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Synthesis and mechanistic studies of curcumin analogs based oximes as potential anticancer agents

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Abstract

The incidence of cancer can be decreased by chemoprevention using either natural or synthetic agents. Apart from synthetic compounds, numerous natural products have exhibited promising potential to inhibit carcinogenesis *in vivo*. In this study, α, β-unsaturated carbonyl based anticancer compounds were used as starting materials to synthesize new oxime analogs. The findings from the antiproliferative assay using seven different human cancer cell lines provided a clear picture of structure-activity relationship. The oxime analogs namely **7a** and **8a** showed strong antiproliferative activity against the cell lines. The mechanistic effects of compounds on EGFR TK kinases and tubulin polymerization, and $BRAF^{V600E}$ were investigated. In addition, the efficacy of compounds in reversing the efflux-mediated resistance developed by cancer cells was also studied. The compounds **5a** and **6a** displayed potent activity on various targets such as $BRAF^{V600E}$ and EGFR TK kinases and also exhibited strong antiproliferative activity against different cell lines hence showing potential of multifunctional anticancer agents.

Keywords: Natural compounds; α, β-unsaturated carbonyl; tubulin polymerization; epidermal growth factor receptor (EGFR); multidrug resistance (MDR).

INTRODUCTION

Cancer is one of the main causes of mortality around the globe. The careful estimation and statistics of World Health Organization (WHO) show that the number of new cases of cancer is likely to increase by around 70% in next 2 decades. Some aspects such as virus infection, chronic inflammation, genetic variation and life style may affect the vulnerability to cancer. Apart from orthodox treatments including radiotherapy and chemotherapy, molecular targeted therapy is evolving to be a prospective technique for cancer therapeutics (*1*). For the individuals who are at high risk of developing cancer, chemoprevention might be an alternate interference to delay or inhibit carcinogenesis (*2*). Although several chemotherapeutic agents have been in the clinic for years, still, there is a long way for chemopreventive agents to be safely administered to humans. The recognition of biomarkers and targets that could help monitor the efficacy of chemopreventive agents is an enormous task (*1*).

The synthesis of novel compounds or alteration or modification in the structure of natural compounds on the basis of designs of a natural compound scaffolding, have delivered us lots of vital new drugs within the agriculture, medicine and food spheres (*3, 4*). Due to their acceptable safety profiles, plant-based natural compounds have continuously gathered the attention of scientists around the world on the basis of being biocompatible. Plant-based compounds are considered as potentially safe and effective anticancer and skin lightening agents (*5*). Curcumin is a natural compound derived from turmeric, a spice that has been used for decades for its several health benefits. The anticancer characteristics of curcumin have been under the spotlight from the past 3-4 decades. Lately, we reported a series of thirty compounds bearing α, β-carbonyl moiety as present in curcumin for potential use in agriculture, food, cosmetics and medicine industry (*6*). The effect of synthesized compounds on seven different human cancer

cell lines was assessed. Some compounds were found to be strong anticancer agents exhibiting 81-82% cytotoxicity. In another study, we found that the oxime analogs showed strong antiproliferative activity against cancer cell lines as compared to curcumin-like oxime ether, cyclohexanone and tetralone analogs (*7*).

In the present study, we extended our previously reported work (*6*) by synthesizing new oxime derivatives of most potent curcumin-like α, β-unsaturated carbonyl-based compounds reported in an earlier study. The anticancer activity of all new oxime derivatives was assessed and the derivatives were subjected to further studies to investigate their effects on tubulin polymerization, EGFR TK kinases and $BRAF^{V600E}$ and were tested *in vitro* for the reversal of efflux-mediated resistance developed by the cancer cells.

EXPERIMENTAL SECTION

Synthesis of new oxime analogs

The previously reported method (*7*) by our group was used to synthesize new oxime analogs using most potent α, β-unsaturated carbonyl based compounds (**1-8**)(*6*) as precursors. Each selected compound (1 mmol) was reacted to hydroxylamine hydrochloride (2 mmol) in 10 mL ethanol to produce the corresponding oxime (**1a-8a**). The reaction mixture was filtered upon completion and rotary evaporator was used to evaporate the solvent. Distilled water (15 mL) and dichloromethane (15 mL x 3) were added to the mixture for the extraction of organic compounds. To dry the organic extracts, anhydrous $MgSO_4$ was added, followed by filtration. TLC was used to check the purity of product. Ethylacetate was used to recrystallize the product yielding solid powder. Some products were purified by column chromatography using ethylacetate: hexane (70:30 v/v) as eluent.

2,6-Bis[4-(diethoxymethyl)benzylidene]cyclohexanone oxime (**1a**)

Yield (41%). mp: 117-118 ˚C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.42 (s, H), 7.47 (s, 2H), 6.99 (d, J=8Hz, 4H), 6.55 (d, J=8Hz, 4H), 5.85 (s, 2H), 3.49 (q, J=7, 8H), 2.29 (t, J=7 Hz, 4H), 1.78 (m, 2H), 1.25 (t, J=7.5, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 166.5, 152.7, 145.2, 135.5, 131.2, 127.5, 125.2, 100.7, 55.5, 28.5, 26.2, 16.8 ; HRMS (ESI) m/z: $[M+H]^+$ calculated 494.6423, found 494.6525, Microanalysis calculated for $C_{30}H_{39}NO_5$ (493.63), C: 72.99%, H: 7.96%. N: 2.84%. Found C: 72.98%, H: 8.15%, N: 2.75%.

2,6-Bis-(4-dimethylamino-2-nitro-benzylidene)-cyclohexanone oxime (**2a**)

Yield (50%). mp: 141-142 ˚C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.37 (s, H), 7.64 (s, 2H), 7.22 (d, J=8Hz, 2H), 6.97 (d, J=8Hz, 2H), 6.82 (s, 2H), 3.15 (s, 12H), 2.55 (t, J=7 Hz, 4H), 1.82 (m, 2H) ; ^{13}C NMR (125 MHz, $CDCl_3$) δ: 169.0, 148.5, 145.2, 144.2, 140.5, 128.5, 117.1, 115.2, 105.1, 46.9, 29.4, 27.1; HRMS (ESI) m/z: $[M+H]^+$ calculated 466.5096, found 466.5037, Microanalysis calculated for $C_{24}H_{27}N_5O_5$ (465.50), C: 61.92%, H: 5.85%, N: 15.04%. Found C: 62.10%, H: 5.87%, N: 15.00%.

3,5-Bis-(4-diethoxymethyl-benzylidene)-tetrahydro-pyran-4-one oxime (**3a**)

Yield (51%). mp: 109-110 ˚C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.41 (s, H), 7.65 (s, 2H), 7.12 (d, J=8Hz, 4H), 6.65 (d, J=8Hz, 4H), 5.55 (s, 2H), 3.69 (q, J=7, 8H), 2.67 (s, 4H), 1.21 (t, J=8, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.9, 150.5, 143.4, 135.1, 130.7, 126.4, 125.3, 100.5, 62.5, 55.2, 16.7; HRMS (ESI) m/z: $[M+H]^+$ calculated 496.6151, found 496.6255, Microanalysis calculated for $C_{29}H_{37}NO_6$ (495.61), C: 70.28%, H: 7.52%, N: 2.83%. Found C: 70.46%, H: 7.57%, N: 2.80%.

3,5-Bis[4-(dimethylamino)2-nitro-benzylidene]tetrahydro-pyran-4-one oxime (**4a**)

Yield (45%). mp: 185- 186 ˚C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.49 (s, H), 7.61 (s, 2H), 7.26 (d, J=8Hz, 2H), 6.94 (d, J=8Hz, 2H), 6.55 (s, 2H), 3.12 (s, 12H), 2.90 (s, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 169.5, 148.1, 146.5, 144.9, 139.2, 128.8, 118.6, 116.2, 102.3, 65.9, 46.8; HRMS (ESI) m/z: $[M+H]^+$ calculated 468.4824, found 468.4729, Microanalysis calculated for $C_{23}H_{25}N_5O_6$ (467.47), C: 59.09%, H: 5.39%, N: 14.98%. Found C: 59.27%, H: 5.52%, N: 14.62%.

3,5-Bis[4-(diethoxymethyl)benzylidene]-1-methyl-piperidin-4-one oxime (**5a**)

Yield (39%). mp: 144-145 ˚C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.45 (s, H), 7.23 (s, 2H), 7.02 (d, J=8Hz, 4H), 6.91 (d, J=8Hz, 4H), 5.57 (s, 2H), 3.20 (q, J=7.5, 8H), 2.72 (s, 4H), 2.14 (s, 3H), 1.18 (t, J=7.0, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 169.0, 148.5, 146.2, 139.3, 135.7, 128.4, 126.5, 100.9, 56.5, 50.9, 40.5, 16.2 ; HRMS (ESI) m/z: $[M+H]^+$ calculated 509.6569, found 509.6452 $[M+H]^+$, Microanalysis calculated for $C_{30}H_{40}N_2O_5$ (508.65), C: 70.84%, H: 7.93%, N: 5.51%. Found C: 70.99%, H: 8.05%, N: 5.49%.

3,5-Bis[4-(dimethylamino)2-nitro-benzylidene]-1-methyl-piperidin-4-one oxime (**6a**)

Yield (42%). mp: 176- 177 ˚C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.40 (s, H), 7.16 (s, 2H), 7.05 (d, J=8Hz, 2H), 6.87 (d, J=8Hz, 2H), 6.72 (s, 2H), 3.18 (s, 12H), 2.99 (s, 4H), 2.17 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 165.6, 148.7, 146.3, 142.1, 141.5, 126.4, 118.0, 115.4, 106.7, 45.2, 42.8, 37.5; HRMS (ESI) m/z: $[M+H]^+$ calculated 481.5243, found 481.5210, Microanalysis calculated for $C_{24}H_{28}N_6O_5$ (480.52), C: 59.99%, H: 5.87%, N: 17.49%. Found C: 60.24%, H: 5.95%, N: 17.25%.

3,5-Bis[4-(diethoxymethyl)benzylidene]piperidin-4-one oxime (**7a**)

Yield (51%). mp: 103-105 ˚C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.35 (s, H), 7.29 (s, 2H), 6.97 (d, J=7.5Hz, 4H), 6.77 (d, J=7.5Hz, 4H), 5.62 (s, 2H), 3.29 (q, J=7.5, 8H), 2.62 (s, 4H), 1.20 (t, J=7.0, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.3, 144.2, 142.7, 138.0, 136.5, 128.7, 125.2, 101.4, 55.5, 48.2, 17.1 ; HRMS (ESI) m/z: $[M+H]^+$ calculated 495.6304, found 495.6329 $[M+H]^+$, Microanalysis calculated for $C_{29}H_{38}N_2O_5$ (494.62), C: 70.42%, H: 7.74%, N: 5.66%. Found C: 70.59%, H: 7.85%, N: 5.52%.

3,5-Bis[4-(dimethylamino)2-nitro-benzylidene]piperidin-4-one oxime (**8a**)

Yield (40%). mp: 181- 182 ˚C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.39 (s, H), 7.22 (s, 2H), 7.05 (d, J=8Hz, 2H), 6.89 (d, J=8Hz, 2H), 6.72 (s, 2H), 3.15 (s, 12H), 3.07 (s, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 169.4, 148.5, 146.3, 142.0, 140.9, 126.4, 117.2, 114.5, 102.4, 48.5, 46.7; HRMS (ESI) m/z: $[M+H]^+$ calculated 467.4977, found 467.5014 $[M+H]^+$, Microanalysis calculated for $C_{23}H_{26}N_6O_5$ (466.49), C: 59.22%, H: 5.62%, N: 18.02%. Found C: 59.29%, H: 5.65%, N: 17.95%.

Biological evaluation

3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay was conducted to determine the effect of new oxime compounds on mammary epithelial cells (MCF-10A) (*6, 7*). Different types of cell lines including HT-29 (colon cancer), PC-3 (prostate cancer), A-549 (epithelial), Panc-1(pancreas cancer), MCF-7 (breast cancer), H-460 (lung cancer) and PaCa-2 (pancreatic carcinoma) were used to perform PI fluorescence assay (*6, 7*) to study the antiproliferative efficacy of compounds. Tubulin Polymerization Assay Kit (Cytoskeleton Inc., Denver, CO, USA), which works on the principle of fluorescent reporter enhancement (*8*), was

used to investigate the effect of compounds on tubulin polymerization. EGFR and BRAF and MDR-reversal assays were performed as reported previously (*7*). Detailed methodologies are provided in supplementary materials for this article.

Statistical analysis

All studies were carried out thrice and data are presented as the mean ± standard error of mean (SEM). Graph Pad Prism 5 software was used to calculate IC_{50} values. One-way analysis of variance (ANOVA) for multiple comparisons was used to analyze the data.

RESULTS AND DISCUSSION

Synthesis of oximes

Previously reported method was used to synthesize new oxime analogs in this study (*7*). Respective oximes (**1a-8a**) were synthesized by reacting selected α, β-unsaturated carbonyl based compound (1 mmol) with hydroxylamine hydrochloride (2 mmol) in 10 mL ethanol (Scheme 1). Different techniques such as microanalysis (CHNS), HRMS, ^{1}H NMR, ^{13}C NMR and melting point (MP) analysis were used to characterize all synthesized compounds and the data are provided in experimental part. The purity grade of all compounds is ≥95%.

Cell viability

The cell viability assay was performed using human mammary gland epithelial cell line (MCF-10A). The synthesized compounds were treated with MCF-10A cells for 96 h and MTT assay was used to determine cell viability. The results are shown as toxicity (%) in Table 1. All compounds exhibited a cell viability of more than 88% and hence were nontoxic.

Antiproliferative activity

Different types of cell lines including HT-29 (colon cancer), PC-3 (prostate cancer), A-549 (epithelial), Panc-1(pancreas cancer), MCF-7 (breast cancer), H-460 (lung cancer) and PaCa-2 (pancreatic carcinoma) were used to perform PI fluorescence assay to study the antiproliferative efficacy of compounds. The trend of cell growth inhibition by individual compound was similar against various cell lines. The IC_{50} for all compounds was calculated using GraphPad Prism software (GraphPad Software, San Diego, CA, USA). According to IC_{50} data, substantial association was observed among compounds in relation to their chemical structures.

The compound **8a** exhibited strongest anticancer activity with IC_{50} of 0.02 μM for Panc-1 cells, PaCa-2, PC-3 and MCF-7, followed by **7a** with IC_{50} of 0.03 μM for MCF-7 cell line. The activity of these two compounds was similar to that of positive control erlotinib. Following **7a** and **8a** were oxime compounds **5a** and **6a** which also exhibited strong anticancer potency (Table 1). Next to follow were two compounds **3a** and **4a** which also displayed potent cancer cell growth inhibition however less in comparison to **5a** and **6a**.

All oxime analogs exhibited strong antiproliferative activity against 7 different cell lines in comparison to parent α, β-unsaturated carbonyl-based compounds (*6*), with IC_{50} in range of 0.02 to 2.7 μM as shown in Table 1. Among oxime analogs same behavior regarding substitution patterns on aromatic rings was witnessed as by parent curcumin-like compounds in previous study (*6*). The anticancer activity of curcumin-related compounds was enhanced by the introduction of 2-nitro-4-dimethylamine combination on aromatic rings. Amongst the investigated compounds, two substitution patterns were present and the results showed that compounds having combination of 4-dimethylamine and 2-nitro possessed extremely potent

anticancer activity in comparison to those having diethoxymethyl group substitution at position 4 of rings.

Previously (*6*), curcumin-like compounds that are used in this study to synthesize new oximes showed unlike behavior as far as linkers were concerned. Amongst curcumin-like compounds (**1-8**), tetrahydropyran-4-one (**3-4**) linker-containing compounds showed the most powerful activities, temperate activity was exhibited by *N*-Methyl-4-piperidone-containing compounds (**5-6**), whereas compounds **7-8** bearing 4-piperidone moiety exhibited strong inhibition potential, nonetheless it was weaker as compared to that of compounds having *N*-Methyl-4-piperidone. Among newly synthesized oxime analogs (**1a-8a**), 4-piperidone (**7a-8a**) linker-containing compounds showed the most potent activities, strong cell proliferation inhibitory potential was shown by *N*-Methyl-4-piperidone-bearing compounds (**5a-6a**), though compounds **3-4** containing tetrahydropyran-4-one linker displayed potent inhibitory potential nevertheless it was weaker to that of compounds having *N*-Methyl-4-piperidone. The resultant difference in the activity of compounds could be attributed to the molecular interactions owing to the presence of oxime moiety in newly synthesized compounds.

Anticancer mechanistic studies such as $BRAF^{V600E}$, tubulin polymerization and EGFR-TK were performed to check the activity of all new compounds, in addition to the *in vitro* investigation of multidrug resistance reversal potential of the compounds.

Tubulin polymerization assay

Tubulin is a globular protein which has appeared to be an important molecular target in drug discovery against cancer. Tubulin has important roles in numerous cell processes including segregation of chromosomes in mitosis, cell signaling and maintenance of skeletal integrity of cell (*9, 10*). A number of compounds have been identified which attach to various parts of the

tubulin protein and thus inhibit the polymerization or depolymerization of microtubules leading to mitotic spindle arrest (*11*). Compounds which are capable of disturbing cellular microtubule tubulin equilibrium are beneficial in the treatment of diseases (*12, 13*). The accomplishment of inhibitors of tubulin polymerization as anticancer agents has inspired interest to recognize new compounds which could be more effective in tumors or targeted tissues.

Figure 1 summarizes the effect of new synthetic compounds on tubulin polymerization. Most compounds exhibited influence on the assembly of tubulin with compounds **3a** and **4a** proving to be strongest inhibitors of tubulin assembly. As per PI fluorescence assay, compounds **5a** and **6a** did not inhibit tubulin assembly suggesting a different mechanism behind the observed cytotoxicity rather than tubulin inhibition. As compared to model antimitotic chemotherapeutic drug docetaxel, none of the compound showed potent microtubule-stabilizing potential (*14*).

EGFR inhibition

The epidermal growth factor receptor (EGFR) belongs to the erbB family of closely linked cell membrane receptors including EGFR (erbB-1 or HER1), erbB-2 (HER2), erbB-3 (HER3), and erbB-4 (HER4) (*15, 16*). EGFR expression, overexpression, or dysregulation is witnessed in many human solid tumors, including colorectal, non-small cell lung (NSCLC), ovarian, breast and head and neck cancers (*17-20*). The activation of EGFR might enhance tumor growth by increasing invasive capacity, by blocking apoptosis, cell adhesion, motility and proliferation (*19*). Small molecule EGFR inhibitors are known to be potent antitumor moieties (*17*). Erlotinib has exhibited potential against numerous human cancer types in clinical trials and has been approved for the treatment of colon cancers and NSCLC (*21*).

Table 2 shows the findings of EGFR-TK assay performed to evaluate the EGFR inhibitory potential of new compounds. The findings of the cancer cell-based assays mentioned

before were complemented by the results from this assay. Potent EGFR inhibition was shown by all compounds with IC_{50} in the range of 0.04 to 2.5 μM. According to Table 2, compounds **5a** and **6a** were discovered to be most active and they inhibited EGFR similar to that of positive control erlotinib (IC_{50} = 0.05±0.02 μM). The activity of compounds **5a** and **6a** were found to be enhanced by the addition of *N*-Methyl-4-piperidone moiety. Although, they did not inhibit tubulin polymerization nonetheless were found to be potent inhibitors of EGFR. The compounds **7a-8a** bearing 4-piperidone moiety also exhibited potent inhibition of EGFR however the activity was marginally weaker in contrast to compounds having *N*-Methyl-4-piperidone linker. The study showed that α, β-unsaturated carbonyl-based oxime compounds are strong inhibitors of EGFR and possess potential to be used as anticancer agents.

$BRAF^{V600E}$ inhibitory activity

High occurrence of BRAF mutations (particularly, the replacement of glutamic acid for valine at position 600, V600E) have been observed in melanomas. This replacement which lies in the kinase activation loop, principally prevents the requirement for phosphorylation and leads to constitutively active BRAF that shows almost a 500-fold upsurge in activity of kinase over wild type isoform (*22-24*). Oncogenic $BRAF^{V600E}$ avoids the necessity for upstream regulation by phosphorylation that leads to a MAPK signaling pathway which is activated in the deficiency of extracellular growth factor signals, bringing augmented survival of cell, progression of tumor and unrestrained proliferation of cell. Mutant BRAF signaling inhibition, by either inhibition of MEK or by direct inhibition of the enzyme, has been revealed to stop the growth of tumor (*25, 26*). Lately, BRAF mutant melanoma treatment with selective BRAF inhibitor has exhibited antitumor activity (*27*), authenticating mutant BRAF as therapeutic target and providing prospects for the development of anti- melanoma drugs. So, inhibitors (having small molecules)

which target V600E mutant BRAF protein kinase are being designed and developed for cancer treatment.

The potential of newly synthesized compounds against $BRAF^{V600E}$ was investigated using an *in vitro* experiment. Table 2 shows the IC_{50} (ranging from 1.3-3.1 μM) of all investigated compounds. All α, β-unsaturated carbonyl-based oxime compounds strongly inhibited $BRAF^{V600E}$. The compounds **5a** and **6a** showed almost same BRAF inhibitory activity and were discovered to be potent inhibitors of cancer cell proliferation and were also observed to be strong EGFR inhibitors. The results from this study show that the compounds have potential to be used as anticancer agents and they also inhibit BRAF enzyme efficiently.

MDR reversal effects

A key factor in the unresponsiveness of several types of chemotherapy is multidrug resistance, the mechanism by which numerous cancers become resistant to chemotherapeutic drugs. The patients are affected with a range of solid tumors and blood cancers such as lower GIT, lung, ovarian and breast cancers. Tumors comprise two different types of malignant cells, some of which are drug-resistant whereas others are drug-sensitive. The cells which are sensitive to drugs are killed by chemotherapeutic agents, but most of drug-resistant cells remain alive. There are chances of chemotherapy to fail once the tumor regrows as the residual tumor cells become resistant. This resistance is considered to be attributed to the occurrence of at least two molecular 'pumps' in plasma membranes of tumor cells that vigorously eject chemotherapeutic agents from the interior of cell. The effects of drug or molecular pathways in the cytoplasm or nucleus are thus evaded by cancer cells. The multidrug resistance-associated protein (MRP) and P-glycoprotein are the two pumps which cause chemoresistance in cancer. These pumps are the targets of numerous anticancer therapeutic regimens due to their importance and function(*28*).

The effect of compounds on drug accumulation in MDR cancer cells was investigated using rhodamine accumulation experiment (Table 2). To examine the toxicity of these compounds, trypan blue assay was performed. At a concentration of 50µg/mL, the compounds were found to be non-toxic to cells. The compounds were not effective on R123 accumulation at a concentration of 5 µg/ml concentration. However, at a concentration of 50µg/mL and 30 min incubation, the compounds were found to revert the multidrug resistance in mouse lymphoma cells. The positive control used in the experiment was verapamil. The results of experiments related to MDR are presented in Table 2. The fluorescence activity ratio (FAR) of 1 or more designated the reversal of MDR by compounds. The MDR mediators link to the transmembrane portion of Pgp, hence inducing a change in structure of Pgp, which then stops the activity of ABC transporters (*29*). The oxime analogs **4a** and **8a** were found to be most potent while compounds **1a** and **2a** did not possess beneficial activity. Against human mdr1 gene-transfected mouse lymphoma cells, all compounds were found to be active. Resistance modifiers and traditional chemotherapeutics can be collectively used in the treatment of MDR cancer cells. Those chemical moieties having both MDR modulatory potential as well as potent anticancer activity can be used for treatment of cancer.

Conclusion

To summarize, a series of α, β-unsaturated cyclohexanone derivatives-based oxime analogs with different substitutions were synthesized, and investigated *in vitro* using human cancer cell lines for their antiproliferative potential. Mechanistic assays were also performed to assess the anticancer potential of new compounds. This study has discovered some new oxime analogs as tubulin polymerization, $BRAF^{V600E}$ and EGFR-TK inhibitors. The compounds **5a** and **6a** were discovered to be most potent in all trials. Thus, we have identified potent anticancer agents and

have collected some useful SAR information that may turn out to be beneficial in synthesizing additional MDR reversal agents with antitumor activity.

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Notes

"No author has Competing Financial Interest"

FIGURE LEGENDS

Figure 1: Effect of selected synthetic compounds on tubulin polymerization activity at concentration of 25 μM. Results are the mean values of three experiments, n=3.While vincristine and docetaxel (3 μM) were used as reference compounds. The y-axis demonstrates tubulin polymerization activity measured at 15 min (arbitrary units) in the growth phase of the tubulin polymerization curve.

Scheme 1: Synthesis scheme of α, β-unsaturated carbonyl based compounds and oxime analogs. Reagents and conditions: (**i**) NaOH, EtOH, Room temperature **(ii)** $NH_2OH.HCl$, pyridine, ethanol, anhyd., reflux.

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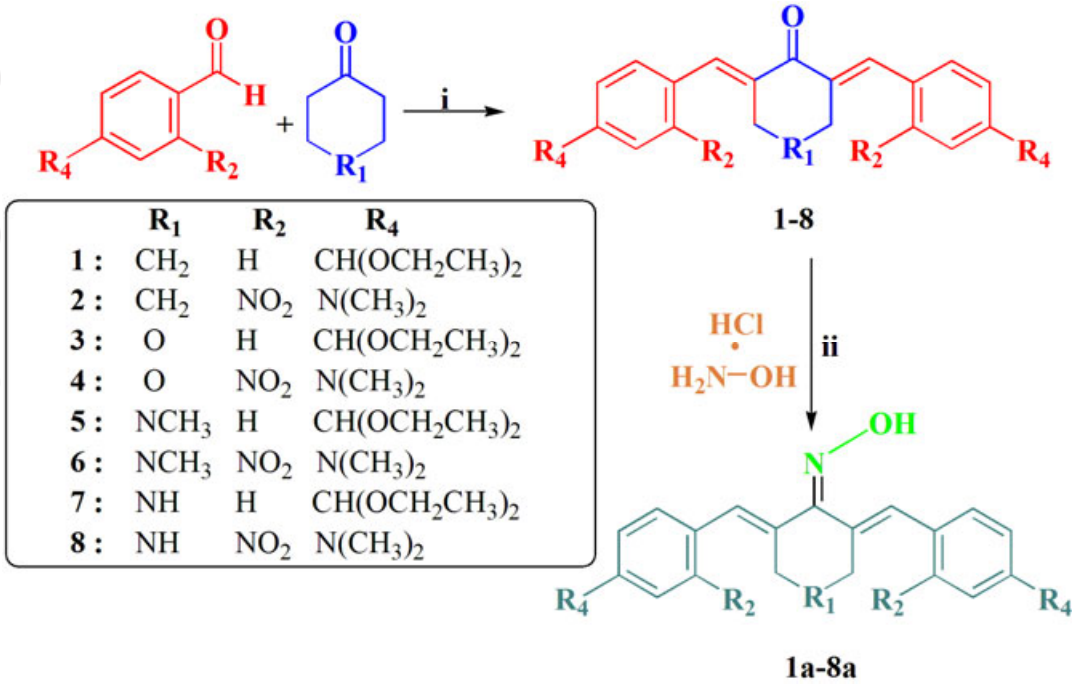

	R_1	R_2	R_4
1 :	CH_2	H	$CH(OCH_2CH_3)_2$
2 :	CH_2	NO_2	$N(CH_3)_2$
3 :	O	H	$CH(OCH_2CH_3)_2$
4 :	O	NO_2	$N(CH_3)_2$
5 :	NCH_3	H	$CH(OCH_2CH_3)_2$
6 :	NCH_3	NO_2	$N(CH_3)_2$
7 :	NH	H	$CH(OCH_2CH_3)_2$
8 :	NH	NO_2	$N(CH_3)_2$

Table 1: Inhibitory effects of new oxime analogues on the growth of normal (MCF-10A) mammary epithelial cells (cell viability) and different types of human cancer cells.

Comp.	Cell viability %	Antiproliferative activity IC_{50} (µM)						
		MCF-7	HT-29	PC-3	A-549	PaCa-2	H-460	Panc-1
1a	90	2.4±0.5	2.6±0.2	2.5±0.1	2.7±1.1	2.4±0.4	2.5±0.5	2.6±0.8
2a	92	2.0±0.9	2.1±0.6	1.9±0.7	2.0±0.5	1.8±0.6	1.9±0.3	2.1±0.6
3a	94	1.4±0.2	1.2±0.9	1.7±0.2	1.8±0.7	1.6±0.5	1.3±0.5	1.8±0.8
4a	91	0.8±0.4	0.5±0.1	0.8±0.2	0.9±0.4	0.7±0.2	0.9±0.6	0.5±0.1
5a	89	0.2±0.09	0.4±0.08	0.2±0.1	0.3±0.1	0.4±0.1	0.2±0.5	0.4±0.07
6a	95	0.09±0.04	0.05±0.04	0.08±0.02	0.09±0.02	0.07±0.05	0.08±0.04	0.1±0.05
7a	95	0.03±0.02	0.04±0.02	0.04±0.02	0.05±0.02	0.04±0.03	0.03±0.01	0.04±0.02
8a	94	0.02±0.01	0.03±0.01	0.02±0.01	0.04±0.03	0.02±0.01	0.04±0.02	0.02±0.01
Erlotinib	-	0.03±0.02	0.02±0.02	0.02±0.01	0.03±0.01	0.04±0.01	0.02±0.01	0.02±0.01

MCF-7 (breast cancer cell line): HT-29 (colon cancer cell line): PC-3 (prostate cancer cell line): A-549 (epithelial): PaCa-2 (pancreatic carcinoma cell line): H-460 (lung cancer cell line): Panc-1 (pancreas cancer cell line).

Table 2: Effects of selected synthetic compounds on EGFR, $BRAF^{V600E}$ and MDR.

Comp.	EGFR inhibition IC_{50} (µM)	BRAF inhibition IC_{50} (µM)	Fluorescence activity ratio (FAR)
1a	2.5±0.8	2.9±0.5	6.5
2a	2.0±0.5	2.2±1.0	9.8
3a	1.9±0.5	3.1±0.5	24.6
4a	1.6±0.9	2.9±0.9	27.5
5a	0.05±0.02	1.5±0.7	17.9
6a	0.04±0.01	1.3±0.5	21.5
7a	0.9±0.1	2.1±0.9	22.9
8a	0.7±0.4	1.9±0.2	28.5
Erlotinib	0.05±0.02	0.07±0.02	25.5
Verapamil	-	-	12.5