European Journal of Medicinal Chemistry 47 (2012) 445–451

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Novel 1,3,4-heterodiazole analogues: Synthesis and *in-vitro* antitumor activity

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ARTICLE INFO

Article history:
Received 29 September 2011
Received in revised form
3 November 2011
Accepted 5 November 2011
Available online 15 November 2011

Keywords:
Heterodiazole
1,3,4-Oxadiazole
Thiadiazole,[3,2-*a*]pyimidine
Antitumor activity

ABSTRACT

The synthesis of some new heterodiazole and their annulated imidazo[2,1-*b*]1,3,4-oxa/thiadiazolone **6a**–**d**, **7a**–**d**; 1,3,4-oxa or thiadiazole[3,2-*a*]pyrimidine diamine **8a**–**d** and 1,3,4-oxa or thiadiazole-3-piperidino-1-propamide **11a**,**b** derivatives have been described. The obtained compounds were evaluated for their *in-vitro* antitumor activity. A single dose (10 μM) of the test compounds were used in the full National Cancer Institute (NCI) 60 cell lines panel assay. Compounds **6c** and **6d** displayed appreciable anticancer activity against leukemia, non-small cell lung, CNS and showed moderate activity against colon, melanoma, and breast cancer cells lines. Compound **6c** possessed remarkable broad-spectrum antitumor activity which almost 4 fold more active than the known drug 5-FU with GI_{50}, TGI, and LC_{50} values of 6.0, 17.4, and 55.1 μM, respectively.

1. Introduction

Cancer is continuing to be a major health problem in developing as well as undeveloped countries [1–5]. The great cancer incidence worldwide increases the search for new, safer and efficient anticancer agents, aiming the prevention or the cure of this illness. In spite of all the efforts to combat cancer, the success of the treatment of certain types of tumors has shown little progress due to their aggressiveness and the mechanisms of malignant cell metastasis. Moreover, there has been wide interest in compounds containing 1,3,4-oxa/thiadiazole scaffold because of their unique chemical structure and the broad-spectrum of their biological properties [6–16]. They displayed interesting *in-vitro* and *in-vivo* antitumor activity [17–19]. Although many classes of drugs are being used for the treatment of cancer, the need for more potent selective antitumor agents is still not precluded.

Literature survey revealed that, *N*-substituted 2-amino-5-aryl-1,3,4-thiadiazole analogues (**1**) specially its phenyl substituent, exhibit significant *in-vitro* antiproliferative activity [20]. Moreover, Levamisole (**2**) appears to be the most effective in patient's drug against small tumor burdens as it acts by stimulating the responsiveness of lymphocytes toward tumor antigens [21]. The imidazo [2,1-*b*]thiazole derivatives of Levamisole (**3**) and the other imidazo [2,1-*b*][1,3,4]thiadiazole analogues (**4**) have been reported as potential antitumor agents [22,23], Fig. 1. In view of above mentioned facts and as an attempt to obtain new potent antitumor agents with good bioavailability and low toxicity, we would like to describe herein the synthesis and anticancer activity of a new series of *N*-substituted 2-amino-5-aryl-1,3,4-oxa or thiadiazole and 7,7a-dihydro-imidazo[2,1-*b*][1,3,4]oxa or thiadiazole analogues.

2. Results and discussion

2.1. Chemistry

The target compounds **6**–**8**, **10**, and **11** were synthesized as outlined in Schemes 1 and 2. 5-Aryl-1,3,4-oxa or thiadiazol-2-amine exemplified in this manuscript (**5a**–**d**) were prepared according to reported methods [24,25]. Acylation of the amino group of **5a**–**d** with chloroacetyl chloride [26] or oxalyl chloride in dry benzene in presence of potassium carbonate followed by cyclization on *N*3 gave the targets (**6a**–**d**) and (**7a**–**d**), respectively. Moreover, reaction of **5a**–**d** with malononitrile in absolute ethanol and calculated amount of triethylamine [27] afforded 2-phenyl-8a*H*-[1,3,4]oxa or thiadiazolo[3,2-*a*]pyrimidine-5,7-diamine **8a**–**d**, Scheme 1. On the other hand, reaction of **5a**,**b** with 3-chloropropionyl chloride using different reaction conditions yielded 3-chloro-*N*-(5-(4-chlorophenyl)-1,3,4-oxa or thiadiazol-2-yl) propanamide (**10a**,**b**) rather than the expected cyclized product tetrahydro[1,3,4]oxa or thiadiazolo[3,2-*a*]pyrimidine-7-one (**9**).

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doi:10.1016/j.ejmech.2011.11.013

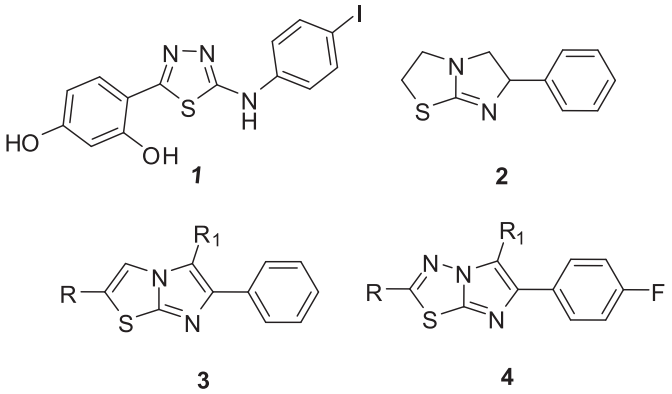


Fig. 1. Structures of some literature active antitumor agents.

Meanwhile, reaction of **10a**,**b** with piperidine in dry acetonitrile [28] afforded **11a**,**b** (Scheme 2). The structures of the obtained compounds was established through spectroscopic (IR, ^{1}H NMR, Mass) as well as elemental analyses data.

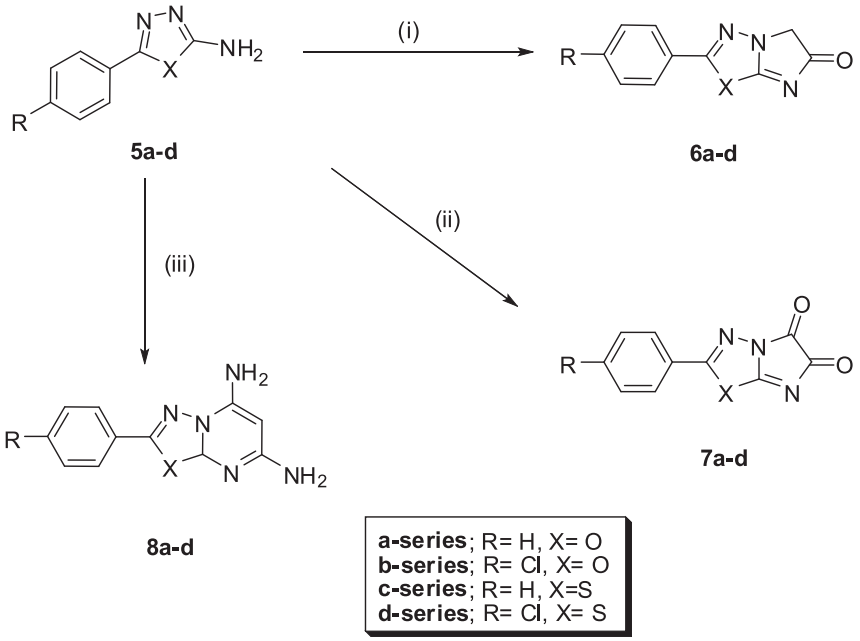
2.2. Preliminary in-vitro antitumor screening

The synthesized compounds (**6a**–**d**, **7a**–**d**, **8a**,**b** and **11a**–**d**) were subjected to the NCI's disease-oriented human cell lines screening assay to be evaluated for their *in-vitro* antitumor activity. A single dose (10 μM) of the test compounds were used in the full NCI 60 cell lines panel assay which includes nine tumor subpanels namely; leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cells [29–32]. The data reported as mean-graph of the percent growth of the treated cells, and presented as percentage growth inhibition (GI %). The obtained results of the tested oxadiazole and thiadiazole analogues **6a**–**d**, **7a**–**d**, **8a,b**, and **11a**–**d**, Table 1; showed distinctive potential pattern of selectivity, as well as broad-spectrum antitumor activity.

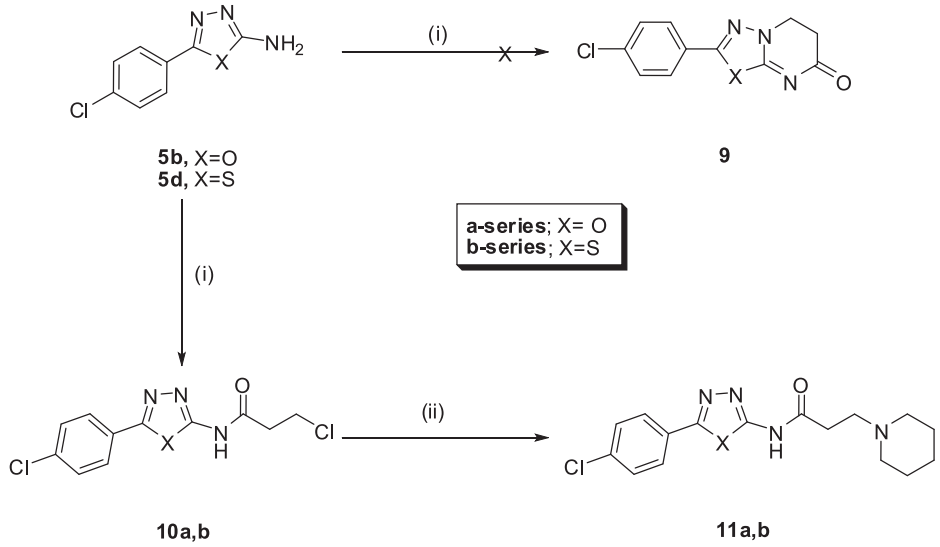
Regarding the activity toward individual cell lines; compound **6c** and **6d** showed selective potency against leukemia cell line CCRF-CEM with GI values of 88.2 and 62.5%, respectively. Meanwhile, leukemia cell line RPMI-8226; non-small cell lung EKVX; CNS cancer SF-295, SNB-75, and U251 proved to be selectively sensitive to **6c** with GI values of 72.2, 94.5, 90.6, 88.3 and 58.3%, respectively. In addition, compounds **6c** and **6d** proved lethal to the melanoma cell line LOX IMVI, ovarian cancer IGORV1, renal cancer cell lines CAKI-1 and UO-31. The same was observed in case of **6c** toward the melanoma cell line MALME-3M. With regard to broad-spectrum antitumor activity; close examination of the data presented in Table 1, revealed that compounds **6c** and **6d** are the most active members of this study, showing effectiveness toward numerous cell lines belong to different tumor subpanels. The same analogy indicated that **8a**, **8b** and **11d** possess moderate antitumor activity. Compounds **7b**, **7d** and **11a** posses' selective potency toward leukemia cell lines, while **6a** posses' selectivity toward renal cancer cell lines. Compound **6c** passed the primary anticancer assay at an arbitrary concentration of 100 μM. Consequently, this active compound was carried over and tested against a panel of 60 different tumor cell lines at a 5-log dose range [29–32]. Three response parameters, GI_{50}, TGI, and LC_{50} were calculated for each cell line, using the known drug 5-Fluorouracil (5-FU) as a positive control. Compound **6c** is almost four fold more active than the positive control 5-FU, with GI_{50}, TGI, and LC_{50} values of 6.0, 17.4, and 55.1 μM, respectively (Fig. 2, Table 2).

2.3. Structure-activity correlation

Structure-activity correlation, based on the number of cell lines proved sensitive toward each of the synthesized individual compounds, revealed that, 7,7a-dihydro-imidazo[2,1-*b*][1,3,4]thiadiazol-5(6*H*)-ones (**6c**,**d**) are more active antitumors than their 7,7a-dihydro-imidazo[2,1-*b*][1,3,4]oxadiazol-5(6*H*)-one (**6a**,**b**) counterparts. The introduction of a carbonyl group at position 6- of compound **6** produced 7,7a-dihydro-imidazo[2,1-*b*][1,3,4]oxadiazole-5,6-diones (**7**) with a dramatic decrease in the antitumor



Scheme 1. Synthesis of the target compounds **6**–**8**: (i) $ClCOCH_2Cl$, K_2CO_3, toluene; (ii) ClCOCOCl, toluene; (iii) $CH_2(CN)_2$, NEt_3, EtOH.



Scheme 2. Synthesis of the target compounds **10** and **11**: (i) $ClCO(CH_2)_2Cl$, DMF; (ii) piperidine, K_2CO_3, acetonitrile.

activity and enhanced selectivity toward leukemia cell lines as shown in compounds **7b** and **7d**. Replacement of the imidazo[2,1-*b*][1,3,4]thiadiazol-5(6*H*)-one nucleus by [1,3,4]oxadiazolo[3,2-*a*]pyrimidine-5,7-diamine produced compound **11** with nearly abolished activity except the 4-chlorophenyl analogue **11d** which retained a moderate broad-spectrum antitumor activity.

3. Conclusion

Compound 2-phenyl-7,7a-dihydro-imidazo[2,1-*b*][1,3,4]thiadiazol-5(6*H*)-one (**6c**), Fig. 2; is a broad-spectrum antitumor agent showing effectiveness toward numerous cell lines belong to different tumor subpanels. Compound **6c** is the most active member of this study with GI_{50}, TGI, and LC_{50} values of 6.0, 17.4, and 55.1 μM, respectively. The synthesized 7,7a-dihydro-imidazo[2,1-*b*][1,3,4]thiadiazol-5(6*H*)-one analogues could be considered as a useful template for future development to obtain more potent antitumor agent(s).

4. Experimental

Unless specified all chemicals were of commercial grade, used without further purification and were obtained from Aldrich Chemical Co. (Milwaukee, WI). Melting points were carried out by the open capillary tube method using a Gallenkamp digital melting point Griffin apparatus 1901 and they are uncorrected. Elemental Microanalyses were recorded using Heraew and Vario El III (elemntar), CHNS analyzer (Germany) at the Micro Analytical Center, Faculty of Science, Cairo University. Infrared Spectra were recorded on Jasco FT.IR plus 460 Japan, and expressed in wave number (cm^{-1}), using potassium bromide discs. ^1H NMR Spectra were carried out using a Varian Gemini 300 MHz spectrophotometer. The chemical shifts were expressed in δ ppm units using trimethylsilane as the internal standard. The exchangeable protons were exchanged by D_2O. Mass Spectra was recorded on Shimadzu QP-2010 plus. All reactions were monitored by thin layer chromatography. Silica gel/TLC-cards DC- Alufolien-Kieselgel with fluorescent indicator 254 nm; layer thickness 0.2 mm; 20 × 20 cm aluminum cards were used. Petroleum ether: ethyl acetate (1:1) or (1:2) was the adopted solvent system. Compounds **5a**–**d** [24,25] were prepared according to reported procedures.

4.1. Chemistry

4.1.1. General procedure for the preparation of 2-phenylimidazo [2,1-b][1,3,4]oxa/thiadiazol-6(5H)-one (**6a**–**d**)

A mixture of **5a**–**d** (10 mmol) and chloroacetyl chloride (15 mmol, 1.69 g, 1.20 mL) was refluxed in dry toluene (30 mL) in the presence of anhydrous potassium carbonate (1.37 g, 10 mmol) for 8 h. The reaction mixture was then filtered while hot and the filtrate was evaporated to dryness *under vacuum*. The residual mass was washed with water, dried and crystallized from aqueous ethanol.

*4.1.1.1. 2-Phenylimidazo [2,1-b][1,3,4]oxadiazol-6(5H)-one (**6a**).* Yield: 73%; mp 125–127 °C; IR (KBr, cm^{-1}): 3062 (CH aromatic), 2924, 2854 (CH aliphatic), 1693 (C=O); ^1H NMR (DMSO-d_6): δ 3.02, 3.15 (*s*, 2H, CH_2), 7.37–8.57 (*m*, 5H, Ar–H); ^{13}C NMR (DMSO): 166.0.155.3, 130.0, 129.3, 129.0, (2) 128.6, (2) 125.6, 65.3; Anal. Calcd. For $C_{10}H_7N_3O_2$ (201.18): C, 59.70; H, 3.51; N, 20.89. Found: C, 59.40; H, 3.12; N, 20.44.

*4.1.1.2. 2-(4-Chlorophenyl)imidazo[2,1-b][1,3,4]oxadiazol-6(5H)-one (**6b**).* Yield: 78%; mp 320–322 °C; IR (KBr, cm^{-1}): 3078 (CH aromatic), 2951, 2858 (CH aliphatic), 1683 (C=O), 833 (C–Cl); ^1H NMR (DMSO-d_6): δ 3.44 (*s*, 2H, CH_2), 7.28–7.93 (*m*, 4H, Ar–H); EIMS, *m/z*: 237 (M^+ + 2, 8.00%), 236 (M^+ + 1, 15.21%), 235 (M^+, 2.68%); Anal. Calcd. For $C_{10}H_6ClN_3O_2$ (235.63): C, 50.97; H, 2.57; N, 17.83. Found: C, 51.18; H, 2.76; N, 18.20.

*4.1.1.3. 2-Phenylimidazo [2,1-b][1,3,4]thiadiazol-6(5H)-one(**6c**).* Yield: 85%; mp 212–214 °C; IR (KBr, cm^{-1}): 3032 (CH aromatic), 2947, 2835 (CH aliphatic), 1705 (C=O); ^1H NMR (DMSO-d_6): δ 4.48 (*s*, 2H, CH_2), 7.47–7.85 (*m*, 3H, Ar–H), 7.94 (dd, 2H, Ar–H); ^{13}C NMR (DMSO): 165.3, 162.2, 158.0, 130.6, 129.8, (2)129.2, (2)126.8, 42.3; EIMS, *m/z*: 219 (M^+ + 2, 0.26%), 218 (M^+ + 1, 1.50%), 217 (M^+, 0.24%); Anal. Calcd. For $C_{10}H_7N_3OS$ (217.25): C, 55.29; H, 3.25; N, 19.34. Found: C, 55.62; H, 3.14; N, 19.71.

*4.1.1.4. 2-(4-Chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-6(5H)-one (**6d**).* Yield: 83%; mp 246–248 °C; IR (KBr, cm^{-1}): 3024 (CH aromatic), 2947, 2831 (CH aliphatic), 1705 (C=O), 759 (C–Cl); ^1H NMR (DMSO-d_6): δ 4.48 (*s*, 2H, CH_2), 7.62 (dd, 2H, Ar–H), 8.00 (dd, 2H, Ar–H); ^{13}C NMR (DMSO): 165.3, 161.0, 158.3, 135.2, 129.3, (2) 128.7, (2)128.5, 42.2; EIMS, *m/z*: 254 (M^+ +3, 5.20%), 252 (M^+ + 1,

Table 1
Percentage growth inhibition (GI %) of *in-vitro* subpanel tumor cell lines at 10 μM concentration of compounds **6**–**11**. Bold values represents to point out the active compounds and lethal effect.

Subpanel tumor cell lines	% Growth Inhibition (GI %)[a]													
	6a	**6b**	**6c**	**6d**	**7a**	**7b**	**7c**	**7d**	**8a**	**8b**	**8c**	**8d**	**11b**	**11d**
Leukemia														
CCRF-CEM	–	–	**88.2**	**62.5**	–	–	–	–	–	–	–	–	–	–
HL-60(TB)	–	–	21.0	–	–	15.0	–	15.2	15.6	–	–	34.1	–	–
K-562	–	17.2	34.8	18.8	–	13.6	–	12.7	–	–	–	26.9	–	26.0
MOLT-4	–	–	37.5	24.0	–	–	–	10.6	10.4	–	–	20.6	–	19.8
RPMI-8226	–	–	**72.2**	43.5	–	nt	12.6	–	–	–	–	nt	–	–
SR	–	–	36.3	32.5	–	12.1	–	11.1	10.4	–	–	19.9	11.9	13.4
Non-small cell lung cancer														
A549/ATCC	–	–	39.2	–	–	–	–	–	–	–	–	–	–	–
EKVX	–	–	**94.5**	–	–	–	–	–	–	–	–	–	20.4	–
NCI-H226	–	–	27.7	11.4	–	–	–	–	–	–	–	13.1	–	–
NCI-H23	–	–	21.6	14.9	–	–	–	–	–	–	–	–	–	–
NCI-322M	–	–	22.1	31.0	12.5	–	10.8	–	–	–	–	–	–	12.9
NCI-H522	–	18.9	46.5	32.4	–	–	12.4	19.7	–	14.0	–	19.9	19.5	20.9
Colon cancer														
HCT-116	–	–	42.6	39.9	–	–	–	–	–	–	–	–	–	–
HCT-15	–	–	44.8	51.2	–	–	–	–	–	–	–	–	–	–
HT29	–	–	36.2	31.1	–	–	–	–	–	–	–	11.8	–	–
KM12	–	–	21.8	38.3	–	–	–	–	–	–	–	–	–	–
CNS cancer														
SF-268	–	–	20.0	16.5	–	–	–	–	–	–	–	–	–	–
SF-295	14.7	–	**90.6**	38.6	–	–	–	–	15.6	–	–	–	11.0	–
SNB-19	–	–	23.2	18.2	–	–	–	–	–	–	–	–	–	–
SNB-75	–	–	**88.3**	–	–	–	–	–	–	–	11.6	–	–	–
U251	–	–	**58.3**	19.9	–	–	–	–	–	–	–	–	12.0	11.9
Melanoma														
LOX IMVI	–	–	**L**	**L**	–	–	–	–	–	–	–	–	–	–
MALME-3M	–	–	**L**	22.0	–	–	–	–	–	–	–	–	10.7	–
M14	–	–	30.2	31.4	–	–	–	–	–	–	–	–	–	–
MDA-MB-435	–	–	30.4	11.7	–	–	–	–	–	–	–	–	–	–
SK-MEL-2	–	–	14.1	–	–	–	–	–	–	10.3	–	27.5	–	–
SK-MEL-28	–	–	10.4	–	–	–	–	–	–	–	–	17.4	–	–
SK-MEL-5	–	–	35.2	21.2	–	–	–	–	–	–	–	–	24.7	–
UACC-257	–	–	48.4	–	15.2	–	15.5	–	–	–	16.4	–	–	–
UACC-62	–	–	27.8	20.7	–	–	–	–	–	–	–	–	–	–
Ovarian cancer														
IGORV1	–	–	**L**	**L**	–	–	–	–	–	–	15.8	22.3	–	–
OVCAR-8	–	–	35.3	39.8	–	–	–	–	–	–	–	–	–	–
NCI/ADR-RES	–	–	17.9	–	–	–	–	–	–	–	–	–	–	–
Renal cancer														
786–0	–	–	25.1	–	–	–	–	–	–	–	–	–	–	–
A498	10.9	–	nt	–	–	–	–	–	–	–	–	–	11.6	12.7
ACHN	–	–	29.5	17.3	–	–	–	–	–	–	–	–	–	–
CAKI-1	21.2	–	**L**	**L**	–	–	–	–	–	20.5	10.7	–	18.8	–
SN12C	–	–	32.9	20.1	–	–	–	–	–	–	–	–	–	–
TK-10	–	–	25.4	–	–	–	–	–	–	–	–	–	–	–
UO-31	20.4	26.8	**L**	**L**	20.5	11.7	25.7	–	27.0	23.8	36.6	21.9	28.6	16.5
Prostate cancer														
PC-3	–	–	30.1	25.7	–	–	10.7	–	–	16.3	–	14.3	–	14.7
Breast cancer														
MCF7	–	–	46.4	25.7	–	–	–	12.1	–	–	–	–	–	17.3
BT-549	–	–	**53.4**	39.5	–	–	–	12.2	–	–	–	–	–	–
T-47D	–	–	33.2	13.0	–	–	–	–	–	–	–	–	–	–
MDA-MB-468	–	–	**61.5**	–	–	–	–	–	–	–	–	–	–	–

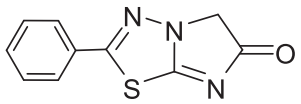
[a] -, GI <10%; nt, not tested; L, compound proved lethal to the cancer cell line.

17.20%); Anal. Calcd. For $C_{10}H_6ClN_3OS$ (251.69): C, 47.72; H, 2.40; N, 16.70. Found: C, 48.08; H, 2.52; N, 16.98.

*4.1.2. General procedure for the preparation of 2-phenylimidazo [2,1-b][1,3,4]oxa/thiadiazol-5,6-dione **7a-d***

To a suspension of the appropriate oxa/thiadiazole **5a**–**d** (10 mmol) in toluene (40 mL) at 60–65 °C, oxalyl chloride (15 mmol, 1.90 g, 1.27 mL) added dropwise with stirring, then refluxed for 6 h. The solid product separated on cooling was filtered, washed twice with 15% potassium carbonate solution (20 mL), dried, and crystallized from methanol.

*4.1.2.1. 2-Phenylimidazo [2,1-b][1,3,4]oxadiazol-5,6-dione (**7a**).* Yield: 69%; mp > 350 °C; IR (KBr, cm^{-1}): 3062 (CH aromatic), 1774, 1708 (C=Os); ^1H NMR (DMSO-d_6): δ 7.46–7.62 (*m*, 3H, Ar–H), 7.75 (dd, 2H, Ar–H); EIMS, *m/z*: 217 ($M^+ + 2$, 0.14%), 215 (M^+, 0.11%); Anal. Calcd. For $C_{10}H_5N_3O_3$ (215.16): C, 55.82; H, 2.34; N, 19.53. Found: C, 55.72; H, 2.54; N, 19.22.



6c: $GI_{50} = 6.0$ μM
$TGI = 17.4$ μM
$LC_{50} = 55.1$ μM

Fig. 2. Structure of the active antitumor agent **6c**.

*4.1.2.2. 2-(4-Chlorophenyl)imidazo[2,1-b][1,3,4]oxadiazol-5,6-dione (**7b**).* Yield: 73%; mp > 350 °C; IR (KBr, cm^{-1}): 3062 (CH aromatic), 1750, 1693 (C=Os), 756 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 8.17 (dd, 2H, Ar–H), 8.32 (dd, 2H, Ar–H); ^{13}C NMR (DMSO): 165.6, 156.1, 149.9, 144.03, 136.2, (2)130.5, (2)128.9, 123.6; EIMS, *m*/*z*: 250 (M^+ + 1, 8.48%), 249 (M^+, 9.32%), 248 (M^+−1, 4.28%); Anal. Calcd. For $C_{10}H_4ClN_3O_3$ (249.61): C, 48.12; H, 1.62; N, 16.83. Found: C, 48.34; H, 1.85; N, 17.10.

*4.1.2.3. 2-Phenylimidazo [2,1-b][1,3,4]thiadiazole-5,6-dione (**7c**).* Yield: 81%; mp 327–329 °C; IR (KBr, cm^{-1}): 3016 (CH aromatic), 1746, 1693 (C=Os); ^{1}H NMR (DMSO-d_6): δ 7.10–7.53 (*m*, 3H, Ar–H), 7.71 (dd, 2H, Ar–H); ^{13}C NMR (DMSO): 160.5, 156.1, 149.3, 135.9, 132.4, (2)129.9, (2) 129.0, 126.3; EIMS, *m*/*z*: 232 (M^+ + 1, 0.17%), 231 (M^+, 0.57%); Anal. Calcd. For $C_{10}H_5N_3O_2S$ (231.23): C, 51.94; H, 2.18; N, 18.17. Found: C, 52.23; H, 2.24; N, 18.58.

*4.1.2.4. 2-(4-Chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5,6-dione (**7d**).* Yield: 88%; mp 204–205 °C; IR (KBr, cm^{-1}): 3012 (CH aromatic), 1748, 1697 (C=Os); ^{1}H NMR (DMSO-d_6): δ 7.37, 7.40 (dd, 2H, Ar–H), 7.88,7.91 (dd, 2H, Ar–H),; ^{13}C NMR (DMSO): 168.8, 161.1, 159.8, 156.8, 135.3, (2) 133.9, (2) 129.3, 127.8; EIMS, *m*/*z*: 267 (M^+ + 2, 16.00%); Anal. Calcd. For $C_{10}H_4ClN_3O_2S$ (265.68): C, 45.21; H, 1.52; N, 15.82. Found: C, 44.92; H, 1.41; N, 15.99.

*4.1.3. General procedure for the preparation of 2-phenyl-8aH-[1,3,4] oxa/thiadiazolo[3,2-a]pyrimidine-5,7-diamine **8a-d***

A mixture of **5a**,**b** (5 mmol), malononitrile (5 mmol, 0.33 g) and triethylamine (2 mL) in absolute ethanol (30 mL) was heated under reflux for 12 h. The precipitate formed was filtered, dried and crystallized from acetic acid.

*4.1.3.1. 2- Phenyl-8aH-[1,3,4]oxadiazolo [3,2-a]pyrimidine-5,7-diamine (**8a**).* Yield: 58%; mp 295–297 °C; IR (KBr, cm^{-1}): 3332, 3244, 3178, (NHs), 3030 (CH aromatic); ^{1}H NMR (DMSO-d_6): δ 7.21–7.92 (*m*, 7H, Ar–H), 8.72 (*s*, 4H, $2NH_2$ exchangeable by D_2O); EIMS, *m*/*z*: 231 (M^+ + 2, 0.44%), 230 (M^+ + 1, 0.76%), 229 (M^+, 0.32%); Anal. Calcd. For $C_{11}H_{11}N_5O$ (229.23): C, 57.63; H, 4.84; N, 30.55. C, 57.69; H, 4.97; N, 31.02.

*4.1.3.2. 2-(4-Chlorophenyl)-8 aH-[1,3,4]oxadiazolo [3,2-a]pyrimidine-5,7-diamine (**8b**).* Yield: 61%; mp 248–250 °C; IR (KBr, cm^{-1}): 3330, 3251, 3112 (NH_2S), 3030 (CH aromatic), 829 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 7.2 (br. *s*, 4H, NH_2s exchangeable by D_2O), 7.52–7.79 (*m*, 6H, Ar–H); EIMS, *m*/*z*: 265 (M^+ + 2, 20.0%), 264 (M^+ + 1, 20.0%), 263 (M^+, 12.0%); Anal. Calcd. For $C_{11}H_{10}ClN_5O$ (263.68): C, 50.10; H, 3.82; N, 26.56. Found: C, 50.35; H, 3.55; N, 26.20.

*4.1.3.3. 2-Phenyl-8aH-[1,3,4]thiadiazolo [3,2-a]pyrimidine-5,7-diamine (**8c**).* Yield: 59%; mp 146–149 °C; IR (KBr, cm^{-1}): 3421, 3348, 3213(NH_2S), 3086 (CH aromatic); ^{1}H NMR (DMSO-d_6): δ 7.22–7.85 (*m*, 7H, Ar–H), EIMS, *m*/*z*: 245 (M^+); Anal. Calcd. For $C_{11}H_{11}N_5S$ (245.30): C, 53.86; H, 4.52; N, 28.55. Found: C, 53.37; H, 4.11; N, 28.89.

*4.1.3.4. 2- (4-Chlorophenyl)-8 aH-[1,3,4]thiadiazolo [3,2-a]pyrimidine-5,7-diamine (**8d**).* Yield: 66%; mp > 350 °C; IR (KBr, cm^{-1}): 3275, 3150(Br., NH_2S), 3089 (CH aromatic), 829 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 7.38 (br. *s*, 4H, NH_2 exchangeable by D_2O), 7.48–7.77 (*m*, 6H, Ar–H); ^{13}C NMR (DMSO): 179.2, 168.7, 155.1, 133.8, 129.7, (2) 128.9, (2)127.7, 120.5, 118.7; Anal. Calcd. For $C_{11}H_{10}ClN_5S$ (279.74): C, 47.23; H, 3.60; N, 25.03. Found: C, 47.55; H, 3.67; N, 24.83.

*4.1.4. General procedure for the preparation of 3-chloro-N-(5-(4-chlorophenyl)-1,3,4-oxa/thiadiazol-2-yl)propanamide **10a**,**b***

A solution of the appropriate oxa or thiadiazole **5b** and/or **5d** (10 mmol) in dry dimethyl formamide (DMF, 10 mL) containing 3-chloropropionyl chloride (15 mmol, 1.90 g, 1.43 mL) was stirred at room temperature (25–30 °C) for 24 h. The solution was poured onto crushed ice (20 mL) and the resulting solid was filtered, washed and crystallized from benzene/methanol (7:3).

*4.1.4.1. 3-Chloro-N-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)propanamide (**10a**).* Yield: 77%; mp 164–166 °C; IR (KBr, cm^{-1}): 3278 (NH), 3062 (CH aromatic), 2900, 2853 (CH aliphatic), 1693 (C=O); ^{1}H NMR (DMSO-d_6): 2.26 (*t*, 2H, $COCH_2$), 3.12 (*t*, 2H, CH_2Cl), 7.57 (dd, 2H, Ar–H), 7.98 (dd, 2H, Ar–H), 10.21 (*s*, 1H, NH exchangeable by D_2O); EIMS, *m*/*z*: 286 (M^+1, 37.49%), 285 (M^+, 11.57%); Anal. Calcd. For $C_{11}H_9Cl_2N_3O_2$ (286.11): C, 46.18; H, 3.17; N, 14.69. Found: C, 46.25; H, 3.31; N, 14.88.

*4.1.4.2. 3-Chloro-N-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)propanamide (**10b**).* Yield: 86%; mp >350 °C; IR (KBr, cm^{-1}): 3163 (NH), 3051 (CH aromatic), 2945, 2854 (CH aliphatic), 1685 (C=O), 833 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.28(*t*, 2H, $COCH_2$), 3.38 (*t*, 2H, CH_2Cl), 7.41 (dd, 2H, Ar–H), 7.91 (dd, 2H, Ar–H), 10.33 (*s*, 1H, NH exchangeable by D_2O); ^{13}C NMR (DMSO): 162.9, 160.9, 158.6, 135.0, 130.5, (2)129.3, (2)128.5, 41.1, 33.5; EIMS, *m*/*z*: 304 (M^+ + 3, 1.57%), 303 (M^+ + 2, 10.62%), 301 (M^+, 15.30%); Anal. Calcd. For $C_{11}H_9Cl_2N_3OS$ (302.17): C, 43.72; H, 3.00; N, 13.91. Found: C, 43.93; H, 3.21; N, 14.24.

Table 2
Compound **6c** median growth inhibitory (GI_{50}, μM), total growth inhibitory (TGI, μM) and median lethal (LC_{50}, μM) concentrations of *in-vitro* subpanel tumor cell lines.

Compound	Activity	Subpanel tumor cell lines[a]									MG-MID[b]
		I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	
6c	GI_{50}	4.1	9.7	9.4	8.7	8.0	7.7	8.9	7.6	6.9	6.0
	TGI	25.9	20.7	24.9	18.8	20.1	33.5	16.6	18.3	23.7	17.4
	LC_{50}	[c]	55.7	67.1	51.3	51.4	83.9	42.3	50.8	71.3	55.1
5-FU	GI_{50}	15.1	[c]	8.4	72.1	70.6	61.4	45.6	22.7	76.4	22.60
	TGI	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]
	LC_{50}	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]

[a] I, leukemia; II, non-small cell lung cancer; III, colon cancer; IV, CNS cancer; V, melanoma; VI, ovarian cancer; VII, renal cancer; VIII, prostate cancer; IX, breast cancer.
[b] Full panel mean-graph midpoint (μM).
[c] Compounds showed values > 100 μM.

*4.1.5. General procedure for the preparation of N-(5-(4-chlorophenyl)-1,3,4-oxa/thiadiazol-2-yl)-3-(piperidin-1-yl)propamide **11a,b***

To a solution of compounds **10a** and/or **10b** (10 mmol) in dry acetonitrile (30 mL) in the presence of anhydrous potassium carbonate (1.37g, 10 mmol), piperidine (12 mmol, 1.02 g,1.18 mL) was added dropwise with stirring. Then, the mixture was refluxed for 24 h. The reaction mixture was filtered while hot, the filtrate was evaporated to dryness .The obtained solid was crystallized from acetone.

*4.1.5.1. N-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-3-(piperidin-1-yl)propamide (**11a**).* Yield: 62%; mp 295–297 °C; IR (KBr, cm^{-1}): 3155 (NH), 3032 (CH aromatic), 2924, 2854 (CH aliphatic), 1761(C=O); ^{1}H NMR (DMSO-d_6): δ 1.14–1.16 (*m*, 2H, CH_2 (C_4)), 1.20–1.22 (*m*, 4H, CH_2 ($C_{3,5}$)), 2.86–3.06 (*m*, 6H, CH_2 ($C_{2,6}$), + $COCH_2$), 3.11 (*t*, 2H, CH_2N, $J = 8.4$), 7.46 (dd, 2H, Ar–H), 7.93 (dd, 2H, Ar–H), 11.64 (*s*, 1H, NH exchangeable by D_2O); EIMS, *m/z*: 334 (M^+, 9.58%), 333 (M^+-1, 2.54%); Anal. Calcd. For $C_{16}H_{19}ClN_4O_2$ (334.80): C, 57.40; H, 5.72; N, 16.73. Found: C, 57.18; H, 5.89; N, 17.06.

*4.1.5.2. N-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)-3-(piperidin-1-yl)propamide (**11b**).* Yield: 68%; mp 143–145 °C; IR (KBr, cm^{-1}): 3178 (NH), 3020 (CH aromatic), 2935, 2854 (CH aliphatic), 1693 (C=O), 829 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 1.51–1.69 (*m*, 2H, CH_2 (C_4)), 1.71–1.77 (*m*, 4H, CH_2 ($C_{3,5}$)), 2.92–3.03 (*m*, 6H, CH_2 ($C_{2,6}$) + $COCH_2$), 3.35 (*t*, 2H, CH_2N, $J = 8.4$), 7.57 (dd, 2H, Ar–H), 7.94 (dd, 2H, Ar–H), 10.2 (*s*, 1H, NH exchangeable by D_2O); EIMS, *m/z*: 354 ($M^+ + 4$, 2.00%), 353 ($M^+ + 3$, 4.12%), 352 ($M^+ + 2$, 3.62%), 351 ($M^+ + 1$, 5.00%), 350 (M^+, 14.37%); Anal. Calcd. For $C_{16}H_{19}ClN_4OS$ (350.86): C, 54.77; H, 5.46; N, 15.97. Found: C, 54.43; H, 5.21; N, 16.02.

4.2. Antitumor screening

Under a sterile condition, cell lines were grown in RPMI 1640 media (Gibco, NY, USA) supplemented with 10% fetal bovine serum (Biocell, CA, USA), 5×10^5 cell/ml was used to test the growth inhibition activity of the synthesized compounds. The concentrations of the compounds ranging from 0.01 to 100 μM were prepared in phosphate buffer saline. Each compound was initially solubilized in dimethyl sulfoxide (DMSO), however, each final dilution contained less than 1% DMSO. Solutions of different concentrations (0.2 ml) were pipetted into separate well of a microtiter tray in duplicate. Cell culture (1.8 ml) containing a cell population of 6×10^4 cells/ml was pippeted into each well. Controls, containing only phosphate buffer saline and DMSO at identical dilutions, were also prepared in the same manner. These cultures were incubated in a humidified incubator at 37 °C. The incubator was supplied with 5% CO_2 atmosphere. After 48 h, cells in each well were diluted 10 times with saline and counted by using a coulter counter. The counts were corrected for the dilution [28–31].

Acknowledgments

Thanks are due to the NCI, Bethesda, MD, USA for performing the antitumor testing of the synthesized compounds.

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