Synthetic studies towards Fiscalin B and Fumiquinazoline G

Project I report

Natália Marina Andrade Barbosa Lopes

Porto, 2017

Synthetic studies towards Fiscalin B and Fumiquinazoline G

Project I

2016/ 2017

Author: Natália Marina Andrade Barbosa Lopes

Advisor: Professora Doutora Maria Emília Sousa

CoAdvisor: Professora Madalena Maria de Magalhães Pinto

Date of Evaluation:

Evaluators:

Classification:

Acknowledgments

In this section I would like to thank to Professora Doutora Maria Emília Sousa for this opportunity to integrate this research project, for her support and guidance that helped me to go ahead with this work.

To Professora Doutora Madalena Pinto, coadvisor and responsible of LQOF for the facilities to develop this project.

To Doutora Solida Long for all the help, experience, patience and cooperation that allowed me to conclude this project.

To Doutora Sara Cravo for all the assistance given throughout the research project.

To my colleague and friend Liliana Moreira for all the support, patience, happiness and hours spent with me in the lab and Joana Moreira for her orientation and knowledge.

To all my friends for all the support.

To my family for supporting me and giving me the opportunity to dedicate several hours beyond the classes to this project.

This work was developed in Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia da Universidade do Porto. This research was partially supported through national funds provided by FCT – Foundation for Science and Technology and European Regional Development Fund (ERDF) and COMPETE under the projects PEst-C/MAR/LA0015/2013, PTDC/MAR-BIO/4694/2014, PTDC/AAG-TEC/0739/2014, and INNOVMAR - Innovation and Sustainability in the Management and Exploitation of Marine Resources, reference NORTE-01-0145-FEDER-000035, Research Line NOVELMAR.

Part of this work was presented in one poster communication:

Poster communication: Lopes, N., Long,S., Resende,D., Kijjoa, A., Silva, A., Pinto, M., Sousa, E. Synthetic Strategies for Fiscalin B and Analogues, IJUP17 – 10th Meeting of Young Researchers of University of Porto, University of Porto, Porto, Portugal, 8-10 February 2017.

Abstract

Marine natural products have been explored through decades. The necessity to find new treatments to several diseases have motivated the study of many marine compounds that have reveal important activities. One of the problems in the investigation of these compounds is the isolation process to furnish sufficient amounts for full characterization. To overcome this problem, research often resort to the total synthesis.

Fiscalin B (**5**) is a 1,4-dialkyl derivative with a pyrazino[2,1-*b*] quinazoline-3,6-dione system produced by *Neosartorya fischeri*, a fungus that grows in humid environments. This compound can inhibit the binding of substance P (SP) to the neurokin (NK-1) receptor. Fumiquinazoline G (**7**), also a pyrazino[2,1-*b*] quinazoline, is isolated from *Aspergillus fumigatus*, a fungus present on the gastrointestinal tract of *Pseudolabrus japonicus*. This compound inhibits the proliferation of tsFT210 and P388 cells revealing antitumor activity.

The main objectives of this project were to synthetize fiscalin B (**5**) and fumiquinazoline G (**7**) through two different approaches: a multistep procedure and by using one pot reaction.

The multistep synthesis was investigated to obtain fiscalin B (**5**). This procedure included four reactions and three amino acids: anthranilic acid (**10**), L-valine (**12**) and D-tryptophan (**11**). With this procedure, it was only possible to conclude successfully two out the four reactions initially planned.

Fiscalin B (**5**) was successful synthetize by the one pot reaction (yield: 10 %). This procedure involved thermal heating during 16 h between anthranilic acid (**10**) and a protect L-valine (**16**) followed by the addiction of D-tryptophan methyl ester (**11**) and microwave heating. The same procedure was tried to obtain fumiquinazoline G (**7**), using a protect D-alanine (**27**) instead of L-valine (**16**), but proved to be unsuccessful. Since the synthesis of fumiquinazoline G (**7**) wasn't accomplish through this one pot reaction method, it was investigate the multistep method, previously used in the synthesis of fiscalin B (**5**). With this procedure, three of the four reactions were successfully accomplished. However, fumiquinazoline G (**7**) could not be synthetize through any of these methods.

In this project, five compounds were synthetized, with NDTALAFORM (**29**) being a new compound, and characterized by infrared and ^{1}H NMR. Although total synthesis of the target marine compounds was not fully accomplished by the multistep procedure, the

synthetic pathway followed can be further investigated to proceed to this target compound.

Keywords: fiscalin B; fumiquinazoline G; total synthesis.

Index

Index of figures

Index of tables

Index of schemes

Abbreviations and symbols

^{13}C NMR= carbon nuclear magnetic resonance

^{1}H NMR= proton nuclear magnetic resonance

Boc= *tert*-butyloxycarbonyl

Bu_3P= tributylphosphine

d= doublet

dd= double doublet

ddd= double double doublet

DIPEA= *N*,*N*-diisopropylethylamine

DMAP= 4-dimethylaminopyridine

dt= doublet of triplets

Et_3N= triethylamine

EtOAc= ethyl acetate

Fmoc= 9-fluorenyl-methyloxycarbonyl

IR = infrared spectroscopy

J = coupling constant

m = multiplet

MeCN= acetonitrile

MeOH = methanol

MW= microwave

NK= neurokinin

Ph_3P= triphenylphosphine

Rf= retention factor

rt= room temperature

SP= substance P

t = triplet

TBTU= 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate

TLC= thin-layer chromatography

UV= ultraviolet

1. Introduction

1.1 The importance of marine natural products

Marine organisms provided numerous novel compounds with sensational multiple pharmacological properties. The necessity of novel therapeutics has gain more importance especially because of the resistance associated to the current therapeutics and the inexistent treatments for incurable diseases. Earth's surface is 70% cover by sea and this environment offers unique conditions for marine species to grow, which contributes to biodiversity of the compounds produced by these species.[1]

The isolation process and the supply is one of the difficulties in obtaining marine compounds. To overcome these problems, new techniques are being implemented such as in synthetic chemistry and fermentation, as well as in biotechnology.

Several molecules of marine origin have been investigated in respect to their effects on different pathologies. Marine species and their compounds have a significant role in the discovery novel components that can be successfully be used in numerous biomedical applications. These include anticancer, antibacterial, anticoagulant, antidiabetic, antifungal, antiviral, anti-inflammatory and antiprotozoal activities.[2,3]

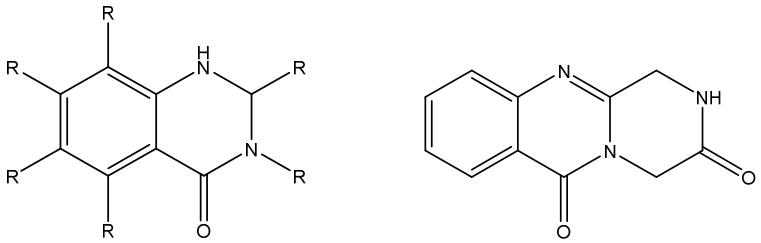
The applications of marine products are not restricted to the search for solutions to pathological problems. These compounds can be used for other purposes, such as in biotechnology. In marine biotechnology engineering, scientific principles are applied to provides goods and services. Marine products have also significant importance in the development of biomaterials, health care diagnostics, aquaculture and seafood safety.[4]

One of the applications of marine microorganisms is the biodegradation of pollutants, being for example *Pseudomonas chlororaphis* of valuable. This bacterium is capable to produce pyoverdine, the compound that can catalyzes the degradation of organotin compounds.[4] Marine products such as proteases can be used for industrial applications. Marine proteases are very different of those found in internal or external organs of invertebrates and vertebrates. Factors as pH and temperature are important for the activity, catalytic efficiency, stability and proper functioning of these enzymes. The fact that fish's proteinases have thermal stability and high activity at low temperatures make these enzymes great candidates for being used in the industry. However, the use of marine proteases is still under experimental stage.[5]

Other application of marine compounds is related to their antifouling effect. Biofouling is the accumulation process of organisms on man-made objects submerged in the marine waters. This can be a severe problem for shipping, offshore aquaculture and coastal industries and several marine natural products are being investigated for this application.[6]

1.2 Fumiquinazolines and fiscalins

Fumiquinazolines and fiscalins are a class of compounds that have in common the quinazolinone structure (**1**) (**figure 1**). Particularly, these groups consist in a fusion of two heterocycles and belong to a subclass know as pyrazinoquinazoline alkaloids (**2**) (**figure 1**). This subclass has the same structure as quinazolinone with a piperazine fused ring.



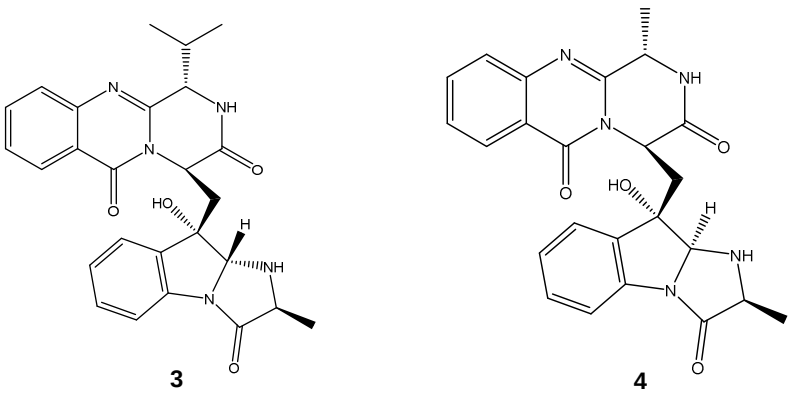
1 **2**

Figure 1: Quinazolinone (**1**) and pyrazinoquinazoline (**2**) structures

The proximity of these groups of compounds is evidenced by the structural similarity between fiscalin A (**3**) and fumiquinazoline A (**4**) (**figure 2**).[7]

Comentário [NL1]: Estrutura alterada

Comentário [NL2]: Estrutura alterada



3 **4**

Figure 2: Fiscalin A (**3**) and fumiquinazoline A (**4**)

1.3 Isolation, structure characterization, and activities of fiscalin B (5)

Fiscalins A (**3**), B (**5**) and C (**6**) (**figures 2** and **3**) were first reported in 1993 when they were found in culture broth produced by *Neosartorya fischeri*.[8] *N.fischeri* is a fungus which grows in humid environments like soil, putrefying vegetation, and organic remains.[9]

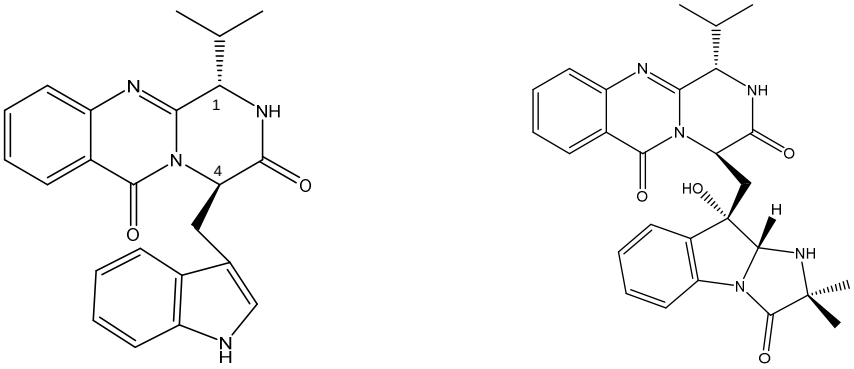


Figure 3: Fiscalin B (**5**) and C (**6**)

Fiscalin B (**5**) is a 1,4-dialkyl derivative of the pyrazino[2,1-*b*] quinazoline-3,6-dione system, containing an indoyl moiety linked to an anthranilic acid derived tricyclic system, and presents two stereogenic centers at C-1 and C-4.[10]

In its first report [10], the structure of fiscalin B (**5**) was determine by X-ray crystallography, spectroscopy and chiral amino acids analysis.

The molecular formula of fiscalin B (**5**) ($C_{23}H_{22}N_4O_2$) was obtained by the mass spectrum by the molecular ion $[M+H]^+$. In this spectrum two major fragments were observed. The first one with $C_{14}H_{15}N_3O_2$ unit with m/z 257.1158 and the second one with C_9H_8N unit with m/z 130.0649 and then the UV (ultraviolet) spectrum indicated the presence of two chromophores: quinazolone and indole. The indole ring was associated with the C_9H_8N unit and quinazolone chromophore was associated with the $C_{14}H_{15}N_3O_2$ unit.[8]

The ^1H NMR (proton nuclear magnetic resonance) spectrum suggested the presence of several units: two D_2O exchangeable protons that belongs to NH protons, units like $(CH_3)_2CH$-CH, -CH_2-CH and the protons of two *ortho* substituted aromatic rings (eight aromatic protons). In ^{13}C NMR (carbon-13 nuclear magnetic resonance) it was observed two carbonyl carbon signals. This data was confirmed by IR absorption bands at 1685 cm^{-1} which belongs to C=O and 3400 and 3279 cm^{-1} which belongs to NH.[8]

The complete characterization of fiscalin B (**5**) was also complemented by COSY (correlated spectroscopy) and HETCOR (heteronuclear correlation) experiments.

Fiscalins A (**3**), B (**5**), and C (**6**) have the ability of inhibit the binding of substance P (SP) to the human neurokinin (NK-1) receptor.[8] These compounds have shown inhibitory constants (Ki) of 57µM, 174 µM, and 68 µM, respectively. SP is a peptide that acts as a

neurotransmitter and neuromodulator. SP is secreted by nerves and inflammatory cells such as macrophages, eosinophils, lymphocytes and dendritic cells.[11]

SP is synthesized in the ribosome as a precursor as a larger protein that is enzymatically converted into the active undecapeptide.[12] This peptide is distributed in the central and peripheral nervous system and regulates cardiovascular and respiratory function besides the activation of the emetic reflex.[12] SP also participates in neurotransmission of pain and noxius stimuli in the spinal cord. In the peripheral system is present in the neurons from gastrointestinal, respiratory and genitourinary tracts.[12] NK-1R is one of the receptors that belongs to tachykinin receptors, like NK-2R and NK-3R. NK-1R is activated by SP, neurokinin A and neurokinin B but the relative affinity of the last two is 100- and 500-folder lower than for SP.[13]

When SP bind to NK-1 receptor is capable of inducing several physiological responses like smooth muscle contraction, salivation and vasodilation. Besides that, SP could be involved in inflammatory response and pain transmission.[8] Increased responses to SP and NK-1R expression are associated to asthma patients and have been reported in the rectum and colon of patients with inflammatory bowel disease. Besides, augmented levels of SP are found in patients with rheumatoid arthritis.[11]

1.4 Isolation, structure characterization and activities of fumiquinazoline G (7)

Fumiquinazoline G (**7**) (**figure 4**) was isolated from a strain of *Aspergillus fumigatus* present on the gastrointestinal tract of *Pseudolabrus japonicus*, with fumiquinazolines A-F.[14]

The molecular formula of fumiquinazoline G (**7**) ($C_{21}H_{18}N_4O_2$) was established from HREIMS (high-resolution electron ionization mass spectrometry) data. In this spectrum two major fragments were observed. The first one with $C_{12}H_{10}N_3O_2$ unit with m/z 228.0773 and the second one with C_9H_8N unit with m/z 130.0656. The ^{1}H and ^{13}C NMR spectra of fumiquinazoline G (**7**) were compared with fumiquinazoline F (**8**). The differences between these compounds were compared with the differences between fumiquinazolines A (**4**) and B (**9**). The structural differences of fumiquinazolines A (**4**) and B (**10**) are similar to the fumiquinazoline F (**8**) and G (**7**) (**figure 4**).[14]

Comentário [NL3]: Estrutura alterada

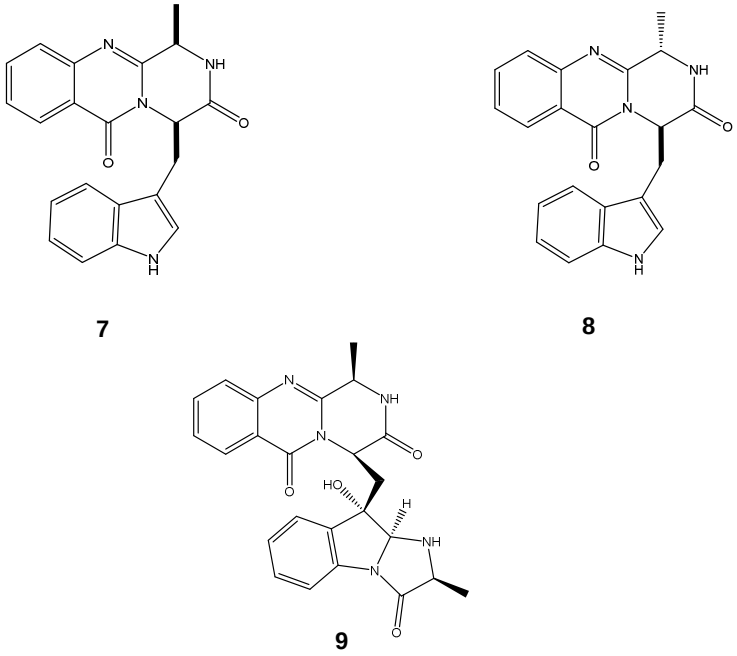


Figure 4: Fumiquinazoline G (**7**), F (**8**) and B (**9**)

Fumiquinazoline G (**7**) has reveal inhibitory activity against the proliferation of tsFT210 cells and also inhibited the proliferation of P388 cells (ED_{50} 13.8 µg/ml). For this reason, fumiquinazoline G (**7**) was proposed as an interesting model to pursuit antitumor agents.[15]

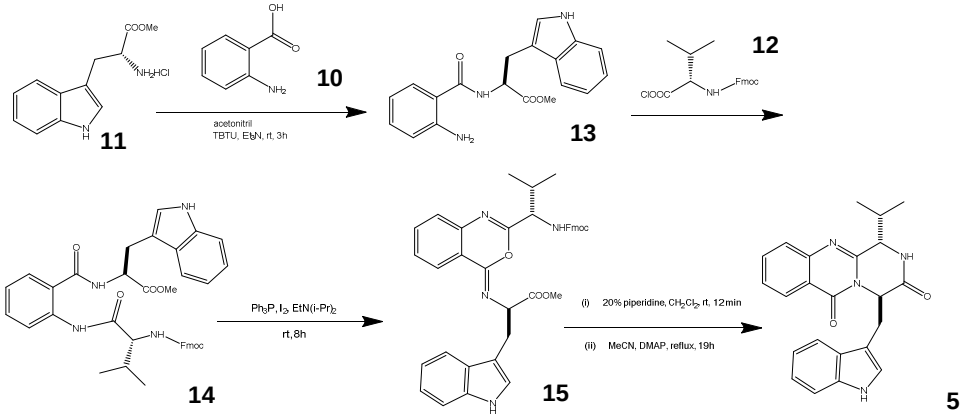
1.5 Synthesis of fiscalin B (5) and fumiquinazoline G (7)

1.5.1 Multistep approach

The synthesis of fiscalin B (**5**) and fumiquinazoline G (**7**) was accomplished in 2000 by Wang and Ganesan.[16] To synthetize fiscalin B (**5**) three amino acids were used: anthranilic acid (**10**), D-tryptophan methyl ester (**11**), and fmoc-L-valine-Cl (**12**).[16]

This synthesis involved four steps. In the first step, there is the direct coupling of D-tryptophan methyl ester (**11**) with anthranilic acid (**10**) to get the first intermediate compound NDT (**13**). The second step consisted in the coupling of compound **12** with compound **13** under the two-phase Schotten-Baumann conditions to get the tripeptide NDTLV (**14**). In the third step occurs the dehydratation promoted by Ph_3P/I_2 and tertiary amine combination, previously used to dehydrate *β*-keto amides to oxazoles, to get the oxazine (**15**). Since the amides have an approximate acidity to a ketone, this approach

was tried and it was successful. The last step to get the fiscalin B (**5**) was promoted through (i) fmoc deprotection by piperidine of the compound **15** and (ii) overnight in reflux with acetonitrile/DMAP (**scheme 1**).[16]



Scheme 1: The multistep approach to obtain fiscalin B (**5**). TBTU= 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate, Et_3N= triethylamine, Fmoc= 9-fluorenyl-methyloxycarbonyl, Ph_3P= triphenylphosphine, $EtN(i\text{-}Pr)_2$= *N*,*N*-diisopropylethylamine, rt= room temperature, h=hour, MeCN= acetonitrile, DMAP= 4-dimethylaminopyridine

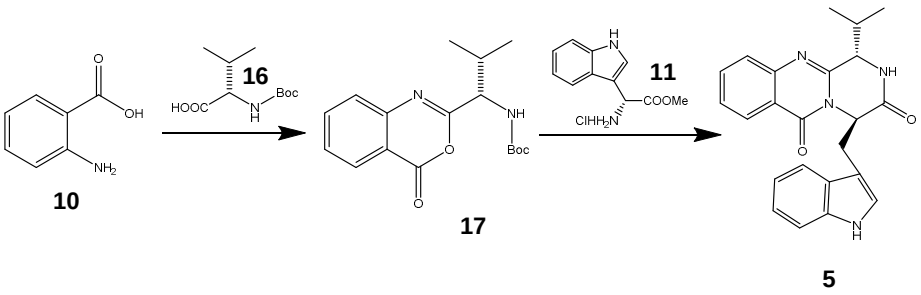
The same approach was followed to obtain fumiquinazoline G (**7**), using fmoc-D-alanine-Cl (**27**) instead fmoc-L-valine-Cl (**12**) and proved to be successful.

1.5.2 Microwave-promoted three-component one-pot reaction

Later, Liu and his colleagues were able to synthetize fiscalin B (**5**) by a microwave-promoted three-component one-pot reaction (**scheme 2**). First it was necessary promoting the thermal heating during 16 h between anthranilic acid (**10**) and boc-L-valine (**16**) to get the intermediary compound- benzoxazine-4-one (**17**).[17]

Then, the addiction of D-tryptophan methyl ester hydrochloride (**11**) and microwave heating allowed to get the fiscalin B (**5**), with only 20 % yield and 50 % ee (enantiomeric excess). This yield can be justified by the steric hindrance created by the isopropyl group from boc- L-valine (**16**).[17]

Comentário [NL4]: Estrutura alterada

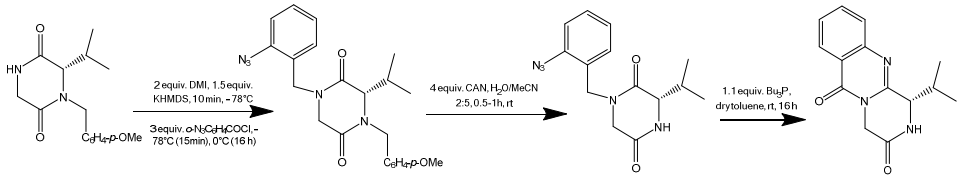


Scheme 2: Synthesis of fiscalin B (**5**) by the microwave-promoted three-component one-pot reaction. Boc= *tert*-butyloxycarbonyl

1.5.3 Other methodologies to synthetize fiscalin B (5)

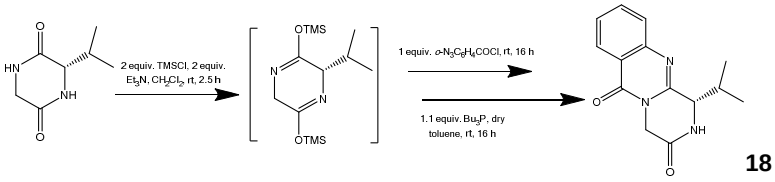
The use of (1*S*)-1-isopropyl (**18**) derivative of 2,4-dihydro-1*H*-pyrazino[2,1*b*] quinazoline-3,6-diones as starting material was another approach to obtain fiscalin B (**5**). The synthesis of compound **18** was achieved by two methods (**scheme 3**).[10]

Method A



18

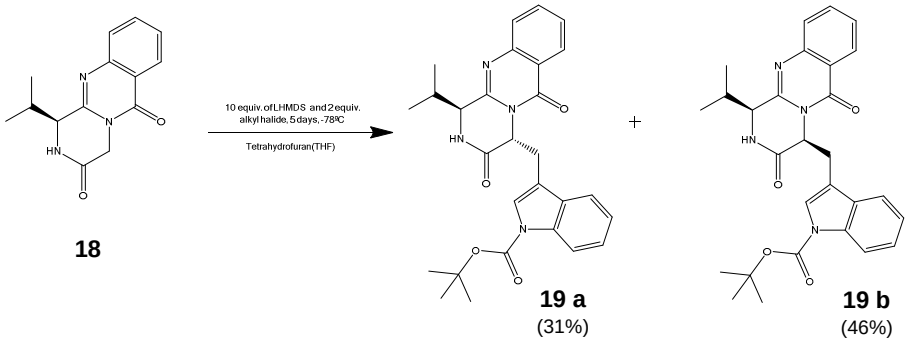
Method B



18

Scheme 3: Different methods to obtain compound **18**. DMI= 1,3-dimethyl-2-imidazolidinone, KHMDS= potassium bis(trimethylsilyl)amide, CAN= ceric ammonium nitrate, Bu_3P= tributylphosphine, TMSCl= trimethylsilyl chloride, Et_3N= triethylamine, rt= room temperature, h= hour

The alkylation of compound **18** with *N*-boc-3-indolylmethyl bromide resulted in an *anti*-isomer (**19 a**) and in a *syn*-isomer (**19 b**) that was the major product (**scheme 4**).



18

19 a (31%)

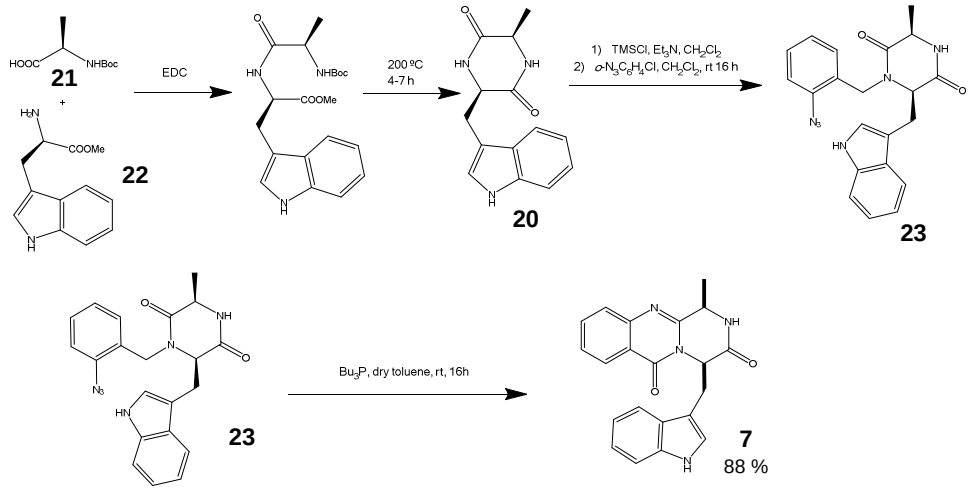
19 b (46%)

Scheme 4: Alkylation of the compound **18**. LHMDS= lithium hexamethyldisilazide

The deprotection of the *anti*-isomer (**19 a**) with boron tribromide gave fiscalin B (**5**) with 88% yield for this reaction.[10]

1.5.4 Other methodologies to synthetize fumiquinazoline G (7): regioselective *N*-acylation

The use of aryl-methylpiperazine-2,5-diones (**20**) as starting materials was another approach to obtain fumiquinazoline G (**7**). Compound **20** can be prepared in two steps by standard methods from boc-D-alanine (**21**) and D-tryptophan methyl ester (**22**). The reaction of compound **20** with chlorotrimethylsilane (TMSCl) in dichloromethane, in the presence of trimethylamine gave highly regioselectivity for compound **23**. The treatment with Bu_3P and dry toluene furnished fumiquinazoline G (**7**) in 88 % yield (**scheme 5**).[18]

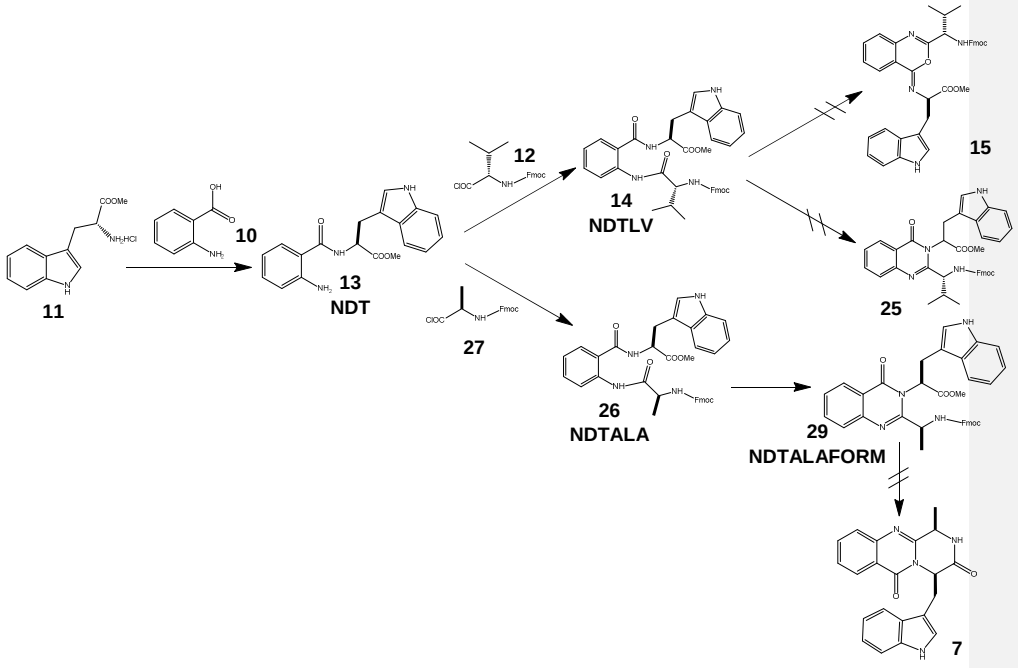


Scheme 5: Regioselective *N*-acylation approach to synthetize fumiquinazoline G (**7**). EDC= *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride, TMSCl= chlorotrimethylsilane, Et_3N= trimethylamine, Bu_3P= tributylphosphine, rt= room temperature

2. Results and discussion

The aim of this project was to synthetize fiscalin B (**5**) and fumiquinazoline G (**7**) using two different approaches: the multistep procedure and the one pot reaction. The multistep procedure was first tried with fiscalin B (**5**). In this procedure, it was synthetized compound **14** and following two different approaches were investigated to get compounds **15**

(Mazurkiewicz-Ganesan approach) and **25**, but both proved to be unsuccessful (**scheme 6**). Since this multistep procedure was unsuccessful to obtain fiscalin B (**5**), the one pot reaction was followed (**scheme 12**) (firstly performed by Dr. Solida Long) and fiscalin B (**5**) was obtained. The same approaches were investigated to obtain fumiquinazoline G (**7**), but both procedures failed in furnishing fumiquinazoline G (**7**). Nevertheless, by the multistep approach precursors **26** and **29** were obtained but approaches to obtain compound **7** failed (**scheme 6**).



Scheme 6: Multistep approach to synthetize fiscalin B (**5**) and fumiquinazoline G (**7**) followed in this project

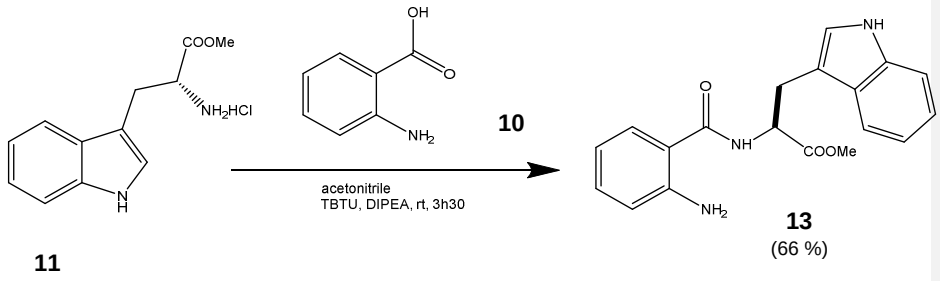
2.1 Attempts to obtain fiscalin B (5) by the multistep procedure

2.1.1 Synthesis of *N*-(2-aminobenzoyl)-D-tryptophan methyl ester (NDT) (13)

The first reaction of the multi-step synthesis of fiscalin B (**5**) consists in the direct coupling of D-tryptophan methyl ester (**11**) with anthranilic acid (**10**) mediated by TBTU (2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate) and DIPEA (*N,N*-diisopropylethylamine) that function as coupling reagents.

In this reaction, anthranilic acid (**10**) was stirred with acetonitrile until complete dissolution and then it was added the coupling reagents TBTU and DIPEA. After half an hour, the reaction was checked by TLC to verified the presence of the anthranilic acid copulated with TBTU. This compound appeared with a brown spot and with a higher retention factor (Rf) relative to the anthranilic acid (**10**) and TBTU. The absence of anthranilic acid (**10**) allowed the addiction of the D-tryptophan methyl ester (**11**). Thirty minutes after the last addition of compound **11**, the presence of a new compound with blue fluorescence and a lower Rf than of compound **10** copulated with TBTU was observed, but unreacted starting materials could be detected. After 3 h, no alterations could be observed and the reaction was considered to be finished (**scheme 7**).

After extraction to remove anthranilic acid (**10**) and D-tryptophan methyl ester (**11**) with Na_2CO_3 and HCl, respectively, the extract was purified by flash chromatography to reach compound **13** in 66 % yield.

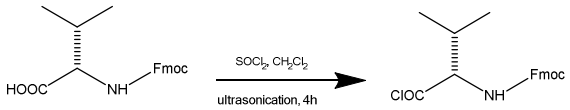


Scheme 7: Coupling reaction between D-tryptophan methyl ester (**11**) with anthranilic acid (**10**) to obtain the intermediate compound NDT (**13**). TBTU= (2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate) and DIPEA= (*N,N*-diisopropylethylamine) rt= room temperature

2.1.2 Synthesis of fmoc-D-valine-chloride (12)

In this reaction, the aim was to convert the carboxyl acid to acid chloride using $SOCl_2$ to activate this protect amino acid **24**. This conversion proceeds through a substitution reaction (SN_2) resulting into compound **12**, HCl and SO_2 (**scheme 8**). Ultrasonic stirring was applied to accelerate the reaction.[19]

Hexane was added to a CH_2Cl_2 solution to allow the insolubilization of the fmoc-D-valine-OH (**24**) with compound **12** remaining soluble in hexane. After filtration, the solvent was evaporated and compound **12** was obtained in a 37% yield.



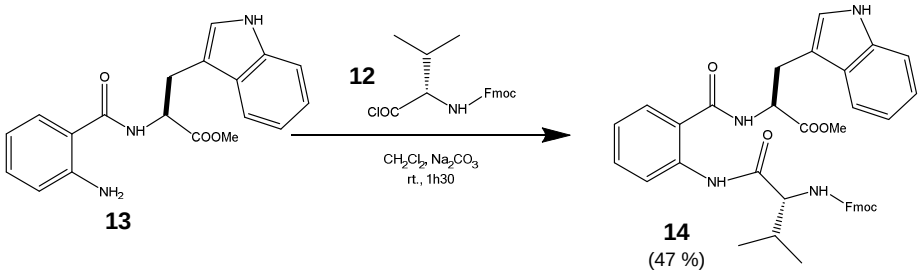
24 **12** (37 %)

Scheme 8: Conversion of fmoc-D-valine-OH (**24**) into fmoc-D-valine-Cl (**12**)

2.1.3 Synthesis of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-L-valyl-2-aminobenzoyl-D-tryptophan methyl ester (NDTLV) (14)

The synthesis of compound **14** was accomplished under the two-phase Schotten-Baumann conditions (**scheme 9**). This method consists in the synthesis of an amides from an amine and an acid chloride. For the reaction to proceed, Na_2CO_3 is added to neutralize the acid that is formed. In these conditions two phases are present: the water phase contains Na_2CO_3 to neutralize the acid and the organic phase contains the starting material and the products.

In this reaction, compounds **12** and **13** were dissolved in anhydrous CH_2Cl_2 with stirring. After 30 min, Na_2CO_3 was added and 1 h after the reaction was finished but a small amount of compound **13** remained (**scheme 9**). The extraction was performed using HCl to eliminate Na_2CO_3. Flash chromatography was further used to purify compound **14** that was obtained with a 47% reaction yield.



14 (47 %)

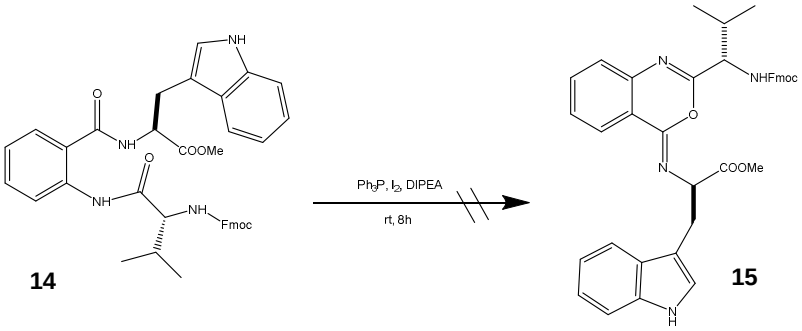
Scheme 9: Synthesis of NDTLV (**14**) using the two-phase Schotten-Baumann conditions

2.1.4 Synthesis of *N*-{2-[(*S*)-1-*N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino- 2-methylpropyl]-4*H*-3,1-benzoxazin-4-ylidene}-D-tryptophan methyl ester (15)

Mazurkiewicz-Ganesan approach

In this reaction, a previously described approach to get oxazoles from dehydration of *β*-keto amides, was applied.[16] For this procedure, Ph_3P, I_2 and a tertiary amine (DIPEA) combination were used for compound **14** to dehydrated and an oxazine ring to be formed, leading to compound **15** (**scheme 10**).

Therefore, NDTLV (**14**) was dissolved in CH_2Cl_2 and then it was added Ph_3P, I_2 and DIPEA. The reaction proceeded during 8 h and after that time compound **14** could still be detected in the reaction, but the reaction was stopped since a new compound with a lower Rf than compound **14** could be detected by TLC, being an indicative of the presence of the desirable compound **15**. Once Ph_3P in an interferent in the purification process, the reaction mixture was evaporated and dissolved in CH_2Cl_2 and then hexane was added. This solvent was used to insolubilize compound **15** from the reaction mixture since Ph_3P is soluble in hexane. After that, the solid was purified by flash chromatography. All the fractions were checked but the compound that was initially observed in the TLC wasn't present leading to the hypothesis that a decomposition in the purification procedure might occurred.



Scheme 10: Approaches to obtain compound **15** using dehydration of *β*-keto amides method

2.1.5 Synthesis of methyl 2-(2-((*R*)-1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-methylpropyl)-4-oxoquinazolin-3(4*H*)-yl)-3-(1*H*-indol-3-yl)propanoate (25)

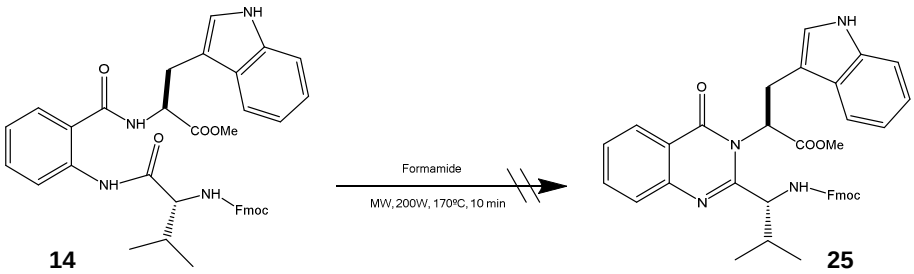
Since the other method was ineffective to get the desirable intermediate compound **15**, a different approach was tried.

Compounds that contain diamine groups were previously described to be subjected to microwave-assisted cyclocondensation to give the desired quinazolinones (**25**). The use of formamide allowed to obtain the best results also using programmed irradiation at 200 W and a fixed temperature of 170 ºC. With this approach, the conversion was completed after 10 min. This method suggests that formamide is the source of ammonia for the introduction of N-3 in the quinazolinone ring.[20]

Comentário [NL5]: Estrutura alterada

Therefore, NDTLV (**14**) was used to proceed to the formation of a quinazolinone intermediate using the same conditions. After 10 min, total consumption of NDTLV (**14**) led to end the reaction (**scheme 11**).

Nevertheless, after purification by preparative chromatography, the desirable compound could not be detected. During this procedure, a conversion of the product to NDTLV (**14**) seemed to occur since it was detected again by TLC analysis. ^{1}H NMR analysis revealed the compound obtained to be NDTLV (**14**).



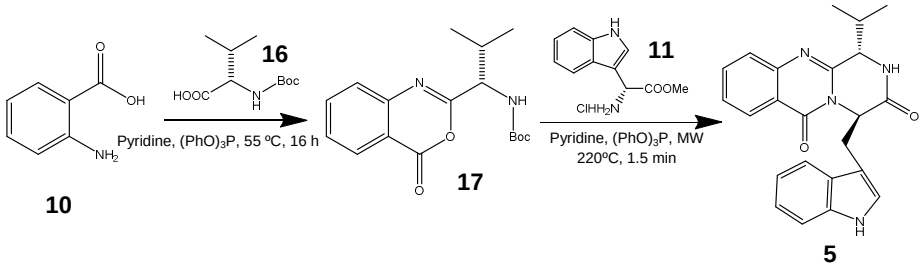
Scheme 11: Approaches to synthesize compound **25** by cyclocondensation with formamide. MW= microwave

2.2 One pot synthesis of fiscalin B (5)

During the multistep procedure, the coupling of anthranilic acid (**10**), protected valine (**16**), and tryptophan methyl ester hydrochloride (**11**) was investigated in parallel to this project and was successfully achieved by the microwave-promoted three-component one-pot reaction (performed by Dr. Solida Long). Anthranilic acid (**10**) reacted with *N*-boc-valine (**16**) to form boc-protected benzoxazin-4-one (**17**) by thermal heating. The addition of tryptophan methyl ester hydrochloride (**11**) led to fiscalin B (**5**) by microwave irradiation (**scheme 12**).

Fiscalin B (**5**) was purified in this project by flash chromatography followed by preparative chromatography.

Comentário [NL6]: Estrutura alterada



Scheme 12: Synthesis of fiscalin B (**5**) achieved by the microwave-promoted three-component one-pot reaction. $(PhO)_3P$= triphenyl phosphite, MW= microwave

The same procedure, the one pot synthesis was applied to obtain fumiquinazoline G (**7**) but proved unsuccessful in obtaining the desirable compound (performed by Dr. Solida Long). Therefore, in the following section, attempts to obtain compound **7** by the multistep procedure are described.

2.3 Attempts to obtain fumiquinazoline G (7) by the multistep procedure

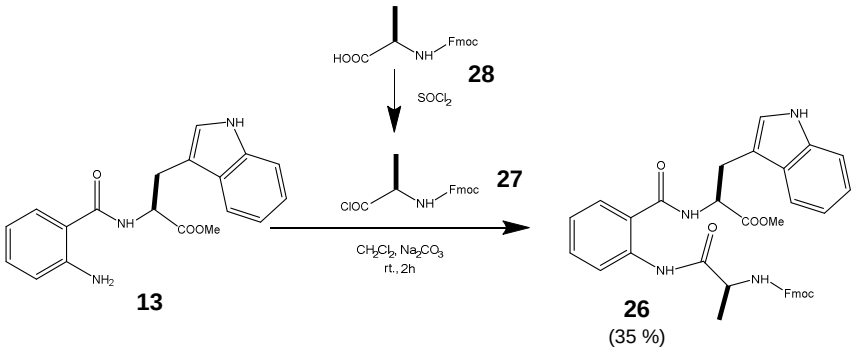
2.3.1 Synthesis of *N*-(2-aminobenzoyl)-D-tryptophan methyl ester (NDT) (13)

A multistep approach was envise to obtain fumiquinazoline G (**7**) as presented previously in **scheme 7**.

2.3.2 Synthesis of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-D-alanyl-2-aminobenzoyl-D-tryptophan methyl ester (NDTALA) (26)

In this reaction, the synthesis of fmoc-D-alanine-Cl (**27**) followed the same procedure as described before, starting from fmoc-D-alanine-OH (**28**) with $SOCl_2$. Following, the synthesis of compound **26** was accomplished also by the two phase Schotten-Baumann conditions (**scheme 13**). The extraction was performed using H_2O and CH_2Cl_2. Compound **26** was purified by flash chromatography. Nevertheless, fractions containing compound **26** remained with traces of NDT (**13**) and the solid thus obtained was further used in the next reaction without further purifications.

Comentário [NL7]: Estrutura alterada

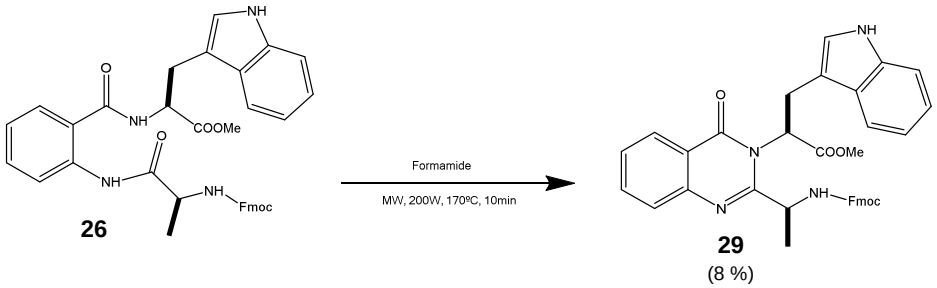


Scheme 13: Synthesis of NDTALA (**26**) using the two-phase Schotten-Baumann conditions. rt= room temperature

2.3.3 Synthesis of methyl (*S*)-2-(2-((*S*)-1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-4-oxoquinazolin-3(4*H*)-yl)-3-(1*H*-indol-3-yl)propanoate (NDTALAFORM) (29)

A similar approach used in **section 2.1.5** was followed (**scheme 14**). It was hypothesized that, in this case, since fmoc-D-alanine-Cl (**27**) doesn't have the isopropyl group, this difference could facilitate the reaction because the steric hindrance is smaller.

After cooling the reaction mixture into ice, the extraction was performed following a purification performed by preparative chromatography to get compound **29** in an 8 % yield for the next reaction. The lower yield can be justified due the conversion of NDTALAFORM (**29**) in NDTALA (**26**) observed during the purification process.



26 **29** (8 %)

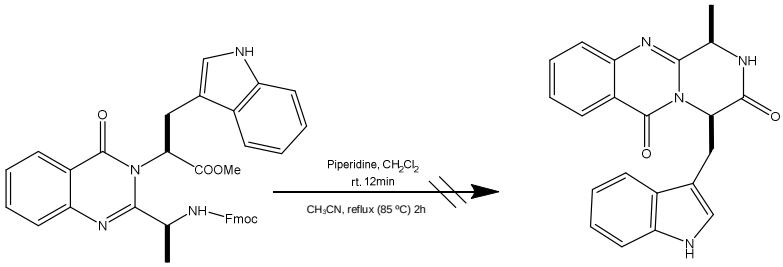
Scheme 14: Synthesis of NDTALAFORM (**29**) using formamide and microwave reaction. MW= microwave

2.3.4 Synthesis of (1*R*,4*R*)-4-(1*H*-indol-3-ylmethyl)-1-methyl-2*H*-pyrazino- [2,1-*b*]quinazoline-3,6(1*H*,4*H*)-dione (fumiquinazoline G) (7)

This final reaction consists in two steps. The first one aims to deprotect compound **29** from the fmoc group. [21] This group can be removed using primary and secondary amines (e.g. piperidine). The second step consists on the cyclization to the third ring to form the final compound **7**.

In this case, piperidine was the amine chosen to remove the fmoc group (**scheme 15**). After 60 min, the reaction was checked by TLC and no changes were observed.

To verified if the problem was with the capacity of piperidine to remove fmoc group, this amine was tested using fmoc-D-alanine-OH (**28**) and checked by TLC and the deprotect D-analine-OH was detected. Thus, the problem with the reaction wasn't the piperidine choice of deprotection method. Nevertheless, more research would be needed to accomplish this final step.



29 **7**

Scheme 15: Approaches to obtain fumiquinazoline G (**7**)

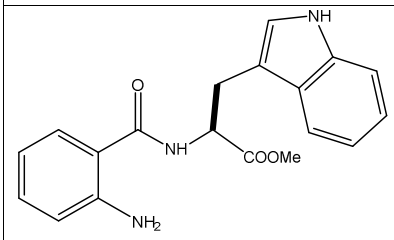
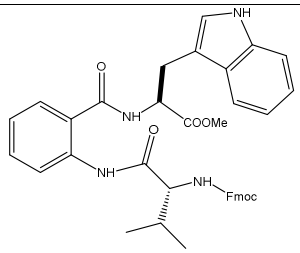
3. Structure elucidation

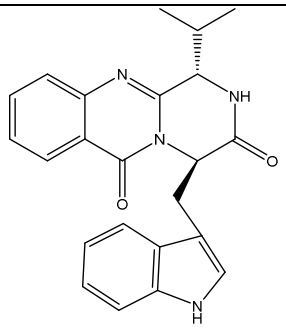
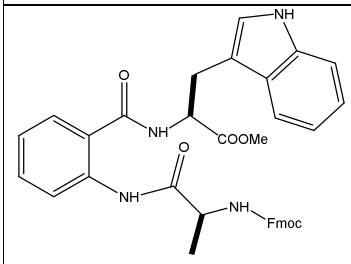
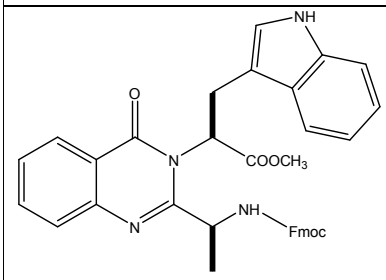
The structure characterization of the compounds was stablished by melting points determination, IR, NMR and polarimetric techniques. In this section, it will be presented IR and ^{1}H NMR data for the compounds synthesized.

In **table 1**, the IR data for compounds **5**, **13**, **14**, **26**, and **29** is presented. In the **tables 2-6**, the ^{1}H NMR of compounds **5**, **13**, **14**, **26**, and **29** is presented

3.1 IR spectroscopy data

Table 1: IR data of the synthesized compounds

Compound	Group	*v* (cm^{-1})
 **NDT (13)**	N-H (amide)	3423
	C=O (ester)	1746
	C=O (amide)	1644
 **NDTLV (14)**	N-H (amide)	3418
	C=O (ester)	1723
	C=O (amide)	1621
	C-N	1350

 **Fiscalin B (5)**	N-H (amide)	3346
	Aromatic	3063, 1596,1471
	C=N	1683
	C-N	1293
 **NDTALA (26)**	N-H (amide)	3329
	C=O (amide)	1647
	C-N	1340
 **NDTALAFORM (29)**	N-H (amine)	3427
	C=N	1663
	C=O (tertiary amide)	1610

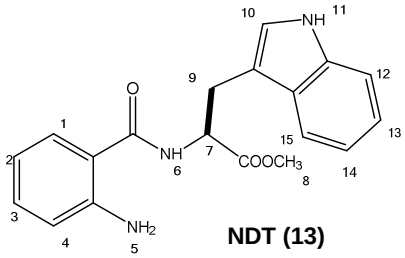
Comentário [NL8]: Estrutura alterada

The IR spectra of NDT (**13**), NDTLV (**14**) and NDTALA (**26**) indicates characteristic bands belonging to major functional groups which are similar between them such as N-H stretch (3329-3423 cm^{-1}) and C=O stretch (1621-1644). The difference between NDT (**13**) and NDTLV (**14**) or NDTALA (**26**) is the coupling of amino acid, fmoc-L-valine-Cl (**12**) and fmoc-D-alanine-Cl (**27**) respectively. These coupling reactions doesn't imply the formation of new functional groups. For this reason, IR spectra of these compounds are very similar.

In the opposite, the IR spectra of NDTALA (**26**) and NDTALAFORM (**29**) have differences that suggest the success of the reaction that allowed to obtain NDTALAFORM (**29**) using NDTALA (**26**) as starting material. The band at 1663 cm^{-1}, that correspond to C=N,

doesn't appear at the IR spectrum of NDTALA (**26**) but it's present in the IR spectrum of NDTALAFORM (**29**). This band demonstrates that the quinazolinone intermediate (**29**) was formed. This same band also appears in the IR spectrum of fiscalin B (**5**) since this compound has the quinazolinone structure.

3.2 ^{1}H NMR spectroscopy data

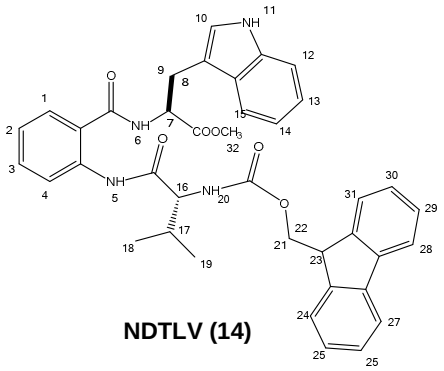


NDT (13)

Table 2: ^{1}H NMR data* of NDT (**13**)

Protons	δH
H-1	7.57 (d, 1H, *J*=8.5)
H-2, H-3 and H-13	7.22-7.16 (m, 3H)
H-4	7.36 (d, 1H, *J*=8.1)
H-6	6.60 (br d, 1H, *J*=1.0)
H-7	5.08 (dd, 1H, *J*=5.3, 2.2)
H-8	3.72 (s, 3H)
H-9	3.43 (d, 2H, *J*=5.4)
H-10	6.56 (t, 1H, *J*=0.8)
H-11	8.11 (br s, 1H)
H-12	7.03 (d, 1H, *J*=2.4)
H-14	7.10 (t, 1H, *J*=7.5)
H-15	6.67 (dd, 1H, *J*=8.7,1.1)

***Values in ppm (δH) relative to $(CH_3)_4Si$ as an internal reference. *J* values are in Hz.

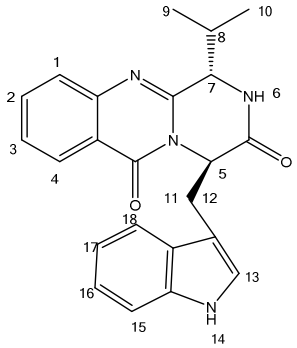


NDTLV (14)

Table 3: ^{1}H NMR data* of NDTLV (**14**)

Protons	δH	Protons	δH
H-1	8.55 (d, 1H, *J*=8.6)	**H-17**	2.20 (m, 1H)
H-2	7.77 (d, 2H, *J*=7.6)	**H-18**	1.02 (d, 3H, *J*=6.8)
H-3		**H-19**	0.97 (d, 3H, *J*=6.8)
H-4	7.63 (br d, 1H, *J*=7.3)	**H-20**	5.56 (d, 1H, *J*=9.0)
H-5	11.01 (br s, 1H)	**H-21**	4.45-4.42 (m, 2H)
H-6	6.99 (br s, 1H)	**H-22**	
H-7	5.09 (dd, 1H, *J*=12.0, 7.49)	**H-23**	4.19 (dd, 1H, *J*=8.9, 5.2)
H-8	3.46 (dd, 1H, *J*= 14.7, 4.5)	**H-24**	7.51-7.25 (m, 8H)
H-9	3.22 (dd, 1H, *J*=14.8, 6.9)	**H-25**	
H-10	6.69 (d, 1H, *J*=8.0)	**H-26**	
H-11	8.57 (br s, 1H)	**H-27**	
H-12	7.67 (br d, 1H, *J*=10.6)	**H-28**	
H-13	7.15 (t, 1H, *J*=7.4)	**H-29**	
H-14	7.07-7.01 (m, 2H)	**H-30**	
H-15		**H-31**	
H-16	4.27 (t, 1H, *J*=6.7)	**H-32**	3.74 (br s, 3H)

*****Values in ppm (δH) relative to $(CH_3)_4Si$ as an internal reference. *J* values are in Hz.



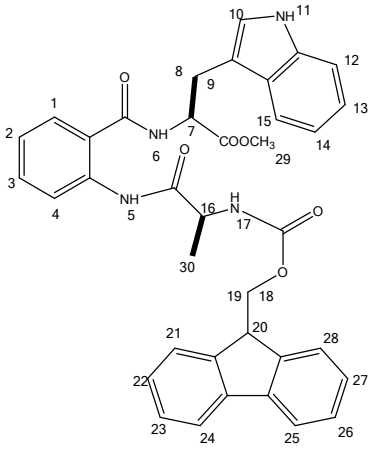
Fiscalin B (5)

Table 4: ^{1}H NMR data* of fiscalin B (**5**)

Comentário [NL9]: Estrutura alterada

Protons	δH	Protons	δH
H-1	7.57 (d, 1H, *J*=3.4)	**H-10**	0.63 (d, 3H, *J*=4.6)
H-2	7.78 (ddd, 1H, *J*=7.7, 8.1, 1.5)	**H-11**	3.72 (d, 1H, *J*=2.7)
H-3	7.55 (t, 1H, *J*=3.4)	**H-12**	3.69 (dd, 1H, *J*=17.8, 4.0)
H-4	8.37 (dd, 1H, *J*=8.0, 1.0)	**H-13**	6.60 (d, 1H, *J*=2.4)
H-5	5.67 (dd, 1H, *J*=5.3, 2.8)	**H-14**	8.03 (br s, 1H)
H-6	5.64 (br s, 1H)	**H-15**	7.28 (d, 1H, *J*=8.2)
H-7	2.69 (d, 1H, *J*=2.5)	**H-16**	7.13 (t, 1H, *J*=7.5)
H-8	2.63 (m, 1H)	**H-17**	6.93 (t, 1H, *J*=7.5)
H-9	0.65 (d, 3H, *J*=4.0)	**H-18**	7.44 (d, 1H, *J*=8.1)

***Values in ppm (δH) relative to $(CH_3)_4Si$ as an internal reference. *J* values are in Hz.

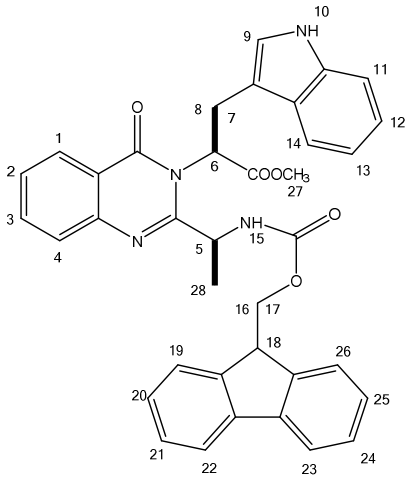


NDTALA (26)

Table 5: ^{1}H NMR data* of NDTALA (**26**)

Protons	δH	Protons	δH
H-1	8.58 (d, 1H, *J*=9.0)	**H-16**	4.35 (m, 1H)
H-2	7.76 (d, 2H, *J*=6.0)	**H-17**	5.56 (s, 1H)
H-3		**H-18**	4.45 (d, 2H, *J*=6.0)
H-4	7.66 (d, 1H, *J*=6.0)	**H-19**	
H-5	11.48 (br s, 1H)	**H-20**	4.27 (d, 1H, *J*=6.0)
H-6	6.96 (s, 1H)	**H-21**	7.49-7.26 (m, 8H)
H-7	5.04 (dt, 1H, *J*= 9.8, 3.8)	**H-22**	
H-8	3.37 (dd, 2H, *J*=3.0)	**H-23**	
H-9		**H-24**	
H-10	6.71 (d, 1H, *J*=9.0)	**H-25**	
H-11	8.13 (br s, 1H)	**H-26**	
H-12	7.59 (d, 1H, *J*=9.0)	**H-27**	
H-13	7.18 (t, 1H, *J*=6.0)	**H-28**	
H-14	7.07 (d, 1H, *J*=9.0)	**H-29**	3.73 (s, 3H)
H-15	7.02 (d, 1H, *J*=9.0)	**H-30**	1.54 (d, 3H, *J*=9.0)

***Values in ppm (δH) relative to $(CH_3)_4Si$ as an internal reference. *J* values are in Hz.



NDTALAFORM (29)

Table 6: ^{1}H NMR data* of NDTALAFORM (**29**)

Protons	δH	Protons	δH
H-1	8.28 (dd, 1H, *J*=6.0, 3.0)	**H-15**	5.24 (br s, 1H)
H-2	7.77 (m, 2H)	**H-16**	2.17 (s, 2H)
H-3		**H-17**	
H-4	7.72 (dd, 1H, *J*=9.0, 3.0)	**H-18**	3.99 (t, 1H, *J*=6.0)
H-5	4.20 (t, 1H, *J*=3.0)	**H-19 and H-26**	7.41 (d, 2H, *J*=6.0)
H-6	5.35 (dd, 1H, *J*=10.5, 4.5)	**H-20**	7.62-7.46 (m, 6H)
H-7	3.75 (d, 1H, *J*=6.0)	**H-21**	
H-8	3.64 (m, 1H)	**H-22**	
H-9	6.83 (d, 1H, *J*=3.0)	**H-23**	
H-10	8.15 (br s, 1H)	**H-24**	
H-11	7.34 (d, 1H, *J*=9.0)	**H-25**	
H-12	7.17 (m, 1H)	**H-27**	3.81 (s, 3H)
H-13	7.08 (dd, 1H, *J*=10.5, 9.0)		
H-14	7.31 (d, 1H, *J*=6.0)	**H-28**	1.26 (s, 3H)

***Values in ppm (δH) relative to $(CH_3)_4Si$ as an internal reference. *J* values are in Hz.

The ^{1}H NMR data for NDT (**13**) reveal twelve signals corresponding to fourteen protons. The most deshielded proton was assigned to the indole proton (H-11) since this proton is an exchangeable proton and is assigned to the singlet at δ_H 8.11 ppm while another amine proton, H-6, shows a doublet at δ_H 6.60 ppm due to the coupling between its proton and proton in position H-7. Six signals corresponding to eight aromatic protons namely H-1, H-2, H-3, H-4, H-12, H-13, H-14 and H-15 were detected. The most deshielded aromatic proton corresponds to H-1 and its signal appears at δ_H 7.57 ppm. These evidences could be justified by the anisotropy of the carbonyl group and aromatic ring that have impact in deshielding this proton. The signal corresponding to H-4 appears at δ_H 7,36 ppm as a doublet, H-2, H-3 and H-13 signals appear as multiplet at δ_H 7-22-7,16 ppm, H-12, H-14 and H-15 signals appear at δ_H 7.03, 7.10 and 6.67 ppm as a doublet, triplet, and double of doublets, respectively.

The signal corresponding to H-10 is observed at δ_H 6.56 ppm due the effect of Sp^2 hybridization and electronegativity of indole nitrogen that makes the signal of H-10 to have a higher chemical shift than typical double bond protons.

For the methyl proton H-8, the signal is observed at δ_H 3.72 ppm integrating three protons as a singlet due to no neighboring proton and connecting to an ester which has electronegativity effect that can withdraw electronic density of electrons. The signal corresponding to H-7 was detected at δ_H 5.08 ppm as double of doublets. This proton is affected by an electronegativity effect of the ester nearby.

The ^{1}H-NMR spectrum of NDT (**13**) and NDTLV (**14**) or NDTALA (**26**) exhibited different characteristic signals for H-5 protons. In the ^{1}H NMR spectrum of NDT (**13**), the signal corresponding to H-5 was not detected. In ^{1}H NMR spectra of NDTLV (**14**) and NDTALA (**26**), H-5 appeared at δ_H 11.01 (br s, 1H) and 11.48 (br s, 1H), respectively. These signals confirm that the coupling of NDT (**13**) with fmoc-L-valine-Cl (**12**) and fmoc-D-alanine-Cl (**27**) was successfully achieved.

NDTLV (**14**) and NDTALA (**26**) are structurally similar. The differences between these compounds is the isopropyl / methyl group present in NDTLV (**14**) and NDTALA (**26**), respectively. These differences are put in evidence by the signal corresponding to H-18 with δ_H 1.02 ppm and H-19 at δ_H 0.97 ppm of NDTLV (**14**) that didn't appear in NDTALA (**26**). Despite of these differences, NDTLV (**14**) showed a similar ^{1}H NMR profile relatively to NDTALA (**26**).

NDTALA (**26**) is the starting material of NDTALAFORM (**29**). The differences between these compounds is the formation of quinazolinone intermediate. The signals

corresponding H-5 at δ_H 11.48 ppm as broad singlet and of H-6 at δ_H 6.96 ppm as singlet are present in NDTALA (**26**). Through the analysis of ^{1}H NMR spectrum of NDTALAFORM (**29**), these signals are no longer present, which suggests that the reaction was successful. Despite of these differences, NDTALA (**26**) showed a similar NMR profile relatively to NDTALAFORM (**29**).

4. Experimental

4.1 General Methods

All the reagents and solvents were purchased from Sigma Aldrich and were used without further purifications.

Microwave reactions were performed using a Teflon 100 ml in a close system (internal reaction temperature measurements with a fiber-optic probe sensor) and were carried out using an Ethos MicroSYNTH 1600 Microwave Labstation from Milestone.

All reactions were monitored by TLC carried out on precoated plates (silica gel, 60 F254 Merck) with 0.2 mm of thickness; 245 and 365 nm ultraviolet lights (UV) were used as visualizing agent.

The purifications of compounds were performed by flash column chromatography using Fluka analytical silica gel 60 (0.04-0.063 mm).

IR spectra were measured on a KBr microplate in a FTIR spectrometer Nicolet iS10 from Thermo Scientific with Smart OMNI-Transmisson accessory (Software OMNIC 8.3).

^{1}H NMR spectra were taken in $CDCl_3$ at room temperature, on Bruker Avance 300 (300.13 MHz for ^{1}H) spectrometer and were performed in the Departamento de Quimica, Universidade de Aveiro. Chemical shifts are expressed in δ (ppm) values relative to tetramethylsilane (TMS) as an internal reference. Compounds were considered to be pure when using two different chromatographic conditions, a single spot appeared in TLC.

One pot reaction synthesis of fiscalin B (**5**) was performed by CEM single mode microwave oven, model Discover SP, at Universidade de Aveiro, by Dr. Diana Resende.

The optical density was determined by ADP410 polarimeter.

Melting points were measured in open capillary tubes on Electrothermal Melting Point Apparatus Ia6304 and are uncorrected.

4.2 Attempts to obtain fiscalin B (5) by the multistep procedure

4.2.1 Synthesis of *N*-(2-aminobenzoyl)-D-tryptophan methyl ester (NDT) (13)

Anthranilic acid (**10**) (0.278 g, 2.03 mmol) was dissolved with stirring in acetonitrile (25 ml). When the dissolution was completed, it was added TBTU (0.920 g, 2.87 mmol) and DIPEA (0.83ml, 4.78 mmol). This mixture was stirred for 30 min. After that, D-tryptophan (**11**) (0.521 g, 2.39 mmol) was added in 10 portions over 100 min at room temperature with stirring. After 3 h and 30 min, the reaction was finished.

The solvent was evaporated under reduce pressure. The crude product was dissolved in CH_2Cl_2 and washed with 3 x 30 ml HCl (1M), 3 x 30 ml Na_2CO_3 (saturate solution) and 3 x 30 ml H_2O. The organic phase was dried by anhydrous sodium sulfate, filtrated and concentrated under reduced pressure. The residue was purified by flash chromatography (eluent 0.5 % MeOH in CH_2Cl_2) to yield compound **13** as a white solid (0.528 g, 65.53 %): mp 132-135 ºC (lit.[16] mp 136-137 ºC); $[\alpha]^{20}_D$= -64.33 (c 0.057, $CHCl_3$); IR ν_{max} (KBr) 3423, 1746, 1644 cm^{-1}; ^1H NMR δ 8.11 (br s, 1H), 7.57 (d, 1H, *J*=8.5), 7.36 (d, 1H, *J*=8.1), 7.22-7.16 (m, 3H), 7.10 (t, 1H, *J*=7.5), 7.03 (d, 1H, *J*=2.4), 6.67 (dd, 1H, *J*=8.7,1.1), 6.60 (br d, 1H, *J*=1.0), 6.56 (t, 1H, *J*=0.8), 5.08 (dd, 1H, *J*=5.3, 2.2), 3.72 (s, 3H), 3.43 (d, 2H, *J*=5.4).

4.2.2 Synthesis of fmoc-D-valine-chloride (12)

Fmoc-D-valine-OH (**24**) (0.688 g, 2 mmol) was dissolved with stirring in anhydrous CH_2Cl_2 (10 ml). $SOCl_2$ (2 ml, 27.6 mmol) was added in portions over 1 h. The reaction was submitted to ultrasonic stirring for more 2 h.

The solvent was evaporated under reduce pressure and the solid thus obtained was dissolved in CH_2Cl_2. Then, hexane was added and after cooling an insolubilization occurred. The white solid was filtrated and identified by TLC to be fmoc-D-valine-OH (**24**). The filtrate was evaporated under reduce pressure to yield compound **12** as a white solid (0.265 g, 36.57 %).

4.2.3 Synthesis of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-L-valyl-2-aminobenzoyl-D-tryptophan methyl ester (NDTLV) (14)

NDT (**13**) (0.184 g, 0.545 mmol) was dissolved with stirring in anhydrous CH_2Cl_2 (10 ml). Fmoc-D-valine-chloride (**12**) (0.265 g, 0.74 mmol) was added to the previous solution and the reaction was maintained with stirring for 30 min. After that, Na_2CO_3 (10 ml, 10 mmol) was added and the reaction continued for 1h. The extraction was performed using 2 x 30

ml CH_2Cl_2 and 10 ml H_2O. The organic layers were gathered and washed with 2 x 20 ml HCl (1M) and 2 x 20 ml H_2O. Then, the organic phase was dried by anhydrous sodium sulfate, filtrated and concentrated under reduced pressure. The residue was purified by flash chromatography (eluent 100% CH_2Cl_2). The fractions containing the desirable product were gathered, the eluent was concentrated and 50 ml of hexane were added. After cooling, white crystals of compound **14** were obtained as a white solid (0.167 g, 46.71 %): mp 205-206 ºC (lit.[16] mp 197 ºC (dec)); $[\alpha]^{20}_{D}$=104.17 (c 0.048, $CHCl_3$); IR ν_{max} (KBr) 3418, 1723, 1621, 1350 cm^{-1}; ^{1}H NMR δ 11.01 (br s, 1H), 8.57 (br s, 1H), 8.55 (d, 1H, *J*=8.6), 7.77 (d, 2H, *J*=7.6), 7.67 (br d, 1H, *J*=10.6), 7.63 (br d, 1H, *J*=7.3), 7.51-7.25 (m, 8H), 7.15 (t, 1H, *J*=7.4), 7.07-7.01 (m, 2H), 6.99 (br s, 1H), 6.69 (d, 1H, *J*=8.0), 5.56 (d, 1H, *J*=9.0), 5.09 (dd, 1H, *J*=12.0, 7.5), 4.45-4.42 (m, 2H), 4.27 (t, 1H, *J*=6.7), 4.19 (dd, 1H, *J*=8.9, 5.2), 3.74 (br s, 3H), 3.46 (dd, 1H, *J*= 14.7, 4.5), 3.22 (dd, 1H, *J*=14.8, 6.9), 2.20 (m, 1H), 1.02 (d, 3H, *J*=6.8), 0.97 (d, 3H, *J*=6.8).

4.2.4 Synthesis of *N*-{2-[(*S*)-1-*N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino- 2-methylpropyl]-4*H*-3,1-benzoxazin-4-ylidene}-D-tryptophan methyl ester (15)

Mazurkiewicz-Ganesan approach

NDTLV (**14**) (0.167 g, 0.25 mmol) was dissolved in CH_2Cl_2 (15 ml) with stirring followed the addiction of Ph_3P (reaction became yellow), iodine (reaction became orange) and DIPEA (reaction became yellow). The reaction continued during 8 h. The reaction mixture was concentrated and 50 ml of hexane were added. The solid thus obtained was filtered and was purified by flash chromatography (hexane: EtOAc 7:3). The fractions collected revealed to contain starting material **14** and Ph_3P and the desirable product could not be detected.

4.2.5 Synthesis of methyl 2-(2-((*R*)-1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-methylpropyl)-4-oxoquinazolin-3(4*H*)-yl)-3-(1*H*-indol-3-yl)propanoate (25)

NDTLV (**14**) (0.0498 g, 0.076 mmol) and formamide (10 ml) were put in a close vessel and the mixture was irradiated in the microwave at constant temperature at 170 ^{o}C for 10 min. At the end of 10 min several compounds could be detected by TLC and NDTLV (**14**) could no longer be detected. The reaction was considered to be finished. $CHCl_3$ (30 ml) and H_2O (30 ml) were added to the reaction mixture and the extraction was performed using 2 x 50 ml HCl. The organic phase was dried by anhydrous sodium sulfate and the solvent evaporated of the solid thus obtained. The purification was performed by preparative chromatography (CH_2Cl_2: acetone 8:2) to furnish the starting material NDTLV (**14**).

4.3 One pot: synthesis of fiscalin B (5)

In a closed vessel were added anthranilic acid (**10**) (0.028 g, 200 mmol), *N*-boc-L-valine (**16**) (0.044 g, 200 mmol), and Ph_3P (63 ml, 220 mmol) were added along with 1ml of dried pyridine. The reaction mixture was heated at 55 ^oC for 16 h. After cooling the mixture to room temperature, D-tryptophane methylester hydrochloride (**11**) (0.051 g, 200 mmol) was added, and the mixture was irradiated in the microwave at constant temperature at 220 ^oC for 1.5 min. The solvent was evaporated and the crude was purified using flash column chromatography using hexane: EtOAc (80:20) as mobile phase. The fractions containing the desired product were gathered, the eluent evaporated and the solid thus obtained submitted to a preparative chromatography ($CHCl_3$: acetone 100: 15). After extraction with CH_2Cl_2, the solvent was evaporated to furnish a yellow solid corresponding to fiscalin B (**5**) (0.022 g, 10 %): mp 168 -169 ^oC (lit.[16] mp 176.5-178.5 ^oC), $[\alpha]_D^{21}$=-248.1 (c 0.043, $CHCl_3$); IR ν_{max} (KBr) 3346, 3063, 1683, 1596, 1471,1293 cm^{-1} ; ^1H NMR δ 8.37 (dd, 1H, *J*=8.0, 1.0), 8.03 (br s, 1H), 7.78 (ddd, 1H, *J*=7.7, 8.1, 1.5), 7.57 (d, 1H, *J*=3.4), 7.55 (t, 1H, *J*=3.4), 7.44 (d, 1H, *J*=8.1), 7.28 (d, 1H, *J*=8.2), 7.13 (t, 1H, *J*=7.5), 6.93 (t, 1H, *J*=7.5), 6.60 (d, 1H, *J*=2.4), 5.67 (dd, 1H, *J*=5.3, 2.8), 5.64 (br s, 1H), 3.72 (d, 1H, *J*=2.7), 3.69 (dd, 1H, *J*=17.8, 4.0), 2.69 (d, 1H, *J*=2.5), 2.63 (m, 1H), 0.65 (d, 3H, *J*=4.0), 0.63 (d, 3H, *J*=4.6)

4.4 Attempts to obtain fumiquinazoline G (7) by multistep procedure

4.4.1 Synthesis of *N*-(2-aminobenzoyl)-D-tryptophan methyl ester (NDT) (13)

Anthranilic acid (**10**) (0.279 g, 2.04 mmol) was dissolved with stirring in acetonitrile (25 ml) following the addiction of TBTU (0.925 g, 2.87 mmol) and DIPEA (0.83 ml, 4.78 mmol). This mixture was stirred for 30 min. After that, D-tryptophan methyl ester (**11**) (0.600 g, 2.36 mmol) was added in 10 portions over 100 minutes at room temperature with stirring. After 3 h, the reaction mixture was kept overnight.

The reaction mixture was evaporated under reduce pressure and the solid thus obtained was dissolved in CH_2Cl_2 and washed with 3 x 30 ml HCl, 3 x 30ml Na_2CO_3 (saturated solution) and 3 x 30ml H_2O. The organic phase was dried by anhydrous sodium sulfate, filtrated and concentrated under reduced pressure. Analyzing by TLC the organic layer it was detected a small amount of D-tryptophan methyl ester (**11**) but the solid thus obtained proceeded to the following reaction without further purifications (0.407 g, 51.19 %): mp

132-135 ºC (lit.[16] mp 136-137 º C); $[\alpha]^{20}_{D}$= -64.33 (c 0.057, $CHCl_3$); IR ν_{max} (KBr) 3423, 1746, 1644 $cm^{-1;}$ ^{1}H NMR δ 8.11 (br s, 1H), 7.57 (d, 1H, *J*=8.5), 7.36 (d, 1H, *J*=8.1), 7.22-7.16 (m, 3H), 7.10 (t, 1H, *J*=7.5), 7.03 (d, 1H, *J*=2.4), 6.67 (dd, 1H, *J*=8.7,1.1), 6.60 (br d, 1H, *J*=1.0), 6.56 (t, 1H, *J*=0.8), 5.08 (dd, 1H, *J*=5.3, 2.2), 3.72 (s, 3H), 3.43 (d, 2H, *J*=5.4).

4.4.2 Synthesis of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-D-alanyl-2-aminobenzoyl-D-tryptophan Methyl Ester (NDTALA) (26)

NDT (**13**) (0.403 g, 1.19 mmol) was dissolved with stirring in anhydrous CH_2Cl_2 (20 ml) and fmoc-D-alanine-chloride (**27**) (0.551 g, 1.67 mmol) was added and the reaction was maintained with stirring for 4 min. After that, Na_2CO_3 (5 ml, 5 mmol) was added and the reaction continued for 2 h. To the reaction mixture it was added 20 ml H_2O and the extraction was performed using 2 x 30ml CH_2Cl_2. The organic layer was collected and it was dried by anhydrous sodium sulfate, filtrated and concentrated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2: MeOH gradient) and then the fractions containing the desirable product were gathered (1 % MeOH in CH_2Cl_2) and the eluent evaporated. The solid obtained was submitted to preparative chromatography (eluent $CHCl_3$: MeOH: EtOAc (95:5:1)). The compound was extracted from silica using 300 ml $CHCl_3$, 300ml EtOAc and 200 ml MeOH to yield compound **26** as a white solid (0.261 g, 34.71%): mp 173-190 ºC (lit.[16] mp 192-194 ºC (dec)), IR ν_{max} (KBr) 3329, 1647, 1340 cm^{-1}; ^{1}H NMR δ 11.48 (br s, 1H), 8.58 (d, 1H, *J*=9.0), 8.13 (br s, 1H), 7.76 (d, 2H, *J*=6.0), 7.66 (d, 1H, *J*=6.0), 7.59 (d, 1H, *J*=9.0), 7.49-7.26 (m, 8H), 7.18 (t, 1H, *J*=6.0), 7.07 (d, 1H, *J*=9.0), 7.02 (d, 1H, *J*=9.0), 6.96 (s, 1H), 6.71 (d, 1H, *J*=9.0), 5.56 (s, 1H), 5.04 (dt, 1H, *J*= 9.8, 3.8), 4.45 (d, 2H, *J*=6.0), 4.35 (m, 1H), 4.27 (d, 1H, *J*=6.0), 3.73 (s, 3H), 3.37 (dd, 2H, *J*=3.0), 1.54 (d, 3H, *J*=9.0).

4.4.3 Synthesis of methyl (*S*)-2-(2-((*S*)-1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-4-oxoquinazolin-3(4*H*)-yl)-3-(1*H*-indol-3-yl)propanoate (NDTALAFORM) (29)

NDTALA (**26**) (0.261 g, 0.415 mmol) and formamide (10 ml) were put in a close vessel and the mixture was irradiated in the microwave at a constant temperature of 170 ^{o}C for 10 min. At the end of 10 min several compounds were observed and NDTALA (**26**) was no longer detected. The reaction mixture was cooled into ice and the extraction was performed using 3 x 100ml $CHCl_3$. The organic layer was dried by anhydrous sodium sulfate, filtrated and concentrated under reduced pressure. The residue was purified by preparative chromatography ($CHCl_3$: acetone 9:1) to give compound **29** as a white solid (0.0206 g, 8.1 %): mp=211-220ºC, $[\alpha]^{20}_{D}$=100.77 (c 0.043, $CHCl_3$); IR ν_{max} (KBr) 3427,

1663, 1610 cm^{-1}; ^1H NMR δ 8.28 (dd, 1H, *J*=6.0, 3.0), 8.15 (br s, 1H), 7.77 (m, 2H), 7.72 (dd, 1H, *J*=9.0, 3.0), 7.62-7.46 (m, 6H), 7.41 (d, 2H, *J*=6.0), 7.34 (d, 1H, *J*=9.0), 7.31 (d, 1H, *J*=6.0), 7.17 (m, 1H), 7.08 (dd, 1H, *J*=10.5, 9.0), 6.83 (d, 1H, *J*=3.0), 5.35 (dd, 1H, *J*=10.5, 4.5), 5.24 (br s, 1H), 4.20 (t, 1H, *J*=3.0), 3.99 (t, 1H, *J*=6.0), 3.81 (s, 3H), 3.75 (d, 1H, *J*=6.0), 3.64 (m, 1H), 2.17 (s, 2H),1.26 (s, 3H).

4.4.4 Synthesis of (1*R*,4*R*)-4-(1*H*-Indol-3-ylmethyl)-1-methyl-2*H*-pyrazino- [2,1-*b*]quinazoline-3,6(1*H*,4*H*)-dione (fumiquinazoline G) (7)

NDTALAFORM (**29**) (0.0225 g, 0.037 mmol), piperidine (0.25 ml), CH_2Cl_2 (1 ml) were put together with stirring for 30 min. After that time, more 0.25 ml of piperidine were added. The reaction was checked by TLC and the formation of new products could not be detected.

5. Conclusions

In this project, two approaches to obtain fiscalin B (**5**) and fumiquinazoline G (**7**) were followed.

A multistep approach was first tried to obtain fiscalin B (**5**), but the target compound could not be obtained. In the third reaction (**section 2.1.4**), during the purification process, the compound obtained seems to decompose to its precursor **14**. When a different approach, using formamide and microwave heating instead of Ph_3P and I_2 were investigated, a decomposed product was also detected.

On the contrary, fiscalin B (**5**) was successfully synthetized by an one pot reaction. This procedure proved to be more effective and faster than the multistep method since fewer reagents and steps are needed to obtain the final compound. However, the yield of one pot reaction was low (10 %) and when applied to obtain fumiquinazoline G (**7**) didn't prove to be successful.

The synthesis of fumiquinazoline G (**7**) was then investigated by the multistep method, the same approach used in the multistep synthesis of fiscalin B (**5**). In this procedure, the third reaction (**section 2.3.3**) was successful, unlike in the synthesis of fiscalin B (**5**). This can be explained by the fact that the purification process was faster and the compound **29** doesn't decompose totally, but the yield of this reaction is low (8 %). However, the final step (**section 2.3.4**) wasn't accomplished. This reaction consisted in the deprotection of fmoc-D-alanine (**27**) and aimed obtaining the quinazoline structure. The deprotection with piperidine wasn't successful and to discard the hypothesis of piperidine being ineffective in this reaction, fmoc-D-alanine-OH (**28**) was submitted to the reaction with piperidine and a deprotect D-alanine-OH was obtained. Future work in investigating the final step of fumiquinazoline G (**7**) synthesis to overcome the deprotection of fmoc group will be necessary.

Compounds **5**, **13**, **14**, **26** and **29** were characterized by ^{1}H NMR, IR, optical density and melting point that allowed to stablish the proposed structures and a new compound was synthetized, NDTALAFORM (**29**) that deserves to be fully characterized.

6. References

1. Montaser, R., Luesch, H. (2011) *Marine natural products: a new wave of drugs?,* Future Medicinal Chemistry, 3(12), 1475-1489.
2. Bailon-Moscoso, N., Cevallos-Solorzano, G., Romero-Benavides, J.C., Orellana, M.I. (2007) *Natural Compounds as Modulators of Cell Cycle Arrest: Application for Anticancer Chemotherapies,* Current genomics, 18(2),106-131.
3. Mayer, A.M., Rodriguez, A.D., Taglialatela-Scafati, O., Fusetani, N. (2013) *Marine pharmacology in 2009-2011: marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous systems, and other miscellaneous mechanisms of action,* Marine drugs, 11(7), 2510-2573.
4. Thakur, N.L., Thakur, A.N. (2006) *Marine technology: an overview,* Indian Journal of Biotechnology, 5, 263-268.
5. Homaeia, A., Lavajoo, F., Sariri, R. (2016) *Development of marine biotechnology as a resource for novel proteases and their role in modern biotechnology*, International Journal of Biological Macromolecules, 88, 542-552.
6. Satheesh, S., Ba-akdah, M.A., Al-Sofyani, A.A. (2016) *Natural antifouling compound production by microbes associated with marine macroorganisms — A review*, Electronic Journal of Biotechnology, 21, 26–35.
7. Snider, B. B., Zeng, H. (2003) *Total Synthesis of (−)-Fumiquinazolines A, B, C, E, H, and I. Approaches to the Synthesis of Fiscalin A,* The Journal of Organic Chemistry, 68 (2), 545-563.
8. Wong, S.M., Musza, L. L., Kydd, G.C., Kullnig, R., Gillum, A. M., Cooper, R. (1993) *Fiscalins: New Substance P Inhibitors Produced by The Fungus Neosartorya fisheri Taxonomy, Fermentation, Structure, and Biological Properties,* The Journal of Antibiotics, 46 (4), 545-553.

9. Girardin, H., Monod, M., Latgé, J.P. (1995) *Molecular Characterization of the Food-Borne Fungus Neosartorya fischeri (Malloch and Cain)*, Applied and Environmental Microbiology, 61 (4), 1378–1383.
10. Hernández, F., Buenadicha, F. L., Avendao, C., Söllhuber, M. (2002) 1-*Alkyl-2,4-dihydro-1H-pyrazino[2,1-b]quinazoline-3,6-diones as glycine templates. Synthesis of fiscalin B,* Tetrahedron Asymmetry**,** 12 (24), 3387-3398.
11. O'Connor, T., O'Connell, J., O'Brien, D., Goode, T., Bredin, C.P., Shanahan, F. (2004) *The Role of Substance P in Inflammatory Disease*, Journal of Cellular Physiology, 201, 167–180.
12. Maggi, C. A. (2000) *Principles of tachykininergic co-transmission in the peripheral and enteric nervous system*, Regulatory Peptides, 93(1–3), 53–64.
13. Gerard, N.P., Garraway, L.A., Eddy, R.L.Jr., Shows, T.B., Iijima, H., Paquet, J.L., Gerard, C. (1991) *Human substance P receptor (NK-1): Organization of the gene, chromosome localization, and functional expression of cDNA clones,* Biochemistry, 30(44), 10640–10646.
14. Takahashi, C., Matsushita, T., Doi, M., Minoura, K., Shingu, T., Kumeda, Y., Numata, A. (1995) *Fumiquinazolines A–G, novel metabolites of a fungus separated from a Pseudolabrus marine fish*, Journal of the Chemical Society*,* Perkin Transactions 18, 2345-2353.
15. Han, X., Xu, X., Cui, C., Gu, Q. (2007) *Alkaloidal compounds produced by a marine-derived fungus, Aspergillus fumigatus H1-04, and their antitumor activities,* Chinese Journal of Medicinal Chemistry,17 (4), 232.
16. Wang, H., Ganesan, A. (2000) *Total synthesis of the fumiquinazoline alkaloids: solution-phase studies*, The Journal of Organic Chemistry, 65, 1022-1030.
17. Liu, J. F., Ye, P., Zhang, B., Bi, G., Sargent, K., Yu, L., Yohannes, D., Baldino, C. M. (2005) *Three-component one-pot total syntheses of glyantrypine, fumiquinazoline F, and fiscalin B promoted by microwave irradiation,* Journal of Organic Chemistry, 70 (16), 6339-6345.
18. Hernández, F., Lumetzberger, A., Avendaño, C., Söllhuber, M. (2001) *Regioselective N-Acylation of 3-Arylmethylpiperazine-2,5-diones: Short Synthesis of (-)-Glyantrypine and (-)-Fumiquinazoline F*, Synlett 2001**,** 2001 (9), 1387-1390.
19. Mansouri, R., Aouf, Z., Lakrout, S., Berredjem, M., Aouf, N. (2016) *A Greener, Efficient and Catalyst-Free Ultrasonic-Assisted Protocol for the N-Fmoc Protection of Amines*, Journal of the Brazilian Chemical Society, 27 (3), 546-550.

20. Kostakis, J.K., Elomri, A., Seguin, E., Iannelli, M., Besson, T. (2007) *Rapid synthesis of 2,3-disubstituted-quinazolin-4-ones enhanced by microwave-assisted decomposition of formamide*, Tetrahedron Letters, 48, 6609-6613.
21. Fields, G.B. (1994) *Methods for removing the Fmoc group*, Methods in Molecular Biology, 35, 17-27.