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ANTIPROLIFERATIVE ACTIVITY OF NEW DERIVATIVE OF 6-MERCAPTOPURINE (6MP), 1-ETHYL 3-((7 H-PURINE-6-YL) DISULFANYL)-2-(2-(6-METHOXYNAPHTHALEN-2-YL) PROPANAMIDO)PROPANOATE (CPDA) AGAINST 60 HUMAN CELL LINES

Mohammed A. Taher
College of pharmacy-university of Baghdad/ clinical laboratory sciences department

ABSTRACT
Possible mutual prodrug of naproxen with 6-Mercaptopurine has been designed to be synthesized for targeting cancer tissues and this was ethyl 3-((7 H-purine-6-yl) disulfanyl) -2- (2- (6-methoxynaphthalen-2-yl) propanamido) propanoate. Cytotoxicity of compound (A) (CpdA) was evaluated against 60 human cell lines by National Cancer Institute. It was found that Cytoxicity of Cpd A was most cytotoxic than 6MP in most cell lines except leukemia histotype in screen for the GI50 end point. Values of TGI of Cpd A like that of 6-MP which were >100 Mμ except cell lines, HOP-92 and NCI-H522 (NSCL) LOX IMVI, MALME-3M , M14, SK-MEL-2 and SK-MEL-5 (melanoma), and TK-10 (renal) where TGI values obtained after CpdA treatment were less than for 6MP. The mechanism responsible for the increase in cytotoxicity observed after treatment with prodrug is still not fully understood. The median GI50 values of breast, colon, renal, NSCL and melanoma cell lines were more sensitive after treatment with CpdA than to 6MP as the GI50 values after treatment with (CpdA) were less than 6MP. In conclusion most of cell lines in different tissue except leukemia were more sensitive toward (CpdA) than 6MP.

KEY WORDS: 6- mercaptopurine, derivative, human cancer cell lines, cytototoxicity.

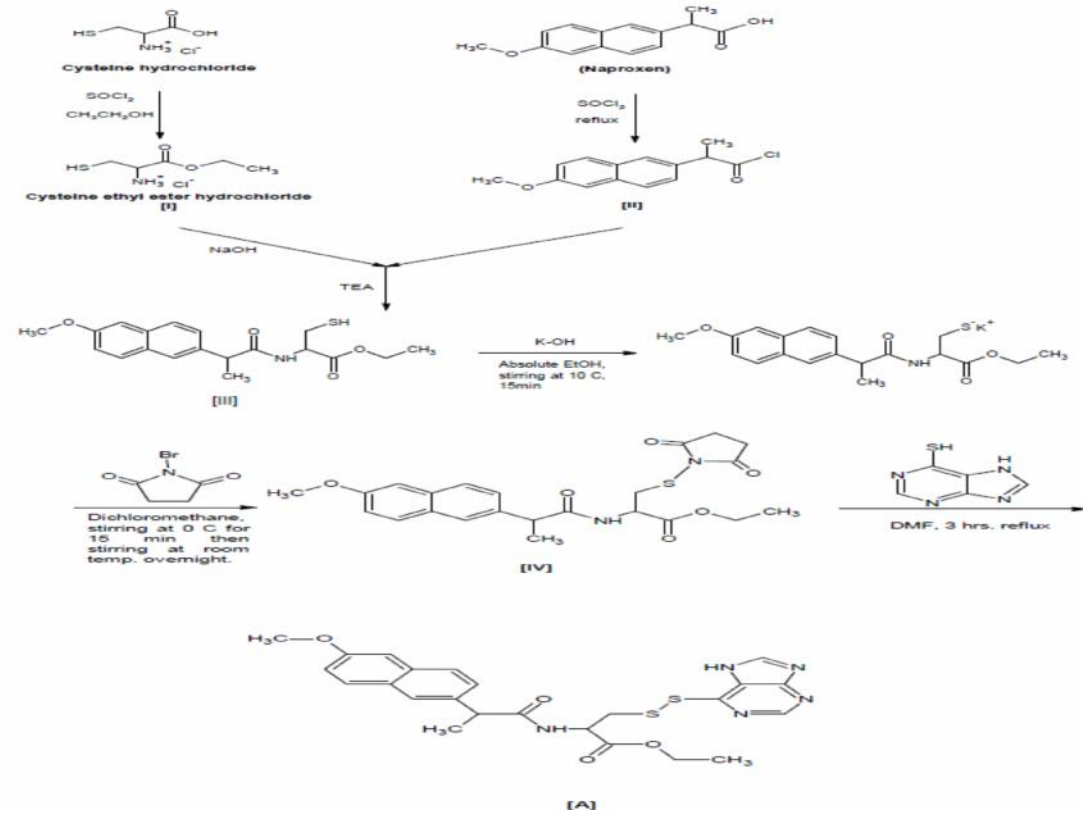
INTRODUCTION

Six-mercaptopurine (6MP) 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-phosphoribosyltransferase (HGPRT)[4] and increased levels of glutathione have been lnked with drug resistance[5]. One promising aspect for improving the distribution of (6MP) appears to be the prodrug approach by which the pharmacokinetic patterns and ultimately therapeutic success can be obtained through introduction of promoities with ideal physiochemical properties. In previous study we synthesized two derivatives of (6MP), 6-[(5-pyridine-yl-1,2,3,4-oxadiaole-2-yl)dithiol]-9H-purine (38) and purine -6yl-benyldithiocarbamate (45).The results showed both compounds were more cytotoxic than 6MP and exhibited high growth –inhibitory activities in renal cancer cell line but compound 45 was more cytotoxic than 38 against ovarian cell line[6].

However mutual prodrug is consisting of two pharmacologically active drugs covalently connected together in which each drug acts as a carrier for the other one. A chemical connection between anticancer agent and NSAID could be regarded as a mutual prodrug in which non-steroidal anti-inflammatory drugs (NSAID) and anticancer agent serves as a promoiety for each other. In addition, several studies provide compelling evidence that NSAIDs have antineoplastic properties and thus might give synergistic action to the former drug. In this study, one possible mutual prodrug of naproxen with 6-Mercaptopurine has been designed to be synthesized for targeting cancer tissues and this was: 1- Ethyl 3-((7 H-purine-6-yl) disulfanyl)-2-(2-(6-methoxynaphthalen-2- yl) propanamido)propanoate (CpdA).

MATERIALS & METHODS

The synthetic procedure for the designed compound A (CpdA) is illustrated in schemes (1). The starting material for generation of the target (CpdA) was cysteine hydrochloride which was converted to cysteine ethyl ester hydrochloride, compound [I], by activation of the carboxylic acid group with thionyl chloride to give its acyl derivative followed by esterification with ethanol (scheme1). Compound [II] was synthesized by refluxing naproxen with thionyl chloride to produce its acyl derivative (naproxen acid chloride). Compound [III] was synthesized by reaction of compound [I] with compound [II] in the presence of sodium hydroxide, in this reaction the amine group of compound [I] was reacted with acyl derivative of compound [II] forming amide bond. After that, compound [III] was converted to potassium salt form and reacted with N-bromosuccinamide to produce compound [IV]. Finally compound [IV] was refluxed with 6-mercaptopurine to produce target (CpdA).



Scheme 1: synthesis of Compound (A)

The NCI screening procedures were described in detail[7]. 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 37oC 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 compound (CpdA) was 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 prodrug: GI50, 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 LC50, 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 by 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 6MP was higher than 100µM, any GI50, TGI, and LD50 value obtained for 6MP and for CpdA that was above 100µM is listed as greater than 100 to simplify the comparison between the 6MP and prodrug.

RESULTS

The in vitro cytotoxicity parameters GI50, TGI and LC50 obtained from CpdA in the NCI's anticancer screening program are listed in table-1. Additionally , these are same in vitro cytotoxicity parameters obtained from thiopurine 6MP are listed in table-2. Treatment with CpdA yielded GI50 values ranging from 1.04 µM to 9.35 µM in the cell lines used to assess the cytotoxicity of CpdA. Furthermore, whereas most TGI values obtained after CpdA treatment where within range of 1.06 µM to >100.The LC50 values are greater than 100Mµ obtained after CpdA treatment. Cell lines that showed a consistency good response toward CpdA treatment at 2 parameters (GI50 and TGI) assessed were HOP-92 and NCI-H522 (non-small cell lung cancer) (NSCL), LOX IMVI, MALME-3M, M14, SK TGI -MEL-2 and SK-MEL-5 (melanoma) and TK-10 (renal).These 8 cell lines have GI50 and TGI less than 8 and 10 respectively. When the responses obtained after CpdA treatment with the respective 6-MP, it was revealed that CpdA treatment yielded GI50 values lower than those obtained after 6-MP treatment in all cell line used, except 16 cell lines (CCRF-

CEM, HL-60(TB), K-562, MOLT-4 and SR (leukemia), HOP-92 and NCI-H522 (NSCL), SF-539 (CNS), LOX IMVI , M14 (melanoma), OVCAR-3, OVCAR-5, and SK-OV-3 (ovarian) and 786-0 and CAK-1 (renal), and DU-145 (prostate). Additionally the CpdA treatment gave TGI values of cell lines (HOP-92 and NCI-H522 (NSCL), LOX IMVI, MALME-3M, M14, SK-MEL-2 and SK-MEL-5 (melanoma) and TK-10 (renal) than those obtained after 6-MP treatment. The median GI50 and TGI values obtained after treatment with CpdA were lower than those obtained after treatment with 6MP treatment (table-3). To examine whether cell lines of different histotypes respond differently to treatment with a prodrug compared with its respective 6MP, the median GI50,TGI and LC50 values for cell lines belonging to the same histotypes were calculated for each drug during treatment and compared between the prodrug and respective 6MP. For the nine different histotypes included in the screen, the median GI50,TGI and LC50 values obtained after CpdA treatment were always lower or equal to those obtained after -MP treatment except the leukemia histotypes (table5).

TABLE 1: The parameters GI50, TGI and LC50 for the CpdA in the NCI anticancer screen. Parameters below or above the highest or lowest drug concentration used are listed as 1.04 or >100 respectively. All values are given in micromolar concentration (µM).

Cell type	Tissue type	GI50	TGI	LC50
CCRF-CEM	Leukemia	8	>100	>100
HL-60(TB)	Leukemia	1.04	>100	>100
K-562	Leukemia	7.2	>100	>100
MOLT-4	Leukemia	3.37	>100	>100
RPMI-8226	Leukemia	1.63	>100	>100
SR	Leukemia	3.32	>100	>100
A549/ATCC	NSCL	4.83	>100	>100
HOP-92	NSCL	7.75	4.82	>100
NCI-H23	NSCL	1.198	>100	>100
NCI-H322M	NSCL	2.18	>100	>100
H-460	NSCL	2.43	>100	>100
NCI-H522	NSCL	2.82	9.91	>100
COLO 205	Colon	1.84	>100	>100
HCT-15	Colon	4.59	>100	>100
HCT-15	Colon	1.84	>100	>100
HT29	Colon	1.23	>100	>100
KM12	Colon	1.34	>100	>100
SW-620	Colon	1.93	>100	>100
SF-268	CNS	2.54	>100	>100
SF-295	CNS	4.81	>100	>100
SF-539	CNS	3.92	>100	>100
SNB-19	CNS	5.64	>100	>100
SNB-75	CNS	2.57	>100	>100
U251	CNS	4.21	>100	>100
LOX IMVI	Melanoma	3.37	1.70	>100
MALME-3M	Melanoma	3.44	9.31	>100
M14	Melanoma	5.37	3.86	>100
SK-MEL-2	Melanoma	5.61	8.40	>100
SK-MEL-28	Melanoma	6.41	>100	>100
SK-MEL-5	Melanoma	2.16	1.06	>100
UACC-257	Melanoma	3.60	>100	>100
UACC-62		3.38	>100	>100
OVCAR-3	Ovarian	3.71	>100	>100
OVCAR-4	Ovarian	7.85	>100	>100
OVCAR-5	Ovarian	9.35	>100	>100
OVCAR-8	Ovarian	2.24	>100	>100
SK-OV-3	Ovarian	3.36	>100	>100
786-0	Renal	2.80	>100	>100
ACHN	Renal	2.00	>100	>100
CAKI-1	Renal	3.59	>100	>100
SN12C	Renal	4.67	>100	>100
TK-10	Renal	1.82	3.55	>100
UO-31	Renal	4.91	>100	>100
PC-3	Prostate	3.47	>100	>100
DU-145	Prostate	9.21	>100	>100
MDA- MB-231/ATCC	Breast	4.41	>100	>100
HS 578T	Breast	1.66	>100	>100

TABLE 2: The parameters GI50,TGI and LC50 obtained for 6MP in the NCI anticancer screen. Parameters below or above the highest or lowest drug concentration used are listed as <0.35 or >100 respectively. All values are given in micromole concentration (μM).

Cell type	Tissue type	GI50	TGI	Lc50
CCRF-CEM	Leukemia	1.26	>100	>100
HL-60(TB)	Leukemia	2.44	>100	>100
K-562	Leukemia	o.35	>100	>100
MOLT-4	Leukemia	1.16	>100	>100
RPMI-8226	Leukemia	1.76	>100	>100
SR	Leukemia	1.24	>100	>100
A549/ATCC	NSCL	30.1	>100	>100
HOP-92	NSCL	2.94	>100	>100
NCI-H23	NSCL	3.89	>100	>100
NCI-H322M	NSCL	9.46	>100	>100
H-460	NSCL	5.53	>100	>100
NCI-H522	NSCL	1.62	>100	>100
COLO 205	Colon	4.94	>100	>100
HCT-15	Colon	2.37	>100	>100
HCT-15	Colon	4.57	>100	>100
HT29	Colon	4.02	>100	>100
KM12	Colon	7.57	>100	>100
SW-620	Colon	6.38	>100	>100
SF-268	CNS	4.11	>100	>100
SF-295	CNS	5.81	>100	>100
SF-539	CNS	2.49	>100	>100
SNB-19	CNS	>100	>100	>100
SNB-75	CNS	8.75	>100	>100
U251	CNS	13.7	>100	>100
LOX IMVI	Melanoma	0.38	>100	>100
MALME-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	0.86	>100	>100
OVCAR-4	Ovarian	8.08	>100	>100
OVCAR-5	Ovarian	8.63	>100	>100
OVCAR-8	Ovarian	2.54	>100	>100
SK-OV-3	Ovarian	1.03	>100	>100
786-0	Renal	2.03	>100	>100
ACHN	Renal	6.27	>100	>100
CAKI-1	Renal	3.21	>100	>100
SN12C	Renal	23.8	>1oo	>1oo
TK-10	Renal	1.94	>100	>100
UO-31	Renal	6.85	>100	>100
PC-3	Prostate	4.39	>100	>100
DU-145	Prostate	2.51	>100	>100
MDA-MB-231/ATCC	Breast	24.4	>100	>100
HS 578T	Breast	14.2	>100	>100

TABLE 3: The median GI50, TGI and LC50 values obtained from 6-MP and CpdA in NCI anticancer screen

Parameters	6MP	CpdA
GI50	6.18	3.84
TGI	>100	5.32
LC50	>100	>100

TABLE 4: The median GI50, TGI and LC50 values obtained from treatment with 6-MP and CpdA in different values are given in micromolar (µM)

Cell line	GI50		TGI		LC50	
	6MP	CpdA	6MP	CpdA	6-MP	CpdA
Leukemia	1.57 (1-9)	4.09 (6-9)	>100 (1-9)	>100 (4-9)	>100 (1-9)	>100(1-9)
NSCL	8.92 (8-9)	3.35 (4-9)	>100 (1-9)	7.36 (3-9)	>100 (1-9)	>100 (1-9)
Colon	4.97 (5-9)	2.12 (1-9)	>100(1-9)	>100 (4-9)	>100(1-9)	>100 (1-9)
CNS	6.97 (6-9)	3.94 (5-9)	>100 (1-9)	>100 (4-9)	>100 (1-9)	>100 (1-9)
Melanoma	4.87 (4-9)	4.16 (7-9)	>100 (1-9)	4.86 (2-9)	>100 (1-9)	>100 (1-9)
Ovarian	4.22 (3-9)	5.30 (8-9)	>100 (1-9)	>100 (4-9)	>100 (1-9)	>100 (1-9)
Renal	7.35 (7-9)	3.29 (3-9)	>100 (1-9)	3.55(1-9)	>100 (1-9)	>100 (1-9)
Prostate	3.45 (2-9)	6.34 (9-9)	>100 (1-9)	>100 (4-9)	>100 (1-9)	>100 (1-9)
Breast	19.3 (9-9)	3.03 (2-9)	>100 (1-9)	>100 (4-9)	>100 (1-9)	>100 (1-9)

DISUCSSION

When the cytotoxicity parameters obtained for CpdA in NCI's anticancer screen were compared with the cytoxicity parameters calculated for 6MP using data obtained from standard agent database, it is evident that the prodrug showed enhanced in virto cytoxicity compared with 6MP. Cytoxicity of Cpd A was most cytotoxic than 6MP in most cell lines except leukemia in screen for the GI50 end point. Values of TGI of CpdA like that of 6MP which were >100 except cell lines ,HOP-92 and NCI-H522 (NSCL) LOX IMVI,MALME-3M , M14, SK-MEL-2 and SK-MEL-5 (melanoma) , and TK-10 (renal) where TGI values obtained were less than for 6MP.Values of LC50 obtained for the CpdA are >100 like those of 6MP.The mechanism responsible for the increase in cytotoxicity observed after treatment with prodrug is still not fully understood. The CpdA is synthesized from combination of anticancer agent 6MP and naproxen (NSAID) may enhance the resulting anticancer activity of prodrug towards most cell lines compared with parent compounds. The active drugs can be released inside the cancer cells (hypoxicmedium) by intracellular thiol such as glutathione that is much higher in tumor cells as compared to normal cells[8] and also by the enzyme Gama-interferon-inducible lysosomal thiol reductase (GILT) which also facilitates cleaving of disulfide bonds within the cancer cells[9]. However the median value obtained for all cell lines after CpdA treatment was lower than those after treatment with 6MP at GI50 and TGI end point and the LC50 values are more than100 µM for both compounds (table-3). Although these findings, in the leukemia cell lines, the highest median GI50 values obtained after treatment by CpdA with equal median TGI and LC50 compared with 6MP (table-4).This indicates that 6MP maintains its antileukemic activity despite high changes in activity against rest of different cell lines. After treatment with CpdA, the panel of breast cancer cells exhibited very low median GI50 value, whereas their median TGI and LC50 were above 100 µM. The high sensitivity of breast cancer cell lines to prodrug should be kept in consideration as currently there is need to more effective with less toxic effect to breast cancer. Colon cancer cell lines were more sensitive to CpdA compared with 6MP. High levels of COX-2 have been noted in carcinomas of colon[10], Anticancer effect of NSAIDs is mainly due to inhibition of COX-2, because COX-2 has a very important role in tumorigenesis and tumor progression. Treatment of colorectal cancer cells, expressing only COX-2, with COX-2 selective inhibitors induced apoptosis[11]. Prostaglandins derived from COX-2, but not COX-1, regulate tumor-induced angiogenesis in mice implanted with human tumors [12].Compound A may have the ability co inhibit COX-2 as naproxen moiety could maintain this effect after incorporation with 6MP.The renal cancer cell lines are highly sensitive to CpdA. The toxicity of 6MP could be in part due to the generation of reactive oxygen species and oxidative stress, and further indicate that in isolated renal cells, 6MP toxicity occurs via multiple pathways [13]. However the GI5o and TGI values of CpdA against renal, NSCL and melanoma cancer cell lines are comparable or better than 6MP suggests that CpdA may possess similar or better antitumor activity than 6MP.

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