New Imidazo[1,2-*c*]pyrimidin-5(6*H*)-Ones Derived from Cytosine: Synthesis, Structure, and Cytotoxic Activity

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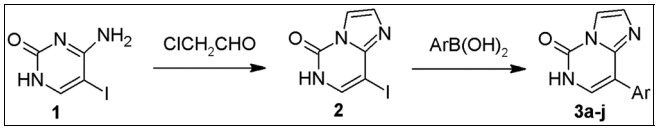
Received January 30, 2014
DOI 10.1002/jhet.2243
Published online 31 July 2014 in Wiley Online Library (wileyonlinelibrary.com).



This work describes the synthesis of 8-iodoimidazo[1,2-*c*]pyrimidin-5(6*H*)-one **2** from 5-iodocytosine **1**. This compound was subjected to Suzuki cross-coupling reaction with aryl and heteroarylboronic acids. After optimization, 10 products **3a–j** were obtained in good yields 61-90%. Cytotoxic activity of all new products was evaluated on seven tumor cell lines including resistant variants and on normal human fibroblasts. Two derivatives showed promising biological activity and good therapeutic index in the case of **3h**.

J. Heterocyclic Chem., **52**, 1382 (2015).

INTRODUCTION

The reaction of 2-aminopyridine, 2-aminopyrimidine, and 4-aminopyrimidine derivatives with α-halogenocarbonyl compounds is well known to produce corresponding imidazo-fused derivatives [1–3]. Imidazo[1,2-*a*]pyridine and imidazo[1,2-*a*]pyrimidine scaffolds were studied for their extensive pharmacological properties, for example, antiviral [4,5], antitubercular [6,7], anticancer [8], cytotoxic [9], antiparasitic [10], antibacterial [2], antifungal [11], cardiotonic [12], and antiinflammatory [13]. Some derivatives act as commercial drugs, from which zolpidem is widely known nonbenzodiazepine hypnotic [14].

Imidazo[1,2-*c*]pyrimidines were studied for similarly large spectrum of biological activities, such as antimicrobial [15], antimycobacterial [16], antitubercular [7], inotropic [17], antiinflammatory, analgesic, antipyretic, and ulcerogenic [18]. These compounds also act as Syk family kinases inhibitors [19,20] and dopamine D4 receptor ligands [21].

Imidazo[1,2-*c*]pyrimidine scaffolds are also formed by reaction of adenine and cytosine bases with α-halogenocarbonyl compounds. Kochetkov and coworkers first reported reaction of 9-methyladenine and 1-methylcytosine with chloroacetaldehyde, which produced corresponding ethenoderivatives [22]. Because this type of reaction became an interesting pathway for synthesis of modified nucleic acid bases with useful biological, fluorescence, and base-pairing properties [23–26].

Biological activity of some imidazo[1,2-*c*]pyrimidin-5(6*H*)-ones has been reported. Compounds with this scaffold are selective $GABA_A$ receptor ligands [27]. Recently, the large family was patented as allosteric modulators of mGluR5 receptors for treatment of neurologic and psychiatric disorders [28]. Nucleosides with this scaffold were tested as antiviral agents [29] and showed activity against hepatitis B virus [30,31] and HIV [32].

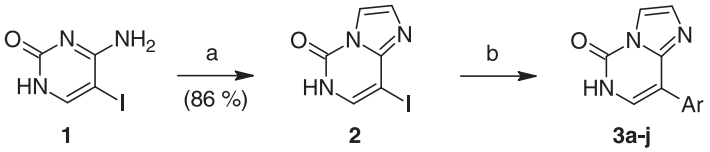
In this article, we present the access for functionalization of imidazo[1,2-*c*]pyrimidin-5(6*H*)-one scaffold by an aryl or heteroaryl ring. 8-Aryl derivatives **3a–j** were obtained from iododerivative **2** by Suzuki–Miyaura cross-coupling reaction (Scheme 1). Cytotoxic activity of all products was evaluated on tumor cell lines and normal human fibroblasts (Table 1).

RESULTS AND DISCUSSION

Chemistry. The starting 8-iodoimidazo[1,2-*c*]pyrimidin-5(6*H*)-one **2** was prepared in 86% yield by cyclization of 5-iodocytosine **1** with chloroacetaldehyde in the presence of sodium acetate. Iodine atom of **2** was substituted by an aryl or heteroaryl ring via Suzuki–Miyaura cross-coupling [33] without any need of protection of parent heterocycle **2** (Scheme 1).

Inspired by coupling of cytosine nucleosides with boronic acids [34], we first selected acetonitrile–water 2 : 1 solvent system with tetrakis(triphenylphosphine)palladium as catalyst. However, these conditions could only be used

Scheme 1. Synthesis of target imidazo[1,2-*c*]pyrimidin-5(6*H*)-ones, yields in parentheses. Reagents and conditions: (a) $ClCH_2CHO$, NaOAc, H_2O, 80°C, 4 h; (b) $ArB(OH)_2$, $Pd(dppf)Cl_2$, $EtOH/H_2O$, Na_2CO_3, reflux, 24 h.



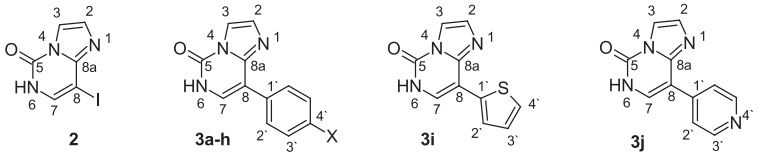
3a) Ar = phenyl (88 %)
3b) Ar = biphenyl-4-yl (81 %)
3c) Ar = 4-methoxyphenyl (90 %)
3d) Ar = 4-chlorophenyl (75 %)
3e) Ar = 4-(trifluoromethyl)phenyl (71 %)
3f) Ar = 4-formylphenyl (83 %)
3g) Ar = 4-carboxyphenyl (74 %)
3h) Ar = 4-cyanophenyl (84 %)
3i) Ar = thiophen-2-yl (61 %)
3j) Ar = pyridin-4-yl (62 %)

Table 1
Summary of cytotoxic activities (IC_{50}, μM).[a]

Compound	CCRF-CEM	CEM-DNR bulk	K562	K562-Tax	A549	HCT116	HCT116p53-/-	MRC-5	BJ
2	46.15	48.99	47.37	44.63	50.00	50.00	18.61	48.33	50.00
3a	50.00	45.69	49.72	45.51	50.00	46.14	35.58	50.00	50.00
3b	8.59	2.84	15.20	8.85	10.13	20.20	15.12	7.00	0.66
3c	45.74	41.56	47.75	39.79	47.31	49.35	35.52	50.00	50.00
3d	36.87	34.84	35.81	37.70	44.69	37.53	35.89	44.45	43.71
3e	28.20	32.89	10.74	34.20	40.42	34.83	25.57	50.00	50.00
3f	32.63	20.28	34.21	23.48	9.04	50.00	18.47	50.00	48.69
3g	50.00	49.72	48.12	48.38	50.00	50.00	18.75	50.00	50.00
3h	1.45	18.84	17.71	28.72	5.07	42.49	8.83	35.27	7.29
3i	44.65	42.78	50.00	43.35	50.00	50.00	18.75	50.00	50.00
3j	50.00	46.94	50.00	46.07	50.00	50.00	18.75	50.00	50.00

[a]Cytotoxic activity was determined by MTT assay following a 3-day incubation. Values represents means of IC_{50} from three independent experiments with standard deviation ranging from 10 to 25% of the average values.

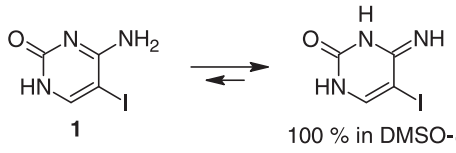
Scheme 2. Numbering of atoms in compounds **2** and **3a–j** for NMR purposes.



for coupling with phenylboronic and biphenyl-4-boronic acid but not for 4-methoxyphenylboronic acid (~30% conversion). When ethanol–water 2 : 1 was used as a solvent, complete conversion was observed in the coupling with 4-methoxyphenylboronic acid but lower (~80%) for coupling with 4-cyanophenylboronic acid. Then, the $Pd(dppf)Cl_2$ was used as catalyst with the aim to obtain complete conversion in case of all used boronic acids. With 3 mol% of this catalyst in ethanol–water 2 : 1, products **3a–i** were coupled in nearly quantitative conversions, detected by thin-layer chromatography. Only in the coupling of **2** with strongly deactivated 4-pyridylboronic acid, 5 mol% of $Pd(dppf)Cl_2$ in ethanol–water 4 : 1 must be used to obtain complete conversion to **3j**. Coupled products **3a–j** were obtained in 61–90% isolated yields.

Products were identified and characterized by IR, MS, and NMR spectroscopy (for atom numbering see Scheme 2). 5-Iodocytosine **1** showed three broad NH protons that mean that it exist as imino tautomer in hexadeuterated dimethyl sulfoxide (DMSO-d_6) (Scheme 3). The same tautomerism was observed in 5-bromo and 5-iodo-*N*-1-sulfonylated cytosine derivatives [35]. Complete assignment of ^1H and ^{13}C chemical shifts in compounds **2** and **3a–j** was carried out on the basis of 1D and 2D (correlation spectroscopy, heteronuclear multiple

Scheme 3. Preferred imino tautomerism of 5-iodocytosine **1**.



quantum coherence, and heteronuclear multiple bond correlation) NMR experiments (Table 2). ^{15}N-NMR spectra of selected derivatives were also measured.

Structure of product **3a** was confirmed by X-ray crystallography. Compound **3a** (Fig. 1) crystallizes in the orthorhombic space group *Pbca*, with eight molecules within the unit cell. To the best of our knowledge, only one analogous structure of imidazo(1,2-*c*)pyrimidin-5(6*H*)-one derivate in position 8 by a carbon atom the 3,4-etheno-5-methoxymethyl-2′-deoxycytidine [36] has been reported so far. Other two partially saturated compounds as for example *N*-methyl-6-(2-chloroethyl)-5,7-dioxo-1,2,3,5,6,7-hexahydroimidazo[1,2-*c*] pyrimidine-8-carboxamide [37] or methyl 2-(1-benzyl-8-formyl-7-methoxy-5-oxo-1,2,3,5-tetrahydroimidazo[1,2-*c*]pyrimidin-3-yl)acetate [38] and two compounds with fused aromatic rings [39,40] that resemble the structural motif of **3a** were also found within the crystallographic database. Typical interatomic separations and angles for each functional group are present within the structure of **3a** [41]. A high degree of π-electron conjugation is interrupted between atoms C2-C3 and C6-N3 where the bond lengths are bit longer than is usually found for analogous distances in aromatic compounds. The torsion of the phenyl ring from the plane defined by the heterocyclic part of the molecule is 45.68(3)°.

Because of the presence of interatomic N2-H2···N3 interactions in the crystal packing of **3a** (Fig. 2), the chains of zig-zag oriented molecules (interplanar angle between two neighboring imidazopyrimidine rings is 41.0 (2)° N2-H2···N3 (Fig. 3) are observed within the crystal lattice.

Biological activity. The cytotoxic activity was analyzed on human cancer cell lines, resistant variants (CCRF-CEM, CEM-DNR, K562, K562-Tax, A549, HCT116, HCT116p53−/−, and A549) and on normal human cells (BJ and MRC-5 – normal cycling fibroblasts) to analyze the therapeutic index that is based on the ratio between the IC_{50} for normal human cells and cancer cell lines. The SAR ought to be evaluated for individual cell lines independently, because it may vary because of different tissue origin and potential molecular targets in particular cell lines. However, in our case, the trend of activity was very similar for all tested cell lines indicating similar or identical target(s), and thus, we will discuss SAR generally (Table 1).

Generally, the cytotoxic activity of the compounds is rather low; only two derivatives **3b** and **3h** are showing promising cytotoxic activity (the lowest $IC_{50} = 1.45/2.84\ \mu M$). Interestingly, **3b** is showing preferential activity to CEM-DNR bulk, daunorubicin-resistant cancer cell line expressing P-glycoprotein (adenosine triphosphate-dependent transporter) and proteins from MRP family and K562-Tax, paclitaxel-resistant cancer cell line, expressing P-glycoprotein only [42]. Similarly, **3h** is showing preferential activity to CCRF-CEM, A549, and HCT116p53−/− cell line with deleted p53 protein and favorable therapeutic index to nontumor cell lines 24.32. Because P-glycoprotein positive cells are highly adenosine triphosphate dependent and the functional p53 protein is contributing to the cells survival in glucose and aminoacid deprived cells as well [43,44], the observed activity is suggesting disruption of energy metabolic pathways of the sensitive tumor cells.

Cell cycle study of the most active compound **3h** has been performed on CCRF-CEM lymphoblasts at 1× and 5× IC_{50} concentration within 24 h treatment. Finally, we did not observe any effect on cell cycle proliferation, DNA and RNA synthesis even in highest concentration (Table 3).

CONCLUSION

In conclusion, 11 new imidazo[1,2-*c*]pyrimidine-5(6*H*)-ones have been synthesized. General conditions for the Suzuki–Miyaura cross-coupling reaction were found, and all compounds were properly characterized. Cytotoxic activity is dependent on the substitution of the aryl ring, introduced by Suzuki–Miyaura cross-coupling reaction. Two derivatives showed promising activity with the lowest $IC_{50} = 1.45\ \mu M$ for **3h** on CCRF-CEM and $IC_{50} = 2.84\ \mu M$ for **3b** on resistant CEM-DNR bulk cell line. Moderate activity of some other derivatives was observed. Compound **3h** showed favorable therapeutic index to nontumor cell lines 24.32.

EXPERIMENTAL

General. The NMR spectra were measured on Bruker Avance (Rheinstetten, Germany) II 400 at 400.13 MHz (^{1}H), 100.62 MHz (^{13}C), 40.54 MHz (^{15}N), and 376.50 MHz (^{19}F) or Bruker Avance 500 at 500.13 MHz (^{1}H) and 125.67 MHz (^{13}C) in DMSO-d_6 (Rheinstetten, Germany) at ambient temperature. The ^{1}H and ^{13}C chemical shifts were referenced to the residual signal of the solvent ($\delta = 2.50$ for ^{1}H and 39.52 for ^{13}C). The ^{15}N and ^{19}F chemical shifts were referred to external nitromethane in a coaxial capillary and internal $CFCl_3$, respectively, both having $\delta = 0.0$.

The X-ray data for colorless crystal of **3a** (Fig. 1) were obtained at 150 K using Oxford Cryostream low-temperature device on a Nonius KappaCCD diffractometer with MoK_α radiation ($\lambda = 0.71073$ Å), a graphite monochromator, and the ϕ and χ scan mode. Data reductions were performed with DENZO-SMN [45]. The absorption was corrected by integration methods [46]. Structures were solved by direct methods (Sir92) [47] and refined by full matrix least square based on F^2 (SHELXL97) [48]. Hydrogen atoms were mostly localized on a difference Fourier map; however, to ensure uniformity of treatment of crystal, all hydrogen

Table 2

^{1}H, ^{13}C, and some ^{15}N chemical shifts (ppm) of compounds **2** and **3a–j**.

Position	2		3a		3b		3c		3d	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1[b]	—	−122.6	—	−129.4	—	−129.2	—	—	—	—
2	7.90	114.1	7.87	112.6	7.89	112.6	7.85	112.5	7.85	112.6
3	7.37	132.0	7.45	132.0	7.49	129.0	7.45	132.0	7.45	132.0
4[b]	—	−190.8	—	−189.4	—	−189.3	—	—	—	—
5	—	146.1	—	145.7	—	145.7	—	145.7	—	145.6
6[b,c]	11.81	−243.3	11.90	−249.1	11.94	−248.9	11.81	—	11.95	—
7	7.58	134.2	7.52	126.5	7.63	126.5	7.44	125.4	7.57	126.9
8	—	59.8	—	110.5	—	110.0	—	110.3	—	109.1
8a	—	145.2	—	144.6	—	144.5	—	144.7	—	144.2
8-1′	—	—	—	133.1	—	132.2	—	125.4		131.9
8-2′	—	—	7.97	127.7	8.11	128.1	7.92	128.8	8.02	129.2
8-3′	—	—	7.42	128.3	7.75	126.6	6.99	113.7	7.44	128.2
8-4′	—	—	7.33	127.5	—	139.1	—	158.7	—	131.9
X	—	—	—	—	[d]	139.6	3.78	54.9	—	—

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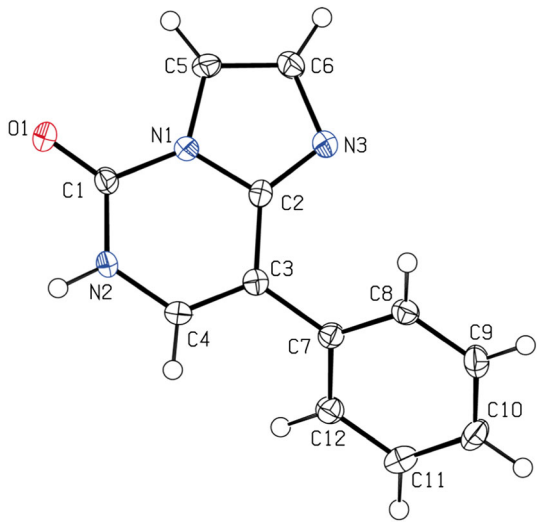

Figure 1. The molecular structure (ORTEP 50% probability level) of **3a**. Selected interatomic distances (Å), angles and interplanar angle (°): O1-C1 1.213(2), C1-N1 1.397(2), N1-C5 1.386(2), C5-C6 1.351(2), C6-N3 1.390(2), N3-C2 1.320(2), C2-C3 1.439(2), C3-C4 1.352(2), C4-N2 1.381(2), N2-C1 1.361(2), C3-C7 1.481(3); C6-N3-C2 105.29(14), N3-C2-N1 110.60(15), C2-N1-C5 107.37(14), C2-N1-C1 126.04(15), N1-C1-N2 112.78(15), C1-N2-C4 124.24(14); C2-C3-C4 versus C8-C7-C12 45.68(3). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

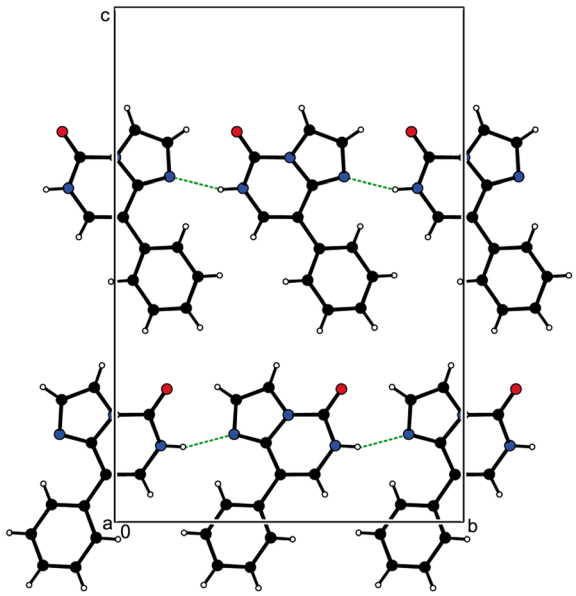

Figure 2. Interatomic N2-H2···N3 interactions of **3a**, view along axis *a*. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

were recalculated into idealized positions (riding model) and assigned temperature factors $H_{iso}(H) = 1.2\ U_{eq}$(pivot atom) with C—H 0.93 Å for hydrogen atoms in aromatic rings and hydrogen atom for N—H bond was localized and assign from Fourier map, standard calculated distance for N—H bond is 0.86 Å.

$R_{int} = \sum |F_o^2 - F_{o,mean}^2| / \sum F_o^2$, $GOF = [\sum (w(F_o^2 - F_c^2)^2)/(N_{diffrs} N_{params})]^{1/2}$ for all data, $R(F) = \sum ||F_o| - |F_c|| / \sum |F_o|$ for observed data, $wR(F^2) = [\sum (w(F_o^2 - F_c^2)^2)/(\sum w(F_o^2)^2)]^{1/2}$ for all data.

Crystallographic data for structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC no. 931506 for **3a**. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EY, UK (fax: +44-1223-336033; E-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

Table 2

(Continued)

3e[a]		3f		3g		3h		3i		
δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	^1J(C,H)
—	—	—	—	—	—	—	—	—	—	
7.85	112.7	7.89	112.7	7.88	112.7	7.88	112.7	7.87	112.8	196.8
7.45	132.0	7.49	132.0	7.48	132.0	7.46	132.0	7.49	132.0	190.5
—	—	—	—	—	—	—	—	—	—	—
—	145.6	—	145.6	—	145.6	—	145.5	—	145.4	—
11.96	—	12.09	—	12.02	—	12.10	—	11.91	—	—
7.69	128.1	7.78	128.5	7.71	127.9	7.79	128.7	7.68	125.0	182.5
—	108.9	—	109.1	—	109.4	—	109.5	—	105.7	—
—	144.1	—	144.0	—	144.2	—	143.9	—	143.4	—
	137.3	—	139.2	—	137.5	—	138.0	—	134.5	—
8.22	128.0	8.30	127.8	8.17	129.3	8.28	127.9	7.53	124.7	168.0
7.72	125.0	7.95	129.5	7.99	127.4	7.87	132.2	7.12	127.1	167.9
—	127.6	—	134.8	—	129.4	—	108.5	7.84	125.4	182.5
—	124.4	11.01	192.6	12.90	167.2	—	119.0	—	—	—

[a](δ ^{19}F) = −60.6.

[b](δ ^{15}N).

[c]^1J (^{15}N, ^{1}H) = 90.1 Hz.

[d]δ (^{1}H/^{13}C) = –/139.7, 7.76/126.7, 7.53/129.1, 7.42/127.6

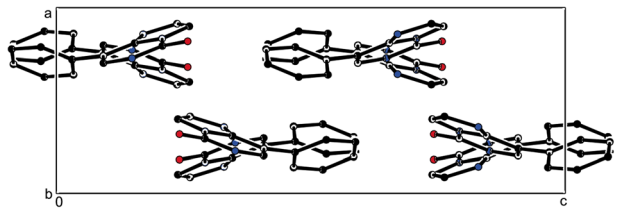

Figure 3. Crystal packing of **3a**, view along axis *b*. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Single crystals of **3a** (obtained by slow evaporation of acetone solution) were of sufficient quality for X-ray diffraction measurement. The crystallographic data and structure refinement parameters for compound **3a** are $C_{12}H_9N_3O$, M = 211.22, orthorhombic, *Pbca*, $a = 7.2310(3)$, $b = 13.4800(9)$, $c = 19.9031(12)$ Å, $\alpha = \beta = \gamma = 90°$, Z = 8, V = 1940.03(19) Å^3, $D_c = 1.446$ g.cm^{-3}, $\mu = 0.097$ mm^{-1}, $T_{min}/T_{max} = 0.985/0.991$; $-8 \le h \le 9$, $-17 \le k \le 14$, $-25 \le l \le 25$; 16,294 reflections measured ($\theta_{max} = 27.50°$), 16,153 independent ($R_{int} = 0.0383$), 1644 with $I > 2\sigma(I)$, 146 parameters, $S = 1.117$, *R1*(obs. data) = 0.0484, *wR2*(all data) = 0.0940; maximum, minimum residual electron density = 0.275, −0.211 eÅ^{-3}.

High-resolution mass spectrometry (HRMS) analyses were measured with Thermo Exactive instrument (Thermo Scientific, Waltham, MA, USA). The injection was performed by autosampler of high-performance liquid chromatography apparatus Accela 1250. The chromatographic preseparation parameters: column Luna C18, 3 µm, 50 × 2mm i.d. (Phenomenex, Torrance, CA), mobile phase acetonitrile/water 50/50 with 0.1% of formic acid, flow rate 200 µL/min, the column temperature 30°C. Sample preparation was obtained using following procedure: The 1 mg of sample was dissolved in 10 mL of solvent acetonitrile/water 5/5 (1 min sonication), and then, 70 µL of this solution and 930 µL of the same solvent were added into vial and mixed before injection of 5 µL. High-resolution mass spectrometer Exactive based on orbitrap mass analyzer was equipped with heated electrospray ionization. The spectrometer was tuned to obtain maximum response for *m/z* 70–600. The source parameters were set to the following values: heated electrospray ionization temperature 250°C, spray voltage +3.0 kV (positive mode), transfer capillary temperature 300°C, and sheath gas/aux gas (nitrogen) flow rates 35/10. The HRMS spectra of target peaks allowed an evaluation of their elemental composition because of high intensities of their protonated molecules. The identification of the respective structures was performed with less than 0.6 ppm difference between experimental and theoretically calculated value.

IR spectra were recorded on Nicolet 6700 Fourier transform infrared spectroscopy over the range of 400–4000 cm^{-1}. Elemental analyses were performed on a Thermo Flash 2000 CHNS experimental organic analyzer. Melting points were determined on Stuart SMP3 apparatus (Barloworld Scientific, Staffordshire, UK). Reaction progress was monitored by thin-layer chromatography (dichloromethane/methanol 5:1 as mobile phase), which was performed on SiO_2 60 F_{254} plates with ultraviolet detection

Table 3

Effects of compound **3h** on cell cycle, apoptosis, and DNA/RNA synthesis in CCRF-CEM lymphoblasts (% of positive cells).

Compound	<G1	G0/G1	S	G2/M	pH3^{Ser10}	BrDU	BrU
Control	8.7	43.0	39.4	17.6	1.8	43.8	40.7
3h 1× IC50	7.2	41.8	41.0	17.2	2.0	44.4	41.6
3h 5× IC50	7.2	44.9	38.3	16.8	2.0	39.7	41.0

at 254 nm. Column chromatography was performed on silica gel 60. All starting materials were commercially available.

Chemistry. ***5-Iodocytosine (1) [49].*** Cytosine (100 g, 0.9 mol) was dissolved in the solution of 80 g of potassium hydroxide in 1500 mL of water. Then, the pulverized iodine (229 g, 0.902 mol) was added, and the mixture was stirred for 70 min at r.t. Thereafter, the reaction mixture was heated to reflux in the course of 70 min and refluxed for 2 h. After cooling to r.t., 30 mL of 20% solution of sodium thiosulfate was added, reaction mixture was stirred overnight, neutralized with 3.5 mL of acetic acid, and filtered and washed with 200 mL of water. Filter cake was suspended in 1250 mL of water and boiled for 10 min. Then cooled overnight and filtered and washed with 200 mL of water and 200 mL of methanol. Product was dried at 60° C/30 mbar. Yield 174.5 g (82%), off-white crystals, mp 238–243°C dec. (lit. 225–245°C dec.[49]), ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.54 (br s, 1H, NH), 7.80 (br s + s, 2H, NH + CH), 10.80 (br s, 1H, NH); ^{13}C-NMR (100.6 MHz, DMSO-d_6): δ 55.3, 149.5, 155.9, 164.7.

8-Iodoimidazo[1,2-c]pyrimidin-5(6H)-one (2). To the mixture of 50 g of 5-iodocytosine (0.211 mol) and 43.3 g (0.528 mol) of sodium acetate in 500 mL of water, 47.8 mL (0.422 mol) of chloroacetaldehyde (57% solution in water) was added. The mixture was stirred for 4 h at 80°C. Then, the mixture was cooled to r.t., product was filtered, washed with 400 mL of water and 100 mL of methanol. Product was dried, yield 47.4 g (86%), brown powder. Analytical sample was recrystallized from acetone and dried at 50–60°C/30 mbar to obtain off-white crystals, mp 230–235°C dec., IR (KBr): 3434, 3218, 3144, 3128, 3099, 2947, 1716, 1604, 1537, 1377, 1331, 1269, 1246, 1165, 1138, 1116, 774, 707, 642, 622, 574, and 546 cm^{-1}. HRMS *m/z* [M+H]$^+$ Calcd. for $C_6H_5IN_3O$: 261.94718, found 261.94704. *Anal*. Calcd. for $C_6H_4IN_3O$: C, 27.61; H, 1.54; N, 16.10. Found: C, 27.82; H, 1.51; N, 15.92.

General procedure for Suzuki–Miyaura cross-coupling. The mixture of 2 g (7.66 mmol) of **2**, 11.50 mmol of appropriate boronic acid, 3.25 g (30.66 mmol) of sodium carbonate, and 0.168 g (0.23 mmol) of Pd(dppf)Cl$_2$ (0.280 g (0.38 mmol) in case of **3j**) was placed to the flask, and argon atmosphere was introduced by three vacuum/argon cycles. First, 40 mL of ethanol and then 20 mL of water were added (48 mL of ethanol and 12 mL of water in case of **3j**), and an additional three vacuum/argon cycles were carried out. Reaction mixture was heated to reflux for 24 h, and then, the product was isolated from reaction mixture (for isolation procedure for **3b** and **3g**, refer to the succeeding texts). Thereafter, the mixture was cooled to r.t., 50 mL of water was added, and then, 6 mL of 35% hydrochloric acid was added slowly. Ethanol was evaporated at 50°C under reduced pressure. A little of active charcoal was added, and the mixture was stirred at 80°C for 10 min. The mixture was filtered over cellite and washed twice with 10 mL of 4% hydrochloric acid at 80°C. After cooling to r.t., pH of the filtrate was adjusted to 8–9 by solid sodium carbonate. Precipitated product was filtered, washed with 100 mL of water, and dried. Analytical samples of all products were recrystallized from acetone (dimethylformamide in case of **3f** and **3g**) and dried at 50–60°C/ 30 mbar. Pure product **3j** was obtained after column chromatography with dichloromethane/methanol 10 : 1 as a mobile phase (for NMR chemical shifts of compounds **3a-j**, refer to Table 2).

Isolation of products 3b and 3g. After refluxing reaction mixture for 24 h (refer to the previous texts) and cooling to r.t., 50 mL of water was added, and ethanol was evaporated at 50°C under reduced pressure. The resulting suspension was filtered off and washed with 50 mL of water. Crude product was dried on air overnight and recrystallized from dimethylformamide (25 mL for **3b**, 50 mL for **3g**). Product **3b** was washed with diethylether (2 × 10 mL), product **3g** with acetone (3 × 10 mL).

8-Phenylimidazo[1,2-c]pyrimidin-5(6H)-one (3a). White crystals, yield 1.27 g (88%). mp 241–243°C, IR (KBr): 3429, 3156, 3113, 3066, 2933, 2820, 2737, 2633, 1731, 1620, 1548, 1511, 1494, 1445, 1411, 1288, 1274, 1247, 1142, 1114, 886, 771, 760, 743, 735, 704, 668, and 648, 574 cm^{-1}. HRMS *m/z* [M+H]$^+$ Calcd. for $C_{12}H_{10}N_3O$: 212.08184, found 212.08173. *Anal*. Calcd. for $C_{12}H_9N_3O$: C, 68.24; H, 4.29; N, 19.89; found: C, 68.24; H, 4.25; N, 19.79.

8-(Biphenyl-4-yl)imidazo[1,2-c]pyrimidin-5(6H)-one (3b). White crystals, yield 1.78 g (81%). mp 280–287°C, IR (KBr): 3438, 3241, 3146, 3127, 3080, 2940, 2898, 1732, 1618, 1544, 1485, 1407, 1286, 1278, 1262, 1243, 1144, 1109, 918, 836, 827, 758, 740, 719, 693, abd 649, 576 cm^{-1}. HRMS *m/z* [M+H]$^+$ Calcd. for $C_{18}H_{14}N_3O$: 288.11314, found 288.11309. *Anal*. Calcd. for $C_{18}H_{13}N_3O$: C, 75.25; H, 4.56; N, 14.63; found: C, 75.58; H, 4.61; N, 14.74.

8-(4-Methoxyphenyl)imidazo[1,2-c]pyrimidin-5(6H)-one (3c). White crystals, yield 1.61 g (90%). mp 248–250°C, IR (KBr): 3435, 3160, 3068, 2999, 2932, 2845, 1723, 1709, 1619, 1610, 1546, 1515, 1462, 1407, 1298, 1276, 1256, 1183, 1141, 1113, 1024, 882, 845, 829, 800, 742, 652, 621, 572, 563, and 519 cm^{-1}. HRMS *m/z* [M+H]$^+$ Calcd. for $C_{13}H_{12}N_3O_2$: 242.09240, found 242.09249. *Anal*. Calcd. for $C_{13}H_{11}N_3O_2$: C, 64.72; H, 4.60; N, 17.42; found: C, 64.79; H, 4.59; N, 17.32.

8-(4-Chlorophenyl)imidazo[1,2-c]pyrimidin-5(6H)-one (3d). White crystals, yield 1.41 g (75%). mp 262–264°C, IR (KBr): 3437, 3146, 3130, 3067, 2743, 1747, 1617, 1549, 1510, 1486, 1412, 1402, 1287, 1266, 1246, 1144, 1112, 1099, 922, 820, 811, 777, 747, 593, 490, and 463 cm^{-1}. HRMS *m/z* [M +H]$^+$ Calcd. for $C_{12}H_9ClN_3O$: 246.04287, found 246.04287. *Anal*. Calcd. for $C_{12}H_8ClN_3O$: C, 58.67; H, 3.28; N, 17.10; Cl, 14.43; found: C, 58.79; H, 3.23; N, 16.98; Cl, 14.55.

8-[4-(Trifluoromethyl)phenyl]imidazo[1,2-c]pyrimidin-5(6H)-one (3e). White crystals, yield 1.52 g (71%). mp 235–236°C, IR (KBr): 3443, 3074, 2935, 2845, 2745, 2638, 1739, 1724, 1619, 1550, 1517, 1414, 1329, 1278, 1246, 1161, 1141, 1132, 1122, 1115, 1070, 1018, 893, 851, 744, 684, 652, 634, 619, 603, 586, and 413 cm^{-1}. HRMS *m/z* [M+H]$^+$ Calcd. for $C_{13}H_9F_3N_3O$: 280.06922, found 280.06938. *Anal*. Calcd. for $C_{13}H_8F_3N_3O$: C, 55.92; H, 2.89; N, 15.05; found: C, 56.02; H, 2.92; N, 14.95.

4-(5-Oxo-5,6-dihydroimidazo[1,2-c]pyrimidin-8-yl)benzaldehyde (3f). Off-white solid, yield 1.52 g (83%). mp 267–270°C, IR (KBr): 3438, 3147, 3131, 3068, 2859, 1748, 1695, 1615, 1607, 1568, 1550, 1503, 1487, 1411, 1395, 1293, 1279, 1248, 1215, 1167, 1143, 1126, 1112, 922, 824, 747, 685, 650, 583, 556, 493, and 476 cm^{-1}. HRMS *m/z* [M+H]$^+$ Calcd for $C_{13}H_{10}N_3O_2$: 240.07675, found 240.07669. *Anal*. Calcd. for $C_{13}H_9N_3O_2$: C, 65.27; H, 3.79; N, 17.56; found: C, 64.98; H, 3.91; N, 17.42.

4-(5-Oxo-5,6-dihydroimidazo[1,2-c]pyrimidin-8-yl)benzoic acid (3g). Off-white solid, yield 1.45 g (74%). mp >350°C dec., IR (KBr): 3435, 3147, 3131, 3064, 2850, 1744, 1701, 1678, 1611, 1548, 1502, 1486, 1437, 1412, 1327, 1291, 1279, 1245, 1184, 1137, 1112, 922, 845, 774, 758, 746, 701, 587, 556, 483, and 442 cm^{-1}. HRMS *m/z* [M+H]$^+$ Calcd. for $C_{13}H_{10}N_3O_3$: 256.07167, found 256.07152. *Anal*. Calcd. for $C_{13}H_9N_3O_3$: C, 61.18; H, 3.55; N, 16.46; found: C, 60.82; H, 3.67; N, 16.35.

4-(5-Oxo-5,6-dihydroimidazo[1,2-c]pyrimidin-8-yl)benzonitrile (3h). White crystals, yield 1.38 g (84%). mp 310–325°C dec., IR (KBr): 3432, 3219, 3175, 3086, 2942, 2227, 1723, 1624, 1606, 1549, 1509, 1413, 1287, 1274, 1243, 1143, 844, 727, 611, 571, 544, and 500 cm^{-1}. HRMS *m/z* $[M+H]^+$ Calcd. for $C_{13}H_9N_4O$: 237.07709, found 237.07699. *Anal*. Calcd. for $C_{13}H_8N_4O$: C, 66.10; H, 3.41; N, 23.72; found: C, 66.42; H, 3.48; N, 23.35.

8-(Thiophen-2-yl)imidazo[1,2-c]pyrimidin-5(6H)-one (3i). White crystals, yield 1.01 g (61%). mp 249–255°C, IR (KBr): 3420, 3157, 3069, 2922, 2847, 2743, 1726, 1709, 1611, 1553, 1519, 1499, 1405, 1287, 1250, 1241, 1141, 1114, 878, 847, 830, 731, 718, 658, 638, 591, and 571 cm^{-1}. HRMS *m/z* $[M+H]^+$ Calcd. for $C_{10}H_8N_3OS$: 218.03826, found 218.03820. *Anal*. Calcd. for $C_{10}H_7N_3OS$: C, 55.29; H, 3.25; N, 19.34; S, 14.76; found: C, 55.09; H, 3.10; N, 19.09; S, 14.56.

8-(Pyridin-4-yl)imidazo[1,2-c]pyrimidin-5(6H)-one (3j). White crystals, yield 1.01 g (62%). mp 260–263°C dec., IR (KBr): 3436, 3137, 3082, 2936, 2736, 1732, 1723, 1620, 1604, 1556, 1501, 1418, 1326, 1284, 1258, 1135, 1117, 1072, 1008, 890, 828, 758, 679, 666, 649, 618, 579, 568, 549, and 497 cm^{-1}. HRMS *m/z* $[M+H]^+$ Calcd. for $C_{11}H_9N_4O$: 213.07709, found 213.07698. *Anal*. Calcd. for $C_{11}H_8N_4O$: C, 62.26; H, 3.80; N, 26.40; found: C, 61.98; H, 3.92; N, 26.56.

Biological activity. ***MTT assay.*** As for the cytotoxic MTT assay [50], all cells were purchased from the American Tissue Culture Collection, unless otherwise indicated: The CCRF-CEM line are highly chemosensitive T-lymphoblastic leukemia cells, K562 cells were derived from patient with acute myeloid leukemia with bcr-abl translocation, A549 line is lung adenocarcinoma, HCT116 is colorectal tumor cell line, and its p53 gene knockdown counterpart (HCT116p53-/-, Horizon Discovery, Cambridge, UK) is a model of human cancers with p53 mutation frequently associated with poor prognosis. The daunorubicin-resistant subline of CCRF-CEM cells (CEM-DNR bulk) and paclitaxel-resistant subline K562-Tax were selected in our laboratory by the cultivation of maternal cell lines in increasing concentrations of daunorubicin or paclitaxel, respectively, and were characterized well in the article by Nosková *et al*. [42]. The CEM-DNR bulk cells overexpress P-glycoprotein, MRP, and LRP protein, while K562-Tax cells overexpress only P-glycoprotein, both proteins belong to family of ABC transporters and are involved in primary and/or acquired multidrug resistance phenomenon. MRC-5 and BJ are nontumor human fibroblasts. The cells were maintained in Nunc/Corning 80 cm^2 plastic tissue culture flasks and cultured in cell culture medium (DMEM/RPMI 1640 with 5 g/L glucose, 2 m*M* glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, 10% fetal calf serum, and $NaHCO_3$). Cell suspensions were prepared and diluted according to the particular cell type and the expected target cell density (25,000–30,000 cells/well based on cell growth characteristics). Cells were added by pipette (80 µL) into 96-well microtiter plates. Inoculates were allowed a preincubation period of 24 h at 37°C and 5% CO_2 for stabilization. Fourfold dilutions, in 20 µL aliquots, of the intended test concentration were added to the microtiter plate wells at time zero. All test compound concentrations were examined in duplicate. Incubation of the cells with the test compounds lasted for 72 h at 37°C, in a 5% CO_2 atmosphere at 100% humidity. At the end of the incubation period, the cells were assayed using MTT. Aliquots (10 µL) of the MTT stock solution were pipetted into each well and incubated for further 1–4 h. After this incubation period, the formazan produced was dissolved by the addition of 100 µL/well of 10% aq SDS (pH 5.5), followed by a further incubation at 37°C overnight. The optical density (OD) was measured at 540 nm with a Labsystem iEMS Reader MF (Labsystem, Helsinki, Finland). Tumor cell survival (IC_{50}) was calculated using the following equation: IC = (ODdrug-exposed well/mean ODcontrol wells) × 100%. The IC_{50} value, the drug concentration lethal to 50% of the tumor cells, was calculated from appropriate dose–response curves.

Cell cycle and apoptosis analysis. Subconfluent CCRF-CEM cells (American Tissue Culture Collection), seeded at the density of 5.10^5 cells/mL in 6-well panels, were cultivated with the 1× or 5× IC_{50} of tested compound in a humidified CO_2 incubator at 37°C in RPMI 1640 cell culture medium containing 10% fetal calf serum, 10 m*M* glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin. Control containing vehicle was harvested at the same time point (24 h). Cells were washed with cold phosphate buffered saline (PBS) and fixed in 70% ethanol overnight at 20°C. The next day, the cells were washed in hypotonic citrate buffer, treated with RNase (50 µg/mL), stained with propidium iodide, and analyzed by flow cytometry using a 488 nm single beam laser (Becton Dickinson, San Jose, CA). Cell cycle was analyzed in the program ModFitLT (Verity), and apoptosis was measured in logarithmic model as a percentage of the particles with propidium content lower than cells in G0/G1 phase (<G1) of the cell cycle. Half of the sample was used for $pH3^{Ser10}$ antibody (Sigma) labeling and subsequent flow cytometry analysis of mitotic cells.

BrDU incorporation analysis. Cells were cultured and treated as for cell cycle analysis. Before harvesting, they were pulse labeled with 10 µ*M* 5-bromo-2-deoxyuridine (BrDU) for 30 min. The cells were trypsinized, fixed with ice-cold 70% ethanol, incubated on ice for 30 min, washed with PBS, and resuspended in 2*M* HCl for 30 min at room temperature to denature their DNA. Following neutralization with 0.1*M* $Na_2B_4O_7$, the cells were washed with PBS containing 0.5% Tween-20 and 1% bovine serum albumin. They were then stained with primary anti-BrdU antibody (Exbio) for 30 min at room temperature in the dark. Cells were than washed with PBS and stained with secondary antimouse-FITC antibody (Sigma). The cells were then washed with PBS and incubated with propidium iodide (0.1 mg/mL) and RNase A (0.5 mg/mL) for 1 h at room temperature in the dark and finally analyzed by flow cytometry using a 488 nm single beam laser (FACSCalibur, Becton Dickinson).

BrU incorporation analysis. Cells were cultured and treated as for cell cycle analysis. Before harvesting, they were pulse labeled with 1 m*M* 5-bromouridine (BrU) for 30 min. The cells were fixed in 1% buffered paraformaldehyde with 0.05% of NP-40 in room temperature for 15 min and then in the refrigerator overnight. They were then washed in 1% glycin in PBS, washed in PBS, and stained with primary anti-BrDU antibody cross-reacting to BrU (Exbio) for 30 min at room temperature in the dark. Cells were than washed with PBS and stained with secondary antimouse-FITC antibody (Sigma). Following the staining, the cells were washed with PBS and fixed with 1% PBS buffered paraformaldehyde with 0.05% of NP-40. The cells were then washed with PBS, incubated with propidium iodide (0.1 mg/mL) and RNase A (0.5 mg/mL) for 1 h at room temperature in the dark, and finally analyzed by flow cytometry using a 488 nm single beam laser (FACSCalibur, Becton Dickinson).

Acknowledgments. This work was supported by Ministry of Industry and Trade of the Czech Republic (project FR-TI1/202) and by grants IGA UP LF_2014_010, CZ.1.05/2.1.00/01.0030 and study stay by CZ.1.07/2.4.00/17.0015. We thank Jan Vaněček for recording of infrared spectra and Dagmar Zvolánková for technical help.

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