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Original article

Lead optimization through VLAK protocol: New annelated pyrrolo-pyrimidine derivatives as antitumor agents

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ABSTRACT

The chemometric protocol VLAK was applied to predict improvement of the biological activity of pyrrolo-pyrimidine derivatives as anticancer agents, by using the NCI ACAM Database as depository of antitumor drugs with a known mechanism of action. Among the selected compounds two of these showed a good increase in the antitumor activity. These new pyrrolo-pyrimidine compounds were demonstrated effective against the full panels of NCI DTP tumour human cell lines. The derivative 8-[3-(piperidino)propyl]-4,10-dimethyl-9-phenyl-6-(methylsulfanyl)-3,4-dihydropyrimido[1,2-c]pyrrolo[3,2-e]pyrimidin-2(8*H*)-one reveled efficacious against the leukemia subpanel, in particular the RPMI cell line resulted the most sensitive (pGI_{50} = 6.68). Moreover the derivative 7-(3-Chloropropyl)-9-methyl-5-(methylsulfanyl)-8-phenyl-3H-imidazo[1,2-c]pyrrolo[3,2-e]pyrimidin-2(7H)-one showed a good antitumor activity against the leukemia subpanel with a low cytotoxic activity, above all against the HCT11 human tumour cell line.

The VLAK protocol revealed a good method to design new molecules with good antitumor activity, starting from low active compounds. Moreover this protocol focused on the pyrrolo-pyrimidine derivatives as useful starting point for further development to obtain more potent antitumor agents.

1. Introduction

In the last years different chemometric approaches and statistical facilities were applied in the attempt to rationalize and to correlate biological activities with molecular descriptors. The Principal Component Analysis (PCA) applied on a molecular descriptors matrix resulted to be a robust method to classify HSP90 inhibitors [1]. Moreover in a previous work the same multivariate approach was revealed effective in the prediction of mechanism of action for anticancer drugs [2]. Recently, we have developed a protocol, named Virtual Lock-And-Key (VLAK), able to predict suitable biological target for molecular structures with unknown biological activity, by means molecular descriptors calculation [3]. The VLAK protocol was applied in the attempt to improve the biological activity of heterocyclic derivatives with poor antitumor effect.

In particular in a previous work the synthetic use of *N*-(BisMethylthio)Methylenamino Acids (BMMAs), in domino reactions, had led to the annelated pyrrolo-pyrimidines depicted in Fig. 1, which showed no relevant antiproliferative activity [4].

Representative compounds of each of these series were selected for screening tests by the National Cancer Institute NCI (Bethesda, USA) in the Developmental Therapeutics Program (DTP). In the primary anticancer screening assay, tested against 3-cell line panel consisting of the MCF7 (Breast), NCI-H460 (Lung), and SF-268 (CNS) the annelated pyrrolo-pyrimidines resulted generally inactive up to 10 μM concentration. The same compounds tested against the human intestinal adenocarcinoma (LoVo cell line), although being less active than the reference drug [doxorubicin (DOXO)], exhibited a moderate antiproliferative activity with IC_{50} = 10.5–41.2 μM (Fig. 1) [4].

It is reported in literature as heterocyclic compounds, with poor biological activity, can improve the anticancer activity against a large spectrum of human tumour cell lines, if properly decorated with selected side chains [5]. Therefore with the aim to enforce the biological activity of the pyrrolo-pyrimidine derivatives (Fig. 1), the VLAK protocol was applied. Thus an *in house* database of structures containing the annelated pyrrolo[3,4-*e*]pyrimidines (**1**–**6**) and pyrrolo[3,2-*e*]pyrimidines (**7**–**10**), suitably functionalized with a large number of side chains (**A**-**T**), was built (Fig. 2) and submitted to VLAK protocol in order to identify new potential anticancer derivatives.

The selected derivatives were synthesized and biologically tested to validate the VLAK methodology and to confirm the chemometric protocol capability as tool for the identification of new potential antitumor compounds.

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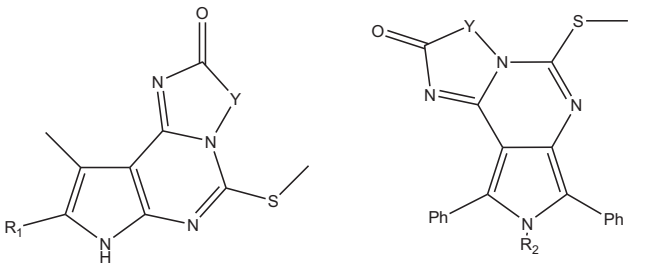


Fig. 1. Annelated pyrrolo[3,2-*e*]pyrimidines and pyrrolo[3,4-*e*]pyrimidines with low antitumor activity against the human intestinal adenocarcinoma (LoVo cell line).

2. Results and discussion

2.1. Chemometric protocol

In order to release a lock it is necessary that the pins of the lock fit to the key profile (Fig. 3). In the VLAK protocol the molecular descriptors are considered the pins of the biological target that, as Fisher model suggested in his famous Lock-and-Key model [6–9], is comparable to a lock.

The first step of the VLAK protocol consists in the conversion of the biological target in a "lock model" in which the keys (the structures) could be "fitted" [3].

The NCI AntiCancer Agent Mechanism (ACAM) Database was chosen as source of biological data for the construction of the VLAK "lock models". It consists in a repository of structures with anticancer activity and a reasonably well-known mechanism of action. Drug screening data are available for each structure as measurement of their growth inhibition ability over a panel of about 60 human tumour cell lines, and all tested molecules are explicitly designed as a training set for neural network and multivariate analysis [10–12]. In particular, this database is constituted by 121 antitumor drugs classified for mechanism of action (Table 1) that in the protocol can be considered like the biological target.

The NCI ACAM Database was chosen because it was demonstrated as the molecular descriptors, processed by multivariate analysis, are able to discriminate the mechanisms of action of the drugs [2]. Thus, for each class of drugs (Alkylating Agents, Antimitotic Agents, Topoisomerase I Inhibitors, Topoisomerase II Inhibitors, RNA/DNA Antimetabolites, and DNA Antimetabolites), a "lock model" was generated starting from own known drugs.

In particular, for each class of drugs with known mechanism of action (MA) (Fig. 4a), the lock model is defined as a sequence of molecular descriptor value ranges. Each range is defined as $\mu Dj(\mathrm{MA}) \pm \sigma Dj(\mathrm{MA})$, where $\mu Dj(\mathrm{MA})$ is the molecular descriptor average value and $\sigma Dj(\mathrm{MA})$ is the standard deviation (Fig. 4b, supporting information SI1).

When the molecular descriptor value Dj of a tested structure X (Fig. 4c) falls within the defined range ($\mu\mathrm{Dj} \pm \sigma\mathrm{Dj}$) $\alpha = 1$ (i.e. D1, D3, and Dj), otherwise $\alpha = 0$ (i.e. D2, D4) (Fig. 4d). At the end each *in house* database structure is converted in a binary sequence. As underlined before, to release a lock all pins must fit; in the proposed protocol, it is supposed that the higher is the number of fitted pins, the higher will be the potential anticancer capability of the investigated compound. Thus, the percentage of affinity A% (Eq. (1)) for each compound belonging to *in house* database was defined for each class (MA) as:

$$A\% = \sum \alpha i,j(\mathrm{MA})/D_{\mathrm{tot}}{*}100 \qquad (1)$$

where $\Sigma\alpha i,j(\mathrm{MA})$ is the sum of all fitted molecular descriptors for the MA class and D_{tot} is the total of the molecular descriptors used in the VLAK protocol.

In supporting information SI2 is reported the output result for all *in house* database against the six lock models representing each class of drugs (MAs). In Table 2 the best ranked derivatives expressed in percentage of affinity (*A%*) are reported: the derivatives **E1**, **H2**, **I1** showed the higher value of *A%* for the class of antimitotic agents (B), while the derivatives **N3** and **O3** for the topoisomerase II inhibitors class (D).

2.2. Chemistry

With these premises, the synthesis of the compounds, selected on the basis of the VLAK protocol [**E1**, **H2**, **I1**, **N3**, **O3**], was successfully achieved through suitable synthetic pathways, as shown in Scheme 1.

The starting annelated pyrrolo-pyrimidines **A1**–**A3** were prepared through the classical approach involving the reaction of 2-amino-3-cyanopyrroles **11a**,**b** with *N*-[bis(methylthio)methylene]amino moiety (BMMA) **12c**,**d** in acetic acid under reflux for an appropriate period of time (0.5–1 h) (Scheme 1) [4].

Derivatives **I1** and **H2** were synthesized in two preparative steps. The reaction of **A1** or **A2** and 1-Bromo-3-Chloropropane, using potassium carbonate as base, gave the chloro derivatives **B1** and **B2** in good yields. These key intermediates, upon heating under reflux with the appropriate amine in *solvent free* condition, gave the targeted compounds **I1** and **H2** (yields 66% and 76%, respectively).

On the other hand compounds **E1**, **N3**, and **O3**, bearing a suitable side-chain were prepared through the key intermediates **C1** and **C3**. Thus, these derivatives were obtained from the sodium salts of **A1** or **A3**, generated *in situ* by using potassium carbonate, and ethyl

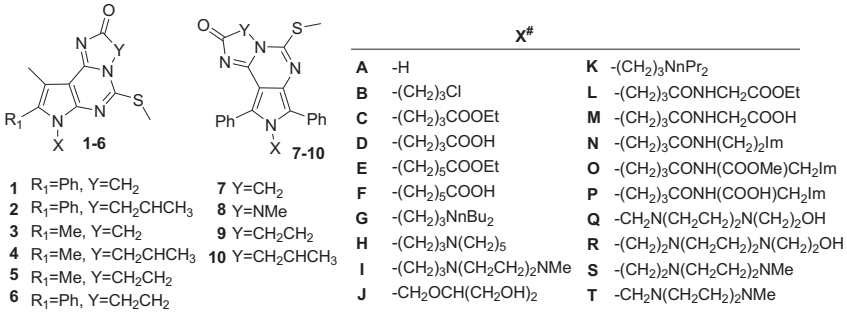


Fig. 2. Annelated pyrrolo[3,2-*e*]pyrimidines (**1**–**6**) and pyrrolo[3,4-*e*]pyrimidines (**7**–**10**) *in house* database submitted to VLAK protocol. #Im = imidazol-5-yl.

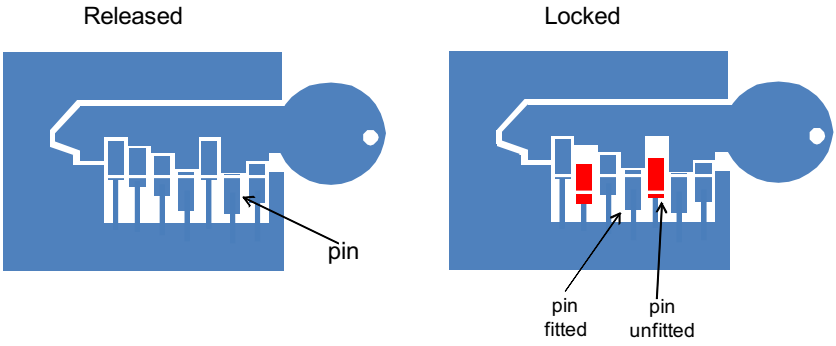


Fig. 3. Lock release mechanism.

4-bromobutyrate in dry DMF. Subsequent hydrolysis with NaOH in EtOH/H_2O mixture quantitatively yielded the corresponding carboxylic acids **D1** and **D3**. Derivative **E1** was synthesized by reacting **D3** with glycine ethyl ester, in dry dichloromethane and triethylamine (TEA), and using dicyclohexylcarbodiimide (DCC) as coupling agent.

The amide derivative **O3** and **N3** were obtained from the same key intermediate **D3** upon reaction with L-histidine methyl ester dihydrochloride or histamine respectively, in dry dichloromethane and TEA, and using DCC as coupling agent.

2.3. Biological screening

All the new pyrrolo-pyrimidines [**E1**, **H2**, **I1**, **N3**, and **O3**] and the intermediates [**B1**, **B2**, **C1**, **C3**, **D1**, and **D3**] were subjected to the NCI's disease-oriented human cell lines screening assay to be evaluated for their *in vitro* antitumor activity.

Derivative **B2** did not pass the selection criteria adopted by DTP screening data and consequently was excluded from the *in vitro* test.

A single dose (10 μM) of the accepted compounds was tested against a panel of approximately 60 tumour human cell lines grouped in nine disease subpanels, including leukemia, non small-cell lung, colon, central nervous system, melanoma, ovarian, renal, prostate, and breast tumour cell lines [13–15] (Table 3, Table G% in supporting information SI4).

In this biological protocol, each cell line is inoculated and pre-incubated on a microtiter plate. Test agents are then added at a single concentration and the culture incubated for 48 h. End-point determinations are made with alamar blue [16]. Results for each tested agent are reported as the percent of growth (G%) of the treated cells when compared to the untreated control cells. Compounds, which reduce the growth of any one of the cell lines to approximately 32% or less, are passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range.

The new pyrrolo-pyrimidine derivatives generally showed not exciting growth inhibition values, with few exceptions: in particular derivative **C3** resulted efficacious against the MDA-MB-468 breast cancer cell line (G% = 55), while **I1** was active against SNB-75 (CNS cancer), SR, and RPMI-8226 (leukemia) showing a G% of 51%, 70%, and 66%, respectively.

Derivatives **H2** and **B1** recorded several G% values significantly under the level of 32% and with a G% average of 39% and 60%, respectively (Table 3). Thus they were passed to five-dose concentration screening.

The antitumor activity of a tested compound, in the five-dose screening, is given by three parameters for each cell line: pGI_{50} value (GI_{50} is the molar concentration of the compound that inhibits 50% net cell growth), pTGI value (TGI is the molar concentration of the compound leading to total inhibition of net cell growth), and pLC_{50} value (LC_{50} is the molar concentration of the compound that induces 50% net cell death). Moreover, a mean graph midpoint (MG_MID) is calculated for each of the mentioned parameters, giving an average activity parameter over all cell lines. For the calculation of the MG_MID, insensitive cell lines are included with the highest concentration tested. The discovery of compounds with new selectivity patterns is one of the targets of the DTP screening program. Selectivity of a compound with respect to a certain cell line of the screen is characterized by a high deviation of the particular cell line parameter compared to the MG_MID value. The pGI_{50}, pTGI, and pLC_{50} values for these compounds are reported in Table 4.

An evaluation of the data reported in Table 4 revealed that **B1** and **H2**, showed antiproliferative activity against all the human tumour cell lines investigated, generally in the low micromolar concentration range.

Considering the MG_MID values, the most active compound was demonstrated to be derivative **H2**, at both GI_{50} (5.56) and TGI_{50} (4.83) level (Table 4).

With respect to the tumour subpanel, **H2** resulted particularly effective against all leukemia cell lines and, in particular, against the RPMI-8226 cell line with pGI_{50} of 6.78 (Fig. 5).

It is also of remarkable interest the low number of cell lines giving $pLC_{50} < 4$, highlighting the low toxicity of such compounds (29/60 for **B1** and 27/55 for **H2**). Moreover the derivative **B1** showed a good antitumor effect against the leukemia subpanel with a low cytotoxic activity above all against the HCT11 tumour cell line.

3. Conclusions

In this work the VLAK protocol was applied for the optimization of annelated pyrrolo-pyrimidine derivatives as antitumor agents. By using the NCI ACAM Database as depository of antitumor drugs with a known mechanism of action, the VLAK protocol was applied on an *in house* database of pyrrolo-pyrimidine derivatives. The selected compounds were synthesized and screened. Two of these (**B1** and **H2**) showed a good increase in the antitumor activity with respect to the starting core structure. In fact the derivative **H2** reveled efficacious against the leukemia subpanel and in particular the RPMI cell line with a $pGI_{50} = 6.68$. Both compounds **H2** and **B1** were demonstrated effective against the full tumour human cell line panels. The VLAK protocol has proved a good method to optimize the search of compounds with improved antiproliferative activity. Moreover this protocol had permitted to identify the pyrrolo-pyrimidine derivatives as useful starting point for further development to obtain more potent antitumor agents.

Table 1
NCI ACAM database drugs.

Name	NSC ID	MA	Name	NSC ID	MA
Busulfan	750	A	Camptothecin derivative	606173	C
Nitrogen mustard	762	A	Camptothecin derivative	606497	C
Chlorambucil	3088	A	Camptothecin derivative	606499	C
Thio-tepa	6396	A	Camptothecin derivative	606985	C
Melphalan	8806	A	Camptothecin derivative	610456	C
Triethylenemelamine	9706	A	Camptothecin derivative	610457	C
Pipobroman	25154	A	Camptothecin derivative	610458	C
Mitomycin C	26980	A	Camptothecin derivative	610459	C
Uracil nitrogen mustard	34462	A	Camptothecin derivative	618939	C
Porfiromycin	56410	A	Camptothecin derivative	629971	C
Fluorodopan	73754	A	Camptothecin derivative	643833	C
CCNU	79037	A	Daunorubicin	82151	D
Methyl CCNU	95441	A	VM-26	122819	D
PCNU	95466	A	Doxorubicin	123127	D
Yoshi-864	102627	A	VP-16	141540	D
cis-Platinum	119875	A[a]	Rubidazone	164011	D
Dianhydrogalactitol	132313	A	m-AMSA	249992	D
Piperazinedione	135758	A	Deoxydoxorubicin	267469	D
Hycanthone	142982	A	*N*,*N*-dibenzyl daunomycin	268242	D
Asaley	167780	A	Menogaril	269148	D
Spirohydantoin mustard	172112	A	Mitoxantrone	301739	D
Chlorozotocin	178248	A	Amonafide	308847	D
AZQ	182986	A	Bisantrene HCL	337766	D
CBDCA	241240	A[a]	Oxanthrazole	349174	D
CHIP	256927	A[a]	Anthrapyrazole derivative	355644	D
Carboxyphthalatoplatinum	271674	A[a]	Pyrazoloacridine	366140	D
Teroxirone	296934	A	Methotrexate	740	E
Hepsulfam	329680	A	5-Fluorouracil	19893	E
Clomesone	338947	A	5-Azacytidine	102816	E
Piperazine ../drugs/mainator	344007	A	Dichlorallyl lawsone	126771	E
Cyclodisone	348948	A	Aminopterin derivative	132483	E
Mitozolamide	353451	A	Aminopterin derivative	134033	E
Cyanomorpholinodoxorubicin	357704	A	Baker's soluble antifol	139105	E[a]
Tetraplatin	363812	A[a]	Pyrazofurin	143095	E
BCNU	409962	A	Ftorafur (pro-drug)	148958	E
Colchicine	757	B	L-alanosine	153353	E
Colchicine derivative	33410	B	Acivicin	163501	E
Vinblastine sulfate	49842	B	Methotrexate derivative	174121	E
Vincristine sulfate	67574	B	Aminopterin derivative	184692	E
Trityl cysteine	83265	B	*N*-(phosphonoacetyl)-L-aspartate (PALA)	224131	E
Taxol	125973	B	5,6-Dihydro-5-azacytidine	264880	E
Maytansine	153858	B	Trimetrexate	352122	E[a]
Rhizoxin	332598	B	Brequinar	368390	E
Thiocolchicine	361792	B	An antifol	623017	E
Dolastatin 10	376128	B	Thioguanine	752	F
Allocolchicine	406042	B	Thiopurine	755	F
Taxol derivative	608832	B	Guanazole	1895	F
Halichondrin B	609395	B	2′-Deoxy-5-fluorouridine	27640	F
Camptothecin	94600	C	Hydroxyurea	32065	F
Camptothecin derivative	95382	C	Pyrazoloimidazole	51143	F
Camptothecin, Na salt	100880	C	Ara-C	63878	F
Camptothecin derivative	107124	C	Beta-TGDR	71261	F
Camptothecin derivative	176323	C	Alpha-TGDR	71851	F
Camptothecin derivative	249910	C	3-HP	95678	F
Camptothecin derivative	295500	C	5-HP	107392	F
Camptothecin derivative	295501	C	Inosine glycodialdehyde	118994	F
Morpholinodoxorubicin	354646	C	5-Aza-2′-deoxycytidine	127716	F
Camptothecin derivative	364830	C	Cyclocytidine	145668	F
Camptothecin derivative	374028	C	Aphidicolin glycinate	303812	F
Aminocamptothecin	603071	C	Macbecin II	330500	F
Camptothecin derivative	606172	C			

MA, mechanism of action; A, Alkylating Agents; B, Antimitotic Agents; C, Topoisomerase I Inhibitors; D, Topoisomerase II Inhibitors; E, RNA/DNA Antimetabolites; F, DNA Antimetabolites.
[a] Excluded in the lock models generation (see Experimental section).

4. Experimental

4.1. Computational details

The compounds contained in the NCI AntiCancer Agents Mechanism Database (NCI ACAM Database) [10–12], were drawn and optimized *in vacuo* by ligprep of MAESTRO SUITE [17]. The NCI ACAM Database entries containing cations or consisting of a mix of two structures were excluded. Thus the starting database was constituted from 114 compounds (supporting information SI3) classified in six mechanism of action: 30, Alkylating Agents; 13, Antimitotic Agents; 24, Topoisomerase I Inhibitors; 15, Topoisomerase II Inhibitors; 16, RNA/DNA Antimetabolites; 16, DNA Antimetabolites (Table 1). CODESSA PRO software was used for the molecular descriptors

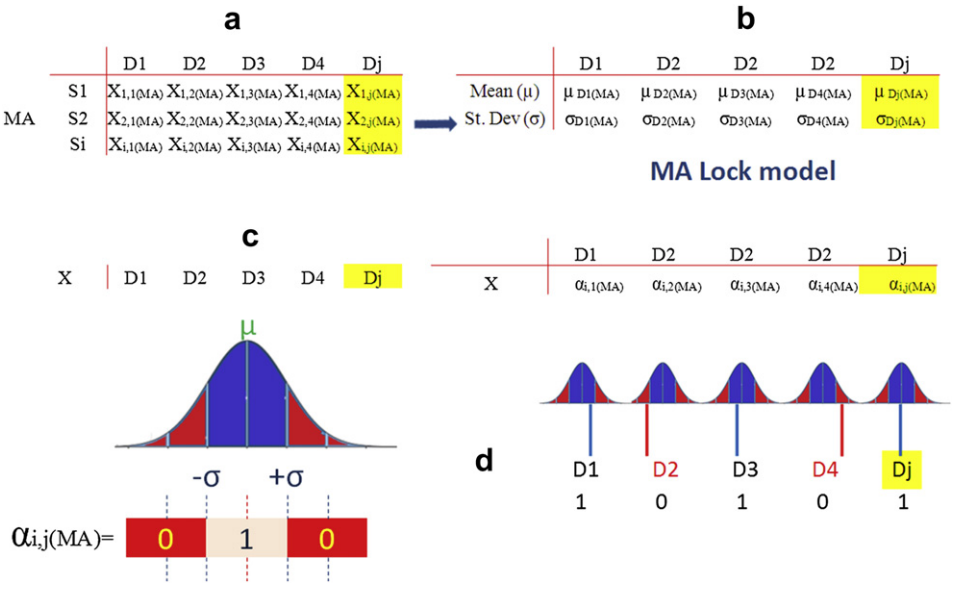


Fig. 4. VLAK protocol applied on ACAM database.

calculation [18]. For all structures 286 molecular descriptor belonging to different classes were calculated (supporting information SI1).

4.2. Chemistry

All melting points (°C) were determined on a Büchi–Tottoli capillary apparatus and are uncorrected; IR spectra were determined in bromoform with a Jasco FT/IR 5300 spectrophotometer; ^{1}H NMR, ^{13}C NMR spectra were recorded in DMSO-d6 solution (TMS as internal reference) at 200 and 50.3 MHz, respectively, using a Bruker AC-E series 200 MHz spectrometer. Microanalyses were in agreement with theoretical values ±0.4%. Thin layer chromatography was performed on precoated (0.25 mm) silica gel GF_{254} plates; compounds were detected with 254 nm UV lamp. Column chromatography was performed with Merck silica gel 230–400 mesh ASTM or with a Biotage FLASH40i chromatography module (prepacked cartridge system).

4.2.1. 7-(3-Chloropropyl)-9-methyl-5-(methylsulfanyl)-8-phenyl-3H-imidazo[1,2-c]pyrrolo[3,2-e]pyrimidin-2(7H)-one (*B1*)

To a stirred suspension of **A1** (1 g, 3.2 mmol) in dry *N*,*N*-dimethylformamide (10 mL), potassium carbonate (1.40 g, 10.1 mmol) and 1-Bromo-3-Chloro-propane (1 mL, 10.0 mmol), were added. The mixture was heated at 80 °C in an oil bath for 1 h, cooled to room temperature and poured into ice and cold water. The crude residue was collected by filtration and, after drying, purified by column chromatography using a dichloromethane/ethyl acetate mixture in gradient as eluent.

Table 2
In house database virtual screening selected hits (*A*%).

Id	MA					
	A	B	C	D	E	F
E1	49.7	**91.6**	59.1	81.5	55.2	32.9
H2	46.9	**91.3**	49.0	68.2	53.5	30.4
I1	46.9	**91.3**	55.6	75.9	59.4	31.8
N3	40.2	71.7	57.0	**90.6**	68.9	34.6
O3	39.9	78.3	42.3	**92.0**	53.1	33.6

MA, mechanism of action; A, Alkylating Agents; B, Antimitotic Agents; C, Topoisomerase I Inhibitors; D, Topoisomerase II Inhibitors; E, RNA/DNA Antimetabolites; F, DNA Antimetabolites. In bold are reported the highest A% values for the selected compounds.

Yield 50.3%. Mp 156.5–157.1 °C. IR: 1656 (CO) cm^{-1}. ^{1}H NMR: δ 1.81–1.99 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.24 (s, 3H, CH_3), 2.71 (s, 2H, CH_2), 3.46 (t, 2H, J = 6.0 Hz, $\underline{CH_2}Cl$), 4.28 (t, 2H, J = 6.0 Hz, NCH_2), 4.33 (s, 3H, CH_3), 7.47–7.62 (m, 5H, Ph). ^{13}C NMR: δ 10.2 (q, CH_3), 13.6 (q, CH_3), 32.2 (t, $CH_2\underline{CH_2}CH_2$), 42.3 (t, C-3), 49.6 (t, CH_2Cl), 59.4 (t, NCH_2), 100.2 (s, C-9a), 111.0 (s, C-9), 128.6 (d, C-4′), 128.8 (d, C-2′, C-6′), 129.7 (s, C-8), 130.2 (d, C-3′, C-5′), 133.8 (s, C-1′), 145.5 (s, C- 6a), 146.7 (s, C-9b), 152.4 (s, C-5), 165.3 (s, C-2). Anal. Cald. for $C_{19}H_{19}ClN_4OS$: C, 58.98; H, 4.95; N, 14.48; S, 8.29. Found: C, 59.09; H, 4.97; N, 14.45; S, 8.39.

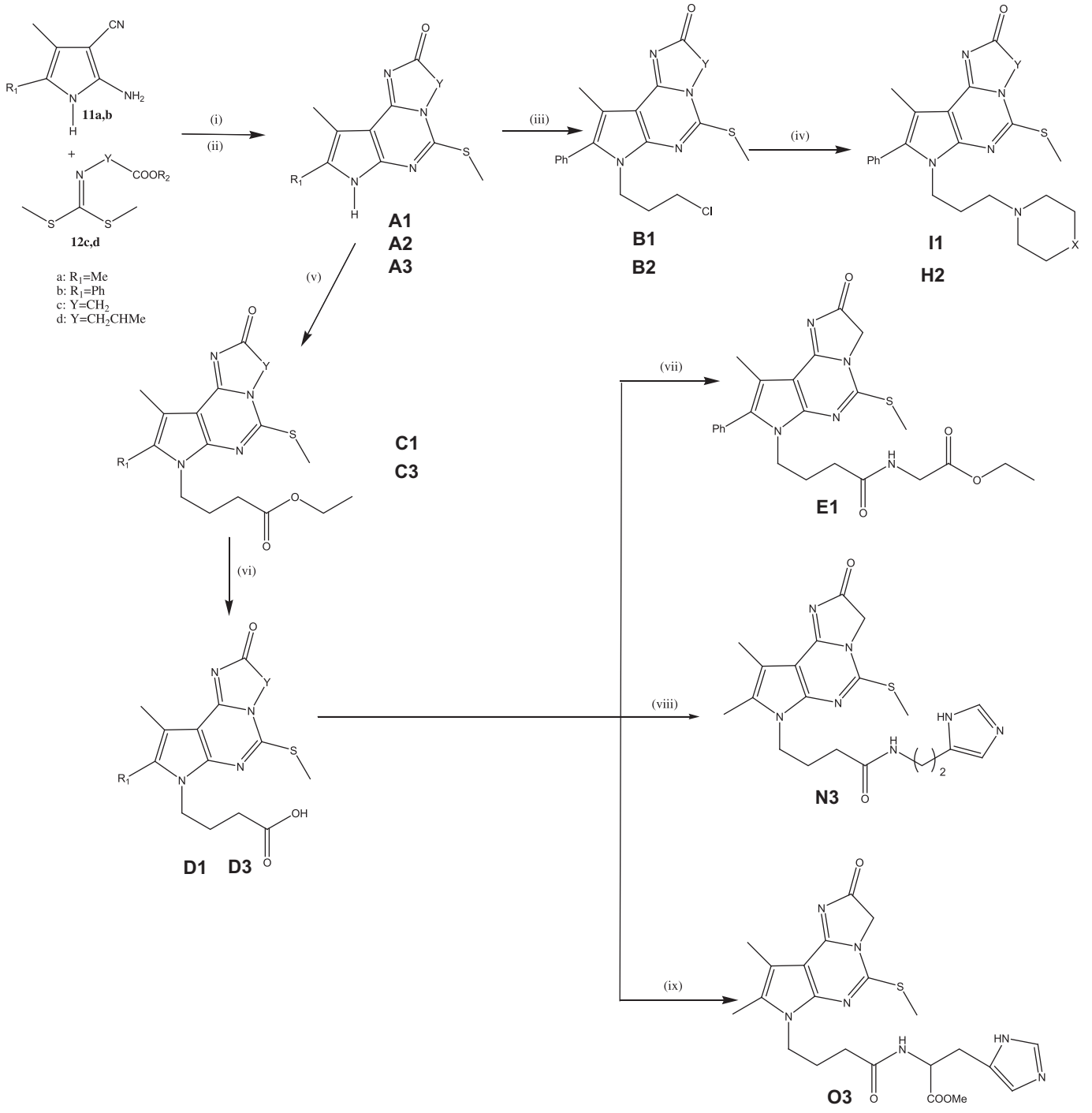
4.2.2. 8-(3-Chloropropyl)-4,10-dimethyl-6-(methylsulfanyl)-9-phenyl-3,4-dihydropyrimido[1,2-c] pyrrolo[3,2-e]pyrimidin-2(8H)-one (*B2*)

To a stirred suspension of **A2** (0.5 g, 1.5 mmol) in dry *N*,*N*-dimethylformamide (5 mL), potassium carbonate (0.62 g, 4.5 mmol) and 1-Bromo-3-Chloro-propane (0.44 mL, 4.5 mmol), were added. The mixture was heated at 80 °C in an oil bath for 1 h, cooled to room temperature and poured into ice and cold water. The crude residue was collected by filtration and, after drying, purified by column chromatography using a dichloromethane/ethyl acetate mixture in gradient as eluent. Recrystallized from diethylether.

Yield 66.6%. Mp 191.7–192.6 °C. IR: 1660 (CO) cm^{-1}. ^{1}H NMR: δ 1.50 (d, 3H, J = 7.4 Hz, CH_3), 1.82–1.98 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.41 (s, 3H, CH_3), 2.60 (dd, 1H, H-3), 2.70 (s, 3H, CH_3), 2.89 (dd, 1H, H-3), 3.34 (t, 2H, J = 6.6Hz, CH_2Cl), 4.07 (t, 2H, J = 6.6 Hz, NCH_2), 4.87–4.93 (m, 1H, H-4), 7.08–7.50 (m, 5H, Ph). ^{13}C NMR: δ 11.5 (q, CH_3), 14.7 (q, CH_3), 17.0 (q, CH_3), 32.8 (t, $CH_2\underline{CH_2}CH_2$), 36.3 (t, C-3), 39.9 (t, CH_2Cl), 41.8 (t, NCH_2), 51.1 (d, C-4), 104.3 (s, C-10a), 113.9 (s, C-10), 126.6 (d, C-4′), 128.8 (d, C-2′, C-6′), 130.4 (d, C-3′, C-5′), 131.0 (s, C-9), 131.5 (s, C-1′), 145.9 (s, C-7a), 154.07 (s, C-10b), 154.13 (s, C-6), 174.3 (s, C-2). Anal. Cald. For $C_{21}H_{23}ClN_4OS$: C, 60.79; H, 5.59; N, 13.50; S, 7.73. Found: C, 60.79; H, 5.59; N, 13.50; O, 3.86; S, 7.73.

4.2.3. 7-[3-(N-methylpiperazine)propyl]-9-methyl-5-(methylsulfanyl)-8-phenyl-3H-imidazo[1,2-c]pyrrolo[3,2-e]pyrimidin-2(7H)-one (*I1*)

To a stirred solution of **B1** (0.12 g, 0.3 mmol) in dry dioxane (5 mL), *N*-methyl-piperazine (0.07 mL, 0.6 mmol) was added. The mixture was heated at reflux for 6 h and, after cooling, the solvent was evaporated under reduced pressure. The crude



Scheme 1. Reagents and conditions: (i) AcOH, Δ; (ii) $NaHCO_3$ or $NaCO_3$; (iii) K_2CO_3, DMF, $Cl(CH_2)_3Br$; (iv) dioxane, *N*-methyl-piperazine or piperidine; (v) K_2CO_3, DMF, ethyl 4-bromobutyrate; (vi) NaOH, EtOH/H_2O; (vii) DCC, DCM, 0 °C, glycine ethyl ester hydrochloride/TEA; (viii) DCC, DCM, 0 °C, histamine/TEA; (ix) DCC, DCM, 0 °C, L-histidine methyl ester hydrochloride/TEA.

residue was purified by column chromatography using dichloromethane/ethyl acetate 98:2 as eluent. Recrystallized from diethyl ether.

Yield 66.5%. Mp 181.4–182.3 °C. IR: 1665 (CO) cm^{-1}. ^1H NMR: δ 1.24 (s, 3H, CH_3), 1.58 (t, 2H, $J = 6.0$ Hz, CH_2N), 2.10–2.22 (m, 10H, CH_2-2″, CH_2-3″, CH_2-5″, CH_2-6″, $CH_2\underline{CH_2}CH_2$), 2.24 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 4.16 (t, 2H, $J = 6.0$ Hz, NCH_2), 4.32 (s, 2H, CH_2); 7.45–7.60 (m, 5H, Ph). ^{13}C NMR: δ 10.2 (q, CH_3), 13.6 (q, CH_3), 26.2 (t, $CH_2\underline{CH_2}CH_2$), 40.8 (t, C-3), 45.5 (t, CH_2N), 49.6 (t, NCH_2), 52.1 (t, CH_2-2″, CH_2-6″), 54.4 (t, CH_2-3″, CH_2-5″), 54.5 (s, CH_3), 100.2 (s, C-9a), 110.4 (s, C-9), 128.5 (d, C-4′), 128.8 (d, C-2′, C-6′), 130.0 (s, C-8), 130.2 (d, C-3′, C-5′), 133.9 (s, C-1′), 146.6 (s, C-6a), 152.1 (s, C-9b), 165.3 (s, C-5), 183.7 (s, C-2). Anal. Cald. for $C_{24}H_{30}N_6OS$: C, 63.97; H, 6.71; N, 18.65; S, 7.11. Found: C, 64.06; H, 6.75; N, 18.61; S, 7.18.

*4.2.4. 8-[3-(piperidino)propyl]-4,10-dimethyl-9-phenyl-6-(methylsulfanyl)-3,4-dihydropyrimido[1,2-c]pyrrolo[3,2-e] pyrimidin-2(8H)-one (**H2**)*

To a stirred solution of **B2** (0.15 g, 0.4 mmol) in dry dioxane (5 mL), piperidine (0.08 mL, 0.8 mmol) was added. The mixture was

Table 3
Overview of one dose screening test results ($G\%_{average}$ for each human tumor cell lines pannel).

Pannel	C3	D3	C1	D1	O3	N3	B1	I1	H2
Leukemia	100	105	84	92	102	102	36	81	3
Non-small cell lung cancer	106	105	101	101	100	100	69	91	53
Colon cancer	122	116	113	106	104	118	48	98	26
CNS cancer	107	108	96	95	96	102	56	89	44
Melanoma	110	110	103	101	104	109	48	100	44
Ovarian cancer	120	110	105	100	107	116	76	95	47
Renal cancer	112	108	109	99	106	110	77	98	63
Prostate cancer	126	108	101	105	101	125	82	101	48
Breast cancer	100	102	101	100	103	105	61	80	15
$G\%_{average}$[a]	110	108	102	100	103	108	60	92	39

[a] Calculated on all *G%* values (table *G%* in SI4).

heated at reflux for 4 h and, after cooling, the solvent was evaporated under reduced pressure. The crude residue was purified by column chromatography using dichloromethane/methanol 98:2 as eluent. Recrystallized from diethyl ether.

Yield 76.3%. Mp 203.6–204.0 °C. IR: 1659 (CO) cm^{-1}. ^1H NMR (SI5): δ 1.23–1.45 (m, 6H, CH_2-3″, CH_2-4″, CH_2-5″), 1.32 (d, 3H, $J = 7.4$ Hz, CH_3), 1.69 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.00–2.40 (m, 7H, H-3, CH_2N, CH_2-2″, CH_2-6″), 2.26 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 2.87 (dd, 1H, H-3), 4.10 (t, 2H, NCH_2), 4.87–4.90 (m, 1H, H-4), 7.45–7.51 (m, 5H, Ph). ^{13}C NMR: δ 10.9 (q, CH_3), 14.8 (q, CH_3), 17 (q, CH_3), 24.2 (t, CH_2), 25.3 (t, CH_2), 30.5 (d, CH), 35.2 (t, CH_2), 35.9 (t, CH_2), 40.5 (t, CH_2), 50.6 (t, CH_2), 52.9 (t, CH_2), 102.9 (s, C-10a), 111.7 (s, C-10), 128.6 (d), 129.9 (d), 130.1 (d), 133.3 (s, C-1′), 145.0 (s, C-7a), 153.0 (s, C-10b), 154.0 (s), 159.0 (s), 173.2 (s). Anal. Cald. for $C_{26}H_{33}N_5OS$: C, 67.36; H, 7.17; N, 15.11; S, 6.91. Found: C, 67.36; H, 7.17; N, 15.11; S, 6.91.

*4.3. General procedure for the preparation of 8-substituted ethyl 4-[9-methyl-5-(methylsulfanyl)-2-oxo-3H-imidazo-[1,2-c]pyrrolo [3,2-e]pyrimidin-7-yl] butyrate (**C1**, **C3**)*

To a stirred suspension of **A1** or **A3** (2.0 mmol) in dry *N,N*-dimethylformamide (5 mL), potassium carbonate (0.85 g, 6.0 mmol) and ethyl-4-bromo-butyrate (0.86 mL, 6.0 mmol) were added. The mixture was heated at 80 °C in an oil bath for 3 h (in the case of **A1**) and for 11 h (in the case of **A3**) and after cooling to room temperature was poured into ice and cold water. The crude residue was collected by filtration and, after drying, purified by column chromatography using dichloromethane/methanol 98:2 as eluent. Recrystallized from diethyl ether.

C1: Yield 84%. Mp 225.4–226.0 °C. IR: 1733 (CO), 1701 (CO) cm^{-1}. ^1H NMR: δ 1.18 (t, 3H, $J = 7.4$ Hz, $COOCH_2\underline{CH_3}$), 1.82–1.94 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.07 (t, 2H, $\underline{CH_2}CO$), 2.35 (s, 3H, CH_3), 2.72 (s, 3H, CH_3), 4.04 (q, 2H, $J = 7.4$ Hz, $COO\underline{CH_2}CH_3$), 4.20 (t, 2H, $J = 7.0$ Hz, NCH_2), 4.23 (s, 2H, H-3), 7.34–7.55 (m, 5H, Ph). ^{13}C NMR: δ 10.4 (q, CH_3), 14.10 (q, CH_3), 14.15 (q, $COOCH_2\underline{CH_3}$), 24.9 (t, $CH_2\underline{CH_2}CH_2$), 31.0 (t, $\underline{CH_2}CO$), 42.1 (t, NCH_2), 49.5 (t, C-3), 60.4 (t, $COO\underline{CH_2}CH_3$), 101.2 (s, C-9a), 112.6 (s, C-9), 128.7 (d, C-4′), 128.8 (d, C-2′, C-6′), 130.1 (d, C-3′, C-5′), 130.4 (s, C-8), 134.6 (s, C-1′), 147.3 (s, C-6a), 152.0 (s, C-9b), 166.0 (s, C-5), 172.3 (s, CO), 183.6 (s, C-2). Anal. Cald. for $C_{22}H_{24}N_4O_3S$: C, 62.25; H, 5.70; N, 13.20; S, 7.55. Found: C, 62.35; H, 5.73; N, 13.15; S, 7.60.

C3: Yield 71%. Mp 163.9–165.0 °C, IR: 1725 (CO), 1697 (CO) cm^{-1}. ^1H NMR: δ 1.24 (t, 3H, $J = 7.0$ Hz, $COOCH_2\underline{CH_3}$), 2.07 (t, 2H, $\underline{CH_2}CO$), 2.27–2.32 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.30 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 4.04 (q, 2H, $J = 7.4$Hz, $COO\underline{CH_2}CH_3$), 4.16 (s, 2H, H-3), 4.19 (t, 2H, $J = 7.0$ Hz, NCH_2). ^{13}C NMR: δ 9.7 (q, CH_3), 9.8 (q, CH_3), 14.0 (q, CH_3), 14.1 (t, $COOCH_2\underline{CH_3}$), 25.1 (t, $CH_2\underline{CH_2}CH_2$), 31.1 (t, $\underline{CH_2}CO$), 41.4 (t, NCH_2), 49.5 (t, C-3), 60.6 (t, $COO\underline{CH_2}CH_3$), 100.9 (s, C-9a), 110.2 (s, C-9), 130.0 (s, C-8), 146.9 (s, C-6a), 151.0 (s, C-9b), 165.0 (s, C-5), 172.5 (s, CO), 183.6 (s, C-2). Anal. Cald. for $C_{17}H_{22}N_4O_3S$: C, 56.34; H, 6.12; N, 15.46; S, 8.85. Found: C, 56.41; H, 6.15; N, 15.47; S, 8.91.

Table 4
Five-dose screening for **B1** and **H2** derivatives.

Panel	Cell line	B1			H2		
		pGI_{50}	pTGI	pLC_{50}	pGI_{50}	pTGI	pLC_{50}
Leukemia	CCRF-CEM	5.29	<4.00	<4.00	5.57	4.92	<4.00
	HL-60(TB)	5.47	<4.00	<4.00	5.74	5.41	5.08
	K-562	5.24	<4.00	<4.00	NT	NT	NT
	MOLT-4	5.41	<4.00	<4.00	5.70	5.29	4.69
	RPMI-8226	5.43	4.18	<4.00	6.78	5.92	5.37
	SR	5.46	<4.00	<4.00	NT	NT	NT
Non-small cell lung cancer	A549/ATCC	5.11	4.18	<4.00	5.53	4.97	4.10
	EKVX	5.16	4.51	<4.00	5.52	4.70	<4.00
	HOP-62	4.96	4.20	<4.00	5.5	4.58	<4.00
	HOP-92	4.94	4.43	<4.00	NT	NT	<4.00
	NCI-H226	4.96	4.53	4.09	5.45	<4.00	<4.00
	NCI-H23	5.08	4.33	<4.00	5.53	<4.00	<4.00
	NCI-H322M	5.01	4.61	4.23	5.21	4.05	<4.00
	NCI-H460	5.43	4.82	4.25	5.49	4.92	4.17
	NCI-H522	5.41	4.72	4.12	NT	NT	NT
Colon cancer	COLO 205	5.38	4.82	4.32	5.90	5.60	5.30
	HCC-2998	5.34	4.71	4.19	5.49	4.79	4.21
	HCT-116	5.50	4.32	<4.00	5.62	<4.00	<4.00
	HCT-15	5.39	4.67	<4.00	5.35	4.55	<4.00
	HT29	5.41	4.71	<4.00	5.79	5.14	<4.00
	KM12	5.38	4.73	4.09	5.50	4.96	4.47
	SW-620	5.32	4.27	<4.00	5.62	4.98	4.49
CNS cancer	SF-268	5.07	4.32	<4.00	5.47	4.85	4.34
	SF-295	5.39	4.57	<4.00	5.59	4.99	4.16
	SF-539	5.47	4.85	4.40	5.19	4.06	<4.00
	SNB-19	5.40	4.75	4.34	5.53	<4.00	<4.00
	SNB-75	5.55	4.93	4.17	5.57	4.66	<4.00
	U251	5.41	4.79	4.27	5.61	4.95	4.47
Melanoma	LOX IMVI	5.38	4.76	4.30	5.52	4.89	4.45
	MALME-3M	5.08	4.63	4.23	5.63	5.14	4.27
	M14	5.48	4.82	<4.00	5.44	4.76	4.19
	MDA-MB-435	5.69	5.33	4.76	5.54	4.98	4.23
	SK-MEL-2	4.98	4.56	4.13	5.67	5.28	4.68
	SK-MEL-28	4.99	4.60	4.20	5.54	4.88	4.44
	SK-MEL-5	5.46	4.85	4.42	5.70	5.31	4.83
	UACC-257	4.90	4.55	4.19	NT	NT	NT
	UACC-62	5.68	4.94	4.42	5.55	4.85	4.42
Ovarian cancer	IGROV1	5.45	4.67	4.02	NT	NT	NT
	OVCAR-3	5.08	4.44	<4.00	5.62	5.07	4.27
	OVCAR-4	5.17	4.35	<4.00	5.59	4.75	<4.00
	OVCAR-5	4.94	4.42	<4.00	4.72	<4.00	<4.00
	OVCAR-8	4.79	4.14	<4.00	5.43	4.51	<4.00
	NCI/ADR-RES	5.37	<4.00	<4.00	4.77	4.13	<4.00
Renal cancer	786-0	5.07	4.21	<4.00	5.45	4.32	<4.00
	A498	5.41	4.82	4.27	5.50	5.04	<4.00
	ACHN	5.02	4.42	<4.00	5.52	4.65	<4.00
	CAKI-1	5.34	4.55	<4.00	NT	4.73	<4.00
	RXF 393	4.95	4.53	4.10	5.82	5.33	4.6
	SN12C	5.20	4.41	<4.00	5.64	4.97	<4.00
	TK-10	5.07	4.11	<4.00	5.54	5.03	<4.00
	UO-31	4.98	4.54	4.11	5.30	4.62	4.12
Prostate cancer	PC-3	5.06	<4.00	<4.00	5.86	5.45	5.04
	DU-145	4.94	4.57	4.21	5.47	4.56	<4.00
Breast cancer	MCF7	5.47	4.78	4.11	5.54	4.95	4.21
	MDA-MB-231/ATCC	5.25	4.62	4.11	5.74	5.02	<4.00
	HS 578T	5.20	4.46	<4.00	5.64	5.1	4.01
	BT-549	5.31	4.45	<4.00	5.58	5.02	4.07
	T-47D	5.58	4.96	4.02	5.70	5.12	<4.00
	MDA-MB-468	5.50	4.88	4.22	5.57	5.00	<4.00
MG_MID		5.26	4.51	4.11	5.56	4.83	4.23

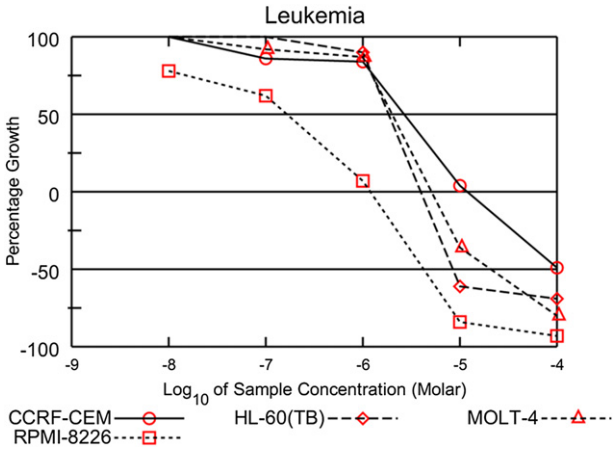


Fig. 5. Dose–response leukemia panel graph for **H2** derivative.

*4.4. General procedure for the preparation of 8-substituted 4-[9-methyl-5-(methylsulfanyl)-2-oxo-3H-imidazo-[1,2-c]pyrrolo[3,2-e] pyrimidin-7-yl] butanoic acid (**D1**, **D3**)*

To a stirred suspension of **C1** or **C3** (1.4 mmol), in dry ethanol (5 mL), a solution of potassium hydroxide (0.23 g, 4.0 mmol) in water (10 mL) was added. The mixture was stirred at room temperature for 12 h (in the case of **C1**) and 24 h (in the case of **C3**). After cooling, the ethanol was evaporated under reduced pressure and the aqueous layer was then carefully adjusted to pH 5. The resulting solid was filtered off, air-dried, and purified by column chromatography using dichloromethane/methanol 98:2 as eluent. Recrystallized from diethyl ether.

*4.4.1. 4-[9-methyl-5-(methylsulfanyl)-2-oxo-8-phenyl-3H-imidazo-[1,2-c]pyrrolo[3,2-e]pyrimidin-7-yl] butanoic acid (**D1**)*

Yield 64%. Mp 212.2–213.5 °C. IR: 3412 (OH), 1753 (CO), and 1697 (CO) cm^{-1}. ^1H NMR: δ 1.60–1.68 (m, 2H, $CH_2\underline{CH_2}CH_2$), 1.99 (t, 2H, $\underline{CH_2}CO$), 2.23 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 4.16 (t, 2H, $J = 7.0$ Hz, NCH_2), 4.22 (s, 2H, H-3), 7.45–7.55 (m, 5H, Ph), 12.05–12.09 (bs, 1H, OH). ^{13}C NMR: δ 10.6 (q, CH_3), 14.0 (q, CH_3), 25.0 (t, $CH_2\underline{CH_2}CH_2$), 30.8 (t, $\underline{CH_2}CO$), 41.5 (t, NCH_2), 49.5 (t, C-3), 101.2 (s, C-9a), 112.6 (s, C-9), 128.5 (d, C-4′), 128.8 (d, C-2′, C-6′), 130.1 (d, C-3′, C-5′), 130.6 (s, C-8), 134.3 (s, C-1′), 147.2 (s, C-6a), 152.8 (s, C-9b), 165.8 (s, C-5), 173.9 (s, CO), 184.1 (s, C-2). Anal. Cald. for $C_{20}H_{20}N_4O_3S$: C, 60.59; H, 5.08; N, 14.13; S, 8.09. Found: C, 60.74; H, 5.14; N, 14.16; S, 8.16.

*4.4.2. 4-[8,9-dimethyl-5-(methylsulfanyl)-2-oxo-3H-imidazo-[1,2-c]pyrrolo[3,2-e]pyrimidin-7-yl] butanoic acid (**D3**)*

Yield 97.3%. Mp 220.6–221.5 °C. IR: 3446 (OH), 1750 (CO), and 1695 (CO) cm^{-1}. ^1H NMR: δ 1.79–1.85 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.00 (t, 2H, $\underline{CH_2}CO$), 2.24 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 4.04 (t, 2H, $J = 7.0$ Hz, NCH_2), 4.20 (s, 3H, CH_3), 4.44 (s, 2H, H-3), 12.05–12.09 (bs, 1H, OH). ^{13}C NMR: δ 9.8 (q, CH_3), δ 10.1 (q, CH_3), 14.1 (q, CH_3), 25.0 (t, $CH_2\underline{CH_2}CH_2$), 30.9 (t, $\underline{CH_2}CO$), 41.3 (t, NCH_2), 49.5 (t, C-3), 101.1 (s, C-9a), 111.4 (s, C-9), 130.3 (s, C-8), 147.0 (s, C-6a), 151.7 (s, C-9b), 165.5 (s, C-5), 173.6 (s, CO), 182.6 (s, C-2). Anal. Cald. for $C_{15}H_{18}N_4O_3S$: C, 53.88; H, 5.43; N, 16.75; S, 9.59. Found: C, 53.96; H, 5.45; N, 16.77; S, 9.65.

*4.4.3. N-[2-(1H-imidazol-4-yl)ethyl]-4-[8,9-dimethyl-5-(methylsulfanyl)-2-oxo-3H-imidazo[1,2-c]pyrrolo[3,2-e]pyrimidin-7-yl]butyramide (**N3**)*

To a stirred suspension of carboxylic acid **D3** (0.3 mmol) in dry dichloromethane (5 mL), cooled to 0 °C, DCC (0.07 g, 0.3 mmol) was added. After 1 h, histamine-free-base (0.05 g, 0.45 mmol) was added and the mixture was stirred at room temperature for 24 h. Evaporation of the solvent under reduced pressure gave a solid that was purified by column chromatography using dichloromethane/methanol 8:2 as eluent. Recrystallized from ethanol.

Yield 63.6%. Mp 189.6–191.0 °C. IR: 1753 (CO), 1695 (CO) cm^{-1}. ^1H NMR: δ 1.86–1.94 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.03 (t, 2H, $J = 7.4$ Hz, $\underline{CH_2}CO$), 2.21 (s, 6H, 2 CH_3), 2.63 (s, 3H, CH_3), 3.22 (t, 2H, $J = 6.6$ Hz, $NHCH_2\underline{CH_2}$), 3.37-3.51 (m, 2H, $NH\underline{CH_2}$), 4.13 (t, 2H, $J = 7.4$ Hz, NCH_2), 4.21 (s, 2H, H-3), 6.76 (s, 1H, CH), 7.50 (s, 1H, CH), 7.85 (t, 1H, $J = 6.04$ Hz, NH). ^{13}C NMR: δ 9.3 (q, CH_3), 9.5 (q, CH_3), 13.5 (q, CH_3), 25.4 (t, $CH_2\underline{CH_2}CH_2$), 26.8 (t, $NHCH_2\underline{CH_2}$), 32.2 (t, $\underline{CH_2}CO$), 38,6 (t, $NH\underline{CH_2}$), 41.1 (t, NCH_2), 49.5 (t, C-3), 99.3 (s, C-9a), 108.3 (s, C-9), 130.1 (s, C-8), 131.2 (C-5′), 134.5 (d, CH) 134.8 (d, CH) 146.1 (s, C-6a), 150.9 (s, C-9b), 164.6 (s, C-5), 171.0 (s, CONH), 173.9 (s, C-2). Anal. Cald. for $C_{20}H_{25}N_7O_2S$: C, 56.19; H, 5.89; N, 22.93; S, 7.50. Found: C, 56.29; H, 5.85; N, 22.98; S, 7.45.

*4.4.4. Methyl 3-(1H-imidazol-4-yl)-2-[8,9-dimethyl-5-(methylsulfanyl)-2-oxo-3H-imidazo-[1,2-c]pyrrolo[3,2-e]pyrimidin-7-yl-butyramide]propanoate (**O3**)*

To a stirred suspension of carboxylic acid **D3** (0.6 mmol) in dry dichloromethane (5 mL), cooled to 0 °C, DCC (0.14 g, 0.66 mmol) was added. After 1 h, L-histidine methyl ester dihydrochloride (0.22 g, 0.9 mmol) and triethylamine (0.13 mL, 0.9 mmol) were added and the mixture was stirred at room temperature for 24 h. Evaporation of the solvent under reduced pressure gave a solid that was purified by column chromatography using dichloromethane/methanol 98:2 as eluent. Recrystallized from ethanol.

Yields 54.7%. Mp 148.1–148.9 °C. IR: 1751 (CO), 1724 (CO), 1696 (CO) cm^{-1}. ^1H NMR: δ 1.81–1.88 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.11 (t, 2H, $J = 7.4$ Hz, $\underline{CH_2}CO$), 2.24 (s, 6H, 2 CH_3), 2.65 (s, 3H, CH_3), 2.90 (t, 1H, $J = 7.0$ Hz, $\underline{CH}CO$), 3.03 (d, 2H, $\underline{CH_2}$), 3.59 (s, 3H, OCH_3), 4.25 (s, 2H, H-3), 4.49 (t, 2H, $J = 7.4$ Hz, NCH_2), 6.90 (s, 1H, CH), 7.80 (s, 1H, CH), 8.35 (d, 1H, NH). ^{13}C NMR: δ 9.3 (q, CH_3), 9.5 (q, CH_3), 13.5 (q, CH_3), 25.3 (t, $CH_2\underline{CH_2}CH_2$), 28.3 (t, $\underline{CH_2}$), 31.8 (t, $\underline{CH_2}CO$), 41.1 (t, NCH_2), 49.5 (t, C-3), 51.8 (d, CH), 52.2 (q, OCH_3), 99.6 (s, C-9a), 107.9 (s, C-9), 116.6 (d, CH), 129.0 (s, C-8), 130.1(s, C-5′), 134.6 (d, CH), 146.0 (s, C-6a), 151.0 (s, C-9b), 164.5 (s, C-5), 171.4 (s, CO), 171.9 (s, CONH), 183.5 (s, C-2). Anal. Cald. for $C_{22}H_{27}N_7O_4S$: C, 54.42; H, 5.60; N, 20.19; S, 6.60. Found: C, 54.55; H, 5.57; N, 20.15; S, 6.62.

*4.4.5. Ethyl N-[4-(9-methyl-5-(methylsulfanyl)-2-oxo-8-phenyl-3H-imidazo[1,2-c]pyrrolo[3,2-e]pyrimidin-7-yl)-butanoyl]glycinate (**E1**)*

To a stirred suspension of carboxylic acid **D1** (0.4 mmol) in dry dichloromethane (5 mL), cooled to 0 °C, DCC (0.093 g, 0.45 mmol) was added. After 1 h, glycine ethyl ester hydrochloride (0.085 g, 0.61 mmol) and triethylamine (0.085 mL, 0.61 mmol) were added and the mixture was stirred at room temperature for 24 h. Evaporation of the solvent under reduced pressure gave a solid that was purified by column chromatography using dichloromethane/methanol 98:2 as eluent. Recrystallized from ethanol.

Yield 78%. 173.1–174.7 °C. IR: 1756 (CO), 1733 (CO), 1695 (CO) cm^{-1}. ^1H NMR (SI5): δ 1.15 (t, 3H, $J = 8.0$ Hz, $COOCH_2\underline{CH_3}$), 1.65–1.72 (m, 2H, $CH_2\underline{CH_2}CH_2$), 1.91 (t, 2H, $\underline{CH_2}CO$), 2.24 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 3.70 (d, 2H, $NH\underline{CH_2}$), 4.04 (q, 2H, $J = 8.0$ Hz, $COO\underline{CH_2}CH_3$), 4.12 (t, 2H, $J = 7.0$ Hz, NCH_2), 4.32 (s, 2H, H-3), 7.39-7.56 (m, 5H, Ph), 8.14 (t, 1H, $J = 6.0$ Hz, CONH). ^{13}C NMR (SI5): δ 10.2 (q, CH_3), 13.6 (q, SCH_3), 14.0 (q, $COOCH_2\underline{CH_3}$), 25.2 (t, $CH_2\underline{CH_2}CH_2$), 31.8 (t, $\underline{CH_2}CO$), 40.7 (t, $NH\underline{CH_2}$), 41.9 (t, NCH_2), 49.6 (t, C-3), 60.3 (t, $COO\underline{CH_2}CH_3$), 100.1 (s, C-9a), 110.6 (s, C-9), 128.5 (d), 128.8 (d), 130.1 (d), 133.9 (s, C-8), 134.6 (s, C-1′), 146.7 (s, C-6a), 152.2 (s, C-9b), 165.3 (s, C-5), 169.8 (s, CONH), 171.4 (s, CO), 183.6 (s, C-2). Anal. Cald. for $C_{24}H_{27}N_5O_4S$: C, 59.86; H, 5.65; N, 14.54; S, 6.66. Found: C, 59.98; H, 5.61; N, 14.57; S, 6.54.

4.5. Biology

The human tumour cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 μL at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line are fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 μl of these different drug dilutions are added to the appropriate microtiter wells already containing 100 μl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed *in situ* by the gentle addition of 50 μl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μl) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (*C*), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

$$[(\mathrm{Ti}-\mathrm{Tz})/(C-\mathrm{Tz})]\times 100 \text{ for concentrations for which } \mathrm{Ti}>/=\mathrm{Tz}$$

$$[(\mathrm{Ti}-\mathrm{Tz})/\mathrm{Tz}]\times 100 \text{ for concentrations for which } \mathrm{Ti}<\mathrm{Tz}.$$

Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50% (GI_{50}) is calculated from $[(\mathrm{Ti}-\mathrm{Tz})/(C-\mathrm{Tz})]\times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from $\mathrm{Ti} = \mathrm{Tz}$. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from $[(\mathrm{Ti}-\mathrm{Tz})/\mathrm{Tz}]\times 100 = -50$. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2012.07.046. These data include MOL files and InChiKeys of the most important compounds described in this article.

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