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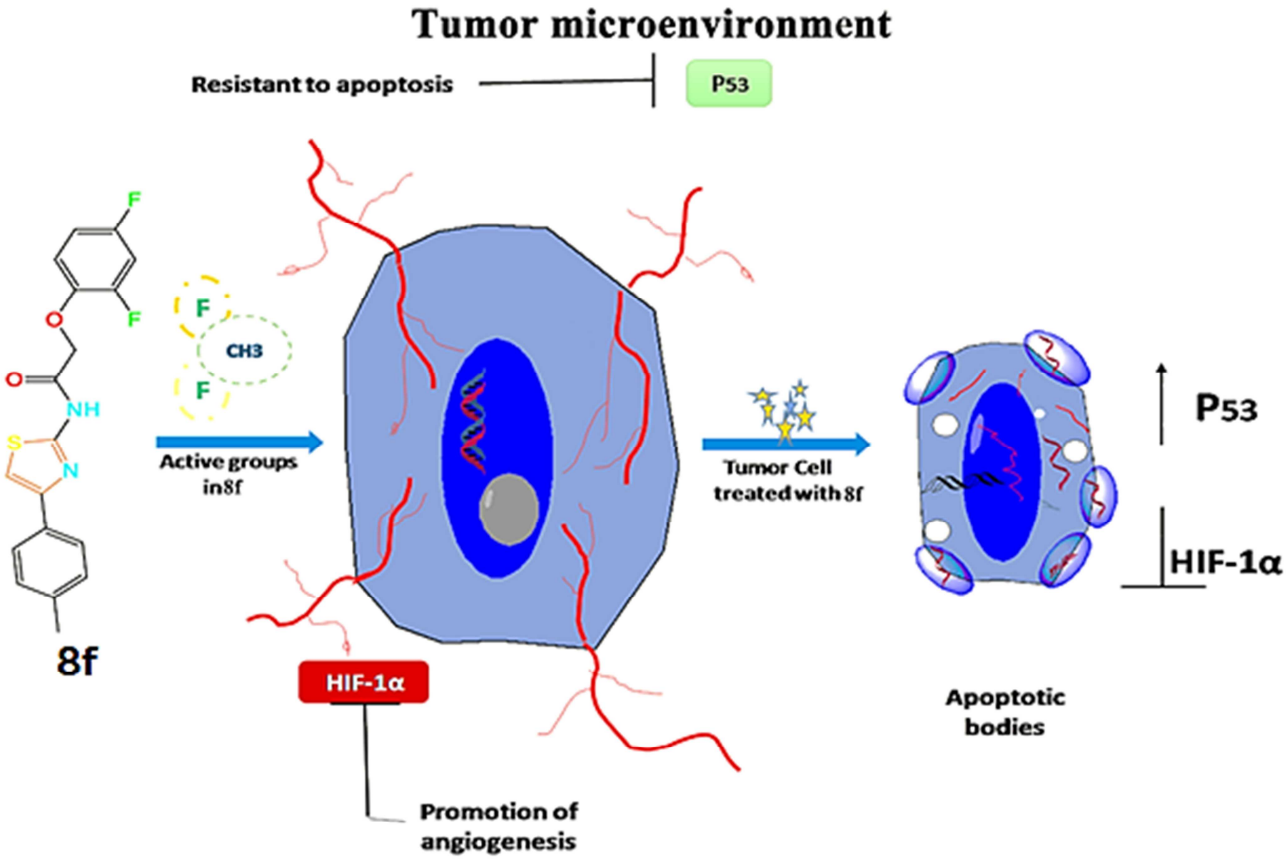
Graphical abstract



The novel 4-phenyl-2-phenoxyacetamide thiazoles modulates the tumor hypoxia leading to the crackdown of neoangiogenesis and evoking the cell death.

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ABSTRACT

Tumor microenvironment is a complex multistep event which involves several hallmarks that transform the normal cell into cancerous cell. Designing the novel antagonistic molecule to reverse the tumor microenvironment with specific target is essential in modern biological studies. The novel 4-phenyl-2-phenoxyacetamide thiazole analogues **8$_{a-ab}$** were synthesized in multistep process, then screened and assessed for cytotoxic and anti-proliferative effects *in vitro* against multiple cancer cells of different origin such as MCF-7, A549, EAC and DLA cells which revealed that compound **8f** with fluoro and methyl substitute has potential cytotoxic efficacy with an average IC_{50} value of~13 µM. The mechanism of cytotoxicity assessed for anti-tumor studies both in ascites and solid tumor models *in-vivo* inferred the regressed tumor activity. This is due to changes in the cause of tumor microenvironment with crackdown of neovascularization and evoking apoptosis process as assessed by CAM, corneal vascularization and apoptotic hallmarks in **8f** treated cells. The molecular gene studies inferred involvement of HIF-1upregulation and stabilization of p53 which are interlinked in signaling as conferred by immunoblot analysis.

Key words: Phenoxy acetic acid; Thiazole; Hypoxia; Tumor Microenvironment; HIF-1a; p53**.**

1. INTRODUCTION

Cancer is the multistep disease condition where divergent tissue mass or a cell expand in an uncoordinated manner with that of bordering normal tissues or a cell and endure to be same even after the termination of the cause that provoked it [1]. Diverse line of demonstration reveals that neoplastic cells intrinsically mutate the normal cells into cancerous one. Communication of neoplastic cells with adjacent cell population by discharging and drawing constant impulse resulting in unending biological vista termed as tumor microenvironment which render provision for maturation of malignant cells [2]. A key environmental provocation linked with tumor metastasis and scarce clinical diagnosis is cancerous cell oxygen deficiency, designated as hypoxia [3]. This condition is known to prompt the genes winding in the regulation of cell proliferation, extracellular matrix (ECM) production, cell adhesion, and other hallmarks of tumor genesis including apoptosis. The mechanism behind these effects is often accomplished through induction of the hypoxia-inducible factor (HIF) family of transcription factors which is regulated by tumor suppressor gene p53 [4]. Thus, tumor microenvironment is accepted as the top cat in the advancement of neoplastic cells and vulnerable target for therapy [5]. Conventional cancer treatment can show earlier positive result, but down the line, diseases may relapse by developing resistance to multiple drugs. This intimates for the search of lost interconnection between tumor development mechanism, hypoxia and current drug development approach. Tumor microenvironment may be a decisive factor for connecting lacking link.

The attention of medicinal chemists is attracted by thiazole ring due to not only their synthetic feasibility but also to their incorporation into the diversity of therapeutic active agents. Thiazole derivatives exemplify an extensive variety of biological potencies including anti-cancer activity

[6-8]. Thiazole, and its derivatives have been given special attention due to their widely potential applications as medicinal drug. For instance, tiazofurin, dasatinib, and bleomycin are known anti-neoplastic drugs. Several phenoxy acid analogues such as methotrexate, nizatidine and alunbirg are known anti-neoplastic drugs [9-11]. Newly, most of the compounds, isolated from the natural products, containing thiazole moiety, exhibit considerable cytotoxicities and anti-tumor potentials [12-15]. Moreover, phenoxy acids and their analogues are related with a diversity of biological activities not only anti-inflammatory, analgesic and anti-oxidant but also anti-cancer activity [16, 17].

Earlier, our lab has reported the BP-1T (benzophenone-thiazole) molecule as potent anti-angiogenic pharamacophore which regulates p53&HIF-1α pathway [18, 19]. These findings have made it clear that conjugation of two moieties will lead to possible anti-cancerous molecule. With this background, we have synthesized active phenoxy acetic acid at the position 4 of potent 2- amino phenyl thiazole moiety using different aryl acids having active groups like; fluoro, difluoro, chloro, nitro, bromo etc, with phenyl thiazole backbone. It has been hoped that the combination of these active groups in the new molecular design would lead to better anti-tumor agents. In this regard, we report the synthesis of newly designed 2-phenoxy-N-(4-phenylthiazol-2-yl)acetamide analogues (**$8_{a\text{-}ab}$**) and evaluated these compounds for the anti-cancer activity.

2. RESULT AND DISCUSSION

2.1. Chemistry

Synthesis of the title compounds **$8_{a\text{-}ab}$** was accomplished by a synthetic procedure as shown in Scheme 1. All the synthesized compounds were established by IR, NMR and mass spectral data. Substituted phenols **$1_{a\text{-}j}$** on esterification with ethyl chloroacetate (**2**) using dry

acetone as a solvent gave substituted ethyl phenoxy acetates **3$_{a-j}$**, which were confirmed by the disappearance of OH stretching and appearance of carbonyl stretching band for the ester group in the IR absorption spectra. The proton NMR observations revealed that broad singlet for OH proton disappeared and a triplet and quartet signals for CH_3 and CH_2 protons respectively appeared. The compounds **3$_{a-j}$** on refluxing with aqueous sodium hydroxide in ethanol gave phenoxy acetic acids **4$_{a-j}$**, which was clearly evident with the appearance of carbonyl group stretching band of a carboxylic acid in the IR spectra. In proton NMR, the appearance of COOH proton and disappearance of triplet and quartet peaks for CH_3 and CH_2 protons, respectively has confirmed the formation of the product. Further, 2-amino-4-phenyl thiazoles (**7$_{a-c}$**) were obtained by the reaction of substituted acetophenones and thiourea in the presence of iodine as a catalyst and ethanol as a solvent to give **7$_{a-c}$**, which was established by the disappearance of carbonyl stretching of acetophenone in the IR spectra. Besides, an increase in one aromatic proton of thiazole ring and the disappearance of one NH_2 proton of thiourea in the NMR spectra also confirms the product. Finally, substituted phenoxy acetic acids (**4$_{a-j}$**), on treatment with 2-amino-4- phenyl thiazoles (**7$_{a-c}$**) in the presence of lutidine and TBTU (O-(benzotriazol-1-yl)-*N*,*N*,*N*^{o},*N*^{o}-tetramethyl uronium tetrafluoroborate) as a coupling reagent, delivered the expected final products **8$_{a-ab}$** in a good yield (80-85%). This was supported by the disappearance of NH_2 and COOH stretching of the compounds **7$_{a-c}$** and **4$_{a-j}$**, respectively in the IR spectra. Also by the disappearance of COOH proton and appearance of CONH proton in the NMR spectra.

2.2. Biology

2.2.1. Compound **8f** *is an effective cytotoxic molecule*

Literature survey reveals that the anti-tumor properties of amino phenyl thiazole and phenoxy acetic acid derivatives are well known [16, 17]. In the current investigation, cytotoxic

potential of the amino phenyl thiazole analogues conjugated with phenoxy acetic acid derivatives was carried out by cell based screening against multiple cancer cell lines, such as MCF-7, A549, EAC and DLA cells *in-vitro*. Out of twenty eight compounds, compound **8f** showed significant cytotoxicity effect against these cell lines with an average IC_{50} value of ~13 µM as listed in table-1 which was inferred by MTT and Tryphan blue assays. In spite of modest anti-proliferative and cytotoxic effect of other compounds of the series as discussed in SAR, two separate cytotoxic assays strongly recommend the compound **8f** as an effective compound which was selected for further studies. In addition to that we tested the potentiality of the compound **8f** against one of the drug resistance cell lines MDA-MB-468 cells which a triple negative breast cancer cell line. The cell lines are resistant to chemotherapy since the lack of biomarkers, which is major factor of failure in establishing a therapeutic strategy [20]. To test whether the synthesized analogues have any effect on these cell lines, all 28 analogues were screened against MDA-MB-468 cell lines, out of these compound **8f** was showed moderate effect against these cancer cells with IC_{50} value of ~10 µM whereas 5-fluorouracil exhibited no significant cytotoxicity as compared to the compound **8f** which is a promisable drug ability which could be further investigated for multidrug resistance [20].

2.2.2. Structure activity Relationship (SAR)

Heterocyclic compounds containing thiazole scaffold as a core unit is known to be pharmacologically active molecules against various pathological conditions including cancer [1-9]. Phenoxy acetic acids have a potential pharmacological activity [16]. Also 2- amino phenyl thiazole derivatives are known to be active anti-cancer agents against many cancer cells [18]. The current study involves the multistep synthesis of 2- amino phenyl thiazole analogues hybrids with phenoxy acetic acid derivatives. Structurally, the title compounds are having a basic

backbone of 2- amino phenyl thiazole with phenoxy acetic acid link [Figure 1]. The IC_{50} values for compounds as depicted in Table-1 which suggest that the compound **8f** with two fluoro groups at ortho and para position in ring A and methyl group at the para position in ring C showed IC_{50} value~13 µM on MCF-7, A549, EAC and DLA cells and~10 µM on MDA-MB-468 a drug resistant cell line as verified by *in-vitro* studies. In addition, the compound **8i** with fluoro group at para position in ring A and methyl group at the para position in ring C exhibited moderate IC_{50} ~19 µM. on MCF-7, A549, EAC and DLA cells and ~24 µM on MDA-MB-468 cells , Other compounds, **8*s*, 8*v*** have a structure similar to **8f** but lacking one methyl group and showed increased IC_{50} ~26 µM & ~35 µM respectively on MCF-7 ,A549, EAC and DLA cells and~32 µM & 41 µM on MDA-MB-468 cells .5-fluorouracil is used as a positive control which showed good anti-neoplastic effect against MCF-7, A549, EAC and DLA cells but did not showed any significant effect against MDA-MB-468 cells. Over all in the title compounds $\mathbf{8_{a\text{-}ab}}$ increasing the number of methyl groups and the number of fluoro groups at the para & ortho position played a significant role in the biological activity of the compounds. Thus, fluoro groups at ortho and para position in ring A of pheoxy acetic acid and a methyl group at the para position in ring C of thiazole are important for the biological activity. The other molecules which contain substituents like chloro and bromo does not show any significant cytotoxicity [Table 1]. Compound **8f** was selected as a lead compound, based on its significant structure activity relationships and increased in IC_{50} values, for further evaluation of its angiogenesis activity.

2.2.3. Compound **8f** *exhibits anti-tumor potentiality*

Ehrlich ascites carcinoma (EAC) and solid tumor have become most widely used experimental animal model for assessing the *in vivo* tumor development and also for angiogenesis studies. The mouse mammary carcinoma cells are known to secrete the ascites fluid

in the exponentially, which is direct nutritional and the growth factors source for tumor development. Therefore, shuttering secretion of ascites by molecules may contribute to tumor reduction [19, 21]. In the current investigation, EAC tumor models were administered with compound **8f** at 25 mg/kg and 50 mg/kg body weight (*bw*) on every alternative day for three doses. The experimental results indicate that compound **8f** was decreased the tumor volume by 74.35% and 80.2% of inhibition in a dose dependent manner [Figure 2a and 2e] which resulted in diminished cell density with six fold reduction in a concentration dependent manner [Figure 2b]. The treatment of compound **8f** markedly counteracted the secretion of ascites with three to four fold decrease in dose dependent manner [Figure 2c] which reflected in extended survivability with 23 and 25 plus days compared to untreated animals [Figure 2d]. The administration of compound **8f** had not shown any symptoms of adverse effect on the normal lymphoid organs which was evident from the morphology of the liver and spleen of treated and untreated groups [Figure 2f and 2g]. For further confirmation, EAC solid tumor models were developed and administered with compound **8f** at 25 mg and *bw* intraperitoneally. The results inferred that compound **8f** has reduced the tumor growth as observed by animal morphology [Figure 3a and 3b] dissected tumor mass up to 10 g compared to control [Figure 3d].The graphical representation of the tumor volume in a dose dependent manner is illustrated in [Figure 3c]. Compound **8f** prolonged lifespan of the animal for 110 days compared to that of untreated animals [Figure 3e].

2.2.4. Compound **8f** *regresses the neoangiogenesis and actuates the tumor cell apoptosis in-vivo.*

Angiogenesis is a central event of tumor development and aggressiveness. It plays a vital role in supplying oxygen, growth factors in tumor cells [22] and confrontation of tumor cells to apoptosis which is one of the significant strategies of cancerous cells for their survivability [23].

Counter attacking physiological conditions is essential to tackle the tumor progression and development. Inhibition of tumor growth by compound **8f** prompted to investigate the anti-angiogenic efficacy on neovascularization by reliable models such as alkali injured rat cornea and $rVEGF_{165}$ induced CAM assay and tumor induced peritoneal angiogenesis assay. The results demonstrate that the compound **8f** restrains the sprouting angiogenesis in cornea to about 60% [Figure 4a and 4b] and with the CAM model to about 80% [Figure 4c and 4d] compared to control. The administration of compound **8f** regressed EAC induced angiogenesis in peritoneum with 30 and 10 % respectively in a concentration dependent manner [Figure 4e and 4f]. Further, compound **8f** altered another hallmark i.e. apoptotic parameters which was assessed by giemsa stain and DNA fragmentation assay. The results demonstrate that the compound **8f** induced the apoptosis in EAC cells by forming membrane blabbing, cell shrinkage and irregular shape which resulted in DNA degradation, a chief event in cell death [Figure 5a and 5c].

2.2.5. Compound **8f** *effectively blocks HIF-1α by p53/MDM2-mediated proteasomal degradation*

During tumor condition, hypoxia microenvironment persists and up regulates the HIF-1-α a transcription factor which promotes the angiogenic event and evades apoptosis of the cells. This process is regulated by down regulation of p53 expression in the tumor cells [21, 23]. The interaction between HIF-1α, p53 and MDM2 has a crucial part in the regulation of HIF-1α. In reaction to hypoxia which will be present in tumor micro environment, HIF-1α is vastly expressed and interacts with p53 thereby, HIF-1α protects the p53 from MDM2-mediated ubiquitination [24] but HIF-1α is negatively regulated by p53 and provides the HIF-1α to MDM2 for ubiquitination process, leading to HIF-1α proteosomal degradation resulting in the tumor inhibition [25-27]. In the current investigation the decrease in HIF-1α expression and increase in the p53 expression after compound **8f** treatment reveals the possible involvement of alteration in

p53/MDM2 mediated stabilization of HIF-1α. HIF -1-α is the key candidate mainly involved in the crackdown of the extracellular matrix (ECM) and sprouting of newer blood vessels in the tumor as well as in the resistance of apoptosis. A decrease in HIF-1 α will contribute the overexpression of p53, a prime transcription factor for induction of programmed cell death. Blocking HIF 1-α and activating p53 expression by molecule in the neoplastic condition may effectively modulate the tumor angiogenesis and apoptosis. Biochemical modulation of tumor inhibition by compound **8f** was assessed by immunoblots. The experimental evidences postulate that compound **8f** effectively decreased the expression of HIF-1α and upregulated the p53 and MDM-2 which is evident from immunoblots [Figure 5b]. VEGF being the predominant activator of angiogenesis mediated tumor establishment, drastically reduced tumor growth after compound **8f** treatment was observed as assessed by ELISA. The compound **8f** directly targets HIF-1α stabilization and there by downstream angiogenic gene expression eventually leading to decreased tumor growth [Figure 5d].

3. Conclusion

In our current research, the 2-amino phenyl thiazole analogues (**$8_{a\text{-}ab}$)** were synthesized then screened for cytotoxic effects *in vitro* against multiple cancer cells of different origin, such as MCF-7, A549, EAC and DLA cells , which revealed that compound **8f** has potential cytotoxic efficacy with an average IC_{50} value of ~13 µM. Compound **8f** also showed cytotoxic effect against a drug resistant MDA-MB-468 cell with the IC_{50} value of ~10 µM The compound **8f** postulated the angiogenic inhibitory modulation in various $rVEGF_{165}$ induced model as assessed by CAM and corneal vascularization. The potent lead compound **8f** was further assessed for anti-tumor studies, both in ascites and solid tumor models *in vivo,* which revealed the regressed tumor activity. The compound **8f** acts against the tumor cells by repressing HIF-1α by p53/MDM-2

mediated degradation .The molecular gene studies inferred involvement of HIF-1α upregulation and stabilization of p53, which are interlinked in signaling as conferred by immunoblot analysis.

4. Materials and methods

4.1. Experimental section

Unless stated otherwise, all reagents and solvents required for the synthesis of the title compounds **8_{a-ab}** were procured from Sigma Aldrich Chemical Co. The purity of the compounds was checked by TLC which was performed on aluminium-backed silica plates and the spots were detected by exposure to UV-lamp at λ= 254 nm. Melting points and boiling point were measured on a Chemiline, Microcontroller Based Melting Point/ Boiling Point-Cl725 Apparatus with a digital thermometer. IR spectra were recorded on the Agilent Technologies Cary 630 FT-IR spectrometer, ^{1}H and ^{13}C NMR spectra were recorded on VNMRS-400 Agilent-NMR spectrophotometer. Chemical shifts are given in parts per million downfield from tetramethylsilane. The mass spectra were obtained with a VG70-70H spectrophotometer and the elemental analysis (C, H, and N) was performed on Elementar Vario EL III elemental analyzer. The results of elemental analyses were within ±0.4%.

Sodium bicarbonate and protease inhibitor cocktail and another reagents and solvents which were required for the biological assays were obtained from Sigma Aldrich, USA, Anti- HIF 1 -α obtained from Santa Cruz, USA, Anti -p53 and anti-β- Actin were obtained from BD bioscience, USA, DMEM medium, whereas anti-biotic- anti-mycotic solution, fetal bovine serum (FBS) were obtained from Invitrogen, USA.

4.1.1. Chemistry

*4.1.1.1. General synthetic procedure for phenoxy acetic ethyl ester derivatives (**3_{a-j}**).*

A mixture of substituted phenols (**1_{a-j}**, 0.05 mol) and ethyl chloroacetate (**2**, 0.075 mol) in dry acetone (40 ml) with anhydrous potassium carbonate (0.075 mol) was refluxed for 10-12 h. The reaction mixture was cooled and solvent removed by distillation. The residual mass was triturated with cold water to remove potassium carbonate, and extracted with ether (3 × 30 ml). The ether layer was washed with 10% sodium hydroxide solution (3 × 30 ml) followed by water (3 × 30 ml) and then dried over anhydrous sodium sulfate and evaporated to afford compounds **(3_{a-j})**. Finally, these compounds were purified by recrystallization and distillation method.

*4.1.1.1.1. Ethyl 2-phenoxyacetate (**3a**)*

Yield 80%; B.P. 100^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1735-1759 (ester, C=O), 1150(ester, C-O); ^{1}H NMR (400 MHz, $CDCl_3$)δ(ppm): 1.32 (t, 3H, CH_3 of ester), 4.24 (q, 2H, CH_2 of ester), 5.01 (s, 2H, OCH_2), 6.77–7.34 (m, 5H, Ar-H); LC–MS m/z 181 [M+1]+. Anal. Calcd. for $C_{10}H_{12}O_3$ (180): C, 66.65; H, 6.71. Found: C, 66.62; H, 6.68 %.

*4.1.1.1.2. Ethyl 2-(2-bromophenoxy)acetate (**3b**)*

Yield 91%; B.P. 279 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1730-1755 (ester, C=O), 1155 (ester, C-O); ^{1}H NMR ($CDCl_3$) δ(ppm): 1.20 (t, 3H, $CH_3$of ester), 4.16 (q, 2H, CH_2 of ester), 4.76 (s, 2H, OCH_2), 6.87-7.23 (m, 4H, Ar-H); LC–MS m/z 259 [M+] and 261 [M+2]. Anal. Calcd. for $C_{10}H_{11}BrO_3$ (259): C, 46.36; H, 4.28. Found: C, 46.15; H, 3.97 %.

*4.1.1.1.3. Ethyl 2-(4-chloro-3-methylphenoxy)acetate (**3c**)*

Yield73%; B.P. 114 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1720-1745 (ester, C=O), 1120 (ester, C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.29 (t, 3H, CH_3 of ester), 2.38 (s, 3H, CH_3), 4.34 (q, 2H, CH_2 of ester), 5.01 (s, 2H, OCH_2), 6.77–7.34 (m, 3H, Ar-H); LC–MS m/z 229 [M+] and 231 [M+2]. Anal. Calcd. for $C_{11}H_{13}ClO_3$ (229): C, 57.78; H, 5.73. Found: C, 57.63; H, 5.65 %.

*4.1.1.1.4. Ethyl 2-(2-methylphenoxy)acetate (**3d**)*

Yield 88%; B.P. 259 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1730-1740 (ester, C=O), 1115 (ester, C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.43 (t, 3H, CH_3 of ester), 2.41 (s, 3H, CH_3), 4.24 (q, 2H, CH_2 of ester), 5.08 (s, 2H, OCH_2), 6.97–7.34 (m, 4H, Ar-H); LC–MS m/z 195 [M+1]+. Anal. Calcd. for $C_{11}H_{14}O_3$ (194): C, 68.02; H, 7.27. Found: C, 67.88; H, 7.21 %.

*4.1.1.1.5. Ethyl 2-(2-isopropylphenoxy)acetate (**3e**)*

Yield 85%; B.P. 300 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1730-1745 (ester, C=O), 1123 (ester, C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.17 (d, 6H, *J*=7 Hz, $2CH_3$), 1.36 (t, 3H, CH_3 of ester), 3.18 (m, 1H, CH), 4.74 (q, 2H, CH_2 of ester), 4.91 (s, 2H, OCH_2), 6.17–7.24 (m, 4H, Ar-H); LC–MS m/z 223 [M+1]+. Anal. Calcd. for $C_{13}H_{18}O_3$ (222): C, 70.24; H, 8.16. Found: C, 70.15; H, 8.11 %.

*4.1.1.1.6. Ethyl 2-(2,4-difluorophenoxy)acetate (**3f**)*

Yield 75%; B.P. 263 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1728-1738 (ester, C=O), 1115 (ester, C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.44 (t, 3H, CH_3 of ester), 4.32 (q, 2H, CH_2 of ester), 4.41 (s, 2H, OCH_2), 6.77–7.44 (m, 3H, Ar-H); LC–MS m/z 217 [M+1]+. Anal. Calcd. for $C_{10}H_{10}F_2O_3$ (216): C, 55.56; H, 4.66. Found: C, 55.48; H, 4.49 %.

*4.1.1.1.7. Ethyl 2-(4-fluorophenoxy)acetate (**3g**)*

Yield 71%; B.P. 145 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1729-1743 (ester, C=O), 1126 (ester, C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.29 (t, 3H, CH_3 of ester), 4.64 (q, 2H, CH_2 of ester), 5.11 (s, 2H, OCH_2), 7.07–7.12 (d, 2H,*J*=7 Hz, Ar-H),7.13-7.14 (d, 2H, *J*=8 Hz, Ar-H); LC–MS m/z 198 [M+]. Anal. Calcd. for $C_{10}H_{11}FO_3$ (197): C, 60.60; H, 5.59. Found: C, 60.58; H, 5.57 %.

*4.1.1.1.8. Ethyl 2-(2,4-diisopropylphenoxy)acetate (**3h**)*

Yield75%; Pasty mass; FT-IR (KBr, ν_{max}cm^{-1}): 1735-1750 (ester, C=O), 1120-1270 (ester, C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.17 (d, 6H, *J*=8 Hz, $2CH_3$),1.25 (d, 6H, *J*=8 Hz,

$2CH_3$),1.29 (t, 3H, CH_3 of ester), 2.38 (m, 1H, CH),3.59 (m, 1H, CH), 4.84 (q, 2H, CH_2 of ester), 4.81 (s, 2H, OCH_2), 6.87–7.84 (m, 3H, Ar-H); LC–MS m/z 265 [M+1]+. Anal. Calcd. for $C_{16}H_{24}O_3$ (264): C, 72.69; H, 9.15. Found: C, 72.53; H, 9.03 %.

*4.1.1.1.9. Ethyl 2-(4-Methylphenoxy)acetate (**3i**)*

Yield 88%; B.P. 141 ^oC; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1731-1760 (ester, C=O), 1127 (ester, C-O); ^1H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.46 (t, 3H, CH_3 of ester), 2.38 (s, 3H, CH_3), 4.55 (q, 2H, CH_2 of ester), 5.32 (s, 2H, CH_2), 6.97–7.14 (d, 4H, *J*=8 Hz, Ar-H), 7.21–7.44 (d, 4H, *J*=8 Hz, Ar-H); LC–MS m/z 195 [M+1]+. Anal. Calcd. for $C_{11}H_{14}O_3$ (194): C, 68.02; H, 7.27. Found: C, 67.89; H, 7.13 %.

*4.1.1.1.10. Ethyl 2-(4-chlorophenoxy)acetate (**3j**)*

Yield 70%; B.P. 178 ^oC; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1733-1751 (ester, C=O), 1131 (ester, C-O); ^1H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.53 (t, 3H, CH_3 of ester), 4.54 (q, 2H, CH_2 of ester), 4.38 (s, 2H, OCH_2), 7.04-7.21 (d, 2H, *J*=8 Hz, Ar-H), 7.33-7.41 (d, 2H, *J*=8 Hz, Ar-H); LC–MS m/z 215 [M+] and 217 [M+2]. Anal. Calcd. for $C_{10}H_{11}ClO_3$ (215): C, 55.96; H, 5.17. Found: C, 55.87; H, 5.13 %.

*4.1.1.2. General synthetic procedure for phenoxyacetic acid analogues (**4**$_{a-j}$).*

Compounds (**3**$_{a-j}$,0.02 mol) were dissolved in ethanol (15 mL), sodium hydroxide (0.035 mol) in water (5 mL) was added, andthe mixture was refluxed for 5–9 h. The reaction mixture was cooled and acidified with 2 N hydrochloric acid. The precipitate was filtered, washed with water, and finally recrystallized from methanol to afford desired compounds (**4**$_{a-j}$). Compound (**4a**) is takenas a representative example to explain physical and characterization data.

*4.1.1.2.1. 2-Phenoxyacetic acid (**4a**)*

Yield 81%; M.P. 98-101ºC; FT-IR (KBr, $\nu_{max}cm^{-1}$):1714-1725 (C=O), 1246 (Ar–O–C), 3377-3388 (OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 4.66 (s, 2H, OCH_2), 6.79-7.35 (m, 5H, Ar-H), 13.11 (s, 1H, COOH); LC–MS m/z 153 [M+1]+. Anal. Calcd. for $C_8H_8O_3$ (152): C, 63.15; H, 5.30. Found: C, 63.12; H, 5.27 %.

*4.1.1.2.2.2-Bromo phenoxy acetic acid (**4b**)*

Yield 91%; M.P. 217-219^{o}C; FT-IR (KBr, $\nu_{max}cm^{-1}$):1715-1730 (C=O), 1250 (Ar–O–C), 3375-3399 (OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 4.71 (s, 2H, OCH_2), 6.89-7.45 (m, 4H, Ar-H), 14.09 (s, 1H, COOH); LC–MS m/z 231 [M+], 233 [M+2]. Anal. Calcd. for C_8H_7BrO (231): C, 41.59; H, 3.05. Found: C, 41.52; H, 3.15 %.

*4.1.1.2.3.2-(4-Chloro-3-methylphenoxy)acetic acid (**4c**)*

Yield 77%; M.P. 115-118 ^{o}C; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1710-1720 (C=O), 1245 (Ar–O–C), 3366-3396 (OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 2.46 (s, 3H, CH_3), 4.56 (s, 2H, OCH_2), 6.61-7.54 (m, 3H, Ar-H), 13.14 (s, 1H, COOH); LC–MS m/z 201 [M+], 203 [M+2]. Anal. Calcd. for $C_9H_9ClO_3$ (201): C, 53.88; H, 4.52. Found: C, 53.73; H, 4.33 %.

*4.1.1.2.4. 2-(2-Methylphenoxy)acetic acid (**4d**)*

Yield 81%; M.P. 142-144 ºC; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1730-1745 (C=O), 1253 (Ar–O–C), 3375-3380 (OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 2.39 (s, 3H, CH_3), 4.66 (s, 2H, OCH_2), 6.88-8.35 (m, 4H, Ar-H), 13.13 (s, 1H, COOH); LC–MS m/z 167 [M+1]+. Anal. Calcd. for $C_9H_{10}O_3$ (166): C, 65.05; H, 6.07. Found: C, 64.91; H, 6.10 %.

*4.1.1.2.5. 2-(2-Isopropylphenoxy)acetic acid (**4e**)*

Yield 69%; M.P. 132-134 ºC; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1715-1735 (C=O), 1249 (Ar–O–C), 3355-3473 (OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.17 (d, 6H, *J*=8 Hz, CH_3), 3.06 (m, H, CH),

4.89 (s, 2H, OCH_2), 6.92-8.01 (m, 4H, Ar-H), 14.02 (s, 1H, COOH); LC–MS m/z 195 [M+1]+. Anal. Calcd. for $C_{11}H_{14}O_3$ (194): C, 68.02; H, 7.27. Found: C, 67.89 H, 7.11 %.

*4.1.1.2.6. 2-(2,4-Difluorophenoxy)acetic acid (**4f**)*

Yield 79%; M.P. 124-127 ºC; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1724-1745 (C=O), 1253 (Ar–O–C), 3375-3391 (OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 4.77 (s, 2H, OCH_2), 6.92-7.38 (m, 3H, Ar-H), 13.20 (s, 1H, COOH); LC–MS m/z 188 [M+]. Anal. Calcd. for $C_8H_6F_2O_3$ (188): C, 51.08; H, 3.21. Found: C, 51.18; H, 3.16 %.

*4.1.1.2.7. 2-(4-Fluorophenoxy)acetic acid (**4g**)*

Yield73%; M.P. 103-106 ºC; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1725-1755 (C=O), 1250 (Ar–O–C), 3345-3380 (OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 4.59 (s, 2H, OCH_2), 7.09-7.25 (d, 2H, *J*=8 Hz, Ar-H),7.31-7.42 (d, 2H, *J*=8 Hz, Ar-H) 13.93 (s, 1H, COOH); LC–MS m/z 170 [M+]. Anal. Calcd. for $C_8H_7FO_3$ (170): C, 56.48; H, 4.15. Found: C, 56.41; H, 4.02 %.

*4.1.1.2.8.2-(2,4-Diisopropylphenoxy)acetic acid (**4h**)*

Yield 78%; M.P. 82-85ºC; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1725-1748 (C=O), 1258 (Ar–O–C), 3348-3399 (OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.15 (d, 6H, *J*=8Hz, $2CH_3$),1.25 (d, 6H, *J*=8Hz, 2CH3), 3.09 (m, H, CH),3.89 (m, H, CH), 4.69 (s, 2H, OCH_2), 5.99-7.45 (m, 3H, Ar-H), 13.26 (s, 1H, OH); LC–MS m/z 237 [M+1]+. Anal. Calcd. for $C_{14}H_{20}O_3$ (236): C, 71.16; H, 8.53. Found: C, 71.06; H, 8.34 %.

*4.1.1.2.9. (4-Methylphenoxy)acetic acid(**4i**)*

Yield 81%; M.P. 142-144 ºC; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1730-1745 (C=O), 1253 (Ar–O–C), 3375-3380 (OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 2.39 (s, 3H, CH_3), 4.66 (s, 2H, OCH_2), 6.88-7.15 (d, 2H, *J*=8 Hz, Ar-H), 7.23-7.35 (d, 2H, *J*=8 Hz, Ar-H), 13.13 (s, 1H, COOH); LC–MS

m/z 167 [M+1]+. Anal. Calcd. for $C_9H_{10}O_3$ (166): C, 65.05; H, 6.07. Found: C, 64.84; H, 6.01 %.

*4.1.1.2.10.4-Chlorophenoxyacetic acid (**4j**)*

Yield 88%; M.P. 155-158ºC; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1701-1725 (C=O), 1243 (Ar–O–C), 3375-3376 (OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 4.58 (s, 2H, OCH_2), 7.09-7.15 (d, 2H, *J*=8 Hz, Ar-H), 7.23-7.45 (d, 2H, *J*=8 Hz, Ar-H), 14.02 (s, 1H, COOH); LC–MS m/z 187 [M+], 189 [M+2]. Anal. Calcd. for $C_8H_7ClO_3$ (187): C, 51.50; H, 3.78. Found: C, 51.42; H, 3.65 %.

*4.1.1.3. General procedure for the synthesis of 2-amino-4-phenylthiazoles (**7$_{a-c}$**)*

2-Amino-4-phenyl thiazoles (**7$_{a-c}$**) were synthesized by using substituted acetophenones (**5$_{a-c}$**, 0.0025 mol) and thiourea (**6**, 0.003 mol). The reaction mixture was refluxed for 14 h in the presence of iodine (0.0062 mol) and ethanol as a solvent. The product ***7$_{a-c}$*** were basified with sodium hydroxide solution to get the white solid. The product was recrystallized from ethanol to get needle like crystals.

*4.1.1.3.1. 2-Amino-4-(4-chlorophenyl)thiazole (**7a**).*

Yield 82%; M.P. 170-172 ^{o}C; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1696 (C=N), 3374-3376 (N-H);^{1}H NMR (400 MHz, DMSO)δ(ppm): 6.91-7.12 (s, 1H, thiazole ring), 7.14-7.22 (d, 2H, *J*=8. Hz, Ar-H), 7.25-7.32 (d, 2H, *J*=8 Hz, Ar-H), 7.45 (bs, 2H, NH_2). LC–MS m/z 210 (M+), 212 (M+2). Anal. Calcd. for $C_9H_7ClN_2S$ (210): C, 51.31; H, 3.35 N, 13.30. Found: C, 51.21; H, 3.17; N, 13.26%.

*4.1.1.3.2. 2-Amino-4-(4-methylphenyl)thiazole (**7b**).*

Yield 88%; M.P. 150-152 ^{o}C; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1692 (C=N), 3373-3375 (N-H); ^{1}H NMR (400 MHz, DMSO) δ(ppm): 2.31 (s, 3H, CH_3), 7.01-7.22 (s, 1H, thiazole ring), 7.25-7.32 (d, 2H, *J*=8.50 Hz, Ar-H), 7.35-7.42 (d, 2H, *J*=8 Hz, Ar-H), 7.50 (bs, 2H, NH_2). LC–MS m/z 191 (M+).

Anal. Calcd. for $C_{10}H_{10}N_2S$ (191): C, 63.13; H, 5.30; N, 14.72. Found: C, 63.15; H, 5.23; N, 14.70%.

*4.1.1.3.3. 2-Amino-4-(4-bromophenyl)thiazole (**7c**).*

Yield 78%; M.P. 184-186 ^oC; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1698 (C=N), 3371-3373 (N-H); ^1H NMR (400 MHz, DMSO) δ(ppm): 6.96-7.22 (s, 1H, thiazole ring), 7.28-7.36 (d, 2H, *J*=8 Hz, Ar-H), 7.37-7.40 (d, 2H, *J*=8.50 Hz, Ar-H), 7.48 (bs, 2H, NH_2). LC–MS m/z 255 (M+), 257(M+2). Anal. Calcd. for $C_9H_7BrN_2S$ (255): C, 42.37; H, 2.77; N, 10.98. Found: C, 42.21; H, 2.52; N, 10.95%.

*4.1.1.4. General synthetic procedure for 4-Phenyl-2-Phenoxyacetamide Thiazoles analogues (**8$_{a-ab}$**).*

phenoxyacetic acids (**4$_{a-j}$**, 2 mmol) in dry DCM (20 ml) was stirred at 25–30 ^oC, and then lutidine (3mmol) was added, followed by the addition of substituted amino-4-phenyl-1,3-thiazoles (2 mmol). The reaction mixture was stirred at the same temperature for 30 min, then cooled to 0–5 ^oC and TBTU (2 mmol) was added over a period of 30 min maintaining the temperature below 5 ^oC. The reaction mass was stirred overnight and monitored by TLC using chloroform: methanol (9:1) as the mobile phase. The purity of the compounds was determined using high performance liquid chromatography (HPLC) by reversed phase agilent zorbax SB-C18 column method, with methanol (50%): acetonitrile (30%): water (20%) as mobile phase. The solvent was evaporated at reduced pressure, quenched by the addition of crushed ice and the obtained solid was filtered, dried and recrystallized from ethanol to afford compounds **8$_{a-ab}$** in good yield [supplementary files].

*4.1.1.4.1. 2-Phenoxy-N-(4-(p-tolyl) thiazol-2-yl) acetamide (**8a**)*

Yield 83%; Purity >88% HPLC; M.P. 154-156 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1679 (C=O), 1700 (C=N), 3173-3176(N-H); ^{1}H NMR (400 MHz, DMSO) δ(ppm): 2.35 (s, 3H, CH_3), 4.84 (s, 2H, CH_2), 6.92-7.77(m, 10H, Ar-H), 12.49 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 21.22, 66.61, 105.94, 114.12, 121.09, 125.73, 129.72, 130.01, 131.99, 150.60, 159.01, 164,57, 169.53; LC–MS m/z (M+) 324. Anal. Calcd. For $C_{18}H_{16}N_2O_2S$ (324): C, 66.65; H, 4.97; N, 8.64. Found C, 66.69; H, 5.07; N, 8.58%.

*4.1.1.4.2.2-(2-Bromophenoxy)-N-(4-(p-tolyl) thiazol-2-yl) acetamide (**8b**).*

Yield 70%;Purity >85% HPLC; M.P. 187-189 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1675 (C=O), 1698 (C=N), 3174-3180 (N-H);^{1}H NMR (400 MHz, DMSO) δ(ppm): 2.47 (s, 3H, CH_3), 4.86 (s, 2H, CH_2), 6.92-7.77(m, 9H, Ar-H), 12.46 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 21.22, 66.62, 106.04, 115.12, 118.40, 125.09, 129.11, 130.01 131.61, 132.69, 150.60, 158.01, 164.57, 169.13; LC–MS m/z 403 (M+) 405 (M+2). Anal. Calcd. For $C_{18}H_{15}BrN_2O_2S$ (403): C, 53.61; H, 3.75; N, 6.95. Found: C, 53.47; H, 3.66; N, 6.88%.

*4.1.1.4.3. 2-(4-Chloro-3-methylphenoxy)-N-(4-(p-tolyl) thiazol-2-yl) acetamide (**8c**).*

Yield 90%;Purity >89% HPLC; M.P. 193-195 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1665 (C=O), 1690 (C=N), 3155-3162 (N-H);^{1}H NMR (400 MHz, DMSO) δ(ppm): 2.35 (s, 6H, $2CH_3$), 4.86 (s, 2H, CH_2), 6.92-7.77(m, 8H, Ar-H), 12.46 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 20.01, 21.22, 66.61, 106.04, 113.70, 125.73, 129.32, 130.01, 131.99, 133.99, 150.60, 159.01, 164,57, 169.13; LC–MS m/z 373 (M+) 375 (M+2). Anal. Calcd. For $C_{19}H1_7ClN_2O_2S$ (373): C, 61.20; H, 4.60; N, 7.51. Found: C, 61.12; H, 4.64; N, 7.39%.

*4.1.1.4.4. N-(4-(P-tolyl) thiazol-2-yl)-2-(2-methylphenoxy) acetamide (**8d**)*

Yield 88%; Purity >84% HPLC; M.P. 156-158 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1670(C=O), 1687 (C=N), 3178-3183 (N-H); ^{1}H NMR (400 MHz, DMSO) δ(ppm): 2.15 (s, 3H, CH_3), 2.34 (s, 3H,

CH_3), 4.63 (s, 2H, CH_2), 6.88-7.96(m, 9H, Ar-H), 12.46 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ15.41, 15.64, 21.32, 66.61, 105.01, 112.12, 120.40, 125.09, 126.21, 126.96, 129.21, 130.01, 131.99, 150.60, 159.01, 164.57, 169.13; LC–MS m/z (M+) 338. Anal. Calcd. For $C_{19}H_{18}N_2O_2S$ (338): C, 67.43; H, 5.36; N, 8.28.Found: C, 67.39; H, 5.32; N, 8.25%.

*4.1.1.4.5.2-(2-Isopropylphenoxy)-N-(4-(p-tolyl) thiazol-2-yl) acetamide (**8e**)*

Yield 83%; Purity >90% HPLC; M.P.130-132 ^{o}C; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1679 (C=O), 1699 (C=N), 3179-3188(N-H); ^{1}H NMR (400 MHz, DMSO) δ(ppm): 1.20 (d, 6H, *J*=8Hz,2CH_3), 2.34 (s, 3H, CH_3),3.57 (m, 1H, CH), 4.83 (s, 2H, CH_2), 6.86 -7.96(m, 9H, Ar-H), 12.48 (bs, 1H, NH); ^{13}C NMR (400 MHz, DMSO): σ 21.33, 23.23, 27.33, 66.82, 105.01, 112.12, 121.01, 125.23, 126.70, 127.12,129.22, 130.01, 131.99, 136.09, 150.60, 155.01, 164.53, 169.13; LC–MS m/z (M+) 366. Anal. Calcd. For $C_{21}H_{22}N_2O_2S$ (366): C, 68.83; H, 6.05; N, 7.64.Found: C, 68.75; H, 5.89; N, 7.60%.

*4.1.1.4.6. 2-(2, 4-Difluorophenoxy)-N-(4-(p-tolyl) thiazol-2-yl) acetamide (**8f**)*

Yield 87%;Purity >96% HPLC; M.P. 136-138 ^{o}C; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1680 (C=O), 1701 (C=N), 3182-3188 (N-H);^{1}H NMR (400 MHz, DMSO) δ(ppm): 2.34 (s, 3H, CH_3), 4.65 (s, 2H, CH_2), 6.67-7.96 (m, 8H, Ar-H), 12.49 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 21.22, 66.64, 105.03, 106.13, 112.07, 117.09, 125.73, 129.53, 130.02, 131.99, 145.23, 150.60, 153.01, 153.78, 164.13, 169.23; LC–MS m/z (M+1) 361. Anal. Calcd. For $C_{18}H_{14}F_2N_2O_2S$ (360): C, 59.99; H, 3.92; N, 7.77. Found C, 59.76; H, 3.89; N, 7.58%.

*4.1.1.4.7. N-(4-(4-Bromophenyl) thiazol-2-yl)-2-(4-chloro-2-methylphenoxy) acetamide (**8g**).*

Yield 84%;Purity >88% HPLC; M.P. 198-200 ^{o}C; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1676 (C=O), 1690 (C=N), 3170- 3175(N-H); ^{1}H NMR (400 MHz, DMSO) δ(ppm): 2.15 (s, 3H, CH_3), 4.66 (s, 2H, CH_2), 6.82-7.78(m, 8H, Ar-H), 12.46 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 14.94,

66.86, 105.44, 111.84, 123.40, 125.09, 126.73, 127.12, 128.03, 129.82, 132.12, 150.60, 156.72, 164.21, 169.26; LC–MS m/z 438 (M+) 440 (M+2) 442 (M+4). Anal. Calcd. For $C_{18}H_{14}BrC_lN_2O_2S$ (438): C, 49.39; H, 3.22; N, 6.40. Found: C, 49.32; H, 3.11; N, 6.28%.

*4.1.1.4.8. N-(4-(4-Bromophenyl) thiazol-2-yl)-2-(4-fluorophenoxy) acetamide (**8h**)*

Yield 87%;Purity >98% HPLC; M.P. 225-227 ^oC; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1671 (C=O), 1697 (C=N), 3179-3183 (N-H);^1H NMR (400 MHz, DMSO) δ(ppm): 4.86 (s, 2H, CH_2), 7.09 -7.98(m, 9H, Ar-H), 12.48 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 66.61, 105. 42, 115.92, 116.43, 123.13, 128.34, 132.04, 133.11, 150.60, 153.74, 155.24, 164.23, 169.26; LC–MS m/z 407 (M+) 409 (M+2). Anal. Calcd. For $C_{17}H_{12}BrFN_2O_2S$ (407): C, 50.14; H, 2.97; N, 6.88. Found: C, 50.03; H, 2.90; N, 6.76%.

*4.1.1.4.9. 2-(4-Fluorophenoxy)-N-(4-(p-tolyl) thiazol-2-yl) acetamide (**8i**)*

Yield 81%;Purity >92% HPLC; M.P.186-188 ^oC; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1678 (C=O), 1693 (C=N), 3181-3184 (N-H); ^1H NMR (400 MHz, DMSO) δ(ppm): 2.34 (s, 3H, CH_3), 4.65 (s, 2H, CH_2), 7.26-7.96 (m, 9H, Ar-H), 12.49 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 21.33, 66.63, 105.02, 115.92, 116.07, 125.71, 129.51, 130.22, 131.99, 150.60, 153.01, 155.22, 164.13, 169.32; LC–MS m/z (M+) 342. Anal. Calcd. For $C_{18}H_{15}FN_2O_2S$ (342): C, 63.14; H, 4.42; N, 8.18. Found C, 63.10; H, 4.32; N, 8.06%.

*4.1.1.4.10. 2-(4-Bromophenoxy)-N-(4-(4-bromophenyl) thiazol-2-yl) acetamide (**8j**)*

Yield 87%;Purity >86% HPLC; M.P. 220-222 ^oC; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1669 (C=O), 1695 (C=N), 3170-3177 (N-H); ^1H NMR (400 MHz, DMSO) δ(ppm): 4.86 (s, 2H, CH_2), 6.99 - 7.96(m, 9H, Ar-H), 12.48 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 66.63, 105.42, 115.93, 118.42, 123.13, 128.33, 132.01, 132.76, 133.12, 150.60, 156.72, 164.22, 169.26; LC–MS m/z

468 (M+) 470 (M+2) 472 (M+4). Anal. Calcd. For $C_{17}H_{12}Br_2N_2O_2S$ (468): C, 43.61; H, 2.58; N, 5.98. Found: C, 43.53; H, 2.46; N, 5.85%.

*4.1.1.4.11. N-(4-(4-Bromophenyl) thiazol-2-yl)-2-phenoxyacetamide (**8k**)*

Yield 89%;Purity >80% HPLC; M.P. 190-192 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1677 (C=O), 1686 (C=N), 3182-3187 (N-H);^{1}H NMR (400 MHz, DMSO) δ(ppm): 4.86 (s, 2H, CH_2), 6.99 -7.96(m, 10H, Ar-H), 12.48 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 66.62, 105. 43, 114.93, 121.45, 123.15, 128.35, 130.15, 132.09, 133.65, 150.60, 157.75, 164.25, 169.26; LC–MS m/z 389 (M+) 391 (M+2). Anal. Calcd. For $C_{17}H_{12}Br_2N_2O_2S$ (389): C, 43.61; H, 2.58; N, 5.98. Found: C, 43.53; H, 2.46; N, 5.75%.

*4.1.1.4.12. N-(4-(4-Bromophenyl) thiazol-2-yl)-2-(o-tolyloxy) acetamide (**8l**)*

Yield 88%;Purity >83% HPLC; M.P. 184-186 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1675 (C=O), 1698 (C=N), 3176-3182 (N-H); ^{1}H NMR (400 MHz, DMSO) δ(ppm): 2.15 (s, 3H, CH_3), 4.63 (s, 2H, CH_2), 6.82-7.96(m, 9H, Ar-H), 12.26 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 16.05, 67.12, 106.03, 112.93, 121.63, 123.72, 126.03, 126.91, 128.33, 131.62, 132.02, 133.11, 151.60, 158.72, 164.22, 169.26; LC–MS m/z 403 (M+) 405 (M+2). Anal. Calcd. For $C_{18}H_{15}BrN_2O_2S$ (403): C, 53.61; H, 3.75; N, 6.95. Found: C, 55.41; H, 3.57; N, 6.89%.

*4.1.1.4.13. N-(4-(4-Bromophenyl) thiazol-2-yl)-2-(2-isopropylphenoxy) acetamide (**8m**)*

Yield 83%;Purity >93% HPLC; M.P. 131-133 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1672 (C=O), 1692 (C=N), 3172-31779 (N-H); ^{1}H NMR (400 MHz, DMSO) δ(ppm): 1.14 (d, 6H, *J*=8Hz, CH_3), 3.05 (m, 1H, CH), 4.63 (s, 2H, CH_2), 6.90 -7.96(m, 9H, Ar-H), 12.48 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 23.62, 27.32, 67.13, 106.01, 113.12, 121.00, 123.73, 126.61,127.12, 128.22, 132.03 , 132.72, 136.61, 150.60, 156.01, 164.5, 169.13; LC–MS m/z 431 (M+) 433

(M+2). Anal. Calcd. For $C_{20}H_{19}BrN_2O_2S$ (431): C, 55.69; H, 4.44; N, 6.49. Found: C, 55.47; H, 4.40; N, 6.27%.

*4.1.1.4.14. N-(4-(4-Bromophenyl) thiazol-2-yl)-2-(2,4-difluorophenoxy) acetamide (**8n**)*

Yield 89%; Purity >94% HPLC; M.P. 176-178 ^{o}C; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1665 (C=O), 1690 (C=N), 3155-3165 (N-H); ^{1}H NMR (400 MHz, DMSO) δ(ppm): 4.63 (s, 2H, CH_2), 6.68 - 7.96(m, 8H, Ar-H), 12.34 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 66.73, 104.99, 106.01, 112.42, 117.62, 123.32, 128.42, 132.02, 132.44, 145.12, 150.22, 153.11, 153.92,164.21, 169.26; LC–MS m/z 425 (M+) 427 (M+2). Anal. Calcd. For $C_{17}H_{11}BrF_2N_2O_2S$ (425): C, 48.02; H, 2.61; N, 6.59. Found: C, 48.07; H, 2.56; N, 6.62%.

*4.1.1.4.15. 2-(4-Bromophenoxy)-N-(4-(4-chlorophenyl) thiazol-2-yl) acetamide (**8o**)*

Yield 83%;Purity >92% HPLC; M.P. 216-218 ^{o}C; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1675 (C=O), 1695 (C=N), 3175-3180 (N-H);^{1}H NMR (400 MHz, DMSO) δ(ppm): 4.89 (s, 2H, CH_2), 6.98 -7.96(m, 9H, Ar-H), 12.48 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 66.71, 105. 92, 115.32, 118.42, 128.71, 129.33, 131.12, 132.62, 134.34, 149.99, 156.92, 163.99, 170.01; LC–MS m/z 424 (M+) 426 (M+2) 428 (M+4). Anal. Calcd. For $C_{17}H_{12}BrClN_2O_2S$ (424): C, 48.19; H, 2.85; N, 6.61. Found: C, 48.11; H, 2.77; N, 6.74%.

*4.1.1.4.16. N-(4-(4-Chlorophenyl) thiazol-2-yl)-2-phenoxyacetamide (**8p**)*

Yield 88%; Purity >89% HPLC; M.P. 197-200 ^{o}C; FT-IR (KBr, $\nu_{max}cm^{-1}$): 1665 (C=O), 1685 (C=N), 3170-3174 (N-H); ^{1}H NMR (400 MHz, DMSO) δ(ppm): 4.85 (s, 2H, CH_2), 6.90-7.96 (m, 10H, Ar-H), 12.46 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 66.61, 105.04, 114.32, 121.02, 128.91, 129.22, 130.01, 131.11, 135.01, 151.00, 159.11, 165, 07, 168.97; LC–MS m/z 345 (M+) 347 (M+2). Anal. Calcd. For $C_{17}H_{13}ClN_2O_2S$ (345): C, 59.22; H, 3.80; N, 8.12. Found: C, 59.16; H, 3.87; N, 8.16%.

*4.1.1.4.17. N-(4-(4-Chlorophenyl) thiazol-2-yl)-2-(2-methylphenoxy) acetamide (**8q**)*

Yield 84%;Purity >81% HPLC; M.P. 191-193 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1676 (C=O), 1692 (C=N), 3180-3189 (N-H); ^{1}H NMR (400 MHz, DMSO) δ(ppm): 2.15 (s, 3H, CH_3), 4.63 (s, 2H, CH_2), 6.85-7.96 (m, 9H, Ar-H), 12.56 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 16.11, 67.01, 105.01, 112.32, 121.31, 126.55, 127.01, 128.92, 129.33, 131.01, 132.12, 134.43, 149.99, 159.33, 164, 57, 169.13; LC–MS m/z 359 (M+) 361 (M+2). Anal. Calcd. For $C_{18}H_{15}ClN_2O_2S$ (359): C, 60.25; H, 4.21; N, 7.81. Found: C, 60.20; H, 4.23; N, 7.72%.

*4.1.1.4.18. N-(4-(4-Chlorophenyl) thiazol-2-yl)-2-(2-isopropylphenoxy) acetamide (**8r**)*

Yield 80%;Purity >89% HPLC; M.P. 137-139 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1677 (C=O), 1695(C=N), 3171-3176 (N-H); ^{1}H NMR (400 MHz, DMSO) δ(ppm): 1.18 (d, 6H, *J*=8Hz, CH_3), 3.05 (m, 1H, CH), 4.63 (s, 2H, CH_2), 6.95 -8.21(m, 9H, Ar-H), 12.67 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 24.03, 28.13, 66.75, 106.03, 113.12, 121.09, 126.64, 127.71, 128.97, 130.01, 131.45, 135,13, 137.24, 151.21, 156.17, 164.52, 169.13; LC–MS m/z 387 (M+) 389 (M+2). Anal. Calcd. For $C_{20}H_{19}ClN_2O_2S$ (387): C, 62.09; H, 4.95; N, 7.24. Found: C, 62.13; H, 4.79; N, 7.18%.

*4.1.1.4.19. N-(4-(4-Chlorophenyl) thiazol-2-yl)-2-(2,4-difluorophenoxy) acetamide (**8s**)*

Yield 85%; Purity >86% HPLC; M.P. 119-121^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1678 (C=O), 1697 (C=N), 3178-3183 (N-H); ^{1}H NMR (400 MHz, DMSO) δ(ppm): 4.63 (s, 2H, CH_2), 6.68-7.96 (m, 8H, Ar-H), 12.46 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 66.81, 104.99, 106.31, 113.11, 117.85, 129.52, 130.32, 131.61, 134.81, 146.01, 151.01, 153.52, 154.11, 164.57, 169.13; LC–MS m/z 381 (M+) 383 (M+2). Anal. Calcd. For $C_{17}H_{11}ClF_2N_2O_2S$ (381): C, 53.62; H, 2.91; N, 7.36. Found: C, 53.45; H, 2.84; N, 7.39%.

*4.1.1.4.20. 2-(4-Chloro-2-methylphenoxy)-N-(4-(4-chlorophenyl) thiazol-2-yl) acetamide (**8t**)*

Yield 88%; Purity >87% HPLC; M.P. 232-235 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1679 (C=O), 1699 (C=N), 3179-3189 (N-H);^{1}H NMR (400 MHz, DMSO) δ(ppm): 2.15 (s, 3H, CH_3), 4.63 (s, 2H, CH_2), 6.85-7.96 (m, 9H, Ar-H), 12.33 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 15.11, 67.31, 105.84, 113.14, 125.62, 126.41, 127.61, 128.90, 129.33, 130.16, 131.41, 134.93, 150.31, 157.32, 164.57, 169.13; LC–MS m/z 393 (M+) 395 (M+2) 397 (M+4). Anal. Calcd. For $C_{18}H_{14}C_{l2}N_2O_2S$ (393): C, 54.97; H, 3.59; N, 7.12. Found: C, 54.81; H, 3.42; N, 7.17%.

*4.1.1.4.21. N-(4-(4-Chlorophenyl) thiazol-2-yl)-2-(4-fluorophenoxy) acetamide (**8u**)*

Yield 86%;Purity >95% HPLC; M.P. 236-238 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1678 (C=O), 1695 (C=N), 3178-3187 (N-H);^{1}H NMR (400 MHz, DMSO) δ(ppm): 4.63 (s, 2H, CH_2), 7.09 - 8.04 (m, 9H, Ar-H), 12.46 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 66.81, 105.08, 115.81, 116.61, 128.41, 130.03, 131.32, 134.41, 150.31, 154.22, 156.22, 166,37, 170.03; LC–MS m/z 363 (M+) 365 (M+2). Anal. Calcd. For $C_{17}H_{12}ClFN_2O_2S$ (363): C, 56.28; H, 3.33; N, 7.72. Found: C, 56.20; H, 3.29; N, 7.56%.

*4.1.1.4.22. N-(4-(4-Chlorophenyl) thiazol-2-yl)-2-(2,4-diisopropylphenoxy) acetamide (**8v**)*

Yield 80%; Purity >98% HPLC; M.P. 133-135 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1676 (C=O), 1695 (C=N), 3179-3189 (N-H); ^{1}H NMR (400 MHz, DMSO) δ(ppm): 1.14-1.20 (d, 6H, *J*=8 Hz, $2CH_3$),(d, 6H, *J*=8 Hz 2CH3), 2.87 (m, 1H, CH), 3.05 (m, 1H, CH), 4.63 (s, 2H, CH_2), 6.76 - 7.96(m, 8H, Ar-H), 12.59 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 23.92, 24.87, 28.33, 34.32, 67.52, 106.01, 112.34, 125.33, 127.13,129.23, 130.13, 131.34, 135.33, 138.42, 141.42, 151.60, 153.67, 165.52, 169.93; LC–MS m/z 429 (M+) 431 (M+2). Anal. Calcd. For $C_{23}H_{25}ClN_2O_2S$ (429): C, 64.40; H, 5.87; N, 6.53. Found: C, 64.26; H, 5.89; N, 6.61%.

*4.1.1.4.23. N-(4-(p-Tolyl) thiazol-2-yl)-2-(4-methylphenoxy) acetamide (**8w**)*

Yield 84%; Purity >92% HPLC; M.P. 173 -175 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1680 (C=O), 1685 (C=N), 3166-3172 (N-H); ^{1}H NMR (400 MHz, DMSO) δ(ppm): 2.29 (s, 6H, 2CH_3), 4.89 (s, 2H, CH_2), 6.89-7.96 (m, 9H, Ar-H), 12.49 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 20.22, 21.45, 66.64, 105.06, 114.33, 125.65, 129.66, 130.01, 130.72, 131.03, 131.82, 150.60, 155.25, 164.13, 169.23; LC–MS m/z 338 (M+). Anal. Calcd. For $C_{19}H_{18}N_2O_2S$ (338): C, 67.43; H, 5.36; N, 8.28. Found C, 67.47; H, 5.22; N, 8.15%.

*4.1.1.4.24. N-(4-(4-Bromophenyl) thiazol-2-yl)-2-(4-methylphenoxy) acetamide (**8x**)*

Yield 89%;Purity >84% HPLC; M.P. 195-197 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1683 (C=O), 1686 (C=N), 3170- 3173 (N-H);^{1}H NMR (400 MHz, DMSO) δ(ppm): 2.25 (s, 3H, CH_3), 4.89 (s, 2H, CH_2), 6.89-7.96 (m, 9H, Ar-H), 12.46 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 21.44, 66.62, 105.04, 114.41, 123.13, 128.03, 130.03, 131.11, 132.11, 132.58, 150.60, 155.13, 164.23, 169.46; LC–MS m/z 403 (M+) 405 (M+2). Anal. Calcd. For $C_{18}H_{15}BrN_2O_2S$ (403): C, 53.61; H, 3.75; N, 6.95. Found: C, 55.54; H, 3.70; N, 6.82%.

*4.1.1.4.25. N-(4-(4-Chlorophenyl) thiazol-2-yl)-2-(4-methylphenoxy) acetamide (**8y**)*

Yield 81%; Purity >83% HPLC; M.P. 216 – 219 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1670 (C=O), 1690 (C=N), 3170-3175 (N-H); ^{1}H NMR (400 MHz, DMSO) δ(ppm): 2.28 (s, 3H, CH_3), 4.89 (s, 2H, CH_2), 6.80 -7.86(m, 9H, Ar-H), 12.38 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 21.42, 66.92, 105.11, 114.41, 128.81,129.22, 130.12 , 130.72, 131.03, 134.54, 150.60, 155.94, 164.52, 169.13; LC–MS m/z 359 (M+) 361 (M+2). Anal. Calcd. For $C_{18}H_{15}ClN_2O_2S$ (359): C, 60.25; H, 4.21; N, 7.81. Found: C, 60.13; H, 4.27; N, 7.79%.

*4.1.1.4.26. 2-(4-Chlorophenoxy)-N-(4-(p-tolyl) thiazol-2-yl) acetamide (**8z**)*

Yield 86%;Purity >85% HPLC; M.P. 173 – 175 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1671 (C=O), 1690 (C=N), 3171-3177 (N-H);^{1}H NMR (400 MHz, DMSO) δ(ppm): 2.34 (s, 3H, CH_3), 4.89 (s, 2H,

CH_2), 7.03 -7.96(m, 9H, Ar-H), 12.69 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): σ 22.02, 66.91, 105.32, 117.33, 125.83,126.84, 129.42, 130.12, 131.22, 132.74, 150.60, 156.72, 164.52, 169.53; LC–MS m/z 359 (M+) 361 (M+2). Anal. Calcd. For $C_{18}H_{15}ClN_2O_2S$ (359): C, 60.25; H, 4.21; N, 7.81. Found: C, 60.02; H, 4.28; N, 7.88%.

*4.1.1.4.27. N-(4-(4-Bromophenyl)thiazol-2-yl)-2-(4-chlorophenoxy)acetamide (**8aa**)*

Yield 84%; Purity >92% HPLC; M.P. 205 – 207 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1675 (C=O), 1680 (C=N), 3176-3182 (N-H); ^{1}H NMR (400 MHz, DMSO) δ(ppm): 4.89 (s, 2H, CH_2), 7.03-7.96 (m, 9H, Ar-H), 12.46 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 66.59, 105.41, 117.43, 123.23, 126.71, 128.43, 131.09, 132.09, 133.03, 150.60, 156.46, 164.34, 169.36; LC–MS m/z 424 (M+) 426 (M+2) 428 (M+4). Anal. Calcd. For $C_{17}H_{12}BrClN_2O_2S$ (424): C, 48.19; H, 2.85; N, 6.61. Found: C, 48.02; H, 2.88; N, 6.48%.

*4.1.1.4.28. 2-(4-Chlorophenoxy)-N-(4-(4-chlorophenyl) thiazol-2-yl) acetamide (**8ab**)*

Yield 80%; Purity >85% HPLC; M.P. 206 – 208 ^{o}C; FT-IR (KBr, ν_{max}cm^{-1}): 1673 (C=O), 1705 (C=N), 3180-3186 (N-H); ^{1}H NMR (400 MHz, DMSO) δ(ppm): 4.89 (s, 2H, CH_2), 7.03 - 7.96(m, 9H, Ar-H), 12.49 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 66.92, 107.12, 117.92, 126.83, 128.94, 129.57, 130.84, 131.20, 134.64, 152.10, 156.44, 164.54, 169.13; LC–MS m/z 379 (M+) 381 (M+2) 383 (M+4). Anal. Calcd. For $C_{17}H_{12}Cl_2N_2O_2S$ (379): C, 53.84; H, 3.19; N, 7.39. Found: C, 53.80; H, 3.13; N, 7.30%.

4.1.2. Biology

4.1.2.1. Animal cell culture and in vitro compound treatment

MCF-7, A549, EAC, DLA and MDA-MB-468 cells were used for the present study and the cells were cultured *in-vitro* as described earlier [28]. All the above cells were treated with increasing concentrations of 2-phenoxy-N-(4-phenylthiazol-2-yl)acetamide analogues (0, 10, 20,

50, and 100 μM in DMSO) for 48 hours and further used for future experiments. Using appropriate vehicle and positive control, every experiment was repeated for three times independently.

4.1.2.2. Trypan blue dye exclusion assay

The effect of 4-phenyl-2-phenoxyacetamide thiazole derivatives **8_{a-ab}** on the cell toxicity against MCF-7, A549, EAC, DLA and MDA-MB-468 cells were assessed by trypan blue dye exclusion assay [29]. DLA cells treated with and without the compounds were pulled out and resuspended in 0.4% trypan blue, and the viable cells were counted using a hemocytometer. The inhibitory concentration (IC_{50}) value was estimated after 48 h of treatment.

4.1.2.3. MTT assay

The effect of 4-phenyl-2-phenoxyacetamide thiazole derivatives **8_{a-ab}** on cell survivality and differentiation of MCF-7, A549, EAC, DLA and MDA-MB-468 cells were assessed by MTT assay, as explained earlier [28]. Referred cells that were treated with or without compounds were cultured for 48 h. MTT reagent (5 mg/mL) was incorporated, and the change in color because of differentiating cells was determined.

4.1.2.4. Animal ethics and determination of LD_{50} value

Swiss female albino mice and Whistler rats weighing between 28‑30g and 100-120g respectively were used throughout the study. The animals were grouped and housed in polyacrylic cages with not more than ten animals per cage with adequate food and water supply. All procedures described were reviewed and approved by the National College of Pharmacy Ethical Committee, Shivamogga, India, in accordance with the CPCSEA guidelines for laboratory animal facility (NCP/IAEC/CL/101/05/ 2012-13). LD_{50} of the compounds **8f** was analyzed and treatment doses were fixed accordingly.

4.1.2.5. Animal tumor models and treatment

EAC ascites and solid tumor was developed as reported earlier [30]. In brief, EAC ascites tumor was developed in the peritoneum cavity of the mice. After the onset of tumor development compound, **8f** was injected intraperitoneally for 3 times on alternative days as reported earlier. Mice were grouped separately (n = 6 each group) and administered with compound **8f** (0, 25, 50 mg/kg body weight (*b.w*), i.p for 3 doses) All the tumor parameters were recorded. The nullification of neovascularization in peritoneum of EAC bearing mice treated with or without compound **8f** mice were photographed and the total vessel length was documented.

EAC cells were harvested from ascites bearing mice and 5 X 10^6 cells were re-injected on the right thigh region of the experimental animal subcutaneously *(s.c)* and cells were cultured *in-vivo* and accommodated under standard laboratory condition by grouping mice separately (n = 6 each group) [30]. The diameters of developing tumor were measured in the case of control and treated animals by using Vernier calipers once in five days. The compound **8f** was deliquesced in DMSO injected alternatively six times intraperitoneally at a concentration of 25 mg/kg body weight. Appropriate vehicle (DMSO) control was also maintained.

4.1.2.6. VEGF-ELISA

The *in vivo* secretion of VEGF levels were quantified by using anti-VEGF antibody by ELISA. In brief, 100 µl of serum were coated in coating buffer at 4°C Incubated with anti-VEGF-A, followed by reincubation with ALP conjugated secondary antibody. VEGF-A was quantified by measuring absorbance at 405 nm by using PNPP as substrate [31]

4.1.2.7. Chorioallanotoic membrane (CAM) assay

Angioprevention effect of compound **8f** was evaluated by treating the recombinant $VEGF_{165}$ ($rVEGF_{165}$) induced *in vivo* CAM. Giriraja Hen's eggs were procured from the Indian

Veterinary Research Institute (IVRI) of Karnataka, India. The eggs were grouped and incubated at 37°C in a humidified and sterile atmosphere for 6 days [32]. The compound **8f** was treated on the growing CAM by cracking the eggshell as reported earlier. After the second day of treatment, the windows were opened analyzed for changes in the microvessel density and photographed using Cannon power shot Sx500 IS camera.

4.1.2.7. Corneal alkali burn Assay

A corneal alkali burn was achieved in the right eye of experimental animal to evaluate the neovascular inhibitory effect of compound **8f** [33].A piece of Whatman #3 filter paper (3-mm diameter) dripped in 4 ml NaOH (1 mol/L) was applied to the center of the cornea for 40 seconds. The cornea was then immediately rinsed with 80 ml of saline for 1 minute, Compound **8f** was administered after the alkali injury.

4.1.2.8. Giemsa staining and DNA fragmentation assay

After the 11th day of ascites tumor implantation, both control and compound **8f** treated mice were sacrificed. EAC cells were collected and washed with phosphate buffered saline (20 mM, PBS) twice and smeared on glass slide, methanol and acetic acid (3:1) were used to fix the cells, and stained by Giemsa solution (0.1%) [34] and visualized using the digital inverted microscope (EVOS). Concurrently genomic DNA was isolated from both control and treated cells as reported earlier and the DNA was analyzed on 1% agarose gel [35] and documented using Bio-rad Gel Documentation^{TM} XR ± Imaging System.

4.1.2.9. Immunoblotting

The whole cell lysate from EAC solid tumor cells treated with or without **8f** was collected and expression of HIF 1-α, p53, MDM-2 and β-actin were assessed from immunoblot technique [36].

4.1.4. Statistical analysis

Values were expressed as mean ± standard deviation (SD). MS excel 8.1 version software was used for data analysis, statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by 2-tailed Student's t-test. Statistical significant values were expressed as $*p < 0.05$ and $**p < 0.01$.

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Conflict of interest statement

The authors declare that there are no conflicts of interest

Scheme 1: Synthesis of 4-phenyl-2-phenoxyacetamide thiazole analogues (**8_{a-ab}**).

Table 1: IC50 values of compounds **8_{a-ab}** calculating based upon trypan blue, MTT at 48 h in MCF-7, A549, EAC DLA and MDA-MB-468 cells.

Fig .1. The basic structure of 2-phenoxy-N-(4-phenylthiazol-2-yl) acetamide analogues.

Fig2: Effect of compound **8f** treated on Ehrlich Ascites Carcinoma (EAC) in mice. Ascites tumor was induced in 6-7 week old Swiss albino mice by injecting EAC cell intraperitoneally. Three doses of compound **8f** (25 mg/kg and 50 mg/kg) each administered to tumor bearing mice

on every alternate day after three days of tumor growth. (a) Decrease in body weight of mice treated with compound **8f** compared with the control mice.(b) Decrease in the cell number of EAC cells treated with compound **8f** in comparison with control. (c) Decrease in ascites secretion after treatment with compound **8f** (d) Cumulative survivability curves of EAC-bearing mice treated with and without compound **8f** (e) Physical morphology of normal, control, compound **8f** (25 mg/kg and 50 mg/kg) treated tumor mice (f) spleens and (g) Livers from normal, control and compound **8f** treated mice depicts that compound **8f** is not cytotoxic to organs.

Fig. 3. Comparison of effect of compound **8f** on progression of solid tumor in mice organs at 35^{th} day of treatment. Solid tumor was induced in 6-7 week old Swiss albino mice by injecting EAC cells subcutaneously into thigh region. six doses of **8f** (25 mg/kg) each administered to tumor bearing mice on every alternate day after solid tumor grew to 100 mm^3 in size (a) Physical appearance of normal, control and compound **8f** treated tumor mice (b) Anti-proliferative effect of compound **8f** on tumor size showed active tumor inhibitory properties of compound **8f** (c) compound **8f** inhibited tumor growth as measured by tumor volume (d) Tumor weight of EAC solid tumor from control and treated with **8f**, Data represented as the mean ±S.D. of three different observations (six animals per treatment group).

Fig. 4: Angiogenesis modulatory effect of compound **8f** on different non tumor and tumor model system**.** (a) Alkali burn rat corneal assay was performed and compound **8f** showed inhibition of neovasularisation in cornea of alkali injured rat eye in comparision with control rat (b) Graphical representation of Micro vessel density of corneal eye of normal ,control and **8f** treated rats (c) *In vivo* CAM photos exhibits the angiopreventive effect of compound **8f** compared to $VEGF_{165}$ treated CAM (d) Graphical representation of MVD count in normal, control, and **8f**

treated *in vivo* CAM (e) Peritoneal angiogenesis as seen by EAC induced neovascularization in compound **8f** treated compared to control and the peritoneum lining of mice was photographed (f) Micro vessel density counts graphical representation of in the peritoneal of control and compound **8f** treated tumor bearing mice.

Fig.5: Compound **8f** suppresses the EAC cell proliferation *in vivo* by inducing apoptosis (a) Giemsa stained DLA cells showing the morphological changes, such as irregular shape, membrane blabbing and formation of apoptotic bodies *in vivo* in **8f** treated cells compared to control cells

(b) Immune blot of **8f** induced gene expression profile with the expression of HIF 1-α, p53 and MDM-2. (c) DNA fragmentation observed in the **8f** treated cells compared against control DNA. d) Reduction of serum VEGF-A by ELISA

Fig. 6. Schematic representation of **8f** induced apoptosis.

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Table 1: IC_{50} values of compounds 8a-ab calculating based upon trypan blue, MTT at 48 h in MCF-7, A549, EAC, DLA and MDA-MB-468 cells.

Compounds		8a	8b	8c	8d	8e	8f	8g	8h	8i	8j	8k	8l	8m	8n	8o	8p	8q	8r	8s	8t	8u	8v	8w	8x	8y	8z	8aa	8ab	5-fluorouracil
MCF7 cells	Trypan blu	43±0.6	39±0.5	41.5 ±0.7	53.2 ±0.3	49 ±0.9	**14 ±0.4**	51.7 ±0.3	56.8 ± 0.9	**18.1± 1.2**	44.5± 0.9	54 ± 1.2	39.7± 0.6	43.5± 0.9	62.1± 0.5	52.1 ±0.6	55.9 ±0.8	62.8 ±0.9	18.1 ±1.2	**25.3± 0.6**	40.1 ±0.5	**35.3 ±1.2**	40.6 ±0.8	41.4 ±0.9	52.4 ±0.2	48.8 ±0.2	43.7± 1.2	41.4 ±0.6	44.1 ±0.3	10.6 ±0.8
	MTT- IC50	43 ± 0.5	39 .5 ±0.7	40.9 ±0.7	53.5 ±0.3	49.2 ±0.7	**14.5 ±0.8**	52.1 ±1.3	55.5 ±0.5	**18.3± 0.3**	44.9± 0.8	54.3± 0.7	40.1± 0.6	42.5± 0.9	62.9± 0.3	52.4 ±1.2	60.3 ±0.3	62.2 ±0.2	18.7 ±1.8	**25.8± 0.3**	40.6 ±1.5	**35.5 ±0.4**	40.1 ±1.2	41.3 ±0.6	52.2 ±1.5	48.7 ±1.2	43.8± 1.5	41.1 ±1.6	44.5 ±1.3	10.2 ±0.6
A549 cells	Trypan blu	41.1±0.8	37.1±0.5	44.5 ±0.3	50.1±1.3	44.7±1.2	**13.2 ±0.8**	51.7 ±0.3	51.7 ± 0.5	**19.1± 0.2**	46.6± 0.9	52.2 ± 1.3	49.8± 0.6	48.5±0.2	59.1± 0.8	54.9±0.8	42.8 ±0.7	64.8 ±0.7	17.1 ±1.4	**26.3± 0.3**	43.1 ±1.5	**36.3 ±0.2**	48.6 ±0.6	48.8±0.7	58.6±1.2	44.8 ±1.2	44.7± 0.2	46.4±0.8	49.1 ±1.3	11.2 ±0.4
	MTT- IC50	41.7±0.7	37.2±0.9	44.9 ±0.9	50.9±0.9	44.2 ±0.2	**13.9 ±0.5**	52.1 ±1.3	52.1± 0.5	**19.3 ± 0.1**	46.5± 0.3	52.9± 0.9	50.1± 0.8	48.2±0.8	58.8± 0.2	54.4 ±1.3	42.9±1.2	64.9±0.9	17.8±0.8	**26.5± 0.6**	43.4±0.5	**36.5 ±1.4**	48.8±0.2	48.3 ±0.9	58.8±0.5	45.72±0.2	44.0± 0.5	46.1 ±0.6	49.9±0.3	11.4 ±0.8
EAC Cells	Trypan blu	49.8± 0.6	48.5±0.2	59.1± 0.8	54.9±0.8	42.8 ±0.7	**14. ±10.2**	47.1 ±1.4	45.3± 0.	**18.1 ±1.5**	41.1±0.8	37.1±0.5	44.5 ±0.3	50.1±1.3	44.7±1.2	51.7 ±0.3	56.8 ± 0.9	63.1± 1.2	49.8± 0.6	**26.5± 0.2**	52.1 ±0.6	**35.9 ±0.8**	62.8 ±0.9	45.1 ±1.2	55.3± 0.6	40.1 ±0.5	42 ±1.2	40.6 ±0.8	52.1 ±0.8	9.6 ±0.3
	MTT- IC50	50.1± 0.8	48.2±0.8	58.8± 0.2	54.4 ±1.3	42.9±1.2	**14.9± 0.8**	47.8±0.8	45.5± 0.	**18.4 ±0.5**	41.7±0.7	37.2±0.9	44.9 ±0.9	50.9±0.9	44.2 ±0.2	52.1 ±1.3	55.5 ±0.5	62.3±0.33	50.1± 0.8	**26.2 ±0.8**	52.4 ±1.2	**35.3 ±0.3**	62.2 ±0.2	45.7 ±1.8	55.8± 0.3	40.6 ±1.5	42.5 ±0.4	40.1 ±1.2	51.9± 0.2	9.2 ±0.9
DLA Cells	Trypan blu	51.7 ±0.3	51.7 ± 0.9	59.1± 0.2	46.6± 0.9	52.2 ± 1.3	**13.9± 0.4**	39.7± 0.6	43.5± 0.9	**18.1± 0.5**	52.1 ±0.6	55.9 ±0.8	62.8 ±0.9	45.1 ±1.2	55.3± 0.6	40.1 ±0.5	53.2 ±0.3	49 ±0.9	40.1 ±0.5	**26.4 ±1.2**	40.6 ±0.8	**35.1 ±0.8**	54 ± 1.2	39.7± 0.6	43.5± 0.9	62.1± 0.5	52.1 ±0.6	55.9 ±0.8	52.1 ±0.4	10.6 ±0.8
	MTT- IC50	52.1 ±1.3	52.1± 0.5	60.3 ± 0.13	46.5± 0.3	52.9± 0.9	**13.4 ±1.2**	40.1± 0.6	42.5± 0.9	**18.9± 0.3**	52.4 ±1.2	60.3 ±0.3	62.2 ±0.2	45.7 ±1.8	55.8± 0.3	40.6 ±1.5	53.5 ±0.3	49.2 ±0.7	40.6 ±1.5	**26.1 ±0.4**	40.1 ±1.2	**35.9± 0.2**	54.3± 0.7	40.1± 0.6	42.5± 0.9	62.9± 0.3	52.4 ±1.2	60.3 ±0.3	53.2 ± 1.2	10.8 ±0.4
MDA-MB 468	Trypan blu	54.5 ±0.3	40.1±1.3	54.7±1.2	51.7 ±0.3	56.8 ± 0.9	**10.2 ±1**	51.7 ±0.3	51.7 ± 0.5	**24.4 ±1**	41.1±0.8	37.1±0.5	44.5 ±0.3	50.1±1.3	44.7±1.2	50.9±0.9	44.2 ±0.2	52.1 ±1.3	55.5 ±0.5	**32.3± 0.3**	52.1 ±0.6	**42.9± 0.8**	41.1±0.8	37.1±0.5	44.5 ±0.3	50.1±1.3	44.7±1.2	51.7 ±0.3	56.8 ± 0.9	44.6 ±0.6
	MTT- IC50	54.9 ±0.9	40.9±0.9	54.2 ±0.2	52.1 ±1.3	55.5 ±0.5	**10.49±0.3**	52.1 ±1.3	52.1± 0.5	**24.9± 0.3**	41.7±0.7	37.2±0.9	44.9 ±0.9	50.9±0.9	44.2 ±0.2	45.1 ±1.2	55.3± 0.6	40.1 ±0.5	53.2 ±0.3	**32.9± 1**	52.4 ±1.2	**42.1± 0.6**	41.7±0.7	37.2±0.9	44.9 ±0.9	50.9±0.9	44.2 ±0.2	52.1 ±1.3	55.5 ±0.5	44.9 ±0.9

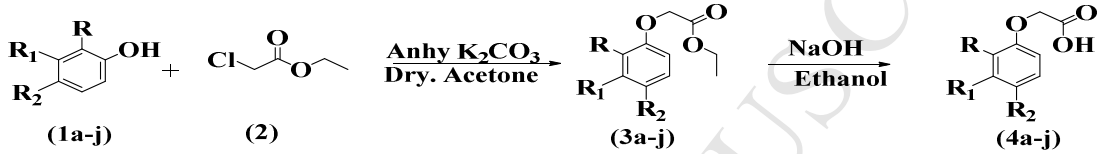
Values are indicate in mean ± SEM and statistical significants values are expressed as *p < 0.05 and **p < 0.01.

Compound **8f** has more potency to show anti-tumor property compared to others series are shown in bold.

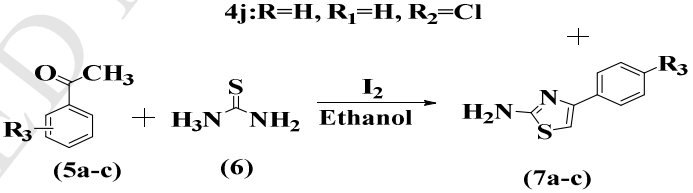
The Novel 4-Phenyl-2-Phenoxyacetamide Thiazoles Modulates the Tumor Hypoxia Leading to the Crackdown of Neoangiogenesis and Evoking the Cell Death.

Yasser Hussein Eissa Mohammed[1,3], Vikas H. Malojirao[2], Prabhu Thirusangu[2], M. Al-Ghorbani, Prabhakar B.T[2], Shukath Ara Khanum[1].

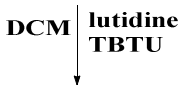
Scheme 1

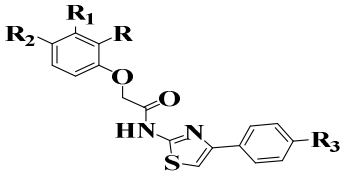


4a:R=H, R_1=H, R_2=H
4b:R=H, R_1=Br, R_2=H
4c:R=H, R_1=CH_3, R_2=Cl
4d:R=CH_3, R_1=H, R_2=H
4e:R=isopropyl, R_1=H, R_2=H
4f:R=F, R_1=H, R_2=F
4g:R=H, R_1=H, R_2=F
4h:R=isopropyl,R_1=H,R_2=isopropyl
4i:R=H, R_1=H, R_2=CH_3
4j:R=H, R_1=H, R_2=Cl



7a:R_3=CH_3
7b:R_3=Br
7c:R_3=Cl





(8a-ab)

8a; R=H, R_1=H, R_2=H, R_3=CH_3
8b; R=H, R_1=H, R_2=Br, R_3=CH_3
8c; R=H, R_1=CH_3, R_2=Cl, R_3=CH_3
8d; R=CH_3, R_1=H, R_2=H, R_3=CH_3
8e; R=isopropyl, R_1=H, R_2=H, R_3=CH_3
8f; R=F, R_1=H, R_2=F, R_3=CH_3
8g; R=H, R_1=CH_3, R_2=Cl, R_3=Br
8h; R=H, R_1=H, R_2=F, R_3=Br
8i; R=H, R_1=H, R_2=F, R_3=CH_3
8j R=H, R_1=H, R_2=Br, R_3=Br
8k; R=H, R_1=H, R_2=H, R_3=Br
8l; R=CH_3, R_1=H, R_2=H, R_3=Br
8m; R=isopropyl, R_1=H, R_2=H, R_3=Br
8n; R=F, R_1=H, R_2=F, R_3=Br
8o; R=H, R_1=H, R_2=Br, R_3=Cl
8p; R=H, R_1=H, R_2=H, R_3=Cl
8q; R=CH_3, R_1=H, R_2=H, R_3=Cl
8r; R=isopropyl, R_1=H, R_2=H, R_3=Cl
8s; R=F, R_1=H, R_2=F, R_3=Cl
8t; R=H, R_1=CH_3, R_2=Cl, R_3=Cl
8u; R=H, R_1=H, R_2=F, R_3=CL
8v; R=isopropyl, R_1=H, R_2=isopropyl, R_3=Cl
8w; R=H, R_1=H, R_2=CH_3, R_3=CH_3
8x; R=H, R_1=H, R_2=CH_3, R_3=Br
8y; R=H, R_1=H, R_2=CH_3, R_3=Cl
8z; R=H, R_1=H, R_2=Cl, R_3=CH_3
8aa; R=H, R_1=H, R_2=Cl, R_3=Br
8ab; R=H, R_1=H, R_2=Cl, R_3=Cl

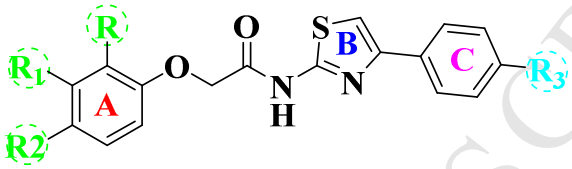


Fig .1. The basic structure of 2-phenoxy-N-(4-phenylthiazol-2-yl)acetamide analogues.

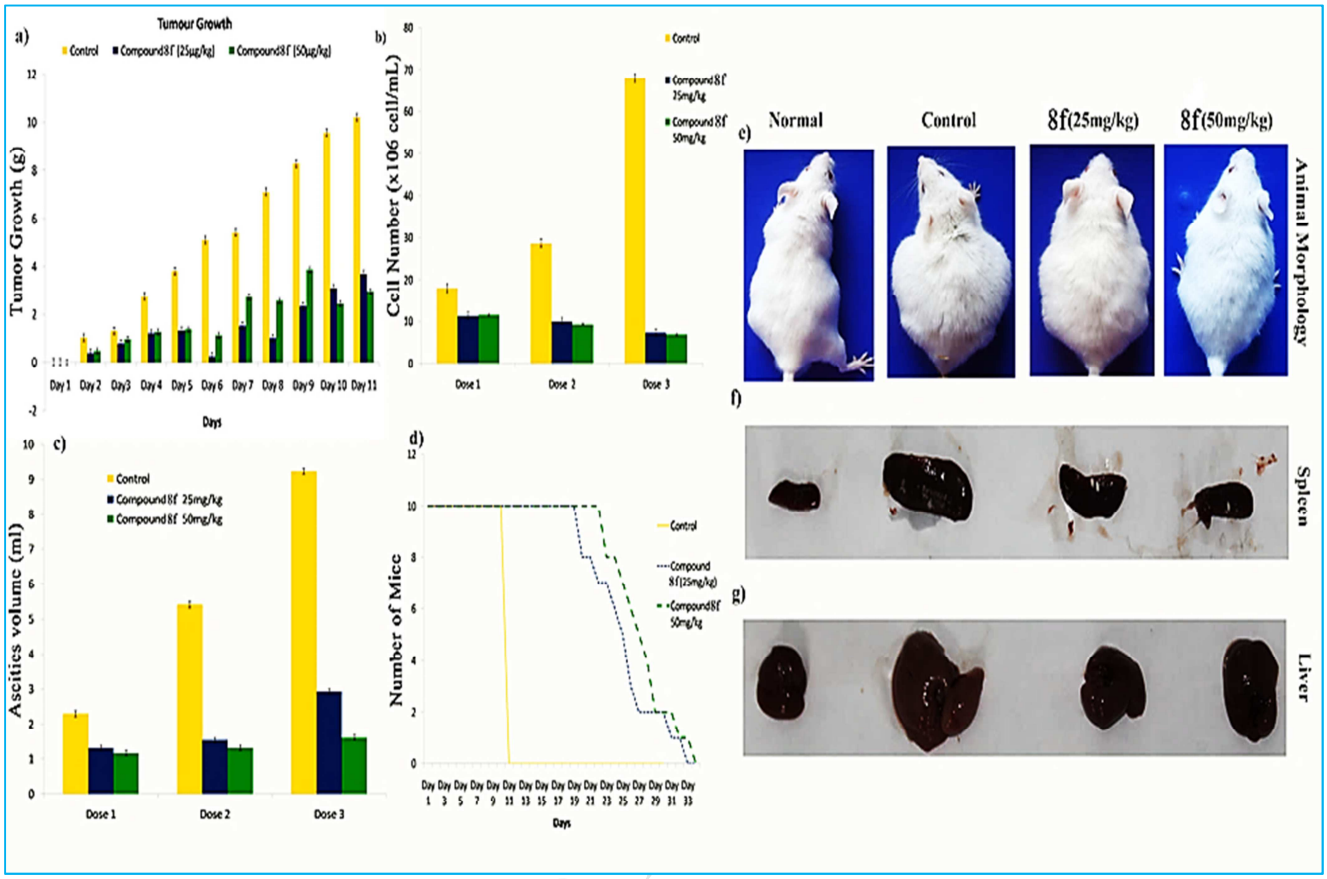


Fig .2. Effect of compound **8f** treated on Ehrlich Ascites Carcinoma (EAC) in mice. Ascites tumor was induced in 6-7 week old Swiss albino mice by injecting EAC cell intraperitoneally. Three doses of compound **8f** (25 mg/kg and 50 mg/kg) each administered to tumor bearing mice on every alternate day after three days of tumor growth. (a) Decrease in body weight of mice treated with compound **8f** compared with the control mice. (b) Decrease in the cell number of EAC cells treated with compound **8f** in comparison with control. (c) Decrease in ascites secretion after treatment with compound **8f** (d) Cumulative survivability curves of EAC-bearing mice treated with and without compound **8f** (e) Physical morphology of normal, control, compound **8f** (25 mg/kg and 50 mg/kg) treated tumor mice (f) spleens and (g) Livers from normal, control and compound **8f** treated mice depicts that compound **8f** is not cytotoxic to organs.

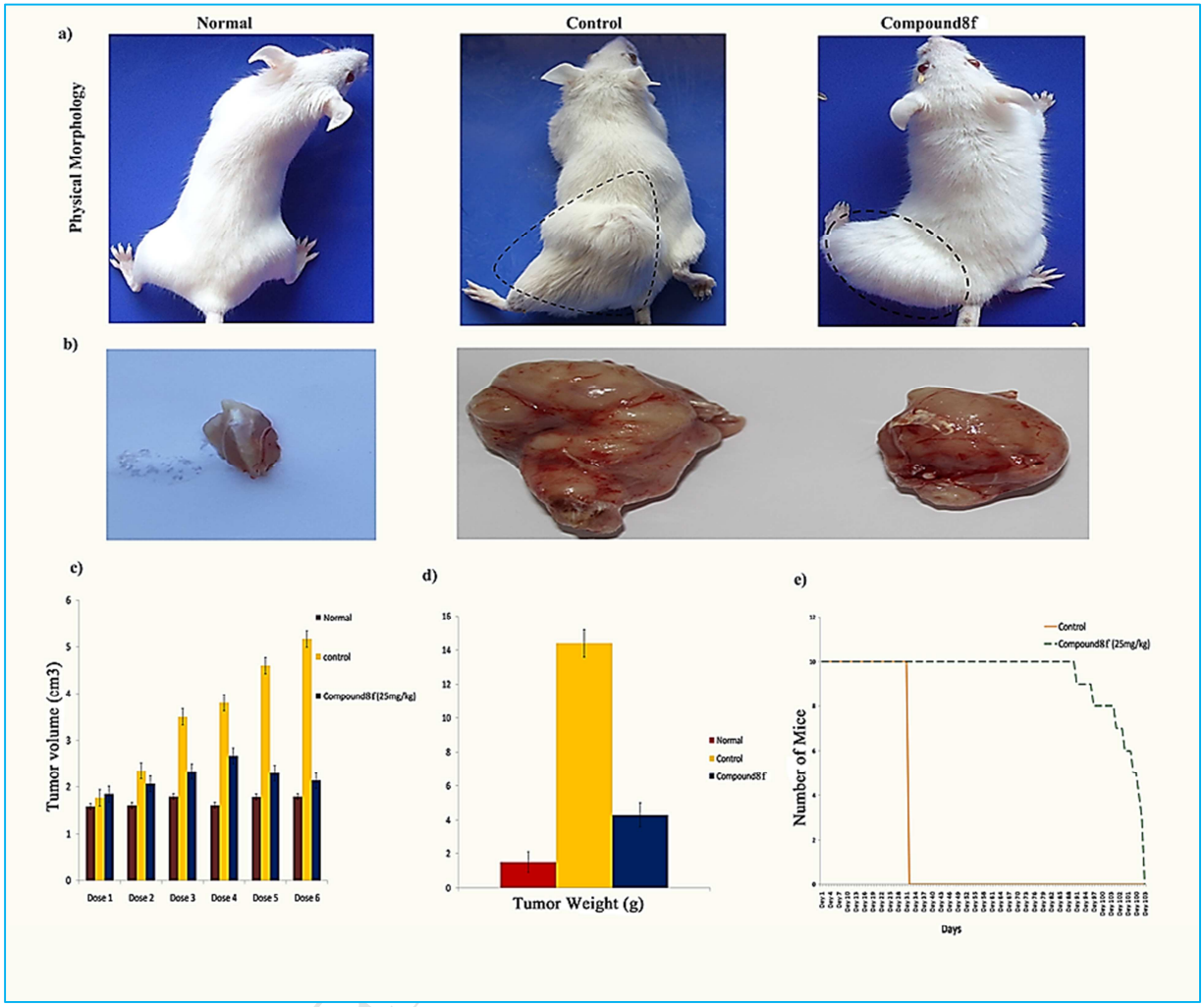


Fig .3. Comparison of effect of compound **8f** on progression of solid tumor in mice organs at 35th day of treatment. Solid tumor was induced in 6-7 week old Swiss albino mice by injecting EAC cells subcutaneously into thigh region. six doses of **8f** (25 mg/kg) each administered to tumor bearing mice on every alternate day after solid tumor grew to 100 mm^3 in size (a) Physical appearance of normal, control and compound **8f** treated tumor mice (b) Anti-proliferative effect of compound **8f** on tumor size shows active tumor inhibitory properties of compound **8f** (c)

compound **8f** inhibited tumor growth as measured by tumor volume (d) Tumor weight of EAC solid tumor from control and treated with **8f**, Data represented as the mean ±S.D. of three different observations (six animals per treatment group).

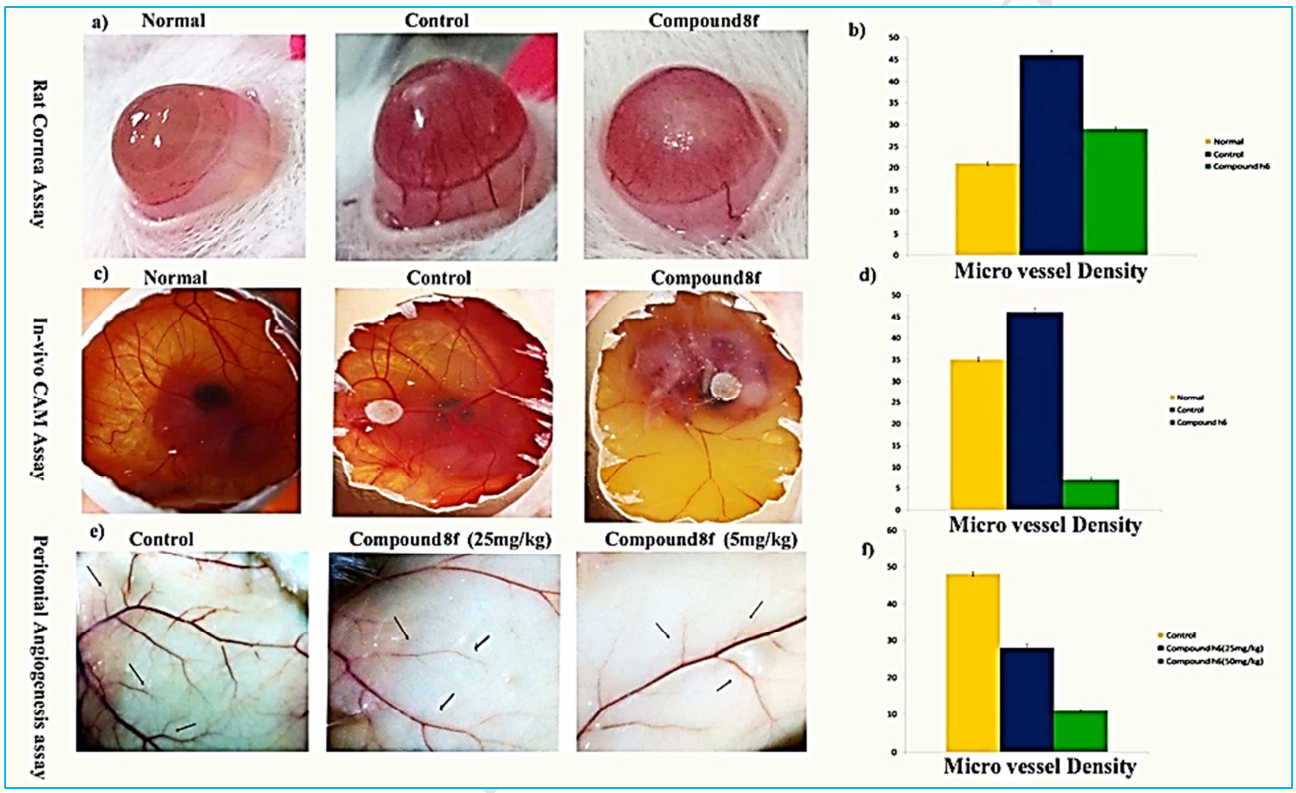


Fig .4. Angiogenesis modulatory effect of compound **8f** on different non tumor and tumor model system**.** (a) Alkali burn rat corneal assay was performed and compound **8f** showed inhibition of neovasularisation in cornea of alkali injured rat eye in comparision with control rat (b) Graphical representation of micro vessel density of corneal eye of normal ,control and **8f** treated rats (c) *In vivo* CAM photos exhibits the angiopreventive effect of compound **8f** compared to $VEGF_{165}$ treated CAM (d) Graphical representation of MVD count in normal, control, and **8f** treated *in vivo* CAM (e) Peritoneal angiogenesis as seen by EAC induced neovascularization in

compound **8f** treated compared to control and the peritoneum lining of mice was photographed (f) Micro vessel density counts graphical representation of in the peritoneal of control and compound **8f** treated tumor bearing mice.

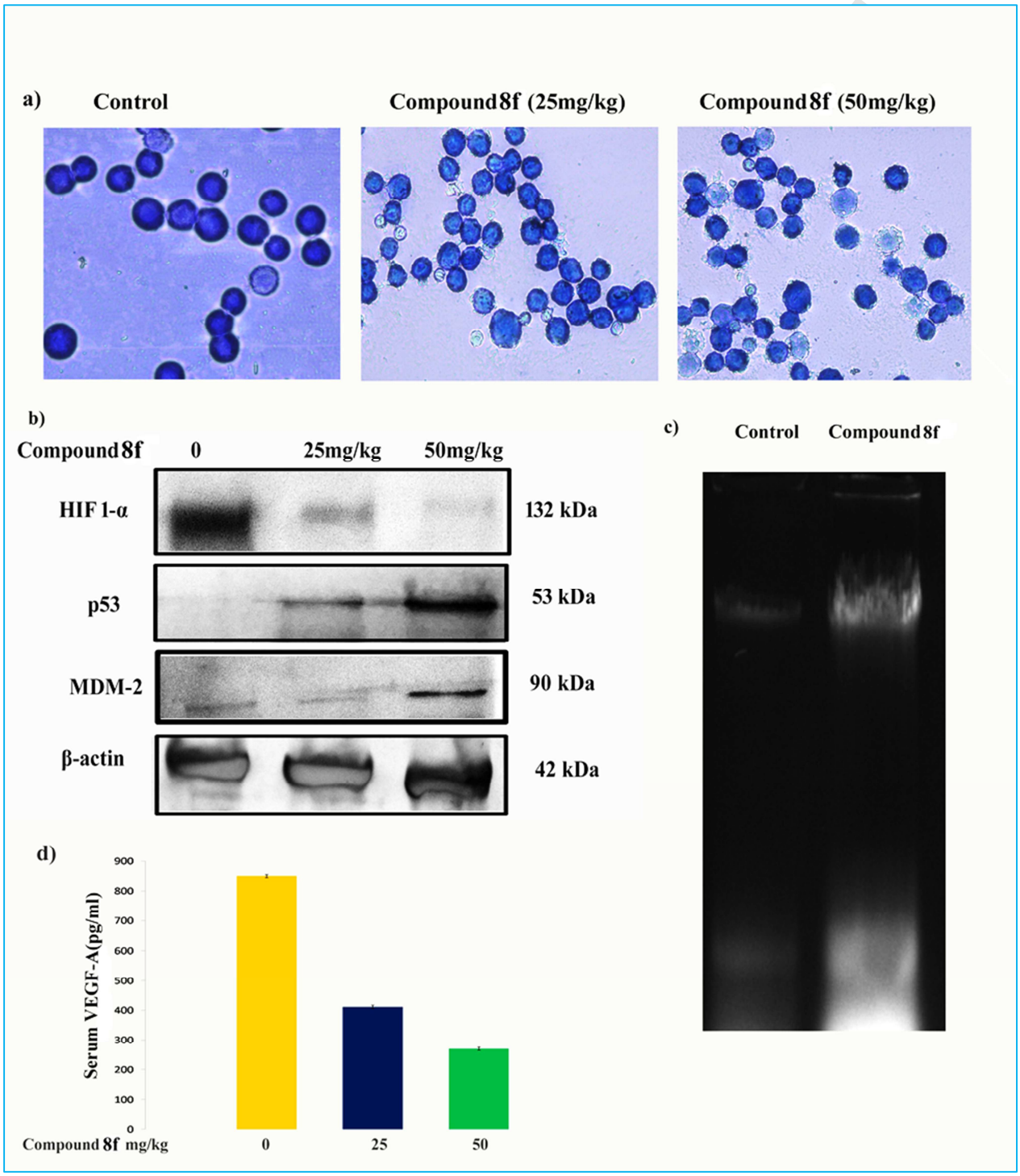


Fig.5: Compound **8f** suppresses the EAC cell proliferation *in vivo* by inducing apoptosis (a) Giemsa stained DLA cells showing the morphological changes, such as irregular shape, membrane blabbing and formation of apoptotic bodies *in vivo* in **8f** treated cells compared to control cells (b) Immune blot of **8f** induced gene expression profile with the expression of HIF 1-α, p53 and MDM-2. (c) DNA fragmentation observed in the **8f** treated cells compared against control DNA. d) Reduction of serum VEGF-A by ELISA.

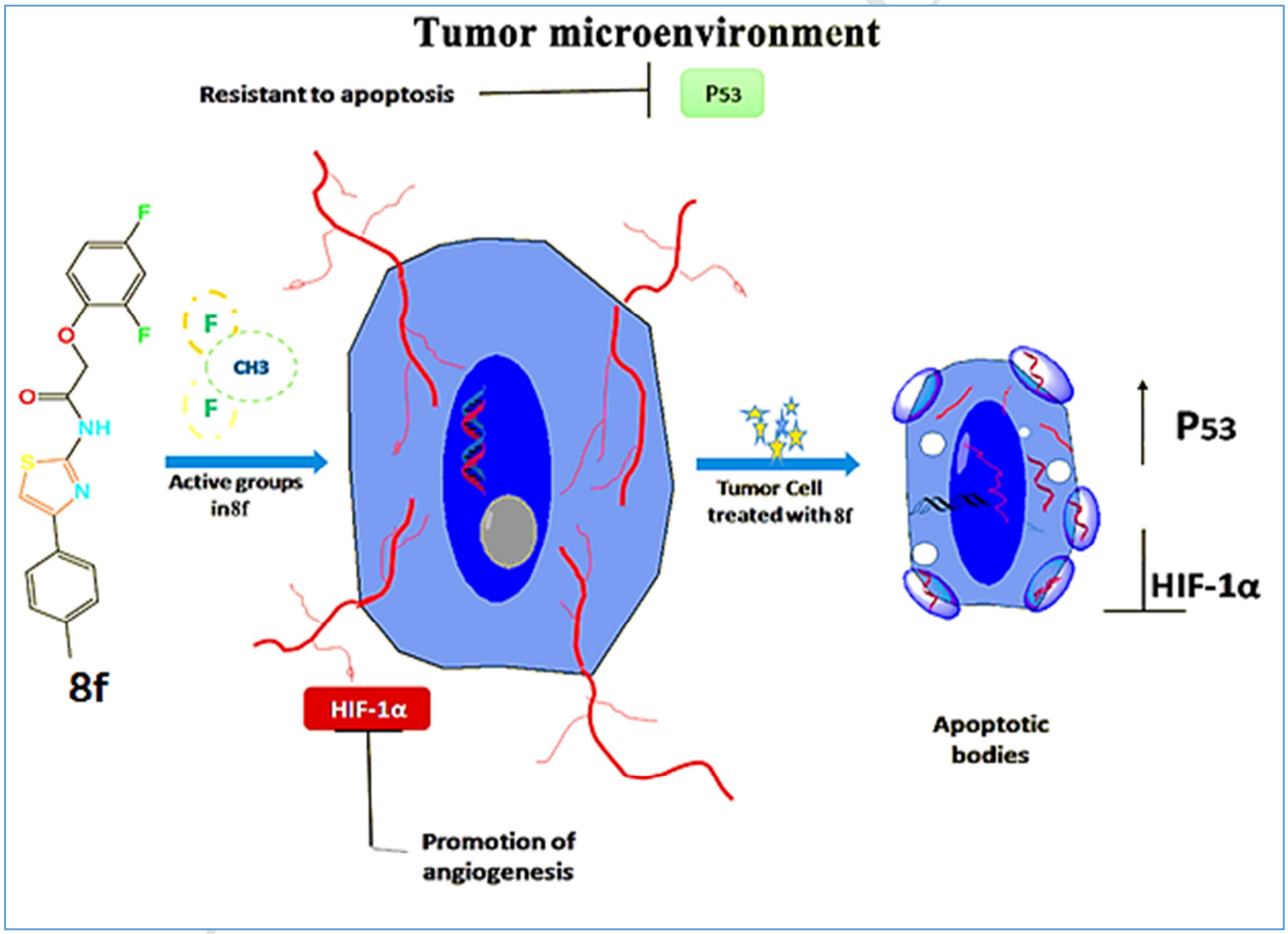


Fig .6. Schematic representation of **8f** induced apoptosis.

Research Highlight

- **Search for new anti-tumor drugs for the treatment of cancer diseases.**
- **A new series of substituted Novel 4-Phenyl-2-Phenoxyacetamide Thiazoles analogous were synthesized.**
- **All the newly synthesized compounds were fully characterized by spectroscopic studies.**
- **Their anti-tumor activity was evaluated.**
- **One compound 8f was reconfirmed as a potent anti-tumor drug by *in vitro* and *in vivo* study.**