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Original Article

SYNTHESIS OF SOME 2,3-DIHYDRO-1,3,4-OXADIAZOLES AND 4,5-DIHYDRO-1,2,4-TRIAZOLES AS ANTICANCER AGENTS

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ABSTRACT

Objective: The main objective of this work was to synthesize and evaluate the novel 2,3-dihydro-1,3,4-oxadiazole and 4,5-dihydro-1,2,4-triazole derivatives for cytotoxic activities.

Methods: The 2,3-dihydro-1,3,4-oxadiazole derivatives 4a-h were synthesized by cyclization of N'-(substituted-benzylidene) isonicotinohydrazide 3a-e in refluxing acetic anhydride. The 2,3-dihydro-1,3,4-oxadiazole derivatives 4a-h were converted into the corresponding 4,5-dihydro-1,2,4-triazoles 5a-h using ammonia. All the synthesized compounds were identified, depending on the physical and spectral data. Title compounds were assessed for their cytotoxic activity against human cancer cell line (MCF-7) by using Sulforhodamine B (SRB) colorimetric assay.

Results: All the synthesized compounds showed characteristic peaks in FTIR, ^{1}HNMR and Mass spectral analysis. The results of the *in vitro* cytotoxic activity revealed that the compound 4c exhibited equipotent cytotoxic activity with an IC_{50} value of 8.04 µM when compared with that of standard drug doxorubicin (IC_{50}= 8.02 µM). The reminder compounds have shown good to moderate cytotoxic activities when compared with that of a reference standard.

Conclusion: We synthesized a series of title compounds in quantitative yields. Most derivatives showed moderate to good cytotoxic activity.

Keywords: 2,3-Dihydro-1,3,4-oxadiazole, 4,5-Dihydro-1,2,4-triazole, MCF-7, Anticancer

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INTRODUCTION

There are numerous literature reports confirming the multidirectional effect of compounds containing the 1,3,4-oxadiazole ring in its structure. 1,3,4-Oxadiazole derivatives showed numerous biological activities [1-14]. In addition, the 1,3,4-oxadiazole derivatives showed anti-proliferative properties against MCF-7 breast cancer cell Line [15-17], ovarian cancer cell lines (ovcar-3 and Hela) [18, 19], human lung cancer cell line (L2987) [20] and colorectal cancer [21]. These compounds have different mechanisms of action, which is very important in view of the observed resistance of tumors to standard drug treatment. Moreover, some of the 1,3,4-oxadiazole derivatives are inhibitors of growth factors, enzymes, kinases and receptors that are mediated in cancer treatment [22-27].

On the other hand, compounds having triazole moieties, such as vorozole, letrozole and anastrozole, appeared to be very effective aromatase inhibitors, which in turn prevented cancer, especially breast cancer [28-32]. Furthermore, certain 1,2,4-triazole derivatives have been reported as antitumor agents [33-35].

Inspired by these finding and in order to develop new anticancer therapeutic agents, we were encouraged to synthesize series of 2,3-dihydro-1,3,4-oxadiazoles and their bioisosters, 4,5-dihydro-1,2,4-triazoles, with incorporation of pyridine moiety, halogens, methoxy and methyl groups which are expected to allows simultaneous modulation of electronic, lipophilic, and steric parameters. These parameters can critically influence both the pharmacodynamic and pharmacokinetic properties of the synthesized compounds and expected to enhance their anticancer activities.

MATERIALS AND METHODS

Melting points are uncorrected and were determined on a Stuart melting point apparatus (Stuart Scientific, Redhill, UK). The FT-IR spectra (KBr) were recorded on Shimadzu FT-IR 110 spectrophotometer (Shimadzu, Koyoto, Japan) by using 1% potassium bromide discs. ^{1}H-NMR spectra were recorded on a Bruker proton 300 MHz (Bruker, Munuch, Germany) spectrometer using DMSO-d_6 as a solvent and tetramethylsilane (TMS) as an internal standard. Chemical shift values are listed in δ scale. Mass spectra were determined using a GC/MS Mat 112 S at the 70ev spectrometer. Elemental analysis (C, H, N) were performed on a Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA) at the microanalytical laboratories of the Faculty of Science, Cairo University. Completion of the reaction was monitored by thin-layer chromatography (TLC) using precoated aluminum sheets silica gel (Merck, 60 F254). Visualization was accomplished with an ultraviolet UV lamp (Merck, Darmstadt, Germany). Synthesized compounds were purified by the re-crystallization process. The purity of the compounds was checked by a single spot in TLC and the solvent system for TLC was determined on a trial and error basis. All the chemicals and solvents used were of commercial grade.

Experimental procedures

Synthesis of N'-(substituted benzylidene) isonicotinohydrazide (3a-h)

Isonicotinohydrazide 1 (0.001 mol), appropriate aromatic aldehyde 2 (0.001 mol), ethanol (30 ml) and a catalytic amount of acetic acid were added to the round-bottomed flask (RBF) and the reaction mixture was refluxed for 3 h. The completion of the reaction was monitored by TLC. The reaction mixture was poured into crushed ice to obtain a solid product. The obtained precipitate was filtered under suction, washed thoroughly with water, dried and recrystallized from methanol.

Synthesis of 1-(2-substituted phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (4 a-h)

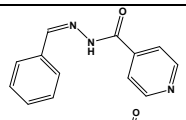
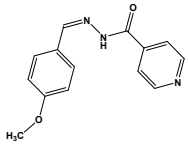
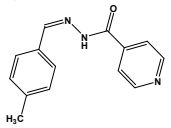
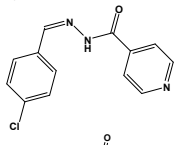
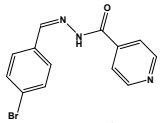
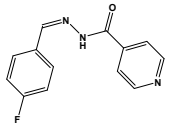
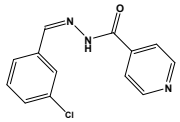
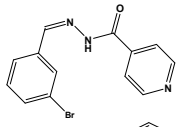
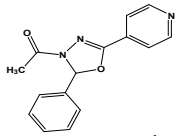
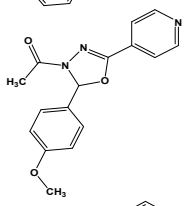
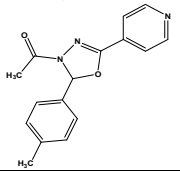
To arylidenearylhydrazides 3a-e (0.01 mol), acetic anhydride (40 ml) was added and the reaction mixture was refluxed for 2 h. The crushed ice was added to the reaction mixture to obtain precipitate, which was filtered off and, washed with 10% aqueous solution of

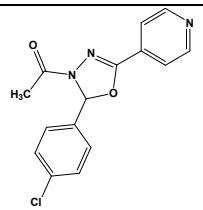
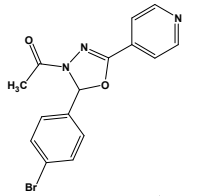
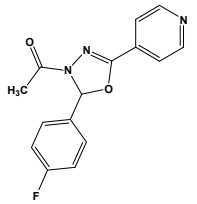
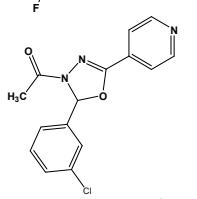
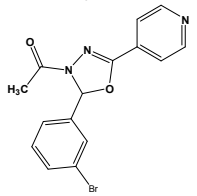
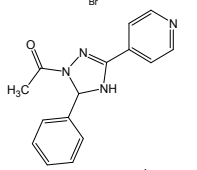
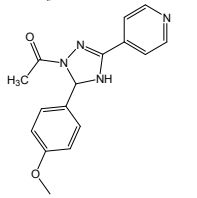
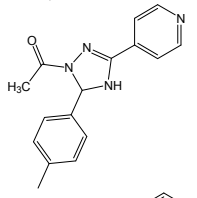
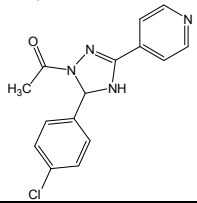
$NaHCO_3$ to remove the acetic acid. The obtained solid was recrystalized from ethanol.

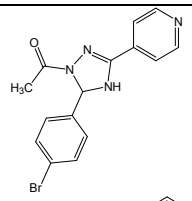
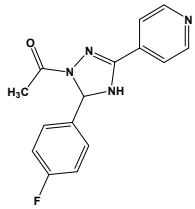
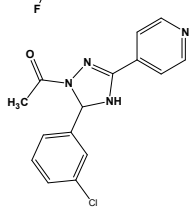
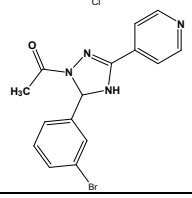
Synthesis of 1-(5-(4-substituted phenyl)-3-(pyridin-4-yl)-4,5-dihydro-1,2,4-triazol-1-yl)ethanones (5a-h)

Compounds 4a-h (0.01 mol) was added during 10 min to stirred and cooled solution of ammonia solution (10 ml) and water (10 ml). The temperature during the addition was kept below 20 °C. Then the reaction mixture was stirred for 2 h at room temperature. The reaction mixture was poured into crushed ice and acidify with hydrochloric acid to obtain precipitate, which was filtered off and, washed with water. The obtained solid was recrystallized from ethanol. IUPAC names and the molecular structures of the synthesized compounds 3a-h, 4a-h and 5a-h are shown in table 1.

Table 1: List of the IUPAC name and the molecular structure of the synthesized compounds (3a-e, 4a-e and 5a-e)

Comp. No.	Molecular structure	IUPAC name
3a		N'-benzylideneisonicotinohydrazide
3b		N'-(4-methoxybenzylidene) isonicotinohydrazide
3c		N'-(4-methylbenzylidene) isonicotinohydrazide
3d		N'-(4-chlorobenzylidene) isonicotinohydrazide
3e		N'-(4-bromobenzylidene) isonicotinohydrazide
3f		N'-(4-fluorobenzylidene) isonicotinohydrazide
3g		N'-(3-chlorobenzylidene) isonicotinohydrazide
3h		N'-(3-bromobenzylidene) isonicotinohydrazide
4a		1-(2-Phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone
4b		1-(2-(4-Methoxyphenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone
4c		1-(5-(Pyridin-4-yl)-2-p-tolyl-1,3,4-oxadiazol-3(2H)-yl)ethanone

4d		1-(2-(4-Chlorophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone
4e		1-(2-(4-Bromophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone
4f		1-(2-(4-Fluorophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone
4g		1-(2-(3-Chlorophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone
4h		1-(2-(3-Bromophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone
5a		1-(5-Phenyl-3-(pyridin-4-yl)-4,5-dihydro-1,2,4-triazol-1-yl)ethanone
5b		1-(5-(4-Methoxyphenyl)-3-(pyridin-4-yl)-4,5-dihydro-1,2,4-triazol-1-yl)ethanone
5c		1-(3-(Pyridin-4-yl)-5-p-tolyl-4,5-dihydro-1,2,4-triazol-1-yl)ethanone
5d		1-(5-(4-Chlorophenyl)-3-(pyridin-4-yl)-4,5-dihydro-1,2,4-triazol-1-yl)ethanone

5e		1-(5-(4-Bromophenyl)-3-(pyridin-4-yl)-4,5-dihydro-1,2,4-triazol-1-yl)ethanone
5f		1-(5-(4-Fluorophenyl)-3-(pyridin-4-yl)-4,5-dihydro-1,2,4-triazol-1-yl)ethanone
5g		1-(5-(3-Chlorophenyl)-3-(pyridin-4-yl)-4,5-dihydro-1,2,4-triazol-1-yl)ethanone
5h		1-(5-(3-Bromophenyl)-3-(pyridin-4-yl)-4,5-dihydro-1,2,4-triazol-1-yl)ethanone

Anticancer activities

The Cytotoxic activity of the synthesized compounds was measured against human mammary carcinoma cell line (MCF7) in the National Cancer Institute, Cairo University. The screening involves a calculation of the percentage growth or the surviving fraction of the drug-treated cell lines compared with untreated control using Sulforhodamine B (SRB) colorimetric assay [36]. Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the compounds to allow attachment of cells to the wall of the plate. Different concentrations of the compound under test (0.0, 1.0, 2.5, 5.0 and 10.0 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in an atmosphere of 5% CO_2. After 48 h, cells were fixed and stained for 30 min with 0.4% (wt/vol) SRB dissolved in 1% acetic acid. Excess unbound dye was removed by four washes with 1% acetic acid and the attached stain was recovered with Tris–EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve of the tumor cell line after the specified compound. The results were described in the table 2.

RESULTS AND DISCUSSION

Chemistry

In this study, N'-(substituted benzylidene) isonicotinohydrazides 3a-h, 2,3-dihydro-1,3,4-oxadiazoles 4a-h and 4,5-dihydro-1,2,4-triazole derivatives 5a-h (table 1) were synthesized, of which compounds 3a-d and 4a-d have been reported before [37].

The synthesis of 1-(2-substituted phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanones was performed in two steps: the synthesis of N'-(substituted benzylidene) isonicotinohydrazides 3a-h from the substituted aromatic aldehyde 2a-h and synthesis of 1-(2-substituted phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl) ethanones 4a-h from the Schiff's bases 3a-h using acetic anhydride (fig. 1). On the other hand, the 2,3-dihydro-1,3,4-oxadiazole rings of compounds 4a-e were transformed into the corresponding 4,5-dihydro-1,2,4-triazole derivatives 5a-e via ring opening of the oxadiazole.

The synthesis of N'-(substitutedbenzylidene) isonicotinohydrazides 3a-h shown to be simple and practical, achieving a good yield, as described in previous studies [37-39]. On the other hand, for the series of 1-(2-substituted phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl) ethanones 4a-h were synthesized according to the previously described procedures [36-39]. Substitutions at the phenyl moiety, reaction time and temperature of the reaction are the main parameters that identify the yield of the products [38, 40]. In this study, the reaction time and temperature of the reaction were adjusted to 3 h and 120 °C respectively to achieve yield in the range from 70% to 82%. Finally, the 1-(2-substituted phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl) ethanones 4a-h were reacted with ammonia to give 1-(5-(4-substituted phenyl)-3-(pyridin-4-yl)-4,5-dihydro-1,2,4-triazol-1-yl)ethanones 5a-h in good yield via ring-opening rearrangement of the oxadiazole.

During the synthesis procedure, thin layer chromatography (TLC) analysis was carried out to verify the formation of the synthesized compounds. The structural elucidation of these compounds was confirmed through 1H NMR, IR and mas spectral data. The IR spectrum for intermediate compounds 3a-h showed characteristic peaks of N-H around (3420 cm^{-1}) and of C-O-NH of amide in range of 1632-1635 cm^{-1}. The IR spectra for compounds 4a-h showed characteristic peaks of carbonyl of CH_3C-O in the range of 1690-1695 cm^{-1}. The IR spectra for final compounds 5a-h showed characteristic peaks of NH in the range of 3205-3212 cm^{-1}. The ^1H NMR spectrum of the compounds 3a-h contains a multiplet in the range of 7.10-7.95 ppm for aromatic protons, a proton of NH appeared as singlet in the around 8.01 ppm which exchanged with D2O while a proton of CH=N appeared as singlet in the range of 8.18-8.21 ppm. The ^1H NMR spectra of the compounds 4a-h contains singlet around 2.26 ppm for CH_3 protons, protons of CH of oxadiazole ring appeared as a singlet in the range of 5.53-5.67 ppm and aromatic protons appeared in the range of 7.20-8.21 ppm. The ^1H NMR spectra of the final compounds 5a-h contains singlet around 6.15 and 2.40 ppm attributed for CH and NH protons of triazole ring, respectively. Mass spectra proved parent peaks of the synthesized compounds confirming the molecular weight.

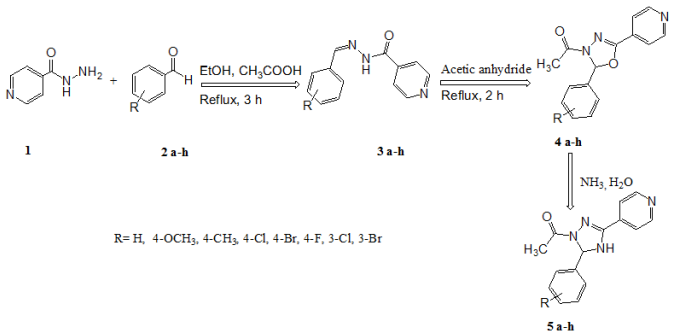


Fig. 1: Synthesis of compounds 3a-e, 4a-e and 5a-e investigated in this work

Spectral characterization of synthesized compounds

N'-benzylideneisonicotinohydrazide (3a): Yield 73%, mp 86-88 ° C. IR spectrum, ν, cm^{-1}: 1633, 1650, 3052, 3420. ^1H NMR (300 MHz, DMSO-d_6): 7.20-7.93 (m, 9H, ArH), 8.01 (s, 1H, NH, exch. With D_2O), 8.20 (s, 1H, CH=N). MS: (m/z) 225 (M^+) observed for $C_{13}H_{13}N_3O$, Anal calcd: C, 69.32; H, 4.92; N, 18.66; found: C, 69.59; H, 5.15; N, 18.92;

N'-(4-methoxybenzylidene)isonicotinohydrazide (3b): Yield 80%, mp 102-104 °C. IR spectrum, ν, cm^{-1}: 1632, 1650, 3061, 3424. ^1H NMR (300 MHz, DMSO-d_6): 3.85 (s, 3H, OCH_3), 7.21-7.89 (m, 8H, ArH), 8.08 (s, 1H, NH, exch. With D_2O), 8.21 (s, 1H,-CH=N). MS: (m/z) 255 (M^+) observed for $C_{14}H_{13}N_3O_2$, Anal calcd: C, 65.87; H, 5.13; N, 16.46; found: C, 65.39; H, 5.19; N, 16.58;

N'-(4-methylbenzylidene)isonicotinohydrazide (3c): Yield 77%, mp 113-115 °C. IR spectrum, ν, cm^{-1}: 1635, 1652, 3063, 3424. ^1H NMR (300 MHz, DMSO-d_6): 2.51 (s, 3H, CH_3), 7.31-7.95 (m, 8H, ArH), 8.10 (s, 1H, NH, exch. With D_2O), 8.18 (s, 1H,-CH=N). MS: (m/z) 239 (M^+) observed for $C_{14}H_{13}N_3O$, Anal calcd: C, 70.28; H, 5.48; N, 17.56; found: C, 70.49; H, 5.29; N, 17.88;

(N'-(4-chlorobenzylidene)isonicotinohydrazide (3d): Yield 72%, mp 105-107 °C. IR spectrum, ν, cm^{-1}: 1634, 1651, 3059, 3422. ^1H NMR (300 MHz, DMSO-d_6): 7.30-7.96 (m, 8H, ArH), 8.00 (s, 1H, NH, exch. With D_2O), 8.18 (s, 1H,-CH=N). MS: (m/z) 259 (M^+) observed for $C_{13}H_{10}ClN_3O$, Anal calcd: C, 60.12; H, 3.88; N, 16.18; found: C, 60.41; H, 4.11; N, 16.52;

N'-(4-bromobenzylidene)isonicotinohydrazide (3e): Yield 74%, mp 101-103 °C. IR spectrum, ν, cm^{-1}: 1635, 1650, 3060, 3420. ^1H NMR (300 MHz, DMSO-d_6): 7.10-7.95 (m, 8H, ArH), 8.02 (s, 1H, NH, exch. With D_2O), 8.19 (s, 1H,-CH=N). MS: (m/z) 305 (M^++2), 303 (M^+) observed for $C_{13}H_{10}BrN_3O$, Anal calcd: C, 51.34; H, 3.31; N, 13.82; found: C, 51.49; H, 3.18; N, 13.56;

N'-(4-fluorobenzylidene)isonicotinohydrazide (3f): Yield 70%, mp 109-111 °C. IR spectrum, ν, cm^{-1}: 1633, 1653, 3064, 3421. ^1H NMR (300 MHz, DMSO-d_6): 7.23-8.12 (m, 8H, ArH), 8.02 (s, 1H, NH, exch. With D_2O), 8.21 (s, 1H,-CH=N). MS: (m/z) 243 (M^+) observed for $C_{13}H_{10}FN_3O$, Anal calcd: C, 64.19; H, 4.14; N, 17.28; found: C, 64.54; H, 3.96; N, 17.55;

(N'-(3-chlorobenzylidene)isonicotinohydrazide (3g): Yield 72%, mp 99-101 °C. IR spectrum, ν, cm^{-1}: 1632, 1650, 3059, 3420. ^1H NMR (300 MHz, DMSO-d_6): 7.28-7.92 (m, 8H, ArH), 8.01 (s, 1H, NH, exch. With D_2O), 8.19 (s, 1H,-CH=N). MS: (m/z) 259 (M^+) observed for $C_{13}H_{10}ClN_3O$, Anal calcd: C, 60.12; H, 3.88; N, 16.18; found: C, 59.90; H, 3.94; N, 16.23;

N'-(2-bromobenzylidene)isonicotinohydrazide (3h): Yield 69%, mp 97-99 °C. IR spectrum, ν, cm^{-1}: 1633, 1652, 3065, 3424. ^1H NMR (300 MHz, DMSO-d_6): 7.19-7.98 (m, 8H, ArH), 8.00 (s, 1H, NH, exch. With D_2O), 8.18 (s, 1H,-CH=N). MS: (m/z) 305 (M^++2), 303 (M^+) observed for $C_{13}H_{10}BrN_3O$, Anal calcd: C, 51.34; H, 3.31; N, 13.82; found: C, 51.21; H, 3.62; N, 13.93;

1-(2-Phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (4a): Yield 70%, mp 112-114 °C. IR spectrum, ν, cm^{-1}: 1690, 1625, 2950. ^1H NMR (300 MHz, DMSO-d_6): 2.27 (s, 3H, CH_3), 5.51 (s, 1H, CH, oxadiazole), 7.10-7.85 (m, 9H, ArH). MS: (m/z) 267 (M^+) observed for $C_{15}H_{13}N_3O_2$, Anal calcd: C, 67.40; H, 4.90; N, 15.72; found: C, 67.80; H, 4.79; N, 15.58;

1-(2-(4-Methoxyphenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (4b): Yield 77 %, mp 127-129 °C. IR spectrum, ν, cm^{-1}: 1691, 1620, 2980. ^1H NMR (300 MHz, DMSO-d_6): 2.27 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 5.53 (s, 1H, CH, oxadiazole), 7.20-7.91 (m, 8H, ArH). MS: (m/z) 297 (M^+) observed for $C_{16}H_{15}N_3O_3$, Anal calcd: C, 64.64; H, 5.09; N, 14.13; found: C, 64.35; H, 5.27; N, 13.89;

1-(5-(Pyridin-4-yl)-2-p-tolyl-1,3,4-oxadiazol-3(2H)-yl)ethanone (4c): Yield 73%, mp 90-92 °C. IR spectrum, ν, cm^{-1}: 1690, 1625, 2982. ^1H NMR (300 MHz, DMSO-d_6): 2.25 (s, 3H, CH_3), 2.51(s, 3H, CH_3), 5.52 (s, 1H, CH, oxadiazole), 7.10-7.91 (m, 8H, ArH). MS: (m/z) 281 (M^+) observed for $C_{16}H_{15}N_3O_2$, Anal calcd: C, 68.31; H, 5.37; N, 14.94; found: C, 68.61; H, 5.78; N, 14.79;

1-(2-(4-Chlorophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (4d): Yield 78%, mp 121-123 °C. IR spectrum, ν, cm^{-1}: 1695, 1620, 2985. ^1H NMR (300 MHz, DMSO-d_6): 2.26 (s, 3H, CH_3), 5.52 (s, 1H, CH, oxadiazole), 7.30-7.96 (m, 8H, ArH). MS: (m/z) 301 (M^+) observed for $C_{15}H_{12}ClN_3O_2$, Anal calcd: C, 59.71; H, 4.01; N, 13.93; found: C, 59.44; H, 4.38; N, 13.65;

1-(2-(4-Bromophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (4e): Yield 82%, mp 160-162 °C. IR spectrum, ν, cm^{-1}: 1690, 1621, 2982. ^1H NMR (300 MHz, DMSO-d_6): 2.26 (s, 3H, CH_3), 5.53 (s, 1H, CH, oxadiazole), 7.20-7.91 (m, 8H, ArH). MS: (m/z) 349 (M^++2), 347 (M^+) observed for $C_{15}H_{12}BrN_3O_2$, Anal calcd: C, 52.04; H, 3.49; N, 12.14; found: C, 52.34; H, 3.58; N, 12.52;

1-(2-(4-Fluorophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (4f): Yield 77%, mp 125-127 °C. IR spectrum, ν, cm^{-1}: 1695, 1620, 2985. ^1H NMR (300 MHz, DMSO-d_6): 2.25 (s, 3H, CH_3), 5.67 (s, 1H, CH, oxadiazole), 7.45-8.21 (m, 8H, ArH). MS: (m/z) 285 (M^+) observed for $C_{15}H_{12}FN_3O_2$, Anal calcd: C, 63.15; H, 4.24; N, 14.73; found: C, 62.91; H, 4.52; N, 14.88;

1-(2-(3-Chlorophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (4g): Yield 76%, mp 117-119 °C. IR spectrum, ν, cm^{-1}: 1696, 1624, 2988. ^1H NMR (300 MHz, DMSO-d_6): 2.26 (s, 3H, CH_3), 5.59 (s, 1H, CH, oxadiazole), 7.42-7.99 (m, 8H, ArH). MS: (m/z) 301 (M^+) observed for $C_{15}H_{12}ClN_3O_2$, Anal calcd: C, 59.71; H, 4.01; N, 13.93; found: C, 59.84; H, 4.27; N, 14.16;

1-(2-(3-Bromophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (4h): Yield 75%, mp 140-142 °C. IR spectrum, ν, cm^{-1}: 1695, 1624, 2986. ^1H NMR (300 MHz, DMSO-d_6): 2.26 (s, 3H, CH_3), 5.62 (s, 1H, CH, oxadiazole), 7.40-8.08 (m, 8H, ArH). MS: (m/z) 349 (M^++2), 347 (M^+) observed for $C_{15}H_{12}BrN_3O_2$, Anal calcd: C, 52.04; H, 3.49; N, 12.14; found: C, 52.19; H, 3.82; N, 11.94;

1-(5-Phenyl-3-(pyridin-4-yl)-4,5-dihydro-1,2,4-triazol-1-yl)ethanone (5a): Yield 62%, mp 110-112 °C. IR spectrum, ν, cm^{-1}: 1699, 1625, 3010, 3210. ^1H NMR (300 MHz, DMSO-d_6): 2.31(s, 3H, CH_3), 2.41(s, 1H, NH, triazole), 6.20 (s, 1H, CH, triazole), 7.15-7.82 (m, 9H, ArH). MS: (m/z) 266 (M^+) observed for $C_{15}H_{14}N_4O$, Anal calcd: C, 67.65; H, 5.30; N, 21.04; found: C, 67.50; H, 5.54; N, 20.86;

1-**(5-(4-Methoxyphenyl)-3-(pyridin-4-yl)-4,5-dihydro-1,2,4-triazol-1-yl)ethanone (5b):** Yield 75%, mp 139-141 °C. IR spectrum, ν, cm^{-1}: 1701, 1625, 2997, 3208. ^1H NMR (300 MHz, DMSO-d_6): 2.31(s, 3H, CH_3), 2.39 (s, 1H, NH, triazole), 3.92 (s, 3H, OCH_3), 6.21 (s, 1H, CH, triazole), 7.32-8.12 (m, 8H, ArH). MS: (m/z) 296 (M^+) observed for $C_{16}H_{16}N_4O_2$, Anal calcd: C, 64.85; H, 5.44; N, 18.91; found: C, 64.66; H, 5.65; N, 19.09;

1-(3-(Pyridin-4-yl)-5-p-tolyl-4,5-dihydro-1,2,4-triazol-1-yl)ethanone (5c): Yield 67%, mp 100-102 °C. IR spectrum, ν, cm^{-1}: 1690, 1625, 2982, 3212. ^1H NMR (300 MHz, DMSO-d_6): 2.24 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 2.40 (s, 1H, NH, triazole), 6.12 (s, 1H, CH, triazole), 7.19-8.01 (m, 8H, ArH). MS: (m/z) 280 (M^+) observed for $C_{16}H_{15}N_4O$, Anal calcd: C, 68.55; H, 5.75; N, 19.99; found: C, 68.81; H, 5.98; N, 19.81;

1-(5-(4-Chlorophenyl)-3-(pyridin-4-yl)-4,5-dihydro-1,2,4-triazol-1-yl)ethanone (5d): Yield 77%, mp 132-134 °C. IR spectrum, ν, cm^{-1}: 1695, 1620, 2985, 3205. ^1H NMR (300 MHz, DMSO-d_6): 2.28 (s, 3H, CH_3), 2.38 (s, 1H, NH, triazole), 6.15 (s, 1H, CH, triazole), 7.38-8.13 (m, 8H, ArH). MS: (m/z) 300 (M^+) observed for $C_{15}H_{13}ClN_4O$, Anal calcd: C, 59.91; H, 4.36; N, 18.63; found: C, 59.76; H, 4.21; N, 18.40;

1-(5-(4-Bromophenyl)-3-(pyridin-4-yl)-4,5-dihydro-1,2,4-triazol-1-yl)ethanone (5e): Yield 79%, mp 136-138 °C. IR spectrum, ν, cm^{-1}: 1700, 1621, 2996, 3210. ^1H NMR (300 MHz, DMSO-d_6): 2.29 (s, 3H, CH_3), 2.40 (s, 1H, NH, triazole), 6.18 (s, 1H, CH, triazole), 7.27-7.98 (m, 8H, ArH). MS: (m/z) 346 (M^++2), 344 (M^+) observed for $C_{15}H_{13}BrN_4O$, Anal calcd: C, 52.19; H, 3.80; N, 16.32; found: C, 51.98; H, 3.98; N, 16.49;

1-(5-(4-Fluorophenyl)-3-(pyridin-4-yl)-4,5-dihydro-1,2,4-triazol-1-yl)ethanone (5f): Yield 77%, mp 141-143 °C. IR spectrum, ν, cm^{-1}: 1705, 1625, 2997, 3214. ^1H NMR (300 MHz, DMSO-d_6): 2.32 (s, 3H, CH_3), 2.41 (s, 1H, NH, triazole), 6.14 (s, 1H, CH, triazole), 7.56-8.42 (m, 8H, ArH). MS: (m/z) 284 (M^+) observed for $C_{15}H_{13}FN_4O$, Anal calcd: C, 63.37; H, 4.61; N, 19.71; found: C, 62.99; H, 4.86; N, 19.49;

1-(5-(3-Chlorophenyl)-3-(pyridin-4-yl)-4,5-dihydro-1,2,4-triazol-1-yl)ethanone (5g): Yield 74%, mp 135-137 °C. IR spectrum, ν, cm^{-1}: 1692, 1622, 2989, 3208. ^1H NMR (300 MHz, DMSO-d_6): 2.27 (s, 3H, CH_3), 2.39 (s, 1H, NH, triazole), 6.13 (s, 1H, CH, triazole), 7.34-8.12 (m, 8H, ArH). MS: (m/z) 300 (M^+) observed for $C_{15}H_{13}ClN_4O$, Anal calcd: C, 59.91; H, 4.36; N, 18.63; found: C, 59.64; H, 4.63; N, 18.83;

1-(5-(3-Bromophenyl)-3-(pyridin-4-yl)-4,5-dihydro-1,2,4-triazol-1-yl)ethanone (5h): Yield 79%, mp 139-141 °C. IR spectrum, ν, cm^{-1}: 1704, 1624, 3005, 3218. ^1H NMR (300 MHz, DMSO-d_6): 2.28 (s, 3H, CH_3), 2.42 (s, 1H, NH, triazole), 6.17 (s, 1H, CH, triazole), 7.33-8.16 (m, 8H, ArH). MS: (m/z) 346 (M^++2), 344 (M^+) observed for $C_{15}H_{13}BrN_4O$, Anal calcd: C, 52.19; H, 3.80; N, 16.32; found: C, 52.43; H, 3.52; N, 16.14;

Cytotoxic activity

Cytotoxic activity results were summarized in table 2. All the synthesized were evaluated to their cytotoxic activity against breast cancer cell line MCF7. Most of the tested compounds exhibited moderate to good cytotoxic activity with percentage inhibition in cell proliferation at an IC_{50} value of 9.84 and 39.21 µM.

With regard to the benzylidene isonicotinohydrazides 3a-h, the compounds 3a-h exhibited moderate to good cytotoxic activity with IC_{50} ranges IC_{50} 17.26-38.01 µM when compared to the reference standard doxorubicin ($IC_{50,}$ 8.02 µM), while the compound 3d showed the best activity among this series with IC_{50} value of 16.28 µM.

Cyclization of 3a-h into the corresponding 2,3-dihydro-1,3,4-oxadiazoles 4a-h resulted in an increased in cytotoxic activity with IC_{50} ranges 8.04-27.08 µM. The compound 4d showed equipotent activity ($IC_{50,}$ 8.04 µM) to that of the reference standard, doxorubicin ($IC_{50,}$ 8.02 µM). Finally, the ring transformation of 2,3-dihydro-1,3,4-oxadiazoles 4a-h into the corresponding 4,5-dihydro-1,2,4-triazoles 5a-h resulted in slightly decrease in cytotoxic activity with IC_{50} ranges 9.78-28.54 µM. The type and position of substituent on phenyl moiety greatly affect anticancer activities.

Table 2: *In vitro* cytotoxic activity of the synthesized compounds against MCF-7 cell line

Compound No	Compound concentration (µM)				IC_{50} (µM)
	5 µM	12.5 µM	25 µM	50 µM	
	Surviving fraction (mean±SEM)[a]				
3a	0.983±0.057	0.695±0.041	0.364±0.041	0.376±0.043	19.52
3b	0.721±0.031	0.465±0.041	0.381±0.029	0.181±0.031	18.22
3c	0.765±0.051	0.569±0.028	0.445±0.027	0.385±0.021	16.28
3d	0.397±0.031	0.281±0.026	0.229±0.015	0.295±0.004	17.26
3e	0.333±0.051	0.312±0.023	0.121±0.035	0.110±0.001	17.43
3f	0.745±0.182	0.478±0.087	0.393±0.065	0.192±0.068	18.65
3g	0.866±0.060	0.651±0.025	0.582±0.030	0.397±0.008	36.45
3h	0.882±0.038	0.655±0.017	0.568±0.011	0.422±0.076	38.01
4a	0.373±0.033	0.281±0.021	0.152±0.032	0.141±0.013	15.21
4b	0.352±0.012	0.226±0.015	0.291±0.045	0.425±0.043	11.20
4c	0.299±0.110	0.281±0.170	0.279±0.024	0.289±0.042	8.04
4d	0.682±0.017	0.495±0.023	0.096±0.034	0.099±0.021	12.01
4e	0.452±0.018	0.262±0.022	0.365±0.021	0.280±0.015	10.22
4f	0.681±0.182	0.485±0.087	0.321±0.065	0.121±0.068	14.73
4g	0.853±0.013	0.517±0.052	0.379±0.043	0.345±0.091	25.62
4h	0.841±0.022	0.678±0.018	0.454±0.027	0.235±0.015	27.08
5a	0.378±0.070	0.287±0.017	0.158±0.050	0.145±0.021	16.23
5b	0.713±0.038	0.482±0.076	0.352±0.008	0.117±0.003	12.54
5c	0.589±0.16	0.487±0.069	0.254±0.081	0.146±0.065	9.78
5d	0.563±0.032	0.312±0.017	0.212±0.011	0.289±0.021	13.09
5e	0.663±0.012	0.412±0.015	0.312±0.045	0.189±0.043	11.59
5f	0.698±0.090	0.435±0.026	0.391±0.072	0.102±0.034	15.25
5g	0.832±0.061	0.644±0.015	0.329±0.071	0.342±0.015	26.62
5h	0.853±0.043	0.617±0.061	0.389±0.070	0.325±0.093	28.54
Doxorubicin	0.314±0.032	0.309±0.016	0.251±0.023	0.266±0.032	8.02

[a]Each value is the mean of three values±standard error.

CONCLUSION

In this study, a series of benzylidene isonicotinohydrazide derivatives 3a-h, 1,3,4-oxadiazol-3(2H)-yl) ethanones 4a-h and 1-(5-(4-substituted phenyl)-3-(pyridin-4-yl)-4,5-dihydro-1,2,4-triazol-1-yl) ethanones 5a-h have been synthesized and evaluated for their antitumor activities. These compounds were characterized using standard spectroscopic and spectrometric techniques, confirming the integrity of these molecules. The biological activities of all of the synthesized compounds were examined against breast cancer MCF7

cell lines. The results of *in vitro* anticancer activity indicated that most of the tested compounds exhibited moderate to good activities. On the other hand, compound **4c** exhibited nearly similar cytotoxic activity (IC_{50}= 8.04 μM) to that of the standard drug doxorubicin (IC_{50}= 8.02 μM).

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AUTHORS CONTRIBUTIONS

All authors had equally contributed to the work.

CONFLICTS OF INTERESTS

Authors declare no conflicts of interest.

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