Volume 9, Issue 8, August – 2024 International Journal of Innovative Science and Research Technology
ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24AUG1311

Synthesis and Characterization of Triazine Derivatives as Important Heterocyclic Compounds and Study their Biological Activities

Thamer Abd Rehan[1]
College of Science, Allkarkh Univeresity for Science

Akram sadeq[2]
Department of Electromechanical Engineering, University of Technology

Ahmad Rahman Salih[3]
College of Dentistry, Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq

Abstract:- Reaction 0f (thioacetamide/ acetemide) with chloroacetyl chloride to produced N-acetyl-2-chloro (thiocetamide/acetamide), then subsequent reaction with thiosemicarbazide to form N-acetyl-2-(2-carbamothioyl hydrazinyl)thioacetamide/acetamide. Ring closure or Cyclization of these compounds in the presence of sodium hydroxide yield N-(3-mercapto-1,4-dihydro-1,2,4 -triazin-5-yl)ethanethioamide/acetamide.

The aim of this contribution is synthesis wide range of triazin derivatives by using different methodologies. These new triazine derivatives will be confirmed by FT-IR, 1HNMR, 13CNMR spectra.

Since this triazine ring are very important core in chemical structure of some therapeutic compounds, will be evaluated for biological activities against two types of bacteria and fungi.

Keywords:- *Heterocycle Compounds, Nitrogen-Containing Heterocycles, Triazine Derivatives, Biological Activities, Antibacterial Activity, Antifungi Activity.*

I. INTRODUCTION

Nitrogen-containing heterocycles are highly important due to their classification as a significant group of synthetic and natural compounds. They exhibit a wide range of physicochemical properties and pharmacological activity [1,2]. Triazine derivatives are a type of nitrogen-containing heterocycle that have demonstrated several biological applications, including analgesic, anti-tuberculosis, anti-fungal, anti-cancer, antiprotozoal, anti-malarial, anti-viral, anti-microbial, and anti-inflammatory activity. Therefore, they are considered a crucial framework in medicinal chemistry due to their heterocyclic nature. [3,4]. Triazine is an aromatic heterocyclic ring compound that serves as an analogue of benzene. It is composed of three nitrogen atoms in place of three carbon atoms, and its chemical formula is $C_3H_3N_3$. [4]. Based on the locations of the nitrogen atoms, triazine possess three isomeric forms: 1,2,3-triazine (I), 1,2,4-triazine (II), and 1,3,5-triazine (III)(fig. 1) [5].

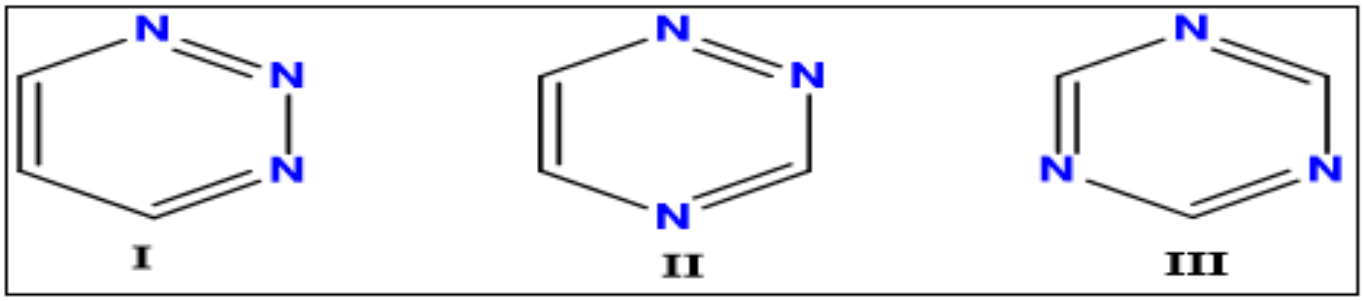

Fig 1 The Isomers Forms of Triazine Ring: 1,2,3-Triazine (I), 1,2,4-Triazine (II), and 1,3,5-Triazine (III).

Numerous substances with a triazine nucleus in their structure have antibacterial activity against a variety of Gram-positive and Gram-negative bacteria and fungi and may be able to partially prevent medication resistance in these microorganisms. As such, they have drawn interest from scientists looking for potent antibiotics that are inexpensive [6]. The triazine ring and its derivatives facilitate the synthesis of compounds with pharmaceutical activity and biological applications such as antimalarial [7,8], anticancer [9,10], antiviral[11], anti-inflammatory [12,13], anti-proliferative [14,15], antitumor [16,17] activities. There are many antibiotics, as well as some inhibitors that contain in their chemical structure the heterocyclic aromatic ring triazine, available on the market, as shown in (fig. 2) [6].

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24AUG1311

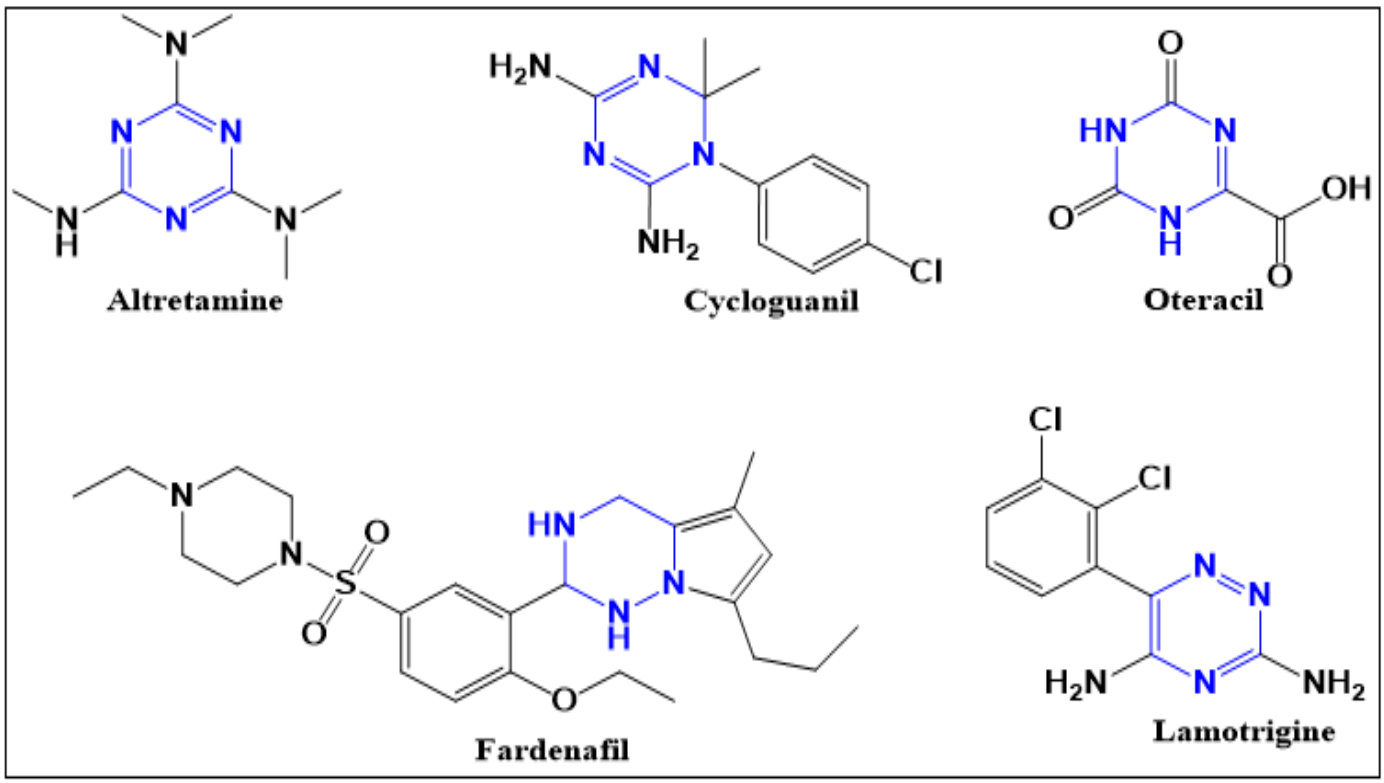

Fig 2 Some Drugs on the Market that Contain a Triazine Ring.

Because of all these biological and pharmacological activities, in this research, 1,2,4-triazine derivatives were prepared through a series of reactions and their biological effectiveness was tested. 1,2,4-triazines and their derivatives occupy a prominent place in modern medicinal chemistry due to their high biological activity. As a result, many physiologically active compounds, both synthetic and natural, include the 1,2,4-triazine ring, a well-known structural motif [18]. Numerous medicinal substances that are sold in pharmacies include a 1,2,4-triazine nucleus in their structure. Examples of these include 6-azacytosine, 6-azauracil, tirapazamine, azaribine, lamotrigine, and ceftriaxone. However, a lot of antibiotics, like toxoflavin, reumycin, and fervenulin, include a 1,2,4-triazine ring [19]. Furthermore, several 1,2,4-triazine derivatives, such as metamitron, ethyl metribuzin, and metribuzin, are employed as agrochemicals [20].

II. EXPERIMENTAL

➢ *Chemicals and Instruments.*

Chemical materials and solvents were purchased from supplying companies. On a Schimadzu FTIR spectrophotometer, the infrared spectra (IR) were obtained using 1% potassium bromide discs. Using DMSOd6 as an internal standard solvent, all of the ^{1}H and ^{13}C NMR spectra were accumulated on a Bruker Ultra-Shield 500MHz spectrometer. The resulting compounds were well purified using column chromatography technique.

➢ *General Procedure*

- *Synthesis of (N-acetyl-2-chloroacetamide (1) and N-thioacetyl-2-chloroacetamide (2))*

Chloroacetyl chloride was added dropwise at room temperature to the (acetamide or thioacetamide) dissolved in dimethylsulfoxid (DMSO). After the addition was completed, the reaction mixture was refluxed for 8 hours. After reaching the end of the reaction using TLC, the mixture was cooled to room temperature and then placed in an ice bath with continuous stirring for 30 minutes until a precipitate formed. The mixture was filtered and the precipitate was washed with distilled water. (Ethyl acetate-Petroleum ether) mixture was used as an eluent in column chromatography to purify the products and obtain the final products (**1** and **2**).

- *Synthesis of N-(((2-carbamothioylhydrazinyl)oxy) carbonyl)acetamide (3) and N-(((2-carbamothioyl hydrazinyl)oxy)carbonyl)thioacetamide (4).*

The reaction mixture (compounds 1 or 2 with potassium carbonate) was refluxed at a temperature of 120 °C for 5 hours using dioxane as a solvent. After that, thiosimecarbazide was added and refluxed for 2 hours. When the end of the reaction was reached, pieces of ice were added to the mixture and stirred for a few minutes until the precipitate appeared, then filtered and the precipitate was washed with distilled water. (Ethyl acetate-Petroleum ether) mixture was used as an eluent in column chromatography to purify the products and obtain the final products (**3** and **4**).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24AUG1311

- *Synthesis of N-(3-thioxo-1,2,3,4-tetrahydro-1,2,4-triazin-5-yl)acetamide (5) N-(3-thioxo-1,2,3,4-tetrahydro-1,2,4-triazin-5-yl)thioacetamide (6)*

Compounds (**3** or **4**) were dissolved in ethanol and then a few drops of a 5% NaOH aqueous solution were added. The reaction mixture was refluxed (80°C) for 12 hours. After the reaction was completed using TLC, the mixture was cooled by placing it in an ice bath, then adding an additional amount of distilled water and stirring for an hour until a precipitate formed. The mixture was filtered and the precipitate was washed with distilled water. (Ethyl acetate-hexan) mixture was used as an eluent in column chromatography to purify the products and obtain the final products (3 and 4).

✓ *N-acetyl-2-chloroacetamide (1)*

FT-IR (KBr/cm^{-1}): 3234 (NH), 2952 and 2891 (CH aliphatic), 1695 and 1670 (C=O), ^{1}H-NMR (DMSO, 500 MHZ) δ: 10.5(s,1H, NH), 4.2 (s, 2H, CH_2-Cl), 2.2 (s, 3H, CH_3). ^{13}C-NMR (DMSO, 00 MHZ) δ: 171.7 and 168.1 (2C=O), 44.2(CH_2), 20 (CH_3).

✓ *N-thioacetyl-2-chloroacetamide (2)*

FT-IR (KBr/cm^{-1}): 2989 and 2887 (CH aliphatic), 1690 and 1675 (C=O), 1450 (C=S). ^{1}H-NMR (DMSO, 500 MHZ) δ: 9.8 (s, 1H, NH), 4.15 (s, 2H, CH_2-Cl), 2.31 (s, 3H, CH_3). ^{13}C-NMR (DMSO, 00 MHZ) δ: 188.3 (C=S), 175.7 (C=O), 42.3 (CH_2), 25.4(CH_3).

✓ *N-(((2-carbamothioylhydrazinyl)oxy)carbonyl)acetamide (3).*

FT-IR (KBr/cm^{-1}): 3477 and 3353 (NH_2), 3327, 3233 and 3155 (NH), 2975 and 2887 (CH aliphatic), ^{1}H-NMR (DMSO, 500 MHZ) δ: 10.7(s,1H, NH-C=O), 10.2 (s, 1H, NH-C=S), 9.5 (s, 1H, NH_2), 5.3 (s, 1H, **NH**-CH_2), 3.5 (s, 1H, CH_2), 2.3 (s,1H, CH_3). ^{13}C-NMR (DMSO, 00 MHZ) δ: 183.5 (C=S), 170.7 and 168.3 (2C=O), 46.3 (CH_2), 20.4(CH_3).

✓ *N-(((2-carbamothioylhydrazinyl)oxy)carbonyl)thioacetamide (4)*

FT-IR (KBr/cm^{-1}): 3332 and 3245 (NH_2), 3234, 3168 and 3130 (NH). ^{1}H-NMR (DMSO, 500 MHZ) δ: 10.8 (s, 1H, S=C-N**H**-C=O), 10.1 (s, 1H, NH-N**H**-C=S), 9.5 (s, 2H, NH_2), 4.3 (s, 1H, N**H**-NH-C=S), 3.6 (s, 2H, CH_2), 2.3 (s, 3H, CH_3). ^{13}C-NMR (DMSO, 00 MHZ) δ: 190.2 and 181.4(2C=S), 176.7 (C=O), 55.3(CH_2), 34.5 (CH_3).

✓ *N-(3-thioxo-1,2,3,4-tetrahydro-1,2,4-triazin-5-yl)acetamide (5).*

FT-IR (KBr/cm^{-1}): 3407, 3367 and 3315(NH), 2952 and 2891(CH aliphatic), 1685 (C=O), 1620 and 1445 (C=C), 1460 (C=S). ^{1}H-NMR (DMSO, 500 MHZ) δ: 9.8 (s, 1H, NH), 9.2 (s, 1H, NH), 8.03 (s, 1H, NH), 6.5 (s, 1H, NH), 6.4 (s, 1H, C=CH), 1.8 (s, 3H, CH_3). ^{13}C-NMR (DMSO, 00 MHZ) δ: 178.3 (C=S), 170.5 (C=O), 124.3 (**C**=CH)**,** 85.3 (C=**C**H)**,** 20.7 (CH_3).

✓ *N-(3-thioxo-1,2,3,4-tetrahydro-1,2,4-triazin-5-yl)thioacetamide (6).*

FT-IR (KBr/cm^{-1}): 3413, 3380 and 3342(NH), 2987 and 2885 (CH aliphatic), 1443 (C=S), ^{1}H-NMR (DMSO, 500 MHZ) δ: 9.3 (s, 1H, NH), 8.7 (s, 1H, NH), 8.03 (s, 1H, NH), 6.5 (s, 1H, NH), 6.4 (s, 1H, C=CH), 1.8 (s, 3H, CH_3). ^{13}C-NMR (DMSO, 00 MHZ) δ: 188.4 (C=S aliphatic), 179.7 (C=S cyclic), 122.3 (**C**=CH), 78.6 (C=**C**H), 22.5 (CH_3).

III. RESULTS AND DISCUSSION

➢ *Triazine derivatives (Figure 3) were prepared through a set of sequential reactions. In the first step, chloroacetyl chloride was reacted with (acetamide and thioacetamide) to form the compounds N-acetyl-2-chloroacetamide (1) and N-thioacetyl-2-chloroacetamide (2), respectively. Synthesis of these compounds was confirmed by their FT-IR spectra through the absence of (NH_2) bands in products (1) and (2). In the second step, (1 and 2) compounds were reacted separately with thiosemicarbazide to produce (3 and 4) derivatives. The FT-IR spectra of (3 , 4) compounds showed new bands at (3363 ,3477,3245 and 3332 cm^{-1}) for symmetric and asymmetric of NH_2 group.*

Finally, compounds (3 and 4) were subjected to ring closure and the formation of 1,2,4-triazine derivatives by treating them with 5% NaOH. The ring closure of compounds (3 and 4) was confirmed by FTIR spectra due to the absence of bands at owing to (NH_2) group.

Table 1 Physical Properties of Synthesized Compounds (1-6)

Com.no.	X	M.F.	M.P (^{0}C)	Color	Yield (%)
1	O	$C_4H_6ClNO_2$	114	Yellow	72
2	S	C_4H_6ClNOS	110	Brown	77
3	O	$C_5H_{10}N_4O_2S$	167	Brown	85
4	S	$C_5H_{10}N_4OS_2$	172	Orange	80
5	O	$C_5H_8N_4OS$	144	Dark yellow	75
6	S	$C_5H_8N_4S_2$	135	White	78

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24AUG1311

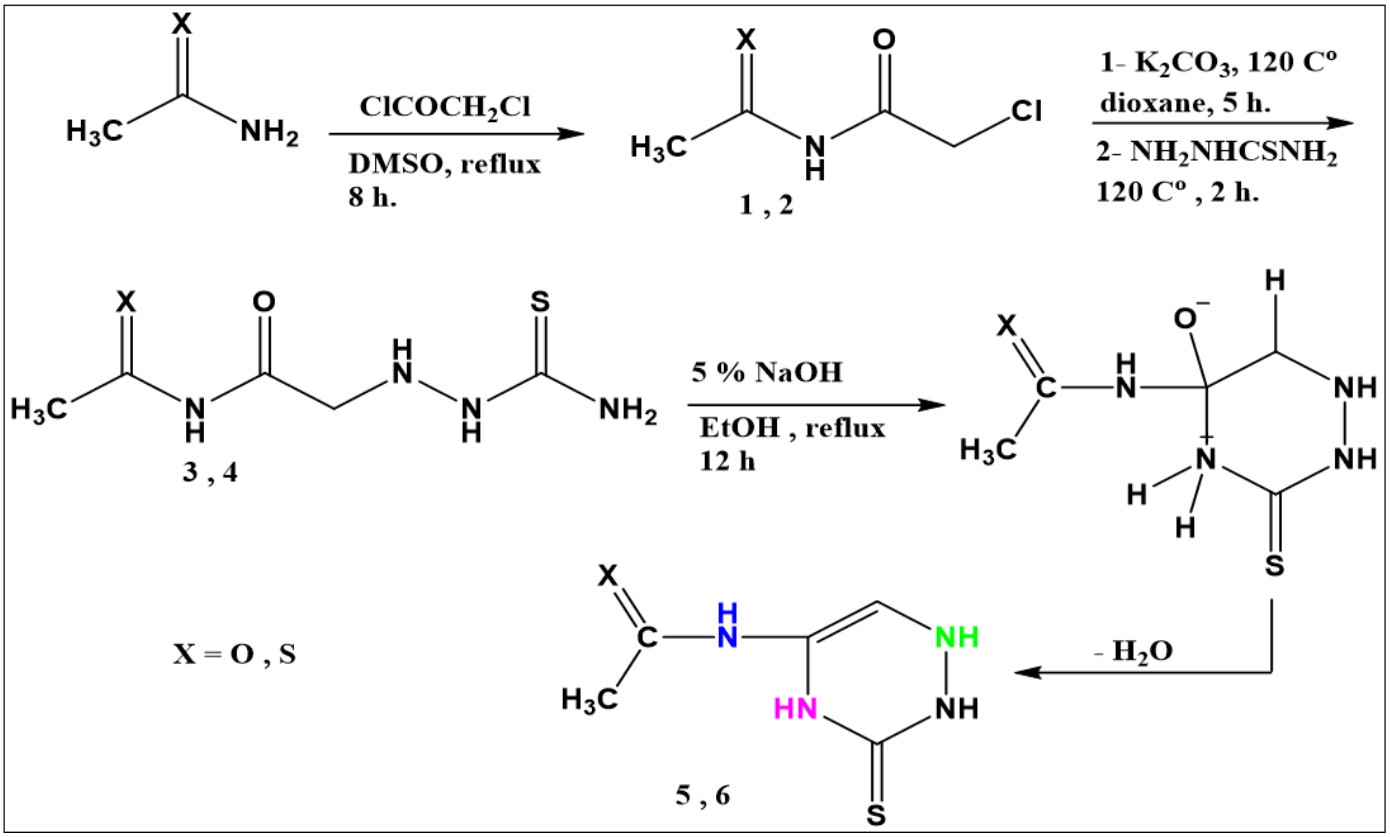

Fig 3 Synthesised Triazine Derivatives

➢ *Biological Activities.*

Gram-positive bacteria undergo adsorption through the lipoteichoic acid layer, characterised by its charged properties and ability to interact with molecules. Conversely, in gram-negative bacteria, the biocide chemicals selectively focus on the non-polar lipid layer. Adsorption hinders the membranes' ability to selectively allow substances to pass through and greatly upsets the biological events within cells by enabling the passage of different chemicals from the surroundings. This study assessed the efficacy of a series of newly synthesised heterocyclic compounds against ***Staphylococcus aureus***, ***Klebsilla pneumonia*** bacteria, and ***Rhizosporium*** fungus. The inhibitory zones were measured using the well diffusion method. In general ***Staphylococcus aureus***, at concentration (0.03 g/ml) was inhibited by all compounds under test and inhibition was ranged from slightly (<10 mm) for compounds (**1,2**) to highly (10-37mm) for compounds (**3, 4, 5 and 6**), ***Klebsilla pneumonia*** at concentration (0.03g/ml) was inhibited by all compounds, inhibition was ranged from (11- 19 mm). While Rhizosporium fungi at concentration (0.03 g/ml) was inhibited by all compounds under test and inhibition was ranged from (10- 39 mm). The data of antimicrobial activity for the described derivatives against two types of pathogenic bacteria and one fungi type were evaluated and are listed in Table (2).

Table 2 The Antimicrobial Activity of Prepared Compounds.

Com. No.	Staphylococcus Aureus (mm)	Klebsilla pneumonia (mm)	Rhizosporium (mm)
1	11	15	14
2	10	12	15
3	11	16	15
4	11	11	18
5	11	14	13
5	12	16	39

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24AUG1311

REFERENCES

[1]. Khoshneviszadeh, M., Ghahremani, M. H., Foroumadi, A., Miri, R., Firuzi, O., Madadkar-Sobhani, A., ... & Shafiee, A. (2013). Design, synthesis and biological evaluation of novel anti-cytokine 1, 2, 4-triazine derivatives. *Bioorganic & medicinal chemistry*, *21*(21), 6708-6717.

[2]. Kumar, R., Sirohi, T. S., Singh, H., Yadav, R., Roy, R. K., Chaudhary, A., & Pandeya, S. N. (2014). 1, 2, 4-triazine analogs as novel class of therapeutic agents. *Mini-Rev. Med. Chem*, *14*, 168-207.

[3]. Khoshneviszadeh, M., Ghahremani, M. H., Foroumadi, A., Miri, R., Firuzi, O., Madadkar-Sobhani, A., ... & Shafiee, A. (2013). Design, synthesis and biological evaluation of novel anti-cytokine 1, 2, 4-triazine derivatives. *Bioorganic & medicinal chemistry*, *21*(21), 6708-6717.

[4]. Singla, P., Luxami, V., & Paul, K. (2015). Triazine as a promising scaffold for its versatile biological behavior. *European journal of medicinal chemistry*, *102*, 39-57.

[5]. Marín-Ocampo, L., Veloza, L. A., Abonia, R., & Sepúlveda-Arias, J. C. (2019). Anti-inflammatory activity of triazine derivatives: A systematic review. *European Journal of Medicinal Chemistry*, *162*, 435-447.

[6]. Liu, H., Long, S., Rakesh, K. P., & Zha, G. F. (2020). Structure-activity relationships (SAR) of triazine derivatives: Promising antimicrobial agents. *European journal of medicinal chemistry*, *185*, 111804.

[7]. Ban, K., Duffy, S., Khakham, Y., Avery, V. M., Hughes, A., Montagnat, O., ... & Baell, J. B. (2010). 3-Alkylthio-1, 2, 4-triazine dimers with potent antimalarial activity. *Bioorganic & medicinal chemistry letters*, *20*(20), 6024-6029.

[8]. Melato, S., Prosperi, D., Coghi, P., Basilico, N., & Monti, D. (2008). A combinatorial approach to 2, 4, 6-trisubstituted triazines with potent antimalarial activity: combining conventional synthesis and microwave-assistance. *ChemMedChem: Chemistry Enabling Drug Discovery*, *3*(6), 873-876.

[9]. Menicagli, R., Samaritani, S., Signore, G., Vaglini, F., & Dalla Via, L. (2004). In vitro cytotoxic activities of 2-alkyl-4, 6-diheteroalkyl-1, 3, 5-triazines: new molecules in anticancer research. *Journal of medicinal chemistry*, *47*(19), 4649-4652.

[10]. Yaguchi, S. I., Fukui, Y., Koshimizu, I., Yoshimi, H., Matsuno, T., Gouda, H., ... & Yamori, T. (2006). Antitumor activity of ZSTK474, a new phosphatidylinositol 3-kinase inhibitor. *Journal of the National Cancer Institute*, *98*(8), 545-556.

[11]. Xiong, Y. Z., Chen, F. E., Balzarini, J., De Clercq, E., & Pannecouque, C. (2008). Non-nucleoside HIV-1 reverse transcriptase inhibitors. Part 11: structural modulations of diaryltriazines with potent anti-HIV activity. *European Journal of Medicinal Chemistry*, *43*(6), 1230-1236.

[12]. Ashour, H. M., Shaaban, O. G., Rizk, O. H., & El-Ashmawy, I. M. (2013). Synthesis and biological evaluation of thieno [2′, 3′: 4, 5] pyrimido [1, 2-b][1, 2, 4] triazines and thieno [2, 3-d][1, 2, 4] triazolo [1, 5-a] pyrimidines as anti-inflammatory and analgesic agents. *European journal of medicinal chemistry*, *62*, 341-351.

[13]. Hynes Jr, J., Dyckman, A. J., Lin, S., Wrobleski, S. T., Wu, H., Gillooly, K. M., ... & Leftheris, K. (2008). Design, synthesis, and anti-inflammatory properties of orally active 4-(phenylamino)-pyrrolo [2, 1-f][1, 2, 4] triazine p38α mitogen-activated protein kinase Inhibitors. *Journal of medicinal chemistry*, *51*(1), 4-16.

[14]. Diana, P., Barraja, P., Lauria, A., Montalbano, A., Almerico, A. M., Dattolo, G., & Cirrincione, G. (2002). Pyrrolo [2, 1-c][1, 2, 4] triazines from 2-diazopyrroles: synthesis and antiproliferative activity. *European journal of medicinal chemistry*, *37*(3), 267-272.

[15]. Krauth, F., Dahse, H. M., Rüttinger, H. H., & Frohberg, P. (2010). Synthesis and characterization of novel 1, 2, 4-triazine derivatives with antiproliferative activity. *Bioorganic & Medicinal Chemistry*, *18*(5), 1816-1821.

[16]. Sztanke, K., Markowski, W., Świeboda, R., & Polak, B. (2010). Lipophilicity of novel antitumour and analgesic active 8-aryl-2, 6, 7, 8-tetrahydroimidazo [2, 1-c][1, 2, 4] triazine-3, 4-dione derivatives determined by reversed-phase HPLC and computational methods. *European journal of medicinal chemistry*, *45*(6), 2644-2649.

[17]. Sztanke, K., Pasternak, K., Rzymowska, J., Sztanke, M., & Kandefer-Szerszeń, M. (2008). Synthesis, structure elucidation and identification of antitumoural properties of novel fused 1, 2, 4-triazine aryl derivatives. *European journal of medicinal chemistry*, *43*(5), 1085-1094.

[18]. Yurttas, L., Ciftci, G. A., Temel, H. E., Saglik, B. N., Demir, B., & Levent, S. (2017). Biological activity evaluation of novel 1, 2, 4-triazine derivatives containing thiazole/benzothiazole rings. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, *17*(13), 1846-1853.

[19]. Kumar, R., Kumar, N., Roy, R. K., & Singh, A. (2017). Triazines—A comprehensive review of their synthesis and diverse biological importance. *Curr. Med. Drug Res*, *1*(1), 173.

[20]. Khodair, A. I., Ahmed, A., Imam, D. R., Kheder, N. A., Elmalki, F., & Hadda, T. B. (2021). Synthesis, antiviral, DFT and molecular docking studies of some novel 1, 2, 4-triazine nucleosides as potential bioactive compounds. *Carbohydrate Research*, *500*, 108246.