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RESEARCH ARTICLE

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Synthesis, cytotoxicity, and antibacterial studies of 2,4,5,6-substituted hexahydro-1*H*-isoindole-1,3(2*H*)-dione

Egehan Yetişkin[1] | **Özlem Gündoğdu**[2] | **Derya Mete**[1] | **Neslihan Çelebioğlu**[3] | **Yunus Kara**[3] | **Gülşah Şanlı-Mohamed**[1]

[1]Department of Chemistry, Faculty of Sciences, İzmir Institute of Technology, İzmir, Turkey

[2]Vocational School of Kaman, Ahi Evran University, Kırşehir, Turkey

[3]Department of Chemistry, Faculty of Sciences, Atatürk University, Erzurum, Turkey

Correspondence

Gülşah Şanlı-Mohamed, Department of Chemistry, Faculty of Science, İzmir Institute of Technology, Urla, İzmir, Turkey.
Email: gulsahsanli@iyte.edu.tr and gulsahsanli@hotmail.com

Yunus Kara, Department of Chemistry, Faculty of Sciences, Atatürk University, Erzurum, Turkey.
Email: yukara@atauni.edu.tr

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Abstract

In this study, synthesis of novel isoindole-1,3-dione analogues bearig halo, hydroxy, and acetoxy groups at the position 4,5,6 of the bicyclic imide ring was performed to examine their potential anticancer effects against some cell lines. A multistep chemical pathway was used to synthesize the derivatives. The cytotoxic effect of trisubstituted isoindole derivatives were evaluated by determining cellular viability using the MTT assay against A549, PC-3, HeLa, Caco-2, and MCF-7 cell lines. The C-2 selective ring-opening products were obtained from the ring-opening reaction of 5-alkyl/aryl-2-hydroxyhexahydro-4*H*-oxireno[2,3-e]isoindole-4,6(5*H*)-diones with nucleophiles such as chloride (Cl^-) and bromide (Br^-) ions. In addition, the ring-opening products halodiols were converted to their related acetates. The anticancer activity of synthesized isoindole-1,3-dione derivatives was investigated against HeLa, A549, MCF-7, PC3, and Caco-2 cells in vitro and resulted in varies cytotoxic effect depend on the group attached to the isoindole molecule. Furthermore, the evaluation of the antimicrobial action of trisubstituted isoindole derivatives against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria was assessed and found out selective inhibition of the both bacterial growth via different trisubstituted isoindole derivatives. The results of this work encourage further research on the potential utilization of trisubstituted isoindole derivatives as cytotoxic and antimicrobial agents.

KEYWORDS

antibacterial activity, cancer cell lines, cytotoxicity, isoindole-1,3-dione derivatives

1 | INTRODUCTION

Inhibitory effects of cantharidines against protein phosphatase 1 and 2A (PP1 and PP2A) are also observed in its analogs, isoindole derivatives (Aggen et al., 1999; Galvis et al., 2013; Hill, Stewart, Sauer, et al., 2007; McCluskey & Sakoff, 2001; McCluskey et al., 2002). The important advantage of isoindole-1,3-dione derivatives over cantharidine is their low toxicity. Therefore derivatives of isoindole-1,3-dione (**3**), have received a great deal of attention because of their potential anticancer properties (Scheme 1). During the last three decades, many research groups have synthesized isoindole-1,3-dione derivatives and extensively investigated their biological activities

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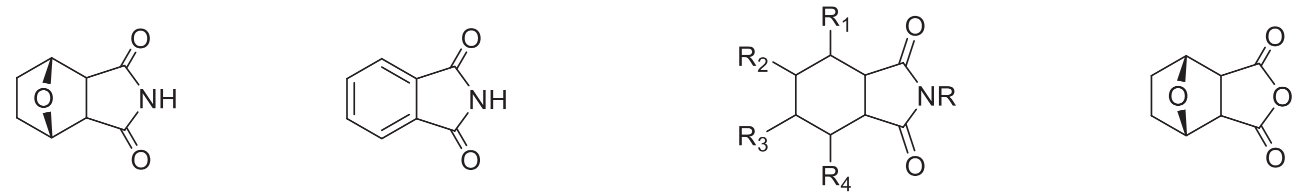
Egehan Yetişkin and Özlem Gündoğdu contributed equally to this study.



Norcantharimide (**1**) Isoindoline-1,3-dione or phthalimide (**2**) Isoindoline-1,3-dione derivative **(3)** Cantharidin (**4**)

SCHEME 1 Some structure of isoindole-1,3-dione derivatives and cantharidine.

(Hill et al., 2007a; Kok et al., 2007; Lamie et al., 2015; Lin et al., 2000, 2004; Wu et al., 2014).

On the other hand, there are three main classes of protein phosphatases: tyrosine-specific, serine/threonine-specific, and dual-specificity phosphatases. We have, however, focused on the tyrosine family as they can affect the control and growth of the cell cycle. Our earlier reports demonstrated that some isoindole-1,3-dione analogues bearing disubstituted and trisubstituted cyclohexane moiety were effective to minimize the original cytotoxicity while maintaining potency toward cancer cell lines in vitro (Hill et al., 2007b; Köse et al., 2017, 2020; Süloğlu et al., 2020; Tan et al., 2020). Additionally, we recently investigated antiproliferative properties of epoxy alcohols antiproliferative properties against different cell lines like MCF-7, A549, HeLa, MCF-7, Caco-2, and PC3 cell lines (Yetişkin et al., 2022). On the other hand, we studied the regioselective ring-opening reactions of epoxy alcohol **11b** in the presence of HX and synthesized tetrasubstituted isoindole derivatives (Tan et al., 2011). Based on these data we synthesized additional compounds in this series and examined some of the tetrasubstituted isoindoline-1,3-diones obtained for cancer preventive activities and antimicrobial properties.

2 | MATERIALS AND METHODS

2.1 | Chemical part

2.1.1 | Chemical materials and apparatus

All chemicals were commercially available and purchased from Merck and Sigma-Aldrich. Solvents were analytical grade and obtained from Sigma-Aldrich and they were used without further purification. Reactions were monitored via thin-layer chromatography (TLC) (McCluskey & Sakoff, 2001). H NMR and (Köse et al., 2020) C NMR spectra were recorded on 400 MHz and 100 MHz with a Varian spectrometer using $CDCl_3$/Acetone-d_6 (Figure S1). Melting points were determined on a capillary melting apparatus (BUCHI 530) and were uncorrected.

2.1.2 | General procedure for allyl alcohols **10**

Allyl alcohols **10a–c** were synthesized as described in literature (Gündoğdu et al., 2022; Tan et al., 2011, 2014).

2.1.3 | General procedure for epoxy alcohols **11a–c**

To a solution of allyl alcohol **11** (1.0 g, 5.12 mmoL) in CH_2Cl_2 (25 mL) was added 77%–79% *m*-CPBA (1.49 g, 6.66 mmoL) at 0°C. The reaction was allowed to slowly warm to room temperature for 6 h of stirring. After this period, the reaction mixture were evaporated under reduced pressure. The resulting residue was purified by column chromatography with EtOAc–petroleum ether (40:60) providing.

2.1.4 | Preparation of 20% HCl (g)/MeOH solution

Methanol (100 mL) was cooled to 0°C and magnetically stirred. In another balloon, the HCl gas released by the dropwise addition of 98% H_2SO_4 onto NaCl(k) was passed through methanol in the other magnetically stirred balloon. This process was continued until the total weight of the dissolved gas was 125 g. In this way, a 20% HCl(g)/MeOH solution was prepared. This prepared solution was kept closed in the −5°C section of the refrigerator. HCl solutions of different concentrations were also prepared in the same way.

2.1.5 | Preparation of 37% HBr(g)/MeOH solution

Methanol (100 mL) was cooled to 0°C and magnetically stirred. Then, NaBr(k) was taken into another balloon and HBr gas, which was released by adding 98% H_2SO_4 dropwise, was passed through methanol in the other magnetically stirred balloon. This process was continued until the total weight of methanol and dissolved gas was 155 g, and in this way a 37% HBr(g)/MeOH solution was prepared. The prepared solution was kept closed in the −5°C section of the refrigerator.

2.1.6 | General procedure for opening reactions of epoxide compounds with HCl

To a solution of epoxy alcohols **11a–c** (500 mg, 2.37 mmoL) in MeOH (25 mL) was added HCl (37%, 3.55 mmoL) and stirred at 0°C for 3 h. Then the reaction solvent was removed in the evaporator. The crude product was purified by crystallization in methanol/petroleum ether and CH_2Cl_2/Hexane.

2.1.7 | General procedure for opening reactions of epoxide compounds with HBr

To a solution of epoxy alcohols **11a–c** (500 mg, 2.37 mmoL) in MeOH (25 mL) was added HBr (47%, 3.55 mmoL) and stirred at 0°C for 3 h. Then the reaction solvent was removed in the evaporator. The crude product was purified by crystallization in methanoL/petroleum ether or CH_2Cl_2/Hexane.

2.1.8 | General procedure for acetate reactions of dihydroxychloro/bromo compounds

The dihydroxy compounds **12a–c**/**13a–c** (1 mmoL) and pyridine (2 mmoL) were stirred in 10 mL of Ac_2O at room temperature for 4 h. The reaction was followed by TLC. The reaction was stopped and ice was added to the flask. After cooling the material, it was extracted with CH_2Cl_2 and dilute HCl. The organic layer was dried over Na_2SO_4, the solvent was removed in the evaporator. The crude product was crystallized or column chromatography with EtOAc/Hexane.

2.2 | Biological part

2.2.1 | Cell culture

HeLa (cervical), A549 (lung), PC3 (prostate), and MCF-7 (breast) cancer cell lines were obtained from Biotechnology and Bioengineering Research Center, İzmir Institute of Technology, and Caco-2 (colon) was provided by Izmir International Biomedicine and Genome Institute. The medium for HeLa and MCF-7 was RPMI 1640 (Lonza) and the PC3, Caco-2, and A549 cells were cultured in Dulbecco's modified Eagle's medium F12 (DMEM F12) (Biological Industries) containing 10% fetal bovine serum (Biological Industries) and 1% penicillin streptomycin (Biological Industries) and 1% l-glutamine (Biological Industries). The cells were incubated in a CO_2 incubator at 37°C, under a humidified atmosphere of 5% CO_2, and passaged by trypsinization and in the growth medium when cells became 80% confluent.

2.2.2 | Cell viability assay

The cytotoxicity of the synthesized molecules evaluated by the colorimetric MTT assay (Tolosa et al., 2015). 3×10^3 of A549, HeLa and MCF-7 cells, 5×10^3 of PC-3 cells, and 8×10^3 of Caco-2 cells per well seeded into 96-well plate and incubated to adhere for 24 h. All compounds were dissolved in sterile dimethyl sulfoxide (DMSO) and diluted at the appropriate concentrations with the culture medium (1000-500-250-100-50-25 μM). Final DMSO concentration was 1% in all conditions. Treated cells were incubated for 24, 48, and 72 h to determine cytotoxic effects. At the end of each incubation time, 10 μL MTT (5 mg/mL PBS) was added to each well. After 3.5 h incubation, centrifuged at 1800 rpm 10 min and supernatant was removed and formazan crystals were dissolved with DMSO. The optical density was determined at 540 nm (Varioscan Flash).

2.2.3 | Apoptosis analysis

FITC Annexin V Apoptosis Detection Kit with PI (BioLegend) was used to determine the apoptotic effects of synthesized isoindole derivatives on HeLa, A549, MCF-7, PC3, and Caco-2 cell lines (Vermes et al., 1995). 5×10^5 cells in 1980 μL was seeded in 6-well plate and incubated to adhere for 24 h at 37°C 5% CO_2. After the incubation period the compounds dissolved in sterile DMSO and added to 20 μL of in each well at their own IC_{50} concentrations. The treated cells were incubated for 48 h in CO_2 incubator at 37°C. Then all media were collected and washed with 1 mL of phosphate-buffered saline (PBS). Cells were harvested by trypsin and all collected cells were again put in their own tubes and centrifuged at 800 rpm for 5 min. The pellet was dissolved in 5 mL of PBS and centrifuged again. Then the pellet was resuspended in 200 μL of binding buffer, 2 μL of Annexin V-FITC, and 5 μL of propidium iodide were added and tubes were incubated for 15 min at room temperature in the dark and analyzed by a flow cytometer (FACSCanto, BD).

2.2.4 | Cell cycle analysis

HeLa, A549, MCF-7, PC3, and Caco-2 cells were seeded 6 well plates at density 5×10^5 cells/well and incubated at 37°C, 5% CO_2 for 24 h. Next day cells were treated with the IC_{50} concentrations of isoindole derivatives and were incubated for 48 h in CO_2 incubator at 37°C. After incubation time, cells harvested by trypsin and suspensions were collected in their own tube. Tubes were centrifuged at 800 rpm for 5 min. The pellet was resuspended with cold PBS on ice then cold ethanol was added during the vortexing process and incubated at −20C° for at least one night. After the tubes were centrifuged at 1200 rpm for 10 min then removed PBS and ethanol. Pellet was resuspended with PBS and centrifuged at 1200 rpm for 10 min again. Supernatant was removed carefully, and the pellet was resuspended in 200 μL 0.1% Triton X-100 in PBS. After 20 μL RNase A (200 μg/mL) was added and cells were incubated for 30 min at 37°C and then 20 μL PI (1 mg/mL) was added and incubated at room temperature for 15 min then analyzed by the flow cytometry.

2.2.5 | Antibacterial analysis

The antibacterial activity of the trisubstituted isoindole derivatives was tested against *Escherichia coli* (Gram-negative) and *Staphylococcu aureus* (Gram-positive) as model microbes (Paramanantham et al., 2019). Initially, the concentration of the bacterial cultures was set to 0.5 McFarland standard, then using a cotton swab, the final bacterial suspensions were adjusted to ~1.25 × 107 CFU/mL for *E. coli* and 1.7 × 107 CFU/mL for *S. aureus*. 90 μL of nutrient broth (NB) and 100 μL of TSB in a 96-well plate were added to the labeled wells in confinement. Drug concentrations were 500, 250, 100, 50, and 25 μg/mL. Finally, 100 μL of the diluted bacterial solution was placed in the wells to reach a total volume of 200 μL and then incubated at 37°C under shaking conditions at 150 rpm for 24 h. After incubation, 100 μL of the culture inoculum was retrieved from each well, serially diluted (10^{-3} to 10^{-6}), and plated on a Mueller Hilton agar at 37 °C. The number of viable bacteria that persisted in the plates was enumerated using colony counter after 24 h incubation. The experiment was conducted in duplicate.

3 | RESULT AND DISCUSSION

3.1 | Chemistry part

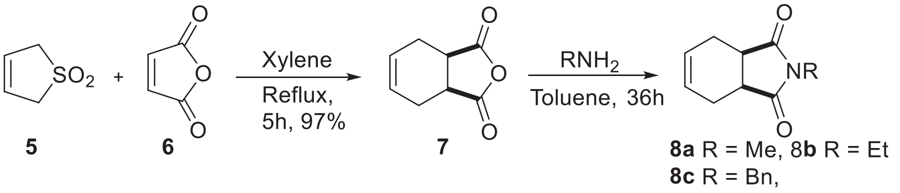
For the synthesis of target isoindole-1,3-dione derivatives, we initiated with the synthesis of 2-alkyl/aryl-5-hydroxy-3a,4,5,7a-tetrahydro-1*H*-isoindole-1,3(2*H*)-dione (**8**) from 3-sulfolene by using a known method as described in the literatüre (Gündoğdu et al., 2022; Tan et al., 2011). The reaction of 3-sulfolene (**5**) with maleic anhydride (**6**) gave cyclic anhydride **7**. Subsequently condensation of primary amines with cyclic anhyride **7** in the presence of a 3:1 mixture of toluene and triethylamine gave the imides **8a–c**. (Scheme 2).

Allyl alcohols 10a-c were synthesized from reduction of hydroperoxides obtained by the reaction of singlet oxygen with bicyclic imides 8a–c (Scheme 3) (Gündoğdu et al., 2022; Tan et al., 2011).

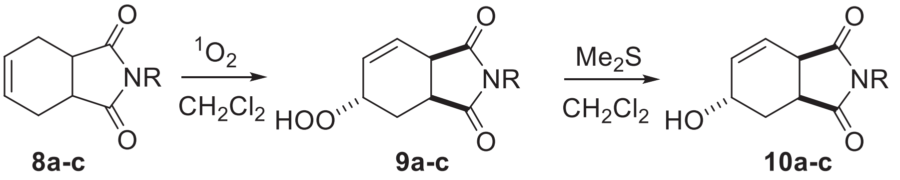
The reaction of alcohols with *m*-CPBA gave *syn*-epoxy alcohols **11a–c** (Scheme 4) (Tan et al., 2011, 2014). The structures of **11a–c** were assigned by ^1H and ^{13}C NMR spectroscopy.

Very recently, C-2 regioselectivity was observed in compound 11b containing the ethyl group bound to the nitrogen atom in the imide ring and explained the C-2 regioselectivity of the product with the assisted of theoretical calculations (Tan et al., 2011). In this context, we decided to synthesize tetrasubstituted new haloisoindole-1, 3-dione 12a, 12c, 13a, and 13c compounds to examine the effect of nitrogen bonded groups on both regioselectivity and biological activity. For this purpose, we performed the reaction of epoxy alcohols 11a–c with HCl and HBr in CH3OH at room temperature. In these reactions, C-2 regioselective ring-opening products were obtained (Scheme 5).

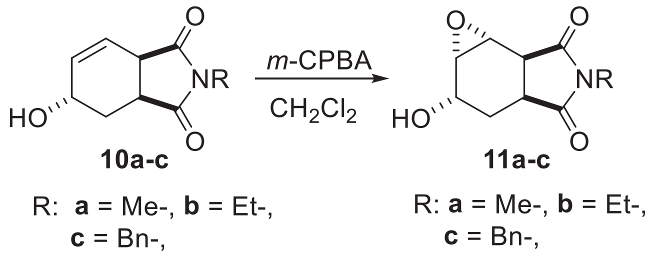
The structures of ring-opening products of epoxy alcohols were assigned by ^1H and ^{13}C NMR spectroscopy. The structures and hence the spectra of the ring-opening products of epoxy alcohols are very similar. Therefore, the structures of the opening products were determined comparatively. The ^1H and ^{13}C NMR spectra of the ring-opening product synthesized in previous studies and whose structure was determined by x-ray were compared with the NMR spectra of the synthesized products and the structure of each product was determined (Gündoğdu et al., 2022). For instance, when the proton signals belonging to groups such as -Bn, −Et, −Me which are attached to the nitrogen (N) atom are excluded, almost the same signal groups are seen for each ring-opening product and resonate in the same region. Therefore, we performed this method to incorporate halogen atoms to the cyclohexane ring. According to the results obtained, it was seen that the groups attached to the nitrogen atom had no effect on the regioselectivity. On the other hand, the anticancer activity of isoindole derivatives containing diacetoxy and chloro/acetoxy groups in the cyclohexane ring was observed in our studies (Köse et al., 2020). In the light of these results, conversion of halodiols to acetate and activity studies



SCHEME 2 Synthesis of imides **8**.



SCHEME 3 Synthesis of allyl alcohols **10**.



SCHEME 4 Synthesis of epoxy alcohol **11**.

of acetates were planned. For this purpose, the epoxide opening products were converted to their diacetates in the presence of halodiols acetic anhydride and pyridine. It was determined that unexpected phthalimide derivatives **2** were formed together with diacetate products in acetylation reactions (Scheme 6).

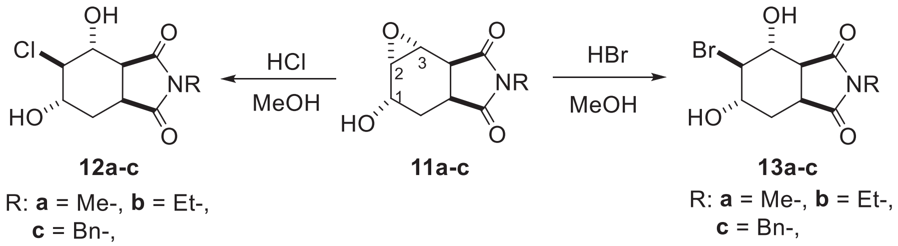
The structures of acetate 14–**15** and phthalimide **2** derivatives were assigned by ^1H and ^{13}C NMR spectroscopy. The formation of the phthalimide derivative **2** was also supported by the additional reaction. When the monoacetate compound, consisting of acetylation of a single-OH group from the acetylation reaction, was subjected to diacetalation again in the presence of Ac_2O and pyridine, the phthalimide derivative **2** was obtained as a single product. According to the results obtained, it was determined that phthalimide (isoindoline-1,3-dione) derivatives **2** were secondary products of diacetate compounds. The formation mechanism of the secondary product formed in the reactions was discussed and the formation mechanism was suggested (Scheme 7).

As can be seen in Scheme 7, three different eliminations occur during the phthalimide formation. Two of these eliminations are two different monomolecular conjugated base eliminations (E1kB). The third elimination is the bimolecular elimination (E2) reaction. The formation of a stable aromatic system is an important factor in the realization of these reactions. The product formation mechanism is an exceptional example that includes three different elimination reactions together.

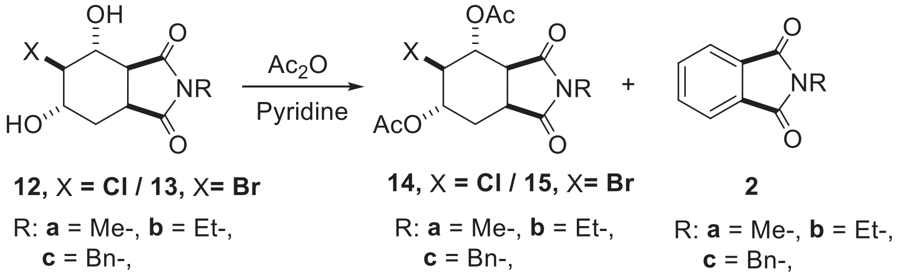
3.2 | Biological part

Reports about the cytotoxicity of trisubstituted isoindole derivatives in a variety of cell lines are rare for wide evaluation purposes of potential cancer treatment and interpretation as candidate anticancer agents. In this study, the cytotoxic effect of trisubstituted isoindole derivatives (drugs) **12a**, **12b**, **12c**, **13a**, **13b**, **and 13c** were evaluated by determining cellular viability using the MTT assay. Figure 1 show the cell viability of A549, PC-3, HeLa, Caco-2, and MCF-7 cell lines exposed to drugs at different concentrations (1000-500-250-100-50-25-10 μM) at 48 h.

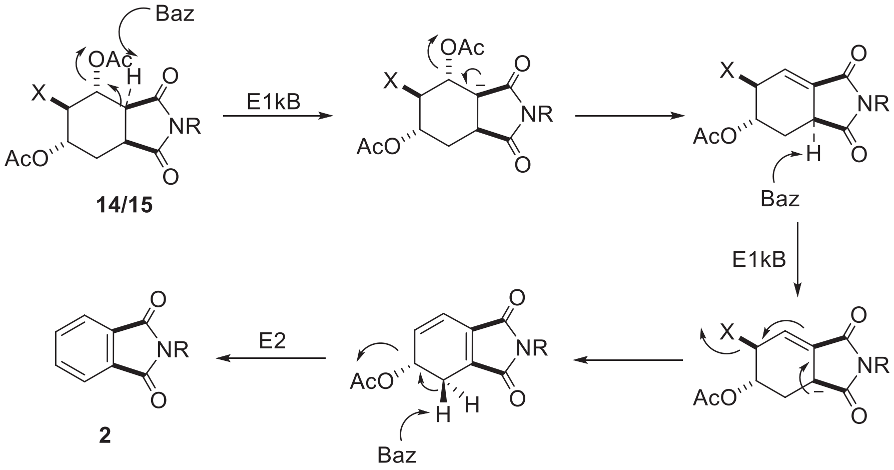
With the MTT test, it was determined that the synthesized drugs had a significant cytotoxic effect in cell lines, and the cellular inhibition rate increase with the increasing concentration of the drugs. The cell viability assay was conducted at 24, 48, and 72 h incubation times with the indicated concentration of the drugs on the same cell lines. The results have shown that the percentage of inhibition increases with the duration of treatment (data not shown). In our studies, 48 h incubation time was chosen to evaluate the cytotoxic effect of trisubstituted isoindole derivatives. According to the experimental results, it was determined that the most effective cytotoxicty in A549 cell lines was created by the active drug **12b**, and it was observed that the molecule **13c** was the most effective in HeLa cell line. Similarly, molecule **13c** has demonstrated the most cytotoxic effect in MCF-7 and CaCo-2 cell lines. Similar to the **13c** molecule on the MCF-7 cell line, the **12c** molecule also has exhibited a good cytotoxic effect. **13a** was found to be the most effective molecule in the PC3 cell line. Cytotoxic effect varies according to the group attached to the isoindole molecule. In **13b** and **12b** molecules, bromine in **13b** are bound and chlorine atom in **12b** are bound to the isoindole molecule. The cytotoxic effect of **12b** was more than **13b** against the A549 cell line as seen in Figure 1. Similar results were obtained some isoindole-1,3-dione derivatives on HeLa, C6 and A549 cancer cell lines in the literature (Köse et al., 2017; Tan et al., 2020).



SCHEME 5 The formation of C-2 type regio-isomer by ring-opening reaction of epoxy alcohols **11a–c** with HCl and HBr.



SCHEME 6 The acetylation reaction of halodiols **12/13**.



SCHEME 7 Proposed mechanism for the formation of phthalimide derivatives.

The cellular viability of **14a**, **14b**, **14c**, **15a**, **15b**, and **15c** isoindole derivatives (drugs) were also tested using an MTT assay in terms of cytotoxic effect. Figure 2 shows the cell viability of A549, PC-3, HeLa, Caco-2, and MCF-7 cell lines exposed to drugs at different concentrations (1000-500-250-100-50-25-10 μM) at 48 h. It is seen that all **14a**, **14b**, **14c**, **15a**, **15b**, and **15c** isoindole derivatives have decreased their cell viability after 48 h incubation at high concentrations. The cytotoxicity behavior of the molecules on each cell line was slightly different as expected. Other studies with differently substituted isoindole derivatives also demonstrated similar cytotoxic potential for HT-29 colon cancer cells of four synthesized isoindoline derivatives (Süloğlu et al., 2020). It has been observed that the cell viability of the A549 cell line declined more than other cell lines against all **14a**, **14b**, **14c**, **15a**, **15b**, and **15c** isoindole derivatives, having less than 20% decrease at high concentrations of the active molecules. After A549, the order of cells showing cytotoxic behavior toward all active drugs was PC-3, Caco-2, HeLa, and MC-7.

When compared to **12a**, **12b**, **12c**, **13a**, **13b**, and **13c** with **14a**, **14b**, **14c**, **15a**, **15b**, and **15c** molecules according to the obtained data, it has seen that **12a**, **12b**, **12c**, **13a**, **13b**, and **13c** compounds were more cytotoxic than **14a-c** and **15a–c** acetate compounds. Because in **12a**, **12b**, **12c**, **13a**, **13b**, and **13c** compounds, hydroxyl (-OH) groups form hydrogen bonds, increasing the solubility in the cell environment. Thus, as it facilitates the entry of the active compounds into the cell, the cytotoxic effect increases more. On the other hand, since acetate groups in **14a–c** and **15a–c** molecules do not form hydrogen bonds like diol compounds, their solubility in the cell decreases, and the effects decrease as they make it difficult to transport to the cell. Compounds containing the benzyl group were active in all cell lines. These results are promising. As isoindole derivatives are known to be potential tyrosine kinase inhibitors and inhibit cell growth, the synthesized compounds have the potential to lead to preclinical and other studies as promising drug candidates.

The apoptotic effect of the synthesized **13a**, **13b**, **and 12b** molecules on A549, HeLa, and PC-3 cell lines was

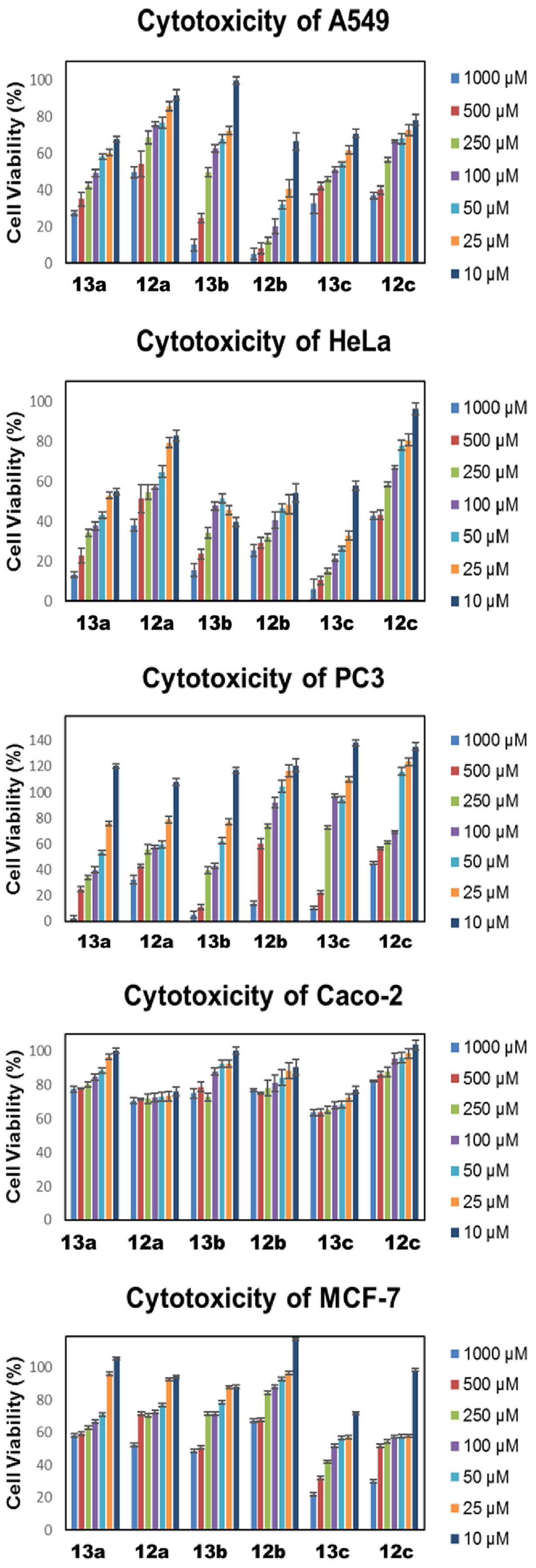


FIGURE 1 The cell viability of A549, PC-3, HeLa, Caco-2, and MCF-7 cell lines exposed to the 12a, 12b, 12c, 13a, 13b, and 13c drugs at different concentrations (1000-500-250-100-50-25-10 μM) at 48 h.

investigated via The Annexin V-FITC kit. According to the results of apoptosis analyzes (Figure 3), there were no increase in apoptosis rates for the PC3 cell line compared to the control group. In the A549 cell line, it was observed that apoptosis rates increased at the highest concentration of **13a** and **13b** molecules. Among the all tested cell lines, HeLa was the most prone to apoptosis. The reason for the ratio of viable cells shown in the results may be related to apoptosis resistance. It is well known that the main cause of apoptosis resistance is reported as autophagy, and tyrosine kinase inhibitors cause autophagy (Kung, 2011).

The antibacterial activity test was carried out for two models of bacterial strains; *E. coli* (Gram-negative) and *S. aureus* (Gram-positive). The results of microbial inhibitory expressed as the number of viable bacteria persisted in the plates enumerated using colony counter after 24 h incubation in the presence of **13a–12c** active compounds (Table 1). Moderate antimicrobial activity was observed in all applied compounds and results were consistent with literature findings (Baluja et al., 2020). The results exhibited **13a** drug displayed a killing effect against both bacterial strains, 70.6% for *S. aureus* and 77% for *E. coli*. In addition to the **13a** compound, the best antibacterial effect was observed using the **13c** drug calculated to be a 61.8% of reduction in cfu/ml after incubation for 24 h against *S. aureus* strain. Besides **13a**, the drug **12c** has decreased the number of viable *E. coli* bacteria strains demonstrating 69% reduction in terms of antibacterial activity. The results demonstrated that the bactericidal activity of the other tested compounds in this study was not effective against the two models of bacterial strains. This may due to the interactions of between cell wall structure of bacterial strains and the tested isoindole derivatives (Srinivas et al., 2006).

4 | CONCLUSION

Here we have reported the synthesis of isoindole-1,3-dione analogues bearing halo, hydroxy, and acetoxy groups at the 4,5,6-position of the bicyclic imide ring. Herein, the cytotoxic effect of trisubstituted isoindole derivatives was evaluated by determining cellular viability using the MTT assay against A549, PC-3, HeLa, Caco-2, and MCF-7 cell lines. The most effective cytotoxicity in A549 cell lines was created by the active drug **12b**, and it was observed that molecule **13c** was the most effective in

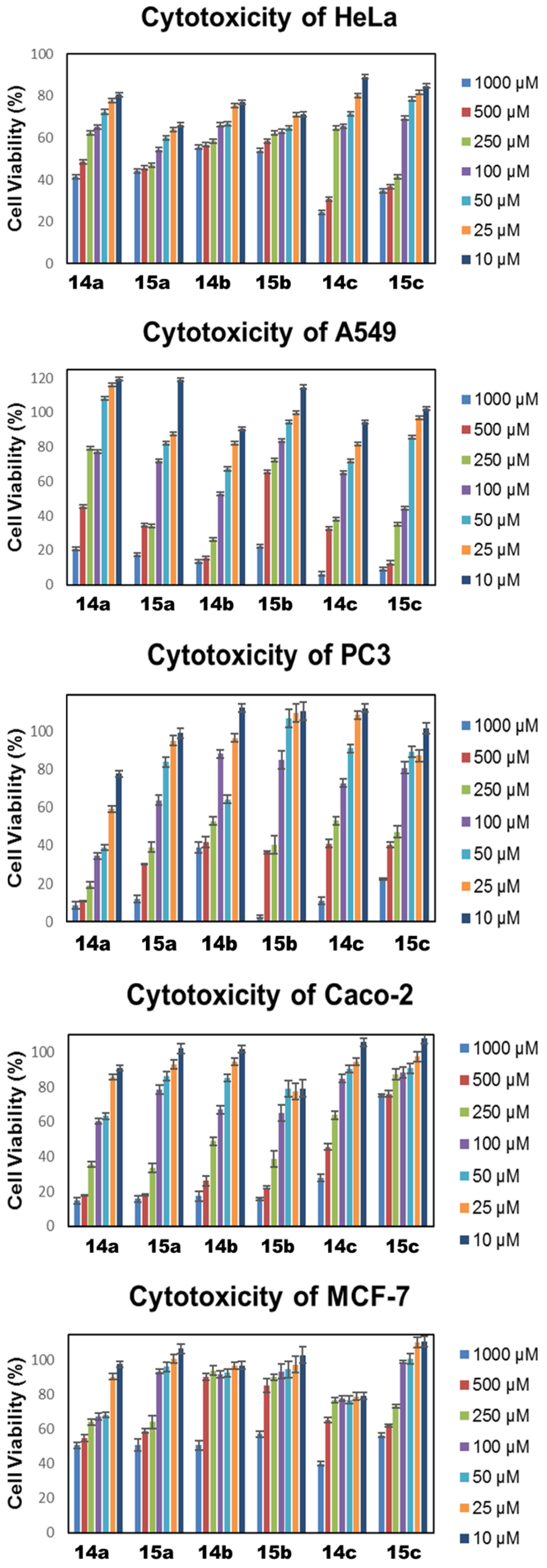


FIGURE 2 The cell viability of A549, PC-3, HeLa, Caco-2, and MCF-7 cell lines exposed to the **14a**, **14b**, **14c**, **15a**, **15b**, and **15c** drugs at different concentrations (1000-500-250-100-50-25-10 µM) at 48 h, respectively.

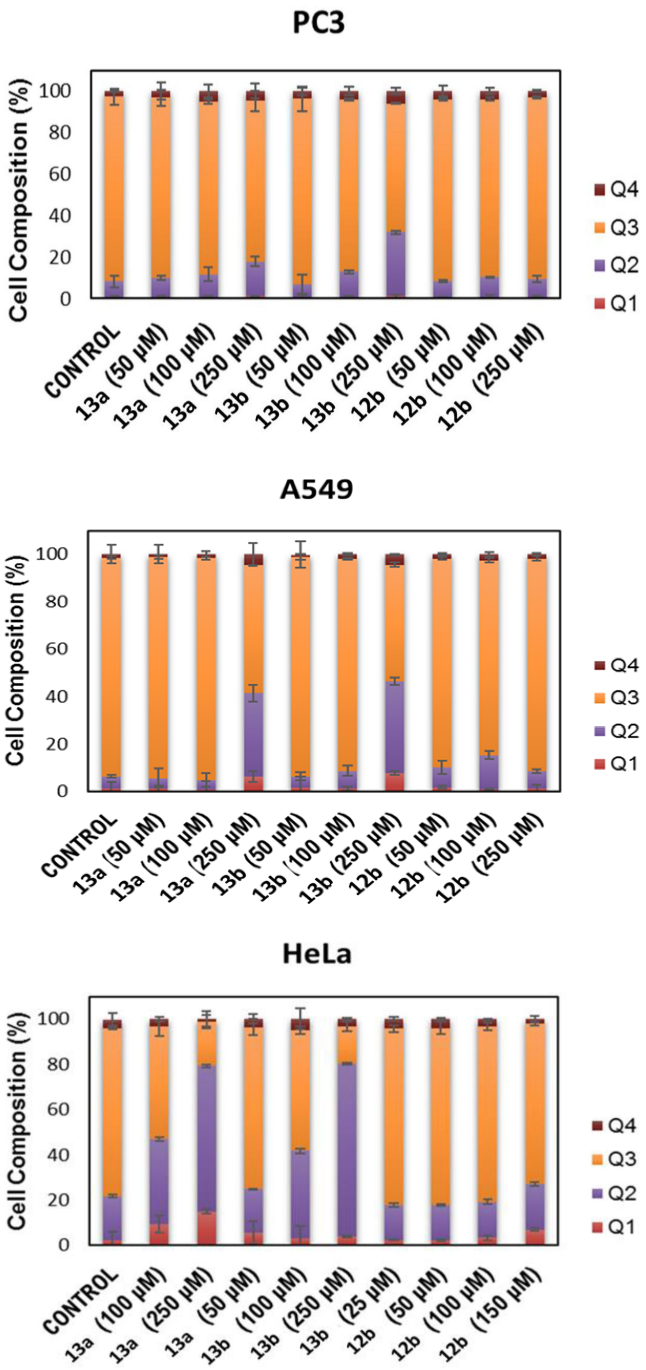


FIGURE 3 The apoptotic effects of **12b**, **13a**, and **13b** compounds on A549, HeLa, and PC-3 cells, each drug were applied at their own IC_{50} concentrations at 48-h incubation time.

TABLE 1 Microbial inhibitory effect expressed as the number of viable *Escherichia coli* and *Staphylococcu aureus* in the presence of **12a**, **12b**, **12c**, **13a**, **13b**, and **13c** drugs after 24 h incubation.

Compounds	***Staphylococcus aureus*** **($\times 10^8$ cfu/mL)**	***Escherichia coli*** **($\times 10^8$ cfu/mL)**
13a	2.4	6.7
12a	8.8	8
13b	3.1	21.6
12b	18.5	13.3
13c	2.1	14.3
12c	34.5	6
DMSO (**control**)	0.4	0.7

the HeLa cell line. The cytotoxic effect varies according to the group attached to the isoindole molecule. The evaluation of the antimicrobial action of trisubstituted isoindole derivatives against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria was also assessed and observed a moderate antimicrobial activity in general. Among the applied molecules, compound **13a** displayed the best killing effect against both bacterial strains, 70.6% for *S. aureus* and 77% for *E. coli* bacterial strains. Besides the **13a** compound, the **13c** drug exhibited a selectively antibacterial effect observed 61.8% reduction against *S. aureus* strain and the drug **12c** selectively decreased *E. coli* bacteria strains viability by 69% reduction in terms of antibacterial activity. The results of this work encourage further research on the potential utilization of trisubstituted isoindole derivatives as cytotoxic and antimicrobial agents.

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CONFLICT OF INTEREST STATEMENT

No potential conflict of interest was reported by the authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Gülşah Şanlı-Mohamed https://orcid.org/0000-0003-0282-4428

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SUPPORTING INFORMATION

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