Chemical Papers (2021) 75:2549–2560
https://doi.org/10.1007/s11696-020-01502-w

ORIGINAL PAPER

Microwave-assisted synthesis of new 2-aryl and 2-alkylimidazolones and evaluation of their in vitro anticancer activity and their in vivo toxicity on zebrafish embryos

Samar Bou Zeid[1,2] · Aline Hamade[1] · Fadia Najjar[1] · Francois Carreaux[2] · Samar Eid[1]

Received: 4 September 2020 / Accepted: 30 December 2020 / Published online: 19 January 2021

Abstract

Herein we describe the synthesis of five new 2-aryl and 2-alkylimidazolone derivatives via an effective one-pot synthetic strategy assisted by microwave irradiations which allowed us to access the desired product in a reduced time reaction compared to the thermal heating and a slightly better yield (48% compared to 45%). The new imidazolone derivatives were evaluated for their anticancer activity in vitro against MCF-7, MDA-MB-231 and HepG2 cell lines. The results showed good cytotoxic effects for some of these derivatives on both MCF-7 and HepG2 cell lines in the range of 5.7–11.3 µM. Among the synthesized derivatives, **2ab** and **2b** showed the strongest activity with IC_{50} values of 7 and 5.7 µM (MCF-7) and 6.2 and 8.6 µM (HepG2), respectively. The cytotoxic activities of these derivatives were moderate compared to those of doxorubicin. However, this product showed higher toxicity in vivo on the development of zebrafish embryos than the synthesized imidazolones. These derivatives at high concentrations exhibited some morphological abnormalities on the embryos.

Keywords Imidazolone derivatives · One-pot synthesis · Microwave irradiations · Anticancer activity · Zebrafish embryos

Introduction

Cancer is a major public health problem worldwide; it is considered the second leading cause of death globally (Wild et al. 2020). In particular, breast cancer is rated as the second most commonly diagnosed cancer and the leading cause of cancer death among females (DeSantis et al. 2017). Hepatocellular carcinoma (HCC) is considered the sixth most frequently diagnosed cancer and the fourth leading cause of death from cancer in the world (Bray et al. 2018). Breast tumors are very heterogeneous and can be classified as tumors expressing estrogen and progesterone receptors (ER+, PR+), tumors expressing amplified human epidermal receptors (HER2+) and tumors not expressing these receptors, called triple-negative breast (ER−, PR−, HER2−) (Dai et al. 2017). As for the HCC, studies have shown an overexpression of HER2 protein in several HCC cell lines (Shi et al. 2019). Considerable efforts have been invested in establishing efficient treatment strategies of which the most commonly used are surgery, radiotherapy and drug treatments (You et al. 2007; Luqmani 2005). In spite of being effective in the treatment of several types of cancer, especially in the case of primary tumors, these strategies can induce tumor heterogeneity resulting in the reduction of the therapeutic agents efficiency and leading to treatment failure and tumor progression (Foo and Michor 2014; Luqmani 2005). Thus, it is necessary to develop new targeted therapies aiming specific cellular mechanisms. Many natural products classified as leads to potential drugs, contribute significantly in the drug discovery and development process, especially in cancer treatment (Newman and

Supplementary Information The online version of this article (https://doi.org/10.1007/s11696-020-01502-w) contains supplementary material, which is available to authorized users.

✉ Fadia Najjar
fnajjar@ul.edu.lb

✉ Francois Carreaux
francois.carreaux@univ-rennes1.fr

✉ Samar Eid
s.eid@ul.edu.lb

[1] Departments of Biology, Chemistry and Biochemistry, Laboratoire D'Innovation Thérapeutique, Faculty of Sciences II, Lebanese University, Fanar, Lebanon

[2] Université de Rennes 1, CNRS, ISCR, UMR 6226, 263 avenue du Général Leclerc, Campus de Beaulieu, 35000 Rennes, France

Cragg 2012). As a matter of fact, marine natural products isolated from marine sponges and organisms, with 1490 new products extracted and identified in 2017 (Carroll et al. 2019), are being explored for their different medical applications (Mayer et al. 2011; Villa and Gerwick 2010). Among these natural marine products, several molecules based on a 2-aminoimidazolone core, such as leucettamine B (Boehm et al. 1993; Chan et al. 1993), have shown interesting biological properties. Leucettamine B (Fig. 1) and its synthetic N-functionalized analogs have manifested significant inhibitory activities against protein kinases, CLK1 [CDC (cyclin-dependent kinase)-like kinase], and DYRKs (dual-specificity tyrosine phosphorylation-regulated kinases) (Burgy et al. 2013; Tahtouh et al. 2012; Debdab et al. 2010, 2011). In the last years the imidazolone scaffold has attracted attention as a potential block for the development of new effective anticancer agents (Hsu et al. 2020; Abdel Gawad et al. 2016; Ling et al. 2013; Xiao et al. 2013). Moreover, analogs of type 2-aryl or 2-alkylimidazolones (Fig. 1) have also been synthesized (Mokale et al. 2014; Voosala et al. 2014; Shi et al. 2012; Fozooni and Tikdari 2008); however, the evaluation for their anticancer activity is limited.

In fact, some synthesized (Z)-2-aryl-5-arylmethylideneimidazolones were reported to have potent cytotoxic effects against epithelial carcinoma cancer cell line A549 (Beloglazkina et al. 2016). Eldehna et al. (2019) also reported the synthesis and biological evaluation of novel 4-benzylidene-2-phenyl-imidazolone-based benzenesulfonamides that showed anti-proliferative activity against colorectal cancer HCT-116 and breast cancer MCF-7 cell lines. The synthetic methodologies adopted to access these imidazolone derivatives have been established on the transformation of oxazolone nuclei into imidazolone unit according to several operating conditions. An efficient multi-component strategy was developed by Kidwai and Devasia (1962), involving the reaction of the ethyl ester of glycine with imidate esters and aromatic aldehydes. Nevertheless, it was less adopted due to the tedious preparation of imidate salts that required the passage of a continuous flow of hydrogen chloride gas over a solution of the nitriles and alcohol in ether or benzene. A new method for synthesizing these imidate salts by generating hydrogen chloride in situ (Yadav and Babu 2005), made the Kidawi's one-pot approach much more interesting. In light of the above evidence and inspired by the biological activity of imidazolone derivatives, an effective one-pot microwave-assisted synthesis was applied to access a library of new 2-aryl and 2-alkylimidazolone derivatives as potential anticancer agents (Scheme 1). This non-conventional heating has emerged as an efficient green method to enhance products yield and to reduce reaction time (Kappe and Dallinger 2009; Strauss and Varma 2006).

The cytotoxic activity of the new compounds was then evaluated in vitro on the breast cancer cell lines MCF-7, MDA-MB-231 and HCC cell line HepG2. Furthermore, we evaluated their toxic effect in vivo using the zebrafish model, considered as a versatile animal model due to the simplicity of its natural habitat, low cost and ease of breeding (Newman et al. 2014). The ex-utero development of its transparent embryos allowed a clear visualization of the development process (Meyers 2018; Link and Megason 2008) and the evaluation of the toxicological effects of the newly synthesized imidazolones on this process.

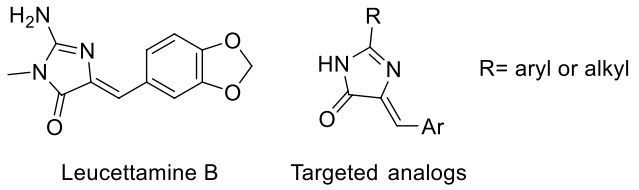


Fig. 1 Structure of leucettamine B and targeted 2-aryl and 2-alkylimidazolones

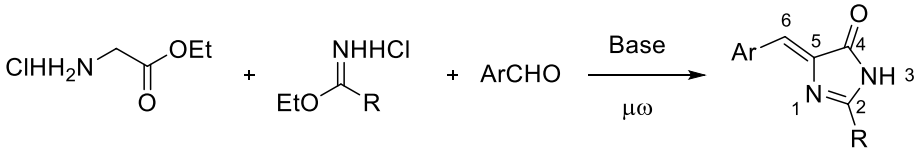
Experimental

Chemistry

Chemicals, reagents and instruments

Solvents and all other chemical reagents were purchased from Acros, Sigma-Aldrich and Fluka and were used without further purification. Solvents were dried by refluxing with the appropriate drying agents and distilled before use. Toluene was distilled over sodium and stored over molecular sieves (3 Å), and ethanol was distilled over calcium hydride (CaH_2). Melting points were measured on a melting point apparatus Stuart SMP10 and were not corrected. ^{1}H NMR spectra were recorded on BRUKER AC 300 and AC 400 (300 and 400 MHz) and ^{13}C NMR spectra on BRUKER AC 300 and AC 400 (75 and 101 MHz) spectrometer. Chemical shifts are expressed in parts per million downfield from tetramethylsilane as an internal standard. Data are given in the following order: δ value, multiplicity (s: singlet; d: doublet; t: triplet; q: quartet; m: multiplet; br: broad), number of protons, coupling constant *J* given in Hertz. All high-resolution mass spectra

Scheme 1 Microwave-assisted synthetic strategy to imidazolone derivatives



(HRMS) were recorded on a Bruker Micro-Tof-Q II or on a Waters Q-Tof 2 at the CRMPO (Centre Régional de Mesures de Physiques de l'Ouest–Rennes–France) using positive ion electrospray. Reactions under microwave irradiations were realized in the apparatus Anton Paar's Monowave® 300 (Scan-Mat Platform, Rennes, France). The microwave instrument consists of a continuous focused microwave power output from 0 to 850 W. All experiments were performed using stirring option at 600 rpm. The target temperature was reached within the required time, and the chosen microwave maximal power was set to 100 W. The reaction temperature is monitored using calibrated infrared sensor and the reaction time. The imidates salts **1a** (Kumar et al. 2016)**, 1b** (Yadav and Babu 2005), **1c** (Yadav and Babu 2005) and **1d** (Shi et al. 2018) were prepared as previously described.

General procedure for the synthesis of 5-benzylidene-2-aryl/2-alkyl-3,5-dihydro-4H-imidazol-4-one 2

Glycine methylester hydrochloride (1.5 eq), the corresponding acetimidate hydrochloride (1.1 eq) and sodium bicarbonate (3 eq) were poured in the microwave vial, flushed with argon, and anhydrous toluene (0.8 mL) was added to the powders. To the suspension was then added the aldehyde (1 eq), the vial was then placed in the Anton Paar monowave reactor, and the reaction was stirred during 15 min at 150 °C and at 100 W of maximum power. After the reaction completion, the suspension was allowed to precipitate at room temperature. The yellow solid obtained was collected by filtration, washed twice with water, twice with cold ethanol and twice with isopropyl ether to afford the corresponding imidazolone as a yellow powder.

(Z)-5-(4-methoxybenzylidene)-2-phenethyl-3,5-dihydro-4H-imidazol-4-one 2aa

General procedure starting from *p*-methoxybenzaldehyde (115 mg, 0.85 mmol) and **1a** (200 mg, 0.936 mmol). Yield, 48%; yellow solid, mp 181–183 °C. ^{1}H NMR (400 MHz, DMSO, δ ppm): 8.16 (d, J = 8.9 Hz, 2H), 7.30 (d, J = 4.4 Hz, 4H), 7.23–7.17 (m, 1H), 7.02 (d, J = 8.9 Hz, 2H), 6.83 (s, 1H), 3.82 (s, 3H), 3.04 (t, J = 7.8 Hz, 2H), 2.84 (t, J = 7.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO, δ ppm): 172.1, 165.2, 161.1, 141.1, 138.6, 134.1, 128.8, 128.8, 127.4, 126.6, 124.5, 114.7, 55.8, 31.9, 31.6. HRMS (ESI+): *m/z* calcd for $C_{19}H_{18}N_2O_2Na$ $[M+Na]^+$: 329.1260, found 329.1259.

(Z)-5-(3-methoxybenzylidene)-2-phenethyl-3,5-dihydro-4H-imidazol-4-one 2ab

General procedure starting from *m*-methoxybenzaldehyde (115 mg, 0.85 mmol) and **1a** (200 mg, 0.936 mmol). Yield, 26%; yellow solid, mp 172–174 °C. ^{1}H NMR (300 MHz, DMSO, δ ppm): 7.89 (s, 1H), 7.71 (d, J = 7.7 Hz, 1H), 7.38–7.27 (m, 5H), 7.24–7.15 (m, 1H), 6.97 (dd, J = 7.9, 2.7 Hz, 1H), 6.82 (s, 1H), 3.78 (s, 3H), 3.04 (t, J = 7.7 Hz, 2H), 2.85 (t, J = 7.7 Hz, 2H). ^{13}C NMR (101 MHz, DMSO, δ ppm): 172.3, 167.1, 159.7, 141.1, 140.7, 135.9, 130.0, 128.8, 128.8, 126.6, 124.9, 124.0, 117.0, 116.1, 55.5, 31.8, 31.3. HRMS (ESI+): *m/z* calcd for $C_{19}H_{18}N_2O_2Na$ $[M+Na]^+$: 329.1260, found 329.1264.

(Z)-5-(4-methoxybenzylidene)-2-(4-methoxyphenyl)-3,5-dihydro-4H-imidazol-4-one 2b

General procedure starting from *p*-methoxybenzaldehyde (114 mg, 0.84 mmol) and **1b** (200 mg, 0.927 mmol). Yield, 70%; yellow solid, mp 262–264 °C. ^{1}H NMR (300 MHz, DMSO, δ ppm): 8.28 (d, J = 8.9 Hz, 2H), 8.12 (d, J = 8.9 Hz, 2H), 7.14 (d, J = 8.9 Hz, 2H), 7.06 (d, J = 8.8 Hz, 2H), 6.91 (s, 1H), 3.87 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (101 MHz, DMSO, δ ppm): 172.6, 163.0, 161.0, 159.7, 139.3, 134.2, 129.6, 127.8, 124.3, 120.9, 114.9, 114.8, 56.0, 55.8. HRMS (ESI+): *m/z* calcd for $C_{18}H_{16}N_2O_3Na$ $[M+Na]^+$: 331.1053, found 331.1056.

(Z)-2-(4-chlorophenyl)-5-(4-methoxybenzylidene)-3,5-dihydro-4H-imidazol-4-one 2c

General procedure starting from *p*-methoxybenzaldehyde (112 mg, 0.826 mmol) and **1c** (200 mg, 0.91 mmol). Yield, 64%; yellow solid, mp 272–274 °C. ^{1}H NMR (300 MHz, DMSO, δ ppm): 8.29 (d, J = 8.8 Hz, 2H), 8.16 (d, J = 8.6 Hz, 2H), 7.67 (d, J = 8.6 Hz, 2H), 7.07 (d, J = 8.8 Hz, 2H), 7.01 (s, 1H), 3.84 (s, 3H). ^{13}C NMR (75 MHz, DMSO, δ ppm): 172.4, 161.4, 159.2, 138.9, 137.4, 134.6, 129.6, 129.4, 127.6, 127.6, 126.2, 114.9, 55.8. HRMS (ESI+): *m/z* calcd for $C_{17}H_{13}N_2O_2ClNa$ $[M+Na]^+$: 335.0557, found 335.0556.

(Z)-2-(4-bromophenyl)-5-(4-methoxybenzylidene)-3,5-dihydro-4H-imidazol-4-one 2d

General procedure starting from *p*-methoxybenzaldehyde (93.5 mg, 0.687 mmol) and **1d** (200 mg, 0.756 mmol), Yield, 79%; yellow solid mp 286–290 °C. ^{1}H NMR (300 MHz, DMSO, δ ppm): 8.29 (d, J = 8.8 Hz, 2H), 8.09 (d, J = 8.6 Hz, 2H), 7.81 (d, J = 8.6 Hz, 2H), 7.07 (d, J = 8.9 Hz, 2H), 7.03 (s, 1H), 3.84 (s, 3H). ^{13}C NMR (75 MHz, DMSO, δ ppm): 172.3, 161.4, 159.2, 138.9, 134.5, 132.5, 129.5, 127.8, 127.5, 126.4, 126.2, 114.9, 55.8. HRMS (ESI+): *m/z* calcd for $C_{17}H_{13}N_2O_2BrNa$ $[M+Na]^+$: 379.0052, found 379.0050.

Biological activity

Cell lines: culture conditions and viability assays

Doxorubicin hydrochloride was obtained from EBEWE Pharma Ges.m.b.H. Nfg.KG A-4866 Unterach, Austria. MCF-7, hormone-dependent human breast cancer, MDA-MB-231, hormone-independent human breast cancer and HepG2 hepatocellular carcinoma cell lines were purchased originally from ATCC (American Type Culture Collection). MCF-7 and MDA-MB-231 cells were routinely maintained in RPMI 1640 medium (Sigma R0883) and HepG2 in DMEM high glucose medium (Sigma D0819), both medium containing 1% penicillin streptomycin (Biowest MS006W) and supplemented with 10% Fetal Bovine Serum (Sigma F9665) and 1% L-Glutamine (Sigma G7513). The cells were cultured at 37 °C in a humidified incubator with 5% CO_2 and were passaged twice a week using 1% trypsin (Sigma T4549). The cytotoxic effects of the imidazolone derivatives were determined using the trypan blue exclusion assay. It is based on the concept of dye exclusion to differentiate viable cells from dead ones. Indeed, cells that possess damaged membranes are stained blue, whereas viable cells do not uptake the dye and remain white. MCF-7, MDA-MB-231 and HepG2 cells were seeded into 24-well culture plates (20,000/well), in a total volume of 500 µL/well. After overnight incubation to ensure cell attachment and well-being, the medium was aspirated and cells were exposed to the synthesized imidazolones in duplicate at final concentrations ranging from 0.1 to 60 µM. Stock solutions of these compounds at 30 mM were prepared in DMSO (Sigma-Aldrich 41,640) and then diluted in culture medium to obtain the desired final concentrations. The highest concentration of DMSO following dilution was 0.2% of the total volume, an amount that was not toxic to the cells. Control cells were grown in culture medium. The vehicle cells were treated with 0.2% of DMSO. Plates were incubated for 24, 48 and 72 h. At the end of each time point, the medium was removed; the cells were collected by the action of trypsin and counted in a hemocytometer using optical microscopy. The results were presented as a percentage of the control. All measurements were taken in duplicate, and each experiment was repeated at least three times independently.

Animals

Adult wild-type zebrafish (Danio rerio) of AB and Casper strain of both sexes were purchased from UMS AMAGEN CNRS INRA (France) and were used after ethical approval. Animals were divided in 5-L thermostated tanks at 28 ± 2 °C (15 fish/tank) in a constant chemical and biological controlled environment according to international standards. Mechanical water filtration and aeration were also kept under constant control. Animals were maintained under a 14–10-h day/night photoperiod cycle and fed three times a day with commercial flakes and live brine shrimp. Natural spawning was induced in the morning by light. Embryos were then collected within 30 min and maintained in an E3 (1*x) medium in an incubator at 25 °C to minimize stress and spontaneous deaths when treating.

E3 medium (60*x) is the adequate medium for zebrafish embryos and was prepared as follows: 34.8 g sodium chloride, 1.6 g potassium chloride, 5.8 g calcium chloride dihydrate, 9.78 g magnesium chloride hexahydrate were dissolved in water with a final volume 2 L to prepare a 60*x stock. This solution was autoclaved before adjusting the pH and diluting, after which it becomes compatible to use for zebrafish embryos.

Zebrafish embryo toxicity assay: exposure to the imidazolone derivatives

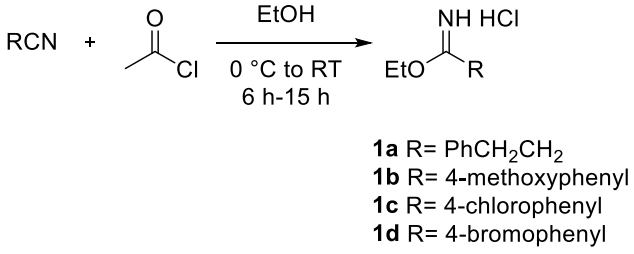
Fertilized eggs of zebrafish between late blastula and early gastrula [2.5–5.25 hpf (hours post-fertilization)] were sorted to ensure only eggs of the same quality were used in the experiments. This was done to minimize spontaneous defects and variability between different conditions. Zebrafish embryos were then transferred to 24-well plates (5–10 embryos per well) and were maintained in 0.5–1 mL of E3 medium. Embryos were then exposed to different concentrations of imidazolone derivatives (1 µM–120 µM). Stock solutions of these compounds at 30 mM were prepared in DMSO and then diluted in E3 medium to obtain the desired final concentrations. The highest concentration of DMSO following dilution was 0.5% of the total volume. A control group (E3 1*x) and a vehicle group (0.5% DMSO) were part of the experiment. Fish embryonic development was observed directly using a stereoscope, and pictures were taken using the AmScope software. The malformations observed and the viability rates were noted and calculated after 24, 48 and 72 h of treatment.

Results and discussion

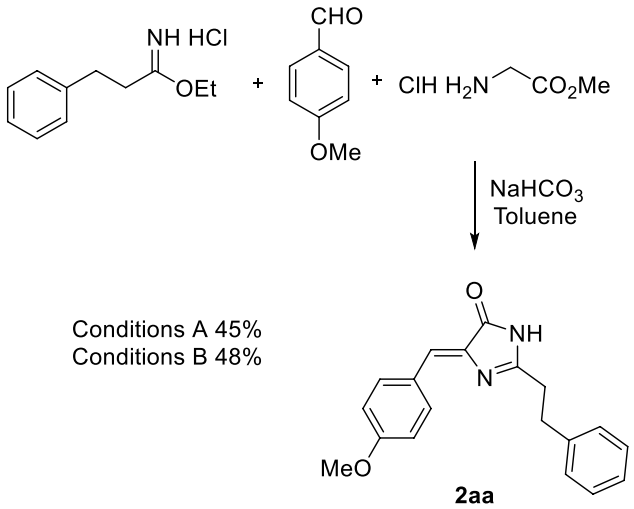
Chemistry

The imidate salts used in this approach were synthesized according to the protocol described by Yadav and Babu (2005). This method is based on the generation of hydrogen chloride in situ following the treatment of acyl chloride with an alcohol (Scheme 2).

The imidate salts were subsequently used in the preparation of 2-aryl and 2-alkylimidazolones according to the strategy described first in 1962 (Kidwai and Devasia 1962), while making modifications to the reaction conditions. It is a



Scheme 2 Preparation of imidate salts



Scheme 3 Optimization of the one-pot process under microwave conditions, reaction conditions: (A) 72 °C 2 h, (B) μω 150 °C, 100 W, 15 min

three-component one-pot reaction involving an imidate salt, a glycine ester and an aromatic aldehyde (Scheme 3). At this stage, two methods of activation of the reaction have been employed. The process was carried out under conventional thermal conditions and also under microwave irradiation in order to compare the two activation modes. In the interest of optimizing the conditions, the reaction was conducted using *p*-methoxybenzaldehyde and compound **1a,** chosen as model compounds, in the presence of sodium hydrogen carbonate as base. In conventional thermal conditions (toluene, 70 °C), the product was isolated with 45% yield (in 97% of conversion) after 2 h of reaction, whereas the same reaction conducted in the same conditions without solvent did not result in the desired product, but rather a degradation of the starting products was observed. Furthermore, the reaction was conducted under microwave irradiations and the conditions were optimized by variation of the heating temperature and the reaction time. In a first attempt, the reaction was carried at 100 °C under 100 W of maximum power and for 15 min to yield the desired product in 88% of conversion. In the next assay the temperature was increased to 150 °C which resulted in a better conversion (99.7%) and better yield (48%) of isolated product compared to thermal heating. Finally in an attempt to achieve a greener process, the reaction was conducted in a solvent-free medium in the same conditions as before; however, a decrease in the conversion to 87% was noted. An increase in the heating temperature to 200 °C in a solvent-free medium caused a degradation of the imidate salt and did not yield the desired product. Therefore, in a final attempt, in a solvent-free medium, the temperature was maintained at 150 °C and the reaction was carried for 30 min. In these conditions the product was obtained in 92% of conversion that was still lower than the conversion obtained in the presence of solvent. The microwave-assisted method in a solvent-based medium has proved to be more efficient and suitable to reduce the reaction time and slightly enhance the yield.

Subsequently, the one-pot approach was generalized to several substrates by varying either the aldehyde or the salt of imidates. The reactions were carried out in the microwave under the same conditions as above (150 °C, 100 W, 15 min) leading to a small library of imidazolone derivatives (Fig. 2). The desired products precipitated in the toluene and were purified by a simple filtration followed by several washes in water and ether. It was not possible to purify them by column chromatography as they were insoluble in several organic solvents, such as dichloromethane, ethyl acetate and partially soluble in methanol and ethanol. All compounds were obtained in a stereospecific way with retention of stereochemistry and the geometry of the exocyclic double bond that was attributed as being *Z* by the shielding effect of the carbonyl C-4 on the olefinic proton H-5 (δH-5 = 6.82–7.05 ppm) (Debdab et al. 2010; Villemin and Martin 1995).

The results showed that the one-pot reaction gives better yields by using imidate salts of aryl type (**2b**, **2c**, **2d**) compared to those of alkyl type (**2aa**, **2ab**). This observation can be explained by the stability of the imidazolones formed from the aryl imidate salts, due to the conjugation of the cyclic double bond with the aromatic nucleus of these salts (Kidwai and Devasia 1962). It could also be explained by the fact that a small quantity of **2aa** and **2ab** products was lost in the precipitation purification and was observed in the filtrate after filtration which was not the case of compounds **2b**, **2c** and **2d**. However, the difference in reactivity observed within these imidates of aryl type seems to be linked to the nature of the substituents on the aromatic ring. An electron–donor group on the aromatic nucleus promoted the intramolecular cyclization of the intermediate, obtained following the addition of glycine to the imidate salt, by activating the attack of the amine on the ester based on the proposed mechanism in Fig. 3. A significant decrease in yield was noticed by variation of the aldehyde (**2aa** compared to **2ab**). This decrease can be attributed to the difference in stability of the final

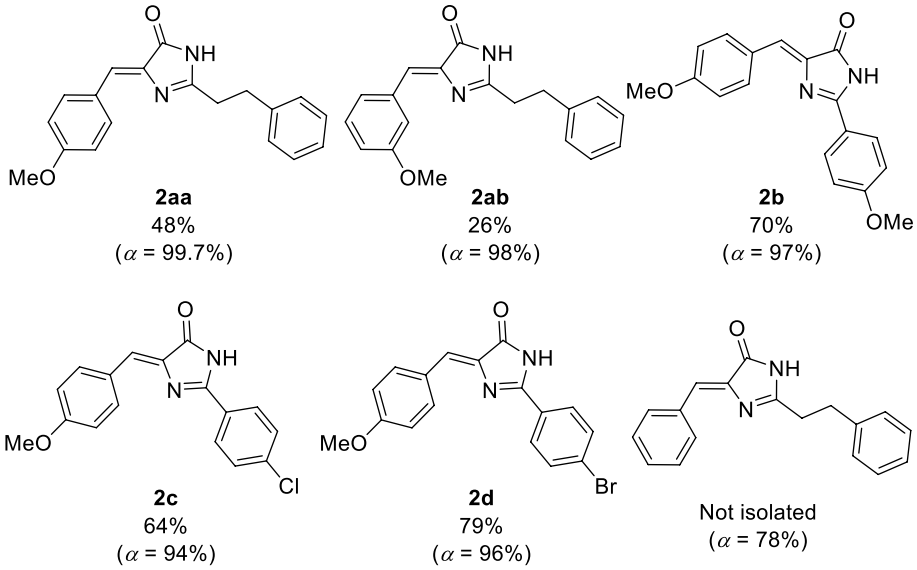


Fig. 2 Yields of the 2-aryl and 2-alkylimidazolones synthesized via one-pot approach; α: conversion percentage

products obtained. Indeed, *p*-methoxybenzaldehyde induces a stability of the final product **2aa** via the conjugation of the exocyclic double bond with the aromatic nucleus, reinforced by the presence of the methoxy group in the para position. This effect is absent in the case of a methoxy group in the meta position (**2ab**). Furthermore, the absence of an activating group on the aromatic nucleus of the aldehyde did not lead to total conversion. Benzaldehyde, used with **1a** under the same conditions as before, resulted in a 78% conversion to the final product. However, attempts to purify this derivative have not been successful.

Cell viability

The anticancer effect of the various synthesized imidazolone derivatives was assessed by means of the cell exclusion test with trypan blue against human breast cancer cell lines MCF-7 (Fig. 4) and MDA-MB-231 (Fig. 5) and against HCC cell lines HepG2 (Fig. 6). Doxorubicin was used as positive control. The IC_{50} (concentration of the compound which caused 50% of cell death) for each cell line was determined based on dose–response curves, concentrations as a function of the viability percentages obtained 72 h post-treatment. The results are summarized in Table 1. It should be noted that the increased lipophilicity of compounds **2c** and **2d** (log *p* values of 3.13–3.4) caused problems in assessing their cytotoxic effect. These compounds started to precipitate in the culture medium even at low concentrations (limit concentration 10 µM); therefore, it was not possible to determine the exact IC_{50} values.

On breast cancer cell lines, the 2-alkyl and 2-arylimidazolone derivatives **2aa**, **2ab** and **2b** (log *p* values of

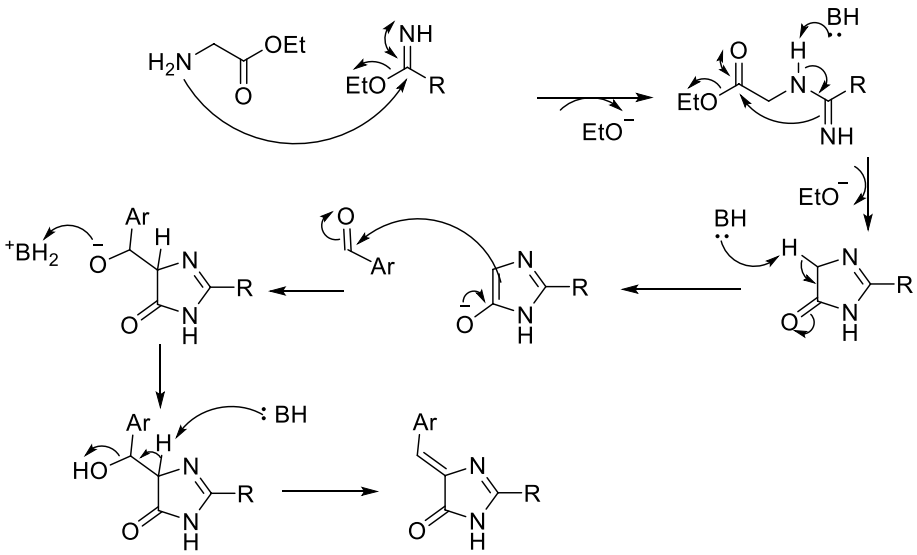


Fig. 3 Proposed reaction mechanism

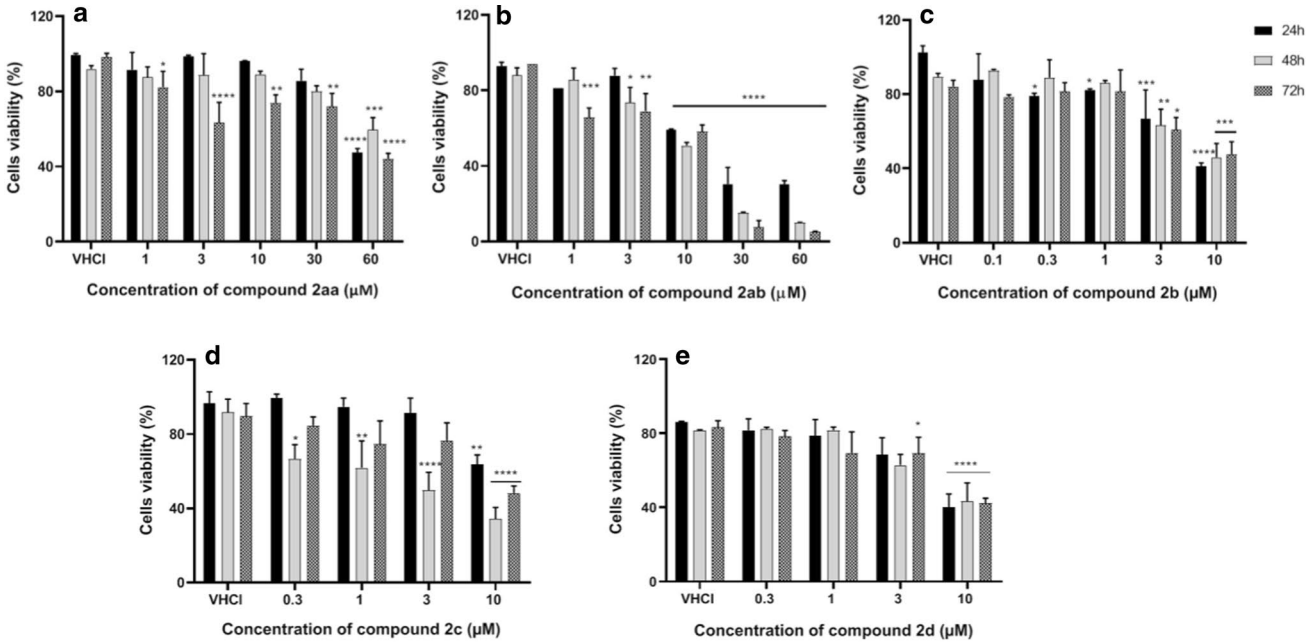


Fig. 4 Percentage of cell viability of the MCF-7 cell line compared to the control following treatment with the different concentrations of compounds **2aa** (**a**), **2ab** (**b**), **2b** (**c**), **2c** (**d**) and **2d** (**e**) after 24, 48 and 72 h. Each experiment was carried out in duplicate. *, **, ***, **** indicate a significant difference compared to the vehicle with $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$, respectively, according to the ANOVA test

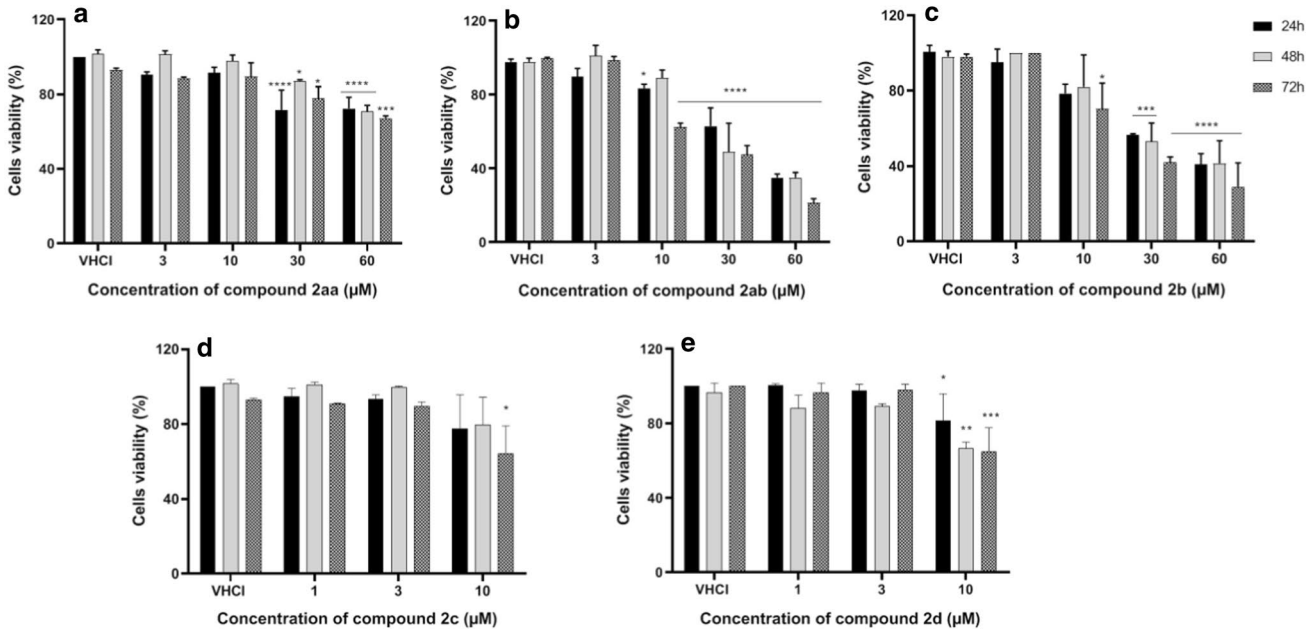


Fig. 5 Percentage of cell viability of the MDA-MB-231 cell line compared to the control following treatment with the different concentrations of compounds **2aa** (**a**), **2ab** (**b**), **2b** (**c**), **2c** (**d**) and **2d** (**e**) after 24, 48 and 72 h. Each experiment was carried out in duplicate. *, **, ***, **** indicate a significant difference compared to the vehicle with $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$, respectively, according to the ANOVA test

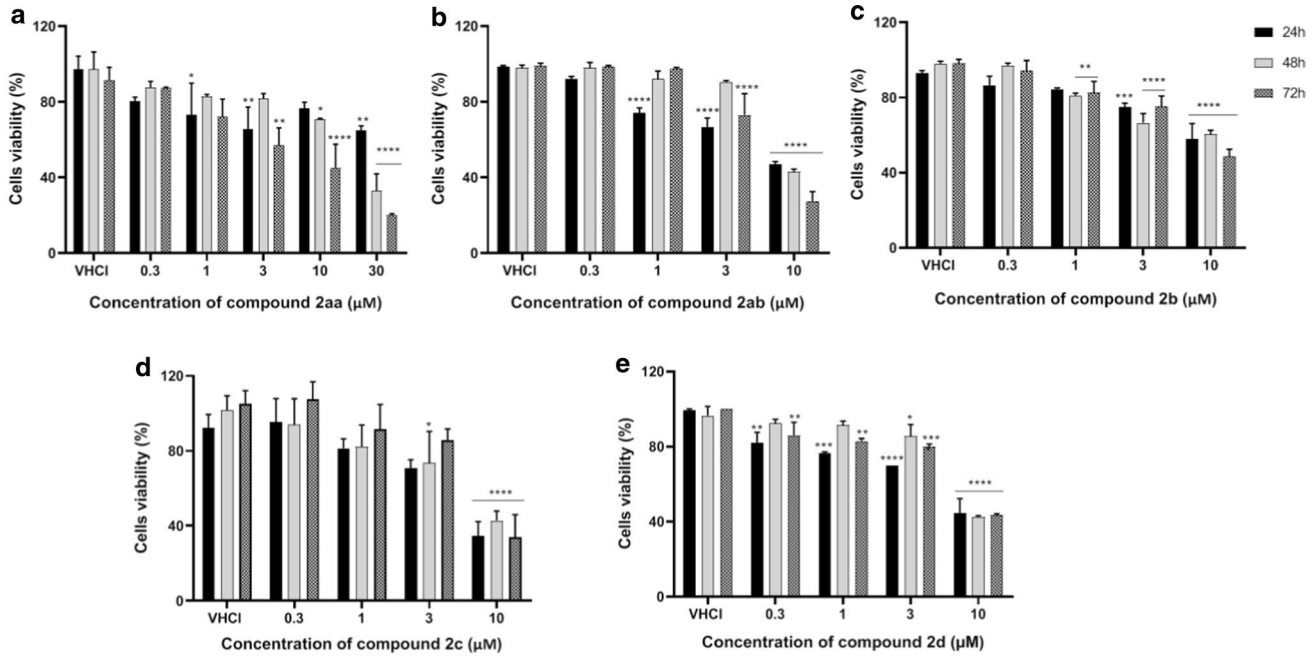


Fig. 6 Percentage of cell viability of the HepG2 cell line compared to the control following treatment with the different concentrations of compounds **2aa** (**a**), **2ab** (**b**), **2b** (**c**), **2c** (**d**) and **2d** (**e**) after 24, 48 and 72 h. Each experiment was carried out in duplicate. *, **, ***, **** indicate a significant difference compared to the vehicle with $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$, respectively, according to the ANOVA test

Table 1 IC_{50} values of compounds against different cancer cell lines

Product	IC_{50}* (μM)		
	MDA-MB-231	MCF-7	HepG2
2aa	> 60	> 30	11.3 ± 2.9
2ab	22 ± 3.99	7 ± 2.39	6.2 ± 1.15
2b	25 ± 3.61	5.7 ± 2.3	8.6 ± 0.83
2c	> 10	> 10	> 10
2d	> 10	> 10	> 10
Doxorubicin	0.01 ± 0.0008	0.022 ± 0.002	0.04 ± 0.0096

*IC_{50} values were derived from single dose–response curves generated from duplicate data points obtained after 72 h of treatment

2.44–2.93) were found to be inactive against the proliferation of the hormone-independent breast cancer line MDA-MB-231. As for the hormone-dependent breast cancer line MCF-7, the results show that the highest anticancer activity was observed for the 2-arylimidazolone compound **2b** ($IC_{50} = 5.7 \pm 2.3$ μM) having a methoxy group in the para position on each of the aromatic nuclei. As for the 2-phenethylimidazolone derivatives, compound **2aa**, having a methoxy substitution in the para position of the benzylidene moiety, did not show any interesting cytotoxic effect ($IC_{50} > 30$ μM).

However, an enhanced cytotoxic activity was noted, when the methoxy group was displaced from para to meta position in compound **2ab**, showing an IC_{50} of 7 ± 2.39 μM.

When tested on the hepatocellular carcinoma cell line HepG2, the 2-phenethylimidazolone derivative **2ab** was found possessing the most cytotoxic activity with an IC_{50} of 6.2 ± 1.15 μM. The displacement of the methoxy substitution from the meta position (**2ab**) to the para position (**2aa**), reduced the cytotoxic activity by half. The 2-arylimidazolone compound **2b** also showed an interesting anticancer effect against this cell line with an IC_{50} of 8.6 ± 0.83 μM. Overall, the 5-benzylidene-2-alkyl and 2-arylimidazolones showed noticeable cytotoxicity against MCF-7 and HepG2 cell lines. In the literature, some 5-benzylidene-2-arylimidazolone derivatives have also shown anticancer activity against epithelial carcinoma cancer cell line A549 with IC_{50} values of 0.44 to 12 μM (Beloglazkina et al. 2016).

The difference of anticancer activity noted between ER+ cell line MCF-7 and HER2+ cell line HepG2 on the one hand and triple-negative cell line MDA-MB-231 on the other hand could be attributed to the different kinases expressions in these cell lines. In fact, it has been reported that CLK2 is amplified and overexpressed in HER2+ and ER+ cell lines and that downregulation of CLK2 inhibited breast cancer growth in cell culture (Yoshida et al. 2015), whereas inhibition of hyperactive CDKs in triple-negative breast cancer resulted in decreased cell migration and invasion (Tarasewicz et al. 2014). These findings could suggest possible selective interactions of the synthesized imidazolone derivatives with specific kinases.

All the imidazolone derivatives were less potent than the doxorubicin drug used as positive control. However, the latter can induce acute and chronic cardiotoxicity that may occur at low doses (Octavia et al. 2012). It also showed toxicity on cardiogenesis, heart formation and survival of zebrafish embryos (Ma et al. 2018; Chang et al. 2014).

Zebrafish embryo toxicity assay

The newly synthesized imidazolone derivatives and doxorubicin drug have been tested for their toxic effect in vivo on the development of zebrafish embryos exposed to increasing concentrations (1–120 µM) of these compounds. Morphological abnormalities and mortality of the embryos were studied. The summary of mortality and observed malformations of zebrafish embryos at 72 hpf with the different compounds are presented in Table 2.

Below concentrations of 10 µM, no incidence of mortality and malformation was observed with all the tested compounds. The mortality rate slightly increased after exposure to **2ab** and **2b** at concentrations higher than 60 µM. Furthermore, treatment with **2aa** starting from 60 µM caused a significantly increased mortality at 48 and 72 hpf. At 72–96 hpf, there were significant differences in the malformation phenotypes in the zebrafish embryos that were exposed to the various compounds. The predominant effects observed after **2ab** (Fig. 7) and **2aa** (Fig. 8) exposure were pericardial edema, yolk sac edema, short and contorted tail. For **2ab**, starting from 30 µM and at 72 hpf, the length of the embryos became much shorter, whereas cardiac edema, yolk sac edema and short yolk sac extension were well noted at 60 µM. In addition, a significant contraction of the tail has been observed at 60 µM (Fig. 7). However, **2aa** compound treatment induced tail contraction at 10 µM and yolk sac and pericardial edema starting from 30 µM (Fig. 8). Furthermore, a delay in zebrafish hatching was observed only for **2aa** compound at high concentrations.

It was not possible to determine the effect of **2c** and **2d** compounds at high concentrations due to their precipitation in the E3 medium. After 72 h of exposure to **2c** and **2d**, there was no significant mortality, neither effects on hatching delay nor malformation in embryonic development, when compared with control group with all concentrations used below 10 µM. Overall, the 5-benzylidene-2-alkyl and 2-arylimidazolones induced significant mortality and abnormalities only at high concentrations. These compounds were less toxic on the development of zebrafish embryos than doxorubicin drug that caused 50% of lethality at 30 µM.

Table 2 Percentage of mortality and types of morphological malformation caused by each analog in zebrafish embryos at 72 hpf

Product	% of mortality (at 72 hpf)	Morphological malformations at 72 hpf			
		Cardiac edema	Yolk sac edema	Curved tail/Short tail	Hatching retardation
2aa					
1	0	−	−	−	−
3	0	−	−	−	−
10	6	−	−	+	−
30	10	+	+	+	−
60	12	+	+	+	−
90	50	−	+	+	+
120	50	−	+	+	+
2ab					
1	0	−	−	−	−
3	0	−	−	−	−
10	5	−	−	−	−
30	5	−	−	−	−
60	13	+	+	+	−
90	15	−	+	+	−
120	13	−	+	+	−
2b					
1	0	−	−	−	−
3	0	−	−	−	−
10	0	−	−	−	−
30	10	−	−	−	−
60	10	−	+	+	−
90	15	+	+	+	−
120	15	+	+	+	−
2d					
1	0	−	−	−	−
3	0	−	−	−	−
10	0	−	−	−	−
2c					
1	0	−	−	−	−
3	0	−	−	−	−
10	0	−	−	−	−
Doxorubicin					
1	0	−	−	−	−
3	0	−	−	−	−
10	20	−	−	−	−
30	50	+	−	+	+
60	100	+	−	+	+
90	100	+	−	+	+
120	100	+	−	+	+

*% of mortality (at 72 hpf) for control and vehicle (0.5% of DMSO) are zero, and none of the morphological malformations at 72 hpf are detectable

Fig. 7 Zebrafish embryos treated with the **2ab** product for 72 h **a** CTRL—control; **b** embryo treated with 3 µM **c** embryo treated with 30 µM; **d** embryo treated with 60 µM; **e** embryo treated with 60 µM; **f** embryo treated with 120 µM. Scale bar 250 µm. ce: cardial edema; yse: yolk sac edema; sy: short yolk sac extension; ct: contracted tail

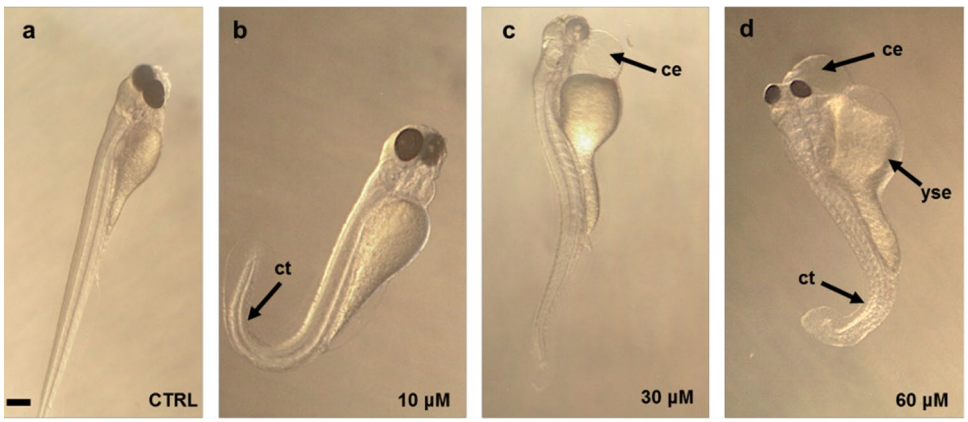


Fig. 8 Photographs of encountered malformations, resulting from the treatment of Casper zebrafish embryos with compounds **2aa** at 96 hpf. **a** CTRL—control; **b** embryo treated with 10 µM **c** embryo treated with 30 µM; **d** embryo treated with 60 µM; scale bar 250 µm. ce: cardial edema; yse: yolk sac edema; ct: contracted tail

Conclusions

We optimized the synthesis of new 2-aryl and 2-alkylimidazolone derivatives, in a one-pot procedure under microwave irradiations. The latter have shown to be more efficient than thermal heating by providing similar or slightly better yields of the imidazolones while reducing the reaction time from 2 h to 15 min. The cytotoxicity of the imidazolone derivatives was evaluated in vitro against tumor cell lines. This family of compounds exhibited interesting anticancer activity against ER+ and PR+ cell line MCF-7 and HER2+ cell line HepG2. Among the synthesized derivatives, compound **2b** expressed the best cytotoxic effect against MCF-7 cell line with an IC_{50} value of 5.7 µM, whereas compound **2ab** was the most active against HepG2 cell line with an IC_{50} of 6.2 µM. Further studies should be conducted to identify the involvement of protein kinases, overexpressed in these types of tumors, in the pathways responsible for the observed cytotoxicity. Besides their cytotoxic activity on cancer cell lines, these compounds showed biological interactions in vivo manifested in morphological abnormalities observed on the development of zebrafish embryos only at high concentrations. Although a limited number of imidazolone derivatives are presented here, it is obvious that a much larger diversity can be achieved through this versatile process to enrich this family of molecules and subsequently conduct a more detailed SAR. This preliminary study showed that these compounds may be considered as promising molecules in the further search of novel active and non-toxic anticancer agents.

Acknowledgements We would like to thank the Lebanese University and the National Council for Scientific Research (CNRS)—Lebanon, for the financial support of Samar Bou Zeid during her thesis and for the financial support of this project. We would also like to thank the Edde Association—Lebanon. The funders had no role in the study design or during data collection and analysis as well as preparation of the manuscript. This work was also supported by the University of Rennes 1 and by the management committee of scientific research at the Lebanese University.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was written by Samar Bou Zeid, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethics approval Adult zebrafish (*Danio rerio*) were used after ethical approval.

References

Abdel Gawad NM, Amin NH, Elsaadi MT, Mohamed FMM, Angeli A, De Luca V, Capasso C, Supuran CT (2016) Synthesis of 4-(thiazol-2-ylamino)-benzenesulfonamides with carbonic anhydrase I, II and IX inhibitory activity and cytotoxic effects against breast cancer cell lines. Bioorganic MedChem 24(13):3043–3051. https://doi.org/10.1016/j.bmc.2016.05.016

Beloglazkina AA, Wobith B, Barskaia ES, Zefirov NA, Majouga AG, Beloglazkina EK, Zyk NV, Kuznetsov SA, Zefirova ON (2016) Synthesis and biological testing of (5Z)-2-aryl-5-arylmethylidene-3,5-dihydro-4H-imidazol-4-ones as antimitotic agents. Med Chem Res 25(6):1239–1249. https://doi.org/10.1007/s00044-016-1566-2

Boehm JC, Gleason JG, Pendrak I, Sarau HM, Schmidt DB, Foley JJ, Kingsbury WD (1993) Synthesis and LTB4 receptor antagonist activities of the naturally occurring LTB4 receptor antagonist leucettamine A and related analogs. J Med Chem 36(22):3333–3340. https://doi.org/10.1021/jm00074a014

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68(6):394–424. https://doi.org/10.3322/caac.21492

Burgy G, Tahtouh T, Durieu E, Foll-Josselin B, Limanton E, Meijer L, Carreaux F, Bazureau J-P (2013) Chemical synthesis and biological validation of immobilized protein kinase inhibitory Leucettines. Eur J Med Chem 62:728–737. https://doi.org/10.1016/j.ejmech.2013.01.035

Carroll AR, Copp BR, Davis RS, Keyzers RA, Prinsep MR (2019) Marine natural products. Nat Prod Rep 36(1):122–173. https://doi.org/10.1039/C8NP00092A

Chan GW, Mong S, Hemling ME, Freyer AJ, Offen PH, DeBrosse CW, Sarau HM, Westley JW (1993) New leukotriene B4 receptor antagonist: leucettamine A and related imidazole alkaloids from the marine sponge leucetta microraphis. J Nat Prod 56(1):116–121. https://doi.org/10.1021/np50091a016

Chang C, Wu SL, Zhao XD, Zhao CT, Li YH (2014) Developmental toxicity of doxorubicin hydrochloride in embryo-larval stages of zebrafish. Biomed Mater Eng 24(1):909–916. https://doi.org/10.3233/BME-130885

Dai X, Cheng H, Bai Z, Li J (2017) Breast cancer cell line classification and its relevance with breast tumor subtyping. J Cancer 8(16):3131–3141. https://doi.org/10.7150/jca.18457

Debdab M, Carreaux F, Renault S, Soundararajan M, Fedorov O, Filippakopoulos P, Lozach O, Babault L, Tahtouh T, Baratte B, Ogawa Y, Hagiwara M, Eisenreich A, Rauch U, Knapp S, Meijer L, Bazureau J-P (2011) Leucettines, a class of potent inhibitors of cdc2-like kinases and dual specificity, tyrosine phosphorylation regulated kinases derived from the marine sponge leucettamine B: modulation of alternative pre-RNA splicing. J Med Chem 54(12):4172–4186. https://doi.org/10.1021/jm200274d

Debdab M, Renault S, Lozach O, Meijer L, Paquin L, Carreaux F, Bazureau J-P (2010) Synthesis and preliminary biological evaluation of new derivatives of the marine alkaloid leucettamine B as kinase inhibitors. Eur J Med Chem 45(2):805–810. https://doi.org/10.1016/j.ejmech.2009.10.009

DeSantis CE, Ma J, Goding Sauer A, Newman LA, Jemal A (2017) Breast cancer statistics, 2017, racial disparity in mortality by state. CA Cancer J Clin 67(6):439–448. https://doi.org/10.3322/caac.21412

Eldehna WM, Abdelrahman MA, Nocentini A, Bua S, Al-Rashood ST, Hassan GS, Bonardi A, Almehizia AA, Alkahtani HM, Alharbi A, Gratteri P, Supuran CT (2019) Synthesis, biological evaluation and in silico studies with 4-benzylidene-2-phenyl-5(4H)-imidazolone-based benzenesulfonamides as novel selective carbonic anhydrase IX inhibitors endowed with anticancer activity. Bioorganic Chem 90:103102. https://doi.org/10.1016/j.bioorg.2019.103102

Foo J, Michor F (2014) Evolution of acquired resistance to anti-cancer therapy. J Theor Biol 355:10–20. https://doi.org/10.1016/j.jtbi.2014.02.025

Fozooni S, Tikdari AM (2008) Microwave-assisted graphite-support synthesis of imidazolones. Catal Lett 120(3):303–306. https://doi.org/10.1007/s10562-007-9285-4

Hsu M-H, Hsieh C-Y, Kapoor M, Chang J-H, Chu H-L, Cheng T-M, Hsu K-C, Lin TE, Tsai F-Y, Horng J-C (2020) Leucettamine B analogs and their carborane derivative as potential anti-cancer agents: design, synthesis, and biological evaluation. Bioorganic Chem 98:103729. https://doi.org/10.1016/j.bioorg.2020.103729

Kappe CO, Dallinger D (2009) Controlled microwave heating in modern organic synthesis: highlights from the 2004–2008 literature. Mol Divers 13(2):71. https://doi.org/10.1007/s11030-009-9138-8

Kidwai AR, Devasia GM (1962) A new method for the synthesis of amino acids. Synthesis of amino acids and their derivatives through 2,4-disubstituted 2-imidazolin-5-ones1. J Org Chem 27(12):4527–4531. https://doi.org/10.1021/jo01059a098

Kumar Y, Shaw M, Thakur R, Kumar A (2016) Copper(II)-mediated aerobic oxidation of benzylimidates: synthesis of primary α-ketoamides. J Org Chem 81(15):6617–6625. https://doi.org/10.1021/acs.joc.6b01262

Ling Y, Wang Z-Q, Xiao Y-A, Zhu C, Shen L, Wang X-M, Hui Y, Wang X-Y (2013) Benzylidene 2-aminoimidazolones derivatives: synthesis and in vitro evaluation of anti-tumor carcinoma activity. Chem Pharm Bull (Tokyo) 61(10):1081–1084. https://doi.org/10.1248/cpb.c13-00340

Link BA, Megason SG (2008) Zebrafish as a model for development. In: Conn PM (ed) Sourcebook of models for biomedical research. Humana Press, Totowa, pp 103–112

Luqmani YA (2005) Mechanisms of drug resistance in cancer chemotherapy. Med Princ Pract 14(Suppl. 1):35–48. https://doi.org/10.1159/000086183

Ma X, Ding Y, Wang Y, Xu X (2018) A Doxorubicin-induced cardiomyopathy model in adult zebrafish. J Vis Exp JoVE. https://doi.org/10.3791/57567

Meyers JR (2018) Zebrafish: development of a vertebrate model organism. Curr Protoc Essent Lab Tech 16(1):e19. https://doi.org/10.1002/cpet.19

Mayer AMS, Rodríguez AD, Berlinck RGS, Fusetani N (2011) Marine pharmacology in 2007–8: Marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous system, and other miscellaneous mechanisms of action. Comp Biochem Physiol Part C Toxicol Pharmacol 153(2):191–222. https://doi.org/10.1016/j.cbpc.2010.08.008

Mokale SN, Lokwani DK, Shinde DB (2014) Synthesis, in-vitro reverse transcriptase inhibitory activity and docking study of some new imidazol-5-one analogs. Med Chem Res 23(8):3752–3764. https://doi.org/10.1007/s00044-014-0954-8

Newman DJ, Cragg GM (2012) Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 75(3):311–335. https://doi.org/10.1021/np200906s

Newman M, Ebrahimie E, Lardelli M (2014) Using the zebrafish model for Alzheimer's disease research. Front Genet. https://doi.org/10.3389/fgene.2014.00189

Octavia Y, Tocchetti CG, Gabrielson KL, Janssens S, Crijns HJ, Moens AL (2012) Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. J Mol Cell Cardiol 52(6):1213–1225. https://doi.org/10.1016/j.yjmcc.2012.03.006

Shi F, Zeng X-N, Wu F-Y, Yan S, Zheng W-F, Tu S-J (2012) Efficient microwave-assisted synthesis and antioxidant activity of 4-arylidene-2-phenyl-1H-imidazol-5(4H)-ones. J Heterocycl Chem 49(1):59–63. https://doi.org/10.1002/jhet.766

Shi J-H, Guo W-Z, Jin Y, Zhang H-P, Pang C, Li J, Line P-D, Zhang S-J (2019) Recognition of HER2 expression in hepatocellular carcinoma and its significance in postoperative tumor recurrence. Cancer Med 8(3):1269–1278. https://doi.org/10.1002/cam4.2006

Shi X, Wang R, Zeng X, Zhang Y, Hu H, Xie C, Wang M (2018) Ruthenium (II)-catalyzed oxidant-free coupling/cyclization of benzimidates and sulfoxonium ylides to form substituted isoquinolines. Adv Synth Catal 360(21):4049–4053. https://doi.org/10.1002/adsc.201800844

Strauss CR, Varma RS (2006) Microwaves in green and sustainable chemistry. In: Larhed M, Olofssonq K (eds) Microwave methods in organic synthesis, topics in current chemistry. Springer, Berlin, pp 199–231

Tahtouh T, Elkins JM, Filippakopoulos P, Soundararajan M, Burgy G, Durieu E, Cochet C, Schmid RS, Lo DC, Delhommel F, Oberholzer AE, Pearl LH, Carreaux F, Bazureau J-P, Knapp S, Meijer L (2012) Selectivity, cocrystal structures, and neuroprotective properties of leucettines, a family of protein kinase inhibitors derived from the marine sponge alkaloid leucettamine B. J Med Chem 55(21):9312–9330. https://doi.org/10.1021/jm301034u

Tarasewicz E, Rivas L, Hamdan R, Dokic D, Parimi V, Bernabe BP, Thomas A, Shea LD, Jeruss JS (2014) Inhibition of CDK-mediated phosphorylation of Smad3 results in decreased oncogenesis in triple negative breast cancer cells. Cell Cycle 13(20):3191–3201. https://doi.org/10.4161/15384101.2014.950126

Villa FA, Gerwick L (2010) Marine natural product drug discovery: leads for treatment of inflammation, cancer, infections, and neurological disorders. Immunopharmacol Immunotoxicol 32(2):228–237. https://doi.org/10.3109/08923970903296136

Villemin D, Martin B (1995) Dry condensation of creatinine with aldehydes under focused microwave irradiation. Synth Commun 25(20):3135–3140. https://doi.org/10.1080/00397919508015462

Voosala C, Yarla NS, Nakka MR, Vidavaluri S (2014) Facile synthesis of 1-(substituted phenyl)-2-phenyl-4-(substituted benzylidine)-imidazole-5-ones and antifungal activity studies against phytopathogens. Med Chem 4(1):1–3. https://doi.org/10.4172/2161-0444.1000156

Wild C, Weiderpass E, Stewart B (eds) (2020) World cancer report: cancer research for cancer prevention. International Agency for Research on Cancer, Lyon, France

Xiao Y-A, Wang Z-Q, Wang X-M, Hui Y, Ling Y, Wang X-Y, He L-Q (2013) Synthesis and in vitro biological evaluation of novel 2-aminoimidazolone derivatives as anti-tumor agents. Chin Chem Lett 24(8):727–730. https://doi.org/10.1016/j.cclet.2013.05.009

Yadav VK, Babu KG (2005) A remarkably efficient Markovnikov hydrochlorination of olefins and transformation of nitriles into imidates by use of AcCl and an alcohol. Eur J Org Chem 2005(2):452–456. https://doi.org/10.1002/ejoc.200400591

Yoshida T, Kim JH, Carver K, Su Y, Weremowicz S, Mulvey L, Yamamoto S, Brennan C, Mei S, Long H, Yao J, Polyak K (2015) CLK2 is an oncogenic kinase and splicing regulator in breast cancer. Cancer Res 75(7):1516–1526. https://doi.org/10.1158/0008-5472.CAN-14-2443

You YN, Lakhani VT, Wells SA (2007) The role of prophylactic surgery in cancer prevention. World J Surg 31(3):450–464. https://doi.org/10.1007/s00268-006-0616-1