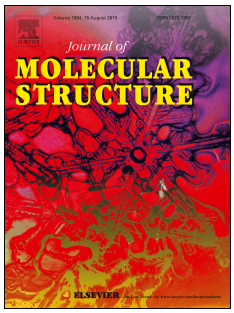

A step-by-step synthesis of triazole-benzimidazole-chalcone hybrids: Anticancer activity in human cells[+]

Amar Djemoui, Abdelkader Naouri, Mohammed Ridha Ouahrani, Djamila Djemoui, Souli Lahecen, Mokhtar Boualem Lahrech, Leila Boukenna, Hélio M.T. Albuquerque, Liza Saher, Djenisa H.A. Rocha, Fátima Liliana Monteiro, Luísa A. Helguero, Khaldoun Bachari, Oualid Talhi, Artur M.S. Silva

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A step-by-step synthesis of Triazole-Benzimidazole-Chalcone hybrids: Anticancer activity in human cells+

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+ Supporting information available

Abstract: Novel series of triazole-benzimidazole-chalcone hybrid compounds have been synthesized via click chemistry, between different azide derivatives and substituted benzimidazole terminal alkynes bearing a chalcone moiety. The starting alkynes are prepared via base-catalyzed nitrogen alkylation of pre-synthetized benzimidazole-chalcone substrates. All the intermediates as well as the final products are fully characterized by 1D and 2D NMR and mass spectrometry techniques. HMBC correlations permits the identification of a unique 1,4-disubstitued triazole-benzimidazole-chalcone isomer. Evaluation of the anti-proliferative potential in breast and prostate cancer cell lines showed that the presence of chloro substituents at the chalcone ring of the triazole-benzimidazole-chalcone skeleton enhanced the cytotoxic effects. The benzyl group linked to the 1,2,3-triazole moiety provides more antiproliferative potential.

Introduction

1,2,3-Triazoles and their derivatives are important nitrogen heterocycles, having relevant applications in both medicinal and technological fields. This kind of heterocycles is well recognized for its biological properties, such as anti-HIV,[1] antifungal,[2] antiplasmodial,[3] antibacterial,[4] anticancer,[5] antileishmanial,[6] anti-inflammatory,[7] antitubercular,[8] antiviral,[9] among others. From a medicinal chemistry point of view, 1,2,3-triazoles have gained relevance due to their implication in binding interactions with different biological targets, maintaining a good pharmacokinetic profile.[10] They are effective amide surrogates in bioactive molecules because of their strong dipole moments which can even be used as linkers, presenting bio-isosteric effects on peptide linkage, aromatic ring, double bonds and imidazole ring. Moreover, the 1,2,3-triazoles are remarkably stable towards hydrolysis, oxidative/reductive conditions, and enzymatic degradation, but reductive cleavage sometimes may occur under forcing conditions leading to the formation of triazolium salts.[11] There are many metal-free methods for the synthesis of 1,2,3-triazoles, however, the most commonly reported strategy for their high yield preparation is the copper(I)-catalysed azide-alkyne cycloaddition (CuAAC) reaction developed by Sharpless et al.,[12] which involves the reaction of an azide with a terminal-alkyne.

Aiming at the construction of a hybrid compound,[13] we focus on using the benzimidazole nucleus, which is another outstanding nitrogen-containing heterocycle. In recent years, benzimidazole nucleus has been associated to several biological properties, namely antimicrobial,[14] antiallergic,[15] anticancer,[16] antiviral,[17] anti-inflammatory,[18] antimalarial,[19] antitubercular,[20] and antiparasitic.[21] The benzimidazole-pyrazole combination has been described to show effective activity against lung cancer cell line (A549) and epidermal growth factor receptor (EGFR) binding affinity.[22a] More recently, Padhy *et al*.,[22b] have also reported the synthesis of *N*-benzylbenzimidazole linked to pyrimidine as moderate anticancer agents on breast cancer cell line (MDA-MB-231). Further synthetic strategies have been designed towards benzothiazole-piperazine-1,2,3-triazole hybrids, being considered as promising active candidates against selected human cancer cell lines.[23a] Besides, the use of click chemistry to construct the flavone-triazole-benzimidazole hybrid was successful and compounds exhibited good antiproliferative and anti-mycobacterial activities.[23b] In relation to the topic of hybrid organic compounds, the chalcone-benzimidazole combination have been studied showing that the *N*-substituted benzimidazole derivatives by an alkyl chain and nitrogen-containing 5- or 6-membered rings enhanced the cytotoxic effects on human breast adenocarcinoma (MCF-7) and human ovarian carcinoma (OVCAR-3) cell lines with IC_{50} around 9-11 µM.[23c] The cytotoxicity of some synthesized chalcone linked-1,2,3-triazoles was tested against four human cancer cell

lines (MCF-7, MIA-PA-Ca-2, A549, HepG2). The most active compound presented IC_{50} of 4-11 µM with better or comparable activity to the reference drug tested. These hybrid compounds have also induced apoptosis, G2/S arrest and triggers mitochondrial potential loss in pancreatic cancer MIA-PA-Ca-2.[23d]

Following similar synthetic pathways towards the elaboration of novel heterocyclic hybrids,[23-26] in this paper we intend to combine, in one molecule, two different nitrogen heterocycles, 1,2,3-triazole and benzimidazole incorporating the functional chalcone moiety, as a Michael acceptor, expecting that this series of 1,2,3-triazole-benzimidazole-chalcone hybrids may present improved and selective biological activities comparing to the isolated parental structures.

Results and Discussion

Synthesis

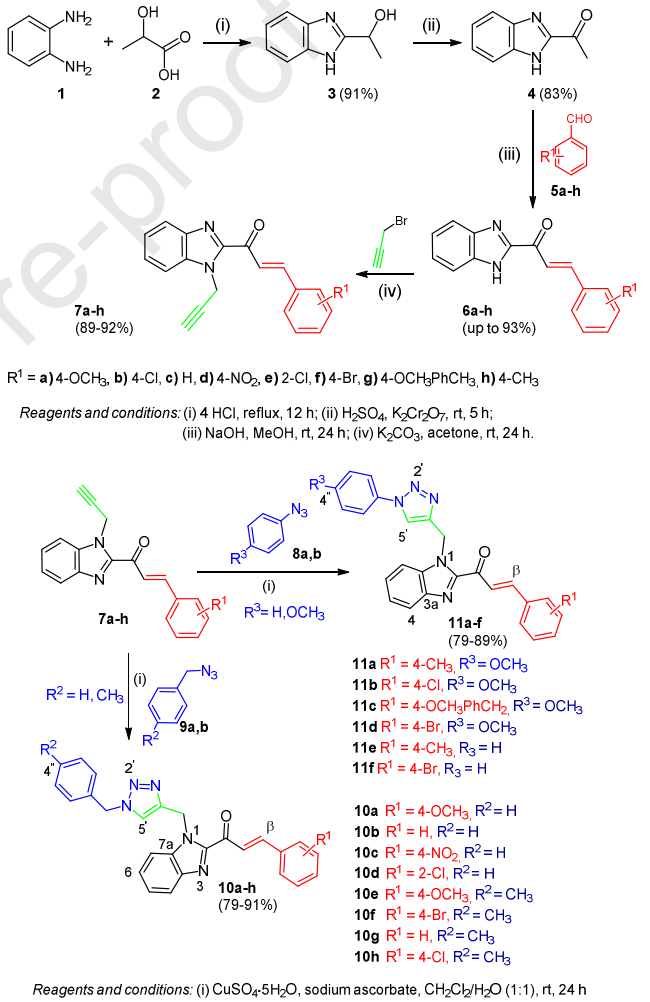
Our synthetic strategy begins by the preparation of the key intermediates of *N*-propargyl-substituted benzimidazole-chalcones **7a-h**, which have been obtained in a four-step reaction sequence. The condensation of *o*-phenylenediamine **1** with lactic acid **2** in hydrochloric acid (HCl, 4N) affords the intermediate **3**, which after the oxidation of its hydroxyl group using potassium dichromate, the desired 2-acetylbenzimidazole **4** is furnished in appreciable yields (83%) (Scheme 1). The synthesis of the benzimidazole-chalcone derivatives **6a-h** was accomplished through the base-catalysed (NaOH) aldol-condensation of 2-acetylbenzimidazole **4** with a series of substituted aromatic aldehydes **5a-h** at room temperature (yield up to 93%, Scheme 1). The final step of the reaction sequence involved the *N*-alkylation of benzimidazole-chalcones **6a-h** with propargyl bromide, affording *N*-propargylated compounds **7a-h** in excellent yields (89-92%) (Scheme 1). ^{1}H-NMR analysis of compounds **6a-h**, showed an N*H* group displayed at δ_H 11.50-13.50 ppm. On the other hand, in the ^{1}H NMR spectra of *N*-propargylated compounds **7a-h,** we observed the disappearance of the signal corresponding to N*H*, and the appearance of two new signals at δ_H 1.20 and 5.00 ppm, being attributed to terminal alkyne and the N-CH_2, respectively.

In parallel, important azide intermediates **8a-b** and **9a-b** are required for our "step-by-step" synthetic strategy and their classical preparation was performed via substitution reactions using sodium azide NaN_3, which also occurred in good yields (67-74%) (*See supporting information*).

The combination of all the parts to construct the target hybrid was achieved via click chemistry, which is the key step towards generating the 1,2,3-triazole-benzimidazole-chalcone structure (Scheme 1). Hence, the reaction of propargyl-compounds **7a-h** with the pre-synthetized azide derivatives (**8a-b** or **9a-b**) was conducted in dichloromethane/water (1:1) as a solvent system catalyzed by $CuSO_4.5H_2O$ and sodium ascorbate at room temperature to deliver a structural variety of 1,2,3-triazole-benzimidazole-chalcone hybrids **10a-h** and **11a-f** in good-to-excellent yield (79-91%) and high purity after simple recrystallisation step.

1D and 2D NMR analysis are used to unequivocally establish the exact structure of the triazole-benzimidazole-chalcone hybrid compounds **10a-h** and **11a-f** (*See supporting information*).

Taking as a representative example, compound **10a**, where it was apparently possible to distinguish between the two methylene groups N(1')-CH_2 and N(1)-CH_2 appearing as singlets at δ_H 5.52 and 5.99 ppm, respectively. The N(1)-CH_2 represents the linking bridge between the triazole and the benzimidazole heterocycles. These protons have shown important HMBC cross-peak correlations with the surrounding quaternary carbons related to both nitrogen-containing rings. Therefore, N(1)-CH_2 could evidently entertain $^3J_{H/C}$ HMBC correlations with C-2 (δ_C 146.9 ppm) and C-7a (δ_C 136.7 ppm) of the benzimidazole part. While, the same one is showing interesting $^2J_{H/C}$ with C-4' (δ_C 143.1 ppm) and $^3J_{H/C}$ with the protonated triazolic carbon C-5' (δ_H 8.12 ppm and δ_C 124.0 ppm determined via HSQC 2D-NMR) (Figure 1, *See supporting information*).



Scheme 1. Synthetic route for the preparation of benzimidazole-chalcone intermediates **7a-h** and triazole-benzimidazole-chalcone hybrids **10a-h** and **11a-f**.

A deep examination of the HMBC spectrum of compound **10a**, sharing a benzyl group on the triazole nucleus, can inform us about the regioselective formation of a 1,4-disubstitued triazole isomer. This is proved when referring to the methylene group N(1')-CH_2 of the 1'-*N*-benzyl substituent, which does not establish any noticeable $^3J_{H/C}$ HMBC connectivity with C-4' (or carbon C-5' in case of 1,5-triazole), thus, the 1,5-disubstituted

triazole isomer is not expected as demonstrated in figure 1. Furthermore, the chalcone moiety can also be observed through the presence of signals corresponding to the protons of the ketone α,β-unsaturated system $H^{\beta}C{=}CH^{\alpha}{-}C{=}O$ represented as two doublets at δ_H 8.06 and 7.80 ppm, respectively with coupling constant *J* = 16 Hz evidencing the *(E)*-configuration.[24e] Additional HMBC connectivities have been considered for compound **10a**, showing the proximity of H^{α} to the carbonyl C=O at δ_C 182.0 ppm. Nearby, H^{β} shows HMBC correlations with the phenyl substituent as illustrated in figure 1. In a similar way, the novel library of triazole-benzimidazole-chalcone hybrid compounds **10a-h** and **11a-f** have been finely characterized (*See supporting information*).

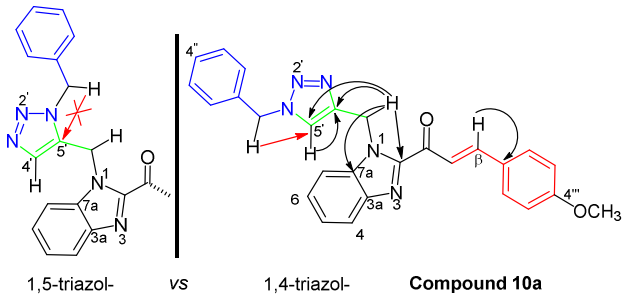


Figure 1. Main HMBC cross-peak correlations observed in the 2D-NMR spectra of the compound **10a**.

Biological assays

The triazole-benzimidazole-chalcone hybrid compounds (**10a**, **10d**, **10e**, **10h**, **11a-b**) were selected to test their cytotoxic effects *in vitro* using two human breast cancer cell lines (T47-D and MDA-MB-231) and one prostate cancer cell line (PC3). The selection was based on compounds' solubility in DMSO and we started the cytotoxic studies by applying a 10 nM–100 µM concentration range with a dilution factor of 10. Thereafter, the 1–100 µM concentration range was expanded to allow calculation of the IC_{50} values for the compounds that exhibited nearly total cytotoxicity at 100 µM concentrations, but no effects at concentrations lower than 1 µM (Table 1). Compound **10h** has no significant effect on MDA-MB-231 but stimulate the cell viability of T47-D cell lines. It is important to observe that compound **10h** showed excellent cytotoxic effect in PC3 cell line being the most active one compared to compounds **10a**, **10d** and **10e** (Table 1). Further results showed that in breast cancer cell lines (MDA-MB-231 and T47-D), compounds **10d** has obviously displayed activity with IC_{50} value ≤ 10 µM.

The preliminary evaluation of *in vitro* antiproliferative activity of compounds **10a**, **10d**, **10e**, **10h** and **11a-b** has allowed identifying the triazole-benzimidazole-chalcones **10a**, **10d**, **10e** as active heterocyclic hybrids. Among them, compound **10d** with a chloro substituent (2-Cl) on the aromatic rings displayed the highest cytotoxicity effects on all cancer cell lines tested. In this group of compounds, **10h** derivative sharing a chloro substituent (4-Cl) on the aromatic rings exerted the highest effects in PC3 cells, with IC_{50} value of 5.64 µM (Table 1).

The positive control used in this study, doxorubicin, yielded IC_{50} values less than 1 µM for PC3 and T47-D and less than 2 µM for MDA-MB-231 (Table 1). The most active compound **10d**, is about 4-fold less active in MDA-MB-231, 48-fold less active in T47-D and 15-fold less active in PC3 than doxorubicin. However, the obtained results do not take into consideration the *in vivo* bioavailability aspect of the triazole-benzimidazole-chalcone hybrid. Further experiences have revealed that the presence of 1-*N*-benzyl-1,2,3-triazole at the triazole-benzimidazole-chalcone scaffold (compounds **10**) favors the cytotoxicity on the tested cell lines, where a chloro-substitution **(10d** and **10h**) at the chalcone ring enhanced the cytotoxic effects. Regarding the structure-activity-relationship (SAR) aspect, the reported chloro-substituted benzimidazole-triazole hybrids have already shown good cytotoxic effects against mouse embryonic fibroblast cell lines NIH/3T3 (IC_{50} 1.63 µM).[23e] In the other hand, benzylated 1,2,3-triazole chalcone hybrid are proved as excellent antiproliferative agents against SK-N-SH cancer cells (IC_{50} 1.53 µM).[23f] In our case, we have noticed that, in all the cancer cell lines tested, derivatives **11a-b** have stimulated the cell viability by 5%. The unique structural difference between compounds **10** and **11** settles in the presence of the benzyl group linked to the 1,2,3-triazole moiety of the whole triazole-benzimidazole-chalcone scaffold. It could be underlined that the absence of the methylene group has provided a counter-effect of the molecules favoring cell proliferation. Such a structure-activity-relationship should be considered for future design and synthesis of novel heterocyclic hybrids bearing a triazole moiety, hence, an adequate choice of the linking groups of a hybrid molecule is primordial.[23]

Table 1. *In vitro* cytotoxicity assessment of compounds **10a**, **10d**, **10e** and **10h** in breast cancer (T47-D and MDA-MB-231) and prostate cancer (PC3) cell lines.

Compounds	IC_{50} (µM)[a]		
	T47-D	MDA-MB-231	PC3
10a	36.7±4,62	51.2±5.17	87.6 ±4,81
10d	**6.23±1,03**	**5.89±1.35**	**10.7±1,25**
10e	38.8±2,58	58.4±0.18	53.2±6,75
10h	>100	>100	**5.64 ±1,33**
Doxorubicin	0.13±0.003	1.51±0.97	**0.73**±0.14

[a] IC_{50} determined using a resazurin-based method after 72 h of incubation. Each value is the mean (IC_{50}±SD) of two independent experiments performed in quadruplicate

Conclusions

In summary, we designed a step-by-step synthetic strategy to build the triazole-benzimidazole-chalcone heterocyclic hybrid bearing varied aromatic substitutions. The novel synthesized compounds are obtained in excellent yields and purities, by using a combination of classical aldol-condensation and click chemistry. 2D NMR techniques were used to unveil the exact molecular structure and prove the regioselective formation of 1,4-disubsituted triazole via HMBC correlations. Further synthetic work on similar nitrogen containing heterocyclic hybrids is undertaken aiming the construction of new libraries for biological screenings. The preliminary anticancer screenings on human cancer cell lines demonstrate that the triazole-benzimidazole-chalcone hybrid sharing a chloro substituent and 1-*N*-benzyl-1,2,3-triazole moiety show the best cytotoxic effect

on all the selected PC-3, MDA-MB-231 and T47-D cell lines. Based on this preliminary cytotoxicity screening, further studies are required to provide more insights about the combination of heterocyclic pharmacophores in a hybrid molecule and their structure-activity-relationship in cancer therapy.

Experimental Section

Chemistry

General: Melting points were measured on a Buchi melting point B-545 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectroscopy data were recorded on a Bruker NMR 400 MHz (for ^{1}H) and 100 MHz (for ^{13}C) spectrometer in [D_6]DMSO as a solvent. Chemical shifts (δ) are reported in ppm and coupling constants (*J* values) are given in Hz. High-resolution mass spectra were recorded on a QTOF THERMO spectrometer. All reactions were monitored by means of TLC on silica or alumina gel plates and the spots were detected with UV light (l=254 nm). Chemicals were purchased from Merck or Sigma–Aldrich and used without prior purification.

General procedure for the synthesis of benzimidazole chalcones derivatives (6a-h): A solution of 2-acetyl-benzimidazole **4** (3.2 g, 20 mmol) and the diversely substituted aromatic aldehydes **5a-h** (24 mmol) in 40 ml of absolute methanol were taken in a flask. Potassium hydroxide (8.0 g, 142.6 mmol) was added and the reaction mixture was stirred for 24 hours at room temperature under TLC monitoring. The mixture was then poured into crushed ice water. The product obtained is filtered and washed with distilled water and finally recrystallized from methanol.

General procedure for the synthesis of terminal alkyne benzimidazole chalcones derivatives (7a-h): To a stirred solution of benzimidazole chalcones derivatives **6a-h** (1 mmol) and propargyl bromide (0.143 g, 1.2 mmol) in acetone (15 mL), potassium carbonate (0.346 g, 2.5 mmol) was added and the mixture is allowed to reflux for 5 hours. After the reaction is completed as indicted by TLC, the solvent was removed, and the crude was recrystallized from ethanol to provide the desired compound as yellow crystals.

General procedure for the synthesis of Triazole-benzimidazole-chalcone hybrid compounds 10a-h and 11a-f: Synthetic phenyl azide derivatives **8a-b** or benzyl azide derivatives **9a-b** (0.21 mmol) and terminal alkyne of benzimidazole chalcones derivatives **7a-h** (0.2 mmol) were taken in CH_2Cl_2 and H_2O (10 mL, 1:1) under continuous stirring for 5 min. After that, $CuSO_4.5H_2O$ (0.035 g, 0.22 mmol) and sodium ascorbate (0.055 g, 0.28 mmol) were added to the reaction mixture, which was allowed to stirring at room temperature for further 24 hours under TLC monitoring. After completion of the reaction, the crude mixture was partitioned between H_2O and CH_2Cl_2. The aqueous layer was extracted with ethyl acetate (3×15 mL). The combined organic phases were dried over magnesium sulfate ($MgSO_4$) and concentrated under reduced pressure. The compounds obtained were recrystallized from absolute ethanol.

Fourteen (14) new triazole-benzimidazole-chalcone hybrid compounds **10a-h** and **11a-f** have been generated; their NMR data and spectra are reported in the supporting information section. In the following, only NMR data of compound **10a** and **11a** are given as representative examples.

(*E*)-1-{1-[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]-1*H*-benzo[*d*]imidazol-2-yl}-3-(4-methoxyphenyl)prop-2-en-1-one (10a): Yellow solid (72.9 mg, 81%); mp 188-190 ℃; ^{1}H-NMR (400 MHz, [D_6]DMSO): δ = 3.84 (s, 3*H*, 4'''-OC*H*$_3$), 5.53 [s, 2H, N(1')-C*H*$_2$], 5.99 [s, 2H, N(1)-C*H*$_2$], 7.06 (d, 2H, H-3''',5''', *J* 8.8 Hz), 7.23-7.34 (m, 5H, H-2'',3'',4'',5'',6''), 7.38 (t, 1H, H-5, *J* 7.6 Hz), 7.47 (t, 1H, H-6, *J* 7.6 Hz), 7.81-7.89 (m, 5H, H-2''',6''', H-α, H-4, H-7), 8.06 (d, 1H, H-β, *J* 16.0 Hz), 8.12 (s, 1H, H-5') ppm. ^{13}C-NMR (100 MHz, [D_6]DMSO): δ = 40.7 [N(1)-*C*H$_2$], 53.2 [N(1')-*C*H$_2$], 55.9 (4'''-OCH$_3$), 112.5 (C-7), 115.2 (C-3''',5'''), 120.9 (C-β), 121.7 (C-4), 124.0 (C-5'), 124.2 (C-5), 126.3 (C-6), 127.4 (C-1'''), 128.3 (C-2'',6''), 128.5 (C-4''), 129.2 (C-3'',5''), 131.4 (C-2''',6'''), 136.4 and 136.7 (C-1'' and C-7a), 141.7 (C-3a), 143.7 (C-4'), 144.7 (C-α), 146.9 (C-2), 162.3 (C-4'''), 182.5 (C=O) ppm. ESI-HRMS: calcd. for $C_{27}H_{24}N_5O_2$ $[M+H]^+$ 450.1930; found 450.1917.

(*E*)-1-{1-[(1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-benzo[*d*]imidazol-2-yl}-3-(4-methylphenyl)prop-2-en-1-one (11a): Yellow Cristal (80 mg, 89%); mp 201-202 ℃; ^{1}H-NMR (400 MHz, [D_6]DMSO): δ = 2.37 (s, 3H, 4'''-C*H*$_3$), 3.80 (s, 3H, 4''-OC*H*$_3$), 6.10 [s, 2H, N(1)-C*H*$_2$], 7.08 (d, 2H, H-3'',5'', *J* 9.0 Hz), 7.31 (d, 2H, H-3''',5''', *J* 7.8 Hz), 7.40 (t, 1H, H-5, *J* 7.7 Hz), 7.50 (t, 1H, H-6, *J* 7.7 Hz), 7.73-7.75 (m, 4H, H-2'',6'' and H-2''',6'''), 7.85-7.92 (m, 3H, H-α, H-7 and H-4), 8.18 (d, 1H, H-β, *J* 16.0 Hz), 8.63 (s, 1H, H-5') ppm. ^{13}C-NMR (100 MHz, [D_6]DMSO): δ = 21.6 (4'''-*C*H$_3$), 40.7 [N(1)-*C*H$_2$], 56.0 (4''-OC*H*$_3$), 112.5 (C-7), 115.2 (C-3'',5''), 121.8 (C-4), 122.0 (C-5'), 122.3 (C-2''.6''), 122.4 (C-β), 124.3 (C-5), 126.4 (C-6), 129.4 (C-2''',6'''), 130.3 (C-3''',5'''), 130.4 (C-1''), 132.1 (C-1'''), 136.8 (C-7a), 141.8 (C-3a and C-4'''), 144.3 (C-4'), 144.6 (C-α), 146.8 (C-2), 159.7 (C-4''), 182.6 (C=O) ppm. ESI-HRMS: calcd. for $C_{27}H_{24}N_5O_2$ $[M+H]^+$ 450.1930; found 450.1916.

Biological assays.

Cell culture: MDA-MB-231 metastatic breast cancer cell line was grown in DMEM culture medium supplemented with 10% fetal bovine serum (FBS) (Gibco™ by Life Technology) and 1% penicillin/streptomycin solution (PEST; Gibco™). T47-D non-metastatic luminal breast cancer and PC3 metastatic prostate cancer cell lines were grown in RPMI culture medium supplemented with 10% FBS and 1% PEST. Cell cultures were maintained at 37 ˚C in a 5% CO_2 humidified atmosphere. Stock solutions of the test compounds were prepared in sterile DMSO to 100 mM final concentration. Thereafter, serial dilutions were prepared in growth medium. Maximum DMSO concentration applied to the cells was 1/1000 to avoid toxic effects associated to higher concentrations of this solvent.

Reagents and test compounds: Compounds **10a**, **10d**, **10e**, **10h**, **11a-b** were dissolved in 100% DMSO to obtain a stock concentration of 100 mm. Thereafter, serial dilutions were prepared in growth medium.

Cell viability assays: Serial dilutions ranged 10 nM-100 µM to cover a wide scale for generation of dose-response curves. In all experiments the solvent DMSO (1/1000) alone was used as negative control. Seven thousand cells/wells were seeded in 96-well plates and allowed to adhere for 24h. Cells were then exposed to the test compounds diluted in culture medium for 48 h (200 µL), after this time 100 µl of the culture medium was replaced by test solution for additional 24 h. Thereafter, cell viability was assessed using 100 µL/well of the rezasurin-based PrestoBlue™ reagent (Life Technologies) according to the manufacturer´s instructions. Values considered were within the linear range of the reading which was after 3h incubation.

The IC_{50} values from at least two independent experiments performed in quadruplicate were calculated using GraphPad Prism software (version 6.00), using the log (inhibitor) *vs.* response (variable slope-four parameters) function. For simplification and comparison among different experiments the figure shows the viability index which was calculated by normalizing the mean absorbance values of each treatment by the mean absorbance of the DMSO control.

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Keywords: 1,2,3-triazole • benzimidazole • chalcones • click chemistry • Anticancer activity

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Highlights:

- Triazole-benzimidazole-chalcone heterocyclic hybrids were prepared via click chemistry with variable substitutions
- 1D and 2D NMR (HMBC correlations) have identified the 1,4-disubstitued triazole-benzimidazole-chalcone as a unique isomer.
- Anti-proliferative potential in breast and prostate cancer cell lines have been evidenced for the triazole-benzimidazole-chalcone hybrid.
- The synthetized triazole-benzimidazole-chalcone hybrid sharing a chloro substituent and 1-*N*-benzyl-1,2,3-triazole moiety enhanced the cytotoxic effects.

Author Contributions Section:

Djemoui A., Naouri A; and TALHI O. conceptualized the work and co-wrote the manuscript.

Ouahrani M.R., Djemoui D. and Lahecen S. performed the synthetic experimental work and wrote the original draft preparation

Lahrech M.B., Boukenna L. and Albuquerque H.M.T. performed the 2D NMR analysis and interpretation

Saher L., Rocha D.H.A., Monteiro F.L. and Helguero L.A. were responsible for the biological screening work

Bachari K. and Silva A.M.S. conceptualized the work and co-wrote the manuscript.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.