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SYNTHESIS OF NOVEL 2-PHENYL-1-BENZOFURAN-3(2H)-ONE DERIVATIVES AS NEW LEADS FOR ANTI-CANCER ACTIVITY

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ABSTRACT: Benzofuranone is a bicyclic ring where a benzene ring fused with a furanone. Synthetic chemistry plays a major in developing a series of potent anti-cancer agents. Bromophenyl acetonitrile was prepared from phenyl acetonitrile by bromination at an alpha position which yielded bromophenyl acetonitrile which was a lachrymator compound. Benzofuranone was synthesized by reacting benzene diols and triols with bromo phenyl acetonitrile yielded an imine derivative are converted to a ketone with treatment with hydrochloric acid then cyclized with sodium acetate. The compounds identity and purity are confirmed by spectral and analytical methods. Benzofuranone derivatives are screened anti-neoplastic activity was performed against human skin cancer cell line G361 at micro molecular concentrations. The compounds IA, IB, ID, IE, IF, IIB, IIC, IIIA, IVB, IVF, VA, VC, VD, VE was found to be with potent activity. These compounds exhibited an excellent activity while compared to reference standard doxorubicin at different micromolar concentrations as such of the synthesized compounds.

INTRODUCTION: Benzofuranones are the most prominently available in nature mostly used in drug discovery and chemical biology; many drugs are derived from the natural sources with very high potent biological activities ¹. Recent worthy literature survey on Natural products has revealed flavonoid luteolin with numerous importances ². Benzofuranones gained importance in pharmaceutical chemistry when it was discovered that furanones are with a high class of anti-cancer activity.

Benzofuran is reported to exhibit a wide spectrum of activity ^{3, 4, 5, 6} such as hypnotic, analgesic, edema-inhibiting, platelet aggregation, anti-convulsant, and anti-inflammatory. Phenolics are the most ubiquitous group of plant drugs and consist of compounds with a hydroxyl group (-OH) attached to an aromatic hydrocarbon.

Flavanoids are a special group of polyphenolic plant secondary metabolites. Flavanoids are located inside the cells are on the surface of the various plant organs & functions in plants ⁷. The basic structure of all flavanoids consists of 2 aromatic rings, the ring A and B are linked by a carbon bridge. For the flavonoids, the three-carbon bridge combines with oxygen ⁸. Flavanoids are divided into several groups based on specific structural features such as flavonoids, isoflavonoids & neoflavonoids ⁷ these flavonoids are differentiated

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by the position of aromatic ring moiety, such as chalcones, flavanoids & aurone. Which are often referred to as minor flavonoids? Recent literature suggests the beneficial health effects of flavonoid utilization. The targeted research areas are mainly on anti-cancer and cardio-protective effects of flavonoids. Epidemiological studies, such as case-control and cohort studies have broadly supported this hypothesis, flavonoids have been subjected to controversy regarding potentially harmful phytoestrogenic effect in estrogen-dependent malignancies like breast and endometrial cancer.

Despite theory, the majority of epidemiological studies had failed to explain the risk of breast cancer, the majority of the studies proved inverse association with flavonoid intake^{9, 10, 11, 12, 13}. Literature review revealed that benzofuranones have antioxidant agents¹⁴, antihypertensive and anti-inflammatory¹⁵ activity against *Plasmodium falciperum* strain¹⁶ bioactive constituents¹⁷ antinociceptive activity¹⁸ active against fungus *Eurotium rubrum* MA 15019 anticholinesterase activity and Alzheimer's disease²⁰ active against K562 human leukaemic cell²¹ butyrylcholinesterase, inhibitors²² potent antimicrobial agents²³ anti-inflammatory activities²⁴ potent NFkB inhibitors²⁵ (Zwergel), anti-cancer, CDK kinase inhibitor, cytotoxicity, anti-angina activity, breast cancer inhibitor, anti-inflammatory, anti-obesity, anti-diabetic, as a part of our research, we synthesized the title compounds, 2-phenyl benzofuranone derivatives. The anti-proliferative activity was tested against human skin cancer cell line G361.

EXPERIMENTAL:

Preparation of Phenyl (Bromo) Acetonitrile:

11.7 gm (0.1mole) was taken in 3 necked round-bottomed flasks in which one of the necks was connected to a flask containing bromine 17.6 gm (0.1mol), to the second neck is fitted with a thermometer such that the tip was dipped in benzyl cyanide solution, the solution was heated up to 110 °C after preheating, third neck was fitted with a tube dipped in beaker of water to absorb excess hydrogen bromide evolved from the reaction, then slowly bromine was added drop by drop to hot benzyl cyanide until the hydrogen bromide gas is completely evolved. Then the reaction mixture quenched into a separating funnel and washed with 5% sodium bicarbonate solution for twice, the

product was extracted by ether and dried by magnesium sulfate. Finally, filtered and distilled gives a crude product of phenyl (Bromo) acetonitrile.

Preparation of 2-(2-Bromo-2-phenylethanimidoyl) benzene-1, 3, 5-triol: A fresh vacuum dried phloroglucinol of (1.5g 0.25mmol) was taken in a three-necked round-bottomed flask and 30ml of Dry ether was added then the solution was cooled to 0 °C then to this phenyl (Bromo) acetonitrile of (2.5 gm 0.25 mmol), lewis acid AlCl₃ (0.5 gm) and then dry hydrochloric acid gas was passed through the solution about three hours and then reaction mixture was kept in ice chest for one day and then dry hydrochloric acid was again pumped in to reaction mixture for 3 h and stored in ice chest for 3 days. After three days reaction mixture forms strong cake then excess ether removed and washed with freshly distilled dry ether for two times, and obtained solid crystals dried and filtered.

The above procedure was repeated by Resorcinol of (2.2 gm 0.2mol) yielded 4-(2-bromo-2-phenylethanimidoyl) benzene-1, 3-diol, orcinol of (1.2 gm 0.1mol) yielded 2-(2-bromo-2-phenylethanimidoyl)-5-methylbenzene-1, 3-diol, benzene-1, 4-diol of (1.1 gm 0.1mol) yielded 2-(2-bromo-2-phenylethanimidoyl) benzene-1, 4-diol, benzene-1,2,4-triol of (1.2 gm 0.01mol) yielded 5-(2-bromo-2-phenylethanimidoyl)benzene-1,2,4-triol.

Preparation of 2-Bromo-2-phenyl-1-(2, 4, 6-trihydroxyphenyl) ethanone:

2-(2-Bromo-2-phenylethanimidoyl) benzene-1, 3, 5-triol (1.0 gm) was taken in a round-bottomed flask and 0.1N of hydrochloric acid (30 ml) was added to the reaction mixture and then refluxed for 2 h, allowed to cool to room temperature and then the reaction mixture was distilled excess solvent was removed, the crude mixture is extracted by dry ether and the left overnight for evaporation at room temperature. A white crystal was obtained which was dried.

The above procedure was repeated by 4-(2-bromo-2-phenylethanimidoyl) benzene-1,3-diol yielded 2-bromo-1-(2,4-dihydroxyphenyl)-2-phenylethanone, 2-(2-bromo-2-phenylethanimidoyl)-5-methyl benzene-1, 3-diol yielded 2-bromo-1-(2, 6-dihydroxy-4-methylphenyl)-2-phenylethanone, 2-

(2-bromo-2-phenylethanimidoyl)benzene-1, 4-diol yielded 2-bromo-1-(2,5-dihydroxyphenyl)-2-phenylethanone, 5-(2-bromo-2-phenylethanimidoyl)benzene-1,2,4-triol yielded 2-bromo-2-phenyl-1-(2,4,5-trihydroxyphenyl) ethanone.

Preparation of 4, 6-dihydroxy-2-phenyl-1-benzofuran-3(2H)-one (IA): 2-bromo-2-phenyl-1-(2,4,6-trihydroxyphenyl) ethanone (0.8 gm) was taken in a round-bottomed flask and sodium acetate (2 gm) [sodium acetate was freshly dried on Bunsen flame fine crystals were prepared was added to the reaction mixture and dissolved in ethanol then refluxed for 10 min, ethanol was distilled and excess solvent was removed, crude was extracted by dry ether and evaporated then

cream white precipitate was obtained which was filtered and dried. Thus obtained product was recrystallized by hot ethanol solution and then TLC Studies were performed.

The above procedure was repeated by 2-bromo-1-(2, 4-dihydroxyphenyl)-2-phenylethanone yielded 6-hydroxy-2-phenyl-1-benzofuran-3(2H)-one (IIA), 2-bromo-1-(2, 6-dihydroxy-4-methylphenyl)-2-phenyl ethanone yielded 4-hydroxy-6-methyl-2-phenyl-1-benzofuran-3(2H)-one (III A), 2-bromo-1-(2,5-dihydroxyphenyl)-2-phenylethanone yielded 5-hydroxy-2-phenyl-1-benzofuran-3(2H)-one (IVA) and 2-bromo-2-phenyl-1-(2, 4, 5-trihydroxyphenyl)ethanone yielded 5,6-dihydroxy-2-phenyl-1-benzofuran-3(2H)-one (VA).

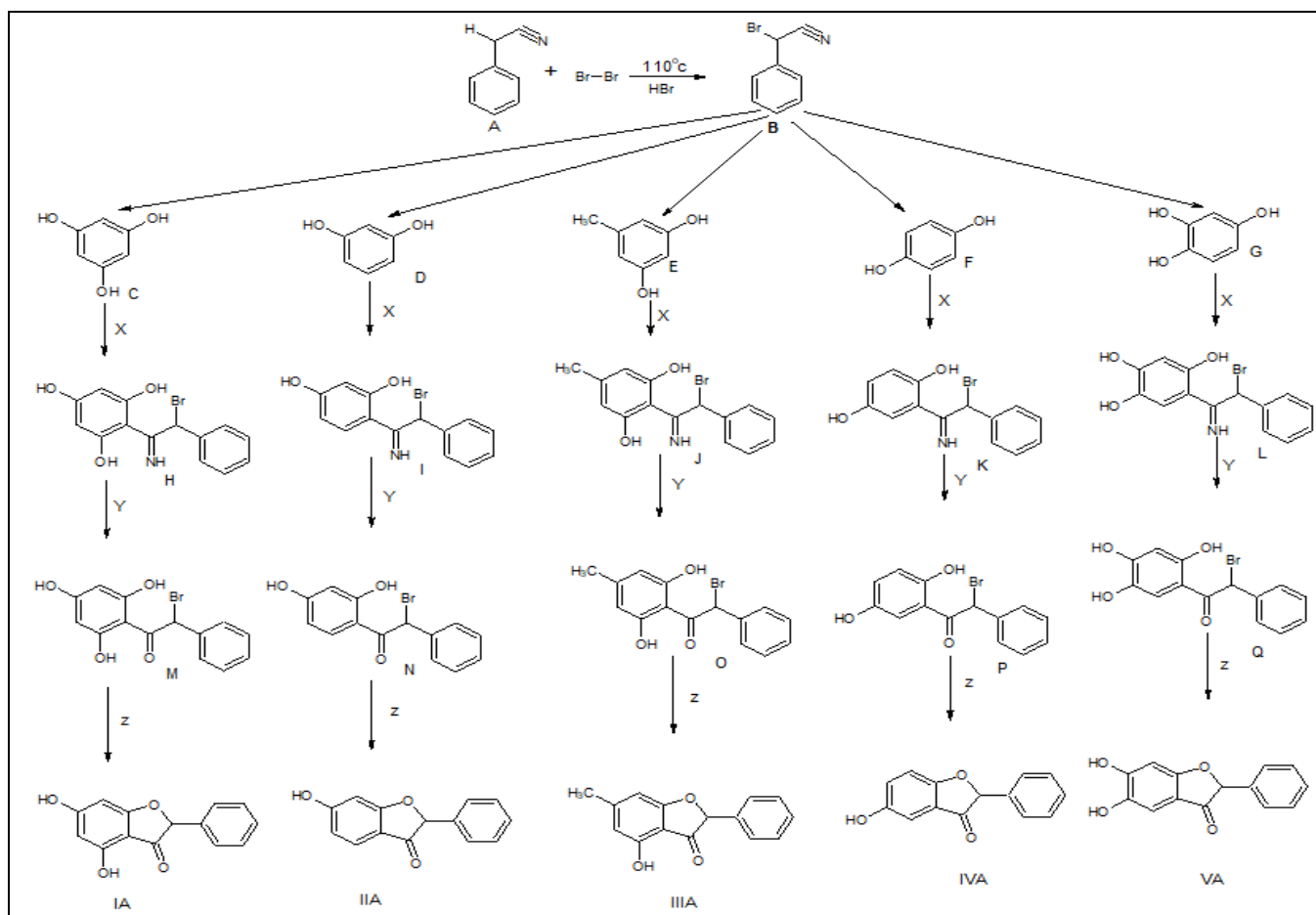


FIG. 1: SCHEME FOR PREPARATION OF BENZOFURANONE DERIVATIVES: X ZnCl₂, DRY HCl, DRY ETHER, AT 0 °C. Y 0.1N HCl Z-SODIUM ACETATE AND ETHANOL. A-Phenyl acetonitrile, B-Phenyl(bromo) acetonitrile, C-Phloroglucinol, D-Resorcinol, E-Orcinol, F-Benzene-1, 4-diol, G-Benzene-1, 2, 5-triol, H 2-(2-bromo-2-phenylethanimidoyl)benzene-1, 3, 5-triol, I 4-(2-bromo-2-phenylethanimidoyl)benzene-1, 3-diol, J 2-(2-bromo-2-phenylethanimidoyl)-5-methylbenzene-1,3-diol, K 2-(2-bromo-2-phenylethanimidoyl)benzene-1,4-diol, L 5-(2-bromo-2-phenylethanimidoyl)benzene-1,2,4-triol, M 2-bromo-2-phenyl-1-(2, 4, 6-trihydroxyphenyl) ethanone, N 2-bromo-1-(2,4-dihydroxyphenyl)-2-phenylethanone, O 2-bromo-1-(2,6-dihydroxy-4-methylphenyl)-2-phenyl ethanone, P 2-bromo-1-(2,5-dihydroxyphenyl)-2-phenylethanone, Q 2-bromo-2-phenyl-1-(2, 4, 5-trihydroxyphenyl) ethanone. IA 4,6-dihydroxy-2-phenyl-1-benzofuran-3(2H)-one, IIA 6-hydroxy-2-phenyl-1-benzofuran-3(2H)-one, IIIA 4-hydroxy-6-methyl-2-phenyl-1-benzofuran-3(2H)-one, IVA 5-hydroxy-2-phenyl-1-benzofuran-3(2H)-one, VA 5, 6-dihydroxy-2-phenyl-1-benzofuran-3(2H)-one.

Preparation of Derivatives for the Benzofuranones:

General Procedure for Nitration: A 5 gm (3.5 ml 0.05mol) of concentrated nitric acid was placed in a 250 ml round-bottomed flask fitted with a thermometer, add small portions 7.4 gm (4 ml) of concentrated sulphuric acid the reaction mixture was kept in an ice-cold bath and cooled, then benzofuranone was added, stirred well and the temperature was controlled. A reflux condenser was fixed for the above reaction mixture and boiled up to 50-55 °C (care to be taken such that

temperature does not increase beyond 55 °C) for about 1 h and then reaction mixture was poured into 100 ml cold water and stirred and then supernatant layer of acid was discarded and the product was washed with ice-cold water for thrice until acid is completely washed out from the product, then transferred in to solution of 5% calcium chloride solution. Then the aqueous layer is separated in separating flask and a trace amount of water is removed by heating on flame²⁸. Thus obtained product was re-crystallized by hot ethanol solution and then TLC studies were performed.

TABLE 1: DETAILED STRUCTURES OF ALL THE DERIVATIVES OBTAINED

	IA	IIA	IIIA	IVA	VA
Benzofuranone derivatives					
Nitration					
Dinitration					
Iodination					
Bromination					
Sulphonation					

General Procedure for Dinitration: A 3.7 ml of concentrated sulphuric acid was placed in 250 ml round-bottomed flask fitted with a thermometer, few fragments of glazed porcelain are added then small portions of (compound-IB, IIB, IIIB, IVB, VB) is added the reaction mixture was refluxed on a water bath for 30 min with occasional stirring and

then reaction mixture was poured into 100 ml cold water and stirred and vacuum filtered dried as much as possible.

Thus obtained product was re-crystallized by hot ethanol solution and then TLC Studies were performed.

General Procedure for Iodination: A small amount of benzofuranone, 2.2 gm of iodine was taken in a three-necked round-bottomed flask fitted with reflux condenser and then to this add 3 ml of nitric acid was added slowly through a separating funnel then the oxides of nitrogen is evolved and the temperature is raised and refluxed for about 15 minutes, and then the solution is poured in to ice-cold water, washed with sodium hydroxide solution, (care has been taken such that reaction mixture is alkaline to litmus) finally washed with water and dried. Thus obtained product was re-crystallized by hot ethanol solution and then TLC studies were performed.

General Procedure for Bromination: A small amount of benzofuranone was taken in a three-necked round-bottomed flask fitted with reflux condenser and gas outlet tube connected to a beaker of water to collect the hydrogen bromide gas, then to this add 3 ml of pyridine (freshly dried over potassium hydroxide) then the apparatus is carefully arranged over a tripod stand with a ice-cold water bath, then bromine was added with most care and then reaction started vigorously and slackens then the temperature is raised to 25 °C finally temperature raised to 70 °C and continued for 1 h until evolution of bromine ceased (no red fumes from the reaction mixture) and then the solution is poured in to ice-cold water, washed sodium hydroxide solution, (care has been taken such that reaction mixture is alkaline to litmus) finally washed with water and dried. Thus obtained product was re-crystallized by hot ethanol solution and then TLC Studies were performed.

General Procedure for Sulphonation: A 25 ml of concentrated sulphuric acid was boiled in a flat bottomed flask, then added 10 ml of oleum (fuming sulphuric acid) and boiled for few minutes and then a small amount of benzofuranone was added and then refluxed for 2 h.

Then the solution was cooled and then neutralized by sodium bicarbonate and to this mixture sodium chloride was until the total solution saturated by sodium chloride, then sodium salt of the product was obtained and then extracted by absolute ethanol, the product is recovered by the evaporation of the solvent and then TLC studies were performed.

Anticancer Activity: The sulforhodamine B (SRB) assay was developed by Skehan and colleagues to measure drug-induced cytotoxicity and cell proliferation for large-scale drug-screening applications. Its principle is based on the ability of the protein-dye sulforhodamine B to bind electrostatically. The activity is pH-dependent on protein basic amino acid residues of trichloroacetic acid-fixed cells. Under mildly acidic conditions it binds to and under mild basic conditions it can be extracted from cells and solubilized for measurement. The signal-to-noise ratio is favorable and the resolution is 1000-2000 cells/well. Its performance is similar when compared to other cytotoxicity assays such as MTT or clonogenic assay. The SRB assay possesses a colorimetric endpoint and is nondestructive and indefinitely stable. These practical advances make the SRB assay an appropriate and sensitive assay to measure drug-induced cytotoxicity even at large-scale application²⁹.

Parameters Reported: GI₅₀, TGI, and LC₅₀

GI₅₀: Growth inhibition of 50 % (GI₅₀) calculated from drug concentration resulting in a 50% reduction in the net protein increase.

TGI: Drug concentration resulting in total growth inhibition (TGI).

LC₅₀: Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment (concentration of drug causing lethality to 50% of the cells as compared to that at the beginning) indicating a net loss of cells following treatment.

RESULTS:

4,6-dihydroxy-2-phenyl-1-benzofuran-3(2H)-one (IA): Yield: 45.62%, Melting point 201 °C, Rf value: 0.66, Mol. formula C₁₄H₁₀O₄, Mol. Weight: 242.22 IR (cm⁻¹) (KBr): 3323.37 (OH); 3224.82 (OH); 1753.06 (C=O); 1171.32 (C-O-C); ¹H NMR (400 MHz, MeOD-d₆): δ= 7.22-7.30 (m 5H Ar-H) 5.96 (d 1H Ar-H J= 1.6Hz), 5.85 (d 1H Ar-H J= 1.6Hz) 5.40 (s 1H CH); ¹³C NMR (100 MHz, MeOD): δ= 88.06 (C-2), 91.51 (C-5), 95.91 (C-7), 97.88 (C-9), 127.41 (C-1''), 128.41 (C-2''), 129.69 (C-2'''), 136.59 (C-1'), 159.98 (C-4), 170.80 (C-8), 176.68 (C-6), 197.32 (C-3). Mass m/z: 243 (M⁺¹), 241 (M¹).

4,6-dihydroxy-2-(4-nitrophenyl)-1-benzofuran-3(2H)-one (IB): Yield: 44.5%, Melting point 224 °C, R_f value: 0.72, Mol. formula C₁₄H₉NO₆, Mol. Weight: 287.22 IR (cm⁻¹) (KBr): 3322.92 (OH); 3224.75 (OH); 1753.06 (C=O); 1171.29 (C-O-C); 1640.52 (NO₂). Mass m/z: 286 (M⁺), 288 (M⁻).

2-(3, 4-dinitrophenyl)-4, 6-dihydroxy-1-benzofuran-3(2H)-one (IC): Yield: 54.5%, Melting point 256 °C, R_f value: 0.34, Mol. formula C₁₄H₈N₂O₈ Mol. Weight: 332.22 IR (cm⁻¹) (KBr): 3393.63 (OH); 3179.85 (OH); 1753.06 (C=O); 1107.40 (C-O-C); 1654.38 (NO₂); 1602.36 (NO₂); Mass m/z: 333 (M⁺), 331 (M⁻).

4, 6-dihydroxy-2-(4-iodophenyl)-1-benzofuran-3(2H)-one (ID): Yield: 74.5%, Melting point 216 °C, R_f value: 0.44, Mol. formula C₁₄H₉IO₄ Mol. Weight: 368.12 IR (cm⁻¹) (KBr): 3424.22 (OH); 3115.02 (OH); 1743.16 (C=O); 1115.37 (C-O-C); 671.05 (I); Mass m/z: 368 (M⁺), 366 (M⁻).

2-(4-bromophenyl)-4,6-dihydroxy-1-benzofuran-3(2H)-one (IE): Yield: 70.40%, Melting point 258 °C, R_f value: 0.54, Mol. formula C₁₄H₉O₄Br Mol. Weight: 318.12 IR (cm⁻¹) (KBr): 3395.99 (OH); 3167.67 (OH); 1753.06 (C=O); 1231.43 (C-O-C); 752.29 (Br); Mass m/z: 319 (M⁺).

4-(4, 6-dihydroxy-3-oxo-2, 3-dihydro-1-benzofuran-2-yl) benzenesulfonic acid (IF): Yield: 72.33%, Melting point 187 °C, R_f value: 0.38, Mol. formula C₁₄H₁₀O₇S Mol. Weight: 322.29 IR (cm⁻¹) (KBr): 3315.50 (OH); 3080.15 (OH); 1670.29 (C=O); 1275.21 (C-O-C); 1355.31 (SO₂H); Mass m/z: 227 (M⁺), 225 (M⁻).

6-hydroxy- 2-phenyl- 1-benzofuran-3(2H)-one (IIA): Yield: 75.62%, Melting point 211 °C, R_f value: 0.38, Mol. formula C₁₄H₁₀O₃ Mol. Weight: 226.22; IR (cm⁻¹) (KBr): 3425.72 (OH); 1685.42 (C=O); 1158.85 (C-O-C).

¹H-NMR (400 MHz, MeOD-d₆): δ= 8.15 (d 1H Ar-H J=8.4) 7.31-7.39 (m 5H Ar-H) 6.05 (d 1H Ar-H J= 1.6Hz), 5.49 (d 1H Ar-H J= 1.6Hz) 5.49 (s 1H CH) ¹³C-NMR (100 MHz, MeOD): δ= 88.06 (C-2), 109.16 (C-5), 95.91 (C-7), 112.06 (C-9), 127.41 (C-1^{''}), 128.41 (C-2^{''}), 129.69 (C-2^{'''}), 136.59 (C-1[']), 124.36 (C-4), 170.80 (C-8), 164.88 (C-6), 197.32 (C-3). Mass m/z: 227 (M⁺), 225 (M⁻).

6-hydroxy- 2-(4-nitrophenyl)- 1-benzofuran-3(2H)-one (IIB): Yield: 64.5%, Melting point 196 °C, R_f value: 0.58, Mol formula C₁₄H₉NO₅ Mol Weight: 271.22; IR (cm⁻¹) (KBr): 3424.22 (OH); 1743.18 (C=O); 1115.37 (C-O-C); 1690.94 (NO₂) Mass m/z : 271 (M⁺), 269 (M⁻).

2-(3, 4-dinitrophenyl)-6-hydroxy-1-benzofuran-3(2H)-one (IIC): Yield: 74.5%, Melting point 236 °C, R_f value: 0.36, Mol. formula C₁₄H₈N₂O₇ Mol. Weight: 316.22; IR (cm⁻¹) (KBr): 3274.17 (OH); 1684.59 (C=O); 1150.86 (C-O-C); 1684.86 (NO₂); 1616.60 (NO₂); Mass m/z : 317 (M⁺), 315 (M⁻).

6-hydroxy- 2-(4-iodophenyl)- 1-benzofuran-3(2H)-one (IID): Yield: 84.5%, Melting point 286 °C, R_f value: 0.54, Mol. formula C₁₄H₉IO₃ Mol. Weight: 352.12; IR (cm⁻¹) (KBr): 3423.23 (OH); 1787.96 (C=O); 1164.58 (C-O-C); 746.39 (I); Mass m/z : 352 (M⁺), 350 (M⁻).

2-(4-bromophenyl)- 6-hydroxy- 1-benzofuran-3(2H)-one (IIE): Yield: 76.40%, Melting point 248°C, R_f value: 0.60, Mol. formula C₁₄H₉O₃Br Mol. Weight: 305.12; IR (cm⁻¹) (KBr): 3441.51 (OH); 1710.71 (C=O); 1349.87 (C-O-C); 758.90 (Br); Mass m/z : 304 (M⁺), 302 (M⁻).

4-(6-hydroxy-3-oxo-2,3-dihydro-1-benzofuran-2-yl) benzenesulfonic acid (IIF): Yield: 92.33%, Melting point 198 °C, R_f value: 0.46, Mol. formula C₁₄H₁₀O₆S Mol. Weight: 306.29; IR (cm⁻¹) (KBr): 3423.25 (OH); 1677.32 (C=O); 1230.45 (C-O-C); 1340.72 (SO₂); Mass m/z : 305 (M⁺), 307 (M⁻).

4-hydroxy-6-methyl-2-phenyl-1-benzofuran-3(2H)-one (IIIA): Yield: 35.62%, Melting point 221°C, R_f value: 0.76, Mol formula C₁₅H₁₂O₃ Mol Weight: 240.22; IR (Cm⁻¹) (KBr): 3422.63 (OH); 2984.60 (CH₃); 1740.40 (C=O); 1173.69 (C-O-C); ¹H NMR (400 MHz, MeOD-d₆):δ= 8.06-8.04 (d 2H Ar-2`H and 6`H J=8) 7.58-7.54 (t 2H Ar-3`H and 5`H J=8) 7.41-7.37 (t 1H Ar-4`H J= 4.8) 5-96 (d 1H Ar-H J= 12.4Hz), 5.85(d 1H Ar-H J= 1.6Hz) 5.40 (s 1H CH) 2.42 (d 3H CH₃ J= 1.6Hz) ¹³C-NMR (100 MHz, MeOD): δ= 52.46 (C-10 CH₃) 88.06 (C-2), 115.88 (C-5), 107.01 (C-7), 112.06 (C-9), 127.41 (C-1^{''}), 128.41 (C-2^{''}), 129.69 (C-2^{'''}), 136.59 (C-1[']), 124.36 (C-4), 170.80 (C-8), 152.98 (C-6), 197.32 (C-3). Mass m/z: 241 (M⁺), 239 (M⁻).

4-hydroxy- 6-methyl- 2-(-4-nitrophenyl)-1-benzofuran-3(2H)-one (IIIB): Yield: 54.5%, Melting point 234°C, R_f value: 0.54, Mol. formula $C_{15}H_{11}NO_5$ Mol. Weight: 285.22; IR (cm^{-1}) (KBr): 3442.78 (OH); 2926.50 (CH_3); 1683.69 (C=O); 1114.30 (C-O-C); 1640.52 (NO_2) Mass m/z: 286 (M^{+1}), 284 (M^{-1}).

2-(-3, 4-dinitrophenyl)- 4-hydroxy- 6-methyl-1-benzofuran-3(2H)-one (IIIC): Yield: 64.5%, Melting point 246°C, R_f value: 0.42, Mol. formula $C_{15}H_{10}N_2O_7$ Mol. Weight: 330.22; IR (cm^{-1}) (KBr): 3442.26 (OH); 2923.78 (CH_3); 1583.65 (C=O); 1193.37 (C-O-C); 1498.69 (NO_2); 1459.42 (NO_2); Mass m/z: 331 (M^{+1}), 329 (M^{-1}).

4-hydroxy- 6-methyl-2- (4-iodophenyl)-1-benzofuran-3(2H)-one (IIID): Yield: 64.5%, Melting point 237°C, R_f value: 0.48, Mol formula $C_{15}H_{11}IO_3$ Mol Weight: 366.12; IR (cm^{-1}) (KBr): 3422.24 (OH); 2924.85 (CH_3); 1596.25 (C=O); 1112.27 (C-O-C); 686.42 (I); Mass m/z: 366 (M^{+1}), 364 (M^{-1}).

2-(4-bromophenyl)- 4-hydroxy- 6-methyl-1-benzofuran-3(2H)-one (IIIE): Yield: 60.80%, Melting point 249°C, R_f value: 0.59, Mol. formula $C_{15}H_{11}O_3Br$ Mol. Weight: 319.12; IR (cm^{-1}) (KBr): 3370.13 (OH); 2966.37 (CH_3); 1624.18 (C=O); 1231.85 (C-O-C); 754.10 (Br); Mass m/z: 366 (M^{+1}), 364 (M^{-1}).

4-(4-hydroxy- 6-methyl-3-oxo-2, 3-dihydro-1-benzo furan-2-yl)benzenesulfonic acid (IIIF): Yield: 86.33%, Melting point 227°C, R_f value: 0.58, Mol. formula $C_{15}H_{12}O_6S$ Mol. Weight: 320.29; IR (cm^{-1}) (KBr): 3443.51 (OH); 2967.42 (CH_3); 1612.15 (C=O); 1277.24 (C-O-C); 1353.71 (SO_2H); Mass m/z : 321 (M^{+1}), 318 (M^{-1}).

5-hydroxy- 2-phenyl- 1-benzofuran- 3(2H)-one (IVA): Yield: 65.62%, Melting point 221°C, R_f value: 0.70, Mol. formula $C_{14}H_{10}O_3$ Mol. Weight: 226.22; IR (cm^{-1}) (KBr): 3422.18 (OH); 1559.05 (C=O); 1250.18 (C-O-C); 1H NMR (400 MHz, MeOD- d_6): δ = 8.22 (d 1H Ar-H $J=8$) 7.57-7.55 (t 2H Ar-2'H and 6'H $J=7.6$) 7.35-7.34 (t =2H Ar-3'H and 5'H $J=7.2$) 6.40-6.39 (d 2H Ar-6'H and 7H), 4.78 (s 1H CH). ^{13}C -NMR (100 MHz, MeOD): δ = 88.06 (C-2), 159.98 (C-5), 100.91 (C-7), 115.88 (C-9), 127.41 (C-1''), 128.41 (C-2''), 129.69 (C-2'''), 136.59 (C-1'), 110.17 (C-4),

170.80 (C-8), 96.51 (C-6), 197.32 (C-3). Mass m/z: 228 (M^{+1}), 225 (M^{-1}).

5-hydroxy- 2-(-4-nitrophenyl)- 1-benzofuran-3(2H)-one (IVB): Yield: 54.5%, Melting point 242°C, R_f value: 0.60, Mol. formula $C_{14}H_9NO_5$ Mol. Weight: 271.22; IR (cm^{-1}) (KBr): 3308.36 (OH); 1671.66 (C=O); 1113.06 (C-O-C); 1616.90 (NO_2) Mass m/z: 271 (M^{+1}), 270 (M^{-1}).

2-(-3, 4-dinitrophenyl)-5-hydroxy-1-benzofuran-3(2H)-one (IVC): Yield: 74.5%, Melting point 276°C, R_f value: 0.47, Mol. formula $C_{14}H_8N_2O_7$ Mol. Weight: 316.22; IR (cm^{-1}) (KBr): 3393.63 (OH); 1654.39 (C=O); 1107.40 (C-O-C); 1602.36 (NO_2); 1510.79 (NO_2); Mass m/z: 317 (M^{+1}), 315 (M^{-1}).

5-hydroxy- 2-(4-iodophenyl)- 1-benzofuran-3(2H)-one (IVD): Yield: 66.5%, Melting point 243°C, R_f value: 0.56, Mol. formula $C_{14}H_9IO_3$ Mol. Weight: 352.12; IR (cm^{-1}) (KBr): 3424.22 (OH); 1743.18 (C=O); 1115.37 (C-O-C); 571.05 (I) Mass m/z : 352 (M^{+1}), 350 (M^{-1}).

2-(4-bromophenyl)- 5-hydroxy- 1-benzofuran-3(2H)-one (IVE): Yield: 85.40%, Melting point 268°C, R_f value: 0.58, Mol. formula $C_{14}H_9O_3Br$ Mol. Weight: 305.12; IR (cm^{-1}) (KBr): 3447.98 (OH); 1735.39 (C=O); 1223.40 (C-O-C); 744.60 (Br); Mass m/z : 304 (M^{+1}), 302 (M^{-1}).

4-(5-hydroxy-3-oxo-2, 3-dihydro-1-benzofuran-2-yl) benzenesulfonic acid (IVF): Yield: 82.33%, Melting point 207°C, R_f value: 0.45, Mol. formula $C_{14}H_{10}O_6S$ Mol. Weight: 306.29; IR (cm^{-1}) (KBr): 3322.92 (OH); 1753.06 (C=O); 1171.29 (C-O-C); 13390.39 (SO_2); Mass m/z : 305 (M^{+1}), 307 (M^{-1}).

5,6-dihydroxy-2-phenyl-1-benzofuran-3(2H)-one (VA): Yield: 65.62%, Melting point 231°C, R_f value: 0.76, Mol. formula $C_{14}H_{10}O_4$ Mol. Weight: 242.22; IR (cm^{-1}) (KBr): 3370.13 (OH); 3095.24 (OH); 1624.18 (C=O); 1231.85 (C-O-C); 1H NMR (400 MHz, MeOD- d_6): δ = 8.48 (s-1H CH) 8.23-8.21 (d 1H Ar-H $J=8$) 7.92-7.91 (d 1H Ar-H $J=6$) 7.57 (s 2H Ar-H) 7.42-31 (m 2H Ar-H), 7.06-7.05(d 2H Ar-H).

^{13}C -NMR (100 MHz, MeOD): δ = 88.06 (C-2), 152.98 (C-5), 100.91 (C-7), 115.88 (C-9), 127.41 (C-1''), 128.41 (C-2''), 129.69 (C-2'''), 159.98 (C-

1'), 110.17 (C-4), 160.04 (C-8), 96.51 (C-6), 197.32 (C-3). Mass m/z: 243 (M^{+1}), 241 (M^{-1}).

5,6-dihydroxy-2-(4-nitrophenyl)-1-benzofuran-3(2H)-one (VB): Yield: 84.5%, Melting point 264°C, R_f value: 0.68, Mol. formula $C_{14}H_9NO_6$ Mol. Weight: 287.22; IR (cm^{-1}) (KBr): 3424.22 (OH); 3115.02 (OH); 1743.18 (C=O); 1280.18 (C-O-C); 1690.94 (NO₂) Mass m/z: 288 (M^{+1}), 286 (M^{-1}).

2-(3, 4-dinitrophenyl)-5, 6-dihydroxy-1-benzofuran-3(2H)-one (VC): Yield: 90.5%, Melting point 186°C, R_f value: 0.51, Mol. formula $C_{14}H_8N_2O_8$ Mol. Weight: 332.22; IR (cm^{-1}) (KBr): 3323.37 (OH); 3224.82 (OH); 1753.06 (C=O); 1171.32 (C-O-C); 1640.58 (NO₂); Mass m/z: 333 (M^{+1}), 331 (M^{-1}).

5, 6-dihydroxy-2-(4-iodophenyl)-1-benzofuran-3(2H)-one (VD): Yield: 54.5%, Melting point 266°C, R_f value: 0.64, Mol. formula $C_{14}H_9IO_4$ Mol. Weight: 368.12; IR (cm^{-1}) (KBr): 3322.92 (OH); 3224.75 (OH); 1753.06 (C=O); 1171.29 (C-O-C); 634.04 (I); Mass m/z : 368 (M^{+1}), 366 (M^{-1}).

2-(4-bromophenyl)-5,6-dihydroxy-1-benzofuran-3(2H)-one (VE): Yield: 58.40%, Melting point 208°C, R_f value: 0.58, Mol. formula $C_{14}H_9O_4Br$ Mol. Weight: 312.12; IR (cm^{-1}) (KBr): 3393.63 (OH); 3279.29 (OH); 1753.06 (C=O); 1107.40 (C-O-C); 695.71 (Br); Mass m/z: 319 (M^{-1}).

4-(5, 6-dihydroxy-3-oxo-2, 3-dihydro-1-benzofuran-2-yl) benzenesulfonic acid (VF): Yield: 62.33%, Melting point 167°C, R_f value: 0.47, Mol. formula $C_{14}H_{10}O_7S$ Br Mol. Weight: 322.29; IR (cm^{-1}) (KBr): 3395.99 (OH); 3187.57 (OH); 1601.36 (C=O); 1231.43 (C-O-C); Mass m/z: 323 (M^{+1}), 321 (M^{-1}).

DISCUSSION:

2-Phenyl Benzofuranone: The reactants phenyl acetonitrile and bromine reacted where phenyl acetonitrile was preheated 110 °C and bromine was added drop by drop where alpha hydrogen was replaced by bromine and HBr gas has been evolved which was trapped in water and after ceasing of HBr gas the reaction has been completed, then after completion of the reaction it was washed with 5% sodium bicarbonate and then extracted by dry ether and dry ether solution also washed with magnesium

sulfate and then solvent is distilled off. Thus obtained product was confirmed by the reported boiling point of 242 °C and a good yield of 90.40% and IR spectrum has confirmed the existence of bromine in the molecule. Bromo (phenyl) acetonitrile was stirred in a three-necked round-bottomed flask immersed in ice-salt mixture which leads 0 °C for the reaction then added 30 ml of dry ether solvent, to this mixture benzene-1,3,5-triol was added slowly then zinc chloride was (Lewis acid) added as a catalyst. Dry hydrochloric acid was prepared and pumped into the reaction mixture for about three hours and kept in ice-chest for three days. After three days flask was removed from the ice chest and supernatant ether layer was removed and then washed with dry ether and then dried.

Which yielded a crude product of 66.10%. 4-(2-Bromo-2-phenylethanimidoyl)benzene-1, 3, 5-triol (1 gm) was dissolved in 0.1N hydrochloric acid and then refluxed for two hours where the imine was oxidized which converts the imine to ketone, then the reaction mixture was distilled and the crude was extracted by dry ether and evaporated which yields a white fine powder with a yield of 56.10%. 2-bromo-2-phenyl-1-(2,4,6-trihydroxyphenyl) ethanone (1gm) was dissolved in freshly distilled ethanol and sodium acetate which was freshly dried on a bunsen flame 2 gm is added and then refluxed for 10 min then ethanol was distilled off and the crude extract was dissolved in dry ether and evaporated yields a cream white crystals of 45.62%.

Nitration: Sulphuric acid and nitric acid were mixed in a flask and then cooled in an ice bath and then 4,6-dihydroxy-2-phenyl-1-benzofuran-3-(2H)-one and care have been taken such that temperature does not rise more than 55 °C such that a avoid multiple nitrations on the phenyl ring. Then slowly refluxed and the product obtained is washed ice-cold water finally extracted with ether and distilled gives a yield of 44.5%.

Dinitration: Sulphuric acid and nitric acid were mixed in a flask and then cooled in an ice bath and then 4, 6-dihydroxy-2-phenyl-1-benzofuran-3-(2H)-one Then vigorously refluxed and dinitrated product 2-(3, 4-dinitrophenyl)-4, 6-dihydroxy-1-benzofuran-3(2H)-one obtained is washed ice-cold water finally extracted with ether and distilled gives a yield of 54.5%.

Iodination: Iodine and nitric acid were mixed in a flask and then cooled in an ice bath and then 4, 6-dihydroxy- 2-phenyl- 1-benzofuran- 3-(2H)-one. Then vigorously refluxed and dinitrated product 4, 6-dihydroxy-2-(4-iodophenyl)- 1-benzofuran-3(2H)-one obtained is washed ice-cold water finally extracted with ether and distilled gives a yield of 74.5%.

Bromination: Bromine and pyridine were mixed in a flask and then cooled in an ice bath and then 4, 6-dihydroxy- 2-phenyl- 1-benzofuran-3-(2H)-one. Then pyridine (Freshly distilled) was added then the reaction temperature rises to 70 °C and HBr gas evolution from the reaction mixture is ceased indicates the completion of reaction gives product 2-(4-bromophenyl)-4, 6-dihydroxy-1-benzofuran-3(2H)-one obtained is washed ice-cold water finally extracted with ether and distilled gives a yield of 70.40%.

Sulphonation: Oleum (Fuming sulphuric acid) was mixed in a flask and then boiled in a water bath and then 4,6-dihydroxy-2-phenyl-1-benzofuran-3-(2H)-one. Then vigorously refluxed gives sulphonic product 4-(4, 6-dihydroxy-3-oxo-2, 3-dihydro-1-benzofuran-2-yl) benzenesulfonic acid obtained is washed ice-cold water finally extracted with ether and distilled gives a yield of 72.33%.

Anticancer Activity: The newly synthesized compounds were screened for their anticancer activity against human skin cancer cell line G361 by sulforhodamine B assay. Doxorubicin was used as a standard reference drug and the results obtained. All compounds showed low anti-proliferative activity. The % growth inhibition of the compounds IA, IB, ID, IE, IF, IIB, IIC, IIIA, IVB, IVF, VA, VC, VD, VE was found to be considered at a concentration of 10^{-4} M. TGI₅₀ (Growth inhibition of 50% cells, calculated from drug concentration resulting in a 50% reduction in the net protein increase).

As 2-phenyl benzofuranone derivatives are the most active compound, it serves as a lead to further optimization in the drug discovery process.

CONCLUSION: We had successfully developed a new series of 2-phenyl-1-benzofuran-3(2H)-one derivatives. Though benzofuranones were existed with prominent anti-cancer activity and in our

developed a new series of phenyl substituted benzofuranones. These compounds are screened for anti-proliferative activity against human skin cancer cell lines.

The compounds IA, IB, ID, IE, IF, IIB, IIC, IIIA, IVB, IVF, VA, VC, VD, VE showed an excellent anti-proliferative activity which serves as a lead to further optimization in the drug discovery process.

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CONFLICT OF INTEREST: Nil

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