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Inhibitory effects of new 1,3-diethyl-8-mercapto xanthine derivatives in human MCF7 and K562 cancer cell lines

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Abstract : A series of new 2-methyl-2-[(1,3-Diethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)thio]-*N*- substituted arylacetamides were synthesized by reacting the 8-merapto-xanthine derivative with appropriate prepared anilide. The structure of the newly prepared compounds were confirmed by ^{1}H NMR, high resolution mass spectrometer and microanalytical analysis. The antitumor activity of these compounds was evaluated on breast cancer (MCF7) and leukemic (K562) cell lines by cell viability assay utilizing the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The result showed that some compounds illustrate different anti-tumor activity while others showed weak anti-tumor activity. For those with anti-tumor activity, the IC50 values range from 9.56-57 microM. Interestingly, compound para-nitro xanthine derivative showed the most encouraging activity against K562 leukemia cells and to a lesser extent against MCf7 breast cancer cells. These findings indicate that the position and the type of the substitution on the phenyl moiety affect the activity of the xanthine derivatives and illustrate that the electron withdrawing groups increases the activity especially at the para position. The computer aid design approach revealed that the anti-tumor activity of these compounds may be attributable to their abilities to effectively bind and block oncogenic tyrosine kinases, particularly bcr/abl.

Keywords : 2-methyl-2-[(1,3-Diethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)thio]-*N*- substituted arylacetamides; 8-merapto-xanthine derivative; Anilide; antitumor activity.

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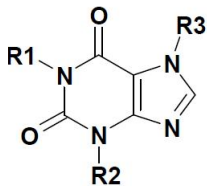
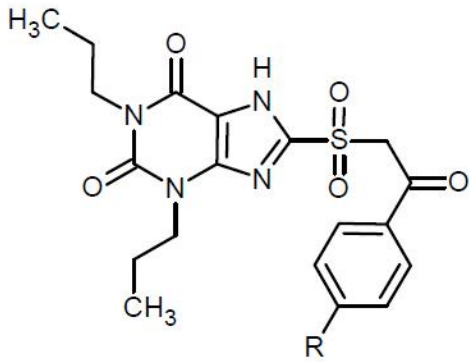
INTRODUCTION

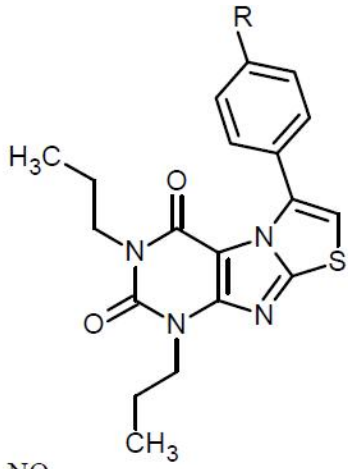
Purine nucleus is reported to be an important class of biologically active structures. Xanthine is a purine base found in most human body tissues and fluids[1]. They have been widely investigated for their biological activities, such as anti-asthmatic e.g. theophylline I, anti-tumor, anti-oxidant, anti-microbial, central nervous system (CNS) stimulant e.g. caffeine II, anti-inflammatory, diuretic and smooth muscle relaxation.



Structure number	R_1	R_2	R_3	Compound Name
I	$-CH_3$	$-CH_3$	-H	Theophylline
II	$-CH_3$	$-CH_3$	$-CH_3$	Caffeine



III



R = H , Cl , Br , CH_3 , NO_2

IV

Nitrogen-containing compounds from terrestrial and marine organisms have been intensively investigated over the last few years for their antitumor activity. These compounds include 1,3,7-trimethylxanthine or caffeine, a natural methylxanthine present in coffee and tea, and its various analogues, referred to as 1,3,7-trialkylxanthines or xanthenes[2-6]. Previous studies revealed that caffeine inhibits the development of tumors induced by various carcinogens in numerous organs including skin, lung, stomach and liver[7-9]. Oral administration of caffeine was also shown to substantially inhibit UVB-induced carcinogenesis in SKH-1 mice[10]. A recent study revealed that caffeine inhibited 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumor[8]. Caffeine was shown to suppress the proliferation of various cancer and transformed cell lines including human neuroblastoma cells, human pancreatic adenocarcinoma cells, human A549 lung adenocarcinoma cells and mouse epidermal JB6 promotion-sensitive (Pþ) cells[11].

1,3,8-trisubstituted xanthines III and 1,3,6-trisubstituted thiazolo[2,3-f]purine-2,4-diones IV were assessed in vitro for their cytotoxic effect against two human malignant cell lines: T-cell leukemia derived SKW-3 and breast cancer derived MDA-MB-231. Some compounds showed interesting anti-tumor activity[12].

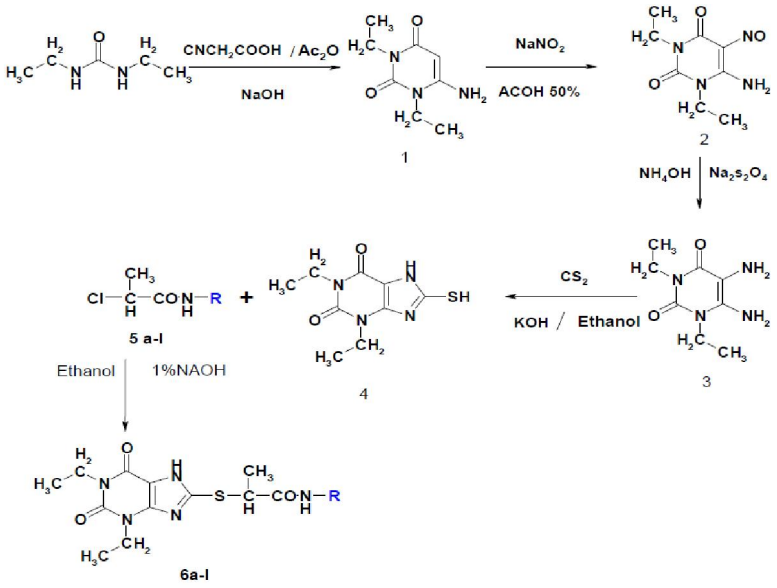
These promising effects of xanthine derivatives on cancer cell lines led to numerous chemical works focusing on the synthesis of new xanthine derivatives with more potent anticancer activity. Accordingly, we envisaged to prepare a set of xanthine de-

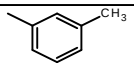
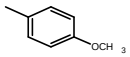
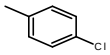
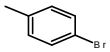
rivatives incorporating *N*- substituted arylacetamides moiety for evaluation of their antitumor activity.

RESULT AND DISCUSSION

Chemistry

1,3 Diethyl-5,6-diaminouracil 3 were synthesized according to previously described methods[13-17]. Thus 1,3-Diethyl urea was condensed with cyanoacetic acid to give the 6 aminouracil 1. Standard nitrosation of compound 1 with sodium nitrite in acetic acid leading to compound 2, was followed by reduction with sodium dithionite to give



s	R
5a, 6a	
5b, 6b	
5c, 6c	
5d, 6d	
5e, 6e	
5f, 6f	
5g, 6g	
5h, 6h	
5i, 6i	
5j, 6j	
5k, 6k	
5l, 6l	

Scheme 1 : Synthesis of xanthine derivatives

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diaminouracil 3.

Finaly, the desired 8-mercapto xanthine derivative 4 was obtained by reacting the diaminouracil with carbon disulfide in ethanol solution in the presence of potassium hydroxide[18]. The N-substituted aryl-2-methyl-2-cloroacetamides 5a-l, were prepared by reacting the appropriate amine, pyridine in chloroform with chloroacetyl chloride[19,20]. The 8-mercapto-cloroacetamides derivatives 6a-l, were obtained by reacting the aqueous solution of compound 4, in sodium hydroxide with the appropriate *N*- substituted arylacetamides 5a-l solutions in ethanol[21], Scheme 1.

The newly synthesized compounds 6a-l was characterized by elemental analyses, MS and NMR spectral data.

Anti-tumor activity

The antitumor activity of the synthesized compounds were characterized by conducting cell viability assay using tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Cultures of the breast cancer cell lines MCF-7 and the Leukemic cell line K562 leukemia were treated first at one concentration of 50 μM and the results are shown in TABLE 1. In the MCF-7 screening test, five compounds showed a potential anti–MCF7activity. Those compounds were able to reduce the viability after 72 hours to less than 50 %. In the case of K562 cells, only four compounds illustrated a potential anti–K562 activity. Interestingly, at 10 μM concentration only compound 6k was able to reduce the K262 cell viability to less than 50% and none of the compound was able to achieve that reduction in the MCF7 cell case. Further we determined the IC_{50} values for the potential compounds against the MCF-7 and K562 (TABLE 2). Unsurprisingly, compound 6k showed the highest potency against K562 and MCF7cells, it scored an IC_{50} values of 9.56 and 17.59 μM against K562 and MCF7, respectively. The IC50 of the other compounds ranged between 33- 59; the values that does not really reflect an interesting anti-cancer activity.

Structure-activity relationship analysis and docking-based explanation

In this study, out of the 12 compounds that were tested for their anticancer activity against the two cell lines only compound 6k showed a very encouraging results. From structure activity relationship point of view, the type of substitution on phenyl side chain seems to play a role in the anticancer activity of the targeted compounds. The relatively low IC50 values obtained for compound 6k reveals the importance of the substitution on the phenyl side chain. The enhanced anti-cancer activity may results from the high electron withdrawing capability of the NO2 group on the phenyl ring, which in turn have improved the reactivity of compound 6k toward its cellular target.

TABLE 1 : Percentage cell survival of MCF-7 and K562 following 72 h exposure to 10 and 50 μM of all compounds

Compound	K562 % survival ± SD		MCF-7 % survival ± SD	
	At 10 μ M	At 50 μ M	At 10 μ M	At 50 μ M
6a	60 ±12.10	49 ±16.92	81 ±4.49	88 ±3.46
6b	76 ± 3.14	63 ±11.10	83 ±5.13	93 ±13.08
6c	62 ±5.41	35 ±5.15	75 ±43.63	42 ±3.20
6d	60 ±12.44	49 ±2.60	81 ±5.51	64 ±4.74
6e	59 ±4.68	46 ±7.42	82 ±7.31	48 ±7.44
6f	70 ±3.67	51 ±3.74	79 ±3.59	81 ±9.75
6g	74 ±13.51	59 ±3.01	75 ±9.23	85 ±3.72
6h	84 ±23.88	74 ±4.95	80 ±5.38	85 ±5.26
6i	70 ±17.66	68 ±1.80	80 ±2.69	87 ±10.13
6j	68 ±2.54	48 ±9.16	84 ±13.85	75 ±15.78
6k	50 ±7.35	33 ±0.20	78 ±0.89	77 ±11.29
6l	67 ±10.30	53 ±5.35	75 ±7.31	71 ±3.59

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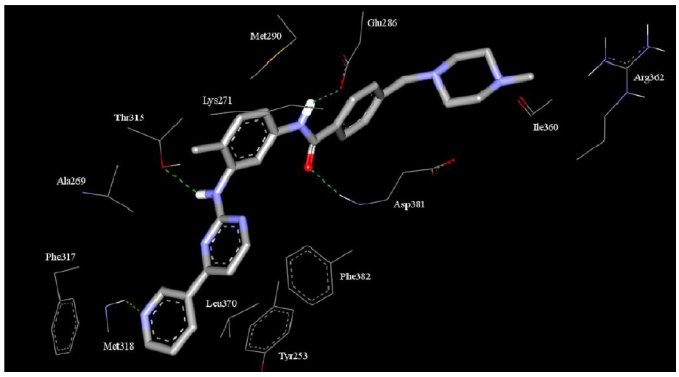
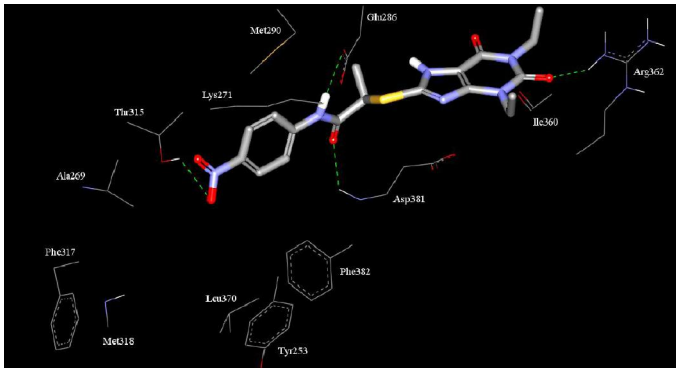
TABLE 2 : Effects of compounds that have shown potential activity on the screening assay MCF-7 and K562. Doxorubicin is used as a positive control

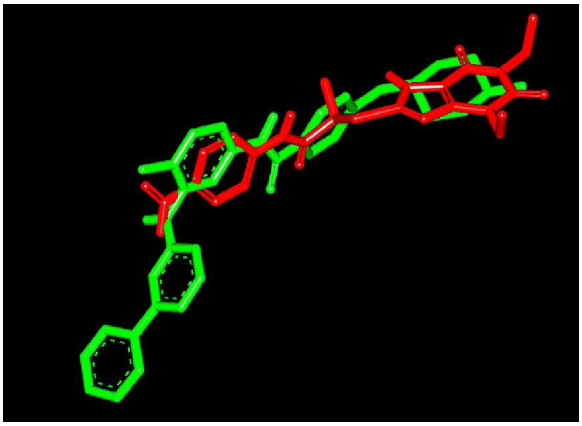
Compound	IC_{50} MCF-7 (µM) ± SD	IC_{50} K562 (µM) ± SD
Doxorubicin	0.31 ±0.01	1.41± 0.31
6c	29.34 ±2.4	33.72 ± 4.6810468
6d	> 100	49.01 ±1.0465180
6e	49.22 ±2.5	41.78 ±7.2831998
6j	56.54 ±1.6	47.5 ± 3.8678740
6k	17.59 ±2.715290	9.57 ±0.6222539
6f	60.07 ±9.9	52.33 ±2.0293964



(A)



(B)



(C)

Figure 1 : (A) X-ray crystallographic structure of imitanib co-crystallized within c-abl kinase domain (PDB code: 1IEP, resolution 2.1 Å), (B) The most potent xanthine analogue docked within the same binding pocket, (C) Superposition of the co-crystalized structure of imitanib over the docked structure of the most potent xanthine analogue.

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The inhibitory effects of our new compounds against K562 and MCF-7 cancer cell lines, which over-express bcr/abl and EGFR tyrosine kinases, respectively[1, 2], combined with the apparent pharmacophoric commonalities between these compounds and the anticancer agent imitanib, prompted us to anticipate that their observed anti-cancer properties are attributable to their abilities to effectively bind and block oncogenic tyrosine kinases, particularly bcr/abl. Figure 1 compares how imitanib binds within the ATP binding pocket of bcr/abl (PDB code: 1IEP, resolution 2.1 Å) with the way the most active analogue (in TABLE 1) docks into the binding pocket of the same protein (the docking experiment was performed using LigandFit docking engine and PMF scoring function). Clearly from the figure, hydrogen-bonding interactions connecting the amidic linker of imitanib with the carboxylic acid side chain of Glu286 and the peptidic NH of Asp381 correlate well with hydrogen-bonding interactions connecting the amidic linker of the most potent analogue with the same amino-acid residues. Similar analogy can be noticed between hydrogen-bonding interactions connecting the hydroxyl of Thr315 with the aromatic NH of imitanib (Figure 1a) and the nitro oxygen of the most potent analogue (Figure 1b). Furthermore, hydrophobic stacking of the methylbenzene linker of imitanib within a narrow corridor comprised of the CH_3S of Met290 and the $(CH_2)_4$ of Lys271 (Figure 1a) compares to fitting the nitro aromatic ring of the most potent analogue within the same corridor (Figure 1b). Finally, the apparent electrostatic attraction connecting the piperazine ring of imitianib with the carboxylate side chain of Asp381 (Figure 1a) compares to the hydrogen-bonding interaction connecting the carbonyl of the xanthine terminal of the most potent analogue with the guanidino NH_2 group of Arg362 in the binding pocket.

However, on the other hand, positioning the pyridinyl-pyrimidine fragment of imatinib within the aromatic hydrophobic pocket of the side chains of Phe382, Tyr253 and Phe317 (Figure 1a) is not represented in the binding interactions of the most potent analogue with the binding pocket, which probably explains the inferior bioactivity of these compounds compared to imitanib.

CONCLUSION

In summary, we have synthesized a series of new 2-methyl-2-[(1,3-Diethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)thio]-*N*- substituted arylacetamides through the reaction of 8-meraptoxanthine derivative with appropriate prepared Anilide. The prepared compounds were tested in vitro for their antitumor activity against breast cancer (MCF-7) and Leukemic (K562) cell lines. The results revealed that one of the synthesized compound 6k exerted significant antiproliferative activity with the aforementioned cancer cell lines, with IC_{50} value of 17.59 μM, against MCF-7 cell line. The same compounds have IC_{50} value of 9.57 μM, against K562 cell line. Also Compound 6c displayed good activity against the same cancer cell lines, with IC_{50} value of 29.34 μM, against MCF-7 cell line. The same compound have IC_{50} value of 33.72 μM, against K562 cell line. In addition, moderate to weak antitumor activity was displayed by all of the prepared compounds against those cell lines. These findings would encourage us to do further studies and testing that prove the usefulness of the prepared compounds as candidate anticancer agents.

EXPERIMENTAL

Materials and equipments

Reagents used for synthesis were purchased from Sigma-Aldrich (Gillingham– Dorset, UK), MERCK (Schuchardt, Germany) and Acros organics (New Jersey, USA). All solvents were obtained from commercial suppliers and used without further purification.

Melting points (mp) were determined on an electrothermal Stuart Scientific melting point apparatus (uk), and were uncorrected.

Thin-layer chromatography (TLC) was carried out using TLC aluminium sheets kieselgel 60 F_{254} (MERCK) and dichloromethane/methanol (9.5:0.5) as a mobile phase and visualization was effected with ultraviolet lamp at short wavelength (λ= 254 nm).

All chemical yields are unoptimized and generally represent the result of a single experiment.

NMR spectra were recorded on a Bruker Avance III 500, 500 MHz spectrometer at Jordan University, collage of Chemistry, Amman, Jordan, see Figure 3.1 DMSO-d_6 was used as a solvent, unless otherwise specified, and the chemical shifts are given in δ (ppm), coupling constants (*J)* are in Hertz (Hz).

High resolution Mass spectrometer was recorded on Bruker Daltonics APEX IV, at Jordan University, collage of Chemistry, Amman, Jordan.

The microanalyses for C, H, N was performed on Euro EA elemental analyzer at the college of pharmacy, Jordan University, Amman, Jordan.

The anticancer evaluation of our target compounds were healed in The Molecular Biology Laboratory, college of medicine, University of Jordan.

General procedure for preparation of 1,3-Diethyl-8-thioxo-3,7,8,9-tetrahydro-1H-purine-2,6-dione (4)

To a stirred solution of 5,6-diamino-1,3-dimethyluracil 3 (3.28 g, 16.5mmol) in ethanol (25 mL), carbon disulfide (1.5 mL, 26.4 mmol) was added. The reaction mixture was refluxed for 4 h, and then cooled. Cold water (25 mL) was added to the reaction mixture with stirring, the precipitate formed was filtered, washed successively with cold water, and then with methanol. The product was dried, and crystallized from water as colorless crystals.

1,3-Diethyl-8-thioxo-3,7,8,9-tetrahydro-1H-purine-2,6-dione (4)

Yield: 61%, mp: 280-283 ^{o}C. ^{1}H NMR (500 MHZ): 3.86 (q, 2H, N1-CH_2CH_3), 1.12 (t, 3H, N1-CH_2CH_3), 3.92 (q, 2H, N3-CH_2CH_3), 1.17 (t, 3H, N3-CH_2CH_3), 11.97 (br s, 1H, N9-H), 13.4 (br s, 1H, N7-H). Anal. Calcd for $C_9H_{12}N_4O_2S$ (240.29): C, 44.99, H, 5.03, N, 23.32, found C, 44.36, H, 4.96, N, 23.10.

2-methyl-2-[(1,3-Diethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-substituted arylacetamides (6 a-l)

To a stirred solution of compound 4 (0.3 gram, 1.28mmol) in aqueous sodium hydroxide 1% w/v (5ml), the prepared Anilide (1.28mmol) dissolved in ethanol (3ml) was added portion wise. The reaction mixture was stirred at the ambient temperature for four hours and then cooled in a refrigerator for 3h. The product was filtered washed with water, then diethyl ether, and dried. All the compounds 6a-l was crystallized from ethanol.

1.2-methyl-2-[(1,3-Diethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-(3-methylphenyl)acetamides 6a

Yield: 60%; mp: 218-220 ^{o}C. ^{1}H NMR (500 MHZ): 3.91(q, 2H, N1-CH_2), 1.11(t,3H, N1-CH_2CH_3), 4.1(q, 2H, N3-CH_2), 1.18 (t, 3H,N1-CH_2CH_3), 4.55 (q, 1H, SCH-), 1.59(d,3H,SCHCH_3),2.23(s,3H, 3'-CH_3), 6.88-7.43(m,4H,Ar-H), 10.3(s, 1H, amide-H), 13.7(br s, 1H, N7-H). HRMS (ESI) MS *m/z:* calcd for $C_{19}H_{23}N_5O_3S$ [M - H] $^{+}$(401.49); found 400.14488. Anal. Calcd for $C_{19}H_{23}N_5O_3S$ (401.49g/mol): C, 56.84, H, 5.77, N, 17.44, found: C, 56.78, H, 5.66, N, 17.21.

2-methyl-2-[(1,3-Diethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-(4-methoxyphenyl)acetamides 6b

Yield: 85%; mp: 262-264 ^{o}C. ^{1}H NMR (500 MHZ): 3.91(q, 2H, N1-CH_2), 1.12(t,3H, N1-CH_2CH_3), 4.09(q, 2H, N3-CH_2), 1.18 (t, 3H,N3-CH_2CH_3), 3.72(s, 3H,-OCH_3), 4.53 (q, 1H, SCH-), 1.59(d,3H,SCHCH_3), 6.88(d, J=8.7 Hz, 2H, 32 ,52 Ar-H), 7.49 (d, J=8.7 Hz, 2H, 22 ,62 Ar-H), 10.2(s, 1H, amide-H), 13.6(br s, 1H, N7-H). HRMS (ESI) MS *m/z:* calcd for $C_{19}H_{23}N_5O_4S$[M - H] $^{+}$ (417.49); found: 416.13980. Anal. Calcd for ($C_{19}H_{23}N_5O_4S$g/mol): C, 54.66, H, 5.55, N, 16.77, found: C, 54.54, H, 5.39, N, 15.608.

N-(4-Chlorophenyl)-2-methyl-2-[(1,3-Diethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamides 6c

Yield: 74%; mp: 220^{o}C. ^{1}H NMR (500 MHZ):3.91(q, 2H, N1-CH_2), 1.11(t,3H, N1-CH_2CH_3), 3.97(q, 2H, N3-CH_2), 1.16 (t, 3H,N1-CH_2CH_3), 4.41 (q, 1H, SCH-),1.53(d,3H,SCHCH_3), 7.36(d,J=8.9 Hz, 2H, 32 ,52 Ar-H), 7.67(d,J=8.9 Hz, 2H, 22 ,62 Ar-H), 11.27(s, 1H, amide- H), 13.6(br s, 1H, N7-H). HRMS (ESI) MS *m/z:* calcd for $C_{18}H_{20}ClN_5O_3S$[M - H] $^{+}$ (421.91); found: 420.09011. Anal. Calcd for ($C_{18}H_{20}ClN_5O_3S$ g/mol):

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C, 51.24, H, 4.78, N, 16.6, found: C, 51.1, H, 4.7, N, 16.5.

N-(2-clorophenyl)-2-methyl-2-[(1,3-Diethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamides 6d

Yield: 88.8%; mp: 243°C. ^{1}H NMR (500 MHZ): 3.91(q, 2H, N1-CH_2), 1.11(t,3H, N1-CH_2CH_3), 4. 1(q, 2H, N3-CH_2), 1.18 (t, 3H,N1-CH_2CH_3), 4.72 (q, 1H, SCH_2), 1.6(d,3H,$SCHCH_3$),7.21-7.73 (m, 4H, Ar-H), 9.9(s, 1H, amide-H), 13.7(br s, 1H, N7-H). HRMS (ESI) MS *m/z:* calcd for $C_{18}H_{20}ClN_5O_3S$[M - H] $^+$ (421.91); found: 420.09011. Anal. Calcd for ($C_{18}H_{20}ClN_5O_3S$ g/mol): C, 51.24, H, 4.78, N, 16.6, found: C, 51.2, H, 4.65, N, 16.1.

N-(4-bromophenyl)-2-methyl-2-[(1,3-Diethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamides 6e

Yield: 85%; mp: 269-272°C. ^{1}H NMR (500 MHZ): 3.91(q, 2H, N1-CH_2), 1.11(t,3H, N1-CH_2CH_3), 3.99(q, 2H, N3-CH_2), 1.16 (t, 3H,N1-CH_2CH_3), 4.58 (q, 1H, SCH_2), 1.59(d,3H,$SCHCH_3$), 7.5(d, 2H, 32 ,52 Ar-H), 7.57(d, 2H, 22 ,62 Ar-H), 10.5(s, 1H, amide-H),13.7(br s, 1H, N7- H). HRMS (ESI) MS *m/z:* calcd for $C_{18}H_{20}BrN_5O_3S$[M - H] $^+$ (466.36); found: 465.03011. Anal. Calcd for ($C_{18}H_{20}BrN_5O_3S$ g/mol): C,46.36, H, 4.32, N, 15.02, found: C, 45.2, H, 4.2, N, 14.9.

2-methyl-2-[(1,3-Diethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-phenylacetamides 6f

Yield: 66%; mp: 243°C. ^{1}H NMR (500 MHZ): 3.91(q, 2H, N1-CH_2), 1.11(t,3H, N1-CH_2CH_3), 3.99(q, 2H, N3-CH_2), 1.16 (t, 3H,N1-CH_2CH_3), 4.56 (q, 1H, SCH_2), 1.6(d,3H,$SCHCH_3$), 7.21-7.6 (m,5H,Ar-H), 10.3(s, 1H, amide-H), 13.7(br s, 1H, N7-H). HRMS (ESI) MS *m/z:* calcd for $C_{18}H_{21}N_5O_3S$[M - H] $^+$ (387.46); found: 386.12918. Anal. Calcd for ($C_{18}H_{21}N_5O_3S$ g/mol): C,51.24, H, 4.78, N, 16.6, found: C, 51.01, H, 4.65, N, 16.42.

2-methyl-2-[(1,3-Diethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-(4-methylphenyl)acetamides 6g

Yield: 92%; mp: 235-240°C. ^{1}H NMR (500 MHZ): 3.9 (q, 2H, N1-CH_2),1.1 (t,3H, N1-CH_2CH_3), 4(q, 2H, N3-CH_2), 1.2 (t, 3H,N1-CH_2CH_3), 4.55 (q, 1H, SCH_2),1.6(d,3H,$SCHCH_3$), 7.13 (d, J=8.7 Hz, 2H, 32 ,52 Ar-H), 7.48 (d, J=8.7 Hz, 2H, 22 ,62 Ar-H), 10.2(s,1H, NH-amide), 13.8 (br s, 1H, N7-H). HRMS (ESI) MS *m/z:* calcd for $C_{19}H_{23}N_5O_3S$[M - H] $^+$ (401.49); found: (400.14441). Anal. Calcd for ($C_{19}H_{23}N_5O_3S$ 401g/mol): C,56.84, H, 5.77, N, 17.44, found: C, 56.78, H, 5.66, N, 17.21.

2-methyl-2-[(1,3-Diethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetylamino-benzoic acid 6h

Yield: 81%; mp: 280°C. ^{1}H NMR (500 MHZ): 3.9 (q, 2H, N1-CH_2), 1.12 (t, 3H, N1-CH_2CH_3), 3.96(q, 2H, N3-CH_2), 1.1 2(t, 3H, N1-CH_2CH_3), 4. 55 (q, 1H, SCH_2), 1.6(d,3H,$SCHCH_3$), 7.8 (d, J=8.7 Hz, 2H, 32 ,52 Ar-H), 8 (d, J=8.7 Hz, 2H, 22 ,62 Ar-H), 10.7(s,1H, NH-amide), 13.8 (br s, 1H, N7-H). HRMS (ESI) MS *m/z:* calcd for $C_{19}H_{21}N_5O_5S$[M - H] $^+$ (431.46); found (430.11895).Anal. Calcd for ($C_{19}H_{21}N_5O_5S$ 431 g/mol): C, 54.53, H, 5.39, N, 16.23, found: C, 52.89, H, 4.91, N, 15.6.

N-(4- Acetylphenyl)-2-methyl-2-[(1,3-Diethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide 6i

Yield: 65%; mp:230-232 °C. ^{1}H NMR (500 MHZ):3.9 (q, 2H, N1-CH_2), 1.1 (t, 3H, N1-CH_2CH_3), 3.95(q, 2H, N3-CH_2), 1.1 (t, 3H, N1-CH_2CH_3), 4. 6 (q, 1H, SCH_2),1.6(d,3H,$SCHCH_3$), 7.75 (d, J=8.7 Hz, 2H, 32 ,52 Ar-H), 7.9 (d, J=8.7 Hz, 2H, 22 ,62 ArH), 10.7(s,1H, NH-amide), 13.8 (br s, 1H, N7-H). HRMS (ESI) MS *m/z:* calcd for $C_{20}H_{23}N_5O_4S$[M - H] $^+$ (429.49); found (428.13987). Anal. Calcd for ($C_{20}H_{23}N_5O_4S$ 429g/mol): C, 55.93, H, 5.4, N, 16.31, found: C, 55.45, H, 5.32, N, 16.18.

N-(3-chlorophenyl)-2-methyl-2-[(1,3-Diethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide 6j

Yield 74%; mp:230 °C. ^{1}H NMR (500 MHZ): 3.9 (q, 2H, N1-CH_2), 1.13 (t, 3H, N1-CH_2CH_3), 3.98(q, 2H, N3-CH_2), 1.13 (t, 3H, N1-CH_2CH_3), 4. 53 (q, 1H, SCH_2), 1.58(d,3H,$SCHCH_3$), 7.21-7.73 (m,4H,Ar-H), 10.6(s,1H, NH-amide), 13.8 (br s, 1H, N7-H). HRMS (ESI) MS *m/z:* calcd for $C_{18}H_{20}ClN_5O_3S$[M - H] $^+$ (421.91); found

(420.0901).Anal. Calcd for ($C_{18}H_{20}ClN_5O_3S$ 421g/mol): C, 51.24, H, 4.78, N, 16.6, found: C, 51.2, H, 4.65, N, 16.1.

2-methyl-2-[(1,3-Diethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-(4-nitrophenyl)acetamide 6k

Yield 74%; mp230-235 °C. ^{1}H NMR (500 MHZ): 3.9 (q, 2H, N1-CH_2),1.1 (t,3H, N1-CH_2CH_3), 3.95 (q, 2H, N3-CH_2), 1.1 (t, 3H,N1-CH_2CH_3), 4.45 (q, 1H, SCH_2), 1.6(d,3H,$SCHCH_3$), 7.85(d, J=8.7 Hz, 2H, 32 ,52 Ar-H), 8.25 (d, J=8.7 Hz, 2H, 22 ,62 Ar-H), 11(s,1H, NH-amide), 13.8(br s, 1H, N7-H). HRMS (ESI) MS *m/z:* calcd for $C_{18}H_{20}N_6O_5S$[M - H] $^+$ (432); found (431).Anal. Calcd for($C_{18}H_{20}N_6O_5S$ 432g/mol): C, 50, H, 4.66, N, 19.43, found: C, 49.1, H, 4.57, N, 19.36.

N-Benzyl-2-[(1,3-Diethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]acetamide 6l

Yield 70%; mp:192 °C. ^{1}H NMR (500 MHZ): 3.91(q, 2H, N1-CH_2), 1.11(t,3H, N1-CH_2CH_3), 3.99(q, 2H, N3-CH_2), 1.16 (t, 3H,N1-CH_2CH_3), 4.56 (q, 1H, SCH_2), 1.6(d,3H,$SCHCH_3$), 4.46 (d, J=7Hz, 2H, $HNCH_2$), 7.21-7.6 (s,5H,Ar-H), 10.3(t, J=6 Hz, 1H, amide-H), 13.7(br s, 1H, N7-H). HRMS (ESI) MS *m/z:* calcd for $C_{19}H_{23}N_5O_3S$[M + H] $^+$ (401.48), found (401.14488). Anal. Calcd for ($C_{19}H_{23}N_5O_3S$ 402g/mol): C, 56.84, H, 55.77, N, 17.44, found: C, 56.72, H, 5.69, N 17.37.

Cell lines and cell culture

The K562 leukemia cell line was obtained from Dr Mona Hassona (Faculty of Science, The University of Jordan) and was cultured in RPMI while the T47D and MCF-7 breast cancer cells were obtained from American Type culture collections (ATCC) and were cultured in DMEM/F12. All media were supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco Invitrogen), 1% of 2mM L-glutamine (Lonza), 50 IU/mL penicillin (Lonza), and 50 µg/mL streptomycin (Lonza) and cells were maintained at 37°C, 5% CO_2 humidified incubator.

Cell proliferation assay

MCF-7, T47D and K562 cells were seeded at a density of 1×10^4, 1×10^4 and 4×10^4 cells per well in 96-well plates in appropriate medium. For anti-MCF7 and anti-K562 screening, the cells were treated with 50 µM concentrations of the tested compounds. For the IC_{50} determination the cells were treated with increasing concentrations of the tested compound (1.56–100 µM). In all assays, the drugs were dissolved in DMSO immediately before the addition to cell cultures and equal amounts of the solvent were added to control cells. Cell viability was assessed, after 3 days of treatment, with tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), obtained from Sigma (Dorset, UK). IC_{50} concentrations were obtained from the dose–response curves using Graph Pad Prism Software 5 (San Diego California USA, www.graphpad.com).

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