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STUDY ON THE SYNDISSERTATION OF NOVEL QUINONE-FUSED HETEROCYCLIC DERIVATIVES USING DOMINO REACTION AND THEIR BIOLOGICAL EVALUATION

SUMMARY OF DISSERTATION ON ORGANIC CHEMISTRY

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INTRODUCTION

1.The urgency of the dissertation

Quinones are ubiquitous biological pigments found in a range of living organisms (bacteria, fungi, higher plants, and in few animals). They have interesting structures and biological activities such as cytotoxicity, anti-malarial, antibacterial, and anti-cancer properties. antibiotics, antifungals, ... Several quinone-based drugs such as doxorubicin and mitomycin C have been used clinically for cancer chemotherapy. Therefore, scientists are continuing to search and develop new compounds with higher anti-tumor potential based on the quinone framework.

The quinone-fused heterocyclic compounds containing oxygen and nitrogen account for a large proportion in this class of substances, typically naphtho[2,3-*b*]furan-4,9-dione, naphtho[2,3-*c*]chromene-7,12-dione, aza-anthraquinone, benzo [*f*]indole-4,9-dione, ... Scientific reports were shown that they have many activities such as antifungal, antibacterial, anti-inflammatory, anti-cancer, anti-HIV, anti-malarial, anti-parasitic… These compounds have been successfully synthesized from various methods such as domino reaction, Michael reaction, Diels-Alder cyclic addition reaction, Claisen reaction, metal catalyzed reaction, redox reaction, coupling reaction, etc. Among these methods, the domino reactions were shown many advantages such as increasing the complexity of the product structure due to the formation of many new bonds, high stereoselectivity and not required to isolate intermediate compounds.

Therefore, we conducted the dissertation "*Study on the syndissertation of novel quinone-fused heterocyclic derivatives using domino reaction and their biological evaluation*" in order to synthesize new quinone-fused heterocyclic compounds containing oxygen and nitrogen heteroatoms, with potential bioactivities as a basis for further studies. This dissertation is scientific and practical significance. The objective of the study is to synthesize some new heterocyclic derivatives of benzo[*g*]chromene-5,10-dione, podophyllotoxin-naphthoquinone, benzo[*a*] pyridazine[3,4-*c*] phenazine through multicomponent domino reactions, and evaluate cytotoxic activity against cancer cell lines of some synthetic compounds.

2. Research objectives of the dissertation

- Research and application domino reaction to synthesize new quinone derivatives containing oxygen and nitrogen heteroatoms.
- Cytotoxic evaluation of synthesized compounds.

3. Research content of the dissertation

- Syndissertation of 3-benzoyl-4*H*-benzo[*g*]chromene-5,10-dione compounds from the domino reaction.
- Syndissertation of podophyllotoxin-naphthoquinone compounds from domino reactions.
- Syndissertation of *N*-arylated-dihydrobenzo[*g*]quinoline-5,10-dione compounds from the domino reaction.
- Syndissertation of benzo[*a*]pyridazino[3,4-*c*]phenazine compounds from the domino reaction.
- Cytotoxic evaluation of some synthesized compounds on cancer cell lines: epidermoid carcinoma (KB), hepatoma carcinoma (HepG2), non-small lung (SK-Lu-1 or A549) and breast (MCF-7) cancer cell lines .

CHAPTER 1. OVERVIEW

Chapter 1 consisting of 23 pages, which presents a literature review on quinone fused heterocyclic compounds containing oxygen and nitrogen and their biological activities; domino reaction and studies on applying domino reaction in the syndissertation of quinone fused heterocyclic compounds.

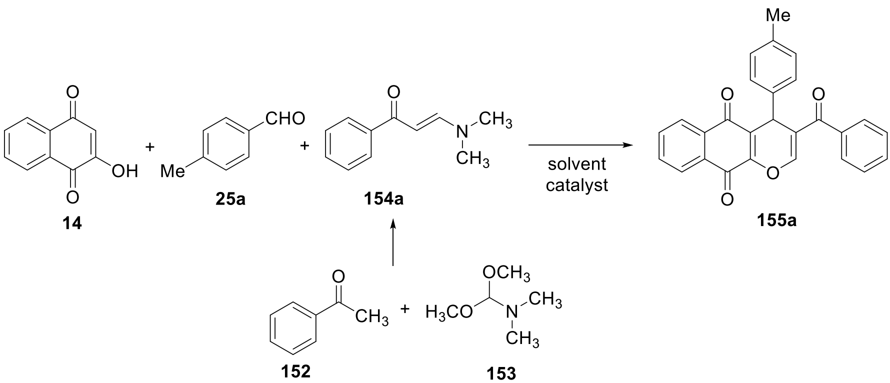
CHAPTER 2. RESEARCH METHODS AND EXPERIENCE

Chapter 2 consisting of 43 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. Syndissertation results of 3-benzoyl-4*H*-benzo[*g*]chromene-5,10-dione derivatives

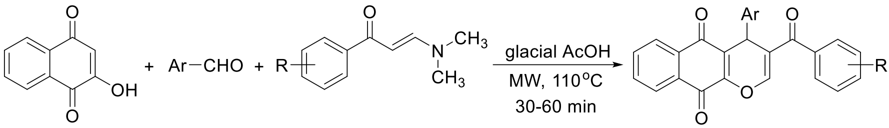
Initially, the reaction of 2-hydroxy-1,4-naphthoquinone (**14**), 4-methylbenzaldehyde (**25a)** and enaminone (**154a)** (formed by the condensation of *N*,*N*-dimethyformamide dimethyl acetal (**153)** with acetophenone (**152**) in toluene at reflux for 1 h) according to scheme 3.1. was chosen for developing the optimal reaction conditions.



Scheme 3.1. Syndissertation of compound **155a**

This reaction was carried out under MW in different solvents (CH_3CN, *t*-BuOH, EtOH, dioxane, toluene, AcOH) at a range of temperatures (80–120°C) in the presence or absence of catalyst. Experimental results showed that the syndissertation of compound **155a** has the highest yield when heating at 110°C in glacial acetic acid under microwave irradiation for 40 min.

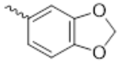
Using the optimized reaction conditions, a series of 3-benzoyl-4*H*-benzo[*g*]chromene-5,10-dione derivatives **155a-w** with different substitutions on the aryl groups were synthesized (scheme 3.2, table 3.2). The reaction yield of compounds is from 60 to 88%. The structures of all synthesized compounds **155a-w** were elucidated using IR, NMR and HRMS spectroscopic techniques.



Scheme 3.1. Syndissertation of compounds **155**

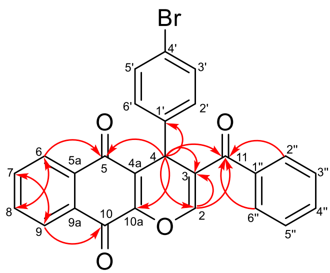
Table 3.2. Compounds **155a-w**

Entry	Product	Ar-	R	Yeild (%)
1	**155a**	4-MeC_6H_4	H	84%
2	**155b**	4-$MeOC_6H_4$	H	63%
3	**155c**	2-FC_6H_4	H	72%
4	**155d**	4-BrC_6H_4	H	88%
5	**155e**	4-$CF_3C_6H_4$	H	68%
6	**155f**	3-MeO-4-OHC_6H_3	H	66%
7	**155g**	C_6H_5	4-Me	61%
8	**155h**	4-MeC_6H_4	4-Me	82%
9	**155i**	3-$MeOC_6H_4$	4-Me	69%
10	**155j**	4-ClC_6H_4	4-Me	75%
11	**155k**	4-BrC_6H_4	4-Me	78%
12	**155l**	C_6H_5	4-F	60%
13	**155m**	4-MeC_6H_4	4-F	83%
14	**155n**		4-F	73%

				
15	**155o**	2-naphthyl	4-F	65%
16	**155p**	3-BrC_6H_4	4-F	75%
17	**155q**	4-BrC_6H_4	4-F	78%
18	**155r**	4-$NO_2C_6H_4$	4-F	68%
19	**155s**	C_6H_5	3-OH	63%
20	**155t**	4-MeC_6H_4	3-OH	77%
21	**155u**	2-FC_6H_4	3-OH	60%
22	**155v**	4-BrC_6H_4	3-OH	65%
23	**155w**	4-$NO_2C_6H_4$	3-OH	64%

On the ^{1}H NMR spectrum of compound **155b**, there is a singlet signal at 5,40 ppm (1H, s, H-4) of the H-4 proton and a singlet signal at 7,51 ppm (1H, s, H-2) of the cyclic H-2 proton chromene. Signal pairs at position δ_H = 8,16 – 8,15 ppm (1H, m, H-9), 8,04 – 8,02 ppm (1H, m, H-6), 7,75 – 7,74 ppm (2H, m, H-7, H-8) is that of the four naphthoquinone cyclic protons. In addition, the signal of 9 protons of two aromatic rings appeared to overlap in the low-field region from 7,58 to 7,35 ppm.

^{13}C NMR spectrum of compound **155b** appeared all of signals of 26 carbon atoms in the molecule, including typical signals of 3 carbonyl carbon atoms at 193,1 ppm (C-11), 182,9 ppm (C- 5), 177,8 ppm (C-10). In the higher field is the signal of the carbon atoms bonded to the oxygen atom in the chromene ring at 150,5 ppm (C-2) and 149,1 ppm (C-10a). The C-4 carbon atom gives a characteristic signal at 34,2 ppm, the remaining signals of the naphthoquinone and aromatic ring carbons appear from 141,3 to 120,3 ppm.



The results of HMBC and HSQC spectral analysis of compound **155b** presented in table 3.3 confirmed the expected structural formula and fully attributed the proton and carbon signals.

Table 3.3. NMR spectral data of compound **155b**

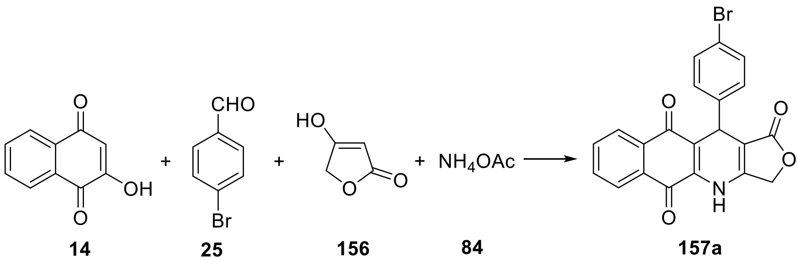
No.	**C (ppm)**	**δ H (ppm) (mult,, J (Hz))**	**Interactions on the HMBC (H →C)**
2	150,1	7,51 (1H, s)	3, 10a, 11
3	120,3	-	-
4	34,2	5,40 (1H, s)	2, 3, 4a, 5, 10a, 11 1', 2', 6'
4a	123,7	-	-
5	182,9	-	-
5a	131,7	-	-
6	126,7	8,04-8,02 (1H, m)	5, 8
7	134,7	7,75-7,74 (1H, m)	5a, 9
8	134,0	7,75-7,74 (1H, m)	6
9	126,6	8,16-8,15 (1H, m)	7, 10
9a	130,6	-	-
10	177,8	-	-
10a	149,1	-	-
11	193,1	-	-
1’	141,3	-	-
2’	130,5	7,35 (1H, d, 8,4 Hz)	4’, 6’
3’	131,9	7,42 (1H, d, 8,4 Hz)	1', 4'
4’	121,6	-	-
5’	131,9	7,42 (1H, d, 8,4 Hz)	1', 4'
6’	130,5	7,35 (1H, d, 8,4 Hz)	2', 4'
1’’	137,5	-	-

2’’	128,8	7,58 (1H, dd, 1,8 Hz, 8,4 Hz)	4", 6", 11
3’’	128,6	7,44 (1H, d, 7,8 Hz)	1’’
4’’	132,5	7,55 (1H, t, 7,2 Hz)	3', 5'
5’’	128,6	7,44 (1H, d, 7,8 Hz)	1’’
6’’	128,8	7,58 (1H, dd, 1,8 Hz, 8,4 Hz)	2", 4", 11

On the high-resolution mass spectrometry of compound **155b**, fragments *m/z* $[M-H]^-$ 469,0097 and 471,0179 were found which match the theoretically calculated mass for the molecular formula $[C_{26}H_{14}BrO_4]^-$ (469,0075 and 471,0055). The structures of other compounds were similarly elucidated.

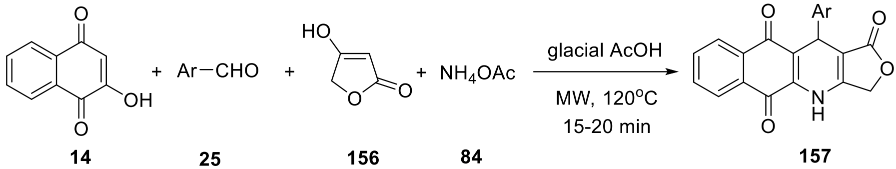
3.2. Syndissertation results of podophyllotoxin-naphthoquinone derivatives

The syndissertation of 11-phenyl-4,11-dihydrobenzo[*g*]furo[3,4-*b*] quinoline-1,5,10(3*H*)-trione **157a** starting from 2-hydroxy-1,4-naphthoquinone (**14**) (1 mmol), tetronic acid (**136**) (1 mmol), benzaldehyde (1 mmol) and ammonium acetate (3 mmol) under microwave irradiation was chosen for the screening of reaction conditions (Scheme 3.4). This reaction was carried out in severent organic solvents such as ethanol, *t*-butanol and glacial acetic acid in the presence of molecular sieve as water absorption at range of 80-130°C. The results showed the reaction in glacial acetic acid at 120°C gave the highest yield (82%) of product **157a**.



Scheme 3.4. Syndissertation of compound **157a**

Based on the optimized reaction conditions (AcOH, 120^{o}C), different substituted aromatic aldehydes were applied to this reaction to obtain a series of podophyllotoxin analogs **157a-i** (Scheme 3.6). The structures of all synthesized compounds **157a-i** were fully characterized by IR, NMR and HRMS spectroscopic techniques.

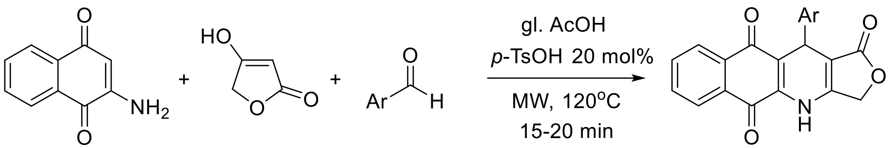


Scheme 3.6. Syndissertation of compound **157** from a four-component domino reaction

Table 3.5. Compounds **157a-i**

Entry	Product	Ar	Time (min)	Yield (%)
1	**157a**	2-FC_6H_4	15	78
2	**157b**	4-FC_6H_4	15	80
3	**157c**	4-BrC_6H_4	15	78
4	**157d**	4-ClC_6H_4	15	81
5	**157e**	4-$CF_3C_6H_4$	15	80
6	**157f**	4-$MeOC_6H_4$	20	85
7	**157g**	3-OMe-4-OHC_6H_3	20	79
8	**157h**	1-naphthyl	20	79
9	**157i**	2-naphthyl	20	89

In addition, compounds **157** were synthesized efficiently *via* microwave-assisted three-component reactions of 2-amino-1,4-naphthoquinone (**78**), tetronic acid (**156**), and (hetero) aromatic aldehydes in glacial acetic acid as a solvent and *p*-toluenesulfonic acid (*p*-TsOH, 20 mol%) as a catalyst (scheme 3.7).



Scheme 3.7. Syndissertation of compound **157** from a three-component domino reaction

Table 3.6 . Compounds **157j-x**

Entry	Product	Ar	Road 1		Road 2	
			Time (min)	Yield (%)	Time (min)	Yield (%)
1	**157j**	3-OMeC_6H_4	20	83	15	86
2	**157k**	3-BrC_6H_4	15	79	15	83
3	**157l**	3-$NO_2C_6H_4$	25	67	15	75
4	**157m**	C_6H_5	15	82	15	84
5	**157n**	4-MeC_6H_4	20	88	15	88
6	**157o**	2-$NO_2C_6H_4$	26	65	15	73
7	**157p**	3-oxo-1,3-dihydro benzofuran-5-yl	20	82	15	85
8	**157q**	2-F-4-MeOC_6H_3	20	39	20	71
9	**157r**	3-F-4-MeOC_6H_3	20	42	20	74
10	**157s**	2,6-F_2-4-MeOC_6H_2	25	37	25	70
11	**157t**	2-CF_3-4-MeOC_6H_3	20	36	20	73
12	**157u**	2-F-4-OHC_6H_3	20	17	20	72
13	**157v**	4-(4-fluorophenoxy)C_6H_4	25	trace	25	69
14	**157x**	4-$CF_3OC_6H_4$	20	69	20	78

Experimental results showed that using the three-component domino reaction to synthesize podophyllotoxin-naphthoquinone compounds according to scheme 3.7 usually gives higher yields and shorter reaction time than using the four-component domino reaction according to scheme 3.6.

Then, we continued to using the three-component domino reaction according to the procedure as shown in diagram 3.7 to synthesize 8 podophyllotoxin-naphthoquinone derivatives **157aa-ah** with high yields (79 – 89%) (table 3.7). The structures of all synthesized compounds **155aa-ah** were elucidated using IR, NMR and HRMS spectroscopic techniques.

Table 3.7. Compounds **157aa-ah**

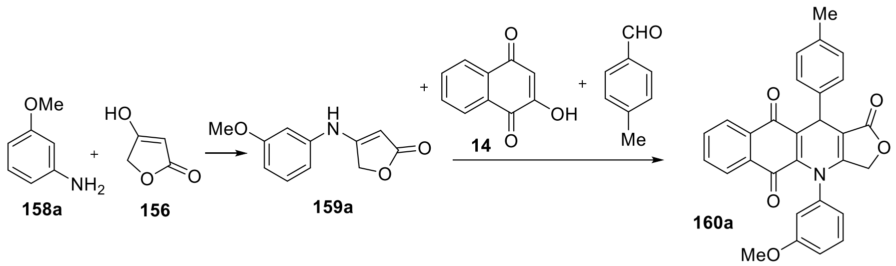
STT	Product	Ar	Reaction time (minutes)	Yield (%)
1	**157aa**	2-OHC_6H_4	20	80
2	**157ab**	4-OHC_6H_4	20	79
3	**157ac**	4-$NO_2C_6H_4$	15	82
4	**157ad**	4-CNC_6H_4	15	89
5	**157ae**	3,4-$(MeO)_2C_6H_3$	15	85
6	**157af**	3,4,5-$(MeO)_3C_6H_2$	15	86
7	**157ag**	pyridin-3-yl	15	81
8	**157ah**	5-Br-pyridin-2-yl	15	83

Thus, by using multicomponent domino reaction, we have successfully synthesized 31 podophyllotoxin-naphthoquinone compounds **157** from 2-hydroxy-1,4-naphthoquinone (**14**) and ammonium acetate (**84**) or 2-amino-1,4-naphthoquinone (**78**), aldehyde with tetronic acid (**156**) in glacial acetic acid as a solvent under microwave irradiation.

3.3. Synthetic results of *N*-arylated-dihydrobenzo[*g*]quinoline-5,10-dione derivatives

In order to incorporate an aryl substituent onto the nitrogen atom of the podophyllotoxin-naphthoquinone framework, we synthesized 4-(3-

methoxyphenyl)-11-(*p*-tolyl)-4,11-dihydrobenzo[*g*]furo[3,4-*b*]quinoline-1, 5,10(3*H)*-trione (**160a)** by a three-component domino reaction as shown in scheme 3.9.



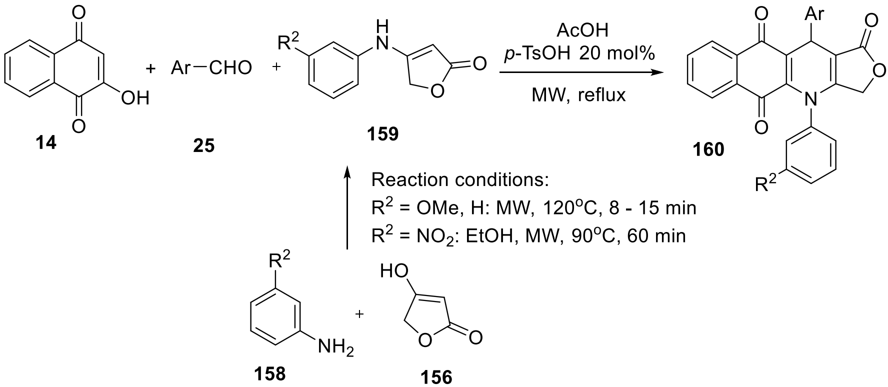
Scheme 3.9. Syndissertation of compound **160a**

Table 3.8. Optimization of the reaction condition for the syndissertation of compound **160a**

Entry	Solvent	Catalyst (mol%)	Reaction time (min)	Yield (%)
1	CH_3CN	–	90	0
2	EtOH	–	90	0
3	Toluene	–	90	0
4	AcOH	–	90	0
5	CH_3CN	TFA (10 mol%)	20	13
6	EtOH	TFA (10 mol%)	20	17
7	Toluene	TFA (10 mol%)	20	Trace
8	AcOH	TFA (10 mol%)	20	43
9	CH_3CN	*p*-TsOH (10 mol%)	20	14
10	EtOH	*p*-TsOH (10 mol%)	20	20
11	Toluene	*p*-TsOH (10 mol%)	20	11
12	AcOH	*p*-TsOH (10 mol%)	20	57
13	AcOH	*p*-TsOH (20 mol%)	20	83
14	AcOH	*p*-TsOH (30 mol%)	20	83

The reaction was investigated in different organic solvents and catalysts as indicated in table 3.8. The result revealed that the highest yield was obtained using glacial acetic acid as a solvent and 20 mol% of *p*-TsOH as a catalyst.

By using the optimised reaction conditions, we have successfully synthesized 17 compounds of *N*-arylated-dihydrobenzo[*g*]quinoline-5,10-dione **160a-q** from 2-hydroxy-1,4-naphthoquinone (**14**), aromatic aldehyde **25**, aniline/aniline derivative **158** and tetronic acid (**156**) under microwave irradiation. Products **160a-q** were afforded in good yields (69–86%) within 20-60 min. The structure of 17 synthesized compounds **160a-q** were fully characterized by IR, NMR and HRMS.



Scheme 3.10. Syndissertation of compounds **160a-q**

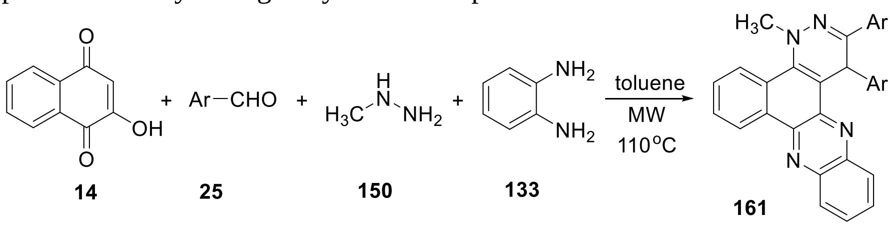
Table 3.9. Compounds **160a-q**

Entry	**Product**	**Ar**	**R^2**	**Time (min)**	**Yield (%)**
1	**160a**	4-MeC_6H_4	3-OMe	20	83
2	**160b**	4-BrC_6H_4	3-OMe	60	85
3	**160c**	4-$CF_3C_6H_4$	3-OMe	60	69
4	**160d**	C_6H_5	3-OMe	60	84
5	**160e**	4-MeC_6H_4	3-NO_2	20	80

6	**160f**	4-BrC_6H_4	3-NO_2	40	83
7	**160g**	4-$CF_3C_6H_4$	3-NO_2	40	80
8	**160h**	C_6H_5	3-NO_2	40	86
9	**160i**	3-OMeC_6H_4	3-NO_2	40	78
10	**160j**	3-$NO_2C_6H_4$	3-NO_2	60	81
11	**160k**	4-MeC_6H_4	H	40	79
12	**160l**	4-BrC_6H_4	H	40	81
13	**160m**	4-$CF_3C_6H_4$	H	40	80
14	**160n**	C_6H_5	H	40	82
15	**160o**	3-OMeC_6H_4	H	40	79
16	**160p**	3-$NO_2C_6H_4$	H	40	82
17	**160q**	4-ClC_6H_4	H	40	81

3.4. Synthetic results of benzo[*a*]pyridazino[3,4-*c*]phenazine derivatives

In addition, we conducted a multicomponent domino reaction from 2-hydroxy-1,4-naphthoquinone (**14**), aromatic or heterocyclic aldehyde **25**, methylhydrazine (**150)** and *o*-phenylenediamine (**133)** according to scheme 3.12 to synthesize a new heterocyclic compound containing phenazine and pyridazine-based heterocyclic radicals, which are structural subunits present in many biologically active compounds.



Scheme 3.12. Syndissertation of compounds **161**

9 New benzo[*a*]pyridazino[3,4-*c*]phenazines **161a-i** were selectively obtained in 45-63% yield after purification by silica gel column chromatography. The proposed molecular structure of all synthesized

compounds **161a-i** were fully characterized by IR, ^{1}H NMR, ^{13}C NMR and HRMS.

Table 3.11. Compounds **161a-i**

Entry	Product	Ar	Yield (%)
1	**161a**	C_6H_5	52
2	**161b**	$4\text{-}ClC_6H_4$	55
3	**161c**	$3\text{-}BrC_6H_4$	63
4	**161d**	$4\text{-}BrC_6H_4$	60
5	**161e**	$4\text{-}NO_2C_6H_4$	48
6	**161f**	$4\text{-}SO_2(CH_3)C_6H_4$	41
7	**161g**	$3\text{-}MeOC_6H_4$	57
8	**161h**	$4\text{-}MeOC_6H_4$	50
9	**161i**	2-naphthyl	45

We also conducted the same reaction with *o*-substituted aromatic aldehydes (Ar = $2\text{-}FC_6H_4$, 2-OMe C_6H_4, $2\text{-}NO_2\text{-}5\text{-}OHC_6H_3$). However, the reaction of these aldehydes, 2-hydroxy-1,4-naphthoquinone (**14)**, methylhydrazine (**150)** and *o*-phenylenediamine (**133)** didn't afford compounds **161**, probably due to steric hindrance between substituted group of aldehyde and nitrogen atom on phenazine core.

3.5. Cytotoxic activity

Podophyllotoxin-naphthoquinones **157,** *N*-arylated-dihydrobenzo[*g*] quinoline-5,10-diones **160** and benzo[*a*]pyridazino[3,4-*c*]phenazines **161** were selected to evaluate the cytotoxic activity against human cancer cell lines including mouth epidermal carcinoma KB, hepatoma carcinoma HepG2, lung cancer SK-Lu-1 or A549, and breast cancer MCF-7, as well as human embryonic kidney cell line Hek-293. Ellipticine was used as positive control.

3.5.1. Cytotoxic activity of N-arylated-dihydrobenzo[g]quinoline-5,10-dione compounds

Table 3.13. Cytotoxicity of compounds **160a-q**

Entry	**Compound**	**IC $_{50}$ (µM)**				
		KB	**HepG2**	**A549**	**MCF-7**	**Hek-293**
1	**160a**	> 20	**15,75 ± 1,08**	> 20	> 20	−
2	**160b**	> 20	> 20	> 20	> 20	−
3	**160c**	> 20	> 20	> 20	> 20	−
4	**160d**	14,97 ± 1,11	2,22 ± 0,22	11,26 ± 0,65	9,21 ± 0,33	3,87 ± 0,15
5	**160e**	> 20	> 20	> 20	> 20	−
6	**160f**	> 20	> 20	> 20	> 20	−
7	**160g**	> 20	> 20	13,48 ± 0,94	> 20	−
8	**160h**	17,22 ± 1,08	12,91 ± 1,08	6,13 ± 0,32	10,39 ± 0,47	−
9	**160i**	> 20	**1,23 ± 0,10**	16,17 ± 1,01	**1,98 ± 0,30**	37,89 ± 4,00
10	**160j**	> 20	> 20	7,75 ± 0,29	> 20	−
11	**160k**	> 20	> 20	> 20	> 20	−
12	**160l**	11,03 ± 1,79	2,97 ± 0,30	11,25 ± 0,70	9,23 ± 0,42	10,15 ± 0,58
13	**160m**	16,41 ± 1,03	9,12 ± 0,68	9,31 ± 0,72	8,30 ± 0,51	15,13 ± 1,00
14	**160n**	8,79 ± 0,36	**0,95 ± 0,12**	9,08 ± 0,36	**0,91 ± 0,12**	10,33 ± 1,54
15	**160o**	12,99 ± 0,62	7,07 ± 0,33	12,32 ± 0,82	11,16 ± 1,11	11,47 ± 1,12
16	**160p**	5,79 ± 0,22	**0,63 ± 0,02**	7,23 ± 0,32	**0,81 ± 0,19**	4,96 ± 0,36
17	**160q**	> 20	> 20	> 20	> 20	−
Ellipticin		1.33	1,33 ± 0,20	1,42 ± 0,20	2,55 ± 0,20	2,35 ± 0,20

According to concentrations of compounds inhibiting cell growth by 50% (IC_{50} values) shown in table 3.13, compounds **160d**, **160h**, **160i** and **160l-p** exhibited low to moderate micromolar IC_{50} values in the different cell lines evaluated (IC_{50} < 18 µM) with the exception of **160i**, which exhibited no appreciable activity in KB cells. Interestingly, compounds **160i**, **160n** and **160p** were particularly toxic in HepG2 and

MCF-7 cells with IC_{50} < 2 μM, that are similar to ellipticine. Besides that, compounds **160d** and **160l** expressed the high inhibitory activity against HepG2 cell line with IC_{50} = 2,22 and 2,97 μM, respectively. In addition, the selective cytotoxicity of the most potent cytotoxic compounds was also evaluated using non-cancerous human embryonic kidney (Hek-293) cells. In general, these compounds showed low to moderate toxic in the Hek-293 with IC_{50} values ranging from 3,87 to 37,89 μM.

3.5.2. Cytotoxic activity of benzo[a]pyridazino[3,4-c]phenazine compounds

Table 3.1. Cytotoxicity of compounds **161a-i**

Entry	**Compound**	**IC_{50} (μM)**				
		KB	**HepG2**	**Lu-1**	**MCF-7**	**Hek-293**
1	**161b**	> 100	> 100	> 100	> 100	> 100
2	**161a**	19,41 ± 0,40	10,48 ± 0,20	18,55 ± 0,25	22,04 ± 0,50	> 100
3	**161c**	> 100	> 100	> 100	> 100	> 100
4	**161d**	> 100	> 100	> 100	> 100	> 100
5	**161e**	> 100	> 100	> 100	> 100	> 100
6	**161f**	62,48 ± 1,80	44,68 ± 1,03	> 100	62,74 ± 1,17	> 100
7	**161g**	64,41 ± 0,80	36,32 ± 0,27	55,96 ± 1,06	70,66 ± 1,49	> 100
8	**161h**	16,70 ± 0,34	14,62 ± 0,15	18,25 ± 0,23	21,83 ± 0,19	73.44 ± 1.34
9	**161i**	> 100	81,42 ± 0,94	> 100	> 100	> 100
Ellipticin		1.26 ± 0.05	1,34 ± 0,04	1,83 ± 0,05	2,48 ± 0,05	6,58 ± 0,04

Results of the cytotoxicity test of benzo[*a*]pyridazino[3,4-*c*]phenazine **161a-i** derivatives showed that compounds with -Cl, -Br, $-NO_2$ groups (**161a, c, d, e)** were inactive against all four cancer cell lines, whereas compound **161f** having SO_2CH_3 group showed less active toward KB, HepG2 and HCF7 cell lines with IC_{50} values ranging from 44 to 63 μM. The introduction of methoxy group to aryl ring (compounds **161g-h**) showed higher inhibitory activity in comparison with compounds **161b-f**.

In particular, compound **161h** (Ar = 4-OMeC_6H_5) and compound **161b** (Ar = C_6H_5) displayed good cytotoxic effects on four tested cell lines with IC_{50} values ranging from 10 to 23 μM. Besides, compound **161i** having naphthyl group showed no activity against these cancer cell lines. All compounds **161a-i** exhibited lower toxic to the Hek-293 cells.

3.5.3. Cytotoxic activity of podophyllotoxin-naphthoquinone compounds

Table 3.15. Cytotoxicity of compounds **157a-p**

Entry	**Compound**	**IC $_{50}$ (µM)**				
		KB	**HepG2**	**Lu-1**	**MCF-7**	**Hek-293**
1	**157a**	**1,19 ± 0,01**	**0,42 ± 0,01**	**0,28 ± 0,01**	> 2,50	10.30 ± 0.10
2	**157b**	**2,16 ± 0,07**	**0,44 ± 0,01**	**0,28 ± 0,03**	> 2,50	5.35 ± 0.21
3	**157c**	**2,14 ± 0,11**	**1,50 ± 0,01**	**0,45 ± 0,01**	> 2,50	8.27 ± 0.14
4	**157d**	**1,46 ± 0,01**	**1,35 ± 0,01**	**0,50 ± 0,01**	> 2,50	5.89 ± 0.12
5	**157e**	**0,80 ± 0,01**	**1,24 ± 0,01**	**0,51 ± 0,01**	> 2,50	7.59 ± 0.17
6	**157f**	**0,88 ± 0,02**	**2,06 ± 0,02**	**0,88 ± 0,05**	> 2,50	8.17 ± 0.04
7	**157g**	**1,90 ± 0,02**	**0,41 ± 0,01**	**0,41 ± 0,01**	> 2,50	1.41 ± 0.01
8	**157h**	> 2,50	> 2,50	> 2,50	> 2,50	14.68 ± 0.12
9	**157i**	> 2,50	**1,68 ± 0,07**	**1,02 ± 0,05**	> 2,50	7.61 ± 0.05
10	**157j**	**1,23 ± 0,01**	**0,46 ± 0,01**	**< 0,040**	> 2,50	9.35 ± 0.44
11	**157k**	**1,71 ± 0,07**	**< 0,036**	**< 0,036**	> 2,50	7.20 ± 0.05
12	**157l**	**1,16 ± 0,01**	**< 0,039**	**< 0,039**	**2,19 ± 0,02**	1.47 ± 0.04
13	**157m**	**1,54 ± 0,01**	**< 0,044**	**< 0,044**	**1,98 ± 0,04**	1.34 ± 0.01
14	**157n**	**> 2,50**	**2,24 ± 0,01**	**1,65 ± 0,03**	**> 2,50**	**21.59 ± 0.40**
15	**157o**	**2,34 ± 0,26**	**> 2,50**	**2,22 ± 0,04**	**> 2,50**	**9.95 ± 0.01**
16	**157p**	**1,86 ± 0,02**	**0,47 ± 0,01**	**0,08 ± 0,01**	> 2,50	4.78 ± 0.14
Ellipticin		1.75 ± 0.03	1,66 ± 0,03	1,54 ± 0,03	1,58 ± 0,03	1,69 ± 0,04

Table 3.16. Cytotoxicity of compounds **157 qx**

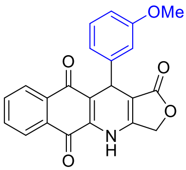
Entry	**Compound**	**IC $_{50}$ (µM)**				
		KB	**HepG2**	**A549**	**MCF-7**	**Hek-293**
1	**157q**	2,54 ± 0,01	3,13 ± 0,01	4,42 ± 0,01	2,65 ± 0,01	21.87 ± 0.01
2	**157r**	**2,36 ± 0,01**	3,24± 0,01	**1,13 ± 0,07**	3,43 ± 0,03	10.04 ± 0.10
3	**157s**	> 5,00	> 5,00	> 5,00	> 5,00	40.06 ± 0.21
4	**157t**	> 5,00	> 5,00	> 5,00	> 5,00	21.91 ± 0.26
5	**157u**	**1,86 ± 0,03**	> 5,00	**1,82 ± 0,01**	2,69 ± 0,03	1.54 ± 0.14
6	**157v**	**0,60 ± 0,01**	**1,05 ± 0,03**	> 5,00	**2,12 ± 0,04**	3.68 ± 0.02
7	**157x**	**1,11 ± 0,01**	**1,11 ± 0,03**	**1,39 ± 0,10**	2,59 ± 0,03	1.08 ± 0.01
Ellipticin		1.53 ± 0.04	1,50 ± 0,03	1,58 ± 0,03	1,83 ± 0,07	6,33 ± 0,04

Table 3.17. Cytotoxicity of compounds **157aa - ah**

Entry	**Compound**	**IC $_{50}$ (µM)**				
		KB	**HepG2**	**A549**	**MCF-7**	**Hek-293**
1	**157aa**	**0,57 ± 0,02**	**0,63 ± 0,02**	**0,43 ± 0,01**	**1,61 ± 0,03**	2.03 ± 0.06
2	**157ab**	> 2,50	> 2,50	**1,59 ± 0,03**	**2,23 ± 0,06**	6.46 ± 0.15
3	**157ac**	> 2,50	> 2,50	**1,12 ± 0,04**	> 2,50	7.17 ± 0.16
4	**157ad**	> 2,50	> 2,50	**2,03 ± 0,05**	> 2,50	> 20
5	**157ae**	**0,52 ± 0,02**	**0,53 ± 0,02**	**1,10 ± 0,02**	**2,28 ± 0,07**	2.23 ± 0.08
6	**157af**	**0,02 ± 0,01**	**0,02 ± 0,01**	**0,62 ± 0,02**	**0,12 ± 0,03**	0.03 ± 0.01
7	**157ag**	**0,62 ± 0,01**	> 2,50	**0,61 ± 0,02**	**1,96 ± 0,04**	1.81 ± 0.05
8	**157ah**	> 2,50	> 2,50	> 2,50	> 2,50	> 20
Ellipticin		1.15 ± 0.01	1,51 ± 0,05	1,60 ± 0,02	1,82 ± 0,03	6,18 ± 0,11

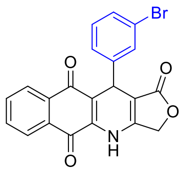
The results showed that most of synthesized podophyllotoxin-naphthoquinone compounds **157** exhibited inhibitory effect on cancer cells tested at different concentrations, 26/31 compounds displayed high potent inhibitory activities with $IC_{50} < 2,5$ µM.

In general, podophyllotoxin-naphthoquinones **157a-x, 127aa-ah** had less cytotoxic inhibitory activity against MCF-7 cancer cell line than other cancer cell lines with IC_{50} > 2,50 μM. Many synthetic podophyllotoxin-naphthoquinone compounds were shown to have higher inhibitory activity than these of the reference ellipticine. In particular, compounds **157j** (Ar = 3-$MeOC_6H_4$), **157k** (Ar = 3-BrC_6H_4), **157l** (Ar = 3-$NO_2C_6H_4$), **157m** (Ar = C_6H_5) and **157af** (Ar = 3,4,5-$(MeO)_3C_6H_2$) were found to be the most potent antineoplastic agents with IC_{50} < 40 nM against HepG2, SK- Lu-1 and KB cells.



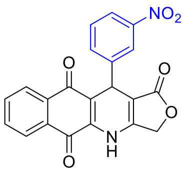
157j

IC_{50} (Lu-1) < 0,04 μM



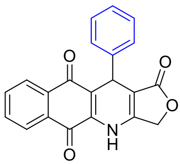
157k

IC_{50} (Lu-1, HepG2) < 0,036 μM



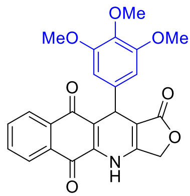
157l

IC_{50} (Lu-1, HepG2) < 0,039 μM



157m

IC_{50} (Lu-1, HepG2) < 0,044 μM



157af

IC_{50} (Lu-1, HepG2) < 0,02 μM

Scheme 3.28. Molecular structure and cytotoxic activity of some compounds **157**

Compounds **157h** (Ar = 1-naphthyl), **157s** (Ar = 2,6-F_2-4-$MeOC_6H_2$), **157t** (Ar = 2-CF_3-4-$MeOC_6H_3$), **157ah** (Ar = 5-Br-pyridin-2-yl) were less cytotoxic to all 4 cancer cell lines than other compounds with IC_{50} > 2,50 μM. In addition, all compounds **157a-ah** (except **157af)** exhibited low toxicity or moderate to Hek-293 cell line with IC_{50} more than 1,08 μM, even many compounds more than 20 μM.

In addition, the most potent compounds **157j** and **157k** were investigated further to assess their ability to influence cell cycle progression and apoptosis in SK-Lu-1 cells. The results confirmed that tested compounds possessed anti-proliferative activity through concentrationdependently inducing a significant G2/M-phase arrest, which is a representative feature shared by tubulin polymerization inhibitors. In addition, compounds **157j** and **157k** enhanced 4-5-fold caspase-3/7 activity as compared to Ellipticine. Tested with Annexin V and PI/dead cell apoptosis®, compounds **157j,k** exhibited the anti-proliferative effect through dose-dependently triggering cellular apoptosis of SK-Lu-1 cells, particularly in the early apoptotic stage.

Additionally, molecular docking studies were performed and showed importance interaction of two compounds against residues in the colchicine-binding-site of tubulin as well. Compounds **157j** and **157k** exhibited activity as a classical tubulin inhibitor, similarly interacted with residues at the zone 1 and 2 of CBS and provided lower affinity against tubulin target than colchicine.

CONCLUSIONS

1. Developed 4 new processes for the successful syndissertation of quinone-fused heterocyclic derivatives through multicomponent domino reactions; synthesized 80 new quinone-fused heterocyclic derivatives, which have not been published in previous documents, including:

- 23 3-Benzoyl-4*H*-benzo[*g*]chromene-5,10-dione compounds **155a-w** (the reaction yield is from 60 to 88%)

- 31 Podophyllotoxin-naphthoquinone compounds **157a-x**, **157aa-ah** (the reaction yield is from 69 to 89%)

- 17 *N*-Arylated-dihydrobenzo[*g*]quinoline-5,10-dione compounds **160a–q** (the reaction yield is from 69 to 86%)

- 09 Benzo[*a*]pyridazino[3,4-*c*]phenazine compounds **161a-i** (the reaction yield is from 41 to 63%)

1. The cytotoxic activity of 57 synthesized compounds was evaluated against cancer cell lines including epidermoid carcinoma (KB), hepatoma carcinoma (HepG2), non-small lung (SK-Lu-1 or A549) and breast (MCF-7) cancer cell lines. The results showed that 43 compounds exhibited cytotoxicity against tested cancer cell lines, of which 25 compounds showed higher cytotoxic activity than those of the reference ellipticine. Compounds **157i**, **157j**, **157k**, **157l**, **157af** displayed the highest cytotoxic activity with IC_{50} < 50 nM.

2. Through research on assay of caspase-3/7 enzymatic activities, assay of morphology, and apoptosis analysis, it has been proven that the compounds podophyllotoxin-naphthoquinone **157j**, **157k** have the ability to trigger apoptosis of Lu-1 human cancer cells, leading to inhibition of cell proliferation. In addition, cell cycle and molecular docking studies initially demonstrated that compounds **157j** and **157k** also have tubulin polymerization inhibitory activity.

NEW CONTRIBUTIONS OF THE DISSERTATION

2. Developed 4 new processes for the successful syndissertation of quinone-fused heterocyclic derivatives through multicomponent domino reactions.

3. Synthesized 80 new quinone-fused heterocyclic derivatives, which have not been published in previous documents, including:

- 23 3-Benzoyl-4*H*-benzo[*g*]chromene-5,10-dione compounds **155a-w**.
- 31 Podophyllotoxin-naphthoquinone compounds **157a-x**, **157aa-ah**.
- 17 *N*-Arylated-dihydrobenzo[*g*]quinoline-5,10-dione compounds **160a–q**.
- 09 Benzo[*a*]pyridazino[3,4-*c*]phenazine compounds **161a-i**.

4. The cytotoxic activity of 57 synthesized compounds was evaluated against cancer cell lines including epidermoid carcinoma (KB), hepatoma carcinoma (HepG2), non-small lung (SK-Lu-1 or A549) and breast (MCF-7) cancer cell lines. The results showed that 43 compounds exhibited cytotoxicity against tested cancer cell lines, of which 25 compounds showed higher cytotoxic activity than those of the reference ellipticine. Compounds **157i**, **157j**, **157k**, **157l**, **157af** displayed the highest cytotoxic activity with $IC_{50} < 50$ nM.

5. The initial findings have indicated the anticancer mechanism of podophyllotoxin-naphthoquinone compounds **157j** and **157k** by arresting the G2/M phase of the cell cycle, activating caspase-3/7 enzymes, and inducing apoptosis. Additionally, the tubulin inhibition mechanism of these two compounds has also been proposed through molecular docking simulations.

LIST OF PUBLICATIONS

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4. Nguyen Ha Thanh, Hoang Thi Phuong, Le Thi Tu Anh, Le Nhat Thuy Giang, **Nguyen Thi Quynh Giang**, Nguyen Tuan Anh, Dang Thi Tuyet Anh and Phan Van Kiem, 2022, Syndissertation and Cytotoxic Evaluation of Fluoro and Trifluoromethyl Substituents Containing Novel Naphthoquinone-Fused Podophyllotoxins, *Natural Product Communications*, Volume 17(10): 1–6.

5. Ha Thanh Nguyen, Giang Le-Nhat-Thuy, Phuong Hoang Thi, **Quynh Giang Nguyen Thi**, Tuan Anh Nguyen, Thu Ha Nguyen Thi, Tuyet Anh Dang Thi, and Tuyen Van Nguyen, 2022, Microwave-Assisted Three-Component Syndissertation of Novel *N*-arylated-Dihydrobenzo[*g*] quinoline-5,10-Diones and Their Potential Cytotoxic Activity, *Chemistry & Biodiversity*, 19(8), e202200359.

6. Giang Le-Nhat-Thuy, Tuyet Anh Dang Thi, **Quynh Giang Nguyen Thi**, Phuong Hoang Thi, Tuan Anh Nguyen, Ha Thanh Nguyen, Thu Ha Nguyen Thi, Hoang Sa Nguyen, Tuyen Van Nguyen, 2021, Syndissertation and biological evaluation of novel benzo[*a*]pyridazino[3,4-*c*]phenazine derivatives, *Bioorganic & Medicinal Chemistry Letters*, Volume 43, 128054.