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LETTERS

Anti-prostate cancer and anti-EGFR activities of new Nilutamide-isoxazole hybrids

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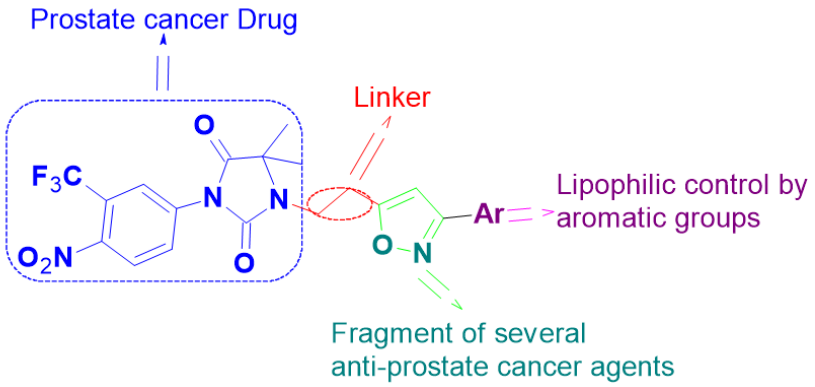
Article

ABSTRACT

Herein, synthesis of new Nilutamide-isoxazoles (**5a-5n**) *via* Cu(I)-promoted one-pot reaction between 1-(but-3-yn-1-yl)-5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)imidazolidine-2,4-dione (**3**) and several aldehydes (**4a-4n**) in benign aq. ^{t}butanol as key approach has been reported. The *in vitro* growth inhibition activity of all these compounds revealed that the majority of compounds were more active against DU-145 in comparison to PC3. Particularly, compounds **5f**, **5h** and **5k** showed greater activity against DU-145 than the standard drug 5-Fluoro Uracil with IC_{50} values <30 μM. whereas compound **5g** showed comparable activity against DU-145 cell line with the positive control. The Epidermal growth factor receptor (EGFR) is well known to be expressed in DU-145 cancer cells, the most potent compounds **5f**, **5h** and **5k** were then screened for their inhibitory potential against tyrosine kinase EGFR and found that compounds **5f** and **5k** showed remarkable inhibition with MIVs 93.4% and 91.3% respectively, while compound **5h** displayed good inhibition (MIV = 84.6%) as compared to the Erlotinib.



Keywords: Nilutamide, Isoxazole, Prostate cancer activity, EGFR inhibition

INTRODUCTION

The prostate cancer is most common nonskin malignant tumor in men and the second foremost reason for cancer-related transience in many developed countries.[1] Despite the fact that we have more than 15 FDA-approved therapies for prostate cancer which include targeted therapy, hormonal therapy, chemotherapy, and immunotherapy,[2] majority of patients still progress to castration-resistant prostate cancer, which has high mortality rate as well as poor prognosis.[3] Additionally, a recurrence in several patients who are diagnosed at advanced stages usually has a detrimental impact on clinical outcomes when standard chemotherapeutic intervention is used.[4-5] Current treatments were linked to many adverse side effects, like the stimulation of tumor spread and neuroendocrine differentiation that led to the failure of treatment.[6-8] Consequently, it is vital to identify precise therapeutic targets in order to make novel and effective treatments for advanced prostate cancer and precise therapeutic targets to deliver fruitful replacements for prostate cancer patients.

Remarkably, Nilutamide, also known as [5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)imidazolidine-2,4-dione] (**1**), was one of the active non-steroidal antiandrogen (NSAA) medications used to treat prostate cancer. It inhibits the effects of testosterone and dihydrotestosterone on the body and thus act selective antagonist of the androgen receptor.[9-10] Since, many prostate cancer cells depend on these hormones for their development and existence, Nilutamide could stop the spread of prostate cancer and lengthen the lives of men who have this disease.[9] However, because other NSAAs like Bicalutamide and Enzalutamide had improved efficacy, acceptability and protection, they have frequently replaced Nilutamide due to few side effects related to it.[10–15] To our knowledge, there have only been two studies on the modification of Nilutamide as *in vitro* anticancer drugs.[16-17]

Due to the relatively simple synthesis, the isoxazole ring has fascinated the interest of pharmacologists and chemists from the research groups all over the world. As a result of low cytotoxicity,

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isoxazole could serve as a common scaffold in creating novel compounds with diverse biological activities.[18] In particular, by conjoining isoxazole ring with varied pharmacophores, several compounds with diverse anticancer properties[19] including prostate cancer activity[19-25] were reported.

Based on all the above findings and in view of usage of pharmacophore hybridization approach[26-27] in the current medicinal chemistry research, herein, we designed and synthesized some new Nilutamide-isoxazole hybrids as EGFR targeting anti-prostate cancer agents. (Figure 1).

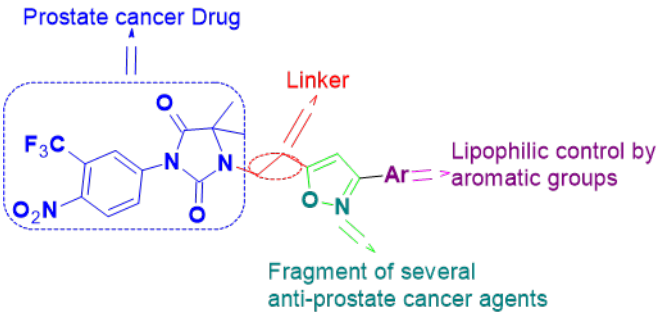
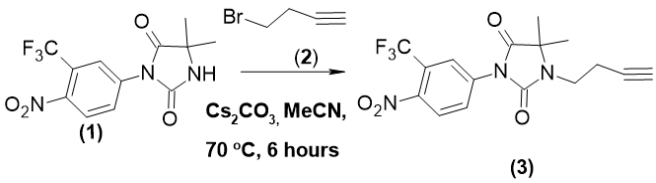


Figure 1. The designed strategy of Nilutamide-isoxazoles

RESULTS AND DISCUSSION

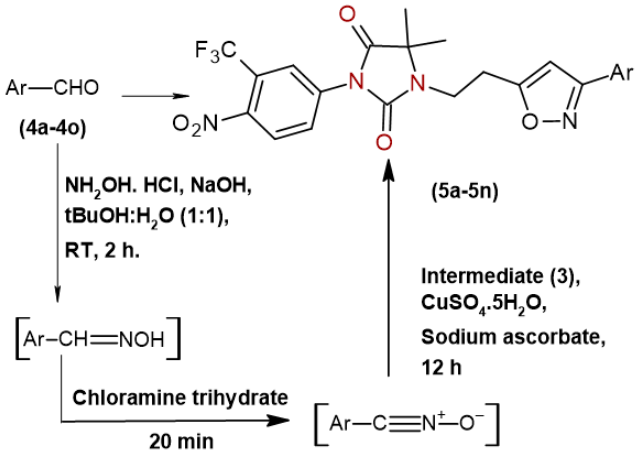
Chemistry

The synthetic approach to designed isoxazole derivatives of Nilutamide (**5a-5n**) was shown in **schemes 1** and **2**. In the step 1, the Nilutamide (**1**) was treated with 4-bromobut-1-yne (**2**) by means of Cs_2CO_3 in MeCN at 70 °C for 6 h to give 1-(but-3-yn-1-yl)-5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl) imidazolidine-2,4-dione (**3**).



Scheme 1. Syntheis of 1-(but-3-yn-1-yl)-5,5-dimethyl-3-(4-nitro-3(trifluoromethyl)phenyl)imidazolidine-2,4-dione (**3**)

In the second step, we extended the synthetic application of the previously reported Cu-promoted one-pot regioselective approach of 3,5-disubstituted isoxazoles.[28] In an aspect, aldehydes (**4a-4n**) were initially transformed into respective *in-situ* aldoximes using NH_2OH. HCl and NaOH in (1:1) aq. ^{t}BuOH at ambient temperature after 2 h, which were then transformed into their *in situ* nitrile oxides by the slow addition of chloramine T trihydrate for 20 min. Finally, the 1,3-dipolar cycloaddition reaction between *in situ* nitrile oxides and 1-(but-3-yn-1-yl)-5,5-dimethyl-3-(4-nitro-3(trifluoromethyl)phenyl) imidazolidine -2,4-dione (**3**) under *in situ* Cu(I) catalysis (formed by combining $CuSO_4$ and sodium ascorbate) for 12 h at same temperature afforded the targeted Nilutamide-isoxazole hybrids (**5a-5n**) (Scheme 2).



Scheme 2. Synthesis of Nilutamide-isoxazoles (**5a-5n**)

In vitro anti-prostate cancer activity

Later, the newly synthesized Nilutamide-isoxazole hybrids (**5a-5n**) were screened for their *in vitro* anti-prostate cancer activity studies against two human prostate cancer cell lines PC3 and DU-145 using MTT assay and results were compared with the chemotherapeutic drug 5-Fluorouracil (5-FU). According to results of table 1, majority of compounds have shown higher potency against DU-145 than the PC-3 and IC_{50} (μM) values were obtained in the range of 21.8 to 62.7. In particular, compounds **5f** (IC_{50} = 21.8±0.6 μM), **5h** (IC_{50} = 26.4±0.5 μM) and **5k** (IC_{50} = 27.7±0.8 μM) had superior potency against DU-145 than the 5-FU (IC_{50} = 38.3±1.3 μM). Except compound **5a**, remaining compounds **5b-5e**, **5g-5j** and **5l-5n** showed promising to good activity against DU-145 in comparison to 5-FU. However, all the compounds showed good to poor activity against PC3 when compared with the positive control.

The nature of the substituent present on the phenyl ring that attached to the 3rd position of isoxazole core moiety affecting the in vitro prostate cancer activity was then analyzed using structure- activity relationship (SAR) studies. In the case of electron-donating substituents, the compound **5d** containing a 4-methoxy substituent showed good activity. Likewise, when we introduced two methoxy substituents at 3 and 5-positions led to compound **5e** had weaker potency than **5d**. Nonetheless, simple phenyl ring compound **5a** and methyl substitutions on the phenyl ring (compounds **5b** and **5c**) have shown weaker activity than the methoxy derivatives **5d** and **5e**.

With respect to electron withdrawing substitutions, the compound **5f** containing 4-Br substituent showed superior activity. The next better activity exhibited by the compound **5h** containing 4-F substituent. However, the activity was decreased, when we introduced two bromine substituents on the phenyl ring (compound **5k**) than the mono-bromo compound **5f**. Overall, the halogen group containing compounds **5f-5h** and **5k-5l** were found to be more active than the remaining compounds (**5i-5j** and **5m-5n**) in the electron-withdrawing group series.

In vitro EGFR tyrosine kinase inhibitory activity

The epidermal growth factor receptor (EGFR) is one of the foremost targets in the development of cancer drugs,[29] because, it is a cell-surface receptor for the members of the EGFR family

and plays a keen role in ductal development of the mammary glands.[30] The over-expression of this EGFR leads to leads many types of cancers.[31-32] Predominantly, in contrast to PC3 cancer cells, DU145 cancer cells are known to have a higher level of EGFR expression.[33] On the other aspect, few isoxazole-based compounds were also reported to anti-EGFR activity.[34-35] Hence, the most potent compounds **5f**, **5h** and **5k** and one of the least active compounds **5a** found in the *in vitro* DU-145 cancer cell activity were then screened for their tyrosine Kinase EGFR inhibition efficacy by means of previous Carmi et al's method[36] and results were compared with the standard drug Erlotinib. The results of table 2, revealed that the compound **5f** and **5k** have shown remarkable inhibition potential which has MIV values 93.4% and 91.3% respectively. In addition, compound **5h** showed good inhibition with MIV 84.6%. However, compound **5a** had poor efficacy in inhibiting EGFR (MIV = 43.5%). These outcomes revealing us that one of the mechanisms of anti-DU-145 cancer cell activity of the investigated compounds, probably would be due to the inhibition of tyrosine kinase EGFR.

Table 1: *In vitro* anticancer activity of newly developed indole-thiazolidine-2,4-dione-isoxazoles (**5a-5n**) with IC_{50} in µM[a]

Compound	Ar	[b]PC3	[c]DU-145
5a	C_6H_5	NI	62.7±3.9
5b	4-MeC_6H_4	NI	45.3±1.8
5c	3,5-di-MeC_6H_3	89.2±2.1	43.5±2.3
5d	4-OMeC_6H_4	79.7±1.6	40.3±1.7
5e	3,5-di-OMeC_6H_3	85.1±2.3	41.6±1.9
5f	4-BrC_6H_4	74.9±1.8	**21.8±0.6**
5g	4-ClC_6H_4	75.3±2.2	39.8±1.6
5h	4-FC_6H_4	78.2±1.7	**26.4±0.5**
5i	4-CNC_6H_4	83.7±2.5	42.6±2.2
5j	4-$NO_2C_6H_4$	80.8±2.3	43.2±2.4
5k	3,5-di-BrC_6H_3	75.7±2.6	**27.7±0.8**
5l	3,5-di-ClC_6H_3	80.2±2.9	41.5±2.1
5m	3,5-di-CNC_6H_3	NI	45.1±2.4
5n	3,5-di-$NO_2C_6H_3$	85.2±3.1	44.2±1.5
5-FU		68.5±1.5	38.3±1.3

[a]Each data represents as mean ±S.D values; [b]PC3: Human prostate cancer cell line.; [c]DU-145: Human prostate cancer cell line; NI = IC_{50} = >100 µM

Table 2. Tyrosine kinase EGFR inhibitory activity of compounds **5a**, **5f**, **5h**, and **5k** at 10 µM

Compound	Mean inhibition of kinase activity (%)[a]
5f	**93.4±0.08**
5h	84.6±0.4
5k	**91.3±0.1**
5a	43.5±0.8
Erlotinib	98.2±0.1

[*] The values are indicated as the mean±SD

EXPERIMENTAL

General information

All the commercially available chemicals were used without purification. The purity of the compounds was evaluated using Merck 60F254 silica gel plates. The ^1H & ^{13}C NMR spectra recorded with a Mercuryplus spectrometer (operating at 300 MHz for ^1H & 100 MHz for ^{13}C) chemical shifts were referenced to TMS. The ESI (electrospray ionization) mass spectra (at an ionizing voltage of 70 eV) were attained using a Shimadzu QP5050A quadrupole mass spectrometer. Elemental analyses were obtained with an Elemental Analyser Perkin–Elmer 240 C apparatus.

Synthesis of 1-(but-3-yn-1-yl)-5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)imidazolidine-2,4-dione (3): To a 100 mL round bottom was added [5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)imidazolidine-2,4-dione] (**1**) (10 mmol), Cs_2CO_3 (15 mmol) and 4-bromo-1-butyne (12 mmol) in 30 mL MeCN and resulting mixture was stirred at 70 °C for 6 h. The progress of the reaction as analyzed by TLC, the reaction mixture was then extracted twice with 30 mL of water/ethyl acetate and the excess of ethyl acetate was dried under anhydrous Na_2SO_4 and then concentrated under vacuum to get the crude product. Finally this crude product was subjected to 60-120 size silica jel column chromatography using hexane-EtOAc (7:3) as eluent to get pure **3** in 81% yield. ^1H NMR (300 MHz, $CDCl_3$) Pale yellow solid; δ 8.10 (s, 1H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.71 (d, *J* = 7.6 Hz, 1H), 4.08 (t, *J* = 7.2 Hz, 2H), 2.29 (s, 1H), 2.20 (t, *J* = 7.2 Hz, 2H), 1.45 (s, 6H) ppm.

Procedure for the synthesis of Nilutamide-isoxazole hybrids (5a-5n): The 0.5 mmol of aldehydes (**4a-4n**) were added to 3 mL of 1:1 ratio of H_2O-^{t}BuOH solvent media containing NH_2OH. HCl (0.5 mmol) and NaOH (0.5 mmol) and the resulting mixture was allowed to siring at room temperature for 2 h. Later, the *in situ* formation of corresponding aldoxime was observed with the help of TLC, then chloramine T trihydrate (0.5 mmol) was added in portions wise for 20 min followed by the addition of $CuSO_4 \cdot 5H_2O$ (0.05 mmol), sodium ascorbate (0.1 mmol) and intermediate **3** (0.5 mmol) and the p^H of the reaction mixture was settled to 6 by adding few drops of 1M NaOH and the stirring was continued for further 12 h under same reaction temperature. At the completion of the reaction as analyzed by TLC, the reaction mixture was decanted into 10 mL of ice cold water and 2 mL of dilute NH_4OH was added to it in order to remove all the unnecessary copper salts. Later, the crude product was obtained using filtration and consequently subjected to column chromatography (60-120 mesh size silica gel) using (2:3) ethyl acetate: hexane as eluent to give pure products (**5a-5n**).

MTT assay

96-well tissue culture microtiter plates were used and each well received 100 µL of complete media containing 1×10^4 cells as an inoculum. Prior to the experiment, the plates were incubated for 18 h at 37 °C in a humidified 5% CO_2 incubator. After removing the medium, each well received 100 µL of fresh medium containing the test chemicals and 5-FU at various concentrations, such as 0.5, 1 and 2 µM. This medium was then incubated at 37 °C for 24 h. The medium was then discarded, and

10 µL of MTT dye was added in its place. For 2 h, plates were incubated at 37 ºC. In 100 µL of extraction buffer, the resultant formazan crystals were solubilized. Using a microplate reader, the optical density (O.D.) was read at 570 nm (Multi-mode Varioskan Instrument-Themo Scientific). Never did the medium contain more DMSO than 0.25%.

Anti EGFR activity

The EGFR tyrosine kinase inhibitory activity of the compounds **5a**, **5f**, **5h** and **5k** was evaluated as described by Carmi et al.[36]

Charectirization data

5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)-1-(2-(3-phenylisoxazol-5-yl)ethyl)imidazolidine-2,4-dione (**5a**): Colorless solid; Yield 79%; M.P. 187-189 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (s, 1H, Ar-H), 8.03 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.78-7.72 (m, 3H). 7.44–7.36 (m, 3H), 6.84 (s, 1H), 4.10 (t, *J* = 7.3 Hz, 2H), 2.33 (t, *J* = 7.3 Hz, 2H), 1.47 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 170.4, 160.7, 158.6, 146.7, 144.6, 132.5, 131.3, 130.9, 129.4, 128.5, 127.9, 126.2, 124.6, 124.2, 97.3, 58.2, 44.6, 24.5, 22.7 ppm; MS (ESI): m/z = 489 $[M+H]^+$; CHN analysis for $C_{23}H_{19}F3N_4O_5$; Cacld (%): C, 56.56; H, 3.92; N, 11.47; Found (%): C, 56.58; H, 3.90; N, 11.50.

5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)-1-(2-(3-(p-tolyl)isoxazol-5-yl)ethyl)imidazolidine-2,4-dione (**5b**): Colorless solid; Yield 77%; M.P. 189-191 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (s, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.76-7.70 (m, 3H), 7.21 (d, *J* = 7.7 Hz, 2H), 6.84 (s, 1H), 4.11 (t, *J* = 7.3 Hz, 2H), 2.39 (s, 3H), 2.32 (t, *J* = 7.3 Hz, 2H), 1.46 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.5, 170.2, 160.4, 157.9, 146.4, 145.1, 140.4, 132.7, 131.1, 129.6, 129.1, 127.6, 127.3, 124.8, 124.5, 97.4, 58.4, 44.5, 24.9, 22.5, 21.5 ppm; MS (ESI): m/z = 503 $[M+H]^+$; CHN analysis for $C_{24}H_{21}F_3N_4O_5$; Calcd (%): C, 57.37; H, 11.34; N, 11.15; Found (%): C, 57.39; H, 11.31; N, 11.17.

1-(2-(3-(3,5-dimethylphenyl)isoxazol-5-yl)ethyl)-5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)imidazolidine-2,4-dione (**5c**): Colorless solid; Yield 74%; M.P. 193-195 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (s, 1H), 8.01 (d, *J* = 7.6 Hz, 1H), 7.72 (d, *J* = 7.6 Hz, 1H), 7.40 (s, 2H), 7.11 (s, 1H), 6.83 (s, 1H), 4.11 (t, *J* = 7.3 Hz, 2H), 2.40 (s, 6H), 2.33 (t, *J* = 7.3 Hz, 2H), 1.46 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.9, 170.4, 160.2, 158.2, 146.6, 144.8, 139.3, 133.2, 132.2, 131.9, 129.2, 127.8, 127.1, 124.6, 124.3, 97.5, 58.2, 44.8, 24.8, 22.6, 21.6 ppm; MS (ESI): m/z = 517 $[M+H]^+$. CHN analysis for $C_{25}H_{23}F_3N_4O_5$; Calcd (%): C, 58.14; H, 4.49; N, 10.85; Found (%): C, 58.12; H, 4.48; N, 10.83.

1-(2-(3-(4-methoxyphenyl)isoxazol-5-yl)ethyl)-5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)imidazolidine-2,4-dione (**5d**): Colorless solid; Yield 73%; M.P. 194-196 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (s, 1H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.73 (d, *J* = 7.6 Hz, 1H), 7.57 (d, *J* = 8.0 Hz, 2H), 6.96 (d, *J* = 8.0 Hz, 2H), 6.83 (s, 1H), 4.12 (t, *J* = 7.3 Hz, 2H), 3.85 (s, 3H), 2.32 (t, *J* = 7.3 Hz, 2H), 1.45 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 170.1, 160.5, 160.1, 158.4, 146.4, 144.7, 133.7, 130.5, 129.7, 128.8, 124.1, 123.9, 122.1, 113.8, 97.2, 58.3, 56.3, 44.7, 24.6, 22.5 ppm; MS (ESI): m/z = 519 $[M+H]^+$; CHN analysis for $C_{24}H_{21}F_3N_4O_6$; Calcd (%): C, 55.60; H, 4.08; N, 10.81; Found (%): C, 55.63; H, 4.06; N, 10.78

1-(2-(3-(3,5-dimethoxyphenyl)isoxazol-5-yl)ethyl)-5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)imidazolidine-2,4-dione (**5e**): Light orange solid; Yield 70%; M.P. 187 M.P. 195-197 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (s, 1H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.74 (d, *J* = 7.6 Hz, 1H), 6.94 (s, 2H), 6.82 (s, 1H), 6.65 (s, 1H), 4.12 (t, *J* = 7.3 Hz, 2H), 3.86 (s, 6H), 2.32 (t, *J* = 7.3 Hz, 2H), 1.45 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.3, 170.5, 160.7, 159.8, 157.6, 146.2, 145.3, 135.6, 132.8, 130.7, 128.8, 124.8, 124.3, 108.5, 103.2, 96.9, 57.9, 56.5, 44.5, 24.5, 22.2 ppm ; MS (ESI): m/z = 549 $[M+H]^+$; CHN analysis for $C_{25}H_{23}F_3N_4O_7$; Calcd (%): C, 54.75; H, 4.23; N, 10.22; Found (%): C, 54.77; H, 4.21; N, 10.24.

1-(2-(3-(4-bromophenyl)isoxazol-5-yl)ethyl)-5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)imidazolidine-2,4-dione (**5f**): Light yellow solid; Yield 80%; M.P. 203-205 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (s, 1H), 8.04 (d, *J* = 7.6 Hz, 1H), 7.75 (d, *J* = 7.6 Hz, 1H), 7.70 (d, *J* = 7.7 Hz, 2H), 7.35 (d, *J* = 7.7 Hz, 2H), 6.84 (s, 1H), 4.13 (t, *J* = 7.3 Hz, 2H), 2.34 (t, *J* = 7.3 Hz, 2H), 1.47 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.3, 170.2, 160.3, 158.5, 146.8, 144.9, 132.7, 131.4, 129.5, 128.9, 128.1 127.4, 126.5, 123.9, 123.5, 97.5, 58.6, 44.5, 24.6, 22.3 ppm; MS (ESI): m/z = 567 $[M+H]^+$; CHN analysis for $C_{23}H_{18}BrF_3N_4O_5$; Calcd (%): C, 48.69; H, 3.20; N, 9.88; Found (%): C, 48.67; H, 3.23; N, 9.87.

1-(2-(3-(4-chlorophenyl)isoxazol-5-yl)ethyl)-5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)imidazolidine-2,4-dione (**5g**): Colorless solid; Yield 82%; M.P. 193-195 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (s, 1H), 8.05 (d, *J* = 7.6 Hz, 1H), 7.74 (d, *J* = 7.6 Hz, 1H), 7.65 (d, *J* = 8.2 Hz, 2H), 7.29 (d, *J* = 8.2 Hz, 2H), 6.85 (s, 1H), 4.14 (t, *J* = 7.3 Hz, 2H), 2.35 (t, *J* = 7.3 Hz, 2H), 1.47 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 170.7, 160.7, 158.9, 146.3, 145.7, 137.2, 132.8, 130.9, 129.8, 129.1, 128.4, 127.6, 125.1, 124.4, 97.7, 58.7, 44.8, 24.7, 22.5 ppm; MS (ESI): m/z = 523 $[M+H]^+$; CHN analysis for $C_{23}H_{18}ClF_3N_4O_5$; Calcd (%): C, 52.83; H, 3.47; 10.90; N, 10.72; Found (%): C, 52.81; H, 3.49; N, 10.75.

1-(2-(3-(4-fluorophenyl)isoxazol-5-yl)ethyl)-5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)imidazolidine-2,4-dione (**5h**): Colorless solid; Yield 84%; M.P. 192-194 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (s, 1H), 8.06 (d, *J* = 7.6 Hz, 1H), 7.74 (d, *J* = 7.6 Hz, 1H), 7.53 (d, *J* = 7.6 Hz, 2H), 7.15 (d, *J* = 7.6 Hz, 2H), 6.85 (s, 1H), 4.15 (t, *J* = 7.2 Hz, 2H), 2.35 (t, *J* = 7.2 Hz, 2H), 1.46 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.3, 170.8, 162.3, 160.6, 159.1, 146.8, 144.7, 133.2, 132.2, 130.8, 129.2, 127.9, 125.6, 124.8, 114.8, 97.7, 58.9, 45.2, 24.9, 22.7 ppm; MS (ESI): m/z = 507 $[M+H]^+$. CHN analysis for $C_{23}H_{18}F_4N_4O_5$; Calcd (%): C, 54.55; H, 3.58; N, 11.06; Found (%): C, 54.58; H, 3.60; N, 11.03.

4-(5-(2-(5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)-2,4-dioxoimidazolidin-1-yl)ethyl)isoxazol-3-yl)benzonitrile (**5i**): Light yellow solid; Yield 81%; M.P. 191-193 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (s, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.77-7.70 (m, 3H), 7.46 (d, J =

8.1 Hz, 2H), 6.84 (s, 1H), 4.12 (t, *J* = 7.2 Hz, 2H), 2.33 (t, *J* = 7.2 Hz, 2H), 1.46 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.3, 170.4, 160.4, 158.7, 145.9, 144.9, 136.6, 134.5, 132.7, 131.1, 129.5, 128.3, 124.9, 124.5, 119.7, 114.5, 97.5, 58.4, 44.6, 24.7, 22.4 ppm; MS (ESI): m/z = 514 $[M+H]^+$; CHN analysis for $C_{24}H_{18}F_3N_5O_5$; Calcd (%): C, 56.14; H, 3.53; N, 13.64; Found (%): C, 56.12; H, 3.57; N, 13.62.

5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)-1-(2-(3-(4-nitrophenyl)isoxazol-5-yl)ethyl)imidazolidine-2,4-dione (**5j**): Light yellow solid; Yield 85%; M.P. 201-203 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (d, *J* = 8.5 Hz, 2H), 8.15 (s, 1H), 8.07 (d, *J* = 7.6 Hz, 1H), 7.89 (d, *J* = 8.65 Hz, 2H), 7.73 (d, *J* = 7.6 Hz, 1H), 6.86 (s, 1H), 4.16 (t, *J* = 7.2 Hz, 2H), 2.36 t, *J* = 7.2 Hz, 2H), 1.48 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.6, 170.6, 160.8, 159.1, 150.2, 146.5, 145.2, 137.1, 133.7, 130.6, 128.5, 127.8, 126.1, 124.7, 124.3, 97.8, 58.8, 45.2, 24.9, 22.8 ppm; MS (ESI): m/z = 556 $[M+Na]^+$; CHN analysis for $C_{23}H_{18}F_3N_5O_7$; Calcd (%): C, 51.79; H, 3.40; N, 13.13; Found (%): C, 51.76; H, 3.43; N, 13.11.

1-(2-(3-(3,5-dibromophenyl)isoxazol-5-yl)ethyl)-5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)imidazolidine-2,4-dione (**5k**): Light orange solid; Yield 78%; M.P. 210-212 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (s, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.86 (s, 1H), 7.73-7.69 (m, 3H), 6.83 (s, 1H), 4.13 (t, *J* = 7.3 Hz, 2H), 2.34 (t, *J* = 7.3 Hz, 2H), 1.46 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.4, 170.6, 161.1, 158.6, 146.2, 144.5, 136.5, 132.7, 131.2, 129.9, 128.5, 128.2, 124.8, 124.4, 122.8, 97.4, 58.3, 44.6, 24.7, 22.6 ppm; MS (ESI): m/z = 647 $[M+H]^+$; CHN analysis for $C_{23}H_{17}Br_2F_3N_4O_5$; Calcd (%): C, 42.75; H, 2.65; N, 8.67; Found (%): C, 42.73; H, 2.61; N, 8.69.

1-(2-(3-(3,5-dichlorophenyl)isoxazol-5-yl)ethyl)-5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)imidazolidine-2,4-dione (**5l**): Colorless solid; Yield 83%; M.P. 196-198 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (s, 1H), 8.04 (d, *J* = 7.6 Hz, 1H), 7.73 (d, *J* = 7.6 Hz, 1H), 7.63 (s, 2H), 7.39 (s, 1H), 6.84 (s, 1H), 4.14 (t, *J* = 7.3 Hz, 2H), 2.35 (t, *J* = 7.3 Hz, 2H), 1.47 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 170.3, 160.7, 158.5, 145.9, 144.7, 135.8, 133.6, 132.4, 130.5, 129.7, 128.5, 126.2, 124.6, 124.3, 97.6, 58.6, 44.8, 24.9, 22.8 ppm; MS (ESI): m/z = 558 $[M+H]^+$; CHN analysis for $C_{23}H_{17}Cl_2F_3N_4O_5$; Calcd (%): C, 49.57; H, 3.07; N, 10.05; Found (%): C, 49.55; H, 3.09; N, 10.07.

5-(5-(2-(5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)-2,4-dioxoimidazolidin-1-yl)ethyl)isoxazol-3-yl)isophthalonitrile (**5m**): Light blue solid; Yield 80%; M.P. 194-196 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (s, 2H), 8.13 (s, 1H), 8.08 (s, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.74 (d, *J* = 7.6 Hz, 1H), 6.85 (s, 1H), 4.15 (t, *J* = 7.3 Hz, 2H), 2.34 (t, *J* = 7.3 Hz, 2H), 1.46 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.6, 170.5, 160.6, 158.7, 146.4, 144.8, 138.2, 137.4, 132.7, 132.1, 130.5, 129.2, 125.8, 125.4, 119.3, 117.6, 97.8, 58.7, 44.9, 24.7, 22.7 ppm; MS (ESI): m/z = 539 $[M+H]^+$; CHN analysis for $C_{25}H_{17}F_3N_6O_5$; Calcd (%): C, 55.77; H, 3.18; N, 15.61; Found (%): C, 55.75; H, 3.19; N, 15.64

1-(2-(3-(3,5-dinitrophenyl)isoxazol-5-yl)ethyl)-5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)imidazolidine-2,4-dione (**5n**): Light yellow solid; Yield 77%; M.P. 209-211 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.02 (s, 2H), 8.78 (s, 1H), 8.15 (s, 1H), 8.06 (d, *J* = 7.6 Hz, 1H), 7.76 (d, *J* = 7.6 Hz, 1H), 6.87 (s, 1H), 4.16 (t, *J* = 7.3 Hz, 2H), 2.37 (t, *J* = 7.3 Hz, 2H), 1.47 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.8, 170.9, 161.3, 159.2, 147.6, 146.7, 145.4, 133.5, 132.9, 132.3, 130.1, 129.5, 125.2, 124.9, 123.3, 98.1, 58.9, 45.6, 24.9, 23.1 ppm; MS (ESI): m/z = 579 $[M+H]^+$; CHN analysis for $C_{23}H_{17}F_3N_6O_9$; Calcd (%): C, 47.76; H, 2.96; N, 14.53; Found (%): C, 47.79; H, 2.94; N, 14.52.

Conclusion

The Cu(I)-promoted synthesis of a new series Nilutamide-isoxazoles (**5a-5n**) deprived of the isolation of unstable and unsafe hydroximoyl chlorides. The *in vitro* anti-prostate cancer activity revealed that many compounds showed more activity against DU-145 with IC_{50} values ranging from 21.8 to 62.7 μM. Specifically, bromo-substituted compounds **5f** (IC_{50} = 21.8 μM) and **5k** (IC_{50} = 27.7 μM) and fluoro substituted compound **5h** (IC_{50} = 26.4 μM) had higher activity against DU-145 than the 5-FU (IC_{50} = 38.3 μM). As well, compounds **5f** and **5k** had significant potency in inhibiting tyrosine Kinase EGFR with MIVs 93.4% and 91.3% respectively, in comparison to the Erlotinib (98.2%). Further anti-prostate cancer mechanistic studies in under progress.

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