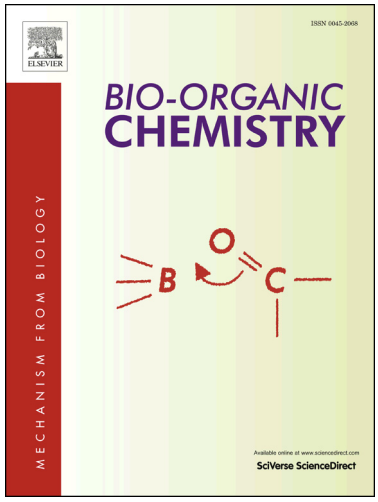


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# Synthesis of novel morpholine conjugated benzophenone analogues and evaluation of antagonistic role against neoplastic development

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**Abstract**

A series of novel 4-benzyl-morpholine-2-carboxylic acid *N'*-[2-(4-benzoyl-phenoxy)-acetyl]-hydrazide derivatives **8a-j** has been synthesized from (4-hydroxy-aryl)-aryl methanones through a multi-step reaction sequence and then evaluated for anti-proliferative activity *in vitro* against various types of neoplastic cells of mouse and human such as DLA, EAC, MCF-7 and A549 cells. From the cytotoxic studies and structural activity relationship of compounds **8a-j**, it is clear that methyl group on the B ring of benzophenone is essential for antiproliferative activity and bromo at ortho position (compound **8b**) and methyl at para position (compound **8f**) on A ring of benzophenone are significant for extensive anti-mitogenic activity. Investigation on clonogenesis and Fluorescence-activated cell sorting suggests that compounds **8b** and **8f** have the potency to exhibit the prolonged activity with cell cycle arrest on G2/M phase against cancer progression. Further, the compounds **8b** and **8f** inhibit murine ascites lymphoma through caspase activated DNase mediated apoptosis.

*Keywords:* Benzophenone; Morpholine; CAD; DLA and MCF-7.

## 1. Introduction

Cancer, a circumstance of an abnormal cell division or mitogencity, is considered the deadliest among the diseases [1-3]. To maintain the tissue or cell homeostasis, normal cells carefully regulate the production and release of growth promoting factors which are responsible for the cellular proliferation through the cell growth and division cycle [4, 5]. In case of cancer, cells, differ from most of the normal cells in a number of biochemical processes, specifically during cell growth, cell division and cell death. Cancer cells, by deregulating cell division and cell death and by up-regulating intracellular self signal, achieve the uncontrollable proliferation [4-6]. Caspases are the important key factors during apoptosis, which activate the caspase activated DNase (CAD). The fragmentation of chromosomal DNA into nucleosomal units through active CAD expression is considered as a prominent biochemical hallmark of apoptotic cell death [7, 8]. As a result, targeting mitogenicity or proliferative efficacy of the tumour cell resulting in apoptotic cell death is viewed as an effective strategy for cancer drug development process. Though, a noticeable number of novel anti-neoplastic cytotoxic molecules have been introduced, they have failed to reach the bedside due to their unknown mechanism, non-specificity and adverse effects [9]. For cancer chemoprevention, there is urgency in the search for non-toxic chemopreventive agents that inhibit mitogenic and cell survival signaling by targeting ap in cancer cells.

Morpholine ring system is a core structure of various synthetic compounds displaying a broad spectrum of therapeutic applications [10-16]. The literature survey revealed that morpholine derivatives proved as an excellent class of anticancer agents against a variety of cancer cell lines, such as human colorectal adenocarcinoma, metastatic human breast cancer,

gastric cancer, mammalian target of rapamycin, non small cell lung cancer, prostate cancer [17, 18].

On the other hand, the proficiency of benzophenone analogues as chemotherapeutic agents, especially as anticancer, is well documented [19-21]. Previously, our group has reported some benzophenone-heterocycle hybrids with good anticancer activity [22-27]. In continuation of our efforts towards the design of new anticancer agents, we considered it worthwhile to pursue further modifications on the benzophenone part by appending morpholine subunit at 2-position on (4-benzoyl-phenoxy)-acetic acid hydrazide (Figure. 1) for inhibition of tumour cell proliferation of mouse and human origin.

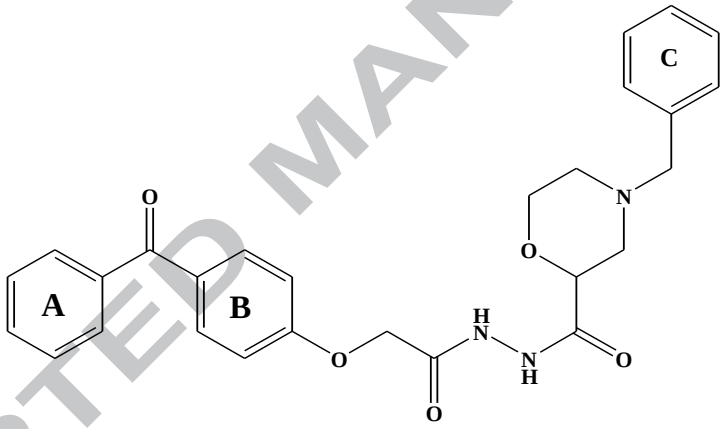


**Figure 1.** Structure of 4-benzyl-morpholine-2-carboxylic acid N'-[2-(4-benzoyl-phenoxy)-acetyl]-hydrazide

## 2. Results and discussion

### *2.1. Chemistry*

Synthesis of the target compounds 4-benzyl-morpholine-2-carboxylic acid *N*'-[2-(4-benzoyl-phenoxy)-acetyl]-hydrazides **8a-j** was performed according to the reactions illustrated in scheme 1. The key starting compounds, phenyl benzoates **3a-j**, were prepared according to the published procedures [23], starting from the commercially available substituted phenols **1a-j** with benzoyl

chlorides **2a-j**. Fries rearrangement of compounds **3a-j** with anhydrous aluminium chloride as a catalyst gave hydroxybenzophenones **4a-j.** Furthermore, acylation of **4a-j** with chloro ethyl acetate afforded the substituted ethyl esters **5a-j**, which were converted to the corresponding acetyldrazides **6a-j** upon treatment with hydrazine hydrate. The corresponding final compounds **8a-j** were successfully synthesized by coupling compounds **6a-j** with 4-benzyl-morpholine-2-carboxylic acid **7** using 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide (EDCI) and 1-hydroxybenzotriazole (HOBt) as coupling agents and triethylamine (TEA) as a base. All the structures of newly synthesized compounds were assigned on the basis of their spectroscopic data; IR, NMR, LC-MS and C,H,N analysis. The spectra of the title compound **6a** were considered as a representative example of the series **6a-j**. In IR spectra, the compound **6a** showed bands at 1615, 1650 and in between 3105-3210 $cm^{-1}$ corresponding to aromatic carbonyl, amide carbonyl and NH-$NH_2$ stretching frequencies respectively. In $^1H$ NMR spectra of compound **6a** showed one singlet at δ 4.63 assigned to $OCH_2$ protons, it also revealed two broad singlets at δ 4.36 and 9.30 assigned to amino and amide protons, as well as multiplet signals appeared in the range δ 6.96-7.68 for aromatic protons. The mass spectra of compound **6a** gave significant stable $M^+$ peak at m/z 285. In IR spectra of compounds **8a** was confirmed by the appearance of one more carbonyl at 1669 $cm^{-1}$ and disappearance of the $NH_2$ absorption peak. In addition, $^1H$ NMR spectra showed disappearance of $NH_2$ protons at 4.36 and an increase in one more NH proton and four aromatic protons with earlier aromatic proton peaks at δ10.14 and 6.95-7.59 respectively, as well as by the appearance of three characteristic bands at 2.64, 3.65 and 4.17 corresponding to seven protons of morpholine ring which clearly evidence the formation of compound **8a**. The mass spectra of compound **8a** gave significant stable ($M^+$) peak at m/z 589. Further, all the target compounds **8a-j** were clearly confirmed by $^{13}C$ NMR.

## 2.2. Pharmacology

### *2.2.1. Evaluation of $IC_{50}$ values of **8a-j** and in vitro selection of lead compounds*

Research conducted on anticancer drug development suggests that the conjugation of oxadiazole [26,27], thiazole [25], benzimidazole [24], coumarin [22,23], pyridine [28] and acetamide [29] with benzophenone has a promising pharmacological activity by targeting specifically intrinsic signaling molecule in programmed cell death, hypoxia inducible factor-1α (HIF-1α) and Vascular endothelial growth factor (VEGF) in tumour vasculature. On the other hand, morpholine derivatives have excellent pharmacological characteristics against the variety of pathological conditions including cancer of the different cells [10-16]. In the present investigation, new potent analogues were synthesized, by integrating morpholine nuclei to benzophenone moiety. Initially, antiproliferative efficacy of benzophenone-morpholine analogues **8a-j** were evaluated against murine cancer cells Dalton's lymphoma ascites (DLA) and Ehrlich ascites carcinoma (EAC) by performing MTT, Trypan blue and LDH leak assays (Table. 1). The average cytotoxicity of **8b** and **8f** was calculated against each cell line by cytotoxic studies. The compounds **8b** and **8f** were found to exhibit a promising anti-neoplastic effect against DLA cells with $IC_{50}$ of ~7.5 µM and ~10.3µM respectively. The similar results were obtained against EAC cells with $IC_{50}$ of ~9.5 µM and ~10.8 µM for compounds **8b** and **8f** respectively [Supplementary 1A & B].

The results prompted us to extend the studies in human cancer cells to revalidate the efficiency of compounds **8b** and **8f** and then cytotoxicity of compounds **8a-j** evaluated against Breast adenocarcinoma (MCF-7) and Lung adenocarcinoma (A549) cells (Table. 2). The study reveals that compounds **8b** and **8f** have potency to show anti-neoplastic property in MCF-7 cells with $IC_{50}$ of ~7.1 µM and ~9.3 µM respectively. Further, the compounds **8b** and **8f** were found to

inhibit A549 cell proliferation with $IC_{50}$ of ~10.1 µM and ~13.5µM respectively [Supplementary 1C & D], which almost parallel to cytotoxic effects of compounds **8b** and **8f** against MCF-7, DLA and EAC cells (Table. 1 & 2). The investigation clearly indicates that in the series of compounds **8a-j**, the compound **8b** with a methyl group at ortho position on B ring and bromo group at the ortho position of A ring in benzophenone and compound **8f** with two methyl groups at the para position of A and at the ortho position of the B ring of benzophenone showed noticeable cytotoxic effects against multiple cancer types such as DLA, EAC, MCF-7 and A549 cells which are evident from cytotoxic studies. Thus, compounds **8b** and **8f** emerged as lead compounds and further investigations were done in DLA and MCF-7 cells for the analysis of prolonged activity against tumour growth.

### *2.2.2. Structure Activity Relationship (SAR) of compounds **8b** and **8f***

Benzophenone and morpholine derivatives have drawn much attention during the past decades due to their wide range of pharmacological activities [22-33]. The extensive research focused on cancer drug development suggests that the drug bearing methyl and bromo groups have a potential pharmacological activity [23,25,26]. Based on the *in vitro* cytotoxic assays, compounds **8b** and **8f** showed the significant minimal inhibitory concentration ($IC_{50}$) against various murine (Table. 1) and human cancer cell lines (Table. 2). Structurally, methyl group at ortho position of B ring and the bromo group at ortho position of A ring in compound **8b** and two methyl groups at ortho position in B ring and at para position of A ring in compound **8f** has a central role in anti-mitogenicity or anti-proliferative effect. But surprisingly, the compounds which have methyl at ortho position of B ring in benzophenone **8a-f** showed the bioactivity at the level of significant to moderate activity where as chloro and flouro at ortho positions of B ring as in **8g-i** fails to achieve the anti-proliferative activity except compound **8j** in which a

methyl group is present at para position of A ring and moreover, compound **8f** having two methyl groups showed a considerable cytotoxicity and these results clearly explain the important of methyl group in benzophenone ring. Though compounds **8b-d** share the methyl universally, they have bromo at ortho, meta and para positions respectively, compound **8b** showed very good activity, whereas compound **8c** moderate and **8d** negligible tumour inhibitory activity against various neoplastic cells, and this firmly confirms that the position of bromo has an essential role in anti-proliferation efficacy. Finally, from the cytotoxic studies and structural activity relationship of compounds **8a-j**, it is clear that, methyl at ortho position of the B ring is fundamental for antiproliferative activity and bromo at the ortho position as in compound **8b** and methyl at the para position as in compound **8f** at the A ring are significant for extensive anti-proliferative activity.

*2.2.3. Compounds **8b** and **8f** exhibit the prolonged anti-neoplastic activity*

Clonogenic or colony formation assay is an appropriate method to investigate the long-term anti-mitogenicity of cytotoxic molecules on cancer cell proliferation. The reticence in colony formation considers as a prolonged cytotoxic effect of the active biomolecule [34]. In this analysis, DLA and MCF-7 cells treated with or without compounds **8b** and **8f** for analyzing long-term effect. Results revealed that compounds **8b** and **8f** visibly diminished the clonogenic efficiency of DLA and MCF-7 cells. Compounds **8b** and **8f** were found to inhibit the colony formation of DLA cells by 81.4% and 73.2%, respectively, and density of the colony formation is remarkably reduced by compounds **8b** and **8f** which are apparent from microscopic analysis of the colonies (Figure. 2A & B). Further, prolonged anti-mitogenic efficacy of compounds **8b** and **8f** reconfirmed against MCF-7 cells represents that about 90.3% and 77.8% of suppression in colony formation and density (Figure. 2C & D).

*2.2.4. The compound **8b** and **8f** induces the cell cycle arrest*

The induction of cell cycle arrest is a common mechanism for inhibition of cancer progression. To study the cell cycle events, DLA and MCF-7 cells were treated with or without compounds **8b** and **8f** and stained with propidium iodide. Results indicate that both compounds **8b** and **8f** have remarkably increased the cell cycle arrest on the G2/M phase in both murine lymphoma and human breast carcinoma cells, which is clear from cell cycle analysis (Figure. 3A-D). These results encouraged to study the physiological effect of compounds **8b** and **8f** on murine ascites tumour *in vivo*.

*2.2.5. Compounds **8b** and **8f** regresses ascites tumour through CAD mediated apoptosis*

To study the pathophysiological response of compounds **8b** and **8f**, *in vivo* murine ascites tumour model developed by culturing DLA cells in the peritoneum and administered with compound **8b** and **8f** at 50 mg/kg (b.w) i.p for three doses. Secreted ascites fluid in the peritoneum induces the establishment of tumour; therefore targeting formation of ascites is a key approach to regulate the ascites tumour development [22,28]. Results inferred that compounds **8b** and **8f** remarkably repressed the proliferation of tumour cells in mice, which is evident from tumour volume (Figure. 4A) and significantly reduced the ascites section with diminished cell density (Figure. 4B & C). The treatment of compounds extended the survivability of animals with >2.6 (**8b**) and 2.4 (**8f**) fold increase which is clear from survivability studies (Table. 3). Molecular events of compounds exhibited tumour regression was assessed by immunoblot, DNA fragmentation and Fluorescence-activated cell sorting analysis. Activation of Caspase-3 is a key biochemical event in apoptosis, which activates CAD, a cellular DNA degrading factor. Induction of activated CAD degrades chromosomal DNA into small DNA fragments which causes the apoptotic cell death [7, 8]. Compounds **8b** and **8f** induce the expression of active

CAD by activating caspase-3 which resulted in DNA fragmentation (Figure.5A & B), which consequently lead to apoptosis (Figure. 5C).

## 3. Conclusion

In summary, a series of morpholine conjugated benzophenone analogues **8a-j** were synthesized and evaluated for *in vitro* anti-proliferative activity against DLA, EAC, MCF-7 and A549 cells. From the current study, structural activity relationship of these compounds suggests that, in compound **8b** a methyl group at ortho position of B ring and the bromo group at ortho position of A ring is fundamental for antiproliferative activity. Also in compound **8f** with two methyl groups at the para position of A ring and another at ortho position of B ring are significantly exhibited extensive anti-mitogenic activity. Investigation on clonogenesis and Fluorescence-activated cell sorting suggests that compounds **8b** and **8f** have the potency to exhibit the prolonged activity with cell cycle arrest on G2/M phase against cancer progression. Further, compound **8b** and **8f** inhibits murine ascites lymphoma through CAD mediated apoptosis.

## 4. Materials and Methods

### *4.1. Chemistry*

Chemicals were procured from Sigma Aldrich Chemical Co. Reactions were monitored by thin layer chromatography (TLC) on silica gel 60 F254 aluminum sheets with visualization of components by UV light. Melting points were measured on a Thomas Hoover capillary melting point apparatus with a digital thermometer. Column chromatography was performed using silica gel (200–300 mesh) eluting with chloroform and methanol. IR spectra were recorded on FT-IR Shimadzu 8300 spectrophotometer, NMR spectra were recorded on a Bruker 400 MHz NMR spectrophotometer using TMS as an internal standard and DMSO-$d_6$ as solvent. The chemical

shift values (δ) are given in ppm relative to TMS as an internal reference. The mass spectra were obtained with a VG70-70H spectrophotometer and important fragments were given with the relative intensities in brackets. Elemental analysis was done by Perkin Elmer 2400 elemental analyzer. All the compounds gave C, H and N analysis within ±0.4% of the theoretical values.

*4.1.1. General procedure for the synthesis of phenyl benzoates (**3a-j**)*

A mixture of substituted phenols (**1a-j**, 0.20 mol) was dissolved in dichloromethane (DCM), triethylamine (TEA, 0.45 mol) was added and the reaction mixture was cooled to 0 °C. A solution of benzoyl chloride derivatives (**2a-j**, 0.21 mol) in DCM was added slowly to the above mixture and stirred for 3 h. Then the reaction mass was diluted with DCM (100 ml), washed with 10% sodium hydroxide solution (3×40 ml), followed by water (3×30 ml), The organic layer was dried over sodium sulfate and the solvent was evaporated to afford crude compounds **3a-d.** Finally, all the compounds were purified by recrystallized with methanol.

*4.1.1.1. Benzoic acid o-tolyl ester (**3a**)* Yield 83%; IR ($cm^{-1}$): 1715 (C=O); $^1$H NMR (DMSO-$d_6$): δ 2.45 (s, 3H, $CH_3$), 7.13-7.60 (m, 9H, Ar-H). LC-MS m/z 213 (M+1). Anal. Calcd. for $C_{14}H_{12}O_2$: C, 79.22; H, 5.70. Found: C, 79.18; H, 5.69%.

*4.1.1.2. 2-Bromo-benzoic acid o-tolyl ester (**3b**)* Yield 81%; IR ($cm^{-1}$): 1720 (C=O); $^1$H NMR (DMSO-$d_6$): δ 2.33 (s, 3H, $CH_3$), 6.72-7.70 (m, 8H, Ar-H). LC-MS m/z 290 ($M^+$) and 292 (M+2). Anal. calcd for $C_{14}H_{11}BrO_2$: C, 57.76; H, 3.81. Found: C, 57.72; H, 3.85%.

*4.1.1.3. 3-Bromo-benzoic acid o-tolyl ester (**3c**)* Yield 84%; IR ($cm^{-1}$): 1720 (C=O); $^1$H NMR (DMSO-$d_6$): δ 2.33 (s, 3H, $CH_3$), 6.72-7.94 (m, 8H, Ar-H). LC-MS m/z 290 ($M^+$) and 292 (M+2). Anal. calcd for $C_{14}H_{11}BrO_2$: C, 57.76; H, 3.81. Found: C, 57.72; H, 3.85%.

*4.1.1.4. 4-Bromo-benzoic acid o-tolyl ester (**3d**)* Yield 81%; IR ($cm^{-1}$): 1720 (C=O); $^1H$ NMR (DMSO-$d_6$): $\delta$ 2.33 (s, 3H, $CH_3$), 6.72-7.70 (m, 8H, Ar-H). LC-MS m/z 290 ($M^+$) and 292 (M+2). Anal. calcd for $C_{14}H_{11}BrO_2$: C, 57.76; H, 3.81. Found: C, 57.72; H, 3.85%.

*4.1.1.5. 2-Chloro-benzoic acid o-tolyl ester (**3e**)* Yield 80%; IR ($cm^{-1}$): 1718 (C=O); $^1H$ NMR (DMSO-$d_6$): δ 2.34 (s, 3H, $CH_3$), 6.72-7.30 (m, 8H, Ar-H). LC-MS m/z 246 ($M^+$) and 248 (M+2). Anal. calcd for $C_{14}H_{11}ClO_2$: C, 68.16; H, 4.49. Found: C, 68.22; H, 4.45%.

*4.1.1.6. 4-Methyl-benzoic acid o-tolyl ester (**3f**)* Yield 89%; IR ($cm^{-1}$): 1725 (C=O); $^1H$ NMR (DMSO-$d_6$): δ 2.35 (s, 6H, $CH_3$), 6.71-7.71 (m, 8H, Ar-H). LC-MS m/z 226 ($M^+$). Anal. Calcd. for $C_{15}H_{14}O_2$: C, 79.62; H, 6.24. Found: C, 79.60; H, 6.29%.

*4.1.1.7. 2-Chloro-6-fluorophenyl-4-fluorobenzoate (**3g**)* Yield 94%; m p 52-54°C; IR (KBr, $cm^{-1}$): 1738 (C=O); $^1H$ NMR (DMSO-d6): δ 7.42-8.28 (m, 7H, Ar-H); LC-MS m/z 267 ($M^+$) and 269 (M+2). Anal. Calcd. for $C_{13}H_7ClF_2O_2$: C, 58.12; H, 2.63. Found: C, 58.22; H, 2.43%.

*4.1.1.8. 2-Chloro-6-fluorophenyl-4-chlrorobenzoate (**3h**)* Yield 95%; m p 52-53 °C; IR (KBr, $cm^{-1}$): 1750 (C=O); $^1H$ NMR (DMSO-d6): δ 7.39- 8.17 (m, 7H, Ar-H); LC-MS m/z 285 ($M^+$), 287 (M+2) and 289 (M+4). Anal. Calcd. for $C_{13}H_7Cl_2FO_2$ (285): C, 54.77; H, 2.47. Found: C, 54.57; H, 2.33%.

*4.1.1.9. 2-Chloro-6-fluorophenyl-4-iodobenzoate (**3i**)* Yield 93%; m p 78-79 °C; IR (KBr, $cm^{-1}$): 1765 (C=O); $^1H$ NMR (DMSO-$d_6$): δ 7.39-8.06 (m, 7H, Ar-H); LC-MS m/z 375 ($M^+$), 377 (M+2). Anal. Calcd. for $C_{13}H_7ClFIO_2$: C, 41.47; H, 1.87. Found: C, 41.29; H, 1.68%.

*4.1.1.10. 2-Chloro-6-fluorophenyl-4-methylbenzoate (**3j**)* Yield: 96%; m p 62-63 °C; IR (KBr, $cm^{-1}$): 1780 (C=O); $^1H$ NMR (DMSO-$d_6$): δ 2.3 (s, 3H, $CH_3$), 7.38-8.07 (m, 7H, Ar-H);

LC-MS m/z 263 ($M^+$) and 265 (M+2). Anal. Calcd. for $C_{14}H_{10}ClFO_2$: C, 63.53; H, 3.81. Found: C, 63.69; H, 3.71%.

*4.1.2. General procedure for the synthesis of (4-hydroxy-phenyl)-phenyl methanones* ***(4a-j)***

Substituted 4-hydroxy benzophenones **4a-j** were synthesized by Fries rearrangement. Compounds **3a-j** (0.063 mol) and anhydrous aluminium chloride (0.126 mol) were blended and the mixture was heated to 150 °C and this temperature was maintained for 2 h. Then the reaction mixture was cooled to room temperature and quenched with 6N hydrochloric acid in the presence of ice water. The reaction mixture was stirred for about 2-3 h, filtered the solid and recrystallized with methanol to obtain desired compounds **4a-j**.

*4.1.2.1. (4-Hydroxy-3-methyl-phenyl)-phenyl-methanone* ***(4a)*** Yield 72%; mp 125-128 $^oC$; IR (KBr, $cm^{-1}$): 1640 (C=O), 3510-3600 (OH); $^1H$ NMR (DMSO-$d_6$): δ 2.35 (s, 3H, $CH_3$), 6.71-7.50 (m, 8H, Ar-H), 12.20 (bs, 1H, OH); LC-MS m/z 212 ($M^+$). Anal. Calcd for $C_{14}H_{12}O_2$: C, 79.22; H, 5.70. Found: C, 79.18; H, 5.69%.

*4.1.2.2. (2-Bromo-phenyl)-(4-hydroxy-3-methyl-phenyl)-methanone* ***(4b)*** Yield 80%; mp 150-152°C; IR (KBr, $cm^{-1}$): 1635 (C=O), 3515-3600 (OH); $^1H$ NMR (DMSO-$d_6$): δ 2.33 (s, 3H, $CH_3$), 6.72-7.70 (m, 7H, Ar-H), 12.10 (bs, 1H, OH); LC-MS m/z 290 ($M^+$) and 292 (M+2). Anal. Calcd. for $C_{14}H_{11}BrO_2$: C, 57.76; H, 3.81. Found: C, 57.72; H, 3.85%.

*4.1.2.3. (3-Bromo-phenyl)-(4-hydroxy-3-methyl-phenyl)-methanone* ***(4c)*** Yield 84%; mp 153-156$^oC$; IR (KBr, $cm^{-1}$): 1635 (C=O), 3515-3600 (OH); $^1H$ NMR (DMSO-$d_6$): δ 2.33 (s, 3H, $CH_3$), 6.72-7.70 (m, 7H, Ar-H), 12.10 (bs, 1H, OH); LC-MS m/z 290 ($M^+$) and 292 (M+2). Anal. Calcd. for $C_{14}H_{11}BrO_2$: C,57.76; H, 3.81; Found: C, 57.72; H, 3.85%.

*4.1.2.4. (4-Bromo-phenyl)-(4-hydroxy-3-methyl-phenyl)-methanone* ***(4d)*** Yield 80%; mp 150-152°C; IR (KBr, $cm^{-1}$): 1635 (C=O), 3515-3600 (OH); $^1H$ NMR (DMSO-$d_6$): δ 2.33 (s, 3H,

$CH_3$), 6.72-7.70 (m, 7H, Ar-H), 12.10 (bs, 1H, OH); LC-MS m/z 290 ($M^+$) and 292 (M+2). Anal. Calcd. for $C_{14}H_{11}BrO_2$: C,57.76; H, 3.81;. Found: C, 57.72; H, 3.85%.

*4.1.2.5. (2-Chloro-phenyl)-(4-hydroxy-3-methyl-phenyl)-methanone* ***(4e)*** Yield 80%; mp 158-160°C; IR (KBr, $cm^{-1}$): 1660 (C=O), 3515-3625 (OH); $^1H$ NMR (DMSO-$d_6$): δ 2.23 (s, 3H, $CH_3$), 6.96-7.65 (m, 7H, Ar-H), 12.10 (bs, 1H, OH); LC-MS m/z 246 ($M^+$) and 248 (M+2). Anal. Calcd. for $C_{14}H_{11}ClO_2$: C, 57.76; H, 3.81;. Found: C, 57.72; H, 3.85%.

*4.1.2.6. (4-Hydroxy-3-methyl-phenyl)-p-tolyl-methanone* ***(4f)*** Yield 78%; mp 155-156 $^oC$; IR (KBr, $cm^{-1}$): 1660 (C=O), 3520-3620 (OH); $^1H$ NMR (DMSO-$d_6$): δ 2.25 (s, 3H, $CH_3$), 2.35 (s, 3H, $CH_3$), 6.71-7.71 (m, 7H, Ar-H), 11.00 (bs, 1H, OH); LC-MS m/z 227 ($M^+$). Anal. Calcd for $C_{15}H_{14}O_2$: C, 79.62; H, 6.24. Found: C, 79.60; H, 6.29%.

*4.1.2.7. (3-Chloro-5-fluoro-4-hydroxyphenyl)4-fluorophenyl methanone* ***(4g)*** Yield 61%; mp 146-147 °C; IR (KBr, $cm^{-1}$): 1671 (C=O), 3545-3635 (OH); $^1H$ NMR (DMSO-$d_6$): δ 7.36-7.82 (m, 6H, Ar-H), 11.64 (bs, 1H, OH); LC-MS m/z 268 ($M^+$) and 270 (M+2). Anal. Calcd. for $C_{13}H_7ClF_2O_2$: C, 58.12; H, 2.63. Found: C, 58.21; H, 2.52%.

*4.1.2.8. (3-Chloro-5-fluoro-4-hydroxyphenyl)4-chlorophenyl methanone* ***(4h)*** Yield 68%; mp 167-169°C; IR: (KBr, $cm^{-1}$): 1660 (C=O), 3525-3625 (OH); $^1H$ NMR (DMSO-$d_6$): δ 7.52-7.94 (m, 6H, Ar-H), 11.60 (bs, 1H, OH); LC-MS m/z 285 ($M^+$), 287 (M+2) and 289 (M+4). Anal. Calcd. for $C_{13}H_7Cl_2FO_2$: C, 54.77; H, 2.47. Found: C, 54.65; H, 2.32%.

*4.1.2.9. (3-Chloro-5-fluoro-4-hydroxyphenyl)4-iodophenyl methanone* ***(4i)*** Yield 65%; mp 182-183°C; IR (KBr, $cm^{-1}$): 1635 (C=O), 3430-3590 (OH); $^1H$ NMR (DMSO-$d_6$): δ 7.46-7.80 (m, 6H, Ar-H), 11.60 (bs, 1H, OH); LC-MS m/z 376 ($M^+$) and 378 (M+2). Anal. Calcd. for $C_{13}H_7ClFIO_2$: C, 41.47; H, 1.87. Found: C, 41.32; H, 1.71%.

*4.1.2.10. (3-Chloro-5-fluoro-4-hydroxyphenyl)4-methylphenyl methanone (**4j**)* Yield 68%; mp 198-199°C; IR (KBr, $cm^{-1}$): 1640 (C=O), 3580-3685 (OH); $^1$H NMR (DMSO-$d_6$): δ 3.03 (s, 3H, $CH_3$), 7.21-7.62 (m, 6H, Ar-H), 11.52 (bs, 1H, OH). LC-MS m/z 264 ($M^+$) and 266 (M+2). Anal. Calcd. for $C_{14}H_{10}ClFO_2$: C, 63.53; H, 3.81. Found: C, 63.41; H, 3.72%.

*4.1.3. General procedure for the synthesis of (4-benzoyl-phenoxy)-acetic acid ethyl esters **(5a-j)***

To a solution of compounds **4a-j** (0.038 mol) in dry DMF (70 ml), potassium carbonate (0.076 mol) and ethyl chloroacetate (0.057 mol) were added and the reaction mass was heated to 60 °C for 6-8 h. The reaction mass was diluted with ethyl acetate (60 ml), potassium carbonate was filtered off and the bed was washed with ethyl acetate (40 ml). The organic layer was washed with water (3×30 ml), brine (2×40 ml), dried over sodium sulfate and concentrated to yield crude compounds **5a-j.** Finally, the crude compounds were purified by recrystallizition with ethanol to obtain desired compounds.

*4.1.3.1. Ethyl [2-benzoyl-4-methylphenoxy]acetate (**5a**)* Yield 83%, mp 61-63$^o$C; IR (KBr, $cm^{-1}$): 1664 (C=O), 1760 (ester, C=O); $^1$H NMR (DMSO-$d_6$): δ 1.26 (t, *J* = 7.0 Hz, 3H, $CH_3$ of ester), 2.34 (s, 3H, $CH_3$), 4.16 (q, *J* = 7.50 Hz, 2H, $CH_2$ of ester), 4.51 (s, 2H, $OCH_2$), 7.12-7.74 (m, 8H, Ar-H); LC-MS m/z 298 ($M^+$). Anal. Calcd. for $C_{18}H_{18}O_4$: C, 72.48; H, 6.04. Found: C, 72.46; H, 6.02%.

*4.1.3.2. Ethyl [2-(2-bromo benzoyl)-4-methylphenoxy]acetate (**5b**)* Yield 81%, mp 65-67$^o$C; IR (KBr, $cm^{-1}$): 1665 (C=O), 1730 (ester, C=O); $^1$H NMR (DMSO-$d_6$): δ 1.21 (t, *J* = 7.0 Hz, 3H, $CH_3$ of ester), 2.34 (s, 3H, $CH_3$), 4.22 (q, *J* = 7.50 Hz, 2H, $CH_2$ of ester), 4.46 (s, 2H, $OCH_2$), 7.21-7.64 (m, 7H, Ar-H); LC-MS: m/z 376 ($M^+$) and 378 (M+2). Anal. Calcd. for $C_{18}H_{17}BrO_4$: C, 57.29; H, 4.50. Found: C, 57.26; H, 4.53%.

*4.1.3.3. Ethyl [2-(3-bromo benzoyl)-4-methylphenoxy]acetate (**5c**)* Yield 80%, mp 55-57°C; IR (KBr, $cm^{-1}$): 1620 (C=O), 1725 (ester, C=O); $^1$H NMR (DMSO-$d_6$): δ 1.23 (t, *J* = 7.0 Hz, 3H, $CH_3$ of ester), 2.28 (s, 3H, $CH_3$), 4.35 (q, *J* = 7.50 Hz, 2H, $CH_2$ of ester), 4.51 (s, 2H, $OCH_2$), 7.08-7.66 (m, 7H, Ar-H); LC-MS: m/z 376 ($M^+$) and 378 (M+2). Anal. Calcd. for $C_{18}H_{17}BrO_4$: C, 57.29; H, 4.50. Found: C, 57.29; H, 4.59%.

*4.1.3.4. Ethyl [2-(4-bromo benzoyl)-4-methylphenoxy]acetate (**5d**)* Yield 86%, mp 59-61°C; IR (KBr, $cm^{-1}$): 1640 (C=O), 1735 (ester, C=O); $^1$H NMR (DMSO-$d_6$): δ 1.37 (t, *J* = 7.0 Hz, 3H, $CH_3$ of ester), 2.31 (s, 3H, $CH_3$), 4.25 (q, *J* = 7.50 Hz, 2H, $CH_2$ of ester), 4.45 (s, 2H, $OCH_2$), 6.96-7.40 (m, 7H, Ar-H); LC-MS: m/z 376 ($M^+$) and 378 (M+2). Anal. Calcd. for $C_{18}H_{17}BrO_4$: C, 57.29; H, 4.50. Found: C, 57.21; H, 4.42%.

*4.1.3.5. Ethyl [2-(2-chloro benzoyl)-4-methylphenoxy]acetate (**5e**)* Yield 84%; mp 52-54°C; (KBr, $cm^{-1}$): 1672 (C=O), 1737 (ester, C=O); $^1$H NMR (DMSO-$d_6$): δ 1.21 (t, *J* = 7.0 Hz, 3H, $CH_3$ of ester), 2.31 (s, 3H, $CH_3$), 4.21 (q, *J* = 7.50 Hz, 2H, $CH_2$ of ester), 4.46 (s, 2H, $CH_2$), 7.25-7.70 (m, 7H, Ar-H); LC-MS: m/z 332 ($M^+$) and 334 (M+2). Anal. Calcd. for $C_{18}H_{17}ClO_4$: C, 64.96; H, 5.11. Found: C, 64.99; H, 5.07%.

*4.1.3.6. Ethyl [2-(4-methyl benzoyl)-4-methylphenoxy]acetate (**5f**)* Yield 79%; mp 57-59°C; (KBr, $cm^{-1}$): 1665 (C=O), 1740 (ester, C=O); $^1$H NMR (DMSO-$d_6$): δ 1.22 (t, *J* = 7.0 Hz, 3H, $CH_3$ of ester), 2.35 (s, 6H, $2CH_3$), 4.25 (q, *J* = 7.50 Hz, 2H, $CH_2$ of ester), 4.45 (s, 2H, $CH_2$), 7.20-7.83 (m, 7H, Ar-H); LC-MS: m/z 311 ($M^+$). Anal. Calcd. for $C_{19}H_{20}O_4$: C, 73.07; H, 6.41. Found: C, 73.04; H, 6.38%.

*4.1.3.7. Ethyl [2-(4-fluorobenzoyl)-2-chloro-6-fluorophenoxy]acetate (**5g**)* Yield: 85%; IR (KBr, $cm^{-1}$): 1660 (C=O), 1730 (ester, C=O); $^1$H NMR (DMSO-$d_6$) δ: 1.22 (t, *J* = 7.0 Hz, 3H, $CH_3$ of ester), 4.21 (q, *J* = 7.50 Hz, 2H, $CH_2$ of ester), 5.03 (s, 2H, $CH_2$), 7.58-7.77 (m, 6H, Ar-H).

LC-MS: m/z: 354 ($M^+$) and 356 (M+2). Anal. Calcd. for $C_{17}H_{13}ClF_2O_4$: C, 57.56; H, 3.69. Found: C, 57.41; H, 3.52 %.

*4.1.3.8. Ethyl [2-(4-chlorobenzoyl)-2-chloro-6-fluorophenoxy]acetate (**5h**)* Yield: 83%; IR (KBr, $cm^{-1}$): 1650 (C=O), 1740 (ester, C=O); $^1H$ NMR (DMSO-$d_6$) δ: 1.21 (t, *J* = 7.0 Hz, 3H, $CH_3$ of ester), 4.19 (q, *J* = 7.50 Hz, 2H, $CH_2$ of ester), 5.02 (s, 2H, $CH_2$), 7.59-7.75 (m, 6H, Ar-H). LC-MS: m/z: 371 ($M^+$), 373 (M+2) and 375 (M+4). Anal. Calcd. for $C_{17}H_{13}Cl_2FO_4$: C, 55.01; H, 3.53. Found: C, 55.19; H, 3.41%.

*4.1.3.9. Ethyl [2-(4-iodobenzoyl)-2-chloro-6-fluorophenoxy]acetate (**5i**)* Yield: 80%; IR (KBr, $cm^{-1}$): 1605 (C=O), 1750 (ester, C=O); $^1H$ NMR (DMSO-$d_6$) δ: 1.22 (t, *J* = 7.0 Hz, 3H, $CH_3$ of ester), 4.21 (q, *J* = 7.50 Hz, 2H, $CH_2$ of ester), 5.06 (s, 2H, $CH_2$), 7.21-7.93 (m, 6H, Ar-H). LC-MS: m/z: 462 ($M^+$) and 464 (M+2). Anal. Calcd. for $C_{17}H_{13}ClFIO_4$: C, 44.13; H, 2.83. Found: C, 44.23; H, 2.72 %.

*4.1.3.10. Ethyl [2-(4-fluorobenzoyl)-2-chloro-6-fluorophenoxy]acetate (**5j**)* Yield: 86%; IR (KBr, $cm^{-1}$): 1610 (C=O), 1765 (ester, C=O); $^1H$ NMR (DMSO-$d_6$) δ: 1.23 (t, *J* = 7.0 Hz, 3H, $CH_3$ of ester), 2.47 (s, 3H, $CH_3$), 4.33 (q, *J* = 7.50 Hz, 2H, $CH_2$ of ester), 5.13 (s, 2H, $CH_2$), 7.16-7.55 (m, 6H, Ar-H). LC-MS: m/z: 350 ($M^+$) and 352 (M+2). Anal. Calcd. for $C_{18}H_{16}ClFO_4$: C, 61.63; H, 4.60. Found: C, 61.51; H, 4.52%.

*4.1.4. General procedure for the synthesis of (4-benzoyl-phenoxy)-acetic acid hydrazides **(6a-j)***

Hydrazine hydrate (0.018 mol) was added to a solution of compounds **5a-j** (0.018 mol) in ethanol (30 ml) and continuously stirred for 2-4 h at room temperature. A white solid was separated out, which was quenched with water (50 ml), filtered and washed with water (50 ml).

Finally, the solid was dried under vacuum and recrystallized with methanol to obtain compounds **6a-j**.

*4.1.4.1. 2-[2-Benzoyl-4-methylphenoxy]acetohydrazide (**6a**)* Yield 75%; mp 179-181$^{o}$C; IR (KBr, cm$^{-1}$): 1615 (C=O), 1650 (amide, C=O), 3105-3210 (NH-NH$_2$); $^{1}$H NMR (DMSO-d$_6$): δ 2.25 (s, 3H, CH$_3$),4.36 (bs, 2H, NH$_2$), 4.63 (s, 2H, CH$_2$), 6.96-7.68 (m, 8H, Ar-H), 9.30 (bs, 1H, NH); LC-MS m/z 285 (M$^{+}$). Anal. Calcd. for $C_{16}H_{16}N_2O_3$: C, 67.60; H, 5.63; N, 9.85. Found: C, 67.62; H, 5.65; N, 9.83%.

*4.1.4.2. 2-[2-(2-Bromobenzoyl)-4-methylphenoxy]acetohydrazide (**6b**)* Yield 72% mp 185-187$^{o}$C; IR: (KBr, cm$^{-1}$): 1625 (C=O), 1660 (amide, C=O), 3115-3220 (NH-NH$_2$); $^{1}$H NMR (DMSO-d$_6$): δ 2.32 (s, 3H, CH$_3$), 4.25 (bs, 2H, NH$_2$), 4.55 (s, 2H, CH$_2$), 7.06-7.55 (m, 7H, Ar-H), 9.22 (bs, 1H, NH); LC-MS m/z 363 (M$^{+}$) and 365 (M+2). Anal. Calcd. For $C_{16}H_{15}BrN_2O_3$: C, 52.89; H, 4.13; N, 7.71. Found: C, 52.87; H, 4.15; N, 7.73%.

*4.1.4.3. 2-[2-(3-Bromobenzoyl)-4-methylphenoxy]acetohydrazide (**6c**)* Yield 70%; mp 170-172$^{o}$C; IR: (KBr, cm$^{-1}$): 1640 (C=O), 1670 (amide, C=O), 3135-3245 (NH-NH$_2$); $^{1}$H NMR (DMSO-d$_6$): δ 2.34 (s, 3H, CH$_3$), 4.25 (bs, 2H, NH$_2$), 4.50 (s, 2H, CH$_2$), 6.94-7.51 (m, 7H, Ar-H), 9.24 (bs, 1H, NH); LC-MS m/z 362 (M$^{+}$) and 364 (M+2). Anal. Calcd. For $C_{16}H_{15}BrN_2O_3$: C, 52.89; H, 4.13; N, 7.71. Found: C, 52.83; H, 4.11; N, 7.71%.

*4.1.4.4. 2-[2-(4-Bromobenzoyl)-4-methylphenoxy]acetohydrazide (**6d**)* Yield 75%; mp 184-186$^{o}$C; IR: (KBr, cm$^{-1}$): 1650 (C=O), 1660 (amide, C=O), 3125-3235 (NH-NH$_2$); $^{1}$H NMR (DMSO-d$_6$): δ 2.25 (s, 3H, CH$_3$), 4.25 (bs, 2H, NH$_2$), 4.45 (s, 2H, CH$_2$), 6.95-7.6 (m, 7H, Ar-H), 9.22 (bs, 1H, NH); LC-MS m/z 362 (M$^{+}$) and 364 (M+2). Anal. Calcd. For $C_{16}H_{15}BrN_2O_3$: C, 52.89; H, 4.13; N, 7.71. Found: C, 52.80; H, 4.22; N, 7.68%.

*4.1.4.5. 2-[2-(2-Chlorobenzoyl)-4-methylphenoxy]acetohydrazide (**6e**)* Yield 75%; mp 167-169$^{o}$C; IR (KBr, cm$^{-1}$): 1622 (C=O), 1658 (amide, C=O), 3112-3218 (NH-NH$_2$); $^1$H NMR (DMSO-d$_6$): δ 2.31 (s, 3H, CH$_3$), 4.23 (bs, 2H, NH$_2$), 4.52 (s, 2H, CH$_2$), 7.13-7.65 (m, 7H, Ar-H), 9.36 (bs, 1H, NH); LC-MS m/z 319 (M$^+$) and 321 (M+2). Anal. Calcd. for $C_{16}H_{15}ClN_2O_3$: C, 60.28; H, 4.70; N, 8.79. Found: C, 60.24; H, 4.74; N, 8.76%.

*4.1.4.6. 2-[2-(4-Methylbenzoyl)-4-methylphenoxy]acetohydrazide (**6f**)* Yield 71%; mp 186-188$^{o}$C; IR: (KBr, cm$^{-1}$): 1630 (C=O), 1670 (amide, C=O), 3120-3220 (NH-NH$_2$); $^1$H NMR (DMSO-d$_6$): δ 2.23 (s, 6H, 2CH$_3$), 4.24 (bs, 2H, NH$_2$), 4.61 (s, 2H, CH$_2$), 7.24-7.88 (m, 7H, Ar-H), 9.35 (bs, 1H, NH); LC-MS m/z 299 (M$^+$). Anal. Calcd. for $C_{17}H_{18}N_2O_3$: C, 68.45; H, 6.04; N, 9.39. Found: C, 68.41; H, 6.0; N, 9.35%.

*4.1.4.7. 2-[4-(4-Fluorobenzoyl)-2-chloro-6-fluorophenoxy]acethydrazide (**6g**)* Yield 76%; mp 107-109 °C; IR (KBr, cm$^{-1}$): 1610 (C=O), 1645 (amide, C=O), 3100-3205 (NH-NH$_2$); $^1$H NMR (DMSO-d$_6$): δ 4.35 (bs, 2H, NH$_2$), 4.69 (s, 2H, CH$_2$), 7.37-7.86 (m, 6H, Ar-H), 9.32 (bs, 1H, NH); LC-MS m/z 342 (M$^+$) and 344 (M+2). Anal. Calcd. for $C_{15}H_{11}ClF_2N_2O_3$: C, 52.88; H, 3.25; N, 8.22. Found: C, 52.75; H, 3.38; N, 8.11%.

*4.1.4.8. 2-[4-(4-Chlorobenzoyl)-2-chloro-6-fluorophenoxy]acethydrazide (**6h**)* Yield: 72%; mp 141-142 °C; IR (KBr, cm$^{-1}$): 1625 (C=O), 1655 (amide, C=O), 3150-3255 (NH-NH$_2$); $^1$H NMR (DMSO-d$_6$): δ 4.25 (bs, 2H, NH$_2$), 4.70 (s, 2H, CH$_2$), 7.16-7.75 (m, 6H, Ar-H), 9.30 (bs, 1H, NH); LC-MS m/z 357 (M$^+$), 359 (M+2) and 361 (M+4). Anal. Calcd. for $C_{15}H_{11}Cl_2FN_2O_3$: C, 50.44; H, 3.10; N, 7.84. Found: C, 50.31; H, 3.22; N, 7.72%.

*4.1.4.9. 2-[4-(4-Iodobenzoyl)-2-chloro-6-fluorophenoxy]acethydrazide (**6i**)* Yield: 74%; mp 120-123°C; IR (KBr, cm$^{-1}$): 1615 (C=O), 1635 (amide, C=O), 3120-3250 (NH-NH$_2$); $^1$H NMR (DMSO-d$_6$): δ 4.38 (bs, 2H, NH$_2$), 4.72 (s, 2H, CH$_2$), 7.15-7.55 (m, 6H, Ar-H), 9.32 (bs, 1H,

NH); LC-MS m/z 448 ($M^+$) and 450 (M+2). Anal. Calcd. for $C_{15}H_{11}ClFIN_2O_3$: C, 40.16; H, 2.47; N, 6.24. Found: C, 40.03; H, 2.33; N, 6.14%.

*4.1.4.10. 2-[4-(4-Methylbenzoyl)-2-chloro-6-fluorophenoxy]acethydrazide (**6j**)* Yield: 78%; mp 78-79 °C; IR (KBr, $cm^{-1}$): 1605 (C=O), 1620 (amide, C=O), 3135-3270 (NH-$NH_2$); $^1$H NMR (DMSO-$d_6$): δ 2.40 (s, 3H, $CH_3$), 4.37 (bs, 2H, $NH_2$), 4.69 (s, 2H, $CH_2$), 7.05-7.69 (m, 6H, Ar-H), 9.31 (bs, 1H, NH); LC-MS m/z 336 ($M^+$) and 338 (M+2). Anal. Calcd. for $C_{16}H_{14}ClFN_2O_3$: C, 57.07; H, 4.19; N, 8.32. Found: C, 57.17; H, 4.11; N, 8.47%.

*4.1.5. General procedure for the synthesis of 4-Benzyl-morpholine-2-carboxylic acid N'-[2-(4-benzoyl-phenoxy)-acetyl]-hydrazides (**8a-j**)*

4-Benzyl-morpholine-2-carboxylic acid **7** (0.001 mol) was dissolved in DCM (15 ml), EDCI (0.0012 mol), HOBt (0.001 mol) and triethylamine (TEA) (0.002 mol) were added to it and the reaction mass stirred at 25°C for 10 min. Compounds **6a-j** (0.001 mol) were added and stirred the reaction mixture for 4-7 h at 25°C. The progress of the reaction mass was checked by TLC. The reaction mass was diluted with DCM (20 ml), quenched with 10% sodium bicarbonate solution (30×3) and separated the layers. The organic layer was washed with water, brine, dried over anhydrous sodium sulphate, filtered and the solvent was evaporated under vacuum. The pale brown gummy mass was purified by column chromatography over silica gel to give the compound **8a-j**.

*4.1.5.1. 4-Benzyl-morpholine-2-carboxylic acid N'-[2-(4-benzoyl-2-methyl-phenoxy)-acetyl]-hydrazide (**8a**)* Yield 68%; IR ($cm^{-1}$): 1648 (C=O), 1669 (amide, C=O), 3245-3347 (NH-NH); $^1$H NMR (DMSO-$d_6$): δ 2.24 (s, 3H, $CH_3$), 2.64 (t, *J* = 8.0 Hz, 4H, $NCH_2$ of morpholine ring), 3.65 (s, 2H, $NCH_2$), 3.65 (t, *J* = 7.80 Hz, 1H, CH of morpholine ring), 4.17 (t, *J* = 7.80 Hz, 2H, $OCH_2$ of morpholine ring), 4.92 (s, 2H, $OCH_2$), 6.95-7.59 (m, 13H, Ar-H), 10.14 (bs, 1H, NH),

10.37 (bs, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): δ 14.23, 52.46, 54.66, 55.15, 63.06, 66.49, 70.55, 71.78, 75.32, 77.15, 127.46, 127.88, 128.21, 129.16, 129.65, 133.57, 134.48, 136.59, 145.26, 153.19, 155.75, 163.43, 166.12, 192.83; LC-MS m/z 589 ($M^+$). Anal. Calcd. for $C_{28}H_{29}N_3O_5$: C, 68.98; H, 6.00; N, 8.62, Found: C, 68.91; H, 6.06; N, 8.71%.

*4.1.5.2. 4-Benzyl-morpholine-2-carboxylic acid N'-{2-[4-(2-bromo-benzoyl)-2-methyl-phenoxy]-acetyl}-hydrazide (**8b**)* Yield 59%; IR ($cm^{-1}$): 1655 (C=O), 1673 (amide, C=O), 3261-3351 (NH-NH); $^1$H NMR (DMSO-$d_6$): δ 2.23 (s, 3H, $CH_3$), 2.60 (t, *J* = 8.0 Hz, 4H, $NCH_2$ of morpholine ring), 3.64 (s, 2H, $NCH_2$), 3.64 (t, *J* = 7.80 Hz, 1H, CH of morpholine ring), 4.08 (t, *J* = 7.80 Hz, 2H, $OCH_2$ of morpholine ring), 4.93 (s, 2H, $OCH_2$), 6.93-7.77 (m, 12H, Ar-H), 10.11(bs, 1H, NH), 10.35 (bs, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): δ 14.17, 52.22, 54.66, 66.64, 71.54, 71.69, 75.05, 77.39, 123.61, 126.23, 127.46, 127.68, 128.53, 129.55, 130.27, 131.61, 134.53, 136.70, 145.11, 152.80, 155.44, 163.13, 166.25, 187.45; LC-MS m/z 565 ($M^+$) and 567 (M+2). Anal. Calcd. for $C_{28}H_{28}BrN_3O_5$: C, 59.37; H, 4.98; N, 7.42, Found: C, 59.48; H, 4.97; N, 7.35%.

*4.1.5.3. 4-Benzyl-morpholine-2-carboxylic acid N'-{2-[4-(3-bromo-benzoyl)-2-methyl-phenoxy]-acetyl}-hydrazide (**8c**)* Yield 63%; IR ($cm^{-1}$): 1652 (C=O), 1673 (amide, C=O), 3268-3358 (NH-NH); $^1$H NMR (DMSO-$d_6$): δ 2.24 (s, 3H, $CH_3$), 2.63 (t, *J* = 8.0 Hz, 4H, $NCH_2$ of morpholine ring), 3.64 (s, 2H, $NCH_2$), 3.64 (t, *J* = 7.80 Hz, 1H, CH of morpholine ring), 4.11 (t, *J* = 7.80 Hz, 2H, $OCH_2$ of morpholine ring), 4.89 (s, 2H, $OCH_2$), 6.96-7.98 (m, 12H, Ar-H), 10.14 (bs, 1H, NH), 10.36 (bs, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): δ 14.17, 51.10, 54.49, 62.92, 71.63, 77.33, 119.33, 123.34, 126.73, 127.50, 127.98, 128.89, 129.05, 130.41, 134.64, 136.86, 137.98, 145.57, 152.69, 155.64, 163.75, 166.46, 183.56, 189.34; LC-MS m/z 565 ($M^+$) and 567

(M+2). Anal. Calcd. for $C_{28}H_{28}BrN_3O_5$: C, 59.37; H, 4.98; N, 7.42, Found: C, 59.30; H, 5.17; N, 7.34%.

*4.1.5.4. 4-Benzyl-morpholine-2-carboxylic acid N'-{2-[4-(4-bromo-benzoyl)-2-methyl-phenoxy]-acetyl}-hydrazide (**8d**)* Yield 65%; IR ($cm^{-1}$): 1655 (C=O), 1675 (amide, C=O), 3266-3357 (NH-NH); $^1$H NMR (DMSO-$d_6$): δ 2.24 (s, 3H, $CH_3$), 2.64 (t, *J* = 8.0 Hz, 4H, $NCH_2$ of morpholine ring), 3.66 (s, 2H, $NCH_2$), 3.66 (t, *J* = 7.80 Hz, 1H, CH of morpholine ring), 4.16 (t, *J* = 7.80 Hz, 2H, $OCH_2$ of morpholine ring), 4.90 (s, 2H, $OCH_2$), 6.97-7.77 (m, 12H, Ar-H), 10.15 (bs, 1H, NH), 10.36 (bs, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): δ 14.05, 52.24, 54.53, 66.60, 75.05, 77.14, 118.34, 126.23, 127.22, 127.75, 128.35, 128.76, 129.55, 130.40, 131.87, 134.12, 135.99, 137.86, 145.06, 153.33, 154.08, 163.75, 166.58, 188.33; LC-MS m/z 565 ($M^+$) and 567 (M+2). Anal. Calcd. for $C_{28}H_{28}BrN_3O_5$: C, 59.37; H, 4.98; N, 7.42, Found: C, 59.47; H, 5.04; N, 7.31%.

*4.1.5.5. 4-Benzyl-morpholine-2-carboxylic acid N'-{2-[4-(2-chloro-benzoyl)-2-methyl-phenoxy]-acetyl}-hydrazide (**8e**)* Yield 67%; IR ($cm^{-1}$): 1645 (C=O), 1666 (amide, C=O), 3250-3340 (NH-NH); $^1$H NMR (DMSO-$d_6$): δ 2.22 (s, 3H, $CH_3$), 2.63 (t, *J* = 8.0 Hz, 4H, $NCH_2$ of morpholine ring), 3.64 (s, 2H, $NCH_2$), 3.64 (t, *J* = 7.80 Hz, 1H, CH of morpholine ring), 4.08 (t, *J* = 7.80 Hz, 2H, $OCH_2$ of morpholine ring), 4.94 (s, 2H, $OCH_2$), 6.94-7.72 (m, 12H, Ar-H), 10.13 (bs, 1H, NH), 10.28 (bs, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): δ 13.78, 52.39, 54.40, 63.18, 66.34, 71.77, 75.43, 77.30, 126.28, 127.50, 127.59, 128.68, 128.76, 129.59, 134.53, 136.56, 137.78, 138.88, 145.06, 153.09, 155.67, 163.80, 168.26, 190.51; LC-MS m/z 521 ($M^+$) and 523 (M+2). Anal. Calcd. for $C_{28}H_{28}ClN_3O_5$: C, 64.43; H, 5.41; N, 8.05, Found: C, 64.32; H, 5.37; N, 7.88%.

*4.1.5.6. 4-Benzyl-morpholine-2-carboxylic acid N'-{2-[2-methyl-4-(4-methyl-benzoyl)-phenoxy]-acetyl}-hydrazide (**8f**)* Yield 56%; IR ($cm^{-1}$): 1645 (C=O), 1664 (amide, C=O), 3252-3343 (NH-NH); $^1$H NMR (DMSO-$d_6$): δ 2.18 (s, 3H, $CH_3$), 2.25 (s, 3H, $CH_3$), 2.87 (t, *J* = 8.0 Hz, 4H, $NCH_2$ of morpholine ring), 3.45 (t, *J* = 7.80 Hz, 1H, CH of morpholine ring), 3.59 (t, 2H, $NCH_2$), 3.90 (t, *J* = 7.80 Hz, 2H, $OCH_2$ of morpholine ring), 4.72 (s, 2H, $OCH_2$), 6.99-7.61 (m, 12H, Ar-H), 9.77 (bs, 1H, NH), 10.02 (bs, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): δ 14.13, 21.63, 52.20, 54.66, 54.85, 62.92, 66.60, 71.63, 71.69, 75.05, 77.33, 127.46, 127.59, 128.35, 129.46, 130.19, 133.65, 134.53, 136.78, 145.06, 153.18, 155.67, 163.75, 166.44, 192.60; LC-MS m/z 502 ($M^+$). Anal. Calcd. for $C_{29}H_{31}N_3O_5$: C, 69.44; H, 6.23; N, 8.38, Found: C, 69.31; H, 6.29; N, 8.40 %.

*4.1.5.7. 4-Benzyl-morpholine-2-carboxylic acid N'-{2-[2-chloro-6-fluoro-4-(4-fluoro-benzoyl)-phenoxy]-acetyl}-hydrazide (**8g**)* Yield 63%; IR ($cm^{-1}$): 1655 (C=O), 1676 (amide, C=O), 3268-3353 (NH-NH); $^1$H NMR (DMSO-$d_6$): δ 2.70 (t, 4H, *J* = 8.0 Hz, $NCH_2$ of morpholine ring), 3.62 (s, 2H, $NCH_2$), 3.62 (t, *J* = 7.80 Hz, 1H, CH of morpholine ring), 4.12 (t, *J* = 7.80 Hz, 2H, $OCH_2$ of morpholine ring), 5.16 (s, 2H, $OCH_2$), 7.05-7.55 (m, 11H, Ar-H), 10.67 (bs, 1H, NH), 10.91 (bs, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): δ 52.48, 54.47, 55.01, 62.75, 66.68, 71.69, 75.05, 77.54, 122.12, 126.05, 127.40, 128.55, 130.27, 131.39, 133.65, 134.50, 136.78, 144.91, 153.70, 155.56, 164.25, 166.44, 191.68; LC-MS m/z 543 ($M^+$) and 545 (M+2). Anal. Calcd. for $C_{27}H_{24}ClF_2N_3O_5$: C, 59.62; H, 4.45; N, 7.73, Found: C, 59.76; H, 4.45; N, 7.62%.

*4.1.5.8. 4-Benzyl-morpholine-2-carboxylic acid N'-{2-[2-chloro-4-(4-chloro-benzoyl)-6-fluoro-phenoxy]-acetyl}-hydrazide (**8h**)* Yield 67%; IR ($cm^{-1}$): 1650 (C=O), 1673 (amide, C=O), 3275-3372 (NH-NH); $^1$H NMR (DMSO-$d_6$): δ 2.68 (t, 4H, *J* = 8.0 Hz, $NCH_2$ of morpholine ring), 3.57 (s, 2H, $NCH_2$), 3.57 (t, *J* = 7.80 Hz, 1H, CH of morpholine ring), 4.09 (t, *J* = 7.80 Hz, 2H, $OCH_2$ of morpholine ring), 5.20 (s, 2H, $OCH_2$), 7.25-7.99 (m, 11H, Ar-H), 10.65 (bs, 1H,

NH), 10.91(bs, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): δ 51.56, 54.30, 54.85, 62.92, 66.60, 71.56, 75.18, 77.33, 127.40, 128.16, 128.35, 129.48, 130.17, 131.64, 133.65, 134.23, 136.58, 145.36, 153.65, 155.67, 163.79, 166.50, 191.60; LC-MS m/z 559 ($M^+$), 561 (M+2) and 563 (M+4). Anal. Calcd. for $C_{27}H_{24}Cl_2FN_3O_5$: C, 57.87; H, 4.32; N, 7.50, Found: C, 57.81; H, 4.26; N, 7.11%.

*4.1.5.9. 4-Benzyl-morpholine-2-carboxylic acid N'-{2-[2-chloro-6-fluoro-4-(4-iodo-benzoyl)-phenoxy]-acetyl}-hydrazide (**8i**)* Yield 65%; IR ($cm^{-1}$): 1655 (C=O), 1675 (amide, C=O), 3280-3365 (NH-NH); $^1$H NMR (DMSO-$d_6$): δ 2.69 (t, 4H, *J* = 8.0 Hz, $NCH_2$ of morpholine ring), 3.62 (s, 2H, $NCH_2$), 3.62 (t, *J* = 7.80 Hz, 1H, CH of morpholine ring), 4.04 (t, *J* = 7.80 Hz, 2H, $OCH_2$ of morpholine ring), 5.20 (s, 2H, $OCH_2$), 7.25-7.99 (m, 11H, Ar-H), 10.65 (bs, 1H, NH), 10.91 (bs, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): δ 52.16, 54.66, 54.45, 63.25, 66.68, 71.63, 74.80, 77.30, 105.51, 127.46, 128.56, 128.27, 129.45, 130.13, 133.65, 134.48, 136.93, 145.12, 153.18, 155.59, 163.75, 166.53, 191.87; LC-MS m/z 652 ($M^+$) and 654 (M+2). Anal. Calcd. for $C_{27}H_{24}ClFIN_3O_5$: C, 49.75; H, 3.71; N, 6.45, Found: C, 49.63; H, 3.75; N, 6.54%.

*4.1.5.10. 4-Benzyl-morpholine-2-carboxylic acid N'-{2-[2-chloro-6-fluoro-4-(4-methyl-benzoyl)-phenoxy]-acetyl}-hydrazide (**8j**)* Yield 62%; IR ($cm^{-1}$): 1650 (C=O), 1678 (amide, C=O), 3275-3360 (NH-NH); $^1$H NMR (DMSO-$d_6$): δ 2.23 (s, 3H, $CH_3$), 2.68 (t, *J* = 8.0 Hz, 4H, $NCH_2$ of morpholine ring), 3.64 (s, 2H, $NCH_2$), 3.64 (t, *J* = 7.80 Hz, 1H, CH of morpholine ring), 4.10 (t, *J* = 7.80 Hz, 2H, $OCH_2$ of morpholine ring), 5.11 (s, 2H, $OCH_2$), 7.16-7.85 (m, 11H, Ar-H), 10.59 (bs, 1H, NH), 10.89 (bs, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): δ 22.16, 52.61, 54.60, 54.85, 62.77, 67.25, 71.40, 75.37, 77.39, 107.49, 127.73, 128.66, 128.30, 129.27, 130.59, 133.65, 134.67, 136.78, 145.25, 153.36, 155.65, 163.85, 166.71, 191.88; LC-MS m/z 541 ($M^+$) and 543

(M+2). Anal. Calcd. for $C_{28}H_{27}ClFN_3O_5$: C, 62.28; H, 5.04; N, 7.78, Found: C, 62.37; H, 5.01; N, 7.69%.

*4.2. Pharmacology*

Various types of tumour cells of different origin, such as DLA and EAC cells (murine) and MCF-7 and A549 cells (human) were used for determining the $IC_{50}$ value of newly synthesized series **8a-j** by MTT, LDH leak and trypan blue assays. Extended anti-mitogenic efficacies of the lead compounds were evaluated by colony formation assay and Fluorescence-activated cell sorting. *In vivo* anti-tumour effect was investigated in the murine ascites tumour model.

*4.2.1. Cell Culture and in vitro treatment*

The DLA, EAC, MCF-7 and A549 cells were grown in DMEM medium (Gibco-Invitrogen, USA), supplemented with 10% FBS (In vitrogen, USA), 100 μg/ml of Antibiotic-antimicotic solution (Sigma-Aldrich, USA) and sodium carbonate (0.37%) in a humidified carbon dioxide ($CO_2$) incubator at 37$^o$C with 5% $CO_2$. The cells were treated with six different concentrations of compounds **8a-j** (0, 5, 10, 25, 50 and 100 μM in DMSO) and reincubated at 37$^o$C for 45 h. MTT assay, LDH leak assay, and trypan blue dye exclusion assays were performed as reported earlier for cytotoxicity analysis [22].

*4.2.2. Colony formation assay*

The colony formation assay has been the gold standard for determining the prolonged anti-mitogenic effects of cytotoxic compounds on cancer cell proliferation *in vitro* and it was performed as described earlier for compounds **8b** and **8f** with minor modification against DLA and MCF-7 [34]. The cells were cultured and exposed with or without compounds **8b** and **8f** at 10 μM concentrations for 2 h. After being rinsed with fresh medium, cells were allowed to grow for 14 days to form colonies, which were then fixed with methanol and stained with crystal violet

(0.4 g/L) and then the colonies were counted in Olympus inverted microscope and a portion of the colonies were photographed. Colony formation inhibition was used to elucidate the long-term effects of compounds **8b** and **8f** on murine lymphoma (DLA) and human breast cells (MCF-7).

*4.2.3. Cell cycle analysis*

Compound induced cell cycle arrest was studied by cell cycle analysis as described earlier [35]. In brief, DLA and MCF-7 cells were cultured *in vitro* and after 24 h, the cells were treated with or without 10 μM of compounds **8b** and **8f** for 48 h. The harvested cells were washed with PBS, fixed with 70% ethanol and RNase-A treatment was given overnight, stained with propidium iodide stain, finally analyzed in a BD Fluorescence-activated cell sorting Verse™ flow cytometer. A minimum of ~10,000 cells was acquired per sample and histograms were plotted and analyzed using WinMDI version 2.9 software.

*4.2.4. Animal Ethics and Determination of $LD_{50}$ value*

Swiss female albino mice weighing between 28-30 g were used throughout the study. All procedures described were reviewed and approved by the National College of Pharmacy Ethical Committee, Shimoga, India, in accordance with the CPCSEA guidelines for laboratory animal facility (NCP/IAEC/CL/101/05/2012-13). $LD_{50}$ of the compounds **8b** and **8f** were evaluated as described earlier and their adverse effects were studied by injecting 50 mg/kg body weight, intraperitoneally (i.p) to healthy Swiss albino mice continuously for 10 days [24].

*4.2.5. Animal tumour models and treatment*

DLA tumours were maintained as ascites by intraperitoneal serial transplantation in mice [24]. A DLA tumour model was developed by injecting $5x10^6$ cells/ mouse i.p. and grouped into three (n = 6). After the $4^{th}$ day of tumour development, the mice bearing DLA were administered with or without compounds **8b** and **8e** (50 mg/kg body weight i.p) for 3 doses on

every alternate day as per $LD_{50}$ studies. On day 10, mice were euthanized, tumour parameters such as tumour volume, ascites secretion, cell density were evaluated and also survivability of tumour bearing mice (n = 10) were monitored. DNAs were isolated from cells of control and treated groups for determining nuclear fragmentation. Fluorescence-activated cell sorting analysis was performed to quantify the compounds **8b** and **8f** induced apoptosis after staining with propidium iodide [27, 35].

*4.2.6. Immunoblots*

The whole cell lysates of plus or minus compounds **8b** and **8e** *in vivo* treated DLA cells were prepared by RIPA buffer with PMSF and Protease inhibitor cocktail and their concentration was determined by using biospectrophotometer. Equal concentrations of lysates were resolved 12% SDS-PAGE and transferred to nylon membrane. Immunoblot analysis was carried out for active CAD (Santa Cruz), cleaved caspase-3 and β-actin (BD Bioscience) as mentioned earlier [35].

*4.2.7. Statistical analysis*

Values were expressed as mean ± standard error (SEM). Statistical significance (5%) was evaluated by one-way analysis of variance (ANOVA) followed by Student's t-test. Statistical significant values were expressed as $p < 0.05$ and $p < 0.01$.

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**Table 1. $IC_{50}$ values of compounds 8a-j calculated based upon MTT, LDH leak and Trypan blue assays in DLA, EAC cells.**

| Compounds | Cancer cells from murine origin | | | | | |
|---|---|---|---|---|---|---|
| | $IC_{50}$ value (µM) against DLA cells | | | $IC_{50}$ value (µM) against EAC cells | | |
| | MTT assay | LDH leak assay | Trypan blue assay | MTT assay | LDH leak assay | Trypan blue assay |
| **Control** | -------- | -------- | -------- | -------- | -------- | -------- |
| **8a** | 66.7±1.3 | 68.4±2.1 | 61.2±1.0 | 63.5±1.4 | 72.0±1.9 | 63.4±2.0 |
| **8b** | **7.0±1.0*** | **8.1±1.5** | **7.4±.1.2** | **9.5±1.1*** | **10.1±1.3** | **9.0±1.4** |
| **8c** | 47.3±3.4 | 52.0±2.4 | 49.4±3.0 | 48.6±2.4 | 57.0±2.1 | 51.6±3.2 |
| **8d** | 78.5±2.4 | 78.7±1.9 | 71.1±2.8 | 69.1±3.2 | 76.4±2.3 | 59.8±2.1 |
| **8e** | 67±3.8 | 70.0±3.2 | 62.6±4.3 | 64.3±2.8 | 70.8±3.0 | 64.4±1.9 |
| **8f** | **9.5±1.4** | **11.2±1.2** | **10.3±1.0*** | **10.2±2.1** | **11.6±1.3** | **10.6±1.6** |
| **8g** | >100 | >100 | >100 | 91.9±3.2 | 88.5±4.3 | 89.8±2.9 |
| **8h** | 91.1±3.8 | 87.3±4.1 | 89.0±1.7 | 95.5±1.8 | 92.2±2.9 | 95.3±2.1 |
| **8i** | >100 | >100 | 98.8±3.2 | >100 | >100 | 95.7±2.1 |
| **8j** | 48.4±3.5 | 58.1±2.7 | 51.4±3.0 | 47.3±2.6 | 54.6±2.4 | 46.2±2.9 |
| **5-FU** | 12.0±1.3 | 13.5±2.1 | 10.7±1.7 | 11.8±2.1 | 12.1±1.2 | 11.3±1.0 |

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

**Table 2. $IC_{50}$ values of compounds 8a-j calculated based upon MTT, LDH leak and Trypan blue assays in MCF-7 and A549 cells.**

| Compounds | Cancer cells from human origin | | | | | |
|---|---|---|---|---|---|---|
| | $IC_{50}$ value (µM) against MCF-7 cells | | | $IC_{50}$ value (µM) against A549 cells | | |
| | MTT assay | LDH leak assay | Trypan blue assay | MTT assay | LDH leak assay | Trypan blue assay |
| **Control** | -------- | -------- | -------- | -------- | -------- | -------- |
| **8a** | 48.5±2.0 | 46.7±1.8 | 48.9±1.4 | 53.4±3.0 | 62.1±2.6 | 54.7±3.2 |
| **8b** | **7.1±0.8**$^{**}$ | **7.3±1.2** | **7.0±0.7**$^{**}$ | **10.1±0.6**$^{**}$ | **11.2±0.9**$^{*}$ | **9.1±1.0**$^{*}$ |
| **8c** | 47.7±1.2 | 54.2±1.5 | 45.6±2.3 | 56.4±2.5 | 58.3±2.1 | 52.9±3.4 |
| **8d** | 75.2±1.5 | 76.3±1.8 | 70.8±1.6 | 76.8±2.2 | 79.6±3.2 | 76.8±1.9 |
| **8e** | 57.0±3.2 | 63.2±3.8 | 57.7±1.2 | 63.3±2.8 | 73.8±3.3 | 64.7±1.7 |
| **8f** | **9.1±0.8**$^{**}$ | **10.3±1.2** | **8.6±1.8** | **13.1±1.1**$^{*}$ | **13.8±1.3** | **13.7±1.2** |
| **8g** | >100 | >100 | 95.8±3.8 | >100 | >100 | >100 |
| **8h** | 87.6±3.1 | 93.7±4.2 | 79.9±3.2 | 89.0±3.2 | 94.5±2.1 | 90/4±1.0 |
| **8i** | >100 | 90.2±1.8 | 87.8±2.4 | >100 | >100 | 92.4±3.2 |
| **8j** | 44.8±3.2 | 49.6±2.8 | 45.2±1.6 | 57.8±3.1 | 63.5±1.2 | 50.7±2.3 |
| **5-FU** | 14.5±1.1 | 14.6±1.3 | 13.1±2.0 | 13.3±1.4 | 14.1±1.2 | 12.3±2.2 |

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

**Table 3. Compound 8b and 8f prolonged the survivability of mice bearing Dalton's ascites tumour.**

| Days | 2 | 4 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 26 | 28 | 30 | 32 | 34 | 36 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Control Mice (n)** | 10 | 10 | 10 | 8 | 0 | - | - | - | - | - | - | - | - | - | - | - | - |
| **8b treated mice (n)** | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 8 | 6 | 5 | 3 | 2 |
| **8f treated mice (n)** | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 8 | 7 | 7 | 6 | 5 | 3 | 0 |

n indicates the number of animals survived on the respective days

**Figure captions**

**Fig. 1.** Structure of 4-benzyl-morpholine-2-carboxylic acid N'-[2-(4-benzoyl-phenoxy)-acetyl]-hydrazide

**Fig. 2. Compounds 8b and 8f exhibits the prolonged anti -mitogenicity against DLA cells and MCF-7 cells.** DLA and MCF-7 cells were cultured and treated with or without compound **8b** and **8f** for 2 h, and incubated for 14 days to evaluate the anti-clonogenic effect. **A)** Inhibition of clonogenesis of DLA cells and microscopic view represents the decrease in colony numbers compared to control. **B)** Percentage of DLA colony formation inhibition. **C)** Suppression of clonogenesis of MCF-7 cells and microscopic view represents the decrease in colony density compared to control. **D)** Percentage of MCF-7 colony formation inhibition.

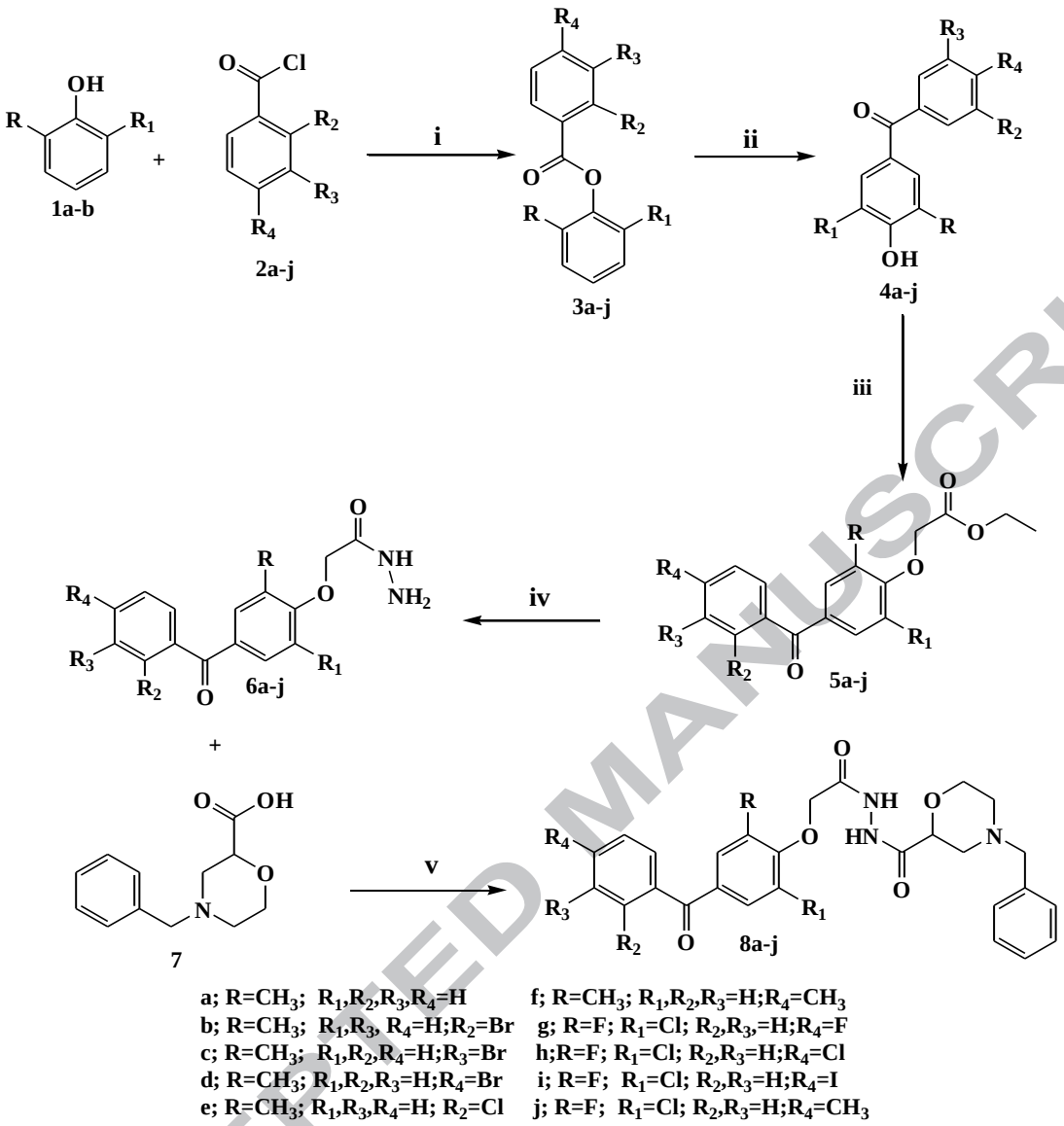
**Fig. 3. Compounds 8b and 8f arrests the cell cycle in G2/M phase in DLA and MCF-7 cells**. DLA and MCF-7 cells were cultured *in vitro* and treated with plus or minus of compounds **8b** and **8f**, stained with propidium iodide and cells were sorted by Fluorescence-activated cell sorting. **A)** Cell cycle arrest on G2/M phase. **B)** Percentage of cells arrested in G2/M phase in DLA cells. **C)** Cell cycle arrest on G2/M phase **D)** Percentage of cells arrested in G2/M phase in MCF-7 cells (*$p < 0.05$ and **$p < 0.01$).

**Fig. 4. Compounds 8b and 8f regresses the ascites lymphoma proliferation *in vivo*.** Ascites lymphoma model was developed by injecting DLA cells in the peritoneum of mice and after onset of the tumour development, mice were administered with compound 8b and 8f at 50 mg/kg b.w for 3 doses to evaluate *in vivo* anti-tumour effect. **A)** Inhibition of tumour volume. B) Reduction of ascites secretion. C) Decrease of cell density.

**Fig. 5. Compounds 8b and 8f promotes the apoptotic cell death by caspase-3 mediated CAD activation. A m**olecular event of compound **8b** and **8f** exhibited tumour regression was analyzed by immunoblot, DNA fragmentation assay and Fluorescence-activated cell sorting. **A)** Activation

of CAD by cleaved caspase-3. **B)** Degradation of nuclear DNA. **C)** Percentage of cells undergoing apoptosis.

**Supplementary 1.** Tumour inhibitory curves depict the cytotoxicity of compound 8b and 8f on A) DLA B) EAC C) MCF-7 and D) A549 cells.




**Scheme 1. Synthesis of oxadiazole-morpholine analogues 8a-j.** Reaction conditions and yield: (i) TEA/$CH_2Cl_2$, stirring at RT for 3 h, yield: 80-96%, (ii) Anhy. $AlCl_3$, 150 °C for 2 h, yield: 61-84%, (iii) $ClCH_2COOC_2H_5$/Dry DMF, Anh. $K_2CO_3$, reflux, 60 $^oC$ for 6-8 h, yield: 79-86%, (iv) $NH_2NH_2.H_2O$/Ethanol, stirring at RT for 2-4 h, yield: 70-78%, (v) EDCI/HOBt, Dry $CH_2Cl_2$, stirring at RT for 4-7 h, yield: 56-68%.

## Graphical Abstract

# Synthesis of novel morpholine conjugated benzophenone analogues and evaluation of anti-mitogenic response against neoplasmic cells of different origin

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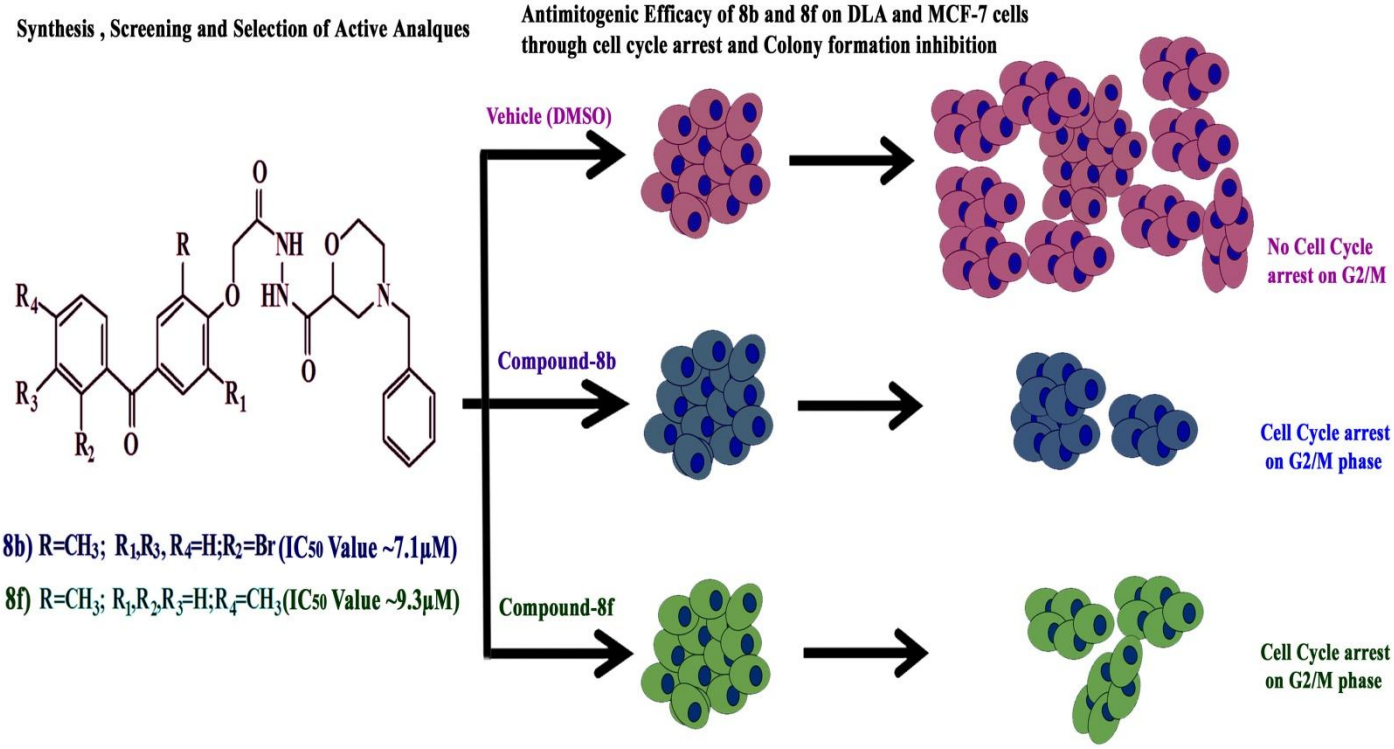

# Highlights

- **A series of novel morpholine conjugated benzophenone analogues 8a-j were synthesized.**
- **Synthesized compounds were characterized by spectral studies & elemental analysis.**
- **Compounds 8a-j were evaluated for *in vitro* anti-proliferative and anti-mitogenic activities.**
- **Compound 8b showed extensive antiproliferative activity and compound 8f exhibited anti-mitogenic activity.**