Inhibitory Effects of New Mercapto Xanthine Derivatives in Human mcf7 and k562 Cancer Cell Lines

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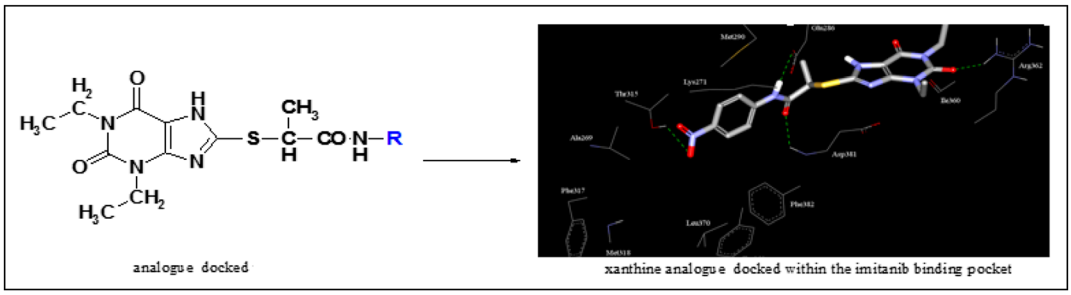


A series of new 2-methyl-2-[(1,3-Diethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)thio]-*N*- substituted arylacetamides were synthesized. The antitumor activity of these purine based compounds were evaluated on breast cancer (MCF7) and leukemic cancer (K562) cell lines via cell viability assay utilizing the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). These results were substantiated using computer docking experiments (LigandFit docking engine and PMF scoring function) which predict that the antitumor activity of these new compounds may be attributable to their abilities to effectively bind and block oncogenic tyrosine kinases, particularly bcr/abl.

INTRODUCTION

Purine nucleus is reported to be an important class of biologically active structures. Xanthine is a purine base that is found in most human body tissues and fluids [1]. They have been widely investigated for their biological activities. Nitrogen-containing compounds from terrestrial and marine organisms have been intensively investigated over the last few years for their antitumor activity, which 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 [2–7]. Previous studies revealed that caffeine inhibits the development of tumors induced by various carcinogens in numerous organs including skin, lung, stomach, and liver [8–10]. Oral administration of caffeine found to be inhibit UVB-induced carcinogenesis in SKH-1 mice [11]. A recent study revealed that caffeine inhibited 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumor [9]. Caffeine was shown to suppress the proliferation of various cancer and transformed cell lines including human neuroblastoma, human pancreatic adenocarcinoma, human A549 lung adenocarcinoma, and mouse epidermal JB6 promotion-sensitive (P) cells, respectively [12–14].

1,3,8-trisubstituted xanthines and 1,3,6-trisubstituted thiazolo[2,3-f]purine-2,4-diones were assessed *in vitro* for their cytotoxic effect against two human malignant cancer cell lines: T-cell leukemia derived SKW-3 and breast cancer derived MDA-MB-231. Some compounds showed interesting anti-tumor activity [15]. These promising anti-cancer effects of xanthine derivatives led us to numerous chemical works focusing on the synthesis of new xanthine derivatives with more potent anti-cancer activity. Accordingly, we envisaged to prepare a set of xanthine derivatives incorporating *N*-substituted arylacetamide moiety and evaluate their antitumor activity against MCF7 breast cancer and K562 leukemia cell lines.

RESULT AND DISCUSSION

Chemistry. 1,3-Diethyl-5,6-diaminouracil **3** were synthesized according to previously described methods [16–20]. 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 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 [21]. The N-substituted aryl-2-methyl-2-cloroacetamides

5a-l were prepared by reacting the appropriate amine pyridine in chloroform with 2-chloropropanoyl chloride [22,23]. 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 arylacetamide **5a-l** solutions in ethanol [24], Scheme 1.

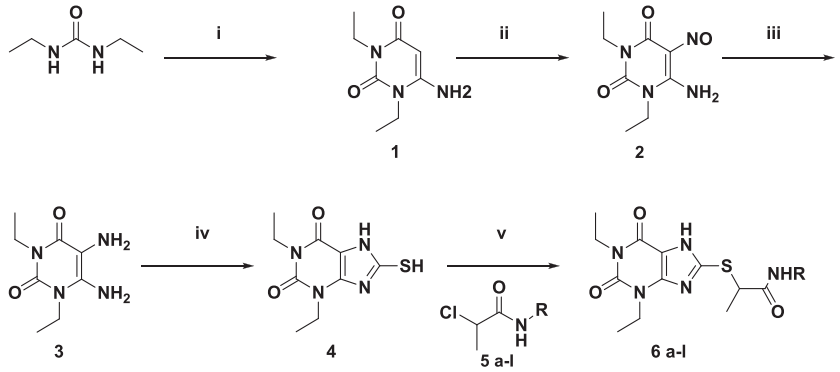
Anti-tumor activity. The anti-tumor activity of the synthesized compounds were evaluated for their cell viability assay using tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Cultures of the breast cancer cell line MCF-7 and the Leukemic cell

Table 2

Effects of compounds that have shown potential activity on the screening assay MCF7 and K562, Doxorubicin is used as a positive control.

Compound	IC_{50} MCF7 (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

Scheme 1. Reagents and condition: (i) $CNCH_2COOH$, Ac_2O, 80°C, 2 h, 70% NaOH; (ii) $NaNO_2$, 50% aq AcOH, 80°C, 45 min; (iii) $Na_2S_2O_4$, 12.5% aq NH4OH, 60°C, 1 h; (iv) CS_2, KOH, EtOH, reflux, 4 h; (iv) 5 a-l, 1% NaOH, EtoH.

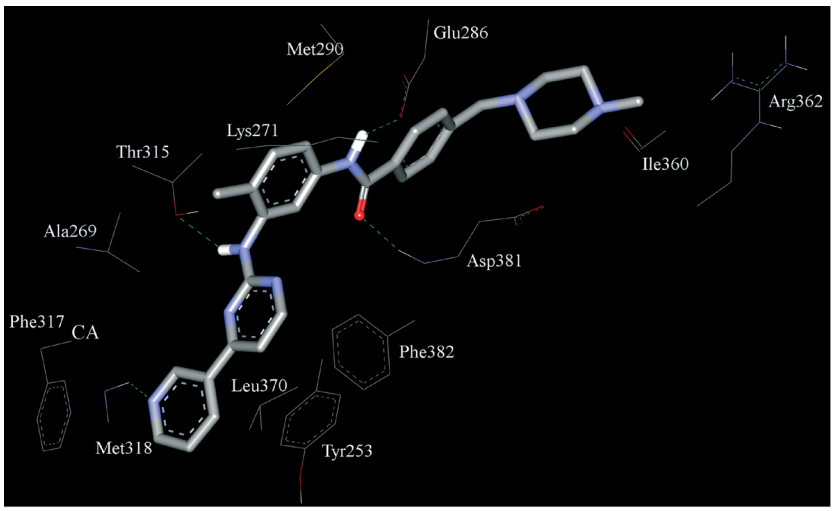


Entry	R	Entry	R	Entry	R
a	3-CH_3Ph	e	4-BrPh	i	4-CH_3OCPh
b	4-OCH_3Ph	f	Ph	j	3-ClPh
c	4-ClPh	g	4-CH_3Ph	k	4-NO_2Ph
d	2-ClPh	h	4-HOOCPh	l	CH_2Ph

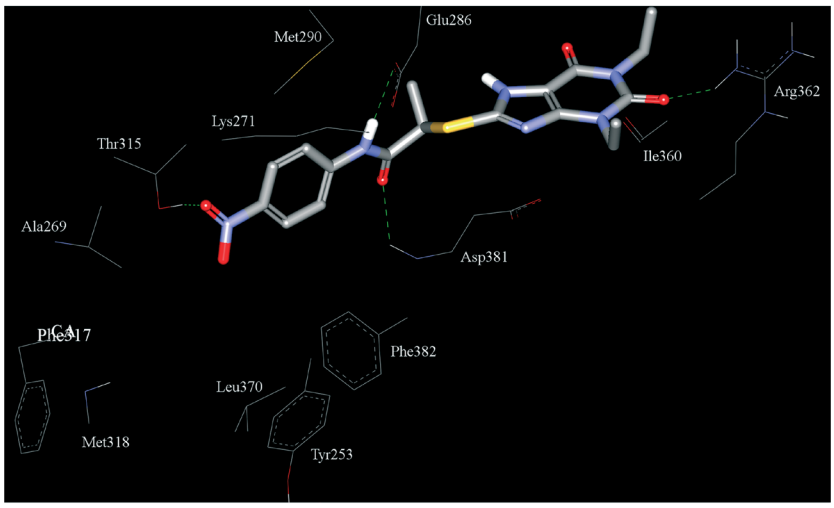
Table 1

Percentage cell survival of MCF7 and K562 following 72 h exposure to 10 and 50 μM of all compounds.

	K562% survival ± SD		MCF7% survival ± SD	
Compound	at 10 μM	at 50 μM	at 10 μM	at 50 μM
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	412 ± 3.20
6d	60 ± 12.44	49 ± 2.60	81 ± 5.51	64 ± 4.74
6e	59 ± 4.68	146 ± 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



(A)



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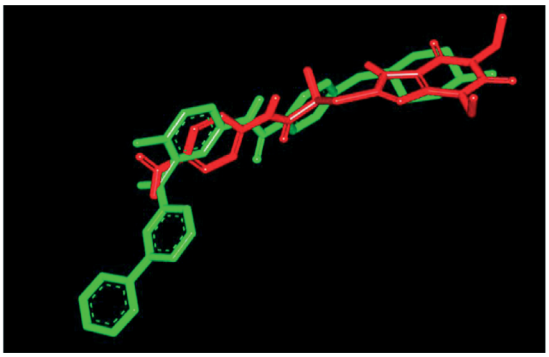

(C)

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. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

line K562 were treated initially at a 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 h to less than 50%. In the case of K562 cells, only four compounds showed a potential anti-K562 activity. At 10 μM concentration, only compound 6k was able to reduce the K262 cell viability to less than 50%, and none of them has shown in the case of MCF7. Further, we determined the IC_{50} values of the potential compounds against the MCF7 and K562 (Table 2). Unsurprisingly, compound 6k showed the highest potency against K562 and MCF7cells; it scored IC_{50} values of 9.56 and 17.59 μM. On the other hand, the IC_{50} of the remaining derivatives ranged between 33 and 59 μM; these values reflected that they were not potent.

From structure activity relationship point of view, the type of substitution on phenyl side chain seems to play a key role in the anti-cancer 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 result from the high-electron withdrawing capability of the NO_2 group on the phenyl ring, which in turn has improved the reactivity of compound 6k toward its cellular target. To understand inhibitory effects of our new synthesized compounds against K562 and MCF7 cancer cell lines that over express tyrosine Kinases, namely, bcr/abl tyrosine kinase and EGFR tyrosine kinase [1,2], we used the anti-cancer agent imitanib in the docking experiment, which is a specific inhibitor of a number of tyrosine kinase enzymes, combined with the apparent pharmacophoric commonalities between imitanib, and our new synthesized compounds 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 (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 (Fig. 1a) and the nitro oxygen of the most potent analogue (Fig. 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 (Fig. 1a) this model can be used to explain the fitting the nitro aromatic ring of the most potent analogue within the same corridor (Fig. 1b). Finally, the apparent electrostatic attraction connecting the piperazine ring of imitianib with the carboxylate side chain of Asp381 (Fig. 1a) compares with 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 (Fig. 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 with 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-merapto-xanthine with appropriate Anilide. The prepared compounds were tested *in vitro* for their antitumor activity against breast (MCF7) and Leukemic (K562) cancer cell lines. The results revealed that compound 6k exerted significant antiproliferative activity with the aforementioned cancer cell lines, with IC_{50} value of 17.59 μM, against MCF7 and IC_{50} value of 9.57 μM, against K562 cell line. Compound 6c displayed good activity, with IC_{50} value of 29.34 μM, against MCF7 cell line and IC_{50} value of 33.72 μM, against K562 cell line. The rest of the compounds showed moderate to weak activities. The findings would encourage us to do further studies and testing that prove the usefulness of the prepared compounds as candidate anti-cancer agents.

EXPERIMENTAL

Materials and equipments. Reagents used for the synthesis were purchased from Sigma-Aldrich (Gillingham– Dorset, UK), MERCK (Schuchardt, Germany) and Acros organics (NJ, 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 aluminum 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 ($\lambda = 254$ 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, college of chemistry, Amman, Jordan, and we used also Agilent DD2 400-MHz spectrometer at Lebniz institute for plant biochemistry NWC department, Halle, Germany; 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, college 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 anti-cancer 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, 16.50 mmol) in ethanol (25 mL), carbon disulfide (1.50 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°C. ^{1}H NMR (500 MH_Z, DMSO): δ 13.4 (br s, 1H, N7-H), 11.97 (br s, 1H, N9-H), 3.92 (q, 2H, N3-CH_2CH_3), 3.86 (q, 2H, N1-CH_2CH_3), 1.17 (t, 3H, N3-CH_2CH_3), 1.12 (t, 3H, N1-CH_2CH_3). *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 arylacetamide (6 a-l). To a stirred solution of compound 4 (0.30 g, 1.28 mmol) in aqueous sodium hydroxide 1% w/v (5.0 ml), the prepared Anilide (1.28 mmol) dissolved in ethanol (3.0 ml) was added portion wise. The reaction mixture was stirred at the ambient temperature for 4 h and then cooled in a refrigerator for 3 h. The product was filtered, washed with water, then diethyl ether, and dried. All the compounds **6a-l** was crystallized from ethanol.

2-methyl-2-[(1,3-Diethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)thio]-N-(3-methylphenyl)acetamide 6a. Yield: 60%; mp: 218–220°C. ^{1}H NMR (500 MH_Z, DMSO): δ 13.7(br s, 1H, N7-H), 10.3(s, 1H, amide-H), 6.88–7.43(m,4H,Ar-H), 4.55 (q, 1H, SCH-), 4.1(q, 2H, N3-CH_2), 3.91(q, 2H, N1-CH_2), 1.11(t,3H, N1-CH_2CH_3), 1.18 (t, 3H,N1-CH_2CH_3), 1.59 (d,3H,$SCHCH_3$), 2.23(s,3H, 3'-CH_3). HRMS (ESI) MS *m/z:* calcd for $C_{19}H_{23}N_5O_3S$ $[M–H]^+$ 401.1522; found 400.1448. *Anal*. Calcd for $C_{19}H_{23}N_5O_3S$ (401.49 g/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)acetamide 6b. Yield: 85%; mp: 262–264°C. ^{1}H NMR (500 MH_Z, DMSO): δ 13.6(br s, 1H, N7-H), 10.2(s, 1H, amide-H), 7.49 (d, J = 8.7 Hz, 2H, 2′,6′ Ar-H), 6.88(d, J = 8.7 Hz, 2H, 3′,5′Ar-H), 4.53 (q, 1H, SCH-), 4.09(q, 2H, N3-CH_2), 3.91(q, 2H, N1-CH_2), 3.72(s, 3H,-OCH_3), 1.59(d,3H,$SCHCH_3$), 1.18 (t, 3H,N3-CH_2CH_3), 1.12(t,3H, N1-CH_2CH_3). HRMS (ESI) MS *m/z:* calcd for $C_{19}H_{23}N_5O_4S[M–H]^+$ (417.1471); found: 416.1398. *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]acetamide 6c. Yield: 74%; mp: 220°C. ^{1}H NMR (500 MH_Z, DMSO): δ 13.6(br s, 1H, N7-H),), 11.27(s, 1H, amide- H), 7.67(d,J = 8.9 Hz, 2H, 2′,6′ Ar-H), 7.36(d,J = 8.9 Hz, 2H, 3′,5′Ar-H), 4.41 (q, 1H, SCH-), 3.97(q, 2H, N3-CH_2), 3.91(q, 2H, N1-CH_2), 1.53(d,3H, $SCHCH_3$), 1.16 (t, 3H,N1-CH_2CH_3), 1.11(t,3H, N1-CH_2CH_3). HRMS (ESI) MS *m/z:* calcd for $C_{18}H_{20}ClN_5O_3S[M–H]^+$ (421.0975); found: 420.0901. *Anal.* Calcd for ($C_{18}H_{20}ClN_5O_3S$ g/mol): 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]acetamide 6d. Yield: 88.8%; mp: 243°C. ^{1}H NMR (500 MH_Z, DMSO): δ 13.7(br s, 1H, N7-H), 9.9(s, 1H, amide-H), 7.21–7.73 (m, 4H, Ar-H), 4.72 (q, 1H, SCH_2), 4. 1(q, 2H, N3-CH_2), 3.91(q, 2H, N1-CH_2), 1.6(d,3H,$SCHCH_3$), 1.18 (t, 3H,N1-CH_2CH_3), 1.11(t,3H, N1-CH_2CH_3). HRMS (ESI) MS *m/z:* calcd for $C_{18}H_{20}ClN_5O_3S[M–H]^+$ (421.0975); found: 420.0901. *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]acetamide 6e. Yield: 85%; mp: 269–272°C. ^{1}H NMR (500 MH_Z, DMSO): δ 13.7(br s, 1H, N7-H), 10.5(s, 1H, amide-H), 7.57(d, 2H, 2′,6′Ar-H), 7.5(d, 2H, 3′,5′Ar-H), 4.58 (q, 1H, SCH_2), 3.99(q, 2H, N3-CH_2), 3.91(q, 2H, N1-CH_2), 1.16 (t, 3H,N1-CH_2CH_3), 1.59(d,3H,$SCHCH_3$), 1.11(t,3H, N1-CH_2CH_3).HRMS (ESI) MS *m/z:* calcd for $C_{18}H_{20}BrN_5O_3S[M–H]^+$ (466.0470); found: 465.0301. *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-phenylacetamide 6f. Yield: 66%; mp: 243°C. ^{1}H NMR (500 MH_Z, DMSO): δ 13.7(br s, 1H, N7-H), 10.3(s, 1H, amide-H), 7.21–7.6 (m,5H,Ar-H), 4.56 (q, 1H, SCH_2), 3.99(q, 2H, N3-CH_2), 3.91(q, 2H, N1-CH_2), 1.6(d,3H, $SCHCH_3$), 1.16 (t, 3H,N1-CH_2CH_3), 1.11(t,3H, N1-CH_2CH_3). HRMS (ESI) MS *m/z:* calcd for $C_{18}H_{21}N_5O_3S[M–H]^+$ (387.1365); found: 386.1291. *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)acetamide 6g. Yield: 92%; mp: 235–240°C. ^{1}H NMR (500 MH_Z, DMSO): δ 13.8 (br s, 1H, N7-H), 10.2(s,1H, NH-amide), 7.48 (d, J = 8.7 Hz, 2H, 2′,6′Ar-H),7.13 (d, J = 8.7 Hz, 2H, 3′,5′Ar-H), 4.55 (q, 1H, SCH_2), 3.99 (q, 2H, N3-CH_2), 3.9 (q, 2H, N1-CH_2), 1.6 (d,3H,$SCHCH_3$), 1.2 (t, 3H,N1-CH_2CH_3), 1.1 (t,3H, N1-CH_2CH_3). HRMS (ESI) MS *m/z:* calcd for $C_{19}H_{23}N_5O_3S[M–H]^+$ (401.1522); found: (400.1444). *Anal.* Calcd for ($C_{19}H_{23}N_5O_3S$ 401 g/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 MH_Z, DMSO): δ 13.8 (br s, 1H, N7-H), 10.7(s,1H, NH-amide), 8.1 (d, J = 8.7 Hz, 2H, 2′,6′Ar-H), 7.8 (d, J = 8.7 Hz, 2H, 3′,5′Ar-H), 4. 55 (q, 1H, SCH_2), 3.96(q, 2H, N3-CH_2), 3.9 (q, 2H, N1-CH_2), 1.6(d,3H, $SCHCH_3$), 1.12 (t, 3H, N1-CH_2CH_3), 1.12(t, 3H, N1-CH_2CH_3). HRMS (ESI) MS *m/z:* calcd for $C_{19}H_{21}N_5O_5S[M–H]^+$ (431.1263); found (430.1189).*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 MH_Z, DMSO): δ 13.8 (br s, 1H, N7-H), 10.7(s,1H, NH-amide), 7.9 (d, J = 8.7 Hz, 2H, 2′,6′ArH), 7.75 (d, J = 8.7 Hz, 2H, 3′,5′Ar-H), 4.6 (q, 1H, SCH_2), 3.95(q, 2H, N3-CH_2), 3.9 (q, 2H, N1-CH_2), 1.6 (d,3H,$SCHCH_3$), 1.1 (t, 3H, N1-CH_2CH_3), 1.1 (t, 3H, N1-CH_2CH_3). HRMS (ESI) MS *m/z:* calcd for $C_{20}H_{23}N_5O_4S[M–H]^+$ (429.1471); found (428.1398). *Anal.* Calcd for ($C_{20}H_{23}N_5O_4S$ 429 g/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 MH_Z, DMSO): δ 13.8 (br s, 1H, N7-H), 10.6(s,1H, NH-amide), 7.21–7.73 (m,4H,Ar-H), 4.53 (q, 1H, SCH_2), 3.98(q, 2H, N3-CH_2), 3.9 (q, 2H, N1-CH_2), 1.58(d,3H,$SCHCH_3$), 1.13 (t, 3H, N1-CH_2CH_3), 1.13 (t, 3H, N1-CH_2CH_3). HRMS (ESI) MS m/z: calcd for $C_{18}H_{20}ClN_5O_3S$ [M–H] $^+$ (421.0975); found (420.0901). *Anal.* Calcd for ($C_{18}H_{20}ClN_5O_3S$ 421 g/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%; mp: 230–235°C. ^{1}H NMR (500 MH_Z, DMSO): δ 13.8(br s, 1H, N7-H), 11 (s,1H, NH-amide), 8.25 (d, J = 8.7 Hz, 2H, 2′,6′ Ar-H), 7.85(d, J = 8.7 Hz, 2H, 3′,5′Ar-H), 4.45 (q, 1H, SCH_2), 3.95 (q, 2H, N3-CH_2), 3.9 (q, 2H, N1-CH_2), 1.6(d,3H,$SCHCH_3$), 1.1 (t,3H, N1-CH_2CH_3), 1.1 (t, 3H,N1-CH_2CH_3). HRMS (ESI) MS *m/z:* calcd for $C_{18}H_{20}N_6O_5S$ [M–H] $^+$ (432.1216); found (431.1147). *Anal.* Calcd for ($C_{18}H_{20}N_6O_5S$ 432 g/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 MH_Z, DMSO): δ 13.7(br s, 1H, N7-H), 10.3(t, J = 6 Hz, 1H, amide-H), 7.21–7.6 (s,5H,Ar-H), 4.56 (q, 1H, SCH_2), 4.46 (d, J = 7 Hz, 2H, $HNCH_2$), 3.99 (q, 2H, N3-CH_2), 3.91(q, 2H, N1-CH_2), 1.6(d,3H,$SCHCH_3$), 1.16 (t, 3H,N1-CH_2CH_3), 1.11(t,3H, N1-CH_2CH_3). HRMS (ESI) MS *m/z:* calcd for $C_{19}H_{23}N_5O_3S$ [M + H] $^+$ (401.1522), found (401.1448). *Anal.* Calcd for ($C_{19}H_{23}N_5O_3S$ 402 g/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 2 m*M* 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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