Egypt. J. Chem. **Vol. 66,** No. 3 pp. 79 - 90 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Synthesis, Anticancer Activity and Molecular Docking Study of some New Thiazolo[2, 3-*a*]Pyrimidinedione-Based Heterocyclic Compounds

Nesrin M. Morsy [1*], Khadiga M. Abu-Zied [2], Hanem M Awad[3]

[1]*Organometallic and Organometalloid Chemistry Department, National Research Centre, Dokki, Cairo, 12622, Egypt.*

[2] *Photochemistry Department, National Research Centre, Dokki, Cairo, 12622, Egypt.*

[3] *Tanning Materials and Leather Technology Department & Regulatory Toxicology Lab, Centre of Excellence, National Research Centre, Dokki 12622, Cairo, Egypt.*

Abstract

New thiazolo[2,3-*a*] pyrimidinedione derivatives were synthesized by two different chemical methods.One method included the addition of a mixture of glacial acetic acid ,acetic anhydride (2 : 1), chloroacetic acid and anhydrous sodium acetate to mercapto-thieno[2,3-*d*]pyrimidinone derivatives **5a** or **5b** under reflux to give thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidinedione derivatives **6a** or **6b** which react with selected aldehydes **7**, **8**, **9a-c**,**10**,**11** at a next step to give our targeted products. The other method is the direct reaction of the solutions of **5a** or **5b** in the presence of the previous reagents except chloroacetic acid under reflux with series of aldehydes yielding the new derivatives **7a**, **7b**, **8a**, **8b**, **9d**, **9e**, **9f**, **9g**, **9h**, **9i**, **10a**, **10b**,**11a** and **11b**. The chemical structure of these compounds was confirmed by various spectroscopic analysis. In vitro cytotoxic activity was investigated for all compounds against HCT-116, HepG2, and MCF-7 cancer cell lines. Two compounds were potent against all cell lines, **8a** with IC50 4.7, 5.6, and 6.2 µM and **9h** with IC50 5.4, 7.8, and 5.6 against HCT-116, HepG2, and MCF-7 respectively. Molecular docking against inosine monophosphate dehydrogenase 2 showed that compound 8a had the top ranked free energy of binding ΔG -8.68 (kcal/mol) and RMSD 1.2 Å.

Keywords: Synthesis; Thiazolo[3,2-*a*]pyrimidinedione; Aldehyde; Anticancer Activity; Molecular Docking.

1. Introduction

The process of discovery and optimization of novel bioactive anticancer agents is very important and has a great attention in scientific research. Heterocyclic compounds have different applications as therapeutic agents [1]. Pyrimidine is a six membered ring with two nitrogen atoms at 1 and 3 position and it is found in naturally occurring substances such as nucleic acids, vitamins, nucleotides, coenzymes, purines, uric acid and pterins[2,3] . Moreover pyrimidine moiety is an important moiety of many marketed drugs such as zidovudine (**I**), stavudine (**II**), 5-flurouracil (**III**), methotrexate (**IV**), imatinib (**V**), dasatinib (**VI**), pazopanib (**VII**), cytarabine (**VIII**), trimethoprime (**IX**), sulfamethazine (**X**) [4] Figure 1.

Thienopyrimidine scaffold has various pharmacological and biological activities [5-7], such as anticancer agents [8-10], antioxidants [11]. Recently, some thiazolo[3,2-*a*] pyrimidine derivatives were reported to inhibit IMPDH with cytotoxic activity [12]. Inosine monophosphate dehydrogenase (IMPDH) plays an important part in a metabolic step in the regulation of cell growth and isolation. This step is NAD-subordinate oxidation of inosine 5′ monophosphate (IMP) to xanthosine 5′ monophosphate, and viewed as the rate-restricting move toward the amalgamation of the guanine nucleotides. [13,14]. The human genome encodes two IMPDH isoenzymes, IMPDH-I on chromosome 7 and IMPDH-II on chromosome 3. Recent studies reported that IMPDH-II expression is elevated in cancer cells. In addition,it has been perceived as a significant objective in antitumor and immuno-suppressive drug design. The inhibition of type II showed significant cytotoxic effect against different cancer cell lines [15-20]. Nowadays, the antitumor potential for the use of IMPDH inhibitors has a great attention [21]. IMPDH2 has a role that is directed

*Corresponding author e-mail: nesrinmorsy@yahoo.com.; (Nesrin M. Morsy).

Receive Date: 19 January 2023, Revise Date: 13 February 2023, Accept Date: 15 February 2023

DOI: 10.21608/EJCHEM.2023.188464.7479

directly to cell differentiation and neoplastic transformation in colorectal cancer [22], breast cancer [23], and hepatic cancer cells [24]. Ability of the tested compounds to bind and inhibit IMPDH2 will reduce the cancer cell differentiation with antiproliferative activity. The discovery of Mycophenolic acid which is a potent inhibitor of IMPDH (EC_{50} = 0.24 µM, Ki = 11 nM) with significant immunosuppressive and anticancer activities [25, 26] provided evidence to support our rationale for targeting IMPDH in the design of isoform-special factors that can be supported by identification of significant interaction needed for targeting high binding affinity. Based on the above and in continuation of our researches [27-49]. we synthesized new compounds with thiazolo [2,3-*a*]pyrimidine scaffold **7a**, **7b**, **8a**, **8b**, **9d**, **9e**, 9**f**, **9g**, **9h**, **9i**, **10a**, **10b**,11**a** and **11b.**

Characterization of the produced structures was done with spectroscopic analysis, ^{1}H-NMR,^{13}C-NMR, MS, elemental analysis. The molecular docking was performed to interpret the biological anticancer activity against HepG2, MCF-7 and HCT-116 cancer cell lines for seventeen compounds.

2. Results and Discussion:

2.1. Chemistry:

New derivatives containing the thienopyrimidinone moiety were synthesized and combined with other heterocyclic, aromatic, and metal complex moieties. Ethyl cyanoacetate **(1)** was allowed to react with sulphur and cyclopentanone **(3a)** or cyclohexanone **(3b)** in the presence of diethyl amine **(2)**; the product was either ethyl 2-amino-5,6-dihydro-4*H*-cyclopent[*b*]-thiophene-3-carboxylate (**4a)** or ethyl-2-amino-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-3-carboxylate (**4b)**. When compounds (**4a)** or (**4b)** was refluxed with potassium thiocyanate and conc. HCl in dioxane, the compounds 2-mercapto-6,7-dihydro-3*H*-cyclopenta- [4,5]thieno[2,3-*d*]pyrimidine-4(5*H*)-one **(5a)** or 2-mercapto-5,6,7,8-tetrahydrobenzo-[4,5]theino- [2,3-*d*]pyrimidine4(3*H*)-one **(5b)** was yielded (Scheme 1).

Thieno[2,3-*d*]thiazolo[2,3-*a*]pyrimidine-3,5-dione scaffold **7a**, **7b**,**8a**, **8b**, **9d**,**9e**,9**f**, **9g**, **9h**, **9i**, **10a**, **10b**,**11a** and **11b** were synthesized using two methods, the first one is the two steps reaction. The first step included addition of glacial acetic acid, chloroacetic acid, acetic anhydride and anhydrous sodium acetate to mercapto-thieno[2,3-*d*]pyrimid-inone derivatives **5a** or **5b** to give thieno[2,3-*d*]-thiazolo[3,2-*a*]pyrimidindione derivatives **6a** or **6b**, then addition of proper aldehyde **7**, **8**, **9a-c**,**10**,**11** under reflux .The second method was proceeded in one step when compounds **5a** and **5b** were added directly to the proper aldehydes **7**, **8**, **9a-c**,**10**,**11** in the presence of glacial acetic acid, acetic anhydride and anhydrous sodium acetate . (Scheme 2).

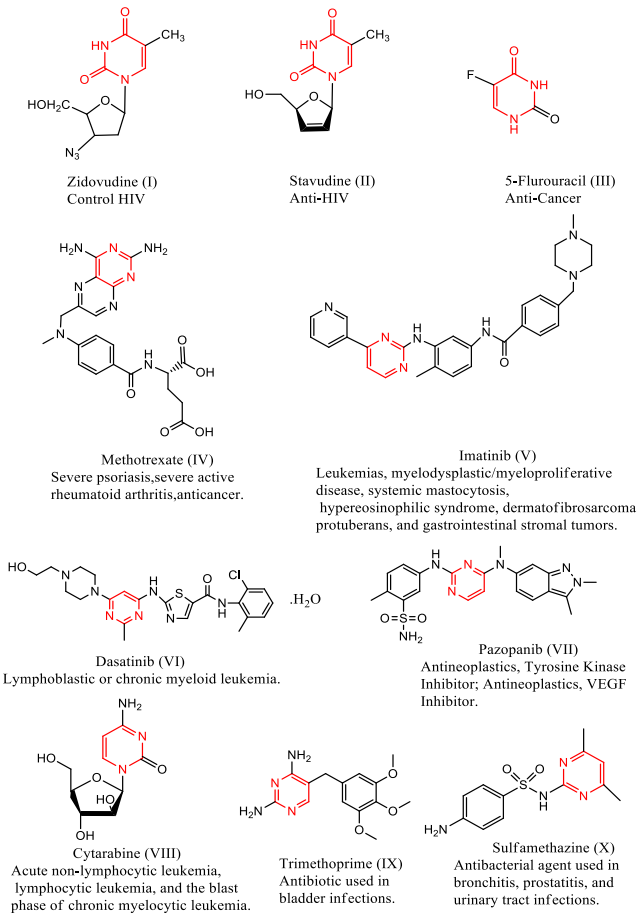
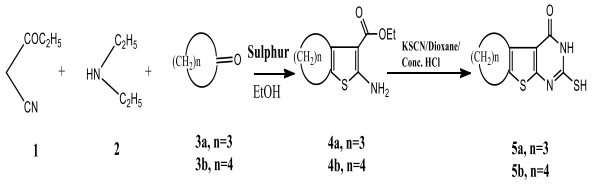


Figure 1 : Drugs containing pyrimidine and pyrimidinone units and its usage.



Scheme 1: Synthesis of mercapto-thienopyrimidine derivatives

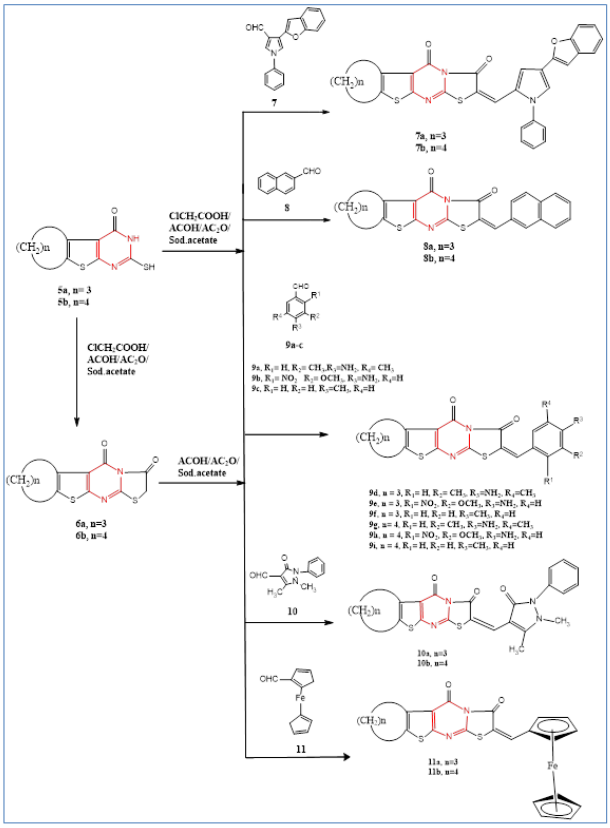
The resulted products **7a**, **7b**,**8a**, **8b**, **9d**,**9e**,**9f**, **9g**, **9h**, **9i**, **10a**, **10b**,11**a** and **11b** were crystallized from the proper solvent and then were examined by different spectroscopic analysis like IR, ^{1}H-NMR, ^{13}C-NMR. Two compounds **7a** and **7b** with 2-((4-(benzofuran-2-yl)-1-phenyl-1*H*-pyrrol-3-yl)methylene)-(cyclo-alkyl)[4,5]thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimi-dine-3,5-(2*H*,6*H*)-dione structure were confirmed by ^{1}H-NMR; The cyclopentyl moiety of **7a** was confirmed

by δ 2.37 ppm (m, 2H, CH_2) , δ 2.83 ppm (t, 2H,CH_2), δ 2.93 ppm (t, 2H,CH_2). The furan ring showed a singlet peak for one proton at δ 6.9 ppm and the ^{13}C-NMR also confirmed that by the appearance of a peak at δ 102.7 for C=C-O of furan. Thiazole ring had a peak at δ 125.4 C=C-S, and another peak for the carbon of carbonyl group of thiazole ring at δ 172.0 -C=O. The N-C=N of pyrimidine appeared at δ 158.3, the C=O group of pyrimidine appeared at 168.6. IR showed the presence of two C=O groups of **7a** and **8a** at 1641cm^{-1},1582 cm^{-1}respectively. The D_2O exchangeable two protons of the -NH_2 group of **9d** and **9e** appeared at δ 6.27 ppm was confirmed by the IR peak at 3423 cm^{-1}. A singlet peak for the three protons of -OCH_3 group of **9e** appeared at δ 3.96 ppm.

While the methyl protons -CH_3 of **9f** appeared as a singlet peak at δ 2.34 ppm. The -CH proton at the double bond that link between the naphthyl group and thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5-dione of **8b** appeared at δ 7,62 ppm as a singlet peak. The IR spectrum showed two carbonyl groups of **8b** at 1582 cm^{-1}. While ^{13}C-NMR spectrum confirmed the cyclohexyl carbons of **8b** at δ 24.5 (5C, CH_2). Two D_2O exchangeable protons for -NH_2 group of **9g** appeared as a singlet peak at δ 6.27 ppm. The carbonyl groups of **9g** were confirmed by IR, where two peaks at 1652 and 1536 cm^{-1} were shown. In addition, ^{13}C-NMR confirmed that a peak at δ 168.6 for C=O, of pyrimidine, and another one at δ 171.3 for N-C=O of thiazole. IR showed two peaks for two carbonyl groups of **10b** at 1637, and 1531 cm^{-1}. The two methyl groups at the pyrazole ring were confirmed by ^{1}H-NMR, where appeared as two singlets at δ 2.57 and 3.11 ppm.

2.2. *In vitro* antiproliferative activity

Lactate dehydrogenase (LDH) is an enzyme that catalyses the reversible change of pyruvate to lactate under anaerobic circumstances. There are five active isoenzymes for LDH in human tissues, one of them which is LDHA is expressed in cancer cells and is used as a biomarker for different cancer types. It is a predictive marker for assessing the response of cancer cells to the therapeutic anticancer agents [50] . IMPDH2 is highly expressed in colorectal cancer, breast cancer, and hepatic cancer cells [22-24]. Ability of the tested compounds to bind and inhibit IMPDH2 will reduce the cancer cell differentiation and this response can be measured by the antiproliferative activity. Seventeen derivatives were examined in vitro for their cytotoxic action in contrast to HCT-116, HepG2 and MCF-7 human malignant growth cells utilizing the LDH assay. The percentage of intact cells were determined and contrasted with those of the standard. Activities of these compounds against the three carcinoma cell lines were contrasted with that of doxorubicin. All derivatives inhibited three malignant growth cells (HCT-116, HepG2 and MCF-7) in a dose-dependent way (Fig. 2-4). In case of HCT-116 human colorectal carcinoma cells, both (Figure 2 -Table 1)



Scheme 2: Synthesis of **7a**, **7b**,**8a**, **8b**, **9d**,**9e**,9**f**, **9g**, **9h**, **9i**, **10a**, **10b**,11**a** and **11b**

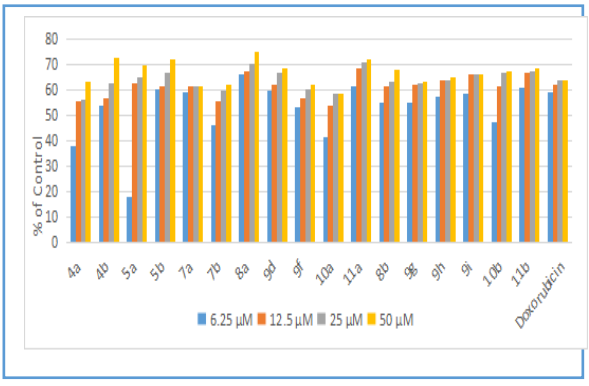


Figure 2: Dose dependent antiproliferative data against HCT-116 cancer cells

most of the tested compounds showed potent cytotoxic effect; **8a** (IC_{50} = 4.7 µM), both compounds **11a** and **11b** showed the same activity (IC_{50} = 5.1 µM), **9d** (IC_{50} = 5.2 µM), **7a** and **9g** (IC_{50} = 5.3 µM), **9h** (IC_{50} = 5.4 µM), **8b** and **9g** (IC_{50} = 5.7 µM) and **9f** (IC_{50} = 5.8 µM) and these results confirmed that **8a** was the most potent when compared to doxorubicin (IC_{50} = 5.2 µM).

On the other hand, results of HepG2 cell line (Figure 3-Table 1) showed that compound **11b** (IC_{50} = 5.6 µM), **8b**(IC_{50} = 5.7 µM), **9h** (IC_{50} = 7.8 µM) and **7b** (IC_{50} = 6.1 µM) when compared to doxorubicin (IC_{50} = 5.7 µM). The results of MCF-7 human breast cancer cells illustrated four compounds (**9h**, **9i**, **9g** and **8b**, with IC_{50} = 5.6, 5.6, 5.9, and 6.2 µM respectively) with promising cytotoxic activity against MCF-7 (**Figure 4** &**Table 1**).

From the above-mentioned results, one can deduce that: two compounds (**8a** and **9h**) have a selective activity against all the three cancer types. Two compounds (**9g** and **9i**) are selectively active on both colon and breast cancer types and not active on liver cancer type. They can be considered as lead compounds that may need further investigation and optimization in the future.

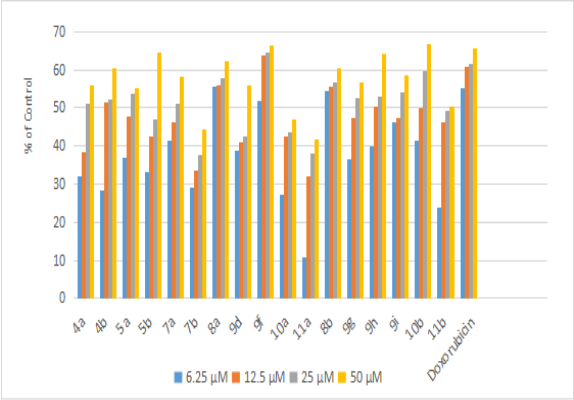


Figure 3: Dose dependent antiproliferative data against HepG2 cancer cells

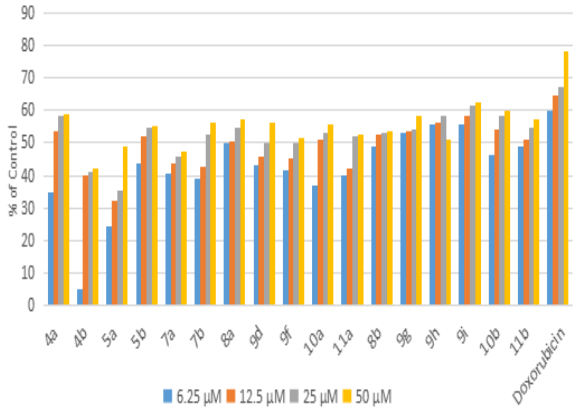


Figure 4: Dose dependent antiproliferative data against MCF-7 cancer cells

Table 1: Cytotoxic results (IC_{50}) of the seventeen compounds against the three cancer cell lines.

Compound	IC_{50} (µM) ± SD		
	HCT-116	HepG-2	MCF-7
4a	11.3 ± 2.1	16.2 ± 2.6	11.6 ± 0.9
4b	5.8 ± 0.5	12.1 ± 1.9	30.4 ± 4.6
5a	9.9 ± 0.9	13.1 ± 1.1	35.5 ± 2.8
5b	5.2 ± 0.3	14.8 ± 2.1	12.1 ± 2.5
7a	5.3 ± 0.3	13.5 ± 2.1	27.3 ± 3.7
7b	11.2 ± 2.1	6.1 ± 0.4	14.7 ± 2.1
8a	4.7 ± 0.3	5.6 ± 0.4	6.2 ± 0.5
9d	5.2 ± 0.5	29.5 ± 4.5	25.1 ± 3.9
9f	5.8 ± 0.4	18.5 ± 3.1	24.9 ± 3.1
10a	11.6 ± 2.1	28.6 ± 3.6	12.2 ± 1.1
11a	5.1 ± 0.4	19.4 ± 3.1	14.8 ± 2.1
8b	5.7 ± 0.4	5.7 ± 0.4	11.8 ± 1.9
9g	5.7 ± 0.4	23.7 ± 3.9	5.9 ± 0.5
9h	5.4 ± 0.3	7.8 ± 0.6	5.6 ± 0.3
9i	5.3 ± 0.3	13.2 ± 1.9	5.6 ± 0.4
10b	10.2 ± 1.9	12.5 ± 2.1	11.5 ± 1.4
11b	5.1 ± 0.5	25.3 ± 4.5	12.2 ± 2.4
Doxorubicin	5.2 ± 0.3	5.7 ± 0.4	5.2 ± 0.4

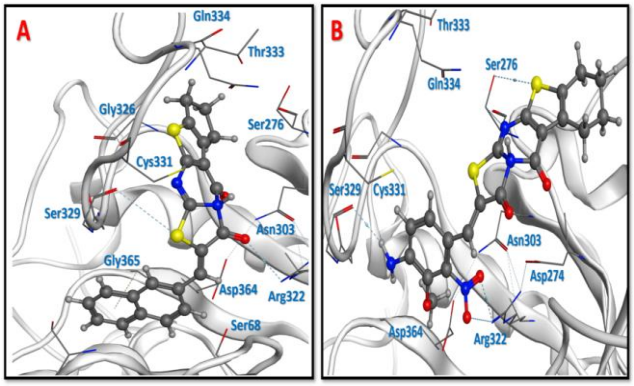


Figure 5: Docking interactions of compounds A) **8a** and B) **9h**. Showing the beat pose for each compound in the catalytic active site of IMPDH-II

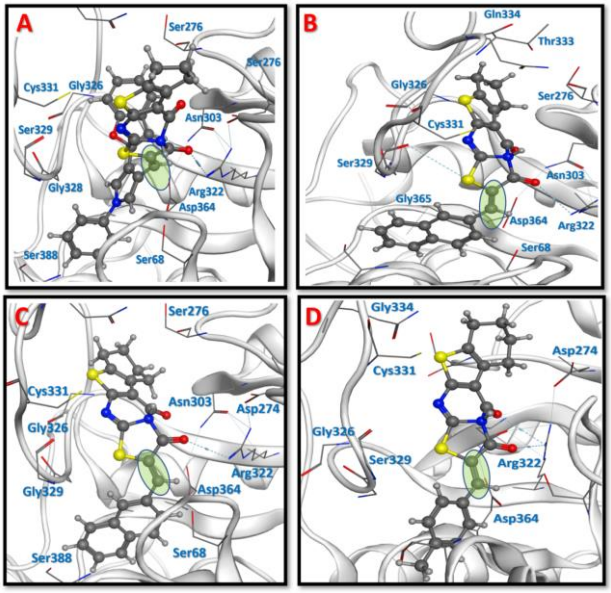


Figure 6: The orientation of the (*Z*) configuration of compounds; A) **7a**, B) **8a**, C) **8b** , D) **9i**. It shows how this resulted in the same interactions with Arg322.

Table 2: Docking results against inosine monophosphate dehydrogenase 2, showing the free energy of binding ΔG (Kcal/mol), Root of mean square deviation RMSD (Å), and the interacted residues in the best pose.

Compound	ΔG (Kcal/mol)	RMSD (Å)	Interacted residues
4a	-5.28	1.91	Ser329, and Ser388
4b	-5.37	2.04	Ser329, and Ser388
5a	-5.42	2.78	Ser329, and Ser388
5b	-5.32	1.86	Gln334
7a	-8.42	2.15	Arg322
7b	-7.57	2.22	Arg322
8a	-8.68	1.21	Arg322, Ser329, and Gly365
9d	-8.47	1.34	Asp364, and Ser276
9f	-6.72	1.31	Arg322
10a	-6.89	2.02	Arg322, and Asp274
11a	-8.01	2.97	Asp274, and Cys331
8b	-8.38	0.93	Arg322
9g	-8.30	1.10	Asp274
9h	-8.60	1.55	Arg322, Ser329, and Ser376
9i	-8.56	1.28	Arg322
10b	-7.94	1.36	Arg322, Gly326, and Ser276
11b	-8.03	1.29	Arg322

3. Experimental

3. 1. Chemistry

All melting points were measured on a Gallen Kamp melting point apparatus and are uncorrected .^{1}H-NMR (500 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Varian spectrometer using DMSO-d_6 as solvent and TMS as an internal standard. Chemical shifts are reported in ppm. Coupling constants (*J*) are expressed in Hz. Mass spectra were recorded on a Varian MAT 112 spectrometer at 70 eV. Elemental analyses were performed at the Micro Analytical Centre, Cairo University, Egypt. Progress of the reactions was monitored by thin-layer chromatography (TLC) using aluminum sheets coated with silica gel F254 (Merck), viewing under a short-wavelength UV lamp effected detection. All evaporations were carried out under reduced pressure at 40°C.

The compounds **4a**,**4b**,**5a**,**5b**,**6a**,**6b** were prepared as reported.[51-54]

Ethyl-2-amino-5,6-dihydro-4*H*-cyclopenta[*b*] thiophene-3-carboxylate (**4a**); M.P.= 111-**112ºC. Ethyl-2-amino-4,5,6,7-tetrahydrobenzo [*b*]thiophene-3-carboxylate**(**4b**); M.P.= 92ºC . **Mercapto-6,7-dihydro-3*H*-cyclopenta[4,5]thieno [2,3-*d*]pyrimidin-4(5*H*)-one (5a); M.P=**.340-342ºC . **2-Mercapto-5,6,7,8-tetrahydrobenzo-[4,5]theino[2,3-*d*]pyrimidine4(3*H*)-one** (**5b**); M.p = >300 ºC.

7,8-Dihydrocyclopenta[4,5]thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5(2*H*,6*H*)-dione (6a); M.P.= >300 ºC.

6,7,8,9-Tetrahydro-2*H*-benzo[4,5]thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5-dione(6b); M.P =.>300ºC.

Synthesis of Thienopyrimidinone Derivatives 7a, **7b**,**8a**, **8b**, **9d**,**9e**,9**f**, **9g**, **9h**, **9i**, **10a**, **10b**,**11a** and **11b:**

Method 1:

A solution of compound **5a** or **5b** (10 mmole) was added to the proper aldehydes (10 mmole) and heated in 50 ml glacial acetic under reflux for 8 h in the presence of chloroacetic acid (1.04 g, 10mmole) , 25 ml acetic anhydride , and anhydrous sodium acetate (4.1 g). The reaction was monitored by using TLC technique and the reaction was stopped after completed. The reaction mixture was poured onto ice-water, the precipitate was collected and recrystallized from the proper solvent.

Method 2 :

A mixture of acetic anhydride, glacial acetic acid, chloroacetic acid, anhydrous sodium acetate was added to (10 mmole) of **5a** or **5b** then refluxed for 4 h., poured into cooled water and the collected precipitate was crystallized from ethanol to give brown precipitate with 70% yield of compound **6a** or **6b**. Aldehydes (10 mmole) were added to a solution of **6a**or **6b** in 25 ml acetic anhydride, 50 ml glacial acetic acid, and anhydrous sodium acetate (4.1 g) refluxed for 3 h., poured into water, filtered off and crystallized from proper solvent to give the same products **7a**, **7b**,**8a**, **8b**, **9d**,**9e**,9**f**, **9g**, **9h**, **9i**, **10a**, **10b**,**11a** and **11b.**as two steps reaction.

2-((4-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrrol-3-yl)methylene)-7,8-dihydrocyclopenta-[4,5]thieno [2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5(2*H*,6*H*)-dione (7a).

Brown Crystals (ethanol), m. p. 205ºC, yield: 60%, IR (KBr)$_{Vmax}$ /cm^{-1} 2856 (CH); 1662, 1641 (2CO) ;^{1}H-NMR (DMSO-d_6, 500 MHZ , δ ppm). 2.37 (m, 2H, CH_2, cyclo-pentane) , 2.83(t, 2H,CH_2, cyclo-pentane), 2.93(t, 2H,CH_2, cyclo-pentane), 6.9 (s, 1H, Furan ring), 7.51(s, 1H, -CH-N-), 7.66(s,1H, -CH-N-), 7.91(s, 1H, -C=CH), 7.5-8.09(m, 9H, aromatic). ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm): 24.9, 25.6, 31.9 (3C,CH_2, cyclpentan), 102.7 (C=C-O furan), 110.3(C=C-N, pyrrole)112.7(-C=C-N-, pyrrole)117.6 (-C=C=O-,pyrimidine), 121.3(1C,phenyl), 122.0 (S-C=CH),123.3,124.7 (2C,phenyl), 125.4 (C=C-S,thiazole),125.4, 125.5, 129.3(3C, phenyl), 135.5 (-C=C-N-,pyrrole),139.4(C=C-S), 140.4 (1C, phenyl), 143.3(-S-C=C-)155.5 (S-C-N),155.5(-O-C=C),158.3 (N-C=N, pyrimidine), 168.6 (C=O, pyrimidine), 172.0 (-C=O, thiazole). MS (*m/z*, 533%) (M^{+}, 70%). Anal. Calcd. (%); $C_{30}H_{19}N_3O_3S_2$ (533.62) : C, 67.52; H, 3.59; N, 7.87; S, 12.02. Found (%) ; C,67.49; H,3.57; N,7.67; S, 12.03.

2-(Naphthalen-2-ylmethylene)-7,8-dihydrocyclopenta[4,5]thieno[2,3-*d*]thiazolo[3,2-*a*]pyr-imidine-3,5(2*H*,6*H*)-dione (8a).

Brown crystals (ethanol), M. P. 316 ºC , yield: 54 %, IR (KBr)$_{Vmax}$ /cm^{-1} 1690; 1582 (2CO).^{1}H-NMR (DMSO-d_6) , 500 MHz , δ ppm : 2.39(m, 2H, CH_2); 2.85 (t, 2H, CH_2); 2.98 (t, 2H, CH_2); 7.8 (s, 1H, -CH=); 7.75-8 (m, 7H, naphthalene.);. ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm): 23.0, 23.4 and 24.5 (3C,CH_2, cyclopent.), 116.0(S-C=CH), 117.6(=C-C=O, pyrimidine), 125.4 (C=C-S) ,125.7, 126.0, 126.4, 127.7, 127.8,128.1,128.2, 133.2, 133.5,133.6. (10C, naphthalene), 143.3 (CH=C-S), 155.5 (S-C-N, pyrimidine), 158.3(N=C-N, pyrimidine), 168.6, O=C-N, pyrimidine), 172.0(N-C=O, thiazole). MS (*m/z*, 402%) (M$^+$, 56%). Anal. Calcd. (%) for $C_{22}H_{14}N_2O_2S_2$ (402.49): C, 65.65; H, 3.51; N, 6.96; S, 15.93. Found (%):C, 65.62 ; H,3.49 ; N,6.97; S,15.90.

2-(4-Amino-3,5-dimethylbenzylidene)-6,7,8,9-tetrahydro-2*H*-benzo[4,5]thieno[2,3-*d*]thiaz-olo[3,2-*a*]pyrimidine-3,5-dione (9d).

Brown crystals (ethanol). M. P. 296 ºC, yield: 52 %, IR (KBr)$_{Vmax}$ /cm^{-1} 3423 (NH_2), 1647,1592 (2 CO). ^{1}H-NMR (DMSO-d_6,500 MHz,δ ppm). 2.39 (s, 2CH_3,3H), 2.49 (m,2H, CH_2) 2.77(t, 2H, CH_2),2.9(t, 2H, CH_2), 6.27 (s, 2H, NH_2, D_2O exchangeable), 6.69 (t, 1H, aromatic), 6,84 (t,1H,aromatic) 7.51 (s, 1H, -CH=).^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm) : 23.0, 25.4 and 24.5 (3C,CH_2, cyclohx.), 117.6 (C=C, pyrimidine), 125.4 (C= C, hex.), 126.0 (C=C-S, thiazole), 139.4 (C=C, hex.), 155.5 (C=C, pyrimidine), 158.3 (N-C=N), 168.6 (C=O, pyrimidine), 171.3 (N-C=O, thiazole). MS (*m/z*, 395%) (M$^+$, 69%). Anal. Calcd. (%): for $C_{20}H_{17}N_3O_2S_2$ (395.50):Cal; C, 60.74; H, 4.33; N, 10.62; S, 16.22. Found (%); C, 60.71 ; H, 4.30; N, 10.65; S, 16.11.

2-(4-Amino-3-methoxy-2-nitrobenzylidene)-6,7,8,9-tetrahydro-2*H*-benzo[4,5]thieno[2,3-d]thiazolo[3,2-*a*]pyrimidine-3,5-dione (9e).

Pale brown crystals (ethanol). M. P. 230ºC, yield: 67 %; IR (KBr)$_{Vmax}$ /cm^{-1} 3480, 3424, (NH_2); 1662; 1526 (2 CO) ;^{1}H-NMR (DMSO-d_6, 500 MHz , δ ppm); 2.5 (m,2H, CH_2); 2.86 (t, 2H, CH_2); 2.92 (t, 2H, CH_2); 3.96 (s, 3H, OCH_3); 6.27(s,2H,NH_2,D_2O exchangeable) 7.25 (s, 1H, -CH=); 7.52 (d, 1H,aromatic, *J* = 15Hz); 7.55 (d,1H,aromatic, *J*=15),8.36(s,1H,-CH=). ^{13}C- NMR (DMSO-d_6, 100 MHz, δ ppm): 23.0, 23.4 and 24.5 (3C,CH_2, cyclopentan), 54.8 (CH3), 116.0 (-S-C=CH), 117.6 (-C=O-N,pyrimidine), 118.3, 120.4, 123.3 (4C, benzene ring), 125.4 (-C=C-S, thiazole), 135.4 (-C-NO2, benzene ring), 136.4 (-C-NH_2, benzene ring),139.4(-C=C-,thiazole), 143.3 (-C=CH-phenyl), 143.6 (=C-C-OCH_3), 155.5 (S-C-N, pyrimidine), 158.3 (N=C-N, pyrimidine), 168.6 (O=C-N, pyrimidine), 172.0 (-N-C=O, thiazole). MS (*m/z*, 442%) (M$^+$, 60%). Anal. Calcd. (%) for $C_{19}H_{14}N_4O_5S_2$ (442.47): Cal; C, 51.58; H, 3.19; N, 12.66; O, 18.08; S, 14.49 Found (%): C, 51.60; H, 3.17; N, 12.64, S, 14.48 .

2-(4-Methylbenzylidene)-7,8-dihydrocyclopenta [4,5]thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimi-dine-3,5(2*H*,6*H*)-dione (9f) .

Pale brown crystals (ethanol). M. P. 172ºC, yield: 67 %, IR (KBr)$_{Vmax}$ /cm^{-1}; 1651; 1539 (2 CO) ;^{1}H-NMR (DMSO, 500 MHz , δ ppm) ; 2.39 (m,2 H, CH_2); 2.34 (s, 3H, CH_3); 2.85 (t, 2H, CH_2); 2.98 (t, 2H, CH_2); 7.18-7.59 (m, 4H, aromatic).^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm) : 24.9, 25.8 and 31.9 (3C,CH_2, cycolpent.),117.6 (C=C, pyrimidine), 125.4 (C= C, pent.), 126.0 (C=C-S, thiazole),139.4 (C=C, pyrimidine), 155.5 (C= C, pent.), 158.3 (N-C=N), 168.6 (C=O, pyrimidine), 171.3 (N-C=O, thiazole). MS (*m/z*, 366.46%) (M$^+$, 77%). Anal. Calcd. (%) for $C_{19}H_{14}N_2O_2S_2$ (366.46): Cal; C, 62.27; H, 3.85; N, 7.64; S, 17.50 Found (%): C, 62.25; H, 3.82; N, 7.65, S, 17.48 .

2-((1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)methylene)-7,8-dihydro-cyclopenta [4,5]thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5-(2*H*,6*H*)-dione (10a).

Brown crystals (ethanol). M. P.283ºC , yield: 59%,IR (KBr)$_{Vmax}$ /cm^{-1} 1649, 1550,1541 (3CO); ^{1}H-NMR (DMSO-d_6, 500 MHz , δ ppm): 2.39 (m, 2 H, CH_2); 2.57 (s, 3H, CH_3); 2.85 (t, 2H, CH_2); 3.98 (t, 2H, CH_2); 3.11 (s, 3H, CH_3); 6.66 (s, 1H, -CH=); 6.9-7.37 (m, 5H, aromatic).^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm) : 23.0, 23.4 and 24.5 (3C,CH_2, cyclohexan), 117.6 (C=C, pyrimidine), 125.4 (C= C, hex.), 126.0 (C=C-S, thiazole),139.4 (C=C, hex.) , 155.5 (C= C, pyrimidine), 158.3 (N=C-N, pyrimidine),163.4 (C=O,pyrazole) 168.6 (C=O, pyrimidine), 171.3 (N-C=O, thiazole) MS (*m/z*, 462.54%) (M$^+$, 80%). Anal. Calcd. (%) for $C_{23}H_{18}N_4O_3S_2$ (462.54) : C, 59.72; H, 3.92; N, 12.11; S, 13.86 Found (%): C, 59.52 ; H, 3.89; N, 12.00; S,13.84.

Cyclopenta-1,3-dien-1-yl(2-((3,5-dioxo-7,8-dihydrocyclopenta[4,5]thieno[2,3-*d*]thiazolo-[3,2-*a*]pyrimidin-2(3*H*,5*H*,6*H*)-ylidene)methyl)cyclopenta-1,3-dien-1-yl)iron (11a) .

Brown crystals (ethanol). M. P. 283ºC , yield: 59%, IR (KBr)$_{Vmax}$ /cm^{-1}; 1649, 1541 (2CO);^{1}H-NMR (DMSO-d_6, 500 MHz, δ ppm) : 2.9 (d, 2 H, CH_2); 2.39 (t, 2H, CH_2); 2.85 (t, 2H, CH_2); 2.98 (t, 2H, CH_2); 6.4 (m, 1H,CH); 2.9-6.5 (m, 9H, ferrocenyl); 6.67 (s, 1H, -CH=).^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm):23.0, 23.4 and 24.5 (3C,CH_2, cyclopent.), 117.6 (C=C, pyrimidine), 125.4 (C= C, hex.), 126.0 (C=C-S, thiazole),139.4 (C=C, hex.) 155.5 (C= C, pyrimidine), 158.3 (N=C-N, pyrimidine), 168.6 (C=O, pyrimidine), 171.3 (N-C=O, thiazole) MS (*m/z*, 460.35%) (M$^+$, 65%). Anal. Calcd. (%) for $C_{22}H_{16}FeN_2O_2S_2$ (460.35): C, 57.40; H, 3.50; Fe,

12.13; N, 6.09; S, 13.93. Found (%): C, 57.41; H, 3.48; Fe, 12.10; N,6.05; S,13.91.

2-((4-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrrol-3-yl)methylene)-6,7,8,9-tetrahydro-2*H*-benzo[4,5]thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5-dione (7b) .

Brown crystals (ethanol). M. P.283 ºC, yield: 59%, IR $(KBr)_{Vmax}$ /cm^{-1} 1649, 1541 (2 CO); ^{1}H-NMR (DMSO, 500 MHz , δ ppm): 2.39 (m, 2 H, CH_2); 2.85 (t, 2H, CH_2); 2.98 (t, 2H, CH_2); 7.14 (t, 1H, CH); 7.32-7-89(m, 9H, aromatic); 7.09 (s,1H,-CH=, furan ring); 7.8 (s,1H,-CH=).^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm): 24.9, 25.6, 31.9 (4C,CH_2, cyclohexane), 102.7 (C=C-O furan), 110.3(C=C-N, pyrrole), 112.7(-C=C-N-, pyrrole)117.6 (-C=C=O-, pyrimidine), 121.3(1C,phenyl),122.0(S-C=CH) 123.3,124.7(2C,phenyl),125.4(C=C-S, thiazole) ,125.4, 125.5, 129.3(3C, phenyl), 135.5 (-C=C-N-,pyrrole), 139.4 (C=C-S), 140.4 (1C, phenyl), 143.3(-S-C=C-), 155.5 (S-C-N),155.5(-O-C=C) , 158.3 (N-C=N, pyrimidine), 168.6 (C=O, pyrimidine), 172.0 (-C=O, thiazole) MS (*m*/*z*, 547.65%) (M^+, 59%) Anal. Calcd. (%) for $C_{31}H_{21}N_3O_3S_2$ (547.65): C, 67.99; H, 3.87; N, 7.67; S, 11.71 Found (%): C, 67.95 ; H, 3.85; N, 7.68; S,11.69.

2-(Naphthalen-2-ylmethylene)-6,7,8,9-tetrahydro-2*H*-benzo[4,5]thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5-dione (8b).

Pale yellow crystals (ethanol). M.P. 309 ºC , yield: 49 %, IR $(KBr)_{Vmax}$ /cm^{-1} 2854 (CH); 1690, 1582 (2CO). ^{1}H-NMR (DMSO-d_6, 500 MHz, δ ppm):1.79 (m, 2H, CH_2); 1.8 (m, 2H, CH_2); 2.34 (t, 2H, CH_2); 2.83 (t, 2H, CH_2); 7,62 (s, 1H, -CH=), 7.48-8.00(m, 7H, aromatic). ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm) : 23.0, 23.4 and 24.5 (4C, CH_2, cyclohex.), 116.0(S-C=CH), 117.6(=C-C=O,pyrimidine), 125.4(C=C-S), 125.7, 126.0, 126.4, 127.7, 127.8, 128.1, 128.2, 133.2, 133.5, 133.6. (10C, aromatic naphthalene), 143.3 (CH=C-S), 155.5 (C= C, pyrimidine), 158.3 (N-C=N), 168.6 (C=O, pyrimidine), 172.3 (N-C=O, thiazole). MS (*m*/*z*, 416%) (M^+, 55%). Anal. Calcd. (%) for $C_{23}H_{16}N_2O_2S_2$ (416.52) : C, 66.32; H, 3.87; N, 6.73; S, 15.40. Found (%): C,66.31; H,3. 86; N, 6.71; S, 15.39.

2-(4-Amino-3,5-dimethylbenzylidene)-6,7,8,9-tetrahydro-2*H*-benzo[4,5]thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5-dione (9g).

Brown crystals (ethanol).M. P. 221 ºC , yield: 65 %,IR $(KBr)_{Vmax}$ /cm^{-1} 3801, 3778(NH_2), 1652, 1536 (2 CO);^{1}H-NMR (DMSO-d_6, 500 MHz, δ ppm):1.76 (m, 2H, CH_2); 1.78 (m, 2H, CH_2); 2,5(s,3H, 2CH3); 2.69 (t, 2H, CH_2); 2.82 (t, 2H, CH_2); 6.27 (s, 2H, NH_2, D_2O excha-ngeable);6.65(2s,1H, aromatic); 7.72 (s,1H, -CH=). ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm) : 23.0, 25.4 and 24.5 (4C,CH_2, cyclohex.), 117.6 (C=C, pyrimidine), 125.4 (C= C, hex.), 126.0 (C=C-S, thiazole),139.4 (C=C, hex.), 155.5 (C= C, pyrimidine), 158.3 (N-C=N), 168.6 (C=O, pyrimidine), 171.3 (N-C=O, thiazole). MS (*m*/*z*, 409.5%) (M^+, 71%). Anal. Calcd. (%) for $C_{21}H_{19}N_3O_2S_2$ (409.52): C, 61.59; H, 4.68; N, 10.26; O, 7.81; S, 15.66. Found (%): C,61.60; H, 4.69; N, 12.24; S, 7.80.

2-(4-Amino-3-methoxy-2-nitrobenzylidene)-6,7,8,9-tetrahydro-2*H*-benzo[4,5]thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5-dione (9h) .

Gray crystals (ethanol). M. P. 199 ºC, yield: 55%, IR $(KBr)_{Vmax}$ /cm^{-1} 3495, 3450 (NH_2), 1643,1543 (2 CO).^{1}HNMR (DMSO-d_6, 500 MHz, δ ppm) :1.75 (m, 2H, CH_2); 2.39 (m, 2H, CH_2); 2.64 (t, 2H, CH_2); 2.5 (t, 2H, CH_2); 3.37 (s, 3H, OCH_3) ;7.32 (d, 1H,aromatic, *J* = 15); 7.94 (d,1H, aromatic, *J*= 15); 8.12 (s, 1H, -CH=). MS (*m*/*z*, 456%) (M^+, 73%). Anal. Calcd. (%) for $C_{20}H_{16}N_4O_5S_2$ (456.06) : C, 52.62; H, 3.53; N, 12.27; S, 14.05. Found: (%) C,52.60; H, 3.53; N, 12.26; S, 14.01.

2-(4-Methylbenzylidene)-6,7,8,9-tetrahydro-2*H*-benzo[4,5]thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5-dione (9i).

Gray crystals (ethanol). M. P. 206 ºC, yield: 70 %, IR $(KBr)_{Vmax}$ /cm^{-1} 1669, 1523.5 (2 CO); ^{1}H-NMR (DMSO-d_6, 500 MHz , δ ppm): 1.66 (m, 2H, CH_2); 1.71 (m, 2H, CH_2); 2.5 (s, 3H, CH_3); 2.60 (t,2H,CH_2); 2.74 (t, 2H, CH_2); 7.43 (d, 1H, aromatic, *J*=5); 7.77 (d, 1H, aromatic, *J* = 6 Hz); 7.9 (s,1H, aromatic); 7.9 (s,1H,-CH=).^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm) : 23.0, 23.4 and 24.5 (4C,CH_2, cyclohex.) , 117.6 (C=C, pyrimidine), 125.4 C= C, hex.), 126.0 (C=C-S, thiazole),139.4 (C=C, hex.), 155.5 (C= C, pyrimidine), 158.3 (N=C-N, pyrimidine), 168.6 (C=O, pyrimidine), 171.3 (N-C=O, thiazole). MS (*m*/*z*, 380.48%) (M^+, 75%) Anal. Calcd. (%) for $C_{20}H_{16}N_2O_2S_2$ (380.48): C, 63.13; H, 4.24; N, 7.36; S, 16.85. Found (%): C, 63.00; H, 4.36; N, 7,34, S, 16.84.

2-((1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)methylene)-6,7,8,9-tetra-hydro-2*H*-benzo[4,5]thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5-dione (10b).

Gray crystals (ethanol). M. P. 206 ºC , yield: 70 %,IR $(KBr)_{Vmax}$ /cm^{-1} 1637, 1550, 1531, (3 CO); ^{1}H-NMR (DMSO-d_6, 500 MHz , δ ppm):1.79 (m, 2H, CH_2); 2.57 (s, 3H, CH_3); 2.64 (t, 2H, CH_2); 2,73 (t, 2H, CH_2); 3.11 (s, 3H,CH_3); 6.9-7.37 (m, 5H, aromatic).^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm): 23.0, 23.4 and 24.5 (4C,CH_2, cyclohex.), 117.6 (C=C, pyrimidine), 125.4 (C=C, hex.), 126.0 (C=C-S, thiazole), 139.4 (C=C, hex.), 155.5 (C=C, pyrimidine), 158.3 (N=C-N, pyrimidine),163.4 (C=O, pyrazole) 168.6 (C=O, pyrimidine), 171.3 (N-C=O, thiazole). MS (*m*/*z*, 476.57) (M^+, 80%) Anal. Calcd. (%) for $C_{24}H_{20}N_4O_3S_2$ (476.57): C, 60.49; H, 4.23; N, 11.76; S, 13.46. Found (%):C, 60.47; H, 4.21; N, 11,75, S, 13.44.

Cyclopenta-1,3-dien-1-yl(2-((3,5-dioxo-3,5,6,7,8,9-hexahydro-2*H*-benzo[4,5]thieno[2,3-*d*]thiazolo [3,2-*a*]pyrimidin-2-ylidene)methyl)cyclopenta-1,3-dien-1-yl)iron (11b).

Gray crystals (ethanol). M. P. 206 ºC , yield: 70 %,IR $(KBr)_{Vmax}$ /cm^{-1}; 1637, 1531, (2CO); ^{1}H-NMR (DMSO-d_6, 500 MHz , δ ppm) :1.79 (m, 2H, CH_2); 2.64 (t, 2H, CH_2); 2.73 (t, 2H, CH_2); 2.9-6.5 (m, 9H, ferrocenyl); 7.28 (s, 1H, -CH=); ^{13}C-NMR (DMSO-d_6,100 MHz, δ ppm): 23.0, 23.4 and 24.5 (4C,CH_2, cyclohex.), 117.6 (C=C, pyrimidine), 125.4 (C= C, hex.), 126.0 (C=C-S, thiazole),139.4 (C=C, hex.) 155.5 (C= C, pyrimidine), 158.3 (N=C-N, pyrimidine), 168.6 (C=O, pyrimidine), 171.3 (N-C=O, thiazole). MS (*m/z*, 474.38) (M^+, 82%). Anal. Calcd. (%) for $C_{23}H_{18}FeN_2O_2S_2$ (474.38): C, 58.23; H, 3.82; Fe, 11.77; N, 5.91; S, 13.52. Found (%): C, 58.20 ; H, 3.80; N, 5,89, S, 13.50.

3.2. Biological Activity:

3.2.1. Materials and Methods

Roswell Park Memorial Institute (RPMI) 1640 medium was purchased from Sigma Chem. Co. (St. Louis, MO, USA). Fetal bovine serum (FBS) and fetal calf serum (FCS) were purchased from Gibco, UK. Dimethyl sulfoxide (DMSO) and methanol were of HPLC grade, and all other reagents and chemicals were of analytical reagent grade.

3.2.2. *In vitro* anticancer activity:

3.2.2.1. Cell culture

HepG-2 (Human liver carcinoma), HCT116 (human colorectal carcinoma), and MCF-7 (human breast adenocarcinoma) were purchased from the American Type Culture Collection (Rockville, MD, USA) and maintained in RPMI-1640 medium which was supplemented with 10% heat-inactivated FBS, 100U/ml penicillin and 100U/ml streptomycin. The cells were grown at 37°C in a humidified atmosphere of 5% CO_2. All experiments were conducted thrice in triplicate (n = 3). All the values were represented as means ± SD.

3.2.2.2. Lactate dehydrogenase (LDH) assay

To determine the effect of each synthesized compound on membrane permeability in HepG2, MCF-7 and HCT-116 cancer cell lines, a lactate dehydrogenase (LDH) release assay was used [55-59]. The cells were seeded in 24-well culture plates at a density of 2×10^5 cells/well in 500 µL volume and allowed to grow for 18h before treatment. After treatment with a series of different concentrations of each compound or Doxorubicin® (positive control), the plates were incubated for 48h. Then, the supernatant (40 µL) was transferred to a new 96 well to determine LDH release and 6% triton X-100 (40 µL) was added to the original plate for determination of total LDH. An aliquot of 0.1 M potassium phosphate buffer (100 µL, pH 7.5) containing 4.6 mM pyruvic acid was mixed to the supernatant using repeated pipetting. Then, 0.1 M potassium phosphate buffer (100 µL, pH 7.5) containing 0.4 mg/mL reduced β-NADH was added to the wells. The kinetic changes were read for 1 min using ELISA microplate reader in absorbance at wavelength 340 nm. This procedure was repeated with 40 µL of the total cell lysate to determine total LDH. The percentage of LDH release was determined by dividing the LDH released into the media by the total LDH following cell lysis in the same well.

3.2.3. Statistical Analysis

All experiments were conducted in triplicate (n = 3). All the values were represented as mean ± SD. Significant differences between the means of parameters as well as IC_{50}s were determined by probit analysis using SPSS software program (SPSS Inc., Chicago, IL).

3.3. Molecular Docking

The crystal structure of human Crystal structure of human type II inosine monophosphate dehydrogenase[13] was downloaded from protein data bank with (pdb 1B3o). It was resolved by X-ray crystallography method with resolution of 2.9 Å and R-value free of 0.277. All coordinates were derived from protein data bank and all interactions were visualized between the conserved residues and the complexed ligand. The MOE docking protocol was applied, in which the triangle method was used as a placement method with timeout of 300 s, and number of return poses as 1000. London dG and affinity dG were used as rescoring methods. RMSD was computed for each docked pose in Å. Force field was selected as a refinement method by applying MMFF94x

4. Conclusion

In this work, some derivatives with thiazolo[2,3-*a*] pyrimidinedione were synthesized by two different synthetic methods and characterized by spectroscopic analysis. The cytotoxic activity of the compounds were evaluated against three cancer cell lines; HepG2, MCF-7 and HCT-116.

The most active compound (**8a**) with the best anticancer activity against HCT-116, HepG-2 and MCF-7 cell lines showed IC_{50} values of 4.7 ± 0.3, 5.6 ± 0.4 and 6.2 ± 0.5 µM respectively, when compared to the values of the standard Doxorubicine with IC_{50} values 5.2 ± 0.3, 5.7 ± 0.4 and 5.2 ± 0.4 µM. Molecular docking of compound **(8a)** showed ΔG -8.68 (Kcal/mol) with interactions with the important residues in the active site; Arg322, Ser329 and Gly365. The discovery of this compound with 2-(naphthalen-2-ylmethylene)-7,8-dihydrocyclopenta-[4,5]thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5-

(2*H*,6*H*)-dione scaffold may need further investigation and future optimization.

5. Acknowledgment

The authors would like to thank National Research Centre for the provided facilities. Also great thanks to Prof. Dr. Mohammed A. Khedr, Department of Pharmaceutical Chemistry (Kuwait University & Helwan University) for performing the modeling studies. This work is dedicated to the spirit of Professor Dr. Ahmed S. Aly, Photochemistry Department, National Research Centre, Dokki, Cairo 12622, Egypt.

6. Conflict of interest

The authors declare that there is no conflict of interest.

7. References:

[1] Bozorov, K.; Zhao, J.-Y.; Elmuradov, B.; Pataer, A.; Aisa, H.A.. Recent developments regarding the use of thieno[2,3-*d*]pyrimidin-4-one derivatives in medicinal chemistry, with a focus on their synthesis and anticancer properties. *Eur. J. Med. Chem.* 2015;102: 552-573. https://doi.org/10.1016/j.ejmech.2015.08.018

[2] Mohana R. S, Sompalle R. Synthetic chemistry of pyrimidines and fused pyrimidines: a review. *Synth. Commun*. 2016; 46: 8, 645-672. https://doi.org/1080/00397911.2016.1165254.

[3] Bánhegyi, P.; Kéri, G.; Örfifi, L.; Szekélyhidi, Z.; Wáczek, F. Vichem Chemie Kutato Kft, Tricyclic benzo [4,5] thieno-[2,3-*d*] pyrimidine-4-yl-amin Derivatives, Their Salts, Process for Producing the Compounds and Their Pharmaceutical Use. U.S. Patent 8,802,849, 2014.

[4] Mahapatra A. , prasad T. and Sharma T. Pyrimidine: a review on anticancer activity with key emphasis on SAR. *Future journal of pharmaceutical Sciences*.:2021; Article number123, 7. https://doi.org/10.1186/s43094-021-00274-8

[5] Prachayasittikul S., Pingaew R., Worachartcheewan A., Sinthupoom N., Prchayasittikul V.,Ruchirawat S.,. Roles of pyridine and pyrimidine derivatives as privileged scaffolds in anticancer agents. *Mini-Rev. Med Chem*. 2017;17: 10, 552–573. http://doi.org/0.2174/1389557516666160923125801.

[6] Abuelhassan, S.; Bakhite, E.A.; Abdel-Rahman, A.E.; El-Mahdy, A.F.M. Synthesis, characterization, and biological activities of some novel thienylpyrido[3\',2\':4,5]thieno[3,2-*d*]pyrimidines and related heterocycles. *J. Heterocycl. Chem*. 2021; 58: 1784–1801 .https://doi.org/10.1002/jhet.4310

[7] Li, H.; Chen, C.; Xu, S.; Cao, X., . Synthesis and Bioevaluation of Thieno[2,3-*d*]pyrimidinone Derivatives as Potential Tumor Cell Growth Inhibitors. *J. Chem*. 2013; 2013:5, 1–6. , Article ID 692074. http://dx.doi.org/10.1155/2013/692074 https://doi.org/10.1080/10426500701407714

[8] Gorja, D.R.; Kumar, K.S.; Mukkanti, K.; Pal, M., . C–C (alkynylation) vs C–O (ether) bond formation under Pd/C–Cu catalysis: Synthesis and pharmacological evaluation of 4-alkynylthieno[2,3-*d*]pyrimidines. Beilstein J. Org. Chem. 2011; 21: 7, 338–345. https://doi.org/10.3762/bjoc.7.44

[9] Mavrova, A.T.; Dimov, S.; Yancheva, D.; Rangelov, M.; Wesselinova, D.; Tsenov, J.A.. Synthesis, anticancer activity and photostability of novel 3-ethyl-2-mercapto-thieno[2,3-*d*]pyrimidin-4(3*H*)-ones. *Eur. J. Med. Chem*. 2016; 123:3, 69–79. http://dx.doi.org/10.1016/j.ejmech.2016.07.022

[10] Sharaky, M.; Kamel, M.; Aziz, M.A.; Omran, M.; Rageh, M.M.; Abouzid, K.A.M.; Shouman, S.A. Design, synthesis and biological evaluation of a new thieno[2,3-*d*]pyrimidine-based urea derivative with potential antitumor activity against tamoxifen sensitive and resistant breast cancer cell lines. *J. Enzym. Inhib. Med. Chem*. 2020; 35: 1, 1641–1656. https://doi.org/10.1080/14756366.2020.1804383.

[11] Elmongy, E.; Khedr, M.; Abotaleb, N.; Abbas, S. Design and synthesis of new thienopyrimidine derivatives along with their antioxidant activity. *Egypt. J. Chem*. 2021; 64: 6857–6867. https://doi.org/10.21608/ejchem.2021.23721.2412

[12] Kundapura U. *In vitro* and *in silico* biological studies of novel thiazolo[3,2-*a*] pyrimidine-6-carboxylate derivatives. Med Chem Res. 2014; 23: 168-180. https://doi.org/10.1007/s00044-013-0606-4

[13] Colby T. D. , Vanderveen K. , Strickler M. D. , Markham G. D. and Goldstein B. M. Crystal structure of human type II inosine monophosphate dehydrogenase: Implications for ligand binding and drug design. *Proc. Natl. Acad. Sci. USA*, 1999; 30: 96, 7, 3531-3536. https://doi.org/10.1073/pnas.96.7.3531

[14] Franklin T. J., Edwards G, Hedge P. Adv Exp. Med. Biol. 1994; 370:155–160

[15] Glesne, D.; Collart, F.; Varkony, T.; Drabkin, H.; Huberman, E. Chromosomal localization and structure of the human type II IMP dehydrogenase gene (IMPDH2). *Genomics* . 1993;16: 1, 274–277. https://doi.org/10.1006/geno.1993.1177

[16] Senda, M.; Natsumeda, Y. Tissue-differential expression of two distinct genes for human IMP

dehydrogenase (E.C.1.1.1.205). *Life Sci*. 1994; 54:24, 1917–1926.https://doi.org/10.1016/0024-3205(94)90150-3

[17]Collart, F.R.; Chubb, C.B.; Mirkin, B.L.; Huberman, E. Increased inosine-50-phosphate dehydrogenase gene expression in solid tumor tissues and tumor cell lines. *Cancer Res.* 1992; 52: 5826–5828. https://aacrjournals.org/cancerres/article/52/20/5826/498248/Increased-Inosine-5-phosphate-Dehydrogenase-Gene

[18] Wang, X.; Yang, K.; Xie, Q.; Wu, Q.; Mack, S.C.; Shi, Y.; Kim, L.J.Y.; Prager, B.C.; Flavahan, W.A.; Liu, X.; Singer M.; Hubert C. G; Miller T. E.; Zhou W.; Huang Z; Fang X; Regev A; Suvà M. L.; Hwang T. H.; Locasale J. W.; Bao S.; Rich J. N. Purine synthesis promotes maintenance of brain tumor initiating cells in glioma. *Nat. Neurosci*. 2017;20: 5, 661–673. https://doi.org/10.1038/nn.4537

[19] Valvezan, A.J.; Turner, M.; Belaid, A.; Lam, H.C.; Miller, S.K.; McNamara, M.C.; Baglini, C.; Housden, B.E.;Perrimon, N.; Kwiatkowski, D.J. mTORC1 Couples Nucleotide Synthesis to Nucleotide Demand Resulting in a Targetable Metabolic Vulnerability. *Cancer Cell.* 2017; 32 5, 624–638. https://doi.org/10.1016/j.ccell.2017.09.013

[20] Huang, F.; Ni, M.; Chalishazar, M.D.; Huffman, K.E.; Kim, J.; Cai, L.; Shi, X.; Cai, F.; Zacharias, L.G.;Ireland, A.S. Inosine Monophosphate Dehydrogenase Dependence in a Subset of Small Cell Lung Cancers. *Cell Metab.* 2018; 28:3 369–382. https://doi.org/10.1016/j.cmet.2018.06.005

[21] Naffouje R., Grover P. ,Yu H., Sendilnathan A., Wolfe K., Majd N., Smith E. P., Takeuchi K., Senda T., Kofuji S. and Sasaki A. T. Anti-Tumor Potential of IMP Dehydrogenase Inhibitors: A Century-Long Story. *Cancers.* 2019; *11: 1346.* https://www.mdpi.com/2072-6694/11/9/1346

[22] Duan, S., Huang, W., Liu, X. IMPDH2 promotes colorectal cancer progression through activation of the PI3K/AKT/mTOR and PI3K/AKT/FOXO1 signaling pathways. *J. Exp. Clin. Cancer Res.* 2018; 37: 304 . https://doi.org/10.1186/s13046-018-0980-3

[23]Sidi Y. Growth inhibition and induction of phenotypic alterations in MCF-7 breast cancer cells by an IMP dehydrogenase inhibitor. *Br. J. Cancer*. 1988; 58: 61–63. https://doi: 10.1038/bjc.1988.162

[24] He Y., Zheng Z., Xu Y., Weng H., Gao Y., Qin K., Rong J., Chen C., Yun M., Zhang J., Ye S. Over-expression of IMPDH2 is associated with tumor progression and poor prognosis in hepatocellular carcinoma. *Am. J. Cancer Res*. 2018; 8: 8, 1604-1614. PMID: 30210928; PMCID: PMC6129487. https://pubmed.ncbi.nlm.nih.gov/30210928/

[25]Carter, S., Franklin, T., Jones, D. Mycophenolic Acid: an Anti-cancer Compound with Unusual Properties. *Nature.* 1969; 223: 848–850. https://doi.org/10.1038/223848a0

[26] Sintchak, M. D., Fleming, M. A., Futer, O., Raybuck, S. A., Chambers, S. P., Caron, P. R., Murcko, M. A., & Wilson, K. P. Structure and mechanism of inosine monophosphate dehydrogenase in complex with the immunosuppressant mycophenolic acid. *Cell.* 1996; 85: 6, 921–930. https://doi.org/10.1016/s0092-8674(00)81275-1

[27] Aly, A.A, Ramadan, M., Morsy, N.M. and Elkanzi, N.A.A. Inclusion of carbonyl groups of benzo [*b*] thiophene-2,5-dione into amidrazones. Synthesis of 1,2,4-triazine-5,6-diones. *J. Het. Chem*. 2017; 54 :3, 2067-2070 . https://doi.org/10.1002/jhet.2805

[28] Aly, A.A., Bräse, S., Hassan, A.A., Mohamed, N.K., Abd El-Haleem, L.E., Nieger, M., Morsy, N.M. and Abdelhafez, El.M.N. New Paracyclophanylthiazoles with Anti-Leukemia Activity: Design, Synthesis, Molecular Docking, and Mechanistic Studies. *Molecules.* 2020; 25: 13, 3089-3118 . https://doi.org/10.3390/molecules25133089.

[29] Aly, A.A, Bräse, S., Hassan, A.A., Mohamed, N.K., Abd El-Haleem, L.E., Nieger, M., Morsy, N.M, Alshammari ,M.B., Ibrahim, M.A.A. and Abdelhafez, El.M.N. Design, Synthesis, and Molecular Docking of Paracyclophanyl-Thiazole Hybrids as Novel CDK1 Inhibitors and Apoptosis Inducing Anti-Melanoma Agents. *Molecules.* 2020; 25: 23, 5569-5597 . https://doi.org/10.3390/molecules25235569

[30] Elkanzi, N. A. A. and Morsy, N.M . A review On Synthesis and Antimicrobial Activity of β-Lactams. Antibacterial Activities and Antifungal Activities. *J. Heter. Let*. 2014; 4: 1, 153-182 .

[31] Elkanzi, N.A.A., Morsy, N.M., Aly, A. A., Brown, A. B. and Ramadan M. New Pyrimidine-2-thiones from Reactions of Amidrazonethiols with Amino-1,1,2-ethenetricarbonitrile and Investigation of Their Antitumor Activity. *J. Heter. Chem*. 2015;53: 6, 1838-1842 . https://doi.org/10.1002/jhet.2495

[32] Elkanzi N. A. A., Aly, A. A., Shawky A. M., El-Sheref E. M., Morsy, N. M. and El-Reedye A. A. M. Amination of Malononitrile Dimer to Amidines: Synthesis of 6-aminopyrimidines. *J. Heterocyclic Chem*. 2016; 53: 6, 1941-1944. . https://doi.org/10.1002/jhet.2510

[33] Elkanzi N. A. A., Morsy, N. M. , Aly A. A., El Malah T. and Shawky,A. M. Green chemistry: microwave-assisted facile synthesis of 6-imino-1,3,4-thiadiazenes from reaction of

thiocarbohydrazones with malononitrile dimer. *J. Sulf. Chem.* 2016; 36: 1, 114-121. https://doi.org/10.1002/jhet.2510

[34] El Sayed M.T., El-Sharief M.A.M.S., Zari, E.S., Morsy, N.M., Elsheakh, A.R., Nayel, M., Voronkov, A., Berishvili, V., Sabry, N.M., Hassan, G.S. and Abdel-Aziz, H.A. Design, synthesis, anti-inflammatory antitumor activities, molecular modeling and molecular dynamics simulations of potential Naprosyn analogs as COX-1 and/or COX-2 inhibitors. *J. Bio. Chem.* 2018; 76: 188-201 . https://doi.org/10.1016/j.bioorg.2017.11.002

[35] El Sayed M.T., El-Sharief M.A.M.S., Zarie E.S., Morsy N.M., Elsheakh A.R., Voronkov, A. and Hassan G.S. Design, Synthesis, anti-inflammatory activities and Molecular docking of Potential novel antipyrine and pyrazolone Analogs as (COX) Inhibitors. *Bio. & Med. Chem. Let.* 2018; 28: 5, 952–957 . https://doi.org/10.1016/j.bmcl.2018.01.043

[36] Elsherif M.A., Hassan A.S., Moustafa, G.O., Awad H.M. and Morsy N.M. Antimicrobial evaluation and molecular properties prediction of pyrazolines incorporating benzofuran and pyrazole moieties. *J. Appl. Pharm. Sci*. 2020; 10: 2, 37-43 . https://doi.org/10.7324/JAPS.2020.102006

[37] Ghonim, A.A and Morsy, N.M. A Facile Synthesis of New Heterocyclic Compounds from Thiourea and Urea, Which Links with Some Hexoses. *Org. Chem.: An Ind. j*. 2017;13: 3, 114.

[38] Ghoneim A.A. and Morsy N.M. Synthesis and structure elucidation of some new azo dye from hydroxyquinolin-2(1*H*)-one derivatives and their antimicrobial evaluation. *J. Iran. Chem. Soc*. 2018;15: 11, 2567–2572, https://doi.org/10.1007/s13738-018-1445-5

[39] Ghoneim A.A. and Morsy, N.M. Design and Synthesis of Novel 4-Amino-2,3-dihydro-2-imino-3-(1-iminododecyl)thiazole-5-Carbonitrile Derivatives as Antimicrobial Agents. *Der Pharm. Chem*. 2020; 9: 3,1-6 . https://www.derpharmachemica.com/archive/dpc-volume-12-issue-3-year-2020.html

[40] Hassan A.S. Morsy, N.M., Awad H.M. and Ragab A. Synthesis, molecular docking, and in silico ADME prediction of some fused pyrazolo[1,5-*a*]pyrimidine and pyrazole derivatives as potential antimicrobial agents. *J. Iran. Chem. Soc*. 2022; 19: 2, 521–545 https://doi.org/10.1007/s13738-021-02319-4

[41] Hassan A. S., Morsy N. M., Aboulthana W. M. , Ragab A. In vitro enzymatic evaluation of some pyrazolo[1,5-*a*]pyrimidine derivatives: Design, synthesis, antioxidant, anti-diabetic, anti-Alzheimer, and anti-arthritic activities with molecular modeling simulation. *Drug Develop.* Res. (2022) published online. https://doi.org/10.1002/ddr.22008.

[42] Morsy N.M., Hassan A.S., Hafez T.S., Mahran M.R.H., Sadawe I.A. and Gbaj A.M., . Synthesis, antitumor activity, enzyme assay, DNA binding and molecular docking of Bis-Schiff bases of pyrazoles. *J. Iran. Chem. Soc.* 2021;18 : 1, 47-59 .
http://dx.doi.org/10.1007/s13738-020-02004-y

[43] Morsy N. M., Abu-Zied Kh. M., Aly A. S., Elgamal A. M.Design, Synthesis, Molecular Docking of some New Polyhydrobenzothieno-thiazolo-pyrimidinedione Glycoside Derivatives with Double Anti-microbial-Anti-inflammatory Action. *Egypt. J. Chem*. 2022;65: 12. 577-598. https://doi.org/10.21608/ejchem.2022.155556.6714.

[44] Mukhtar S.S., Hassan A.S., Morsy N.M., Hafez T.S., Hassaneen H.M. and Saleh F.M. Overview on Synthesis, Reactions, Applications, and Biological Activities of Schiff Bases. *Egypt. J. Chem*. 2021; 64: 11, 6541-6554 . DOI: 10.21608/ejchem.2021.79736.3920

[45] Mukhtar S.S., Hassan A.S., Morsy N.M., Hafez T.S., Saleh F.M. and Hassaneen H.M. Design, synthesis, molecular predication and biological evaluation of pyrazole-azomethine conjugates as antimicrobial agents. *Synth. Commun*. 2021; 51: 10, 1564-1580 . https://doi.org/10.1080/00397911.2021.1894338

[46] Mukhtar S. S., Morsy N. M., Hassan A. S., Hafez T. S., Hassaneen H. M., Saleh F. M. A Review of Chalcones: Synthesis, Reactions, and Biological Importance. *Egyp. J. Chem*. 2022; 65: 8, .379-395. https://doi.org/10.21608/ejchem.2022.112735.5125

[47] Yosef H.A.A., Elkanzi N.A.A. and Morsy N.M. Design and Synthesis of some novel fused triheterocyclic thiazolopyrimidine derivatives incorporating a benzoquinoline moiety. *Heter. Let*.. 2015; 5: 4, 563-578.

[48] Yosef H.A.A, Morsy N.M., Mahran, M.R.H. and Aboul-Enein H.Y. Preparation and Reactions of Optically Active Cyanohydrins Using the (*R*)-Hydroxynitrile Lyase from *prunus amygdalus*. *J.Iran.Chem.Soc.* 2007; 4: 1,46-58. . https://link.springer.com/content/pdf/10.1007/BF03245802.pdf

[49] Yosef H.A.A., Morsy, N.M., Mahran, M.R.H. and Shaker, N.O. Chemistry of optically active cyanohydrins - Part 3:[1] preparation and reactions of (R)-2-hydroxy-2-(naphthalen-1-yl) ethane-nitrile using (R)-hydroxynitrile lyase from Prunus amygdalus. Antitumor and antimicrobialevaluation of the new products. Egy. J. Chem. 2014; 57: 5,6 387-410 . https://ejchem.journals.ekb.eg/article_1057_9bfd3196fec8f6af27faf9f5cc4f93e8.pdf

[50] Miao, P., Sheng, S., Sun, X., Liu, J., & Huang, G. Lactate dehydrogenase a in cancer: A promising target for diagnosis and therapy. IUBMB Life 2013; 65: 11, 904–910. https://doi.org/10.1002/iub.1216

[51] Gewald A. K., Schinke E., Bottcher H. Heterocyclen aus CH-aciden Nitrilen, VIII. 2-Amino-thiophene aus methylenaktiven Nitrilen, Carbonylverbindungen und Schwefel .Chem. Ber. 1966; 99: 94-100. https://doi.org/10.1002/cber.19660990116.

[52] Abu-Zied Kh. M. Synthesis and Reactions of Novel Thienopyrimidine and Thiazolothienopyrimidine Derivatives. phosphorus, *Sulfur and Silicon*. 2007; 182 : 9, 2179 -2191.
https://doi.org/10.1080/10426500701407714.

[53] Aly A. S., Abu-Zied Kh. M. and Gaafar A. M., Facile Synthesis of Some Thieno[2,3-*d*] pyrimidine Derivatives. *Phosphorus Sulfur and Silicon* 2008; 183: 3063-3078.
https://doi.org/10.1080/10426500802059984

[54] Aly A. S., Abu-Zied Kh. M. and Gaafar A. M. Synthesis and Reactions of some Novel azolothienopyrimidines and thienopyrimido as triazines derivatives. *Phosphorus Sulfur and Silicon* 2007;182: 447-474. https://doi.org/10.1080/10426500600977304

[55] Soliman H. A , Mubarak A.Y., El-Mekabaty A., Awad H. M , Elmorsy S. S. Eco-friendly synthesis of amidochloroalkylnaphthols and its related oxazepinones with biological evaluation. *Monatshefte für Chemie-Chemical Monthly*. 2016;147: 809–816. https://doi.org/10.1007/s00706-015-1536-2

[56] Younis A., Fathy U., El-katebA. A., Awad H. M.; . Ultrasonic assisted synthesis of novel anticancer chalcones using water as green solvent. *Der Pharm. Chemica* 2016; 8: 17, 129-136.
https://www.derpharmachemica.com/pharma-chemica/ultrasonic-assisted-synthesis-of-novel-anticancer-chalcones-using-water-as-green-solvent.pdf

[57] Kassem A. F. , Nassar I. F. ,Abdel-Aal M. T., Awad H. M. , El-Sayed W. A. Synthesis and Anticancer Activity of New ((Furan-2-yl)-1,3,4-thiadiazolyl)-1,3,4-oxadiazole Acyclic Sugar Derivatives. *Chemical & pharmaceutical bulletin* 2019; 67: 8, 888-895. . https://doi.org/10.1248/cpb.c19-00280

[68] Flefel E. M. , El-Sofany W. I., Awad H. M , El-Shahat M. First Synthesis for Bis-Spirothiazolidine Derivatives as a Novel Heterocyclic Framework and Their Biological Activity. *Mini Reviews in Medicinal Chemistry*. 2020; 20:2, 152-160. https://doi.org/10.2174/1389557519666190920114852

[59] Abdel Rahman A. A. H., Nassar I. F. , Shaban A. K. F. , EL-Kady D. S. , Awad H. M. El Sayed W. A. Synthesis, docking studies into cdk-2 and anticancer activity of new derivatives based pyrimidine scaffold and their derived glycosides. *Mini Reviews in Medicinal Chemistry*, , 2019;19:13, 1093-1110. DOI: 10.2174/1389557519666190312165717