Environmentally And Green Approaches To The Synthesis Of New Series Of Quinazolinones Including 1,2,3-Triazole Moiety With High Anti-Cancer Effectiveness

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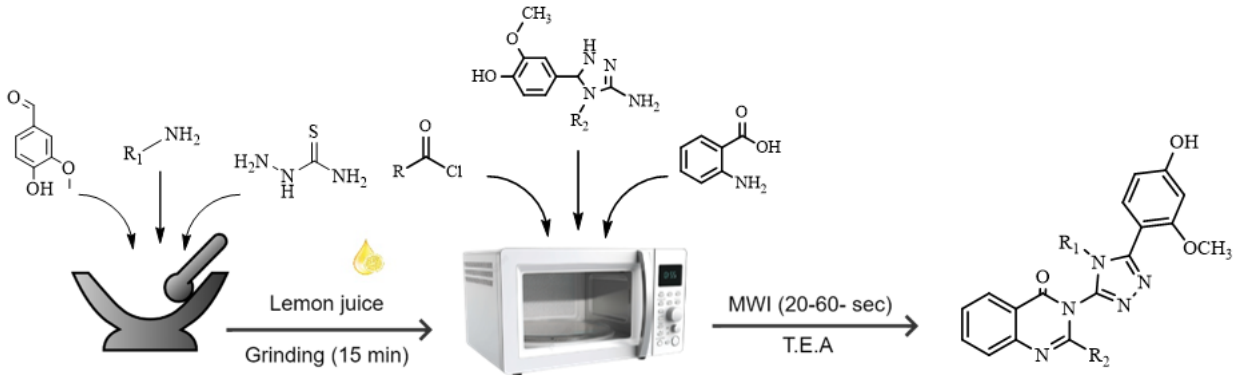
Abstract
Fused Heterocyclic compounds an important skeleton for pharmaceutical is the most privileged and predominate of organic chemistry. They have formidable medical value and pharmaceutical effectiveness. Actually, in this study the mean units building **(1** and **2)** represented by N-substituted 1,2,4-triazole have been successfully prepared through click-multicomponent reaction among glycine (creatinine) thiosemicarbazide and vanillin using the grinding technique as a green approach for only (15 min) in acidic media from freshly prepared lemon juice as a highly selective and biocatalyst. Then, a green and ecofriendly strategy used to afford a new series of fused quinazolinones **(3-10)** using the above units building **(1** and **2)** via also click-Multicomponent reactions with anthranilic acid and substituted acid chloride in basic media from triethylamine and the reaction was accelerated by microwave radiation for (20-60 sec) at (450 Watt) in good to excellent yield. The anticancer evaluation has been done successfully for compounds **(4** and **7)** against breast and lung cancer cells (human cells) and also they shown high growth inhibition with the low cytotoxicity.

Graphical Abstract



Keywords: Quinazolinone, click-multicomponent reactions, Lemon juice, 1,2,4-Triazole, Green chemistry, Grinding technique, microwave irradiation

Introduction

Clear green approach with environmentally accepted and renewable raw material which offer environmental and economic advantage are very necessary in organic synthesis comparing with that of the traditional scientific process.
Recently, this green approach used widely in heterocyclic modification which possess potential application in medical and pharmaceutical field. Quinazolinone are one of the most important types of the above heterocyclic compounds that receive a great deal of attention due to it's potential application as anti-cancer(Jampilek et al., 2009; Potewar et al., 2008), anti-bacterial(Li et al., 2022), anti-microbial(Mabkhot et al., 2014; Schwalbe et al., 2007), analgesic(Rezaeinasab et al., 2022), anti-inflammatory(Rakesh et al., 2015), anti-hypertensive(Maleki, 2014), anti-diabetes(Moghadam Farid et al., 2023), anti-fungal(Fan et al., 2019), anti-malaria(Birhan et al., 2015) and may other biological activity. Whereas the 1,2,4-triazole ring system possess wide applications in many fields as anti-fungal(Elzoheiry et al., 2022; Shettar et al., 2021), anti-virial(Burman et al., 2022), anti-migraines(Chokshi et al., 2019), anti-cancer(Park et al., 2021; Takahashi et al., 2011) and tested as active drug for treatment of epilepsy in children(Pfaller et al., 2021).
Herein, in this presentation we report a high selective preparation approach of a new series of fused quinazoline known including N-substituted 1,2,4-triazole moiety represented by compounds **(3-10)** through a green approach represented by grinding and microwave irradiation and also accelerated by biocatalysts efficient, selective acid represented by lemon juice in the first stage of this process with the high yield and without environmental pollution.

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. H-NMR spectra were recorded using a 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 taken using an Agilent GC-MS (Turkey). (CHN) was determined with (PerkinElmer Diamond, heating rate: 0.01-100 °C min-1, balancing) Horizontal differential type, air, inert gas, vacuum (10-2 Torr), purge gas flow rate: 0-1000ml.min-1). A household microwave oven (Silver Crest SMWC,700 A Germany) with a power setting of (450 watts) was used for irradiation (MWI). TLC was carried out on silica gel-coated glass plates (20x20) involving $CaSO_4$ (13%) as binding material with an 8:2 solvent system of benzene: methanol, and the spots were seen by exposing them to iodine vapours.

Preparation of fresh lemon juice: -

Fresh lemon fruit was purchased locally, carefully sliced with a knife, and manually pressed to obtain the crude juice, which was then filtered through filter paper to remove any solid elements and yield clear juice. The PH of the juice was determined (between 3-3.4), and it was then used as an acid catalyst.

Preparation of the units building (1, 2): - (El-Saghier et al., 2019; Sachdeva et al., 2013)

An equimolar (0.001 mole) of vanillin (0.152g), thiosemicarbazide (0.091g), and once with glycine (0.075g) to afford compound (1), and once with creatinine (0.113 g) to afford compound (2) were well grinding for (15 min) in acidic media from lemon juice (1 ml). The reaction monitored with T.L.C (using solvent system benzene: methanol in ratio (8:2). The crude product was then thoroughly washed with cold water (5x5) to remove the acid, dried, and recrystallized from ethanol to get the compounds (1 and 2).

Preparation of fused quinazolinone (3-10): -(Mohammadkhani & Heravi, 2020)

Method A: via microwave radiation

In a beaker (5 ml) equimolar (0.001 mole) of unit building **(1** or **2)** with anthranilic acid and variety acidic chloride and basic media from T.E.A (3 drops) were well mixed and irradiated by microwave domestic oven for (20-60 sec) and (450 Watt) through wet reaction (0.5 ml H_2O) followed by cooling and washed thoroughly with cold water to remove the excess of base which tested by litmos paper, drying then recrystallized from (EtOH-water) to afford the compound **(3-10)**

Method B: via traditional method

Equimolar (0.001 mole) of the units building **(1** or **2)** anthranilic acid and substituted acid chloride with a few drops of T.E.A (3 drops) as basic catalyst where dissolve in ethanol (20 ml) and poured in round bottom flask (50 ml) equipped with condenser then the reaction mixture reflexed for (4 hrs.), followed by cooling, poured in a beaker equipped with ice-water, shaking very well the crude products filtered off, washing with cold water, followed by drying, then recrystallized from (EtOH-water) to afford a compound **(3-10)**.

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 ºC. 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)acid, 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.

2-(3-amino-5-(4-hydroxy-3-methoxyphenyl)-1,5-dihydro-4H-1,2,4-triazol-4-yl)-1-methyl-1,5-dihydro-4H-imidazol-4-one (2) Yield (70%), white powder, mp: 197-200 ºC. Elem. Anal. ($C_{11}H_{14}N_4O_4$, 304.13), Calcd: C, 51.31; H, 5.30; N, 27.62; O, 15.77. Found: C, 51.303; H, 5.285; N, 27.613; O, 15.798. IR (KBr, cm^{-1}): 3428 (OH)phenol, 3252 (NH_2), 3146 (NH), 1611 (C=O) imidazole, 1585 (C=N), 1277 (C-N), (C-O-C) asy, 1106, 1025. ^{1}H-NMR (DMSO-d6), δ ppm: OCH_3 (s,3.84,3H), CH-triazole (s,6.79,2H), NH_2 (s,7.02, 2H); CH_2-imidazole (s,7.05,2H); H-aromatic(m,7.48-7.98,3H); NH-triazole (s,8.14,1H); OH-phenol (s, 9.48, 1H). MS (m/z): 304; Base peal: 55.

2-(5-(4-hydroxy-3-methoxyphenyl)-3-(4-oxo-2-phenylquinazolin-3(4H)-yl)-1,5-dihydro-4H-1,2,4-triazol-4-yl)acetic acid (3) Yield (82%), pale yellow crystals, mp: 173-177 ºC. Elem. Anal. ($C_{25}H_{19}N_5O_5$, 471.15), Calcd: C, 63.69; H, 4.49; N, 14.85; O, 16.97. Found: C, 63.81; H, 4.43; N, 14.83; O, 16.93. IR (KBr, cm^{-1}): 3233 (OH), 3116 (NH), 1681 (N-C=O), 1640 (C=N), 1226 (C-N), (C-O-C), 1160, 1027. ^{1}H-NMR (DMSO-d6), δ ppm: OCH_3 (s, 0.98, 3H), CH_2-acid (s, 3.85, 2H); CH_2-imidazole (s, 6.65, 2H), CH-triazole (s, 6.85, 1H); H-aromatic (m, 7.21-874, 12H); NH-triazole (s, 9.25, 1H), OH-phenol (s, 11.13, 1H); OH-acid (s, 12.19, 1H).

2-(3-(2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl)-5-(4-hydroxy-3-methoxyphenyl)-1,5-dihydro-4H-1,2,4-triazol-4-yl)acetic acid (4) Yield (88%), deep yellow crystals, mp: 178-181 ºC. Elem. Anal. ($C_{25}H_{18}ClN_5O_5$,505.12), Calcd: C, 59.35; H, 3.98; N, 13.84; O, 15.81; Cl, 7.01. Found: C, 59.38; H, 3.97; N, 13.82; O, 15.85; Cl, 6.98. IR (KBr, cm^{-1}): 3341 (OH), 3116 (NH), 1666 (N-C=O), 1581 (C=N), 1251 (C-N), (C-O-C), 1159, 1089. ^{1}H-NMR (DMSO-d6), δ ppm: OCH_3

(s, 3.49, 3H); CH_2-acid (s, 4.0, 2H); CH_2-imidazole (s, 7.21, 2H); CH-triazole (s, 7.23, 1H); H-aromatic (m, 7.65-8.06, 7H), P-chloro phenyl (AB-system) (d-d, 8.07- 8.67, 4H); NH-triazole (s, 8.68, 1H); OH-phenol (s, 12.15, 1H); OH-acid (s, 13.80, 1H). MS (m/z): 505; Base peal: 151.

2-(3-(2-(4-(chlorocarbonyl)phenyl)-4-oxoquinazolin-3(4H)-yl)-5-(4-hydroxy-2-methoxy phenyl)-4H-1,2,4-triazol-4-yl)acetic acid (5) Yield (82%), yellow crystals, mp: 130-133 ºC. (KBr, cm^{-1}): 3422 (OH), 3321 (NH), 1680 (N-C=O), 1614 (C=N), 1260 (C-N), (C-O-C), 1105, 1016.

2-(3-(4-hydroxy-2-methoxyphenyl)-5-(2-methyl-4-oxoquinazolin-3(4H)-yl)-4H-1,2,4-triazol-4-yl)acetic acid (6) Yield (76%), pale brown powder, mp: 188-190 ºC. IR (KBr, cm^{-1}): 3472 (OH), 3428 (NH), 1611 (N-C=O), 1585 (C=N), 1277 (C-N), (C-O-C), 1164, 1025

3-(5-(4-hydroxy-3-methoxyphenyl)-4-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-4,5-dihydro-1H-1,2,4-triazol-3-yl)-2-phenylquinazolin-4(3H)-one (7) Yield (90%), orange crystals, mp: 117-120ºC. Elem. Anal. ($C_{27}H_{21}N_7O_4$, 509.18), Calcd: C, 63.65; H, 4.55; N, 19.24; O, 12.56. Found: C, 63.71; H, 4.42; N, 19.13; O, 12.54. IR (KBr, cm^{-1}): 3257 (OH), 3117 (NH), 1681 (N-C=O), 1640 (C=N), 1226 (C-N), (C-O-C), 1159, 1080. ^{1}H-NMR (DMSO-d6), δ ppm: N-CH_3 (s, 1.19, 3H); OCH_3 (s, 3.84, 3H); CH_2-imidazol (s, 6.78, 2H); CH-triazole (s, 6.80, 1H), H-aromatic (m, 7.03-8.74, 12H); OH-phenol (s, 12.17, 1H). MS (m/z): 509; Base peal: 105.

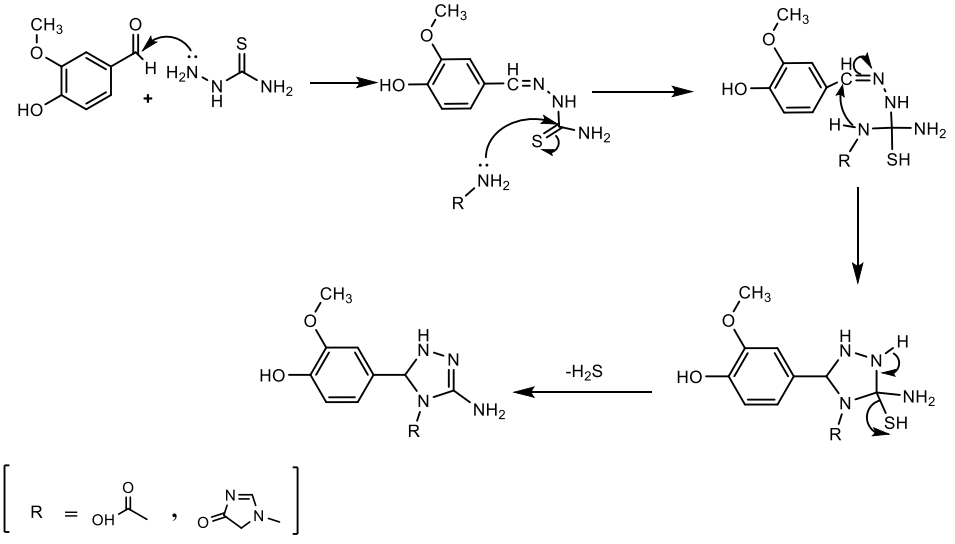
2-(4-chlorophenyl)-3-(5-(4-hydroxy-3-methoxyphenyl)-4-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-4,5-dihydro-1H-1,2,4-triazol-3-yl)quinazolin-4(3H)-one(8) Yield(74%), yellow crystals, mp: 105-108 ºC. Elem. Anal. ($C_{27}H_{20}ClN_7O_4$, 543.14), Calcd: C, 59.62; H, 4.08; N, 18.02; O, 11.76; Cl, 6.52. Found: C, 59.64; H, 4.09; N, 18.01; O, 11.75; Cl, 6.51. IR (KBr, cm−1): 3419 (OH), 3120 (NH), 1661 (N-C=O), 1558 (C=N), 1217 (C-N), (C-O-C), 1162, 1092. ^{1}H-NMR (DMSO-d6), δ ppm: N-CH_3 (s, 1.25, 3H), OCH_3 (s, 3.83, 3H); CH_2-imidazole (s, 7.21, 2H); CH-triazole (s, 7.23, 1H), H-aromatic (m, 7.65- 8.06, 7H); p-Chloro-phenyl (AB system) (d-d, 8.07-8.68, 4H) OH-phenol (s, 12.18, 1H).

3-(5-(4-hydroxy-2-methoxyphenyl)-4-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-4H-1,2,4-triazol-3-yl)-4-oxo-3,4-dihydroquinazoline-2-carbonyl chloride (9) Yield (81%), pale yellow crystals, mp: 132-135 ºC. Elem. Anal. ($C_{28}H_{20}ClN_7O_5$, 569.12), IR (KBr, cm−1): 3476 (OH), 3422 (NH), 1679 (N-C=O), 1611 (C=N), 1260 (C-N), (C-O-C), 1104, 1015

3-(5-(4-hydroxy-2-methoxyphenyl)-4-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-4H-1,2,4-triazol-3-yl)-2-methylquinazolin-4(3H)-one (10) Yield (71%), pale brown powder, mp: 114-117 ºC. Elem. Anal. ($C_{22}H_{19}N_7O_4$, 521.18), IR (KBr, cm−1): 3415 (OH), 3166 (NH), 1613 (N-C=O), 1585 (C=N), 1280 (C-N), (C-O-C), 1166, 1030.

Results and Discussion

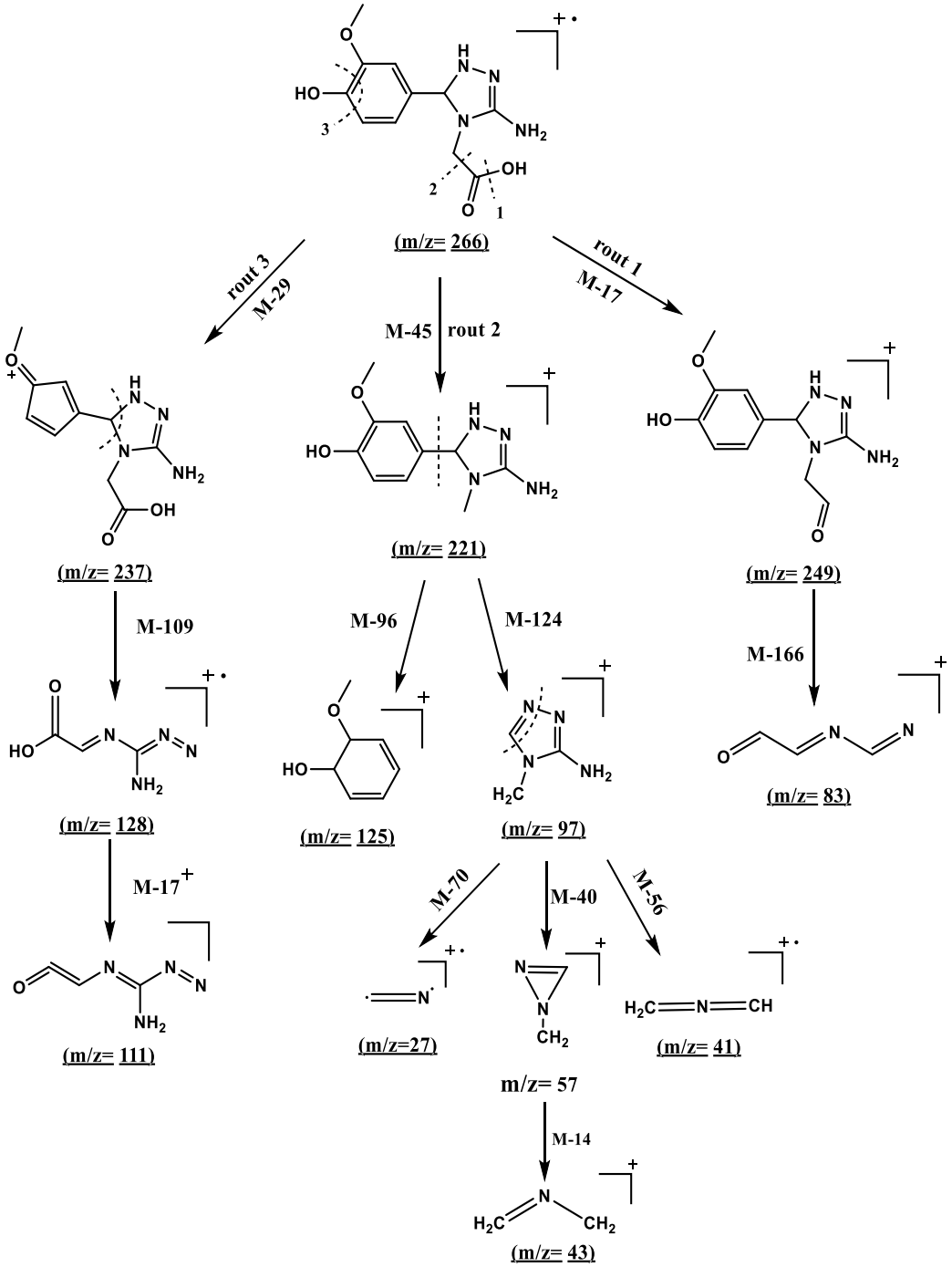
An accepted reaction of glycine (creatinine), vanillin and thiosemicarbazide have been accomplished in presence of efficient, selective and biocatalyst lemon juice to provide the units building N-substituted 1,2,4-triazole in a good yield as described in the following Scheme(El-Saghier et al., 2019).



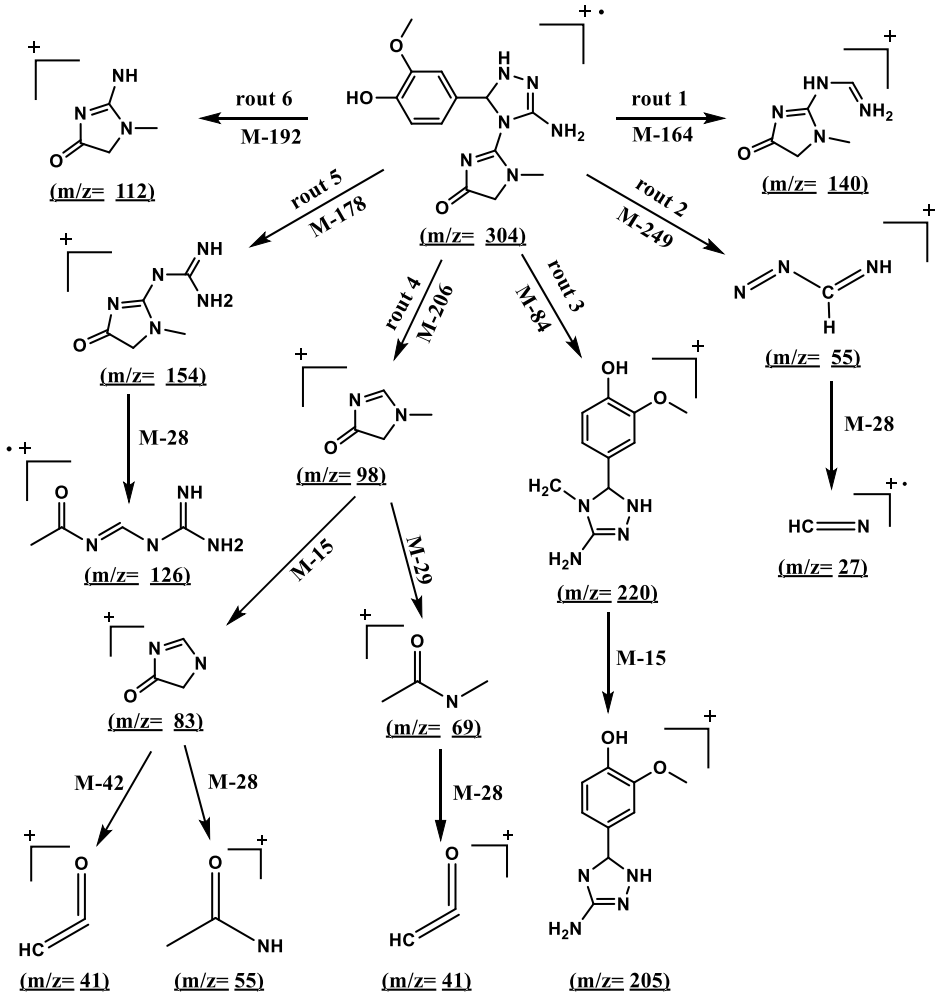
Scheme 1: Synthesis mechanism of 1,2,4-triazole (**1** and **2**)

This reaction proceeded through one-pot multicomponent reaction which is also called click reaction as a supreme type of modern organic synthesis, and actually this compound prepared by using the grinding technique for (15 min) in acidic media of neutral, renewable and environmentally lemon juice followed by spectral identification which shown in FT-IR

spectroscopy stretching vibration at (3475 cm^{-1}), (3255 cm^{-1}) refer to the OH and NH_2 functional groups, this finding encouraged us to further identification, so they give in ^{1}HNMR spectroscopy absorption peak at (δ ppm) (11.28), (9.48), (7.02) refer to OH-acid in units building **(1)**, OH-phenol and NH_2 functional groups in the two units (1 and 2) respectively. Whereas they shown in GC-Mass spectroscopy (m/z) value at 266 and 304 that refer to the unit's molecular weights as shown in the following fragmentation pattern.

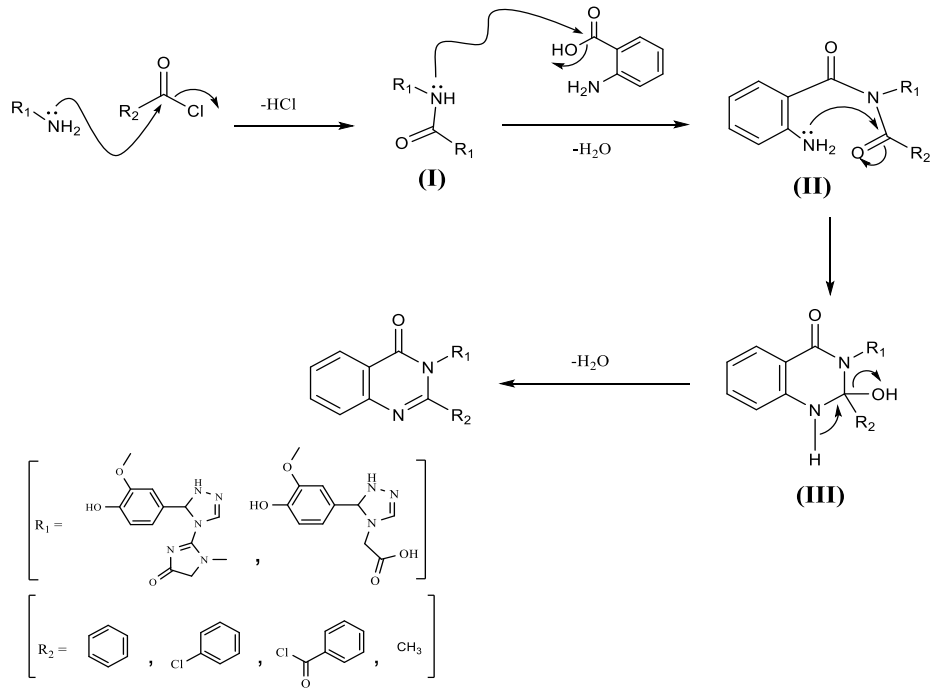


Scheme 2: fragmentation pattern of compound **(1)**



Scheme 3: fragmentation pattern of compound **(2)**

Finally, these compounds were easily wet three component reaction with anthranilic acid and substituted acid chloride in basic media of T.E.A and accelerated by microwave irradiation to obtain the fused quinazolinone derivative **(3-10)**, Scheme (4) (Mohammadkhani & Heravi, 2020)

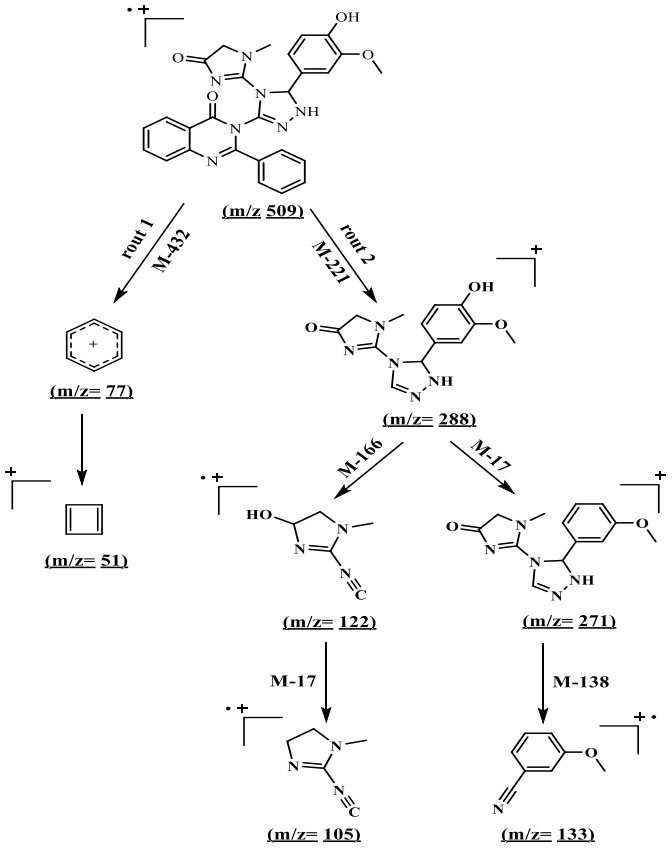


Scheme 4: Synthesis mechanism of compounds (3-10)

This tandem reaction displayed abroad substrate scope with a good functional group to, selectivity producing various fused quinazolinone in a good-excellent yield.

It is believed that the reaction proceeded according to the sequential attack that began from the attacks of the primary amino group in unit building **(1** or **2)** on the carbonyl of acid chloride to give the corresponding amide with losing of hydrogen chloride molecule then a condensation reaction between the amide and anthranilic acid to form the corresponding imide which suffer from intracyclization reaction between the primary amino group of anthranilic ring and the imide carbonyl group to afford compounds **(3-10)** with losing of H_2O molecule

Interestingly, the fused quinazolinone structure encourage us to study it's biological activity against breast and lung cancer and actually after structure provement using FT-IR, ^{1}H-NMR, GC-Mass spectroscopy which came in agreements with the expected structures as given in the experimental section especially in ^{1}H-NMR and the following Scheme described the fragmentation pattern for compounds **(7)**.



Scheme 3: fragmentation pattern of compound **(7)**

Basically, the anti-cancer evaluation was done for compounds (**4** and **7**) against breast and lung cancer cells and the obtained results revealed that these two compounds have a high growth inhibition with a low cytotoxicity especially compounds **(2)** as given by IC50, OD and cytotoxicity value and the following diagrams and pictures which described all results obtained.

Types of cancer cell lines

- **Normal Human Fibroblast** (**NHF):** - Normal human derived adipose tissue (NHF) cell line (Safi *et al*., 2019).
- **Michigan Cancer Foundation-7 (MCF-7): -** Is a breast cancer cell line isolated in 1970 from a 69-year-old White woman. MCF-7, referring to the institute in Detroit where the cell line was established in 1973 by Herbert Soule and co-workers (Soule *et al*., 1973).
- **Ahmed Murtada Jabria 2013 (AMJ-13): -** Breast cancer cell line has been established from an Iraqi breast cancer patient. was established from the primary tumor of a 70-year-old Iraqi woman with a histological diagnosis of infiltrating ductal carcinoma (Al-Shammari *et al*., 2015).

Maintenance of cell cultures

cell lines, was maintained in MEM supplemented with 10% Fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 50% confluence twice a week, and incubated at 37 °C.

Cytotoxicity Assays

To determine the cytotoxic effect, the MTT cell viability assay was conducted on 96-well plates(Adil et al., 2020). Cell lines were seeded at 1 × 104cells/well. After 24 hrs. or a confluent monolayer was achieved, cells were treated with tested compound. Cell viability was measured after 72h of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT (and incubating the cells for 1.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking (Abdullah et al., 2020). The absorbency was determined on a microplate reader at 492 nm (test wavelength); the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation (Al-Shammari et al., 2020): -

Cell Viability =Absorbance of treated cell / Absorbance of non-treated cell **X** 100 %

Cytotoxicity = 100 – cell viability

Statistical analysis:

The obtained data were statically analyzed using an unpaired t-test with GraphPad Prism 6 (Mohammed et al., 2019). The values were presented as the mean ± SD of triplicate measurements (Al-Ziaydi et al., 2020).

Results

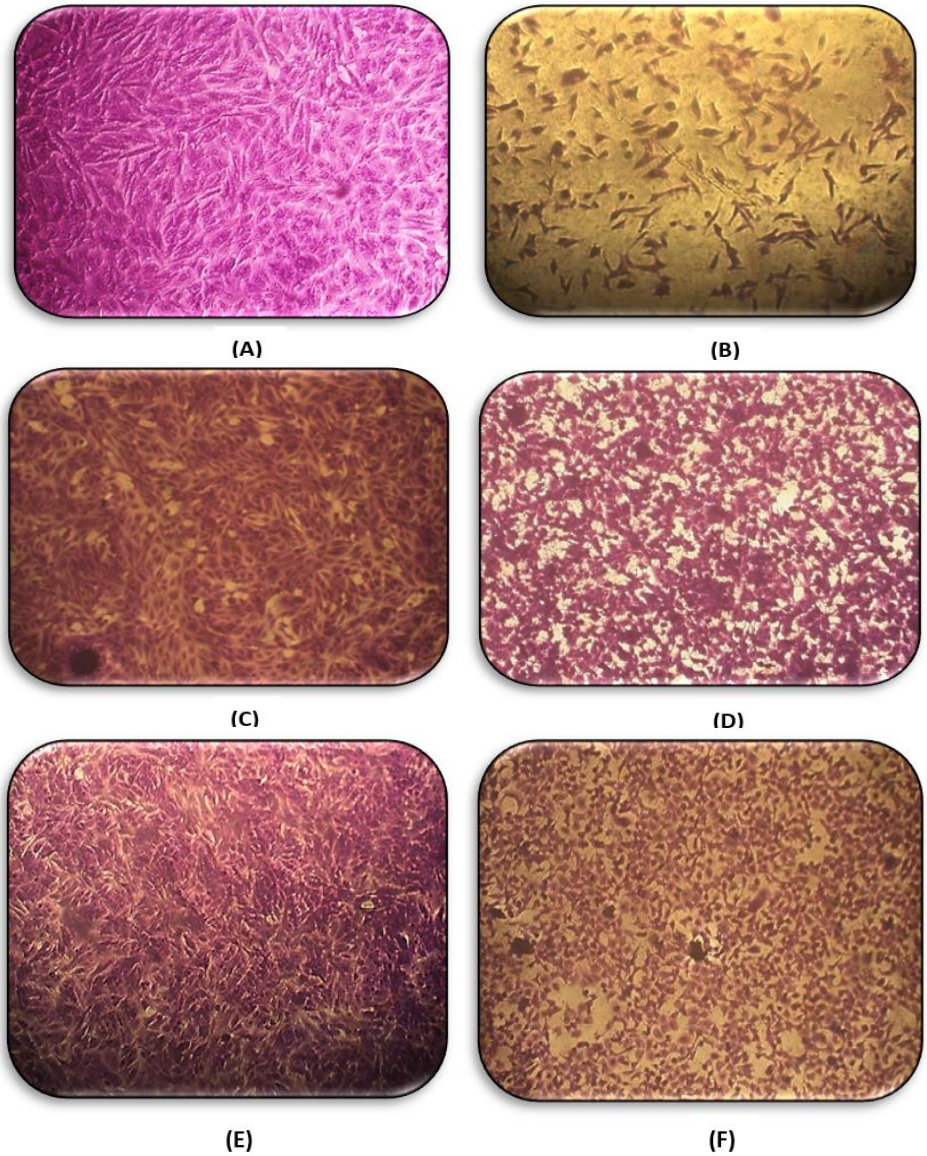


Fig 1: (A) untreated (MCF-7) cells with compound **(4)**. **(B)** treated (MCF-7) cells with compound **(4). (C)** untreated (NHF) cells with compound **(4)**. **(D)** treated (NHF) cells with compound **(4). (E)** untreated (AMJ13) cells with compound **(7)**. **(F)** treated (AMJ13) cells with compound **(7).**

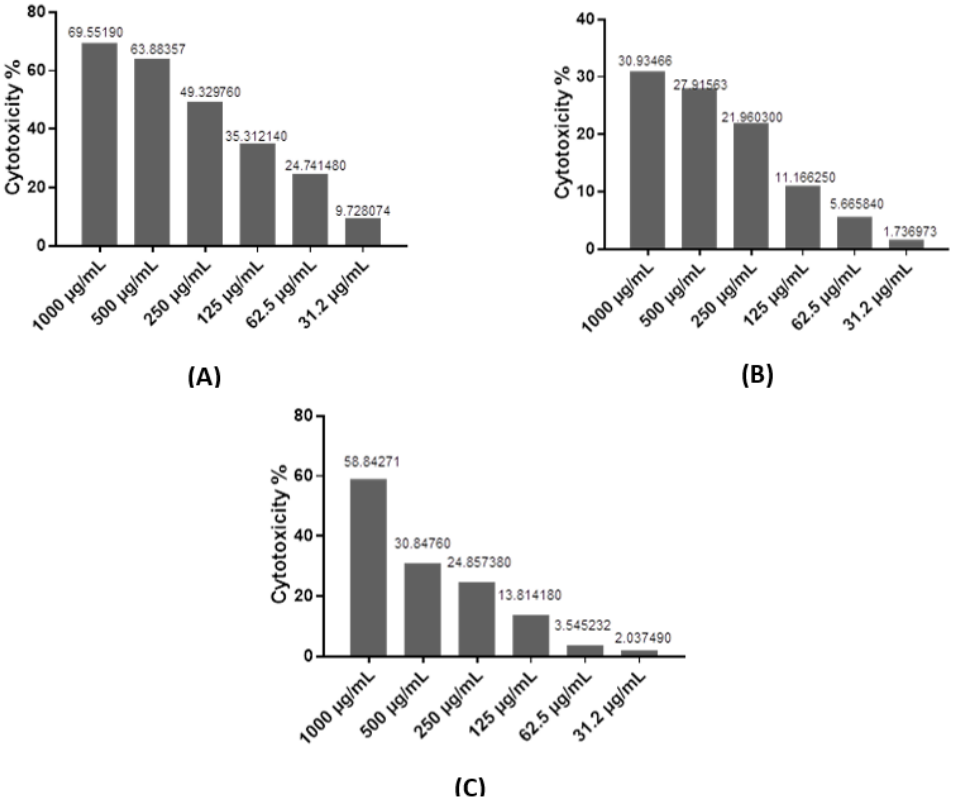


Fig 2: (A) Cytotoxicity chart for compound **(4)** against (MCF-7) cells. **(B)** Cytotoxicity chart for compound **(4)** against (NHF) cells. **(C)** Cytotoxicity chart for compound **(7)** against (AMJ13) cells.

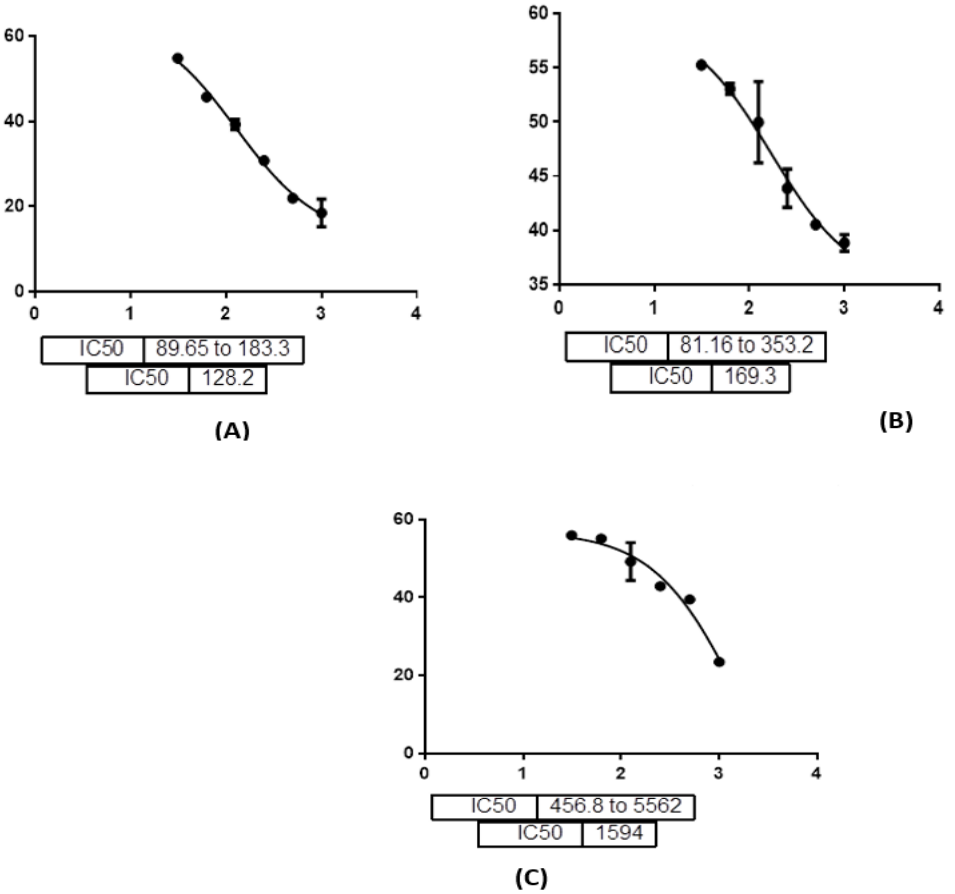


Fig 3: (A) IC50 chart for compound **(4)** against (MCF-7) cells. **(B)** IC50 chart for compound **(4)** against (NHF) cells. **(C)** IC50 chart for compound **(7)** against (AMJ13) cells.

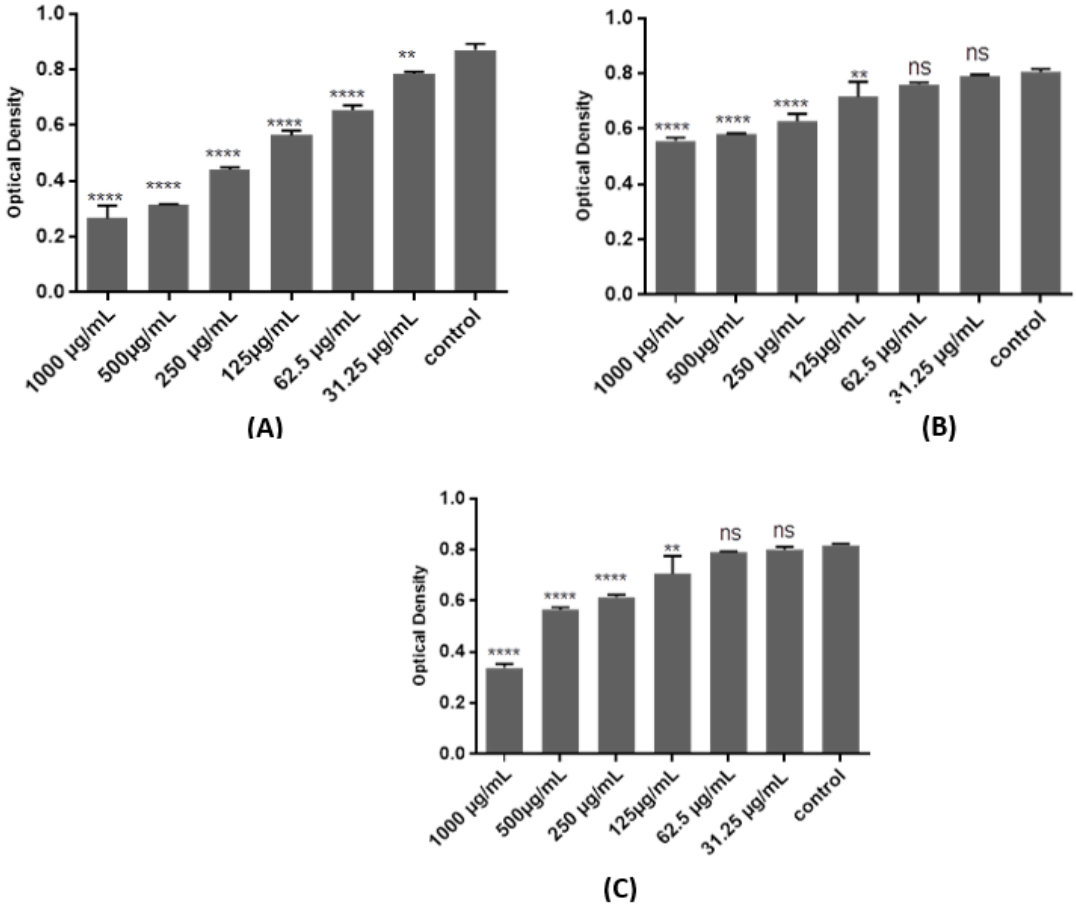


Fig 4: (A) OD chart for compound **(4)** against (MCF-7) cells. **(B)** OD chart for compound **(4)** against (NHF) cells. **(C)** OD chart for compound **(7)** against (AMJ13) cells.

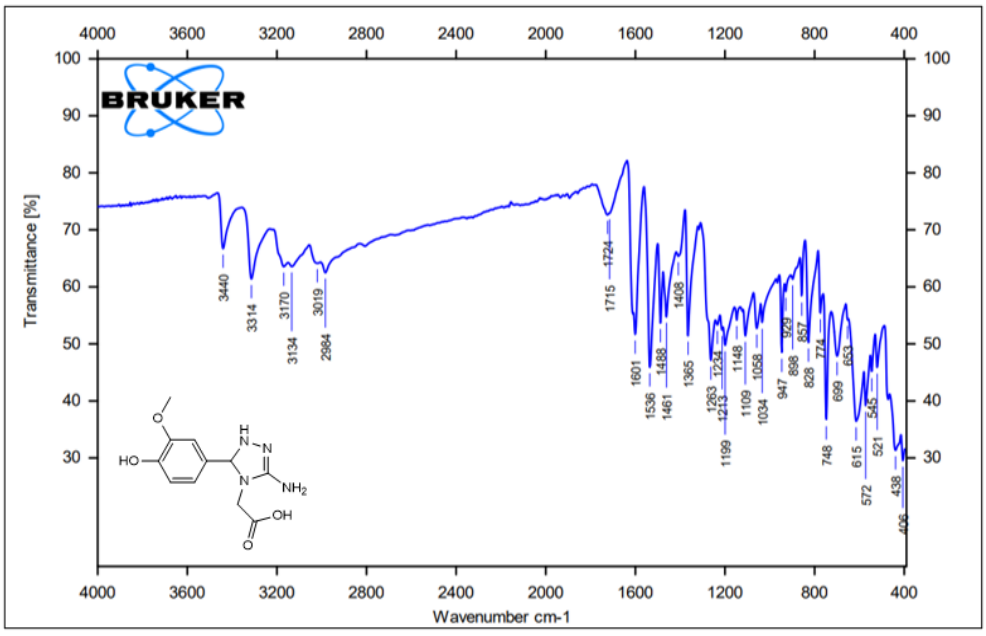


Fig (5): FT-IR for compound **(1)**

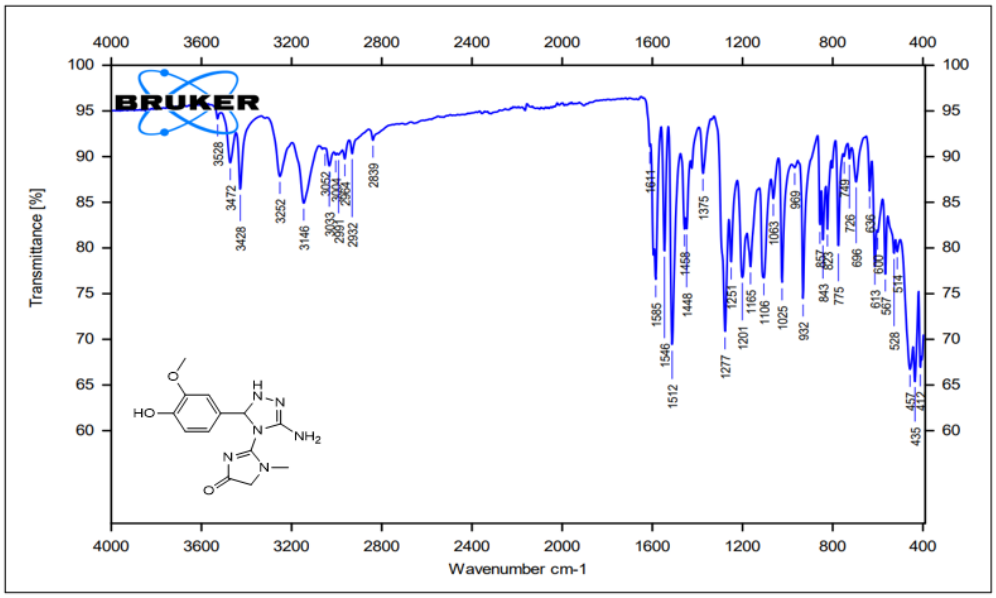


Fig (6): FT-IR for compound **(2)**

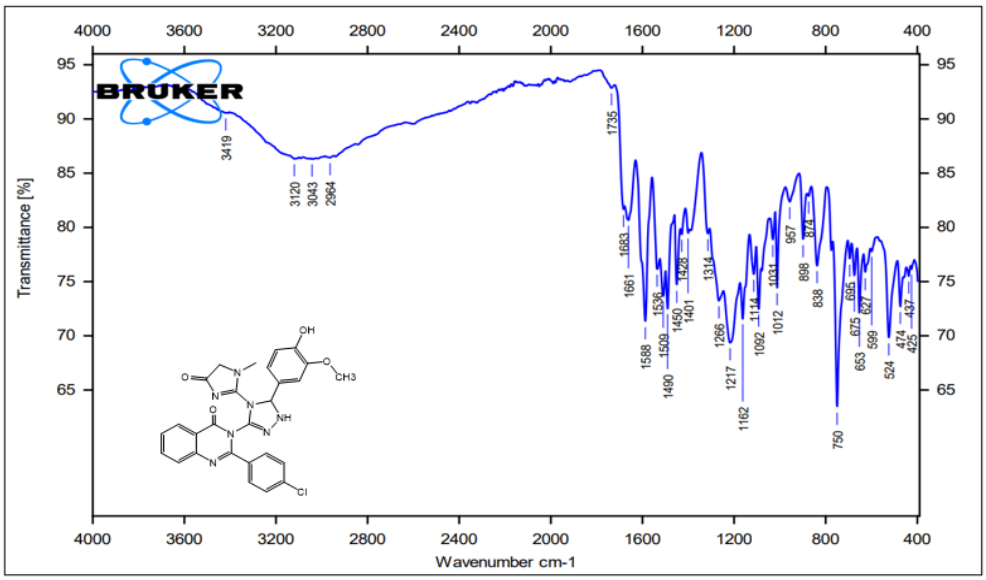


Fig (7): FT-IR for compound **(8)**

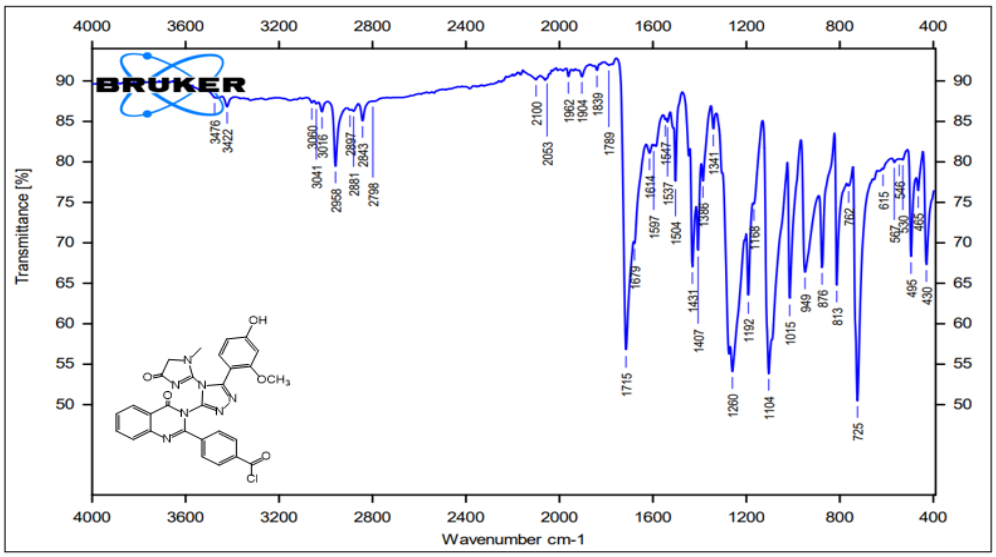


Fig (8): FT-IR for compound **(9)**

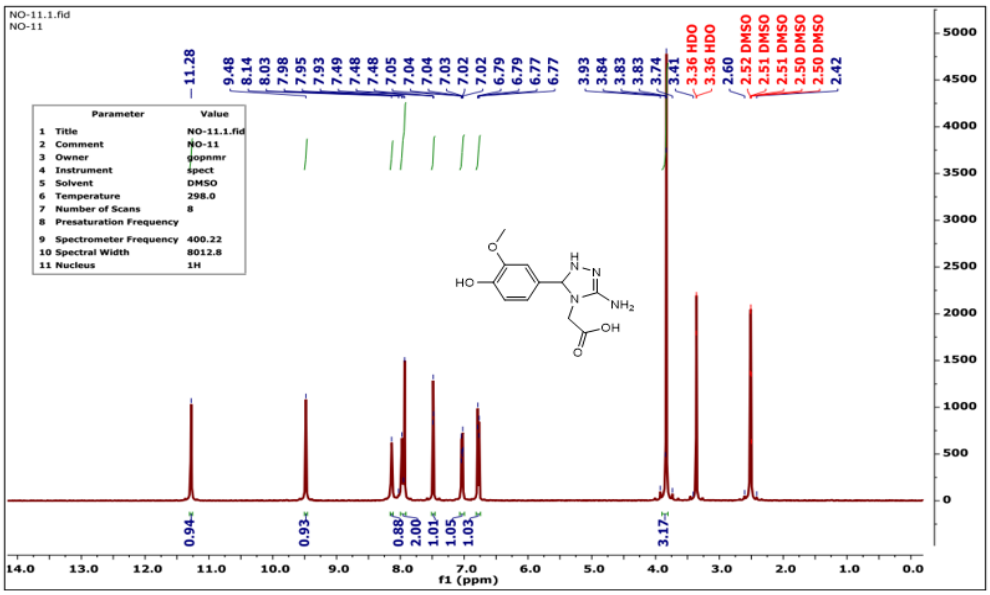


Fig (9): ^{1}H-NMR for compound **(1)**

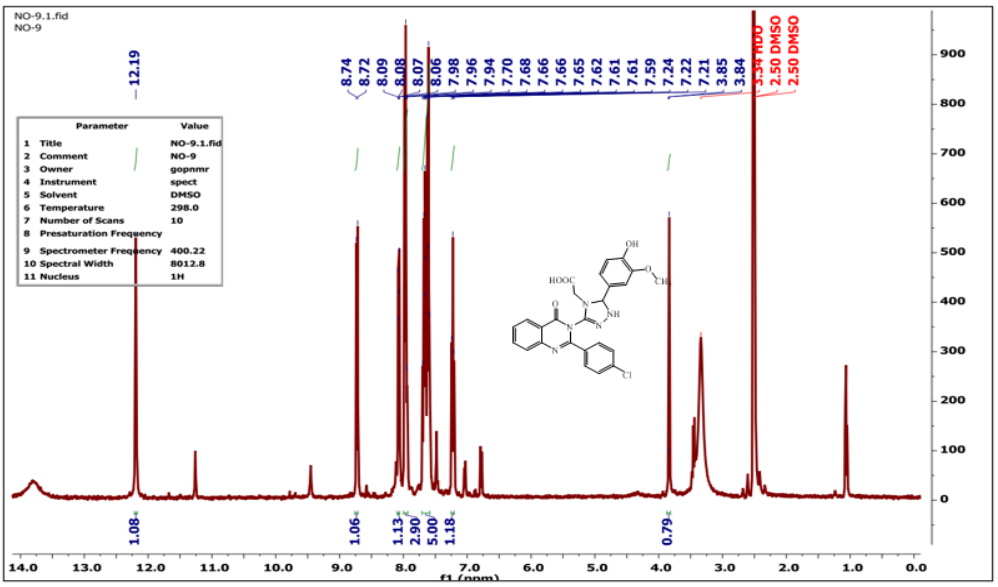


Fig (10): ^{1}H-NMR for compound **(4)**

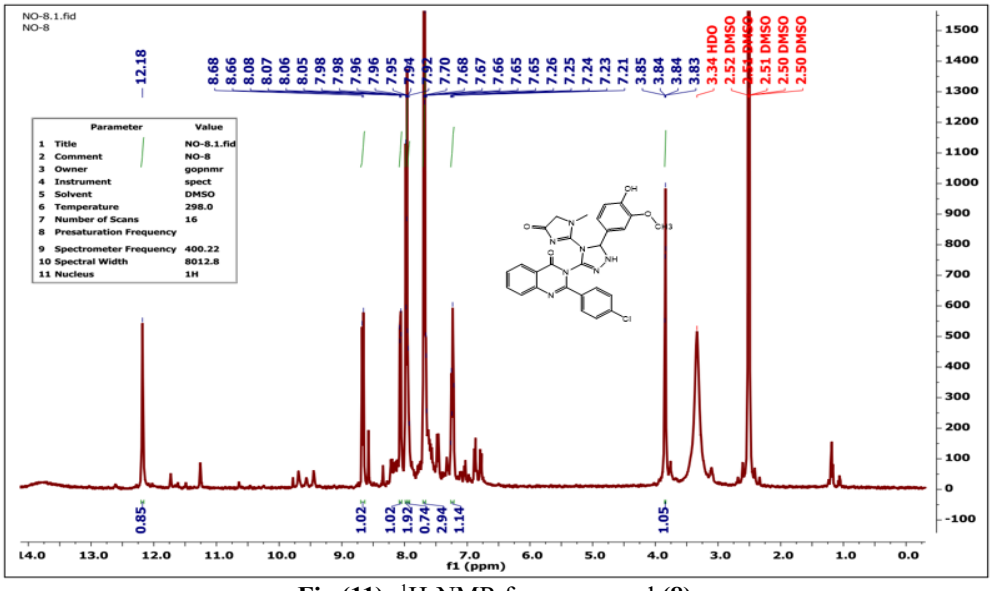


Fig (11): ^{1}H-NMR for compound **(8)**

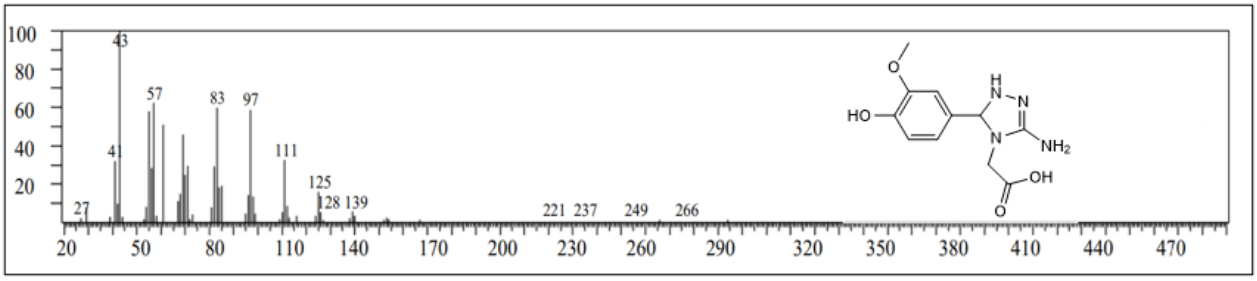


Fig (12): GC-Mass for compound **(1)**

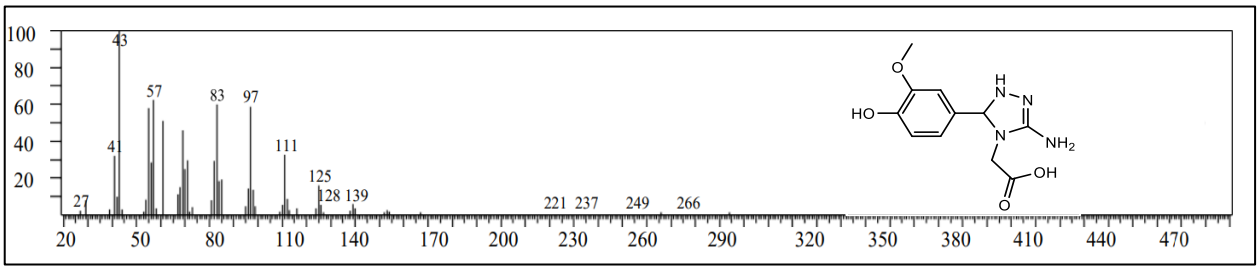


Fig (13): GC-Mass for compound **(2)**

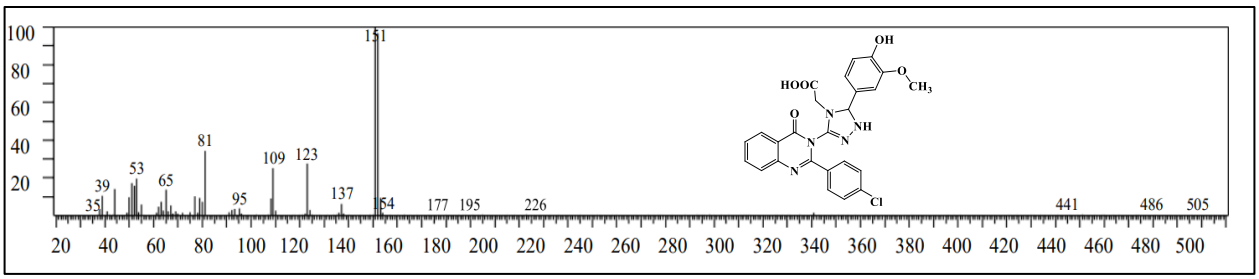


Fig (14): GC-Mass for compound **(4)**

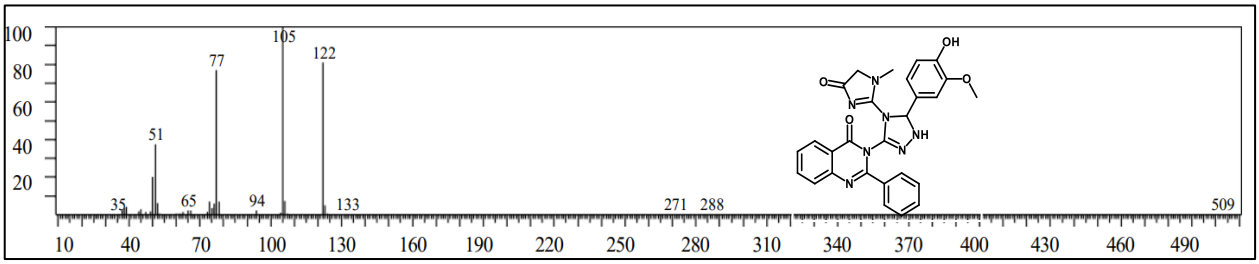


Fig (15): GC-Mass for compound **(5)**

Conclusion

Efficient, simple, fast, selective, clean and eco-friendly approach used to afford all compounds in this presentation through grinding and microwave radiation techniques in acidic media from neutral and renewable lemon juice, firstly and later in basic media from T.E.A. The compounds **(4)** and **(7)** shown a high anti-cancer effect against breast and lung cancer cells due to its supreme structure with 1,2,4-triazole and fused quinazolinone moiety.

Reference

1. Abdullah, S. A., Al-Shammari, A. M., & Lateef, S. A. (2020). Attenuated measles vaccine strain have potent oncolytic activity against Iraqi patient derived breast cancer cell line. *Saudi J Biol Sci*, *27*(3), 865-872.
2. Adil, B., Al-Shammari, A., & Murbat, H. (2020). Breast Cancer Treatment Using Cold Atmospheric Plasma Generated by the FE-DBD Scheme. *Clinical Plasma Medicine*, *19-20*, 100103.
3. Al-Shammari, A. M., Alshami, M. A., Umran, M. A., Almukhtar, A. A., Yaseen, N. Y., Raad, K., & Hussien, A. A. (2015). Establishment and characterization of a receptor-negative, hormone-nonresponsive breast cancer cell line from an Iraqi patient. *Breast Cancer (Dove Med Press)*, *7*, 223-230.
4. Al-Shammari, A. M., Jalill, R. D. A., & Hussein, M. F. (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*(3), 1691-1702.

5. Al-Ziaydi, A. G., Al-Shammari, A. M., Hamzah, M. I., Kadhim, H. S., & Jabir, M. S. (2020). Newcastle disease virus suppress glycolysis pathway and induce breast cancer cells death. *Virusdisease*, *31*(3), 341-348.
6. Birhan, Y. S., Bekhit, A. A., & Hymete, A. (2015). In vivo antimalarial evaluation of some 2,3-disubstituted-4(3H)-quinazolinone derivatives. *BMC Res Notes*, *8*, 589.
7. Burman, B., Drutman, S. B., Fury, M. G., Wong, R. J., Katabi, N., Ho, A. L., & Pfister, D. G. (2022). Pharmacodynamic and therapeutic pilot studies of single-agent ribavirin in patients with human papillomavirus–related malignancies. *Oral Oncology*, *128*, 105806.
8. Chokshi, A., Vaishya, R., Inavolu, R., & Potta, T. (2019). Intranasal spray formulation containing rizatriptan benzoate for the treatment of migraine. *International Journal of Pharmaceutics*, *571*, 118702.
9. El-Saghier, A., Mohamed, M., Abd-Allah, O., Kadry, A., Ibrahim, T., & Bekhit, A. (2019). Green synthesis, antileishmanial activity evaluation, and in silico studies of new amino acid-coupled 1,2,4-triazoles. *Medicinal Chemistry Research*, *28*.
10. Elzoheiry, M. A., Elmehankar, M. S., Aboukamar, W. A., El-Gamal, R., Sheta, H., Zenezan, D., Nabih, N., & Elhenawy, A. A. (2022). Fluconazole as Schistosoma mansoni cytochrome P450 inhibitor: In vivo murine experimental study. *Experimental parasitology*, *239*, 108291.
11. Fan, Z., Shi, J., Luo, N., Ding, M., & Bao, X. (2019). Synthesis, Crystal Structure, and Agricultural Antimicrobial Evaluation of Novel Quinazoline Thioether Derivatives Incorporating the 1,2,4-Triazolo[4,3-a]pyridine Moiety. *Journal of Agricultural and Food Chemistry*, *67*(42), 11598-11606.
12. Jampilek, J., Musiol, R., Finster, J., Pesko, M., Carroll, J., Kralova, K., Vejsova, M., #039, Mahony, J., Coffey, A., Dohnal, J., & Polanski, J. (2009). Investigating Biological Activity Spectrum for Novel Styrylquinazoline Analogues. *Molecules*, *14*(10), 4246-4265.
13. Li, Z., Zhao, L., Bian, Y., Li, Y., Qu, J., & Song, F. (2022). The Antibacterial Activity of Quinazoline and Quinazolinone Hybrids. *Curr Top Med Chem*, *22*(12), 1035-1044.
14. Mabkhot, Y. N., Al-Har, M. S., Barakat, A., Aldawsari, F. D., Aldalbahi, A., & Ul-Haq, Z. (2014). Synthesis, antimicrobial and molecular docking studies of quinazolin-4(3H)-one derivatives. *Molecules*, *19*(7), 8725-8739.
15. Maleki, A. (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 [10.1039/C4RA10856F]. *RSC Advances*, *4*(109), 64169-64173.
16. Moghadam Farid, S., Iraji, A., Mojtabavi, S., Ghasemi, M., Faramarzi, M. A., Mahdavi, M., Barazandeh Tehrani, M., Akbarzadeh, T., & Saeedi, M. (2023). Quinazolinone-1,2,3-triazole-acetamide conjugates as potent α-glucosidase inhibitors: synthesis, enzyme inhibition, kinetic analysis, and molecular docking study. *RSC Med Chem*, *14*(3), 520-533.
17. Mohammadkhani, L., & Heravi, M. M. (2020). Microwave-assisted synthesis of quinazolines and quinazolinones: an overview. *Frontiers in Chemistry*, *8*, 580086.
18. Mohammed, M. S., Al-Taee, M. F., & Al-Shammari, A. M. (2019). Caspase Dependent and Independent Anti-hematological Malignancy Activity of AMHA1 Attenuated Newcastle Disease Virus. *Int J Mol Cell Med*, *8*(3), 211-223.
19. Park, H. G., Kim, J. H., Dancer, A. N., Kothapalli, K. S., & Brenna, J. T. (2021). The aromatase inhibitor letrozole restores FADS2 function in ER+ MCF7 human breast cancer cells. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *171*, 102312.
20. Pfaller, M. A., Shortridge, D., Harris, K. A., Garrison, M. W., DeRyke, C. A., DePestel, D. D., Moise, P. A., & Sader, H. S. (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*, 321-326.
21. Potewar, T. M., Ingale, S. A., & Srinivasan, K. V. (2008). Synthesis of tryptanthrin and deoxyvasicinone by a regioselective lithiation-intramolecular electrophilic reaction approach. *Arkivoc*, *14*, 100-108.
22. Rakesh, K. P., Manukumar, H. M., & Gowda, D. C. (2015). Schiff's bases of quinazolinone derivatives: Synthesis and SAR studies of a novel series of potential anti-inflammatory and antioxidants. *Bioorg Med Chem Lett*, *25*(5), 1072-1077.
23. Rezaeinasab, R., Jafari, E., & Khodarahmi, G. (2022). Quinazolinone-based hybrids with diverse biological activities: A mini-review. *J Res Med Sci*, *27*, 68.
24. Sachdeva, H., Dwivedi, D., & Saroj, R. (2013). Alum catalyzed simple, efficient, and green synthesis of 2-[3-amino-5-methyl-5-(pyridin-3-yl)-1,5-dihydro-4H-1,2,4-triazol-4-yl]propanoic acid derivatives in aqueous media. *ScientificWorldJournal*, *2013*, 716389.
25. Safi, I. N., Mohammed Ali Hussein, B., & Al-Shammari, A. M. (2019). In vitro periodontal ligament cell expansion by co-culture method and formation of multi-layered periodontal ligament-derived cell sheets. *Regenerative Therapy*, *11*, 225-239.
26. Schwalbe, R., Steele-Moore, L., & Goodwin, A. C. (2007). *Antimicrobial susceptibility testing protocols*. Crc Press.
27. Shettar, A., Shankar, V. K., Ajjarapu, S., Kulkarni, V. I., Repka, M. A., & Murthy, S. N. (2021). Development and characterization of Novel topical oil/PEG creams of voriconazole for the treatment of fungal infections. *Journal of Drug Delivery Science and Technology*, *66*, 102928.
28. Soule, H. D., Vazguez, J., Long, A., Albert, S., & Brennan, M. (1973). A human cell line from a pleural effusion derived from a breast carcinoma. *J Natl Cancer Inst*, *51*(5), 1409-1416.

29. Takahashi, K., Yamagishi, G., Hiramatsu, T., Hosoya, A., Onoe, K., Doi, H., Nagata, H., Wada, Y., Onoe, H., & Watanabe, Y. (2011). Practical synthesis of precursor of [N-methyl-11C] vorozole, an efficient PET tracer targeting aromatase in the brain. *Bioorganic & Medicinal Chemistry*, *19*(4), 1464-1470.