Received: 18 November 2017 | Revised: 26 January 2018

DOI: 10.1002/hc.21412

RESEARCH ARTICLE

WILEY Heteroatom Chemistry

An easy synthetic protocol for imidazo-1,4-oxazines and evaluation of their toxicities

Burak Kuzu[1,2] | **Hasan Genç**[3] | **Mehmet Taşpinar**[4] | **Meltem Tan**[1,2] | **Nurettin Menges**[1,2]

[1]Pharmaceutical Chemistry Section, Faculty of Pharmacy, Van Yüzüncü Yil University, Van, Turkey

[2]YYU TEKNOKENT, R&D Lab. SAFF Chemical Reagent, Van, Turkey

[3]Faculty of Education, Van Yüzüncü Yıl University, Van, Turkey

[4]School of Medicine, Van Yüzüncü Yil University, Van, Turkey

Correspondence
Nurettin Menges and Meltem Tan, Pharmaceutical Chemistry Section, Faculty of Pharmacy, Van Yüzüncü Yil University, Van, Turkey.
Emails: nurettinmenges@yyu.edu.tr (N.M.); meltemtan@yyu.edu.tr (M.T.)

Funding information
YYU-BAP, Grant/Award Number: TAP-2016-5610 and TAP-2017-6092; TÜBİTAK, Grant/Award Number: 115Z894

Abstract

Imidazo-1,5-alkynyl alcohol derivatives were synthesized, and they were cyclized to imidazo-1,4-oxazines by means of cesium carbonate. Propargyl-allene isomerization was examined, and the reaction mechanism was proposed. Moreover, cytotoxicity of synthesized molecules against LN405 cell lines was investigated by means of structure-activity relationship (SAR). With SAR study, toxicities of some functional groups have been shown. In addition, two lead compounds were tested against DNA damaging.

KEYWORDS

allene, COMET, cyclization of alkyne, heterocyclic compounds, SAR

1 | INTRODUCTION

The design and synthesis of desired and tolerable methods to attain complex molecules from appropriate starting materials are still challenging work for lots of scientific areas such as modern synthetic and medicinal chemistry. The methods which include synthesis of compounds containing nitrogen and oxygen are of growing importance due to the importance of some biological activities.[1a–c] Some important heterocyclic compounds such as antituberculosis agent **1** (PA-824), antibiotic, sold under the trade name Levaquin **2**, and acortatarin A **3** which is an inhibitor for the production of reactive oxygen species (ROS)[2] include oxazine cores (Figure 1).

In a few decades, tremendous efforts have been devoted to the development of new, efficient, atom-economic, and no-catalyst methods to achieve important heterobicyclic molecules.[3b–e] One of these protocol covers 1,4-oxazine rings. There have been some synthetic methods for 1,4-oxazines and its bicyclic derivatives in the literature. Kerwin and coworkers have developed

Contract grant sponsor: Scientific and Technological Research Council of Turkey.

Contract grant number: 115Z894.

Contract grant sponsor: Van Yüzüncü Yil University Coordinator of Scientific Research Projects.

Contract grant number: TAP-2016-5610.

Contract grant number: TAP-2017-6092.

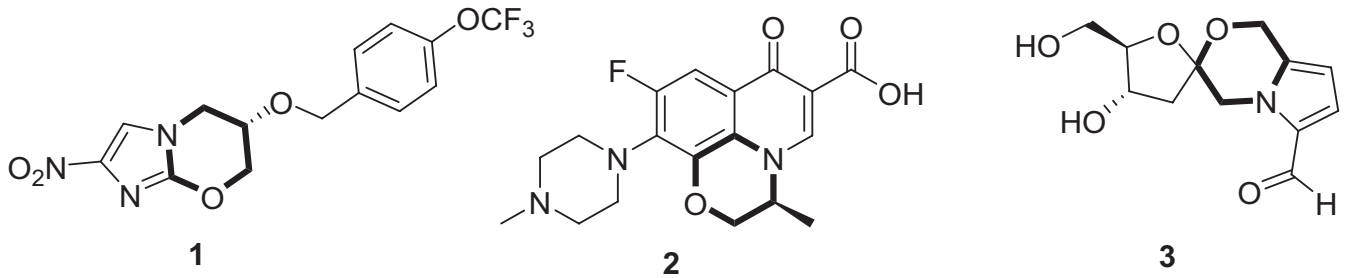
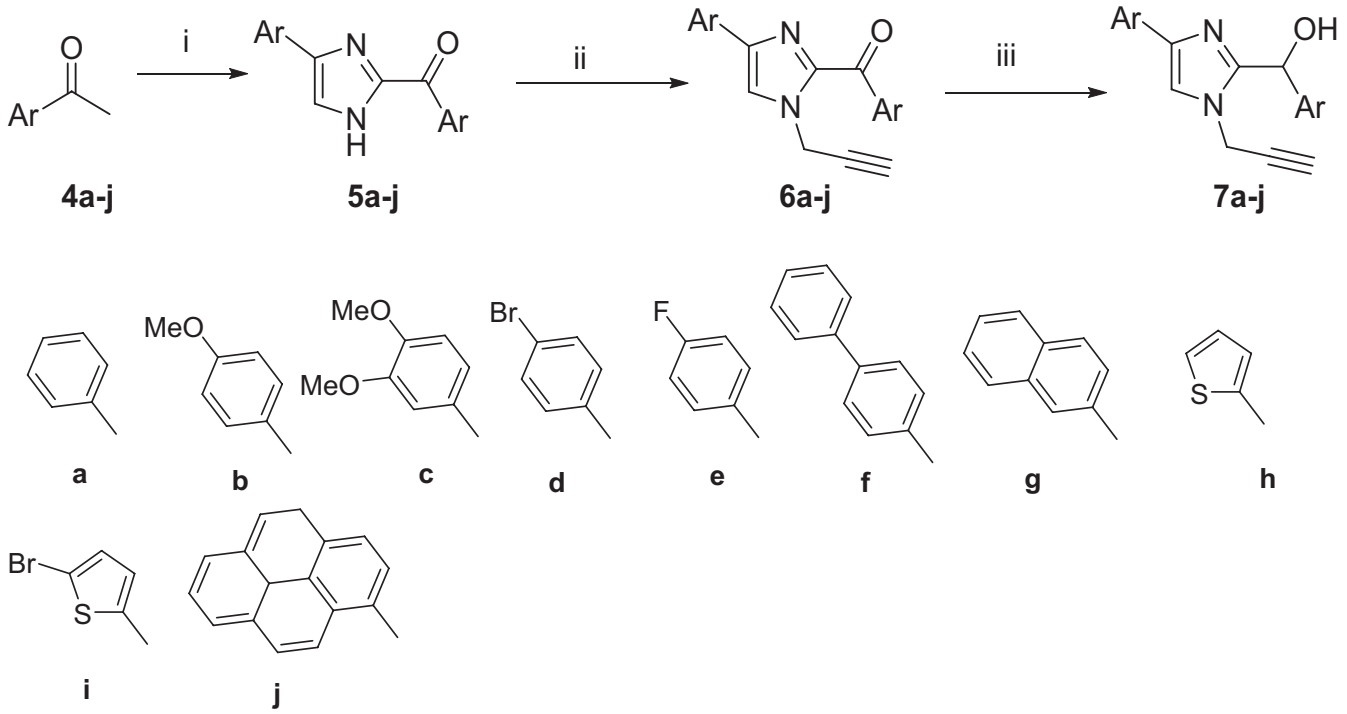


FIGURE 1 Some important molecules which bear oxazine ring



SCHEME 1 Obtaining of starting materials. (i): (1) SeO_2, 1,4-dioxane, reflux, (2) NH_4OAc, EtOH, rt. (ii) propargyl bromide, NaH, DMF, 0°C. (iii) $NaBH_4$, MeOH, rt

a method for cyclization of 1,4-alkynyl alcohols using metal catalyst to reach 1,4-oxazine-imidazole derivatives.[3a] Wang et al[4] have reported a method for hydroalkoxylation of 1,5-alkynyl alcohols with NaH to get 1,4-oxazines. Muthusubramanian et al[5a] have published a paper in which imidazolo-1,4-oxazine derivatives occurred via hydroalkoxylation of 1,5-alkynyl alcohols by lithium *t*-butoxide (*t*-BuOLi). On the other hand, 1,5-alkynyl alcohols were also utilized to synthesize 1,4-diazepinone-imidazole ring system via CuI.[5b] In this study, we have utilized 1,5-alkynyl alcohols to form 1,4-oxazine-imidazole derivatives with safer base, cesium carbonate. Formerly used bases such as NaH and *t*-BuOLi are toxic and highly water-sensitive. To remove these disadvantages, we have synthesized 1,4-oxazine-imidazoles using cesium carbonate in ethanol.

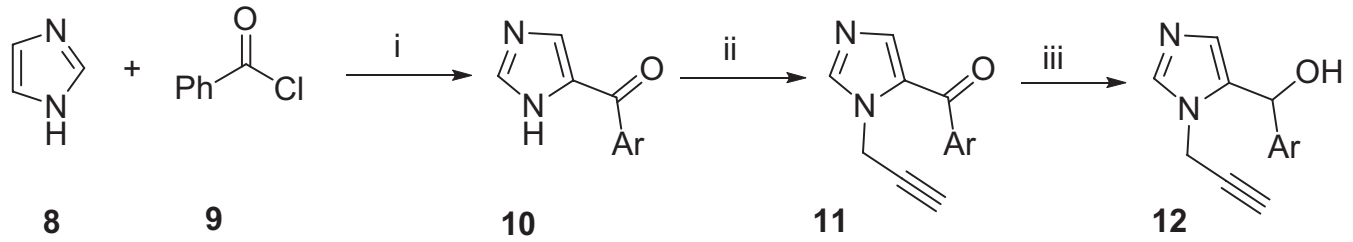
2 | RESULTS AND DISCUSSION

2.1 | Chemistry

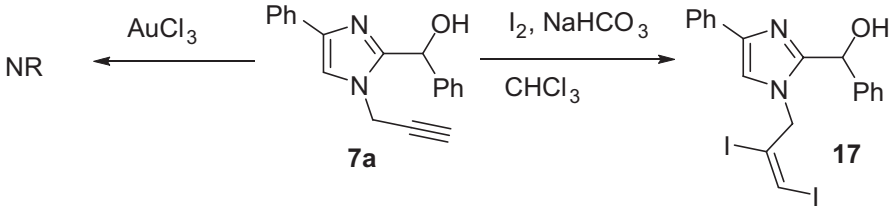
Propargylation of NH group and reduction in carbonyl group at C-2 position of the imidazole ring gave starting materials **7a-j**, and these molecules were used to furnish the imidazo-1,4-oxazine derivatives (Scheme 1).

Another route that we have followed to take starting materials was acylation of the unsubstituted imidazole ring. This reaction gave us the imidazole ring derivatized with benzoyl at C-5 position (Scheme 2). Propargylation and reduction reactions were then applied to **10** resulting in N-propargylated imidazole derivative **12**.

Starting materials were attempted to be cyclized with some of common catalysts and reagents such as $AuCl_3$ and I_2 which are well known for hydroalkoxylation in the literature



SCHEME 2 Another route for hydroalkoxylation of imidazole. (i) pyridine, NaOH, reflux, (ii) and (iii) $NaBH_4$, MeOH, rt



SCHEME 3 Attempts for cyclization of hydroalkoxylation

(Scheme 3).[6a–e] Unfortunately, gold (III) chloride did not give any result. On the other hand, iodine gave a reaction with alkyne unit to give compound **17.**

The addition of various O-H nucleophiles to C-C multiple bonds represents one of the great methods for preparing such oxygen-containing heterocycles. However, due to so many factors including the relatively high bond enthalpies of most O-H σ-bonds and the modest reactivity of electron-rich olefins with nucleophiles, an efficient intramolecular hydrofunctionalization reaction remains a challenge. Unfortunately, poor nucleophilicity of OH group brings some challenges to cyclize with multiple bonds. So, propargyl group should be converted to a more reactive group so that cyclization of propargyl group with OH takes place. Our previous studies have uncovered that propargyl group can be isomerized to allene, more reactive than propargyl against nucleophiles.[3c] This strategy has been also used by Muthusubramanian[5] and Wang[4] in which *t*-BuOLi and NaH have been applied. These bases are hard, toxic, flammable, water-reactive and corrosive. With these disadvantages, we have chosen cesium carbonate as a base to convert the propargyl group into the allene. This reactive intermediate (**16a**) was also isolated in good yield (Scheme 4). Two cyclization routes were carried out under basic condition. One of them was cyclization of C-2-substituted N-propargylated imidazoles **7a-j**(**I**), and the other was C-5-substituted N-propargylated imidazole **12**(**II**). In both cases, we have achieved to form imidazo-1,4-oxazines in good yields (Scheme 4). In the reaction pathway of I, we could not isolate **15c** which bears 3,4-dimethoxyphenyl rings. This skeleton might be exposed to oxidation under open air. Crude NMR data for **15c** presented an unidentified mixture.

Allene is a very reactive molecule, and its reactivity can be seen when analyzed its ^{13}C-NMR spectrum. We have therefore isolated allene isomer **16a**, and its NMR data showed that middle carbon (C-2), terminal carbon (C-3), and internal carbon (C-1) resonated at 202.8, 87.4, and 98.1 ppm, respectively (Figure 2). Allene protons between C-1 and C-3 protons have long-range couplings with 5.6 Hz. C-1 proton and C-3 protons resonated at 8.22 and 5.62 ppm, respectively.[3c] There are two possible reactions between allene unit and OH group which are exo-dig and endo-dig cyclization. However, the observed chemical shift of middle carbon gives information to predict the selectivity of the cyclization reaction. NBO charges are −0.087, 0.045, and −0.416 for C-1, C-2, and C-3, respectively.[7] These values show that C-2 of allene unit is the most reactive side (Figure 2).

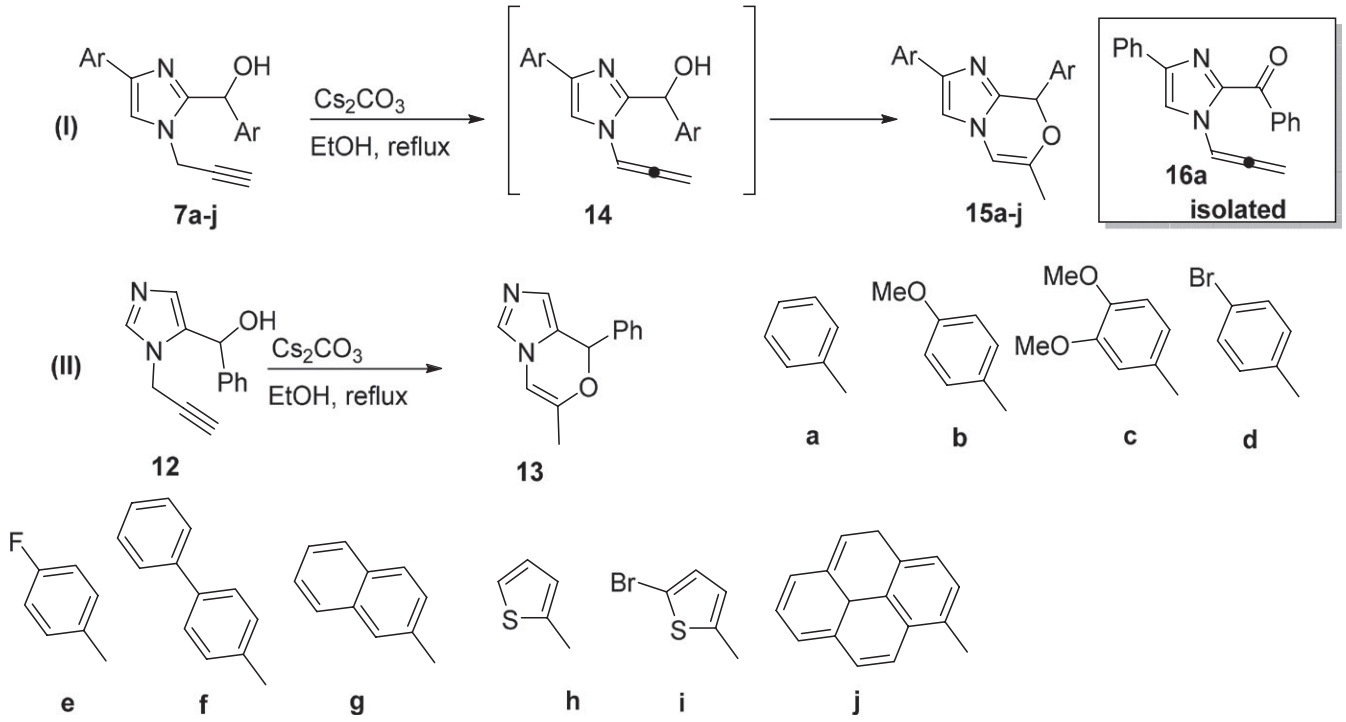
With this respect, we have proposed the reaction mechanism as seen in Scheme 5.

Allene-propargyl isomerization occurred by a base (I). Then, OH group attacks the most reactive carbon of the allene unit to produce cyclic intermediate (II). Proton abstraction of methylenic carbon gives imidazooxazine derivatives **15**.

2.2 | MTT assay: toxicity test and SAR studies

To evaluate the cytotoxicities with structure-activity relationship (SAR), synthesized molecules were added into LN405 cell lines. As shown in Figure 3, we have chosen phenyl, substituted phenyl, its fused analogs, and thiophene derivatives.

We have first analyzed phenyl-substituted imidazo-1,4-oxazine **13**. It did not show any toxicity against LN405 cell lines. Then, we have observed that **15a** was no effect on the cells, as well. After negative results, we have wanted to check substituent effects and OMe, F, Br, and Ph groups were selected. 4-Methoxy-substituted imidazo-1,4-oxazine derivative **15b** did present very little toxic effect. 4-Bromine-substituted derivative **15d** decreased cell



SCHEME 4 Cyclization reaction with cesium carbonate

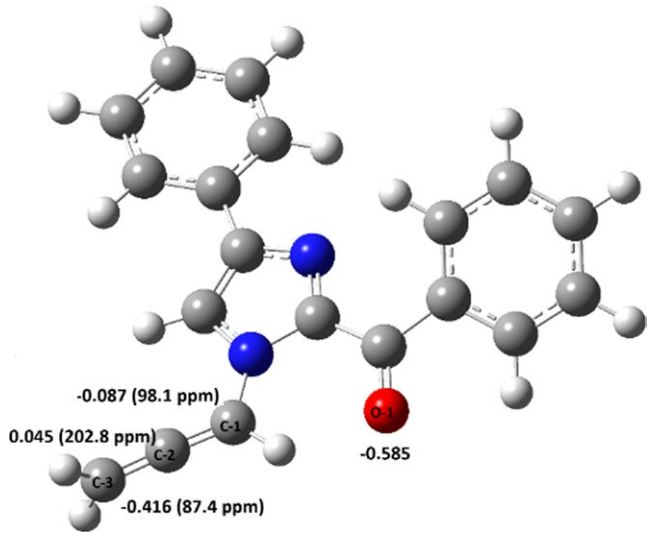
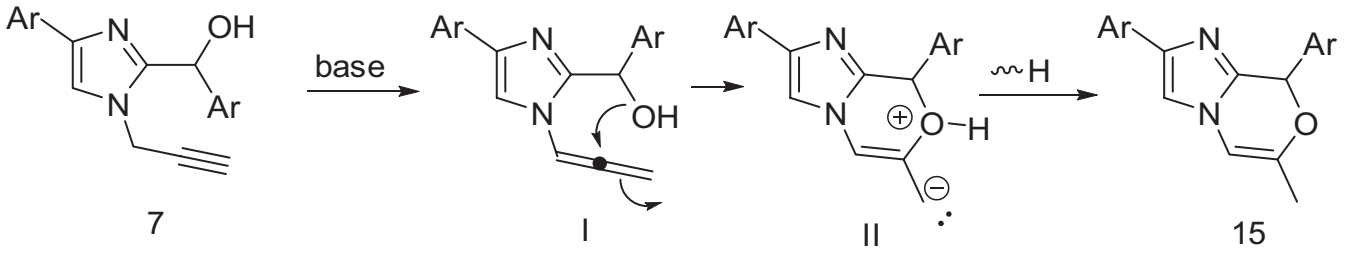


FIGURE 2 NBO charges of allene carbon atoms of **16a** (values indicate NBO charges, and values in brackets indicate observed ^{13}C-NMR chemical shifts of those carbons)

viability up to 91%. 4-Fluorine-substituted compound **15e** decreased cell viability up to 77% (Figure 3). Fluorine is a unique atom for drug design. Different types of interactions between fluorine atoms and functional groups of proteins which have not observed with the other halogens have been reported. In addition, in biological environments, compounds with a fluorine substituent behave more differently than other halogen-substituted compounds.[8] An unusual effect of the 4-fluorine-substituted compound **15e** can be explained with F-bonding/interactions.[8–9] Such F-H hydrogen bonds, π-interactions with aromatic or guanidine groups, multipolar interactions, and/or F-nitrogen interactions (halogen bonding) are reported.[8]

Biphenyl-substituted imidazo-1,4-oxazine **15f** presented the most toxicity of the selected compounds. This result might indicate that π-π interactions have occurred in the hydrophobic packet resulting in the most toxicity. To understand the limitations of a bulky phenyl group, we have analyzed the toxicities of the naphthalene and the pyrene derivatives **15g** and **15j**, respectively (Figure 3). Significant loss of toxicity potency also occurred with the replacement of the biphenyl moiety by one or more fused phenyl groups. This result might point that the hydrophobic selectivity pocket might not have enough space to bind a larger group or that the naphthalene or pyrene group could not possess a conformation/orientation that could give maximal hydrophobic interactions. This observation uncovered that bulky group and its orientation might be important for exogen-protein interactions in the studied skeleton.

Replacement of phenyl ring by thiophene, which is a heterocyclic aromatic ring having a donor atom, showed little increase in the toxicity against the cells. While thiophene-substituted imidazo-1,4-oxazine derivative **15h** showed cell viability down to 92%, cell viability of its brominated derivative **15i** was calculated 96%. Probably, thiophene has hydrogen bonding interaction with protein in the packet which might give more biological effect. Toxicity of brominated thiophene **15i** is lower than unsubstituted thiophene **15h**.



SCHEME 5 Proposed reaction mechanism

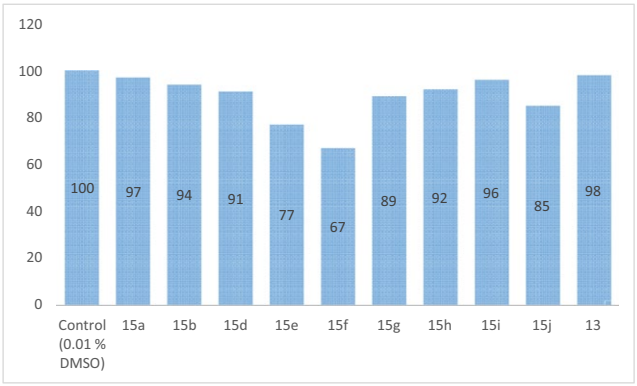


FIGURE 3 Cytotoxicity against LN405 Cell lines after 72 h. Numbers show percentage of cell viability at 20 mmol L^{-1} in DMSO

This might be due to the higher molecular refractivity of **15i** than that of **15h** that blocks the entering of the molecule into the packet of protein effectively. Finally, we have observed that all molecules which have a lower concentration of 20 mmol L^{-1} in DMSO did not present any toxicity against LN405 cell lines. This outcome points out that those molecules are safe at a mentioned concentration according to toxicity tests.

2.3 | COMET assay: genotoxicity tests

MTT assay emerged some lead compounds, and we have evaluated their genotoxicity effects against LN405 cell lines. Lead compounds were chosen as **15e** and **15f** which are the most toxic compounds against LN405 cell lines. DNA-damaging effect of **15e** and **15f** was analyzed using COMET assay. There was no statistically significant result between control and **15f** in terms of DNA damage ($P = 0528$). But, the statistical differences between control and **15e** and between **15f** and **15e** in terms of DNA damage effects in cells were determined (respectively, $P < 0001$ and $P < 0001$). As a result, the cytotoxicity effect of **15f** at 20 mmol L^{-1} may not be due to DNA damaging. Further investigations should be proceeded to reveal how the cytotoxic effect of this compound at 20 mmol L^{-1} is displayed. On the other hand, **15e** has two fluorine atoms at the para position of the phenyl ring. The fluorine atom is an electronegative atom, and due to the electronegativity of it, **15e** might bind DNA easier than the others. For this reason, it can be said that the cytotoxic effect of **15e** at 20 mmol L^{-1} might occur due to DNA damaging.

To sum up, an atom-economic and safer method has been revealed for imidazo-1,4-oxazines. In addition, reactive intermediate, allene isomer, which is responsible for cyclization, was isolated. Moreover, theoretical NBO charges of allene unit and observed NMR data were analyzed. Allene isomer is more reactive than its propargyl isomer and its middle carbon resonated at 202.8 ppm. With these outcomes, a proposed reaction mechanism was plotted. Furthermore, cytotoxicities of all synthesized molecules were studied, and toxicities of some important functional groups have been discussed. With SAR study, we uncovered that phenyl ring at exact orientation is responsible for higher toxicity against LN405 cell lines. Furthermore, fluorine atom at the para position of phenyl ring exhibited also genotoxicity which might be due to specific interactions between guanidine ring and fluorine which are unique for fluorine atom.

3 | EXPERIMENTAL

3.1 | General

All reagents used were commercially available unless otherwise stated, and all solvents were used as received. A Varian 400 spectrometer was used to acquire 400 MHz ^{1}H NMR and 100 MHz ^{13}C NMR spectra. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) in chloroform-d or d_6-DMSO. HRMS spectra were recorded using a THERMO ITQ900 LC/MS spectrometer with ESI method. All spectroscopic analysis were conducted at Van Yüzüncü Yil University, Scientific Research and Application Center. Spectra of all synthesized compounds was given in Supporting information.

3.2 | General procedure for 6a-j and 11

Imidazole skeletons were synthesized following our previous article.[10] The imidazole derivatives (**10** and **5a-j**)

(1 mmol) were dissolved in the dry DMF (15 mL), and NaH (1.6 mmol) was added to the solution at 0°C. After the solution was stirred for 30 minutes, propargyl bromide (1.4 mmol) was added to the cooled solution. The reaction was controlled by TLC and was ended. The mixture was extracted with ethyl acetate (10 × 3 mL) and water (30 mL). Then, organic layer was treated with anhydrous $MgSO_4$. Then, the ethyl acetate was evaporated, and the obtained compound was purified with column chromatography (5:1, hexane:ethyl acetate).

3.3 | General procedure for 7a-j and 12

The obtained propargylated imidazole derivatives (**11** and **6a-i)** (1 mmol) were dissolved in ethanol, and $NaBH_4$ (2 mmol) was added to the solution at room temperature. The solution was stirred for 2 hours, and the reaction completion was controlled by TLC. After completion of the reaction, water (20 mL) was added to reaction flask. Then, the obtained solid compound was dried at room temperature. The solid compound was used for next step without any purification.

Note: Compound **7j** was not isolated because of some unidentified impurities (further cyclization reaction was progressed in the reaction mixture, See section 3.4)

3.4 | General procedure for 15a-j and 13

N-propargylated C-2- and C-4-substituted imidazole derivative (**12** and **7a-i)** (1 mmol) was dissolved in ethanol (5 mL), and Cs_2CO_3 (1 mmol) was added to this solution. Then, the mixture was refluxed for 2 hours. Then, completion of the reaction was controlled by TLC. After that, the reaction was extracted with ethyl acetate (10 × 3 mL) and water (20 mL). The organic layer was dried with $MgSO_4$, and organic phase was evaporated. Viscous liquid was washed with diethyl ether to get final product.

For **15j**: Compound **6j** (1 mmol) was reacted with $NaBH_4$ (2 mmol) in ethanol (10 mL) and after completion of the reaction, judged by TLC, Cs_2CO_3 (1 mmol) was added to the reaction mixture. Then, the reaction medium was heated at reflux temperature of ethanol. Crude product was concentrated under vacuum and was extracted with ethyl acetate (10 × 3 mL) and water (20 mL). The organic layer was dried with $MgSO_4$, and organic phase was evaporated. Viscous liquid was washed with diethyl ether to get final product.

3.5 | Phenyl[4-phenyl-1-(prop-2-yn-1-yl)-1H-imidazol-2-yl]methanone (6a)

Yellow solid, m.p.: 95-97°C; yield: 71%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.51-8.49 (m, 2H, Ar-H), 7.87-7.85 (m, 2H, Ar-H), 7.74 (s, 1H, Ar-H), 7.64-7.59 (m, 1H, Ar-H), 7.55-7.51 (m, 2H, Ar-H), 7.45-7.40 (m, 2H, Ar-H), 7.35-7.30 (m, 1H, Ar-H), 5.33 (d, J = 2.65 Hz, 2H, CH_2), 2.57 (t, J = 2.65 Hz, 1H, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 183.6, 141.9, 141.8, 136.9, 133.1, 133.0, 131.3, 128.7, 128.1, 127.7, 125.3, 120.9, 77.1, 75.1, 38.7.

3.6 | (4-Methoxyphenyl)[4-(4-methoxyphenyl)-1-(prop-2-yn-1-yl)-1H-imidazol-2-yl]methanone (6b)

Light yellow solid, m.p.: 83-85°C; yield: 76%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.56-8.52 (m, AA′BB′ system, 2H, H-15, H-19), 7.80-7.76 (m, AA′BB′ system, 2H, H-10, H-14), 7.64 (s, 1H, H-2), 7.01-6.93 (m, AA′BB′ system, 4H, Ar-H), 5.33 (d, $J_{22,24}$ = 2.58 Hz, 2H, H-22), 3.89 (s, 3H, OMe), 3.83 (s, 3H, OMe), 2.54 (t, $J_{22,20}$ = 2.58 Hz, 1H, H-22). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 182.0, 163.6, 159.2, 142.0, 141.5, 133.7, 129.8, 126.5, 126.0, 119.5, 114.0, 113.4, 77.3, 74.8, 55.5, 55.3, 38.5.

3.7 | (3,4-Dimethoxyphenyl)[4-(3,4-dimethoxyphenyl)-1-(prop-2-yn-1-yl)-1H-imidazol-2-yl]methanone (6c)

Yellow solid, m.p.: 91-93°C; yield: 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.29 (dd, $J_{15,16}$ = 2.01 Hz, $J_{15,19}$ = 8.43 Hz, 1H, H-15), 8.14 (d, $J_{19,15}$ = 2.01 Hz, 1H, H-19), 7.65 (s, 1H, H-2), 7.40 (d, $J_{14,10}$ = 1.97 Hz, 1H, H-14), 7.36 (dd, $J_{10,14}$ = 1.97 Hz, $J_{11,10}$ = 8.25 Hz, 1H, H-10), 6.96 (d, J = 8.43 Hz, 1H, h-16), 5.32 (d, $J_{22,24}$ = 2.58 Hz, 2H, H-20), 3.97 (s, 3H, OMe), 3.96 (s, 3H, OMe), 3.94 (s, 3H, OMe), 3.90 (m, 3H, OMe), 2.54 (t, $J_{24,22}$ = 2.58 Hz, 1H, H-24). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 181.7, 153.4, 149.1, 148.7, 148.3, 142.0, 141.5, 129.6, 126.6, 117.8, 113.4, 111.3, 110.0, 108.4, 77.2, 74.8, 56.1, 56.0, 55.9, 55.8, 38.6. HRMS (ESI) (M + H): $C_{23}H_{23}N_2O_5$: 407.1607, found: 407.1600.

3.8 | (4-Bromophenyl)[4-(4-bromophenyl)-1-(prop-2-yn-1-yl)-1H-imidazol-2-yl]methanone (6d)

Yellow solid, m.p.: 140-141°C; yield: 85%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.36-8.33 (m, AA′BB′ system, 2H, Ar-H), 7.77 (s, 1H, H-2), 7.72-7.69 (m, AA′BB′ system, 2H, Ar-H), 7.67-7.64 (m, 2H, Ar-H), 7.54-7.51 (m, AA′BB′ system, 2H, Ar-H), 5.35 (d, $J_{22,24}$ = 2.59 Hz, 2H, H-22), 2.58 (t, $J_{24,22}$ = 2.59 Hz, 1H, H-24). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 182.3, 141.7, 140.9, 135.5, 132.8, 131.8, 131.4, 128.5, 126.7, 121.6, 121.1, 76.7, 75.4, 38.9.

3.9 | (4-Fluorophenyl)[4-(4-fluorophenyl)-1-(prop-2-yn-1-yl)-1H-imidazol-2-yl]methanone (6e)

Yellow solid, m.p.: 110-111°C; yield: 93%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.57-8.52 (m, AA′BB′ system, 2H, Ar-H), 7.83-7.71 (m, AA′BB′ system, 2H, Ar-H), 7.71 (s, 1H, H-2), 7.21-7.15 (m, 2H, Ar-H), 7.13-7.07 (m, AA′BB′ system 2H, Ar-H), 5.34 (d, $J_{22,24}$ = 2.61 Hz, 2H, H-22), 2.57 (t, $J_{24,22}$ = 2.61 Hz, 1H, H-24). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 181.8, 167.1, 164.6, 163.7, 161.2, 141.7, 141.0, 134.1, 134.0, 133.1, 133.0, 129.2, 129.1, 126.9, 126.8, 120.5, 115.7, 115.5, 115.4, 115.1, 76.9, 75.2, 38.8.

3.10 | (1,1′-Biphenyl]-4-yl)[4-(1,1′-biphenyl-4-yl)-1-(prop-2-yn-1-yl)-1H-imidazol-2-yl]methanone (6f)

Light brown solid, m.p.: 160-162°C; yield: 71%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.62-8.59 (m, AA′BB′ 2H, Ar-H), 7.98-7.94 (m, AA′BB′ system, 2H, Ar-H), 7.83 (s, 1H, H-2), 7.78-7.75 (m, AA′BB′ system, 2H, Ar-H), 7.71-7.64 (m, 6H, Ar-H), 7.52-7.36 (m, 6H, Ar-H), 5.40 (d, $J_{32,34}$ = 2.60 Hz, 2H, H-32), 2.59 (t, $J_{34,32}$ = 2.60 Hz, 1H, H-34). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 183.1, 145.6, 142.1, 141.5, 140.7, 140.3, 140.1, 135.6, 132.1, 131.9, 128.9, 128.8, 128.2, 127.4, 127.3, 127.2, 127.0, 126.8, 125.7, 120.9, 77.2, 75.2, 38.8.

3.11 | Naphthalen-2-yl[4-(naphthalen-2-yl)-1-(prop-2-yn-1-yl)-1H-imidazol-2-yl]methanone (6g)

Viscous liquid, brown color. Yield: 79%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.25 (s, 1H, Ar-H), 8.44 (dd, *J* = 1.71, 8.64 Hz, 1H, Ar-H), 8.37 (s, 1H, Ar-H), 8.06 (d, *J* = 8.05 Hz, 1H, Ar-H), 8.01-7.96 (m, 2H, Ar-H), 7.93-7.89 (m, 4H, Ar-H), 7.86-7.84 (m, 1H, Ar-H), 7.65-7.56 (m, 2H, Ar-H), 7.52-7.45 (m, 2H, Ar-H), 5.41 (d, $J_{10,12}$ = 2.58 Hz, 2H, H-10), 2.60 (t, $J_{12,10}$ = 2.58 Hz, 1H, H-12). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 183.5, 142.3, 141.8, 135.6, 134.2, 134.1, 133.6, 133.0, 132.4, 130.5, 130.1, 128.6, 128.4, 128.2, 127.9, 127.8, 127.7, 126.5, 126.3, 126.2, 123.7, 123.6, 121.2,76.7, 75.2, 38.8.

3.12 | [1-(Prop-2-yn-1-yl)-4-(thiophen-2-yl)-1H-imidazol-2-yl](thiophen-2-yl)methanone (6h)

Brown solid, m.p.: 100-102°C; yield: 80%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.58 (dd, $J_{17,15}$ = 1.23 Hz, $J_{17,16}$ = 3.88 Hz, 1H, H-17), 7.71 (dd, $J_{15,17}$ = 1.23 Hz, $J_{15,16}$ = 4.93 Hz, 1H, H-15), 7.57 (s, 1H, H-7), 7.34 (dd, $J_{5,2}$ = 1.17 Hz, $J_{5,1}$ = 3.57 Hz, 1H, H-5), 7.25 (dd, $J_{2,5}$ = 1.17 Hz, $J_{2,1}$ = 5.06 Hz, 1H, H-2), 7.16 (dd, $J_{16,17}$ = 3.88 Hz, $J_{16,15}$ = 4.93 Hz, 1H, H-16), 7.04 (dd, $J_{1,5}$ = 3.57 Hz, $J_{1,2}$ = 5.06 Hz, 1H, H-1), 5.30 (d, $J_{18,20}$ = 2.61 Hz, 2H, H-18), 2.55 (t, $J_{20,18}$ = 2.61 Hz, 1H, H-20). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 174.5, 141.5, 140.7, 137.1, 136.7, 136.6, 135.9, 128.0, 127.7, 124.5, 123.1, 120.3, 76.8, 75.3, 38.6.

3.13 | (5-Bromothiophen-2-yl)[4-(5-bromothiophen-2-yl)-1-(prop-2-yn-1-yl)-1H-imidazol-2-yl]methanone (6i)

Orange solid, m.p.: 157-159°C; yield: 74%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.18 (d, $J_{14,15}$ = 4.15, 1H, H-14), 7.59 (s, 1H, H-7), 7.15 (d, $J_{15,14}$ = 4.15 Hz, 1H, H-15), 7.09 (d, $J_{5,1}$ = 3.82 Hz, 1H, H-5), 7.01 (d, $J_{1,5}$ = 3.82 Hz, 1H, H-1), 5.34 (d, $J_{20,22}$ = 2.58 Hz, 2H, H-20), 2.57 (t, $J_{22,20}$ = 2.58 Hz, 1H, H-22). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 172.9, 141.6, 140.3, 138.1, 136.4, 136.3, 130.8, 130.5, 125.7, 123.2, 120.2, 111.6, 76.3, 75.6, 38.6. HRMS (ESI) (*M* + H): $C_{15}H_9Br_2N_2OS_2$: 454.8523, found: 454.8518.

3.14 | [1-(Prop-2-yn-1-yl)-4-(pyren-2-yl)-1H-imidazol-2-yl](pyren-2-yl)methanone (6j)

Light brown solid, m.p.: 194-196°C; yield: 64%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.88 (d, *J* = 9.35 Hz, 1H, Ar-H), 8.61-8.54 (m, 2H, Ar-H), 8.44-8.23 (m, 10H, Ar-H), 8.15-8.12 (m, 2H, Ar-H), 8.10 (bs, 2H, Ar-H), 8.01-7.94 (m, 2H, Ar-H), 5.68 (d, *J* = 2.49 Hz, 2H, CH_2), 3.71 (*t*, *J* = 2.49 Hz, 1H, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 186.8, 143.4, 141.7, 133.3, 132.9, 131.3, 131.1, 130.7, 130.6, 130.5, 129.7, 129.2, 129.1, 128.0, 127.8, 127.7, 127.3, 127.2, 126.8, 126.7, 126.5, 125.4, 125.3, 124.9, 124.7, 124.3, 124.2, 124.0, 79.2, 77.2, 38.8. HRMS (ESI) (*M* + H): $C_{36}H_{23}N_2O$: 499.1810, found: 499.1827.

3.15 | Phenyl[4-phenyl-1-(prop-2-yn-1-yl)-1H-imidazol-2-yl]methanol (7a)

Light brown solid, m.p.: 130-132°C; yield: 95%. ^{1}H NMR (400 MHz, d_6-DMSO) δ = 7.74-7.65 (m, 3H, Ar-H), 7.37-7.18 (m, 8H, Ar-H), 6.44 (bs, 1H, OH), 5.96 (bs, 1H, CH-O), 4.90 (d, *J* = 17.76 Hz, AB system, 1H, CH_2), 4.49 (d, *J* = 17.76 Hz, AB system, 1H, CH_2), 2.48 (bs, 1H, CH). ^{13}C NMR (100 MHz, d_6-DMSO) δ = 149.1, 141.9, 139.1, 134.5, 128.9, 128.5, 127.6, 126.8, 126.5, 124.7, 117.3, 78.8, 76.5, 68.7, 35.7.

3.16 | (4-Methoxyphenyl)[4-(4-methoxyphenyl)-1-(prop-2-yn-1-yl)-1H-imidazol-2-yl]methanol (7b)

White solid, m.p.: 150-152°C; yield: 94%. ^{1}H NMR (400 MHz, d_6-DMSO) δ = 7.65-7.63 (m, AA′BB′ system, 2H, Ar-H), 7.50 (s, 1H, H-2), 7.28-7.26 (m, AA′BB′ system,

2H, Ar-H), 6.90-6.87 (m, 4H, Ar-H), 6.30 (bs, 1H, H-6), 5.87 (s, 1H, H-9), 4.86 (dd, $J_{22,24}$ = 2.53 Hz, $J_{22,22}$ = 17.79 Hz, 1H, AB system, H-22), 4.75 (dd, J = 2.54 Hz, 17.79 Hz, 1H, AB system, H-22), 3.73 (s, 3H, OMe), 3.71 (s, 3H, OMe), 3.39 (t, $J_{24,22}$ = 2.53 Hz, 1H, H-24). ^{13}C NMR (100 MHz, d_6-DMSO) δ = 158.8, 158.3, 149.0, 139.1, 134.1, 127.7, 127.4, 125.9, 115.9, 114.3, 113.9, 79.0, 76.4, 68.4, 55.5, 55.4 35.5.

3.17 | (3,4-Dimethoxyphenyl)[4-(3,4-dimethoxyphenyl)-1-(prop-2-yn-1-yl)-1H-imidazol-2-yl]methanol (7c)

White solid, m.p.: 178-180°C; yield: 88%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.33 (d, $J_{14,10}$ = 1.83 Hz, 1H, H-19), 7.24 (dd, $J_{14,10}$ = 1.83 Hz, $J_{11,10}$ = 8.27 Hz, 1H, AB system, H-16), 7.18 (s, 1H, H-2), 6.89-6.77 (m, 4H, Ar-H), 5.99 (s, 1H, H-6), 4.38 (dd, $J_{22,24}$ = 2.54 Hz, $J_{22,22}$ = 17.78 Hz, 1H, AB system, H-22), 4.31 (dd, J = 2.54 Hz, 17.78 Hz, 1H, AB system, H-22), 3.95 (s, 3H, OMe), 3.89 (s, 3H, OMe), 3.84 (s, 3H, OMe), 3.78 (s, 3H, OMe), 2.30 (t, $J_{24,22}$ = 2.54 Hz, 1H, H-24). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 149.1, 149.0, 148.6, 148.5, 148.1, 139.3, 132.5, 126.6, 118.5, 117.1, 115.4, 111.2, 110.9, 109.3, 108.3, 76.5, 74.3, 68.8, 55.9 (2C), 55.8 (2C), 35.8. HRMS (ESI) (M + H): $C_{23}H_{25}N_2O_5$: 409.1763, found: 409.1751.

3.18 | (4-Bromophenyl)[4-(4-bromophenyl)-1-(prop-2-yn-1-yl)-1H-imidazol-2-yl]methanol (7d)

Light yellow solid, m.p.: 187-189°C; yield: 82%. ^{1}H NMR (400 MHz, d_6-DMSO) δ = 7.50-7.44 (m, AA′BB′ system, 4H, Ar-H), 7.43-7.40 (m, AA′BB′ system, 2H, Ar-H), 7.22-7.18 (m, 2H, Ar-H), 7.17 (s, 1H, H-2), 5.99 (bs, 1H, H-7), 4.44 (dd, $J_{22,24}$ = 2.59 Hz, $J_{22,22}$ = 17.77 Hz, 1H, AB system, H-22), 4.26 (dd, $J_{22,24}$ = 2.59 Hz, $J_{22,22}$ = 17.77 Hz, 1H, AB system, H-22), 2.26 (t, $J_{24,22}$ = 2.59, 1H, H-24). ^{13}C NMR (100 MHz, d_6-DMSO) δ = 148.9, 141.3, 138.0, 131.8, 131.4, 128.9, 126.7, 120.8, 119.6, 118.1, 78.7, 76.7, 67.9, 35.7.

3.19 | (4-Fluorophenyl)[4-(4-fluorophenyl)-1-(prop-2-yn-1-yl)-1H-imidazol-2-yl]methanol (7e)

Light orange solid, m.p.: 150-152°C, yield: 96%. ^{1}H NMR (400 MHz, d_6-DMSO) δ = 7.76-7.73 (m, AA′BB′ system, 2H, Ar-H), 7.65 (s, 1H, H-2), 7.42-7.39 (m, AA′BB′ system, 2H, Ar-H), 7.18-7.12 (m, 4H, AA′BB′ system, Ar-H), 6.48 (d, $J_{6,9}$ = 4.92 Hz, 1H, H-6), 5.95 (d, $J_{9,6}$ = 4.92 Hz, 1H, H-9), 4.91 (dd, $J_{22,24}$ = 2.55 Hz, $J_{22,22}$ = 17.83, 1H, AB system, H-22), 4.82 (dd, $J_{22,24}$ = 2.55 Hz, $J_{22,22}$ = 17.83, 1H, AB system, H-22), 3.40 (t, $J_{24,22}$ = 2.55 Hz, 1H, H-24). ^{13}C NMR (100 MHz, d_6-DMSO) δ = 162.8 (d, J = 38.3 Hz), 160.4 (d, J = 38.2 Hz), 149.0, 138.4, 138.2 (d, J = 2.97 Hz), 131.1 (d, J = 2.93 Hz), 128.6 (d, J = 8.20 Hz), 126.5 (d, J = 7.91 Hz), 117.2, 115.7 (J = 21.39 Hz), 115.2 (J = 21.35 Hz), 78.8, 76.5, 68.0, 35.6.

3.20 | (1,1′-Biphenyl-4-yl)[4-(1,1′-biphenyl-4-yl)-1-(prop-2-yn-1-yl)-1H-imidazol-2-yl]methanol (7f)

Light yellow solid, m.p.: 149-152°C; yield: 91.3%. ^{1}H NMR (400 MHz, d_6-DMSO) δ = 7.83-7.80 (m, 2H, Ar-H), 7.66-7.64 (m, AA′BB′ system, 4H, Ar-H), 7.59-7.56 (m, AA′BB′ system, 4H, Ar-H), 7.48-7.41 (m, 6H, Ar-H), 7.35-7.30 (m, 2H, Ar-H), 7.30 (s, 1H, H-10), 6.21 (bs, 1H, H-7), 4.47 (dd, $J_{10,12}$ = 2.57 Hz, $J_{10,10}$ = 17.78 Hz, 1H, H-10), 4.38 (dd, $J_{10,12}$ = 2.57 Hz, $J_{10,10}$ = 17.78 Hz, 1H, H-10), 2.24 (t, $J_{12,10}$ = 2.57 Hz, H-34).^{13}C NMR (100 MHz, d_6-DMSO) δ =148.8, 140.8, 140.7, 140.6, 139.6, 139.1, 138.9, 132.4, 128.8, 128.7, 127.4, 127.3, 127.3, 127.2, 127.1, 126.9, 126.6, 125.2, 116.4, 76.4, 74.4, 68.9, 35.9.

3.21 | Naphthalen-2-yl[4-(naphthalen-2-yl)-1-(prop-2-yn-1-yl)-1H-imidazol-2-yl]methanol (7g)

Light brown solid, m.p.: 199-202°C; yield: 94.1%. ^{1}H NMR (400 MHz, d_6-DMSO) δ = 8.28 (bs, 1H, Ar-H), 7.99 (bs, 1H, Ar-H), 7.94-7.83 (m, 8H, Ar-H), 7.53-7.39 (m, 5H, Ar-H), 6.66 (d, $J_{9,7}$ = 4.20 Hz, 1H, H-9), 6.20 (d, $J_{7,9}$ = 4.20 Hz, 1H, H-7), 5.01 (dd, $J_{10,12}$ = 2.54 Hz, $J_{10,10}$ = 17.80 Hz, 1H, AB system, H-10), 4.88 (dd, $J_{10,12}$ = 2.54 Hz, $J_{10,10}$ = 17.80 Hz, 1H, AB system, H-10), 3.41 (t, $J_{12,10}$ = 2.54 Hz, 1H, H-12). ^{13}C NMR (100 MHz, d_6-DMSO) δ = 149.3, 139.5, 139.2, 133.8, 133.2, 132.8, 132.4, 128.4, 128.2, 128.1, 128.0, 127.9, 126.7, 126.6, 126.3, 125.2, 124.6, 123.9, 122.3, 118.2, 78.9, 76.7, 68.9, 35.9.

3.22 | [1-(Prop-2-yn-1-yl)-4-(thiophen-2-yl)-1H-imidazol-2-yl](thiophen-2-yl)methanol (7h)

Brown solid, m.p.: 145-146°C; yield: 80%. ^{1}H NMR (400 MHz, d_6-DMSO) δ = 7.57 (s, 1H, H-7), 7.47 (dd, $J_{15,17}$ = 1.26 Hz, $J_{15,16}$ = 5.05 Hz, 1H, H-15), 7.34 (dd, $J_{2,5}$ = 1.16, $J_{2,1}$ = 5.06, 1H, H-2), 7.28 (dd, $J_{5,2}$ = 1.16 Hz, $J_{5,1}$ = 3.52 Hz, 1H, H-5), 7.02 (dd, $J_{1,5}$ = 3.52 Hz, $J_{1,2}$ = 5.06 Hz, 1H, H-1), 6.96 (dd, $J_{16,17}$ = 3.51 Hz, $J_{16,15}$ = 5.06 Hz, 1H, H-16), 6.86 (dt, $J_{17,15}$ = 1.26 Hz, $J_{17,16}$ = 3.52 Hz, 1H, H-17), 6.74 (d, $J_{11,13}$ = 4.4 Hz, 1H, H-3), 6.13 (d, $J_{11,13}$ = 4.4 Hz, 1H, H-11), 4.94 (dd, $J_{18,20}$ = 2.56, 17.78 Hz, 1H, AB system, H-18), 4.85 (dd, $J_{18,20}$ = 2.56, 17.78 Hz, 1H, AB system, H-18), 3.45 (t, $J_{20,18}$ = 2.56 Hz, 1H, H-20). ^{13}C NMR (100 MHz, d_6-DMSO) δ = 148.1, 145.9, 138.4, 134.8, 128.1, 127.1, 126.1, 124.8, 123.9, 122.0, 116.6, 78.7, 76.7, 65.7, 35.9.

3.23 | (5-Bromothiophen-2-yl)[4-(5-bromothiophen-2-yl)-1-(prop-2-yn-1-yl)-1H-imidazol-2-yl]methanol (7i)

Viscous liquid dark brown, yield: 92%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.15 (s, 1H, H-7), 6.95 (d, J = 3.83, 1H, Ar-H), 6.94 (d, J = 3.8 0 Hz, 1H, Ar-H), 6.85 (d, $J_{15,14}$ = 3.80 Hz, 1H, H-15), 6.46 (dd, $J_{14,16}$ = 1.32 Hz, $J_{14,15}$ = 3.79 Hz, 1H, H-14), 6.03 (d, $J_{11,12}$ = 1.32 Hz, 1H, H-11), 4.68 (dd, $J_{20,22}$ = 2.61 Hz, $J_{20,20}$ = 17.81 Hz, 1H, H-20), 4.5 (dd, $J_{20,22}$ = 2.61 Hz, $J_{20,20}$ = 17.81 Hz, 1H, H-20), 2.38 (t, $J_{22,20}$ = 2.61 Hz, 1H, H-22). ^{13}C NMR (100 MHz, $CDCl_3$) δ=147.2, 145.2, 137.5, 133.7, 130.4, 129.7, 124.7, 122.9, 116.3, 112.7, 110.4, 75.9, 75.0, 65.8, 36.4. HRMS (ESI) (M + H): $C_{15}H_{11}Br_2N_2OS_2$: 456.8680, found: 456.8666.

3.24 | Phenyl[1-(prop-2-yn-1-yl)-1H-imidazol-5-yl]methanone (11)

Brown viscous liquid, yield: 89%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.28-8.26 (m, 2H, Ar-H), 7.60-7.56 (m, 1H, Ar-H), 7.50-7.47 (m, 3H, Ar-H), 7.28 (d, J = 1.04 Hz, 1H, Ar-H), 5.33 (d, $J_{7,9}$ = 2.61 Hz, 2H, H-7), 2.53 (t, $J_{9,7}$ = 2.61 Hz, 1H, H-9). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 184.2, 142.2, 137.0, 132.9, 130.9, 130.8, 129.6, 128.1, 124.9, 76.9, 74.9, 38.5. HRMS (ESI) (M + H): $C_{13}H_{11}N_2O$: 211.0871, found: 211.0869.

3.25 | Phenyl[1-(prop-2-yn-1-yl)-1H-imidazol-5-yl]methanol (12)

Light brown viscous liquid, yield: 93%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.31 (d, J = 4.35 Hz, 4H, Ar-H), 7.26 (t, J = 4.35 Hz, 1H, Ar-H), 7.01 (d, $J_{1,4}$ = 1.34 Hz, 1H, H-1), 6.84 (d, $J_{4,1}$ = 1.34 Hz, 1H, H-4), 5.96 (bs, 1H, H-6), 4.69 (dd, $J_{7,9}$ = 2.60 Hz, $J_{7,7}$ = 17.75 Hz, 1H, H-7), 4.37 (dd, $J_{7,9}$ = 2.60 Hz, $J_{7,7}$ = 17.75 Hz, 1H, H-7), 2.29 (t, $J_{9,7}$ = 2.60 Hz, 1H, H-9). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 148.6, 140.4, 128.4, 127.5, 126.5, 126.0, 120.4, 74.1, 68.8, 35.7. HRMS (ESI) (M + H): $C_{13}H_{13}N_2O$: 213.1028, found: 213.1019.

3.26 | 6-Methyl-2,8-diphenyl-8H-imidazo[2,1-c][1,4]oxazine (15a)

Light red solid, m.p.: 158-160°C, yield: 92%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.32 (bs, 1H, Ar-H), 7.87-7.85 (m, 2H, Ar-H), 7.82-7.80 (m, 2H, Ar-H), 7.73 (bs, 1H, Ar-H), 7.63 (dd, J = 1.71, 8.56 Hz, 1H, Ar-H), 7.51-7.47 (m, 2H, Ar-H), 7.46-7.43 (m, 1H, Ar-H), 7.32 (bs, 1H, Ar-H), 6.60 (bs, 1H, CH-O), 6.29 (q, J = 1.13 Hz, 1H, C=CH), 1.92 (d, J = 1.13 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 142.5, 141.7, 139.1, 134.9, 133.8, 131.1, 128.9, 127.7, 126.7, 125.6, 123.3, 111.1, 102.0, 75.7, 17.5.

3.27 | 2,8-Bis(4-methoxyphenyl)-6-methyl-8H-imidazo[2,1-c][1,4]oxazine (15b)

Brown solid, m.p.: 126-128°C; yield: 80%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.71-7.67 (m, 2H, AA′BB′, Ar-H), 7.30-7.26 (m, 2H, AA′BB′ system, Ar-H), 7.08 (s, 1H, H-2), 6.92-6.86 (m, 4H, Ar-H), 6.31 (bs, 1H, H-6), 6.25 (d, $J_{22,24}$ = 1.20, 1H, H-22), 3.82 (s, 3H, OMe), 3.79 (s, 3H, OMe), 1.87 (d, $J_{24,22}$ = 1.20, 3H, H-24). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 159.9, 158.6, 142.2, 141.5, 138.9, 129.7, 128.8, 126.7, 126.1, 113.9, 113.8, 109.5, 101.8, 75.3, 55.3, 55.2, 17.4.

3.28 | 2,8-Bis(4-bromophenyl)-6-methyl-8H-imidazo[2,1-c][1,4]oxazine (15d)

Light red solid, m.p.: 155-157°C; yield: 65%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.62-7.58 (m, 2H, AA′BB′ system, Ar-H), 7.52-7.45 (m, 4H, AA′BB′ system, Ar-H), 7.27-7.24 (m, 2H, AA′BB′ system, Ar-H), 7.16 (s, 1H, H-2), 6.29 (bs, 1H, H-7), 6.25 (q, $J_{32,34}$ = 1.18 Hz, 1H, H-22), 1.90 (d, $J_{34,32}$ = 1.18 Hz, 3H, H-34). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 142.6, 140.8, 138.6, 136.3, 132.6, 131.7, 131.6, 129.2, 128.9, 126.5, 126.4, 123.0, 120.6, 110.8, 101.9, 74.8, 17.4.

3.29 | 2,8-Bis(4-fluorophenyl)-6-methyl-8H-imidazo[2,1-c][1,4]oxazine (15e)

Red solid, m.p.: 96-98°C; yield: 83.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.71-7.68 (m, 2H, AA′BB′ system, Ar-H), 7.39-7.35 (m, 2H, AA′BB′ system, Ar-H), 7.13 (s, 1H, H-2), 7.07-7.02 (m, 4H, AA′BB′ system, Ar-H), 6.34 (bs, 1H, H-6), 6.28 (q, $J_{22,24}$ = 1.22 Hz, 1H, H-22), 1.90 (d, $J_{24,22}$ = 1.22, 3H, H-24). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 164.2, 163.2, 161.7, 160.8, 142.5, 140.9, 138.8, 133.1, 129.9, 129.2, 126.5, 115.6, 115.4, 110.3, 101.9, 74.8, 17.4.

3.30 | 2,8-Di(1,1′-biphenyl-4-yl)-6-methyl-8H-imidazo[2,1-c][1,4]oxazine (15f)

White solid, m.p.: 224-226°C; yield: 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.86-7.84 (m, 2H, AA′BB′ system, Ar-H), 7.65-7.61 (m, 6H, AA′BB′ system, Ar-H), 7.60-7.57 (m, 3H, Ar-H), 7.51-7.48 (m, 2H, Ar-H), 7.48-7.44 (m, 2H, Ar-H), 7.44-7.42 (m, 1H, Ar-H), 7.37-7.33 (m, 2H, Ar-H), 7.24 (s, 1H, H-2), 6.46 (s, 1H, H-6), 6.30 (q, $J_{32,34}$ = 1.20 Hz, 1H, H-32), 1.95 (d, $J_{34,32}$ = 1.20 Hz, 3H, H-34). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 141.6, 140.8, 140.6, 139.6, 138.9, 136.4, 128.8, 128.7, 127.9, 127.6,

127.4, 127.3, 127.2, 127.1, 126.9, 125.4, 125.3, 110.7, 101.9, 75.3, 17.5.

3.31 | 6-Methyl-2,8-di(naphthalen-2-yl)-8H-imidazo[2,1-c][1,4]oxazine (15g)

Light red solid, m.p.: 162-164°C; yield: 82%. ^{1}H NMR (400 MHz, d_6-DMSO) δ = 8.21 (bs, 1H, Ar-H), 7.97-7.91 (m, 3H, Ar-H), 7.88 (s, 1H, Ar-H), 7.86-7.82 (m, 5H, Ar-H), 7.58-7.50 (m, 3H, Ar-H), 7.45-7.38 (m, 2H, Ar-H), 6.78 (q, $J_{28,30}$ = 0.91 Hz, 1H, Ar-H), 6.62 (bs, 1H, H-7), 1.90 (d, $J_{30,28}$ = 0.91 Hz, 3H, H-30). ^{13}C NMR (100 MHz, d_6-DMSO) δ = 142.6, 140.8, 139.5, 135.6, 133.7, 133.4, 132.9, 132.4, 131.8, 128.8, 128.6, 128.5, 128.2, 128.1, 128.0, 127.1, 127.0, 126.8, 125.8, 125.6, 123.9, 122.4, 113.0, 102.7, 75.5, 17.3.

3.32 | 6-Methyl-2,8-di(thiophen-2-yl)-8H-imidazo[2,1-c][1,4]oxazine (15h)

Viscous liquid, dark brown, yield: 80%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7. 32 (dd, $J_{15,17}$ = 1.67 Hz, $J_{15,16}$ = 4.64 Hz, 1H, H-15), 7.27 (dd, $J_{5,2}$ = 1.16 Hz, $J_{5,1}$ = 3.57 Hz, 1H, H-2), 7.18 (dd, $J_{2,5}$ = 1.16 Hz, $J_{2,1}$ = 5.07 Hz, 1H, H-2), 7.09 (s, 1H, H-7), 7.02 (dd, $J_{1,5}$ = 3.57 Hz, $J_{1,2}$ = 5.07 Hz, 1H, H-1), 6.95-6.92 (m, 2H, H-16,17), 6.60 (bs, 1H, H-27), 6.27 (q, $J_{18,20}$ = 1.20 Hz, 1H, H-18), 1.89 (d, $J_{20,18}$ = 1.20 Hz, 3H, H-20). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 142.3, 140,4, 138.2, 137.2, 136.8, 127.5, 126.9, 126.8, 126.7, 123.5, 122.2, 110.1, 101.9, 71.2, 17.4.

3.33 | 2,8-Bis(5-bromothiophen-2-yl)-6-methyl-8H-imidazo[2,1-c][1,4]oxazine (15i)

Viscous liquid, dark brown, yield: 63%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.04 (s, 1H, H-7), 7.01 (d, $J_{5,1}$ = 3.84 Hz, 1H, H-5), 6.96 (d, $J_{1,5}$ = 3.84 Hz, 1H, H-1), 6.89 (d, $J_{15,14}$ = 3.81 Hz, 1H, H-15), 6.68 (dd, $J_{14,30}$ = 0.98 Hz, $J_{14,15}$ = 3.81 Hz, 1H, H-14), 6.48 (d, J = 0.98 Hz, 1H, H-11), 6.26 (q, $J_{44,46}$ = 1.22 Hz, 1H, H-44), 1.91 (d, $J_{46,44}$ = 1.22 Hz, 3H, H-46). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 142.6, 141.5, 137.6, 136.1, 130.4, 129.5, 127.6, 127.4, 122.4, 113.9, 110.3, 110.2, 101.8, 71.1, 17.4. HRMS (ESI) (M + H): $C_{15}H_{11}Br_2N_2OS_2$: 456.8680, found: 456.8671.

3.34 | 6-Methyl-2,8-di(pyren-2-yl)-8H-imidazo[2,1-c][1,4]oxazine (15j)

Brown solid, m.p.: 212-215°C; yield: 89%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.89 (d, J = 9.30 Hz, 1H, Ar-H), 8.67 (d, J = 9.30 Hz, 1H, Ar-H), 8.30-8.22 (m, 5H, Ar-H), 8.17-8.05 (m, 8H, Ar-H), 8.04 (bs, 2H, Ar-H), 7.99-7.95 (m, 1H, Ar-H), 7.65 (d, J = 7.95 Hz, 1H, H-22), 7.52-7.48 (m, 1H, H-24), 6.49 (q, J = 1.21 Hz, 1H, H-39), 1.80 (d, $J_{42,24}$ = 1.21 Hz, 3H, H-42). ^{13}C NMR (100 MHz, $CDCl_3$) δ =143.0, 141.7, 139.0, 132.1, 131.4, 131.2, 131.0, 130.9, 130.5, 130.1, 129.5, 129.0, 128.1, 128.1, 127.5, 127.4, 127.3, 127.1, 127.0, 126.1, 125.8, 125.7, 125.6, 125.5, 125.4, 125.3, 125.2, 124.9, 124.8, 124.7, 124.4, 123.6, 114.2, 101.9, 73.9, 17.4. HRMS (ESI) (M + H): $C_{39}H_{25}N_2O$: 537.1967, found: 537.1942.

3.35 | 6-Methyl-8-phenyl-8H-imidazo[5,1-c][1,4]oxazine (13)

Viscous liquid, dark brown, yield: 73%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.36-7.33 (m, 5H, Ar-H), 7.02 (d, $J_{4,1}$ = 1.31 Hz, 1H, H-4), 6.85 (d, $J_{1,4}$ = 1.31 Hz, 1H, H-1), 6.22 (q, $J_{15,16}$ = 1.23 Hz, 1H, H-15), 6.21 (bs, 1H, H-6), 1.87 (d, $J_{16,15}$ = 1.23 Hz, 3H, H-16). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 142.8, 138.9, 137.2, 128.8, 128.7, 128.6, 127.2, 115.1, 102.0, 75.9, 17.2. HRMS (ESI) (M + H): $C_{13}H_{13}N_2O$: 213.2600, found: 213.2589.

3.36 | Phenyl[4-phenyl-1-(propa-1,2-dien-1-yl)-1H-imidazol-2-yl]methanone (16a)

Viscous liquid, dark brown, yield: 88%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.46-8.44 (m, 2H, Ar-H), 8.22 (t, J = 6.6 Hz, 1H), 7.86-7.84 (m, 2H, Ar-H), 7.66 (bs, 1H), 7.61 (tt, J = 1.30, 7.39 Hz, 1H, Ar-H), 7.53 (tt, J = 1.80, 7.74 Hz, 1H, Ar-H), 7.40 (tt, J = 1.80, 7.74 Hz, 2H, Ar-H), 7.31 (tt, J = 1.30, 7.39 Hz, 1H, Ar-H), 5.61 (d, J = 6.6 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 202.8, 183.9, 142.4, 141.7, 137.0, 133.0, 132.8, 131.3, 128.7, 128.0, 127.8, 125.3, 118.1, 98.1, 87.4. HRMS (ESI) (M + H): $C_{19}H_{15}N_2O$: 287.1184, found: 287.1171.

3.37 | (*E*)/(*Z*)-[1-(2,3-diiodoallyl)-4-phenyl-1H-imidazol-2-yl]phenylmethanol (17)

This molecule was synthesized via reaction with 1 eq **7a**, 1.1 eq $NaHCO_3$, and 1.1 eq molecular iodine in chloroform (3 mL). The final mixture was stirred in room temperature for 18 hours. To complete the reaction, a solution of $Na_2S_2O_4$ (5 mL) was added, and the mixture was extracted with ethyl acetate. Organic phase was separated, dried, and evaporated to get the addition product.

Yellow solid, m.p.: 120-122°C; yield: 88%. ^{1}H NMR (400 MHz, d_6-DMSO) δ = 7.74-7.71 (m, 2H, Ar-H), 7.52 (t, $J_{12,10}$ = 1.11 Hz, 1H, H-12), 7.46 (s, 1H, H-2), 7.45-7.42 (m, 2H, Ar-H), 7.35-7.30 (m, 4H, Ar-H), 7.26 (tt, J = 1.13, 7.31 Hz, 1H, Ar-H), 7.17 (tt, J = 1.20, 7.36 Hz, 1H, Ar-H), 6.33 (d, $J_{6,9}$ = 4.96, 1H, H-6), 5.90 (d, $J_{9,6}$ = 4.96 Hz, 1H, H-9), 4.94 (dd, $J_{10,12}$ = 1.11 Hz, $J_{10,10}$ = 15.84 Hz, 1H, H-10), 4.78 (dd, J = 1.11, 15.84 Hz, 1H, H-10) ^{13}C NMR (100 MHz, d_6-DMSO) δ = 149.8, 142.0, 139.1, 134.6, 128.9, 128.4, 127.6, 126.9, 126.8, 124.7, 117.3, 99.4, 87.0, 68.4,

57.0. HRMS (ESI) (M + H): $C_{19}H_{17}I_2N_2O$: 542.9430, found: 542.9415.

3.38 | MTT toxicity method

MTT assay was used to determine the drug sensitivity of the LN405 glioma cell line. The synthesized molecules stock solutions (10 mol L^{-1}) were prepared in DMSO and diluted in the culture medium prior to use. Cells were plated in 96-well plates at 8×10^3 cells/well density in 100 µL medium and incubated overnight for attachment to the surface of the plate. The cells were treated with synthesized molecules at various concentrations (0.1, 0.02, 0.01, 0.001, and 0.0001 mol L^{-1}). Control cells were treated with the same amount of DMSO at synthesized molecules for each experiment. These plates were incubated for 72 hours. Stock MTT solution was prepared in sterile PBS and filtered through a 0.22-µm filter. At the end of the incubation period, the medium was discarded, and a new medium containing 10-fold diluted MTT stock solution (5 mg/mL) was added. After 3-hour incubation time at 37°C, 5% CO_2, MTT solution was gently discarded, and the lysis solution was added into each well for solubilization of the formazan crystals and shaken for 15 minutes for homogenization. The absorbance of each well at 570 nm was measured in a microplate reader. Growth inhibition was compared between the treated with synthesized molecules samples and the untreated controls and analyzed using GraphPad Prism 5 software (San Diego, CA). All the experiments were performed at least four times. The sensitivity of the treated cells with synthesized molecules was measured by IC_{50}. The molecules concentration causing a 50% inhibition in LN405 glioma cell activity was defined as IC_{50}.

3.39 | COMET assay genotoxicity test

Single cell gel electrophoresis (COMET) DNA damage was determined by COMET assay after LN405 cells were treated with compounds. The applied method is similar to published protocol in our article.[11] These cells were plated in 25-cm^2 flasks at a density of 0.7×10^5 cells/mL and incubated overnight for attachment to the surface of the flask. After overnight incubation, compounds with were added to the cells at 0.1 µmol L^{-1} concentration. Approximately 30%-35% death ratio, which does not arrive at the level of IC_{50} dose in cells, was determined at this concentration according to control group for 72 hours. Therefore, this concentration value was chosen to detect DNA damage in COMET assay. The cells that treated with compounds were incubated for 72 hours. After incubation time, the cells were treated with trypsin and then centrifuged at 2833.5 × g for 5 minutes at 4°C, and the supernatant was discarded. These cells were washed and diluted with PBS (Ca^{2+} and Mg^{2+} free) yielding a concentration of 5×10^5 cells/mL. All the steps of this experiment were carried out under dim light so as to prevent any additional DNA damage. Each cleaned microscope slide was precoated with a layer of 1% normal melting point agarose (Vivantis LE Agarose) in PBS (Ca^{2+} and Mg^{2+} free) and then dried at room temperature at least overnight. Approximately 10^4 cells (18 µL) were mixed with 0.75% low melting point agarose (Sigma) (50 µL) at 37°C, and this suspension was dropped onto the first agarose layer. The slides were allowed to solidify for 25 minutes at 4°C. The coverslips were removed, and the slides were immersed in freshly prepared cold lysis buffer containing 2.5 mol L^{-1} NaCl, 0.1 mol L^{-1} EDTA, 0.01 mol L^{-1} Tris, 1% sodium N-lauroyl sarcosine (adjusting pH to 10); 1% Triton X-100 and 9 mmol L^{-1} DL-dithiothreitol, prechilled at 4°C, were added for 1 hour. The slides were then incubated at 37°C in lysis buffer with 20 µg/mL of Proteinase K (Vivantis) for 2 hours. The slides were removed from the lysis buffer, drained, and placed in a horizontal electrophoresis unit filled with fresh neutral electrophoresis buffer (Tris-borate-EDTA [TBE]), containing 10 mmol L^{-1} Tris, 80 mmol L^{-1} boric acid, 0.5 mol L^{-1} EDTA (pH = 8.3) at 4°C for 20 minutes incubation so as to allow the DNA to unwind. Electrophoresis was performed at room temperature, at 25 V for 20 minutes. After electrophoresis, the slides were air-dried and subsequently stained with 50 µL of 8 µg/mL ethidium bromide and covered with a coverslip. The images of more than 1000 randomly chosen cells were visually analyzed. Observations were made at a magnification of 400× using a fluorescent microscope (Olympus, Japan). Each image was classified according to the intensity of the fluorescence in the comet tail, and given a value of 0, 1, 2, 3, or 4 (from undamaged class 0 to maximally damaged class 4).

ACKNOWLEDGMENTS

Financial support from the Scientific and Technological Research Council of Turkey (TUBITAK, Grant No. 115Z894), Van Yüzüncü Yil University Coordinator of Scientific Research Projects (TAP-2016-5610 and TAP-2017-6092), is gratefully acknowledged.

ORCID

Nurettin Menges http://orcid.org/0000-0002-5990-6275

REFERENCES

[1] a) E. Yoo, D. B. Salunk, D. Sil, X. Guo, A. C. D. Salyer, A. R. Hermanson, M. Kumar, S. S. Malladi, R. Balakrishna, W. H. Thompson, H. Tanji, U. Ohto, T. Shimizu, S. A. David, *J. Med. Chem.* **2014**, *57*, 7955. b) A. Shakoori, J. B. Bremner, A. C. Willis, R. Haritakun, P. A. Keller, *J. Org. Chem.* **2013**, *78*, 7639.

c) J. A. Wiles, A. Hashimoto, J. A. Thanassi, J. Cheng, C. D. Incarvito, M. Deshpande, M. J. Pucci, B. J. Bradbury, *J. Med. Chem.* **2006**, *49*, 39.

[2] X. G. Tong, L. L. Zhou, Y. H. Wang, C. Xia, Y. Wang, M. Liang, F. F. Hou, Y. X. Cheng, *Org. Lett.* **2010**, *12*, 1844.

[3] a) C. Laroche, S. M. Kerwin, *J. Org. Chem.* **2009**, *74*, 9229. b) N. Menges, E. Şahin, *ACS Sustainable Chem. Eng.* **2014**, *2*, 226. c) N. Menges, O. Sari, Y. Abdullayev, S. S. Erdem, M. Balci, *J. Org. Chem.* **2013**, *78*, 5184. d) M. S. Özer, N. Menges, S. Keskin, E. Şahin, M. Balci, *Org. Lett.* **2016**, *18*, 408. e) S. Guven, M. S. Ozer, S. Kaya, N. Menges, M. Balci, *Org. Lett.* **2015**, *17*, 2660.

[4] J. K. Vandavasi, W. P. Hu, H. Y. Chen, G. C. Senadi, C. Y. Chen, J. J. Wang, *Org. Lett.* **2012**, *12*, 3134.

[5] a) M. Nagaraj, S. Muthusubramanian, *J. Chem. Sci.* **2016**, *128*, 451. b) M. Nagaraj, M. Boominathan, D. Perumal, S. Muthusubramanian, N. Bhuvanesh, *J. Org. Chem.* **2012**, *77*, 6319.

[6] a) M. H. Larsen, K. N. Hauk, S. K. Hashmi, *J. Am. Chem. Soc.* **2015**, *137*, 10668. b) Q. Wang, S. Aparaj, N. G. Akhmedov, J. L. Petersen, X. Shi, *Org. Lett.* **2012**, *14*, 1334. c) S. Hosseyni, L. Wojtas, M. Li, X. Shi, *J. Am. Chem. Soc.* **2016**, *138*, 3994. d) R. S. Vasconcelos, L. F. Silva, A. Giannis, *J. Org. Chem.* **2011**, *76*, 1499. e) F. Shibahara, A. Kitogawa, E. Yamaguchi, T. Murai, *Org. Lett.* **2006**, *8*, 5621.

[7] a) M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, Gaussian 09, Revision D.01, Gaussian, Inc., Wallingford CT **2009**; b) Optimization of compound **16** was done by using DFT/B3LYP-6-311G (d,p) and charges of atoms indicate Natural Bond Orbital (NBO) methods

[8] P. Zhou, J. W. Zou, F. F. Tian, Z. C. Shang, *J. Chem. Inf. Model.* **2009**, *49*, 2344.

[9] X. Fang, Y. Yin, B. Wang, L. Yao, Y. T. Chen, T. Schröter, A. Weiser, J. Pocas, G. Wayne, M. D. Cameron, L. Lin, C. Ruiz, S. Khan, S. C. Schürer, A. Pachori, P. LoGrasso, Y. Feng, *J. Med. Chem.* **2010**, *53*, 5727.

[10] B. Kuzu, M. Tan, Z. Ekmekci, N. Menges, *J. Lumin.* **2017**, *192*, 1096.

[11] M. Taspinar, S. Ozdemir, M. Ozkan, D. Oztuna, H. Canpinar, J. A. Rey, A. Sunguroğlu, J. S. Castresana, H. C. Uğur, *Tumour Biol.* **2013**, *34*, 1935.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Kuzu B, Genç H, Taşpinar M, Tan M, Menges N. An easy synthetic protocol for imidazo-1,4-oxazines and evaluation of their toxicities. *Heteroatom Chem*. 2018;e21412. https://doi.org/10.1002/hc.21412