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Apoptosis effects of phenyl tagged *isoxazole-benzoxadiazole* hybrids On MDA MB-231 cancer cell line

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Abstract: In this report, a series of phenyl tagged isoxazole-benzoxadiazole hybrids 4a–4g were synthesized by substituting in phenyl ring C4' with -OCH_3, -CH_3, -F, -Cl, -Br and C3' with -NO_2. Anticancer potential of 4a–4g on MDA MB-231 human cancer cell line was evaluated by using MTT assay. The order of anticancer activity of derived hybrids with phenyl ring substitution is, p-Br>p-Cl, p-F, m-NO_2, p-CH_3, >p-OCH_3, parent hybrid. Among them, 4g; hybrid with p-Br exhibits highest anticancer behavior. The reported haptophoric hybrids may provide a useful insight for designing effective chemotherapy drugs for treating MDA MB 231 cancer cells.

Keywords: anticancer, electron withdrawing groups, isoxazole-benzoxadiazole hybrids, MDA MB 231 cell line, phenyl tagged isoxazole.

1. INTRODUCTION

The molecular structure of *isoxazoles* can be differentiated and understood accurately as *oxazoles* type of *azoles* with an 'O' atom next to 'N' atom [Scheme 1].Although, *isoxazoles* are the derivatives of *azoles*, they display differential and specific biological receptions due to the presence of hetero atoms 'O' adjacent to 'N'. Several *isoxazole* moiety based drugs are commercially available for treating various diseases.[1] Numerous *isoxazole* analogues are reported to exhibit anticancer behavior, [2]-[8] whereas only *(N-[4-trifluoromethylphenyl]-methyl**isoxazol**-4-carboxamide);*leflunomide; [9] (drug bank id: DB010970)has been approved by FDA as a potential drug for treating triple negative breast cancer cells (TNBC). TNBC cells respond to the immunomodulation [10] effect of drug, inhibits the release of dihydroorotate dehydrogenase (DHODH) enzyme, consequently curtails the pyrimidine synthesis pathway in TNBC there by enhances the cancer cell death and hence the growth of tumor. [11] Another, *isoxazole* analogue, *(alpha S, 5S)-alpha-amino-3-chloro-4,5-dihydro-5-*

isoxazole*acetic acid;* acivicin [12, 13] found to be with antitumor activity was discarded in clinical trial due its inadmissible neurotoxic effects [14].

1,2,5-oxadiazole (furazan) is an *azole* class of heterocyclic, five-atom ring compound of an 'O' atom with two vicinal 'N' atoms; (N-O-N) bonded at third and fourth positions through 'C' atoms. Benzofurazan (2, 1,3-benzoxadiazole) is fused benzene and furazan rings [Scheme 1]. Though, very few benzofurazan scaffold based drugs [15-16] are marketed, none of them are found be reported for cancer treatment. However, many benzofurazan analogues [17-19] are reported to have potent anticancer activity.

Attachment of phenyl ring to the 5-position of isoxazole scaffold [20] is observed to be an advantageous in enhancing the anti-proliferative *function based on the (i) electronic (withdrawing/donating) nature of substituent, (ii) size of substituent, and (iii) position (o, m, p) of substitution, in the phenyl ring.* [20, 21] Substitution of electron withdrawing group (ewg) / donating group (edg) at C4' of phenyl ring was found to alter proliferation. Ewg/edg varies the lipophilicity of isoxazole and enhanced lipophilic effect leads to high permeability into the cellular membrane [22]. In general, presence of *ewg* in C4' of phenyl ring tagged with isoxazole shows enhanced activity of antiproliferation. Both, isoxazole (Table 1), [23-30] and *benzoxadiazole* (Table 2), [31]-[47] moieties are found to display effective anticancer activity towards *MDA MB-231* [23-47] cancer cell lines. In order to utilize the synergestic effect of aforementioned azole moieties, developing synthetic route for a stable isoxazole – benzoxadiazole hybrid structure is necessary.

Hence, based on the, (i) demonstrated characteristics of *isoxazol and benzoxadiazole* moieties and relevant anticancer activities reported in the literature, it is obvious that both the moieties exhibit substantial anticancer activity towards various cancer cellines, (ii) attachment of phenyl group alters the anticancer performance of the molecule. With this understanding it is worthwhile to design a feasible schematic route for the de novo synthesis of hybrid structure "*phenyl tagged isoxazole-benzoxadiazole*" derivatives by attaching the phenyl and benzofurazan groups to the central *isoxazole* moiety [Scheme 1]. Aromaticity index of both haptophoric moieties isoxazole and furazan is found to be similar as ~ 53 [48] which could be facilitating the stability of hybrid product.

The *phenyl tagged isoxazole-benzoxadiazole* derivatives ***4a***–***4g*** [Scheme 1] were obtained through Claisen–Schmidt condensation reaction procedure with different reactants and reaction conditions for substituting in C4' [$-OCH_3$, $-CH_3$, -F, -Cl, -Br] and C3' [$-NO_2$] of phenyl ring. The substituted products of "*phenyl tagged isoxazole-benzoxadiazole*" hybrids were evaluated for their anticancer potential on *MDA MB-231*cell lines.

Experimental Procedure

2. MATERIALS AND METHODS:

Analytical grade reagents, chemicals, and solvents were used without further purification. Percolated silica gel plates (silica gel 0.25mm) were used for thin layer chromatography. Mixture of petroleum ether, ethyl acetate (8:2) mixture was used for developing solvent system and the spots were visualized by ultraviolet light and/ or iodine vapors. The FT-IR spectra were obtained using AVATOR-330 instrument. The resulting frequencies of all sharp bands between 4000 – 400 cm^{-1} were observed and analyzed. The ^{1}H NMR and ^{13}C NMR spectral data were recorded using BRUCKERAVANCE 111 spectrometer operating at 400

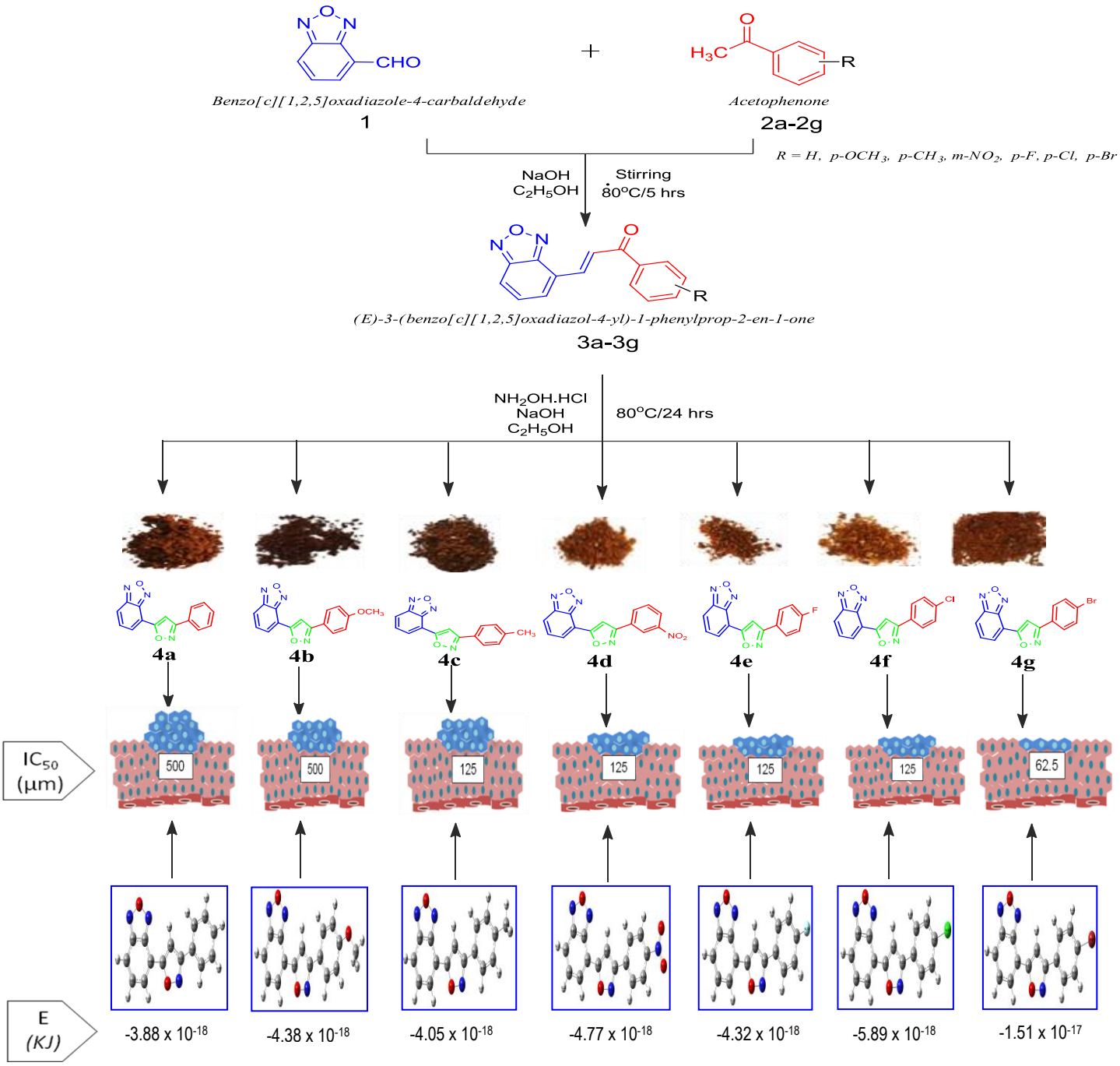
MHZ. For NMR analysis $CDCl_3$ was used as a solvent and tetra methyl silane as the internal standard. Melting points of synthesized products were estimated using open capillary apparatus. Quantum chemical calculations were performed on the 3D structural data of molecules by applying DFT method using the Gaussian 09 program suite [49] at the Becke-3-Lee-Yang-Par (B3LYP) level [50, 51] combined with the standard 6-311G(d,p) basis set. During optimization procedure, all the parameters were allowed to relax in order to obtain the stable structure with minimum energy. The optimized energy of the title compounds was ascertained from the structure optimization procedure.

A. Synthesis of 3a; the chalcone intermediates, the relevant reagent used for synthesizing used for preparing chalcone intermediates ***3b-3g*** *are listed below:* Claisen–Schmidt condensation reaction procedure was adopted to prepare a stable chalcone intermediates ***3a-3g;*** Scheme 1. In order to prepare ***3a***, benzo[C][1,2,5]oxadiazole-4-carbaldehyde; ***1*** (2g,0.01mol) and substituted acetophenone; ***2a*** (1.6g,0.01mol) were added to 25ml of ethanol and mixed well. To that, 5ml of 10% NaOH was poured gently and stirred for 5hrs at room temperature. Progress and completion of reaction was observed and identified using TLC. The obtained blend was diluted with ice water (50ml), acidified with 10% aq. HCl and stored in icebox for 24h to precipitate the product. The obtained chalcone intermediate 3-(benzo[c][1,2,5]oxadiazol-4-yl)-1-phenylprop-2-en-1-one***;*** ***3a*** was recrystallized from ethanol. The chalcone intermediates ***3b-3g*** also were prepared through the Claisen–Schmidt condensation reaction procedure explained above with a change in reagent with respect to the product targeted intermediates.

- ***3b*** *– 3-(benzo[c][1,2,5]oxadiazol-4-yl)-1-(4-methoxyphenyl)prop-2-en-1-one*
- ***3c*** *– 3-(benzo[c][1,2,5]oxadiazol-4-yl)-1-(p-tolyl)prop-2-en-1-one*
- ***3d*** *– 3-(benzo[c][1,2,5]oxadiazol-4-yl)-1-(3-nitrophenyl)prop-2-en-1-one*
- ***3e*** *– 3-(benzo[c][1,2,5]oxadiazol-4-yl)-1-(4-fluorophenyl)prop-2-en-1-one*
- ***3f*** *– 3-(benzo[c][1,2,5]oxadiazol-4-yl)-1-(4-chlorophenyl)prop-2-en-1-one*
- ***3g*** *–3-(benzo[c][1,2,5]oxadiazol-4-yl)-1-(4-bromophenyl)prop-2-en-1-one.*

*B. Synthesis of 4a-4g; "phenyl tagged isoxazole-benzoxadiazole" from (****3a-3g****):* The hybrid structures "*phenyl tagged isoxazole-benzoxadiazole*" derivatives ***4a***–***4g***were derived from a stable chalcone intermediates***3a-3g;*** Scheme 1. In order to prepare compound ***4a;*** *phenyl tagged isoxazole-benzoxadiazole*, from ***3a,*** 10ml of water, 50ml of ethanol, and 3ml of 10% aqNaOH were added to the mixture of 3-(benzo[c][1,2,5]oxadiazole-4-yl)-1-(4-phenyl)prop-2-en-1-one (1g,0.004mol) and hydroxylamine hydrochloride (0.3g,0.004mol) and refluxed for 24hr at 80°C. After the completion of reaction that is identified using TLC, the reaction blend was allowed to cool and then poured over crushed ice, neutralized with HCl and kept in refrigerator overnight. Few additional drops of Con.HCl was added to neutralize completely, the brown precipitate was filtered and washed with excess amount of water. The solid separation was filtered, dried and crystalized from ethanol to get pure compound;***4a***. The pureness of compound was analyzed using TLC. The isoxazole derivatives ***4b-4g*** also were prepared through the procedure explained above with a change in reagent with respect to the product targeted. The relevant reagent used for synthesizing isoxazole derivatives ***4b-4g*** are listed below,

- ***4b*** *– 4-(3-(4-methoxyphenyl)isoxazol-5-yl)benzo[c][1,2,5]oxadiazole*
- ***4c*** *– 4-(3-(p-tolyl)isoxazol-5-yl)benzo[c][1,2,5]oxadiazole*
- ***4d*** *– 4-(3-(3-nitrophenyl)isoxazol-5-yl)benzo[c][1,2,5]oxadiazole*
- ***4e*** *– 4-(3-(4-fluorophenyl)isoxazol-5-yl)benzo[c][1,2,5]oxadiazole*
- ***4f*** *– 4-(3-(4-chlorophenyl)isoxazol-5-yl)benzo[c][1,2,5]oxadiazole*
- ***4g*** *–4-(3-(4-bromophenyl)isoxazol-5-yl)benzo[c][1,2,5]oxadiazole*



Scheme 1: Synthesis of phenyl tagged *isoxazole-benzoxadiazole* hybrids; 4a–*4g*

3. RESULTS AND DISCUSSIONS

Confirmation of hybrid structure formation: As demonstrated in the Scheme 1, either the reactants or intermediate contains isoxazole moiety. Hence, the synthetic scheme was designed in such a way to form isoxazole as a linker between *phenyl and benzoxadiazole* (Scheme 1). The *isoxazole* ring formation in the *phenyl tagged isoxazole-benzoxadiazole* hybrids; ***4a-4g*** by the rearrangement of 'C' and 'N' atoms of chalcone intermediate ***3*** was confirmed by FTIR, ^{1}H and ^{13}C NMR spectral investigations (4a-4g). The presence of *isoxazole* rings in the *phenyl tagged isoxazole-benzoxadiazole* hybrids; ***4a-4g*** was identified

and confirmed from the signature peaks of *isoxazole.* The IR band of 'C-O-N' of *isoxazole* is observed in the range of 1228cm^{-1} to 1343cm^{-1}. In specific, peaks at 1228cm^{-1} (***4a***; R=H), 1249cm^{-1} (***4b***; R=*p*-OCH_3), 1267cm^{-1} (***4c***; R=*p*-CH_3), 1343cm^{-1} (***4d***; R=*m*-NO_2), 1277cm^{-1} (***4e***; R=*p*-F), 1259cm^{-1} (***4f***; R=*p*-Cl), 1272cm^{-1} (***4g***; R=*p*-Br) (4g). In ^{1}H NMR spectra, peaks for $H_{2"}$ proton associated with *isoxazole* ring appears at δ5.65ppm–7.26ppm. δ7.02ppm (***4a***; R=H), 6.80 ppm (***4b***; R=*p*-OCH_3),δ6.69 ppm (***4c***; R=*p*-CH_3), δ7.26 ppm (***4d***; R=*m*-NO_2), δ7.11 ppm (***4e***; R=*p*-F), δ6.95 ppm (***4f***; R=*p*-Cl), δ5.65 ppm (***4g***; R=*p*-Br). In ^{13}C NMR spectra, peaks for $C_{2"}$ and C=N carbons associated with *isoxazole* ring appears in the range δ90.1ppm–103.9ppm and δ140.2ppm–164.6ppm. δ99.9 ppm ($C_{2"}$); δ158.4 ppm (C=N) (***4a***; R=H), δ103.8 ppm ($C_{2"}$); δ163.4 ppm (C=N) (***4b***; R=*p*-OCH_3), δ103.9 ppm ($C_{2"}$); δ163.6 ppm (C=N) (***4c***; R=*p*-CH_3), δ99.9 ppm ($C_{2"}$); δ156.3 ppm (C=N) (***4d***; R=*m*-NO_2), δ90.1 ppm ($C_{2"}$); δ164.6 ppm (C=N) (***4e***; R=*p*-F), δ90.1 ppm ($C_{2"}$); δ140.2 ppm (C=N) (***4f***; R=*p*-Cl), δ103.9 ppm ($C_{2"}$); δ 149.2 ppm (C=N) (***4g***; R=*p*-Br). The slight shifting in the spectral values of *isoxazole* ring in IR spectra ('C-O-N'), ^{1}H NMR ($H_{2"}$) and in ^{13}C NMR ($C_{2"}$, C=N) could be attributed to the electronic effects of substituents in the C4' (-OCH_3, -CH_3, -F, -Cl, -Br) C3' (-NO_2) positions of phenyl ring (4a-4g). The formation of *phenyl tagged isoxazole-benzoxadiazole* hybrid structure with *isoxazole* ring linker is further confirmed from the mass spectral data of ***4c;*** *4-(3-(p-tolyl)isoxazol-5-yl)benzo[c][1,2,5]oxadiazole* (4c). In 4c, the peak at *m/z* [M+1]= 278 represents the mass of the skeleton of the derivative; *4-(3-phenylisoxazol-5-yl)benzo[c][1,2,5]oxadiazole* (***4a***; R=H)*;* the peak at *m/z* = 14 is for the substitution of methyl group in the C4' position of phenyl ring (*p*-CH_3).

A. 4-(3-phenylisoxazol-5-yl)benzo[c][1,2,5]oxadiazole (4a); Brown solid; Yield 65.3%; mp.196-198°c; IR (KBr, cm^{-1}): 1554(C=N), 1444(C=C), 1228 (C-O-N, (isoxazole)), 3056 (aromatic C-H); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.02(s, 1H, (isoxazole), ($H_{2"}$)), 7.45-7.51(m, 4H, Ar-H), 7.54-7.58(t, 1H, Ar-H), 7.91(d, 1H, Ar-H, (H_2), 7.95-8.17(d, 2H, Ar-H); ^{13}C (100 MHz, $CDCl_3$, δ, ppm): 99.4(isoxazole, ($C_{2"}$)), 115.9-131.7 (Ar-C), 147.1(C_6), 149.5(C_1), 158.4(C=N, (isoxazole), ($C_{3"}$)), 171.2(C-O, ($C_{1"}$)); Anal Calcd; ($C_{15}H_9N_3O_2$): C, 68.44; H, 3.45; N, 15.96%; Found: C, 68.42; H, 3.40; N, 15.84%.

B. 4-(3-(4-methoxyphenyl)isoxazol-5-yl)benzo[c][1,2,5]oxadiazole (4b); Brown solid; Yield 67%; mp.150-152°c; IR (KBr, cm^{-1}): 1594(C=N), 1456(C=C), 1249 (C-O-N, (isoxazole)), 3070 (aromatic C-H), 2920(Aliphatic C-H, OCH_3); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) : 6.80(d, 1H, (isoxazole), ($H_{2"}$)), 7.01-7.03(d, 2H, Ar-H), 7.35(q, 1H, Ar-H), 7.57-7.62(t, 3H, Ar-H), 7.91-7.93(q, 1H, Ar-H, (H_2), 3.84(s, 3H, -OCH_3); ^{13}C (100 MHz, $CDCl_3$, δ, ppm): 103.8(isoxazole, ($C_{2"}$)), 114.7-131.8(Ar-c), 148.3(C_6), 149.8(C_1), 163.4 (C=N, (isoxazole), ($C_{3"}$)),163.6(C-O,($C_{1"}$)), 55.5(-OCH_3); Anal. Calcd ($C_{16}H_{11}N_3O_3$): C, 65.53; H, 3.78; N, 14.33%; Found: C, 65.52; H, 3.74; N, 14.23%.

C. 4-(3-(p-tolyl)isoxazol-5-yl)benzo[c][1,2,5]oxadiazole (4c); Brown solid; Yield 67%; mp.124-126°c; IR (KBr, cm^{-1}): 1548(C=N), 1416 (C=C), 1267 (C-O-N, (isoxazole)), 3032 (aromatic C-H), 2920(Aliphatic C-H, CH_3); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 6.69(d, 1H, (isoxazole), ($H_{2"}$)), 6.79-7.25(t, 3H, Ar-H), 7.49(t, 1H , Ar-H), 7.71-7.73(d, 2H, Ar-H), 8.03(d, 1H, Ar-H, (H_2), 2.33(t, 3H, -CH_3); ^{13}C (100 MHz, $CDCl_3$, δ, ppm): 103.9(isoxazole, ($C_{2"}$)), 115.2-131.5(Ar-C), 142.8(C_6), 149.3(C_1), 163.6(C=N, (isoxazole), ($C_{3"}$)), 171.3(C-O, ($C_{1"}$)), 21.34(CH_3), ; mass(m/z): 278.0[M+1]; Anal. Calcd ($C_{16}H_{11}N_3O_2$): C, 69.31; H, 4.00; N, 15.15%; Found: C, 69.29; H, 3.95; N, 15.05%.

D. 4-(3-(3-nitrophenyl)isoxazol-5-yl)benzo[c][1,2,5]oxadiazole (4d); Brown solid; Yield 69%; mp.156-158°c; IR (KBr, cm^{-1}): 1526(C=N), 1443(C=C), 1343 (C-O-N (isoxazole)), 3081 (aromatic C-H); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.26(d, 1H, (isoxazole), ($H_{2"}$)), 7.36(s, 1H, Ar-H), 7.51-7.55(d, 1H, Ar-H), 7.68-7.71(d, 1H, Ar-H), 8.39-8.46(q, 3H, Ar-H), 8.91(s, 1H, Ar-H, (H_2); ^{13}C (100 MHz, $CDCl_3$, δ, ppm): 99.9(isoxazole, ($C_{2"}$)), 115.8-131.7(Ar-c), 147.1(C_6), 149.1(C_1), 156.3(C=N, (isoxazole), ($C_{3"}$)), 171.1(C-O, ($C_{1"}$)); Anal. Calcd ($C_{15}H_8N_4O_4$): C, 58.45; H, 2.62; N, 18.18%; Found: C, 58.43; H, 2.58; N, 18.06%.

E. 4-(3-(4-fluorophenyl)isoxazol-5-yl)benzo[c][1,2,5]oxadiazole (4e); Brown solid; Yield 65%; mp.168-170°c; IR (KBr, cm^{-1}): 1588(C=N), 1413(C=C), 1277 (C-O-N, (isoxazole)), 3042 (aromatic C-H); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.11(d, 1H, (isoxazole), ($H_{2"}$)), 7.26-7.36(m, 3H Ar-H), 7.60(t, 1H, Ar-H), 7.95-7.99(m, 2H, Ar-H), 8.09(d, 1H, Ar-H, (H_2); ^{13}C (100 MHz, $CDCl_3$, δ, ppm): 90.1 (isoxazole, ($C_{2"}$)), 113.7-131.7(Ar-c), 145.9(C_6), 149.8(C_1), 164.6(C=N, (isoxazole), ($C_{3"}$)), 167.1(C-O, ($C_{1"}$)); Anal. Calcd ($C_{15}H_8FN_3O_2$): C, 64.06; H, 2.87; N, 14.94%; Found: C, 64.04; H, 2.84; N, 14.84%.

F. 4-(3-(4-chlorophenyl)isoxazol-5-yl)benzo[c][1,2,5]oxadiazole (4f); Brown solid; Yield 68%; mp.178-180°c; IR (KBr, cm^{-1}): 1584(C=N), 1485(C=C), 1259 (C-O-N, (isoxazole)), 3058 (aromatic C-H); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 6.95 (d,1H, (isoxazole), ($H_{2"}$)), 7.35(d, 1H, Ar-H), 7.39-7.44(m, 3H, Ar-H), 7.88-7.99(q, 2H, Ar-H), 8.01(d, 1H, Ar-H, (H_2); ^{13}C (100 MHz, $CDCl_3$, δ, ppm) : 90.1(isoxazole, ($C_{2"}$)), 114.7-131.2(Ar-C), 139.9(C_6), 149.4(C_1), 140.2(C=N, (isoxazole), ($C_{3"}$)), 170.0(C-O, ($C_{1"}$)); Anal. Calcd ($C_{15}H_8ClN_3O_2$): C, 60.52; H, 2.71; N, 14.12%; Found: C, 60.59; H, 2.67; N, 14.10%.

G. 4-(3-(4-bromophenyl)isoxazol-5-yl)benzo[c][1,2,5]oxadiazole (4g); Brown solid; Yield 42%; mp.198-200°c; IR (KBr, cm^{-1}) : 1580(C=N), 1420(C=C), 1272 (C-O-N,(isoxazole)), 3082 (aromatic C-H); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 5.65(s, 1H, (isoxazole), ($H_{2"}$)), 7.26(s, 1H, Ar-H), 7.52(d, 1H, Ar-H), 7.60-7.82(m, 4H, Ar-H), 8.06(d, 1H, Ar-H, (H_2); ^{13}C (100 MHz, $CDCl_3$, δ, ppm): 103.9(isoxazole, ($C_{2"}$)), 115.6-131.8(Ar-c), 132.3(C_6), 144.3(C_1), 149.2(C=N, (isoxazole),($C_{3"}$)), 155.7(C-O, ($C_{1"}$)); Anal. Calcd ($C_{15}H_8BrN_3O_2$): C, 52.66; H, 2.36; N, 12.28%; Found: C, 52.78; H, 2.33; N, 12.21%.

4. ANTICANCER EFFECT OF PHENYL TAGGED ISOXAZOLE-BENZOXADIAZOLE HYBRIDS (4a–4g):

All *phenyl tagged isoxazole-benzoxadiazole* hybrids; ***4a–4g*** were evaluated for their anticancer activity towards MDA-MB 231 cell line using MTT assay. Anticancer behavior was quantified using the IC_{50} value which is the concentration of hybrid molecule required for reaching half maximal inhibitory Table 3. Among the hybrids ***4a-4g***, parent hybrid without any substitution in the phenyl ring attached with central isoxazole scaffold and with *p*-OCH_3 showed the least potent anticancer activity (IC_{50} = 500µM). However, hybrid structures with *p*-CH_3, *m*-NO_2, *p*-F, *p*-Cl, displayed moderate effect (IC_{50} = 125µM) and hybrid with *p*-Br exhibited strong antiproliferation effect on *MDA-MB 231* cell line (Table 3). From the results it is clear that the substitution in the tagged aryl ring impacts the antiproliferation activity of hybrids.

Compound	Substituent	Electronic effect	IC_{50} (µM)
4a	-	-	500
4b	*p*-OCH_3	donating	500
4c	*p*-CH_3	donating	125
4d	*m*-NO_2	withdrawing	125
4e	*p*-Cl	withdrawing	125
4g	*p*-Br	withdrawing	62.5

Compound	Energy of optimized structure (*KJ*)	Substituent	Mulliken charges of atoms in substituents

Table 1: Electronic effects and IC_{50} values of various substituents in phenyl ring

Further, it is obvious that the substitution of "ewg" in the *Para* position of terminal aryl results in the increased inhibitory activity. The order of anticancer activity of derived hybrids with phenyl ring substitution is, *p*-Br>*p*-Cl, *p*-F, *m*-NO_2, *p*-CH_3, >*p*-OCH_3, parent hybrid. Electron-withdrawing groups (NO_2, F, Cl, and Br) in the para and meta position of phenyl ring are advantageous for raising the anti-proliferation effect. Although, 'Br' is bulkier than 'F' and 'Cl' with lesser electron withdrawing efficiency, low IC_{50} (62.5µM) value of ***4g*** suggests that the bulkier 'Br' atom in C4' position plays a vital role to inhibit proliferation of *MDA MB 231* cells [52].

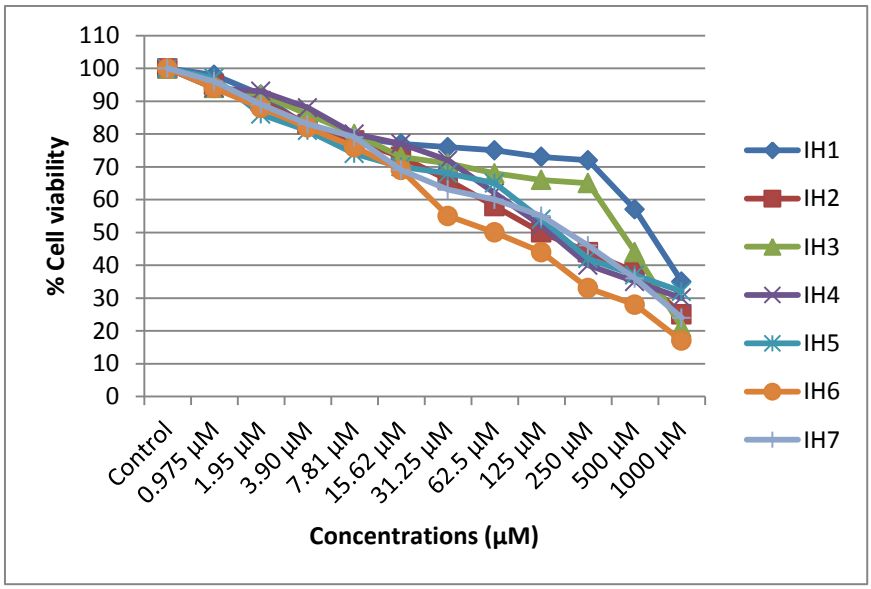


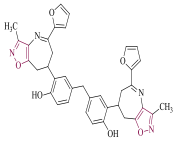
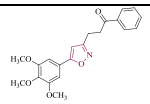
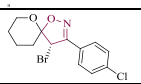
Fig.1. In vitro breast cancer screening of the compounds **IH1** (4a), **IH2** (4c), **IH3** (4b), **IH4** (4f), **IH5** (4e), **IH6** (4g) and **IH7** (4d) in MDA-MB 231 human breast cancer cell lines after 48h exposure.

4a	-3.88 x 10^{-18}	-	0.098 (H27)
4b	-4.38 x 10^{-18}	*p*-OCH_3	-0.345 (O27) -0.134 (C28) 0.132 (H29) 0.114 (H30) 0.114 (H31)
4c	-4.05 x 10^{-18}	*p*-CH_3	-0.256 (C27) 0.125 (H28) 0.108 (H29) 0.126 (H30)
4d	-4.77 x 10^{-18}	*m*-NO_2	0.169 (N27) -0.266 (O28) -0.265 (O29)
4e	-4.32 x 10^{-18}	*p*-F	-0.228 (F27)
4f	-5.89 x 10^{-18}	*p*-Cl	-0.064 (Cl27)
4g	-1.51 x 10^{-17}	*p*-Br	-0.018 (Br27)

Table 2: Energy of optimized structures and Mulliken charge values

Energy (Table 4) of optimized structures computed through quantum chemical calculations infers that '***4g'*** the compound with *'Br' substituent in C4' position of phenyl ring [4-(3-(4-bromophenyl)isoxazol-5-yl)benzo[c][1,2,5]oxadiazole]* is the most stable derivative with minimum energy. The estimated Mulliken charge values infers that, *'Br27' has relatively least partial atomic charge -0.018* [53] among all the substituent atoms, which facilitates the higher anticancer activity of ***4g***. Hence, the derivative ***4g;*** possess relatively the optimum electron density and stability attributes that enables ***4g*** to exhibit strong anticancer drug effect [54] than other derivatives ***4a-4f*** (Scheme 1). The antiproliferation; IC_{50} values of various *isoxazole* analogues (Table 1) [23-30] and *benzoxadiazole* analogues (Table 2) [31-47] on *MDA-MB 231* cell lines [23-47] are listed in Table 1 and Table 2 for comparison. Although, the IC_{50} value of ***4g*** is not significant in comparison with the literature value, it is found to be impressive and effective among the tested compounds ***4a-4g***. Hence, further research on enhancing the anticancer property of ***4g*** is a potential direction for further investigation towards the drug discovery for cancer.

Table 3: Comparative analysis of IC_{50} values of *isoxazole* analogues on *MDA-MB 231* cell lines

***Isoxazole* analogues**	**Structures**	**IC_{50}(µM)**	**Reference**
4,4'-methylenebis(2-(5-(furan-2-yl)-3-methyl-7,8-dihydro-6H-isoxazolo[4,5-b]azepin-7-yl)phenol)2-(3,4-dimethoxyphenyl)-5-(3-nitrophenyl)-1,3,4-oxadiazole		10.32 ± 0.43	[23]
1-phenyl-3-(5-(3,4,5-trimethoxyphenyl)isoxazol-3-yl)propan-1-one		2.49	[24]
(4S)-4-bromo-3-(4-chlorophenyl)-1,6-dioxa-2-azaspiro[4.5]dec-2-ene		53.1±0.64	[25]

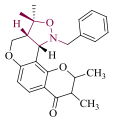
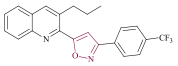
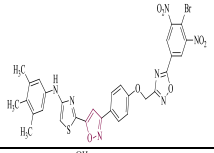
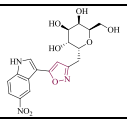
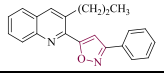
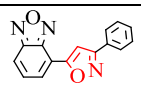
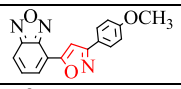
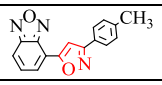
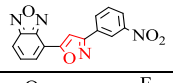
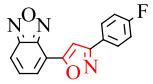
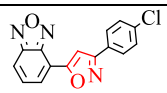
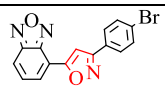
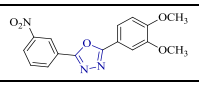
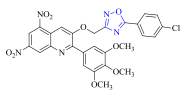
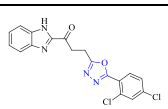
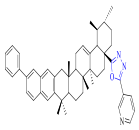
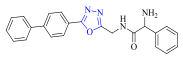
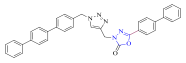
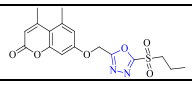
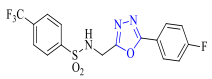
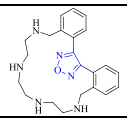
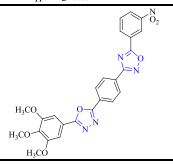
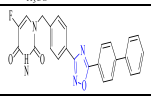
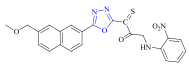
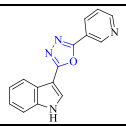
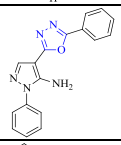
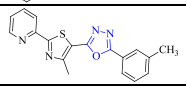
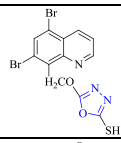
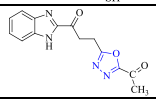
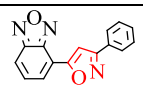
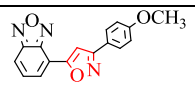
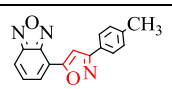
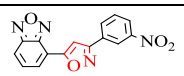
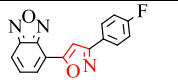
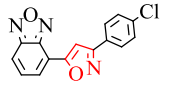
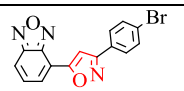
(3aR,11cS)-1-benzyl-3,3,9,10-tetramethyl-1,3a,4,9,10,11c-hexahydropyrano[2',3':5,6]chromeno[4,3-c]isoxazol-8(3H)-one		14.45	[26]
5-(3-propylquinolin-2-yl)-3-(4-(trifluoromethyl)phenyl)isoxazole		12	[27]
2-(3-(4-((5-(4-bromo-3,5-dinitrophenyl)-1,2,4-oxadiazol-3-yl)methoxy)phenyl)isoxazol-5-yl)-N-(3,4,5-trimethylphenyl)thiazol-4-amine		1.76±0.44	[28]
(2R,3R,4R,5R,6R)-2-((5-(1H-indol-3-yl)isoxazol-3-yl)methyl)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol		22.3	[29]
3-phenyl-5-(3-propylquinolin-2-yl)isoxazole		47.2 ± 0.12	[30]
4-(5-Phenylisoxazol-3-yl) benzo[c][1,2,5]oxadiazole*[4a]*		500	this work
4-(5-(4-Methoxyphenyl) isoxazol-3-yl) benzo[c][1,2,5]oxadiazole*[4b]*		500	this work
4-(5-p-tolyl) isoxazol-3-yl) benzo[c][1,2,5]oxadiazole*[4c]*		125	this work
4-(5-(3-nitrophenyl) isoxazol-3-yl) benzo[c][1,2,5]oxadiazole*[4d]*		125	this work
4-(5-(4-fluorophenyl) isoxazol-3-yl) benzo[c][1,2,5]oxadiazole*[4e]*		125	this work
4-(5-(4-chlorophenyl) isoxazol-3-yl) benzo[c][1,2,5]oxadiazole*[4f]*		125	this work
4-(5-(4-bromophenyl) isoxazol-3-yl) benzo[c][1,2,5]oxadiazole*[4g]*		62.5	this work

Table 4: Comparative analysis of IC_{50} values of *benzoxadiazole* analogues *MDA-MB 231* cell lines

***Benzoxadiazole* analogues**	**Structures**	**IC_{50}(μM)**	**Reference**
2-(3,4-dimethoxyphenyl)-5-(3-nitrophenyl)-1,3,4-oxadiazole		38.29	[31]
3-(((5,7-dinitro-2-(3,4,5-trimethoxyphenyl)quinolin-3-yl)oxy)methyl)-5-(3,5-dinitrophenyl)-1,2,4-oxadiazole		1.10 ± 0.36	[32]
1-(1H-Benzo[d]imidazol-2-yl)-3-(5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl)propan-1-one		1.00	[33]

2-((1S,2R,4aS,6aS,6bR,16aS)-1,2,6a,6b,9,9,16a-heptamethyl-13-phenyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,16,16a,16b,17,18b-octadecahydronaphtho[2,3-b]picen-4a-yl)-5-(pyridin-3-yl)-1,3,4-oxadiazole		8.35 ± 0.25	[34]
N-((5-([1,1'-biphenyl]-4-yl)-1,3,4-oxadiazol-2-yl)methyl)-2-amino-2-phenylacetamide		283 ± 12.3	[35]
5-([1,1'-biphenyl]-4-yl)-3-((1-([1,1':4',1''-terphenyl]-4-ylmethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,3,4-oxadiazol-2(3H)-one		1.75 ± 0.2	[36]
4,5-dimethyl-7-((5-(propylsulfonyl)-1,3,4-oxadiazol-2-yl)methoxy)-2H-chromen-2-one		7.07	[37]
N-((5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-4-(trifluoromethyl)benzenesulfonamide		62.40	[38]
5,6,7,8,9,10,11,12,13,14,15,16-dodecahydrodibenzo[l,p][1,2,5]oxadiazolo[3,4-n][1,4,7,10]tetraazacyclooctadecine		25	[39]
5-(3-nitrophenyl)-3-(4-(5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl)phenyl)-1,2,4-oxadiazole		1.11 ± 0.18	[40]
1-(4-(5-([1,1'-biphenyl]-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)-5-fluoropyrimidine-2,4(1H,3H)-dione		8.37	[41]
1-(5-(7-(methoxymethyl)naphthalen-2-yl)-1,3,4-oxadiazol-2-yl)-3-((2-nitrophenyl)amino)-1-thioxopropan-2-one		10.22 ± 0.58	[42]
2-(1H-indol-3-yl)-5-(pyridin-3-yl)-1,3,4-oxadiazole		1-10	[43]
1-phenyl-4-(5-phenyl-1,3,4-oxadiazol-2-yl)-1H-pyrazol-5-amine		47.75	[44]
2-(4-methyl-2-(pyridin-2-yl)thiazol-5-yl)-5-(m-tolyl)-1,3,4-oxadiazole		36.35 ± 1.25	[45]
5-((5,7-dibromoquinolin-8-yl)methoxy)-1,3,4-oxadiazole-2-thiol		25.8	[46]
3-(5-acetyl-1,3,4-oxadiazol-2-yl)-1-(1H-benzo[d]imidazol-2-yl)propan-1-one		10.1	[47]
4-(5-Phenylisoxazol-3-yl) benzo[c][1,2,5]oxadiazole*[4a]*		500	this work
4-(5-(4-Methoxyphenyl) isoxazol-3-yl) benzo[c][1,2,5]oxadiazole*[4b]*		500	this work
4-(5-p-tolyl) isoxazol-3-yl) benzo[c][1,2,5]oxadiazole*[4c]*		125	this work

4-(5-(3-nitrophenyl) isoxazol-3-yl) benzo[c][1,2,5]oxadiazole *[4d]*		125	this work
4-(5-(4-fluorophenyl) isoxazol-3-yl) benzo[c][1,2,5]oxadiazole *[4e]*		125	this work
4-(5-(4-chlorophenyl) isoxazol-3-yl) benzo[c][1,2,5]oxadiazole *[4f]*		125	this work
4-(5-(4-bromophenyl) isoxazol-3-yl) benzo[c][1,2,5]oxadiazole *[4g]*		62.5	this work

5. CONCLUSION

We reported the synthesis, structure elucidation and anticancer studies of 7 *phenyl tagged isoxazole-benzoxadiazole hybrids;* ***4a–4g*** with the variation in substituents as *p*-OCH_3, *p*-CH_3, *p*-F, *p*-Cl, *p*-Br and *m*-NO_2 of phenyl ring. The (i) hybrid with *p*-Br exhibited strong antiproliferation, (ii) hybrid structures with *p*-CH_3, *m*-NO_2, *p*-F, *p*-Cl, displayed moderate effect (IC_{50} = 125µM), and (iii) parent hybrid without any substitution in the phenyl ring and with *p*-OCH_3 showed the least potent anticancer activity (IC_{50} = 500µM) on *MDA-MB 231* cell line. From the results it is clear that the substitution in the tagged aryl ring impacts the antiproliferation activity of hybrids. Further, it is obvious that the substitution of "electron withdrawing group" in the *para* position of terminal aryl results in the increased inhibitory activity. The order of anticancer activity of derived hybrids with phenyl ring substitution is, *p*-Br>*p*-Cl, *p*-F, *m*-NO_2, *p*-CH_3, >*p*-OCH_3, parent hybrid. Although, 'Br' is bulkier than 'F' and 'Cl' with lesser electron withdrawing efficiency, low IC_{50} (62.5µM) value of ***4g*** suggests that the bulkier 'Br' atom in C4' position plays a vital role to inhibit proliferation of *MDA MB 231* cells. ***4g*** possess relatively the optimum electron density and stability attributes that enables ***4g*** to exhibit strong anticancer drug effect than other derivatives ***4a-4f.*** Further investigation on enhancing the anticancer drug property of ***4g*** is in progress in our laboratory.

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