Iraqi National Journal of Chemistry

Journal homepage:
http://iqnjc.com/Default.aspx

# Lemon Juice as Highly Selective, Efficient, and Renewable Catalyst to Achieve New Series of Heterocyclic Compounds via Click-Multicomponent Reaction

**Jaseim M. Mostafa; Shaymaa K. Younis**

**University of Mosul; College of Science; Department of Chemistry**

**E-mail: chemjasem95@gmail.com**

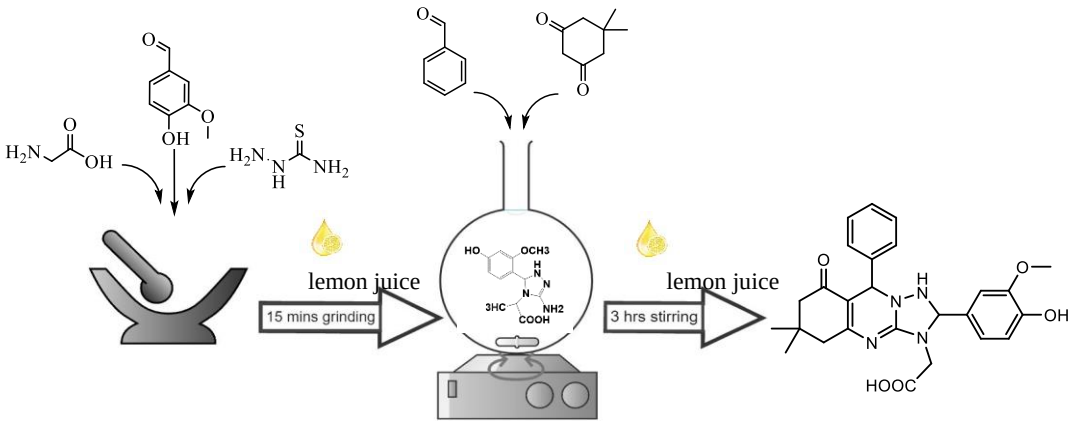
**shaymaakhazaal@uomosul.com**

**Abstract**

Recently, lemon juice has been extensively explored as a cheap, easily available, non-toxic, biodegradable, usable, reusable, highly selective, biocatalyst, and green solvent in organic synthesis, especially nitrogen-containing heterocyclic compounds. a simple, conventional, green, and environmentally friendly one-pot procedure for modification N-substituted 1,2,4-triazole (1) using lemon juice as a renewable and natural catalyst via click multicomponent reaction among glycine, thiosemicarbazide, and vanillin has been successfully done. This supreme unite building is used later as an active precursor, usually through the Biginelli reaction as a click three-component reaction with dimedone and substituted benzaldehyde in acidic media from lemon juice too to afford poly fused heterocyclic compounds **(2-7)**. The available spectral and elemental analysis (FT-IR, 1H-NMR, GC-Mass, and CHN) confirmed the synthesized compound's structure. In contrast, the biological activity as an anti-lung cancer agent has been evaluated only for compound (3), and the results were impressive.

**Keywords:** Lemon juice, Click-multicomponent reaction, Fused heterocyclic compounds, 1,2,4-Triazole, Biginelli reaction.

## Graphical Abstract



## Introduction

Heterocyclic compounds are acquiring a great deal of attention in recent years because of their wide medical applications (Mohamed et al. 2021a).

Actually, the heterocyclic compounds with pyrimidine nucleus exhibits potential applications in the medical, pharmaceutical and biological fields(Sochacka-Ćwikła et al. 2020) as anti-cancer (Shahzad et al. 2019; Kaur et al. 2015), anti-microbial (Zhuang and Ma 2020), anti-bacterial (Su et al. 2021) and active drug against cardiovascular disease (Maleki 2014) as well as anti-virus (Schwalbe, Steele-Moore, and Goodwin 2007) and anti-histamine (Rahaman et al. 2009).

On the other hand, the heterocyclic compound, including the 1,2,4-triazole moiety also showed high effectiveness as anti-fungi (Elzoheiry et al. 2022), anti-virus (Burman et al. 2022), anti-migraine (Chokshi et al. 2019), anti-cancer (Takahashi et al. 2011). It used in the treatment of epilepsy in children (Pfaller et al. 2021).

In this presentation, heterocyclic compounds with both pyrimidine and 1,2,4 triazole moiety have been prepared starting with the simple effective and ecofriendly path way to afford the unit building (2-(3-amino-5-(4-hydroxy-3-methoxyphenyl)-1,5-dihydro-4H-1,2,4-triazol-4-yl)acetic acid)(1) via click-three component reaction among vanillin, glycine and thiosemicarbazide in acidic media from lemon juice using solvent free grinding technique as a green reaction for (15 min) at room temperature. Then, this unit building was underwent the Biginelli reaction with both of dimedone and substituted benzaldehyde and also in acidic condition from lemon juice through click-multicomponent reactions to afford a new series of poly fused heterocyclic compounds represented by compounds (2-(2-(4-hydroxy-3-methoxyphenyl)-6,6-dimethyl-8-oxo-9-phenyl-1,2,5,7,8,9-hexahydro-[1,2,4]triazolo[5,1-b]quinazolin-3(6H)-yl)acetic acid) **(2-7)**.

## Materials and methods

Melting points (M.P.) were determined using the SMP30-Stuart melting point apparatus. FT-IR spectra were recorded on a (KBr) disk using a Pye Unicom sp 2000. 1H-NMR spectra were scanned using Bruker Bio Spin GmbH Spectrophotometer (400 MHz) (Turkey) with TMS as the internal standard and DMSO-d6 and CDCl3 as the solvents. The mass spectra (GC-Mass) were obtained using Agilent GC-MS spectrophotometer (Turkey). (CHN) were determined using (PerkinElmer Diamond, heating rate: 0.01-100 °C. min-1, balance Type: horizontal differential type, atmosphere: air, inert gas, vacuum (10-2 Torr), purge gas flow rate: 0-1000ml.min-¹). For microwave irradiation (MWI), a home microwave oven (Silver Crest SMWC,700 A Germany) with a power setting of (450 watts) was employed. TLC was performed on silica gel involving $CaSO_4$ (13%) as binding materal using the solvent system (benzene: Methanol) in ratio 8:2 and the spots were visualized by exposing them to iodine vapours.

## Preparation of lemon juice as biocatalyst: -

Fresh lemon fruit was purchased from a local shop, cut carefully with knife then manually pressed to get the crude juice which then filtered via filter paper to remove all solid materials and get clear juice. The juice PH was determined (between 3-3.4) followed by using it as acid catalyst.

**Synthesis of the unit building 2-(3-amino-5-(4-hydroxy-3-methoxyphenyl)-1,5-dihydro-4H-1,2,4-triazol-4-yl) acetic acid (1)**(El-Saghier et al. 2019)

**Method A:**

In small porcelain mortar an equimolar (0.001 mole) of vanillin (0.152g), thiosemicarbazide (0.091g) and glycine (0.075g) were well grinding for (15 min) in acidic media from lemon juice (1ml) with monitored the reaction with T.L.C (using solvent system benzene: methanol in ratio 8:2). The crude product then washed thoroughly with cold water (5x5) to remove the acid, dried followed by recrystallization from ethanol to afford the compound **(1)**.

**Method B: Via traditional method**

In round bottom flask (25ml) equipped with condenser, an equimolar (0.001 mole) of vanillin (0.152 g), thiosemicarbazide (0.091 g) and the glycine (0.075 g) were dissolved in aqueous ethanol solution (2ml ethanol 97% + 12 ml $H_2O$) in acidic media from lemon juice (1ml). The reaction mixture then reflexed for (2 to 3 hours) followed by cooling then poured the crude products into beaker equipped with ice water with constant stirring, filtered and washed thoroughly with water to get rid the acid followed by recrystallization from ethanol to afford the compound **(1)** with the same physical properties with low percentage yield.

**2-(3-amino-5-(4-hydroxy-3-methoxyphenyl)-1,5-dihydro-4H-1,2,4-triazol-4-yl)acetic acid (1).** Yield (76%), off white powder, mp: 189-191 $^{o}$C. Elem. Anal. ($C_{13}H_{16}N_6O_3$, 266.10), Calcd: C, 49.62; H, 5.30; N, 21.04; O, 24.04. Found: C, 49.573; H, 5.263; N, 20.98; O, 24.184. IR (KBr, cm−1): 3440 (OH)acid, 3314 (OH)phenol, 3170 ($NH_2$), 3134 (NH), 1715 (C=O), 1601 (C=N), 1263 (C-N), (C-O-C)asy, 1199, 1058 . $^1$H-NMR (DMSO-d6), δ ppm: $OCH_3$ (s,3.84,3H), $CH_2$-acid (s,6.77,2H), CH-triazole (s, 6.79,1H), $NH_2$ (s,7.02,2H); H-aromatic (m,7.48-8.03,3H); NH-triazole (s,8.14,1H); OH-phenol (s,9.48,1H); OH-acid (s,11.28,1H). MS (m/z): 266; Base peal: 43.

**Synthesis of poly fused heterocyclic compounds 2-(2-(4-hydroxy-3-methoxyphenyl)-6,6-dimethyl-8-oxo-9-phenyl-1,2,5,7,8,9-hexahydro-1,2,4-triazolo[5,1-b]quinazolin-3(6H)-yl) acetic acid (2-7)**(Mohamed et al. 2021b)

Via Biginelli reaction which take place through using an equimolar (0.001 mole) of the unit building **(1)** (0,266g), dimedone (0.144 g) and substituted benzaldehyde in the presence of lemon juice as a biocatalyst. in round bottom flask (25ml) equipped with

magnetic bar. The reaction mixture was then stirred in water path at (75 $^{o}$C) for (2 hours) with monitoring the reaction with T.L.C using solvent system (benzene: methanol in a ratio of 8:2), followed by cooling then poured into beaker equipped with ice-water with constant shaking followed by filtration and washed thoroughly with water, dried and recrystallized from ethanol to achieve the compound **(2-7)**

**2-(2-(4-hydroxy-3-methoxyphenyl)-6,6-dimethyl-8-oxo-9-phenyl-1,2,5,7,8,9-hexahydro-[1,2,4]triazolo[5,1-b]quinazolin-3(6H)-yl)acetic acid (2).** Yield (75%), white powder, mp: 179-182 $^{o}$C. Elem. Anal. ($C_{26}H_{28}N_4O_5$, 476.21), Calcd: C, 65.53: H, 5.92: N, 11.79: O, 16.79. Found: C, 65.510: H, 5.930: N, 11.781: O, 16.779. IR (KBr, cm−1): 3528 (O-H)acid, 3437 (O-H)phenol, 3276 (NH), 1754 (C=O)acid, 1672 (C=O), 1578 (C=N), 1278 (C-N), (C-O-C)asy, 1114, 1030 . $^{1}$H-NMR (DMSO-d6), δ ppm: Dimedone (m, 1.01-2.36, 10H), OCH3 (s, 3.84, 3H); $CH_3$-acid (s, 6.78, 2H), CH-triazole (s, 6.80, 1H); H-aromatic (m, 7.03-8.06, 9H); NH-triazole (s,8.09,1H), OH-phenol (s, 9.43, 1H); OH-acid (s, 11.24, 1H).

**2-(2-(4-hydroxy-3-methoxyphenyl)-6,6-dimethyl-9-(4-nitrophenyl)-8-oxo-1,2,5,7,8,9-hexahydro-[1,2,4]triazolo[5,1-b]quinazolin-3(6H)-yl)acetic acid (3).** Yield (85%), yellow powder, mp: 190-191 $^{o}$C. Elem. Anal. ($C_{26}H_{27}N_5O_7$, 521.19), Calcd: C, 59.88: H, 5.22: N, 13.43: O, 21.47. found: C, 59.73: H, 5.178: N,13.28: O, 21.420. IR (KBr, cm−1): 3491 (O-H)acid, 3363 (O-H)phenol, 3139 (NH), 1700 (C=O)acid, 1674 (C=O), 1577 (C=N), 1250 (C-N), (C-O-C)asy, 1154, 1099. 1H-NMR (DMSO-d6), δ ppm: Dimedone (m, 0.86-2.35,10H); $OCH_3$ (s,3.85, 3H); $CH_2$-acid (s, 6.10, 2H); CH-triazole (s,6.77, 1H); H-aromatic (m, 7.03-8.40, 8H); NH-triazole (s, 944, 1H); OH-phenol (s,11.25, 1H; OH-acid (s, 11.71, 1H). MS (m/z): 521; Base peal: 83.

**2-(9-(2-chlorophenyl)-2-(4-hydroxy-3-methoxyphenyl)-6,6-dimethyl-8-oxo-1,2,5,7,8,9-hexahydro-[1,2,4]triazolo[5,1-b]quinazolin-3(6H)-yl)acetic acid (4).** Yield (73%), pale yellow crystals, mp: 186-187 $^{o}$C. Elem. Anal. ($C_{26}H_{27}ClN_4O_5$, 510.17), Calcd: C, 61.12: H, 5.33: N, 10.96: O, 15.6: Cl, 6.94. found: C, 61.010: H, 5.290: N, 10.240: O, 15.430: Cl, 6.83. IR (KBr, cm−1): 3394 (O-H)acid, 3235 (O-H)phenol, 3123 (NH), 1720 (C=O)acid, 1642 (C=O), 1602 (C=N), 1381 (C-N), (C-O-C)asy, 1141, 1071; 745 (C-CL). 1H-NMR (DMSO-d6), δ ppm: Dimedone (m, 0.86-2.48, 10H); $OCH_3$ (s, 3.83, 3H), $CH_2$acid (s, 4.58, 2H); CH-triazole (s, 6.91, 1H); H-aromatic (m, 7.08-8.30, 8H); NH-triazole (s, 8.39, 1H); OH-phenol (s,8.48, 1H); OH-acid (s, 11.62, 1H). MS (m/z): 510; Base peal: 68.

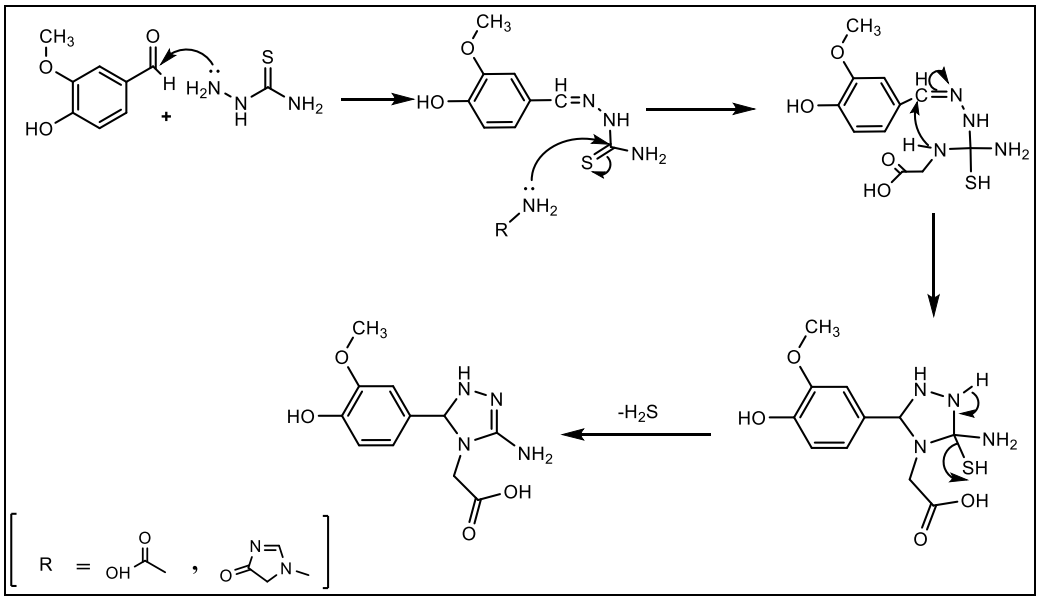
**2-(2-(4-hydroxy-3-methoxyphenyl)-6,6-dimethyl-9-(3-nitrophenyl)-8-oxo-1,2,5,7,8,9-hexahydro-[1,2,4]triazolo[5,1-b]quinazolin-3(6H)-yl)acetic acid (5).** Yield (84%), pale yellow powder, mp: 198-200 ºC. IR (KBr, cm−1): 3434 (O-H)acid, 3363 (O-H)phenol, 3139 (NH), 1729 (C=O)acid, 1670 (C=O), 1586 (C=N), 1285 (C-N), (C-O-C)asy, 1167, 1045.

**2-(2-(4-hydroxy-3-methoxyphenyl)-6,6-dimethyl-8-oxo-9-(p-tolyl)-1,2,5,7,8,9-hexahydro-[1,2,4]triazolo[5,1-b]quinazolin-3(6H)-yl)acetic acid (6).** Yield (57%), brown powder, mp: 159-162 ºC. IR (KBr, cm−1): 3427 (O-H)acid, 3252 (O-H)phenol, 3149 (NH), 1714 (C=O-OH), 1650 (C=O), 1582 (C=N), 1270 (C-N), (C-O-C)asy, 1197, 1028.

**2-(2-(4-hydroxy-3-methoxyphenyl)-9-(4-methoxyphenyl)-6,6-dimethyl-8-oxo-1,2,5,7,8,9-hexahydro-[1,2,4]triazolo[5,1-b]quinazolin-3(6H)-yl)acetic acid (7).** Yield (65%), brown powder, mp: 163-165 ºC. IR (KBr, cm−1): 3456 (O-H)acid, 3318 (O-H)phenol, 3101 (NH), 1715 (C=O)acid, 1611 (C=O), 1590 (C=N), 1284 (C-N), (C-O-C)asy, 1169, 1035.

## Results and Discussion

We have established effective click-multicomponent reaction protocol from available and cheap materials to achieve a highly effective unit building represented by N-substituted 1,2,4-triazole in the presence of lemon juice as a biodegradable, renewable, efficient, inexpensive acid catalysts as shown in the following mechanism(El-Saghier et al. 2019): -

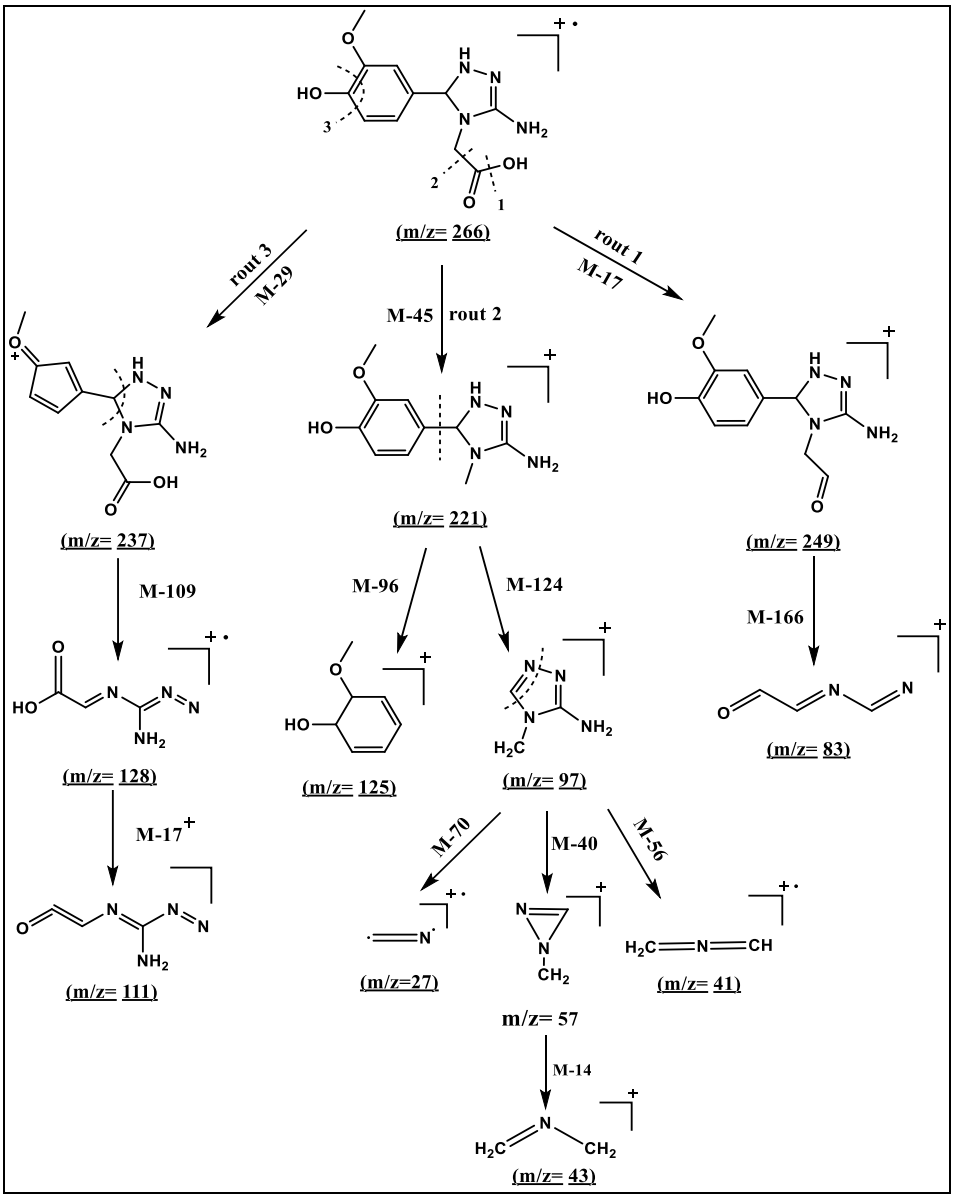



**Scheme 1:** Synthesis mechanism of 1,2,4-triazole **(1)**

This unit building has been obtained through simple filtration and recrystallization with no need for column chromatography for purification, which reduce the waste as well as environmental pollution. Furthermore, the green grinding procedure was comparing with the traditional methods, then choosing the best between them. Basically, this unit building was identified by FT-IR spectroscopy that shown stretching vibration for the acid-OH, phenol-OH, $NH_2$ and acid-C=O group that gives preliminary proof of the purpose proposed synthetic formula.

Whereas in $^1$H-NMR, it's shown clear singlet peak at δ(ppm) (11.28) and (9.48) refer to the OH-acid and OH-phenol respectively which give an improvement about the expected synthetic formula.

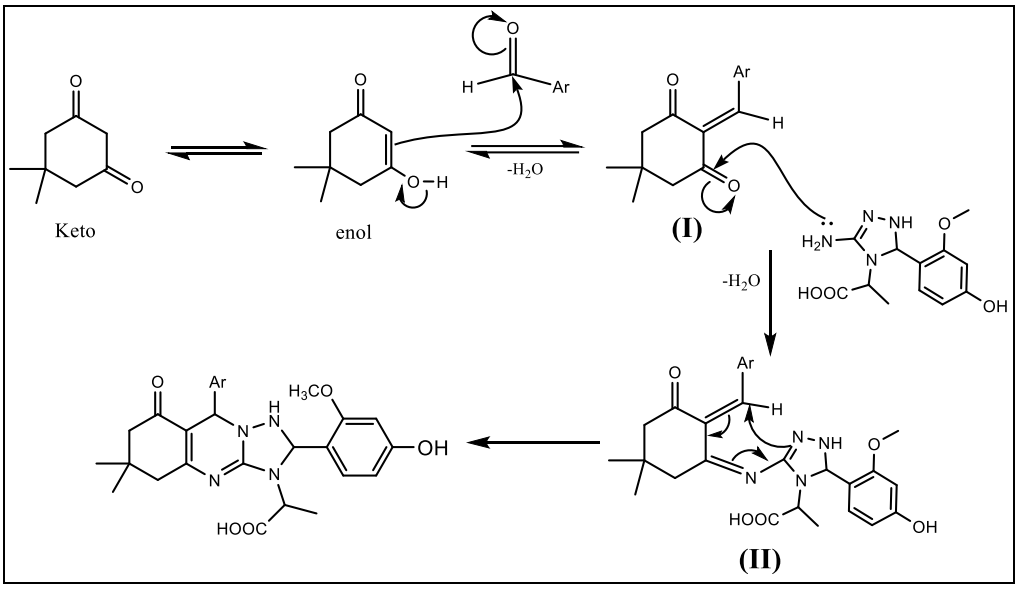
For more identification, the GC-Mass spectroscopy for the unit building **(1)** has been done successfully because this technique gives m/z=266 which came in agreement with the unit building molecule weight and actually with Base peak=43 and also this technique shown the fragments with different mass which describe the fragmentation pattern of the units building as shown in following scheme: -




**Scheme 2:** fragmentation pattern of 1,2,4-triazole compound **(1)**

Finally, the CHN analysis has been done and also it gives additional evidence on the validity of the proposed scientific formula.

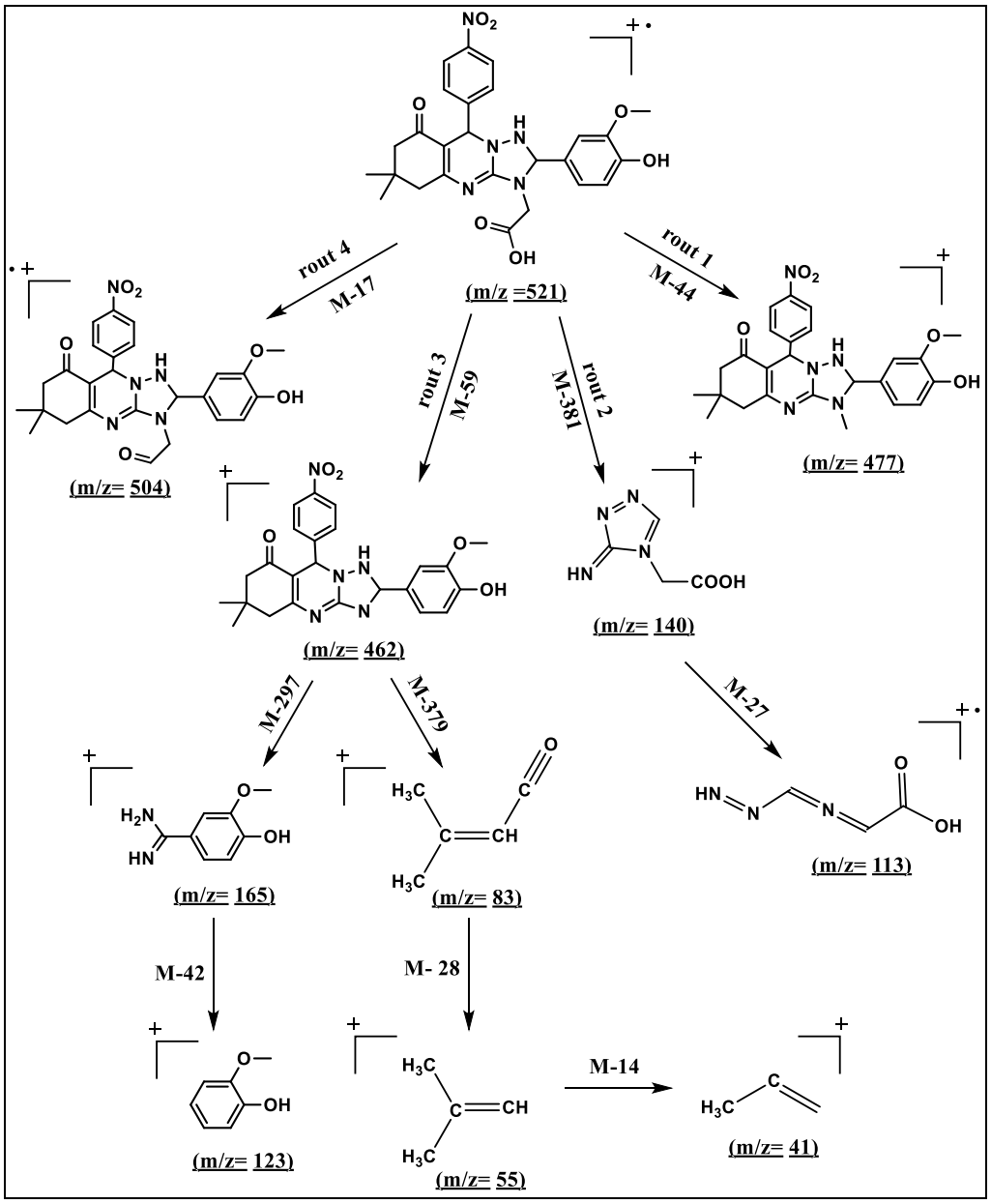
After approving the correctness of the synthetic formula of the unit building, it's then underwent the Biginelli reaction as primary aromatic amine source with dimedone and substituted benzaldehyde in acidic media from lemon juice to afford poly fused heterocyclic compounds as explained in the following mechanism below (Mohamed et al. 2021b): -




**Scheme 3:** Synthesis mechanism of poly fused heterocyclic compounds **(2-7)**

These compounds have been identified via spectral method. So, in FT-IR the appearance of cyclo ketone absorption band and disappearing of the acid-carbonyl absorption band shown evidence about the suggested structure, whereas in $^{1}$H-NMR for compounds **(2,3,5)** shown absorption peak at refer to the only OH-phenol in addition to the other absorption peaks listed.

Actually, and for further identification we choose compound **(3, 5)** to study is structured through GC-Mass spectroscopy. The fragmentation pathway which gives its exact molecular weight at (m/e= 521 and 510) respectively and they show base peak at (m/z= 83 and 68) respectively and the following Scheme describes the fragmentation pattern for compound **(3)**.




**Scheme 4:** fragmentation pattern of the compound **(3)**

Besides that, CHN analysis has been done for compounds **(2,3,5)** and also, they given identical value with those of calculated one which came in agreements with suggested structure.

## The anti-cancer evaluation

Because all the prepared compound bearing active fused heterocyclic system and after improving the right structure for this compound, we selected compound **(3)** and evaluated it's biological activity as anti-lung cancer (A549 cell line), and it's cytotoxicity with six concentrations(Adil, Al-Shammari, and Murbat 2020) followed by tested its IC50(Al-Shammari, Jalill, and Hussein 2020) and the range of inhibition. The following pictures and diagrams describe the high inhibition of lung cancer cell

with low toxicity. The pictures bellow shows the high effectiveness of compound (3) against lung cancer cells as growth inhibition(Al-Ziaydi et al. 2020).

## A549 lung cell line

A549 cells are adenocarcinomic human alveolar basal epithelial cells, and constitute a cell line that was first developed in 1972 by D. J. Giard, et al. through the removal and culturing of cancerous lung tissue in the explanted tumor of a 58-year-old Caucasian male. The cells are used as models for the study of lung cancer and the development of drug therapies (Cooper et al. 2016; Giard et al. 1973).

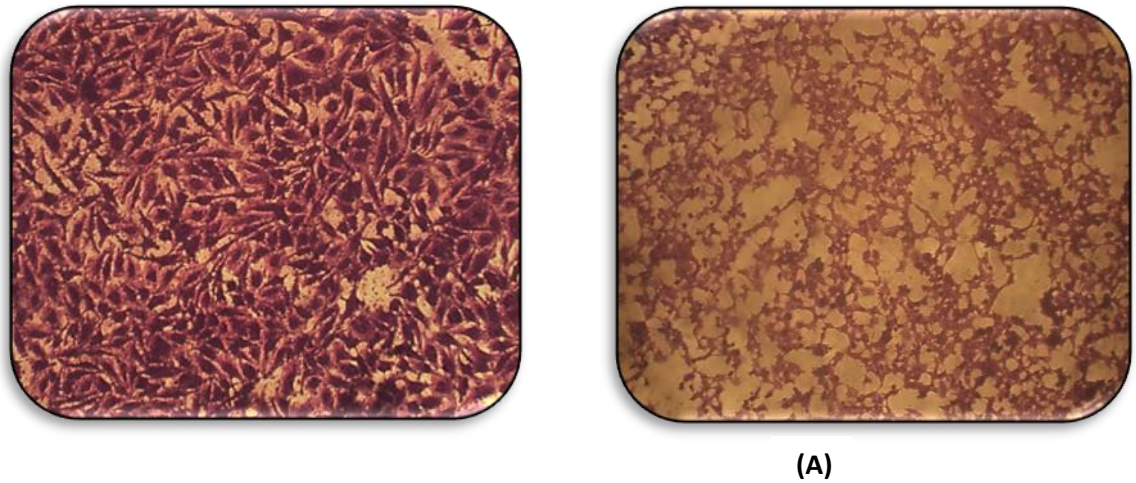



**Fig 1: (A)** untreated (A549) cells with compound **(3)**. **(B)** treated (A549) cells with compound **(3)**

Whereas, the following diagram show the low cytotoxicity even at high concentration.

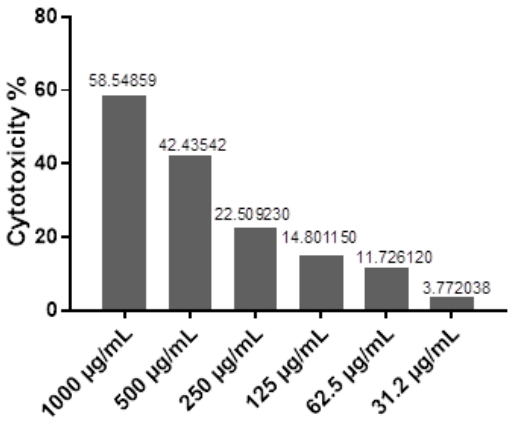



**Fig 2:** Cytotoxicity chart for compound **(3)** against (A549) cells

On the other hand, it shown IC50= 941.9, this is very accepted results, especially since the permissible rang is (486,6-1893).

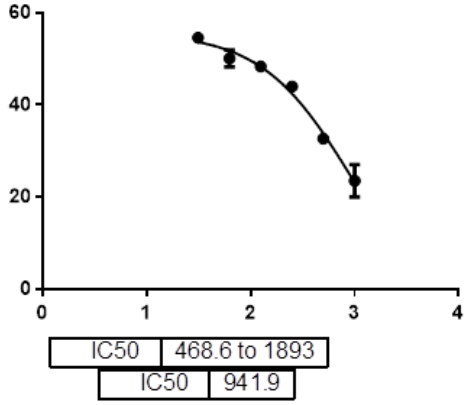



**Fig 3:** IC50 chart for compound **(3)** against (A549) cells

Finally, the OD for compound (3) gave harmonious results with reference model as shown below.

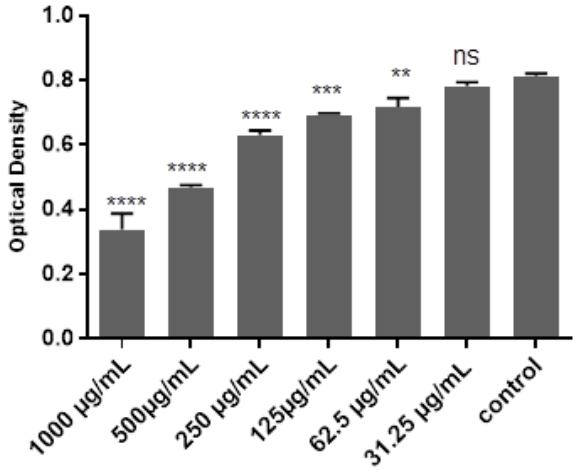



**Fig 4:** Optical density chart for compound **(3)** against (A549) cells

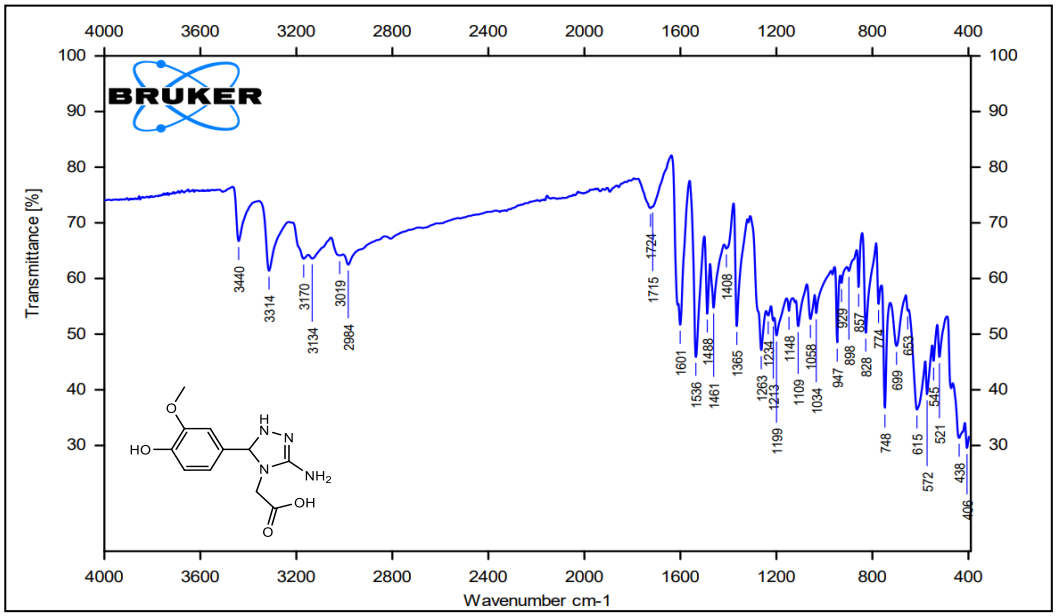



**Fig (5):** FT-IR for compound **(1)**

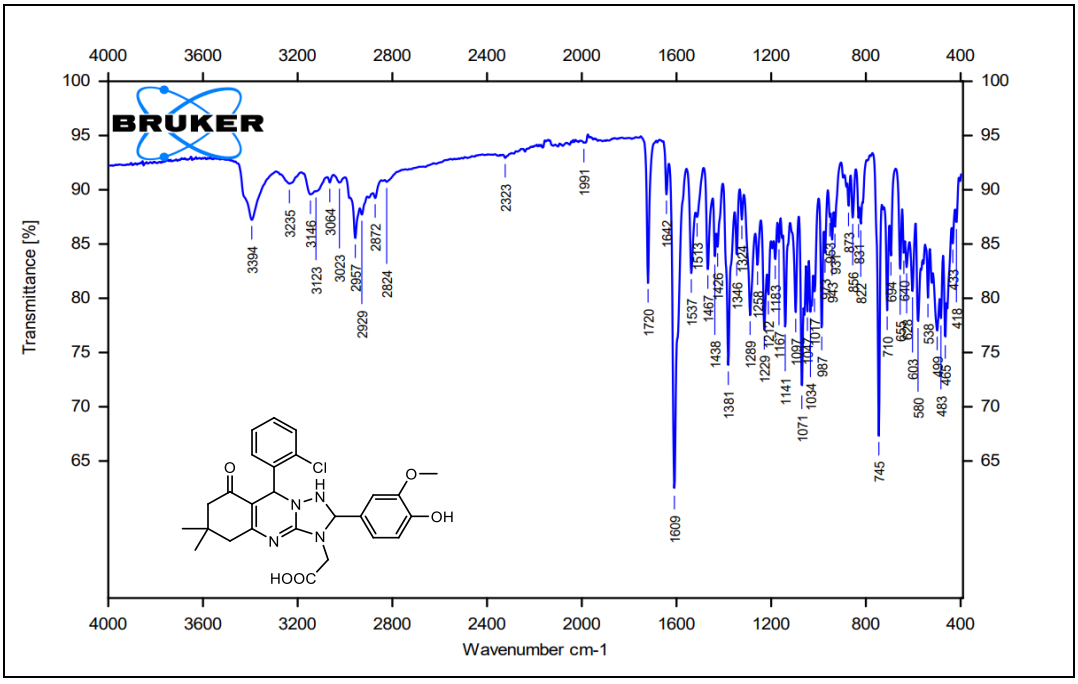



**Fig (6):** FT-IR for compound **(5)**

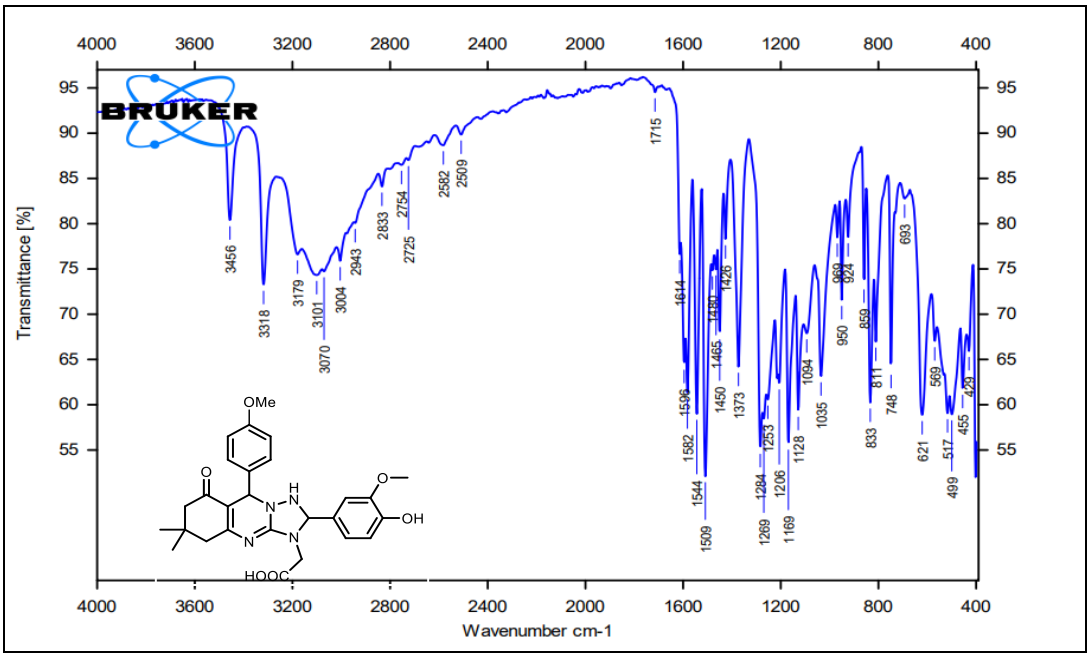



**Fig (7):** FT-IR for compound **(7)**

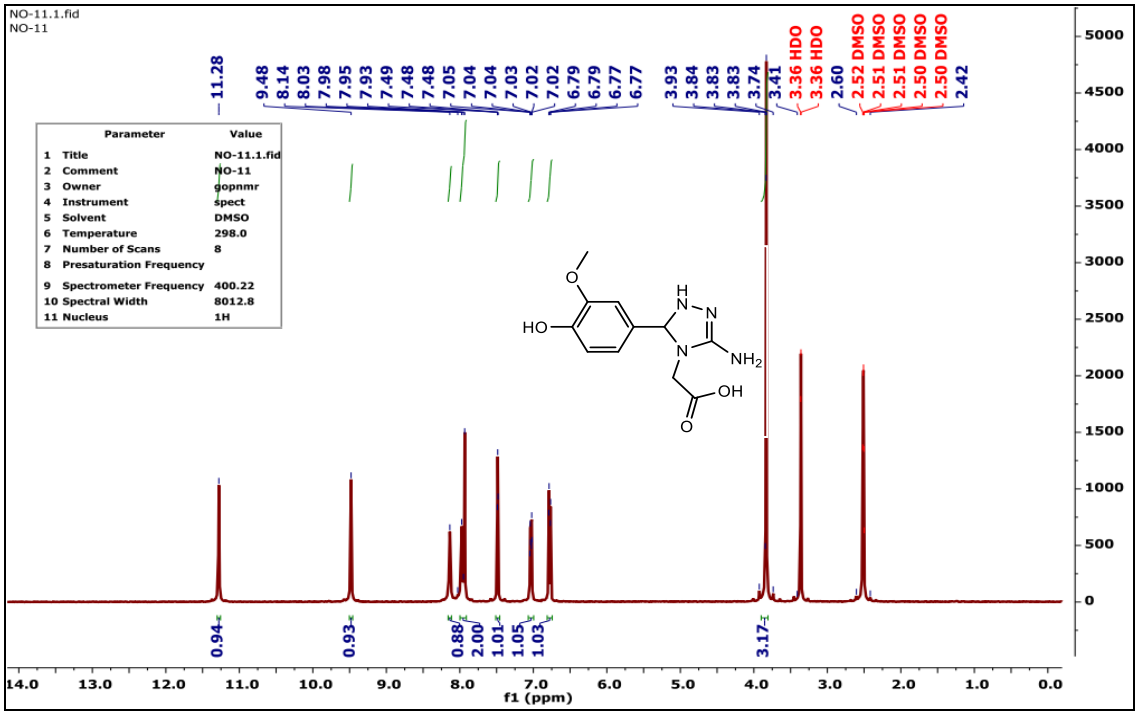



**Fig (8):** $^{1}$H-NMR for compound **(1)**

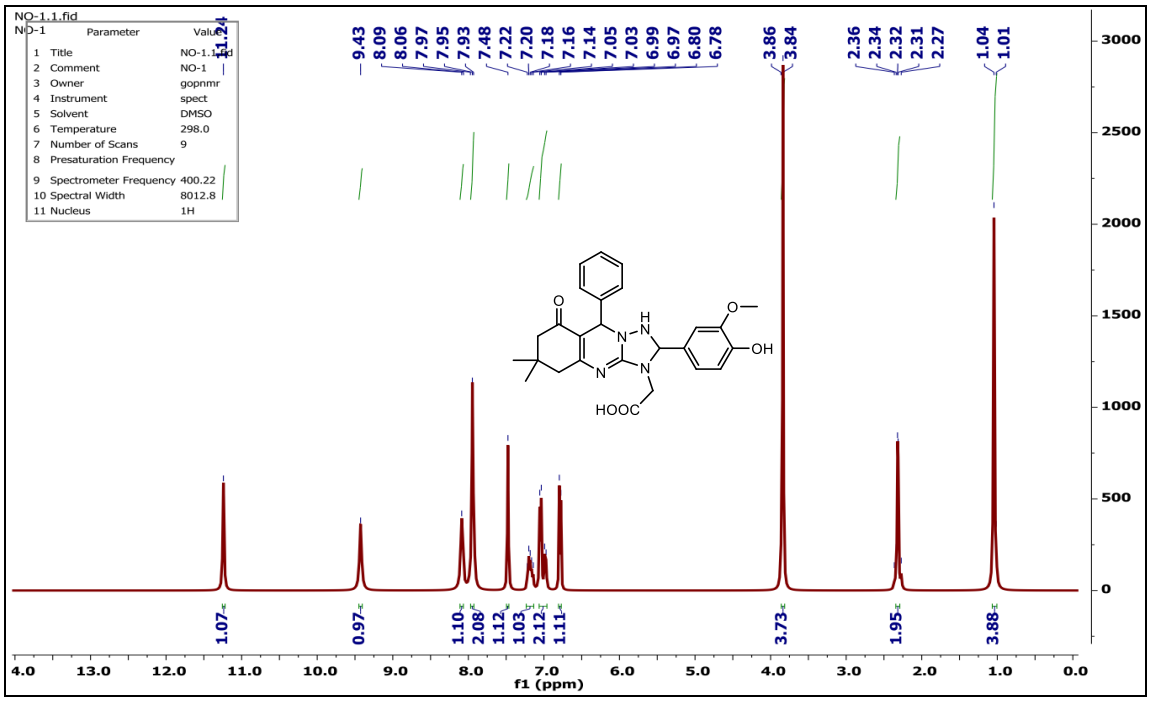



**Fig (9):** $^{1}$H-NMR for compound **(2)**

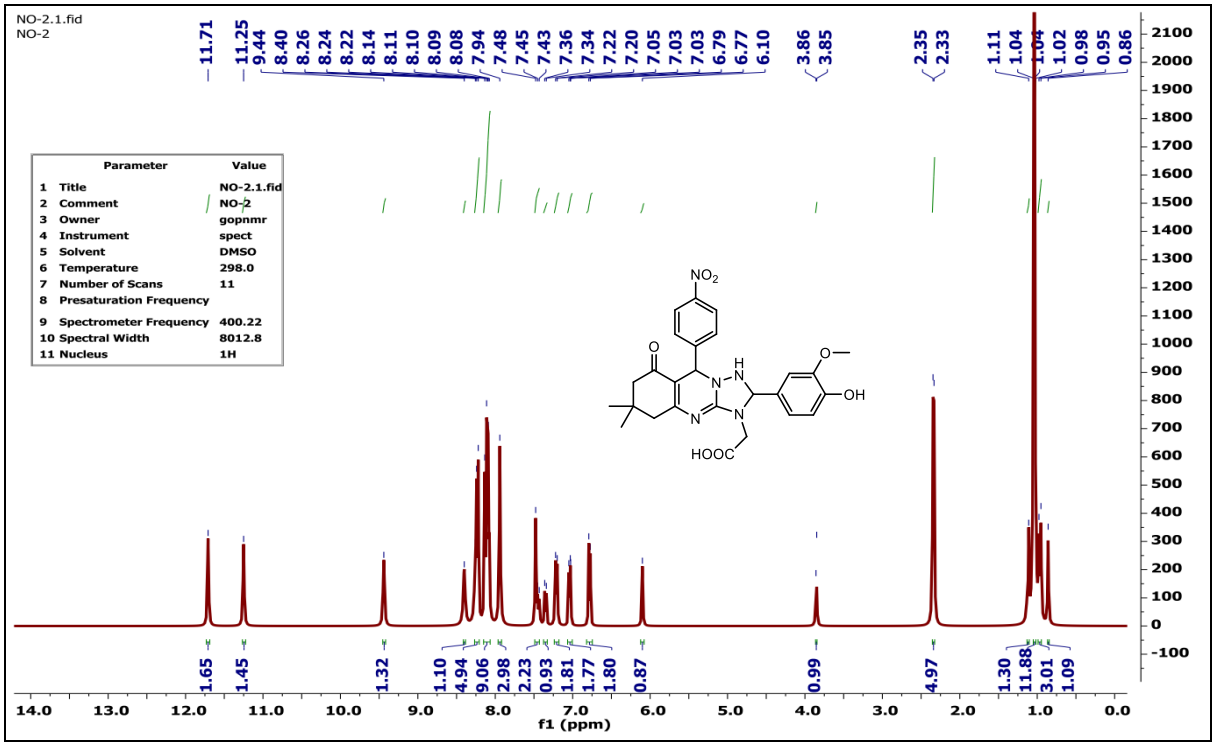



**Fig (10):** $^{1}$H-NMR for compound **(3)**

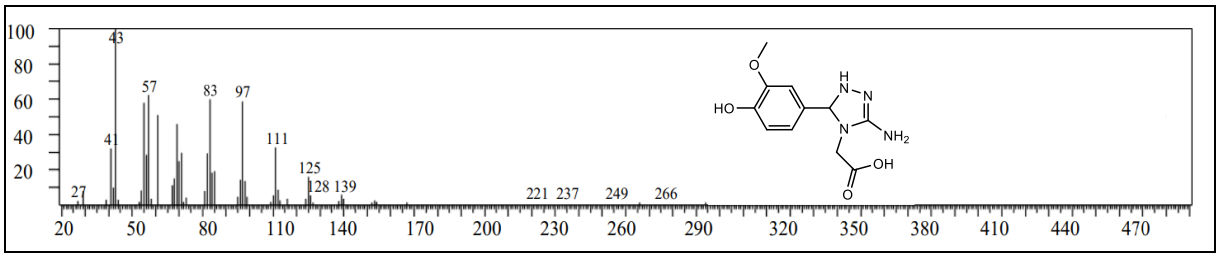


**Fig (11):** GC-Mass for compound **(1)**

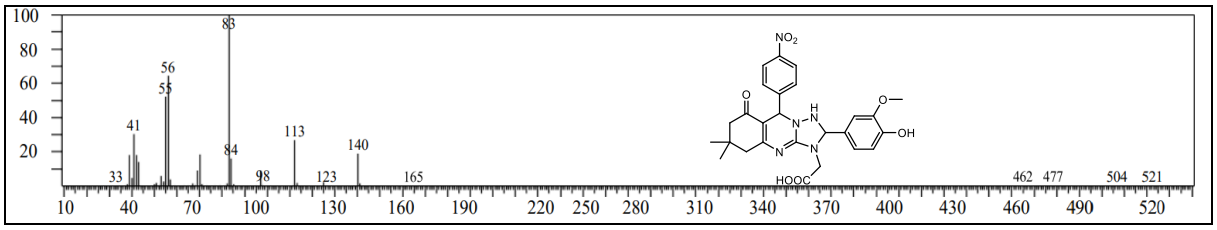


**Fig (12):** GC-Mass for compound **(3)**

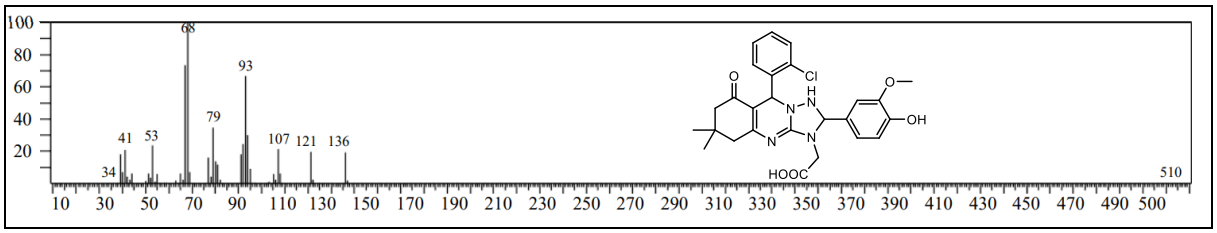


**Fig (13):** GC-Mass for compound **(5)**

## Conclusion

Highly efficient, simple, fast and clean approaches have been achieved in this presentation via using natural acid catalyst represented by lemon juice and a green chemistry technique represented by grinding and one-pot multicomponent reaction that gave products with a high percentage yield without more purification. Furthermore, this compound possess predictably anti-cancer effects due to its active structure, which were high growth inhibition for lung cancer cells with low cytotoxicity.

## References

Adil, Ban, Ahmed Al-Shammari, and Hamid Murbat. 2020. 'Breast Cancer Treatment Using Cold Atmospheric Plasma Generated by the FE-DBD Scheme', *Clinical Plasma Medicine*, 19-20: 100103.

Al-Shammari, A. M., R. D. A. Jalill, and M. F. Hussein. 2020. 'Combined therapy of oncolytic Newcastle disease virus and rhizomes extract of Rheum ribes enhances cancer virotherapy in vitro and in vivo', *Mol Biol Rep*, 47: 1691-702.

Al-Ziaydi, A. G., A. M. Al-Shammari, M. I. Hamzah, H. S. Kadhim, and M. S. Jabir. 2020. 'Newcastle disease virus suppress glycolysis pathway and induce breast cancer cells death', *Virusdisease*, 31: 341-48.

Burman, Bharat, Scott B Drutman, Matthew G Fury, Richard J Wong, Nora Katabi, Alan L Ho, and David G Pfister. 2022. 'Pharmacodynamic and therapeutic pilot studies of single-agent ribavirin in patients with human papillomavirus–related malignancies', *Oral Oncology*, 128: 105806.

Chokshi, Ashish, Ravi Vaishya, Rachana Inavolu, and Thrimoorthy Potta. 2019. 'Intranasal spray formulation containing rizatriptan benzoate for the treatment of migraine', *International Journal of Pharmaceutics*, 571: 118702.

Cooper, J. R., M. B. Abdullatif, E. C. Burnett, K. E. Kempsell, F. Conforti, H. Tolley, J. E. Collins, and D. E. Davies. 2016. 'Long Term Culture of the A549 Cancer Cell Line Promotes Multilamellar Body Formation and Differentiation towards an Alveolar Type II Pneumocyte Phenotype', *PLoS One*, 11: e0164438.

El-Saghier, Ahmed, Mounier Mohamed, Omyma Abd-Allah, Asmaa Kadry, Tamer Ibrahim, and Adnan Bekhit. 2019. 'Green synthesis, antileishmanial activity evaluation, and in silico studies of new amino acid-coupled 1,2,4-triazoles', *Medicinal Chemistry Research*, 28.

Elzoheiry, Manal A, Manar S Elmehankar, Wafaa A Aboukamar, Randa El-Gamal, Heba Sheta, Dina Zenezan, Nairmen Nabih, and Abeer A Elhenawy. 2022. 'Fluconazole as Schistosoma mansoni cytochrome P450 inhibitor: In vivo murine experimental study', *Experimental parasitology*, 239: 108291.

Giard, D. J., S. A. Aaronson, G. J. Todaro, P. Arnstein, J. H. Kersey, H. Dosik, and W. P. Parks. 1973. 'In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors', *J Natl Cancer Inst*, 51: 1417-23.

Kaur, R., P. Kaur, S. Sharma, G. Singh, S. Mehndiratta, P. M. Bedi, and K. Nepali. 2015. 'Anti-cancer pyrimidines in diverse scaffolds: a review of patent literature', *Recent Pat Anticancer Drug Discov*, 10: 23-71.

Maleki, Ali. 2014. 'One-pot three-component synthesis of pyrido[2′,1′:2,3]imidazo[4,5-c]isoquinolines using Fe3O4@SiO2–OSO3H as an efficient heterogeneous nanocatalyst', *RSC Advances*, 4: 64169-73.

Mohamed, M. A. A., A. A. Bekhit, O. A. Abd Allah, A. M. Kadry, T. M. Ibrahim, S. A. Bekhit, K. Amagase, and A. M. M. El-Saghier. 2021a. 'Synthesis and antimicrobial activity of some novel 1,2-dihydro-[1,2,4]triazolo[1,5-a]pyrimidines bearing amino acid moiety', *RSC Adv*, 11: 2905-16.

Mohamed, Mounir, Adnan Bekhit, Omyma Abd Allah, Asmaa Kadry, Tamer Ibrahim, Salma Bekhit, Kikuko Amagase, and Ahmed El-Saghier. 2021b. 'Synthesis and antimicrobial activity of some novel 1,2-dihydro-[1,2,4]triazolo[1,5- a ]pyrimidines bearing amino acid moiety', *RSC Advances*, 11: 2905-16.

Pfaller, Michael A, Dee Shortridge, Kelly A Harris, Mark W Garrison, C Andrew DeRyke, Daryl D DePestel, Pamela A Moise, and Helio S Sader. 2021. 'Ceftolozane-tazobactam activity against clinical isolates of Pseudomonas aeruginosa from ICU patients with pneumonia: United States, 2015–2018', *International Journal of Infectious Diseases*, 112: 321-26.

Rahaman, S. A., Y. Rajendra Pasad, P. Kumar, and B. Kumar. 2009. 'Synthesis and anti-histaminic activity of some novel pyrimidines', *Saudi Pharm J*, 17: 255-8.

Schwalbe, Richard, Lynn Steele-Moore, and Avery C Goodwin. 2007. *Antimicrobial susceptibility testing protocols* (Crc Press).

Shahzad, Sohail Anjum, Muhammad Yar, Zulfiqar Ali Khan, Lubna Shahzadi, Syed Ali Raza Naqvi, Adeem Mahmood, Sami Ullah, Ahson Jabbar Shaikh, Tauqir Ali Sherazi, Adebayo Tajudeen Bale, Jędrzej Kukułowicz, and Marek Bajda. 2019. 'Identification of 1,2,4-triazoles as new thymidine phosphorylase inhibitors: Future anti-tumor drugs', *Bioorganic Chemistry*, 85: 209-20.

Sochacka-Ćwikła, A., A. Regiec, M. Zimecki, J. Artym, E. Zaczyńska, M. Kocięba, I. Kochanowska, I. Bryndal, A. Pyra, and M. Mączyński. 2020. 'Synthesis and Biological Activity of New 7-Amino-oxazolo[5,4-d]Pyrimidine Derivatives', *Molecules*, 25.

Su, S., M. Chen, X. Tang, F. Peng, T. Liu, Q. Zhou, W. Zhan, M. He, C. Xie, and W. Xue. 2021. 'Design, Synthesis and Antibacterial Activity of Novel Pyrimidine-Containing 4H-Chromen-4-One Derivatives*', *Chem Biodivers*, 18: e2100186.

Takahashi, Kayo, Gen Yamagishi, Toshiyuki Hiramatsu, Ayako Hosoya, Kayo Onoe, Hisashi Doi, Hiroko Nagata, Yasuhiro Wada, Hirotaka Onoe, and Yasuyoshi Watanabe. 2011. 'Practical synthesis of precursor of [N-methyl-11C] vorozole, an efficient PET tracer targeting aromatase in the brain', *Bioorganic & Medicinal Chemistry*, 19: 1464-70.

Zhuang, J., and S. Ma. 2020. 'Recent Development of Pyrimidine-Containing Antimicrobial Agents', *ChemMedChem*, 15: 1875-86.