**). Prolonged heating of slurry of salts **19a-c** in ACN leads to a *Hantzsch* thiazole synthesis, giving novel tricyclic thiazoles **20a-c**. The following investigation led to the finding that this reaction can be performed also in DMF at room temperature in one pot fashion, without an isolation of intermediary salt. Attempts to change the reactivity towards ECR by use of mild thiophile, trimethyl phosphite, led to *N*-methylated thiazole **21**, whose structure was elucidated using 2D NMR experiments. Finally, ECR product formation was achieved by transformation of **19a** in the presence of a base. The use of $KHCO_3$ in chlorinated solvents, recommended by *Eschenmoser*[12] led to ambiguous results. The use of the same base (3 eq.) and the substitution of the solvent with polar aprotic DMF resulted in the formation of the ECR product **22** with a 62 % yield.



***Scheme 9** – Condition-dependent transformations of salts **19a-c**.*

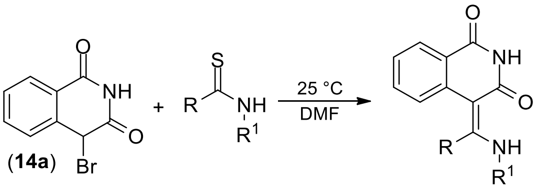
On the other hand, the reaction of secondary thioamides requires only mild thiophile for ECR (**Scheme 10**) and the corresponding aminomethylidene-1,4-dihydroisoquinolin-3(2*H*)-ones **23a-f** were obtained under optimized reaction conditions. Reaction yield (13 – 65 %) depends mainly on the substitution of the *N*-phenyl ring, same trends were observed as in the case of 3-bromoxindole (**1a**). Higher temperatures, stronger bases, and thiophiles decrease the reaction yield, or even cause complete decomposition of the starting materials.



23a: R^1: Ph; R^2: OMe (62 %)
23b: R^1: Ph; R^2: Me (50 %)
23c: R^1: Ph; R^2: H (65 %)
23d: R^1: Ph; R^2: Cl (32 %)
23e: R^1: Ph; R^2: CF_3 (13 %)
23f: R^1: Me; R^2: H (51 %)

***Scheme 10** – ECR of **13** with sec. thioamides under optimized conditions.*

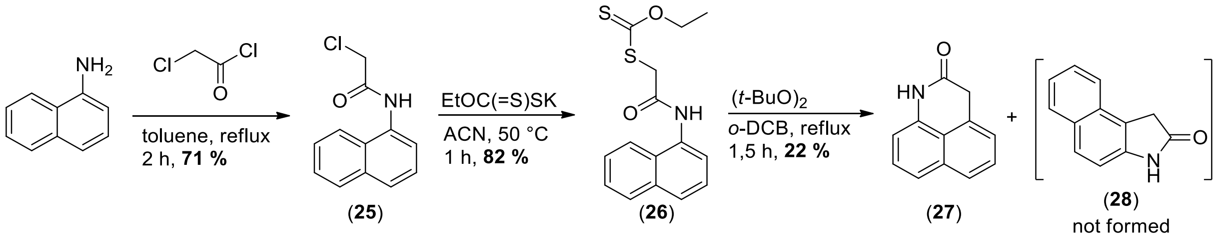
Next, I did a modification of lactam **13** by substituting the quaternary carbon with two electron-donating methyl groups with an electron-withdrawing carbonyl group (**13** → **14a**). The starting isoquinoline-1,3(2*H*,4*H*)-dione was synthesized from homophthalic acid, and subsequently brominated using NBS to yield 4-bromoisoquinoline-1,3(2*H*,4*H*)-dione (**14a**) in 65 % yield. The reaction of this brominated compound with thiobenzamide (**2a**) and thioacetamide in DMF proceeded without a formation of isolable intermediary thioiminium salt, resulting in the formation of ECR products **24a-c** (**Scheme 11**) with good yields (75 – 91%). No formation of thiazole was observed, unlike in the reactions involving compound **13**. The presence of an additional base or thiophile did not positively impact the reaction outcome.



24a: R: Ph; R^1: H (75 %)
24b: R: Me; R^1: H (78 %)
24c: R, R^1: Ph (91 %)

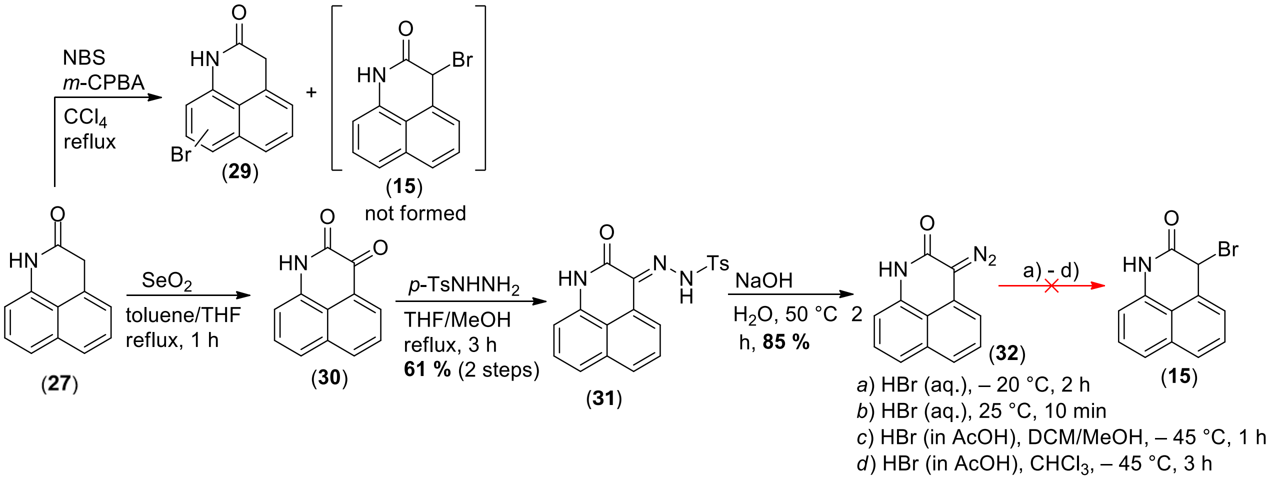
***Scheme 11** – ECR of **14** with thioamides.*

The failure of the *Schmidt* rearrangement of acenaphthenone as well as reaction pathway involving aminolysis of the intermediary lactone inspired me to develop a new method for the synthesis of amide **27**. This was achieved (**Scheme 12**) by adapting a recently reported[13] cyclization method that utilizes a radical generated from substituted xanthates.



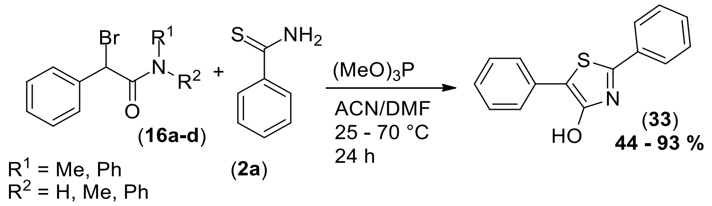
***Scheme 12** – Novel synthetic route for amide **27**.*

Attempts to brominate **27**, however, resulted in the formation of an aromatic core bromination product **29** (judged by NMR). An alternative strategy to synthesize the desired bromoderivative, including α-oxidation to an α-ketoamide **30**, condensation to the corresponding tosylhydrazone **31**, and subsequent elimination to form a diazo compound **31**, was also unsuccessful. The reaction failed at the final step, where the aim was to substitute the diazo group of **32** with a bromine atom (**Scheme 13**).



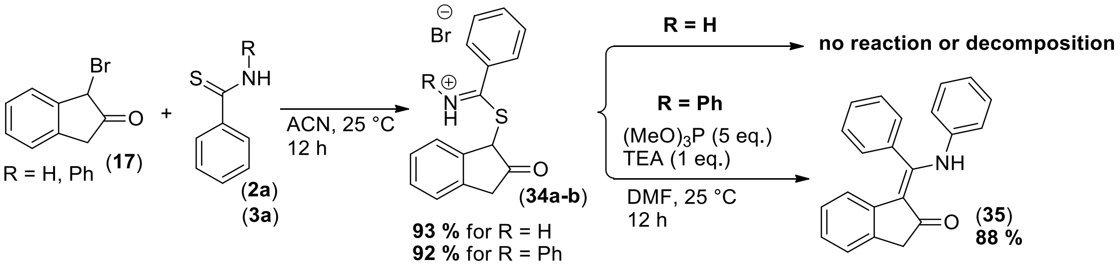
***Scheme 13** – Unsuccessful attempts for synthesizing derivative **15**.*

Further simplification of the structure of lactams **13** and **14** by breaking the Ar–N bond or removing the >C(CH$_3$)$_2$ or >C=O bridge gives acyclic α-bromo(phenyl)acetamides **16a-d**. When α-bromoamide **16a** was reacted with thiobenzamide (**2a**) in the absence of any base the only product was 2,5-diphenyl-1,3-thiazol-4-ol (**33**). This indicates efficient amide group cleavage (which gives aniline; pK_a = 4.6), after the initial closure of the thiazole ring. The addition of thiophile, trimethyl phosphite, did not shift the reaction towards the expected ECR but rather reduced the yield of compound **33**. The same reactivity was observed when *N*-Me, *N*,*N*-diMe a *N*-Me-*N*-Ph analogues of **16a** were employed, with yields ranging from 44 % to 93 % (**Scheme 14**).



*Scheme 14 – Reactions of acyclic bromoamides **16a-d** giving thiazole **33**.*

Next, the reactivity of 1-bromoindan-2-one (**17**) was investigated. The reaction of **17** with both thiobenzamide (**2a**) and thiobenzanilide (**3a**) in ACN at room temperature produced stable and isolable thioiminium salts **34a-b**, with yields of 92 % and 93 %, respectively (**Scheme 15**). Salt (**34a**) is stable in the presence of a mild base, thiophile and at elevated temperature. On the other hand, the salt **34b**, derived from thiobenzanilide (**3a**) tends to be much more reactive. Screening experiments and the following optimization revealed that transformation into ECR product **35** can take place in the presence of mixture TEA and trimethyl phosphite in high yield. This reactivity of salts **34a-b** aligns with the „normal" behavior in ECR.

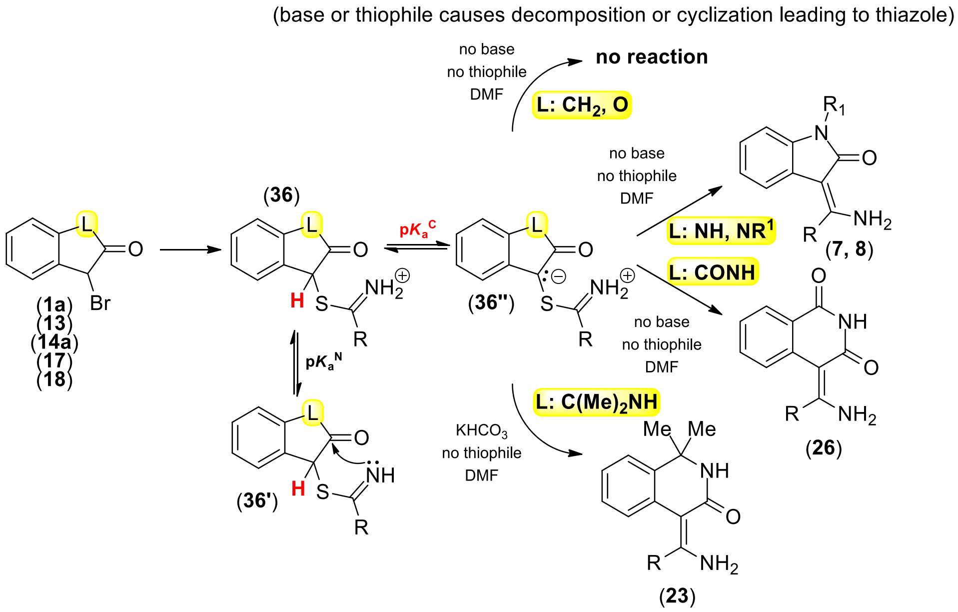


*Scheme 15 – Formation of indanone-derived salts **34a-b**.*

The reactivity of 3-bromobenzofuran-2(3H)-one (**18**) was tested with thiobenzamide (**2a**) and thiobenzanilide (**3a**) in various solvents, but no stable α-thioiminium salts were formed, leading to decomposition. A series of experiments using bases and thiophilic agents in ACN and DMF failed to induce the expected transformation via the ECR mechanism, instead resulting in complex product mixtures.

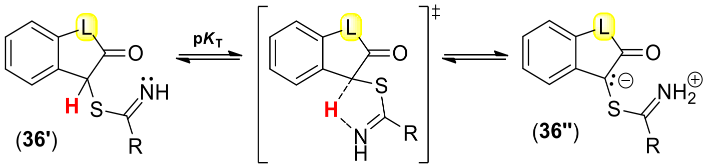
Summary

The reaction of α-bromoamides with thioamides can proceed through two primary pathways: the *Hantzsch* thiazole synthesis and the ECR. Our research group previously observed[4,6] ambivalent reactivity of 3-bromooxindole (**1a**), meaning it can form either a thiazole (kinetic control) or an ECR product (thermodynamic control), with the reaction being influenced by solvent polarity and temperature. The structure-reactivity relationships discussed thus far in the context of the ECR indicate that, unlike ketone **17** and lactone **18** (L: CH_2 and O), not only the previously described 3-bromooxindole (**1a**) but also other α-brominated benzolactams **13** and **14a** possess a unique ability to yield reaction products with primary thioamides, typically without the need for added thiophilic agents or bases (**Scheme 16**). The addition of these agents, which is usual[1,2] in most of the ECR protocols, has a detrimental effect on the reaction, either reducing the ECR yield or causing the decomposition of starting materials. The choice of the solvent is also crucial. Instead of the chlorinated solvents (DCM, $CHCl_3$) commonly recommended in the literature[14], polar aprotic solvents - especially DMF and in some cases, ACN - proved to be more effective.



Scheme 16 – Summary of ECR using α-bromoamides as starting compounds.

Comparison with the reactivity of analogous α-bromo(phenyl)acetamides **16a-d** reveals that the presence of the Ph–CHBr–CO–N moiety is not the only factor sufficient for a successful ECR. The crucial aspect is the incorporation of this fragment into a cyclic structure. The reaction of acyclic α-bromoamides **16a-d** consistently yields 2,5-diaryl-4-hydroxythiazole **33**. The success of the ECR depends on the acidity of the hydrogen in the intermediary α-thioiminium salt (general structure **36**) at both the α-carbon (pK_a^C) and the nitrogen of the thioiminium group (pK_a^N). The key species leading to the ECR is the zwitterionic intermediate **36“** (**Scheme 17**).



Scheme 17 – Formation of zwitterionic intermediate 36“ via 5-membered TS.

Dissociation of a proton from **36** leads to imidothioate **36‘**. In this species, the imino group is no longer sufficiently reactive for an internal attack by carbanion. Instead, it becomes a newly formed nucleophilic center capable of intramolecular attack on the carbonyl group (leading to thiazole formation). However, **36‘** can be efficiently converted into the zwitterionic species **36“** through an intramolecular proton transfer from carbon to nitrogen *via* a thermodynamically favorable five-membered transition state. The equilibrium between these two intermediates is quantified by the constant K_T ($pK_T = pK^C - pK^N$), with higher K_T values (lower pK^T) making transformation via ECR more favorable. The energy profile of this equilibrium, including the kinetics of its establishment, was modeled using quantum chemical methods (**Figure 2**, right side). The results correlate well with the observed reactivity.

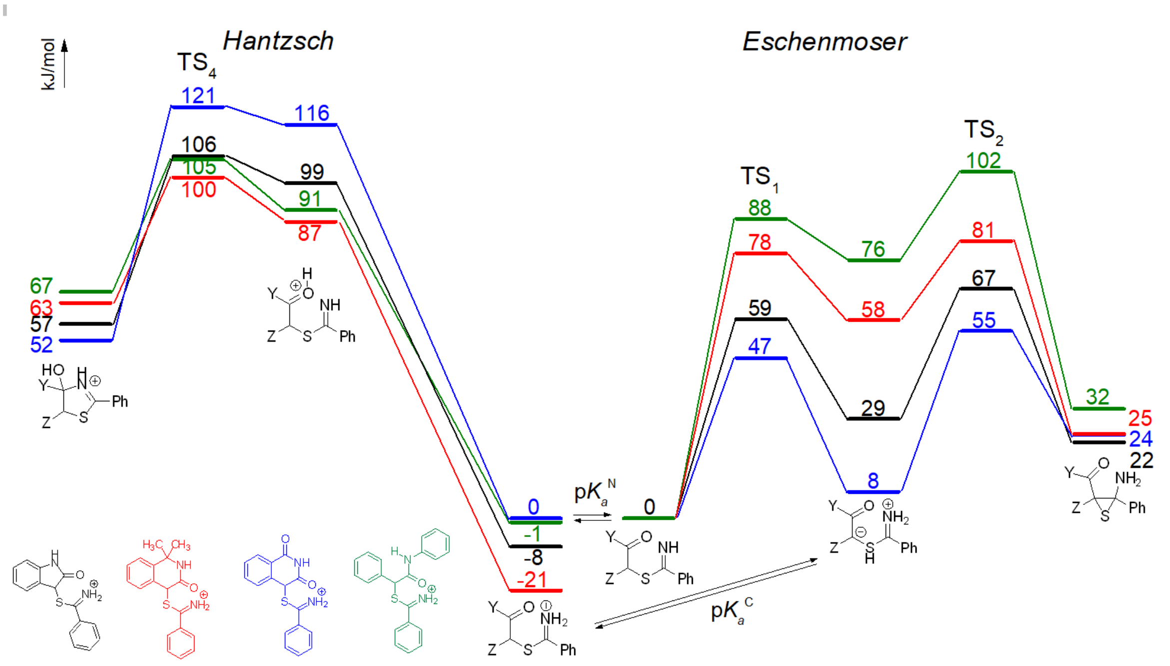
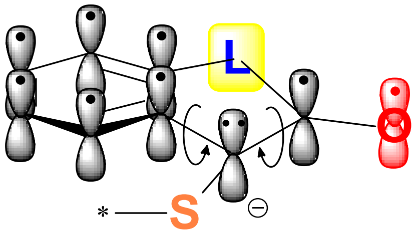


Figure 2 *- Comparison of energy profiles (relative Gibbs energies at 298 K in kJ·mol^{-1} for the ECR (right) and Hantzsch (left) pathways for salts derived from* ***1a****,* ***13****,* ***14****, and* ***16a*** *calculated at the B3LYP-D3/6-311++G(d,p) level of theory in N,N-dimethylformamide (SMD).*

The energy diagram clearly shows a trend of gradually increasing pK_T, leading to a lower equilibrium concentration of the key intermediate **36“**. ECR occurs most readily with salts derived from 4-bromoisoquinolinedione (**14a**), as the carbonyl group in the *ortho* position significantly acidifies the hydrogen on the α-carbon through both –*I* and –*M* effects. For salts derived from 3-bromooxindole (**1a**) and 1,1-dimethyl-4-bromoisoquinolinone (**13**), where the +*I* effect of the methyl groups is present, the acidity at the α-carbon gradually decreases, with the lowest acidity observed in salts derived from acyclic amides, **16a-d**.

In the case of α-thioiminium salts derived from acyclic α-bromoamides **16a-d**, lower acidity is caused by the absence of an *ortho* substituent, as well as by entropic factors and a lower degree of conjugation. Carbanions derived from cyclic lactams are planar (and rigid), with their stabilization through resonance with the adjacent ring and carbonyl being highly effective. For isoquinoline-1,3-dione, the carbonyl group at the 1-position also participates in conjugation. In contrast, for the more flexible amide carbanion (where the linker L is absent), the extent of resonance stabilization is reduced (**Figure 3**).



L: NH, NR^1, NHC(CH$_3$)$_2$, NHC=O → rigid conjugated system

L: absent → free rotation across C–C lowers degree of conjugation

Figure 3 *- Visualization of the conjugated system in zwitterion* ***36“***.

2.3. Application of ECR in the synthesis of kinase inhibitors

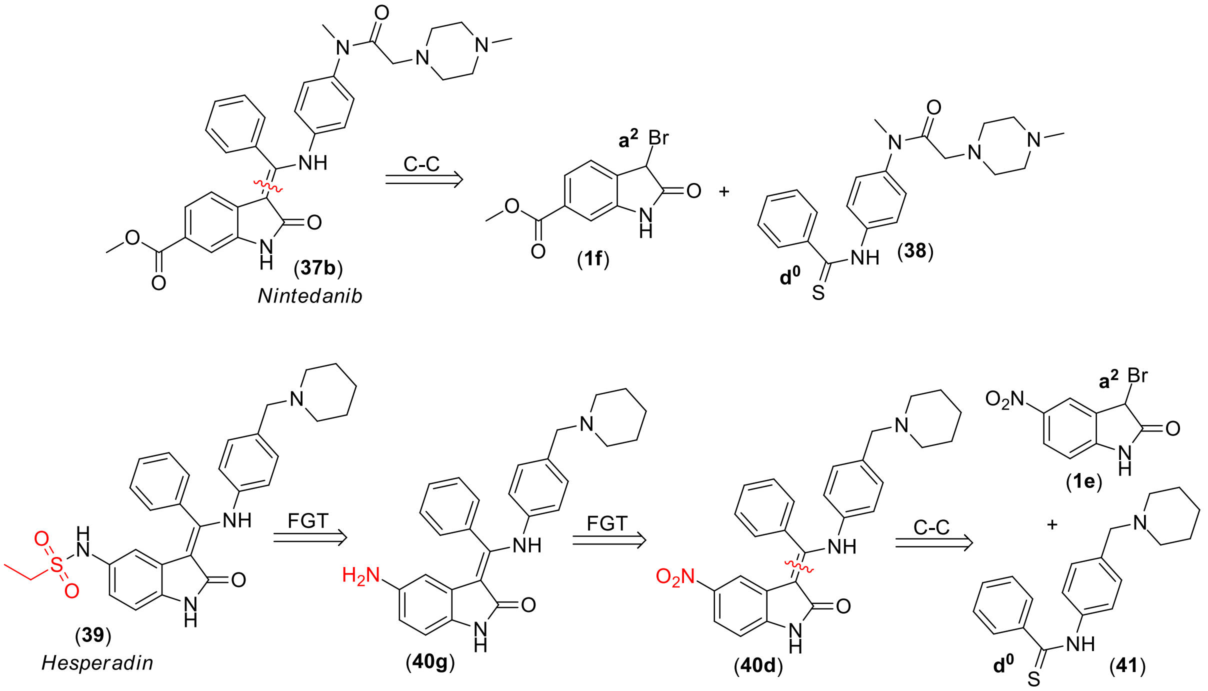
2.3.1. Synthesis of kinase inhibitors with oxindole pharmacophore

Protein kinases are crucial enzymes involved in the development of various diseases, including asthma, autoimmune disorders, cardiovascular conditions, inflammatory diseases, neurological disorders, and especially cancer. Inhibition of kinase domains is increasingly being employed as a therapeutic strategy for a wide range of the aforementioned diseases. In 2020, there were 52 US FDA-approved kinase inhibitors[15], predominantly for cancer treatment.

Notably, *Sunitinib* and *Nintedanib*, both containing a substituted 3-aminomethylidenoxindole moiety as pharmacophore, are currently used in clinical practice, with others like *Semaxanib* and *Hesperadin* in clinical trials. *Nintedanib*, marketed as *Ofev* and *Vargatef* since 2014[16], targets fibroblast growth factor receptors (FGFR 1-3) and is primarily used to treat[17] idiopathic pulmonary fibrosis. *Hesperadin*, an Aurora B kinase inhibitor, has demonstrated efficacy against various tumors.[18]

The results obtained[19] from the reactions of 3-bromoindoles (**1a-e**) with simple thiobenzanilides (**3a-j**), as described in the previous chapter, have inspired me to explore the application potential of ECR in the synthesis of structurally complex compounds of medicinal interest – *Nintedanib* and *Hesperadin*.

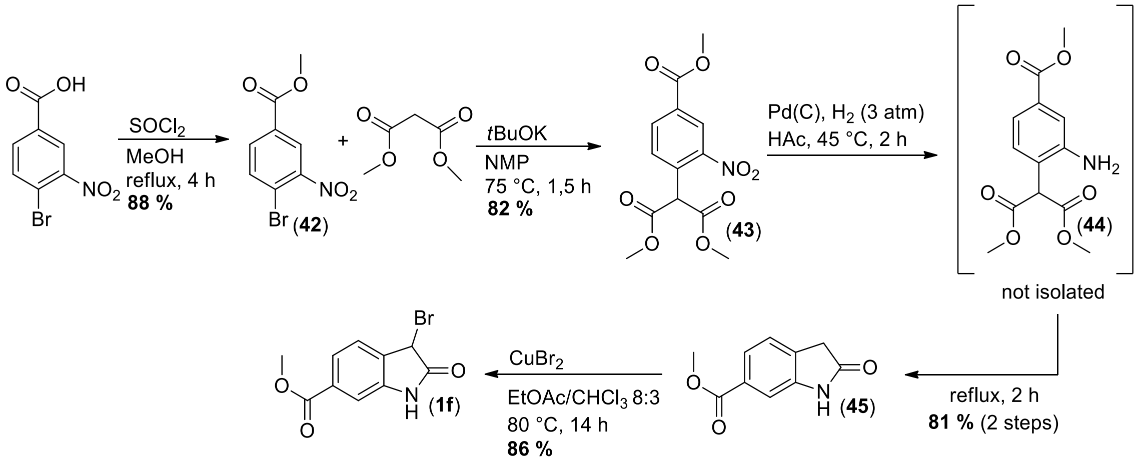
Synthesis of *Nintedanib* (**37b**) was designed with a convergent synthetic approach, with a disconnection strategy leading directly to the ECR fragments, specifically methyl 3-bromooxindole-6-carboxylate (**1f**, a^2 synthon) and a thiobenzanilide derivative (**38**, d^0 synthon). For *Hesperadin* (**39**), a strategy that involves two functional group transformations (FGTs) leading to a nitro derivative **40d** was selected, followed by a disconnection of the double bond, resulting in 5-nitro-3-bromooxindole (**1e**) and a thioamide **41** (**Scheme 18**).



***Scheme 18** – Retrosynthesis of Nintedanib (37b) and Hesperadin (39)*

Synthesis of starting compounds

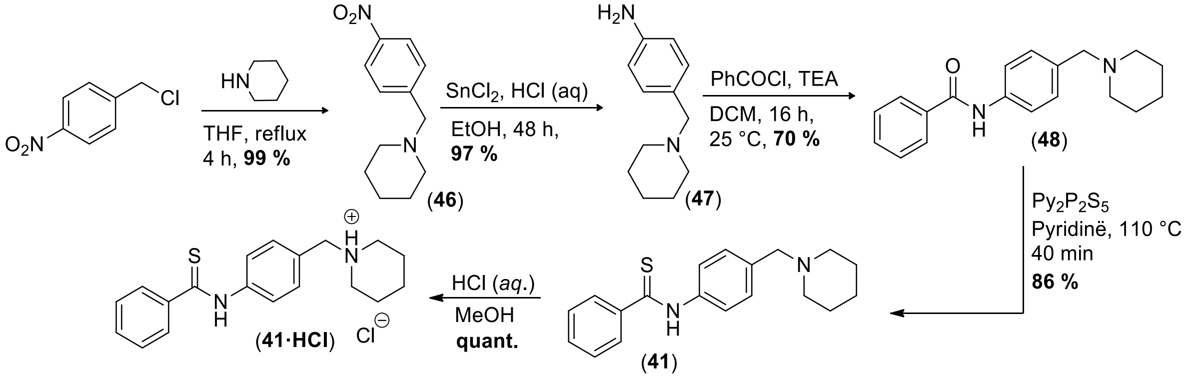
First, I turned my attention to the synthesis of starting methyl-3-bromooxindole-6-carboxylate (**1f**). Unfortunately, the reaction route used[20] for 3-bromoxindoles **1a-e** (starting from isatins) is not suitable for substrates bearing a hydrolyzable group (*e.g.* COOMe). Therefore, I designed an alternative procedure involving direct bromination of methyl oxindole-6-carboxylate (**45**), a compound readily available from 4-bromo-3-nitrocarboxylic acid. Bromination using bromine, NBS, and dioxane dibromide gave mainly di- or even polybrominated products. Optimization led to the finding that $CuBr_2$ can be used as a mild bromination agent. Reaction performed in EtOAc/$CHCl_3$ mixture gave bromoderivative **1f** in high yield after simple crystallization (**Scheme 19**).



***Scheme 19** – Reaction route leading to methyl-3-bromooxindole-6-carboxylate (**1f**).*

Having the starting 3-bromoxindoles (**1e, f**) in hand, I began working on the synthesis of key thioamides **38** and **41**.

For the **41**, the chosen synthetic route involved the thionation of amide **48** (**Scheme 20**), which can be readily obtained from 4-nitrobenzyl chloride. The thionation process was optimized (**Table 3**), initially employing P_4S_{10} in pyridine. In contrast to the total conversion of the starting **48**, the chemoselectivity was unsatisfactory, resulting only in a moderate isolated yield of **41**. The addition of a base[21] (Na_2CO_3) led to decomposition. Attempts using *Lawesson*'s reagent also failed to achieve satisfactory thionation due to the low chemoselectivity. An independently prepared[8] pyridine-phosphorus sulfide adduct ($Py_2P_2S_5$) in ACN reacted very slowly with the amide **48**; however, when pyridine was used as the solvent, the conversion of **48** was rapid and highly selective, yielding thioamide **41** with an isolated yield of 86%. For reasons discussed later, this compound was converted to its hydrochloride salt (**41·HCl**) with a quantitative yield.

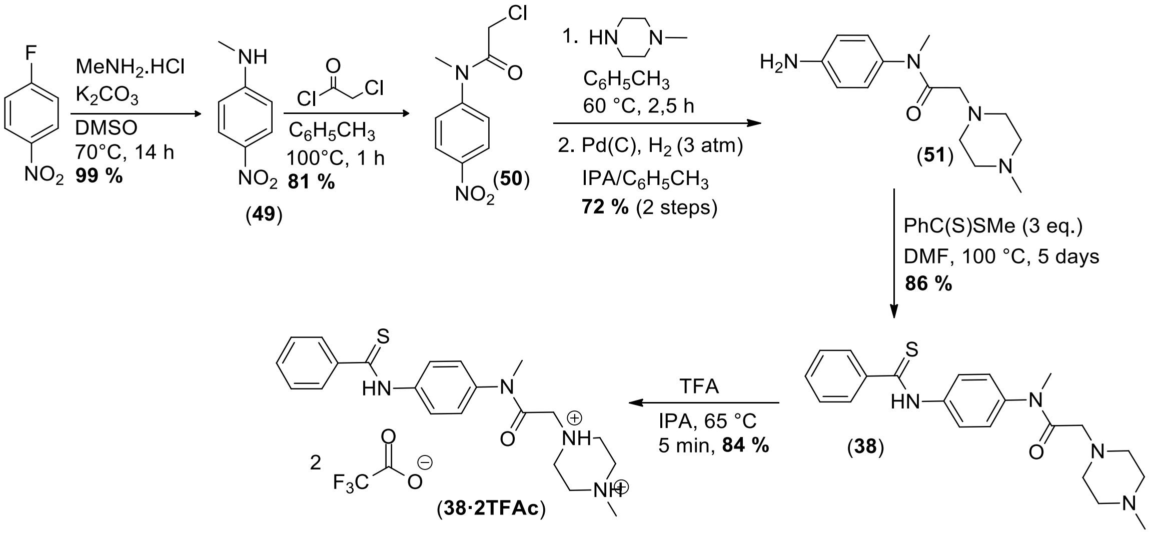


***Scheme 20** – Synthesis of thioamide **41**.*

Entry	Reagent	Eq.	Solvent	*t* [h]	Conversion 48 [%] (GC)	Assay of 41 [%] (GC)	Yield [%]
1	P_4S_{10}	1	C_6H_5N	1	quant.	75	62
2	P_4S_{10}/Na_2CO_3	0,5	THF	5	quant.	0	/
3	*Lawesson.* r.	0,5	$C_6H_5CH_3$	6	64	58	/
4	*Lawesson.* r.	0,5	$C_6H_5CH_3$	30	97	72	51
5	$Py_2P_2S_5$	0,5	ACN	14	15	10	/
6	$Py_2P_2S_5$	0,5	ACN	60	27	12	/
7	$Py_2P_2S_5$	0,5	C_6H_5N	0,75	quant.	95	86

***Table 3** – Reagent scope for thionation of amide **48**.*

Compared to **41**, the synthesis of thioamide **38** was more challenging. This compound cannot be prepared by thionation of the corresponding amide, as it would contain two amidic groups. For this reason, I decided to utilize the thioacylation of aniline **51** as a key reaction step (**Scheme 21**). Synthesis of starting aniline was adapted from patent literature, synthesis of **51** is referred to in many sources, as it serves as an intermediate in the original synthesis of *Nintedanib* (**37**).



***Scheme 21** – Synthesis of thioamide **38**.*

The reagents for the thioacylation of aniline **51** were selected based on their synthetic accessibility and reactivity towards arylamines. To avoid the use of unstable thiobenzoyl chloride[22] and dithiobenzoic acid[23], I made attempts to use methyl dithiobenzoate (**52a**), *S*-benzothiazol-2-yl dithiobenzoate (**52b**), redox system utilizing dibenzyldisulfide (**52c**)/DMSO/iodine, and dithiobenzoyldisulfide (**52d**) for this purpose (**Table 4**).

Although the literature reports claim that dithiobenzoate **52a** can thioacylate more nucleophillic alkylamines under DMAP[24] and TfOH[25] catalysis, aniline **51** was unreactive under these conditions. No reaction was observed when using *Lewis* acids in aprotic solvents. In highly polar DMF at elevated temperatures, a 55 % conversion of **51** was achieved with 1 equivalent of the reagent **52a**. When the excess of **52a** was used (2 eq.), a quantitative conversion of **51** was observed, and thioamide **38** was isolated in an excellent yield of 86%. Further increasing the excess of the reagent had no additional positive effect on the reaction. In the more polar solvent DMSO, issues with chemoselectivity were encountered. The *S*-benzothiazolyl analogue **52b** demonstrated the ability to achieve results similar to **52a** (1,5 eq. in DCM/DMF mixture[26]) but in a shorter reaction time and under milder conditions. Attempts to use the **52c**/DMSO/iodine system resulted in complete decomposition of the starting material, as did the use[27] of **52d** in DMSO.

Entry	Reagent	Eq.	Catalyst (mol%)	Solvent	t_R [h]	T [°C]	Conversion 51 (^{1}H NMR)	Assay 38 [%] (^{1}H NMR)	Yield [%]
1	**53a**	1	DMAP (10)	DCM	70	25	<5	n.d.	/
2	**53a**	1	CF_3SO_3H (5)	MeOH	60	25	<5	n.d.	/
3	**53a**	1	$ZnCl_2$ (20)	$C_6H_5CH_3$	24	80	<5	n.d.	/
4	**53a**	1	$La(OTf)_3$ (5)	$CHCl_3$	72	60	<5	n.d.	/
5	**53a**	1	/	DMF	60	100	55	50	/
6	**53a**	2	/	DMF	60	100	quant.	>95	86
7	**53a**	4	/	DMF	40	100	quant.	>95	81
8	**53a**	1	/	DMSO	60	100	50	35	/
9	**53a**	1	DBU (100)	DMSO	60	100	60	40	/
10	**53a**	1	DBU (100)	DMF	60	100	20	15	/
11	**53b**	1,5	/	DCM/DMF	12	60	quant.	>95	86
12	**53c**	0,5	I_2 (10)	DMF	24	100	>95	n.d.	/
13	**53d**	1	/	DMSO	24	100	>95	n.d.	/

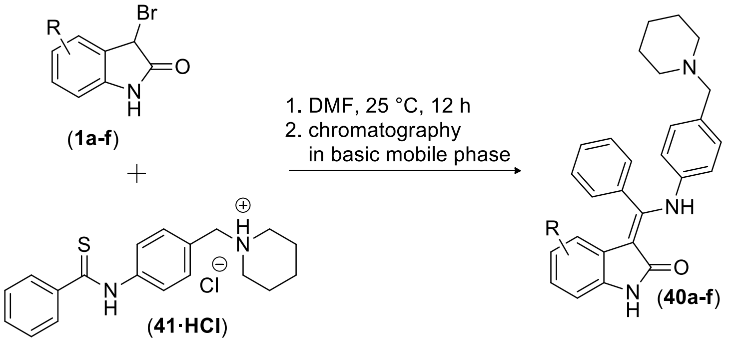
Table 4 *– Reagent scope for thioacylation of aniline* ***51****.*

Given the superior availability of dithiobenzoate **52a**, it was selected for upscaling. The reaction, conducted under optimized conditions on a 15 mmol scale, yielded **38** with 86 % of the theoretical yield. For reasons discussed in the following chapter, the compound was converted to a stoichiometric bis(trifluoracetate) (**38·2TFAc**), which is well soluble in DMF.

ECR of thioamides 38 and 41

Reaction of compound **1a** with thioamide **41** in DMF at room temperature failed to yield ECR product **40a**, giving isoindigo preferentially. Thioamide **41** was recovered in quantitative yield. This suggests that the preferred reaction pathway is the base-assisted transformation of **1a** to **9**. To suppress the basicity of the piperazine and piperidine moieties in thioamides **38** and **41**, they were converted into their salts (**38·2TFAc** and **41·HCl**), as discussed previously.

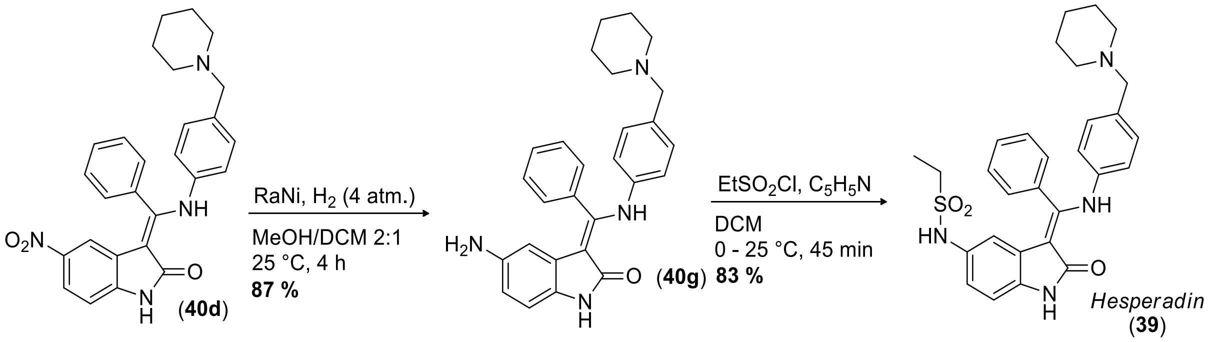
Using **41·HCl**, the ECR proceeded smoothly in DMF at 25 °C with various substituted 3-bromooxindoles (**1a-f**), leading to the formation of products **40a-f** in yields ranging from 76 to 97 % (**Scheme 22** and **Table 5**). The highest yield was achieved for the unsubstituted derivative **40a** (97 %). No clear trend was observed in the influence of electronic effects on the reaction yield.



Compd.	R	Yield [%]
40a	H	97
40b	5-Me	71
40c	5-Br	83
40d	5-NO_2	76
40e	6-Cl	93
40f	6-COOMe	80

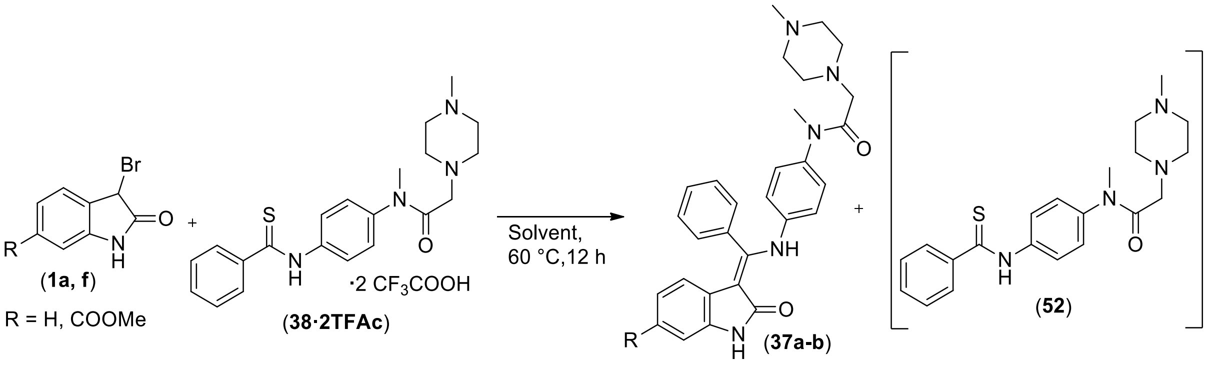
***Scheme 22 and Table 5** – ECR of **41·HCl** and **1a-f**.*

The derivative **40d** was utilized as a key intermediate in the synthesis of *Hesperadin* (**39**) (**Scheme 23**). The transformation was inspired by patent literature[28] and involved the reduction of the nitro group using hydrogen under *Raney* nickel catalysis followed by the esylation of resulting aniline **40g**, conducted at low temperature to prevent unwanted esylation of the oxindole nitrogen. *Hesperadin* (**39**) was obtained in 72 % yield (over two steps).



***Scheme 23** – Synthesis of Hesperadin (39) from **40d**.*

After the successful synthesis of *Hesperadin*, attention was turned to the use of **38·2TFAc** for the synthesis of *Nintedanib* (**37b**). Unfortunately, the reaction of **1f** with **38·2TFAc** failed to produce the desired chemically pure ECR product **37b**. Although the formation of ECR product **37b** was confirmed by ESI-MS, all attempts to separate it from the residual starting material **38** by column chromatography or crystallization were unsuccessful, probably due to the very close polarity of **37b** and **38**. Subsequent exploration of the solvent scope (**Scheme 24**) revealed that the ECR of **38·2TFAc** with **1a** proceeds in polar aprotic solvents, except for DMSO (**Table 6**). However, only in ACN, it was possible to achieve a quantitative conversion of **38·2TFAc**.



***Scheme 24** – Solvent scope for the reaction of **1a, f** with **38·2TFAc**.*

Similar results were obtained for the reaction of **1f** with **38·2TFAc** with the exception that in the reaction conducted without an inert atmosphere, the formation of a thioamide **38·2TFAc** desulfurization product **52** was observed. This side reaction was effectively suppressed by performing the reaction under an inert atmosphere and in a degassed solvent.

Entry	R	Solvent	Crude reaction mixture comp. (ESI-MS [%]) Thioamide 38	Amide 52	ECR prod. 37a/37b	Isolated yield [%]
1	H	Acetone	9	2	89	67
2	H	ACN	n.d.	3	97	74
3	H	DMSO	4	75	21	/
4	H	DMF	16	0	84	/
5	H	THF	27	1	72	/
6	COOMe	ACN	2	16	82	74
7	COOMe	ACN*	0	7	93	85
8	COOMe	DMF	29	2	69	/
9	COOMe	DMSO	2	86	13	/

*degassed ACN was used and reaction was performed under inert atmosphere

***Table 6** – ECR of thioamide **38** with **1a, f**.*

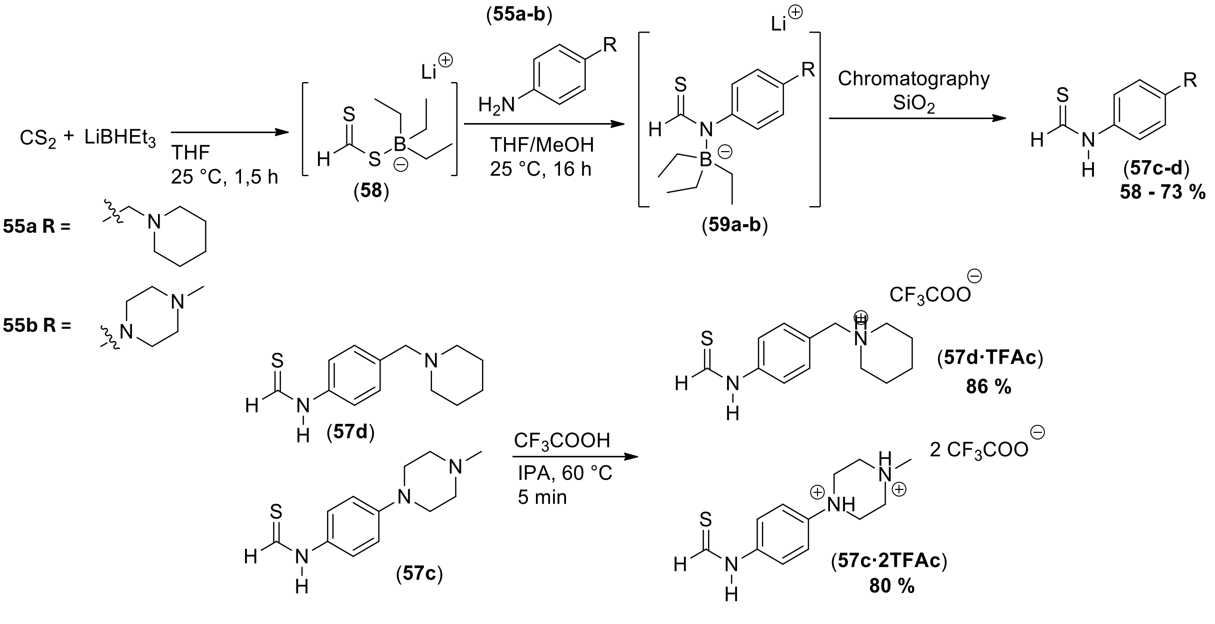
The application of the optimized conditions on a gram scale (using 2.5 mmol of **38·2TFAc**) resulted in the successful synthesis of *Nintedanib* (**37b**) with an 81% yield.

2.3.2. Synthesis of kinase inhibitors with isoquinoline-1,3-dione pharmacophore

Ongoing research has revealed that 4-bromoisoquinoline-1,3-dione (**14a**), whose reactivity was discussed in the previous section, and its 6-bromo analogue **14b**, can also react with thioformanilide (**57a**) in the manner of ECR. This finding encouraged me to apply this reaction in the synthesis of experimental inhibitors of cyclin-dependent kinases (CDK4) **53**[29,30] and their advanced precursors. Typically, such compounds are prepared[36] by condensing isoquinoline-1,3-dione with orthoformates, followed by vinyl nucleophilic substitution using arylamines.

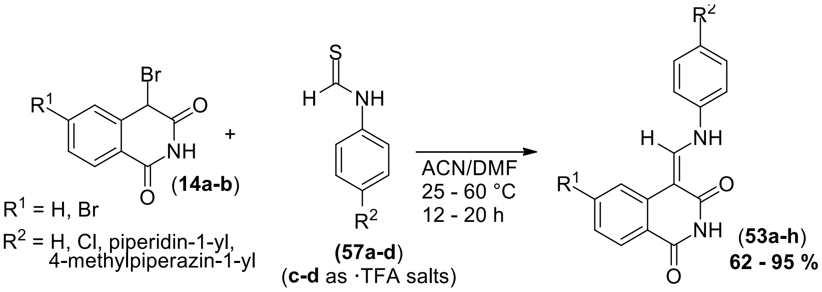
To conduct the ECR, it became necessary to deal with the synthesis of thioformanilide derivatives **57c-d**. However, the first attempts to utilize corresponding formanilides failed in the stage of amide carbonyl thionation. Consequently, alternative approaches to synthesizing the target compounds **57c-d** *via* direct thioformylation of the corresponding arylamines were investigated. Unfortunately, anilines **55a-b** proved unreactive towards *in situ* generated[33] methyl dithioformate, even at elevated temperature.

Literature reports[34] claim that salts of dithioformic acid are capable of thioformylate various arylamines inspired me to develop a new thioformylation method, where the thioacylating species **58** is generated from carbon disulfide and Superhydride® ($LiEt_3BH$), with the entire reaction carried out in a one-pot setup. Formed complexes **59a-b** decomposed quantitatively during chromatography to desired products,. Using this method, key thioformanilides **57c-d** were obtained in yields of 73 % (**57c**) and 58 % (**57d**). These were subsequently converted into trifluoroacetate salts **57c·2TFAc** and **57d·TFAc** in yields of 86 % or 80 % respectively (**Scheme 25**).



Scheme 25 – Synthesis of key thioformanilides ***57c-d*** *utilizing novel thioformylation protocol.*

Building on my previous findings, I opted to use polar aprotic solvents, MeCN, and DMF for the ECR (**Scheme 26**). Optimization revealed that for simple thioformanilides, DMF is the preferred solvent, with a 20 mol% excess of **14a** used to ensure complete consumption of starting thioformanilide. These conditions were adopted for the following reactions, except those involving thioformanilides bearing piperidine and piperazine substituents (**57c-d, Table** 7).



***Scheme 26** – ECR of **14a, b** with thioformanilides **57a-d**.*

The trifluoroacetate salt **57d·TFAc** reacts slowly with **14a** in DMF, and despite the occurrence of the expected ECR (as indicated by MS), it was impossible to obtain pure **53c** through column chromatography due to harsh separation from the starting **57d**. For thioformanilides containing basic nitrogen (**57c·2TFAc** and **57d·TFAc**), better results were achieved in hot ACN (at 60 °C), with yields ranging from 43 % to 93 %.

Compd.	R^1	R^2	Solvent	T [°C]	Yield [%] (lit. [%])[a]
53a	H	H	DMF	25	86 (92[32])
53b	H	Cl	DMF	25	73 (79[32])
53c	H	piperidin-1-ylmethyl	DMF	25	chrom. unsep.
53c	H	piperidin-1-ylmethyl	ACN	60	93 (51[35])
53d	H	4-methylpiperazin-1-yl	ACN	60	76 (90[35])
53e	Br	H	DMF	25	69
53f	Br	Cl	DMF	25	62
53g	Br	piperidin-1-ylmethyl	ACN	60	79 (63[35])
53h	Br	4-methylpiperazin-1-yl	ACN	60	95 (55[35])

[a] yield of condensation reaction using 4-(methoxymethylidene)isoquinolin-1,3(2*H*,4*H*)-dione and corresponding amines[32,35]

***Table** 7 – ECR of **14a-b** with thioformanilides **57a-d**.*

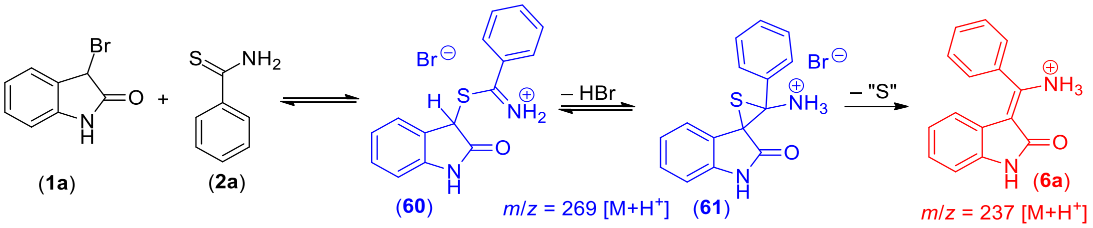
The most successful results were obtained with compounds **53c** and **53h**, which were previously identified as potent CDK4 inhibitors (IC_{50} = 3.3 and 1.4 µM, respectively). The earlier methods for synthesizing these compounds were significantly less efficient, yielding only 51 %[32] and 55 %[35], respectively. Additionally, this work represents the first report on the use of primary thioformanilides as the nucleophilic component in the ECR.

2.4. Exploring reaction mechanism using advanced MS techniques

The widely accepted^{1,2} general mechanism of the ECR involves two distinct reaction steps. In the first step, an α-thioiminium salt **60** is formed, which subsequently undergoes transformation into the final product *via* thiirane intermediate **61** (**Scheme 27**).

In addition to the previously discussed deprotonation of the α-carbon of the intermediary α-thioiminium salt **61**, the step involving sulfur extrusion is also crucial to the mechanism. Our research group previously discovered$^{4-6}$ that in the case of the reaction of **1a** with **2a**, sulfur extrusion occurs atypically without the assistance of an external thiophile, and as my observations suggest, this is also the case for other α-bromolactams (**13, 14a-b**).

The existing literature agrees that a key intermediate in the sulfur extrusion step is a thiirane intermediate. However, its existence has never been experimentally confirmed and is supported only by indirect evidence.$^{36-39}$ Therefore, I attempted to elucidate a mechanism involving sulfur extrusion from salt **60**, which is not mediated by an external thiophile, using advanced mass spectrometric instrumentation and methods.



***Scheme 27** – Proposed mechanism of ECR.*

Intermediates **60** and **61** are isomers and thus cannot be distinguished by "classical" ESI. Mobility spectrometry (IMS), which separates ions based on size and shape^{40}, can be used for this purpose. The mobilogram of the intermediate ions reveals the presence of two isomers with distinct inverse mobilities (1/K_0) (**Figure 4a**). Real-time monitoring of the reaction revealed significantly different kinetics of these distinct species (**Figure 4b**).

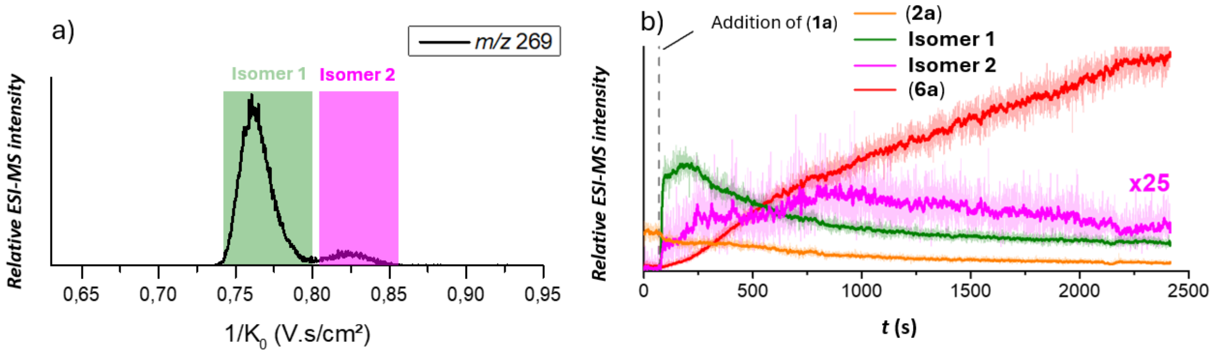


***Figure 4** – **a)** Ion mobility separation of two isomeric structures with m/z = 279; ions detected at inverse mobilities 0.74-0.78 V·s/cm² **b)** time evolution of the intensities of the individual isomers and the product **6a**. The relative intensity of **Isomer 2** was multiplied by 25.*

Subsequent CID experiments did not clarify the structure of these species due to their nearly identical fragmentation patterns. The structure of the major isomer was assigned using ion spectroscopy, namely cryogenic infrared photodissociation (IRPD)[41], with flow chemistry employed to generate the signal of reactive species at maximum intensity. The experimental IRPD spectrum best matches with theoretical spectrum of α-thioiminium salt.

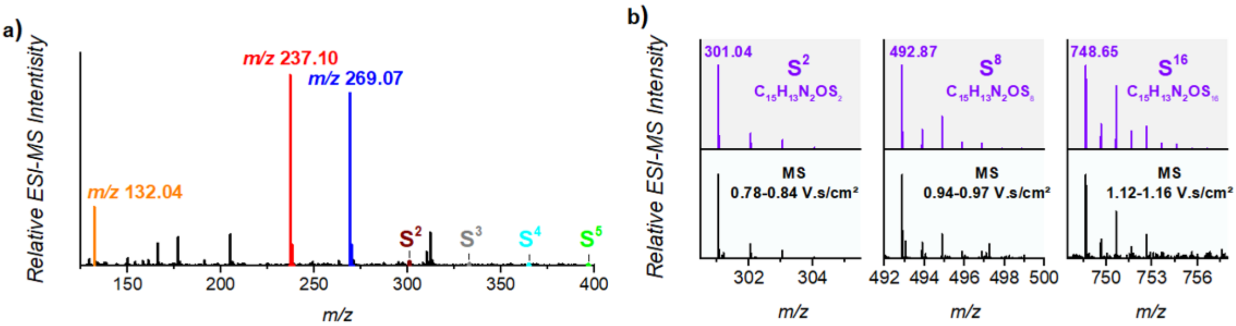
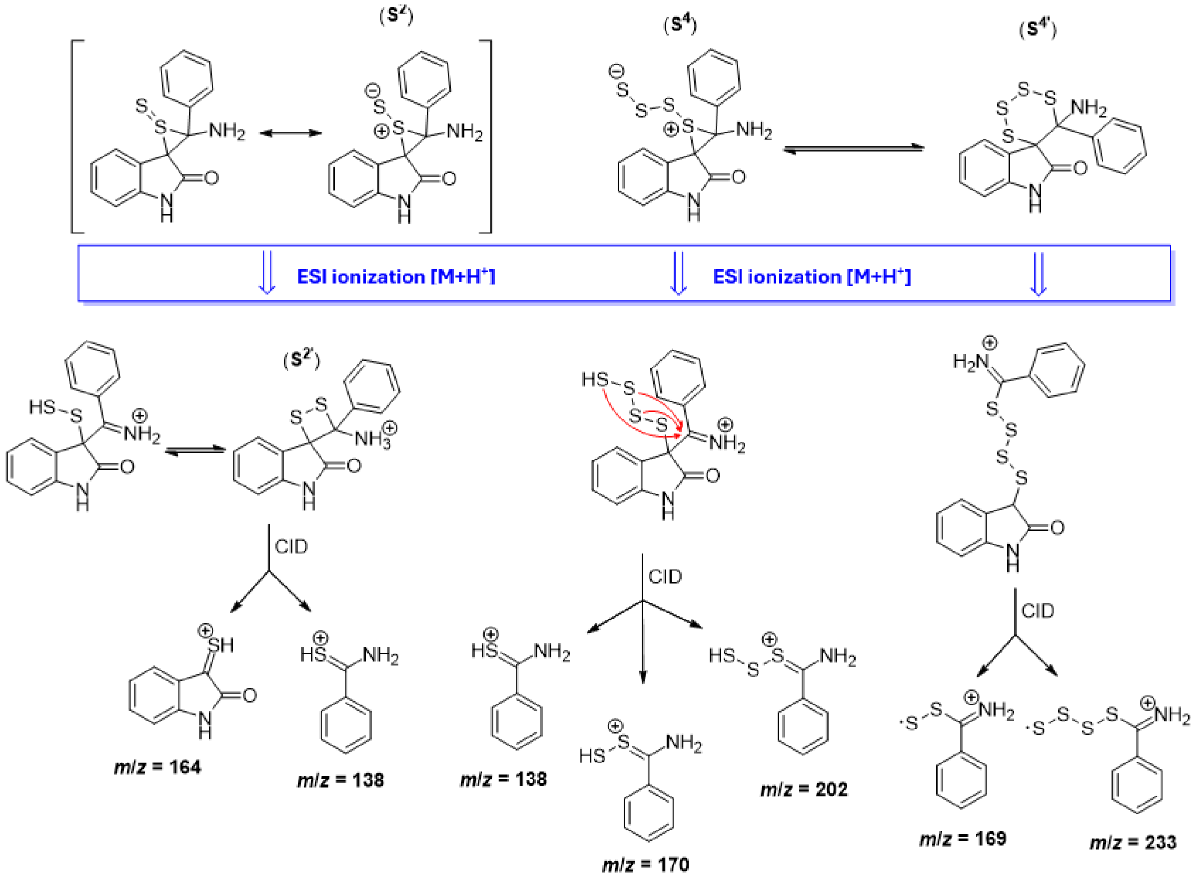


Figure 5 *– **a)** Representative source spectrum of the reaction between **1a** and **2a** (5 mM/25 mM) in DMF, with visualization of key intermediates **b)** Theoretical (top) and experimental (bottom) isotopic patterns of selected compounds with the empirical formula* $C_{15}H_{13}N_2OS_n$ *(n = 2, 8, 16).*



Scheme 28 *– Fragmentation mechanism concerning breaking of C-S bong in polysulfidic species* S^n.

Among already discussed species, additional intermediates crucial to the reaction mechanism were detected using sensitive ESI-MS instrumentation (**Figure 5a**). The composition indicates the retention of all atoms as in **60**/**61** while the sulfur content increases progressively (up to 19 sulfur atoms). This composition was further confirmed based on their exact masses using HRMS and the match between experimental isotopic patterns and the calculated spectra (**Figure 5b**).

The structure of the $\mathbf{S^n}$ intermediates was further elucidated by CID experiments. Each $\mathbf{S^n}$ intermediate loses thiooxindole or its *S*-thioanalogue, fragmenting into thiobenzamide and thiobenzanilide-*S*-sulfides. These fragments retain varying numbers of sulfur atoms on both the thiooxindole and thiobenzamide moieties. This fragmentation behavior can be attributed to the formation of a thiirane-*S*-sulfide ($\mathbf{S^2}$), which is likely protonated during ESI, resulting in open structures (**see Scheme 28** above). Upon collisional activation, a nucleophilic attack by one of the sulfur atoms on the iminium group occurs. For the $\mathbf{S^2}$ intermediate, the nucleophilic attack exclusively involves the terminal sulfur atom, leading to the cleavage of the S–S bond and the formation of protonated thiobenzamide and 3-thioxo-2-oxindole. Intermediates containing more sulfur atoms fragment in a similar manner, with any sulfur atom—except the one bound to the oxindole—being capable of initiating the fragmentation.

Another valuable insight into the reaction mechanism brought the study of the distribution of reactive intermediates **60/61**, $\mathbf{S^n}$, and product **6a** as a function of the concentration of the reacting components **1a** and **2a**, conducted using a flow chemistry. The flow setup comprised five syringe pumps (**Figure 6a**), flow rates of the syringe pumps containing 3-bromoxindole (**1a**), thiobenzamide (**2a**) and DMF were programmed to maintain the 1:1 ratio between the two reactants.

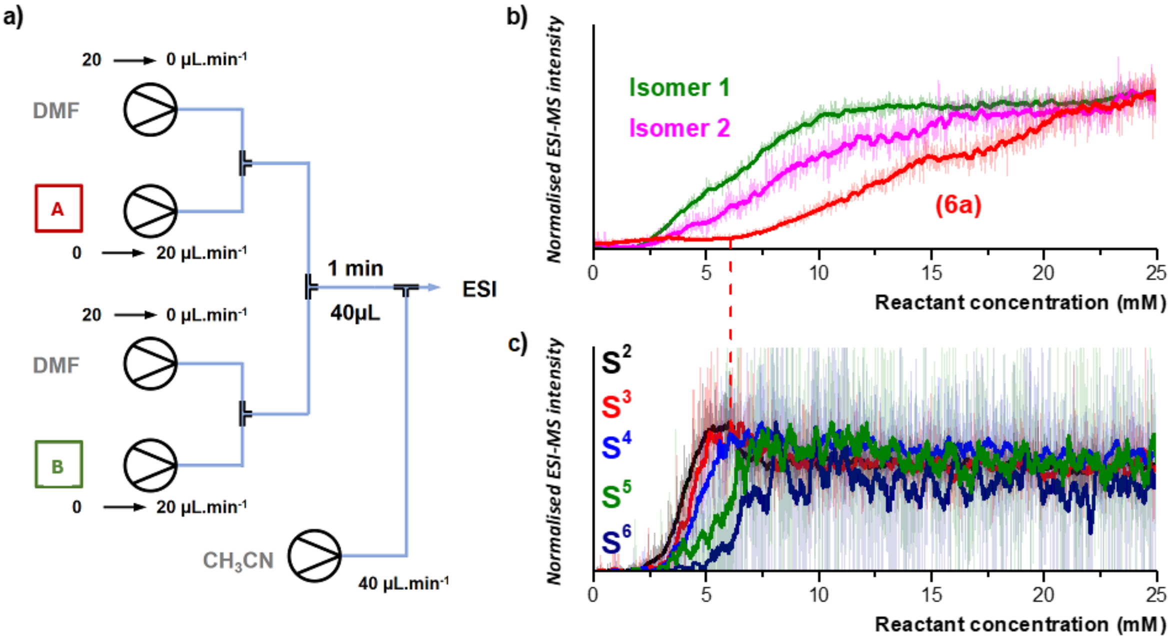


***Figure 6** – **a)** Schematic representation of the flow setup **b)** Normalized time evolution of m/z 269 isomers and product **6a**. c) Time evolution of $\mathbf{S^n}$ intermediates, where n = 2 – 6.*

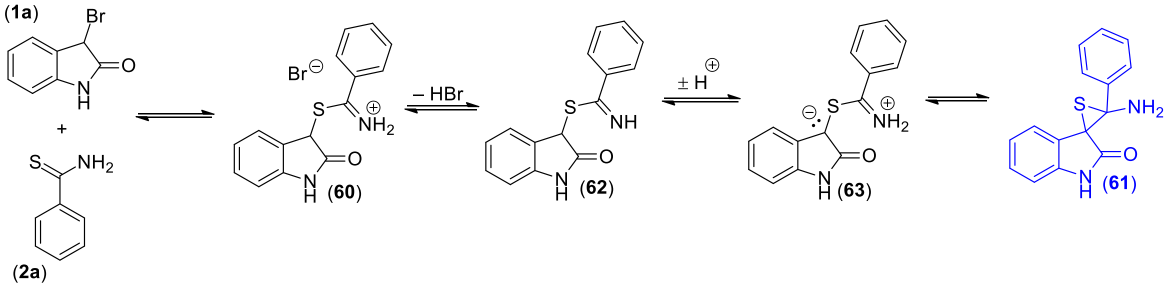
From the graph showing the distribution of reactive intermediates and product **6a** as a function of reactant concentration (**Figure 6b**), it is evident that the concentration of product **6a** remains negligible until approximately 6 mM is reached, despite the presence of species **60** and **61**. Data analysis for intermediates $\mathbf{S^2}$–$\mathbf{S^6}$ (**Figure 6c**) revealed that the overcoming of this „gap“ correlates closely with the concentration maxima of polysulfide intermediates $\mathbf{S^2}$ and $\mathbf{S^3}$, indicating their key autocatalytic role in the desulfurization process.

During the ECR of **1a** and **2a** carried out at higher concentrations (typically >0.5 M), a byproduct containing large amounts of sulfur always precipitated. This byproduct was generally considered an unwanted side product and removed during the work-up of the reaction mixture. However, to study the reaction mechanism, this by-product was isolated, purified, and analyzed by elemental analysis and DIP-APCI.[42] Elemental analysis confirmed a high sulfur content (73 %) but also revealed a significant amount of CHN elements, experimentally determined composition best corresponds to the molecular formula $C_{15}H_{14}N_2OS_{20}$. The obtained compound also exhibited zero solubility in toluene, ruling out the presence of elemental sulfur in the sample. Pyrolysis during the DIP-APCI analysis led to the formation of oligomeric sulfur chains (S_4–S_{11}), and additionally, a significant amount of the ECR product **6a** was also detected.

The results discussed above suggest that the byproduct of the reaction occurring without the presence of a thiophile is not exclusively elemental sulfur, as existing literature sources suggest[37–39] for similar examples of thiophile-free sulfur extrusions, as sulfur chains with more than eight sulfur atoms were detected in the APCI experiment. The catalytic cycle of $\mathbf{S^n}$ intermediates may be terminated by their precipitation from the reaction medium, due to their high molecular weight and the associated poor solubility (e.g., the molecular weight of $C_{15}H_{14}N_2OS_{20}$ is 879 g/mol).

Summary

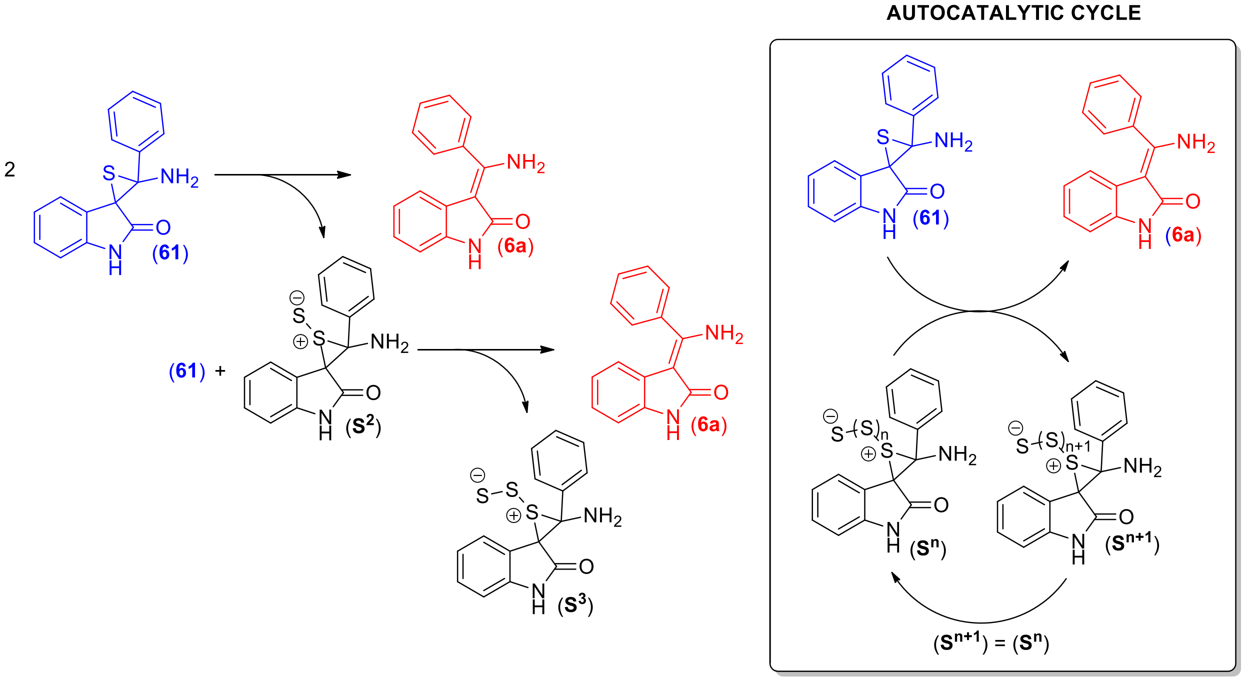
The mechanism of the ECR can be proposed as follows: In the initial step, the reaction between components **1a** and **2a** leads to the formation of the α-thioiminium salt **60**, which exists in an acid-base equilibrium with the free imidothioate **62**. The deprotonation of the salt **60** or an intramolecular proton transfer in **62** results in the formation of the zwitterionic species **63**, which subsequently cyclizes to form thiirane **61** (**Scheme 29**). Under the conditions of ESI-MS measurements, the **60** is a stable intermediate that accumulates in the solution, as confirmed by IRPD analysis. This mechanism is consistent with the widely accepted mechanism of the ECR.[1]



Scheme 29 *– Formation of thiirane* ***61****.*

The most significant aspect of the mechanism involves the subsequent transformation of the thiirane intermediate **61**, which undergoes a bimolecular reaction in which the sulfur atom possesses ambivalent reactivity (acts both as a nucleophile and as an acceptor of a lone pair). This step results in the formation of the key intermediate **S^2** and the product **6a**. The high nucleophilicity of the terminal, negatively charged sulfur in **S^2** drives a rapid reaction with another molecule of **61**, leading to the formation of intermediate **S^3** (**Scheme 30**). This reaction sequence is critical, as it leads to the progressive elongation of the sulfur chain, eventually reaching lengths of up to 20 atoms, as confirmed by mass spectrometry.

These species play a crucial role in ECR as a "sulfur scavenger". Each reaction step, associated with the elongation of the sulfur chain, simultaneously results in the formation of a molecule of the product **6a**. This process can be schematically represented as an autocatalytic cycle.



Scheme 30 *– Formation of* ***S^2****/****S^3*** *intermediates and role of* ***S^n*** *species as „sulfur scavenger“ in ECR; represented as autocatalytic cycle.*

3. Conclusion

α-Brominated benzolactams represent original substrates for the ECR, with the best results achieved atypically in reactions with primary thioamides. Their exceptional reactivity is further enhanced by the absence of thiophilic agents and, in most cases, a catalytic base, simplifying the separation of reaction products. The optimized ECR variant I developed was successfully utilized for synthesizing both established and experimental kinase inhibitors.

Key outcomes of this research include:

a) Elucidation of structure-reactivity relationships in reactions of various α-bromolactams with primary and secondary thioamides. General principles governing the ECR were established, especially related to the acidity of the intermediate α-thioiminium salt, influenced by the structure of the starting α-bromoamide. These findings are supported by DFT calculations, which align well with observed reactivity.

b) Application of this synthetic approach in the synthesis of biologically active compounds, including the successful synthesis of kinase inhibitors *Nintedanib* (**37b**) and *Hesperadin* (**39**), with yields comparable to the best published methods.

c) Experimental confirmation of the desulfurization step in the ECR. For the first time, HRMS-ESI and IRPD provided mass and ion spectra of reactive intermediates, including newly discovered polysulfidic intermediates $\mathbf{S^n}$, which play a crucial role in the desulfurization step. The autocatalytic role of these species was confirmed by the use of advanced flow experiments.

4. References

(1) Shiosaki, K. The Eschenmoser Coupling Reaction. In Comprehensive Organic Synthesis – Selectivity, Strategy and Efficiency in Modern Organic Chemistry; Trost, B. M.; Fleming, I., Eds.; Pergamon Press: Oxford, 1993; Vol. 2; Pp 865–892.

(2) Hussaini, S. R.; Chamala, R. R.; Wang, Z. The Eschenmoser Sulfide Contraction Method and Its Application in the Synthesis of Natural Products. *Tetrahedron* **2015**, *71* (36), 6017–6086.

(3) Woodward, R. B. The Total Synthesis of Vitamin B_{12}. *Pure Appl. Chem.* **1973**, *33* (1).

(4) Kammel, R.; Tarabová, D.; Růžičková, Z.; Hanusek, J. Reaction of a Brominated Benzolactone/Lactam with 4-Methoxythiobenzamide and Thiourea: An Eschenmoser Coupling Reaction, Ring Transformation, or Dimerization? *Tetrahedron Lett.* **2015**, *56* (20), 2548–2550.

(5) Kammel, R.; Tarabová, D.; Brož, B.; Hladíková, V.; Hanusek, J. Formation of 3-[Amino(Aryl)-Methylidene]-1,3-Dihydro-2 H -Indol-2-Ones Involving Ring Transformation of 2-Aryl-5-(2-Aminophenyl)-4-Hydroxy-1,3-Thiazoles. *Tetrahedron* **2017**, *73* (14), 1861–1866.

(6) Kammel, R.; Tarabová, D.; Machalický, O.; Nepraš, M.; Frumarová, B.; Hanusek, J. Synthesis, Characterization and Spectral Properties of New, Highly Fluorescent, 4-Hydroxythiazoles. *Dyes and Pigments* **2016**, *128*, 101–110.

(7) Manaka, A.; Sato, M. Synthesis of Aromatic Thioamide from Nitrile Without Handling of Gaseous Hydrogen Sulfide. *Synth. Commun.* **2005**, *35* (5), 761–764.

(8) Bergman, J.; Pettersson, B.; Hasimbegovic, V.; Svensson, P. H. Thionations Using a P_4S_{10} −Pyridine Complex in Solvents Such as Acetonitrile and Dimethyl Sulfone. *J. Org. Chem.* **2011**, *76* (6), 1546–1553.

(9) L. Marek. Diplomová Práce: Syntéza Inhibitorů Kináz s 3-[Amino(Aryl)Methyliden]-1,3-Dihydro-2*H*-Indol-2-Onovým Skeletem; Univerzita Pardubice, **2019**.

(10) Hansch, Corwin.; Leo, A.; Taft, R. W. A Survey of Hammett Substituent Constants and Resonance and Field Parameters. *Chem. Rev.* **1991**, *91* (2), 165–195.

(11) Huang, P.; Ou, W. A Direct, Versatile, and Chemoselective Synthesis of Vinylogous Bis- and Monourethanes/Amides and β-Keto Esters by Aza-Knoevenagel-Type Reactions of Tertiary Amides with Enolates. *Eur. J. Org. Chem.* **2017**, *3*, 582–592.

(12) Roth, M.; Dubs, P.; Götschi, E.; Eschenmoser, A. Sulfidkontraktion via Alkylative Kupplung: Eine Methode Zur Darstellung von β-Dicarbonylderivaten. Über Synthetische Methoden, 1. Mitteilung. *Helv. Chim. Acta* **1971**, *54* (2), 710–734.

(13) Quiclet-Sire, B.; Zard, S. Z. An Expedient Synthesis of Homophthalimides. *Chem. Commun.* **2002**, *20*, 2306–2307.

(14) Koduri, N. D.; Hileman, B.; Cox, J. D.; Scott, H.; Hoang, P.; Robbins, A.; Bowers, K.; Tsebaot, L.; Miao, K.; Castaneda, M.; Coffin, M.; Wei, G.; Claridge, T. D. W.; Roberts, K. P.; Hussaini, S. R. Acceleration of the Eschenmoser Coupling Reaction by Sonication: Efficient Synthesis of Enaminones. *RSC Adv.* **2013**, *3* (1), 181–188.

(15) Roskoski, R. Properties of FDA-Approved Small Molecule Protein Kinase Inhibitors: A 2020 Update. *Pharm. Res.* **2020**, *152*, 104609.

(16) Roth, G. J.; Binder, R.; Colbatzky, F.; Dallinger, C.; Schlenker-Herceg, R.; Hilberg, F.; Wollin, S.-L.; Kaiser, R. Nintedanib: From Discovery to the Clinic. *J. Med. Chem.* **2015**, *58* (3), 1053–1063.

(17) Richeldi, L.; Du Bois, R. M.; Raghu, G.; Azuma, A.; Brown, K. K.; Costabel, U.; Cottin, V.; Flaherty, K. R.; Hansell, D. M.; Inoue, Y.; Kim, D. S.; Kolb, M.; Nicholson, A. G.; Noble, P. W.; Selman, M.; Taniguchi, H.; Brun, M.; Le Maulf, F.; Girard, M.; Stowasser, S.; Schlenker-Herceg, R.; Disse, B.; Collard, H. R. Efficacy and Safety of Nintedanib in Idiopathic Pulmonary Fibrosis. *N. Engl. J. Med.* **2014**, *370* (22), 2071–2082.

(18) Morahan, B. J.; Abrie, C.; Al-Hasani, K.; Batty, M. B.; Corey, V.; Cowell, A. N.; Niemand, J.; Winzeler, E. A.; Birkholtz, L.-M.; Doerig, C.; Garcia-Bustos, J. F. Human Aurora Kinase Inhibitor Hesperadin Reveals Epistatic Interaction between Plasmodium Falciparum PfArk1 and PfNek1 Kinases. *Commun. Biol.* **2020**, *3* (1), 701.

(19) Marek, L.; Váňa, J.; Svoboda, J.; Hanusek, J. Synthesis of the Kinase Inhibitors Nintedanib, Hesperadin, and Their Analogues Using the Eschenmoser Coupling Reaction. *J. Org. Chem.* **2021**, *86* (15), 10621–10629.

(20) Tacconi, G.; Righetti, P. P.; Desimoni, G. Heterocycles with Potential Heterodienes. II. Bifunctional Nitrones: Synthesis of New Heterocycles with Both Nitrone and Heterodiene Functions. *J. Prakt. Chem.* **1980**, *322* (4), 679–684.

(21) Batjargal, S.; Huang, Y.; Wang, Y. J.; Petersson, E. J. Synthesis of Thioester Peptides for the Incorporation of Thioamides into Proteins by Native Chemical Ligation. *J. Pept. Sci.* **2014**, *20* (2), 87–91.

(22) Staudinger, H.; Siegwart, J. Ueber Thiobenzoylchlorid. *Helv. Chim. Acta* **1920**, *3* (1), 824–833.

(23) Kato, S.; Mizuta, M. Studies of Organosulfur Compounds. VII. The Preparation and Reaction of Some Crystalline Piperidinium Salts of Aliphatic Dithio Acid. *Bull. Chem. Soc. Jpn.* **1972**, *45* (11), 3492–3493.

(24) Csomós, P.; Fodor, L.; Sohár, P.; Bernáth, G. Synthesis of Thiazino[6,5-b]Indole Derivatives, Analogues of the Phytoalexin Cyclobrassinin. A New Method for Preparation of 3-Aminomethylindole. *Tetrahedron* **2005**, *61* (39), 9257–9262.

(25) Fodor, L.; Csomós, P.; Fülöp, F.; Csámpai, A.; Sohár, P. Preparation and Ring Transformation of Isomeric β-Lactam Derivatives of Bicyclic 1,3-Thiazines. *Tetrahedron* **2013**, *69* (1), 410–417.

(26) Park, Chan-O; Mallucci, Patrick; Lee, Joung-Hee. A Convenient Method for the Synthesis of Thiobenzamide Derivatives and O-Thiobenzoates by Use of 2-Benzothiazolyl Dithiobenzoate as Effective Thiobenzoylation Reagent. *Bull. Korean Chem. Soc.* **2002**, *23* (7), 1029–1030.

(27) Kato, S.; Shibahashi, H.; Katada, T.; Takagi, T.; Noda, I.; Mizuta, M.; Goto, M. Preparation and Some Reactions of Bis(Thioacyl) Sulfides. *Liebigs Ann. Chem.* **1982**, *7*, 1229–1244.

(28) Walter R.; Heckel A.; Roth G.J.; Kley J.; Schnapp G.; Lenter M.; Meel J. C. A.; Spevak W.; Weyer C. U.; Sulfonylamino Substituted 3-(Aminomethylide)-2-Indolinones As Cell Proliferation Inhibitors. Patent WO0236564a1, **200**2.

(29) Tsou, H.-R.; Otteng, M.; Tran, T.; Floyd, M. B.; Reich, M.; Birnberg, G.; Kutterer, K.; Ayral-Kaloustian, S.; Ravi, M.; Nilakantan, R.; Grillo, M.; McGinnis, J. P.; Rabindran, S. K. 4-(Phenylaminomethylene)Isoquinoline-1,3(2*H*, 4*H*)-Diones as Potent and Selective Inhibitors of the Cyclin-Dependent Kinase 4 (CDK4). *J. Med. Chem*. **2008**, *51* (12), 3507–3525.

(30) Tsou H.-R.; Ayral-Kaloustian S.; Birnberg G. H.; Floyd M. B.; Kaplan J.; Kutterer K. M. K.; Liu X.; Nilakantan R.; Otteng M. A.; Tang Z.; Zask A.; Reich M.; Tran T.. Substituted Isoquinoline-1,3(2*H*,4*H*)-Diones, 1-Thioxo-1,4-Dihydro-2*H*-Isoquinoline-3-Ones And 1,4-Dihydro-3(2h)-Isoquinolones And Use Thereof As Kinase Inhibitor. Patent WO2007075783 A3, **2007**.

(31) Tsou, H.-R.; Liu, X.; Birnberg, G.; Kaplan, J.; Otteng, M.; Tran, T.; Kutterer, K.; Tang, Z.; Suayan, R.; Zask, A.; Ravi, M.; Bretz, A.; Grillo, M.; McGinnis, J. P.; Rabindran, S. K.; Ayral-Kaloustian, S.; Mansour, T. S. Discovery of 4-(Benzylaminomethylene)Isoquinoline-1,3-(2*H*,4*H*)-Diones and 4-[(Pyridylmethyl)-Aminomethylene]Isoquinoline-1,3-(2*H*, 4*H*)-Diones as Potent and Selective Inhibitors of the Cyclin-Dependent Kinase 4. *J. Med. Chem.* **2009**, *52* (8), 2289–2310.

(32) Wolfbeis, O. S.; Trummer, I.; Knierzinger, A. Synthesen von Fluoreszenzfarbstoffen, 10 2-Substituierte Pyrano[2,3-c]Isochinolin-3,6-dione Und Merocyaninfarbstoffe Aus Homophthalsäureimiden. *Liebigs Ann. Chem.* **1981**, *5*, 811–818.

(33) Seyferth, D.; Womack, G. B.; Cowie, M.; Hames, B. W. Reactions of Dithio Esters and O-Alkyl Thioesters with Diiron Nonacarbonyl. Unexpectedly Different Products Formed from RC(S)OR' and HC(S)OR'. *Organometallics* **1984**, *3* (12), 1891–1897.

(34) Muraoka, M.; Yamamoto, T.; Enomoto, K.; Takeshima, T. Synthesis of 2-Alkoxycarbonyl Enamino Thioaldehydes and Selenaldehydes (as Pentacarbonyltungsten(0) Complexes). Improved Synthesis of Simple and 2-Cyano Enamino Thioaldehydes and Some Chemical Reactions of These Compounds. *J. Chem. Soc., Perkin Trans.* 1 **1989**, *7*, 1241.

(35) Mayer, S. C.; Banker, A. L.; Boschelli, F.; Di, L.; Johnson, M.; Kenny, C. H.; Krishnamurthy, G.; Kutterer, K.; Moy, F.; Petusky, S.; Ravi, M.; Tkach, D.; Tsou, H.-R.; Xu, W. Lead Identification to Generate Isoquinolinedione Inhibitors of Insulin-like Growth Factor Receptor (IGF-1R) for Potential Use in Cancer Treatment. *Bioorg. Med. Chem. Lett.* **2008**, *18* (12), 3641–3645.

(36) Huisgen, R.; Reinertshofer, J. Nitroso-acyl-amine Und Diazo-ester VIII. Aliphatische Nitroso-lactame Und Cyclische Diazoester. Justus Liebigs Ann. Chem. **1952**, 575 (2), 197–216.

(37) Lutz, E.; Biellmann, J.-F. Mechanism of Sulfur Extrusion from Thiirane. *Tetrahedron Lett.* **1985**, *26* (23), 2789–2792.

(38) Chew, W.; Harpp, D. N. Thermal Decomposition of 3',3'-Dichlorospiro[Fluorene-9,2'-Thiirane]: Kinetics and Mechanism. *J. Org. Chem.* **1993**, *58* (16), 4405–4410.

(39) Chew, W.; Harpp, D. N. Mechanism of Sulfur Extrusion in 2,2 Dichloro-3-[9-Fluorenyl] Episulfide. *Tetrahedron Lett.* **1992**, *33* (1), 45–48.

(40) Gabelica, V.; Marklund, E. Fundamentals of Ion Mobility Spectrometry. *Curr. Opin. Chem. Biol.* **2018**, *42*, 51–59.

(41) Jašík, J.; Žabka, J.; Roithová, J.; Gerlich, D. Infrared Spectroscopy of Trapped Molecular Dications below 4K. *Int. J. Mass Spectr.* **2013**, *354–355*, 204–210.

(42) Lattimer, R. P.; Polce, M. J. Direct Probe CI-MS and APCI-MS for Direct Materials Analysis. *J. Anal. Appl. Pyrol.* **2011**, *92* (2), 355–360.

5. List of published works

Publications related to the topic of dissertation:

Duez, Q.; Marek, L.; Váňa, J.; Hanusek, J.; Roithová, J. Autocatalysis in Eschenmoser Coupling Reactions. *Chem. Eur. J.* **2024**, 30 (9), e202303619.

Marek, L.; Váňa, J.; Svoboda, J.; Svobodová, M.; Hanusek, J. Efficient Synthesis of (*Z*)-4-((Substituted phenylamino)methylidene)isoquinoline-1,3(2*H*,4*H*)-diones Using the Eschenmoser Coupling Reaction. *Synthesis* **2024**, 56 (03), 518-526.

Marek, L.; Váňa, J.; Svoboda, J.; Hanusek, J. Eschenmoser coupling reactions starting from primary thioamides. When do they work and when not? *Beilstein J. Org. Chem.* **2023,** 19, 808–819.

Marek, L.; Kolman, L.; Váňa, J.; Svoboda, J.; Hanusek. J. Synthesis of (*Z*)-3-[amino(phenyl)methylidene]-1,3-dihydro-2*H*-indol-2-ones using an Eschenmoser coupling reaction. *Beilstein J. Org. Chem.* **2021***,* 17, 527–539.

Marek, L.; Váňa, J.; Svoboda, J.; Hanusek, J. Synthesis of the Kinase Inhibitors Nintedanib, Hesperadin, and Their Analogues Using the Eschenmoser Coupling Reaction. *J. Org. Chem.* **2021**, 86 (15), 10621–10629.

Published work unrelated to the topic of dissertation:

Svobodová, M.; Svoboda, J.; Li, B.H.; Bertolasi, V.; Socha, L.; Sedlák, M.; Marek, L.; Synthesis and Characterization of New Boron Compounds Using Reaction of Diazonium Tetraphenylborate with Enaminoamides. *Molecules* **2022**, 27 (2), 367.

Korábková, T.; Bartáček, J.; Marek, L.; Hanusek, J.; Růžička, A.; Váňa, J.; Switchable cyclopalladation of substrates containing two directing groups: on the way to non-symmetrical [2.2]-dipalladaparacyclophanes. *Dalton Trans.* **2023**, 52, 11113-11119.