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Anti-leukemia Activity of 7-hydroxy-2-substituted-methyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one Derivatives

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The synthesis of new 7-hydroxy-2-substituted-methyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-ones derivatives, designed as structural bicyclic analogues of the iron chelator deferiprone, is described. They were tested for their ability to inhibit proliferation in human Bcr-Abl$^+$ leukemia cells.

Keywords: Anti-leukemia; Iron chelator; Oxazolo[3,2-*a*]pyrimidin-5-one

INTRODUCTION

For a few decades, iron has been shown to favour neoplastic cell growth and display carcinogenic activity, due to its catalytic effect on the formation of hydroxy radicals, suppression of the activity of host defense cells and promotion of cancer cell multiplication.[1] Primarily neoplasms develop at body sites of excessive iron deposits, while the invaded host attempts to withhold iron from the tumor cells via sequestration of the metal in newly formed ferritin. The host also endeavours to withdraw iron from cancer cells via macrophage synthesis of nitric oxide.[2,3] In various tumor cell lines, some of us recently showed that the pro-tumoral effect of iron may be, in part, related to its ability to rescue cells from NO-mediated growth inhibition and apoptosis.[4] More specifically, the role of iron was then studied in chronic myeloid leukemia (CML), through therapeutic iron-level control. CML is the most common human leukemia, characterized by the presence of a chromosomal translocation (t9;22) inducing the apparition of a new translocated gene (Bcr-Abl) encoding a fusion protein (p210$^{Bcr-Abl}$), with an elevated tyrosine-kinase activity.[5] In CML derived cell lines iron was found to be required for various cell functions including cell differentiation, regulation of respiratory enzymes, and DNA reparation.[6] Consequently, iron depletion was described as an alternative approach for anti-leukemia therapy.[7]

Studies have demonstrated that iron chelators can effectively inhibit the growth of some neoplasms, including leukemia and neuroblastoma.[8] During the past twenty years, considerable research was conducted to design appropriate iron chelators,[9] leading to the development of compounds related to 3-hydroxypyridin-4-ones. For example deferiprone[10,11] and mimosine[12] are currently candidates for the development of orally active iron chelators. In this work we describe the synthesis and preliminary anti-leukemia activity of new 7-hydroxy-2-substituted-methyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-ones derivatives **1a–g**, **2a–c**, **3b** and **5a**, designed as analogues of deferiprone (Figure 1).

They were tested for their ability to inhibit the proliferation of Bcr-Abl$^+$ human leukemia cells.

MATERIALS AND METHODS

Chemistry

Melting points were determined with an SM-LUX-POL Leitz hot-stage microscope and are reported uncorrected. Infrared (IR) spectra were determined in KBr discs on a BRUKER IFS-25 spectrometer. NMR spectra were recorded on a BRUKER AC 200 spectrometer (200 MHz). Chemical shifts refer to

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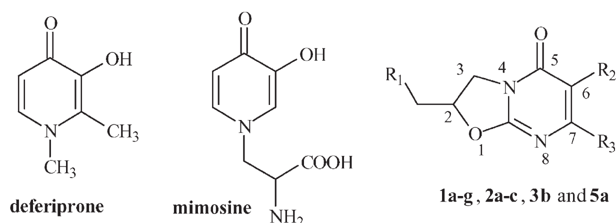

FIGURE 1 Structure of deferiprone, mimosine and 2-substituted-methyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-ones (**1a–g**), (**2a–c**), (**3b**) and (**5a**).

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tetramethylsilane which was used as an internal reference. OH appeared as singlet exchangeable with D_2O. Elemental analyses were conducted by CNRS, Vernaison, France and the results were within ± 0.3% of their calculated values.

General Procedure for the Preparation of 7-hydroxy-2-substituted-methyl-5H-oxazolo[3,2-a]pyrimidin-5-ones (1a–g)

To a solution of $NaOC_2H_5$ (27 mmol of Na metal, 70 ml of EtOH) was added diethyl malonate or diethylmethyl malonate (30.3 mmol) and 5-substituted-2-amino-2-oxazoline (**6**) (26 mmol). The reaction mixture was stirred at reflux for 6 h, then evaporated under reduced pressure. The precipitate was dissolved in 100 ml of ice-water, filtered and brought to pH 5 with diluted HCl. The residue was filtered, washed with cold water, dried and recrystallized from EtOH. In the case of compounds **1e–g**, after acidification with HCl the solution was evaporated to dryness and the residue purified on a silica gel column with chloroform–methanol (90/10, v/v) as eluent to afford the desired compounds.

7-Hydroxy-6-methyl-2-phenoxymethyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one, **1a**

As white crystals (45% yield), mp > 260°C. IR: 3440 (OH), 1655 cm^{-1} (CO); ^{1}H-NMR (d_6-DMSO) δ 11.17 (1H, s, OH), 7.28 (2H, t, $J = 7.85$ Hz, H-3′ and H-5′), 6.95 (1H, t, $J = 7.85$ Hz, H-4′), 6.92 (2H, d, 7.85 Hz, H-2′ and H-6′), 5.33 (1H, m, H-2), 4.30 (3H, m, H-3 and OCH_2), 3.95 (1H, dd, $J = 11.00$ and 6.75 Hz, OCH_2), 1.71 (3H, s, CH_3). ^{13}C-NMR (d_6-DMSO) δ 171.7 (CO), 166.8 (C-7), 163.1 (C-8a), 162.1 (C-1′), 134.7 (C-3′ and C-5′), 126.4 (C-4′), 119.8 (C-2′ and C-6′), 94.6 (C-6), 82.4 (C-2), 73.1 (OCH_2), 49.1 (C-3), 13.0 (CH_3). Elemental analysis ($C_{14}H_{14}N_2O_4$) C, H, N.

7-Hydroxy-2-phenoxymethyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one, **1b**

As white crystals (64% yield), mp 230°C. IR: 3410 (OH), 1665 cm^{-1} (CO); ^{1}H-NMR (d_6-DMSO) δ 11.39 (1H, s, OH), 7.29 (2H, t, $J = 7.85$ Hz, H-3′ and H-5′), 6.95 (1H, t, $J = 7.85$ Hz, H-4′), 6.93 (2H, d, 7.85 Hz, H-2′ and H-6′), 5.36 (1H, m, H-2), 5.02 (1H, s, H-6), 4.31 (3H, m, H-3 and OCH_2), 3.95 (1H, dd, $J = 10.95$ and 6.75 Hz, OCH_2). Elemental analysis ($C_{13}H_{12}N_2O_4$) C, H, N.

7-Hydroxy-6-methyl-2-[(2-ethoxyphenoxy)-methyl]-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one, **1c**

As white crystals (54% yield), mp > 260°C. IR: 3400 (OH), 1665 cm^{-1} (CO); ^{1}H-NMR (d_6-DMSO) δ 11.10 (1H, s, OH), 6.89 (4H, m, ArH), 5.33 (1H, m, H-2), 4.28 (3H, m, H-3 and OCH_2), 4.07 (1H, dd, $J = 10.85$ and 5.95 Hz, OCH_2), 3.87 (2H, q, $J = 6.90$ Hz, CH_2), 1.71 (3H, s, CH_3), 1.18 (3H, t, $J = 6.90$ Hz, CH_3). Elemental analysis ($C_{16}H_{18}N_2O_5$) C, H, N.

7-Hydroxy-2-[(2-ethoxyphenoxy)methyl]-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one, **1d**

As white crystals (21% yield), mp 188°C. IR: 3315 (OH), 1690 cm^{-1} (CO); ^{1}H-NMR (d_6-DMSO) δ 11.42 (1H, s, OH), 6.91 (4H, m, ArH), 5.35 (1H, m, H-2), 4.99 (1H, s, H-6), 4.26 (3H, m, H-3 and OCH_2), 4.07 (1H, dd, $J = 11.10$ and 5.60 Hz, OCH_2), 3.90 (2H, q, $J = 6.60$ Hz, CH_2), 1.22 (3H, t, $J = 6.60$ Hz, CH_3). Elemental analysis ($C_{15}H_{16}N_2O_5$) C, H, N.

7-Hydroxy-6-methyl-2-methoxymethyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one, **1e**

As white crystals (20% yield), mp 197°C. IR: 3405 (OH), 1680 cm^{-1} (CO); ^{1}H-NMR (d_6-DMSO) δ 11.26 (1H, s, OH), 5.12 (1H, m, H-2), 4.13 (1H, dd, $J = 10.80$ and 8.10 Hz, H-3a), 3.82 (1H, dd, $J = 10.80$ and 6.50 Hz, H-3b), 3.65 (2H, m, OCH_2), 3.31 (3H, s, OCH_3), 1.68 (3H, s, CH_3). Elemental analysis ($C_9H_{12}N_2O_4$) C, H, N.

7-Hydroxy-6-methyl-2-(*N,N*-diethylamino)-methyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one, **1f**

As white crystals (25% yield), mp 137°C. IR: 3400 (OH), 1700 cm^{-1} (CO); ^{1}H-NMR (d_6-DMSO) δ 10.85 (1H, s, OH), 5.02 (1H, m, H-2), 4.05 (1H, dd, $J = 9.55$ and 8.90 Hz, H-3a), 3.70 (1H, dd, $J = 9.55$ and 6.80 Hz, H-3b), 2.73 (2H, d, $J = 5.20$ Hz, NCH_2), 2.50 (4H, q, $J = 7.0$ Hz, CH_2), 1.28 (3H, s, CH_3), 0.92 (6H, t, $J = 7.0$ Hz, CH_3). Elemental analysis ($C_{12}H_{19}N_3O_3$) C, H, N.

7-Hydroxy-6-methyl-2-piperidinylmethyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one, **1g**

As white crystals (21% yield), mp 180°C. IR: 3420 (OH), 1715 cm^{-1} (CO); ^{1}H-NMR (d_6-DMSO) δ 10.88 (1H, s, OH), 5.10 (1H, m, H-2), 4.06 (1H, dd, $J = 9.50$ and 7.65 Hz, H-3a), 3.69 (1H, dd, $J = 9.50$ and 7.10 Hz, H-3b), 2.64 (2H, d, $J = 5.20$ Hz, NCH_2), 2.45 (4H, m, CH_2 pip.), 1.62 (3H, s, CH_3), 1.45 (5H, m, CH_2 pip.), 1.18 (1H, CH_2 pip.). Elemental analysis ($C_{13}H_{19}N_3O_3$) C, H, N.

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General Procedure for the Preparation of 7-acetoxy-2-substituted-methyl-5H-oxazolo[3,2-a]pyrimidin-5-ones (2a–c)

A solution of 7-hydroxy-2-substituted-methyl-*5H*-oxazolo[3,2-*a*]pyrimidin-5-one (**1a–c**) (4 mmol) and ZnI_2 (10 mg) in 6 ml of acetic anhydride was heated at 110°C for 2 h. The reaction mixture was evaporated to dryness, and after cooling, triturated with diethyl ether. The residue was then extracted with ethyl acetate, the organic layer was washed with a saturated aqueous sodium hydrogen carbonate, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by chromatography on a silica gel column, eluting with chloroform–methanol (90/10, v/v) to yield compounds **2a–c**.

7-Acetoxy-6-methyl-2-phenoxymethyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one, **2a**

As white crystals (27% yield), mp 116°C. IR: 1760, 1665 cm^{-1} (CO); ^1H-NMR (d_6-DMSO) δ 7.29 (2H, t, $J = 7.95$ Hz, H-3′ and H-5′), 6.95 (1H, t, $J = 7.95$ Hz, H-4′), 6.92 (2H, d, 7.95 Hz, H-2′ and H-6′), 5.42 (1H, m, H-2), 4.34 (3H, m, H-3 and OCH_2), 4.04 (1H, dd, $J = 11.25$ and 6.75 Hz, OCH_2), 2.27 (3H, s, CH_3), 1.74 (3H, s, CH_3). ^{13}C NMR (d_6-DMSO) δ 166.9 (CO), 161.1 (CO), 160.1 (C-7), 157.9 (C-8a), 156.8 (C-1′), 129.2 (C-3′ and C-5′), 120.9 (C-4′), 114.2 (C-2′ and C-6′), 103.0 (C-6), 77.6 (C-2), 67.4 (OCH_2), 43.8 (C-3), 20.0 (CH_3), 8.1 (CH_3). Elemental analysis ($C_{16}H_{16}N_2O_5$) C, H, N.

7-Acetoxy-2-phenoxymethyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one, **2b**

As white crystals (31% yield), mp 65°C. IR: 1775, 1690 cm^{-1} (CO); ^1H-NMR (d_6-DMSO) δ 7.30 (2H, t, $J = 8.10$ Hz, H-3′ and H-5′), 6.98 (1H, t, $J = 8.10$ Hz, H-4′), 6.93 (2H, d, 8.10 Hz, H-2′ and H-6′), 5.81 (1H, s, H-6), 5.45 (1H, m, H-2), 4.35 (3H, m, H-3 and OCH_2), 4.05 (1H, dd, $J = 11.20$ and 6.80 Hz, OCH_2), 2.24 (3H, s, CH_3). Elemental analysis ($C_{15}H_{14}N_2O_5$) C, H, N.

7-Acetoxy-6-methyl-2-[(2-ethoxyphenoxy)-methyl]-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one, **2c**

As yellow crystals (53% yield), mp 75°C. IR: 1770, 1680 cm^{-1} (CO); ^1H-NMR (d_6-DMSO) δ 6.88–6.79 (4H, ArH), 5.23 (1H, m, H-2), 4.43–4.15 (4H, m, H-3 and OCH_2), 3.91 (2H, q, $J = 6.95$ Hz, CH_2), 2.24 (3H, s, CH_3), 1.83 (3H, s, CH_3), 1.30 (3H, t, $J = 6.95$ Hz, CH_3). Elemental analysis ($C_{18}H_{20}N_2O_6$) C, H, N.

7-Chloro-2-phenoxymethyl-5H-oxazolo[3,2-a]pyrimidin-5-one (3b)

Compound **1b** (2 mmol) was refluxed with phosphorous oxychloride (20 ml) for 4 h. Subsequently, the excess of phosphorous oxychloride was distilled under vacuum and the residue was stirred with a saturated aqueous sodium hydrogen carbonate solution, filtered, washed with water and dried to give white crystals (61% yield) **3b**. mp 65°C. IR: 1660 cm^{-1} (CO); ^1H-NMR (d_6-DMSO) δ 7.30 (2H, t, $J = 7.55$ Hz, H-3′ and H-5′), 6.96 (1H, t, $J = 7.55$ Hz, H-4′), 6.93 (2H, d, 7.55 Hz, H-2′ and H-6′), 6.22 (1H, s, H-6), 5.44 (1H, m, H-2), 4.30 (3H, m, H-3 and OCH_2), 4.02 (1H, dd, $J = 10.85$ and 6.60 Hz, OCH_2). Elemental analysis ($C_{13}H_{11}ClN_2O_3$) C, H, N.

7-[(2,3-Epoxy)propoxy]-6-methyl-2-phenoxymethyl-5H-oxazolo[3,2-a]pyrimidin-5-one (4a)

To a solution of compound **1a** (7.1 mmol) in 130 ml of dry acetone were added anhydrous potassium carbonate (14.2 mmol) and epibromohydrin (7.8 mmol). The reaction mixture was refluxed for 5 h, after which the solvent was evaporated and the residue taken up in chloroform and water. The organic layer was separated, dried over Na_2SO_4 and evaporated under reduced pressure. The oily residue was purified by chromatography on a silica gel column with chloroform–methanol (90/10, v/v) as eluent to give a pale yellow oil (16% yield) **4a**. IR: 1670 cm^{-1} (CO); ^1H-NMR (d_6-DMSO) δ 7.29 (2H, t, $J = 8.00$ Hz, H-3′ and H-5′), 6.94 (1H, t, $J = 8.00$ Hz, H-4′), 6.92 (2H, d, $J = 8.00$ Hz, H-2′ and H-6′), 5.39 (1H, m, H-2), 4.52 (1H, dd, $J = 12.20$ and 2.50 Hz, H-1a″), 4.31 (3H, m, H-3 and OCH_2), 4.00 (2H, m, OCH_2 and H-1b″), 3.27 (1H, m, H-2″), 2.78 (1H, dd, $J = 5.00$ and 4.65 Hz, H-3a″), 2.65 (1H, dd, $J = 5.00$ and 2.65 Hz, H-3b″), 1.76 (3H, s, CH_3). ^{13}C-NMR (d_6-DMSO) δ 165.8 (CO), 161.3 (C-7), 157.9 (C-8a), 157.2 (C-1′), 129.6 (C-3′ and C-5′), 121.3 (C-4′), 114.7 (C-2′ and C-6′), 91.9 (C-6), 77.7 (C-2), 67.9 (OCH_2), 67.4 (OCH_2), 49.5 (OCH epox.), 44.1 (C-3), 43.8 (OCH_2 epox.), 7.7 (CH_3). Elemental analysis ($C_{17}H_{18}N_2O_5$) C, H, N.

7-[3-(N,N-diethylamino)-2-hydroxypropoxy]-6-methyl-2-phenoxymethyl-5H-oxazolo[3,2-a]pyrimidin-5-one (5a)

A mixture of **4a** (0.6 mmol) and 5 ml of diethylamine was heated at 70–80°C for 6 h. The excess of diethylamine was evaporated and the residue was taken up in water before extraction with dichloromethane. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The oily residue was purified by chromatography on a silica gel column with chloroform–methanol (90/10, v/v) as eluent to give a pale yellow oil (35% yield) **5a**. IR: 3395 (OH), 1670 cm^{-1} (CO); ^1H-NMR (d_6-DMSO) δ 7.29 (2H, t, $J = 8.00$ Hz, H-3′ and H-5′), 6.94 (1H, t, $J = 8.00$ Hz, H-4′), 6.92 (2H, d, $J = 8.00$ Hz, H-2′ and H-6′), 5.41 (1H, m, H-2), 4.72 (1H, bs, OH), 4.30 (4H, m, H-6, H-1a″ and OCH_2), 4.03 (2H, m, H-1b″ and OCH_2), 3.78 (1H, m, H-2″), 2.44 (6H, m, NCH_2), 1.76 (3H, s, CH_3), 0.91 (6H, t, $J = 7.05$ Hz, CH_3). ^{13}C-NMR (d_6-DMSO) δ 166.6 (CO), 161.4 (C-7), 158.1 (C-8a), 157.3

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(C-1′), 129.8 (C-3′ and C-5′), 121.4 (C-4′), 114.8 (C-2′ and C-6′), 91.8 (C-6), 77.7 (C-2), 69.5 (OCH_2), 68.1 (OCH_2), 67.3 (CHOH), 55.9 (NCH_2), 47.5 (NCH_2), 44.0 (C-3), 12.0 (CH_3), 7.8 (= C-CH_3). Elemental analysis ($C_{21}H_{29}N_3O_5$) C, H, N.

Pharmacology

Cells

MO7E cells are derived from Bcr-Abl$^-$ human myeloid cells and require, as for freshly isolated CD34$^+$ precursor cells, the presence of exogenous growth factor, interleukin-3 (IL-3) for their in vitro survival and growth. Following their transfection with Bcr-Abl gene,[13] these cells were transformed into a tumorigenic cell line (MBA4) that does not require exogenous growth factor (IL-3) for their continuous in vitro growth. In this work, MO7E and MBA4 cells were therefore used respectively as factor-dependent and Bcr-Abl-transformed counterpart of the same hematopoietic cells.

Cell Cultures

Cells were grown at 37°C and 5% CO_2 atmosphere, in αMEM medium (Gibco-BRL), supplemented with 10% fetal calf serum (FCS, Eurobio), penicillin 100 U mL^{-1}, streptomycin 100 μg mL^{-1}, 1 mM *L*-glutamine (Gibco-BRL) and 10 ng mL^{-1} of IL-3 (Sandoz Laboratories) for MO7E cells. Cells were seeded at 2.5×10^5 cells mL^{-1} in 24-wells plates (Falcon). Compounds were added at day 0, and cells were harvested 24, 48 and 72 h later, washed in phosphate buffer saline (Eurobio) and analysed for viability by trypan blue exclusion as described elsewhere.[4] In addition, 50 μl of 2.5×10^5 cells/ml were plated in 96-wells plates (Falcon) at day 0. The test compounds were solubilized in DMSO (Sigma-Aldrich) then diluted in culture medium for final concentrations of 25, 10, 5 and 1 μM. The compounds were then added to cells at different concentrations, diluted in 50 μL of culture medium. Cells were incubated at 37°C, 5% CO_2. At 24, 48 and 72 h culture, 20 μL/well of MTS (Cell titer 96® aqueous one solution cell proliferation assay, Promega) were added to cells and absorbance at 490 nm was recorded 3 h later in an ELISA plate reader. Each experiment was done in quadruplicate and results show the mean from three distinct experiments.

RESULTS AND DISCUSSION

Chemistry

5-Substituted-2-amino-2-oxazolines **6**, prepared from the corresponding epoxides **7**,[14] were used as starting materials in the synthesis of the 7-hydroxy-2-substituted-methyl-*5H*-oxazolo[3,2-*a*]pyrimidin-5-ones **1a–g**. Pyrimidine ring closure was achieved by heating **6** with diethyl malonate or diethyl methylmalonate in refluxing ethanol in the presence of sodium ethanolate to give the 7-hydroxy-2-substituted-methyl-*5H*-oxazolo[3,2-*a*]pyrimidin-5-ones **1a–g** as racemic compounds (Scheme 1).[15]

The structural assignment of **1** was made on the basis of the spectroscopic data (IR and NMR). The IR spectra of compounds **1** exhibited a carbonyl band at 1655–1715 cm^{-1} and the large band at 3315–3440 cm^{-1} which suggested the presence of an hydroxy group. The ^1H NMR spectra of **1a–g** showed a peak at $\delta = 10.85–11.42$ ppm assigned to a mobile enolic hydroxy proton. 2-Acetoxy derivatives **2a–c** were prepared in 27–53% yields by acetylation of the hydroxy compounds **1a–c** with acetic anhydride in presence of a catalytic amount of ZnI_2. The structure of **2b** was established by X-ray crystallography (Figure 2).[16]

The bicyclic oxazolopyrimidine system was found quite planar. Moreover, in the pyrimidine ring the measured bond lengths are shortened because of a π-electronic delocalization, indicating a pseudo-aromatic character for the hexagonal cycle.[17] Consequently, the enol function in parent compounds **1** could be compared to the 3-hydroxy of deferiprone which is implicated in metal complexation. In order to improve the chelating potential, various C-2 substitutions were investigated. For example, chlorodeshydroxylation of compound **1b** with phosphorous oxychloride led to the 7-chloro-2-phenoxymethyl-*5H*-oxazolo[3,2-*a*]pyrimidin-5-one **3b** in 61% yield. All attempts to displace the chlorine atom of **3b** with amines were unsuccessful. Finally, starting from mimosine as a lead compound, we designed **5a** with a lateral chain containing several lone paired heteroatoms. Pyrimidin-5-one **1a** was reacted with epibromohydrin in the presence of 4 equivalents of K_2CO_3 as a base in refluxing acetone for 6 h. Flash chromatography on a silica gel column led to the expected 7-[(2,3-epoxy)propoxy]-6-methyl-2-phenoxymethyl-*5H*-oxazolo[3,2-*a*]pyrimidin-5-one **4a**. Finally, **4a** was treated with diethylamine, to yield the β-hydroxyamino derivative **5a** (Scheme 1).

Pharmacology

Compounds **1a–g**, **2a–c**, **3b** and **5a** were assessed for their ability to inhibit the *in vitro* proliferation of leukemic Bcr-Abl$^+$ MBA4 cells and IL-3-dependent Bcr-Abl$^-$ MO7E cells. As depicted in Table I, at 25 μM, all increased the percentage of growth inhibition. Nevertheless, overall results indicated that MBA4 cells were more sensitive to the studied compounds than MO7E cells.

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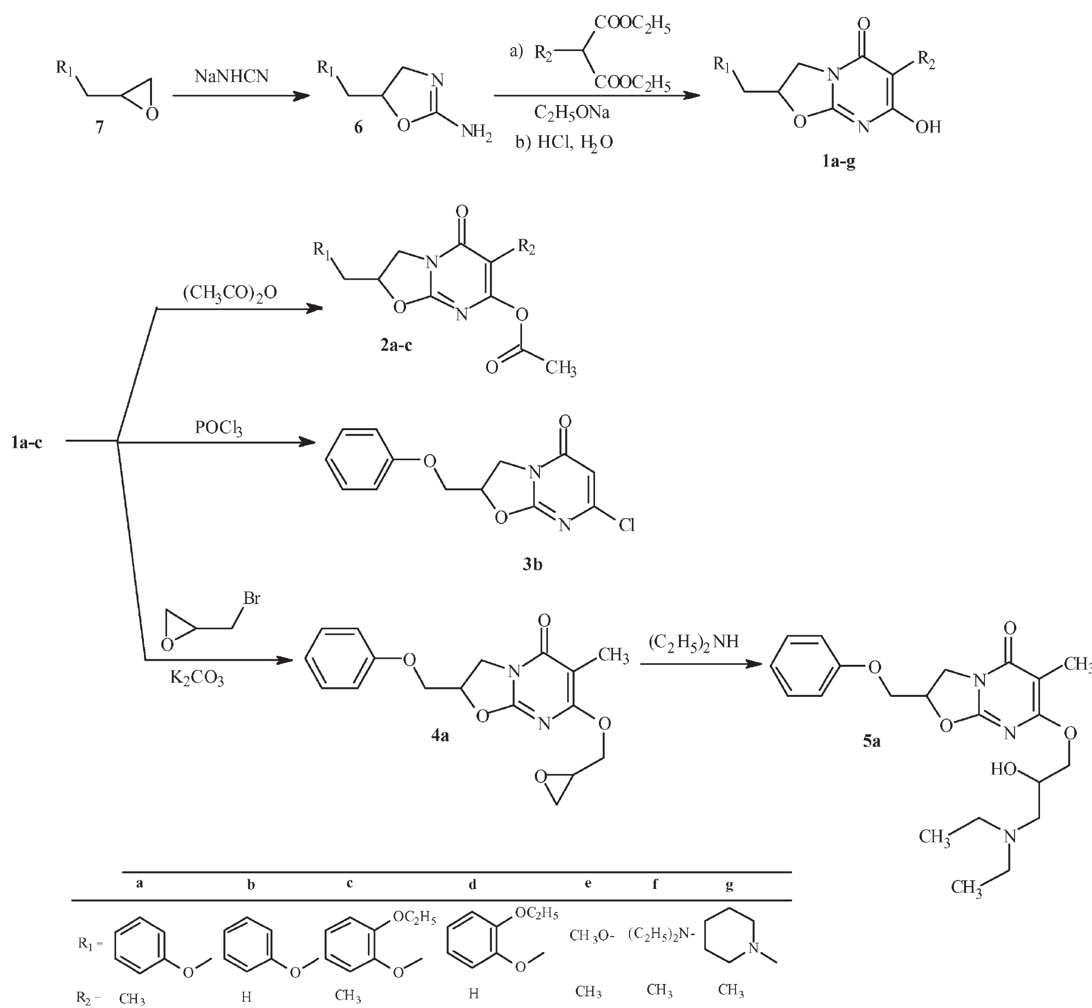

SCHEME 1 Synthesis of compounds (**1a–g**), (**2a–c**), (**3b**), (**4a**) and (**5a**).

Interestingly, some compounds discriminated between the cells as they preferentially inhibited Bcr-Abl$^+$ MBA4 cell growth, without any evidence for MO7E cell inhibition. This is clearly shown for compounds **1c**, **1d**, **1e**, **1f**, **1g**, **2a** and **2b**, which inhibited MB4A cells, but had low (**1c**, **1f**) if any effect (**1d**, **1g**, **2a**, **2b**) on the MO7E cells. Finally, only **1b**, **3b** and **5a** presented more important inhibitory effects on MO7E than on MBA4 cells. As CML cells display higher sensitivity to iron deprivation than

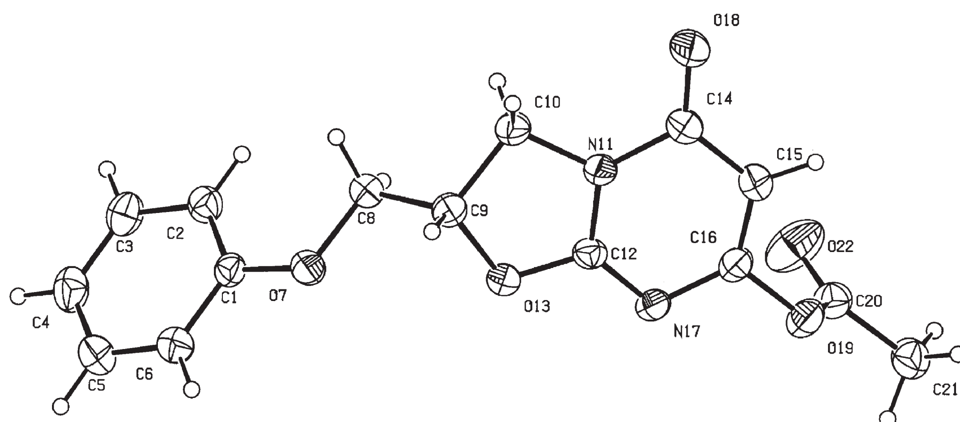

FIGURE 2 Side view of the crystal structure of **2b** with our numbering scheme, displacement ellipsoids are drawn at the 30% probability level.

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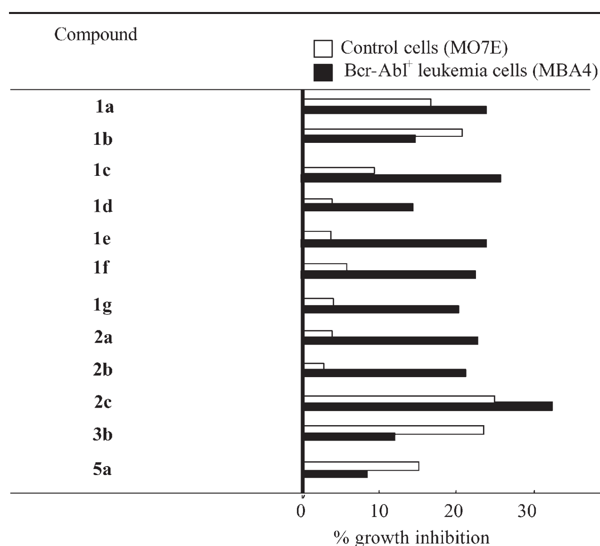
TABLE I Biological effects of the novel synthesized compounds at 25 μM*



* Means from three distinct experiments, each done in quadruplicate (SD±8%).

other hematopoietic cells,[7] the MBA4 cells inhibition of compounds **1c**, **1d**, **1e**, **1f**, **1g**, **2a** and **2b** could be, in part, related to an iron chelator effect.

In terms of structure–activity relationships only preliminary results were attained, the number of tested compounds being too limited to draw a definitive conclusion. As illustrated with compounds **1a–g**, no particular influence was linked to the nature of the substituent on the 2-position. On the other hand, in compounds **1** and **2** the C-2 substitution of oxygenated moieties, well suited for metal coordination, led to the most efficient compounds. From the X-ray data established for **2b**, the intramolecular distance O(22)...N(17) was 3.300(2)Å, a well suited length for iron chelation, in comparison with the iron chelating site in related potent ligands.[18]

From this work it appears that some new 7-hydroxy-2-substituted-methyl-5*H*-oxazolo[3,2-*a*] pyrimidin-5-ones showed anti-leukemia activity illustrated by preferential proliferation inhibition of Bcr-Abl$^+$ human leukemia cells. Although CML cells display higher sensitivity to iron deprivation than other hematopoietic cells, further studies will be necessary to determine if the mechanism of action could be related to an eventual iron chelator potency.

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