SYNTHESIS OF 1,5-DISUBSTITUTED 1,2,3-TRIAZOLE VIA MULTI-COMPONENT REACTION

Vo Viet Dai, Do Thi Phuong Thu, Nguyen Tran Nguyen*

*The University of Da Nang, University of Science and Education; *ntnguyen@ued.udn.vn*

Abstract - 1,5-Disubstituted 1,2,3-triazoles are synthesized viametal-free multi-component reaction from primary amine, ketones and 4-nitrophenyl azide. The structure of three target compounds including:1-(4-methoxybenzyl)-5-(4-methylphenyl)-1H-1,2,3-triazole (1), 1-(4-methoxybenzyl)-5-(4-nitrophenyl)-1H-1,2,3-triazole (2) and 1-(4-methoxybenzyl)-5-phenyl-1H-1,2,3-triazole (3) is elucidated and identified by infrared spectroscopy, nuclear magnetic resonance spectroscopic analysis and high resolution mass spectrum. The formation of heterocyclic aromatic 1,2,3-triazole ring is approved by the appearance of singlet peak at 7.71 ppm in 1H NMR corresponding with triazolyl proton. In addition, antibacterial and antifungal activities of products are also tested with three Gram-positive bacteria (*B. subtilis, S. aureus* and *L. fermentum*), three Gram-negative bacteria (*P. aeruginosa, E.coli, S. enterica*), and *Candida albicans* fungus.

Key words - multi-component reaction; 1,5-disubstituted 1,2,3-triazole; substituted 1,2,3-triazole

1. Introduction

The 1,2,3-triazole containing compounds showed large biological activity spectrum such as antimalarial [1], antibiotic [2], anticancer [3], anti-HIV [4], and antifungal activities [5]. Therefore, 1,2,3-triazoles and their derivatives have attracted more attention in order to develop new drug candidates. The most popular protocols to obtain 1,4- and 1,5-disubstituted 1,2,3-triazoles are the metal-catalyzed azide-alkyne cycloaddition reactions [6]. However, the application of the above mentioned methods, especially in medicinal chemistry is limited by the toxicity of the heavy metals. In addition, it is noticed that biological activities and binding affinities of 1,5-disubstituted 1,2,3-triazoles towards some biological targets could be improved as compared to 1,4-disubstituted derivatives [7, 8]. Nevertheless, the synthetic methods towards 1,5-disubstituted 1,2,3-triazoles are quite rare and, therefore, it is extremely necessary to build up a metal-free method to synthesize these promising biologically active compounds. In 1965, Pocar and his colleagues carried out the reaction of an isolated imine/enamine, which was derived from propylamine and acetone, with 4-nitrophenyl azide and the product was 1-propyl-5-methyl-1,2,3-triazole obtained by accidentally in moderate yield [9]. However, the isolation of imine/enamine from the reaction mixture and then use it for doing reaction with 4-nitrophenyl azide will take more time and chemicals, and therefore, one-pot multicomponent reaction should be considered. In 2016, based on the results of Pocar, J. Thomas reported a general metal-free route towards the synthesis of 1,2,3-triazoles from primary amines and ketones [10]. This method was then applied to the synthesis of artemisinin derivaties and their anticancer as well as anti-HIV activities were also evaluated [11, 12]. In addition, synthesis of enantiomerically pure 1,2,3-triazoles derived from amino esters based on the above mentioned route was also reported by Wim Dehaen group [13]. It is clear that method developed by J. Thomas and Wim Dehaen helped to widen the diversity of 1,2,3-triazole containing compounds.This paper will report the synthesis of three 1,5-disubstituted 1,2,3-triazoles from primary amine, ketone and 4-nitrophenyl azide,*via* multi-component reaction [10] and biological activities of the desired products also concerned.

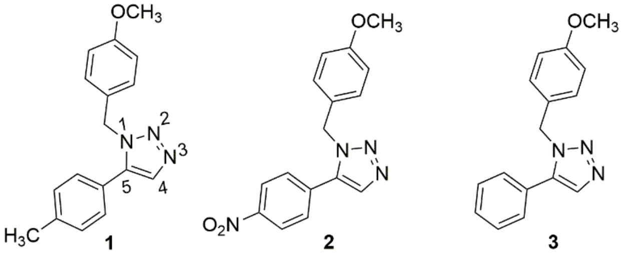


***Figure 1**. Chemical structures of compounds 1-3*

2. Experimental

Material

For column chromatography, silica gel 60 (0.063–0.200 mm) (E. M. Merck) was used as the stationary phase. All chemicals received from commercial sources (Acros, Merck) were used without further purification.

General procedures

NMR spectra were acquired with Bruker Avance 500 MHz spectrometer at Chemistry Department, VNU – HUS and chemical shifts (δ) are reported in parts per million (ppm) referenced to tetramethylsilane (TMS) or the internal (NMR) solvent signals. Exact mass measurements were acquired on QTOF LC/MS Agilent 6530 spectrometer at Vietnam Academy of Science and Technology (VAST). IR spectra were recorded on 1S-FTIR Shimadzu spectrometer at Chemistry Department, VNU – HUS. All biological activity testing were carried out at Vietnam Academy of Science and Technology (VAST).

General procedure for synthesis of 1,5-disubstituted 1,2,3-triazole [10]

Ketone (1.16 mmol), primary amine (1.74 mmol), 4-nitrophenyl azide [14] (1.26 mmol), 4 Å molecular sieves (100 mg), acid catalyst CH_3COOH (1 – 2 drops) and toluene (1.2 mL) were added to a reaction tube equipped with a stirring bar and the reaction mixture was stirred at 100°C for 12 hours. The reaction was followed by thin layer chromatography with the mobile phase of hexane/ethyl acetate = 6/4. The crude product was then purified by column chromatography with the eluent of CH_2Cl_2 and followed by a mixture of hexane and ethyl acetate to obtain pure product.

*Synthesis of 1-(4-methoxybenzyl)-5-(4-methylphenyl)-1H-1,2,3-triazole (**1**)*

4-Methylacetophenone (168 mg, 1.26 mmol), 4-methoxybenzylamine (240 mg, 1.74 mmol), 4-nitrophenyl azide (204 mg, 1.26 mmol), 4 Å molecular sieves (100 mg), CH_3COOH (1 - 2 drops), toluene (1.2 mL). Product was obtained as dark yellow semi-solid after purification by column chromatography. Yield: 88%. IR cm^{-1}: 3122.75, 2931.80, 1612.49, 1558.49, 1514.12, 1442.75. ^1H NMR (500 MHz, $CDCl_3$, TMS): δ 7.70 (s, 1H, triazole), 7.23 (d, J = 7.8Hz, 2H, benzene), 7.15 (d, J = 8.1Hz, 2H, benzene), 7.03 (d, J = 8.6 Hz, 2H, benzene), 6.80 (d, J = 8.7 Hz, 2H, benzene), 5.46 (s, 2H, CH_2), 3.77 (s, 3H, OCH_3), 2.41 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$, TMS): δ 159.57, 139.82, 138.25, 133.03, 129.78, 128.98, 128.85, 127.66, 123.99, 114.29, 55.42, 51.52, 21.48. HRMS Calc. for $C_{17}H_{17}N_3O$: 279.1372, found: 280.1444 [M + H^+].

*Synthesis of 1-(4-methoxybenzyl)-5-(4-nitrophenyl)-1H-1,2,3-triazole (**2**)*

4-Nitroacetophenone (207 mg, 1.26mmol), 4-methoxybenzylamine (240 mg, 1.74 mmol), 4-nitrophenyl azide (204 mg, 1.26 mmol), 4 Å molecular sieves (100 mg), CH_3COOH (1− 2 drops) and toluene (1.2 mL). Product was obtained as off-white solid after purification by column chromatography. Yield: 90%. IR cm^{-1}: 3118.90, 2918.30, 1600.92, 1520, 1452.40. ^1H NMR (500 MHz, $CDCl_3$, TMS): δ 8.27 (d, J = 8.3 Hz, 2H, benzene), 7.81 (s, 1H, triazole), 7.44 (d, J = 8.4 Hz, 2H, benzene), 6.98 (d, J = 8.4 Hz, 2H, benzene), 6.80 (d, J = 8.7 Hz, 2H, benzene), 5.53 (s, 2H, CH_2), 3.77 (s, 3H, OCH_3). ^{13}C NMR (125 MHz, $CDCl_3$, TMS): δ 159.80, 148.37, 135.97, 134.17, 133.55, 129.91, 128.61, 126.86, 124.22, 114.50, 55.42, 52.12. HRMS Calc. for $C_{16}H_{14}N_4O_3$: 310.1066, found: 311.1041 [M + H^+].

*Synthesis of 1-(4-methoxybenzyl)-5-phenyl-1H-1,2,3-triazole (**3**)*

Acetophenone (150 mg, 1.26 mmol), 4-methoxybenzylamine (240 mg, 1.74 mmol), 4-nitrophenyl azide (204 mg, 1.26 mmol), 4 Å molecular sieves (100 mg), CH_3COOH (1 – 2 drops) and toluene (1.2mL). Product was obtained as brown semi-solid after purification by column chromatography. Yield: 93%. IR cm^{-1}: 3126.61, 2954.95, 1610.56, 1583.56, 1520.19, 1458.18. ^1H NMR (500 MHz, $CDCl_3$, TMS): δ 7.71 (s, 1H, triazole), 7.44-7.42 (m, 3H, benzene), 7.28-7.26 (m, 2H, benzene), 7.01 (d, J = 8.7 Hz, 2H, benzene), 6.79 (d, J = 8.6 Hz, 2H, benzene), 5.47 (s, 2H, CH_2), 3.75 (s, 3H, OCH_3).^{13}C NMR (125 MHz, $CDCl_3$, TMS): δ 159.39, 137.92, 133.21, 129.46, 128.92, 128.67, 127.50, 126.97, 114.12, 55.24, 51.35. HRMS Calc. for $C_{16}H_{15}N_3O$: 265.1215, found: 266.1287 [M + H^+].

3. Result and discussion

Based on the invention of Pocar in 1965 [9], it is clear that isolated imine/enamine could react with 4-nitrophenyl azide to obtain 1,5-disubstituted 1,2,3-triazole in good yield. The Shiff base imine could be resulted from ketone like acetophenone and primary amine like 4-methoxybenzylamine *via* a reversible reaction: ketone + amine ⇌ imine + H_2O. The imine formation reaction generally takes place under acidic condition and therefore, the catalytic amount of CH_3COOH was added to the reaction mixture (Figure 2.). In addition, in order to increase the yield, 4 Å molecular sieves were also added to the sealed tube to remove water that was produced during the reaction [10].

In this paper, acetophenone, 4-methoxybenzylamine and 4-nitrophenyl azide were starting materials chosen to optimize the temperature condition for the triazole synthesis (Figure 2). It is well documented that imine is the thermodynamically controlled product formed by the reaction between aldehyde or ketone with primary amine. Moreover, imine can be converted into enamine through tautomerization in which a hydrogen atom and a double bond will be shifted [15]. Triazole product was not produced if the one-pot reaction was carried out at room temperature even if it was magnetically stirred up to 48 hours.

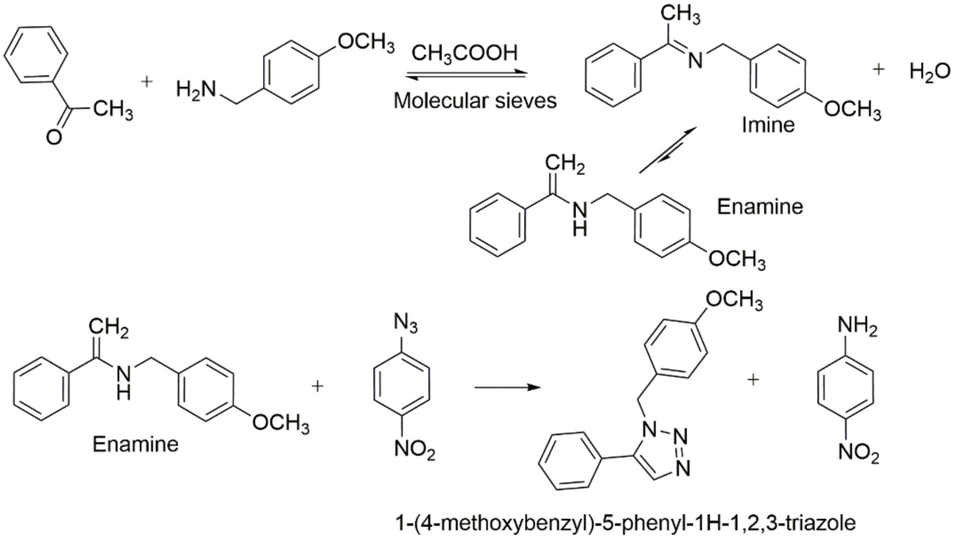


***Figure 2.** Synthesis of 1-(4-methoxybenzyl)-5-phenyl-1H-1,2,3-triazole from acetophenone, 4-methoxybenzylamine and 4-nitrophenyl azide*

However, heating the reaction mixture at 60°C in 48 hours resulted in product 1-(4-methoxybenzyl)-5-phenyl-1H-1,2,3-triazole in 60 to 65% yield. When the reaction was heated up to 100°C, the yield could be reached approximately 93%. Therefore, the experimental work has shown that imine was formed *via* the thermodynamically controlled reaction [10].

The formation of 1,2,3-triazole ring was confirmed by the presence of an absorption band at the wavenumber of 3126 cm^{-1}, in the FTIR spectrum of 1-(4-methoxybenzyl)-5-phenyl-1H-1,2,3-triazole, which arise from the =C−H stretching of the triazole.

In addition, the stretching vibration of C=C bond of the triazole resulted from the absorption of infrared energy corresponding with the wavenumber of 1610 cm^{-1}. Moreover, the N=N stretching of 1,5-disubstituted 1,2,3-triazole was represented by absorption peak at 1583 cm^{-1}. Similarly, the above mentioned characteristic peaks were also observed in the FTIR spectra of other triazole compounds.

The presence of the characteristic singlet peak at the chemical shift of δ 7.71 ppm in ^{1}H NMR spectra of all three products corresponded with triazolyl proton at position 4. ^{1}H NMR spectra of triazole **1** and **2** appeared four doublet peaks in the region of δ 6.5 to 8.0 ppm due to the presence of two 1,4-disubstituted benzene rings. Methylene groups were linked to both benzene and triazole aromatic ring, and therefore, singlets located at around 5.5 ppm were characteristic of two protons of the above group. Moreover, three protons of methoxy groups ($-OCH_3$) were shown by singlet peak at approximately 3.7 ppm. The high resolution mass spectral analysis were found to be in accordance with the molecular weights of three triazole compounds.

Antibacterial activity

All products were examined for their antibacterial activity against *P.* aeruginosa, *E.coli*, *S. enterica*, *B. subtilis*, *S. aureus* and *L. fermentum*. The antibacterial activity of three compounds was compared with standard drug ampicillin. The half maximal inhibitory concentration (IC50) values were summarized in Table 1. The results obtained from antibacterial studies presented that all compounds possessed weak activities against tested bacteria with IC50 values higher than 128 μg/mL.

Table 1. *Antibacterial and antifungal activities of three triazoles*

Triazole	IC$_{50}$ (μg/mL)						
	Gram (+)			Gram (-)			Fungus
	S. aureus	*B. subtilis*	*L. fermentum*	*S. enterica*	*E.coli*	*P. aeruginosa*	*Candida albicans*
1	*>128*	*>128*	*>128*	*>128*	*>128*	*>128*	68.41
2	*>128*	*>128*	*>128*	*>128*	*>128*	*>128*	>128
3	*>128*	*>128*	*>128*	*>128*	*>128*	*>128*	52.41

Antifungal activity

All three compounds were tested for their antifungal activity against fungus *Candida albicans* and Amphotericin B was used as the standard drug. The half maximal inhibitory concentration (IC50) values against *Candida albicans* were calculated and summarized in Table 1. The results showed that triazoles **1** and **3** exhibited better activity against *Candida albicans*, with the IC50 values of 68.41 μg/mL and 52.41 μg/mL, respectively, as compared to triazole **2**.

4. Conclusions

Three 1,5-disubstituted 1,2,3-triazoles were synthesized *via* one-pot three-component reaction and evaluated for their biological activity against *P.* aeruginosa, *E.coli*, *S. enterica*, *B. subtilis*, *S. Aureus*, *L. Fermentum* and *Candina albicans*. The reaction was approved to be thermodynamically controlled process in which the yield was increased from 65% to 93% when the reaction was heated from 60°C to 90°C. Compound **1** and **3** exhibited significant antifungal activity against *Candida albicans* and therefore, they are promising molecules for further biological testing.

Acknowledgements: Authors would like to thank Prof. Dr Mac Dinh Hung, Chemistry Department, VNU – HUS for NMR measurement.

REFERENCES

[1] M. D’hooghe, S. Vandekerckhove, K. Mollet, K. Vervisch, S. Dekeukeleire, L. Lehoucq, C. Lategan, P. J. Smith, K. Chibale, N. De Kimpe, “Synthesis of 2-amino-3-arylpropan-1-ols and 1-(2,3-diaminopropyl)-1,2,3-triazoles and evaluation of their antimalarial activity”, *Beilstein J. Org. Chem*., **7**, 2011, 1745–1752.

[2] M. Aufort, J. Herscovici, P. Bouhours, N. Moreau, C. Girard, “Synthesis and antibiotic activity of a small molecules library of 1,2,3-triazole derivatives”, *Bioorganic Med. Chem. Lett.*, 18(3),2008, 195-1198.

[3] A. J. Salmon, M. L. Williams, Q. K. Wu, J. Morizzi, D. Gregg, S. A. Charman, D. Vullo, C. T. Supuran, “Metallocene-based inhibitors of cancer-associated carbonic anhydrase enzymes IX and XII”, *J. Med. Chem*, 55(11), 2012, 5506-5517.

[4] M. Whiting, J. Muldoon, Y. C. Lin, S. M. Silverman, W. Lindstrom, A. J. Olson, H. C. Kolb, M. Finn, K. B. Sharpless, J. H. Elder, “Inhibitors of HIV-1 protease by using in situ click chemistry”, *Angew. Chem. Int. Ed. Engl.*, 45, 2006, 1435-1439.

[5] N. G. Aher, V. S. Pore, N. N. Mishra, A. Kumar, P. K. Shukla, A. Sharma, M. K. Bhat, “Synthesis and antifungal activity of 1,2,3-triazole containing fluconazole analogues”, *Bioorganic Med. Chem. Lett.*,19(3), 2009, 759-763.

[6] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, “A stepwise Huisgen cycloaddition process: Copper(I)-catalyzed regioselective “ligation” of azides and terminal alkynes”, *Angew. Chem. Int. Ed. Engl.*, 41, 2002, 2596-2599.

[7] P. Schmieder, R. Kühne, J. Rademann, “Metal-Free, Regioselective Triazole Ligations that Deliver Locked cis Peptide Mimetics”, *Angew. Chem. Int. Ed. Engl.*, 48(27), 2009, 5042-5045.

[8] M. Empting, O. Avrutina, R. Meusinger, S. Fabritz, M. Reinwarth, M. Biesalski, S. Voigt, G. Buntkowsky, H. Kolmar, ““Triazole bridge”: disulfide-bond replacement by ruthenium-catalyzed formation of 1,5-disubstituted 1,2,3-triazoles”, *Angew. Chem. Int. Ed. Engl.*, 50(22), 2011, 5207-5211.

[9] G. Bianchetti, P. Dalla Croce, D. Pocar, “Studies on the schiff bases - enamines equilibrium”, *Tetrahedron Lett*., 6,1965, 2043-2045.

[10] J. Thomas, S. Jana, J. John, S. Liekens and W. Dehaen, “A general metal-free route towards the synthesis of 1,2,3-triazoles from readily available primary amines and ketones”, *Chem. Commun*., 52, 2016, 2885-2888.

[11] S. Janaa, S. Iramb, J. Thomas, S. Liekens and W. Dehaen, “Synthesis and anticancer activity evaluation of novel aza-artemisinin derivatives”, *Bioorg. Med. Chem*., 25, 2017, 3671-3676.

[12] S. Jana, S. Iram, J. Thomas, M. Q. Hayat, C. Pannecouque and W. Dehaen, “Application of the triazolization reaction to afford dihydroartemisinin derivatives with anti-HIV activity”, *Molecules*,

22, 2017, 303.

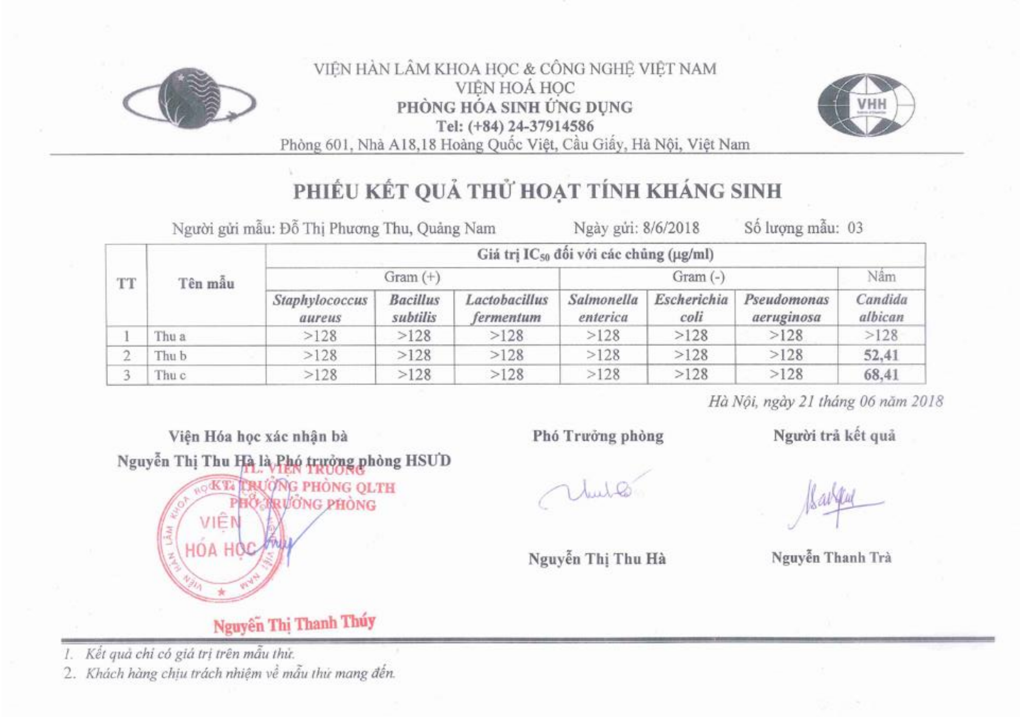
[13] G. S. -Dorta, S. Jana, L. Borkova, J. Thomas and W. Dehaen, "Straightforward synthesis of enantiomerically pure 1,2,3-triazoles derived from amino esters", *Org. Biomol. Chem.*, 16, 2018, 3168-3176.

[14] N. Faucher, Y. Ambroise, J.-C. Cintrat, E. Doris, F. Pillon, B. Rousseau (), "Highly Chemoselective Hydrogenolysis of Iodoarenes", *J. Org. Chem.*, 67, 2002, 932-934.

[15] A. Aryangat, *The MCAT Chemistry Book*, 2015, 169-170.

SUPPLEMENTARY MATERIALS

1. Biological activity testing results



VIỆN HÀN LÂM KHOA HỌC & CÔNG NGHỆ VIỆT NAM
VIỆN HOÁ HỌC
PHÒNG HÓA SINH ỨNG DỤNG
Tel: (+84) 24-37914586
Phòng 601, Nhà A18,18 Hoàng Quốc Việt, Cầu Giấy, Hà Nội, Việt Nam

PHIẾU KẾT QUẢ THỬ HOẠT TÍNH KHÁNG SINH

Người gửi mẫu: Đỗ Thị Phương Thu, Quảng Nam Ngày gửi: 8/6/2018 Số lượng mẫu: 03

TT	**Tên mẫu**	**Giá trị IC_{50} đối với các chủng (μg/ml)**						
		Gram (+)			Gram (-)			Nấm
		Staphylococcus aureus	***Bacillus subtilis***	***Lactobacillus fermentum***	***Salmonella enterica***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Candida albican***
1	Thu a	>128	>128	>128	>128	>128	>128	>128
2	Thu b	>128	>128	>128	>128	>128	>128	**52,41**
3	Thu c	>128	>128	>128	>128	>128	>128	**68,41**

Hà Nội, ngày 21 tháng 06 năm 2018

Viện Hóa học xác nhận bà
Nguyễn Thị Thu Hà là Phó trưởng phòng HSƯD

TL. VIỆN TRƯỞNG
KT. TRƯỞNG PHÒNG QLTH
PHÓ TRƯỞNG PHÒNG

Nguyễn Thị Thanh Thúy

Phó Trưởng phòng

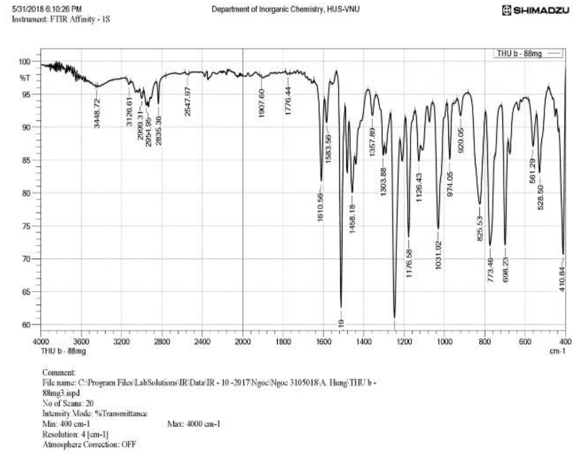
Nguyễn Thị Thu Hà

Người trả kết quả

Nguyễn Thanh Trà

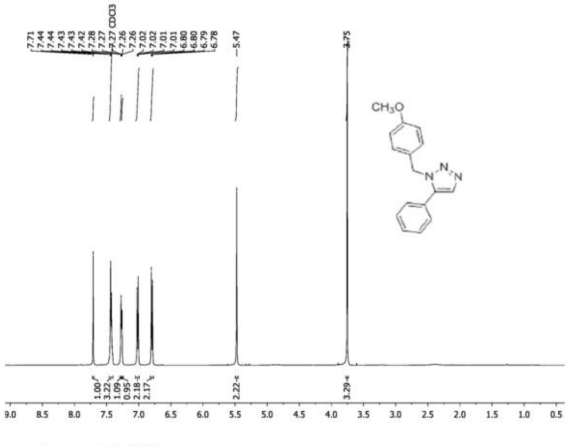
1. *Kết quả chỉ có giá trị trên mẫu thử.*
2. *Khách hàng chịu trách nhiệm về mẫu thử mang đến.*

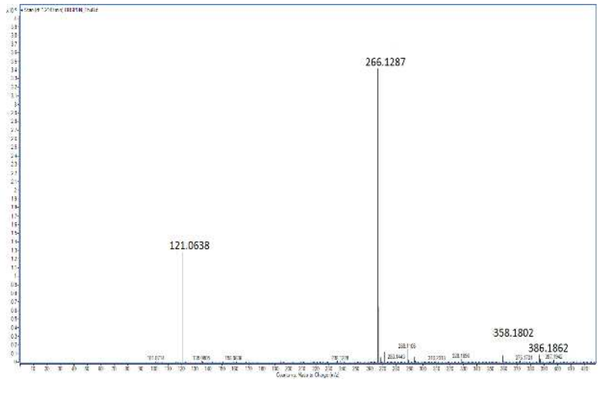
2. FTIR, HRMS, 1H NMR, 13C NMR of 1-(4-methoxybenzyl)-5-phenyl-1H-1,2,3-triazole

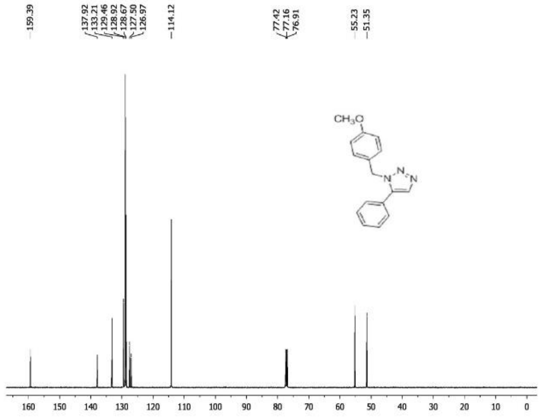


5/31/2018 6:10:26 PM
Instrument: FTIR Affinity - 1S
Department of Inorganic Chemistry, HUS-VNU

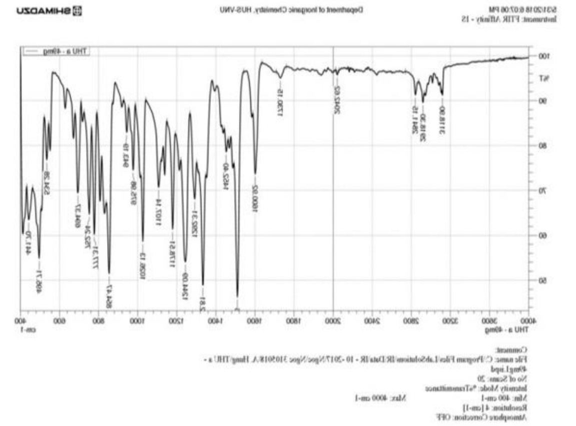
Comment:
File name: C:\Program Files\LabSolutions\IR\Data\IR - 10 -2017\Ngoc\Ngoc 3105018\A. Hung\THU b - 88mg3.ispd
No of Scans: 20
Intensity Mode: %Transmittance
Min: 400 cm-1 Max: 4000 cm-1
Resolution: 4 [cm-1]
Atmosphere Correction: OFF

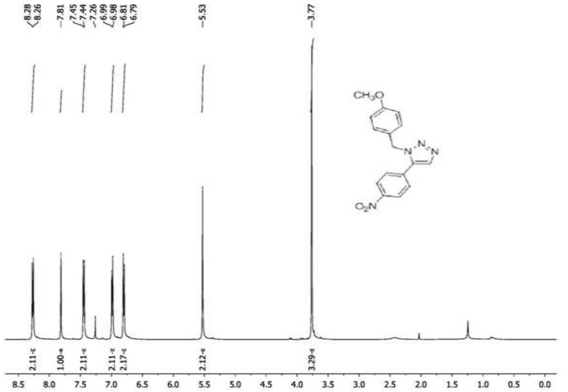


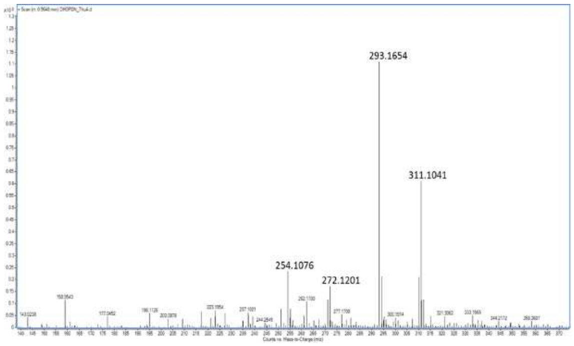


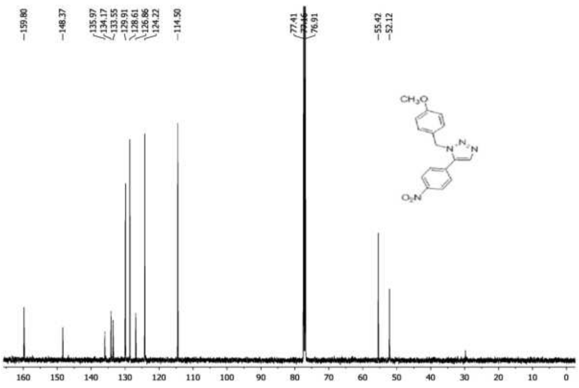


3. FTIR, HRMS, 1H NMR, 13C NMR of 1-(4-methoxybenzyl)-5-(4-nitrophenyl)-1H-1,2,3-triazole

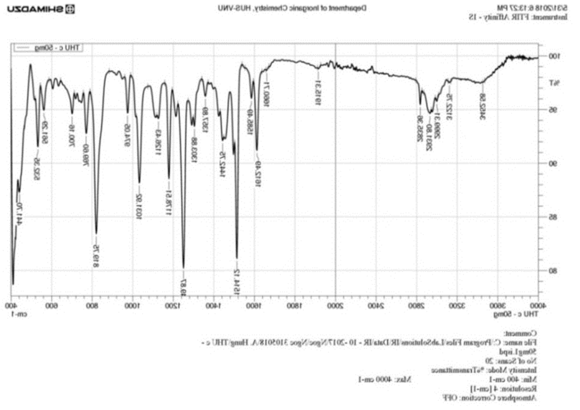


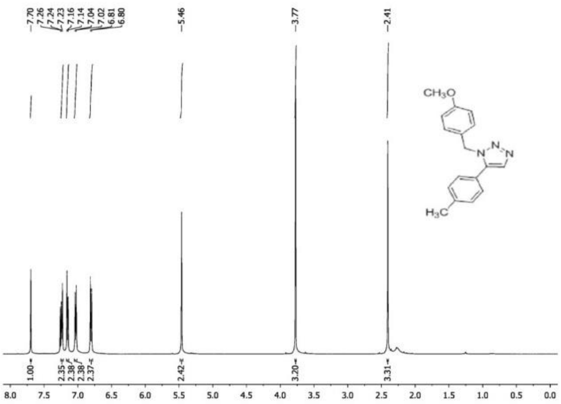


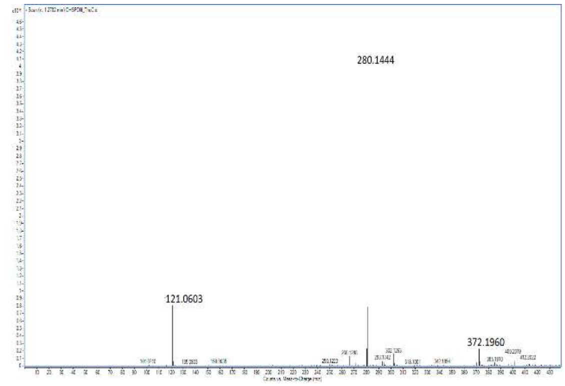


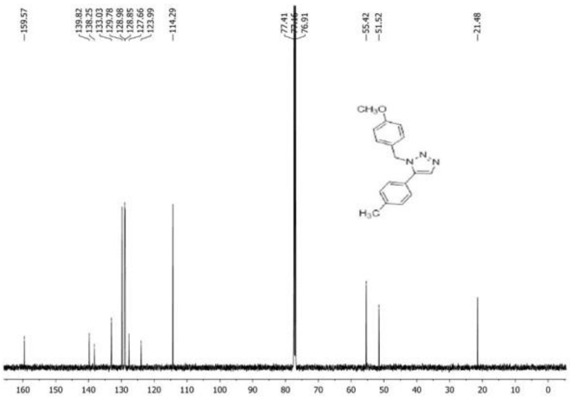


4. FTIR, HRMS, 1H NMR, 13C NMR of 1-(4-methoxybenzyl)-5-(4-methylphenyl)-1H-1,2,3-triazole









(The Board of Editors received the paper on 30/4/2020, its review was completed on 15/6/2020)