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Graphical Abstract

Oxazolidinone derivatives: Cytoxazone-Linezolid hybrids induces apoptosis and senescence in DU145 prostate cancer cells

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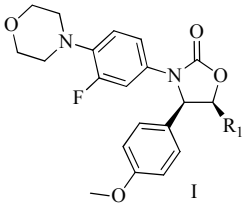
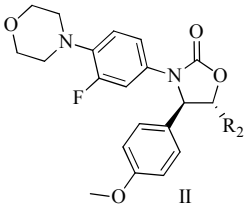
A novel series of oxazolidinones having *p*-methoxy phenyl group at C-4 position have been synthesized. Compound **17** induces cellular senescence and apoptosis in DU145 prostate cancer cells.



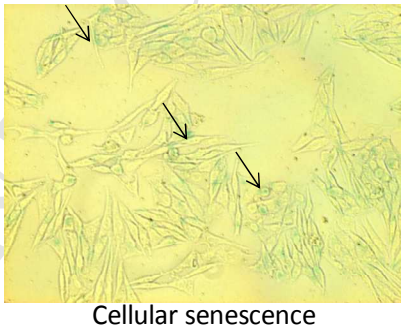
I

9 $R_1 = CH_2OH$
10 $R_1 = CH_2OCO(CH_2)_2OCOCH=CH_2$
11 $R_1 = CH_2OCOC_6H_5$



II

13 $R_2 = CH=CH_2$, **14** $R_2 = CH_2OH$
15 $R_2 = CH_2OCOC_6H_5$
16 $R_2 = CH_2OCO(CH_2)_2OCOCH=CH_2$
17 $R_2 = CH_2CH_3$, **18** $R_2 = CH_2CH_2OH$
20 $R_2 = CH_2NH_2$, **21** $R_2 = CH_2NHCOCH_3$



Cellular senescence

- A novel series of oxazolidinones have been synthesized.
- The series of compounds were evaluated for their anticancer potential.
- Couple of compounds exhibited significant inhibitory activity against cancer cells.
- Compound **17** induces cellular senescence and apoptosis of DU145 cells.
- This study finds oxazolidinones as novel anticancer agents.

Oxazolidinone derivatives: Cytoxazone-Linezolid hybrids induces apoptosis and senescence in DU145 prostate cancer cells

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Abstract: In this study, we report the synthesis of novel oxazolidinone derivatives derived from linezolid **3** having *p*-methoxyphenyl group at C-4 position. *Invitro* evaluation for their anticancer activity toward cultured A549, DU145, HELA, and MCF7 were carried out. The series of compounds prepared displayed wide range of cytotoxicity in MTT assays (10 to 70 μM) across the cell lines tested. Of the all tested compounds **16** and **17** displayed good anticancer potential against A549 (lung) and DU145 (prostate) cancer cells. Further, to determine their anticancer potential, in the present study we have assessed effect of **17** on DU145 cells growth in *in vitro* assays. The results clearly demonstrated that the exposure of DU145 cells to **17** inhibits cell proliferation and induces apoptosis by activation of caspase -3 and -9. Long term exposure of DU145 cells to **17** induced cellular senescence confirmed by senescence marker β-galactosidase staining of cells on post exposure to **17**. The results from this current report support that the oxazolidinone derivatives with ethyl and acryl substitutions showed promising anticancer activity which will be helpful to develop further novel anticancer agents with better therapeutic potential.

Key words: oxazolidinones, cytoxazone, linezolid, anticancer, apoptosis, senescence.

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1. Introduction:

The leading cause of death in the world is cancer. Over 8.0 million people died of cancer and many more going to get effected with this disease in future [1]. The preliminary cancer treatment options remains either alone or in combination of radio therapy, surgery and chemotherapy. For the last several years continuing efforts are being made by industry and academia to develop useful chemotherapeutic agents. In this connection many class of compounds have been identified for their antitumor activity.

Oxazolidinone derivatives have a novel mechanism of action on cancer, HIV, monoamine oxidise, glutamate receptor antagonist, and metabotropic [2]. These are also new class of antibacterial agents and demonstrate potent *in vitro* and *in vivo* activity against important human pathogens, including multiple antibiotic-resistant strains of gram positive organisms [3]. Design of new oxazolidinones with different structural modifications for better therapeutic activity is a very active ongoing research [4]. Some of the oxazolidinones like "3-nitro-5–methyl-2-oxazolidone, 3-(2-hydroxy-3-(2-nitro-1H-imidazol-1-yl)propyl)-2-oxazolidinone, (4-(4-(bis(2-chloroethyl)amino)-2,5-dimethoxyphenyl)methylene aminophenyl)-2-oxazolidinone" are showing anticancer activity and are in early clinical trials [5]. Posizolid **1**, torezolid **2**, linezolid **3,** cytoxazone **4** and *epi*-cytoxazone **5** are showing interesting biological activities having the basic common oxazolidinone structure (Figure 1). Posizolid **1** is an antibiotic under investigation by AstraZeneca for the treatment of bacterial infections [6]. Torezolid **2** has been used for complicated skin infections [7]. Linezolid **3** is the first example of new class of synthetic antibacterial compounds which block protein biosynthesis and used for the treatment of serious infections caused by Gram-positive bacteria [8]. Until now a number of structural modifications of linezolid **3** have been reported. Many research groups involved in the introduction of heterocyclic moieties replacing the rings A-, B-, or C [9] (Figure 2). Cytoxazone **4** is a natural product shows cytokine-modulating activity by inhibiting the signalling pathway of Th_2 cells [10] and its C-5-epimer *epi*-cytoxazone **5**, which is a synthetic derivative, is also showing interesting activity [11]. One of the basic structural differences between cytoxazone **4** and other above compounds is the presence of *p*-methoxyphenyl group at C-4 position in the oxazolidinone ring. We envisaged that the hybrid structures **I** & **II** of linezolid **3** & cytoxazone **4** and linezolid **3** & *epi*-cytoxazone **5** may be of some importance for new leads, since such kind of oxazolidinones to the best of our knowledge have not been synthesized. In addition a substituent at C-4 position creates a new stereo centre which gives the possibility of erythro

and threo oxazolidinones and helps in studying the relation of stereochemistry with therapeutic action. In this context here in we describe the synthesis and anticancer activity of new hybrids having the *p*-methoxyphenyl functionality at the C-4 position.

2. Results and discussion

2.1. Chemistry

The retrosynthetic analysis for our new hybrid structures **I** (**9**, **10**, **11**) and **II** (**13**, **14**, **15**, **16**, **17**, **18**, **20**, **21**) were shown in Scheme 1. The required starting materials for our synthesis are cytoxazone **4** and *epi*-cytoxazone precursor **12** which have been synthesized using our earlier procedure [12]. The basic core structures of erythro **I** and threo **II** can be prepared by *N*-arylation of oxazolidinones **4** & **12** with iodo derivative **6** to give **9** & **13** and further manipulations of **9** & **13** should give the compounds **10** to **21**.

The desired iodo compound **6** (Scheme 2) for the synthesis of main scaffold for Buchwald coupling can be synthesized from **7** which in turn can be prepared from commercially available 1,2-difluoro-4-nitrobenzene [13]. The amine in compound **7** was converted to iodo by diazotization followed by iodination [14] to afford the compound **7** in 85 % yield. Treatment of cytoxazone **4** with TBSCl afforded the compound **8** in 94 % yield. The coupling between oxazolidinone **8** (Scheme 3) and iodo compound **6** in Buchwald conditions followed by deprotection of TBS-group with TBAF solution furnished the compound **9** in 80 % yields [15]. The compound **9** was treated with benzoic anhydride to give the compound **11** in 90 % yield. When **9** was treated with acrylic acid, DCC and DMAP interestingly it gave the compound **10** exclusively in 87 % [16].

For preparing threo derivatives, the coupling (Scheme 4) between oxazolidinone **12** and iodo compound **6** have been achieved by Buchwald conditions to yield the scaffold **13** in 90% yield. The olefin in compound **13** was oxidised by O_3 in dichloromethane at -78 ºC and then reduced by the $NaBH_4$ in methanol to afford the compound **14** in 87% yield. The compound **14** was made bezoyl derivative **15** (Scheme 5) by treating with benzoic anhydride and triethylamine in 77% yield. The compound **14** was made acryloyl derivative using acrylic acid, DCC and DMAP to give the compound **16** in 85%. The reduction of olefin in compound **13** (Scheme 6) with H_2 and Pd/C in MeOH gave the compound **17** in 85% yield and oxidation of compound **13** with BH_3.DMS gave the compound **18** in 75% yield

The alcohol functionality in compound **14** (Scheme 7) was converted into amine by following transformations. The hydroxyl in **14** was initially converted into mesyl derivative by treatment with methanesulfonyl chloride and triethylamine. The mesyl compound was then treated with NaN_3 in DMF at 100 °C to afford the azide **19** in 86% yield. Then the reduction of azide with H_2, Pd/C in methanol gave the amine **20** in 73% yield. The amine in compound **20** was converted into acetyl derivative by treating with acetic anhydride and triethylamine to afford the compound **21** in 85%.

2.2. Biological study

The series of compounds synthesized were subjected to random screening against selected cell lines to determine their anticancer potential. The IC_{50} values of epimers **4** & **5**, **9** & **14**, **10** & **16** and **11** & **15** have been compared, but significant difference is observed only in **10** & **16**. Of all the compounds analyzed, **16** and **17** displayed more cytotoxic effect against DU145 with an IC_{50} value of 8.69±0.38 and 9.47±0.18 µM respectively (Table 1). Thus, the effect of this compound on prostate cancer cell line DU145 was further tested to study its mechanism of action. As the **17** compound was found to be cytotoxic against DU145 cell line, the change in morphology induced by this compound was also observed. The inherent morphology of cells was changed and eventually detached from surface upon exposure at higher concentration of the compound (Figure 3). Further to access the long term effect of **17** on the anchorage-independent proliferative capacity of the prostate cancer cell line DU145, a colony formation assay (soft agar assay) mimicking *in vivo* environment was performed [17]. The results of the assay clearly indicate that the compound **17** has an inhibitory effect on colony formation ability of proliferating cancer cells (Figure 4). A dose dependent inhibition of colony formation ability of cells suggests that the compound **17** effect anchorage independent growth of DU145 cells. In order to confirm whether **17** cause any cell death of DU145 cells, cell death and cell cycle analysis was performed using a Flow Cytometer. Upon treatment with **17,** DU145 cells accumulated in G0/G1 phase of cell cycle (Figure 5). The results showing growth arrest in sub G1 indicates cell death upon exposure to **17**. The observed cell death may be due to the apoptosis induced by activation of caspases (Table 2). Therefore to determine whether the observed cell death is due to activation of caspases which are prerequisite for the induction of apoptosis eventually leading to cell death. Therefore, we have measured activation of key caspases by **17**. The results have confirmed the activation both caspase-3 and caspase-9 in DU145 cells treated with **17** for 48 h continuously. The

activation of procaspase-3 is a distinguishing feature of apoptotic cells. Thus, the presence of activated caspase-3 was experimentally shown using a fluorogenic substrate Ac-DEVD-AMC. A dose-dependent increase in the activity of caspase was observed with different concentrations of **17** as compared to those DU145 cells which were treated with DMSO (Figure 6A). Similarly, activation of caspase-9 was also shown using a fluorogenic substrate Ac-DEVD-AFC. Activation of caspase-9 is mitochondrial dependant which in turn activates caspase-3 executing apoptotic cell death in cancer (Figure 6B). Doxorubicin was used as positive control for measurement of caspase activation. Taken together the results clearly show that the compound **17** induced cell death thorough mitochondrial intrinsic pathway. To further ascertain this claim, the effect of compound **17** on the mitochondrial membrane potential was observed using a dye called JC-1. The unique property of this dye helps to determine the changes in the mitochondrial membrane potential. Healthy cells are known to show abundant red fluorescence (Rhodamine) due to the localization of JC-1 in the inner mitochondrial membrane matrix. Upon treatment with compound **17**, a change in the mitochondrial membrane potential was observed as a result of the dye released from the mitochondrial matrix into the cytosol leading to a shift from red to green fluorescence (FITC) (Figure 7). The loss of mitochondrial membrane potential is known to release cytochrome c in turn activating caspase-9. In summary these observations suggest that the exposure of DU145 cells to **17** leads to mitochondrial dependent induction of apoptosis eventually leading to cell death.

Though we observed little extent of apoptosis in DU145 cells exposed to **17,** proliferation assays show strong effect on total growth of cells. Certain small molecules possess the ability to induce senescence in healthy cells. To test whether **17** had a similar effect on prostate cancer cell line DU145, senescence SA-βgal assay was performed. This is a cytochemical assay wherein the chromogenic substrate 5-bromo-4-chloro-3-indoyl β-D-galactopyranoside (X-gal) yields an insoluble blue compound when cleaved by β-galactosidase increased in senescent cell. Interestingly, this activity is not observed in healthy or quiescent cells, thus, making SA-βgal a biomarker for senescent cells. In the present study as observed in figure 7 up on treatment with **17** increased SA-βgal staining in DU145 cells strongly suggest that **17** induces senescence of DU145 cells there by inhibiting their growth (Figure 8).

3. Conclusion

In conclusion, a series of novel oxazolidinone derivatives has been synthesised and characterized. Evaluation for their anticancer activity toward cultured A549, DU145, HELA, and MCF7 were carried out. From the series of compounds synthesized in the present study, compound **17** affecting prostate cancer cell proliferation partly by direct targeting cell cycle progression and inducing cellular senescence. This from all above experiments it is evident that the cytoxazone-linezolid hybrids with acryl or ethyl substitutions showed more promising anticancer potential than the other substitutions reported. We hypothesize that the compound **17** may target many pro-survival pathways in cancer cells shows aptness to consider this scaffold as basis to develop novel an anticancer agent.

4. Experimental protocols

4.1. General

TLC was performed on Merck Kiesel gel 60; F254 plates (layer thickness 0.25 mm). Column chromatography was performed on silica gel (60–120 mesh) using ethyl acetate and hexane mixture as eluent. Melting points were determined on a Fisher John's melting point apparatus. IR spectra were recorded on a Perkin–Elmer RX-1 FT-IR system. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker Avance-300 MHz, Varian-400, and 500 MHz spectrometers. ^{1}H NMR data are expressed as chemical shifts in ppm followed by multiplicity (s-singlet; d-doublet; t-triplet; q-quartet; m-multiplet), number of proton (s) and coupling constant (s) *J* (Hz). ^{13}C NMR chemical shifts are expressed in ppm. Optical rotations were measured with JASCO digital polarimeter. Accurate mass measurement was performed on Q STAR mass spectrometer (Applied Biosystems, USA).

*4.2. Synthesis of 4-(2-fluoro-4-iodophenyl)morpholine (**6**)*:

To a solution of *p*-TsOH·H_2O (11.63 g, 61.22 mmol) in acetonitlile (40 mL) was added the compound **7** (4 g, 20.4 mmol). The resulting suspension was cooled to 10–15 °C and to this was added, gradually, a solution of $NaNO_2$ (2.81 g, 40.81 mmol) and KI (8.46 g, 51 mmol) in water (10 mL). The reaction mixture was stirred for 10 minutes then allowed to come to 20 °C and stirred until the starting material consumed. To the reaction mixture was then added water (100 mL), $NaHCO_3$ (1 M; until pH = 9–10) and $Na_2S_2O_3$ (2M, 40 mL). The aqueous layer was extracted with ethyl acetate (2 X 50 mL) and the combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4. The solvent was removed on a rotary evaporator and the residue was purified by column chromatography (ethyl

acetate/hexane, 1:4) to give compound **6** (5.325 g, 85%) as white solid. mp: 85–87 °C, FT-IR (neat) ν_{max} cm^{-1} 2960, 2853, 1558, 1490, 1372, 1334, 1296, 1255, 1234, 1165, 1112, 922, 856, 805, 751, 573. ^{1}H NMR ($CDCl_3$, 500 MHz) : δ 7.39-7.33 (m, 2H), 6.66 (t, 1H, *J* = 8.6 Hz), 3.87-3.84 (m, 4H), 3.07-3.04 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 155.2 (d, *J* = 251.3 Hz), 139.9 (d, *J* = 8.2 Hz), 133.5 (d, *J* = 3.8 Hz), 125.1 (d, *J* = 23.6 Hz), 122.6 (d, *J* = 7.6 Hz), 120.2 (d, *J* = 2.7 Hz), 66.8, 50.59, 50.55. ESIMS m/z: 308 $[M+H]^+$, HRMS (ESI) Calcd for $C_{10}H_{12}ONFI$ $[M+H]^+$ 307.99272, found 307.99321.

*4.3. Synthesis of (4R,5R)-5-(((tert-butyldimethylsilyl)oxy)methyl)-4-(4-methoxyphenyl)oxazolidin-2-one (**8**):*

To the solution of the compound **4** (0.2 g, 0.896 mmol) in dichloromethane (5 mL) was added imidazole (0.121 g, 1.793 mmol), TBSCl (0.163 g, 1.075 mmol) and DMAP (cat) at 0 ºC and stirred at room temperature for 1 h. The reaction mixture was then diluted with water and extracted with dichloromethane. The organic layer was concentrated under reduced pressure and purified through column chromatography (ethyl acetate/hexane, 1:1) to afford the compound **8** (0.28 g, 94%) as colourless oil. $[\alpha]^{30}_D$ = -44.4 (*c*=0.6, $CHCl_3$); FT-IR (neat) ν_{max} cm^{-1} 3274, 2930, 2856, 1747, 1611, 1513, 1464, 1248, 1177, 1076, 833, 775, 664. ^{1}H NMR ($CDCl_3$, 500 MHz) : δ 7.18 (d, 2H, *J* = 8.6 Hz), 6.88 (d, 2H, *J* = 8.6 Hz), 5.43 (bs, 1H), 4.93 (d, 1H, *J* = 8.2 Hz), 4.82 (m, 1H), 3.81 (s, 3H), 3.48 (dd, 1H, *J* = 5.9 Hz, 10.9 Hz), 3.27 (dd, 1H, *J* = 6.1 Hz, 10.9 Hz), 0.80 (s, 9H), -0.08 (s, 3H), -0.13 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 159.8, 159.1, 128.3, 127.9, 114.0, 80.4, 61.4, 57.9, 55.3, 25.7, 18.1, -5.6, -5.7. ESIMS m/z: $[M+H]^+$ 338, HRMS (ESI) Calcd for $C_{17}H_{28}O_4NSi$ $[M+H]^+$ 338.18 found 338.18.

*4.4. Synthesis of. (4R,5R)-3-(3-fluoro-4-morpholinophenyl)-5-(hydroxymethyl)-4-(4-methoxyphenyl)oxazolidin-2-one (**9**)*:

To a solution of compound **8** (0.2 g, 0.593 mmol) in 1,4-dioxane (5 mL) was added compound **6** (0.18 g, 0.593 mmol), CuI (0.005 g, 0.029 mmol, 5.0 mol %), and Cs_2CO_3 (0.385 g, 1.186 mmol) evacuated, and backfilled with argon then *N,N'*-dimethylethylenediamine (cat) was added under argon. The reaction mixture was stirred at 110 °C for 3 h. The resulting bright yellow suspension was allowed to reach room temperature and then filtered through a pad of celite eluting with ethyl acetate (2 X 10 mL).The filtrate was concentrated. The residue was dissolved in dry tetrahydrofuran (5 mL) was added 1M. solution of TBAF (1.186 mL, 1.186 mmol) and stirred for 2 h at room

temperature. The reaction mixture was then diluted with water and extracted with ethyl acetate. The organic layer was concentrated under reduced pressure and purified through column chromatography (ethyl acetate/hexane, 1:1) to afford the compound **9** (0.19 g, 80% for two steps) as colourless oil. $[\alpha]^{30}_{D}$ = -20.6 (*c*=0.26, $CHCl_3$); FT-IR (neat) ν_{max} cm^{-1} 3395, 2927, 2855, 1737, 1611, 1513, 1447, 1378, 1249, 1176, 1047, 908, 847, 752, 664. ^{1}H NMR ($CDCl_3$, 500 MHz) : δ 7.31 (dd, 1H, *J* = 14.3 Hz, 2.5 Hz,), 7.14 (d, 2H, *J* = 8.5 Hz), 7.00 (ddd, 1H, *J* = 8.8 Hz, 2.5 Hz, 1.0 Hz), 6.88 (d, 2H, *J* = 8.8 Hz), 6.78 (t, 1H, *J* = 9.1 Hz), 5.35 (d, 1H, *J* = 8.3 Hz), 4.92 (ddd, 1H, *J* = 12.3 Hz, 7.6 Hz, 4.5 Hz), 3.84-3.80 (m, 4H), 3.79 (s, 3H), 3.44 (m, 1H), 3.31 (m, 2H), 3.0-2.96 (m, 4H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 160.0, 155.2 (d, *J* = 247.0 Hz), 154.8, 136.5 (d, *J* = 8.1 Hz), 131.9 (d, *J* = 10.8), 128.1, 125.3, 118.5 (d, *J* = 3.6 Hz), 115.9 (d, *J* = 2.7 Hz), 114.6, 109.2 (d, *J* = 26.3 Hz), 77.5, 77.3, 66.9, 61.9, 55.2, 50.8. ESIMS m/z: $[M+H]^+$ 403, HRMS (ESI) Calcd for $C_{21}H_{24}O_5N_2F$ $[M+H]^+$ 403.16561 found 403.16569.

*4.5. Synthesis of. 3-(((4R,5R)-3-(3-fluoro-4-morpholinophenyl)-4-(4-methoxyphenyl)-2-oxooxazolidin-5-yl)methoxy)-3-oxopropyl acrylate (**10**):*

To a stirred solution of compound **9** (0.04 g, 0.099 mmol) in dry dichloromethane (5 mL) were added acrylic acid (0.014 g, 0.199 mmol), DCC (0.044g, 0.199 mmol) and DMAP (cat) at 0 ºC. The reaction mixture was allowed to room temperature and stirred for 1 h. The reaction was quenched with saturated aq. $NaHCO_3$ solution and the two resulting layers were separated. The aqueous layer was extracted with dichloromethane (2 X 50 mL) and the combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4. The solvent was removed on a rotary evaporator and the residue was purified by column chromatography (ethyl acetate/hexane, 3:7) to give the compound **10** (0.045 g, 87%) as colourless oil. $[\alpha]^{30}_{D}$ = -82.8 (*c*=0.27, $CHCl_3$); FT-IR(neat) ν_{max} cm^{-1} 2924, 2854, 1741, 1612, 1513, 1449, 1404, 1334, 1251, 1173, 1030, 939, 810, 764. ^{1}H NMR ($CDCl_3$, 300 MHz) : δ 7.31 (dd, 1H, *J* = 14.3Hz, 2.6 Hz), 7.14 (d, 2H, *J* = 8.6 Hz), 7.01 (dd, 1H, *J* = 8.8 Hz, 1.5 Hz), 6.88 (d, 2H, *J* = 8.4 Hz), 6.78 (t, 1H, *J* = 9.0 Hz), 6.41 (dd, 1H, *J* = 17.3 Hz, 1.3 Hz), 6.11 (dd, 1H, *J* = 17.3 Hz, 10.3 Hz), 5.85 (dd, 1H, *J* = 10.3 Hz, 1.1 Hz), 5.34 (d, 1H, *J* = 8.1 Hz), 5.01 (ddd, 1H, *J* = 12.8 Hz, 8.4 Hz, 3.9 Hz,), 4.39 (t, 2H, *J* = 6.2 Hz), 3.93 (dd, 1H, *J* = 12.2 Hz, 3.7 Hz), 3.85-3.77 (m, 5H), 3.79 (s, 3H), 3.02-2.95 (m, 4H), 2.68 (t, 2H, *J* = 6.2 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 169.9, 165.8, 160.2, 155.2 (d, *J* = 246.1 Hz), 154.5, 136.6 (d, *J* = 8.1 Hz), 131.7 (d, *J* = 9.9 Hz), 131.3, 128.1, 127.9, 124.8, 118.5 (d, *J* = 4.5 Hz), 115.9 (d, *J* = 2.7 Hz), 114.7, 109.1 (d, *J* = 25.4 Hz), 74.6, 66.8, 63.2, 61.9, 59.5, 55.2, 50.81, 50.80,

33.5. ESIMS m/z: $[M+H]^+$ 529, HRMS (ESI) Calcd for $C_{27}H_{30}O_8N_2F$ $[M+H]^+$ 529.19676 found 529.19725.

*4.6. Synthesis of ((4R,5R)-3-(3-fluoro-4-morpholinophenyl)-4-(4-methoxyphenyl)-2-oxooxazolidin-5-yl)methyl benzoate (**11**):*

To the solution of the compound **9** (0.05 g, 0.098 mmol) in dichloromethane (10 mL) was added triethylamine (0.04 mL, 0.296 mmol), benzoic anhydride (0.042 g, 0.186 mmol) and DMAP (cat) at 0 ºC and then allowed to keep the reaction mixture at room temperature for 1 h. The reaction mixture was then diluted with water and extracted with dichloromethane. The organic layer was concentrated under reduced pressure and purified through column chromatography (ethyl acetate/hexane, 3:7) to afford the compound **11** (0.054 g, 90%) as colourless oil. $[\alpha]^{30}_D$ = -60.3 (*c*=0.19, $CHCl_3$); FT-IR (neat) ν_{max} cm^{-1} 2922, 2852, 1753, 1724, 1514, 1449, 1395, 1269, 1251, 1176, 1116, 1028, 770, 713. ^{1}H NMR ($CDCl_3$, 500 MHz) : δ 7.99 (d, 2H, *J* = 7.3 Hz), 7.58 (t, 1H, *J* = 7.3 Hz), 7.44 (t, 2H, *J* = 7.6 Hz), 7.34 (dd, 1H, *J* = 14.3 Hz, 2.5 Hz), 7.18 (d, 2H, *J* = 8.5 Hz), 7.03 (dd, 1H, *J* = 8.6 Hz, 1.9 Hz), 6.87 (d, 2H, *J* = 8.5 Hz), 6.79 (t, 1H, *J* = 9.1 Hz), 5.41 (d, 1H, *J* = 8.2 Hz), 5.16 (td, 1H, *J* = 3.8 Hz, 3.9 Hz, 4.1 Hz), 4.17 (dd, 1H, *J* = 12.3 Hz, 3.9 Hz), 3.98 (dd, 1H, *J* = 12.3 Hz, 7.9 Hz), 3.85-3.80 (m, 4H), 3.77 (s, 3H), 3.01-2.97 (m, 4H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 166.6, 160.6, 155.2 (d, *J* = 247.9 Hz), 154.2, 134.5 (d, *J* = 9.9 Hz), 133.4, 131.5 (d, *J* = 15.4 Hz), 129.7, 129.1, 128.4, 128.1, 127.6, 116.3 (d, *J* = 3.6 Hz), 116.0 (d, *J* = 3.6 Hz), 114.7, 109.8 (d, *J* = 24.5 Hz), 74.9, 66.9, 63.2, 62.1, 55.2, 50.8. ESIMS m/z: $[M+H]^+$ 507, HRMS (ESI) Calcd for $C_{28}H_{28}O_6N_2F$ $[M+H]^+$ 507.19 found 507.19.

*4.7. Synthesis of (4R,5R)-3-(3-fluoro-4-morpholinophenyl)-4-(4-methoxyphenyl)-5-vinyloxazolidin-2-one (**13**):*

To a solution of compound **6** (0.8 g, 3.65 mmol) in 1,4-dioxane (10 mL) was added compound **12** (1.12 g, 3.65 mmol), CuI (0.034 g, 0.182 mmol, 5.0 mol %), and Cs_2CO_3 (2.37 g, 7.30 mmol) evacuated for a short while and backfilled with argon then *N,N'*-dimethylethylenediamine (10 mol%) was added under argon. The reaction mixture was stirred at 110 ºC for 3 h. The resulting bright yellow suspension was allowed to reach room temperature and then filtered through a pad of celite eluting with ethyl acetate (2 X 20 mL). The filtrate was concentrated and the residue was purified by silica gel column chromatography (ethyl acetate/hexane, 7:3) to afford the pure compound **13** (1.308 g, 90%) as a light brown syrup. $[\alpha]^{30}_D$ = -8.18 (c=2.4, CHCl3); FT-IR(neat) ν_{max} cm^{-1} 2928, 2854,

1753, 1612, 1513, 1375, 1249, 1227, 1175, 1116, 1030, 938, 833, 772. ^{1}H NMR ($CDCl_3$, 500 MHz) : δ 7.21-7.14 (m, 3H), 6.95 (dd, 1H, *J* = 8.8 Hz, 2.2Hz), 6.87 (d, 1H, *J* = 8.6 Hz), 6.77 (t, 1H, *J* = 9.0 Hz), 6.01 (m, 1H), 5.43-5.36 (m, 2H), 4.88 (d, 1H, *J* = 6.8 Hz), 4.70 (m, 1H), 3.83-3.79 (m, 4H), 3.78 (s, 3H), 3.00-2.95 (m, 4H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 159.9, 155.2, 155.1 (d, *J* = 246.1 Hz), 136.8 (d, *J* = 8.1 Hz), 132.9, 131.7 (d, *J* = 9.9 Hz), 128.4, 127.7, 119.9, 118.4 (d, *J* = 3.6 Hz), 117.1 (d, *J* = 2.7 Hz), 114.6, 110.1 (d, *J* = 25.4 Hz), 82.4, 66.8, 66.1, 55.2, 50.77, 50.74. ESIMS m/z: $[M+H]^+$ 399, HRMS (ESI) Calcd for $C_{22}H_{24}O_4N_2F$ $[M+H]^+$ 399.1714 found 399.1713.

*4.8. Synthesis of (4R,5S)-3-(3-fluoro-4-morpholinophenyl)-5-(hydroxymethyl)-4-(4-methoxyphenyl)oxazolidin-2-one (**14**):*

To a solution of compound **13** (0.5 g, 1.256 mmol) in dry dichloromethane (10 mL) at –78 °C ozone gas was passed for 30 minutes (until solution turns to light blue color). The reaction mixture was treated with dimethyl sulphide then concentrated under reduced pressure. The crude reaction mixture dissolved in methanol (5 mL), to it was added $NaBH_4$ (0.19 g, 5.025 mmol) at 0 ºC and stirred the reaction mixture for 2 h at 0 °C. Then the reaction mixture was quenched with saturated NH_4Cl solution and solvents were removed under reduced pressure. Crude compound was partitioned between dichloromethane and water. Aqueous layer was again extracted with dichloromethane. Combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure followed by silica gel column chromatography (ethyl acetate/hexane, 7:3) to afford the pure compound **14** (0.439 g, 87%) as colourless oil. $[\alpha]_D^{30}$ = -15.7 (*c*=2.1, $CHCl_3$); FT-IR (neat) ν_{max} cm^{-1} 3395, 3013, 2960, 2836, 1741, 1612, 1512, 1377, 1227, 1175, 1032, 904, 745, 664. ^{1}H NMR ($CDCl_3$, 300 MHz) : δ 7.25-7.15 (m, 3H), 6.98 (d, 1H, *J* = 8.8 Hz), 6.88 (d, 2H, *J* = 8.4 Hz), 6.78 (t, 1H, *J* = 9.0 Hz), 5.21 (d, 1H, *J* = 6.6 Hz), 4.38 (m, 1H), 4.01 (m, 1H), 3.85-3.72 (m, 5H), 3.78 (s, 3H), 3.02-2.95 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.8, 155.6, 155.1 (d, *J* = 245.8 Hz), 136.9 (d, *J* = 8.7 Hz), 131.5 (d, *J* = 9.8 Hz), 129.3, 127.8, 118.4 (d, *J* = 3.8 Hz), 117.4 (d, *J* = 2.7 Hz), 114.7, 110.4 (d, *J* = 25.2 Hz), 82.0, 66.8, 61.3, 61.2, 55.2, 50.77, 50.74. ESIMS m/z: $[M+H]^+$ 403, HRMS (ESI) Calcd for $C_{21}H_{24}O_5N_2F$ $[M+H]^+$ 403.16638 found 403.16689.

*4.9. Synthesis of ((4R,5S)-3-(3-fluoro-4-morpholinophenyl)-4-(4-methoxyphenyl)-2-oxooxazolidin-5-yl)methyl benzoate (**15**):*

To the solution of the compound **14** (0.05 g, 0.098 mmol) in dichloromethane (10 mL) was added triethylamine (0.04 mL, 0.296 mmol), benzoic anhydride (0.042 g, 0.186 mmol) and

DMAP (cat) at 0 ºC and then allowed to keep the reaction mixture at room temperature for 1 h. The reaction mixture was then diluted with water and extracted with dichloromethane. The organic layer was concentrated under reduced pressure and purified through column chromatography (ethyl acetate/hexane, 3:7) to give the compound **15** (0.047 g, 77%) as colourless oil. [α] $^{30}_{D}$ = +35.02 (*c*=2.2, $CHCl_3$); FT-IR (neat) ν_{max} cm^{-1} 2959, 2836, 1754, 1723, 1611, 1513, 1449, 1377, 1268, 1249, 1116, 1028, 772, 712. ^{1}H NMR ($CDCl_3$, 500 MHz) : δ 7.99 (d, 2H, *J* = 8.0 Hz), 7.58 (t, 1H, *J* = 7.47 Hz), 7.42 (t, 2H, *J* = 7.7 Hz), 7.25-7.19 (m, 3H), 6.97 (dd, 1H, *J* = 8.6 Hz, 2.5 Hz), 6.89 (d, 2H, *J* = 8.6 Hz), 6.77 (t, 1H, *J* = 9.0 Hz), 5.08 (d, 1H, *J* = 5.0 Hz), 4.68-4.60 (m, 3H), 3.83-3.80 (m, 4H), 3.79 (s, 3H), 3.00-2.97 (m, 4H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 166.0, 160.1, 155.1 (d, *J* = 247.0 Hz), 154.8, 136.9 (d, *J* = 9.0 Hz), 133.5, 131.5 (d, *J* = 9.9 Hz), 129.7, 129.1, 128.9, 128.5, 127.5, 118.5 (d, *J* = 3.6 Hz), 116.9 (d, *J* = 3.6 Hz), 114.8, 110.0 (d, *J* = 25.4 Hz), 79.1, 66.8, 63.7, 62.6, 55.2, 50.77, 50.74. ESIMS m/z: $[M+H]^+$ 507, HRMS (ESI) Calcd for $C_{28}H_{28}O_6N_2F$ $[M+H]^+$ 507.19259 found 507.1926.

*4.10. Synthesis of 3-(((4R,5S)-3-(3-fluoro-4-morpholinophenyl)-4-(4-methoxyphenyl)-2-oxooxazolidin-5-yl)methoxy)-3-oxopropyl acrylate (**16**):*

To a stirred solution of compound **14** (0.04 g, 0.099 mmol) in dry dichloromethane (5 mL) were added acrylic acid (0.014 g, 0.199 mmol), DCC (0.044 g, 0.199 mmol) and DMAP (cat) at 0 ºC. The reaction mixture was allowed to room temperature and stirred for 1 h. The reaction was quenched with saturated aq. $NaHCO_3$ solution and the two resulting layers were separated. The aqueous layer was extracted with dichloromethane (2 X 50 mL) and the combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4. The solvent was removed on a rotary evaporator and the residue was purified by column chromatography (ethyl acetate/hexane, 3:7) to afford the compound **16** (0.044 g, 85%) as colourless oil. [α] $^{30}_{D}$ = -0.4 (*c*=1.8, $CHCl_3$); FT-IR (neat) ν_{max} cm^{-1} 2928, 2855, 1743, 1613, 1513, 1448, 1378, 1248, 1169, 1029, 938, 809, 747, 664. ^{1}H NMR ($CDCl_3$, 500 MHz) : δ 7.23-7.17 (m, 3H), 6.97 (ddd, 1H, *J* = 8.8 Hz, 2.5 Hz, 1.0 Hz), 6.89 (d, 2H, *J* = 8.6 Hz), 6.79 (t, 1H, *J* = 9.0 Hz), 6.40 (dd, 1H, *J* = 17.3 Hz, 1.3 Hz), 6.09 (dd, 1H, *J* = 17.3 Hz, 10.3 Hz), 5.84 (dd, 1H, *J* = 10.3 Hz, 1.3 Hz), 5.02 (d, 1H, *J* = 6.1 Hz), 4.53 (m, 1H), 4.48-4.38 (m, 4H), 3.83-3.80 (m, 4H), 3.79 (s, 3H), 3.01-2.97 (m, 4H), 2.75 (t, 2H, *J* = 6.1 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.2, 165.7, 160.1, 155.1 (d, *J* = 246.9 Hz), 154.7, 137.0 (d, *J* = 8.7 Hz), 131.4, 131.3(d, *J* = 9.9 Hz), 128.8, 127.8, 127.6, 118.5 (d, *J* = 3.8 Hz), 117.0 (d, *J* = 2.7 Hz), 114.8, 110.1 (d, *J* = 25.2 Hz), 78.8, 66.8, 63.2, 62.3, 59.5, 55.2, 50.77, 50.74, 33.7.

ESIMS m/z: $[M+H]^+$ 529, HRMS (ESI) Calcd for $C_{27}H_{30}O_8N_2F$ $[M+H]^+$ 529.19807 found 529.19852.

*4.11. Synthesis of (4R,5R)-5-ethyl-3-(3-fluoro-4-morpholinophenyl)-4-(4-methoxyphenyl) oxazolidin-2-one (**17**):*

To a solution of compound **13** (0.06 g, 0.15 mmol) in methanol (2 mL) was added 10 % Pd/C (cat) and stirred the reaction mixture under H_2 atmosphere for 1 h. The reaction mixture was filtered through a pad of celite and concentrated under reduced pressure. The residue was purified by column chromatography (ethyl acetate/hexane, 1:4) to afford the pure compound **17** (0.051 g, 85%) as a colourless syrup. $[\alpha]^{30}_D$ = - 37.41(*c*=2.05, $CHCl_3$); FT-IR(neat) ν_{max} cm^{-1} 2964, 2934, 2854, 1746, 1612, 1512, 1446, 1376, 1337, 1248, 1175, 1116, 1031, 938, 775, 730, 665. ^{1}H NMR ($CDCl_3$, 500 MHz) : δ 7.22-7.16 (m, 3H), 6.97 (dd, 1H, *J* = 8.8 Hz, 1.6 Hz), 6.87 (d, 2H, *J* = 8.6 Hz), 6.77 (t, 1H, *J* = 9.0 Hz), 4.80 (d, 1H, *J* = 6.2 Hz), 4.25 (m, 1H), 3.83-3.79 (m, 4H), 3.78 (s, 3H), 3.00-2.96 (m, 4H), 1.90-1.83 (m, 2H), 1.07 (t, 3H, *J* = 7.3 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.8, 155.4, 155.1 (d, *J* = 246.4 Hz), 136.6 (d, *J* = 8.8 Hz), 132.0 (d, *J* = 9.9 Hz), 129.6, 127.7, 118.4 (d, *J* = 3.8 Hz), 117.0 (d, *J* = 2.7 Hz), 114.7, 110.0 (d, *J* = 25.3 Hz), 83.3, 66.8, 65.7, 55.2, 50.85, 50.80, 27.1, 9.0. ESIMS m/z: $[M+H]^+$ 401, HRMS (ESI) Calcd for $C_{22}H_{26}O_4N_2F$ $[M+H]^+$ 401.1871 found 401.1876.

*4.12. Synthesis of (4R,5R)-3-(3-fluoro-4-morpholinophenyl)-5-(2-hydroxyethyl)-4-(4-methoxyphenyl)oxazolidin-2-one (**18**):*

To a solution of compound **13** (0.06 g, 0.15 mmol) in dry tetrahydrofuran (10 mL) was added $BH_3.S(CH_3)_2$ complex (0.024 mL, 0.301 mmol) at 0 °C under nitrogen. The mixture was allowed to warm to room temperature over 3 h and then evaporated under reduced pressure. sodium hydroxide solution (3M, 0.75 mmol) and 30% aqueous H_2O_2 (0.75 mmol) were added successively to a solution of the residue in tetrahydrofuran (10 mL) at 0 °C. The turbid solution was warmed to room temperature over 2 h and then quenched with water (20 mL) and extracted with ethyl acetate (3 X 10 mL). The ethyl acetate extracts were dried (Na_2SO_4), and then concentrated in vacuo. The residue was purified by column chromatography (ethyl acetate/hexane, 1:1) to give compound **18** (0.047 g, 75%) as colourless oil. $[\alpha]^{30}_D$ = -18.79 (c= 0.36, CHCl3); FT-IR(neat) ν_{max} cm^{-1} 3432, 2925, 2855, 1741, 1612, 1513,1446, 1378, 1248, 1229, 1175, 1113, 1030, 835, 750, 664. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.21 (d, 2H, *J* = 8.6 Hz), 7.17 (dd, 1H, *J* = 14.1 Hz, 2.5 Hz), 6.96 (ddd, 1H, *J* = 8.6 Hz, 2.5 Hz, 1.0 Hz), 6.87

(d, 2H, *J* = 8.6 Hz), 6.77 (t, 1H, *J* = 9.0 Hz), 4.95 (d, 1H, *J* = 6.4 Hz), 4.51 (m, 1H), 3.87 (m, 2H), 3.83-3.77 (m, 5H), 3.78 (s, 3H), 3.0-2.96 (m, 4H), 2.08 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.9, 155.3, 155.1 (d, *J* = 246.1 Hz), 136.8 (d, *J* = 9.0 Hz), 131.8 (d, *J* = 9.9 Hz), 129.1, 127.8, 118.4 (d, *J* = 4.5 Hz), 117.2 (d, *J* = 3.6 Hz), 114.7, 110.2 (d, *J* = 25.4 Hz), 79.8, 66.8, 66.1, 58.5, 55.2, 50.8, 50.7, 36.2. ESIMS m/z: $[M+H]^+$ 417, HRMS (ESI) Calcd for $C_{22}H_{26}O_5N_2F$ $[M+H]^+$ 417.1801 found 417.1803.

*4.13. Synthesis of (4R, 5R)-5-(azidomethyl)-3-(3-fluoro-4-morpholinophenyl)-4-(4-methoxyphenyl)oxazolidin-2-one (**19**):*

To the solution of the compound **14** (0.19 g, 0.472 mmol) in dichloromethane (10 mL) was added triethylamine (0.196 mL, 1.417 mmol), methanesulfonyl chloride (0.054 ml, 0.708 mmol) and DMAP (cat) at 0 °C and then allowed to keep the reaction mixture at room temperature for 1h. The reaction mixture was then diluted with water and extracted with dichloromethane. The residue was dissolved in dry *N*,*N*-dimethylformamide (4 mL) and sodium azide (0.403 g, 0.945 mmol) was added. The reaction mixture was heated at 100 °C for 2 h. The reaction mixture was then allowed to room temperature, diluted with water and extracted with dichloromethane (2 X 10 mL) and the combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4. The solvent was removed on a rotary evaporator and the residue was purified by column chromatography (ethyl acetate/hexane, 3:7) to give compound **19** (0.173 g, 86% for two steps) as yellow oil. $[\alpha]^{30}_D$ = -32 (*c*= 0.9, $CHCl_3$); FT-IR(neat) ν_{max} cm^{-1} 2921, 2853, 2106, 1754, 1612, 1513, 1444, 1378, 1249, 1116, 1030, 938, 752, 665. ^{1}H NMR ($CDCl_3$, 500 MHz) : δ 7.23-7.18 (m, 3H), 6.98 (dd, 1H, *J* = 8.6 Hz, 1.6 Hz), 6.88 (d, 2H, *J* = 8.6 Hz), 6.79 (t, 1H, *J* = 9.1 Hz), 5.04 (d, 1H, *J*=5.9 Hz), 4.44 (m, 1H), 3.83-3.80 (m, 4H), 3.78 (s, 3H), 3.74 (dd, 1H, *J* = 13.4 Hz, 4.1 Hz), 3.60 (dd, 1H, *J* = 13.4 Hz, 4.1 Hz), 3.01-2.97 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 160.0, 155.1 (d, *J* = 246.9 Hz), 154.5, 137.0 (d, *J* = 8.7 Hz), 131.3 (d, *J* = 9.8 Hz), 128.8, 127.6, 118.5 (d, *J* = 3.8 Hz), 117.2 (d, *J* = 2.7 Hz), 114.8, 110.2 (d, *J* = 25.2 Hz), 79.6, 66.8, 62.6, 55.2, 51.8, 50.76, 50.73, 29.6. ESIMS m/z: 428$[M+H]^+$, HRMS (ESI) Calcd for $C_{21}H_{23}O_4NF$ $[M+H]^+$ 428.17286 found 428.17229.

*4.14. Synthesis of (4R,5R)-5-(aminomethyl)-3-(3-fluoro-4-morpholinophenyl)-4-(4-methoxyphenyl)oxazolidin-2-one (**20**):*

To a solution of compound **19** (0.15 g, 0.351 mmol) in methanol (5 mL) was added 10 % Pd/C (cat) and stirred the reaction mixture under H_2 atmosphere for 3 h. The reaction mixture

was filtered through celite and concentrated under reduced pressure. The crude was purified by column chromatography (Chloroform/methanol, 9:1) to afford the compound **20** (0.102 g, 73%) as colourless oil. $[\alpha]^{30}_{D}$ = +3.0 (*c*=2.5, $CHCl_3$); FT-IR(neat) ν_{max} cm^{-1} 2959, 2835, 1749, 1612, 1513, 1445, 1378, 1334, 1229, 1115, 1030, 905, 833, 772, 729, 665. ^{1}H NMR ($CDCl_3$, 500 MHz) : δ 7.27-7.17 (m, 3H), 6.97 (dd, 1H, *J* = 8.6 Hz, 2.5 Hz), 6.86 (d, 2H, *J* = 8.6 Hz), 6.77 (t, 1H, *J* = 9.1 Hz), 5.06 (d, 1H, *J* = 6.4 Hz), 4.34 (m, 1H), 4.21 (m, 1H), 3.83-3.78 (m, 4H), 3.77 (s, 3H), 3.01-2.95 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.8, 155.2, 155.1 (d, *J* = 246.4 Hz), 136.8, 131.6 (d, *J* = 9.8 Hz), 129.3, 127.8, 118.4 (d, *J* = 3.8 Hz), 117.2 (d, *J* = 2.7 Hz), 114.7, 110.2 (d, *J* = 25.2 Hz), 82.5, 66.8, 62.7, 55.2, 50.7, 43.3. ESIMS m/z: $[M+H]^+$ 402, HRMS (ESI) Calcd for $C_{21}H_{25}O_4N_3F$ $[M+H]^+$ 402.18236 found 402.1817.

*4.15. Synthesis of N-(((4R,5R)-3-(3-fluoro-4-morpholinophenyl)-4-(4-methoxyphenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (**21**):*

To the solution of the compound **20** (0.06 g, 0.149 mmol) in dichloromethane (5 mL) was added triethylamine (0.062 mL, 0.448 mmol), acetic anhydride (0.022 g, 0.224 mmol) and DMAP (cat) at 0 ºC and then allowed to keep the reaction mixture at room temperature for 1 h. The reaction mixture was then diluted with water and extracted with dichloromethane. The organic layer was concentrated under reduced pressure and purified through column chromatography (ethyl acetate/hexane, 3:7) to afford the compound **21** (0.056 g, 85%) as colourless syrup. $[\alpha]^{30}$D = -26.7 (c=1, $CHCl_3$); FT-IR (neat) ν_{max} cm^{-1} 3323, 2961, 2835, 1750, 1659, 1612, 1513, 1376, 1248, 1176, 1114, 1031, 914, 772, 729, 665. ^{1}H NMR ($CDCl_3$, 500 MHz) : δ 7.24 (d, 2H, *J* = 8.6 Hz), 7.18 (dd, 1H, *J* = 14.1 Hz, 2.5 Hz), 6.92 (dd, 1H, *J* = 8.8 Hz, 1.8 Hz), 6.86 (d, 2H, *J* = 8.6 Hz), 6.77 (t, 1H, *J* = 9.0 Hz), 6.08 (t, 1H, *J* = 6.1 Hz), 5.01 (d, 1H, *J* = 6.5 Hz), 3.85 (m, 1H), 3.83-3.80 (m, 4H), 3.78 (s, 3H), 3.61-3.55 (m, 1H), 3.00-2.96 (m, 4H), 2.04 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.0, 159.9, 155.9 (d, *J* = 246.4 Hz), 155.1, 136.9 (d, *J* = 8.8 Hz), 132.6 (d, *J* = 8.2 Hz), 128.6, 127.8, 118.4 (d, *J* = 3.2 Hz), 117.1 (d, *J* = 2.7 Hz), 114.7, 110.2 (d, *J* = 25.2 Hz), 80.7, 66.8, 62.5, 55.2, 50.78, 50.74, 40.1, 23.2. ESIMS m/z: 444 $[M+H]^+$, HRMS (ESI) Calcd for $C_{23}H_{27}O_5N_3F$ $[M+H]^+$ 444.19293 found 444.19259.

4.16. Cell Based Assays

4.16.1. Cytotoxicity evaluation against different cancer cell lines

Cellular viability in presence of the compound was tested by performing a Sulforhodamine B Assay (SRB) [18]. Briefly, the cell line of interest was seeded in flat bottom 96- well plate (5000 cells/100 µL) in a medium containing 10% serum followed by incubating the plate for 18-20 h in an incubator continuously supplied with 5 % CO_2, allowing cells to adhere to the surface of the wells. After 18 h the cells were treated with the compound at desired concentration. Working dilutions of 500 µM concentration of the compounds were prepared, of which 2 µl aliquot was added to the each well, thereby making the final concentration of compound 0 to 100 µM. DMSO and doxorubicin (as standard control anticancer drug) were taken as vehicle and positive controls respectively. Further, the plates were incubated for another 48 h in an incubator maintained at 37 ºC with a constant supply of 5% CO_2. After 48 h of treatment cells were fixed using 10% TCA for 1 h at 4 ºC. The plate was rinsed carefully with MQ water removing TCA and air dried. Then 0.057% SRB solution was added to plates and incubated for 30 minutes before washing to remove unbound SRB using 1% acetic acid. The plates were then air dried and the absorbance was measured using Perkin –Elmer Multimode Reader at 510 nm. To measure the absorbance, 100 µL of 10 mM Tris Base was added to each well to solubilise the SRB. The measure of absorbance is directly proportional to cell growth and is thus used to calculate the IC_{50} values. In the present study, four types of cancer cell lines *i.e.* lung cancer (A549); breast cancer (MCF-7), prostate cancer (DU145) and cervical cancer (HeLa) cell lines were tested for the cytotoxic effect of the series of compounds. Based on the IC_{50} values obtained, the compound **17** was selected for further assays to ascertain its effect on prostate cancer cell line (DU145).

4.16.2. Change in Morphology

As the compound **17** was found to be cytotoxic to DU145 cells, its effect on the morphology of cells was further ascertained. A 24 well plate was seeded with cells in a manner previously described and incubated for 18-20 h. Then the cells were treated with increasing concentrations of **17**. After another 48 h of incubation, the experiment was terminated and the cells were observed under the microscope and images were captured using Olympus Xi71 microscope.

4.16.3. Colony formation assay

The long term effect of **17** on the anchorage-independent growth of DU145 cells was analyzed by soft agar assays as reported previously with minor modifications [17]. In the soft agar assay, base agar was prepared by mixing 1% of agarose (Bacto Agar: Becton, Dickinson, Sparks, MD) with 2 × DMEM with 20% FBS and 2X antibiotics in 6-well plates

in order to achieve final concentration of 0.5% of agar in 1X growth medium with 10% serum concentration. After the solidification of the base agar, 2.5×10^4 cells were mixed with cultivation medium containing compound at varying concentrations and agar solution to obtain a final concentration of 0.35% agar. This was spread on top of the base agar previously solidified. The plate was incubated for 9 days with periodic replenishment every 3 days with medium and compound. Over period of time plates were monitored regularly for appearance of colonies. After 9 days of incubation the plates were stained with 0.005% crystal violet solution until colonies turned purple in colour. The excessive stain was washed off using MQ water and the colonies were photographed and counted using a microscope.

4.16.4. Determination of caspase-3 and -9 activities

Caspase-3 and -9 activities were measured by treating the DU145 cells with **17** using specific fluorogenic substrates Ac-DEVD-AMC and Ac-LEHD-AFC. DMSO and doxorubicin were taken as negative and positive controls respectively. After 48 h treatment of cells with **17**, harvested cells were lysed directly in caspase lysis buffer (50 mM HEPES, 5mM CHAPS, 5 mM DTT, pH 7.5). The lysates were incubated with the respective substrate (Ac-DEVD-AFC/Ac-LEHD-AMC) in 20mM HEPES (pH 7.5), 0.1% CHAPS, 2mM EDTA and 5 mM DTT at 37 °C for 2 h. The release of AFC and AMC was analyzed by a fluorometer using an excitation/emission wavelength of 400/505 nm (for AFC) and 380/460 nm (for AMC) which is directly proportional to caspase-9 and-3 activity respectively. The observed fluorescence values were normalized with total protein concentration estimated by Bradford method and the relative caspase activities were calculated as the ratio of values between mock treated (DMSO) and treated cells.

4.16.5. Senescence Assay

Small molecules displaying anticancer potential may induce senescence in cancer cells preventing their proliferation [17]. Thus, we have studied the ability of **17** to induce senescence in healthy DU145 cells. Induction of cellular senescence was monitored by measuring senescence-associated β-galactosidase (SA-βgal) activity (pH 6.0) assay in DU145 cells exposed to **17**. A 24 well plate was seeded with cells as previously described and treated with the compound and subsequently incubated for 48 h. After 48 h, the media was aspirated and the cells were washed with PBS (2 × 1 min). Cells were fixed by adding enough volume of fixation solution (2% formaldehyde and 0.2% glutaraldehyde in PBS solution) to submerge the cells in solution. After incubation for 5 minutes at room temperature the

fixation solution was removed and the cells were washed twice with PBS (2 × 1 min). Then the fixed cells were stained with freshly prepared staining solution (40 mM citric acid/Na phosphate buffer, 5 mM $K_4[Fe(CN)_6]3H_2O$, 5 mM $K_3[Fe(CN)_6]$, 150 mM sodium chloride, 2 mM magnesium chloride and 1 mg ml − 1 X-gal in distilled water) for overnight at 37 °C. The excess stain was removed by repeated washings with PBS and plate was allowed for drying at room temperature. The positively stained cells for SA-βgal levels were observed and photographed under a Olympus Xi71 microscope.

4.16.6. PI uptake for analysis of cell death

Cell death induced by **17** was determined as a measure of PI uptake. Cells were harvested after treatment with compound at desired concentration and fixed in 70% ethanol at -20 °C for overnight. All cells in the form of a pellet re-suspended in PI solution (RNase -0.1 mg/mL, Triton X-100-0.05%, PI-50 µg/mL) and incubated for 1 h in dark at room temperature. The excess PI solution was washed away by repeated washings with PBS buffer. The resultant PI uptake was analyzed by fluorescence activated cell sorting (FACS Calibur System; BD Bio- science, Erembodegem, Belgium) in a FL-2 fluorescence detector (10000 events were recorded for each condition). Flow cytometry data was analyzed using FCS express 4 software (De Novo Software, Los Angeles, CA).

4.16.7. Mitochondrial Membrane Potential assay:

The ability of **17** to induce apoptosis via caspase activation was analysed by determining the caspase activities in the treated cells. Mitochondrion plays an important role in activating these caspases. The detection of changes in mitochondrial inner membrane electrochemical potential in living cells was done using the cationic, lipophilic dye, JC-1 (5, 5', 6, 6'-tetracholoro-1, 1', 3, 3'-tetraethylbenzimidazolocarbocyanine iodide). In healthy cells the JC-1 dye is concentrated in the mitochondrial matrix and form of red fluorescent aggregates (J-aggregates). When the cells become apoptotic, the mitochondrial membrane potential gets affected and the JC-1 dye is released into the cytosol in the form of monomers, leading to a shift from red fluorescence (Rhodamine) to green fluorescence (FITC). In the assay conducted, we observed whether compound **17** shows any effect on the mitochondrial membrane. The DU145 cells were seeded in 24 well plate and incubated for 18 h at 37 °C with constant supply of 5% CO_2. When the cells had adhered to the surface of the well, they were treated with either **17** or DMSO as control. After 24 h of post treatment with **17**, the

cells were stained with JC-1 dye (0.625% MilliQ water). The working dye solution was prepared by mixing equal volumes of the stock dye complete growth medium. The media in the wells was replaced with dye mix and incubated for 20 minutes at 37 °C in a humidified incubator. After aspiration of staining solution cells were washed with growth medium for two times and covered with growth medium. The resultant fluorescence was observed under the microscope (Olympus Xi71, Japan).

Acknowledgments

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Figure Captions:

Table 1: Four representative cell lines were tested with the series of compounds to determine their cytotoxicity. Table shows the IC_{50} values of the compounds against the cell lines.

Table 2: Compound **17** induced G0/G1 phase cell cycle arrest in DU145 cells. Cells were treated with varying concentrations of **17** (5, 10, 15, 20 and 25 μM) for 48 h and cell cycle progression was examined by flow cytometry. Table shows the percentage cell fractions in G0/G1, S and G2/M phases of **17** treated DU145 cells.

Figure 1: Some of the active oxazolidinone derivatives.

Figure 2: Structure of linezolid **3** and new proposed structures (**I**, **II**)

Figure 3: DU145 cell were treated with compound **17** at indicated concentration or DMSO. Upon exposure of DU145 cells to **17** the extent of change in cell morphology of cells is observed with increasing concentration.

Figure 4: Long term effect of compound **17** on the number of colony-forming DU145 cells. DU145 cells were treated with desired concentration of **17** (0−25 μM) and allowed to grow for 9 days to form colonies. Representative images of the colony-forming assay are shown here. Number of colonies and their size formed by DU145 in soft agar is decreased on exposure to **17**

Figure 5: Cell cycle analysis of DU145 cells treated with **17**. Cells were treated with either DMSO or **17** and the DNA content was measured by propidium iodide staining. To determine the distribution of cells in various phases of cell cycle was taken as control. The percentage of cells increased in G0/G1 phase up on exposure to **17** suggesting its role in G0/G1 arrest.

Figure 6: Effect of **17** on activation of caspases inducing apoptosis in DU145 cells. Treatment of DU145 cells with different concentration of **17** for 48 h induced activation of caspases-3 (A) and caspase-9 (B) significantly in concentration dependent manner. Doxorubicin was used as control for activation of both caspases. All experiments were carried out in triplicates and mean values are presented here.

Figure 7: Effect of **17** on mitochondrial membrane potential inducing activation of caspase 9 was determined by staining the cells exposed to **17** with JC-1 dye. Cell treated with DMSO show red fluorescence as the JC-1 dye is localized and aggregated in the mitochondrial membrane. In cells exposed to **17**, green fluorescence is increased where as red aggregated

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Scheme 1: Retrosynthetic analysis of new proposed structures **(I & II)**

Scheme 2: Synthesis of key intermediate **6**

Scheme 3: Synthesis of analogues **9**, **10**, **11**

Scheme 4: Synthesis of analogues **5, 13, 14**

Scheme 5: Synthesis of analogues **15, 16**

Scheme 6: Synthesis of analogues **17, 18**

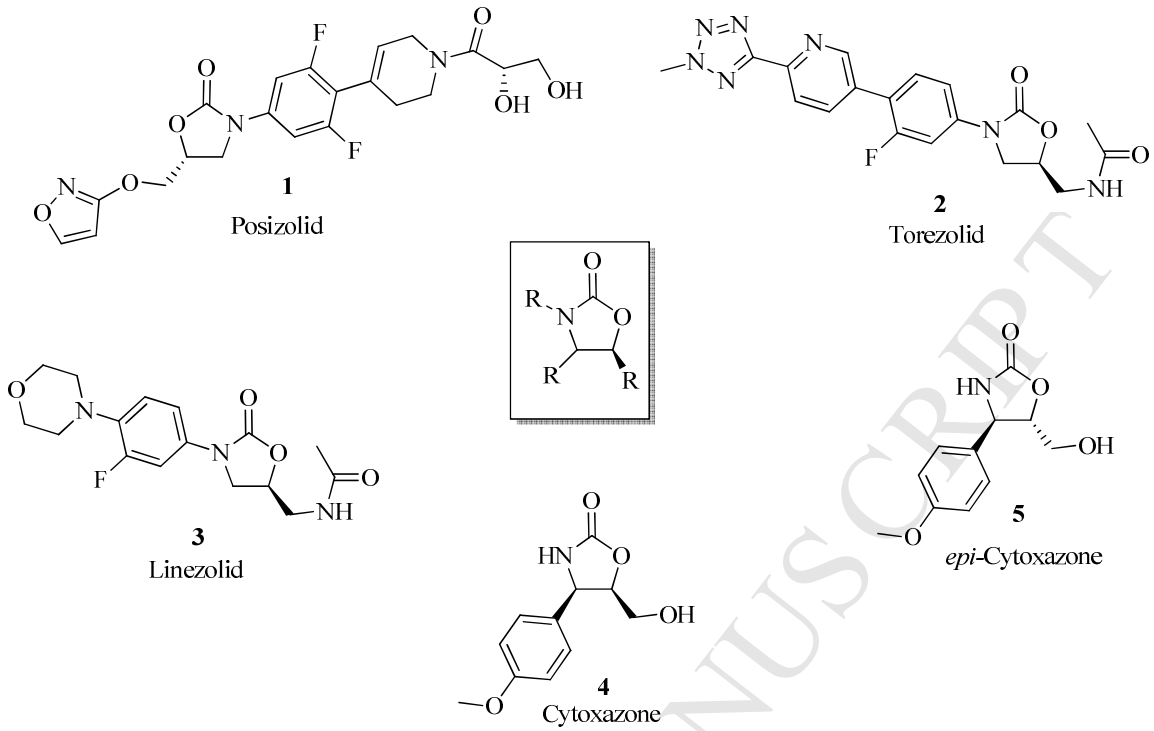
Scheme 7: Synthesis of analogues **20, 21**

Table 1: Four representative cell lines were tested with the series of compounds to determine their cytotoxicity. Table shows the IC_{50} values of the compounds against the cell lines.

Compound No.	A549		DU145		HELA		MCF 7	
	IC_{50}	Std. Dev	IC_{50}	Std. Dev	IC_{50}	Std. Dev	IC_{50}	Std. Dev
4	25.53	2.74	46.45	1.38	26.02	3.3	82.42	10.35
9	43.17	7.87	38.51	4.63	35.89	12.04	36.11	7.45
10	42.4	11.59	31.76	2.55	28.61	7.58	61.54	6.93
11	24.81	4.2	30.78	5.76	19.19	0.7	69.42	33.88
5	31.15	12.24	40.62	24.24	56.69	25.69	>100	0
12	21.31	7.57	32.8	3.53	39.77	2.15	>100	0
13	24.45	11.49	48.16	20.14	48.58	17.55	69.96	8.73
14	24.64	8.74	24.85	12.93	86.56	25.33	>100	0
15	23.87	7.72	21.13	15.58	57.65	2.69	74.03	14.5
16	14.15	1.46	9.47	0.18	42.66	5.84	38.22	10.13
17	13.4	1.22	8.69	0.38	23.31	2.04	>100	0
18	30.31	2.78	69.52	30.51	43.17	8.86	>100	0
20	21.16	5.99	33.03	7.28	>100	0	>100	0
21	54.4	20.64	26.84	8.72	51.27	6.33	>100	0
Doxorubicin	8.4	0.12	6.7	0.1	10.89	0.09	8.62	0.19

Table 2: Compound **17** induced G0/G1 phase cell cycle arrest in DU145 cells. Cells were treated with varying concentrations of **17** (5, 10, 15, 20 and 25 µM) for 48 h and cell cycle progression was examined by flow cytometry. Table shows the percentage cell fractions in G0/G1, S and G2/M phases of **17** treated DU145 cells.

	G0/G1	S	G2/M
DMSO	70.54	5.77	23.09
5 µM	75.65	4.61	19.45
10 µM	75.29	3.85	20.49
15 µM	79.14	3.56	16.7
20 µM	80.5	1.93	16.19
25 µM	91.11	0.79	6.54



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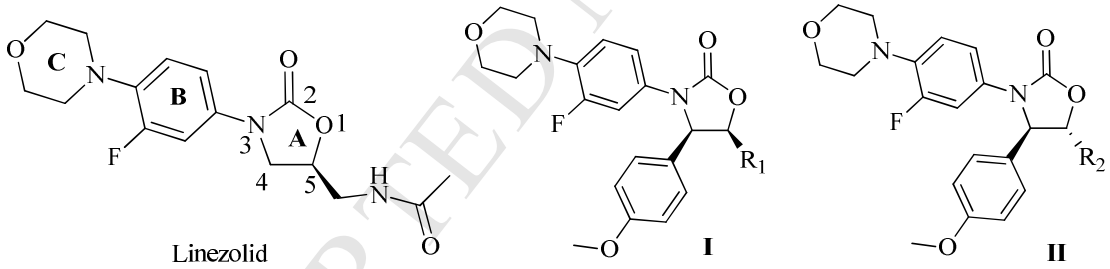

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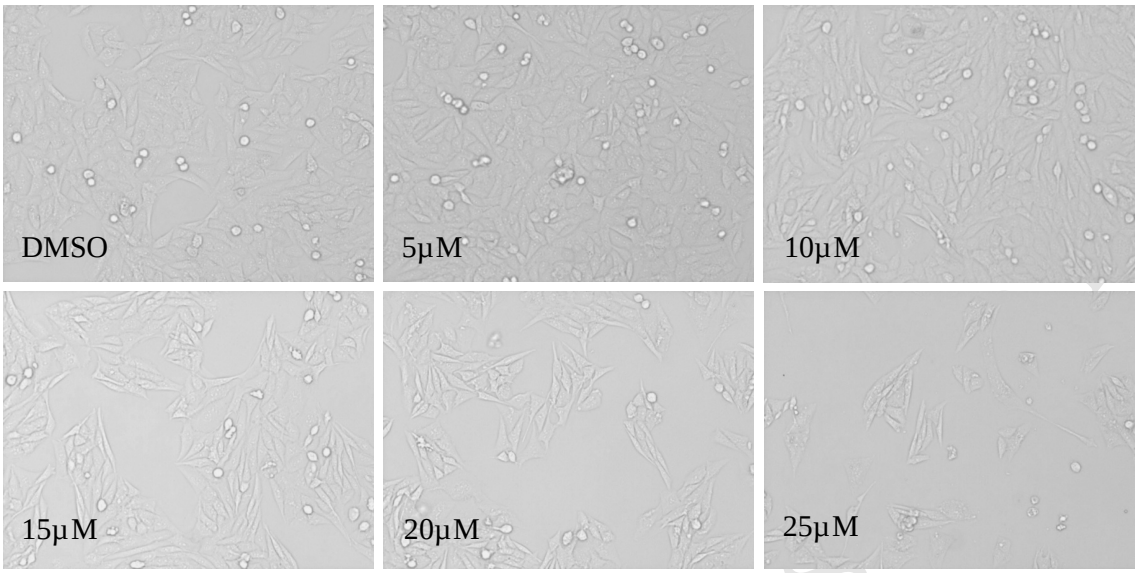

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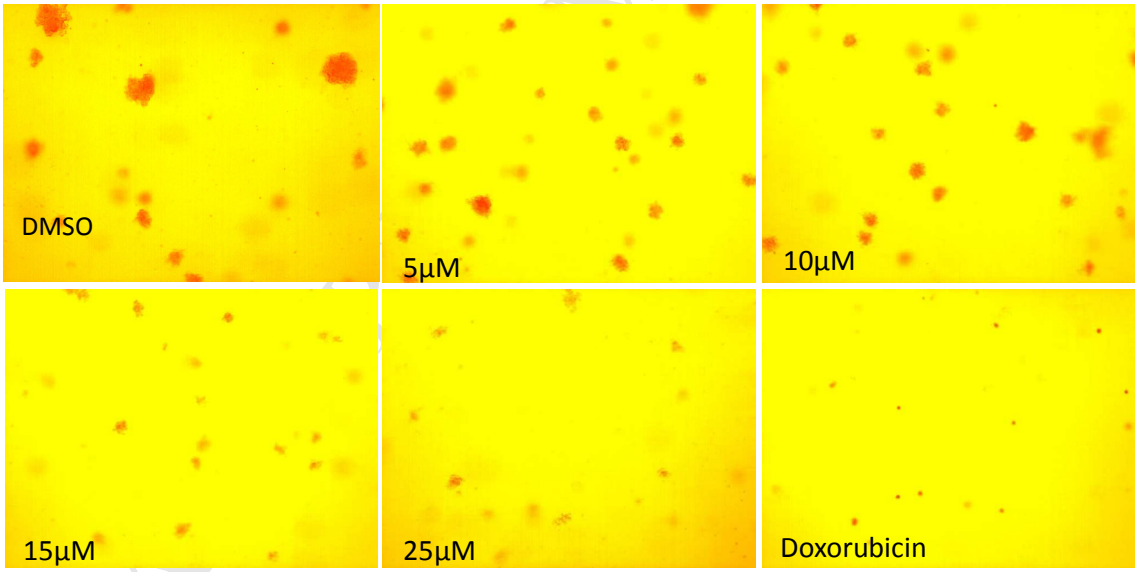

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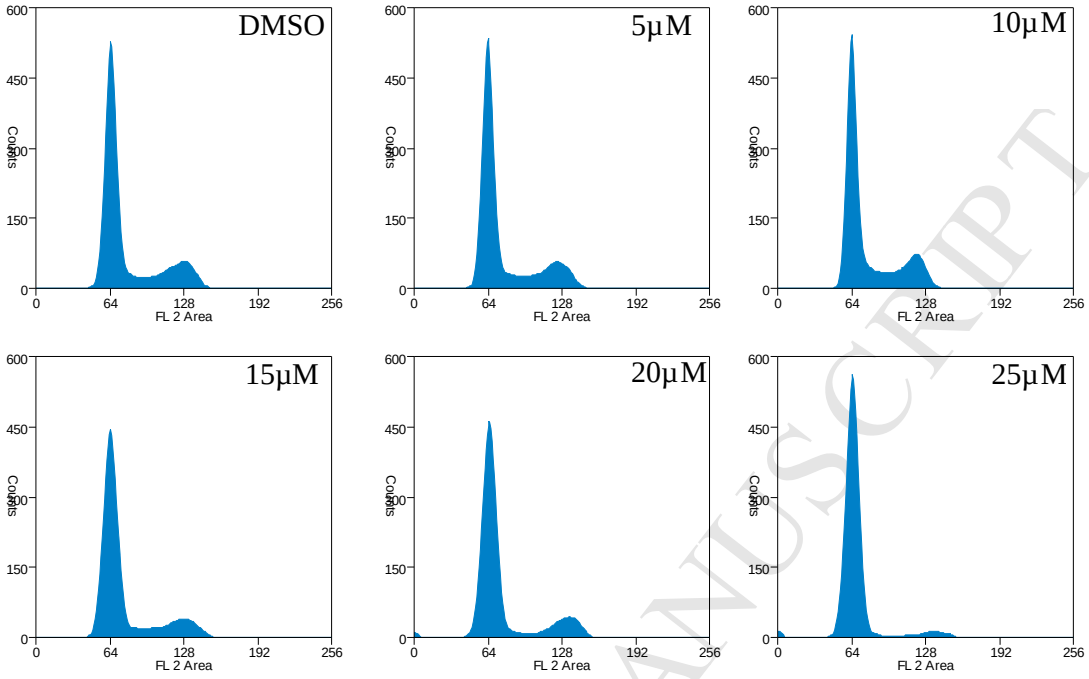

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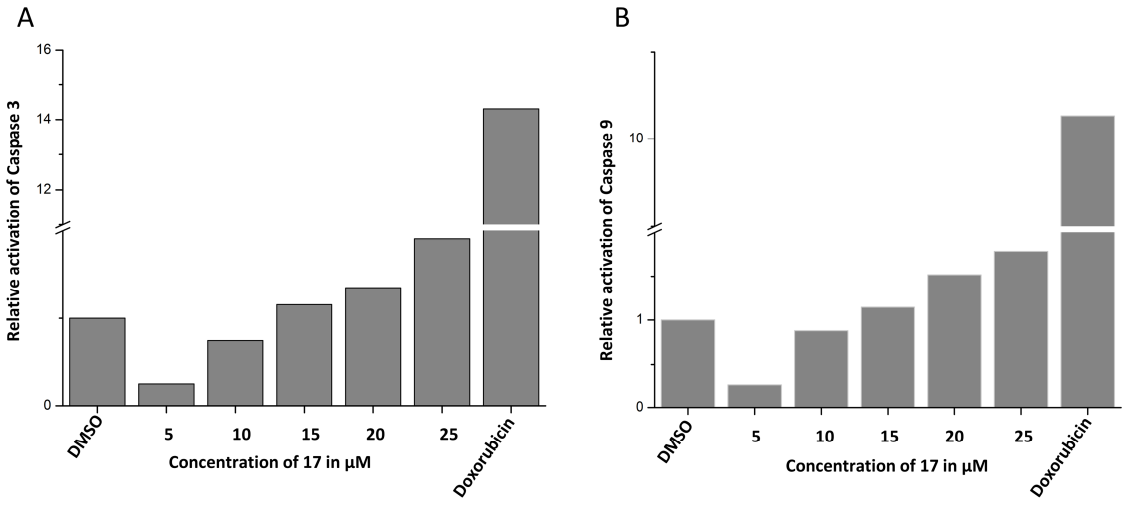

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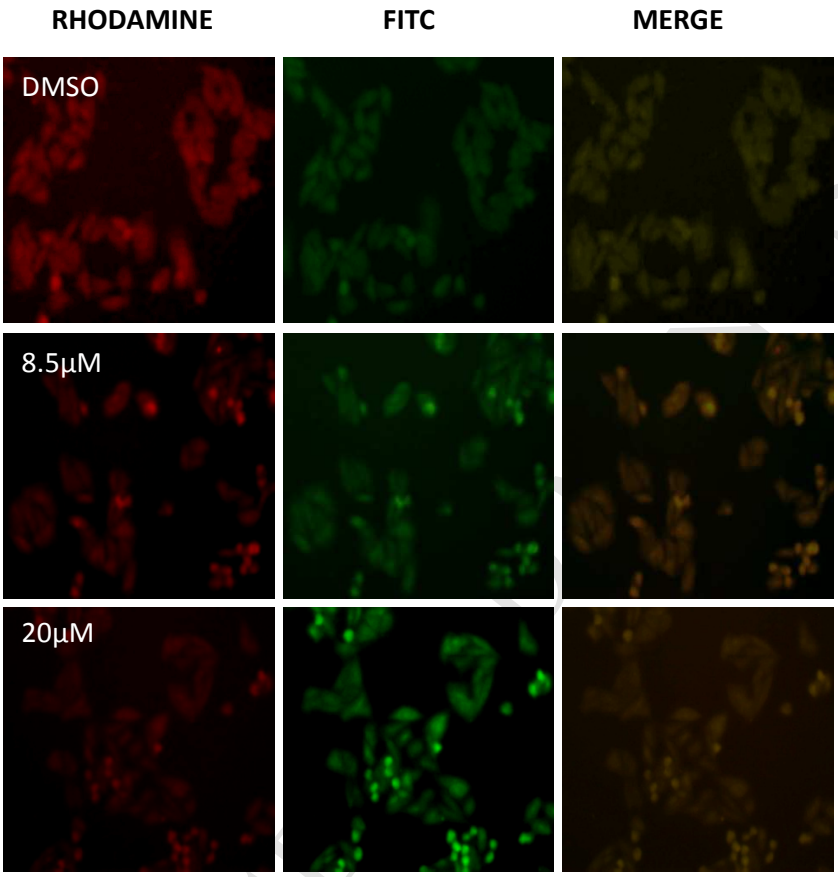

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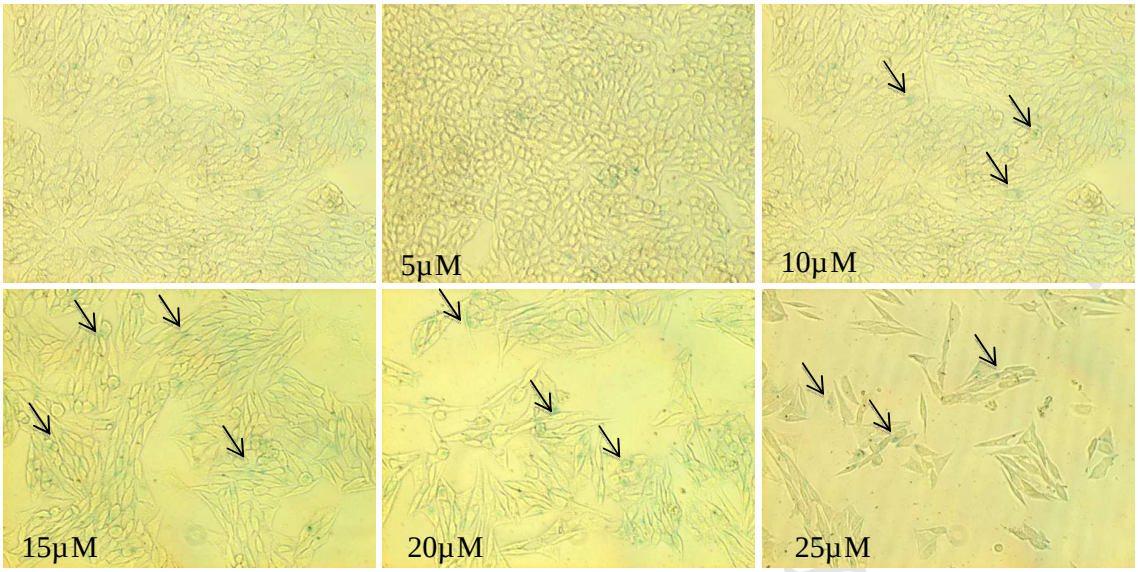

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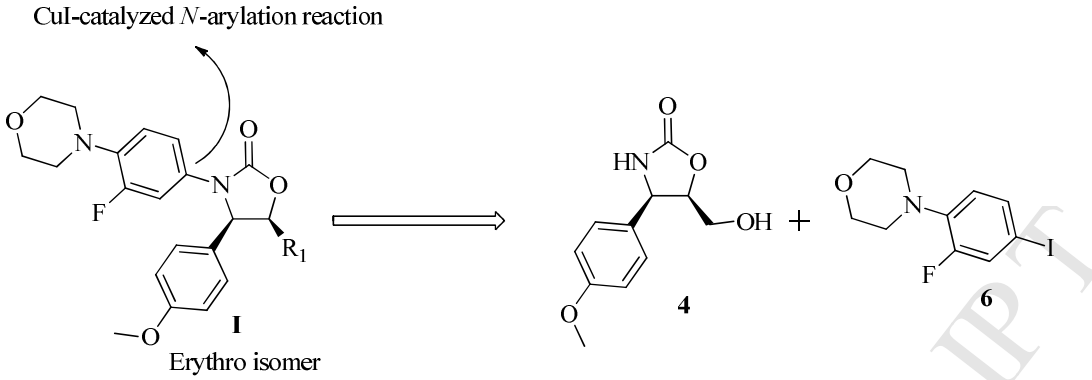
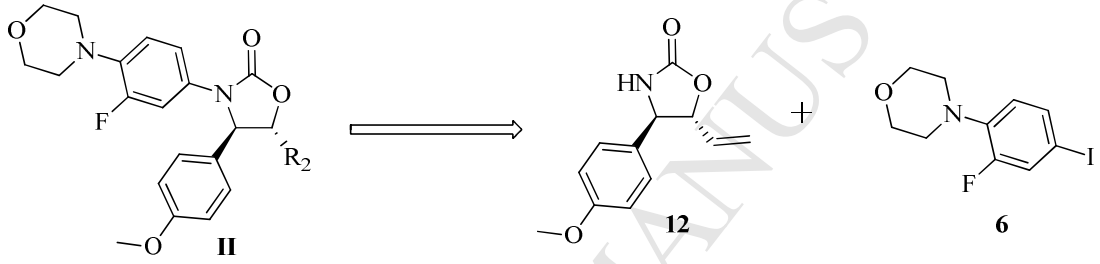
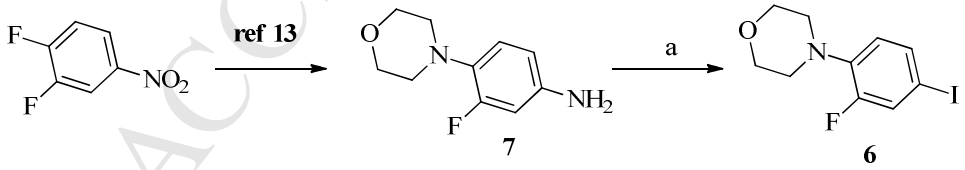


9 $R_1 = CH_2OH$
10 $R_1 = CH_2OCOCH_2CH_2OCOCH{=}CH_2$
11 $R_1 = CH_2OCOC_6H_5$

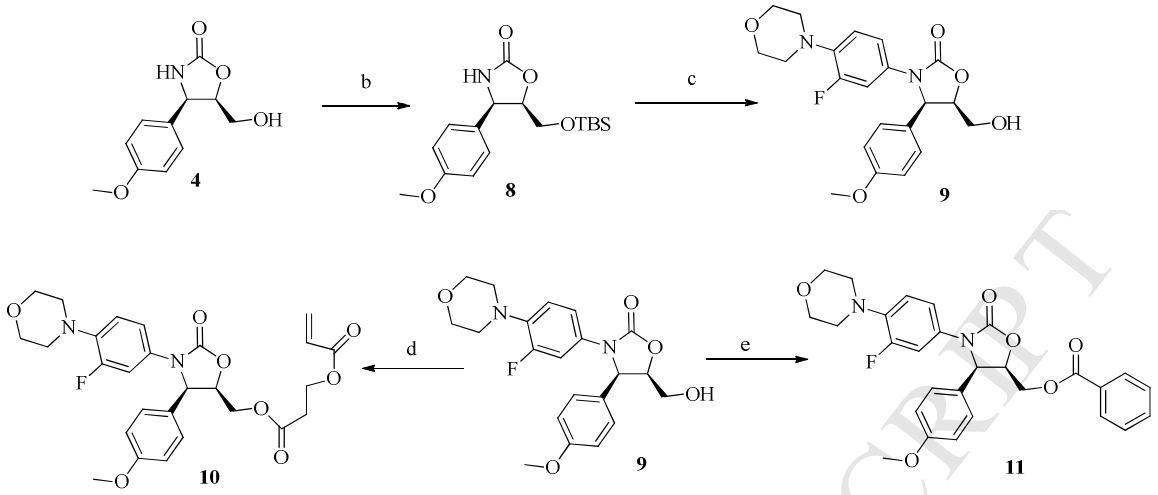


13 $R_2 = CH{=}CH_2$
14 $R_2 = CH_2OH$
15 $R_2 = CH_2OCOC_6H_5$
16 $R_2 = CH_2OCOCH_2CH_2OCOCH{=}CH_2$
17 $R_2 = CH_2CH_3$
18 $R_2 = CH_2CH_2OH$
20 $R_2 = CH_2NH_2$
21 $R_2 = CH_2NHCOCH_3$

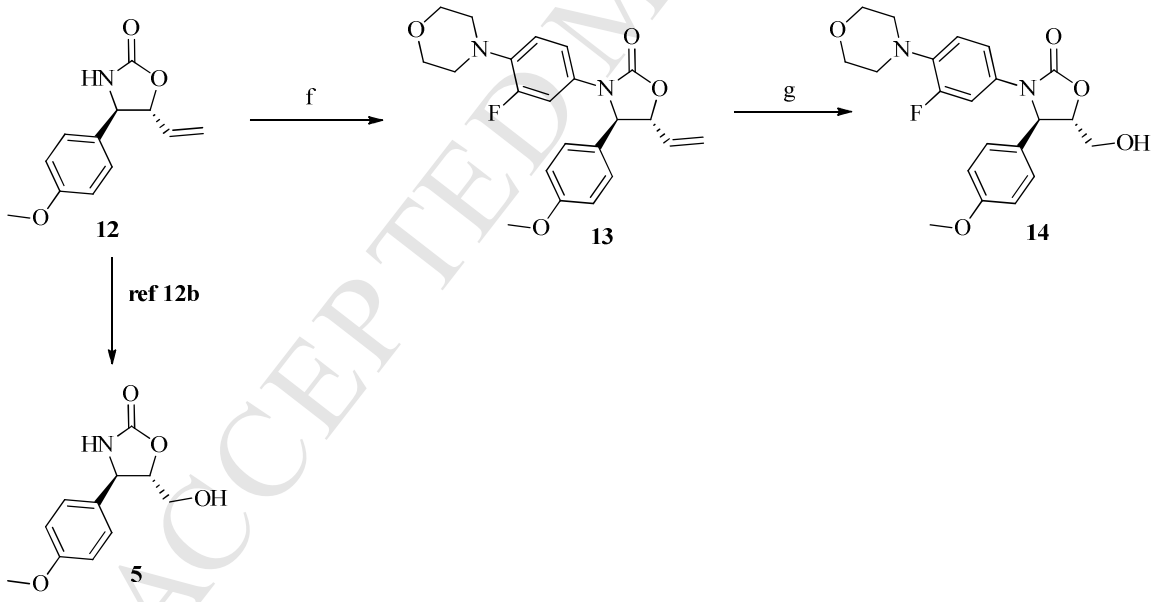
Scheme 1: Retrosynthetic analysis of new proposed structures **(I & II)**



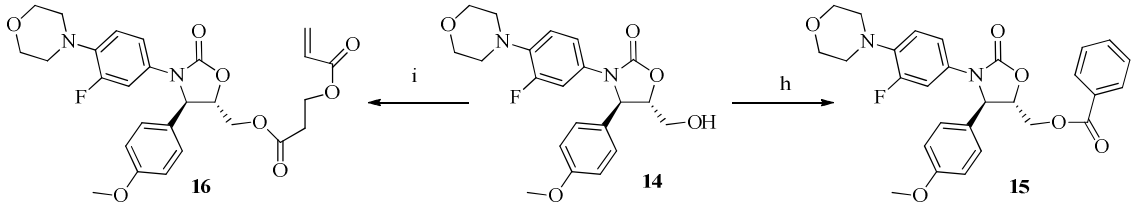
Scheme 2: Synthesis of key intermediate **6**. Reagents and conditions (a) $NaNO_2$, PTSA, KI, H_2O, CH_3CN, 10-25 °C, 1 h, 85%.



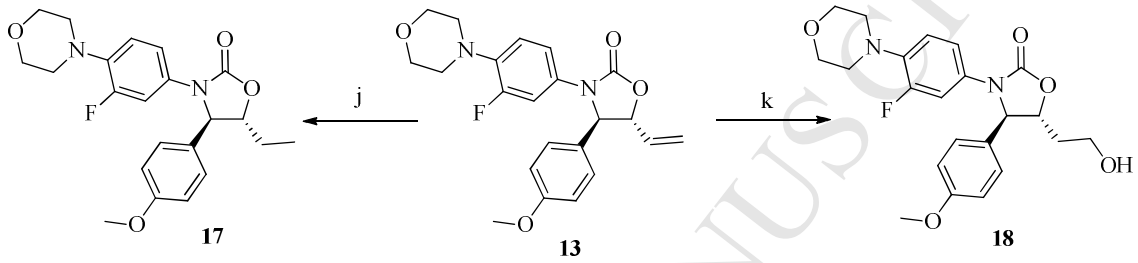
Scheme 3: Synthesis of analogues **9**, **10**, **11.** Reagents and conditions (b) TBSCl, imidazole, 0-25 °C, 1 h, 94%; (c) **6,** CuI, Cs_2CO_3, 1,4-dioxane, *N*,*N*'-dimethylethylenediamine, 100 °C, 4 h, then TBAF, 0-25 °C, 30 min, 80% over two steps; (d) acrylic acid, DCC, DMAP, CH_2Cl_2, 0-25 °C, 87 %; (e) benzoic anhydride, Et_3N, CH_2Cl_2, DMAP, 0-25 °C, 1 h, 90%.



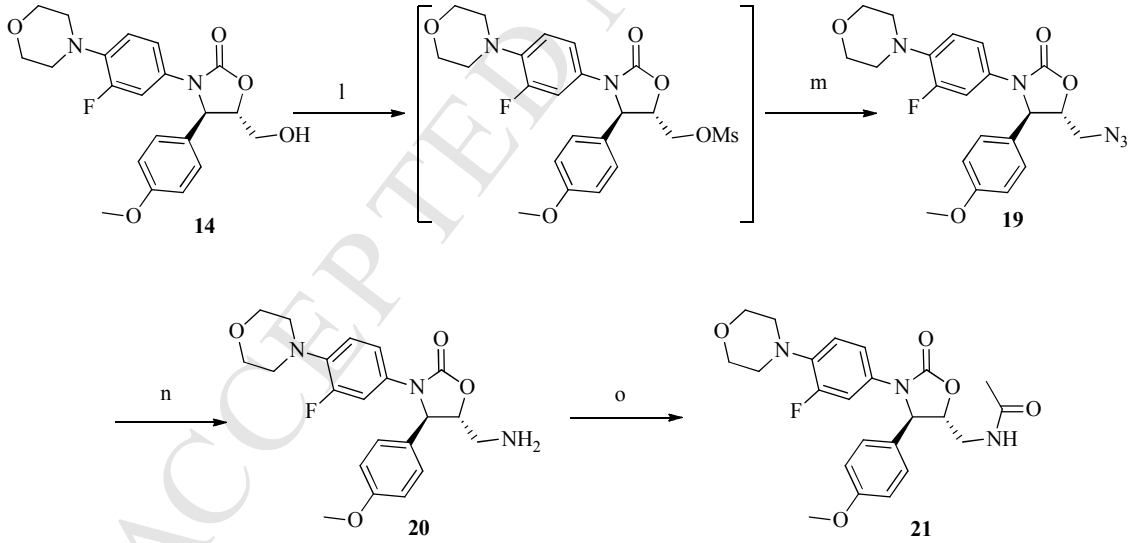
Scheme 4: Synthesis of analogues **5, 13, 14.** Reagents and conditions (f) **6,** CuI, Cs_2CO_3, 1,4-dioxane, *N*,*N*'-dimethylethylenediamine, 100 °C, 4 h, 90%; (g) O_3, CH_2Cl_2, -78 °C then $NaBH_4$, MeOH, 87%.



Scheme 5: Synthesis of analogues **15**, **16.** Reagents and conditions (h) benzoic anhydride, Et_3N, CH_2Cl_2, DMAP, 0-25 ºC, 1 h, 77%; (i) acrylic acid, DCC, DMAP, CH_2Cl_2, 0-25 ºC, 85%.



Scheme 6: Synthesis of analogues **17**, **18**. Reagents and conditions (j) H_2, Pd-C, MeOH, rt, 16 h, 85%; (k) BH_3.DMS, THF, 0-25 ºC, 2 h, H_2O_2, NaOH, 75%.



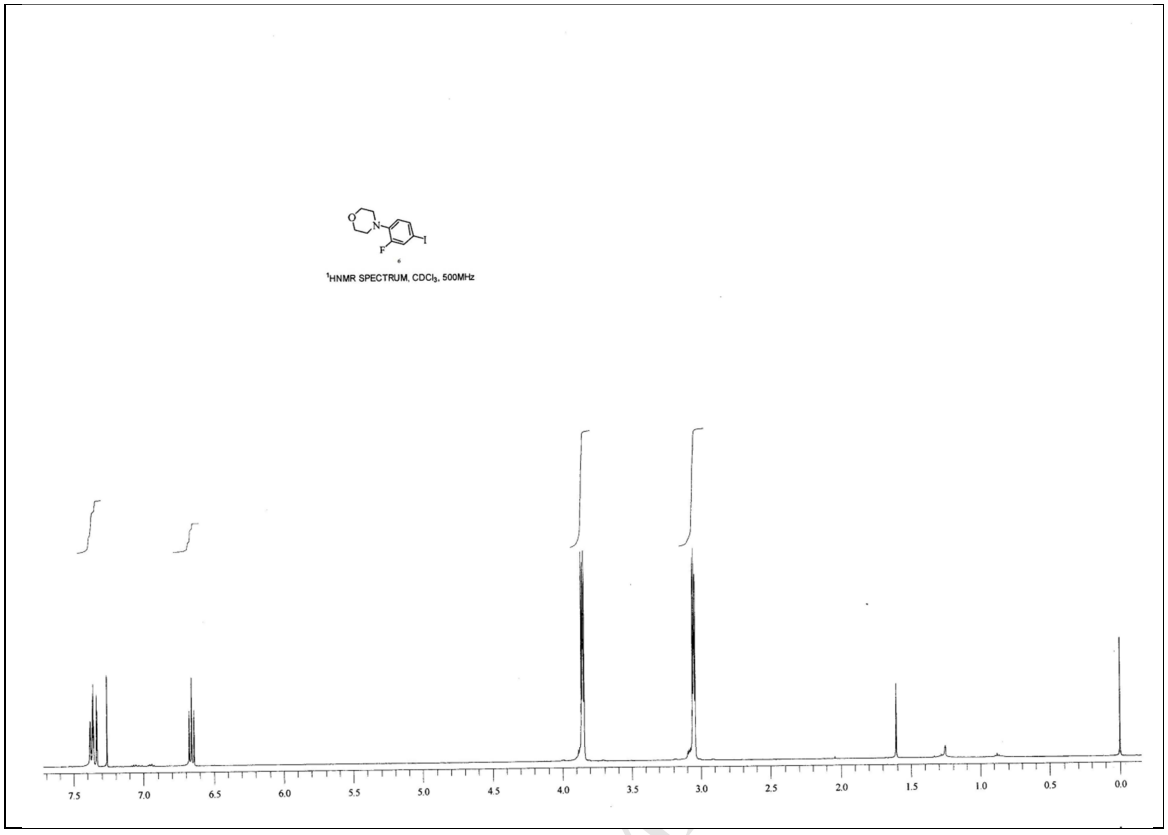
Scheme 7: Synthesis of analogues **20**, **21**. Reagents and conditions l) (i) MsCl, Et_3N, CH_2Cl_2,0-25 ºC, 1 h, (ii) NaN_3, DMF, 100 ºC, 2 h, 86%; (n) H_2-Pd/C, MeOH, rt, 16 h, 73%; (o) Ac_2O, Et_3N, CH_2Cl_2, 0-25 ºC, 1 h, 85%.

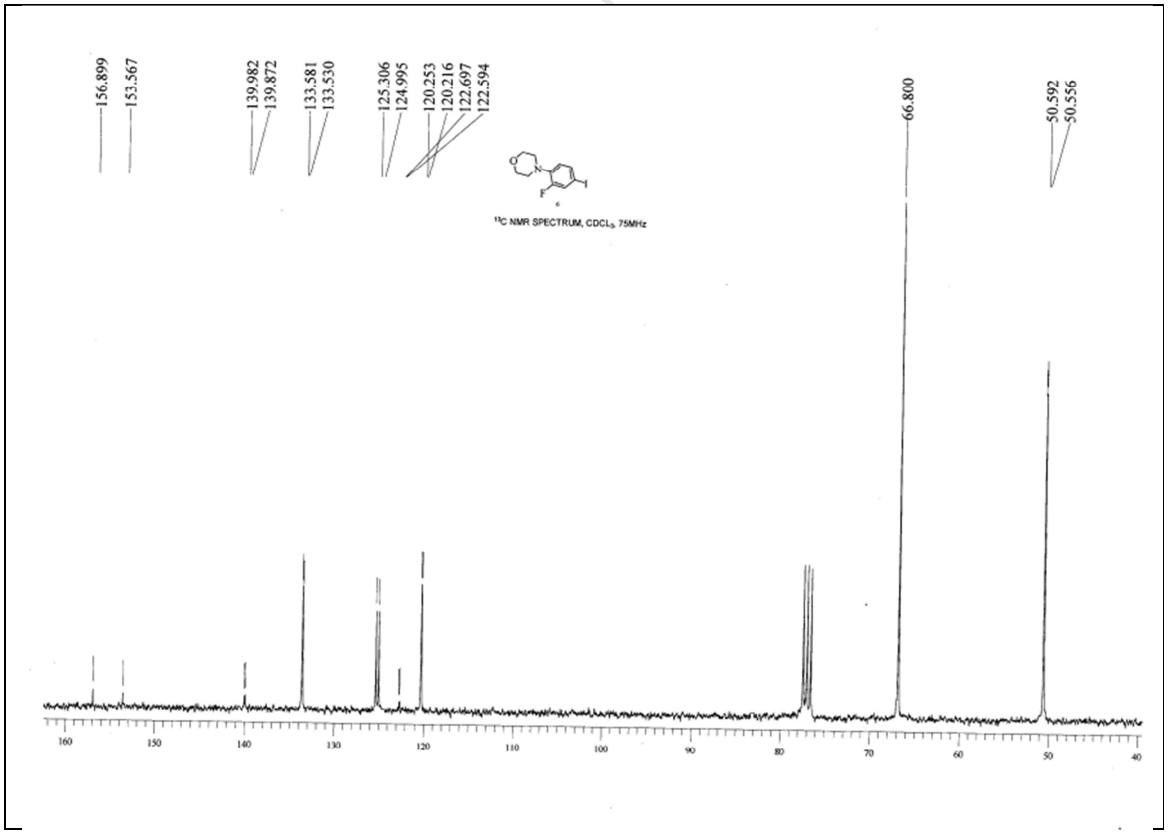
Supplementary Data

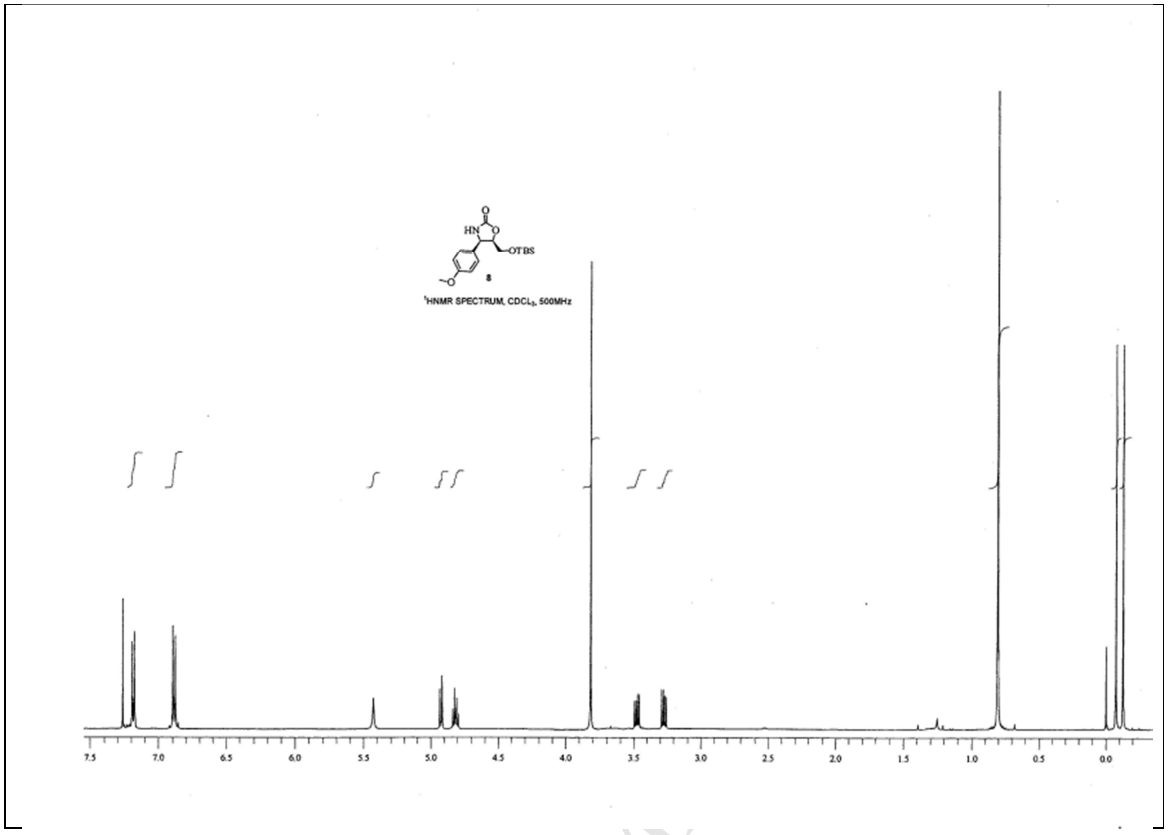
Oxazolidinone derivatives: Cytoxazone-Linezolid hybrids induces apoptosis and senescence in DU145 prostate cancer cells

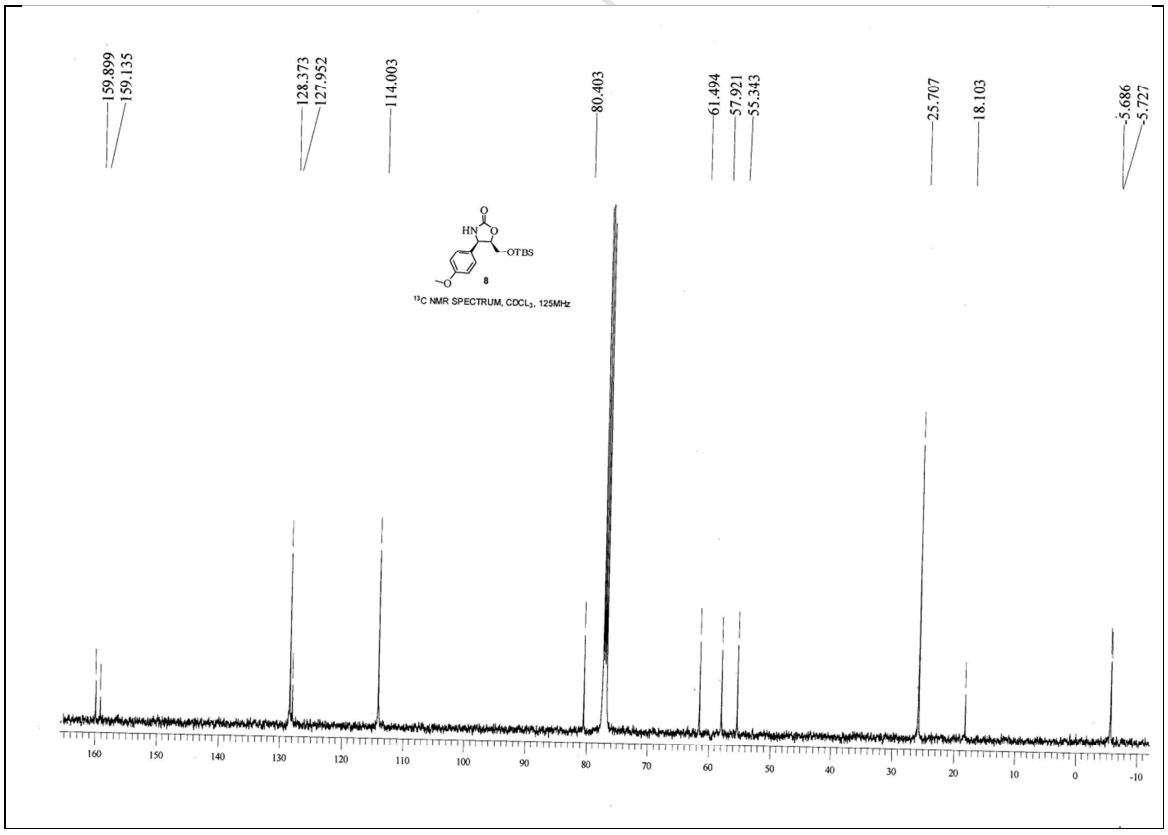
Annavareddi Naresh[a], Maddimsetti Venkateswara Rao[a], Sudha Sravanti Kotapalli[b], Ramesh Ummanni[b]*, Batchu Venkateswara Rao[a]*

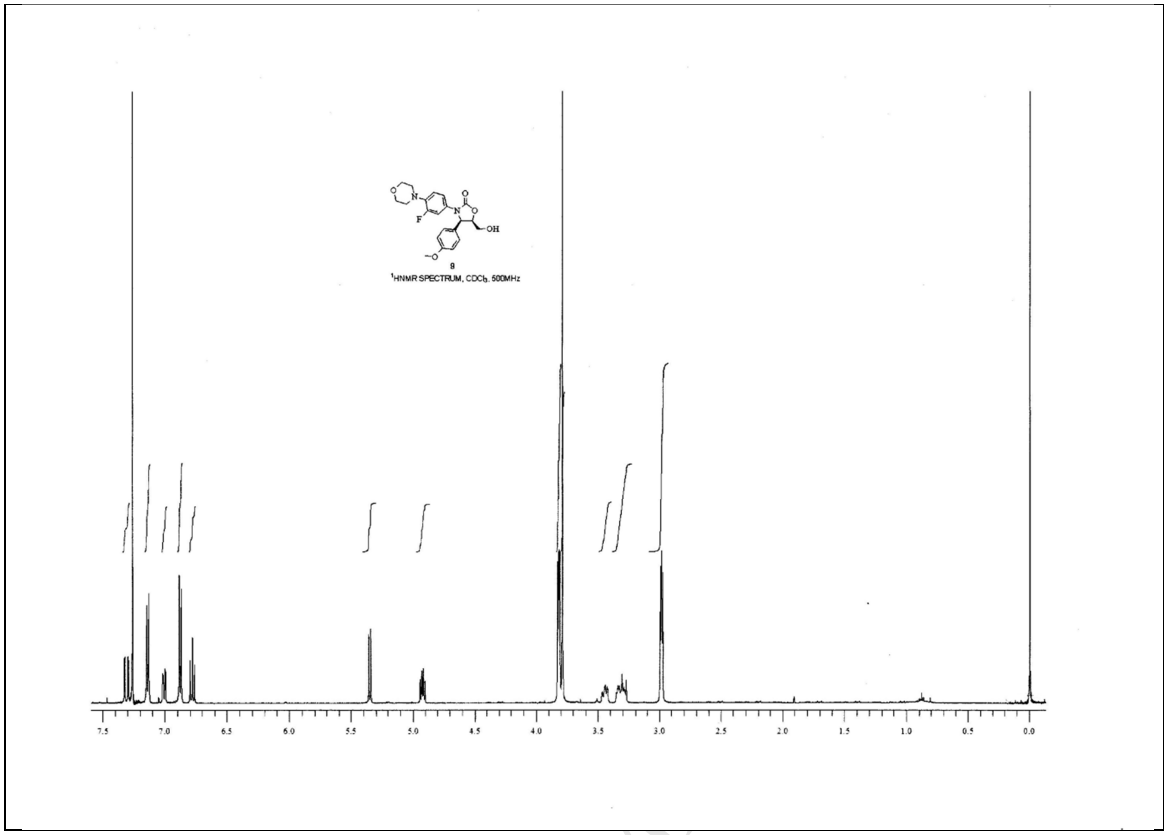
Copies of ^{1}H NMR and ^{13}C NMR spectra of **6, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21**.

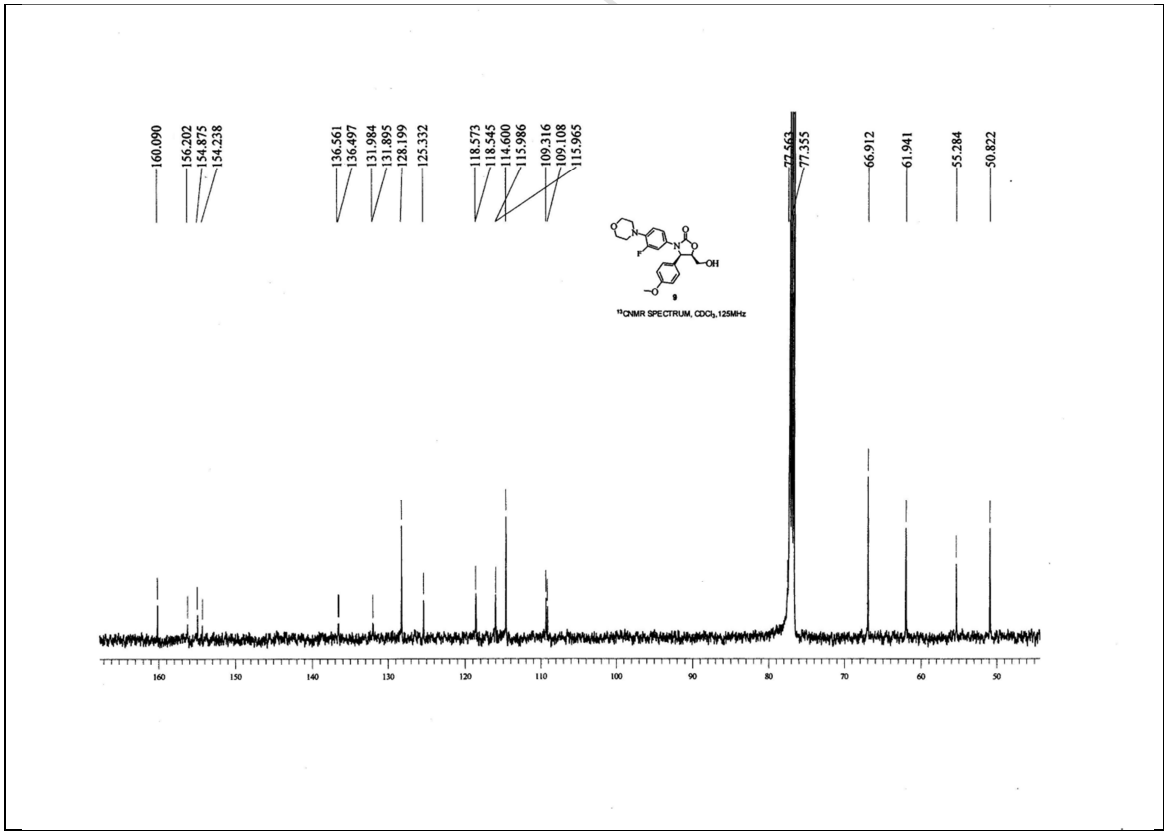

^{1}HNMR SPECTRUM, $CDCl_3$, 500MHz
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

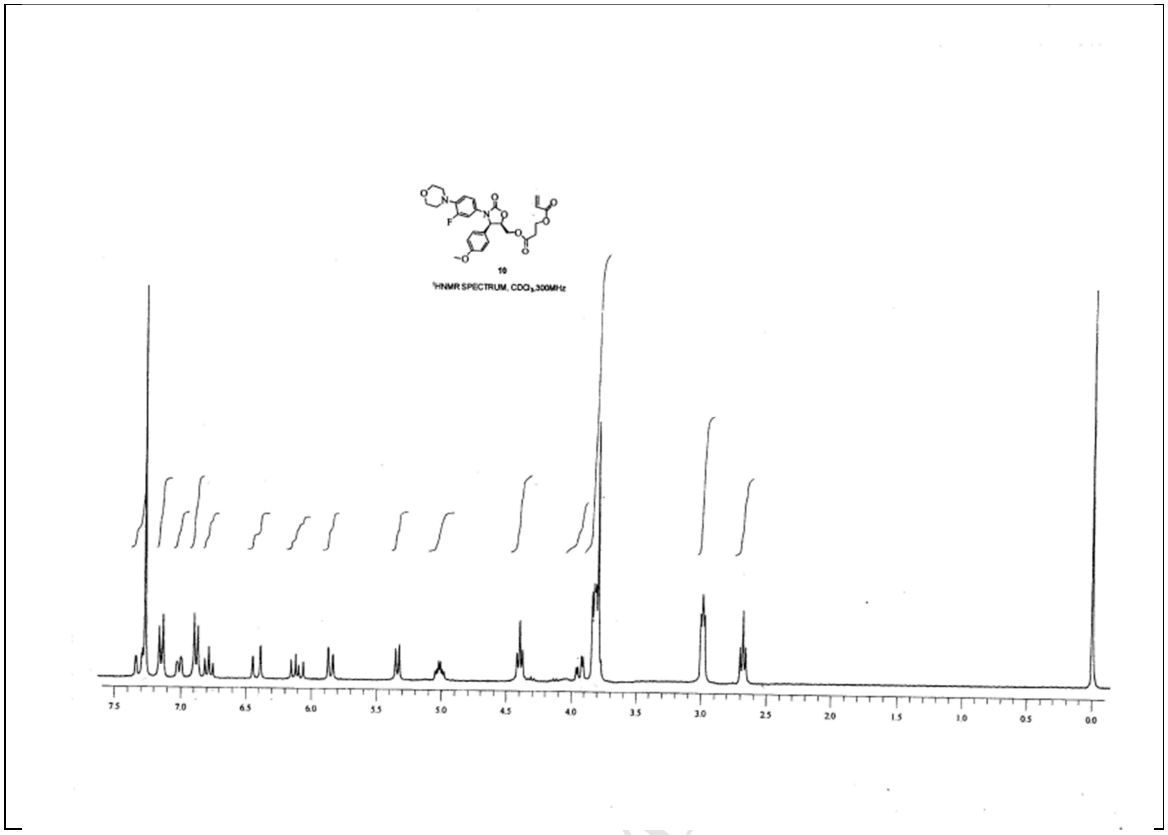

156.899
153.567
139.982
139.872
133.581
133.530
125.306
124.995
120.253
120.216
122.697
122.594
66.800
50.592
50.556
^{13}C NMR SPECTRUM, $CDCl_3$, 75MHz
160
150
140
130
120
110
100
90
80
70
60
50
40

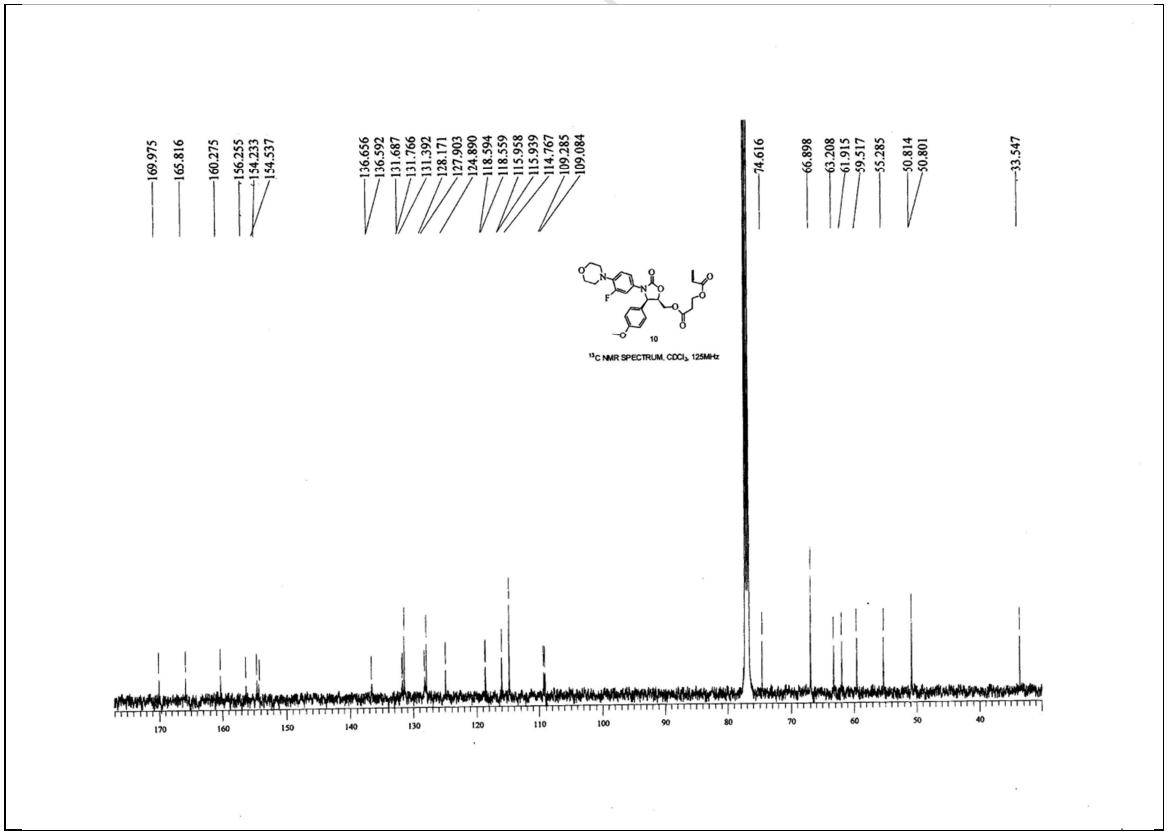

HN
O
O
OTBS
O
8
¹HNMR SPECTRUM, CDCl₃, 500MHz
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

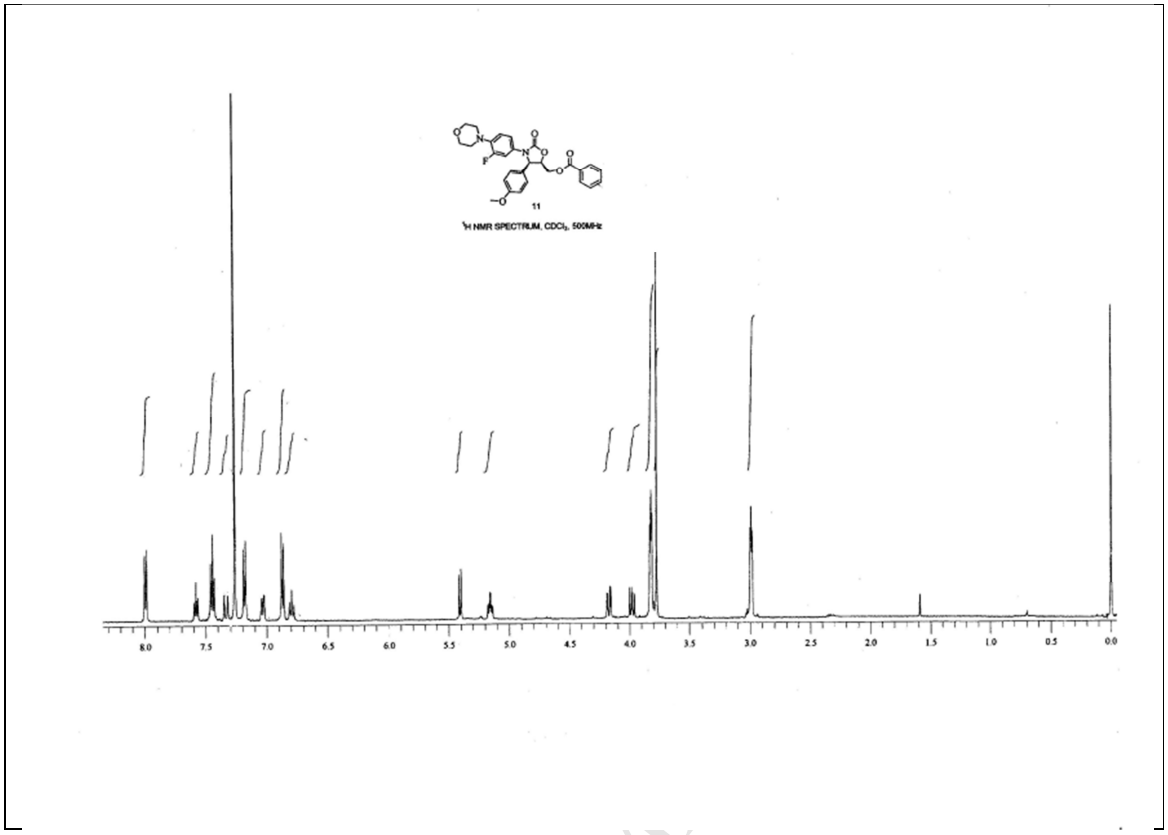

159.899
159.135
128.373
127.952
114.003
80.403
61.494
57.921
55.343
25.707
18.103
-5.686
-5.727
HN
O
O
OTBS
O
8
¹³C NMR SPECTRUM, CDCl₃, 125MHz
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

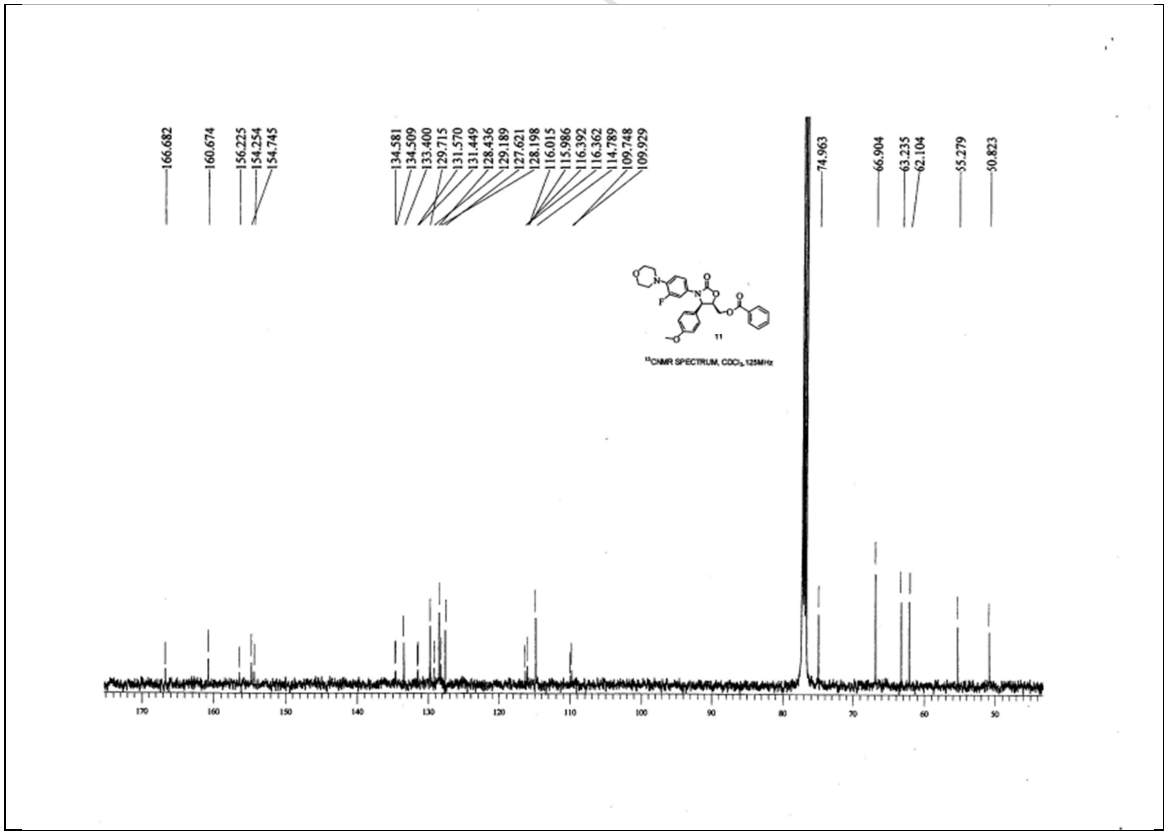

9
^{1}HNMR SPECTRUM, $CDCl_3$, 500MHz

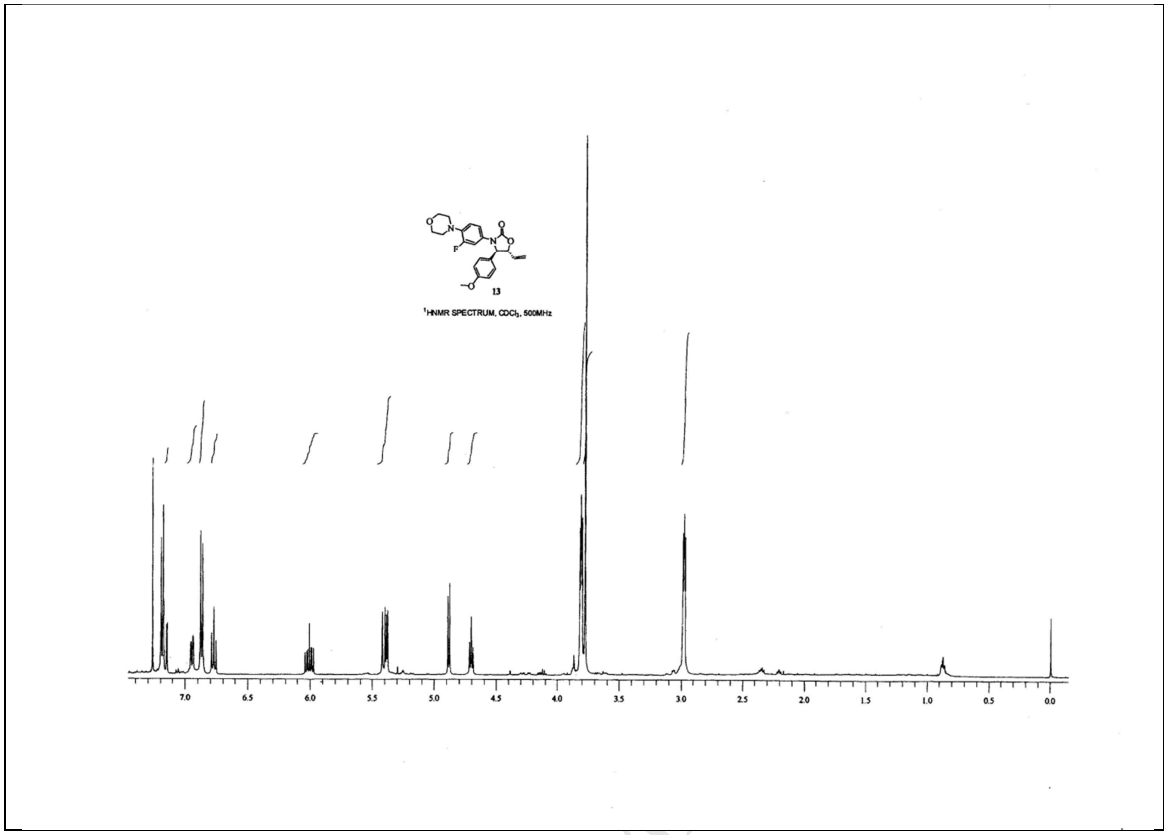

160.090
156.202
154.875
154.238
136.561
136.497
131.984
131.895
128.199
125.332
118.573
118.545
114.600
115.986
109.316
109.108
115.965
77.563
77.355
66.912
61.941
55.284
50.822
9
^{13}CNMR SPECTRUM, $CDCl_3$, 125MHz

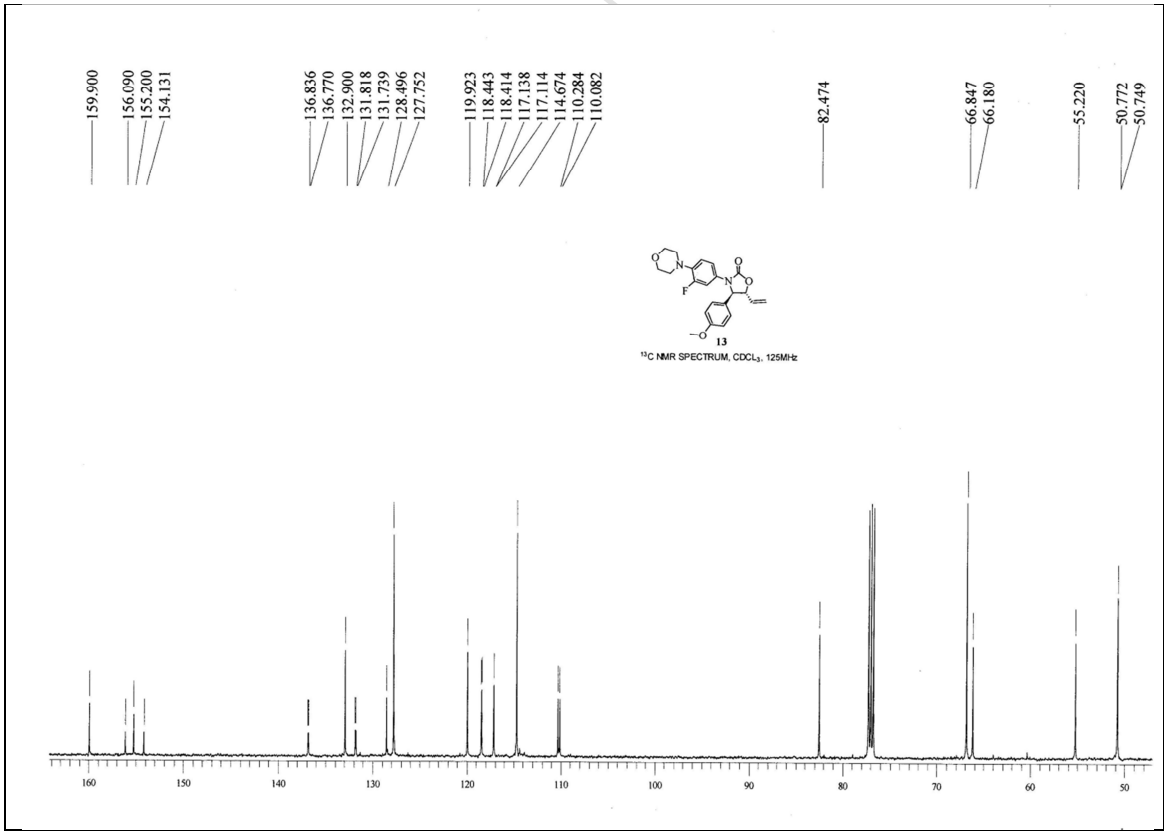

10
^{1}HNMR SPECTRUM, $CDCl_3$ 300MHz
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

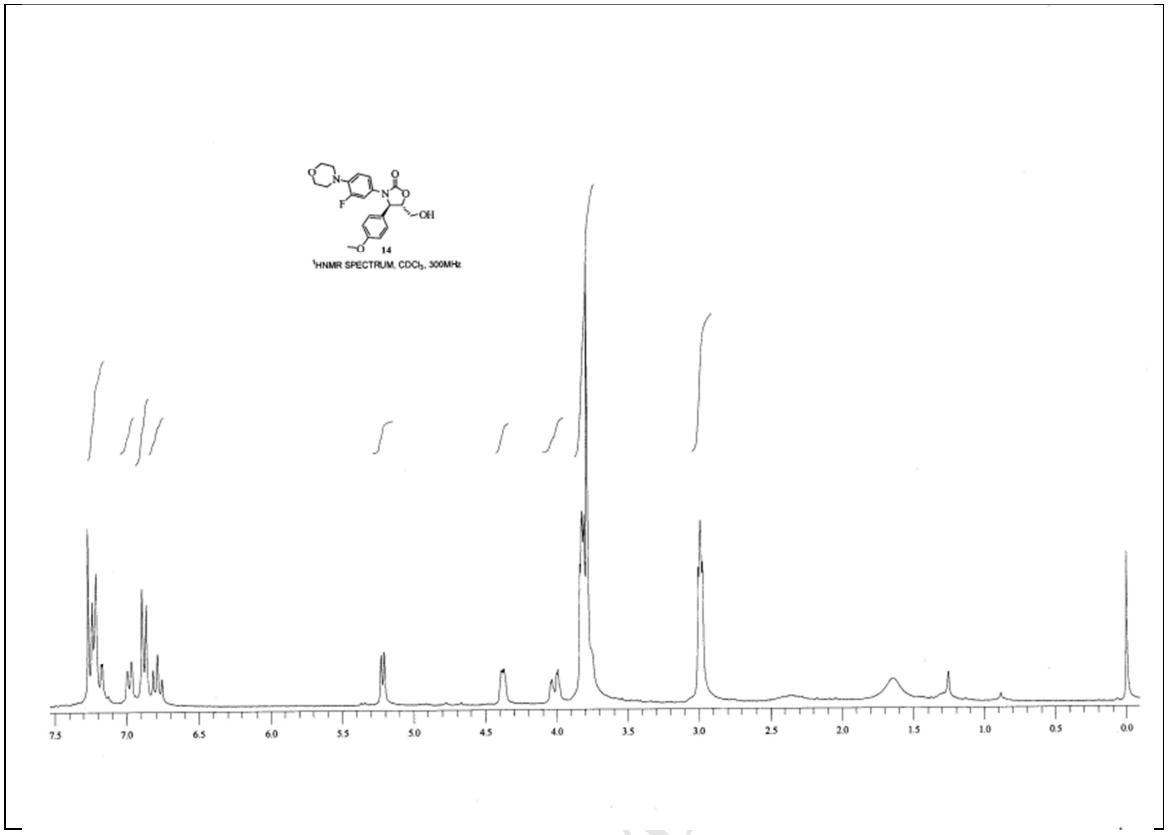

169.975
165.816
160.275
156.255
154.233
154.537
136.656
136.592
131.687
131.766
131.392
128.171
127.903
124.890
118.594
118.559
115.958
115.939
114.767
109.285
109.084
74.616
66.898
63.208
61.915
59.517
55.285
50.814
50.801
33.547
10
^{13}C NMR SPECTRUM, $CDCl_3$ 125MHz
170
160
150
140
130
120
110
100
90
80
70
60
50
40

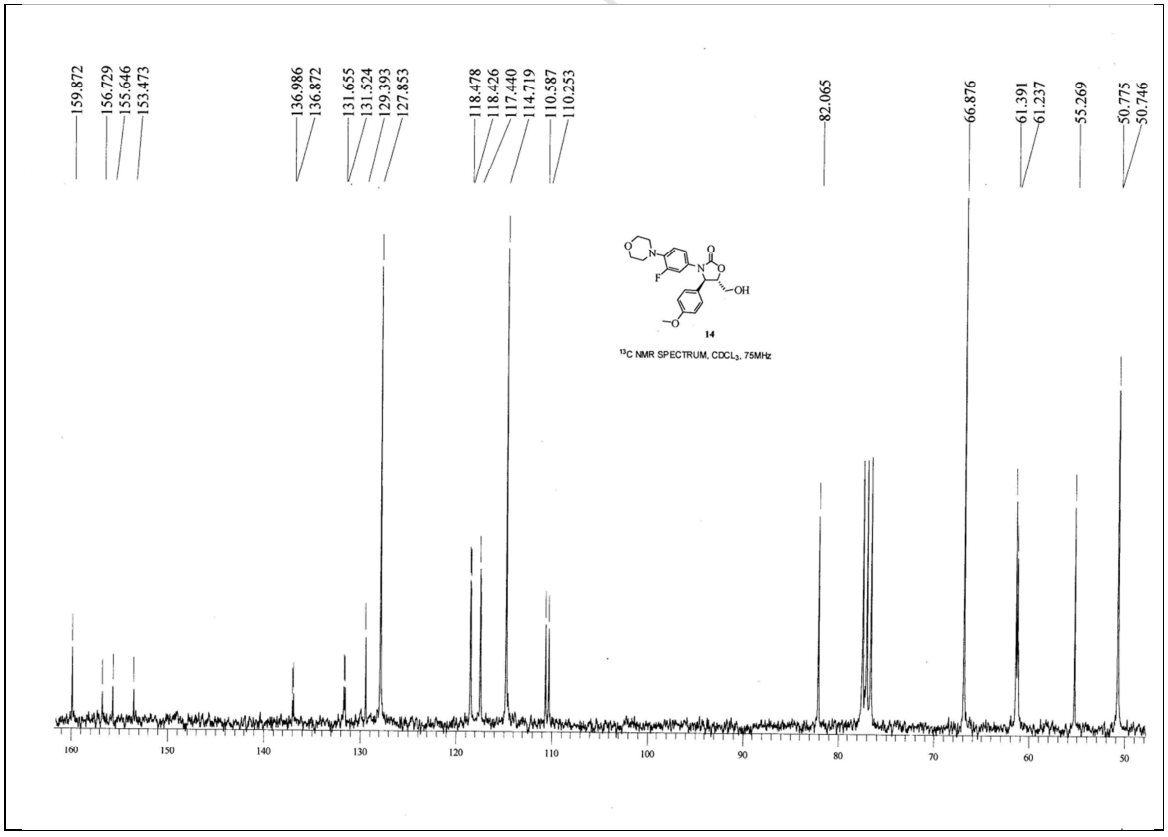

11
^{1}H NMR SPECTRUM, CDCl$_3$, 500MHz
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

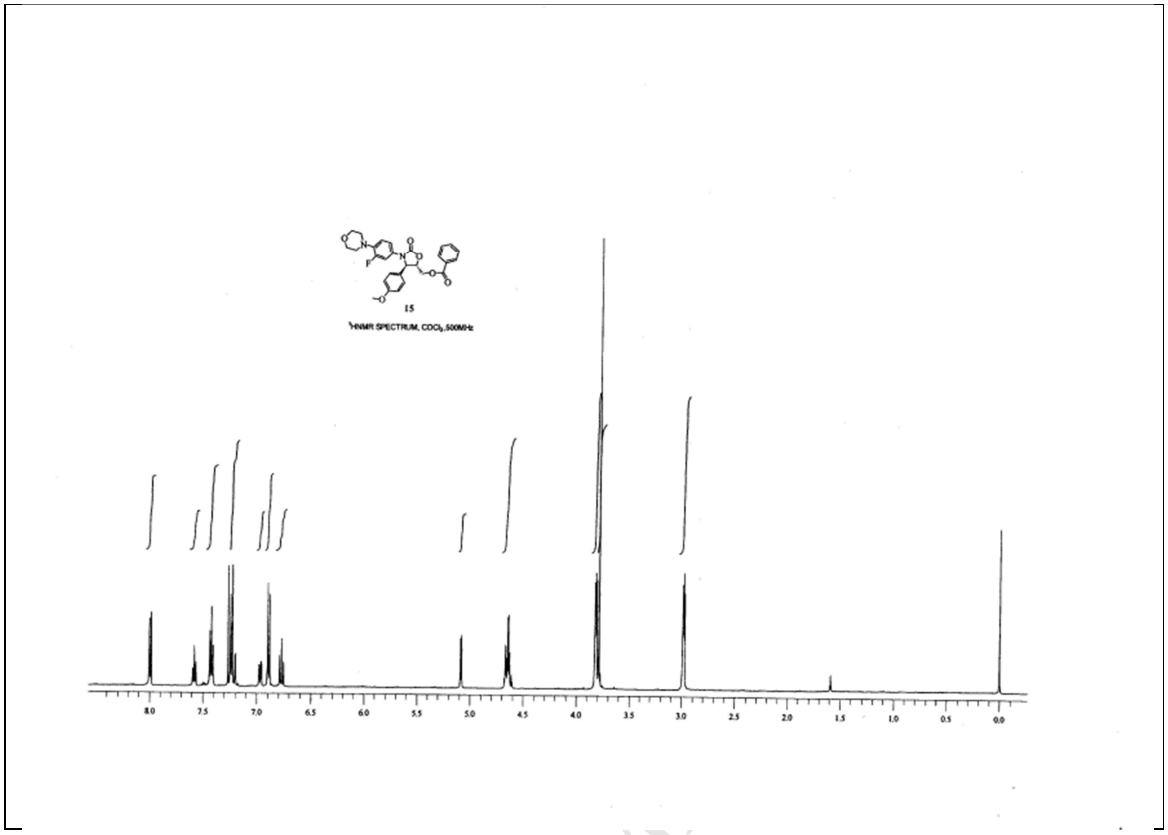

166.682
160.674
156.225
154.254
154.745
134.581
134.509
133.400
129.715
131.570
131.449
128.436
129.189
127.621
128.198
116.015
115.986
116.392
116.362
114.789
109.748
109.929
74.963
66.904
63.235
62.104
55.279
50.823
11
^{13}CNMR SPECTRUM, CDCl$_3$, 125MHz
170
160
150
140
130
120
110
100
90
80
70
60
50

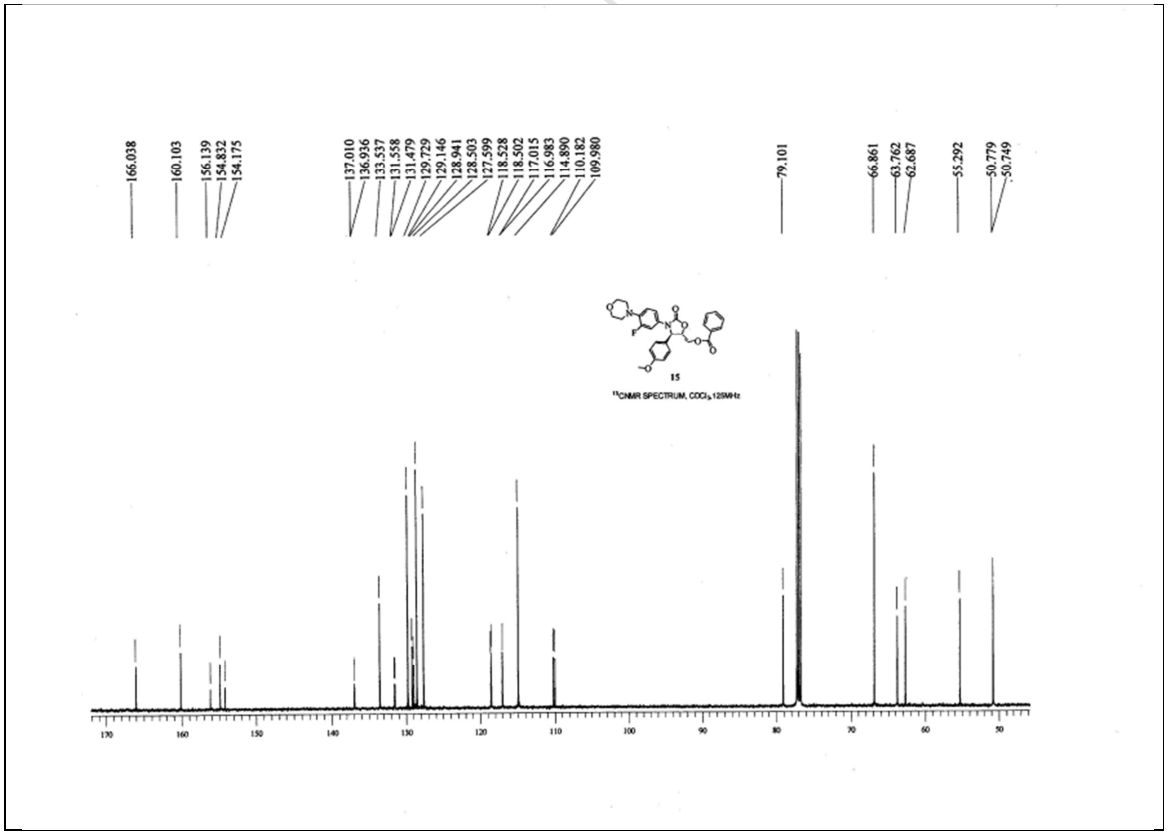

13
^{1}HNMR SPECTRUM, CDCl$_3$, 500MHz
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

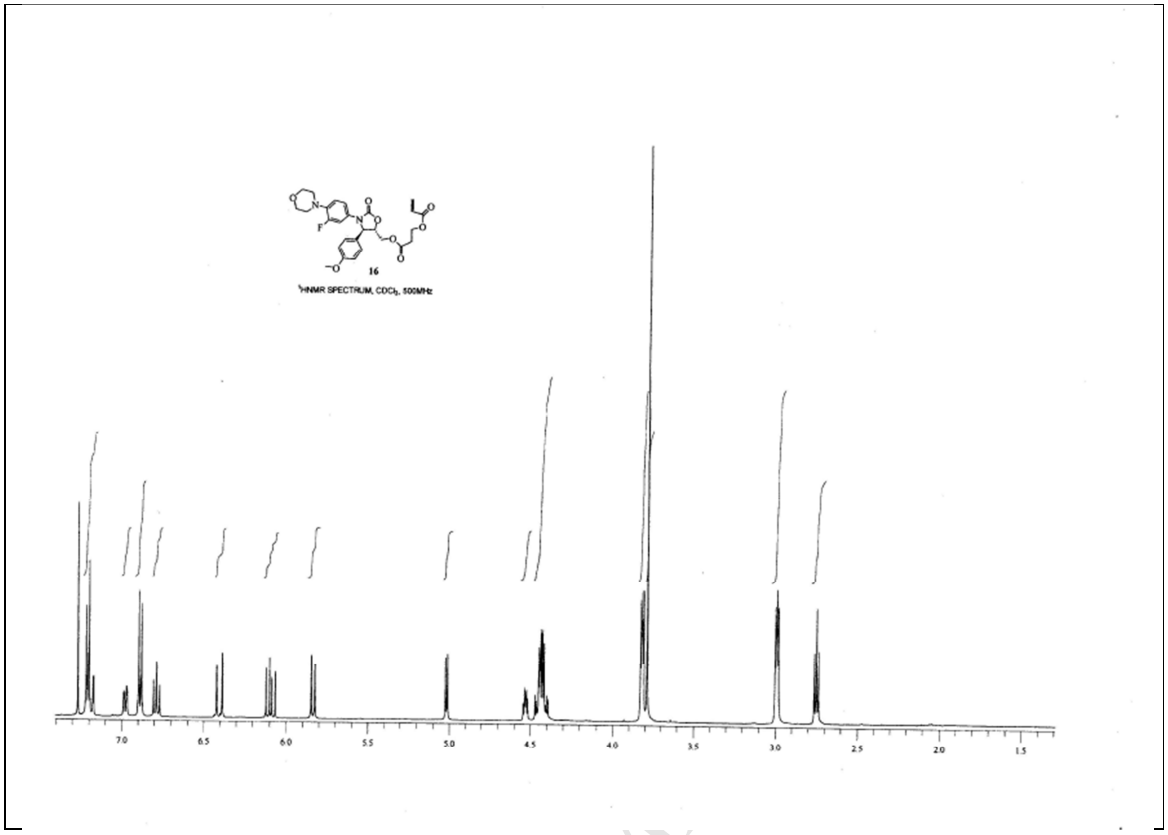

159.900
156.090
155.200
154.131
136.836
136.770
132.900
131.818
131.739
128.496
127.752
119.923
118.443
118.414
117.138
117.114
114.674
110.284
110.082
82.474
66.847
66.180
55.220
50.772
50.749
13
^{13}C NMR SPECTRUM, CDCl$_3$, 125MHz
160
150
140
130
120
110
100
90
80
70
60
50

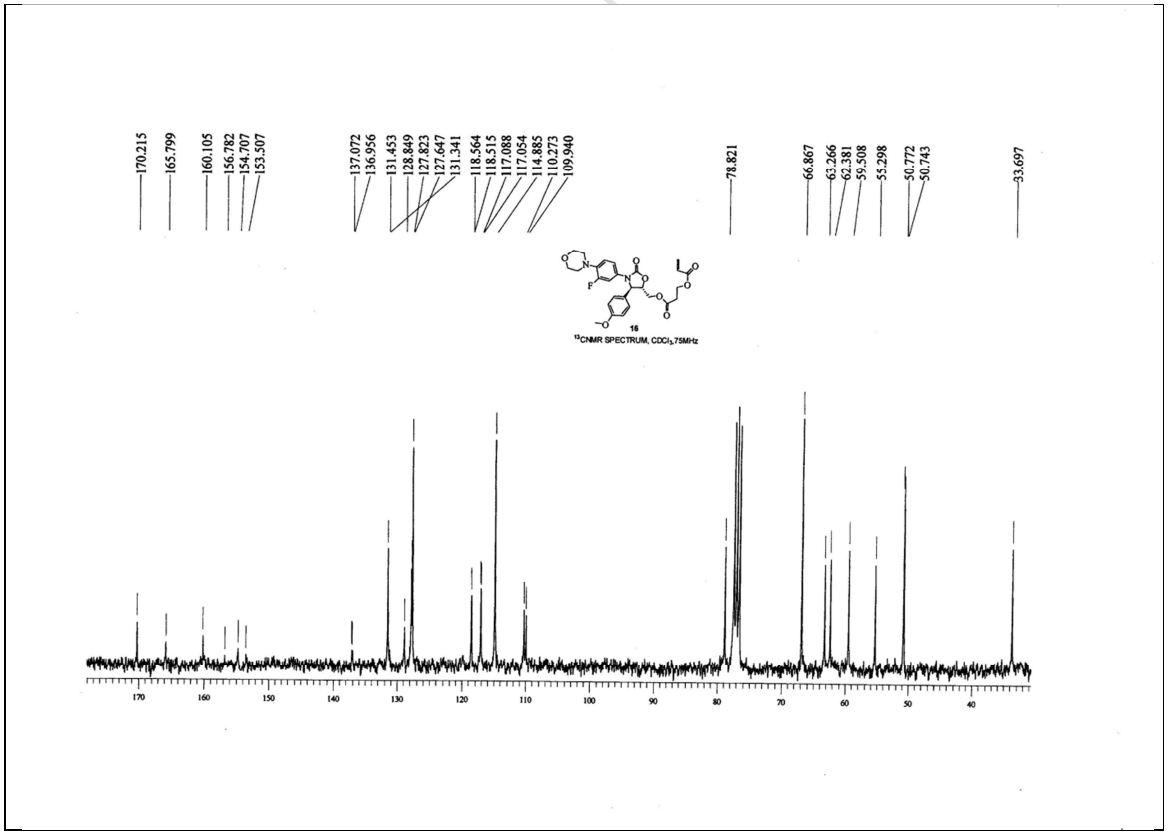

14
^{1}HNMR SPECTRUM, $CDCl_3$, 300MHz
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0


159.872
156.729
155.646
153.473
136.986
136.872
131.655
131.524
129.393
127.853
118.478
118.426
117.440
114.719
110.587
110.253
82.065
66.876
61.391
61.237
55.269
50.775
50.746
14
^{13}C NMR SPECTRUM, $CDCl_3$, 75MHz
160
150
140
130
120
110
100
90
80
70
60
50


15
¹HNMR SPECTRUM, CDCl₃,500MHz
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0


166.038
160.103
156.139
154.832
154.175
137.010
136.936
133.537
131.558
131.479
129.729
129.146
128.941
128.503
127.599
118.528
118.502
117.015
116.983
114.890
110.182
109.980
79.101
66.861
63.762
62.687
55.292
50.779
50.749
15
¹³CNMR SPECTRUM, CDCl₃,125MHz
170
160
150
140
130
120
110
100
90
80
70
60
50


16
^{1}HNMR SPECTRUM, $CDCl_3$, 500MHz
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5


170.215
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156.782
154.707
153.507
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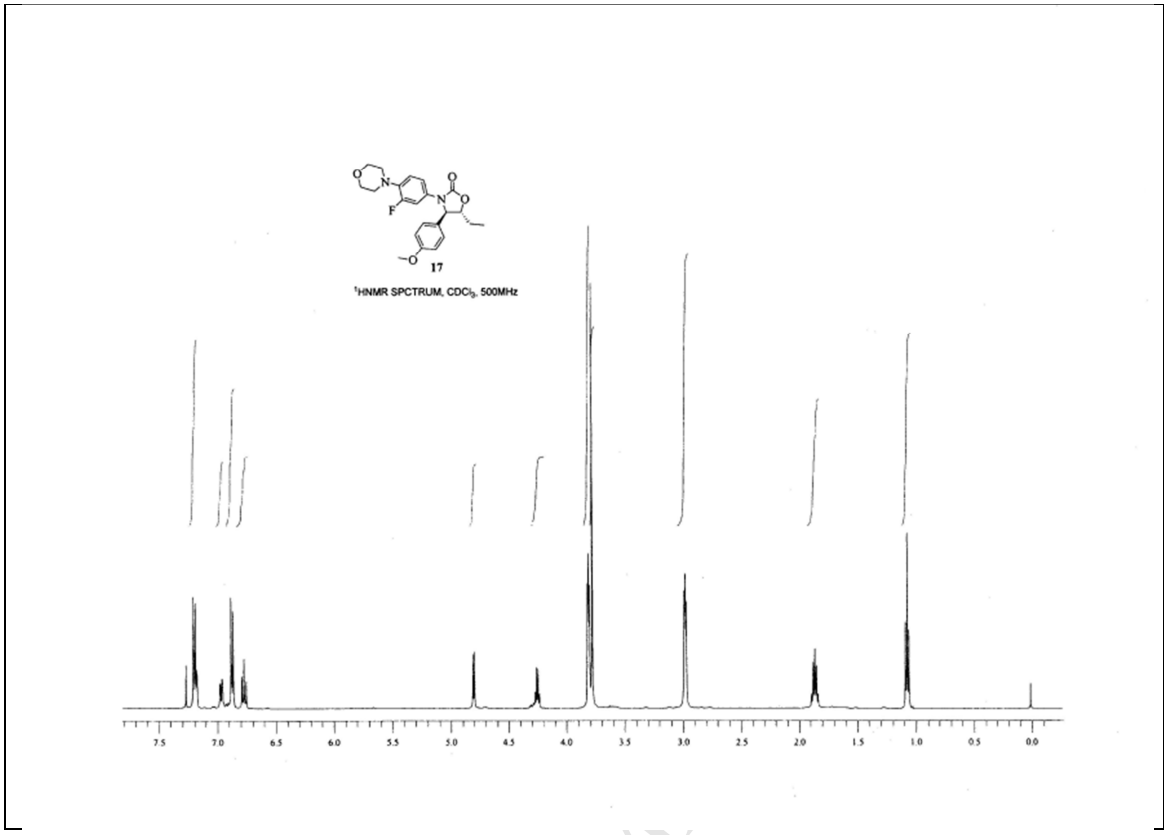
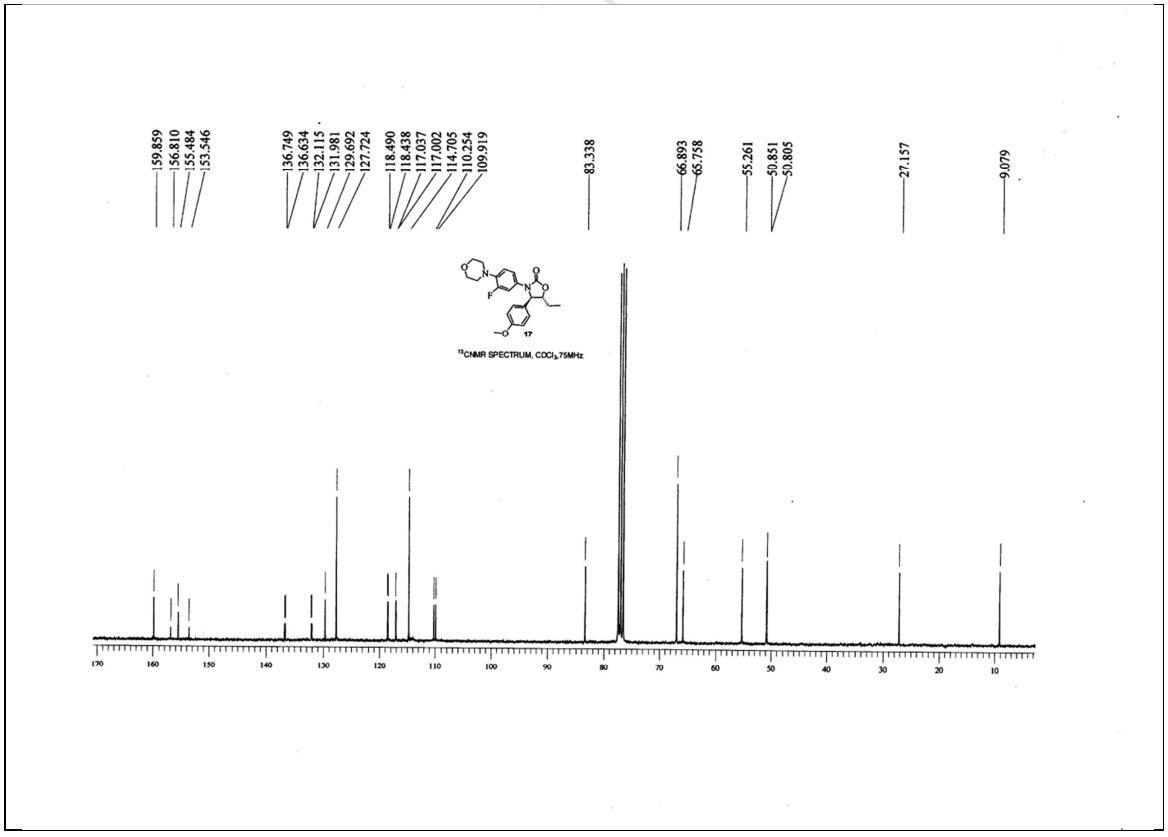
136.956
131.453
128.849
127.823
127.647
131.341
118.564
118.515
117.088
117.054
114.885
110.273
109.940
78.821
66.867
63.266
62.381
59.508
55.298
50.772
50.743
33.697
16
^{13}CNMR SPECTRUM, $CDCl_3$,75MHz
170
160
150
140
130
120
110
100
90
80
70
60
50
40


17
^{1}HNMR SPCTRUM, $CDCl_3$, 500MHz
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0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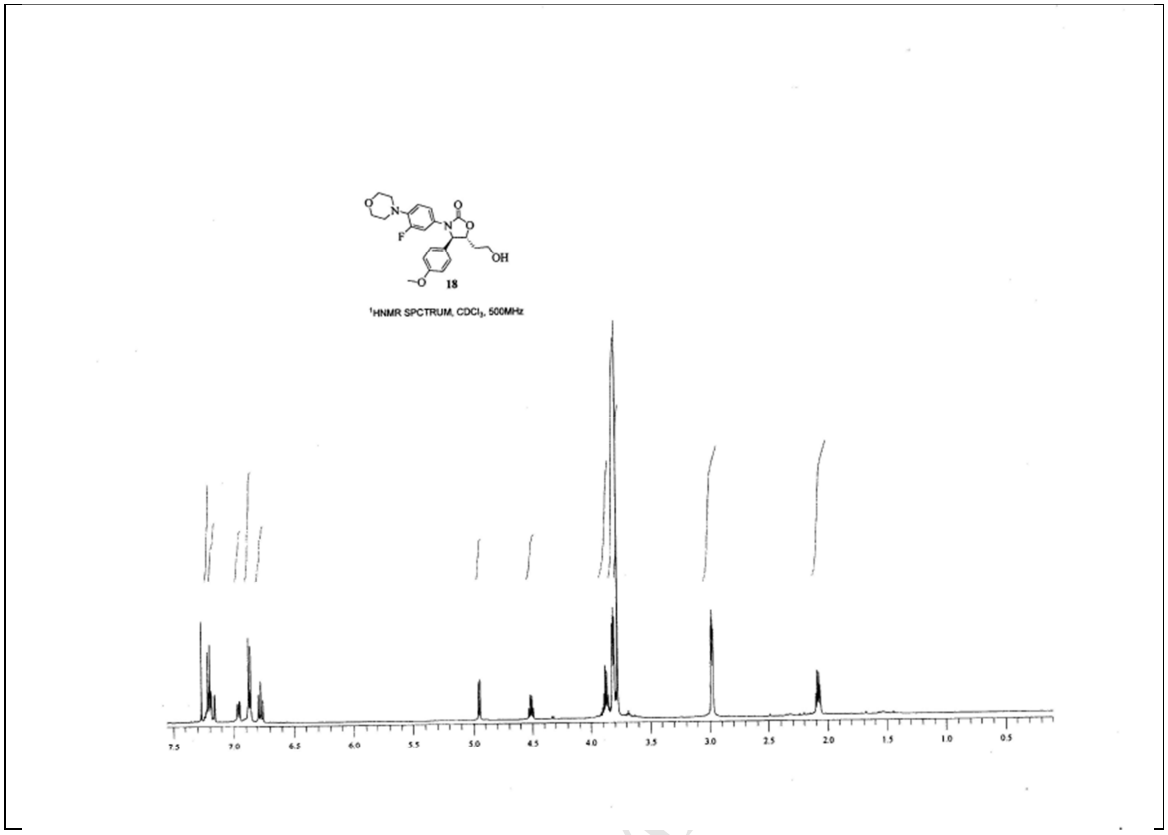
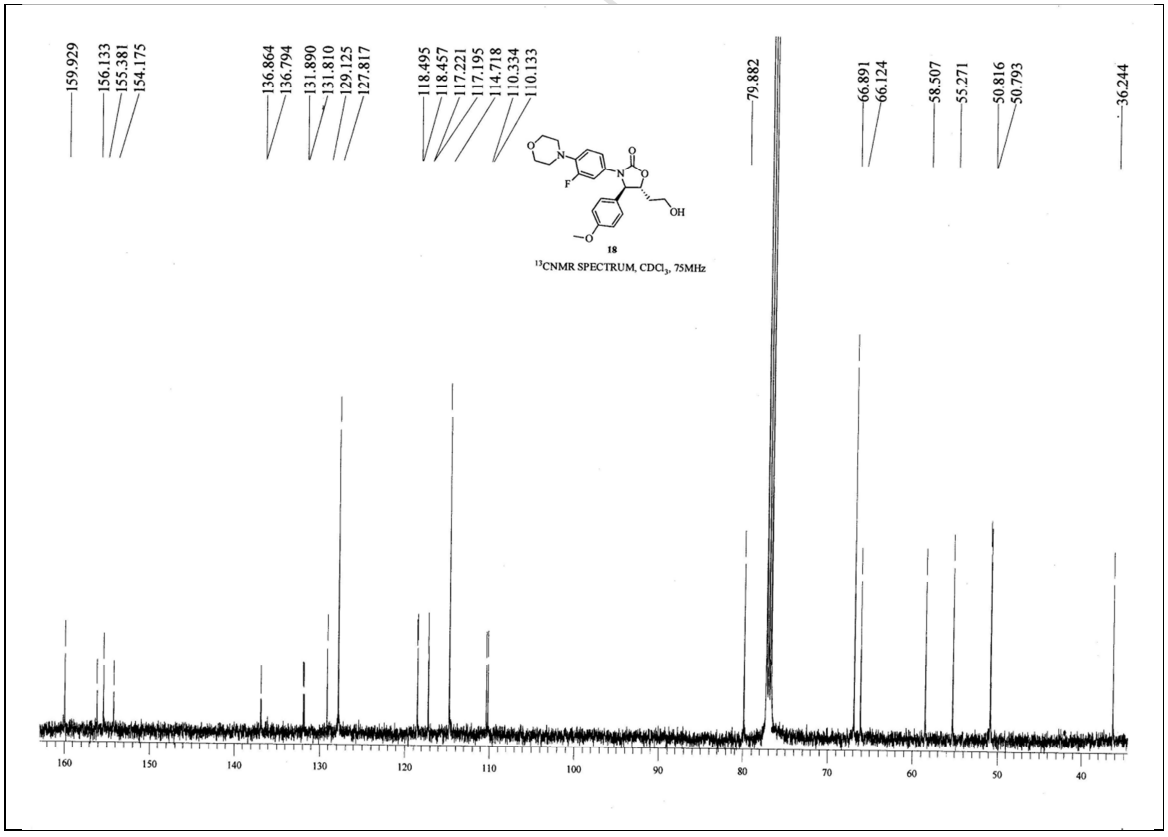
156.810
155.484
153.546
136.749
136.634
132.115
131.981
129.692
127.724
118.490
118.438
117.037
117.002
114.705
110.254
109.919
83.338
66.893
65.758
55.261
50.851
50.805
27.157
9.079
17
^{13}CNMR SPECTRUM, $CDCl_3$,75MHz
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10


18
^{1}HNMR SPCTRUM, $CDCl_3$, 500MHz


159.929
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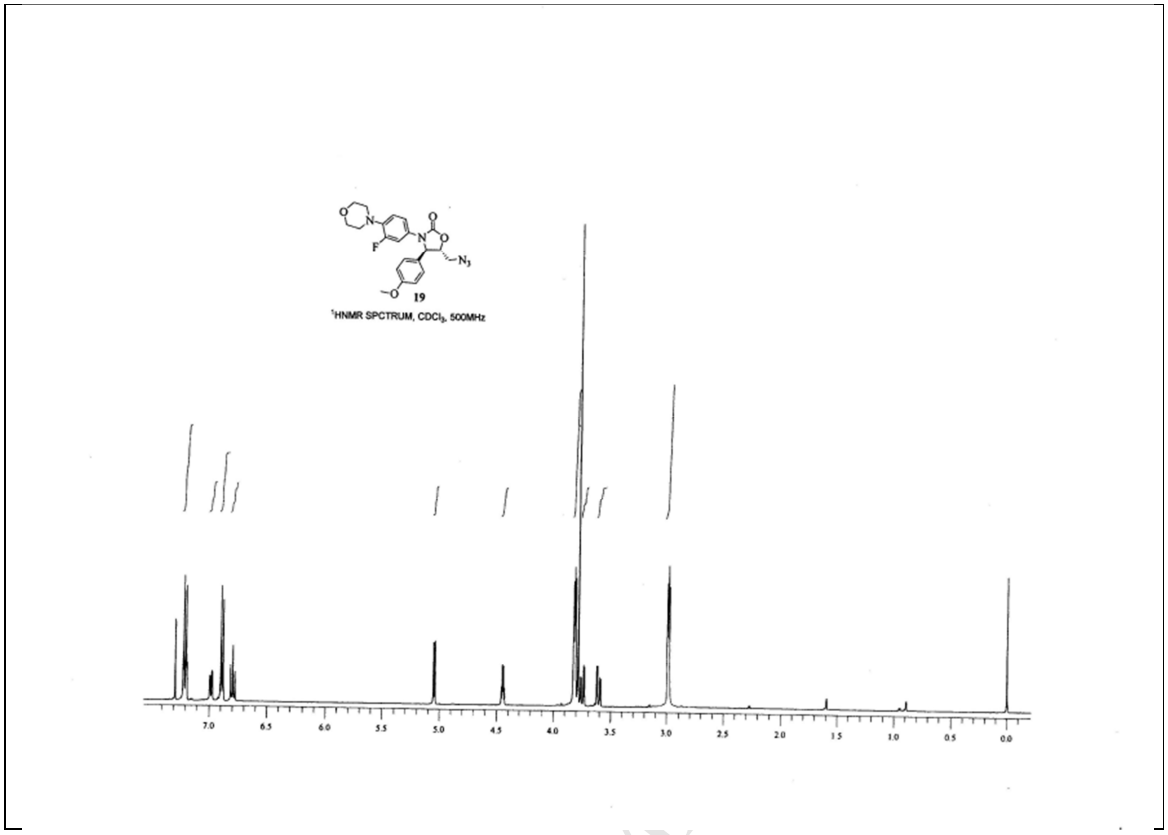
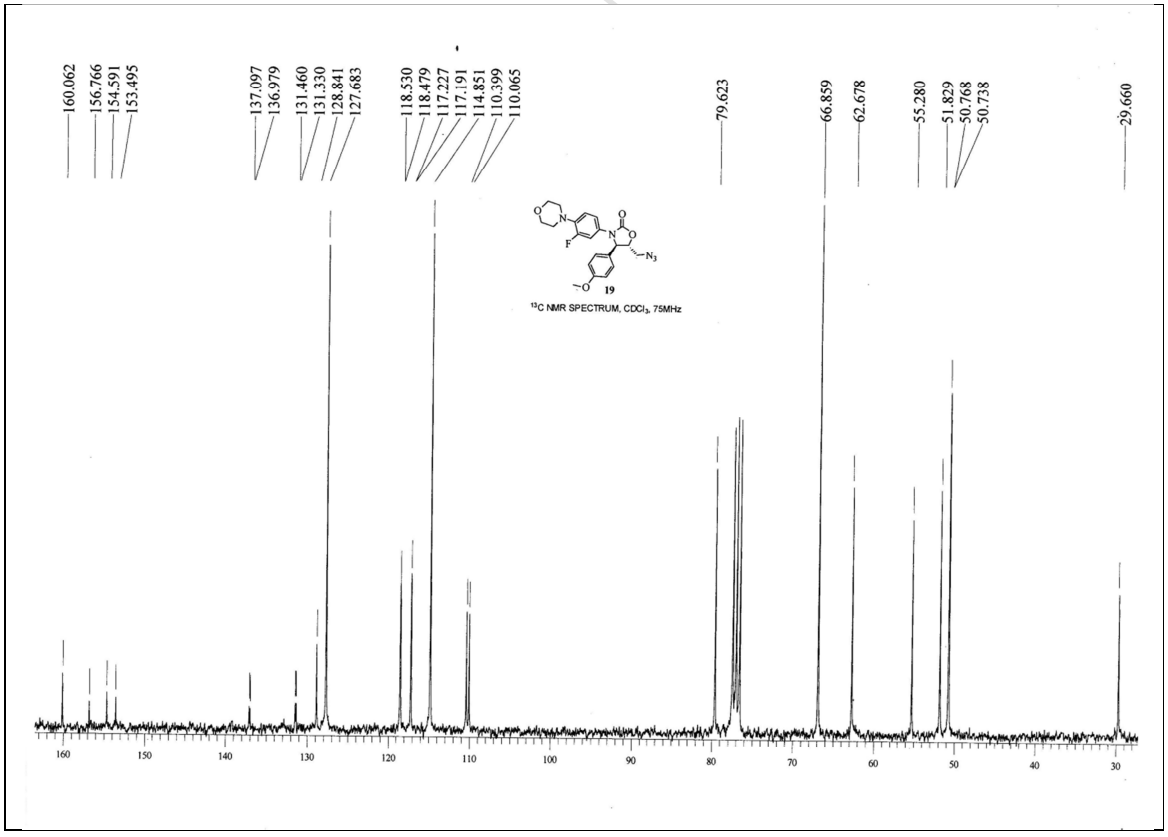
154.175
136.864
136.794
131.890
131.810
129.125
127.817
118.495
118.457
117.221
117.195
114.718
110.334
110.133
79.882
66.891
66.124
58.507
55.271
50.816
50.793
36.244
18
^{13}CNMR SPECTRUM, $CDCl_3$, 75MHz


19
^{1}HNMR SPCTRUM, $CDCl_3$, 500MHz
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0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156.766
154.591
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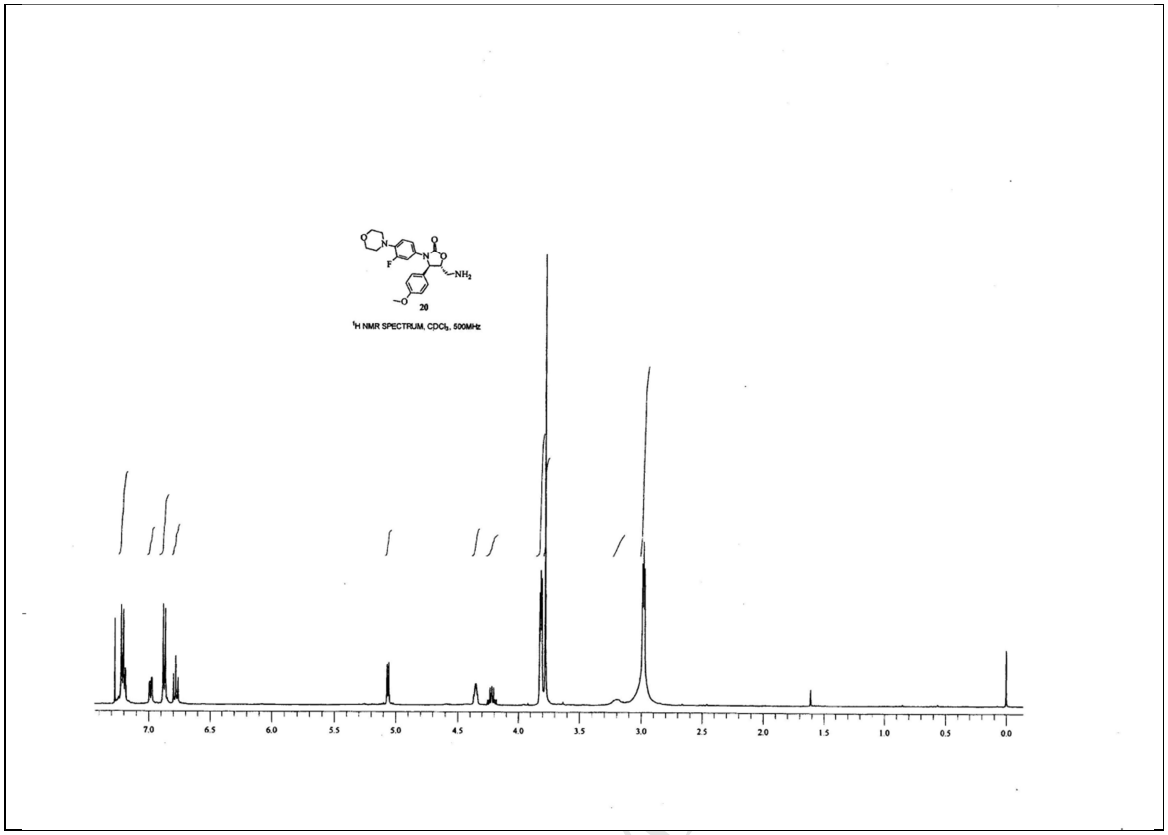
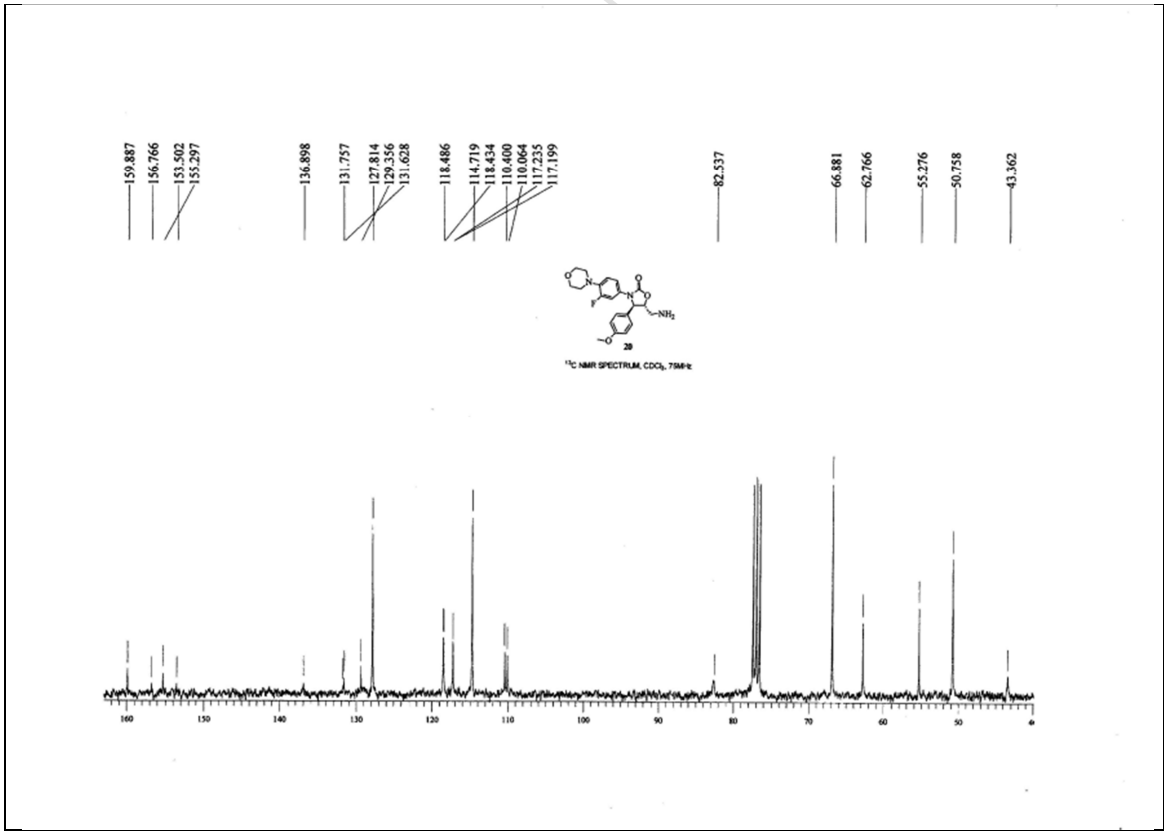
137.097
136.979
131.460
131.330
128.841
127.683
118.530
118.479
117.227
117.191
114.851
110.399
110.065
79.623
66.859
62.678
55.280
51.829
50.768
50.738
29.660
19
^{13}C NMR SPECTRUM, $CDCl_3$, 75MHz
160
150
140
130
120
110
100
90
80
70
60
50
40
30


20
^{1}H NMR SPECTRUM, CDCl$_3$, 500MHz
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0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156.766
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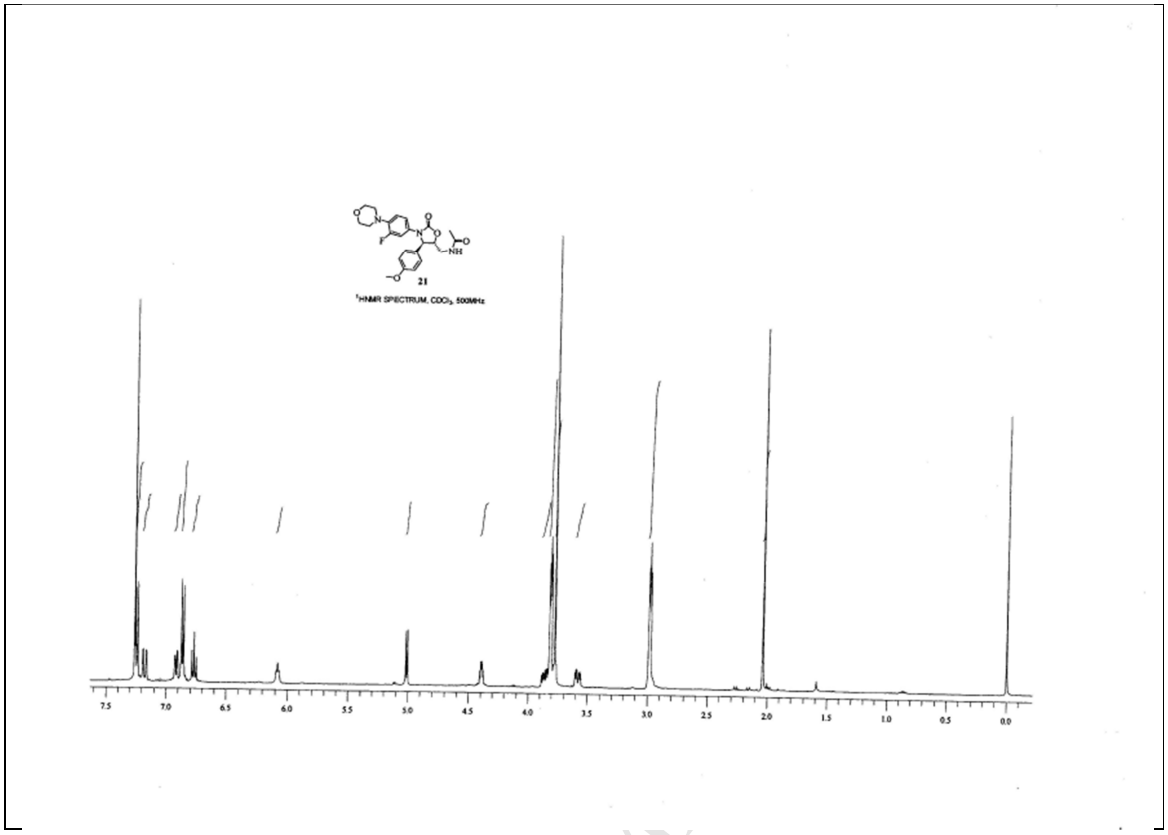
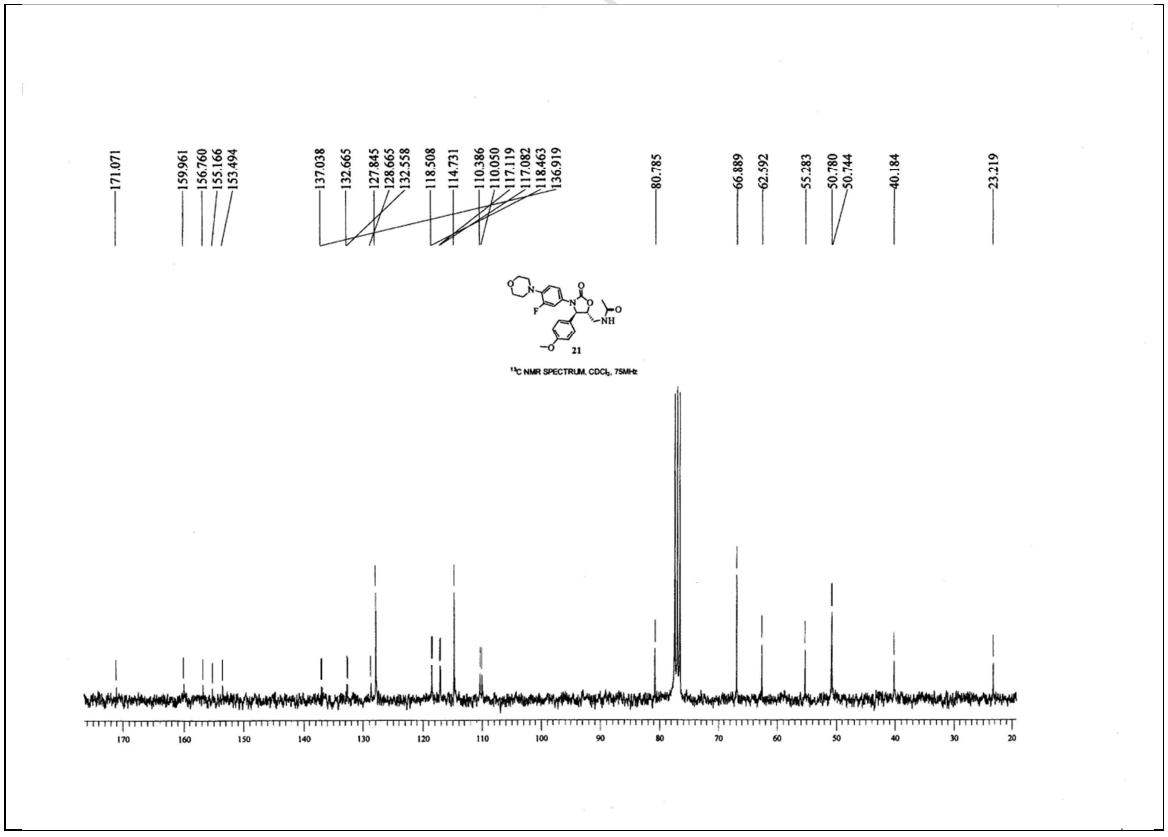
155.297
136.898
131.757
127.814
129.356
131.628
118.486
114.719
118.434
110.400
110.064
117.235
117.199
82.537
66.881
62.766
55.276
50.758
43.362
20
^{13}C NMR SPECTRUM, CDCl$_3$, 75MHz
160
150
140
130
120
110
100
90
80
70
60
50


21
^{1}HNMR SPECTRUM, $CDCl_3$, 500MHz
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0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156.760
155.166
153.494
137.038
132.665
127.845
128.665
132.558
118.508
114.731
110.386
110.050
117.119
117.082
118.463
136.919
80.785
66.889
62.592
55.283
50.780
50.744
40.184
23.219
21
^{13}C NMR SPECTRUM, $CDCl_3$, 75MHz
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20