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Synthesis and evaluation of some new imidazolone bearing phthalyl and tosyl amino acids moieties as anti-cancer agents supported by molecular docking

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Abstract

A facile and convenient synthesis of imidazolone-incorporated phthalyl and/or *p*-tosyl amino acid derivatives was described. The reaction of oxazolone derivative **2** with *p*-phenylenediamine followed by coupling of the free amino group in imidazolone **3** with various protected amino acids such as threonine, valine, alanine, serine, glutamine, glycine, leucine, phenylalanine, arginine, methionine, and aspartic acid using *N,N*-dicyclohexylcarbodiimide (DCC) as the dehydrating agent afforded the desired compounds **4a-j** and **6a-j**. Deprotection of the amino group in *N*-phthaloyl derivatives **4a-j** was accomplished with good yields through refluxing with an ethanolic solution of hydrazine hydrate to afford unprotected aminoacyl derivatives **5a-j**. All chemical structures of imidazolone derivatives were elucidated by elemental analysis as well as spectral data (IR, MS, and ^{1}H NMR spectra). The anti-cancer activity of some synthesized compounds was evaluated against carcinogenic human cell lines, namely, human prostate cancer (PC3) and mammary gland breast cancer (MCF-7), and some of them showed promising cytotoxicity comparable with the standard drug doxorubicin (DOX). A molecular docking study between various receptors and the target compounds was performed, and it confirmed a good correlation between their strength of receptor-binding and anti-cancer activities.

Keywords: Amino acids; Imidazolone; Cyclization; Anti-cancer agents; Molecular docking.

1. Introduction

Cancer is a global problem that is anticipated to significantly worsen our current global health catastrophe. Worldwide, there was 19.3 million new cancer cases, plus 9.9 million cancer deaths in 2020. Breast cancer (BC) and prostate cancer (PC) are among the most common cancers among the roughly 200 different types of human cancers because of their distinctive characteristics, which include their origin, gene expression patterns, acquired mutations, modified transcriptional and signalling networks, metabolic activities, the impact of their microenvironments, host immune responses, and overall health[1]. BC is a prevalent type of cancer among women in both the developing and developed world and it is the most common type of cancer that kills women. According to reports, survival rates vary from more than 80% in developed countries to less than 40% in low-income countries. in 2020, PC will be the second most common and fifth significant cause of cancer-related death among men. PC is responsible for 1.4 million of the total new instances of male cancer and 375,000 of all male cancer deaths globally[1].

Oxazolones incorporated heterocyclic compounds have drawn much attention due to their biological and pharmaceutical activities, which include anticancer [2,3], immunomodulatory activity [4], anti-angiogenic [5], antioxidant [6,7],anti-convulsant [8], antibacterial [9], anti-diabetic [10], antifungal [11], sedative [12],tyrosinase inhibitor [13], as well as analgesic and anti-inflammatory

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[6,12].The reactivity of oxazolones towards various amines, including *p*-phenylenediamine, was demonstrated to produce imidazolyl-based heterocycles [14,15]. Diverse biological properties, including anti-cancer [16], anti-bacterial [17-20], anti-fungal [21], anti-inflammatory [22-24], cardio-activity [25], and angiotensin II receptor antagonistic activity [26] are displayed by imidazolone-based heterocyclic compounds [27].Trisubstituted imidazolones exhibit high levels of apoptosis in human leukemia cells [28] as they operate as inhibitors of the insulin-like growth factor 1 receptor, which is implicated in the formation and growth of numerous tumors. Thus, they can be used for cancer treatment[29]as well as for their effectiveness to prevent prostate cancer (PC3) from migration [30].Additionally, imidazolones manifest a wide range of bioactivities, including leishmanicidal and immunomodulatory effects [31]. (Figure 1) showed some of structures for imidazolone derivatives with approved anti-tumor activity [28,32-34].

In addition, *N*-protected amino acids are significant intermediates incorporated in the synthesis of enormous bioactive organic compounds, including peptides [35], therapeutic chemicals [36], and polymer substances [37,38]. Among that, they are utilized as anti-tumor[39],anti-bacterial [40], anti-inflammatory, and immunomodulatory activities [40,41]. Therefore, and in accordance with the above observations, we were encouraged to develop and synthesize novel imidazolone-incorporated amino acid derivatives with the aim of obtaining some new heterocyclic systems with potentially enhanced biological properties and promising anti-tumor activity.

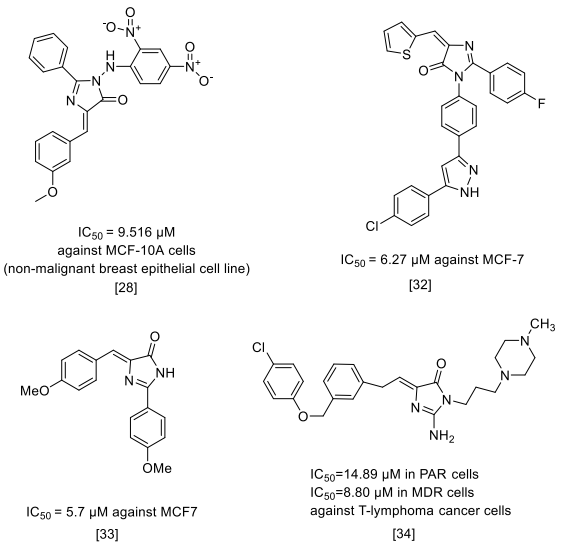


Figure 1. Imidazolone-tethering compounds with potent anti-tumor activity

2. Materials and Methods

Materials

Our whole chemical supply came from Sigma-Aldrich and solvents were bought from El-Nasr Chemicals. Additionally, we used silica gel polyester sheets for thin-layer chromatography (TLC) on all of the products (Kieselgel 60 F254, 0.20 mm, Merck). All melting points are measured in degrees Celsius by the melting point apparatus Gallen-Kamp and are uncorrected. On Fourier transformer IR spectrophotometer smart OMNIC-transmission, Nicolet iS10, at Faculty of Science, Benha University, we captured IR spectra (KBr). At the Microanalytical Center of the Faculty of Science at Zagazig University, the ^{1}H NMR spectra were detected on a Bruker NMR 400 MHz spectrophotometer using tetramethylsilane (TMS) as an internal reference and DMSO-d_6 as the solvent. Mass spectrums were carried out on direct Inlet part to mass analyzer in Thermo Scientific GCMS model ISQ at the regional center for Mycology and Biotechnology (RCMB), Al-Azhar university, Nasr City, Cairo. Anti-cancer activities were carried out in the Pharmacognosy Department of the Faculty of Pharmacy at Mansoura University.

Synthesis

2-((4-(4-methoxybenzylidene)-5-oxo-4,5-dihydrooxazol-2-yl)methyl)isoindoline-1,3-dione (2)

N-phthaloylglycylglycine (**1**, 10.0 mmol), *p*-anisaldehyde (10.0 mmol), and anhydrous sodium acetate (15.0 mmol) were combined and refluxed for 5 h in acetic anhydride (30 mL). After cooling, the reaction mixture was filtered, and the separated solid was recrystallized from ethanol to give oxazolone **2**. Yield, 65%; mp 270-272 °C; IR (cm^{-1}): υ_{max}3045 (C-H aromatic), 2943 (C-H aliphatic), 1775, 1720 and 1657 (C=O of cyclic imide and azlactone) and1628 (C=N). ^{1}H NMR (DMSO-d_6) δ 7.90-7.83 (m, 8H, Ar-H), 7.80 (s, 1H, =CH), 4.20 (s, 2H, CH_2), 3.90 (s, 3H, OCH_3). Mass spectrum showed$M^{\cdot+}$ at 362 (27.08 %) and the base peak at 118 (100%), and other fragments are 122 (56.85%), 149 (51.97%), 262 (70.61%), 272 (52.14%), 288 (52.71%), 293 (51.17%), 315 (58.28%).

2-((1-(4-Aminophenyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-2-yl)methyl)isoindoline-1,3-dione (3)

A mixture of compound **2** (10.0 mmol), *p*-phenylenediamine (10.0 mmol), and freshly fused sodium acetate (15.0 mmol) were all refluxed in 25 mL of acetic acid (glacial) for 8 h. The solution was poured into ice-cold water after it had cooled. Filtration was used to collect the product and recrystallized from ethanol/water. Yield, 80%; mp>

360ºC ; IR (cm^{-1}): υ_{max}3423, 3265 (NH_2), 3064 (C-H aromatic), 2920, 2852 (C-H aliphatic), 1771, 1717 and 1669 (C=Oof cyclic imides and imidazolone) and 1628 (C=N). ^{1}H NMR (DMSO-d_6) δ 9.88 (s, 2H, NH_2exchangeable with D_2O), 7.75-7.46 (m, 12H, Ar-H), 7.30 (s, 1H, =CH), 3.84 (s, 2H, CH_2), 3.60 (s, 3H, OCH_3). Mass spectrum showed molecular ion peak ($M^{\cdot+}$) at 452 (10.7 %) and the base peak at 222 (100%) and other fragments are 195 (65.46 %), 197 (90.84%), 202 (69.07%), 207 (71.82%), 231 (80.26%), 232 (65.79%), 306 (81.79%), 412 (69.39%).

General procedure for the synthesis of *N*-phthaloyl amino acid-imidazolone hybrids (4a-j)

A solution of *N*-phthaloyl amino acids (10.0 mmol) incorporated glycine, L-alanine, L-phenylalanine, L-valine, L-glutamine, DL-methionine, L-threonine, L-arginine, L-aspartic acid, and L-leucine were added to a well-stirred solution **3** (10.0 mmol) in dry THF (30 mL) and then DCC (20.0 mmol) were added at 0 °C. The reaction mixture was stirred for 24 h at 0°C and for an additional 24 h at room temperature. the remaining *N,N*-dicyclohexylurea (DCU) was filtered. The filtrate was vacuum evaporated, and the resulting product was dissolved in ethanol (25 mL), and the solution was then re-filtered to remove any remaining DCU. By evaporating the filtrate *in vacuo* and recrystallizing the compounds in the suitable solvents, the products **4a-j** were produced.

2-(1,3-Dioxoisoindolin-2-yl)-N-(4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)acetamide (4a)

Yield, 70%; mp 308-310°C; IR (cm^{-1}): υ_{max}: 3441 (NH), 3064 (C-H aromatic), 2933, 2854 (C-H aliphatic), , 1776, 1721, 1680 (C=O′s) and 1637 (C=N).^{1}H NMR (DMSO-d_6) δ 9.91 (s, 1H, NH), 8.40-7.33 (m, 16H, Ar-H), 7.30 (s, 1H, =CH), 4.41 (s, 2H, N-CH_2), 4.09 (s, 2H, CH_2) and 3.86 (s, 3H, OCH_3).

2-(1,3-Dioxoisoindolin-2-yl)-N-(4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)propenamide (4b)

Yield, 72%; mp 314-316°C ; IR (cm^{-1}): υ_{max} 3441 (NH), 3023(C-H aromatic), 2934, 2855 (C-H aliphatic), 1778, 1708, 1675(C=O′s) and 1637(C=N). ^{1}H NMR (DMSO-d_6) δ 9.91 (s, 1H, NH), 8.50-7.34 (m, 16H, Ar-H), 7.31 (s, 1H, =CH), 4.41 (m, 1H, N-CH), 4.09 (s, 2H, CH_2) 3.84 (s, 3H, OCH_3) and 1.20 (d, J=6.2Hz, 3H, CH_3).

2-(1,3-Dioxoisoindolin-2-yl)-N-(4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-3-phenylpropanamide (4c)

Yield, 74%; mp 328-330°C ; IR (cm^{-1}): υ_{max} 3428 (NH), 3058(C-H aromatic), 2933, 2855 (C-H aliphatic), 1776, 1711, 1669 (C=O′s) and1634 (C=N).^{1}H NMR (DMSO-d_6) δ 9.93 (s, 1H, NH), 8.58-7.35 (m, 21H, Ar-H), 7.33 (s, 1H, =CH), 4.41(t, J=1.5,7.7Hz, 1H, N-CH), 4.09 (s, 2H, CH_2) 3.86 (s, 3H, OCH_3) and 2.30 (dxd, J= 6.6, 12.7Hz, 2H, CH_2).

2-(1,3-Dioxoisoindolin-2-yl)-N-(4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-3-methylbutanamide (4d)

Yield, 70%; mp 262-264°C; IR (cm^{-1}): υ_{max} 3436 (NH), 3043 (C-H aromatic), 2933, 2853 (C-H aliphatic), 1772, 1712,1662 (C=O′s)and 1636 (C=N).^{1}H NMR (DMSO-d_6) δ 9.98 (s, 1H, NH), 7.85-7.46 (m, 16H, Ar-H), 7.30 (s, 1H, =CH), 4.71(d, J= 7.1Hz, H, N-CH), 4.11 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3),1.96-1.62 (m, 1H, CH) and 1.23-1.01 (d, J= 6.5Hz, 6H, $2CH_3$).

2-(1,3-Dioxoisoindolin-2-yl)-N-(4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-4-(methylthio)butanamide (4e)

Yield, 65%; mp 174-176°C ; IR (cm^{-1}): υ_{max} 3436(NH), 3046 (C-H aromatic), 2930, 2852 (C-H aliphatic), 1775, 1711, 1663 (C=O), 1636 (C=N). ^{1}H NMR (DMSO-d_6) δ 10.10 (s, 1H, NHexchangeable with D_2O), 7.92-7.55 (m, 16H, Ar-H), 7.50 (s, 1H, =CH), 4.65 (s, H, N-CH), 4.09 (s, 2H, CH_2) 3.84 (s, 3H, OCH_3), 2.58 (t, J= 1.9, 7.4Hz, 2H, CH_2S), 2.08(q, 2H, CH_2)and 1.91 (s, 3H, SCH_3).

2-(1,3-Dioxoisoindolin-2-yl)-N-(4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-3-hydroxybutanamide (4f)

Yield, 70%; mp 198-200°C; IR (cm^{-1}): υ_{max} 3425 (OH), 3285 (NH), 3055 (C-H aromatic), 2929, 2852 (C-H aliphatic), 1772, 1710, 1686 (C=O′s) and 1630(C=N); ^{1}H NMR (DMSO-d_6) δ 9.95 (s, 1H, NH), 8.26-7.47 (m, 16H, Ar-H), 7.41 (s, 1H, =CH), 5.50 (s, 1H, OH),4.41 (m, 1H, O-CH),4.16 (d, J= 6.3Hz, 1H, N-CH), 3.95 (s, 2H, CH_2), 3.88 (s, 3H, OCH_3) and 1.23 (d, J= 6.6Hz, 3H, CH_3). Mass spectrum showed molecular ion peak ($M^{\cdot+}$ +1) at 684 (65.46 %) and the base peak at 150 (100%) and other fragments are 171 (56.17%), 221 (60.57%), 273 (57.89%), 280 (70.85%), 302 (78.44%), 510 (84.02%).

3-(1,3-Dioxoisoindolin-2-yl)-4-((4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-

imidazol-1-yl)phenyl)amino)-4-oxobutanoic acid (4g)

Yield, 75%; mp 242-244°C; IR (cm^{-1}): υ_{max} 3432 (OH), 3276 (NH), 3061 (C-H aromatic), 2932, 2855 (C-H aliphatic), 1775, 1710, 1689 (C=O′s), 1630 (C=N). ^{1}H NMR (DMSO-d_6) δ 10.20 (s, 1H, COOH), 9.89 (s, 1H, NH), 8.18-7.47 (m, 16H, Ar-H), 7.46 (s, 1H, =CH), 4.41(t, J= 1.7, 6.5Hz, 1H, N-CH),3.92 (s, 2H, CH_2), 3.56 (s, 3H, OCH_3) and 1.96 (d, J= 7.2Hz, 2H, CH_2).Mass spectrum showed molecular ion peak ($M^{\cdot+}$ +1) at 698 (13.36%) and the base peak at 127 (100%) and other fragments are 164 (53.53%), 354 (53.91%), 389 (65.05%), 391 (59.47%), 491 (75.88%), 499 (53.86%), 542 (56.53%).

2-(1,3-Dioxoisoindolin-2-yl)-N-(4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-4-methylpentanamide (4h)

Yield, 65%; mp 140-142 °C; IR (cm^{-1}): υ_{max} 3441 (NH), 3044 (C-H aromatic), 2988 (C-H aliphatic), 1772, 1709, 1666 (C=O′s)and 1630 (C=N). ^{1}H NMR (DMSO-d_6) δ 9.87 (s, 1H, NH), 8.10 -7.35 (m, 16H, Ar-H), 7.30 (s, 1H, =CH), 4.41 (t, J= 1.5, 6.8Hz, 1H, N-CH), 4.08 (s, 2H, CH_2) and 3.82 (s, 3H, OCH_3),1.96 (m, 1H, CH), 1.70 (t, J= 1.2, 6.4Hz, 2H, CH_2) and 1.21 (d, J= 7.1Hz, 6H, $2CH_3$).

2-(1,3-Dioxoisoindolin-2-yl)-N1-(4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)pentanediamide (4i)

Yield, 67%; mp 310-312 °C; IR (cm^{-1}): υ_{max} 3424-3300 (NH′s), 3061 (C-H aromatic), 2931, (C-H aliphatic), in range of 1778, 1709, 1683 (C=O′s)and 1636(C=N). ^{1}H NMR (DMSO-d_6) δ 9.95 (s, H, NH), 7.91-7.28 (m, 16H, Ar-H), 7.25 (s, 1H, =CH), 5.73 (s, 2H, NH_2), 4.41 (t, J= 1.9, 6.9Hz, 1H, N-CH), 4.15 (s, 2H, CH_2), 3.91 (s, 3H, OCH_3), 2.00 (t, J= 1.1, 6.8Hz, 2H, CH_2) and 1.71 (m, 2H, CH_2).

2-(1,3-Dioxoisoindolin-2-yl)-N-(4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxy benzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-5-guanidinopentanamide (4j)

Yield, 75%; mp 196-198 °C ; IR (cm^{-1}): υ_{max} 3425, 3329 (NH′s), 3037 (C-H aromatic), 2928, 2850 (C-H aliphatic), 1776,1708, 1675 (C=O′s) and 1627(C=N).^{1}H NMR (DMSO-d_6) δ 9.99 (s, 1H, =NH exchangeable with D_2O), 8.90 (s, 2H, NH_2 exchangeable with D_2O),8.59 (s, 1H, NH exchangeable with D_2O), 7.92-7.67 (m, 16H, Ar-H), 7.47 (s, 1H, =CH), 4.41 (s, 1H, N-CH), 4.30 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3), 2.50 (t, J= 1.4, 7.2Hz, 2H, CH_2), 2.21 (q, 2H, CH_2)and 2.00 (m, 2H, CH_2).

General procedure for the synthesis of amino acid-imidazolone hybrids (5a-j).

A solution of compounds **4a-j** (10.0 mmol) in absolute ethanol (20 mL) was treated with hydrazine hydrate (10.0 mmol) and allowed to reflux for 2 h then left for 24 h at room temperature. A solid material was produced as a result of the solvent being evaporated in vacuo; this solid was then added to water (10 mL), the solution was then acidified with acetic acid until it reached a pH 6, it was then heated for an hour on a steam bath, the suspension was then diluted with water (15 mL), and it was then filtered off. After concentrating and cooling the solution, the solid obtained was filtered off, crystallized with the proper solvent to yield **5a-j.**

2-Amino-N-(4-(2-(aminomethyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)acetamide(5a)

Yield, 60%; mp 320-322 °C; IR (cm^{-1}): υ_{max} 3452 (NH), 3273, 3168 (NH_2), 3050 (C-H aromatic), 2926, 3855 (C-H aliphatic), 1690 (C=O of imidazolone), 1662 (C=O of amide)and 1607 (C=N).^{1}H NMR (DMSO-d_6) δ 9.83 (s, 1H, NH), 8.21 (s, 2H, NH_2), 8.04 (s, 2H, NH_2), 7.89-7.47 (m, 8H, Ar-H), 7.40 (s, 1H, =CH), 3.93 (s, 2H, N-CH_2), 3.91 (s, 2H, CH_2) and 3.89 (s, 3H, OCH_3).

2-Amino-N-(4-(2-(aminomethyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-ylphenyl)propanamide(5b)

Yield, 60%; mp 308-310°C ; IR (cm^{-1}): υ_{max} 3444 (NH), 3295, 3169(NH_2), 3060(C-H aromatic), 2929, 2854 (C-H aliphatic), 1706 (C=O of imidazolone), 1674 (C=O of amide) and 1650 (C=N). ^{1}H NMR (DMSO-d_6) δ 9.81(s, 1H, NH), 8.22 (s, 2H, NH_2), 8.02 (s, 2H, NH_2), 7.90-7.47 (m, 8H, Ar-H), 7.35 (s, 1H, =CH), 3.97 (m, 1H, N-CH), 3.92 (s, 2H, CH_2), 3.81 (s, 3H, OCH_3) and 1.20 (d, J= 7.7Hz, 3H, CH_3).

2-Amino-N-(4-(2-(aminomethyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-3-phenylpropanamide (5c)

Yield, 60%; mp 292-294 °C ; IR (cm^{-1}): υ_{max} 3432 (NH), 3296, 3167(NH_2), 3050 (C-H aromatic), 2925, 2854 (C-H aliphatic), 1701 (C=O of imidazolone), 1662 (C=O of amide) and 1630 (C=N).^{1}H NMR (DMSO-d_6) δ 9.85 (s, 1H, NH), 8.23 (s, 2H, NH_2), 8.09 (s, 2H, NH_2), 8.08-7.47 (m, 13H, Ar-H), 7.20 (s, 1H, =CH), 3.93 (t, J= 1.3, 6.5Hz, 1H, N-CH), 3.91 (s, 2H, CH_2), 3.89 (s, 3H, OCH_3), and2.20 (dxd, J= 7.9, 15.4Hz, 2H, CH_2).Mass spectrum showed molecular ion peak ($M^{\cdot+}$ +2) at 472.3 (27.82%) and the base peak at 104 (100%) and other fragments are 162 (63.58%), 216 (93.41%), 222 (55.65%), 449 (59.67%).

2-Amino-N-(4-(2-(aminomethyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-3-methylbutanamide (5d)

Yield, 70%; mp> 360 °C ; IR (cm^{-1}): υ_{max} 3441 (NH),3214, 3165 (NH_2), 3038 (C-H aromatic), 2927, 2855 (C-H aliphatic), 1703 (C=O of imidazolone), 1669 (C=O of amide) and 1609 (C=N).^{1}H NMR (DMSO-d_6) δ 9.87 (s, 1H, NH), 8.20 (s, 2H, NH_2), 8.16 (s, 2H, NH_2), 8.10-7.47 (m, 8H, Ar-H), 7.40 (s, 1H, =CH), 3.86 (d, J= 7.9Hz, 1H, N-CH), 3.85 (s, 2H, CH_2), 3.71 (s, 3H, OCH_3),1.96 (m, 1H, CH) and 1.22 (d, J= 6.5Hz, 6H, $2CH_3$).

2-Amino-N-(4-(2-(aminomethyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-4-(methylthio)butanamide(5e)

Yield, 70%; mp 294-296 °C; IR (cm^{-1}): υ_{max} 3447 (NH), 3268, 3165 (NH_2), 3096 (C-H aromatic), 2921, 2856 (C-H aliphatic), 1704 (C=O of imidazolone), 1665 (C=O of amide)and 1627 (C=N).^{1}H NMR (DMSO-d_6) δ 9.86 (s, 1H, NH), 8.22 (s, 2H, NH_2), 8.10 (s, 2H, NH_2),7.73-7.46 (m, 8H, Ar-H), 7.40 (s, 1H, =CH), 4.05(t, J= 1.7, 6.6Hz, 1H, N-CH),3.80 (s, 2H, CH_2), 3.71 (s, 3H, OCH_3),2.02 (t, J= 1.5, 7.1Hz, 2H, CH_2S), 1.76 (m, 2H, CH_2) and 1.73 (s, 3H, SCH_3).

2-Amino-N-(4-(2-(aminomethyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-3-hydroxybutanamide (5f)

Yield, 75%; mp 282-284 °C ; IR (cm^{-1}): υ_{max} 3429 (OH), 3350 (NH), 3298, 3168 (NH_2),3079 (C-H aromatic), 2926, 2854 (C-H aliphatic), 1707 (C=O of imidazolone), 1661 (C=O of amide) and 1639 (C=N). ^{1}H NMR (DMSO-d_6) δ 9.85 (s, 1H, NH exchangeable with D_2O), 8.24 (s, 2H, NH_2exchangeable with D_2O), 8.22 (s, 2H, NH_2 exchangeable with D_2O), 8.08-7.45 (m, 8H, Ar-H), 7.40 (s, 1H, =CH), 4.00 (m, 1H, O-CH),3.91 (d, J=6.1Hz, 1H, N-CH), 3.80 (s, 2H, CH_2), 3.71 (s, 3H, OCH_3), 3.50 (s, 1H, OH exchangeable with D_2O) and 1.21 (d, J= 7.5Hz, 3H, CH_3).

3-Amino-4-((4-(2-(aminomethyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)amino)-4-oxobutanoic acid (5g)

Yield, 70%; mp>360°C ; IR (cm^{-1}): υ_{max}: 3421-3250 (OH, NH), 3258, 3160 (NH_2), 3040(C-H aromatic), 2929, 2853(C-H aliphatic), 1702 (C=O of imidazolone), 1660 (C=O of amide) and1633 (C=N).^{1}H NMR (DMSO-d_6) δ 10.01 (s, 1H, COOH),9.96 (s, 1H, NH), 9.86 (s, 2H, NH_2)9.73 (s, 2H, NH_2), 8.09-7.47 (m, 8H, Ar-H), 7.21 (s, 1H, =CH), 4.00 (t, J= 1.8, 7.7Hz, 1H, N-CH), 3.71 (s, 2H, CH_2), 3.50 (s, 3H, OCH_3) and 1.96 (d, J= 7.8Hz, 2H, CH_2).

2-Amino-N-(4-(2-(aminomethyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-4-methylpentanamide (5h)

Yield, 60%; mp 320-322 °C ; IR (cm^{-1}): υ_{max}: 3435 (NH), 3298, 3168 (NH_2), 3038(C-H aromatic), 2925, 2853 (C-H aliphatic), 1703 (C=O of imidazolone), 1665 (C=O of amide) and 1609 (C=N). ^{1}H NMR (DMSO-d_6) δ 9.85 (s, 1H, NH), 8.21 (s, 2H, NH_2), 8.10 (s, 2H, NH_2), 7.79-7.44 (m, 8H, Ar-H), 7.30 (s, 1H, =CH), 4.42 (t, J= 1.0, 7.7Hz, 1H, N-CH), 3.91 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3), 1.96 (m, 1H, CH), 1.71 (t, J= 1.4, 7.3Hz, 2H, CH_2) and 1.21 (d, J= 6.7Hz, 6H, $2CH_3$).

2-Amino-N^1-(4-(2-(aminomethyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)pentanediamide(5i)

Yield, 75%; mp> 360 °C ; IR (cm^{-1}): υ_{max} 3426 (NH), 3275, 3170 (NH_2), 3040 (C-H aromatic), 2923, 2850 (C-H aliphatic),1703 (C=O of imidazolone), 1662 (C=O of amide) and 1633 (C=N). ^{1}H NMR (DMSO-d_6) δ 9.86 (s, 1H, NH exchangeable with D_2O), 8.30 (s, 2H, NH_2 exchangeable with D_2O), 8.24 (s, 2H, NH_2 exchangeable with D_2O), 8.10 (s, 2H, NH_2 exchangeable with D_2O),8.09 -7.46 (m, 8H, Ar-H), 7.40 (s, 1H, =CH), 3.91(t, J= 1.3, 7.6Hz, 1H, N-CH),3.80 (s, 2H, CH_2),3.45 (s, 3H, OCH_3), 2.00 (t, J= 1.9, 6.4Hz, 2H, CH_2) and 1.71 (m, 2H, CH_2).

2-Amino-N-(4-(2-(aminomethyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-5-guanidinopentanamide (5j)

Yield, 70%; 288-290°C; IR (cm^{-1}): υ_{max} 3432, 3305 (NH′s), 3270, 3165 (NH_2), 3080 (C-H aromatic), 2924, 2855 (C-H aliphatic), 1713 (C=O of imidazolone), 1662 (C=O of amide) and 1639 (C=N). ^{1}H NMR (DMSO-d_6) δ11.53 (s, 1H, =NH), 9.84 (s, 1H, NH), 9.80 (s, 1H, NH), 8.30 (s, 2H, NH_2), 8.20 (s, 2H, NH_2),8.08 (s, 2H, NH_2), 8.07-7.47 (m, 8H, Ar-H), 7.40 (s, 1H, =CH), 4.41 (t, J= 1.5, 7.2Hz, 1H, N-CH), 4.15 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3),2.50 (t, J=1.1, 6.9Hz, 2H, CH_2), 2.21 (q, 2H, CH_2) and 2.00 (m, 2H, CH_2).

General procedure for the synthesis of *N*-(*p*-tosyl)-amino acid-imidazolone hybrids (6a-j).

N-(*p*-Tosyl)-amino acids (10.0 mmol) including glycine, DL-alanine, L-phenylalanine, L-valine, L-serine, DL-methionine, L-threonine, L-arginine, L-aspartic acid, and L-leucine were added to a well-stirred solution **3** (10.0 mmol) in dry THF (30 mL) and then DCC (20.0 mmol) were added at 0°C. The reaction mixture was stirred for 24 h at 0°C and for an additional 24 h at room temperature. Filtration was used to remove any remaining DCCfrom the mixture. Then filtrate was vacuum evaporated, and the resulting product was dissolved in ethanol (25 mL), and the solution was then re-filtered to remove any remaining DCU. By evaporating the filtrate in vacuo and recrystallizing the compounds from the proper solvents, the products **6a-j** were produced.

N-(4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-2-(4-methylphenylsulfonamido)acetamide (6a)
Yield, 80%; 130-132°C ; IR (cm^{-1}): υ_{max}: 3321 (NH), 3055 (C-H aromatic), 2932, 2855 (C-H aliphatic), 1775, 1723, 1670 (C=O′s), 1636 (C=N)) and1381 (SO_2).^{1}H NMR (DMSO-d_6) δ 9.95 (s, 1H, NH), 8.20 (s, 1H, NH), 7.87-7.28 (m, 16H, Ar-H), 7.21 (s, 1H, =CH), 4.41 (dxd, J= 7.0, 13.7Hz, 2H, -CH_2CO), 3.98(s, 2H, CH_2), 3.89 (s, 3H, OCH_3) and 2.30 (s, 3H, CH_3).

N-(4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-2-(4-methylphenylsulfonamido)propenamide (6b)
Yield, 73%; 104-106°C; IR (cm^{-1}): υ_{max}: 3403(NH~OH), 3064 (C-H aromatic), 2931, 2855 (C-H aliphatic), 1771, 1708, 1657 (C=O′s), 1636 (C=N) and 1379 (SO_2).^{1}H NMR (DMSO-d_6) δ 9.92 (s, 1H, NH), 8.50 (s, 1H, NH), 7.85-7.24 (m, 16H, Ar-H), 7.20 (s, 1H, =CH), 4.41 (m, 1H, -CHCO), 3.95 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 2.30 (s, 3H, CH_3)and 1.21 (d, J= 7.8Hz, 3H, CH_3).

N-(4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-2-(4-methylphenylsulfonamido)-3-phenylpropanamide (6c)
Yield, 71%; 174-176°C; IR (cm^{-1}): υ_{max}: 3328 (NH), 3032 (C-H aromatic), 2930, 2852 (C-H aliphatic), 1775, 1722, 1675 (C=O′s), 1627 (C=N) and 1382 (SO_2). ^{1}H NMR (DMSO-d_6) δ 9.97 (s, 1H, NH), 8.11 (s, 1H, NH), 7.81-7.21 (m, 21H, Ar-H), 7.20 (s, 1H, =CH), 4.41 (t, J=1.1, 6.9Hz, 1H, -CHCO), 3.96 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3), 2.30 (s, 3H, CH_3) and 2.20 (dxd, J= 7.0, 15.1Hz, 2H, CH_2).

N-(4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-3-methyl-2-(4-methylphenylsulfonamido)butanamide (6d)
Yield, 68%; 120-122°C; IR (cm^{-1}): υ_{max}: 3590 (NH~OH), 3096 (C-H aromatic), 2950, 2856 (C-H Aliphatic), 1771, 1720, 1669 (C=O′s), 1613 (C=N) and 1381 (SO_2).^{1}H NMR (DMSO-d_6) δ 9.98 (s, 1H, NH), 8.30 (s, 1H, NH), 7.87-7.30 (m, 16H, Ar-H), 7.28 (s, 1H, =CH), 4.41 (dxd, J= 6.0, 12.8Hz, 1H, -CHCO), 3.99 (s, 2H, CH_2), 3.89 (s, 3H, OCH_3), 2.30 (s, 3H, CH_3),1.75 (m, 1H, CH) and 1.24 (d, J= 7.2Hz, 6H, 2CH_3).Mass spectrum showed molecular ion peak ($M^{\cdot+}$) at 705 (24.9%) and the base peak at 481(100%) and other fragments are 93 (57.28%), 293 (49.30%), 110 (38.75%), 612 (39.44%).

N-(4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-2-(4-methylphenylsulfonamido)-4-(methylthio)butanamide (6e)
Yield, 73%; 150-152°C ; IR (cm^{-1}): υ_{max} 3385 (NH~OH), 3064 (C-H aromatic), 2926, 2854 (C-H aliphatic), 1776, 1722, 1658 (C=O′s), 1628 (C=N)) and 1380 (SO_2).^{1}H NMR (DMSO-d_6) δ 9.90 (s, 1H, NH), 8.60 (s, 1H, NH), 7.93-7.13 (m, 16H, Ar-H), 7.11 (s, 1H, =CH), 4.41 (t, J= 1.2, 7.8Hz, 1H, -CHCO), 4.30 (s, 2H, CH_2), 3.91 (s, 3H, OCH_3), 2.50 (t, J= 1.5, 6.9Hz, 2H, CH_2S), 2.30 (s, 3H, CH_3),2.00 (m, 2H, CH_2) and 1.91 (s, 3H, SCH_3).Mass spectrum showed molecular ion peak ($M^{\cdot+}$) at 737 (34.98%) and the base peak at 407 (100%) and other fragments are 123 (56.85%), 221 (64.53%), 343 (73.69%), 467 (60.84%), 493 (58.31%), 684 (74.62%).

N-(4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-3-hydroxy-2-(4-methylphenylsulfonamido)butanamide (6f)
Yield, 70%; 278-280°C ; IR (cm^{-1}): υ_{max} 3385 (OH), 3323(NH), 3061 (C-H aromatic), 2930, 2854 (C-H aliphatic), 1775, 1722, 1657 (C=O′s), 1628 (C=N) and 1385 (SO_2). ^{1}H NMR (DMSO-d_6) δ 9.94 (s, 1H, NH), 8.58 (s, 1H, NH), 7.82-7.12 (m, 16H, Ar-H), 7.11 (s, 1H, =CH), 5.70 (s, 1H, OH),4.41 (dxd, J= 7.9, 13.8Hz, 1H, -CHCO), 4.00 (m,1H, CH), 3.88 (s, 2H, CH_2), 3.60 (s, 3H, OCH_3), 2.30 (s, 3H, CH_3)and 1.23 (d, J= 7.6Hz, 3H, CH_3).

4-((4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)amino)-3-(4-methylphenylsulfonamido)-4-oxobutanoic acid (6g)
Yield, 84%; 282-284°C ; IR (cm^{-1}): υ_{max} 3386 (OH), 3267 (NH), 3065 (C-H aromatic), 2931, 2855 (C-H aliphatic), 1776, 1722, 1665 (C=O′s), 1623 (C=N) and 1383 (SO_2).^{1}H NMR (DMSO-d_6) δ 10.10 (s, 1H, COOH),9.91 (s, 1H, NH), 8.60 (s, 1H, NH), 7.92-7.13 (m, 16H, Ar-H), 7.11 (s, 1H, =CH), 4.41 (t, J= 1.9, 7.7Hz, 1H, -CHCO), 4.31 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3), 2.30 (s, 3H, CH_3)and 1.96 (d, J= 7.5Hz, 2H, CH_2).

N-(4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-4-methyl-2-(4-methylphenylsulfonamido)pentanamide (6h)
Yield, 80%; 158-160°C ; IR (cm^{-1}): υ_{max} 3376 (NH), 3055 (C-H aromatic), 2932, 2855 (C-H aliphatic), 1771, 1710, 1669 (C=O′s), 1636 (C=N)) and 1379 (SO_2).^{1}H NMR (DMSO-d_6) δ 9.96 (s, 1H, NH

exchangeable with D_2O), 8.20 (s, 1H, NH exchangeable with D_2O), 7.90-7.40 (m, 16H, Ar-H), 7.12 (s, 1H, =CH), 4.41 (t, J= 1.5, 7.3Hz, 1H, -CHCO), 3.91 (s, 2H, CH_2), 3.60 (s, 3H, OCH_3), 2.30 (s, 3H, CH_3), 1.96 (m, 1H, CH), 1.71 (t, J= 1.2, 6.3Hz, 2H, CH_2) and1.21 (d, J= 7.4Hz, 6H, $2CH_3$).

N-(4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-3-hydroxy-2-(4-methylphenylsulfonamido)propenamide (6i)

Yield, 71%; 162-164°C ; IR (cm^{-1}): υ_{max} 3362 (OH), 3350, 3262(NH′s), 3058 (C-H aromatic), 2929, 2852 (C-H Aliphatic), 1775, 1719, 1654 (C=O′s), 1628 (C=N) and 1387 (SO_2).^{1}H NMR (DMSO-d_6) δ 9.91 (s, 1H, NH exchangeable with D_2O), 8.60 (s, 1H, NH exchangeable with D_2O), 7.90-7.34 (m, 16H, Ar-H), 7.27 (s, 1H, =CH), 5.70 (s, 1H, OH exchangeable with D_2O),4.41 (t, J= 1.8, 7.2Hz, 1H, -CHCO), 4.08 (s, 2H, CH_2), 3.70 (s, 3H, OCH_3),2.71 (d, J= 7.9Hz, 2H, OCH_2) and 2.30 (s, 3H, CH_3).

N-(4-(2-((1,3-dioxoisoindolin-2-yl)methyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)-5-guanidino-2-(4-methylphenylsulfonamido)pentanamide (6j)

Yield, 69%; 96-98°C ; IR (cm^{-1}): υ_{max} 3452 (NH/OH), 3350 (NH), 3275, 3180 (NH_2), 3058 (C-H aromatic), 2931, 2854 (C-H aliphatic), 1775, 1724, 1665 (C=O′s), 1638 (C=N)) and 1384 (SO_2).^{1}H NMR (DMSO-d_6) δ 9.99 (s, 1H, =NH), 9.92 (s, 1H, NH), 8.90 (s, 2H, NH_2), 8.59 (s, 1H, NH), 8.20 (s, 1H, NH), 7.92-7.32 (m, 16H, Ar-H), 7.20 (s, 1H, =CH), 4.41 (t, J= 1.7, 6.9Hz, 1H, -CHCO), 4.31 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3), 2.50 (t, J= 1.4, 7.9Hz 2H, CH_2), 2.30 (s, 3H, CH_3),2.21 (q, 2H, CH_2) and 2.00 (m, 2H, CH_2).

Cytotoxicity and anti-tumorevaluation

Carcinogenic human cell lines, namely, human prostate cancer (PC3) and Mammary gland breast cancer (MCF-7), were used to determine the inhibitory effects ofour novel synthesized compounds on cell growth using the MTT assay.The mitochondrial succinate dehydrogenase enzyme is responsible for turning the yellow tetrazolium bromide into a purple formazan derivative in this colorimetric assay.Cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/mL penicillin and 100µg/ml streptomycin at 37 °C in a 5% CO_2 incubator. The cell lines were seeds in a 96-well plate at a density of 1.0 x 10^4 cells/well. at 37° C for 48 h under 5% CO_2. After incubation the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5mg/mL was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100 µL is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800 , USA). The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) X 100.

Molecular docking

The crystal structures in three-dimensional coordinates for the macromolecules PIM-1 kinase (PDB ID: 2OBJ), and *c-MYC* G4 (PDB ID: 6AU4) were retrieved from the protein data bank (www.rscb.org). The most active compounds (**3, 4d, 6e, 6h**) and **DOX** (reference anti-cancer drug) were docked separately into the active pocket of these receptors. All calculations for the docking and visualizations were performed using molecular operating environment (MOE) 2015.10 software package.

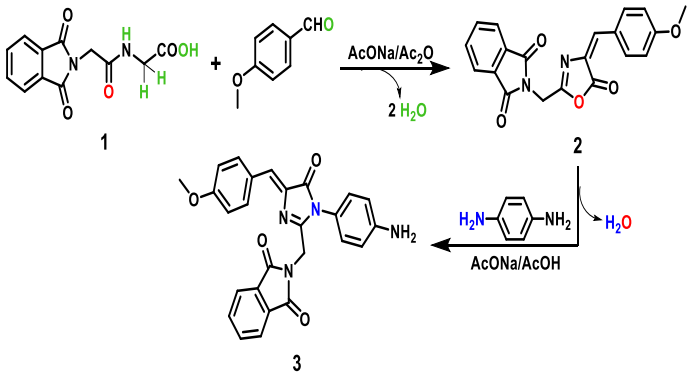
The protein and DNA structures were fixed for any missing atoms or residues by the preparation module in MOE. Using default parameters, this included applying 3D-protonation, partial charges through the MMFF94 (modified) forcefield, and minimizing the structures to a chosen gradient. The binding pockets of PIM-1 kinase and *c-MYC* G4 were defined using the dummy atoms that were generated at the binding area using the site finder option. The docking algorithm was configured to use the triangle matcher placement approach with rigid receptor refinement. The triangular match algorithm was programmed to create 100 poses, but the induced fit refinement approach produced five poses. Also, the default GBVI/WSA dG technique was used as a docking function. The docking conformations with the lowest binding energies (higher absolute values) were chosen. The docking protocol was validated via redocking ligands into the pocket of receptors. Only docking poses with RMSD values less than 2.0 Å were considered successful.

3.Results and discussion

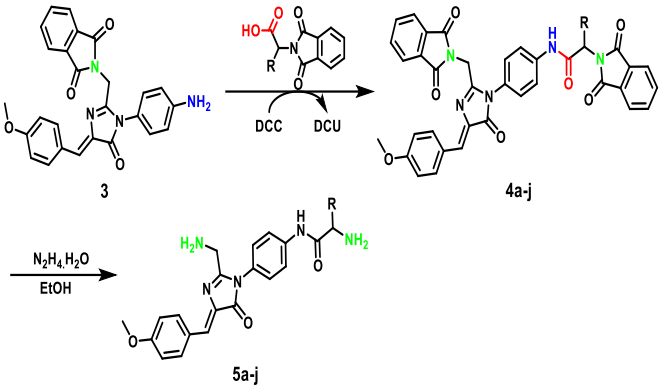
Hybrids of heterocyclic systems and amino acids represent an excellent option for controlling tumors. The reactive molecule 2-{[4-(4-methoxybenzylidene)-5-oxo-4,5-dihydrooxazol-2-yl]methyl}isoindoline-1,3-dione (**2**) was obtained in a good yield *via* refluxing *N*-phthaloylglycylglycine (**1**), and *p*-anisaldehyde in anhydrous sodium acetate/acetic anhydride mixture (*Scheme 1*). The structure of oxazolone **2** was confirmed on the basis of its spectral data. Its IR spectrum showed absorption bands at ύ 1775 cm^{-1} due to C=O group of azlactone, besides the other characteristic peaks. ^{1}H NMR spectrum showed signals for aromatic/methine protons at δ7.90-7.83 ppm, two singlet methylene protons at 4.0 ppm in addition to methoxy protons at

2.4 ppm. Also, the mass spectrum showed molecular ion peak ($M^{\cdot+}$) at 362 (27.08 %). The second step included the treatment of **2** with *p*-phenylenediamine in sodium acetate/ glacial acetic acid mixture to give the key functionalized imidazolone derivative **3** (*Scheme 1*). Its ^{1}H NMR spectrum exhibitedadditional exchangeable signal at 9.88ppm due to NH_2 while its mass spectrum showed molecular ion peak $M^{\cdot+}$ at 452 (10.7%).The expected mechanism for synthesis of compound **3** was showen in (*scheme 4*).

Imidazolone**3** was incorporated with a number of *N*-protected amino acids as reactive key precursors to create a new series of amino acid derivatives. Imidazolone **3** reacted with phthaloyl derivatives of amino acids such as glycine, DL-alanine, L-phenylalanine, L-valine, L-glutamine, DL-methionine, L-threonine, L-arginine, L-aspartic acid, and L-leucine in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC) as dehydrating agent to furnish the corresponding imidazolone derivatives **4a-j, (***Scheme 2*). Deprotection of amino group in *N*-phthaloyl derivatives **4a-j** was accomplished in good yields through their refluxing with ethanolic solution of hydrazine hydrate for 2 hours to afford the unprotected aminoacyl derivatives **5a-j,** (*Scheme 2*). The deprotection process is confirmed by the appearance of the amino group's absorption band in compounds **5a-j**. Similarly, imidazolone **3** reacted with *p*-tosyl derivatives of the same amino acids to furnish imidazolone derivatives **6a-j,** (*Scheme 3*).

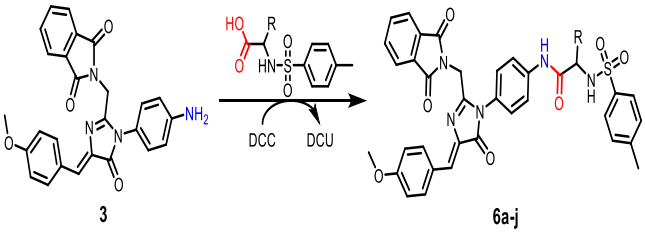


Scheme 1. Synthetic route for the functionalized imidazolone derivative 3.

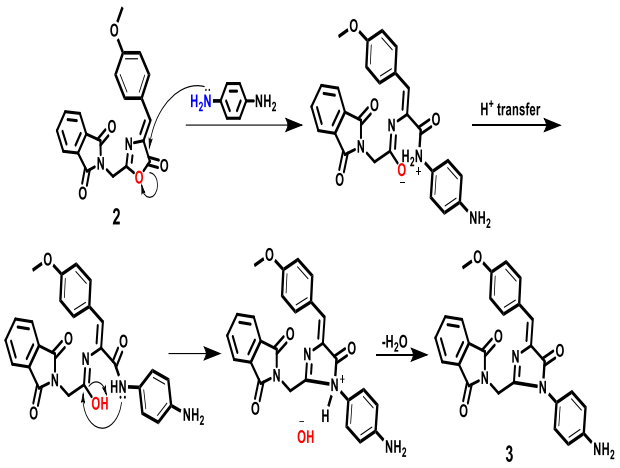


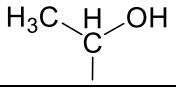
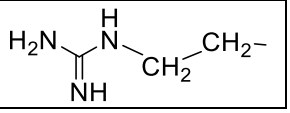
4/5	R	4/5	R
a	H	**f**	H_3C H OH C
b	CH_3-	**g**	HOOC−CH_2−
c	Ph-CH_2-	**h**	$(H_3C)_2$−CH−CH_2−
d	$(CH_3)_2$CH-	**i**	O H_2N CH_2 CH_2-
e	CH_3-SCH_2-CH_2-	**j**	H_2N H N NH CH_2 CH_2−

Scheme 2. Synthesis of phthaloyl-protected and unprotected amino acid-imidazolone hybrids.



Scheme 3. Synthesis of *p*-tosyl-protected amino acids-imidazolone hybrids.



6	R	6	R
a	H	f	
b	CH_3-	g	$HOOC-CH_2-$
c	Ph-CH_2-	h	$(H_3C)_2-CH-CH_2-$
d	$(CH_3)_2CH$-	i	$HO-CH_2-$
e	CH_3-SCH_2-CH_2-	j	

Scheme 4. The mechanistic pathway for the synthesis of compound 3.

In vitro anti-tumor activity

In comparison to the standard doxorubicin (DOX) as anticancer drug, the synthesized compounds displayed varying degrees of inhibitory action toward the evaluated human tumor cell lines.

Among the studied series, compounds **6e** and **6h** showed the greatest cytotoxic effect toward PC3 and MCF-7 with IC_{50} ranges between 5 and 15 mg/mL. The highest antitumor activity against MCF-7 was shown by compounds **3, 5c, 5f, 5i, 6d, 6e, 6h, 6i** with IC_{50} values between very strong and moderate and the highest antitumor activity against PC3 was shown by compounds **3, 5c, 5f, 6e, 6h** with IC_{50} values between very strong and moderate. Other substances, on the other hand, displayed antitumor activity that ranged from moderate to weak toward carcinogenic human cell lines.

As shown in Table 1, the combination of oxazolone derivative **2** with para phenylene diamine which give imidazolone derivative **3** give higher cytotoxic effect than starting oxazolone **2**, also the combination of tosyl amino acid specially tosyl methionine and tosyl leucine with imidazolone derivatives which give derivatives **6e** and **6h**, improve their cytotoxic effect in comparison with starting imidazolone**3.**

Compounds with strong electron-donating groups (CH_3 and OH) present on the compounds **5f, 6d, 6e, 6h, 6i** were found to have the most effective anticancer activity when analyzing the structure-activity relationship (SAR) between the activity and synthesized analogues. These earlier findings suggested that amino acids were essential for enhancing biological processes. Long-chain aliphatic hydrocarbon-containing amino acids demonstrated more powerful anticancer agentsalso electron donating groups in amino acids effect on anti-cancer activity, The anticancer activity increases as the number of amino acid groups that donate electrons increases.

Table 1. The *in vitro* anti-cancer activity (IC_{50}) of the synthesized compounds.

Compd. No	IC_{50} (µM)		Compd. No	IC_{50} (µM)	
	MCF-7	PC3		MCF-7	PC3
2	74.16±3.5	91.56±4.6	5i	47.28±2.5	79.98±3.8
3	12.82±0.9	29.81±2.1	6d	23.16±1.7	54.43±3.1
4d	86.90±3.9	>100	6e	**5.80±0.3**	**9.12±0.7**
4e	63.84±3.3	83.56±4.0	6h	8.32±0.5	15.03±1.2
4j	56.15±3.1	60.86±3.3	6i	18.22±1.3	65.28±3.5
5c	34.06±2.1	44.81±2.4	DOX	**4.17±0.2**	**8.87±0.6**
5f	41.80±2.3	49.49±2.8	----	----	----

Molecular docking studies

a) Binding with PIM-1 kinase:

To check the binding-ability of compounds with PIM-1 kinase, six compounds (**3, 4d, 6e, 6h, DOX,** and **VRV**) were docked separately in the active pocket of this enzyme. Strong binding with such enzyme suggests the chance to be used as inhibitors and hence the mode of action for their activity as anti-tumor agents. By screening the data depicted in **Table 2**, it can be noticed that compound **4d** showed binding affinity to PIM-1 kinase without forming any hydrogen bonds **(Figure 2B,E)**,only one arene-H interaction with Leu174 controls the binding effectiveness.

Among all docked compounds, **6e** exhibited the strongest binding profile (BE = - 9.625 Kcal/mol) *via*forming three hydrogen bonds with Lys67, Asn172 and Lys169 residues. Also, it could form two arene-H interactions with Phe49, Val126 that controlled the molecule flexibility to fit the protein pocket (**Figure 2C,F**). On the other hand, **VRV** showed the weakest binding mode with BE = - 6.461 Kcal/mol although it could form H-bond and H-arene interaction. This may be attributed to its small rigid structure and minor flexible functionality (**Figure 3C,F**). Compound **3** has a similar behavior to that of **VRV** with one more hydrogen bond and enhanced binding strength (**Figure 2A,D** and **Table 2**).

Moreover, **DOX** (**Figure 3B,E**) bound to the active pocket of PIM-1 kinase with a smaller energy value than that of **6h** (**Figure 3A,D**), although the former could form four hydrogen bonds **(Table 2**). Collectively, it can be deduced that the residues Phe49, Lys67, Lys169 and Leu174 represent the crucial amino acid residues for effective binding with PIM-1 kinase. Also, there is a partial correlation between the compounds' anti-cancer activities and their bindingwith this enzyme.

Table 2. Binding data of imidazolones, DOX and VRV with PIM-1 kinase

Ligand	BE* (Kcal/mol)	RMSD**	PIM-1 kinase interactions	
			H-Bond	Arene-H
3	- 7.835	1.189	Lys67, Asp131	Leu174
4d	- 8.580	1.178	-	Leu174
6e	- 9.625	1.826	Lys67, Asn172, Lys169	Phe49, Val126
6h	- 9.406	1.953	Lys169	-
DOX	- 8.142	1.266	Lys67, Phe49, Asp128, Leu44	-
VRV	- 6.461	0.925	Lys67	Leu174

BE* = Binding energy, RMSD** = Root mean-square deviation

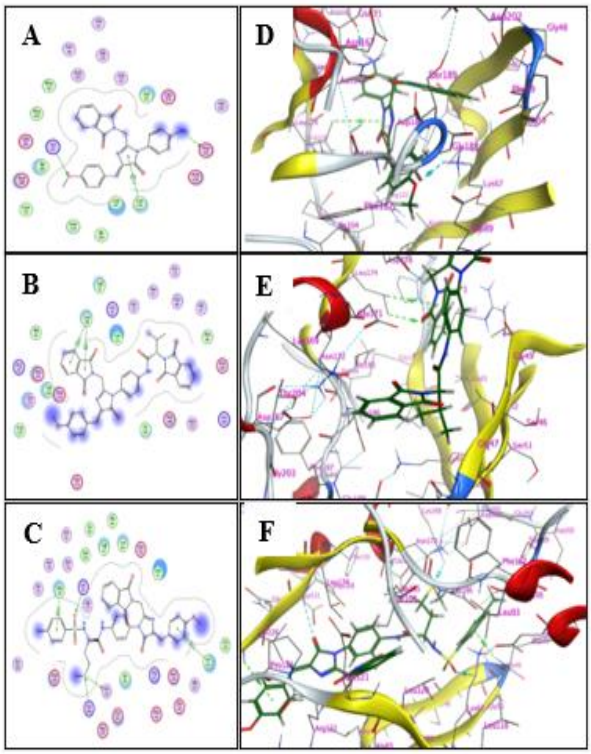


Figure 2. (A-C) 2D-binding profiles of *PIM-1 kinase* with 3, 4d, and 6e, respectively showing H-bonds and arene-H interactions. (D-F) In-depth 3D-binding modes of *PIM-1 kinase* with 3, 4d, and 6e, respectively.

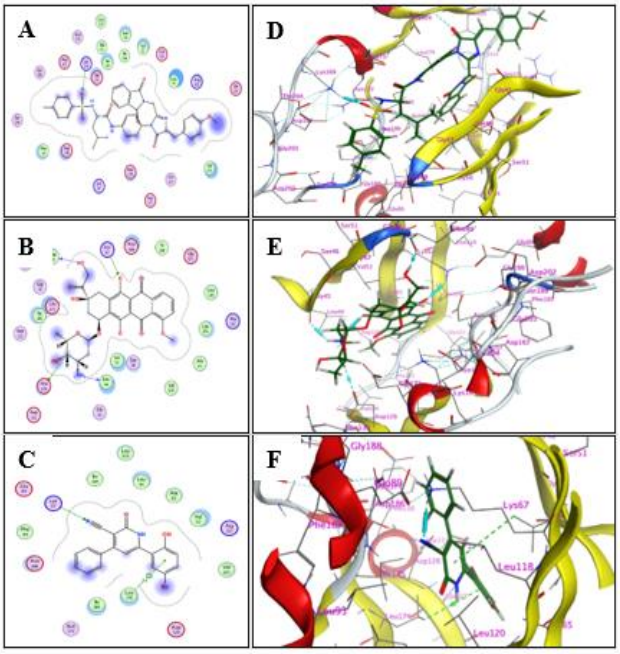


Figure 3. (A-C) 2D-binding profiles of *PIM-1 kinase* with 6h, DOX, and VRV, respectively showing H-bonds and arene-H interactions. (D-F) In-depth 3D-binding modes of *PIM-1 kinase* with 6h, DOX, and VRV, respectively.

b) Binding with c-MYC G-quadruplex (G4):

The *c-MYC* represents an important oncogenerelated to multiple pathways in the occurrence of cancer and significantly affects the cell metabolism, growth, proliferation, and apoptosis. In about 80% of human cancer cells, elevatedexpression of *c-MYC* is observed and induces tumorigenesis. Hence,it would be an effective pathwayfor controlling cancer *via* the inhibition of *c-MYC* G4. Therefore, compounds (**3, 4d, 6e, 6h,** and **Quindoline**) were subjected to docking into the crystal structure of *c-MYC* G4 that may help in predicting the mode of action of these derivatives as anti-cancer agents.

The results for docking and the *c-MYC*-binding data were depicted in **Figures 4,5** and **Table3**.Compound **3** (BE = - 7.975 Kcal/mol) showed a comparable binding with **quindoline** because of their structure similarity although it could form only one hydrogen bond with A22 nucleotide (**Figure 4A,D**) while quindoline formed two H-bonds (**Figure 5B,D**).

Compound **4d** showeda better binding efficiency than quindoline *via* forming one hydrogen bond with G5 and intermolecular arene-H with G19 (**Figure 4B,E and Table 3**). Besides, **6e** (**Figure 4C,F**) exhibited the strongest binding tendency to the pocket of *c-MYC* G4 (BE = - 9.430 Kcal/mol) by forming two H-bonds and one arene-H interaction that help in a great stabilization of the G-quadruplex structure. The derivative **6h** bound to *c-MYC* G4 with a smaller value than **6e**.

The investigated compounds showed an approximately perfect correlation between their anti-cancer activities and binding effectiveness with c-MYC G4. This may suggest that these compounds acquired their activity against cancer cells by inhibiting the replication of the cell-DNA through the stabilization of c-MYC G4.

Table 3. Binding data of imidazolones and quindoline with *c-MYC* G4

Ligand	BE (Kcal/mol)	RMSD	*c-MYC* G4 interactions		
			H-Bond	Arene-H	Arene-Arene
3	- 7.975	1.773	A22	-	A21, G6, G10
4d	- 8.935	1.842	G5	G19	-
6e	- 9.430	1.745	T20, G19	G6	-
6h	- 9.321	1.665	A21	-	A21, G10, G15
Quindoline	- 7.856	1.818	G6, T20	-	G6, A21

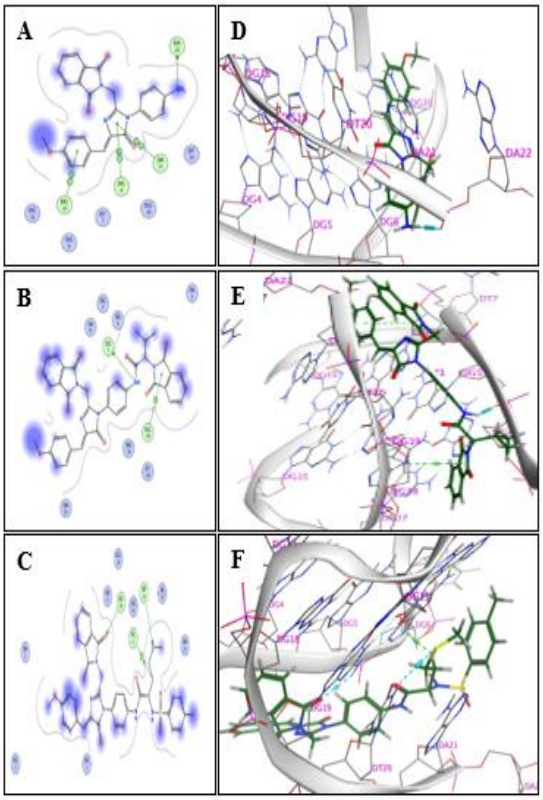


Figure 4. (A-C) 2D-binding profiles of *c-MYC G4* with 3, 4d, and 6e, respectively showing H-bonds and arene-H interactions. (D-F) In-depth 3D-binding modes of *c-MYC G4* with 3, 4d, and 6e, respectively.

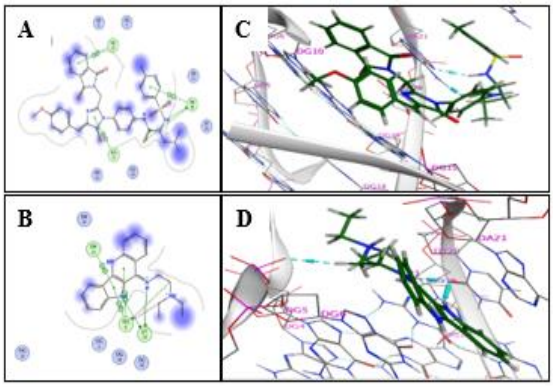


Figure 5. (A,B) 2D-binding profiles of *c-MYC G4* with 6h and quindoline, respectively showing H-bonds and arene-H interactions. (C,D) In-depth 3D-binding modes of *c-MYC G4* with 6h and quindoline, respectively.

Conclusion

In conclusion, we developed and produced a novel class of amino acids that were joined to imidazolone analogues.Using the MTT technique, they were examined for their in vitro anticancer abilities.A significant number of synthetic derivatives based on their IC_{50} values showed strong cytotoxic action.The aromatic and hydrophobic amino acids, valine, leucine, serine, methionine, phenylalanine, and threonine that contain groups which donate electrons are more likely to favor anticancer action, according to research on the structure-activity

relationship (SAR).In addition, the performed molecular docking study confirmed a good correlation between the strength of molecules receptor-binding and their anti-cancer activities. Our findings here could serve as a starting point for the creation of potent imidazolone conjugated amino acids as anti-cancer treatments.

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Institutional review board statement
The study was conducted and approved according to the guidelines of the declaration of the ethical committee of the Faculty of Science, Benha University (BUFS-REC-2023-72 chm).

Conflicts of Interest
The authors declare no conflicts of interest regarding the publication of this paper.

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