MINISTRY OF EDUCATION AND TRAINING

VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY

Tran Van Ket

USING DOMINO REACTIONS SYNTHESIZE NOVEL PODOPHYLLOTOXIN, PYRIMIDINE DERIVATIVES AND THEIR BIOLOGICAL EVALUATION

SUMMARY OF DISSERTATION ON SCIENCES OF MATTER

Major: Organic Chemistry

Code: 9 44 01 14

Ha Noi - 2025

The dissertation is completed at: Graduate University of Science and Technology, Vietnam Academy Science and Technology

Supervisors:

1. Supervisor 1: Prof. Dr. Nguyen Van Tuyen - Graduate University of Science and technology, Vietnam Academy of Science and Technology

2. Supervisor 2: Dr. Vu Ngoc Doan - Military Technology Academy, Ministry of Defense

Referee 1: Assoc. Prof. Dr. Phan Van Kiem

Referee 2: Assoc. Prof. Dr. Mac Dinh Hung

Referee 3: Assoc. Prof. Dr. Pham Thi Tham

The dissertation will be examined by Examination Board of Graduate University of Science and Technology, Vietnam Academy of Science and Technology at pm, , 2025

The dissertation can be found at:
1. Graduate University of Science and Technology Library
2. National Library

INTRODUCTION

1. The urgency of the dissertation

In cancer treatment methods, chemotherapy is consistently regarded as an effective option, as this approach is capable of slowing tumor growth, alleviating the symptoms of the disease, and ensuring better management of the pathological condition. However, this treatment method is also associated with certain limitations, especially for patients with advanced-stage cancer, when the body's resistance is diminished, leading to adverse effects that directly impact the quality of life of patients.

The 4-azapodophyllotoxin compounds are capable of inhibiting the growth of various cancer cell lines through mechanisms such as: inducing apoptosis, arresting the cell cycle at the G2/M phase, and inhibiting the polymerization of tubulin. Notably, they exhibit reduced toxicity and fewer side effects compared to the original podophyllotoxin. Additionally, derivatives containing the pyrano[2,3-d]pyrimidine framework are nitrogen heterocycles known for their prominent biological activities, including anticancer, antituberculosis, antibacterial, antifungal properties, and, particularly, strong antioxidant capabilities. They are also reported to inhibit various enzymes, including α-amylase, α-glucosidase, xanthine oxidase, and acetylcholinesterase (AchE).

Based on the aforementioned reasons, I have chosen the topic "Using domino reactions synthesize podophyllotoxin and pyrimidine derivatives and their biological evaluation" as the subject of my research during my doctoral studies. The aim of this research is to investigate and synthesize new derivatives of podophyllotoxin and pyrimidines with potential biological activity.

2. Research objectives of the dissertation

- Research and application domino reaction to synthesize new podophyllotoxin derivatives and combining it with click reactions to synthesize pyrimidine derivatives

- Cytotoxic evaluation and inhibition of α-glucosidase and AchE enzymes and molecular docking simulations of synthesized compounds.

3. Research content of the dissertation

- Synthesis of podophyllotoxin derivatives containing heterocycles
- Synthesis of hybrid isatin-podophyllotoxin compounds
- Synthesis of hybrid podophyllotoxin-isatin-AZT and podophyllotoxin-isatin-Erlotinib compounds
- Synthesis of indole-pyrano[2,3-d]pyrimidine derivatives
- Evaluation of cytotoxic activity against cancer cells and inhibitory ability on α-glucosidase and AchE enzymes of the synthesized compounds
- Molecular docking studies to confirm the mechanisms of the synthesized derivatives against the tested agents

CHAPTER 1. OVERVIEW

Chapter 1 consisting of 26 pages, which presents a literature review on quinoline quinone, pyrano pyrimidine and isatin compounds and their biological activities; domino reaction and studies on applying domino reaction in the syndissertation of quinoline quinone, isatin, pyrimidine compounds, which Cytotoxic evaluation and inhibition of α-glucosidase and AchE enzymes.

CHAPTER 2. RESEARCH METHODS AND EXPERIENCE

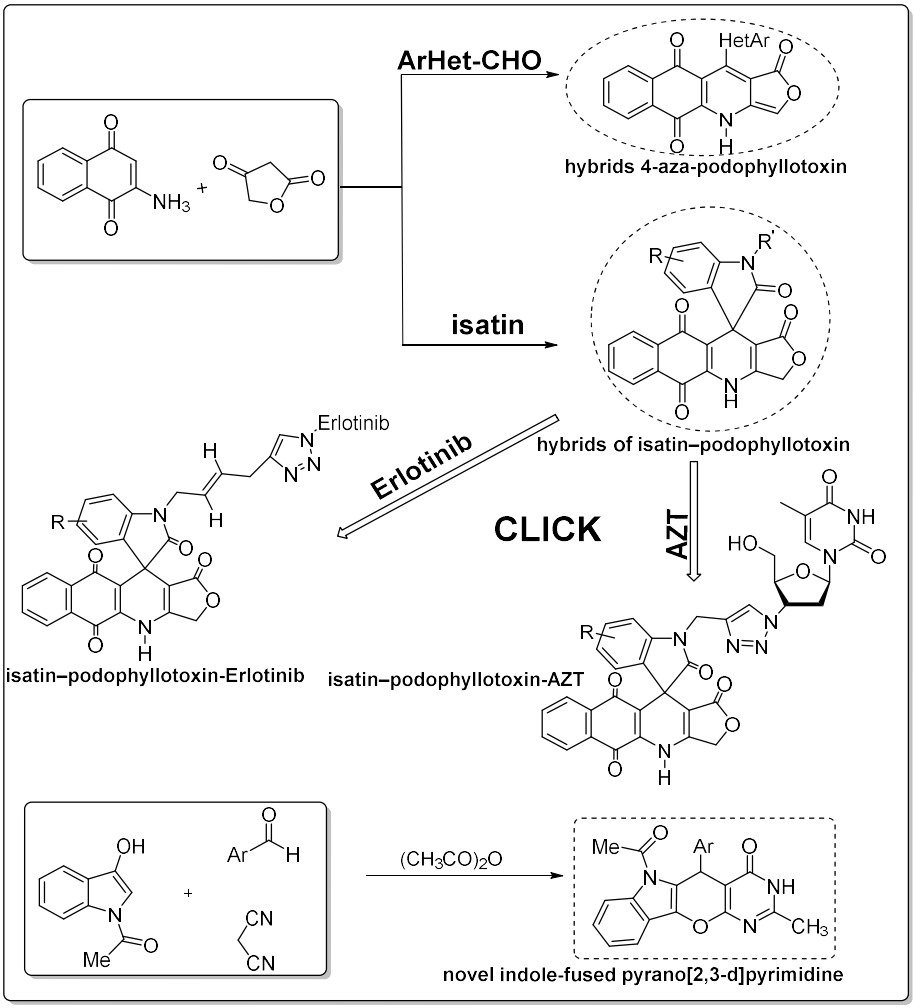
Chapter 2 consisting of 47 pages, presents the research methods, syndissertation process, purification, reaction yield, and physical properties of synthesized compounds such as morphology, color, melting point and their spectral data (IR, ^{1}H NMR, ^{13}C NMR , HSQC, HMBC and HRMS).

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Target of the dissertation

The mission objectives on the way to design and synthesize new derivatives of podophyllotoxin and pyrimidine done domino reactions, starting from initial materials containing activities hydrogen groups (2-

hydroxyl-1,4-naphthoquinone, 2-amino-1,4-naphthoquinone, N-acetylindoxyl) and ketone compounds (aromatic aldehydes, isatin, derivatives, tetronic acid) below microwave conditions. This will be combined with click reactions to form 1,2,3-triazole bridges with components such as AZT and Erlotinib. The synthesized compounds will be tested, biologically evaluated, and subjected to molecular docking studies.



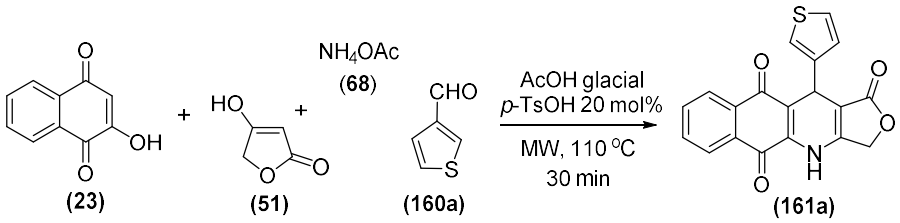
Scheme 3.1. Synthesis target and strategy of the dissertation

3.2. Synthesis of 4-aza podophyllotoxin derivatives containing heterocycles

In the overview section, the compound 4-aza-podophyllotoxin was displayed. When substituting rings A and B while keeping ring D unchanged, studies have consistently shown that the anticancer activity has been improved, particularly in the case of naphthoquinone. However, modifications in ring E have not been widely recorded due to interactions between ring E and ring C, which directly influence cytotoxic activity. Replacing ring E of podophyllotoxin with heterocycles containing oxygen, sulfur, or nitrogen atoms, which have high electronegativity, is expected to create stronger interactions between these heterocycles and ring C. This could potentially enhance the cytotoxic activity of this class of compounds.

a. Using four-component domino reaction

From the initial materials: 2-hydroxyl-1,4-naphthoquinone (**23**) (1 mmol), 3-thiophen aldehyde (**160**) (1 mmol), tetronic acid (**51**) (1 mmol), and ammonium acetate (**68**) (3.0 mmol) with the assistance of a microwave reactor, the thesis successfully synthesized the compound 11-(thiophen-3-yl)-4,11-dihydrobenzo[g]furo[3,4-b]quinolin-1,5,10(3H)-trione (161a) according to the following scheme:



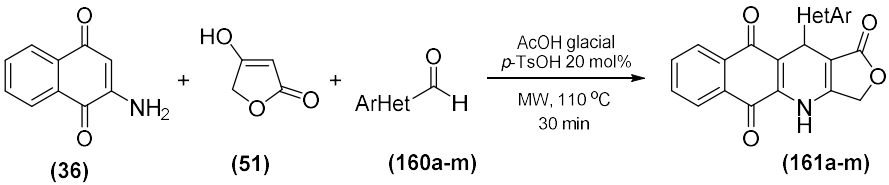
*Scheme 3.2. Syndissertation of compounds **161a** by 4 components*

The structure of compound (**161a**) was determined by IR, NMR, and HRMS spectra. Based on compound (**161a**), the thesis synthesized 13 derivatives, which are obtainable in Table 3.1.

*Table 3.1. 4-aza-podophyllotoxin with hectorocyclic compuonds **161a-m***

Entry	Products	Melting point (^{0}C)	Yield (%)	
			4 compounds	3 compounds
1	**161a**	298 - 299	29	64
2	**161b**	345 - 346	25	62
3	**161c**	304 – 305	20	60
4	**161d**	371 - 372	20	62
5	**161e**	295 - 296	33	70
6	**161f**	295 - 296	32	68
7	**161g**	302 - 303	33	69
8	**161h**	383 - 384	28	64
9	**161i**	262 - 263	27	63
10	**161j**	310 - 311	20	61
11	**161k**	245 - 246	34	74
12	**161l**	288 -289	32	72
13	**161m**	289 - 290	33	72

b. Using three-component domino reaction

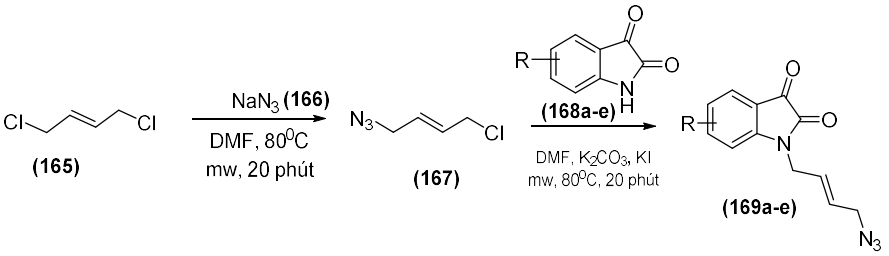


*Scheme 3.3. Syndissertation of compounds **161a** by 3 components*

The experimental results presented in Table 3.3 demonstrate that the use of a three-component domino reaction for synthesizing heterocyclic-containing 4-aza-podophyllotoxin derivatives (**161a-m**) achieves an efficiency twice that of the method employing four reagents below the same experimental conditions.

3.3. Syndissertation of isatin-podophyllotoxin derivatives

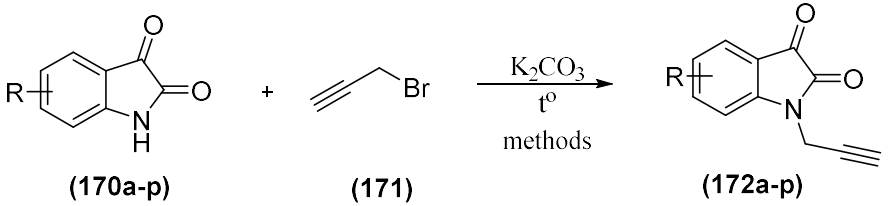
Based on the reference documents, the thesis has chosen to synthesize derivatives (**169a-n**) with the conditions shown in Scheme 3.4. The reason for this choice is because each reaction takes place sequentially under the same temperature conditions and the solvent is DMF. The reaction in the next step does not need to be isolated or extracted, only the catalysts K_2CO_3 and KI bases need to be added. The products will be purified and isolated, ready for the next reactions.



Scheme 3.4. Syndissertation of N- azidobut-2-en-isatin derivavities

To confirm the successful synthesis of the product, the thesis selected a typical derivative of the series, isatin, as the starting material. After the purification and isolation process, the product was preliminarily tested by IR and 1H-NMR spectra.

From the reference documents, the thesis has conducted a survey of different conditions of the alkylation process and selected the reaction between isatin (170a) and propagyl (171) as the subject of research according to Scheme 3.5.



Scheme 3.5. Alkylation reaction of isatin with bromide

For the reason that K_2CO_3 has good solubility and is a mild base

catalyst, the research team chose this catalyst as the subject of investigation in a reaction vessel containing 3 ml of each solvent. The reaction temperature was selected at 25 ^oC, 50 ^oC, 75 ^oC and 100 ^oC to suit the experimental conditions. The methods of investigation included: reflux, ultrasound, microwave were studied and investigated independently.

Table 3.2. Reaction conditions of alkylation

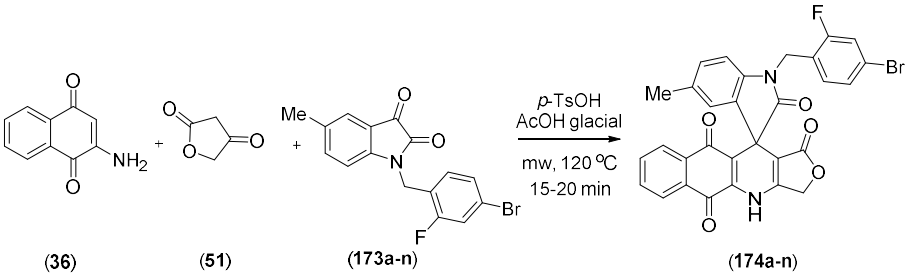
Temperature (^oC)		**Time (mins)**	**Yield (%)**	**Color**	**Solvent**
Microwave	25	60	14.3	Light	Acetone
	50	40	62.8	Light	Acetonitrile
	75	20	90.7	Dark	DMF
	100	15	N/A	Black	DMSO
Refux	25	120	5.0	Light	Acetone
	50	90	20.6	Light	Acetonitrile
	75	45	67.9	Dark	DMF
	100	20	N/A	Black	DMSO
Sonication	25	120	11.2	Light	Acetone
	50	90	34.6	Light	Acetonitrile
	75	45	78.4	Dark	DMF
	100	20	26.2	Dark	DMSO

In the Table 3.2, the most optimal conditions for this type of reaction: the appropriate temperature for the reaction to give the best efficiency is around 75^oC and the solvent DMF combined with the microwave method. The products of the alkylation process obtained all exist in solid form, are yellow, red or orange in color, easily isolated by column chromatography, insoluble in water, soluble in organic solvents such as: methanol, ethanol, acetone, toluene, acetic acid, DMF or DMSO... Because these are the raw materials for the reaction in the next step, the evaluation and determination of the structure of these derivatives will be carried out with the products after completing the domino reaction.

3.3.2. Domino reaction

The trend of combining two active compounds to enhance biological and pharmacological efficacy has garnered significant attention from scientists in recent times. It has now become a popular method in the screening and discovery of new compounds, including spiro compounds.

Multicomponent domino reactions are an efficient synthetic approach that facilitates the creation of diverse and complex compounds with various stereocenters from distinct biologically active components.



*Scheme 3.6. Syndissertation of isatin-podophyllotoxin compounds **174a-n***

On the IR, 1H-NMR, and 13C-NMR spectral data of compound (**174m**), it has been confirmed that the structure of this compound is entirely in line with the initial design and expectations. Based on this, the dissertation has conducted an investigation into the parameters for the optimization of the reaction, aiming to select the best conditions for synthesizing derivatives of this class of compounds.

To determine the optimal conditions for the aforementioned three-component domino reaction, the dissertation selected compound (**174m**) for investigation. Initially, (1 mmol) of 2-amino-1,4-naphthoquinone (**36**) was mixed with 1 mmol of tetronic acid (**51**) and 1 mmol of compound (**173m**), with a reaction time of 30 minutes in a microwave device, using two types of catalysts: tosyl acid (p-TsOH) and *L*-proline. Various solvents such as CH3CN, toluene, xylene, dioxane, and CH3COOH were utilized at corresponding temperature levels as outlined in Table 3.3.

*Table 3.3. The effect of the solvent on the synthesis yield of compound **174m***

Entry	Solvents	Temperature (°C)	Yield (%)	
			***p*-TsOH**	***L*-proline**
1	CH_3CN	80	45	50
2	Toluene	110	30	35
3	Xylen	120	40	35
4	Dioxan	100	35	30
5	CH_3COOH	110	85	80

The results in Table 3.3, it can be observed that conducting the reaction in anhydrous CH_3COOH with *p*-TsOH as the catalyst is the most suitable choice. Furthermore, CH_3COOH is highly soluble in water, which facilitates the extraction and isolation of the product after the reaction. The two types of acid catalysts investigated showed no significant differences; while *p*-TsOH selectively catalyzes the coupling reactions between ketones and anilines, L-proline is a versatile catalyst that supports various reactions such as Aldol condensation, Mannich reactions, Michael addition, Knoevenagel condensation, Hantzsch reactions, and Ullmann reactions.

By using anhydrous CH3COOH as the solvent and p-TsOH as the catalyst at a temperature of 110 °C, the dissertation successfully synthesized 14 derivatives (174a-n), which are solid powders ranging in color from light red to dark red, and are well soluble in CH2Cl2 and MeOH, as illustrated in Scheme 3.6. The physical parameters and properties of the products are presented in Table 3.4.

Table 3.4. Hybrid isatin-podophyllotoxin derivaties

Entry	Products	R	R'	Color	Melting point (°C)	Yield (%)
1	**174a**	H	H	Red	356 - 357	85
2	**174b**	5-Cl	H	Red	365 -366	70

3	**174c**	5-Me	H	Red	361 - 362	83
4	**174d**	5-Br	H	Red	310 - 311	82
5	**174e**	7-Me	H	Orange	367 - 368	74
6	**174f**	5,7-dibromo	H	Orange	364 - 365	73
7	**174g**	5,7-dimethyl	H	Orange	372 - 373	75
8	**174h**	H	benzyl	Red	320 - 321	76
9	**174i**	H	4-CF_3-benzyl	Orange	335 - 336	87
10	**174j**	H	4-Cl-benzyl	Orange	292 - 293	78
11	**174k**	H	4-bromo-2-flo-benzyl	Orange	350 - 351	76
12	**174l**	5-Cl	4-CF_3-benzyl	Red	354 - 355	85
13	**174m**	5-CH_3	4-bromo-2-flo-benzyl	Red	337 - 338	80
14	**174n**	5,6-dibromo	4-CF_3-benzyl	Orange	278 - 279	72

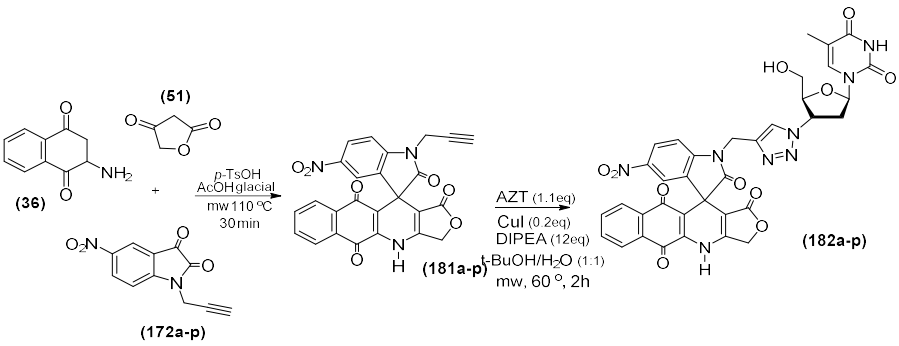
3.4. Synthesis of podophylotoxin-isatin-AZT derivaties (182a-p)

AZT (3′-azido-2′,3′-dideoxythymidine) is a well-known medication widely used in the treatment of patients with HIV. Before being approved by the FDA for treating acquired immunodeficiency syndrome (AIDS), it was demonstrated to inhibit the growth of various types of human cancer cells. Notably, when combined with other chemotherapeutic agents, it showed positive efficacy in clinical treatment for stage I and stage II patients suffering from colorectal cancer, leukemia, lymphoma, and other progressive malignancies.

Studies on the combination of AZT with other anticancer agents such as quinoline, coumarin, and sulfo-coumarin via a 1,2,3-triazole linkage have yielded positive results (IC50 ranging from 9-100 μM). Specifically,

the research findings indicate that these hybrid compounds also possess the ability to inhibit the growth of malignant tumors and prevent metastasis by inhibiting the activity of the enzyme telomerase (EC 2.7.7.49), which regulates transcription through the Wnt/β-catenin signaling pathway.

Building on the successful synthesis of derivatives between isatin and podophyllotoxin as presented in section 3.3.2, the dissertation proceeds to design and synthesize derivatives of podophyllotoxin-isatin with AZT by combining domino reactions and click reactions, as illustrated in Scheme 3.7.



Scheme 3.7. Synthesis of podophyllotoxin-isatin -AZT derivaties

The process was carried out in three stages. First, an alkylation reaction between isatin and propargyl was performed to obtain the compounds (**172a-p**) (as detailed in section 3.3.1). Next, a three-component domino reaction was conducted involving 2-amino-1,4-naphthoquinone (**36**), the isatin derivatives (**172a-p**), and tetronic acid (**51**) in anhydrous acetic acid, using *p*-TsOH as the catalyst. Finally, click reaction was performed in *tert*-BuOH/H2O (1:1), utilizing Cu(I) as the catalyst at a temperature of 60°C with assistance from microwave equipment. The reaction occurred rapidly, providing good yields, and the products could be easily isolated by column chromatography, while the reaction progress was monitored by TLC using a solvent system of n-hexane:EtOAc = 1:1.

The initial compound (**182a**) was selected for analysis and structural

determination using IR, NMR, and HRMS spectroscopic methods.

Based on the synthesis results of the derivative (182a), the dissertation successfully synthesized 16 derivatives (182a-p) of this compound class with yields from 62% to 83%, as presented in Table 3.5.

*Table 3.5. Hybrid of podophylotoxin-isatin-AZT (**182a-p**)*

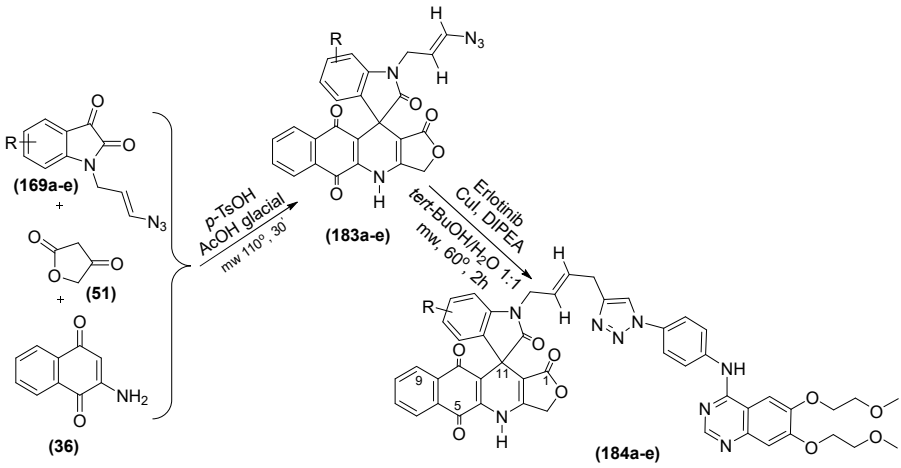
Entry	Products	R	Color	Melting point (^{0}C)	Yield (%)
1	**182a**	5-NO_2	Orange	272 - 273	62
2	**182b**	H	Dark brown	230 -231	71
3	**182c**	7-Me	Dark red	282 - 283	74
4	**182d**	5-Cl	Orange	320 - 321	68
5	**182e**	5-F	Orange	276 - 277	77
6	**182f**	5-Br	Dark brown	295 - 296	81
7	**182g**	5-OCH_3	Dark brown	285 - 286	78
8	**182h**	5,6-diflour	Dark orange	246 - 247	66
9	**182i**	7-F	Orange	239 - 240	82
10	**182j**	5-OCF_3	Dark brown	131 - 132	65
11	**182k**	5,7-dibromo	Dark red	160 - 161	72
12	**182l**	4,6-dichloro	Brown	280 - 281	83
13	**182m**	5-iotdua	Orange	206 - 207	67
14	**182n**	7-CF_3	Spray brown	250 - 251	74
15	**182o**	5-CH_3	Light red	355 - 356	71
16	**182p**	5,7-dimethyl	Orange	268 - 269	69

3.5. Synthesis of podophylotoxin-isatin-Erlotinib deravetives (184a-e)

Erlotinib is a small molecule protein tyrosine kinase inhibitor approved by the FDA for the treatment of metastatic non-small cell lung cancer (NSCLC), pancreatic cancer, and esophageal cancer. However, the increasing incidence of drug resistance and side effects associated with erlotinib underscores the need for more effective solutions.

Incorporating the 1,2,3-triazole group into erlotinib has significantly enhanced its antitumor efficacy, as the 1,2,3-triazole ring possesses a large dipole moment, enabling numerous non-covalent interactions with various substituent groups. This modification could potentially decrease drug resistance and mitigate the side effects caused by erlotinib.

Building on these practical considerations, the dissertation aims to design and synthesize novel derivatives of podophyllotoxin-isatin with erlotinib, according to Scheme 3.13. The goal is to create a compound that can both inhibit tubulin and target protein tyrosine kinase, which will be assessed through in vitro tests against cancer cell lines.



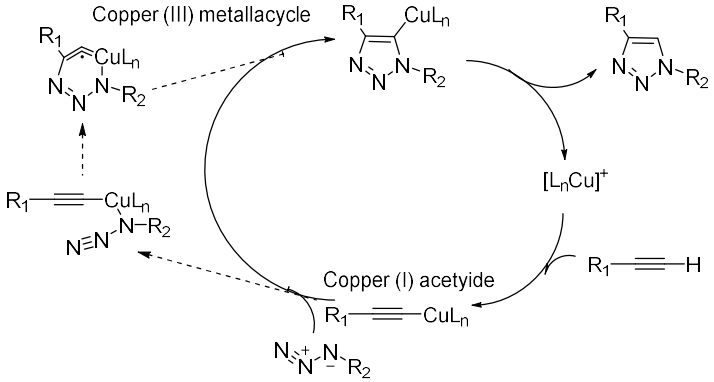
Scheme 3.8. Syndissertation of podophylotoxin-isatin – Erlotinib **(184a-e)**

The structure of the products (184a-e) was determined using IR and NMR spectroscopy. Based on the recorded spectral data, it was confirmed that the structure of the obtained products aligns with the intended coupling design. Consequently, the thesis successfully synthesized five new derivatives of podophyllotoxin-isatin-erlotinib, with the results presented in Table 3.6.

*Table 3.6. Compounds **184a-e***

Entry	Products	R	Color	Melting point (°C)	Yield (%)
1	**184a**	5-Br	Orange	261 - 262	41
2	**184b**	5,7-dibromo	Brown	242 -243	36
3	**184c**	7-F	Spray browr	224 – 225	48
4	**184d**	5, 7-dimethyl	Spray	290 - 291	55
5	**184e**	4,6-diflour	Yellow	266 - 267	32

The mechanism of the click reaction with Cu(I) catalyst is illustrated in Scheme 3.9.



Scheme 3.9. Mechanism of click reaction with Cu(I) catalyst

In this mechanism, copper (I) acetylide ions are initially generated, which then react with an azide reagent to form an intermediate copper (III) metallacycle through a cycloaddition process. The final stages involve protonation and ring contraction to yield the 1,4-substituted product of 1,2,3-triazole [141]. The ligand DIPEA acts as a base, deprotonating the newly formed triazole ring, releasing the final product and regenerating the copper catalyst.

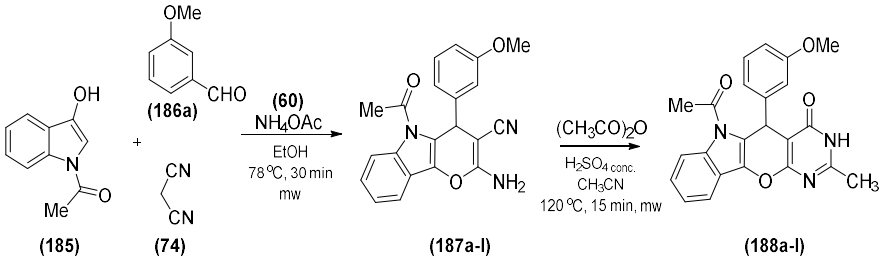
3.6. Synthesis of Indole-Pyrano[2,3-d] Pyrimidine Derivatives

With the objective of designing and synthesizing new derivatives

capable of selectively inhibiting α-glucosidase and acetylcholinesterase (AChE) enzymes, the dissertation focused on integrating indole into the pyrano[2,3-d]pyrimidine framework through a domino reaction.

By purifying and isolating the intermediate compound (187a), the research team conducted ^1H-NMR and ^13C-NMR spectroscopy to preliminarily ascertain the structure of this intermediate, laying the groundwork for further studies.

Following the preliminary structural evaluation of the intermediate (187a), a nucleophilic addition reaction was performed in an acidic environment. Through an intramolecular cyclization and dehydration process, the product was formed. The structure of compound (188a) was confirmed through IR, NMR, and HRMS spectral analysis.



Scheme 3.10: Synthesis of Indole pyrano[2,3-d] pyrimidine Derivatives

Compound (188a) was chosen for analysis, and its structure was established using IR, NMR, and HRMS spectroscopy.

The dissertation investigated and optimized the reaction conditions, with the results presented in Table 3.7.

Bảng 3.7. Conditions of on the synthesis compound ***188a***

Entry	Solvents		Catalysts (20% mol)		Temperature (°C)		Yield (%)	
	Step 1	**Step 2**	**Step 1**	**Step 2**	**Step 1**	**Step 2**	**Reflux**	**mw**
1	EtOH	EtOH	DMAP	p-TsOH	70	100	N/A	
2	CH3CN	EtOH	Et_3N	H_2SO_4	80	110		30

3	EtOH	CH3CN	NH_4OAc	H_2SO_4	80	120		68
4	CH3CN	*t*-BuOH	AcOH	H_2SO_4	90	130		35
5	*t*-BuOH	Toluene	p-TsOH	H_2SO_4	90	140		N/A
6	Toluene	Dioxane	NH_4OAc	p-TsOH	100	100	Multiple	
7	AcOH	CH_3CN	DMAP	Et_3N	110	110		N/A
8	Dioxane	AcOH	p-TsOH	NH_4OAc	120	120	N/A	

Results on the Table 3.7 indicate that the suitable solvents for steps 1 (80°C) and 2 (120°C) were EtOH and CH_3CN, respectively, alongside NH_4OAc as the catalyst for the Knoevenagel condensation in step 1, and H_2SO_4 to provide an acidic environment for the hydrolysis and cyclization in step 2.

Based on the established reaction conditions, the dissertation successfully synthesized 12 hybrid derivatives of indole-pyrano[2,3-*d*]pyrimidine (**188a-l**). The synthesis results and yields of the derivatives are summarized in Table 3.8.

*Table 3.8. Hybrid indole-pyrano[2,3-d]pyrimidine compounds (**188a-l**)*

Entry	Products	Color	Melting point (°C)	Yield (%)
1	**188a**	White	370 - 371	68
2	**188b**	White	374 - 375	72
3	**188c**	Spray	372 - 373	75
4	**188d**	Light Yellow	382 - 383	78
5	**188e**	Brown	376 - 377	70
6	**188f**	White	388 - 389	70
7	**188g**	Brown	383 - 384	65
8	**188h**	Spray brown	382 - 383	66
9	**188i**	Black	385 - 386	68
10	**188j**	Dark brown	386 - 387	65

Entry	Products	Color	Melting point (°C)	Yield (%)
11	**188k**	Brown	384 - 385	68
12	**188l**	Spray	381 - 382	75

Results displayed in the Table 3.8, it can be observed that the roles of electron-withdrawing groups (-Hal, -NO_2, -CN) and electron-donating groups (-OCH_3, -CH_3) on aromatic aldehydes had minimal impact on the reaction yields.

Based on the advantages of the domino reaction in synthesizing polycyclic compounds, the dissertation successfully synthesized 12 new derivatives of the indole-pyrano[2,3-*d*]pyrimidine hybrid class through a two-step process from starting materials including N-acetylindoxyl (**185**), aromatic aldehydes (**186a-l**), malononitrile (**74**), and acetic anhydride ($(CH_3CO)_2O$); in EtOH and CH_3CN solvents using NH_4OAc and H_2SO_4 as catalysts, with reaction yields ranging from 65% to 78%.

3.7. Evaluation of biological activity of compounds

3.7.1. Cytotoxicity activity of compounds 161a-m

From the experimental data, it can be seen that all derivatives exhibited high to medium cytotoxicity activity against all tested cancer cell lines, with IC_{50} values from 0.16 to 14.22 μM. Among these, the cytotoxicity activity of three compounds (**161a, h, k**) was better than that of ellipticine against all four tested cancer cell lines.

3.7.2. The results of cytotoxicity activity of compounds 182a-p

The data showed that 15/16 compounds in this class did not record an IC_{50} value. Only compound (**182n**) exhibited weak cytotoxic activity on two cancer cell lines: KB and MCF-7, with IC_{50} values of 15.82 ± 0.02 and 15.64 ± 0.11 μM, respectively. The results demonstrated compounds (**182**) had poor or no cancer cell cytotoxic activity (according to the National Cancer Institute (NCI)).

3.7.3. Cytotoxic activity of the 184a-e compound series

Experimental data showed that only derivative (**184e**) exhibited strong inhibitory activity against the KB, A549, and HepG2 cell lines. This value was twice as high as the IC_{50} value of ellipticine. However, its cytotoxicity against the non-cancerous Hek-293 cell line had an IC_{50} value of 3.32 μM, which was half that of ellipticine.

3.7.4. Cytotoxic activity test of derivatives 174a-n

The experimental results presented that all derivatives in this class (**174a-n**) displayed reasonable to strong inhibitory activity against four cancer cell lines, with IC_{50} values from 0.90 to 51.68 μM. When the bromo group was introduced into the aromatic ring of isatin without alkylation at the N-1 position, the inhibitory activity against the tested cancer cell lines was enhanced.

3.7.5. Cell Cyclic Analysis of Compound (174f) on A549 Cells

Results from Table 3.9 indicate that treatment with product (**174f**) caused a significant rise of cell accumulation in S phase, accompanied by a decrease of cells in G0/G1 phase. These results suggested that the tested compound could dosedependently arrest the cell cycle of A549 cells at S phase, thereby inhibiting cancer cell growth.

Table 3.9. Percentage of cells by phases of product 174f in A549 cell line

Sample	Percentage cell by phases (%)		
	% G0/G1	**% S**	**% G2/M**
Control (DMSO 0.5%)	32.70	52.96	10.84
174f (0.45 μM)	36.01	47.96	13.49
174f (0.9 μM)	26.63	67.93	4.17
174f (1.8 μM)	20.81	63.88	13.61

3.7.6. Apoptosis of Compound 174f

The results in Table 3.10 show treatment of cells with product (**174f**) at concentrations of 0.45 mM, 0.9 mM, and 1.8 mM resulted in a dose-

dependent increase in apoptotic cell accumulation, from 2.48% (control) to 3.02%, 8.09%, and 19.4%, respectively. Therefore, the results indicated that product (**174f**) inhibited A549 cancer cell growth through inducing cellular apoptosis of A549 cells in a dose-dependent manner, particularly in the early apoptotic stage.

Table 3.10. Percentage of Apoptotic Cells

Sample	% necrotic cells	% early apoptosis cells	% late apoptosis cells	% total apoptosis cells
Control	0.46	1.39	1.09	2.48
Camptothecin (2 μM)	1.99	30.71	11.05	41.76
174f (0.45 μM)	0.64	1.76	1.26	3.02
174f (0.9 μM)	0.94	3.13	1.83	8.09
174f (1.8 μM)	2.53	12.68	6.72	19.4

3.7.7. Evaluation of α-Glucosidase inhibitory activity (188a-l)

Most of the compounds exhibited significant α-glucosidase inhibitory activity with IC_{50} values from 2.49 ± 0.02 to 202.09 ± 15.72 μM, except for compound (**188h**), which showed no activity. Notably, certain compounds demonstrated inhibition 93 to 27 times stronger than the reference compound acarbose (IC_{50} = 231.30 ± 6.55 μM).

3.7.8. Acetylcholinesterase (AchE) inhibitory activity Assay (188a-l)

The results indicated that only two derivatives, (**188a**) and (**188k**), exhibited moderate AchE inhibitory activity, with IC_{50} values of 16.99 ± 1.12 and 5.39 ± 0.51 μM, respectively. The remaining derivatives showed weak or no inhibitory activity. Clearly, the addition of substituents to the phenyl ring reduced AchE inhibitory activity compared to the initial phenyl ring (**188k**).

3.8. Molecular Docking Study Results

3.8.1. Molecular Docking Study of Compound (174f)

The thesis conducts a study on molecular docking of CDK2/cyclin A

and CDK/p25 to explore the interaction mechanisms between compound (**174f**) and the complexes of these CDKs, along with the apoptosis-inducing agent procaspase/caspase 6.

Results: Podophyllotoxin-isatin derivatives were found to be associated with multiple stacking interactions with Ala31, Val64, Leu134, and particularly Phe80. Additionally, hydrogen bonding from the NH and CO groups, as well as from the 5,7-dibromoisatin moiety, towards the oxygen backbone of Glu12, Leu83, and the side chain oxygen of Asp86, significantly stabilizes the adaptability of (**174f**) at the ATP binding site of CDK2/cyclin A and CDK5/p25. The binding energy calculated for (**174f**) is -27.35 kcal/mol, which is lower than that of the natural ATP ligand (-35.84 kcal/mol).

After docking compound (**174f**) at the same site, it was discovered that this derivative also forms a bidentate complex with the Zn ion through two C=O groups of isatin and the lactone ring, as well as the 5,7-dibromoisatin groups. These results suggest that the synthetic compound may activate procaspase in a manner similar to that of PAC-1. However, there is a notable difference in the coordination geometry between compound (**174f**) and PAC-1 when forming complexes with the Zn ion, with compound (**174f**) exhibiting a lower binding energy (-19.10 kcal/mol) compared to PAC-1 (-23.81 kcal/mol).

Prediction of physiccochemical and ADMET properties of Compound 174f

Based on several drug-like rules, we identified that this compound accomplished the Lipinski and Pfizer rules but did not obey the GSK rule. Among the physicochemical properties calculated, lipophilicity (log P), solubility (log S), and number of rings (nRig) are out of drug-like ranges. In addition, it is provisionally classified as BCS class II, which may need some modifications to improve aqueous solubility

Table 3.11. Prediction of 174f compound toxicity using ADMETlab 2.0

Entry	Toxicity	Predicted value	Unit
1	AMES toxicity	No	

Entry	Toxicity	Predicted value	Unit
2	hERG inhibitor	No	
3	Hepatotoxicity	Yes	
4	Skin sensitization	No	
5	Rat oral acute toxicity	0.832	Low toxicity, > 500 mg/kg^{-1}

The ADMET-related properties have been evaluated, showing that the primary concern will be the involvement of cytochrome P450 enzymes. Compound (174f) is predicted to be a substrate for several first-pass metabolism enzymes such as CYP3A4, CYP1A2, CYP2C9, and others. Therefore, this compound will have low oral bioavailability. In terms of toxicity, as shown in Table 3.11, (**174f**) does not show interaction with human hERG-related genes, which are the primary cause of cardiac toxicity. Additionally, this compound shows low toxicity to the skin and respiratory system but causes liver toxicity. Its acute oral toxicity in mice is determined to be low, with a lethal dose > 500 mg/kg.

3.8.2. Series of 4-aza-podophyllotoxin compounds (161a-m)

Podophyllotoxin has long been recognized as an agent that destabilizes microtubules by binding to the colchicine site on tubulin, thereby inhibiting tubulin polymerization and preventing microtubule formation. Therefore, this thesis employed molecular docking methods to evaluate the interaction of compounds (**161a-m**) with tubulin, using colchicine as a positive control.

When docking 13 derivatives of the (**161a-m**) series into the colchicine-binding site of tubulin, all compounds were found to bind effectively to the colchicine active site on tubulin. Most synthesized compounds retained key interactions, such as hydrogen bonding between the quinone carbonyl group and Val181, Ala180, and hydrophobic interactions primarily involving pi-alkyl stacking with most amino acids in region 2, such as Lys352, Ala317, Ala316, Leu255, Ala250, and others. Furthermore, all

compounds formed additional hydrogen bonds with Thr179, which is not observed in colchicine, resulting in stronger binding to the active site.

Compound (**161h**) exhibited the highest binding energy (-17.86 kcal/mol), attributed to strong hydrogen bonding with amino acids in region 1 and hydrophobic interactions similar to compound (**161k**). The results also showed the highest Hbond and Hphob values for compounds (**161h**) and (**161k**) compared to other derivatives, consistent with their ranking order.

All compounds binding to tubulin demonstrated hydrophobic interactions and van der Waals forces, enhancing their ability to bind to tubulin. The docking results for the (**161a-m**) series align with the cytotoxicity data discussed in section 3.7.2.

In predicting absorption, distribution, metabolism, and excretion (ADMET) properties, the results indicated that both compounds have high intestinal absorption in humans and low skin permeability, with a short half-life.

Table 3.12.. Toxicity prediction of compounds 161h and 161k

Entry	Toxicity	161h	161k
1	hERG blockers	Inactive (0.002)	Inactive (0.009)
2	Human hepatotoxicity	Negative (0.425)	Negative (0.12)
3	AMES toxicity	No (0.324)	No (0.273)
4	Rat oral acute toxicity	No (0.413)	No (0.436)
5	FDA maximum daily dose	Negative (0.11)	Negative (0.235)
6	Skin sensitization	No (0.497)	Yes (0.747)
7	Respiratory toxicity	Yes (0.687)	Yes (0.893)
8	Eye irritation	No (0.263)	No (0.135)

Simulation results from Table 3.12 indicate: both compounds are not hERG channel blockers, have low cardiotoxic effects, no hepatotoxicity, no genotoxicity, and no eye irritation. Compound (**161k**) may cause skin and respiratory reactions, while compound (**161h**) shows minimal interaction with body organs, except for the respiratory system.

CONCLUSIONS AND RECOMMENDATIONS

1. - Four successful synthesis protocols have been proposed for 48 new derivatives of podophyllotoxin, with structures confirmed by modern spectroscopic methods such as IR, NMR, and HRMS. Specifically:
   - ➢ 27 compounds were synthesized via domino reactions, including:
   - ✓ 13 derivatives of 4-aza-podophyllotoxin (**161a-m**), with yields of 60-74%
   - ✓ 14 derivatives of podophyllotoxin-isatin (**174a-n**), with yields of 70-85%
   - ➢ 21 compounds were synthesized combination of domino and click reactions:
   - ✓ 16 derivatives of podophyllotoxin-isatin-AZT (**182a-p**), with yields of 62-85%
   - ✓ 5 derivatives of podophyllotoxin-isatin-Erlotinib (**184a-e**), with yields of 32-55%

   - Cytotoxicity testing on human cancer cell lines (KB, HepG2, A549, MCF-7) and normal HEK-293 cells revealed: 29/48 compounds demonstrating potential cytotoxic effects against cancer cells, specifically five compounds: (**161h**), (**161k**), (**161g**), (**174f**), and (**184e**), exhibited IC_{50} values lower than or equivalent to the control substance under the same experimental conditions.

   - Compound (**174f**) was selected for further testing to evaluate its ability to inhibit the cell cycle and induce apoptosis in A549 lung cancer cells. The results indicated that this compound arrested the cell cycle in the S phase and induced early apoptosis in the tested cells.
2. Domino reaction was applied successfully to synthesize 12 indole-pyrano[2,3-*d*]pyrimidine compounds (**188a-l**) with yields from 65% to 78%. The structures of these products were confirmed using modern spectroscopic methods (IR, NMR, HRMS). The results of enzyme inhibition assays against α-glucosidase and Acetylcholinesterase (AchE) revealed that this class of compounds showed good inhibition of α-glucosidase (11/12 compounds, notably (**188l**), (**188e**), and (**188d**). However, they exhibited moderate to weak inhibition against AchE (2/12 derivatives showed moderate inhibition).
3. ADMET and molecular docking studies were conducted on compound (**174f**) and the class of compounds (**161a-m**) to better understand their mechanisms of action on target enzymes and to support more effective drug design. The results showed:

- Compound (**174f**) induced apoptosis in tested cells (A549) by activating procaspases/caspases and CDK2/cyclinA and CDK5/p25 complexes.
- Compounds (**161h** and **161k**) inhibited tubulin polymerization, similar to the mechanism of podophyllotoxin.

Recommendation

Compound (**174f**) has shown the ability to inhibit the cell cycle in the S phase, induce early apoptosis, and exhibits low toxicity, adhering to drug-like properties. Therefore, it has the potential to be developed into an anticancer drug. Further in-depth studies and trials are highly recommended.

NEW CONTRIBUTIONS OF THE DISSERTATION

1. Proposed 5 new processes and formation mechanisms for synthesizing podophyllotoxin and pyrimidine compounds through domino reactions and their combination with Click reactions.
2. 60 new derivatives were synthesized successfully, which have not been previously reported in the literature, including:
   - ✓ 13 podophyllotoxin hybrid compounds (**161a-m**).
   - ✓ 14 podophyllotoxin-isatin compounds (**174a–n**).
   - ✓ 16 podophyllotoxin-isatin-AZT compounds (**182a-p**).
   - ✓ 05 podophyllotoxin-isatin-Erlotinib hybrid compounds (**184a-e**).
   - ✓ 12 indole pyrano[2,3-d]pyrimidine compounds (**188a-l**).
3. Cytotoxicity tests revealed that 05 compounds (**161h, 161k, 174f, 174n, 184e**) have IC_{50} values lower than or equivalent to the reference compound, while 03 compounds (**188l; 188e; 188d**) showed IC_{50} values 27 to 93 times higher than the reference compound in the α-glucosidase inhibition assay.
4. Molecular docking studies and cell cycle inhibition, apoptosis assays identified that compound (**174f**) has the ability to inhibit the cell cycle in the S phase, activate early apoptosis, and exhibit low toxicity, showing potential for drug development. Two compounds (**161h**) and (**161k**) demonstrated low toxicity and tubulin inhibition activity similar to podophyllotoxin.

LIST OF PUBLISHED ARTICLES RELATED TO THE THESIS

1. Ha Thanh Nguyen, Anh Nguyen Tuan, Tuyet Anh Dang Thi, **Ket Tran Van**, Giang Le-Nhat-Thuy, Phuong Hoang Thi, Quynh Giang Nguyen Thi, Cham Ba Thi, Hung Tran Quang, Tuyen Van Nguyen, 2024, Synthesis, in vitro A-Glucosidase, and acetylcholinesterase inhibitory activities of novel Indol-Fused Pyrano[2,3-*d*]Pyrimidine compounds, *Bioorganic & Medicinal Chemistry Letters*, 98, p 129566.
https://doi.org/10.1016/j.bmcl.2023.129566
2. Ha Thanh Nguyen, **Ket Tran Van**, Hai Pham-The, Julien Braire, Phuong Hoang Thi, Tuan Anh Nguyen, Quynh Giang Nguyen Thi, Tuyet Anh Dang Thi, Giang Le-Nhat-Thuy, Tu Anh Le Thi, Doan Vu Ngoc, and Tuyen Nguyen Van, 2024, Synthesis, molecular docking analysis and in vitro evaluation of new heterocyclic hybrids of 4-azapodophyllotoxin as potent cytotoxic agents, *RSC Advances*, 14(3), pp 1838-1853.
https://doi.org/10.1039/d3ra07396c
3. Ha Thanh Nguyen, **Ket Tran Van**, Hai Pham-The, Quang-Bao Le, Giang Le-Nhat-Thuy, Tuyet Anh Dang Thi, Phuong Hoang Thi, Quynh Giang Nguyen Thi, Anh Nguyen Tuan, Doan Vu Ngoc, and Tuyen Van Nguyen, 2025, Synthesis, cytotoxicity, apoptosis-inducing activity and molecular docking studies of novel isatin-podophyllotoxin hybrids, *RSC Advances,* 15(4), pp.2825-2839
https://doi.org/10.1039/d4ra08691k