Med Chem Res
DOI 10.1007/s00044-017-1993-8

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Imatinib derivatives as inhibitors of K562 cells in chronic myeloid leukemia

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Received: 27 April 2017 / Accepted: 13 July 2017

Abstract Imatinib was the first representative of the class of Breakpoint cluster region-Abelson murine leukemia viral oncogene homolog (BCR-ABL) tyrosine kinase inhibitors used for the treatment of chronic myeloid leukemia. Second-generation and third-generation drugs have been introduced in this therapy, affording increased patient survival. However, all BCR-ABL tyrosine kinase inhibitors have been shown to induce resistance, necessitating a search for new therapeutic options. The sunitinib, another tyrosine kinase inhibitor used in the treatment of renal cell carcinoma and gastrointestinal stromal tumors is an isatin derivative. Isatin nucleus is highly versatile for the preparation of new substances, and several tyrosine kinase inhibitors examples have been obtained using it. This work aimed to design, synthesize, and biological evaluation of new compounds using the K562 cell line, which constitutively expresses the active BCR-ABL enzyme. Three new series of imatinib derivatives have been planned from the imatinib, and all have a phenylaminopyrimidine group as the main pharmacophore. Sunitinib was used as a structural prototype to planning the series 1 (**8a–e**) of hybrids between sunitinib and imatinib. Series 2 and 3 are 2-oxo-2-phenyacetamide and 2,2-difluoro-2-phenylacetamide derivatives, respectively. Isatins were used as the starting materials for all series. Compounds were synthesized using simple methodologies and were obtained in high purities. The compounds were tested against K562 cells, and four showed a reduction in cell viability, with EC_{50} values ranging from 0.37 to 2.02 μM, some of which are close to the imatinib standard (0.21 μM).

Keywords Tyrosine kinase inhibitors · Imatinib · Sunitinib · Isatin · Phenylacetamide

Electronic supplementary material The online version of this article (doi:10.1007/s00044-017-1993-8) contains supplementary material, which is available to authorized users.

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Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder of pluripotent stem cells, initially described by John Hughes Bennett in 1845 (Bennett 1845; Geary 2000; Degos 2001; Joske 2008). In the late 1960s, Nowell and Hungerford identified an abnormal chromosome present in human leukemic cells (Geary 2000; Nowell and Hungerford 1961; Majid and Ahmad 2015; Ali 2016). This chromosome, called Philadelphia (Ph), was the first example of a specific chromosomal abnormality related to malignancies (Rowley 1973; Abraham et al. 2016), being the result of a translocation between chromosome arms 9

Published online: 04 September 2017

and 22, which is found in 95% of patients with CML (Jabbour and Kantarjian 2016). This oncogene is responsible for coding a protein with high tyrosine kinase activity owing to the presence of the Breakpoint cluster region-Abelson murine leukemia viral oncogene homolog (BCR-ABL) domain (Nowell and Hungerford 1961; Druker et al. 2001) that is responsible for the cell transformation and the disease pathogenesis (Lugo et al. 1990). Consequently, the elucidation of its molecular mechanism was of great importance for advancing CML chemotherapy, where the inhibition of BCR-ABL tyrosine kinase represents an interesting strategy, since it is present only in positive Ph patients (Geary 2000; Rafiyath et al. 2013).

The first selective BCR-ABL tyrosine kinase inhibitor (TKI), imatinib (**1**; Fig. 1), was approved for use in 2001 by the Food and Drug Administration, revolutionizing the treatment of CML (Jabbour et al. 2007). Imatinib has a phenylaminopyrimidine group (PAP, Fig. 1) as its pharmacophore, which prevents ATP from binding to the Abl domain, via hydrogen bonds and van der Waals interactions (Manley et al. 2002; Asaki et al. 2006; Eck and Manley 2009; Mughal et al. 2013; Iqbal and Iqbal 2014). However, despite all its benefits, failure of the treatment associated with a resistance mechanism has been observed in some patients (Iqbal and Iqbal 2014). This fact led to the development of new TKIs, including second- (nilotinib, **2**; and dasatinib, **3**) and third- (bosutinib, **4**; and ponatinib, **5**) generation inhibitors (Fig. 1) (Azevedo et al. 2017). However, not all patients are successfully treated using those drugs because of the development of resistance related to mutations or serious side effects, including toxicity (Jabbour et al. 2010; Rosti et al. 2012). Furthermore, the European Leukemia net (ELN) and the USA National Comprehensive Cancer Network (NCCN) recommend, for optimally responding patients, following the TKI therapy indefinitely (Baccarani et al. 2013; NCCN 2017) to prevent interruption, which could cause cancer recurrence (Cortes et al. 2004). For this reason, new anticancer drugs with higher efficacy and lower toxicity are in constant demand (Liu et al. 2015).

In 2011, sunitinib (**6**), another TKI was used in the treatment of renal cell carcinoma (RCC) and gastrointestinal stromal tumors (Moreno et al. 2010). This anticancer drug is an isatin derivative (**7**) (Fig. 2) presenting a benzoheterocycle that is highly versatile for the preparation of various other molecules with potential pharmacological activity (Silva et al. 2001; Rane et al. 2016; Ibrahim et al. 2016). Moreover,

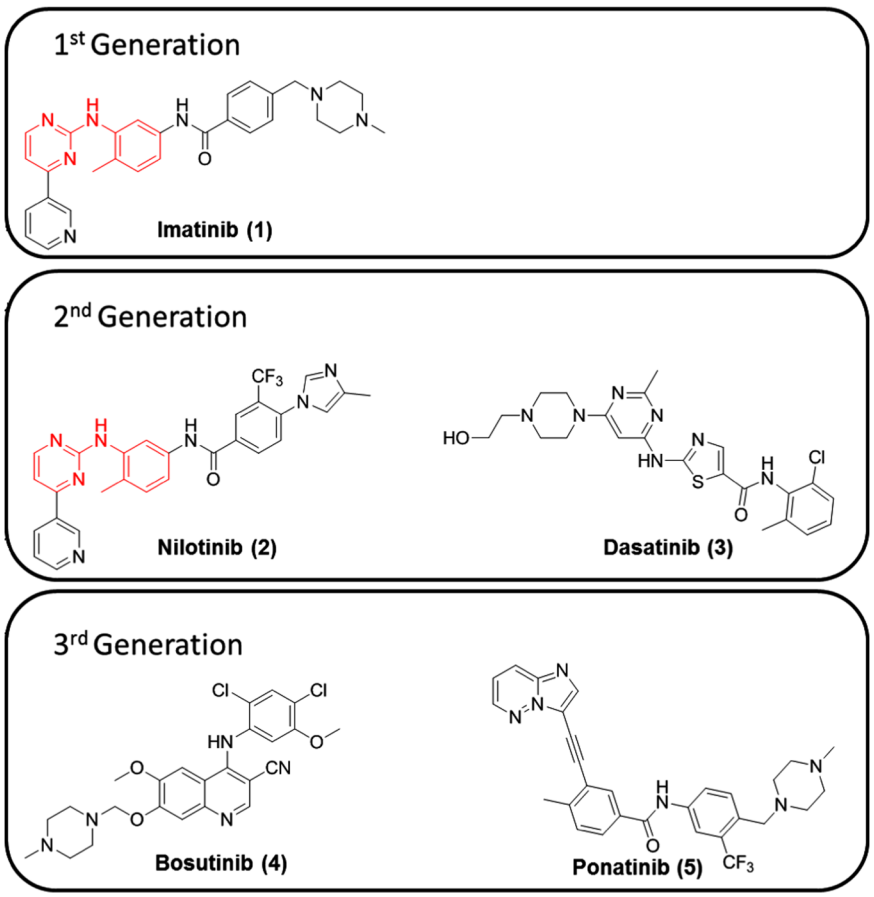


Fig. 1 Three generations of tyrosine kinase inhibitors (imatinib, **1**; nilotinib, **2**; dasatinib, **3**; bosutinib, **4**; ponatinib, **5**) used in the treatment of chronic myeloid leukemia (CML). The phenylaminopyrimidine group (PAP) is highlighted in *red* in **1** and **2** (color figure online)

several derivatives of isatin with CML activity have been described (Sabet et al. 2010; Aboul-Fadl et al. 2012).

Hence, in the present work, we have carried out the synthesis of three series of imatinib derivatives **8a–e**, **9a–e**, and **10a–e** followed by their cytotoxic activity evaluation on the K562 cell line, which constitutively expresses the active BCR-ABL enzyme. The three series of new derivatives have been planned from the imatinib, and the sunitinib was been used as a structural prototype to planning the series 1 (**8a–e**); all of them have the PAP group as their main pharmacophore fragment. Series 1 (**8a–e**) has hybrids between sunitinib and imatinib. Isatins were used as starting materials for all series; series 2 has 2-oxo-2-phenylacetamides (**9a–e**), and series 3 has 2,2-difluoro-2-phenylacetamides (**10a–e**) (Fig. 3).

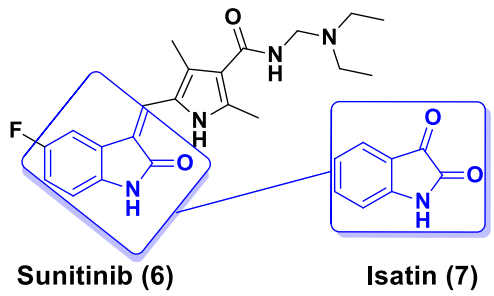


Fig. 2 Sunitinib (**6**), a tyrosine kinase inhibitor used in the treatment of renal cell carcinoma (RCC) and gastrointestinal stromal tumors, which has an isatin nucleus (**7**), highlighted in *blue* (color figure online)

Material and methods

Chemistry

Reagents utilized in most cases were purchased from Sigma-Aldrich Co. and used without further purification.

Solvents were purchased from Tedia and Vetec, dried as described using appropriate techniques for each type of solvent, and stored under nitrogen atmosphere.

Analyzes were monitored by thin layer chromatography (TLC) using silica gel sheets supported on aluminum indicators by ultraviolet light (254 and 366 nm)—Kieselgel 60 F254 Merck.

Mass spectra from a system coupled to an electron impact gas chromatograph (GC-MS) were obtained at 70 eV on an Agilent 6890 apparatus with an Agilent 5973 mass spectrometer. Fragmentation values and molecular ions were described by the relation between the atomic mass unit and the load thereof (*m/z*), and relative abundances were expressed as percentages. The column used was an Agilent 122-5532 DB-5MS (5% diphenyl: 95% dimethyl polysiloxane), and the runs were conducted using a temperature ramp from 50 to 350 °C.

Melting points were determined in a Büchi B-545 apparatus, and the values were not corrected.

Low-resolution mass spectra were achieved by electrospray ionization (MS-ESI) in a Micromass ZQ4000 apparatus. The molecular ion was described by the relation between the atomic mass unit and the load thereof (*m/z*), and relative abundances were expressed as percentages.

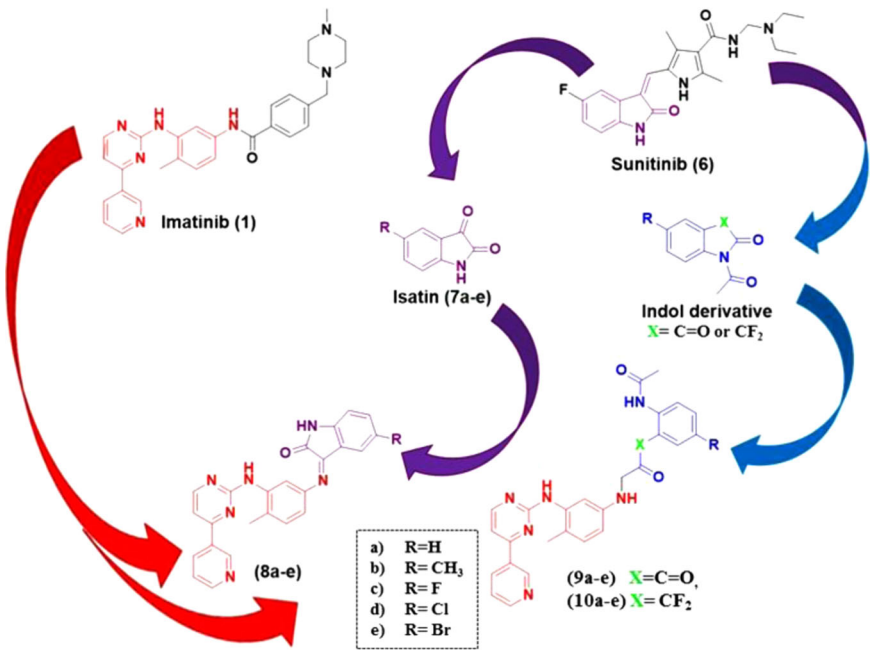


Fig. 3 The three series of new derivatives planned from the imatinib and sunitinib structures; all of them have the phenylaminopyrimidine group (PAP) highlighted in *red* and isatin derivatives highlighted in *blue* or *pink*. Series 1 (**8a–e**) has hybrids between sunitinib and imatinib, series 2 has 2-oxo-2-phenylacetamides (**9a–e**), and series 3 has 2,2-difluoro-2-phenylacetamides (**10a–e**) (color figure online)

Infrared spectra were recorded in a Thermo Scientific spectrophotometer, Nicolet 6700 model. The values of the absorptions are reported in wave numbers, using reciprocal centimeters (cm^{-1}) as a unit.

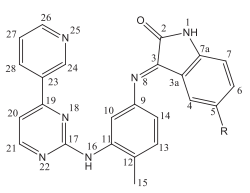
Nuclear magnetic resonance (NMR) spectra were obtained at 400 MHz for hydrogen, 100 MHz for carbon, 400 MHz for phosphorus and 376 MHz for fluorine. Trimethylsilane (TMS) was used as an internal reference standard for hydrogen and carbon (0 ppm). Chemical shifts were reported in dimensionless units (δ) representing parts per million (ppm). The relative areas of the signals were obtained by electronic integration, and their multiplicities were described as single signal (s), double signal (d), triple signal (t), multiple signal (m), large signal (ls) and double signal (dd).

High-resolution mass spectra (HRMS) were registered using a mass spectrometer by electron ionization (EI-MS, digitalizing ES + capillary), in a Maxis 3 G apparatus.

Analytical high-performance liquid chromatography (HPLC) analyzes were obtained in a Shimadzu device (VP). Data acquisition and control were performed using Shimadzu CLASS-VP software version 6.13 SP2. Chromatographic runs were from 190 to 800 nm. The eluents used were (A) methanol:water (1:2) and (B) acetonitrile with isocratic elution of 45 min of 25% (B); the flow of the mobile phase was 1.7 mL/min, and the injected volume was 20 µL. Separation was obtained in a Shimpack MRC-C8 column, 250 × 6 mm, with particle diameters of 5 µm.

*General procedure for the synthesis of **8 (a–e)***

To a 100-mL flask equipped with a condenser, Dean-Stark apparatus and mechanical stirrer were added 1 mmol of the corresponding isatins (**7a–e**) and 1 mmol (277 mg) PAP (**11**) in 50 mL of toluene. The reaction mixture was maintained under reflux for 24 h. The completion of the reaction was monitored by TLC, and then the medium was cooled to room temperature. A solid was obtained, filtered and washed with cold toluene.



Preparation of 3-((4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)imino)indolin-2-one (**8a**) Yield: 96%, mp: 184–185 °C, infrared (IR) (cm^{-1}): 3444 (N–H); 3068 (C-H *sp*2); 1727 (C=O); 1614 (C=N); 832; 795; 755 (aromatic ring), ^{1}H NMR (400 MHz; dimethyl sulfoxide (DMSO)-d_6, δ, ppm): 2.32 (s, 3 H, H-15); 6.73 (m, 3 H, H-4, H-6, H-7); 6.90 (d, $J = 7.8$ Hz, 1 H, H-5); 7.27 (d, $J =$ 1.8 Hz, 1 H, H-10); 7.35 (m, 3 H, H-13, H-14, H-27); 7.42 (d, $J = 5.1$ Hz, 1 H, H-20); 8.31 (dt, $J = 1.8$ e 8.0 Hz, 1 H, H-28); 8.49 (d, $J = 5.2$ Hz, 1 H, H-21); 8.65 (dd, $J = 1.4$ e 4.7 Hz, 1 H, H-26); 8.99 (s, 1 H, H-16); 9.21 (d, $J = 1.7$ Hz, 1 H, H-24); 10.96 (s, 1 H, H-1), ^{13}C NMR (100 MHz; DMSO-d_6, δ, ppm): 17.59 (CH_3, C-15); 107.65 (C-20); 111.30 (C-7); 113.52 (C-10); 113.72 (C-3a); 115.64 (C-5); 121.55 (C-14); 123.55 (C-27); 125.64 (C-4); 128.47 (C-12); 131.16 (C-13); 131.99 (C-6); 133.99 (C-23); 134.25 (C-28); 138.72 (C-11); 146.82 (C-7a); 147.95 (C-9); 148.26 (C-3); 151.29 (C-24); 154.74 (C-19); 159.34 (C-26); 160.98 (C-17); 161.41 (C-21); 163.52 (C-2), MS-ESI ([M + Na] +, *m/z*, %): 443 (100), HRMS—theoretical value ($C_{24}H_{18}N_6O$): 406.1542, value obtained: 406.1540, HPLC (%, nm): 97 (261).

5-methyl-3-((4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl) amino)phenyl)imino)indolin-2-one (**8b**) Yield: 62%, mp: 195–197 °C, IR (cm^{-1}): 2984 (C-H *sp*2); 1727 (C=C); 1616 (C=N); 798; 749; 705 (aromatic ring), ^{1}H NMR (400 MHz; DMSO-d_6, δ, ppm): 1.88 (s, 3 H, 5-CH_3); 2.31 (s, 3 H, H-15); 6.55 (s, 1 H, H-4); 6.73 (dd, $J = 2.1$ e 7.8 Hz, 1 H, H-6); 6.78 (d, $J = 8{,}1$ Hz, 1 H, H-7); 7.13 (dd, $J = 1.0$ e 8.6 Hz, 1 H, H-13); 7.22 (d, $J = 2.0$ Hz, 1 H, H-27); 7.35 (d, $J = 8.1$ Hz, 1 H, H-14); 7.39 (m, 2 H, H-20 e H-10); 8.31 (dt, $J = 1.9$ e 8.0 Hz, 1 H, H-28); 8.48 (d, $J = 5.1$ Hz, 1 H, H-21); 8.65 (dd, $J = 1.6$ e 4.7 Hz, 1 H, H-26); 9.05 (s, 1 H, H-16); 9.21 (d, $J = 1.6$ Hz, 1 H, H-24); 10.85 (s, 1 H, H-1), ^{13}C NMR (100 MHz; DMSO-d_6, δ, ppm): 17.67 (CH_3, C-15); 20.29 (CH_3,C-5); 107.64 (C-20); 111.07 (C-7); 113.66 (C-10); 113.73 (C-3a); 115.62 (C-5); 123.54 (C-14); 126.00 (C-27); 128.70 (C-4); 130.30 (C-12); 131.13 (C-13); 132.00 (C-6); 134.00 (C-23); 134.60 (C-28); 138.64 (C-11); 144.55 (C-7a); 147.93 (C-9); 148.13 130 (C-3); 151.27 (C-24); 154.81 (C-19); 159.31 (C-26); 160.94 (C-17); 161.45 (C-21); 163.61 (C-2), MS-ESI ([M − 1] +, *m/z*, %): 405 (100), HRMS-theoretical value ($C_{25}H_{20}N_6O$): 420.1699, value obtained: 420.1702, HPLC (%, nm): 88 (261).

5-fluoro-3-((4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl) amino)phenyl)imino)indolin-2-one (**8c**) Yield: 68%, mp: 237–239 °C, IR (cm^{-1}): 3452; 3256 (N–H); 2853 (CH_3); 1739 (C=O); 1620 (C=N); 867; 822; 784 (aromatic ring), ^{1}H NMR (400 MHz; DMSO-d_6, δ, ppm): 2.32 (s, 3 H, H-15); 6.52 (dd, $J = 2.4$ e 8.5 Hz, H-4); 6.75 (dd, $J = 2.1$ e 7.9 Hz, 1 H, H-10); 6.89 (dd, $J = 4.3$ e 8.6 Hz, 1 H, H-7); 7.20 (td, $J = 2.5$ e 8.9 Hz, 1 H, H-6); 7.27 (d, $J = 1.8$ Hz, 1 H, H-27); 7.37 (m, 3 H, H-13, H-14 e H-20); 8.31 (dt, $J = 1.8$ e 8.0 Hz, 1 H, H-28); 8.44 (d, $J = 5.1$ Hz, 1 H, H-21); 8.64 (dd, $J = 1.1$ e 4.6 Hz, 1 H, H-26); 9.05 (s, 1 H, H-16); 9.20 (d, $J = 1.6$ Hz, 1 H, H-24); 10.99 (s, 1 H, H-1), ^{13}C NMR (100 MHz; DMSO-d_6, δ, ppm): 17.72 (CH_3, C-15); 107.86 (C-20) 112.43 (d, $J = 12.8$ Hz, C-7); 112.52 (d, $J = 20.4$ Hz, C-4);

113.88 (C-10); 113.97 (C-27); 116.07 (d, $J = 8.0$ Hz, C-3a); 120.76 (d, $J = 23.6$ Hz, C-6) 123.63 (C-14); 129.10 (C-12); 131.37 (C-13); 132.07 (C-23); 134.09 (C-28); 138.76 (C-11); 143.28 (d, $J = 1.1$ Hz, C-7a); 147.69 (C-9); 148.06 (C-19); 151.37 (C-24); 154.33 (d, $J = 2.3$ Hz, C-3); 156.87 (d, $J =$ 236.05 Hz, C-5); 159.27 (C-26); 161.07 (C-17); 161.66 (C-21); 163.67 (C-2), NMR ^{19}F (376 MHz; DMSO-d_6, δ, ppm): −121.15 (Ar–F), MS-ESI ([M − 1] + , *m/z*, %): 423 (100), HRMS—theoretical value ($C_{24}H_{17}FN_6O$): 424.1448, value obtained: 424.1447, HPLC (%, nm): 96 (261).

5-chloro-3-((4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl) amino)phenyl)imino)indolin-2-one (**8d**) Yield: 75%, mp: 247–248 °C, IR (cm^{-1}): 3449 (N–H); 3115 (C–H *sp*2); 1740 (C=O); 1623 (C=N); 819; 792; 747 (aromatic ring), ^{1}H NMR (400 MHz; DMSO-d_6, δ, ppm): 2.34 (s, 3 H, H-15); 6.73 (d, $J = 2.0$ Hz, 1 H, H-4); 6.76 (dd, $J = 7.9$ e 2.0 Hz, 1 H, H-7); 6.92 (d, $J = 8.4$ Hz, 1 H, H-6); 7.31 (d, $J =$ 1.9 Hz, 1 H, H-10); 7.39 (m, 4 H, H-13, H-14, H-20 e H-27); 8.32 (dt, $J = 8.0$ e 1.8 Hz, 1 H, H-28); 8.49 (d, $J = 5.1$ Hz, 1 H, H-21); 8.65 (dd, $J = 4.6$ e 1.2 Hz, 1 H, H-26); 9.07 (s, 1 H, H-16); 9.22 (d, $J = 1.4$ Hz,1 H, H-24); 11.12 (s, 1 H, H-1), ^{13}C NMR (100 MHz; DMSO-d_6, δ, ppm): 17.79 (CH_3, C-15); 107.90 (C-20); 112.99 (C-7); 113.58 (C-10); 113.66 (C-3a); 116.86 (C-5); 123.63 (C-14); 125.15 (C-27); 125.40 (C-4); 128.98 (C-12); 131.35 (C-13); 132.11 (C-6); 133.70 (C-23); 134.14 (C-28); 138.84 (C-11); 145.67 (C-7a); 147.75 (C-9); 148.09 (C-3); 151.36 (C-24); 153.83 (C-19); 159.40 (C-26); 161.00 (C-17); 161.66 (C-21); 163.36 (C-2), MS-ESI ([M − 1] + , *m/z*, %): 439 (100), HRMS—theoretical value ($C_{24}H_{17}ClN_6O$): 440.1152, value obtained: 440.1173. HPLC (%. nm): 98 (261).

5-bromo-3-((4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl) amino)phenyl)imino)indolin-2-one (**8e**) Yield: 77%, mp: 163–164 °C, IR (cm^{-1}): 3450; 3223 (N–H); 3115 (C–H *sp*2); 1741 (C=O); 1606 (C=N) 817; 787; 740 (aromatic ring), ^{1}H NMR (500 MHz; DMSO-d_6, δ, ppm): 2.32 (s, 3 H, H-15); 6.73 (d, $J = 6.3$ Hz, 1 H, H-4); 6.85 (m, 2 H, H-6 e H-7); 7.30 (s, 1 H, H-10); 7.39 (m, 3 H, H-13, H-14 e H-20); 7.48 (d, $J = 8.4$ Hz, 1 H, H-27); 8.32 (d, $J = 7.8$ Hz, 1 H, H-28); 8.49 (d, $J = 4.9$ Hz, 1 H, H-21); 8.64 (d, $J = 2.9$ Hz, 1 H, H-26); 9.05 (s, 1 H, H-16); 9.21 (s,1 H, H-24); 11.11 (s, 1 H, H-1), ^{13}C NMR (100 MHz; DMSO-d_6, δ, ppm): 17.79 (CH_3, C-15); 107.90 (C-20); 113.04 (C-7); 113.45 (C-10); 117.34 (C-3a); 123.61 (C-5); 125.30 (C-14); 127.92 (C-27); 128.16 (C-4); 128.88 (C-12); 131.29 (C-13); 132.11 (C-6); 134.13 (C-23); 134.46 (C-28); 138.82 (C-11); 146.01 (C-7a); 147.72 (C-9); 148.09 (C-3); 151.34 (C-24); 153.67 (C-19); 159.42 (C-26); 160.96 (C-17); 161.65 (C-21); 163.18 (C-2), MS-ESI ([M + Na] + , *m/z*, %): 508 (99); 506 (100), HRMS—theoretical value ($C_{24}H_{17}BrN_6O$): 484.0647, value obtained: 484.0634, HPLC (%, nm): 89 (261).

General procedure for the synthesis of ***12 (a–e)***

To a 100-mL flask equipped with a reflux condenser were added 5-substituted isatin (**7a–e**) (1 mol) and 20 moles of previously distilled acetic anhydride. The reaction mixture was kept under magnetic stirring at reflux for 2–4 h until the reaction was complete. The completion of the reaction was monitored by TLC. The reaction mixture was cooled in the freezer for 2 h, and after this time, the precipitate was filtered under vacuum, washed with hexane and dried at room temperature. The solids were recrystallized with hexane and ethyl acetate (1:1). The derivatives were characterized by GC-MS, and melting points were compared with the literature (Boechat et al. 2008; James et al. 1989).

1-acetylindoline-2,3-dione (**12a**) Yield: 82%, Mp: 139–140 °C (Lit. 143 °C), GC-MS (70 eV, *m/z*, %): 189 (14); 147 (23); 146 (100); 119 (6); 90 (23).

1-acetylb-5-methylindoline-2,3-dione (**12b**) Yield: 89%, Mp: 173–174 °C (Lit. 173 °C), GC-MS (70 eV, *m/z*, %): 203 (17); 161 (51); 160 (100); 133 (20); 104 (26).

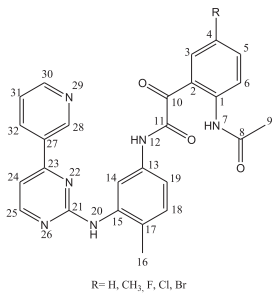
1-acetyl-5-fluoroindoline-2,3-dione (**12c**) Yield: 72%, Mp: 148–150 °C (Lit. 149 °C), GC-MS (70 eV, *m/z*, %): 207 (14); 165 (50); 164 (100); 137 (16); 108 (64).

1-acetyl-5-chloroindoline-2,3-dione (**12d**) Yield: 87%, Mp: 242–243 °C (Lit. 245 °C), GC-MS (70 eV, *m/z*, %): 223 (9); 182 (35); 181 (53); 180 (100); 153 (19); 124 (41).

1-acetyl-5-bromoindoline-2,3-dione (**12e**) Yield: 85%, Mp: 169–170 °C (Lit. 173 °C), GC-MS (70 eV, *m/z*, %): 268 (22); 267 (22); 227 (64); 226 (100); 225 (64); 224 (100); 170 (33).

General procedure for the synthesis of ***9 (a–e)***

To a 100-mL flask were added 1 mmol of the corresponding *N*-acetylisatins (**12a–e**) and 1 mmol (277 mg) of PAP (**11**) in 50 mL of acetonitrile. The reaction mixture was maintained under mechanical stirring for 24 h. The completion of the reaction was monitored by TLC, and the product was precipitated in the medium. The solid was isolated by filtration and washed with cool acetonitrile.



2-(2-acetamidophenyl)-N-(4-methyl-3-((4-(pyridin-3-yl) pyrimidin-2-yl)amino)phenyl)-2-oxoacetamide (**9a**) Yield: 58%, mp: 211–214 °C, IR (cm^{-1}): 3246 (N–H); 3061 (C–H *sp*2); 2249 (CH_3); 1691; 1670 (C=O); 875; 821; 763 (aromatic ring), ^{1}H NMR (400 MHz; DMSO-d_6, δ, ppm): 2.03 (s, 3 H, H-9); 2.24 (s, 3 H, H-16); 8.15 (s, 3 H, H-19); 7.23 (d, $J = 8.4$ Hz, 1 H, H-18); 7.28 (td, $J = 1.0$ e 7.7 Hz, 1 H, H-4); 7.44 (d, $J = 5.12$ Hz, 1 H, H-14); 7.51 (dd, $J = 4.7$ e 7.9 Hz, 1 H, H-31); 7.79 (m, 1 H, H-6); 7.64 (d, $J = 1.4$ e 7.8 Hz, 1 H, H-5); 7.72 (d, $J = 7.7$ Hz, 1 H, H-3); 8.46 (dt, $J = 2.0$ e 7.9 Hz, 1 H, H-32); 8.52 (d, $J = 5.16$ Hz, 1 H, H-25); 8.70 (dd, $J = 1.4$ e 4.7 Hz, 1 H, H-30); 9.00 (s, 1 H, H-20); 9.29 (d, $J = 1.7$ Hz, 1 H, H-28); 10.64 (s, 1 H, H-7); 10.69 (s, 1 H, H-12), ^{13}C NMR (100 MHz; DMSO-d_6, δ, ppm): 17.69 (C-16); 23.82 (C-9); 107.65 (C-24); 116.30 (C-14); 116.64 (C-19); 121.79 (C-6); 123.79 (C-2); 123.81 (C-31); 124.51 (C-4); 128.18 (C-17); 130.30 (C-18); 131.41 (C-3); 132.19 (C-27); 134.03 (C-5); 134.47 (C-1); 135.90 (C-32); 137.99 (C-13); 138.26 (C-15); 148.19 (C-28); 151.36 (C-30); 159.52 (C-25); 161.05 (C-23); 161.57 (C-11); 161.76 (C-21); 168.95 (C-8); 190.23 (C-10), MS-ESI ([M + 1] + , *m/z*, %): 465 (100), HRMS—theoretical value ($C_{26}H_{22}N_6O_3$): 466.1753, value obtained: 446.1754, HPLC (%, nm): 100 (261).

2-(2-acetamido-5-methylphenyl)-N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)-2-oxoacetamide (**9b**) Yield: 51%, mp: 226–228 °C, IR (cm^{-1}): 3242 (N–H); 3066 (C–H *sp*2); 1687; 1670 (C=O); 827; 800; 787 (aromatic ring), ^{1}H NMR (400 MHz; DMSO-d_6, δ, ppm): 2.00 (s, 3 H, H-9); 2.24 (s, 3 H, H-16); 2.31 (CH_3, C-4); 7.23 (d, $J = 8.3$ Hz, 1 H, H-18); 7.44 (m, 3 H, H-31, H-24 e H-14); 7.50 (m, 2 H, H-3 e H-5); 7.66 (d, $J = 8.2$ Hz, 1 H, H-6); 8.15 (d, $J = 1.6$ Hz, 1 H, H-19); 8.46 (dt, $J = 1.4$ e 6.2 Hz, 1 H, H-32); 8.51 (d, $J = 5.1$ Hz, 1-H, H-25); 8.70 (dd, $J = 1.4$ e 4.7 Hz, 1 H, H-30); 8.98 (s, 1 H, H-20); 9.29 (d, $J = 1.7$ Hz, 1 H, H-28); 10.52 (s, 1 H, H-7); 10.65 (s, 1 H, H-12), ^{13}C NMR (100 MHz; DMSO-d_6, δ, ppm): 17.65 (C-16); 20.16 (C-9); 23.70 (CH_3, C-4); 107.63 (C-24); 116.29 (C-14); 116.63 (C-19); 122.02 (C-6); 123.76 (C-31); 124.68 (C-4); 128.15 (C-17); 130.26 (C-18); 131.18 (C-27); 132.18 (C-3); 133.03 (C-5); 134.43 (C-1); 134.55 (C-32); 135.91 (C-15); 137.97 (C-13); 148.17 (C-28); 151.30 (C-30); 159.47 (C-25); 161.04 (C-23); 161.56 (C-11); 161.82 (C-21); 168.78 (C-8); 190.31 (C-10), MS-ESI ([M + 1] + , *m/z*, %): 479 (100), HRMS—theoretical value ($C_{27}H_{24}N_6O_3$): 480.1910, value obtained: 480.1897, HPLC (%, nm): 97 (261).

2-(2-acetamido-5-fluorophenyl)-N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)-2-oxoacetamide (**9c**) Yield: 63%, mp: 231–233 °C, IR (cm^{-1}): 3329 (N–H); 3065 (C–H *sp*2); 1687; 1669 (C=O); 832; 815; 801; 787 (aromatic ring), ^{1}H NMR (400 MHz; DMSO-d_6, δ, ppm): 1.95 (s, 3 H, H-9); 2.24 (s, 3 H, H-16); 7.22 (d, $J = 8.2$ Hz, 1 H, H-18); 7.43 (d, $J = 5.1$ Hz, 1 H, H-24); 7.48 (m, 5 H, H-14, H-31, H-6, H-5 e H-3); 8.19 (s, 1 H, H-19); 8.47 (d, $J = 7.8$ Hz, 1 H, H-32); 8.52 (d, $J = 5.4$ Hz, 1 H, H-25); 8.70 (d, $J = 4.1$ Hz, 1 H, H-30); 8.99 (s, 1 H, H-20); 9.30 (s, 1 H, H-28); 10.49 (s, 1 H, H-7); 10.62 (s, 1 H, H-12), ^{13}C NMR (100 MHz; DMSO-d_6, δ, ppm): 17.59 (CH_3, C-16); 23.05 (CH3, C-9); 107.51 (C-24); 136.17 (d, $J = 23.8$ Hz, C-5); 116.38 (C-14); 116.72 (C-31); 119.66 (d, $J = 22.3$ Hz, C-3); 123.71 (C-19); 124.59 (d, $J = 7.5$ Hz, C-2); 128.06 (C-17); 128.82 (d, $J = 6.5$ Hz, C-6); 130.11 (C-18); 132.11 (C-27); 133.04 (d, $J = 2.3$ Hz, C-1); 134.40 (C-32); 135.87 (C-15); 137.81 (C-13); 148.08 (C-28); 151.27 (C-30); 157.96 (d, $J = 241.1$ Hz, C-4); 159.40 (C-25); 160.32 (C-21); 160.98 (C-11); 161.45 (C-23); 168.74 (C-8); 187.26 (C-10), NMR ^{19}F (376 MHz; DMSO-d_6, δ, ppm): −118,00 (Ar–F), MS-ESI ([M + Na] + , *m/z*, %): 523 (100), HRMS—theoretical value ($C_{26}H_{21}FN_6O_3$): 484.1659, value obtained: 484.1656, HPLC (%, nm): 98 (261).

2-(2-acetamido-5-chlorophenyl)-N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)-2-oxoacetamide (**9d**) Yield: 54%, mp: 217–218 °C, IR (cm^{-1}): 3442; 3252 (N–H); 3110 (C–H *sp*2); 1687; 1672; 1662 (C=O); 829; 818; 797 (aromatic ring), ^{1}H NMR (400 MHz; DMSO-d_6, δ, ppm): 1.97 (s, 3 H, H-9); 2.23 (s, 3 H, H-16); 7.22 (d, $J = 5.7$ Hz, 1 H, H-18); 7.43 (d, $J = 5.1$ Hz, 1 H, H-24); 7.50 (m, 3 H, H-5, H-31 e H-14); 7.66 (m, 2 H, H-3 e H-6); 8.17 (d, $J = 1.1$ Hz, 1 H, H-19); 8.47 (dt, $J = 1.8$ e 8.0 Hz, 1 H, H-32); 8.51 (d, $J = 5.1$ Hz, 1 H, H-25); 8.69 (dd, $J = 1.4$ e 4.7 Hz, 1 H, H-30); 8.97 (s, 1 H, H-20); 9.28 (d, $J = 1.7$ Hz, 1 H, H-28); 10.55 (s, 1 H, H-7); 10.60 (s, 1 H, H-12), ^{13}C NMR (100 MHz; DMSO-d_6, δ, ppm): 17.63 (C-16); 23.31 (C-9); 107.58 (24); 116.46 (C-14); 116.79 (C-19); 123.75 (C-6); 123.97 (C-31); 127.72 (C-4); 128.16 (C-17); 129.66 (C-18); 130.18 (C-27); 132.17 (C-3); 132.55 (C-5); 134.44 (C-1); 135.67 (C-32); 138.88 (C-15); 137.90 (C-13); 148.13 (C-28); 151.28 (C-30); 159.44 (C-25); 160.23 (C-21); 161.05 (C-11); 161.54 (C-23); 168.84 (C-8); 187.23 (C-10), MS-ESI ([M + 1] + , *m/z*, %): 479 (100), HRMS—theoretical value ($C_{26}H_{21}ClN_6O_3$): 500.1364, value obtained: 500.1362, HPLC (%, nm): 98 (261).

2-(2-acetamido-5-bromophenyl)-N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)-2-oxoacetamide (**9e**) Yield: 64%, mp: 211–212 °C, IR (cm^{-1}): 3247; 3110 (N–H); 3071 (C–H *sp*2); 1688; 1674; 1662 (C=O); 852; 818; 797 (aromatic ring), ^{1}H NMR (400 MHz; DMSO-d_6, δ, ppm): 1.96 (s, 3 H, H-9); 2.23 (s, 3 H, H-16); 7.21 (d, $J = 8.4$ Hz, 1 H, H-18); 7.47 (m, 4 H, H-14, H-24, H-32 e H-5); 7.77 (m, 2 H, H-6 e H-3); 8.16 (d, $J = 1.6$ Hz, 1 H, H-19); 8.46 (dt, $J = 2.0$ e 8.0 Hz, 1 H, H-32); 8.51 (d, $J = 5.2$ Hz, 1

H, H-25); 8.69 (dd, $J = 1.6$ e 4.8 Hz, 1 H, H-30); 8.97 (s, 1 H, H-20); 9.27 (d, $J = 1.6$ Hz, 1 H, H-28); 10.54 (s, 1 H, H-7); 10.29 (s, 1 H, H-12), ^{13}C NMR (100 MHz; DMSO-d_6, δ, ppm): 17.64 (C-16); 23.34 (C-9); 107.59 (C-24); 115.55 (C-14); 116.47 (C-19); 116.79 (C-6); 123.77 (C-2); 124.20 (C-31); 128.18 (C-17); 128.44 (C-4); 130.19 (C-18); 132.18 (C-27); 132.49 (C-27); 134.45 (C-5); 135.45 (C-1); 135.88 (C-32); 136.08 (C-13); 137.91 (C-15); 148.13 (C-28); 151.30 (C-30); 159.44 (C-25); 160.23 (C-21); 161.06 (C-11); 161.55 (C-23); 168.84 (C-8); 187.15 (C-10), MS-ESI ([M − 1] + , *m/z*, %): 543 (100), HRMS—theoretical value ($C_{26}H_{21}BrN_6O_3$): 544.0859, value obtained: 544. 0852, HPLC (%, nm): 98 (261).

*General procedure for the synthesis of **13** (**a–e**)*

To a 250-mL flask were added 1 mol of (**12a–e**) and 50 mL of fresh distilled dichloromethane (CH_2Cl_2). Next, 4 moles of DAST were added, and the reaction mixture was maintained under magnetic stirring at room temperature for 4 h. The completion of the reaction was monitored by TLC, and the mixture was poured onto ice. The organic layer was washed with distilled water (3 × 30 mL), and the dichloromethane solution was dried with anhydrous sodium sulfate, dried and filtered. The solvent was evaporated in a rotavapor, and the product was vacuum dried. Derivatives were characterized by GC-MS and melting points are compared with the literature (Cheah et al. 2008).

1-acetyl-3,3-difluoroindolin-2-one (**13a**) Yield: 91%, Mp: 108–111 °C (Lit. 109–111 °C), GC-MS: (70 eV, *m/z*, %): 211 (13); 169 (100); 168 (10);141 (63); 114 (10).

1-acetyl-3,3-difluoro-5-methylindolin-2-one (**13b**) Yield: 95%, Mp: 70–73 °C (Lit. 73–76 °C), GC-MS: (70 eV, *m/z*, %): 225 (12); 184 (9); 183 (100); 182 (8); 155 (73).

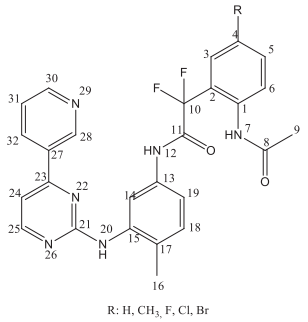
1-acetyl-3,3,5-trifluoroindolin-2-one (**13c**) Yield: 95%, Mp: 72–75 °C (Lit. 73–76 °C), GC-MS: (70 eV, *m/z*, %): 229 (13); 187 (100); 159 (64); 131 (13).

1-acetyl-5-chloro-3,3-difluoroindolin-2-one (**13d**) Yield: 85%, Mp: 133–135 °C (Lit. 134–136 °C), GC-MS: (70 eV, *m/z*, %): 245 (14); 205 (32); 203 (100); 175 (55).

1-acetyl-5-bromo-3,3-difluoroindolin-2-one (**13e**) Yield: 70%, Mp: 143–145 °C (Lit. 144–145 °C), GC-MS: (70 eV, *m/z*, %): 290 (13); 288 (13); 248 (99); 246 (100); 220 (34); 218 (35); 74 (15).

*General procedure for the synthesis of **10** (**a–e**)*

To a 100-mL flask were added 1 mol of (**13a–e**) and 1 mmol (277 mg) of PAP (**11**) in 50 mL of acetonitrile. The reaction mixture was maintained at room temperature under mechanical stirring for 24 h. The completion of the reaction was monitored by TLC, and the product was precipitated in the reaction mixture. The solid was filtered and washed with cool acetonitrile.



2-(2-acetamidophenyl)-2,2-difluoro-N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)acetamide (**10a**) Yield: 58%, mp: 211–214 °C, IR (cm^{-1}): 3392; 3307(N–H); 1689; 1662 (C=O); 862; 794; 765 (aromatic ring), ^{1}H NMR (400 MHz; DMSO-d_6, δ, ppm): 1.96 (s, 3 H, H-9); 2.22 (s, 3 H, H-16); 7.22 (d, $J = 8.3$ Hz, 1 H, H-18); 7.37 (m, 2 H, H-14 e H-4); 7.43 (d, $J = 5.1$ Hz, 1 H, H-24); 7.46 (dd, $J = 4.8$ e 7.9 Hz, 1 H, H-31); 7.56 (t, $J = 7.4$ Hz, 1 H, H-5); 7.62 (d, $J = 7.8$ Hz, 1 H, H-3); 7.68 (d, $J = 7.6$ Hz, 1 H, H-6); 7.99 (d, $J = 1.7$ Hz, 1 H, H-19); 8.43 (dt, $J = 1.8$ e 8.0 Hz, 1 H, H-32); 8.50 (d, $J = 5.1$ Hz, 1 H, H-25); 8.68 (dd, $J = 1.4$ e 4.7 Hz, 1 H, H-30); 8.97 (s, 1 H, H-20); 9.26 (d, $J = 1.8$ Hz, 1 H, H-28); 9.38 (s, 1 H, H-12); 10.68 (s, 1 H, H-7), ^{13}C NMR (100 MHz; DMSO-d_6, δ, ppm): 17.59 (CH3, C-16); 23.31 (C-9); 107.60 (C-24); 114.31 (t, $J = 252.3$ Hz, C-10); 117.22 (C-19); 117.61 (C-14); 123.66 (C-31); 125.44 (C-3); 126.33 (t, $J = 7.5$ Hz, C-1); 127.42 (C-5); 128.96 (C-2); 130.14 (C-17); 131.47 (C-18); 132.06 (C-4); 134.30 (C-27); 134.82 (C-32); 135.73 (C-6); 137.91 (C-15); 148.09 (C-13); 151.29 (C-28); 159.39 (C-30); 160.97 (C-25); 161.52 (C-23); 161.79 (C-11); 162.09 (C-21); 168.68 (C-8), NMR ^{19}F (376 MHz; DMSO-d_6, δ, ppm): −99.00 (CF_2), MS-ESI ([M + Na] + , *m/z*, %): 512 (100), HRMS—theoretical value ($C_{26}H_{22}F_2N_6O_2$): 488.1772, value obtained: 488.1754, HPLC (%, nm): 99 (261).

2-(2-acetamido-5-methylphenyl)-2,2-difluoro-N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)acetamide (**10b**) Yield: 45%, mp: 225–227 °C, IR (cm^{-1}): 3222 (N–H); 2984 (C–H *sp*2); 1693; 1682 (C=O); 823; 804; 790 (aromatic ring), ^{1}H NMR (400 MHz; DMSO-d_6, δ, ppm); 1.96 (s, 3 H, H-9); 2.22 (s, 3 H, H-16); 7.22 (d, $J = 8.3$ Hz,

1 H, H-18); 7.37 (m, 2 H, H-14 e H-4); 7.43 (d, $J = 5.1$ Hz, 1 H, H-24); 7.46 (dd, $J = 4.8$ e 7.9 Hz, 1 H, H-31); 7.56 (t, $J = 7.4$ Hz, 1 H, H-5); 7.62 (d, $J = 7.8$ Hz, 1 H, H-3); 7.68 (d, $J = 7.6$ Hz, 1 H, H-6); 7.99 (d, $J = 1.7$ Hz, 1 H, H-19); 8.43 (dt, $J = 1.8$ e 8.0 Hz, 1 H, H-32); 8.50 (d, $J = 5.1$ Hz, 1 H, H-25); 8.68 (dd, $J = 1.4$ e 4.7 Hz, 1 H, H-30); 8.97 (s, 1 H, H-20); 9.26 (d, $J = 1.8$ Hz, 1 H, H-28); 9.38 (s, 1 H, H-12); 10.68 (s, 1 H, H-7), ^{13}C NMR (100 MHz; DMSO-d_6, δ, ppm): 17.68 (CH_3, C-16); 20.52 (CH_3, C-9); 23.29 (CH3, C-4); 107.66 (C-24); 114.35 (t, $J = 251.9$ Hz, C-10); 117.26 (C-19); 117.66 (C-14); 123.76 (C-31); 126.59 (t, $J = 7.8$ Hz, C-1); 126.85 (C-5); 127.78 (C-3); 128.99 (C-17); 130.22 (C-18); 132.07 (d, $J = 13.1$ Hz, C-4); 133.11 (C-27); 134.39 (C-32); 134.98 (C-6); 135.09 (C-15); 137.96 (C-13); 148.17 (C-28); 151.37 (C-30); 159.48 (C-25); 161.05 (C-23); 161.58 (C-21); 161.83 (t, $J = 30.3$ Hz, C-11); 168.82 (C-8), NMR ^{19}F (376 MHz; DMSO-d_6, δ, ppm): −99.01 (CF_2), MS-ESI ([M−1] + , *m/z*, %): 501 (100), HRMS—theoretical value ($C_{27}H_{24}F_2N_6O_2$): 502.1929, value obtained: 502.1925, HPLC (%, nm): 98 (261).

2-(2-acetamido-5-fluorophenyl)-2,2-difluoro-N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)acetamide (**10c**) Yield: 76%, mp: 133–135 °C, IR (cm^{-1}): 2073 (C–H *sp*2); 1693; 1678 (C=O); 835; 809; 789 (aromatic ring), ^{1}H NMR (400 MHz; DMSO-d_6, δ, ppm): 1.96 (s, 3 H, H-9); 2.22 (s, 3 H, H-16); 7.22 (d, $J = 8.3$ Hz, 1 H, H-18); 7.37 (m, 2 H, H-14 e H-4); 7.43 (d, $J = 5.1$ Hz, 1 H, H-24); 7.46 (dd, $J = 4.8$ e 7.9 Hz, 1 H, H-31); 7.56 (t, $J = 7.4$ Hz, 1 H, H-5); 7.62 (d, $J = 7.8$ Hz, 1 H, H-3); 7.68 (d, $J = 7.6$ Hz, 1 H, H-6); 7.99 (d, $J = 1.7$ Hz, 1 H, H-19); 8.43 (dt, $J = 1.8$ e 8.0 Hz, 1 H, H-32); 8.50 (d, $J = 5.1$ Hz, 1 H, H-25); 8.68 (dd, $J = 1.4$ e 4.7 Hz, 1 H, H-30); 8.97 (s, 1 H, H-20); 9.26 (d, $J = 1.8$ Hz, 1 H, H-28); 9.38 (s, 1 H, H-12); 10.68 (s, 1 H, H-7), ^{13}C NMR (100 MHz; DMSO-d_6, δ, ppm): 17.69 (C-16); 23.87 (C-9); 108.73 (C-24); 114.46 (t, $J = 254.2$ Hz, C-10); 116.38 (d, $J = 5.3$ Hz, C-2); 116.45 (C-14); 117.04 (C-19); 118.97 (d, $J = 22.7$ Hz, C-3); 124.24 (C-31); 128.27 (C-17); 128.55 (d, $J = 8.4$ Hz, C-6); 128.27 (C-18); 132.42 (d, $J = 21.9$ Hz, C-5); 133.09 (C-27); 133.72(d, $J = 3.6$ Hz, C-1); 135.01 (C-32); 135.62 (C-15); 139.09 (C-13); 149.20 (C-28); 152.13 (C-30); 159.58 (d, $J = 242.7$ Hz, C-4); 160.12 (C-25); 160.24 (C-11); 161.76 (C-23); 162.91 (C-21); 168.97 (C-8), NMR ^{19}F (376 MHz; DMSO-d_6, δ, ppm): −99.11 (CF_2); −115.28 (Ar–F), MS-ESI ([M − 1] + , *m/z*, %): 505 (100), HRMS—theoretical value ($C_{26}H_{21}F_3N_6O_2$): 506.1678, value obtained: 506.1677, HPLC (%, nm): 97 (261).

2-(2-acetamido-5-chlorophenyl)-2,2-difluoro-N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)acetamide (**10d**) Yield: 45%, mp: 218–220 °C, IR (cm^{-1}): 3442; 3338; 3263 (N–H); 1714; 1668 (C=O); 812; 801; 776 (aromatic ring), ^{1}H NMR (400 MHz; DMSO-d_6, δ, ppm); 1.95 (s, 3 H, H-9); 2.22 (s, 3 H, H-16); 7.23 (d, $J = 8.0$ Hz, 1 H, H-18); 7.36 (dd, $J = 2.8$ e 8.2 Hz, 1 H, H-5); 7.44 (d, $J = 5.1$ Hz, 1 H, H-24); 7.48 (dd, $J = 4.8$ e 7.9 Hz, 1 H, H-31); 7.65 (s, 2 H, H-3 e H-14); 7.71 (s, 1 H, H-6); 7.97 (d, $J = 1.7$ Hz, 1 H, H-19); 8.43 (dt, $J = 1.7$ e 8.0 Hz, 1 H, H-32); 8.51 (d, $J = 5.1$ Hz, 1 H, H- 25); 8.69 (dd, $J = 1.4$ e 4.7 Hz, 1 H, H-30); 8.97 (s, 1 H, H-20); 9.26 (d, $J = 1.8$ Hz, 1 H, H-28); 9.41 (s, 1 H, H-12); 10.67 (s, 1 H, H-7), ^{13}C NMR (100 MHz; DMSO-d_6, δ, ppm): 17.65 (CH_3, C-16); 23.28 (CH3, C-9); 107.68 (C-24); 113.44 (t, $J = 254.0$ Hz, C-10); 117.27 (C-19); 117.66 (C-14); 123.72 (C-31); 126.21 (t, $J = 8.0$ Hz, C-1); 128.37 (t, $J = 23.4$ Hz, C-2) 129.10 (C-5); 129.33 (C-3); 129.64 (C-17); 130.24 (C-18); 131.50 (C-4); 132.11 (C-27); 134.38 (C-32); 134.77 (C-6); 134.82 (C-15); 138.00 (C-13); 148.13 (C-28); 151.34 (C-30); 159.44 (C-25); 161.02 (C-23); 161.20 (t, $J = 30.0$ Hz, C-11); 161.58 (C-21); 168.85 (C-8), NMR ^{19}F (376 MHz; DMSO-d_6, δ, ppm): −99.00 (CF_2), MS-ESI ([M + Na] + , *m/z*, %): 546 (100), HRMS—theoretical value ($C_{26}H_{21}ClF_2N_6O_2$): 522.1383, value obtained: 522.1383, HPLC (%, nm): 99 (261).

2-(2-acetamido-5-bromophenyl)-2,2-difluoro-N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)acetamide (**10e**) Yield: 72%, mp: 237–238 °C, IR (cm^{-1}): 3441; 3278 (N–H); 1688; 1683 (C=O); 806; 792 (aromatic ring), ^{1}H NMR (400 MHz; DMSO-d_6, δ, ppm); 1.94 (s, 3 H, H-9); 2.22 (s, 3 H, H-16); 7.22 (d, $J = 8.4$ Hz, 1 H, H-18); 7.33 (dd, $J = 1.8$ e 8.0 Hz, 1 H, H-6); 7.43 (d, $J = 4.8$ Hz, 1 H, H-24); 7.45 (dd, $J = 8.0$ Hz e $J = 4.6$ Hz, 1 H, H-31); 7.59 (d, $J = 8.4$ Hz, 1 H, H-5); 7.76 (dd, $J = 1.6$ e 2.72 Hz, 1 H, H-3); 7.82 (d, $J = 2.4$ Hz, 1 H, H-14); 7.97 (d, $J = 1.6$ Hz, 1 H, H-19); 8.42 (d, $J = 8.0$ Hz, 1 H, H-32); 8.50 (d, $J = 4.8$ Hz, 1 H, H-25); 8.68 (dd, $J = 0.8$ e 1.2 Hz, 1 H, H-30); 8.96 (s, 1 H, H-20); 9.25 (d, $J = 1.6$ Hz, 1 H, H-28), 9.41 (s, H, H-12); 10.69 (s, 1 H, H-7), ^{13}C NMR (100 MHz; DMSO-d_6, δ, ppm): 17.65 (CH_3, C-16); 23.32 (CH_3, C-9); 107.67 (C-24); 113.37 (t, $J = 253.3$ Hz, C-10); 117.25 (C-19); 117.64 (C-14); 123.73 (C-31); 128.45 (t, $J = 23.7$ Hz, C-2); 128.00 (t, $J = 9.0$ Hz, C-3); 129.10 (C-5); 129.44 (C-17); 130.24 (C-18); 132.10 (C-27); 134.46 (C-32); 134.75 (C-6); 135.25 (C-15); 137.99 (C-13); 148.12 (C-28); 151.33 (C-30); 159.45 (C-25); 161.01 (C-23); 161.22 (t, $J = 30.3$ Hz, C-11); 161.56 (C-21); 168.78 (C-8), NMR ^{19}F (376 MHz; DMSO-d_6, δ, ppm): −99.01 (CF_2), MS-ESI ([M − 1] + , *m/z*, %): 565 (100), HRMS—theoretical value ($C_{26}H_{21}BrF_2N_6O_2$): 566.0877, value obtained: 566.0872, HPLC (%, nm): 99 (261).

Biological evaluation

Cell lines

The K562 human chronic myeloid cell line expressing the BCR-ABL protein was grown in RPMI-1640 culture medium (SIGMA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco). Human kidney epithelial cells WSS-1 [WS-1] (ATCC® CRL-2029™) were growth in high glucose DMEM (VitroCel, Campinas, SP, Brazil) supplemented with 10% FBS (Vitrocel). Both cell lineages were maintained at 37 °C under 5% CO_2 in a water jacket CO_2 incubator (Forma series II incubator from Thermo Scientific).

Cytotoxicity assay in K562 cells

The cell viability was measured using the colorimetric MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; GE Healthcare, Buckinghamshire, UK). The cells (2×10^4 cells/well) were incubated with the compounds at concentrations ranging from 0.05 to 5.0 µM for 72 h at 37 °C. The MTT solution (5 mg/mL) was added in the last four hours of incubation. After dissolving the formazan product using DMSO, the plates were read using a microtiter plate reader (SpectraMAX® 190 Microplate Reader; Molecular Devices) at 570 nm. The optical density (OD) of cells with no compound (control) was referred to as 100% viability. The OD of cells incubated with the compounds was referred to as a percentage of viability in relation to the control. Tests were performed in triplicate.

Cytotoxicity assay in WSS-1 cells

The cytotoxic effects of imatinib and its derivatives on WSS-1 control cells were evaluated by a resazurin-based viability assay (Neves et al. 2016). Briefly, WSS-1 cells suspended in DMEM were seeded into 96-well microplates at 1×10^4 cells/well. 20 h later, cells were incubated with 0.1–1.000 µM of compounds and kept under a humidified atmosphere (5% CO_2, 37 °C) for 72 h. Four hours before the end of the experiment, resazurin (0.01 mg/mL) was added to each well. The fluorescence of resorufin was registered ($\lambda_{ex} = 560$ nm; $\lambda_{em} = 590$ nm) immediately and 4 h after resazurin addition in a FlexStation 3 Benchtop multimode microplate reader (Molecular Devices, Sunnyvale, CA, USA). The cell viability was calculated by subtracting the initial from the final fluorescence reading and was expressed as percentage of control (DMSO 1%). All assays were performed in triplicate.

Statistical analysis

The EC_{50} values were calculated by a four-parameter logistic curve function using GraphPad Prism v. 6.01 (GraphPad Software, La Jolla, CA, USA, www.graphpad.com).

Results and discussion

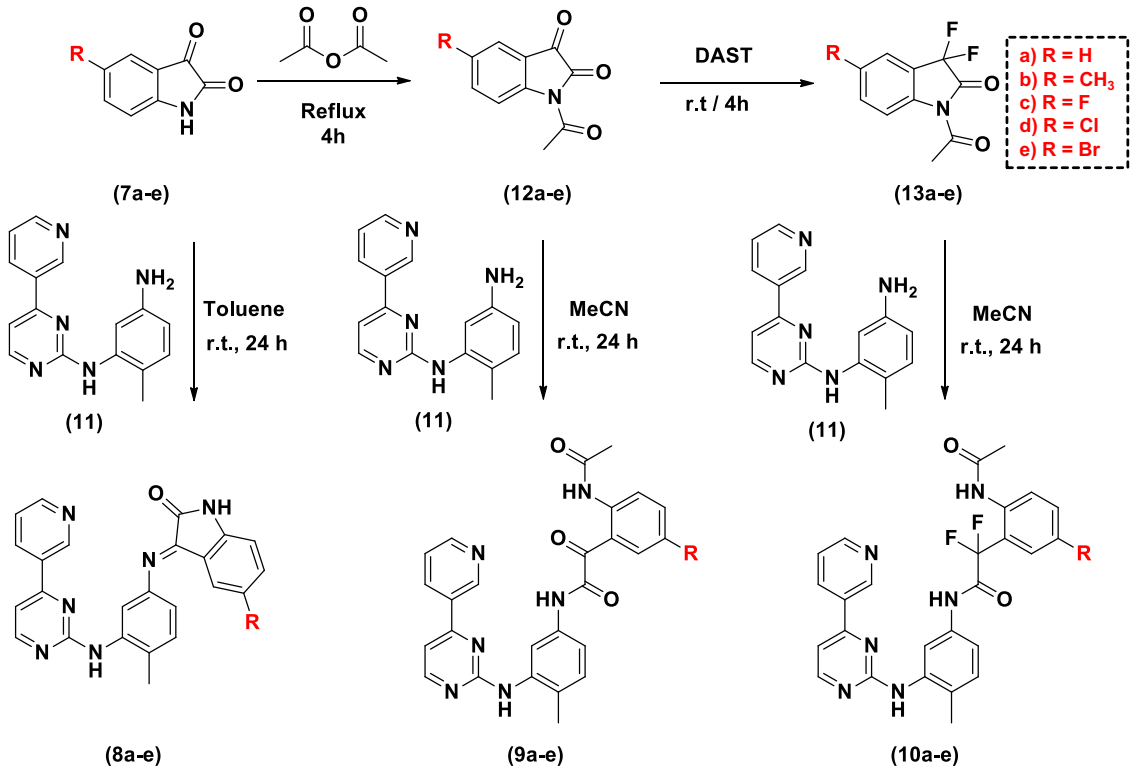
Chemistry

Fifteen new molecules presenting the PAP group, as the pharmacophore moiety of imatinib, (Choi et al. 2010; Druker and Lydon 2000) and isatin derivatives were synthesized and are discussed in three groups according to their structural similarities (Scheme 1).

The first series planned through molecular hybridization from imatinib and sunitinib (**8a–e**) was synthesized by reacting isatins (**7a–e**) with a PAP derivative **11**. The intermediate **11** was obtained through a donation from Cristália S.A. with 99% purity. However, we have previously described a variation of the classical synthetic route (Zimmermann et al. 1996a, b; Rewcastle et al. 2000; Manley et al. 2002; Feng et al. 2013; Boechat et al. 2013). The imine products were formed through a substitution reaction at the C-3 carbonyl group of **7a–e**, with yields between 77 and 96%. The NMR spectra show signal duplication in the ratio of 9:1 due to the formation of both *E* and *Z* isomers, where the *E* isomer is the thermodynamically most stable and, consequently, more abundant (supplementary material, Figure S). Furthermore, similar results are described in the literature (Subari et al. 2010; Ikotun et al. 2012; Aslam et al. 2015).

Compounds of the second series **9a–e** were prepared in two steps, using 5-substituted isatins (**7a–e**) as starting materials (Scheme 1). The *N*-acetylations of **7a–e** were obtained in good yield (72–89%) through a solvolysis process using acetic anhydride. The compounds **12a–e** are already described in the literature (Popp and Piccirilli 1971; Boechat and Pinto 2000; Cheah et al. 2008; Obafemi et al. 2012; Jeankumar et al. 2014). The preceding acetylation step is required to produce the series **9a–e**, leaving the C-2 carbonyl group susceptible to nucleophilic attack by amines (Silva et al. 2001; Boechat et al. 2007). Consequently, the reaction between the *N*-acetylisatins **12a–e** with 6-methyl-*N*-1-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine (**11**) via nucleophilic addition to the C-2 carbonyl provides the respective products in yields ranging from 51 to 64%.

The series **10a–e** was also obtained from *N*-acetylisatin derivatives **12a–e**, using methodology described by our group. The intermediates **12a–e** were treated with diethylaminosulfur trifluoride (DAST) to produce the *gem*-difluorinated compounds **13a–e** in yields of 70–95%.



Scheme 1 Synthetic route for the preparation of compounds **8a–e**, **9a–e**, and **10a–e**

These compounds have already been obtained by Boechat and Coworkers (2008). Then, the reactions between intermediates **13a–e** and **11** provided the products **10a–e** in yields of 45–72% (Scheme 1) (Boechat and Pinto 2000; Boechat et al. 2008, 2013).

The chemical structures of series **8a–e**, **9a–e**, and **10a–e** were elucidated by ^{1}H and ^{13}C NMR, IR spectroscopy, high-performance liquid chromatography (HPLC), mass spectrometry by electrospray ionization (MS-ESI), and HRMS.

Biological evaluation

Cytotoxic effects in K562 and WSS-1 cells

Cell viability was analyzed by the MTT method after incubation with all compounds **8a–e**, **9a–e**, and **10a–e**, and the results were compared to imatinib activity (Fig. 4).

The K562 cellular viability results for the series **8a–e**, **9a–e**, and **10a–e** are described in Fig. 4. Interestingly, none of the imine derivatives **8** (**a–e**) could reduce cell viability significantly, even at higher concentrations (Fig. 4). Among those compounds, **8a** was the most active but still inhibited only 20% of cell viability.

Among the synthesized compounds, the series **9a–e** was the most active against the viability of K562 cells. **9d** (R=Cl) had the best cytotoxic activity, reducing the cell viability in a similar fashion to imatinib, followed by **9b** (R=CH_3), **9e** (R=Br), **9a** (R=H), and **9c** (R=F). In addition, comparable effects to imatinib were also observed for the compounds **9d** and **9e**, with EC_{50} values of 0.37 and 0.56 µM, respectively (Table 1). However, compounds **9a** (R=H), and **9c** (R=F), even at higher concentrations (5 µM), did not achieve the effectiveness of imatinib, presenting decreases of cell viability of approximately 50 and 10%, correspondingly.

Compounds **10a–e** presented no effect in reducing the cellular viability of the K562 cell line when they were incubated at concentrations ranging from 0.05 to 5 µM. In that series, the highest drop in cell viability was observed for compound **10a** (40%), followed by **10b**, **10d**, **10e**, and **10c**. However, when compared with imatinib (80%), that series was not active (Fig. 4).

Consequently, the most active compounds (**9d**, **9b**, **9e**, and **9a**) were tested on the WSS-1 lineage (human cells) to establish the selectivity index. For those compounds, which were able to reduce cell viability by over 50%, EC_{50} values were determined on K562 and WSS-1 cells, and thus, the selectivity index was calculated (Table 1). Compounds **9a**, **9b**, **9d**, and **9e** showed EC_{50} values between 0.56 and 2.02. Nevertheless, those molecules showed lower selectivity indexes when compared to imatinib, mostly due to a higher toxicity to the WSS-1 lineage, with the notable exception of compound **9b** (Table 1).

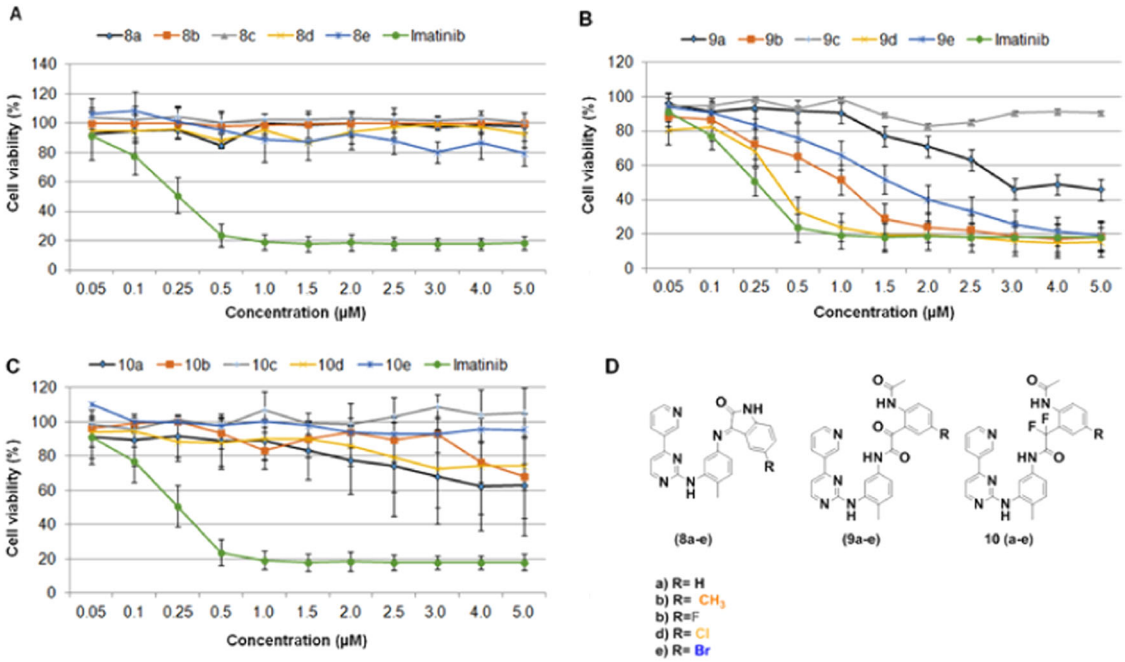


Fig. 4 Cells K562 inhibition assays of derivatives **8a–e** (**a**), **9a–e** (**b**), and **10a–e** (**c**). (**d**) The structures of compounds **8a–e**, **9a–e**, and **10a–e**

Table 1 EC_{50} values on K562 and WSS-1 cells and the selectivity index of compounds **9a**, **9b**, **9d**, and **9e**

Compounds	EC50 (mean ± standard error) (μM)		Selectivity index: WSS-1/K562
	K562	WSS-1	
9a	2.02 ± 0.11	6.88 ± 0.65	3.39
9b	0.86 ± 0.06	34.87 ± 1.72	40.78
9d	0.37 ± 0.05	10.71 ± 0.88	29.18
9e	0.56 ± 0.06	10.83 ± 0.59	19.20
Imatinib	0.21 ± 0.03	33.93 ± 0.79	160.80

Overall, the comparison between the **9a–e** and **10a–e** series shows that the presence of the carbonyl group instead of $-CF_2$ as a spacer between the phenyl ring and the PAP amide group drastically increases the activity of the molecules. In addition, the substitution of the PAP amide group for the ring of the isatin derivatives **7a–e** provides inactive imines **8a–e**, indicating the importance of that moiety for activity.

Conclusions

Fifteen new imatinib derivatives were synthesized via an effective synthetic route with high purity. Biological evaluations emphasized the importance of the 2-oxo-2-phenylacetamide group in imatinib derivatives (**9a–e**). However, the 2,2-difluoro-2-phenylacetamide series (**10a–e**) and the imines **8a–e** presented no effect in reducing the cellular viability of the K562 cell line. Compound **9d** (0.37 μM) is equipotent to imatinib (0.21 μM), followed by **9e** (0.56 μM), **9b** (0.86 μM), and **9a** (2.02 μM) with good cellular viability reduction. These results provide a solid basis for conducting further studies aimed at identifying new antiproliferative drug candidates.

Acknowledgements We are grateful to Coordination for the Improvement of Higher Education Personnel (CAPES), National Council for Scientific and Technological Development (CNPq) and Foundation for Research Support of the State of Rio de Janeiro (FAPERJ) for funding this work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Aboul-Fadl T, Radwan A, Attia MI, Al-Dhfyan A, Abdel-Aziz H (2012) Schiff bases of indoline-2,3-dione (isatin) with potential antiproliferative activity. Chem Cent J 6:49. doi:10.1186/1752-153X-6-49

Abraham SA, Hopcroft LEM, Carrick E, Drotar ME, Dunn K, Williamson AJK, Korfi K, Baquero P, Park LE, Scott MT, Pellicano F, Pierce A, Copland M, Nourse C, Grimmond SM, Vetrie D, Whetton AD, Holyoake TL (2016) Dual targeting of p53 and c-MYC selectively eliminates leukemic stem cells. Nature 534:341–346. doi:10.1038/nature18288

Ali MAM (2016) Chronic myeloid leukemia in the era of tyrosine kinase inhibitors: an evolving paradigm of molecularly targeted therapy. Mol Diagn Ther 20:315–333. doi:10.1007/s40291-016-0208-1

Asaki T, Sugiyama Y, Hamamoto T, Higashioka M, Umehara M, Naito H, Niwa T (2006) Design and synthesis of 3-substituted benzamide derivatives as Bcr-Abl kinase inhibitors. Bioorg Med Chem 16:1421–1425. doi:10.1016/j.bmcl.2005.11.042

Aslam NA, Babu SA, Rani S, Mahajan S, Solanki J, Yasuda M, Baba A (2015) Diastereoselective construction of 3-aminooxindoles with adjacent stereocenters: stereocontrolled addition of γ-Substituted allylindiums to isatin ketimines. Eur J Org Chem 2015:4168–4189. doi:10.1002/ejoc.201500340

Azevedo LD, Bastos MM, Oliveira AP, Boechat N (2017) Sínteses e propriedades de fármacos inibidores da tirosina quinase BCR-ABL, utilizados no tratamento da leucemia mieloide crônica. Química Nova. doi:10.21577/0100-4042.20170027 (In press)

Baccarani M, Deininger MW, Rosti G, Hochhaus A, Soverini S, Apperley JF, Cervantes F, Clark RE, Cortes JE, Guilhot F, Hjorth-Hansen H, Hughes TP, Kantarjian HM, Kim DW, Larson RA, Lipton JH, Mahon FX, Martinelli G, Mayer J, Muller MC, Niederwieser D, Pane F, Radich JP, Rousselot P, Saglio G, Saubele S, Schiffer C, Silver R, Simonsson B, Steegmann JL, Goldman JM, Hehlmann R (2013) European leukemia net recommendations for the management of chronic myeloid leukemia: 2013. Blood 122:872–884. doi:10.1182/blood-2013-05-501569

Bennett JH (1845) Case of hypertrophy of the spleen and liver in which death took place from suppuration of the blood. Edin Med Surg J 64:413–423

Boechat N, Kover WB, Bastos MM, Pinto AC, Maciel LC, Mayer LMU, Silva FSQ, SÁ PM, Mendonça JS, Wardell SMSV, Arruda MSL (2008) N-Acyl-3,3-difluoro-2-oxoindoles as versatile intermediates for the preparation of different 2,2-difluorophenylacetic derivatives. Journal of the Brazilian Chemical Society 19:445–457

Boechat N, Bastos MM, Duarte SL, Costa JCS, Mafra JCM, Daniel LCC (2013) Mesilato de imatinibe: uma otimização em sua síntese imatinib mesylate: an optimization in its synthesis. Rev Virtual Quim 5:222–234. doi:10.5935/1984-6835.20130023

Boechat N, Kover WB, Bastos MM, Romeiro NC, Silva ASC, Santos FC, Valverde AL, Azevedo MLG, Wollinger W, Souza TML, Souza SLO, Frugulhetti ICPP (2007) Design, synthesis, and biological evaluation of new 3-hydroxy-2-oxo-3-trifluoromethylindole as potential HIV-1 reverse transcriptase inhibitors. Med Chem Res 15:492–510. doi:10.1007/s00044-007-9004-0

Boechat N, Pinto AC (2000) Gem-difluoro derivative of phenylacetamide and phenylacetic acid and their pharmaceutical uses. US6034266 (A) filled 17 Sept 1996, issued 7 Mar 2000

Cheah WC, Black DS, Goh WK, Kumar N (2008) Synthesis of anti-bacterial peptidomimetics derived from N-acylisatins. Tetrahedron Lett 18:2965–2968. doi:10.1016/j.tetlet.2008.03.007

Choi HG, Sim T, Gray N, Zhou W, Chang JW, Zhang J, Weisberg E (2010) Fused heterocyclic compounds and their uses. WO2010144909 (A1) filled 14 Jun 2010, issued 16 Dec 2010

Cortes J, O'Brien S, Kantarjian H (2004) Discontinuation of imatinib therapy after achieving a molecular response. Blood 104:2204–2205. doi:10.1182/blood-2004-04-1335

Degos L (2001) John hughes bennett, rudolph virchow… and alfred donné: the first description of leukemia. Hematol J 2:1. doi:10.1038/sj/thj/6200090

Druker BJ, Lydon NB (2000) Lessons learned from the development of an abl tyrosine kinase inhibitor for chronic myelogenous leukemia. J Clin Invest 105:3–7. doi:10.1172/JCI9083

Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, Capdeville R, Talpaz M (2001) Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 14:1038–1042. doi:10.1056/NEJM200104053441402

Eck MJ, Manley PW (2009) The interplay of structural information and functional studies in kinase drug design: insights from BCR-Abl. Curr Opin Cell Biol 21:288–295. doi:10.1016/j.ceb.2009.01.014

Feng Z, Chen X, Liu H, Meng C (2013) Amine pyrimidine derivates, and production method thereof, and medicament composition and use. CN101423513 B filled 29 Oct 2007, issued 27 Mar 2013

Geary CG (2000) The story of chronic myeloid leukemia. Br J Haematol 110:2–11. doi:10.1046/j.1365-2141.2000.02137.x

Ibrahim HS, Abou-Seri SM, Abdel-Aziz HA (2016) 3-Hydrazinoindolin-2-one derivatives: chemical classification and investigation of their targets as anticancer agents. Eur J Med Chem 122:366–381. doi:10.1016/j.ejmech.2016.06.034

Ikotun AA, Adelani PO, Egharevba GO (2012) 1-Benzyl-3-[(4-methylphenyl)imino]indolin-2-one. Acta Crystallogr Sect E 68:o2098. doi:10.1107/S1600536812024506

Iqbal N, Iqbal N (2014) Imatinib: a breakthrough of targeted therapy in cancer. Chemother Res Pr 2014:357027. doi:10.1155/2014/357027

Jabbour E, Cortes JE, Giles FJ, O'Brien S, Kantarjian HM (2007) Current and emerging treatment options in chronic myeloid leukemia. Cancer 109:2171–2181. doi:10.1002/cncr.22661

Jabbour E, Deininger M, Hochhaus A (2010) Spotlight review management of adverse events associated with tyrosine kinase inhibitors in the treatment of chronic myeloid leukemia. Leukemia 25:201–210. doi:10.1038/leu.2010.215

Jabbour E, Kantarjian H (2016) Chronic myeloid leukemia: 2016 update on diagnosis, therapy, and monitoring. Am J Hematol 91:252–265. doi:10.1002/ajh.24275

James RA, Kohn CA, Rees AH, Verschuren RE (1989) Some derivatives of benz[f]azepine. Part III. J Heterocycl Chem 26:793–795. doi:10.1002/jhet.5570260353

Jeankumar VU, Alokam R, Sridevi JP, Suryadevara P, Matikonda SS, Peddi S, Alvala SSM, Yogeeswari P, Sriram D (2014) Discovery and structure optimization of a series of isatin derivatives as *Mycobacterium tuberculosis* chorismate mutase inhibitors. Chem Biol Drug Des 83:498–506. doi:10.1111/cbdd.12265

Joske DJL (2008) Chronic myeloid leukemia: The evolution of gene-targeted therapy. Med J Aust 189:277–282

Liu X, Kung A, Malinoski B, Prakash GKS, Zhang C (2015) Development of alkyne-containing pyrazolopyrimidines to overcome drug resistance of Bcr-Abl Kinase. J Med Chem 58:9228–9237. doi:10.1021/acs.jmedchem.5b01125

Lugo TG, Pendergast AM, Muller AJ, Witte ON (1990) Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. Science 247:1079–1082. doi:10.1126/science.2408149

Majid SJ, Ahmad RM (2015) Chronic myeloid leukemia manifesting on palate: a rare case report with a brief review of literature. Int J Curr Res 7:13996–13999

Manley PW, Cowan-Jacob SW, Buchdunger E, Fabbro D, Fendrich G, Furet P, Meyer T, Zimmermann J (2002) Imatinib: a selective tyrosine kinase inhibitor. Eur J Cancer 38:S19–S27. doi:10.1016/S0959-8049(02)80599-8

Moreno BH, Garralda EC, Hitt R (2010) Tyrosine kinase inhibitors in treating soft tissue sarcomas: sunitinib in non-GIST sarcomas. Clin Transl Oncol 12:468–472. doi:10.1007/s12094-010-0539-z

Mughal A, Aslam HM, Khan AMH, Saleem S, Umah R, Saleem M (2013) Bcr-Abl tyrosine kinase inhibitors- current status. Infect Agent Cancer 8:23. doi:10.1186/1750-9378-8-23

NCCN Clinical Practice Guidelines in Oncology. Chronic Myelogenous Leukemia (2017) 105. http://www.nccn.org/professionals/physician_gls/pdf/cml.pdf. Accessed 1 Jan 2017

Neves BJ, Dantas RF, Senger MR, Melo-Filho CC, Valente WC, de Almeida AC, Rezende-Neto JM, Lima EF, Paveley R, Furnham N, Muratov E, Kamentsky L, Carpenter AE, Braga RC, Silva-Junior FP, Andrade CH (2016) Discovery of new anti-schistosomal hits by integration of qsar-based virtual screening and high content screening. J Med Chem 15:7075–7088. doi:10.1021/acs.jmedchem.5b02038

Nowell PC, Hungerford DA (1961) Chromosome studies in human leukemia. II. Chronic granulocytic leukemia. J Natl Cancer Inst 27:1013–1035. doi:10.1093/jnci/27.5.1013

Obafemi CA, Taiwo FO, Iwalewa EO, Akinpelu DA (2012) Synthesis, antibacterial and anti-inflammatory activities of some 2-phenylglyoxylic acid derivatives. Int J Life Sci Pharma Res 2:22–36

Popp FD, Piccirilli RM (1971) The reaction of N-acetylisatin with amines. J Heterocycl Chem 8:473–475. doi:10.1002/jhet.5570080319

Rafiyath S, Wei G, Liu D (2013) Targeting BCR-ABL for molecular therapy of chronic myelogenous leukemia. Handb Ther Biomark Cancer 233–265. doi:10.1201/b15029-9

Rane R, Karunanidhi S, Jain K, Shaikh M, Hampannavar G, Karpoormath R (2016) A recent perspective on discovery and development of diverse therapeutic agents inspired from isatin alkaloids. Curr Top Med Chem 16:1262–1289. doi:10.2174/1568026615666150915112334

Rewcastle G, Denny W, Showalter HH (2000) Synthesis of 4-(phenylamino)pyrimidine derivatives as ATP-competitive protein kinase inhibitors with potential for cancer chemotherapy. Curr Org Chem 4:679–706. doi:10.2174/1385272003376094

Rosti G, Castagnetti F, Gugliotta G, Palandri F, Baccarani M (2012) Physician's guide to the clinical management of adverse events on nilotinib therapy for the treatment of CML. Cancer Treat Rev 38:241–248. doi:10.1016/j.ctrv.2011.07.004

Rowley JD (1973) A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. Nature 243:290–293. doi:10.1038/243290a0

Sabet R, Mohammadpour M, Sadeghi A, Fassihi (2010) QSAR study of isatin analogs as in vitro anti-cancer agents. Eur J Med Chem 45:1113–1118. doi:10.1016/j.ejmech.2009.12.010

Silva JFM, Garden SJ, Pinto AC (2001) The chemistry of Isatins: a review from 1975 to 1999. J Braz Chem Soc 12:273–324. doi:10.1590/S0103-50532001000300002

Subari AA, Bouhfid R, Zouihri H, Essassi EM (2010) 3-(Phenylimino)indolin-2-one. Acta Crystallographica. Acta Crystallogr Sect E 66:o453. doi:10.1107/S160053681000259X

Zimmermann J, Buchdunger E, Mett H, Meyer T, Lydon NB, Traxler P (1996a) Phenylamino-pyrimidine (PAP)—derivatives: a new class of potent and highly selective PDGF-receptor autophosphorylation inhibitors. Bioorg Med Chem Lett 6:1221–1226. doi:10.1016/0960-894X(96)00197-7

Zimmermann J, Caravatti G, Mett H, Meyer T, Müller M, Lydon NB, Fabbro D (1996b) Phenylamino-pyrimidine (PAP) derivatives: a new class of potent and selective inhibitors of protein kinase C (PKC). Arch Pharm 7:371–376