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LETTERS

Synthesis of Indole-Oxadiazole coupled isoxazole hybrids as potent EGFR targeting anticancer agents

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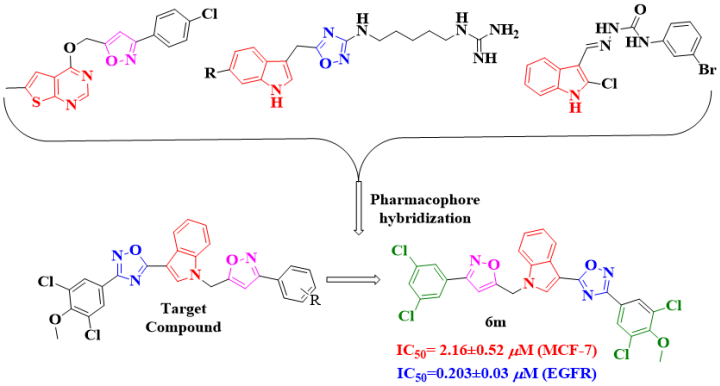
Article

ABSTRACT

The synthesis of new indole-oxadiazole coupled isoxazole hybrids (**6a–o**) synthesized by the Cu(I)-catalyzed reaction of in situ generated nitrile oxides with 3-(3,5-dichloro-4-methoxyphenyl)-5-(1-(prop-2-yn-1-yl)-1H-indol-3-yl)-1,2,4-oxadiazole in good yields have been reported here. The chemical structures of all newly synthesized hybrids were confirmed by ^{1}H-NMR, ^{13}C-NMR, and Mass spectra. All synthesized compounds were screened for their *in vitro* cytotoxicity against two breast cancer cell lines MCF-7 and MDA-MB-231 respectively. All the derivatives were more active against MCF7 than MDA-MB-231 cancer cells and few compounds showed better activity than the standard erlotinib. The ability of more potent compounds to inhibit EGFR tyrosine kinase, one of the key enzymes involved in breast carcinomas was evaluated by in vitro enzymatic assay and it was found that the compound (6g) and (6m) had more inhibitory activity IC_{50} values 0.311±0.05 and 0.203±0.03 μM than erlotinib (IC_{50}=0.421±0.03 μM).



Keywords: Cytotoxicity, EGFR, Indole, Isoxazole, 1,2,4-oxadiazol, hybrid drugs, heterocyclic drugs

INTRODUCTION

According to the GLOBOCAN 2020 estimates, cancer is the second largest cause of death around the world.[1] Breast cancer in women accounted for 11.7% of the 19.3 million new cases of cancer in 2020, according to the latest estimates of the global cancer burden.[2] Epidermal growth factor receptor (EGFR) and herceptin are both members of the same family. It is known that EGFR helps cells move and invade other cells. So, these receptors are shown to be important anticancer targets not only in non-small cell lung carcinomas but also in breast cancer. Both inherited and sporadic breast cancers have been found to have mutations in the EGFR gene.[3] Now, EGFR-targeted medicine is being looked at as a possible way to inhibit breast cancer cells proliferation.[4] A lot of drugs that target tyrosine kinase are being looked at to see if they can be used as EGFR inhibitors.[5,6]

In recent years, it has been interesting for medicinal chemists to use parts of biologically active molecules to make new molecules with a bigger range of biological effects. The advantage of using these small molecules, which are called "privileged scaffolds," is that their interactions with biological systems have been thoroughly studied. This means that predictions can be made about how well these subunits will interact with receptors that have not yet been studied.[7] On the other hand, the indole skeleton is one of the most ideal structures because it has strong antitumor activity and is often found in both active ingredients and natural products.[8–11] Li et al. found that Compound I (Figure 1) had strong anticancer activity against a panel of cancer cell lines. Tests of Compound I mechanism showed that its growth-inhibiting effects are caused by its ability to stop EGFR activity, as shown by the Western blot.[12] Compound II blocked the action of EGFR (T790M), EGFR (L858R), and c-MET with IC50 values of 0.094, 0.099, and 0.595 μM. Compound **III** (Figure 1) was able to inhibit EGFR (IC_{50} = 2.80 μM) and PDGFR (IC_{50} = 6.16 μM).[13] Song *et al*. have found that a number of indole derivatives can inhibit EGFR/VEGFR.[14]

In addition, other heterocycles such as isoxazole derivatives were said to be powerful anticancer EGFR-TKIs. For example, the isoxazole derivative IV (Figure 1) was very good at inhibiting cancer cells proliferation,[15] and the isoxazole derivatives V were good at inhibiting cancer cells expressing EGFR-TK (Figure 1).[16] In the same way, 1,2,4-oxadiazole is the only oxadiazole that can be found in natural products. For example, Carbone M. et al. recovered two alkaloids called Phidianidine A and Phidianidine

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B (VI) from the sea slug Opisthobranch Phidiana militaris.[17] These alkaloids had strong cytotoxic activity in vitro against both cancerous and healthy mammalian cells.[18,19] Later, a big change happened when 3,5-diaryl substituted 1,2,4-oxadiazole derivatives were found to cause apoptosis.[20] Since then, many anticancer drugs based on 1,2,4-oxadiazole have been made.[21–23]

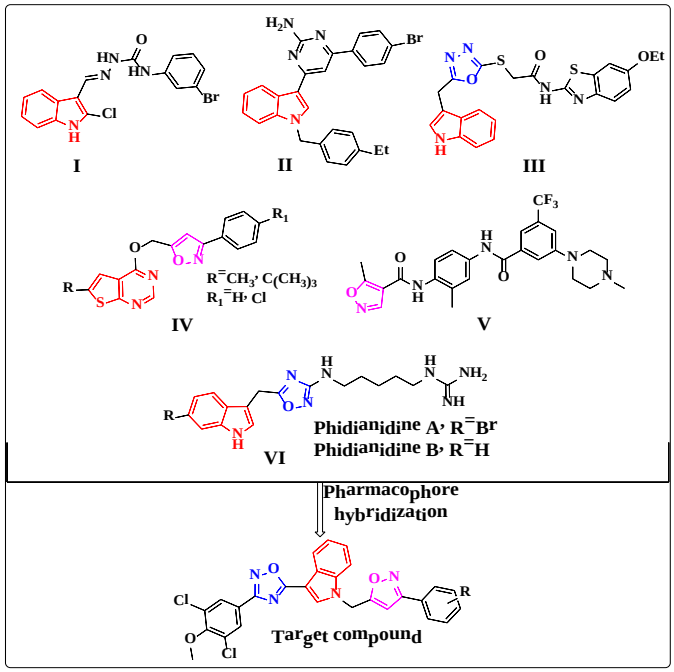


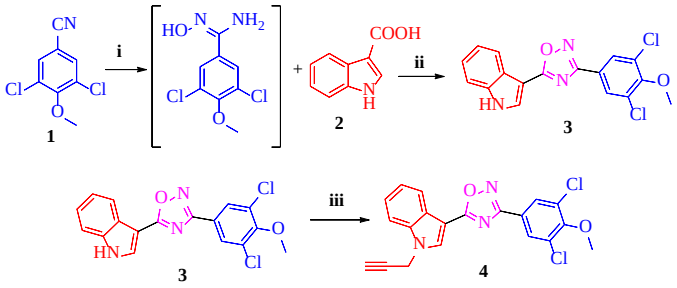
Figure 1. Planned approach of indole-1,2,4-oxadiazole-isoxazole hybrids.

In recent medicinal chemistry, a pharmacophore hybridization approach has been found to be one of the most successful ways to develop new bioactive compounds. When two different bioactive chemicals with similar pharmacophoric functions or different ways of working are put together, the results are often better.[24–26] Based on how well indole, 1,2,4-oxadiazole, and isoxazole-based compounds inhibit proliferation of cancer cells, we used a pharmacophore hybridization method to combine indole, 1,2,4-oxadiazole, and isoxazole pharmacophores as single indole-1,2,4-oxadiazole-isoxazole hybrids. The core structure and SAR studies of indole-1,2,4-oxadiazole-isoxazole hybrids with their in vitro anticancer and EGFR activity, might lead to the creation of new anticancer molecules.

RESULTS AND DISCUSSION

Chemistry

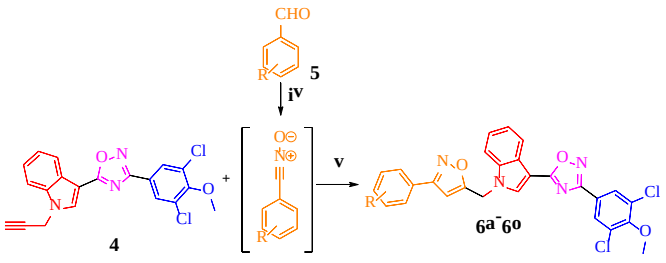
The overall synthetic technique used to produce 3-(3,5-dichloro-4-methoxyphenyl)-5-(1-((3-(aryl)isoxazol-5-yl)methyl)-1H-indol-3-yl)-1,2,4-oxadiazole (6a-6o) is divided into two phases (**Scheme 1** and **2**). In the first step, 3,5-dichloro-4-methoxybenzonitrile (1) was treated with $NH_2OH.HCl$ through Et3N in dry DCM for 8 hours at room temperature to yield in situ 3,5-dichloro-N'-hydroxy-4-methoxybenzimidamide, which was then treated with 1H-indole-3-carboxylic acid (**2**) using vilsmeier's reagent at the same reaction temperature for an additional 7 hours to yield the desired 3-(3,5-dichloro-4-methoxyphenyl)-5-(1H-indol-3-yl)-1,2,4-oxadiazole (**3**).[27] Compound (3) was treated for 6 hours with propargyl bromide in DMF using K_2CO_3 to yield intermediate 3-(3,5-dichloro-4-methoxyphenyl)-5-(1-(prop-2-yn-1-yl)-1H-indol-3-yl)-1,2,4-oxadiazole (4) (**Scheme 1**).[28]



Scheme 1. Reagents and conditions: (i) $NH_2OH.HCl$, TEA, DCM, RT, 8h; (ii) Vilsmeir reagents, RT, 7h. (iii) Propargyl bromide, K_2CO_3, DMF, 80 °C, 6h.

Further, other aldehydes **5** were converted to the corresponding aldoximes using hydroxylammonium chloride and 1M NaOH in t-BuOH:H_2O at room temperature. These aldoximes were converted to the corresponding nitrile oxide using chloramine-T trihydrate. The 1,3-dipolar cycloaddition of in situ generated nitrile oxide and 3-(3,5-dichloro-4-methoxyphenyl)-5-(1-(prop-2-yn-1-yl)-1H-indol-3-yl)-1,2,4-oxadiazole (4) in the presence of copper(I) catalyst at room temperature yielded 3,5-disubstitued isoxazoles (**6a-6o**) in good yields (**Scheme 2**).[29]

All the spectral data of the synthesized compounds were in full agreement with the proposed structures and were also discussed for a representative compound, **6d**. In the ^{1}H-NMR spectrum, the signals are at δ 7.75–6.99 (11H, Ar-H), 6.72 (1H, isoxazole-H), 5.13 (2H, N-CH_2), 3.87 (3H, OCH_3), and 3.81 (3H, OCH_3), and in the ^{13}C-NMR spectrum, the signals are at 159.70 & 159.15 (2C-OCH_3), 55.27 (O-CH_3), and 42.50 (N-CH_2). The [M+H] ion peak at m/z 547 in the ESI mass spectrum confirmed the structure of compound **6d**. The elemental analysis (C, H, and N) data (C, 61.48; H, 3.71; N, 10.21) confirmed the purity of compound **6d**.



Scheme 2. Reagents and conditions: (iv) $NH_2OH.HCl$, NaOH, $TsN(Cl)Na.3H_2O$, 2h; (v) CuI, DMF, RT, 8-10h.

In vitro cytotoxicity

The indole-1,2,4-oxadiazole conjugated isoxazole were evaluated for their anticancer activity against two breast cancer

cell lines, MCF-7 and MDA-MB-231 and were compared with erlotinib as the standard. The results of the anticancer activity screening are summarized in **Table 1**.[30] The compounds have stronger action against MCF-7 than MDA-MB-231 cancer cells. Those compounds demonstrated IC_{50} values ranging from 2.16±0.52 μM to 61.61±1.23 μM, while the standard drugs displayed values ranging from 4.28 ± 0.11 (MCF-7) and 7.46 ± 0.39 μM (MDA-MB-231) respectively. Among the screened compounds, five compounds like 4-chloro phenyl on the isoxazole ring (**6e**), 4-fluoro phenyl on the isoxazole ring (**6g**), 3-chloro phenyl on the isoxazole ring (**6l**), 2-bromo phenyl on the isoxazole ring (**6h**), 4-chloro phenyl on the isoxazole ring (**6i**), 3,5-dichloro phenyl on the isoxazole ring (**6m**), and 4-cyano phenyl on the isoxazole ring (**6o**) were found to be active against MCF-7 cancer cell line. Predominantly, the compounds (6m) (MCF-7; IC_{50}= 2.16±0.52 μM) and (**6g**) (MCF-7; IC_{50}= 3.21±0.48 μM) showed more potent activity compared to the standard drug erlotinib (**Figure 2**).

Kinase inhibition

The epidermal growth factor (EGFR) plays an important role in cell survival, growth, differentiation, and tumorigenesis. Accordingly, the inhibitory potential of a variety of indole-1,2,4-oxadiazole conjugated isoxazole based derivatives against the tyrosine kinase EGFR was investigated.[21] In view of this, the inhibitory activity against the tyrosine kinase EGFR was tested for our five potent compounds (**6e**, **6g**, **6l**, **6m**, and **6o**), and the results were correlated with their in vitro cytotoxicity data. In detail, the compound **6g** (IC_{50}: 0.311±0.05 µM) showed ≈ 1.35 fold more potent activity than erlotinib (IC_{50}: 0.421±0.03 μM). Similarly, compound 6m (IC_{50}: 0.217±0.03 μM) showed ≈ 2.0 fold more potent activity as compared to erlotinib, while the compounds **6o** (IC_{50}: 0.548±0.07 μM) and 6e (IC_{50}: 0.682±0.09 μM) have shown equipotent activity compared to standard. Remaining compound **6l** (IC_{50}: 0.864±0.08 µM) showed good EGFR inhibitory activity as compared to reference erlotinib.

Table 1. In vitro cytotoxicity activity with IC_{50} in μM.[a]

Comp.	R	MCF-7	MDA-MB-231	MCF-10A
6a	H	21.43± 1.21	61.61±1.23	-
6b	4-Me	19.87± 1.02	54.38± 1.21	-
6c	3-Me	23.47±1.12	57.87± 1.66	-
6d	4-OMe	17.38 ± 0.98	29.50± 1.16	-
6e	4-Cl	4.88± 0.63	13.81±1.12	17.88±1.15
6f	4-Br	13.11 ± 1.02	19.87±1.05	-
6g	4-F	3.21 ± 0.48	9.43±0.94	14.77±1.12
6h	4-NO_2	12.66±0.96	20.33±1.02	
6i	3,5-diMe	18.16± 1.09	47.93±1.31	
6j	2,3-diMe	20.66±1.22	51.35±1.11	
6k	3-NO_2	15.19±1.32	48.39±1.08	-
6l	3-Cl	5.83±0.83	14.21 ± 0.97	18.61±1.08
6m	3,5-diCl	2.16±0.52	8.33± 0.81	13.54 ±1.01
6n	3,5-diNO_2	11.18±0.95	18.31±0.94	-
6o	4-CN	5.32±0.69	15.08±0.83	21.54±1.18
Standard	Erlotinib	**4.28 ± 0.11**	**7.46 ± 0.39**	-

[a]=Values are mean ± SD of three replicates. "-"= not tested.

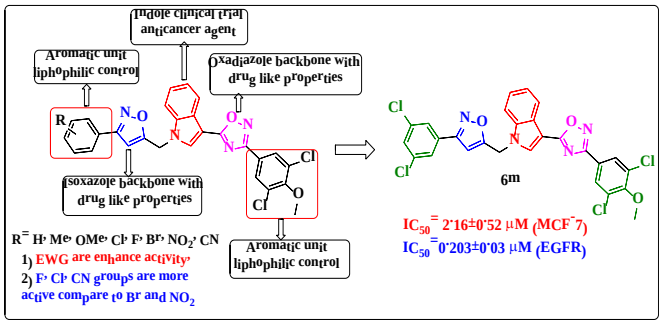


Figure 2. SAR of target Indole-1,2,4-Oxadiazole-Isoxazole derivatives.

Table 2: EGFR inhibitory activity of potent compounds.

Compound	IC_{50}(µM)
6e	0.682±0.09
6g	0.311±0.05
6l	0.864±0.08
6m	0.203±0.03
6o	0.548±0.07
Erlotinib	**0.421±0.03**

EXPERIMENTAL SECTION

Synthesis of 3-(3,5-dichloro-4-methoxyphenyl)-5-(1*H*-indol-3-yl)-1,2,4-oxadiazole (3): A mixture of 3,5-dichloro-4-methoxybenzonitrile (5g, 0.025 mol), $NH_2OH.HCl$ (0.025 mol) and triethylamine (5 mL) in dry DCM (50 mL) was stirred at room temperature for 8 hours. After the aromatic carboxylic acids (0.037 mol) and vilsmeier reagent (0.03 mol) were added and resulting mixture stirred for further 7 hours at same temperature. The completion of reaction was confirmed by TLC, then the reaction mixture was washed successively with saturated $NaHCO_3$ (50 ml) and brine (30 ml). The organic layer was dried (Na_2SO_4), filtered and the solvent was removed to give the crude product, which 1,2,4-oxadiazoles (**3**) were purified by short column chromatography (petroleum ether/ethyl acetate 8:2). ^{1}H-NMR (400 MHz, DMSO) δ 11.50 (s, 1H, -NH), 7.75 (d, J= 8.0Hz, 1H), 7.50 (d, J=8.0 Hz, 1H), 7.35 (s, 2H), 7.24-7.16 (m, 2H), 7.10-7.03 (m, 1H), 3.82 (s, 3H, -OCH_3); EI-MS m/z 360 [M+H].

Synthesis of 3-(3,5-dichloro-4-methoxyphenyl)-5-(1-(prop-2-yn-1-yl)-1H-indol-3-yl)-1,2,4-oxadiazole (4):

A mixture of 3-(3,5-dichloro-4-methoxyphenyl)-5-(1H-indol-3-yl)-1,2,4-oxadiazole (3) (4g, 0.011 mol), K_2CO_3 (0.033 mol) and propargyl bromide (0.016 mol) in DMF (50 mL) was stirred at room temperature for 6h. The completion of the reaction as monitored by TLC, the mixture was diluted with water (40 mL) and extracted with ethyl acetate (2 × 30 mL). The combined organic layer was washed with brine (2 × 30 mL), then dried under anhydrous Na_2SO_4 and finally concentrated under vacuum to afford compound (4) (81%). ^{1}H-NMR (400 MHz, DMSO) δ 7.73 (d, *J*= 8.0Hz, 1H), 7.55 (d, *J*=8.0 Hz, 1H), 7.38 (s, 2H), 7.26-7.22 (m, 1H), 7.16 (s, 1H), 7.11-7.05 (m, 1H), 4.15 (d, *J*=4.0 Hz, 2H, -CH_2), 3.81 (s, 3H, -OCH_3), 2.03 (t, *J*=4.0 Hz, 1H, -CH); EI-MS m/z 398 [M+H].

Synthesis of 3-(3,5-dichloro-4-methoxyphenyl)-5-(1-((3-(aryl)isoxazol-5-yl)methyl)-1H-indol-3-yl)-1,2,4-oxadiazole (6a-6o). Aldehyde (0.5g) was added to a solution of hydroxylamine hydrochloride (4.0 mmol) in 15 mL of 1:1 ^{t}BuOH : H_2O. To this was added NaOH (4.0 mmol), and the mixture was stirred for 30 min at ambient temperature. Chloramine-T trihydrate (4.0 mmol) was added in small portions over 10 min, followed by CuI (10mol %). 3-(3,5-dichloro-4-methoxyphenyl)-5-(1-(prop-2-yn-1-yl)-1*H*-indol-3-yl) -1,2,4-oxadiazole (0.5g) was added, p^{H} was adjusted to 6 by addition of a few drops of 1M NaOH, and stirring was continued for 8-10h. The reaction mixture was poured into cold water (50 mL), and 5 mL of dilute NH_4OH were added to remove all copper salts. Isoxazole was collected by filtration, redissolved, and passed through a short plug of silica gel (35% ethyl acetate–hexane) affording pure product.

3-(3,5-dichloro-4-methoxyphenyl)-5-(1-((3-phenylisoxazol-5-yl)methyl)-1H-indol-3-yl)-1,2,4-oxadiazole (6a): ^{1}H NMR (400 MHz, DMSO) δ 7.75 (d, *J*= 8.0Hz, 1H), 7.56 (d, *J*=8.0 Hz, 1H),7.50-7.45 (m, 2H), 7.35 (s, 2H), 7.28-7.20 (m, 4H), 7.14 (s, 1H), 7.11-7.07 (m, 1H), 6.78 (s, 1H), 5.12 (s, 2H), 3.82 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, DMSO) δ 165.40, 161.66, 160.30, 159.04, 156.38, 135.22, 130.46, 129.51, 128.78(2C), 128.04(2C), 127.08(2C), 126.65, 126.12(2C), 123.78, 122.63, 122.08, 121.59, 120.83, 110.36, 98.72, 96.92, 56.20, 42.25; ESI-MS m/z: 517 [M+H]. Anal.Cal for $C_{27}H_{18}Cl_2N_4O_3$; C, 62.68; H, 3.51; N, 10.83; found C, 62.65; H, 3.53; N, 10.86.

3-(3,5-dichloro-4-methoxyphenyl)-5-(1-((3-(p-tolyl)isoxazol-5-yl)methyl)-1H-indol-3-yl)-1,2,4-oxadiazole (6b): ^{1}H NMR (400 MHz, DMSO) δ 7.73 (d, *J*= 8.0Hz, 1H), 7.61 (d, *J*= 8.0Hz, 2H), 7.53 (d, *J*=8.0 Hz, 1H), 7.40 (d, *J*= 8.0Hz, 2H), 7.31 (s, 2H), 7.26-7.22 (m, 1H), 7.13 (s, 1H), 7.10-7.07 5(m, 1H), 6.73 (s, 1H), 5.13 (s, 2H), 3.81 (s, 3H, -OCH_3), 2.37 (s, 3H, -CH_3); ^{13}C NMR (100 MHz, DMSO) δ 165.60, 161.40, 160.10, 159.19, 156.29, 139.59, 135.30, 130.42, 129.11(2C), 128.20(2C), 127.40(2C), 126.27, 125.59(2C), 123.30, 122.70, 122.16, 121.61, 120.29, 110.49, 98.70, 96.50, 56.43, 42.37, 21.54; ESI-MS m/z: 531 [M+H]. Anal.Cal for $C_{28}H_{20}Cl_2N_4O_3$; C, 63.29; H, 3.79; N, 10.54; found C, 63.33; H, 3.83; N, 10.57.

3-(3,5-dichloro-4-methoxyphenyl)-5-(1-((3-(m-tolyl)isoxazol-5-yl)methyl)-1H-indol-3-yl)-1,2,4-oxadiazole (6c): ^{1}H NMR (400 MHz, DMSO) δ 7.74 (d, *J*= 8.0Hz, 1H), 7.54 (d, *J*=8.0 Hz, 1H), 7.46-7.41(m, 2H), 7.35 (s, 2H), 7.28-7.22 (m, 3H), 7.13 (s, 1H), 7.10-7.04 (m, 1H), 6.79 (s, 1H), 5.13 (s, 2H), 3.81 (s, 3H, -OCH_3), 2.29 (s, 3H, -CH_3); ^{13}C NMR (100 MHz, DMSO) 165.80, 161.36, 160.10, 159.09, 156.14, 137.22, 135.35, 132.30, 131.25, 129.76(2C), 128.97, 128.37, 127.27, 126.27(2C), 124.13, 123.58, 122.84, 122.20, 121.35, 120.29, 110.75, 98.24, 96.25, 56.53, 42.61, 21.13; ESI-MS m/z: 531 [M+H]. Anal.Cal for $C_{28}H_{20}Cl_2N_4O_3$; C, 63.29; H, 3.79; N, 10.54; found C, 63.32; H, 3.83; N, 10.58.

3-(3,5-dichloro-4-methoxyphenyl)-5-(1-((3-(4-methoxyphenyl) isoxazol-5-yl)methyl)-1H-indol-3-yl)-1,2,4-oxadiazole (6d): ^{1}H NMR (400 MHz, DMSO) δ 7.75 (d, *J*= 8.0Hz, 1H), 7.69 (d, *J*=8.0 Hz, 2H), 7.53 (d, *J*=8.0 Hz, 1H), 7.32 (s, 2H), 7.21-7.10 (m, 2H), 7.09-7.03 (m, 1H), 6.99 (d, *J*=8.0 Hz, 2H), 6.72 (s, 1H), 5.13 (s, 2H), 3.87 (s, 3H, -OCH_3), 3.81 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, DMSO) δ 165.67, 161.44, 160.15, 159.70, 159.15, 156.13, 135.32, 129.67(2C), 128.91(2C), 126.64, 126.11(2C), 123.82, 122.95, 122.08, 121.65, 121.13, 120.22, 114.60(2C), 110.68, 98.55, 96.82, 56.47, 55.27, 42.50; ESI-MS m/z: 547 [M+H]. Anal.Cal for $C_{28}H_{20}Cl_2N_4O_4$; C, 61.44; H, 3.68; N, 10.24; found C, 61.48; H, 3.71; N, 10.21.

5-(1-((3-(4-chlorophenyl)isoxazol-5-yl)methyl)-1H-indol-3-yl)-3-(3,5-dichloro-4-methoxyphenyl)-1,2,4-oxadiazole (6e): ^{1}H NMR (400 MHz, DMSO) δ 7.75 (d, *J*= 8.0Hz, 1H), 7.65 (d, *J*= 8.0Hz, 2H), 7.53 (d, *J*=8.0 Hz, 1H), 7.41 (d, *J*= 8.0Hz, 2H), 7.32 (s, 2H), 7.25-7.15(m, 2H), 7.10-7.03 (m, 1H), 6.77 (s, 1H), 5.13 (s, 2H), 3.81 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, DMSO) δ 165.39, 161.72, 160.18, 159.11, 156.20, 135.73, 135.17, 129.95, 129.07(2C), 128.21(2C), 127.33(2C), 126.39, 126.00(2C), 123.57, 122.58, 122.07, 121.44, 120.67, 110.34, 98.83, 96.64, 56.19, 42.47; ESI-MS m/z: 551 [M+H]. Anal.Cal for $C_{27}H_{17}Cl_3N_4O_3$; C, 58.77; H, 3.11; N, 10.15; found C, 58.75; H, 3.14; N, 10.17.

5-(1-((3-(4-bromophenyl)isoxazol-5-yl)methyl)-1H-indol-3-yl)-3-(3,5-dichloro-4-methoxyphenyl)-1,2,4-oxadiazole (6f): ^{1}H NMR (400 MHz, DMSO) δ 7.75 (d, *J*= 8.0Hz, 1H), 7.56 (d, *J*=8.0 Hz, 1H),7.48-7.43 (m, 4H), 7.35 (s, 2H), 7.23-7.19 (m, 1H), 7.14 (s, 1H), 7.10-7.05 (m, 1H), 6.76 (s, 1H), 5.13 (s, 2H), 3.81 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, DMSO) δ 165.60, 161.56, 160.29, 159.01, 156.09, 135.34, 131.44(2C), 129.17(2C), 127.44, 126.65(2C), 126.27, 125.83(2C), 124.80, 123.68, 122.69, 122.16, 121.39, 120.74, 110.47, 98.49, 96.33, 56.56, 42.47; ESI-MS m/z: 594 [M+H] & 596 [M+3H]. Anal.Cal for $C_{27}H_{17}BrCl_2N_4O_3$; C, 54.39; H, 2.87; N, 9.40; found C, 54.35; H, 2.82; N, 9.45.

3-(3,5-dichloro-4-methoxyphenyl)-5-(1-((3-(4-fluorophenyl) isoxazol-5-yl)methyl)-1H-indol-3-yl)-1,2,4-oxadiazole (6g): ^{1}H NMR (400 MHz, DMSO) δ 7.99 (d, *J*= 8.0Hz, 2H), 7.75 (d, *J*= 8.0Hz, 1H), 7.65 (d, *J*= 8.0Hz, 2H), 7.52 (d, *J*=8.0 Hz, 1H), 7.33 (s, 2H), 7.24-7.20 (m, 1H), 7.15 (s, 1H), 7.10-7.05 (m, 1H), 6.81 (s, 1H), 5.13 (s, 2H), 3.82 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, DMSO) δ 165.60, 161.72, 161.08, 160.11, 159.09, 156.09, 135.46, 130.27(2C), 129.76(2C), 127.96, 126.63, 126.27(2C), 123.35, 122.75, 122.16, 121.53, 120.75, 115.53(2C), 110.52, 98.91, 96.49, 56.48, 42.46; ESI-MS m/z: 535 [M+H]. Anal.Cal for $C_{27}H_{17}Cl_2FN_4O_3$; C, 60.57; H, 3.20; N, 10.47; found C, 60.54; H, 3.24; N, 10.45.

3-(3,5-dichloro-4-methoxyphenyl)-5-(1-((3-(4-nitrophenyl) isoxazol-5-yl)methyl)-1H-indol-3-yl)-1,2,4-oxadiazole (6h): ^{1}H NMR (400 MHz, DMSO) δ 8.40 (d, *J*= 8.0Hz, 2H), 8.20 (d, *J*= 8.0Hz, 2H), 7.75 (d, *J*= 8.0Hz, 1H), 7.55 (d, *J*=8.0 Hz, 1H), 7.36 (s, 2H), 7.26-7.20 (m, 2H), 7.12-7.05 (m, 1H), 6.82 (s, 1H), 5.13 (s, 2H), 3.81 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, DMSO) δ 165.38, 161.67, 160.16, 159.05, 156.24, 149.70, 136.35, 135.66, 129.62(2C), 128.01, 127.21(2C), 126.70(2C), 126.19, 125.70(2C), 125.20, 123.29, 122.61, 122.09, 121.57, 120.79, 110.74, 98.77, 96.46, 56.20, 42.64; ESI-MS m/z: 562 [M+H]. Anal.Cal for $C_{27}H_{17}Cl_2N_5O_5$; C, 57.67; H, 3.05; N, 12.45; found C, 57.69; H, 3.02; N, 12.48.

3-(3,5-dichloro-4-methoxyphenyl)-5-(1-((3-(3,5-dimethylphenyl) isoxazol-5-yl)methyl)-1H-indol-3-yl)-1,2,4-oxadiazole (6i): ^{1}H NMR (400 MHz, DMSO) δ 7.74 (d, *J*= 8.0Hz, 1H), 7.59 (s, 2H), 7.52 (d, *J*=8.0 Hz, 1H), 7.35 (s, 2H), 7.29-7.20 (m, 2H), 7.18-7.06 (m, 2H), 6.72 (s, 1H), 5.15 (s, 2H), 3.82 (s, 3H, -OCH_3), 2.34 (s, 6H, $2CH_3$); ^{13}C NMR (100 MHz, DMSO) δ 165.56, 161.54, 160.12, 159.51, 156.22, 137.04(2C), 135.36, 132.00, 131.12, 129.74(2C), 126.66, 126.13(2C), 125.57(2C), 123.88, 122.91, 122.09, 121.53, 120.23, 110.59, 98.69, 96.77, 56.11, 42.49, 21.30(2C), ESI-MS m/z: 545 [M+H]. Anal.Cal for $C_{29}H_{22}Cl_2N_4O_3$; C, 63.86; H, 4.07; N, 10.27; found C, 63.89; H, 4.03; N, 10.30.

3-(3,5-dichloro-4-methoxyphenyl)-5-(1-((3-(2,3-dimethylphenyl) isoxazol-5-yl)methyl)-1H-indol-3-yl)-1,2,4-oxadiazole (6j): ^{1}H NMR (400 MHz, DMSO) δ 7.74 (d, *J*= 8.0Hz, 1H), 7.54 (d, *J*=8.0 Hz, 1H), 7.48-7.40 (m, 1H), 7.33 (s, 2H), 7.24-7.20 (m, 4H), 7.10-7.04 (m, 1H), 6.77 (s, 1H), 5.15 (s, 2H), 3.81 (s, 3H, -OCH_3), 2.30 (s, 3H, -CH_3), 1.98 (s, 3H, -CH_3); ^{13}C NMR (100 MHz, DMSO) δ 165.59, 161.87, 160.41, 159.13, 156.16, 137.50, 136.90, 135.10, 133.22, 131.30, 129.27(2C), 126.64, 126.11(2C), 125.36, 123.67, 122.95, 122.08, 121.13, 120.22, 110.66, 98.68, 96.90, 56.42, 42.47, 19.46, 16.24; ESI-MS m/z: 545 [M+H]. Anal.Cal for $C_{29}H_{22}Cl_2N_4O_3$; C, 63.86; H, 4.07; N, 10.27; found C, 63.89; H, 4.04; N, 10.29.

3-(3,5-dichloro-4-methoxyphenyl)-5-(1-((3-(3-nitrophenyl)isoxazol-5-yl)methyl)-1H-indol-3-yl)-1,2,4-oxadiazole (6k): ^{1}H NMR (400 MHz, DMSO) δ 8.48 (s, 1H), 8.23-8.19 (m, 3H), 7.76 (d, *J*= 8.0Hz, 1H), 7.55 (d, *J*=8.0 Hz, 1H), 7.32 (s, 2H), 7.25-7.20 (m, 1H), 7.13 (s, 1H), 7.09-7.05 (m, 1H), 6.81 (s, 1H), 5.13 (s, 2H), 3.82 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, DMSO) δ 165.78, 161.70, 160.50, 159.22, 156.44, 147.48, 135.76, 134.46, 132.11, 129.58(2C), 128.36, 127.69, 126.65, 126.11(2C), 124.80, 123.82, 122.76, 122.07, 121.35, 120.72, 110.58, 98.33, 96.71, 56.28, 42.62; ESI-MS m/z: 562 [M+H]. Anal.Cal for $C_{27}H_{17}Cl_2N_5O_5$; C, 57.67; H, 3.05; N, 12.45; found C, 57.63; H, 3.07; N, 12.48.

5-(1-((3-(3-chlorophenyl)isoxazol-5-yl)methyl)-1H-indol-3-yl)-3-(3,5-dichloro-4-methoxyphenyl)-1,2,4-oxadiazole (6l): ^{1}H NMR (400 MHz, DMSO) δ 7.82 (s, 1H), 7.75 (d, *J*= 8.0Hz, 1H), 7.56 (d, *J*=8.0 Hz, 1H),7.49-7.43 (m, 2H), 7.35 (s, 2H), 7.28-7.23 (m, 2H), 7.13 (s, 1H), 7.09-7.04 (m, 1H), 6.79 (s, 1H), 5.13 (s, 2H), 3.81 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, DMSO) δ 165.42, 161.55, 160.42, 159.28, 156.27, 135.52, 132.71, 132.21, 129.40, 128.67(2C), 128.06, 126.27(2C), 125.54, 124.52, 123.06, 122.65, 122.16, 121.29, 120.29, 110.62, 98.49, 96.69, 56.21, 42.31; ESI-MS m/z: 551 [M+H]. Anal.Cal for $C_{27}H_{17}Cl_3N_4O_3$; C, 58.77; H, 3.11; N, 10.15; found C, 58.72; H, 3.15; N, 10.18.

3-(3,5-dichloro-4-methoxyphenyl)-5-(1-((3-(3,5-dichlorophenyl) isoxazol-5-yl)methyl)-1H-indol-3-yl)-1,2,4-oxadiazole (6m): ^{1}H NMR (400 MHz, DMSO) δ 7.75 (d, *J*= 8.0Hz, 1H), 7.70 (s, 2H), 7.56 (d, *J*=8.0 Hz, 1H), 7.43(s, 1H), 7.35 (s, 2H), 7.28-7.20 (m, 1H), 7.15 (s, 1H), 7.10-7.05 (m, 1H), 6.79 (s, 1H), 5.13 (s, 2H), 3.81 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, DMSO) δ 165.55, 161.77, 160.54, 159.18, 156.35, 135.64, 133.65(2C), 131.97, 130.74, 129.58(2C), 126.65, 126.00(2C), 125.15(2C), 123.02, 122.54, 122.15, 121.09, 120.56, 110.85, 98.70, 96.60, 56.56, 42.47; ESI-MS m/z: 584 [M+H]. Anal.Cal for $C_{27}H_{16}Cl_4N_4O_3$; C, 55.32; H, 2.75; N, 9.56; found C, 55.35; H, 2.77; N, 9.52.

3-(3,5-dichloro-4-methoxyphenyl)-5-(1-((3-(3,5-dinitrophenyl) isoxazol-5-yl)methyl)-1H-indol-3-yl)-1,2,4-oxadiazole (6n): ^{1}H NMR (400 MHz, DMSO) δ 8.48 (s, 2H), 8.21 (s, H), 7.77 (d, *J*= 8.0Hz, 1H), 7.57 (d, *J*=8.0 Hz, 1H), 7.34 (s, 2H), 7.25-7.20 (m, 1H), 7.15 (s, 1H), 7.10-7.06 (m, 1H), 6.80 (s, 1H), 5.13 (s, 2H), 3.83 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, DMSO) δ 165.74, 161.51, 160.21, 159.35, 156.09, 146.31(2C), 135.69, 132.89(2C), 131.15, 129.30(2C), 126.65, 126.12(2C), 123.78, 122.58, 122.08, 121.86, 121.29, 120.22, 110.53, 98.49, 96.77, 56.74, 42.47; ESI-MS m/z: 607 [M+H]. Anal.Cal for $C_{27}H_{16}Cl_2N_6O_7$; C, 53.39; H, 2.66; N, 13.84; found C, 53.35; H, 2.62; N, 13.87.

4-(5-((3-(3-(3,5-dichloro-4-methoxyphenyl)-1,2,4-oxadiazol-5-yl)-1H-indol-1-yl) methyl)isoxazol-3-yl)benzonitrile (6o): ^{1}H NMR (400 MHz, DMSO) δ 7.75 (d, *J*= 8.0Hz, 1H), 7.72 (d, *J*=8.0 Hz, 2H), 7.56 (d, *J*=8.0 Hz, 1H), 7.43 (d, *J*=8.0 Hz, 2H), 7.32 (s, 2H), 7.25-7.20 (m, 1H), 7.13 (s, 1H), 7.08-7.02 (m, 1H), 6.79 (s, 1H), 5.13 (s, 2H), 3.81 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, DMSO) δ 165.49, 161.56, 160.39, 159.34, 156.26, 135.93, 135.09, 132.54(2C), 129.55(2C), 128.40(2C), 126.64, 126.08(2C), 123.64, 122.95, 122.08, 121.22, 120.22, 119.08, 114.75, 110.40, 98.62, 96.72, 56.38, 42.52; ESI-MS m/z: 542 [M+H]. Anal.Cal for $C_{28}H_{17}Cl_2N_5O_3$; C, 62.01; H, 3.16; N, 12.91; found C, 62.04; H, 3.12; N, 12.95.

Conclusion

A series of indole-1,2,4-oxadiazole-isoxazole were prepared and tested for their anticancer potential against two breast cancer cell lines MCF-7 and MDA-MB-231. More potent compounds were found to possess anti EGFR tyrosine kinase activity, comparable with the standard drug erlotinib. All the compounds have stronger action against MCF-7 than MDA-MB-231 cancer cell. Among all compounds **6e**, **6g**, **6l**, **6m**, and **6o** have shown more potent activity compared to remaining compounds. Compounds **6g** and **6m** have shown more potent activity than standard erlotinib (4.28 ± 0.11 μM) with IC_{50} values 3.21±0.48 and 2.16±0.52 μM respectively. Further, EGFR inhibitory activity of above potent compounds revealed that compounds **6g** and **6m** demonstrated better potency than erlotinib (0.421±0.03 μM), with IC_{50} values of 0.311±0.05 and 0.203±0.03 μM, respectively and remaining compounds have shown good activity. These results are suggesting that a simple modification of compounds **6g** and **6m** can be better candidates for future investigations to produce new drugs.

Supplementary Material

Supplementary material (NMR spectra of compounds) of article can be found, in the online version, at journal site.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest, academic or financial, for publication of this article.

List of abbreviations:

EGFR=Epidermal Growth Factor Receptor
VEGFR=Vascular endothelial growth factor Receptor
DMF= Dimethylformamide
TEA= Triethylamine
DCM= Dichloromethane
TsN(Cl)Na.3H$_2$O = Tosylchloramide sodium trihydrate
^{1}H NMR= Proton nuclear magnetic resonance
^{13}C NMR= Carbon-13 nuclear magnetic resonance
DMSO=Dimethyl sulfoxide
EI-MS= Electron Ionization-Mass Spectrometry

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