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Synthesis and Biological Activity of Triazole Derivatives of Osajin

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In the present study, osajin-1,2,3-triazole hybrids were designed, synthesized and evaluated for their anti-proliferative activity against MCF-7, PC-3 and Hela cell lines. Many of the synthesized hybrid derivatives were found potent than the parent compound, osajin (**1**). All the semi-synthesized derivatives (**3a-j**) were characterized by using mass and NMR spectroscopic techniques. Among the newly synthesized compounds, **3c**, **3d**, and **3e** were shown promising activities against the tested cell lines compared with doxorubicin standard. In addition, molecular docking studies of the synthesized compounds have shown a good correlation with *in silico* molecular docking analysis by exhibiting strong interactions with the inhibitor HERA-protein.

Keywords: Osajin, Triazole derivatives, Anti-proliferative activity, DFT.

INTRODUCTION

Cancer remains a life threatening disease and ranks second to the heart disease worldwide As per recent statistical data by International Agency for Research on Cancer (IARC), The global cancer burden is estimated that about 13.2 million deaths each year and moreover and approximately 21.4 million new cases by 2030 [1,2]. To date, chemotherapy remains frontline therapy of choice for combating cancer even after several decades of drug development efforts. Moreover, clinical effectiveness of available anti-cancer agents has been suffering by side effects including drug resistance and toxicity [3]. Hence, there is a need for the development of new therapeutic agents with lesser side effects. In this context, natural products are well known and recognized as a rich source of bioactive compounds and proven in terms of chemotherapy agents as 74% of marketed drugs are originated from natural sources [4]. Best examples includes vinblastine, vincristine, vinorelbine, docetaxel, etoposide, teniposide, paclitaxel, topotecan and irinotecan are either based on plant/marine sources or its derivatives.

As part of our ongoing programme in exploring the traditional flora for identifying the potential leads [5], we have isolated osajin (**1**) in good yields, which encouraged us to design and synthesize its derivatives. As per the recent reports, osajin (**1**) displayed growth inhibitory activity against human cancer cell lines [6] and as a PDE 5 inhibitor [7]. Recently, Ribaudo *et al*. [8] reported that semi-synthetic derivatives of osajin were also showed the PDE5 inhibitor activity. Recently, concept of hybrid molecules in which incorporation of various biologically active molecules are covalently linked together, have emerged as novel approach in discovery of new cytotoxic agents as they are supposed to minimize undesirable effects and offer better biological profiles compared to parent compounds [9,10].

It is well known fact that 1,2,3-triazole pharmacophore is found in a number of biologically active compounds exhibiting wide range of pharmacological activities such as anti-HIV, anti-biotics, antiviral and anticancer [11,12]. The copper (I) catalyzed 1,2,3-triazole formation from azides and terminal acetylenes is a powerful tool for the generation of privileged scaffolds due to its high degree of dependability, complete specificity and biocompatibility of the reactants [11]. Thus, present study was devised to synthesize a series of osajin-1,2,3-triazole hybrids using click reaction protocol and investigate their anti-cancer activities against panel of cancer cell lines.

EXPERIMENTAL

Plant material: The whole plant material (leaves, stem and bark) of *Walsura trifoliata* was collected from the forest of Tirumala in Chittoor district, India in the month of January 2021 and identication was done by Prof.Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupathi, India. A voucher specimen of the plant was deposited in the herbarium, Department of Botany, Sri Venkateswara University, Tirupathi, India with accession number 536.

The NMR spectra were recorded on a Bruker 300 MHz spectrometer for ^{1}H and 75 MHz for ^{13}C respectively, using TMS as internal standard. Mass spectra were performed on a Agilent Technologies 6510 Q-TOF Mass spectrometer. The chromatography was executed with silica gel (100-200 mesh, Qingdao Marine Chemical, Inc., Qingdao, China) using mixtures of ethyl acetate and hexane as eluents. Reactions, which required the use of anhydrous, inert atmosphere techniques, were carried outunder nitrogen atmosphere. Commercially available reagents, solvents and starting materials were used without further purification. Analytical TLC was performed on recoated Merck plates (60 F_{254}, 0.2 mm) with the solvent system EtOAc/hexane and compounds were viewed under a UV lamp and sprayed with 10%H_2SO_4 followed by heating.

Isolation of osajin: The areal parts of *Walsura trifoliata* (3 kg) were powdered and extracted with methanol at room temperature for 48 h. The resulting methanol extract was evaporated to dryness under reduced pressure to give syrupy residue (250 g). This residue was then suspended in H_2O (500 mL) and extracted with ethyl acetate to give 25 g of ethyl acetate soluble portion, which was further subjected to column chromatography (silica gel, 100-200 mesh, eluting with $CHCl_3$ and $CHCl_3$/MeOH order of increasing polarity) to give 30 fractions. All the column fractions were systematically analyzed by TLC and fractions with similar TLC patterns were combined to give five major fractions (F1, F2, F3, F4 and F5). The TLC examinations of above obtained fractions by using different mobile phase of [hexane/ethyl acetate, 80:20, chloroform/acetone 80:20 and chloroform /methanol, 80:20)], to give 5 major fractions (F_1 to F_5) were obtained, after comparison of all the TLC, finally merged the similar TLC fractions, and the resultant fractions were subjected to repeated column chromatography resulted in the isolation of eight compounds. Fraction F1 was further subjected to column chromatography by using hexane: ethyl acetate (60:40) to get the osajin (compound **1**) (1 g).

Synthesis of propargyl osajin (2): To a stirred suspension of osajin (**1**) (1.0 mol) and K_2CO_3 (1.2 mmol) in acetone was added propargyl bromide (1.1 mmol) under N_2 atmosphere. Reaction mass was allowed to stirr from 11-13 h and monitored by TLC. After completion of reaction, Filtered the reaction mass by using vacuum and evoparated the solvent under vacuum finally to get the crude material. A 2 mL water and 2 mL ethyl acetate was added to the obtained crude material, separated the ethyl acetate layer. Aqueous layer again washed with EtOAc (2 × 5 mL). Combined the total organic layer was separated and distilled under plant vacuum at 1 bar. Resulting crude mass was performed by column chromatography eluent EtOAc/petroleum ether (1.5:8.5) to get the required compound **2** (yield: 508 mg, 93%). Yellow powder.^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 13.09 (1H, s), 7.90 (1H, s), 7.47 (2H, d, *J* = 8.6 Hz), 7.04 (2H, d, *J* = 8.6 Hz), 6.73 (1H, d, *J* = 9.9 Hz), 5.62 (1H, d, *J* = 9.9 Hz), 5.18 (1H, t, *J* = 7.3 Hz), 5.35 (2H, s), 4.71 (1H, d, *J* = 2.2 Hz), 1.81 (3H, s), 1.68 (3H, s), 1.47 (6H, s). HRMS (ESI+) *m/z*: 443.1823 $[M+H]^+$ (calcd. for $C_{28}H_{27}O_5[M+H]^+$, 443.1858).

Synthesis of triazole derivatives (3a-j): The solution of alkynes and corresponding azide (1.2 equiv.) were taken in 2 mL dry THF followed by addition of CuI. The reaction solution was stirred for 6-8 h at room temperature and the reaction was monitored by TLC. After the consumption of starting material, reaction solutions were filtered through celite bed. The filtrate was quenched with water (10 mL) and product mixture was extraction with ethyl acetate (2 × 20 mL). The organic layer was evaporated under reduced pressure using rotavapor to get the crude residue, which was purified by column chromatography using silica gel (60-120 mesh, hexane/ethyl acetate (60:40) to get the desired products (**3a-j**). All the synthesized triazole derivatives were confirmed by the spectral analysis (FTIR, ^{1}H & ^{13}C NMR and mass spectroscopy).

(1-(2-Chloro-3-methylphenyl)-1*H*-1,2,3-triazol-4-yl)-methyl osajin (3a): Pale yellow powder, yield: 92%, m.p.: 264 ºC. ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.09 (1H, s), 7.93 (1H, s), 7.83 (1H, s), 7.56 (1H, dd, *J* = 2.2, 8.3 Hz), 7.49 (2H, d, *J* = 8.3 Hz), 7.30-7.27 (2H, m), 7.10 (2H, d, *J* = 8.3 Hz), 6.74 (1H, d, *J* = 9.8, Hz), 5.63 (1H, d, *J* = 9.8 Hz), 5.36 (2H, s), 5.18 (1H, t, *J* = 6.0 Hz), 3.41 (2H, d, *J* = 6.7 Hz), 2.21 (3H, s), 1.82 (3H, s), 1.69 (3H, s), 1.47 (6H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.11, 158.21, 156.87, 154.84, 154.63, 152.57, 144.15, 137.41, 136.04, 132.82, 131.66, 130.92, 130.19, 128.01, 127.12, 124.84, 124.50, 123.96, 122.94, 121.90, 121.88, 115.78, 114.90, 107.43, 105.84, 77.78, 62.05, 28.17, 25.74, 21.25, 17.86. HRMS (ESI+) *m/z*: 610.2136 $[M+H]^+$ (calcd. for $C_{35}H_{32}N_3O_5Cl$ $[M+H]^+$, 610.2108).

(1-(2-Chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl osajin (3b): Pale yellow powder, yield: 93%, m.p.: 228 ºC. ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.09 (1H, s), 8.18 (1H,d, *J* = 2.7 Hz), 7.98 (1H, triplet of doublet, *J* = 7.7, 1.5 Hz), 7.91 (1H, s), 7.49 (2H, d, *J* = 8.8 Hz), 7.43-7.47 (1H, m), 7.34-7.29 (2H, m), 7.11 (2H, d, *J* = 8.8 Hz), 6.74 (1H, d, *J* = 9.9 Hz), 5.63 (1H, d, *J* = 9.9 Hz), 5.35 (2H, s), 5.18 (1H, t, *J* = 5.7 Hz), 3.40 (1H, d, *J* = 7.3 Hz), 1.82 (3H, s), 1.69 (3H, s), 1.47 (6H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.13, 158.21, 156.86, 154.85, 152.59, 151.64, 149.54, 145.84, 144.41, 131.68, 130.19, 128.01, 125.23, 124.89, 122.97, 121.90, 121.50, 117.15, 115.80, 114.91, 107.43, 105.84, 100.11, 99.69, 78.09, 61.9, 28.17, 25.76, 21.25, 17.88. HRMS (ESI+) *m/z*: 580.2272 $[M+H]^+$ (calcd. for $C_{34}H_{31}N_3O_5Cl$ $[M+H]^+$, 580.2247).

(1-(3-Chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl osajin (3c): Pale yellow powder, yield: 87%, m.p.: 250 ºC. ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.07 (1H, s), 8.07 (1H, s), 7.91 (1H, s), 7.81 (1H, t, *J* = 1.9, 3.9 Hz), 7.66 (1H, dq, *J* = 1.9, 3.9, 7.9 Hz), 7.49 (2H, d, *J* = 8.5 Hz), 7.46-7.42 (2H, m), 7.09 (2H, d, *J* = 8.5 Hz), 6.74 (1H, d, *J* = 9.9 Hz), 5.63 (1H, d, *J* = 9.9 Hz), 5.34 (2H, s), 5.18 (1H, t, *J* = 5.9 Hz), 3.40 (1H, d, *J* = 7.3 Hz), 1.81 (3H, s), 1.68 (3H, s), 1.47 (6H, s). ^{13}C NMR (100 MHz,

$CDCl_3$): δ 180.5, 179.3, 158.2, 156.8, 154.8, 148.7, 144.5, 144.4, 131.6, 130.1, 128.0, 125.2, 124.8, 122.9, 121.9, 121.5, 115.8, 114.9, 107.4, 107.0, 99.6, 98.4, 77.7, 61.9, 61.2, 40.8, 28.1, 25.7, 21.2, 17.8. HRMS (ESI+) *m/z*: 618.1843 $[M+Na]^+$ (calcd. for $C_{34}H_{30}N_3O_5Cl$ $[M+Na]^+$, 618.1771).

(1-(4-Chloro-2,5-dimethoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methyl osajin (3d): Colourless gum, yield: 91%, ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.09 (1H, s), 8.26 (1H, s), 7.91 (1H, s), 7.51 (1H, s), 7.48 (2H, d, *J* = 8.6 Hz), 7.14 (1H, s), 7.09 (2H, d, *J* = 8.8 Hz), 6.73 (1H, d, *J* = 9.9 Hz), 5.63 (1H, d, *J* = 9.9 Hz), 5.34 (2H, s), 5.18 (1H, t, *J* = 5.9,8.5 Hz), 3.93 (3H, s), 3.87 (3H, s), 3.40 (1H, d, *J* = 7.3 Hz), 1.81 (3H, s), 1.68 (3H, s), 1.47 (6H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.1, 158.2, 156.8, 154.8, 152.5, 149.4, 144.1, 114.86, 143.6, 131.6, 130.1, 127.9, 127.1, 124.9, 124.7, 123.8,123.2, 122.9, 121.9, 115.8, 114.9, 114.8, 109.0, 107.4, 105.8, 77.7, 62.0, 56.8, 56.7, 29.6, 28.1, 25.7, 21.2, 17.8. HRMS (ESI+) *m/z*: 678.2017 $[M+Na]^+$ (calcd. for $C_{34}H_{34}N_3O_7Cl$ $[M+ Na]^+$, 678.1983).

(1-(3-Trifluoromethylphenyl)-1*H*-1,2,3-triazol-4-yl)-methyl osajin (3e): Pale yellow gum, yield: 95%, ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.07 (1H, s), 8.13 (1H, s), 8.05 (1H, s), 7.98 (1H, d, *J* = 6.9 Hz), 7.91 (1H, s), 7.74-7.68 (2H, m), 7.49 (2H, d, *J* = 8.6 Hz), 7.09 (2H, d, *J* = 8.6 Hz), 6.75 (1H, d, *J* = 10.0 Hz), 5.63 (1H, d, *J* = 10.0 Hz), 5.36 (2H, s), 5.17 (1H, t, *J* = 5.9,7.1 Hz), 3.40 (1H, d, *J* = 7.3 Hz), 1.82 (3H, s), 1.69 (3H, s), 1.47 (6H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.1, 158.2, 156.9, 154.9, 154.6, 152.5, 145.4, 141.3, 139.5, 139.3, 137.2, 136.7, 135.1, 131.6, 130.5, 130.2, 128.0, 125.5, 124.0, 123.6, 121.9, 120.7, 117.5, 115.8, 114.9, 107.4, 105.8, 77.8, 62.0, 29.6, 28.1, 25.7, 21.2, 17.8. HRMS (ESI+) *m/z*: 630.2216 $[M+H]^+$ (calcd. for $C_{35}H_{31}N_3O_5F_3$ $[M+H]^+$, 652.2068).

(1-(4-Iodophenyl) -1*H*-1,2,3-triazol-4-yl)methyl osajin (3f): Yellow gum, yield: 83%, ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.07 (1H, s), 8.05 (1H, s), 7.90 (1H, s), 7.86 (2H, d, *J* = 8.6 Hz, s), 7.51 (2H, d, *J* = 8.5 Hz), 7.48 (2H, t, *J* = 8.6 Hz), 7.08 (2H, d, *J* = 8.6 Hz), 6.74 (1H, d, *J* = 9.9 Hz), 5.63 (1H, d, *J* = 10.0 Hz), 5.34 (2H, s), 5.18 (1H, t, *J* = 6.1 Hz), 3.40 (1H, d, *J* = 7.1 Hz), 1.81 (3H, s), 1.69 (3H, s), 1.46 (6H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.13, 158.14, 157.68, 156.90, 157.68, 154.89, 152.57, 138.87, 131.67, 130.24, 128.01, 127.54, 122.97, 122.08, 121.93, 120.61, 115.81, 114.88, 113.98, 107.46, 105.87, 101.8, 98.43, 77.80, 28.19, 25.75, 22.16, 17.87. HRMS (ESI+) *m/z*: 710.1127 $[M+Na]^+$ (calcd. for $C_{35}H_{30}N_3O_5I$ $[M+Na]^+$, 710.1194).

(1-(2-Trifluoromethyl phenyl)-1*H*-1,2,3-triazol-4-yl)-methyl osajin (3g): Yellow gum, yield: 89%, ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.08 (1H, s), 7.92 (1H, s), 7.91 (1H, s), 7.87 (1H, d, *J* = 7.7 Hz), 7.75 (1H, t, *J* = 7.4 Hz), 7.69 (1H, t, *J* = 7.4 Hz), 7.58 (1H, d, *J* = 7.7 Hz), 7.49 (2H, d, *J* = 8.8), 7.10 (2H, d, *J* = 8.1 Hz), 6.73 (1H, d, *J* = 10.0 Hz), 5.62 (1H, d, *J* = 10.0 Hz), 5.36 (2H, s), 5.18 (1H, t, *J* = 6.2 Hz), 3.40 (1H, d, *J* = 7.3 Hz), 1.81 (3H, s), 1.69 (3H, s), 1.47 (6H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.1, 158.2, 156.8, ,154.9, 154.6, 152.5, 144.1, 134.7, 133.0, 131.6, 130.5, 130.1, 129.0, 127.9, 127.2, 125.4, 123.9, 123.0, 121.9, 115.8, 114.9, 107.4, 105.8, 105.4, 77.7, 62.0, 28.1, 25.7, 21.2, 17.8. HRMS (ESI+) *m/z*: 652.2035 $[M+Na]^+$ (calcd. for $C_{35}H_{30}N_3O_5F_3$ $[M+Na]^+$, 652.2117).

(1-(4-Nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methyl osajin (3h): Pale yellow gum, yield: 91%, ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.05 (1H, s), 8.43 (2H, d, *J* = 8.5 Hz), 8.20 (1H, s), 8.00 (2H, d, *J* = 8.6 Hz), 7.91 (1H, s), 7.49 (2H, d, *J* = 8.5 Hz), 7.09 (2H, d, *J* = 8.5 Hz), 6.73 (1H, d, *J* = 10.0 Hz), 5.63 (1H, d, *J* = 10.0 Hz), 5.36 (2H, s), 5.18 (1H, t, *J* = 7.1 Hz), 3.40 (2H, d, *J* = 7.1 Hz), 1.81 (3H, s), 1.69 (3H, s), 1.47 (6H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.31, 158.14, 157.68, 156.95, 155.79, 142.43, 139.81, 138.63, 137.11, 136.47, 130.29, 127.32, 124.02, 122.97, 121.91, 121.25, 121.03, 115.81, 114.88, 107.45, 105.44, 98.43, 77.80, 62.03, 29.67, 27.77, 25.75, 21.27. HRMS (ESI+) *m/z*: 607.2192 $[M+H]^+$ (calcd. for $C_{34}H_{31}N_4O_7$ $[M+Na]^+$, 607.2213).

(1-(4-Trifluoromethylphenyl)-1*H*-1,2,3-triazol-4-yl)-methyl osajin (3i): Pale yellow gum, yield: 84%, ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.09 (1H, s), 8.07 (1H, s), 7.92 (1H, s), 7.75 (2H, d, *J* = 8.08 Hz), 7.51-7.45 (4H, m), 7.17 (2H, d, *J* = 8.6 Hz), 6.74 (1H, d, *J* = 10.0 Hz), 5.63 (1H, d, *J* = 10.0 Hz), 5.26 (2H, s), 5.17 (1H, t, *J* = 7.3 Hz), 3.40 (1H, d, *J* = 7.1 Hz), 1.82 (3H, s), 1.69 (3H, s), 1.47 (6H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.43, 158.59, 157.46, 156.87, 156.35, 156.11, 152.13, 144.50, 136.34, 135.02, 132.92, 131.39, 128.85, 127.70, 126.32, 126.07, 125.82, 125.05, 123.65, 122.37, 121.47, 117.65, 108.06, 106.56,77.78, 61.87, 31.90, 27.09, 26.71, 25.26, 22.66. HRMS (ESI+) *m/z*: 630.2215 $[M+H]^+$ (calcd. for $C_{35}H_{31}N_3O_5F_3$ $[M+Na]^+$, 630.2242).

(1-(4-Chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl osajin (3j): Yellow gum, yield: 89%, ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.08 (1H, s), 7.91 (1H, s), 7.56 (2H, d, *J* = 8.6 Hz), 7.53-7.49 (3H, m), 7.16 (2H, d, *J* = 8.6 Hz), 6.74 (1H, d, *J* = 10.0 Hz), 5.64 (1H, d, *J* = 10.0 Hz), 5.25 (2H, s), 5.18 (1H, t, *J* = 7.3 Hz), 3.41 (1H, d, *J* = 7.1 Hz), 1.81 (3H, s), 1.69 (3H, s), 1.47 (6H, s). HRMS (ESI+) *m/z*: 618.1771 $[M+Na]^+$ (calcd. for $C_{34}H_{30}N_3O_5Cl$ $[M+Na]^+$, 618.1695).

in vitro Anticancer activity of compounds

Maintanance of cell lines: The human breast cancer cell line (MCF-7), prostate cancer cell line (PC-3) and cervical cancer cell line (HeLa) were procured from King Institute of Preventive Medicine, Chennai, India. The cell lines were grown in culture flask using minimum essential medium supplemented with 3% L-glutamine, 10% foetal bovine serum, penicillin (100 IU/mL), streptomycin (100 µg/mL) and amphotericin B along with 7.5% sodium bicarbonate in a T25 mL cultured vented flask and incubated at 37 ºC in 5% CO_2 incubator. After 3 days, about 80-90% confluent monolayer (adherent) formation was confirmed by inverted microscope. Then, it was sub-cultured by using TPVG solution along with minimum essential medium and used for further study.

Anticancer activity of title compounds: Growth inhibition of MCF-7, PC-3 and HeLa cells by the synthesized compounds were determined by using cytotoxic assay. The cells were harvested and seeded in a 96 well plates and the plates were incubated for 24 h at 37 ºC in 5% CO_2 for attachment of cells. After 12 h, different concentrations of title compounds such as 10 µg/mL, 20 µg/mL, 30 µg/mL were added to the cells and incubated for 24 h. After incubation, the medium was replaced with phenol red and FBS free medium and 15 µL of

MTT (5 mg/mL) dye was added per well and wrapped with aluminium foil and the plate was incubated again for 4 h. After incubation medium was aspirated and 100 µL of DMSO were added to each well to solubilize the formazan crystals. The optical density (OD) was measured at the wavelength of 570 nm. The percentage of cell inhibition was determined by the following formula:

$$\text{Cell viabiity (\%)} = \frac{OD_{test}}{OD_{control}} \times 100$$

in silico Studies

Molecular docking: The three-dimensional structure of Human estrogen receptor Alpha (HERA) (PDB ID: 2IOG) and Doxorubicin (PDB ID: DM2) was downloaded from the RCSB protein Data Bank. The atomic coordinates of the protein was estranged and geometry optimization was done using Argus Lab 4.0.1. All the ligands were converted into Pdbqt file format and atomic coordinates were generated using Pyrx2010.12. The active binding sites of target protein were analyzed using the Drug Discovery Studio version 3.0 and 3D Ligand site virtual tools . The structures of target protein, native compound and reference compound are shown in Fig. 1.

RESULTS AND DISCUSSION

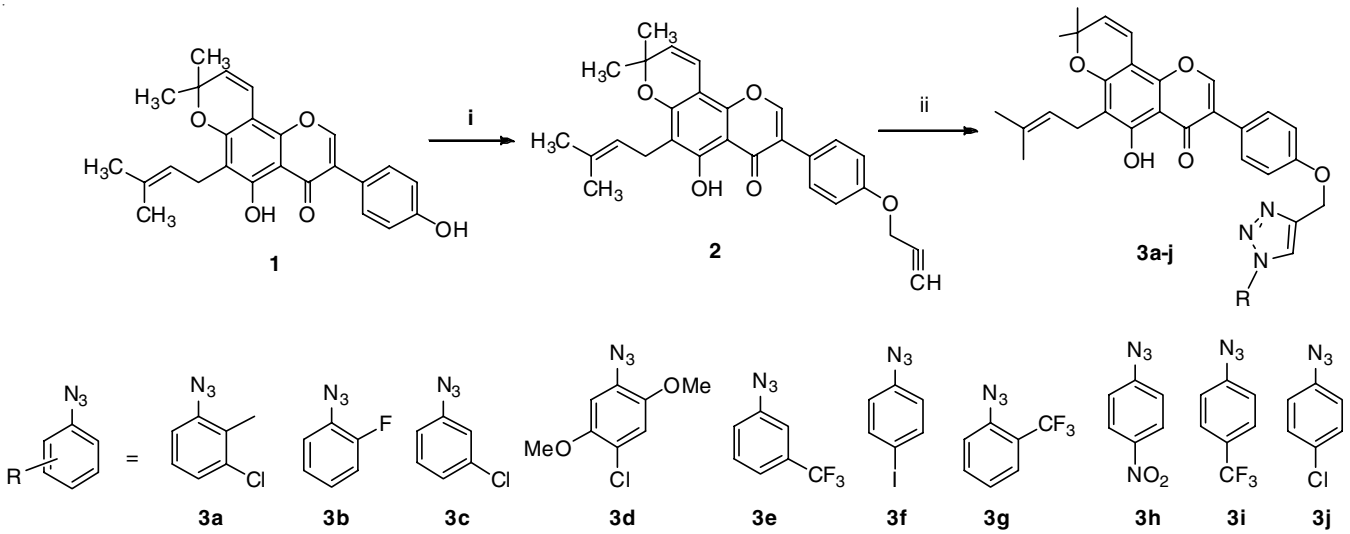
Synthesis: The natural isoflavone, osajin was isolated from *Walsura trifoliata* and used as the starting material for all the semi-synthesized compounds. Initially, hydroxyl group was further transformed to propargyl derivative **2** through standard alkylation using propargyl bromide and K_2CO_3 in acetone. Thus, propargyl derivative **2** was subjected to various azide precursors to afford the target compounds (**3a-j**) in good yields (**Scheme-I**). All triazolic compounds were purified through silica gel column chromatography and fully characterized by IR, NMR and high-resolution mass spectral (HRMS) analysis. In ^{1}H NMR spectra, a characteristic signal for the hydrogen present in triazolic ring was observed within the δ 7.91 ppm). The compounds **3a-j** were achieved within 6 h with yields ranging from 83-95 %.

Anticancer activity: All the newly synthesized derivatives **3a-j** were studied for anti-proliferation against panel of cancer cell lines (MCF-7, PC-3 and Hela) using MTT assay method [13]. Doxorubicin was used as a reference drug for this study and experiments were carried out in triplicate. All the compounds exhibited good to poor anti-TB activity with MIC values in the range of 6.25 to >25 µg/mL. Among all the synthesized compounds, **3c**, **3d** and **3i** exhibited potent activity potent anti-cancer activity with the minimum IC_{50} values compared to the standard (Table-1). Similarly, compounds **3a** and **3h** also displayed same significant anti-cancer activity against the tested cell lines (Table-1).

TABLE-1
CYTOTOXIC ACTIVITIES DATA OF THE SYNTHESIZED TRIAZOLE DERIVATIVES OF OSAJIN (**3a-j**)

Compound	IC_{50} (µg/mL)		
	PC-3	MCF-7	Hela
Osajin	12.68 ± 2.46	11.68 ± 1.89	11.04 ± 1.02
3a	13.02 ± 1.38	12.86 ± 2.32	13.94 ± 1.40
3b	12.14 ± 2.32	12.06 ± 1.46	11.84 ± 1.96
3c	11.80 ± 1.72	10.60 ± 1.88	6.80 ± 1.08
3d	9.62 ± 1.44	6.36 ± 3.10	8.26 ± 1.42
3e	9.48 ± 1.86	8.62 ± 1.40	9.14 ± 1.72
3f	13.92 ± 1.52	12.92 ± 2.66	13.18 ± 2.02
3g	11.66 ± 1.14	11.26 ± 2.28	10.66 ± 2.08
3h	9.06 ± 2.18	9.88 ± 2.98	9.06 ± 1.10
3i	10.14 ± 2.02	10.10 ± 3.02	7.12 ± 1.06
3j	13.24 ± 1.18	13.02 ± 2.66	12.68 ± 1.46
Doxorubicin	14.46 ± 2.12	14.08 ± 1.20	15.38 ± 1.32

***in silico* Studies:** The molecular *in silico* docking studies for all the the derivatives were carried out and docking results of the compounds (**3a-j**) have shown significant binding modes against HERA protein. All the compounds showed a higher dock scores than the reference compound (-6.7) [14-16]. The H-bonds, binding affinities and energy profiles of compounds (**3a-j**), osajin and doxorubicin towards the active site amino acids of the enzyme are summarized in Table-2 and their 3D modelled interactions of the lead title compounds with HERA



Scheme-I: Synthesis of the title compounds; Reagents and conditions: (i) acetone, K_2CO_3, proparjyl bromide, 12 h; (ii) CuI, aromatic azide, THF

TABLE-2
ENERGY PROFILES OF THE SYNTHESIZED TRIAZOLE DERIVATIVES OF OSAJIN (**3a-j**)

Compound	Binding energy	Binding interaction	Bond length (Å)	Bond angle (°)	Bond type
Osajin	-8.7	Pro 324 OC ….HO	2.5	106.6	H-don
		Pro 324 OC ….HO	1.9	113.0	H-don
3a	-8.1	Leu 320 CB ….HO	2.4	97.6	H-don
		Leu 320 CB ….HO	2.0	136.1	H-don
		Arg 394 CZ ….NN	2.3	108.8	H-acc
3b	-8.5	Asp 321 CA ….HO	2.1	99.7	H-don
		Leu 320 CB ….HO	2.0	87.9	H-don
		Arg 394 CZ ….NN	2.2	100.4	H-acc
3c	-9.1	Arg 394 CZ ….NN	2.1	108.6	H-acc
		Trp 393 HC ….OC	2.4	107.1	H-acc
		Glu 443 OC ….HO	2.5	121.9	H-don
3d	-9.3	Arg 394 CZ ….NN	2.1	96.0	H-acc
		Trp 393 HC ….OC	2.2	87.8	H-acc
3e	-9.3	Arg 394 CZ ….NN	2.5	78.3	H-acc
		Trp 393 HC ….OC	2.6	114.3	H-acc
3f	-7.6	Pro 324 OC ….HO	2.8	136.3	H-don
3g	-8.9	Arg 394 CZ ….NN	2.1	106.1	H-acc
		Trp 393 HC ….OC	2.6	86.6	H-acc
3h	-9.3	Arg 394 CZ ….NN	2.2	118.1	H-acc
		Trp 393 HC ….OC	2.2	126.3	H-acc
3i	-9.3	Arg 394 CZ ….NN	2.2	93.1	H-acc
3j	-7.7	Ile 326 HC ….HO	2.7	101.7	H-don
Doxorubicin	-6.7	Trp 393 HC ….HC	2.7	131.7	H-don

protein are shown in Fig. 1. Thus, these interactions provide support to *in vitro* anticancer activity of the compounds on MCF-7, PC-3 and HeLa cell lines. Hence, the present investigation demonstrates that the synthesized compounds will be the promising next generation chemotherapeutic drugs, which can be effectively used in the treatment of breast cancer, prostate cancer, cervical cancer and other related disorders. Amongst the synthesized compounds, compound **3j** has shown the hydrophobic interactions against HERA protein.

Conclusion

A series of osajin derivatives containing 1,2,3-triazole ring (**3a-j**) were synthesized and characterized by ^{1}H & ^{13}C NMR and mass spectrometry. All the newly synthesized compounds were evaluated for their *in vitro* anti-cancer activities against panel of cancer cell lines Only, three compounds **3d**, **3e** and **3h** exhibited potent anti-cancer activity against the tested cell lines. Further, anti-cancer activities of all the compounds have shown a good correlation with *in silico* molecular docking

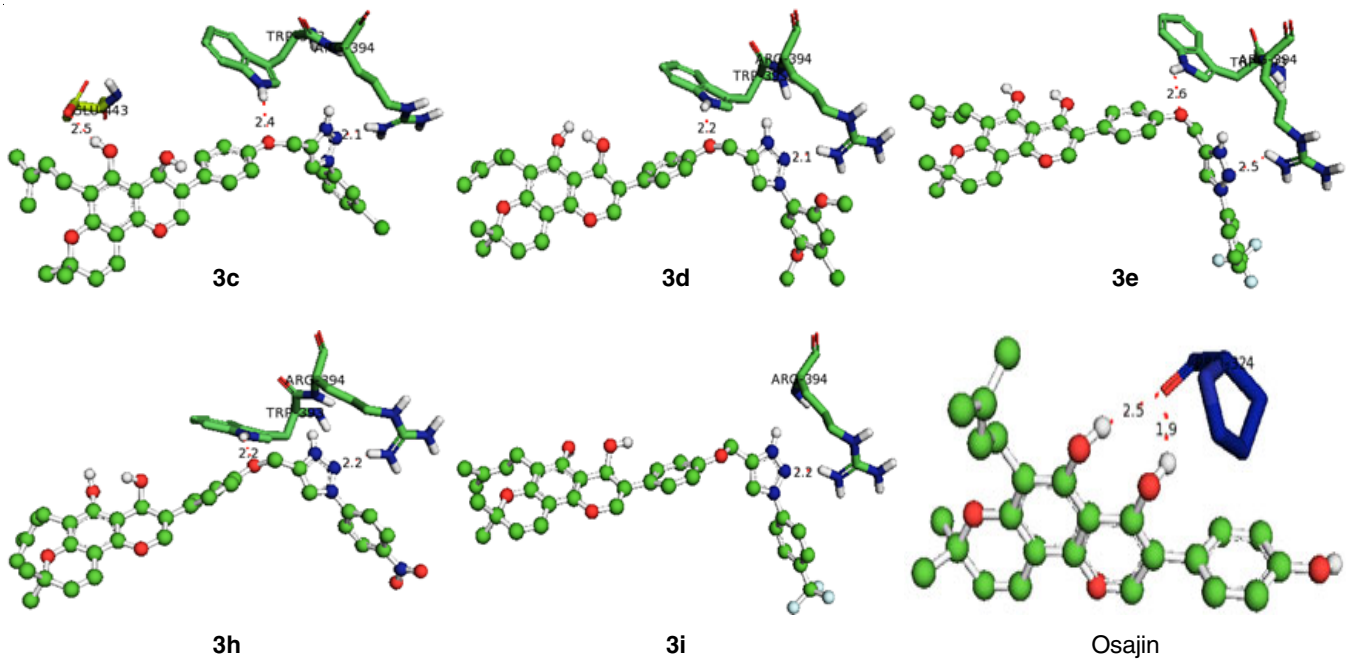


Fig. 1. Diagrammatic representation of 3D modelled binding modes of the lead compounds and osajin with the binding domain of Human estrogen receptor alpha protein

analysis and some compounds exhibiting strong interactions with the protein. This study could provide a roadmap to design and synthesis of new anti-cancer agents to overcome various problems associated with the currently available drugs in the market.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. http://pressroom.cancer.org/releases?item=290 (accessed March 27, 2019).
2. M.J. Thun, J.O. Delancey, M.M. Center, A. Jemal and E.M. Ward, *Carcinogenesis*, **31**, 100 (2010); https://doi.org/10.1093/carcin/bgp263
3. D. Schrama, R.A. Reisfeld and J.C. Becker, *Nat. Rev. Drug Discov*., **5**, 147 (2006); https://doi.org/10.1038/nrd1957
4. D.J. Newman and G.M. Cragg, *J. Nat. Prod*., **70**, 461 (2007); https://doi.org/10.1021/np068054v
5. L.V. Ramana, M.S. Appa Rao, K.M.C. Apparao and B.N. Rao, *Asian J. Chem*., **31**, 2042 (2019); https://doi.org/10.14233/ajchem.2019.22064
6. I.H. Son, I.M. Chung, S.I. Lee, H.D. Yang and H.I. Moon, *Bioorg. Med. Chem. Lett*., **17**, 4753 (2007); https://doi.org/10.1016/j.bmcl.2007.06.060
7. N. Chaichamnong, P. Temkitthawon, N. Khorana, P. Pitpakdeeanan, P. Taepavarapruk, N. Nuengchamnong, Y. Siriwattanasathien, A. Suksamrarn and K. Ingkaninan, *Planta Med*., **84**, 1134 (2018); https://doi.org/10.1055/a-0619-5547
8. G. Ribaudo, M.A. Pagano, V. Pavan, M. Redaelli, M. Zorzan, R. Pezzani, C. Mucignat-Caretta, T. Vendrame, S. Bova and G. Zagotto, *Fitoterpia*, **105**, 132 (2015); https://doi.org/10.1016/j.fitote.2015.06.020
9. S. Fortin and G. Bérubé, *Expert Opin. Drug Discov*., **8**, 1029 (2013); https://doi.org/10.1517/17460441.2013.798296
10. B. Poornima, B. Siva, G. Shankaraiah, A. Venkanna, S. Ramakrishna, V.L. Nayak, C. Venkat Rao and K.S. Babu, *Eur. J. Med. Chem*., **92**, 449 (2015); https://doi.org/10.1016/j.ejmech.2014.12.040
11. Q. Zhang, Y. Lu, Y. Ding, J. Zhai, Q. Ji, Q. Ma, M. Yang, H. Fan, J. Long, Z. Tong, Y. Shi, Y. Jia, B. Han, W. Zhang, C. Qiu, X. Ma, Q. Li, Q. Shi, H. Zhang, D. Li, J. Zhang, J. Lin, L.-Y. Li, Y. Gao and Y. Chen, *J. Med. Chem.*, **55**, 8757 (2012); https://doi.org/10.1021/jm301064b
12. S.I. Presolski, V.P. Hong and M.G. Finn, *Curr. Protoc. Chem. Biol*., **3**, 153 (2011); https://doi.org/10.1002/9780470559277.ch110148
13. M. Botta, S. Armaroli, D. Castagnolo, G. Fontana, E. Bombardelli and P. Pera, *Bioorg. Med. Chem. Lett*., **17**, 1579 (2007); https://doi.org/10.1016/j.bmcl.2006.12.101
14. G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell and A.J. Olson, *J. Comput. Chem*., **30**, 2785 (2009); https://doi.org/10.1002/jcc.21256
15. E. TerHaar, J.T. Coll, D.A. Austen, H.-M. Hsiao, L. Swenson and J. Jain, *Nat. Struct. Biol.*, **8**, 593 (2001); https://doi.org/10.1038/89624
16. E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng and T.E. Ferrin, *J. Comput. Chem.*, **25**, 1605 (2004); https://doi.org/10.1002/jcc.20084