Bioorganic & Medicinal Chemistry Letters xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and in vitro antiproliferative evaluation of novel *N*-alkylated 6-isobutyl- and propyl pyrimidine derivatives

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ARTICLE INFO

Article history:
Received 26 February 2014
Revised 16 April 2014
Accepted 21 April 2014
Available online xxxx

Keywords:
N-Alkylated pyrimidine derivatives
C-6-isobutyl pyrimidine
C-6-propyl pyrimidine
In silico analysis
Antiproliferative evaluation

ABSTRACT

A series of novel *N*-alkylated C-6-isobutyl- or -propyl pyrimidine derivatives were synthesized and their antiproliferative effect was evaluated on a panel of tumor cell lines including leukemia cell line K562 and normal diploid human fibroblasts. *N*-methoxymethylated 5-methylpyrimidin-2,4-dione with di (benzyloxy)isobutyl at C-6 (**14b**) showed the strongest effect on the cell growth at micromolar concentrations. Mechanisms of action for the lipophilic compound **14b** predicted in silico, pointed to its anticancer and antimetastatic potential exerted through inhibition of DNA or RNA polymerases and adhesion molecules. The latter mechanism has been supported in vitro for adherent tumor cell lines.

Pyrimidines are integral DNA and RNA building blocks and thus play an essential role in biological processes. As a consequence, these compounds have considerable chemical and pharmacological importance.[1] Scaffolds containing the pyrimidine heterocycle exhibit various biological effects including antiinflammatory, analgesic, antipyretic, antiarrhythmic, antimicrobial, anticancer and antiviral activities.[2] As deoxynucleoside kinases are included in the activation of deoxynucleoside analogs with biological and therapeutic properties, they are largely targeted for the treatment of viral diseases and cancer.[3] In particular, mitochondrial (mt) thymidine kinase (TK-2) belongs to the family of mammalian deoxynucleoside kinases (dNKs) and its role is implicated in the phosphorylation of pyrimidine nucleosides required for the mitochondrial DNA (mtDNA) whose synthesis is maintained during the whole cell cycle.[4] Besides the activation of nucleoside analogs with pharmacological properties, dNKs have a fundamental role in the salvage pathway of deoxynucleotide synthesis. Extensive studies have been carried out on the use of small side-chain 5-substituted uracil analogs as enzyme inhibitors in cancer and viral chemotherapy by phosphorylation with different efficacy using human and other mammalian nucleoside kinases including thymidine kinases TK-1 and/or TK-2; viral kinases such as herpes simplex virus types 1 and 2 (HSV-1 TK and HSV-2 TK).[5] Besides, C-6 fluoroalkylated pyrimidines revealed pronounced cytostatic activities[6] while thymine with 6-(2,3-dihydroxypropyl) and 6-(1,3-dihydroxyisobutyl) side-chain and its *N*-methylated structural analog have been developed as tracer molecules for monitoring HSV-1 TK expression by positron emission tomography (PET).[7] Moreover, it was found that that *N*-1 and/or *N*-3-alkylated pyrimidine derivatives exert a wide range of antiviral activity.[8] Pronounced biological activities of C-5 and/or C-6 substituted pyrimidine derivatives substantiated by previous reports in relation to their antiproliferative effects, provide a good rationale for expanded study of the chemistry and biological activities of this class.[9] Furthermore, in chemotherapeutical regimens pyrimidine nucleoside analogs are suitable for combinational treatments with other cell cycle inhibitors targeting different signaling pathways, mainly due to their DNA damaging properties that induce the S-phase cell cycle arrest. Such approach might enhance cytotoxicity of drugs and improve clinical response of patients.[3,10]

In this study we report on the in vitro antiproliferative activity and supposed mechanisms of biological action and toxicity of pyrimidine derivatives substituted with acyclic side-chain at position C-6 and alkylated with methoxymethyl (MOM) or trimethylsilylethoxymethyl (SEM) group at *N*-1 and/or *N*-3 position of pyrimidine ring. Synthesis of the key pyrimidine precursor **1a** with

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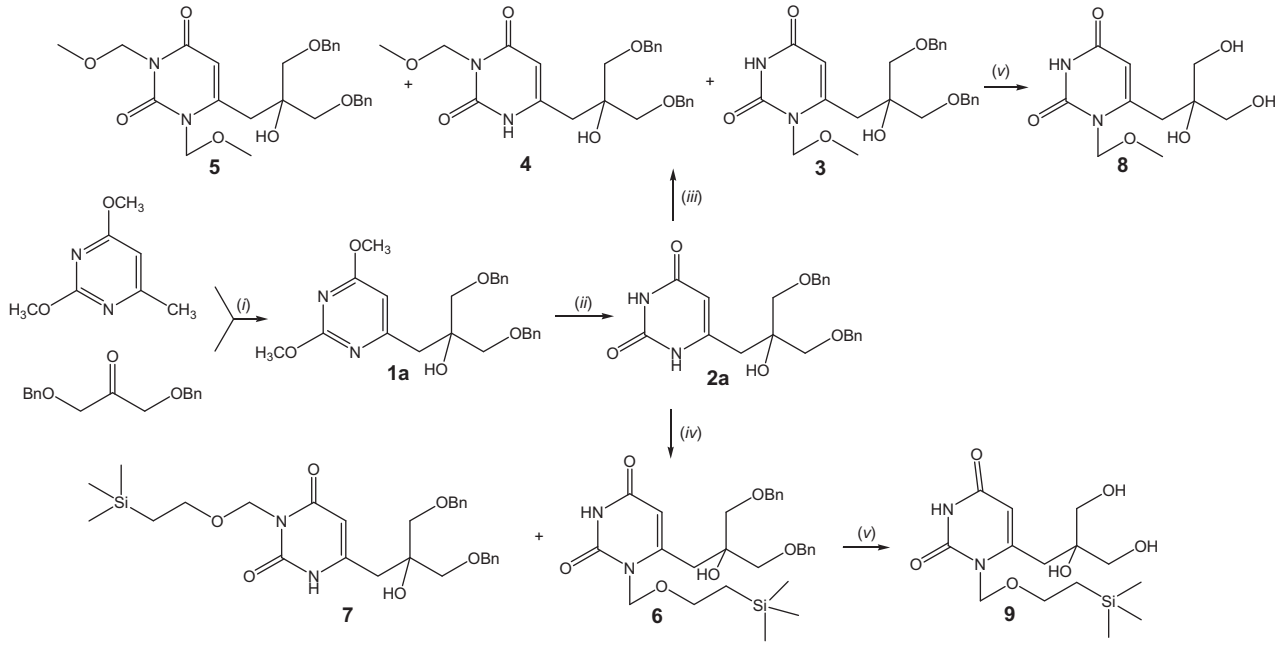
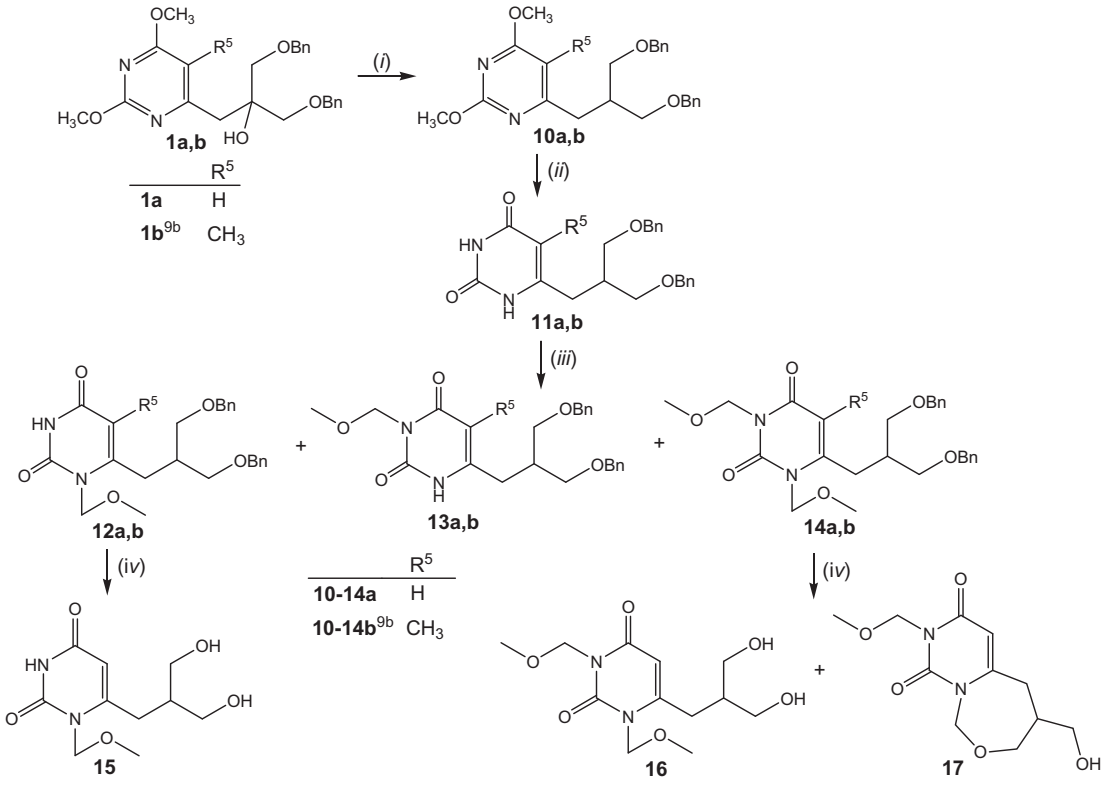
http://dx.doi.org/10.1016/j.bmcl.2014.04.079
0960-894X/

6-(1,3-dibenzyloxy-2-hydroxyisobutyl) side-chain was performed by treatment of 2,4-dimethoxy-6-methylpyrimidine with lithium diispropylamide (LDA) in THF at −55 °C to afford the corresponding lithiated precursor, which reacted in situ with 1,3-dibenzyloxy-2-propanone to give **1a** (Scheme 1). Deprotection of 2,4-dimethoxy groups in **1a** was accomplished using trimethylsilyl iodide generated in situ from trimethylsilyl chloride and sodium iodide to afford pyrimidin-2,4-dione **2a** which was subsequently submitted to *N*-alkylation reaction. Methoxymethylation reaction of **2a** with K_2CO_3 and methoxymethyl chloride in DMF afforded *N*-1-MOM (**3**), *N*-3-MOM (**4**) and *N*,*N*-1,3-diMOM (**5**) 6-(1,3-dibenzyloxy-2-hydroxyisobutyl)pyrimidine derivatives. *N*-1-SEM (**6**) and *N*-3-SEM (**7**) regioisomers were obtained in reaction of silylated pyrimidine derivative **2a** with trimethylsilylethoxymethyl chloride (SEMCl) in 1,1,1,3,3,3-hexamethyldisylazane (HMDS). Debenzylation of *N*-1 regioisomers **3** and **6** with boron trichloride gave *N*-1-MOM (**8**) and *N*-1-SEM (**9**) 6-(1,3-dihydroxy-2-hydroxyisobutyl)pyrimidine derivatives, respectively. Synthesis of *N*-MOM-5-methylpyrimidine derivatives **12b**–**14b** was performed according to the previously reported procedure starting from 6-(1,3-dibenzyloxy-2-hydroxyisobutyl)-5-methylpyrimidine (**1b**).[11] Reaction of C-5 unsubstituted pyrimidine derivative **1a** with methyl oxalyl chloride (MOC) gave oxalate, which was submitted to Barton–McCombie deoxygenation using tributyltin hydride ($SnBu_3H$) and 2,2′-azobis(isobutyronitrile) (AIBN) to give pyrimidine derivative **10a** containing di(benzyloxy)isobutyl side-chain at C-6 (Scheme 2). Deprotection of dimethoxy groups in **10a** was accomplished with trimethylsilyl iodide to obtain pyrimidin-2,4-dione **11a** (Scheme 2). Methoxymethylation reaction of **11a** with MOMCl gave *N*-1-MOM (**12a**), *N*-3-MOM (**13a**) and *N*,*N*-1,3-diMOM (**14a**) pyrimidine derivatives. Debenzylation of **12a** and **14a** was carried out using BCl_3 to afford *N*-1-MOM (**15**) and *N*,*N*-1,3-diMOM (**16**), respectively, with 6-(1,3-dihydroxyisobutyl) side-chain. However, debenzylation of **14a** accompanied by intramolecular cyclization gave bicyclic compound **17** as minor product (Scheme 2). Lithiation reaction of the 2,4-dimethoxy-6-methylpyrimidine and its 5-methylated derivative, using LDA and benzyloxyacetaldehyde in THF at −55 °C afforded **18a** and **18b**, respectively, with C-6 3-benzyloxy-2-hydroxypropyl side-chain (Scheme 3). Reaction of **18a** and **18b** with in situ formed trymethylsilyl iodide gave pyrimidine 2,4-dione derivatives **19a** and **19b**, respectively, and 2,4-dimethoxypyrimidine **20b** with free hydroxyl functionality in C-6 side-chain. Reaction of silylated pyrimidine derivatives **19a** and **19b** with SEMCl afforded *N*-1-SEM pyrimidine derivatives **21a** and **21b**, respectively. Subsequently, debenzylation of **21a** and **21b** with BCl_3 gave target *N*-1-SEM pyrimidine derivatives **22a** and **22b**, respectively, with free hydroxyl groups. Methoxymethylation reaction of **19b** with MOMCl gave *N*-1-MOM (**23b**) and *N*,*N*-1,3-diMOM (**24b**) pyrimidine derivatives. Debenzylation reaction of **23b** afforded *N*-1-MOM (**25b**) pyrimidine with 6-(1,3-dihydroxypropyl) side-chain.

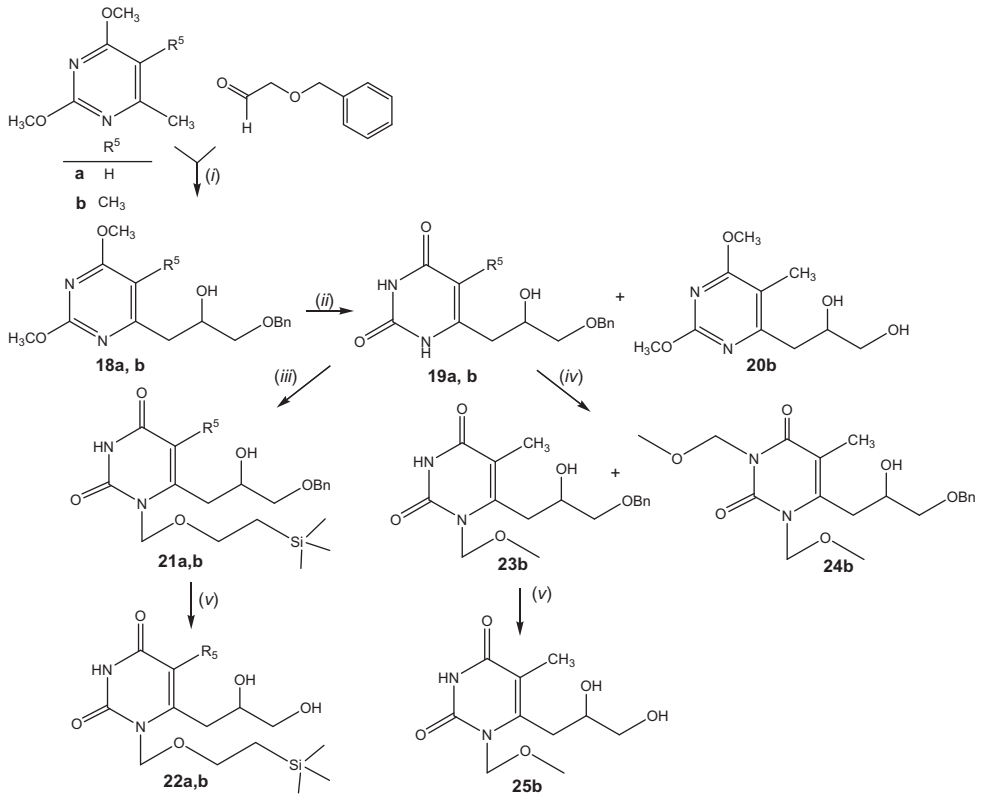
Among 23 compounds tested for antiproliferative activity in vitro, five compounds 2,4-dimethoxypyrimidine **1b** and **10a** and pyrimidin-2,4-diones **12b**, **13b** and **14b** with di(benzyloxy)isobutyl side-chain at C-6 (Scheme 2) showed antiproliferative effects on five selected cancer cell lines and diploid fibroblast-like WI38 or MCR cell lines (Table 1, Table S1 in Supplementary data). Among C-5 unsubstituted pyrimidines, only non-*N*-alkylated 2,4-dimethoxypyrimidine **10a** exhibited cytostatic activity. Furthermore, in the series of tested pyrimidin-2,4-dione derivatives, *N*-alkylated *N*-1-MOM (**12b**), *N*-3-MOM (**13b**) and *N*,*N*-1,3-diMOM (**14b**) 5-methylpyrimidines displayed antiproliferative effect. The strongest concentration-dependent effect on the growth of all tested cells at micromolar concentrations (0.1–1 μM) was exerted by *N*,*N*-1,3-diMOM pyrimidin-2,4-dione **14b**. Therefore, antiproliferative test for compound **14b** was performed on the apoptosis resistant non-adherent, Philadelphia chromosome-positive chronic myeloid leukemia (Ph+ CML) cell line K562 in comparison with commercial kinase inhibitor nilotinib (Tasigna®). Tasigna® is used in standard treatment of Ph+ leukemia due to increased potency, decreased toxicity and greater cellular and tissue penetration than imatinib (Gleevec®), with a similar target profile.[14] Here presented results showed higher antiproliferative effect of compound **14b** on K562 leukemia cells in comparison with Tasigna® (Table 2) even though Tasigna® exhibited lower cytotoxicity on normal human fibroblasts (BJ).



Scheme 1. Reagents and conditions: (i) LDA, THF, −55 °C, 20 h; (ii) TMSCl, NaI, rt, 19 h, Ar; (iii) K_2CO_3, DMF, MOMCl, rt; (iv) HMDS, SEMCl, reflux, 17 h; and (v) BCl_3, CH_2Cl_2, −55 °C, 4 h, Ar.



Scheme 2. Reagents and conditions: (i) MOC, DMAP, CH_3CN, rt, 20 h; Bu_3SnH, AIBN, toluene, reflux, 3 h; (ii) TMSCl, NaI, rt, 19 h, Ar; (iii) K_2CO_3, DMF, MOMCl, rt; and (iv) BCl_3, CH_2Cl_2, −55 °C, 4 h, Ar.



Scheme 3. Reagents and conditions: (i) LDA, THF, −55 °C, 10 h; (ii) TMSCl, NaI, rt, 19 h, Ar; (iii) HMDS, SEMCl, reflux, 17 h; (iv) K_2CO_3, DMF, MOMCl, rt; and (v) BCl_3, CH_2Cl_2, −55 °C, 4 h, Ar.

Table 1
The growth-inhibition effects of compounds **1b**, **10a** and **12b**–**14b** on selected tumor cell lines and normal human fibroblasts in vitro

Compd	IC_{50}[a] (μM)						
	MCF-7	SW620	MiaPaCa-2	HepG2	HeLa	WI38	MCR
1b[12]	4.2	3.5	6.1	2.4	3.7	3.8	NT
10a	0.3	>100	NT	0.3	3.5	NT	18.5
12b	72.6	72.1	55.9	NT	29.7	30.8	NT
13b	21.6	25.6	27.3	NT	17.0	19.4	NT
14b	0.4	0.5	0.5	NT	0.3	0.3	NT

[a] IC_{50}; 50% inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%. NT, non-tested. The cell growth rate was evaluated by performing the MTT assay.[13]

Table 2
The inhibition effects of compound **14b** and Tasigna® on the growth of Ph+ CML cell line K562 and normal human fibroblasts BJ in vitro

Compd	IC_{50}[a] (μM)	
	K562	BJ
Tasigna®	2.4	12.8
14b	0.6	5.3

[a] IC_{50}; 50% inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%.

Observed antiproliferative effects might be partially explained with relation to calculated *n*-octanol/water partition coefficients log*P*. It has been revealed that the active compounds **1b**, **10a**, **12b**–**14b**, all containing di(benzyloxymethyl) unit, are generally more lipophilic than the inactive compounds (Table S2). The greater lipophilic character may contribute to their considerable cellular uptake and/or interactions with cellular target(s).[15]

According to PASS,[16] a tool for estimation of potential pharmacological effects and biological targets, the synthesized molecules were predicted to have antiviral activity (Pa ⩾0.5 in Tables S3 and S4) what is in accordance with the observed antiviral activities of some C-6 substituted thymine and uracil derivates.[8] However, different target molecules for pyrimidin-2,4-dione (Table S3) and 2,4-dimethoxypyrimidine derivatives (Table S4) were suggested as the most probable by PASS. Although both classes have shown antiproliferative effects (Table 1), their modes of action at molecular level may differ according to PASS.

Inhibitions of DNA and RNA polymerases as well as cell adhesion molecules (Pa ~0.3, Pi ~0.1) were indicated for pyrimidin-2,4-diones **12b**, **13b** and **14b** (Table S3). On the contrary, compounds **1b** and **10a** with 2,4-dimethoxypyrimidine scaffold were predicted to have membrane permeability inhibitor capacity (Pa >0.4; Pi ~0.15) and to be Hsp 27 (β1) antagonist (Pa ~0.5, Pi ~0.018) (Table S4). Since the most active compound **14b** was indicated as cell adhesion molecule inhibitor (Pa ~0.4; Pi ~0.05) and antimetastatic agent (Pa ~0.5, Pi ~0.03) (Table S3), this compound was subsequently tested by use of adhesion assay (Fig. 1).

Predicted inhibition of cell adhesion might explain the strong antiproliferative effect observed on adherent tumor cell lines (Table 1). It has already been shown that binding of tumor cells to extracellular matrix proteins might promote tumor invasiveness, that is, binding of metastatic colon cancer cells to fibrinogen or breast cancer cells to fibronectin.[17] Adhesion analysis for adherent tumor cells on the fibronectin matrix confirmed that adhesion molecules are indeed, targeted by compound **14b** (Fig. 1). It was evident that adhesion of all tested cell lines was strongly affected by treatment with compound **14b**.

Prior to treatment with compound **14b**, the adhesion rates of tested cell lines were different and the highest percentages of adhesion were observed for normal fibroblasts BJ and MCF-7, while

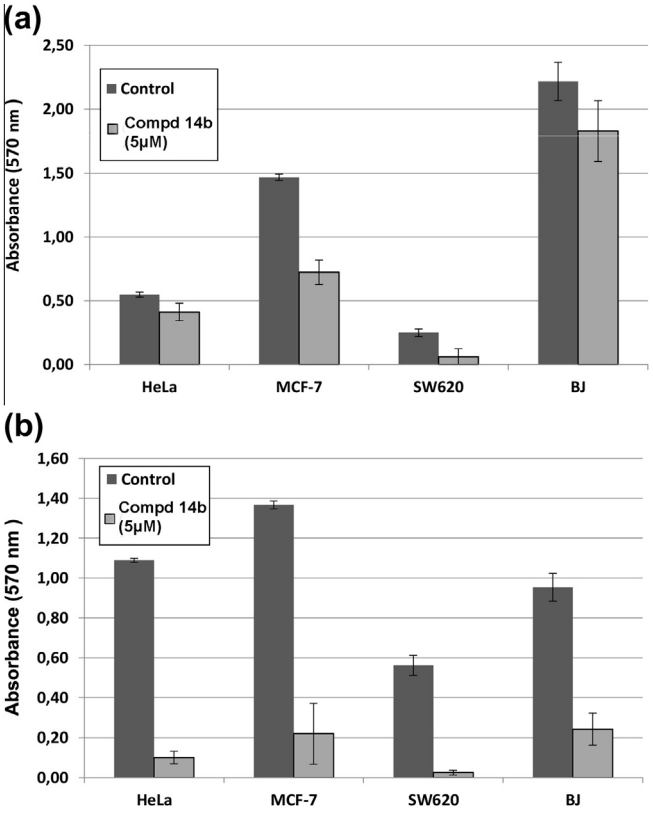


Figure 1. The results of adhesion assay. Tumor cell lines HeLa, MCF-7, SW620 and normal human fibroblast BJ were treated with **14b** at 5 μM for (a) 2 h and (b) 24 h.

the lowest percentage of adhesion was observed for the metastatic SW620 cell line (Fig. 1). Upon 24 h treatment with compound **14b** the strongest effect was detected on SW620 cells where almost no cells were able to adhere to the matrix, while the weakest effect, even though significant, was perceived on normal human fibroblasts (Fig. 1). These differences observed in the adhesion assay of compound **14b** were also visible in the cell cycle analysis (Table 3).

In SW620 cells with mutated tumor-suppressor gene p53,[18] an increase of cells arrested in the S and G2/M phases has been observed upon 24 and 48 h treatment with compound **14b** instead of standard chemotherapy-induced G1 arrest which occurs in p53 functional cells.[19] Indeed, in normal human fibroblasts with wild type (wt) p53 gene treated with compound **14b** the arrest of cells occurred in G1 and ended in subG1 arrest which is indicative of apoptosis. A similar effect has been observed in MCF-7 cells bearing a wt p53[20] after 48 h treatment, where higher concentrations of compound **14b** induced an increase of cells in the G1 phase. Interestingly, compound **14b** showed no effect on the cell cycle of leukemia cell line K562 even though it strongly inhibited their growth (Table 4). Contrary, Tasigna® induced a strong G1 arrest of K562 cells (at significance $p < 0.05$) that is in accordance with the literature data.[20] Therefore, it may be assumed that compound **14b** exerts its antiproliferative effect on leukemia cells by some other mechanism such as inhibition of DNA or RNA polymerases or through a toxic effect on the cell membrane due to its lipophilic properties.[21]

In conclusion, novel *N*-alkylated C-6-isobutyl or -propyl pyrimidine derivatives were successfully synthesized. Lipophilic 2,4-dimethoxypyrimidine (**1b** and **10a**) and *N*-1-MOM (**12b**), *N*-3-MOM (**13b**) and *N*,*N*-1,3-diMOM (**14b**) pyrimidin-2,4-dione derivatives all with di(benzyloxy)isobutyl side-chain at C-6

Table 3
Cell cycle analysis for compound **14b** on adherent tumor cell lines

Cell line/treatment	Cell cycle phase (%)											
	subG1	G1	S	G2/M	subG1	G1	S	G2/M	subG1	G1	S	G2/M
	SW620				WI38				MCF-7			
Control[a]	6.4	56.1	17.1	18.1	36.7	41.7	11.8	9.1	50.2	23.5	13.7	8.1
0.1 μM[a]	4.3	51.5*	19.6*	21.7*	21.9*	61.7*	8.9*	6.9*	55.5	25.5	9.7	7.1
1 μM[a]	5.2	55.9	16.1	20.1	21*	70.1*	2.4*	5.1*	67.1*	14.8*	8.1*	8.7
Control[b]	5.0	57.6	21.8	14.4	27.9	53.9	8.6	8.7	73.97	13.6	6.4	10.3
0.1 μM[b]	6.8	49.7*	25.6*	15.5	38.5*	48.5*	6.8	5.7	50.5*	23.8*	11.9*	9.5
1 μM[b]	6.7	53.1	22	15.8	47.9*	45.5*	3.4*	3.5*	78.9	11.5	4.9	3.8

[a] 24 h Treatment.
[b] 48 h Treatment. The results are shown as percentage of cells in a particular cell cycle phase (%). Statistically relevant results ($p > 0.05$) are denoted with an asterisk (*).

Table 4
Flow cytometric analysis of the cell cycle of K562 cells upon the treatments with compound **14b**

Compd **14b**	Cell percentage (% ± standard deviation)			
	subG1	G1	S	G2/M
Control[a]	2.4 ± 0.0	49.5 ± 3.1	27.5 ± 5.1	21.7 ± 2.4
0.1 μM[a]	2.2 ± 0.3	48.4 ± 5.1	28.6 ± 7.1	22.2 ± 0.4
1 μM[a]	3.1 ± 0.0	53.0 ± 1.3	26.6 ± 5.5	18.4 ± 3.9
Control[b]	4.7 ± 0.6	47.5 ± 2.2	24.0 ± 2.9	23.4 ± 1.0
0.1 μM[b]	3.0 ± 0.2	49.8 ± 0.6	24.1 ± 0.0	23.5 ± 0.3
1 μM[b]	4.2 ± 1.6	51.8 ± 1.5	25.9 ± 7.0	17.4 ± 6.5
Tasigna®	subG1	G1	S	G2/M
Control[a]	1.8 ± 0.3	48.4 ± 0.1	28.9 ± 0.9	21.8 ± 0.5
0.1 μM[a]	3.7 ± 0.2*	85.0 ± 1.1*	7.0 ± 1.1*	4.4 ± 0.1*
0.5 μM[a]	3.3 ± 0.4*	85.8 ± 2.1*	5.6 ± 0.1*	4.8 ± 1.3*
Control[b]	3.0 ± 0.7	51.2 ± 2.4	21.7 ± 2.0	26.1 ± 0.1
0.1 μM[b]	9.2 ± 0.0*	70.0 ± 1.7*	16.5 ± 0.3*	5.1 ± 1.2*
0.5 μM[b]	15.0 ± 3.1*	64.4 ± 3.9*	18.2 ± 5.0*	3.9 ± 4.2*

[a] 24 h Treatment.
[b] 48 h Treatment. Statistically significant changes ($p < 0.05$) are denoted with an asterisk (*).

showed antiproliferative activity on tested tumor cell lines. Among these compounds, pyrimidin-2,4-dione derivate **14b** exerted pronounced inhibitory effect (IC_{50} ~0.4 μM) on all tested cancer cell lines. Furthermore, this compound exhibited higher antiproliferative effect (IC_{50} = 0.6 μM) on leukemia cell line K562 in comparison with commercial kinase inhibitor Tasigna® (IC_{50} = 2.4 μM) that is used in standard treatment of Ph+ leukemia. Pyrimidin-2,4-dione derivatives **12b**–**14b** have been recognized as antimetastatic agents as they may target adhesion molecules, that was supported in vitro for compound **14b** on tumor cells.

Acknowledgments

Support of this study by the Ministry of Science, Education and Sports of the Republic of Croatia (Projects #125-0982464-2925, #335-0000000-3532 and #098-0982464-2511) is gratefully acknowledged.

Supplementary data

Supplementary data (procedures for syntheses of compounds and their characterization data, methods for in silico analysis, antitumor activity assay and cell cycle analysis) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.04.079.

References and notes

1. (a) Boncel, S.; Gondela, A.; Walczak, K. *Curr. Org. Synth.* **2008**, *5*, 365; (b) Amr, A. E.; Ashraf, M. M.; Salwa, F. M.; Nagla, A. A.; Hammam, A. G. *Bioorg. Med. Chem.* **2006**, *14*, 5481.
2. (a) Amin, K. M.; Hanna, M. M.; Abo-Youssef, H. E.; George, R. F. *Eur. J. Med. Chem.* **2009**, *44*, 4572; (b) Kumar, N.; Chauhan, A.; Drabu, S. *Biomed. Pharmacother.* **2011**, *65*, 375; (c) Keri, R. S.; Hosamani, K. M.; Shingalapur, R. V.; Hugar, M. H. *Eur. J. Med. Chem.* **2010**, *45*, 2597; (d) Bryzgalov, A. O.; Dolgikh, M. P.; Sorokina, I. V.; Tolstikova, T. G.; Sedova, V. F.; Shkurko, O. P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1418; (e) Al-Abdullah, E. S.; Al-Obaid, A. R.; Al-Deeb, O. A.; Habib, E. E.; El-Emam, A. A. *Eur. J. Med. Chem.* **2011**, *46*, 4642; (f) Kim, S.; Park, D. H.; Kim, T. H.; Hwang, M.; Shim, J. *FEBS J.* **2009**, *276*, 4715.
3. Van Rompay, A. R.; Johansson, M.; Karlsson, A. *Pharmacol. Ther.* **2003**, *100*, 119.
4. Hernandez, A.-I.; Familiar, O.; Negri, A.; Rodriguez-Barrios, F.; Gago, F.; Karlsson, A.; Camarasa, M.-J.; Balzarini, J.; Perez-Perez, M.-J. *J. Med. Chem.* **2006**, *49*, 7766.
5. (a) Kundu, N. G.; Mahanty, J. S.; Spears, C. P. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1497; (b) Kumar, R.; Nath, M.; Tyrrell, J. *J. Med. Chem.* **2002**, *45*, 2032.
6. (a) Prekupec, S.; Makuc, D.; Plavec, J.; Šuman, L.; Kralj, M.; Pavelić, K.; Balzarini, J.; De Clercq, E.; Mintas, M.; Raić-Malić, S. *J. Med. Chem.* **2007**, *50*, 3037; (b) Krištafor, S.; Gazivoda Kraljević, T.; Makuc, D.; Plavec, J.; Šuman, L.; Kralj, M.; Raić-Malić, S. *Molecules* **2009**, *14*, 4866; (c) Prekupec, S.; Makuc, D.; Plavec, J.; Kraljević, S.; Kralj, M.; Pavelić, K.; Andrei, G.; Snoeck, R.; Balzarini, J.; De Clercq, E.; Raić-Malić, S.; Mintas, M. *Antiviral Chem. Chemother.* **2005**, *16*, 327.
7. (a) Müller, U.; Martić, M.; Gazivoda Kraljević, T.; Krištafor, S.; Ross, T. L.; Ranadheera, C.; Müller, A.; Born, M.; Krämer, S. D.; Raić-Malić, S.; Ametamey, S. M. *Nucl. Med. Biol.* **2012**, *39*, 235; (b) Krištafor, S.; Novaković, I.; Gazivoda Kraljević, T.; Kraljević Pavelić, S.; Lučin, P.; Westermaier, Y.; Pernot, L.; Scapozza, L.; Ametamey, S. M.; Raić-Malić, S. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6161; (c) Raić-Malić, S.; Johayem, A.; Ametamey, S. M.; Batinac, S.; De Clercq, E.; Folkers, G.; Scapozza, L. *Nucleosides Nucleotides* **2004**, *23*, 1707; (d) Johayem, A.; Raić-Malić, S.; Lazzati, K.; Schubiger, P. A.; Scapozza, L.; Ametamey, S. M. *Chem. Biodivers.* **2006**, *3*, 274.
8. Loksha, Y. M.; Globisch, D.; Loddo, R.; Collu, G.; La Colla, P.; Pedersen, E. B. *ChemMedChem* **2010**, *5*, 1847.
9. (a) Kundu, N. G.; Das, P.; Balzarini, J.; De Clercq, E. *Bioorg. Med. Chem.* **1997**, *5*, 2011; (b) Gazivoda Kraljević, T.; Krištafor, S.; Šuman, L.; Kralj, M.; Ametamey, S. M.; Cetina, M.; Raić-Malić, S. *Bioorg. Med. Chem.* **2010**, *18*, 2704.
10. Ibrahim, D. A.; El-Metwally, A. M. *Eur. J. Med. Chem.* **2010**, *45*, 1158.
11. Gazivoda Kraljević, T.; Petrović, M.; Krištafor, S.; Makuc, D.; Plavec, J.; Ross, T. L.; Ametamey, S. M.; Raić-Malić, S. *Molecules* **2011**, *16*, 5113.
12. Krištafor, S.; Gazivoda Kraljević, T.; Ametamey, S. M.; Cetina, M.; Ratkaj, I.; Tandara Haček, R.; Kraljević Pavelić, S.; Raić-Malić, S. *Chem. Biodivers.* **2011**, *8*, 1455.
13. Gazivoda, T.; Raić-Malić, S.; Krištafor, V.; Makuc, D.; Plavec, J.; Bratulic, S.; Kraljević Pavelić, S.; Pavelić, K.; Naesens, L.; Andrei, G.; Snoeck, R.; Balzarini, J.; Mintas, M. *Bioorg. Med. Chem.* **2008**, *16*, 5624.
14. (a) Thomas, X. *World J. Stem Cells* **2012**, *4*, 44; (b) Sabha, N.; Au, K.; Agnihotri, S.; Singh, S.; Mangat, R.; Guha, A.; Zadeh, G. *PloS One* **2012**, *7*, e39412.
15. (a) Stepanić, V.; Koštrun, S.; Malnar, I.; Hlevnjak, M.; Butković, K.; Ćaleta, I.; Dukši, M.; Kragol, G.; Makaruha-Stegić, O.; Mikac, L.; Ralić, J.; Tatić, I.; Tavčar, B.; Valko, K.; Zulfikari, S.; Munić, V. *J. Med. Chem.* **2011**, *54*, 719; (b) Fantin, V. R.; Berardi, M. J.; Scorrano, L.; Korsmeyer, S. J.; Leder, P. *Cancer Cell* **2002**, *2*, 29.
16. Filimonov, D. A.; Poroikov, V. V. In *Chemoinformatics Approaches to Virtual Screening*; AlexandreVarnek, Alexander Tropsha, Eds.; RSC: Cambridge (UK), 2008; p 182.
17. (a) Alves, C. S.; Konstantopoulos, K. *PloS One* **2012**, *7*, e41472; (b) Bartsch, J. E.; Staren, E. D.; Appert, H. E. *J. Surg. Res.* **2003**, *110*, 287.
18. Boyer, J.; McLean, E.; Aroori, S.; Wilson, P.; McCulla, A.; Carey, P.; Longley, D.; Johnston, P. *Clin. Cancer Res.* **2004**, *10*, 2158.
19. (a) Amundson, S. A.; Myers, T. G.; Fornace, A. J., Jr. *Oncogene* **1998**, *17*, 3287; (b) Bunz, F.; Hwang, P. M.; Torrance, C.; Waldman, T.; Zhang, Y.; Dillehay, L.; Williams, J.; Lengauer, C.; Kinzler, K. W.; Vogelstein, B. *J. Clin. Invest.* **1999**, *104*, 263.
20. Lu, X.; Errington, J.; Curtin, N. J.; Lunec, J.; Newell, D. R. *Clin. Cancer Res.* **2001**, *7*, 2114.
21. Wang, C.; Zhou, Y.; Li, S.; Li, H.; Tian, L.; Wang, H.; Shang, D. *Life Sci.* **2013**, *92*, 1004.