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SYNTHESIS OF NEW TWO DERIVATIVES OF 6-MERCAPTOPURINE (6MP) 6-[5-PYRIDINE-4-YL- 1, 2, 3, 4-OXADIAZOLE-2-YL)DITHIOL]-9H-PURINE (38) AND 9H-PURINE-6-YL-BENYLDITHIOCARBAMATE (45) WITH CYTOTOXICITY RESULTS FROM THE NATIONAL CANCER INSTITUTE'S ANTICANCER DRUG SCREEN

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ABSTRACT

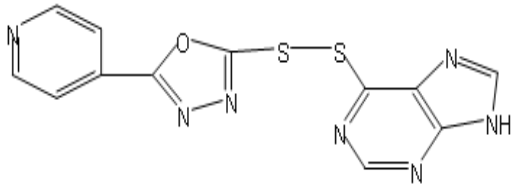
6-[(5-pyridine-yl-1, 2, 3, 4-oxadiazole-2-yl)dithiol]-9H-purine (38) and 9H-purine-6yl-benzyldithiocarbamate (45) are synthesized as possible prodrugs for 6-mercaptopurine(6-MP). The generation of the compounds 38, 42, 45 and 48 were accomplished following multistep reaction procedures. The reaction and purity of the products were checked by TLC, the structure of the final compounds and their intermediates were confirmed by their melting points, infra red spectroscopy and whether differences elemental microanalysis. To determine exist among in sensitivity different tissue types toward treatment with 38 and 45. The cytotoxicity of the two compounds and their intermediates were confirmed by their melting points, infra red spectroscopy and elemental microanalysis. The cytotoxicity of two derivatives (38 and 45) was assessed in National Cancer Institute's anticancer screening program, and the results were compared with the cytotoxicity of 6-MP obtained in the same screen. The results show that the compound 38 and 45 were more cytotoxic than 6-MP. Additionally the prodrugs are less effective against leukemia cell line than 6-MP. Both derivatives exhibited high growth-inhibitory activities in renal cell line. However, compound 45 is more cytotoxic than 38 against ovarian cell line.

INTRODUCTION: Six-mercaptopurine (6-MP) is used in the maintenance therapy of acute lymphoblastic leukemia and it also displays activity against acute and chronic myelogeneousleukemias [1, 2]. The clinical use of the thiopurines against solid tumors has been limited by severe bone marrow toxicity [3].

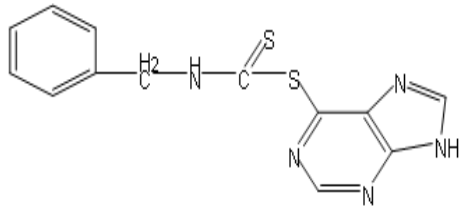
Resistance to the thiopurines was observed and it may be caused by deficiency or complete lack of enzyme hypoxanthine guanine-phosphoribosyltransfersae (HGPRT) [4] and increased levels of glutathione have been linked with drug resistance [5]. One promising aspect for improving the distribution of (6-MP) appears to be the prodrug approach by which the pharmacokinetic patterns and ultimately therapeutic success can be obtained through introduction of promoities with the ideal physiochemical properties. It had been synthesized two prodrugs cis-AVTP [cis-6-(2-acetyl vinyl thiopurine] and trans-AVTP [trans-6-(2-acetyl vinyl thioguanine] [6] and assessed in the National Cancer Institute (NCI), then the results were compared with cytotoxicities of 6-MP and 6- thioguanine (6-TG).

The results show that that cis-AVTP and trans-AVTP exhibit both distinct and similar cytotoxicities toward different histotypes. Also it was found that the cytotoxic activity of both cis –AVTP and trans-AVTG correlated best with that of another thiopurine

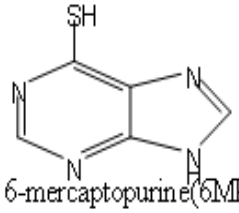
conjugate. The aim of this study is to synthesize and evaluate the cytotoxicity of target compounds 6-[(5-pyridine-4-yl-1, 3, 4-oxadiazole-2-yl) dithio]-9H-purine (38) and 9H-purine-6-yl-benzyldithiocarbmate (45) against different cell line histotypes compared with the parent compound (6MP).



6-[(5-pyridine-4-yl-1,3,4-oxadizole-2yl)dithio]-9H-purine.(38)



9H-purine-6-yl-benzyldithiocarbamate(45)



6-mercaptopurine(6MP)

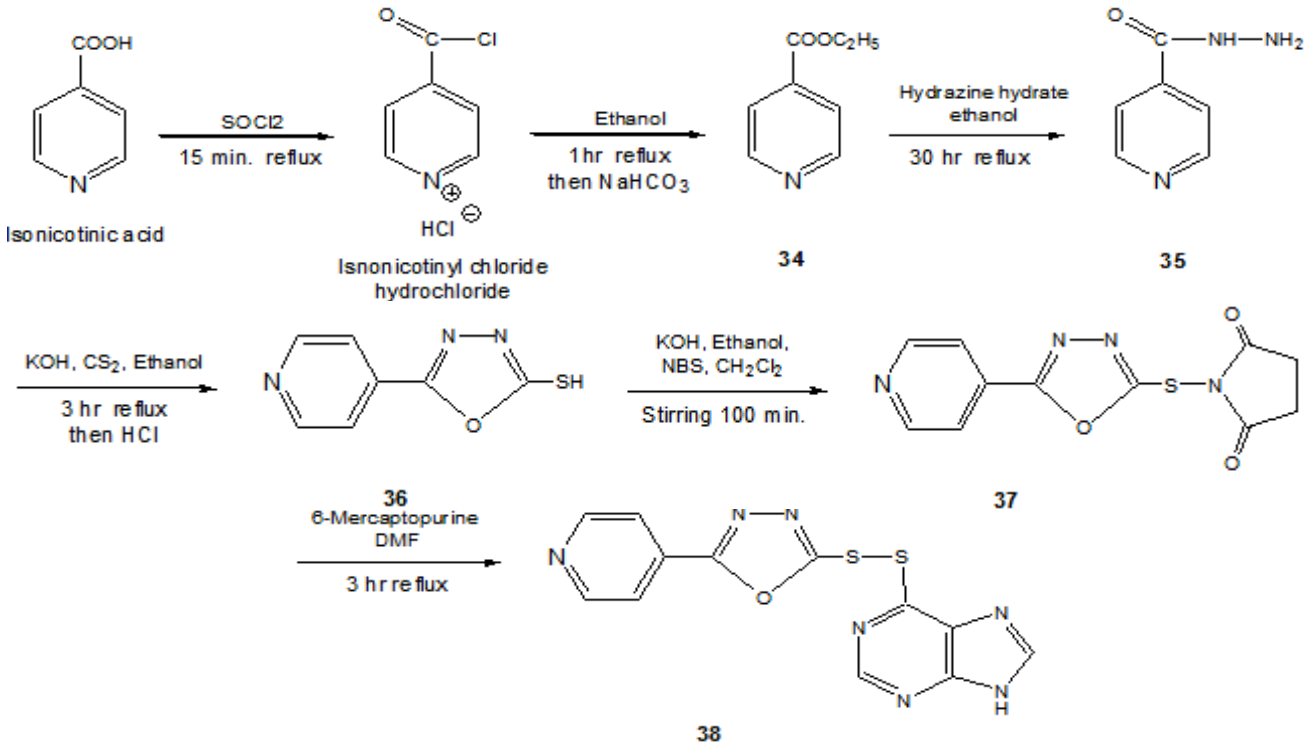
SCHEME 1: STRUCTURES OF 6-[(5-PYRIDINE-YL- 1, 2, 3, 4-OXADIAZOLE- 2- YL)DITHIOL]- 9H- PURINE(38), 9H-PURINE-6YL-BENZYLDITHIOCARBAMATE(45) AND 6-MERCAPTOPURINE (6MP)

RESULTS AND DISCUSSION:

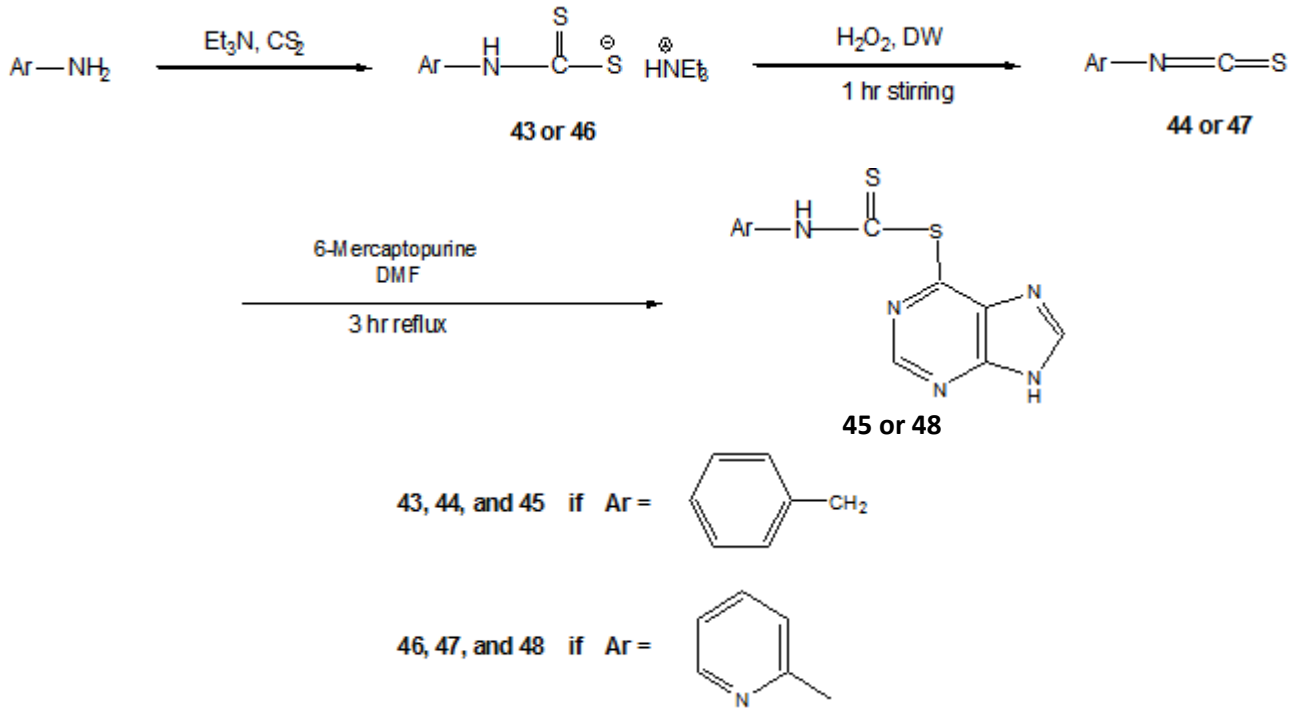
Chemistry: The synthetic procedures for the designed target compounds 38 and 45 are illustrated in **schemes (2) and (3)** respectively. The starting material for the generation of target compound 38 was Isoniazid which was refluxed with carbon disulfide in the presence of potassium hydroxide to give the mercapto- 1, 3, 4-oxadiazole derivative, compound 36, which was treated with N-bromosuccinamide to give compound 37, the reaction between 6-mercaptopurine and compound 37 in dimethyl formide (DMF) liberated the target compound 38.The liberation of dithiocarbamate derivatives compounds 45 was shown in scheme 3.

Amine containing compound, benzylamine was reacted with carbon disulfide in the presence of triethylamine to give compound 46. The isothiocynate derivative, 44 was obtained by the oxidation of compounds 43 using hydrogen peroxide. The final compound 45 was then obtained from the reaction between 6-mercaptopurine and compound.

The synthesized compounds have been identified by their melting points, IR spectra and CHN microanalysis.



SCHEME 2: SYNTHESIS OF COMPOUND 38



SCHEME 3: SYNTHESIS OF COMPOUNDS 45 AND 48

Cytotoxic activity: When the cytotoxicity parameters obtained for compound 45 and 38 in NCI's anticancer screen (**table 1 and 2**) are compared with the cytotoxicity parameters calculated for 6MP using data obtained from the standard agent database (**table 3**).

TABLE 1: THE PARAMETERS GI_{50}, TGI AND LC_{50} OBTAINED FOR COMPOUND 45 IN THE NCI ANTICANCER SCREEN. PARAMETERS BELOW OR ABOVE THE HIGHEST OR LOWEST DRUG CONCENTRATION USED ARE LISTED AS 0.247 OR >100 RESPECTIVELY. ALL VALUES ARE GIVEN IN MICROMOLAR CONCENTRATION (µM)

Cell type	Tissue type	GI_{50}	TGI	LC_{50}
CCR-CEM	Leukemia	1.64	>100	>100
RPMI-8226	Leukemia	0.950	>100	>100
A549/ATCC	Non-small cell lung carcinoma	22.3	>100	>100
HOP-62	Non-small cell lung carcinoma	0.382	>100	>100
HOP-92	Non-small cell lung carcinoma	0.621	>100	>100
NCI-H23	Non-small cell lung carcinoma	2.65	>100	>100
NCI-H322M	Non-small cell lung carcinoma	2.68	>100	>100
NCI-H460	Non-small cell lung carcinoma	5.55	>100	>100
NCI-H522	Non-small cell lung carcinoma	0.795	>100	>100
COLO 205	Colon cancer	3.40	15.4	>100
HCT-116	Colon cancer	1.24	>100	>100
HCT-15	Colon cancer	3.71	>100	>100
HT 29	Colon cancer	4.48	>100	>100
KM12	Colon cancer	3.85	>100	>100
SW-620	Colon cancer	3.54	>100	>100
SF-268	CNS cancer	5.19	>100	>100
SF-295	CNS cancer	0.994	>100	>100
SF-539	CNS cancer	0.860	>100	>100
SNB-19	CNS cancer	48	>100	>100
SNB-75	CNS cancer	13.1	>100	>100
U251	CNS cancer	10.2	>100	>100
LOX IMVI	Melanoma	0.32	19.9	>100
MALME-3M	Melanoma	6.67	>100	>100

M14	Melanoma	0.402	94.7	>100
SK-MEL-28	Melanoma	82.3	>100	>100
SK-MEL-5	Melanoma	3.38	>100	>100
UACC-257	Melanoma	6.93	>100	>100
UACC-62	Melanoma	0.579	>100	>100
OVCAR-3	Ovarian cancer	0.694	>100	>100
OVCAR-4	Ovarian cancer	29.1	>100	>100
OVCAR-5	Ovarian cancer	8.68	>100	>100
OVCAR-8	Ovarian cancer	2.97	>100	>100
SK-OV-3	Ovarian cancer	0.402	>100	>100
786-0	Renal cancer	0.479	>100	>100
ACHN	Renal cancer	4.10	>100	>100
CAK-1	Renal cancer	0.247	25.4	>100
SN12C	Renal cancer	4.94	>100	>100
TK-10	Renal cancer	0.388	>100	>100
UO-31	Renal cancer	0.944	>100	>100
PC-3	Prostate cancer	3.66	>100	>100
DU-145	Prostate caner	0.568	>100	>100
MDA-MB-231/ATCC	Breast cancer	2.32	>100	>100
HS 578T	Breast cancer	16.4	>100	>100
BT-549	Breast cancer	2.98	>100	>100

TABLE 2: THE PARAMETERS GI_{50}, TGI AND LC_{50} OBTAINED FOR COMPOUND 38 IN THE NCI ANTICANCER SCREEN. PARAMETERS BELOW OR ABOVE THE HIGHEST OR LOWEST DRUG CONCENTRATION USED ARE LISTED AS 0.297 OR >100 RESPECTIVELY. ALL VALUES ARE GIVEN IN MICROMOLAR CONCENTRATION (μM)

Cell type	Tissue type	GI50	TGI	LC50
CCR-CEM	Leukemia	1.24	>100	>100
RPMI-8226	Leukemia	1.32	>100	>100
A549/ATCC	Non-small cell lung carcinoma	23	>100	>100
HOP-62	Non-small cell lung carcinoma	2.52	>100	>100
HOP-92	Non-small cell lung carcinoma	0.582	>100	>100
NCI-H23	Non-small cell lung carcinoma	2.73	>100	>100
NCI-H322M	Non-small cell lung carcinoma	2.82	>100	>100
NCI-H460	Non-small cell lung carcinoma	5.15	>100	>100
NCI-H522	Non-small cell lung carcinoma	1.63	>100	>100
COLO 205	Colon cancer	3.30	23.8	>100
HCT-116	Colon cancer	2.00	>100	>100
HCT-15	Colon cancer	2.80	>100	>100
HT 29	Colon cancer	2.42	>100	>100
KM12	Colon cancer	2.61	>100	>100
SW-620	Colon cancer	3.38	>100	>100
SF-268	CNS cancer	2.18	>100	>100
SF-295	CNS cancer	0.51	12.8	>100
SF-539	CNS cancer	1.84	>100	>100
SNB-19	CNS cancer	78.4	>100	>100
SNB-75	CNS cancer	0.672	>100	>100
U251	CNS cancer	7.22	>100	>100
LOX IMVI	Melanoma	0.297	17.3	>100
MALE-3M	Melanoma	2.08	>100	>100
M14	Melanoma	0.405	>100	>100

SK-MEL-28	Melanoma	43.7	>100	>100
SK-MEL-5	Melanoma	3.46	>100	>100
UACC-257	Melanoma	9.34	>100	>100
UACC-62	Melanoma	2.02	>100	>100
OVCAR-3	Ovarian cancer	0.319	1.73	>100
OVCAR-4	Ovarian cancer	0.51	>100	>100
OVCAR-5	Ovarian cancer	3.10	>100	>100
OVCAR-8	Ovarian cancer	3.26	>100	>100
SK-OV-3	Ovarian cancer	0.450	>100	>100
786-0	Renal cancer	0.986	>100	>100
ACHN	Renal cancer	4.08	>100	>100
CAK-1	Renal cancer	0.472	5.17	>100
SN12C	Renal cancer	6.60	>100	>100
TK-10	Renal cancer	0.486	>100	>100
UO-31	Renal cancer	0.742	>100	>100
PC-3	Prostate cancer	3.48	>100	>100
DU-145	Prostate caner	0.455	>100	>100
MDA-MB-231/ATCC	Breast cancer	4.45	>100	>100
HS578T	Breast cancer	2.56	>100	>100
BT-549	Breast cancer	9.47	>100	>100

TABLE 3: THE PARAMETERS GI_{50}, TGI, AND LC_{50} OBTAINED FOR 6MP IN THE NCI ANTICANCER SCREEN. PARAMETERS BELOW OR ABOVE THE HIGHEST OR LOWEST DRUG CONCENTRATION USED ARE LISTED AS <0.38 OR >100 RESPECTIVELY. ALL VALUES ARE GIVEN IN MICROMOLAR CONCENTRATION (μM).

Cell type	Tissue type	GI_{50}	TGI	LC_{50}
CCR-CEM	Leukemia	1.26	>100	>100
RPMI-8226	Leukemia	1.76	>100	>100
A549/ATCC	Non-small cell lung carcinoma	30.1	>100	}100
HOP-62	Non-small cell lung carcinoma	1.75	>100	>100
HOP-92	Non-small cell lung carcinoma	2.94	>100	>100
NCI-H23	Non-small cell lung carcinoma	3.89	>100	>100
NCI-H322M	Non-small cell lung carcinoma	9.46	>100	>100
NCI-H460	Non-small cell lung carcinoma	5.53	>100	>100
NCI-H522	Non-small cell lung carcinoma	1.62	>100	>100
COLO 205	Colon cancer	4.94	>100	>100
HCT-116	Colon cancer	2.37	>100	>100
HCT-15	Colon cancer	4.57	>100	>100
HT 29	Colon cancer	4.02	>100	>100
KM12	Colon cancer	7.57	>100	>100
SW-620	Colon cancer	6.38	>100	>100
SF-268	CNS cancer	4.11	>100	>100
SF-295	CNS cancer	5.81	>100	>100
SF-539	CNS cancer	2.49	>100	>100
SNB-19	CNS cancer	>100	>100	>100
SNB-75	CNS cancer	8.75	>100	>100
U251	CNS cancer	13.7	>100	>100
LOX IMVI	Melanoma	0.38	>100	>100
MALE-3M	Melanoma	3.66	>100	>100
M14	Melanoma	0.67	>100	>100
SK-MEL-28	Melanoma	>100	>100	>100

SK-MEL-5	Melanoma	8.36	>100	>100
UACC-257	Melanoma	14.4	>100	>100
UACC-62	Melanoma	1.76	>100	>100
OVCAR-3	Ovarian cancer	0.86	>100	>100
OVCAR-4	Ovarian cancer	8.05	>10	>100
OVCAR-5	Ovarian cancer	8.63	>100	>100
OVCAR-8	Ovarian cancer	2.54	>100	>100
SK-OV-3	Ovarian cancer	1.03	>100	>100
786-0	Renal cancer	2.03	>100	>100
ACHN	Renal cancer	6.27	>100	>100
CAK-1	Renal cancer	3.21	>100	>100
SN12C	Renal cancer	23.8	>100	>100
TK-10	Renal cancer	1.94	>100	>100
UO-31	Renal cancer	6.85	>100	>100
PC-3	Prostate cancer	4.39	>100	>100
DU-145	Prostate caner	2.51	>100	>100
MDA-MB-231/ATCC	Breast cancer	24.4	>100	>100
HS 578T	Breast cancer	14.2	>100	>100
BT-549	Breast cancer	>100	>100	>100

It is evident that the prodrugs (45 and 38) showed enhanced in vitro cytotoxicity compared with 6MP for endpoint GI_{50}. Compound 38 is more cytotoxic than 45 in all cell lines except cell line panels (RPMI-822, A549/ATCC, HOP-62, NCI-H23, NCI-H322M, NCI-H522, HCT-116, SF-539, SNB-19, M14, SK-MEL-5, UACC-257, OVCAR-8, SK-OV-3, 786-0, CAK-1, SN12C, TK-10, MDA-MB-231/ATCC, BT-549) for endpoint GI50. Compound 45 is more cytotoxic than 38 in cell lines (COLO 205, and M14) for endpoint TGI and less cytotoxic in cell lines (LOX IMVI, OVCAR-3 and CAK-1) for endpoint TGI. Both of them have similar cytotoxicity for endpoint LC_{50}.

However, the median value obtained for all cell lines after MP32 treatment was lower than those of 6MP and nearly equal to those of MP31 (**table 4**). The present kinetic studies showed that prodrugs 45 and 38 liberated the drug 6MP at pH 6 faster than at pH 7.4 9 (**table 5**); this will make these compounds good candidates as prodrug for targeting cancer cells since the PH there approximates 6; while the prodrugs are more stable at pH 7.4, pH of normal cells. In addition the present study indicated that compound 45 and 38 generate 6MP in presence of glutathione (**table 6**); this also make those prodrugs useful prodrugs for targeting 6MP to the tumor cells, since the levels of glutathione is relatively higher in cancer cells than in normal cells.

Several findings are worth consideration when the responses of the different histotypes toward compound 45 and 38 treatments are examined. **Table 7** showed that after 38, 45 and 6MP treatment the median of TGI and LC_{50} are above the 100µM, so the comparison of the compounds mostly will be restricted on GI_{50} value. Leukemia cells had median GI_{50} value after 6MP treatment is less than that of both 38 and 45; the leukemia cell panel included 2 types of cell lines (CCRF-CEM and RPMI-8226).

In the light of the fact that 6MP successfully used as an antileukemic agent, these low GI_{50} but high TGI and LC_{50} values obtained for leukemic cells show that at least in some cases, a clinically useful drug can have high TGI and LC_{50} values based upon the widespread use of 6MP as an antileukemic agent , it would be of considerable interest to examine whether the prodrugs (45 and 38) also exhibit antileukemic activity in vivo and whether the antileukemic activity of any one of these derivatives surpasses or equals to that of 6MP.

TABLE 4: THE MEDIAN GI50, TGI, AND LC50 VALUES OBTAINED FROM 6MP, 45 AND 38 IN THE NCI ANTICANCER SCREEN

Parameters	MP6	Compound 45	Compound 38
GI50	4.48	2.48	2.42
TGI	>100	>100	>100
LC50	>100	>100	>100

Table (5): The rate constant of hydrolysis of compounds 38, and 45 at pH 6 and pH 7.4 at 37°C, and μ=1.

Compound	PH	K_{obs} x10^{-3} (min^{-3})	$t_{1/2}$ (min)
38	6	14.520	47.71
	7.4	1.083	639.65
45	6	Stable for 3 days	Stable for 3 days
	7.4	Stable for 3 days	Stable for 3 days

Table (6): Effect of the glutathione (0.02mg/ml) on the rate constant of hydrolysis of compounds 38, and 45 at pH 6, 37 °C and μ=1.

Compound	K_{obs} x10^{-2} (min^{-1})	$t_{1/2}$ (min)
38	24.043	2.88
45	0.875	79.20

TABLE 7: THE MEDIAN GI_{50}, TGI, AND LC_{50} VALUES OBTAINED AFTER TREATMENT WITH 6MP, 45 AND 38 IN DIFFERENT HISTOTYPES VALUES ARE GIVEN IN MICROMOLAR CONCENTRATION (μM).

Cell line	GI_{50}			TGI			LC_{50}		
	Compound 38	Compound 45	6MP	Compound 38	Compound 45	6MP	Compound 38	Compound 45	6MP
Leukemia	2.56(6)	1.29(2)	1.01(1)	>100 (1-9)	>100 (1-9)	>100(1-9)	>100 (1-9)	>100 (1-9)	>100 (1-9)
NSCL	2.73(8)	2.65(4)	3.89(5)	>100 (1-9)	>100 (1-9)	>100(1-9)	>100 1-9)	>100 (1-9)	>100 (1-9)
Colon	2.7(7)	3.62(8)	4.76(7)	>100 (1-9)	>100 (1-9)	>100(1-9)	>100 (1-9)	>100(1-9)	>100 (1-9)
CNS	2.01(4)	7.69(9)	7.28(8)	>100 (1-9)	>100 (1-9)	>100(1-9)	>100 (1-9)	>100(1-9)	>100 (1-9)
Melanoma	2.08(5)	3.38(7)	3.66(4)	>100 (1-9)	>100 (1-9)	>100(1-9)	>100 (1-9)	>100(1-9)	>100 (1-9)
Ovarian	0.51(1)	2.97(5)	2.45(2)	>100 (1-9)	>100 (1-9)	>100(1-9)	>100 (1-9)	>100(1-9)	>100 (1-9)
Renal	0.864(2)	0.71(1)	4.74(6)	>100 (1-9)	>100 (1-9)	>100(1-9)	>100 (1-9)	>100(1-9)	>100 (1-9)
Prostate	1.96(3)	2.11(3)	3.45(3)	>100 (1-9)	>100 (1-9)	>100(1-9)	>100 (1-9)	>100(1-9)	>100 (1-9)
Breast	4.45(9)	2.98(6)	24.42 (9)	>100 (1-9)	>100 (1-9)	>100(1-9)	>100 (1-9)	>100(1-9)	>100 (1-9)

The response of renal cancer cell line panels toward treatment with 38 and 45 is of considerable significance. The cell line had the first and second lowest GI50 values toward 45 and 38 respectively (Table 7) ,suggesting that it is very sensitive to 38 and 45.The sensitivity of renal cancer cells is of importance because no chemotherapeutic agent is effective against renal cell carcinoma [7, 8].

It had been shown that incubation of isolated rat renal cortical cells with 6MP at 0.5 and 1mM concentrations for 2 h significantly increases cell death as measured by the release of lactate dehydrogenase compared with buffer- only incubations. This increase in lactate dehydrogenase could be partially blocked by allopurinol, an inhibitor of xanthine oxidase that catalyzes the oxidation of 6MP to thiouric acid and

superoxide anion [9]. These findings suggest that the toxicity of 6MP could be in part, due to the generation of reactive oxygen species and oxidative stress, and further indicate that isolated renal cells, 6MP toxicity occurs via multiple pathways. Thus 38 and 45 activity may also occur via multiple pathways. Ovarian cancer cells are more sensitive toward the 38 when the ovarian cell line had the first lowest GI50 value but the opposite for ovarian cell line after treatment with 45.The sensitivity of ovarian cancer cells toward 38 is of importance because , there is need for new chemotherapeutic drug effective against ovarian cancer [10].

Experimental:

Synthesis of 2-(4-pyridyl)-5-mercapto-1, 3, 4-oxadiazole, Compound(36): To a solution containing ethanol (200 ml) and potassium hydroxide (2.8 g, 50 mmole), dissolved in water (10 ml), Isonicotinyl Hydrazine (6.85 g, 50mmole) was added, carbon disulfide (4.56 g, 60 mmol) was added and the mixture was refluxed for 3 hours. The solvent was evaporated to small volume in vacuum and the residue was dissolved in water. A precipitate was obtained upon the addition of the solution to ice containing HCl (5 ml). The precipitate was filtered, dried and recrystalized from ethanol to give yellow crystalline powder of compound 36, percent yield (45%) and melting point 270-273^{o}C (reported 272^{o}C) [11]. IR (*v*, cm^{-1}): 2590 C-SH stretching vibration, 1620 (C=N-), 1595 (C=C aromatic), 830 and 726 4-substituted pyridine.

Synthesis of N-[{2-(4-pyridyl)-1, 3, 4-oxidiazole-5-yl} thio] succinamide, Compound (37): A solution of potassium hydroxide (1.13 g, 20.178 mmole) in absolute ethanol (5 ml) was added to a solution of compound 36 (3.6 g, 20.11 mmole) in absolute ethanol (30 ml). The resulted precipitate was filtered, dried and suspended in methylene chloride (40 ml), cooled to 0^{o}C. Then a similarly cooled suspension of N-bromosuccinamide (3.58 g, 20.11 mmole) in methylene chloride (20 ml) was added. After stirring at 0 ^{o}C for 10 minutes, the suspension was stirred for additional 90 minutes at room temperature. The insoluble material was then filtered to give potassium bromide, and the filtrate was evaporated to dryness *in vacuo*, the residue was recrystallized from ethanol-water to give crystals of compound 37, percent yield (57%). melting point (110-113). IR (*v*, cm^{-1}): 1780 and 1702 C=O stretching vibration of 5 member cyclic imide, 1620 (C=N-), 1595 (C=C aromatic), 830 and 726 4-substitutedpyridine. .

Synthesis of 6-[{5-(4-pyridyl)-1, 3, 4-oxidiazole-2-yl} dithio]-9Hpurine, Compound (38): A solution of compound 37 (1.55g, 5.875 mmole) and 6-mercaptopurine monohydrate (1 g, 5.875 mmole) in DMF (20 ml) was refluxed for 3 hours. A precipitate was obtained by the addition of distilled water. The precipitate was filtered, dried and recrystalized from methanol to give compound 38, percent yield (90%), melting point(215-218 decomp.). Anal. % calcd. for $C_{12}H_7N_7OS_2C$: 43.76, H: 2.14, N : 29.77; found: C:44.06, H: 2.34, N: 29.44. (IR (*v*, cm^{-1}): 3445 and 3512, N-H stretching vibration of purine, 1620 (C=N-), 1595 (C=C aromatic), 830 and 726 4-substituted pyridine, 1386 C-H bending vibration of purine.

Synthesis of Triethylammonium-N-benzyl dithio-carbamate (Compound 43): To stir mixture of benzylamine (10 g, 93 mmole) and triethylamine (9.43 g, 93 mmole) cooled in ice bath, carbon disulfide (7.1g, 93 mmole) was added from dropping funnel over a period of about 10 minutes with continuous stirring. The resultant precipitate was washed with ether (30 ml x 2) and recrystallized from ethanol-ether to give yellow crystals of compound 43. Percent yield (53%) melting point (110-112^{o}C) reported (112^{o}C) [12].

Synthesis of Benzyl Isothiocyanate (Compound 44): Hydrogen peroxide (4.3 ml, 30% v/v) was added dropwise to stirred solution of compound 43 (9 g, 31.6 mmole) in distilled water (50 ml). The reaction mixture was stirred for 1 hour at room temperature; crease oil substance was formed. This oily substance was collected by decantation of water, washed with distilled water and dried to give compound 44. Percent yield (55%) boiling point (242-245^{o}C) reported (243^{o}C) [13]. IR (*v*, cm^{-1}): 1700-2000 Shape of typical mono-substituted benzene, 1557 Phenyl nucleus stretching vibration, 1028 N=C=S stretching vibration.

Synthesis of 9H-purine-6-yl-benzyl dithiocarbamate (Compound 45): A mixture of compound 44 (0.82 g, 5.875 mmole) and 6-mercaptopurine monohydrate (1 g, 5.875 mmole) in DMF (20 ml) was refluxed for 3

hours. A precipitate was obtained by diluting the reaction mixture with distilled water. The precipitate was filtered, washed with cold solution of sodium bicarbonate (10 ml, 5%) and dried to give compound 45. Percent yield (87%), melting point (280-282 Decomp). Anal. % calcd. For $C_{13}H_{11}N_5S_2$; C: 51.82, H: 3.65, N: 23.25; found: C: 52.13, H: 4.01, N: 23.39. IR (*v*, cm^{-1}): 3446 and 3332 N-H stretching vibration of purine, 3100N-S stretching vib. HN-(C=S)S, 1700-2000 shape of typical mono-substituted benzene,1557 Phenyl nucleus stretching vibration, 1411, 1331 and 1276 N-(C=S)-S stretching vibration corresponding to amide I, II, and III.

Hydrolysis of 6-mercaptopurine Derivatives at pH 6 and pH 7.4: The hydrolysis of the 6-mercaptopurine derivatives was studied in aqueous phosphate buffer solution of the pH 6 and 7.4 at 37^{o}C. The total buffer concentration was 0.1M and the ionic strength (m) of 1.0 was maintained for each buffer by adding calculated amount of sodium chloride. The rate of hydrolysis was followed spectrophotometrically by recording 6-mercaptopurine absorbance increase accompanying the hydrolysis at 324 nm and 316 nm for pH 6 and 7.4, respectively.

The reactions were initiated by adding 5 ml of stock solutions of the derivatives in methanol (0.1 mg/ml) to preheated buffer solution to give final concentration of derivatives of 0.01 mg/ml. The solutions were kept in a water-bath at 37^{o}C and at appropriate time interval samples were withdrawn and the absorbances were recorded. The observed pseudo-first order rate constants were determined from the slopes of the linear plots of log (A¥-At) *vs* time, where A¥ and At are the absorbance reading at *infinity* and time *t*, respectively.

The NCI's Anticancer Drug screening: The NCI screening procedures were described in detail [14]. Briefly, cell suspensions that were diluted according to the particular cell type and the expected target cell density (5000-40,000) cells per well based on cell growth characteristics) were added by pipette (100 µL) into 96-well microtiter plates. Inoculates were allowed a pre-incubation period of 24h at 37^{o}C for stabilization. Dilutions at twice the intended test concentration were added at time zero in 100- µL aliquots to the microtiter plate wells. Usually, test compounds (45 and 38) were evaluated at five 10- fold dilutions .In routine testing, the highest well concentration is 10^{-4} M, but for the standard agents the highest well concentration used depended on the agent. Incubations lasted for 48 h in 5% Co2 atmosphere and 100% humidity. The cells were assayed by using the sulforhodamine B assay [7].

A plate reader was used to read the optical densities, and a microcomputer processed the optical densities into the special concentration parameters defined later. Three dose response parameters were calculated for the prodrugs: GI_{50}, the drug concentration resulting in a 50% reduction in the protein increase compared with control cells during the incubation. TGI, the drug concentration resulting in total growth inhibition and LC_{50}, the concentration of drug resulting in 50% reduction in measured protein at the end of the drug treatment compared with that at the beginning, thus indicating a net loss cells following treatment.

These three parameters were calculated if the level of cytotoxicity was reached, whereas if the effect was not reached or was exceeded, the value was listed as greater or less than the maximum or minimum concentration tested.

The thiopurine 6-Mercaptopurine (6MP) in NCI's standard agent database and, thus its in vitro cytotoxicity has been determined using the assay described above. The results from these assays can be accessed from NCI's website (http://itbwork.net. nci.nhi.gov).

Because the highest drug dilution used to assess the cytotoxicity of 6-MP was higher than 100µM, any GI_{50}, TGI, and LD_{50} value obtained for 6MP and prodrugs (38 and 45) that was above 100µM is listed as greater than 100 to simplify the comparison between the 6MP and prodrugs.

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