UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DA FARMÁCIA

Trabalho de Conclusão de Curso de Farmácia

# Synthesis of substituted 4-methyl-quinolines as potential cruzain inhibitors toward anti-*Trypanosoma cruzi* activity

Eduardo da Silveira dos Santos

Porto Alegre, Junho de 2016

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Trabalho de Conclusão de Curso em Farmácia

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Departamento de Produção de Matéria-Prima

Porto Alegre, Junho de 2016

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Este artigo foi elaborado segundos as normas da revista *Bioorganic & Medicinal Chemistry* apresentada em anexo.

**Synthesis of substituted 4-methyl-quinolines as potential cruzain inhibitors toward anti-*Trypanosoma cruzi* activity**

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**ABSTRACT**

A new series of 4-methyl-quinolines was synthesized as potential cruzain inhibitors using Doebner-Von Miller synthesis. The compounds (1a-f) are undergoing analysis in order to guarantee their purities and further explore their biological activity.

*Keywords*: Quinoline, Doebner-Von Miller, Cruzain, Trypanosoma cruzi, Chagas disease.

## 1. Introduction

Quinolines play an important role towards the development and application of new pharmaceutical agents. Due to their remarkable biological activities, quinolines have been launched as antimalarial, antiviral, antibacterial, antifungal, antiprotozoal, anthelmintic, anesthetic, antiasthmatic, anticancer, and cardiotonic drugs[1]. Quinoline moiety containing compounds have lead this class of molecule to a list of top five most common six-membered aromatic nitrogen heterocycles approved by FDA [2]; as well as substituted quinolines have been proved to be ubiquitous motifs in a variety class of molecules regarding drug discovery[3].

Chagas disease, or American trypanosomiasis, is listed as one of the 17 neglected diseases appointed by WHO, affecting approximately 10 million people in Latin America, as well as the leading cause of cardiomyopathy[4]. The infection is causes by *Trypanosoma cruzi,* a flagellate parasite transmitted after triatomine bug. The clinical arsenal used against it is restricted to the two available drugs: benzonidazole and nifurtimox. Both treatments face a significant problem[5] because of toxic effects after long exposure time, as well as the need for high doses due to the emergence of drug resistance[6].

Quinolines have been synthesized and had their biological activity evaluated regarding Chagas disease. Tryciclic quinolines obtained via a multicomponent reaction proved to be active against *Trypanosoma cruzi* by non-specific metabolic pathway.[7] It is believed that hybrids containing quinolinic pharmacophore are able to form iron complexes resulting in deprivation of the fundamental nutrient for cell growth and division[8]. Among the potential targets for the development of new anti-*T. cruzi* drugs, such as lanosterol 14 α-demethyalse[9], tryphanothione reductase[10]; there is another unique enzyme to be further explored which is the major cysteine protease cruzain, a fundamental enzyme for the development of the parasite life cycle within the

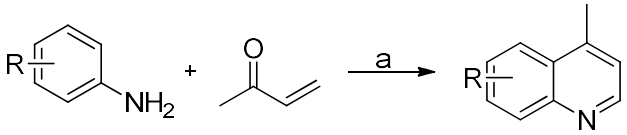
host cell[11-12]. Few years back, a new class of non-peptidic inhibitors of cruzain was reported, the molecules obtained through substrate-activity screening alongside x-ray structure had shown the pivotal role of quinoline moiety on the discovery of promising inhibitors. [13]

Several traditional and modified approaches have been described towards the synthesis of quinolines[1]. Combes, Conrad-Limpach and Skraup synthesis are examples of them. In 1881, Doebner and Von Miller proposed an alternative method to the Skraup protocol. They replaced the glycerol with an α,β-unsaturated ketone, but keeping the aniline in the reaction medium. This protocol offers the usage of a variety of catalysts such as Lewis and Brönsted acids, moreover, microwave irradiation can be applied in order to synthesize them.

Herein, we report the synthesis of novel substituted quinolines as promising cruzain inhibitors aiming the anti-*T. cruzi* activity through Doëbner-Von Miller reaction employing six substitute anilines, and methyl vinyl ketone assisted by ρ-toluenesulfonic acid as Brönsted acid.

## 2. Results and discussion

The synthetic route adopted for the preparation of target compounds 1a-f was outlined in scheme 1. The starting anilines were stirred for over a day up the boiling point of the solvent used to afford the quinolines.




a: p-TsOH, DCE, 85 °C, 48 h

**1a: R = 6-Br** **1d: R = 6-F, 8-$NO_2$**
**1b: R = 8-I** **1e: R = 6-$OCH_3$**
**1c: R = 6-F, 5-$NO_2$** **1f: R = 6-Cl**

Not all compounds were able to be isolated and have their structures fully elucidated due to lack of available time and facilities during the chronogram initially planned. The compound 1a, however, underwent a purification and NMR analysis, and hence served as standard for the other reactions. It is important to highlight the fact that the compounds 1b-d were identificated after a crude NMR analysis were performed, showing their unique proton signals, but some compounds were not purified neither had their NMR spectra obtained. Moreover, the compounds 1e-f, were purified by column chromatography, and were not elucidated by NMR methods as well; however it was assumed that those compounds were afforded after TLC plates were run. The only available method to follow up the synthetic approach was though TLC obtained after column chromatography, compared with 1a's TLC fashion, as mentioned before. The $^1$H NMR and IR spectra are available at Supplementary data section.

The $^1$H NMR spectrum of 1a shows a low-field signal at 8.77 ppm, and coupling constant of approximately 4 Hz, typical of the 2-position hydrogen. This signal also can be observed at the crude samples, indicating that desired molecule was synthesized*[1].

The evaluation of biological activity is underway, and these molecules are part of preliminary analysis of structure activity-relationship between quinolines and cruzaine according to unpublished

data based on computational screening. The synthetic route elected in order to afford them provides a methyl group in position 4, which allows the quinoline core to be further functionalized.

The methyl group positioned at the fourth carbon allows for a possible increase in complexity; for example it can potentially be oxidized from the alcohol up to the carboxylic acid. The presence of these functional groups gives the opportunity for optimization of the interactions between the target. This aim can be reached by adding a linkage chain, allowing the molecule to have another pharmacophore group position in order to increase drug-enzyme interaction.*[2]

## 3. Conclusion

We reported herein a synthesis of a series of quinoline derivatives. This work is in its early stages, and is fundamental to conclude the chemical part in order to move forward for the biological part, and eventually summarize a structure-activity relationship and further explore the cruzain's role in Chagas disease and drug candidates.

## 4. Experimental section

$^1$H NMR spectrum was recorded on Agilent 500 MHz spectrometer. Chemical shifts are reported in δ ppm values and were internally referenced to TMS (δ 0.00) for $CDCl_3$. Infrared (FT-IR) spectra were recorded on Perkin Elmer Spectrum BX instrument for thin film (in acetone) or neat samples. All reactions were carried out with commercially available reagents and solvents and were used without any further purification.

### 4.1 General procedure for the synthesis of 1a-f

The substituted anilines (0,1 g), methyl vinyl ketone (2.5 mol/eq.), and ρ-toluenesulfonic acid (1 mol/eq.) were added to a schlenk tube in 5 mL of 1,2-dichloroethane and stirred for 48 h in oil bath at approximately 85 °C. Then the reaction was quenched with saturated $NaHCO_3$ solution and extracted with $CH_2Cl_2$ (20 mL x 2). The combined organic extracts were dried with $Na_2SO_4$, filtered, and evaporated under reduced pressure, the dark crude was purified by column chromatography on silica gel (mobile phase: dichloromethane:diethyl ether, 95:5)

4.1.1. 1a

Yellow powder. FT-IR ($cm^{-1}$): 2925, 1594, 1493, 824. $^1$H NMR (500 MHzm $CDCl_3$): δ 8.77 (d, *J*=4.2 Hz, 1 H, H2), 8.14 (d, *J*=2.1 Hz, 1 H, H5), 7.97 (d, *J*=9, 1 H, H8), 7.77 (dd, *J*=9.0, 2.1, 1 H, H7), 7.25 (d, *J*=4.2 Hz, 1 H, H3), 2.67 (s, 3H, CH3).

4.1.2. 1b

*Undergoing analysis*

4.1.3. 1c

*Undergoing analysis*

4.1.4. 1d

*Undergoing analysis*

4.1.5. 1e

*Undergoing analysis*

4.1.6. 1f

*Undergoing analysis*

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**Supplementary data**

Suplementary data related to this article can be found at Appendix A.

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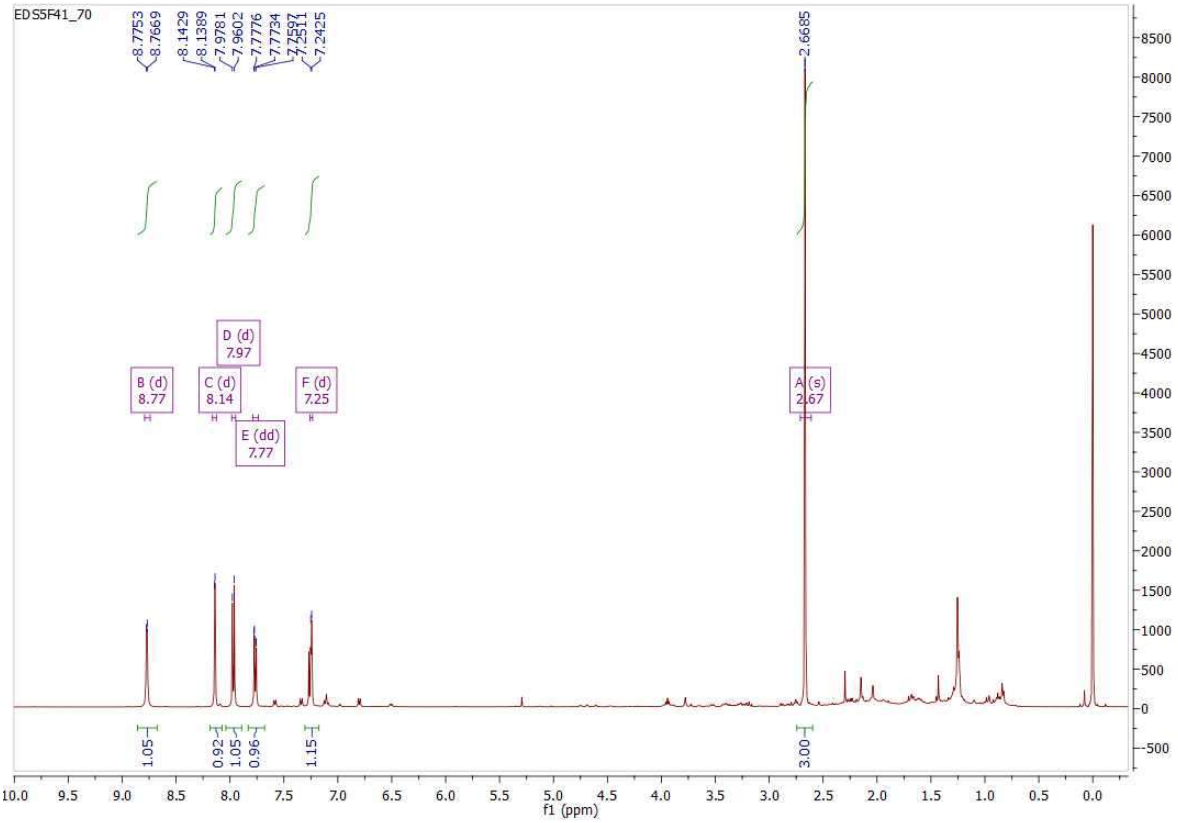
**SUPPORTING INFORMATION**

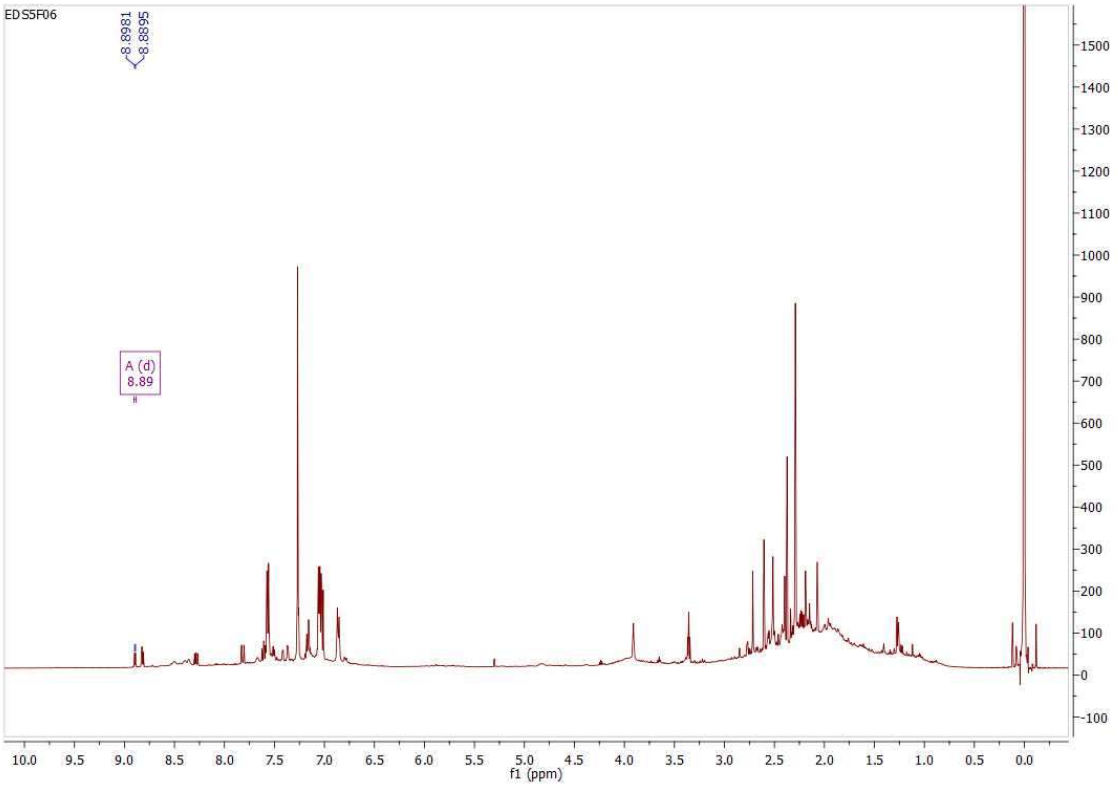
**Appendix A**

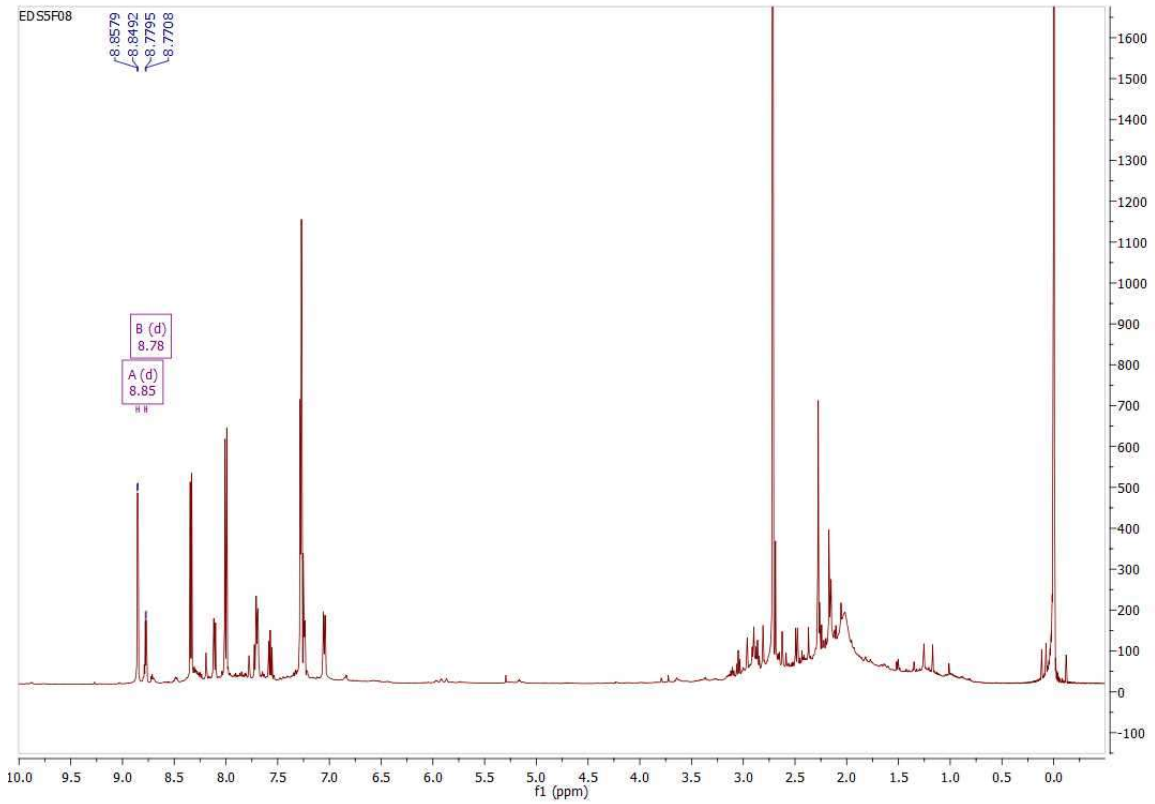
Table of Contentes

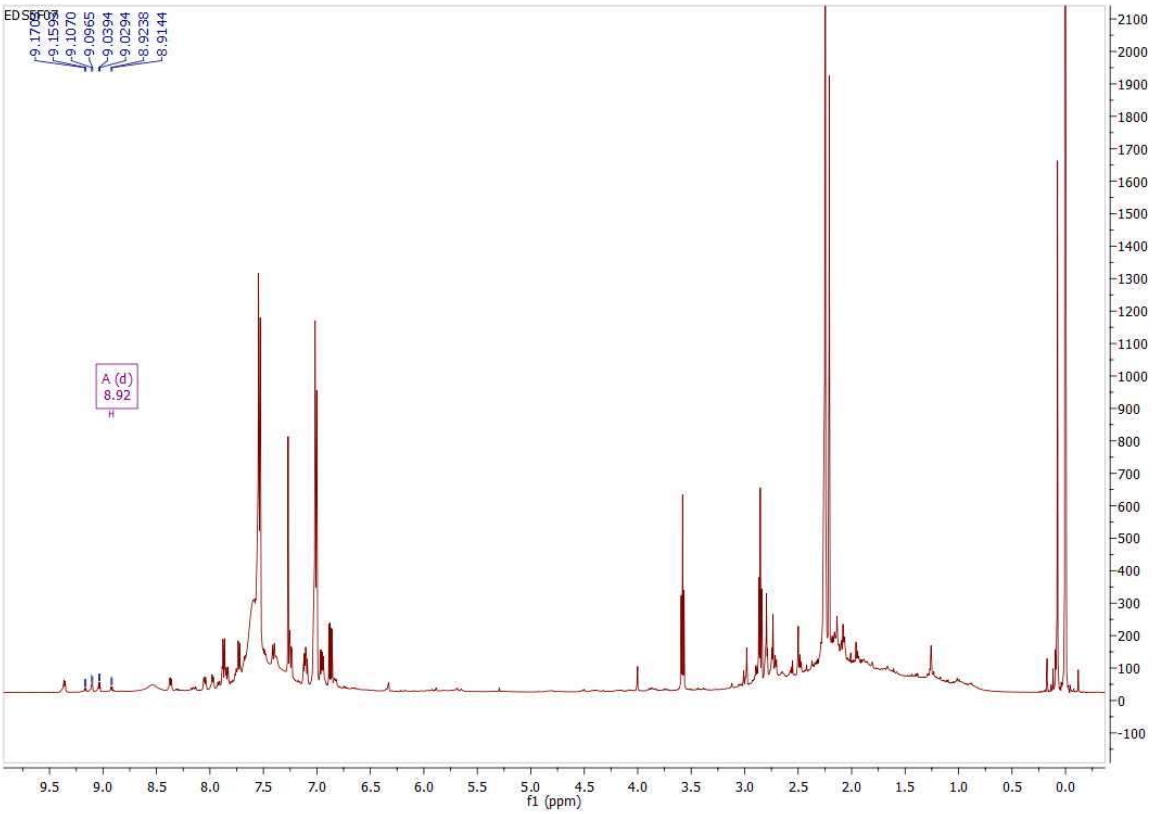
## 1 $^{1}$H Spectra for Numbered Compound 1a



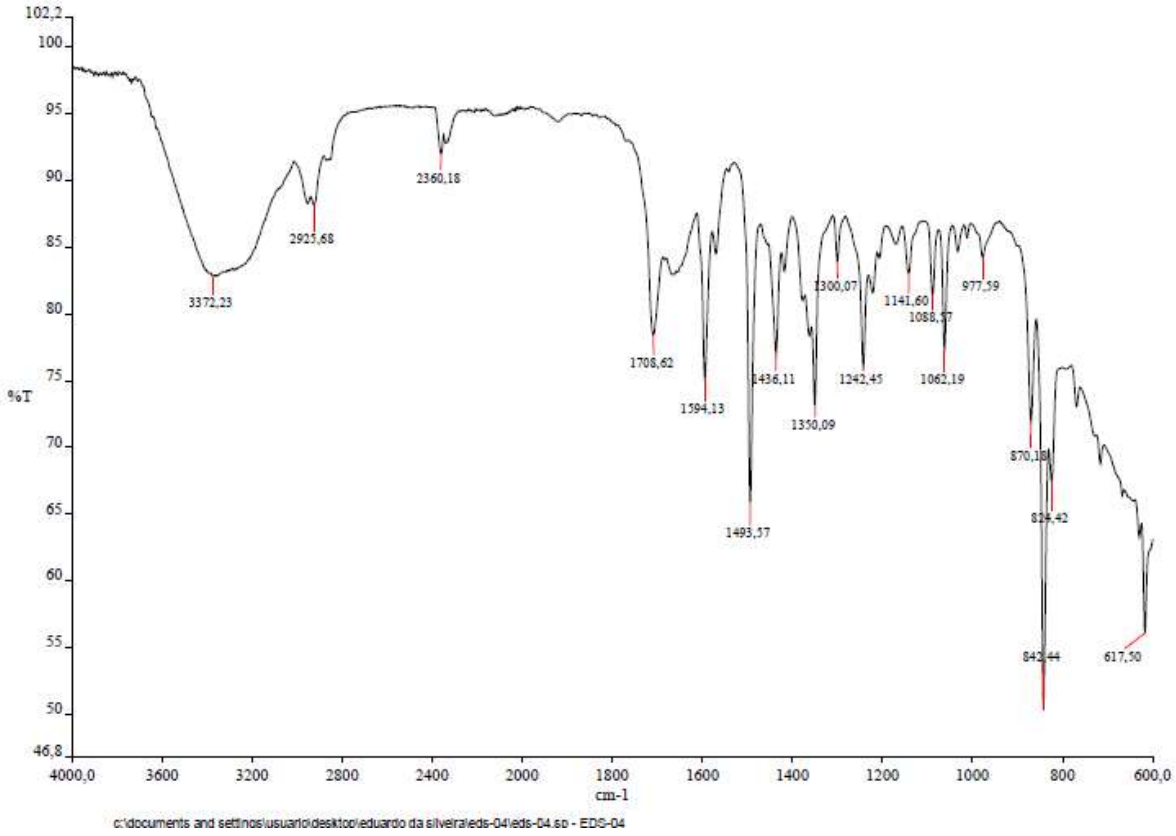

## 2 Crude $^{1}$H Spectra for Numbered Compouds 1b-d



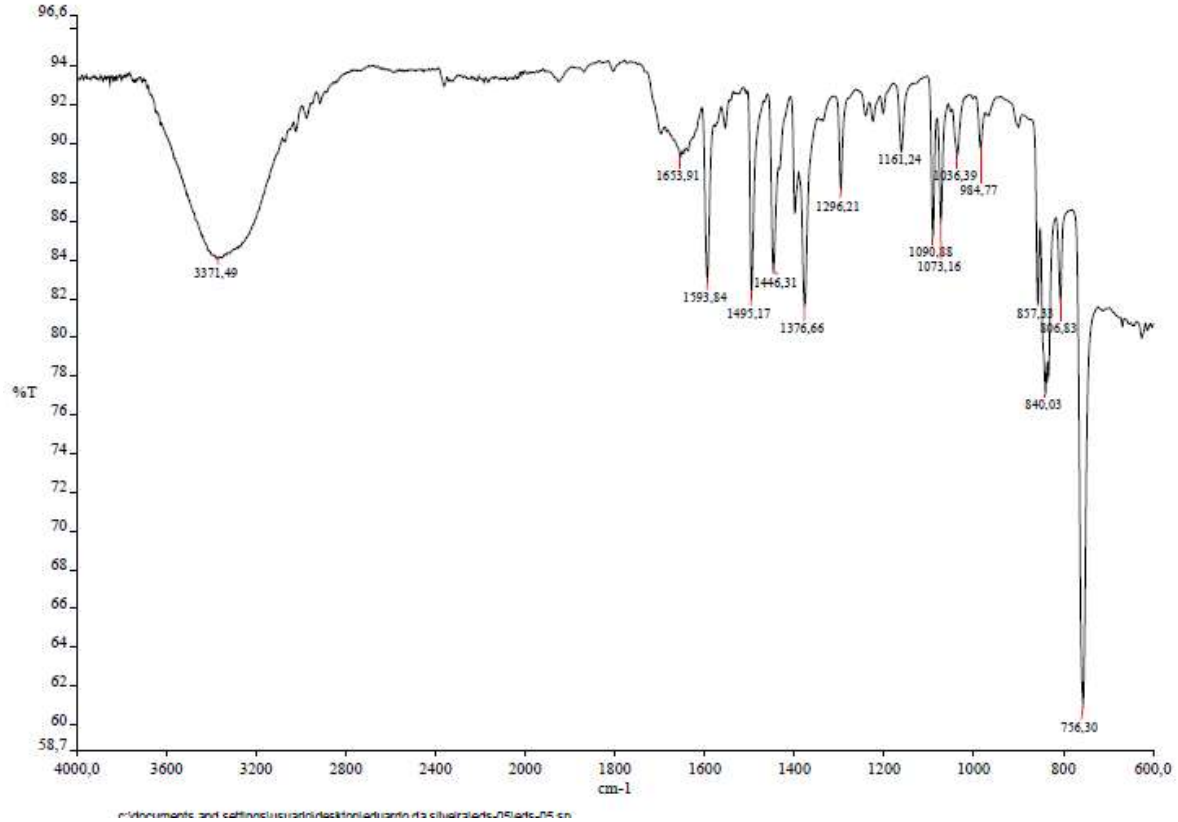

EDS5F08
8.8579
8.8492
8.7795
8.7708
B (d)
8.78
A (d)
8.85
f1 (ppm)

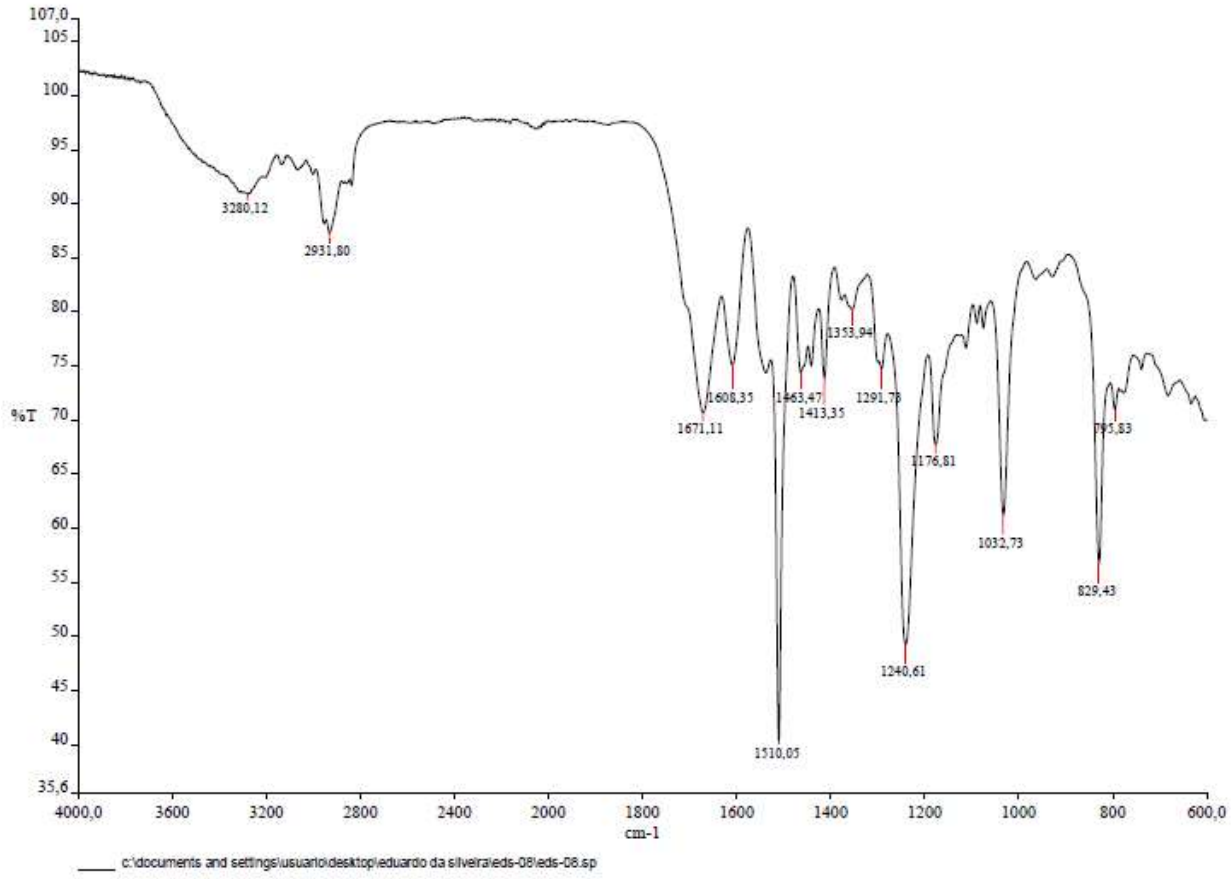

9.1070
9.0965
9.0394
9.0294
8.9238
8.9144
A (d)
8.92
f1 (ppm)

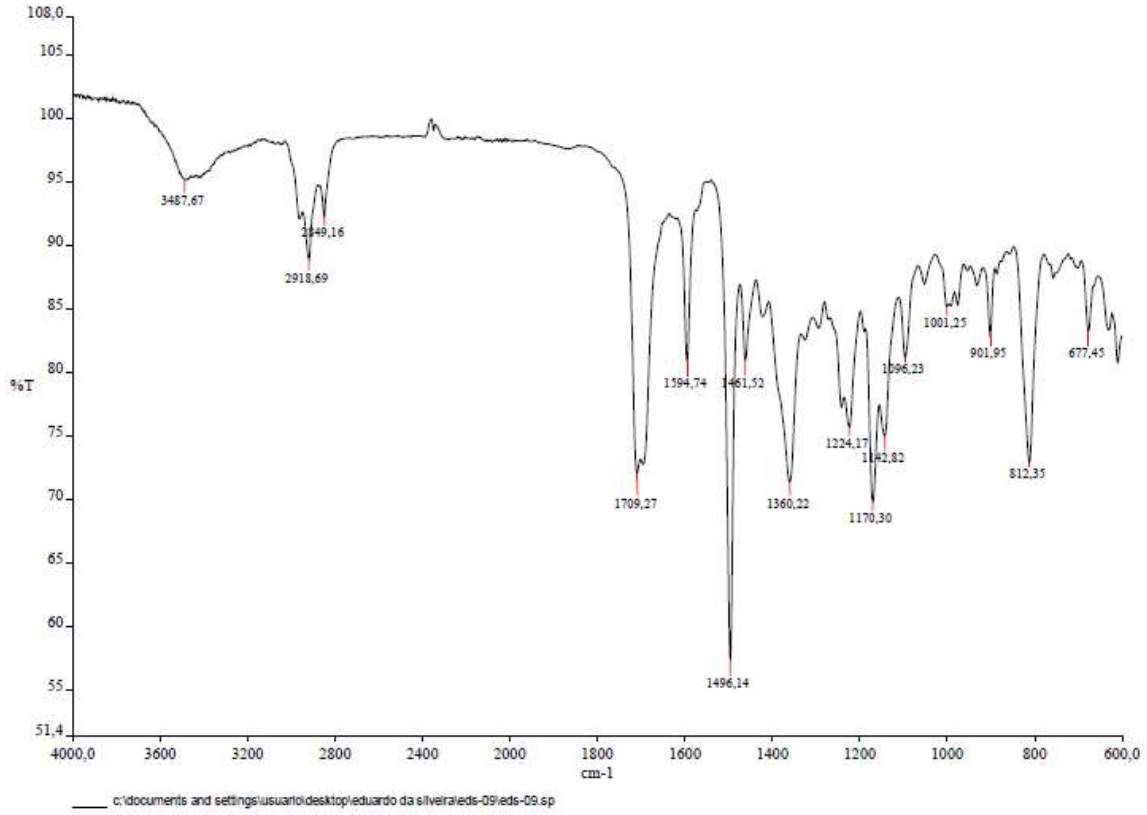
## 3. FT-IR Spectra for Numbered Compound 1a



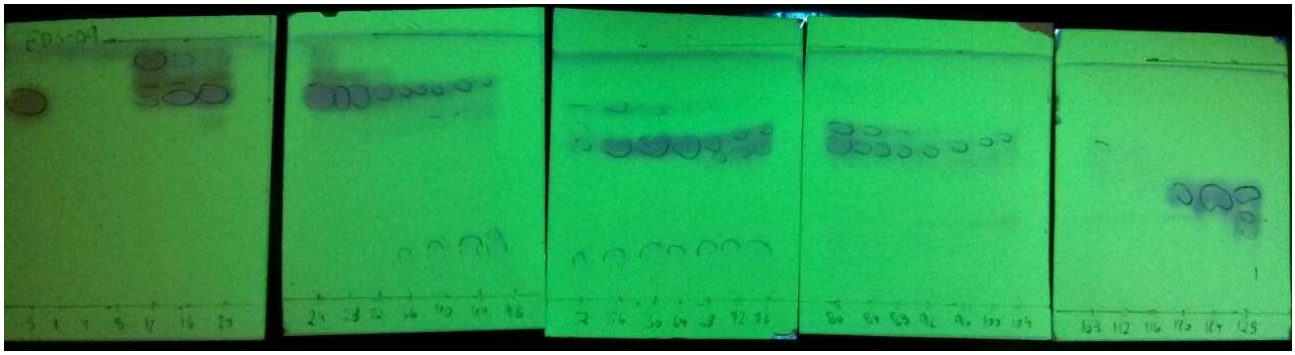
4. **FT-IR Spectra for Numbered Compound 1b, e-f**

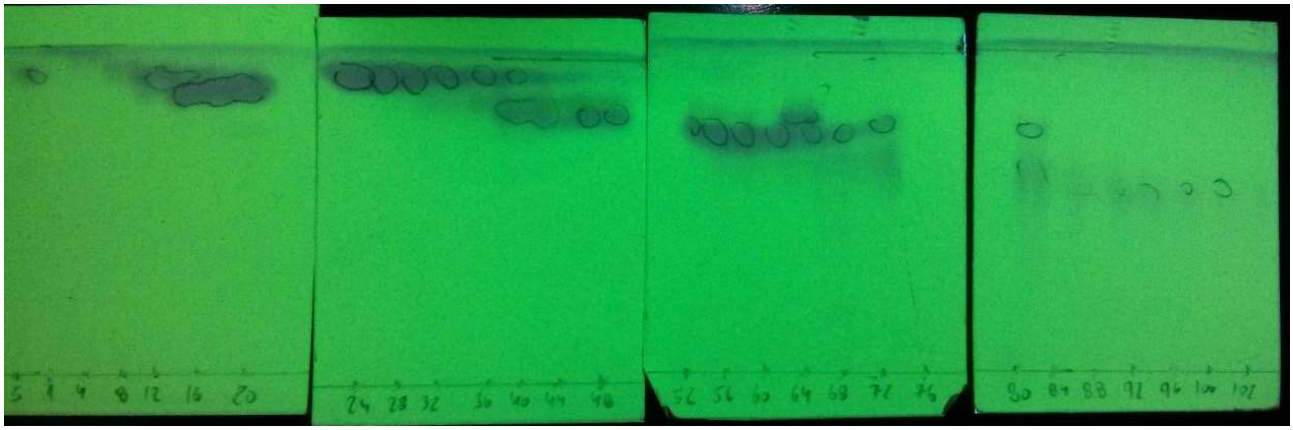





## 5. **TLC plates figures of compounds 1e-f**





# ANEXO

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