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Synthesis of some new isoxazole-piperidine-1,2,3-triazoles as *in vitro* anticancer agents

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The synthesis of some new isoxazole-piperidine-1,2,3-triazoles (4a-4j) have been achieved using Sharpless Cu(I) catalyzed [3+2] cycloaddition as a key approach. The *in vitro* anticancer screening of all the compounds against four human cancer cell lines including MCF-7, HeLa, A549 and IMR32 has revealed that the compounds **4c** and **4f** exhibited promising activity against all the cell lines as compared to etoposide. Rest of the compounds have shown good to zero activity against specific cell line when compared with the positive control. Predominantly, the compound **4c** is showing superior activity against IMR32 which posses IC_{50} value 3.2±0.3 μM.

Keywords: Isoindole, 1,2,3-triazole, Anticancer activity

1,2,3-Triazoles are most important classes of nitrogen containing heterocycles that can form various non covalent interactions which includes hydrogen bonding, hydrophobic interactions, van der Waals forces and dipole-dipole attractions with various protein targets in biological system. Accordingly, 1,2,3-triazole hybrids exhibit remarkable applications in medicinal chemistry that includes anticancer[1,2], antiviral[3,4], antibacterial[5,6], antifungal[7,8], antitubercular[9,10] and antimalarial[11,12] activities. Some of the 1,2,3-triazole compounds like cefatrizine (**1**), carboxyamidotriazole (**2**) and compound **3** (Fig. 1) are under clinical trials for cancer therapy.

In the context of the development new anticancer drug with little side effects and high efficacy in the current medicinal chemistry community and based on the outstanding role of 1,2,3-triazoles[13,14] and isoxazoles[15] in several anticancer active compounds, herein, we reported the synthesis of some new isoxazole-piperidine-1,2,3-triazoles as in vitro anticancer agents.

Experimental Details

The entire synthesis of isoxazole-piperidine-1,2,3-triazoles (4a-j) is shown in Scheme 1. Initially, 3-(piperidin-4-yl) isoxazole (**1**) was treated with chloroacetyl chloride using Et_3N in DCM at 0-5°C for 30 min to give 2-chloro-1-(4-(isoxazol-3-yl)piperidin-1-yl)ethan-1-one (**2**). Later, the intermediate **2** was subjected to azidation using NaN_3 in acetone-H_2O media at 30°C for 3 h to give 2-azido-1-(4-(isoxazol-3-yl) piperidin-1-yl) ethan-1-one (**3**). Finally, the Cu-catalyzed [3+2] cycloaddition reaction between intermediate **3** and different alkynes in MeOH-H_2O media at the same temperature for 9 h provided the desired isoxazole-piperidine-1,2,3-triazoles (**4a-j**) shown in Table 1, in moderate to good yields.

Results and Discussion

Chemistry

In the present investigation, the synthesis of new class of triazole tethered isoxazole piperidine hybrid heterocycles has been achieved for the first time in three good yielding steps employing Huisgen 1,3-dipolar cycloaddition reaction *via* a click chemistry approach. The synthetic strategy planned for the preparation of desired target **4** is summarized in Scheme 1**.** Our synthetic methodology commenced with commercially available isoxazole **1**, which was subjected to the sequence of transformation as outlined. Thus, isoxazole derivative **1** was treated with chloroacetyl chloride in presence of Et_3N in CH_2Cl_2 at 0–5 °C to furnish *N*-acylated derivative **2** in good yield. Compound **2** was further reacted with NaN_3 in mixture of solvents (water : acetone, 1:3) at ambient temperature to afford azide **3**. Spectral data of the desired compounds are shown below.

4a: MF: $C_{23}H_{27}N_5O_4$, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.67 – 7.05 (m, 6H), 6.44 (s, 1H), 5.14 (t, *J* = 48.4 Hz, 4H), 3.75 (d, *J* = 56.4 Hz, 2H), 3.37 (t,

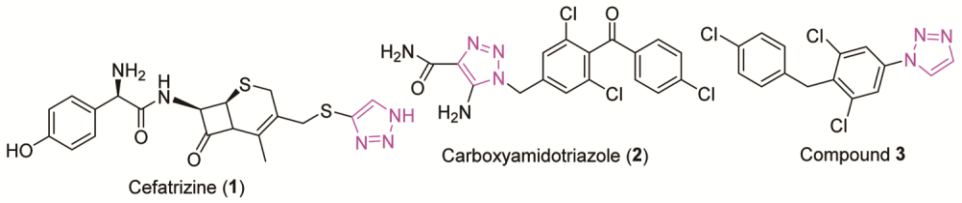
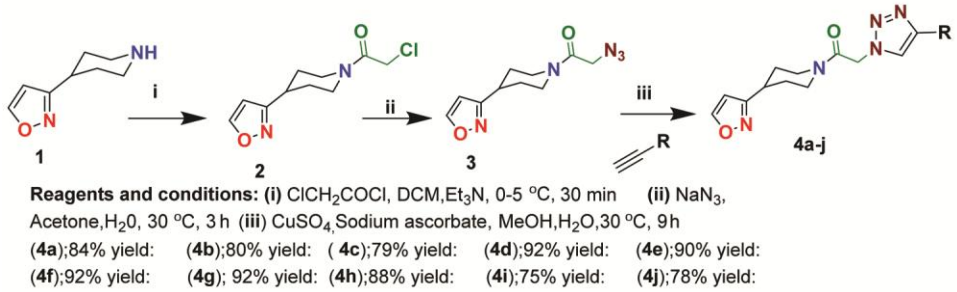


Fig. 1 — 1,2,3-Triazole based molecules under clinical trial for cancer therapy



Scheme 1 — Synthesis of isoxazole-piperidine-1, 2, 3-triazoles (4a-j)

J = 43.3 Hz, 4H), 2.63 – 1.68 (m, 6H), 1.06 (s, 3H).^{13}CNMR (100 MHz, $CDCl_3$) δ 176.12, 175.99, 169.31, 158.31, 143.47, 139.09, 130.57, 130.57, 128.82, 128.82, 127.71,106.76, 53.34. LC-MS: *m/z* 437.21 $[M]^+$.

4b: MF: $C_{21}H_{22}ClN_5O_4$, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.48 (d, *J* = 66.5 Hz, 1H), 7.40 – 7.05 (m, 4H), 6.52 (s, 1H), 5.30 (s, 2H), 5.10 (s, 1H), 4.73 (s, 1H), 3.89 (s, 2H), 3.38 (dd, *J* = 72.7, 13.5 Hz, 5H), 2.28 (s, 2H), 1.98 (s, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ 176.12, 171.78, 169.31, 158.31, 143.47, 133.79, 132.91, 131.74, 130.38, 130.12, 127.97, 121.45, 106.76, 50.88, 48.18, 43.68. LC-MS: *m/z* 443.14 $[M]^+$.

4c: MF: $C_{23}H_{27}N_5O_6$,^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (s, 1H), 7.55 (s, 1H), 7.11 (t, *J* = 53.8 Hz, 3H), 6.46 (s, 1H), 5.20 (d, *J* = 85.2 Hz, 3H), 4.24 (d, *J* = 156.8 Hz, 2H), 3.95 (d, *J* = 126.4 Hz, 9H), 3.31 (dd, *J* = 71.4, 58.8 Hz, 4H), 2.00 (dd, *J* = 263.7, 24.0 Hz, 4H).^{13}C NMR (100 MHz, $CDCl_3$) δ 176.12, 171.41, 169.31, 158.31, 148.54, 146.83, 143.47, 128.87, 121.56, 113.67, 111.58, 106.76, 56.79, 50.88, 48.18, 40.49.LC-MS: *m/z* 469.20 $[M]^+$.

4d: MF: $C_{25}H_{31}N_5O_4$,^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.55 (s, 1H), 7.31 (d, *J* = 28.8 Hz, 4H), 6.49 (s, 1H), 5.52 – 4.60 (m, 4H), 3.96 – 3.37 (m, 7H), 2.26 (dt, *J* = 27.1, 9.0 Hz, 4H), 1.39 (s, 9H).^{13}C NMR (100MHz, $CDCl_3$) δ 176.12, 171.41, 169.31, 158.31, 152.48, 143.47, 131.41, 130.10, 130.10, 121.45, 106.76, 50.88.LC-MS: *m/z* 465.24 $[M]^+$.

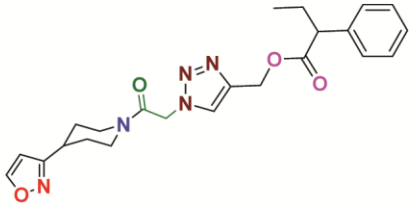
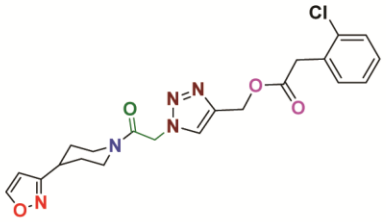
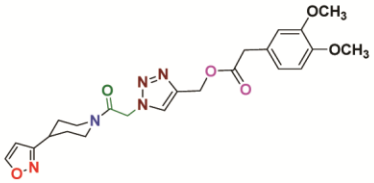
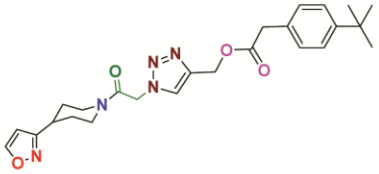
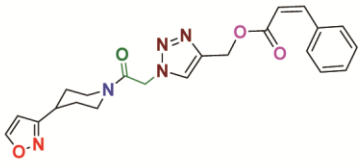
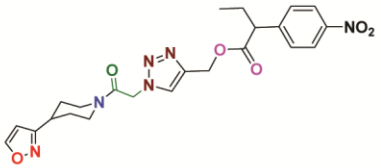
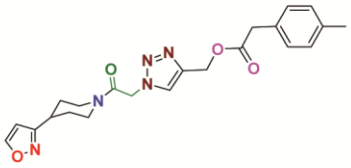
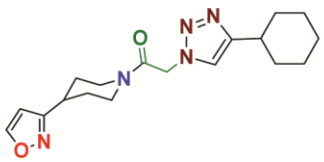
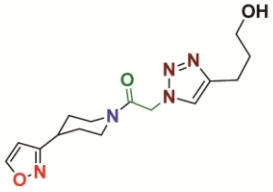
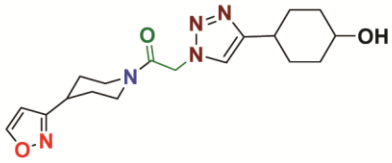
4e: MF: $C_{22}H_{23}N_5O_4$, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.53 (d, *J* = 15.8 Hz, 3H), 7.37 (s, 3H), 7.25 (d, *J* = 10.8 Hz, 1H), 6.35 (s, 2H), 5.72 – 4.36 (m, 4H), 3.36 (dt, *J* = 27.1, 12.3 Hz, 5H), 2.11 (dt, *J* = 108.8, 11.7 Hz, 4H).^{13}C NMR (100 MHz, $CDCl_3$) δ 176.12, 169.31, 168.85, 158.31,143.47, 135.07, 129.46, 129.02, 128.06, 121.45, 117.93, 106.76, 51.72.LC-MS: *m/z* 421.18 $[M]^+$.

4f: MF: $C_{23}H_{26}N_6O_6$,^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (dd, *J* = 173.7, 82.1 Hz, 5H), 6.86 (d, *J* = 187.0 Hz, 2H), 5.71 – 4.26 (m, 4H), 4.24 – 3.71 (m, 2H), 3.30 (dt, *J* = 25.1, 9.4 Hz, 4H), 2.13 (dt, *J* = 54.9, 10.5 Hz, 6H), 1.02 (d, *J* = 13.2 Hz, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 176.12, 175.99, 169.31, 158.31, 147.80, 147.48, 143.47, 131.08, 131.08, 122.92, 106.76, 53.34, 50.64, 48.18. LC-MS: *m/z* 482.19 $[M]^+$.

4g: MF: $C_{22}H_{25}N_5O_4$, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 2H), 7.65 – 6.83 (m, 10H), 6.47 (s, 1H), 5.40 – 4.73 (m, 8H), 4.06 – 3.35(m, 14H), 2.63 – 2.25 (m, 10H), 2.10 (d, *J* = 13.9 Hz, 4H).^{13}C NMR (100 MHz, $CDCl_3$) δ 176.12, 171.40, 169.31, 158.31, 143.47, 136.85, 131.17, 129.94, 121.45, 106.76, 83.08, 50.88, 40.95. LC-MS: *m/z* 423.19 $[M]^+$.

4h: MF: $C_{18}H_{25}N_5O_2$,^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 117.0 Hz, 2H), 6.46 (s, 1H), 5.09 (s, 1H), 3.66 (dt, *J* = 94.0, 15.0 Hz, 5H), 2.77 (d, *J* = 23.7 Hz, 1H), 2.50 – 2.14 (m, 4H), 2.10 – 1.15 (m, 11H).^{13}C NMR (100 MHz, $CDCl_3$) δ 176.12, 169.31, 158.31, 153.87, 125.72, 106.76, 48.18, 43.68, 43.68, 37.20. LC-MS: *m/z* 343.20 $[M]^+$.

Table 1 — Structures of compounds **4a-4j**

Entry	Structure	Entry	Structure
4a		4b	
4c		4d	
4e		4f	
4g		4h	
4i		4j	

4i: MF: $C_{15}H_{21}N_5O_3$, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.55 (s, 1H), 6.46 (s, 1H), 5.06 (d, *J* = 31.3 Hz, 2H), 4.08 – 3.17 (m, 7H), 3.03 – 2.23 (m, 4H), 1.94 (dd, *J* = 55.3, 12.8 Hz, 4H), 0.68 (s, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 176.12,169.31, 158.31, 149.33, 126.34, 106.76, 62.82, 48.18, 23.63.LC-MS: *m/z* 319.16 $[M]^+$.

4j: MF: $C_{18}H_{25}N_5O_3$, ^{1}H NMR (400 MHz, chloroform) δ 7.92 (d, *J* = 7.5 Hz, 1H), 7.55 (s, 1H), 6.48 (d, *J* = 7.5 Hz, 1H), 4.93 (d, *J* = 58.6 Hz, 2H), 3.76 (dddd, *J* = 27.8, 9.7, 8.6, 4.2 Hz, 4H), 3.44 – 3.17 (m, 2H), 2.91 (p, *J* = 8.0 Hz, 1H), 2.61 – 2.23 (m, 4H), 2.12 (dddd, *J* = 12.4, 8.5, 6.3, 4.5 Hz, 4H), 1.82 (dt, *J* = 7.9, 5.8 Hz, 2H), 1.61 (dt, *J* = 7.3, 5.8 Hz, 2H), 1.09 (s, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 176.12 (s), 169.31 (s), 158.31 (s), 153.87 (s), 125.71 (s), 106.76 (s), 69.52 (s), 48.17 (s), 43.68 (s), 37.33 (s), 32.48 (d, *J* = 131.6 Hz), 29.00 (d, *J* = 60.1 Hz). LC-MS: *m/z* 359.42 $[M]^+$.

In vitro anti-cancer activity

The 1,2,3-triazole hybrids (**4a-4j**) have screened for their *in vitro* anticancer activity against four human cancer cell lines MCF-7, HeLa, A549 and IMR 32 using Etoposide as standard. When we observed the results (Table 2), the compounds **4f** (having 4-NO_2 substituent) has shown good activity with IC_{50} (μM) values 4.5 ± 1.4 (MCF-7), 5.2±1.6 (HeLa), 9.3±2.4 (A549) and 4.3±0.5 (IMR32) and **4c** (having two methoxy substituent's) has shown better activity with IC_{50}(μM) values 4.1±1.2 (MCF-7), 4.4±1.3 (HeLa), 8.6±2.2 (A549) and 3.2±0.3 (IMR32)

Table 2 — *In vitro* anticancer activity of synthesized compounds (**4a-4i**) with IC50 in μM[a]

Compound	[b] MCF-7	[c] HeLa	[d] A549	[e] IMR32
4a	11.1±4.7	13.9±7.s5	15.8±9.9	14.5±5.4
4b	ND	10.8±9.86	13.2±6.8	16.8±8.8
4c	4.1±1.2	4.4± 1.3	8.6±2.2	3.2±0.3
4d	10.1±4.2	12.4 ± 2.1	16.8±0.9	13.8± 0.2
4e	12. 4±4.8	20.8±6.4	20.8±6.4	17.4±2.1
4f	4.5 ± 1.4	5.2±1.6	9.3±2,4	4.3±0.5
4g	16.1 ± 1.1	12.7 ± 2.2	16.2±0.9	ND
4h	11.3 ± 5.5	8.8 ± 2.5	7.3±1.6	5.4±0.5
4i	ND	14.2±8.1	18.5±9.8	18.2±8.1
4j	16.1 ± 1.1	16.8± 0.2	29.2±6.4	20.8±2.2
Etoposide	3.1± 0.2	2.3±0.1	6.1±0.5	2.51±0.1

ND = Not determine. [a] Each data represents as mean ±S.D values from three different experiments performed in triplicates. [b] MCF-7: human breast cancer cell line. [c] HeLa: human cervical cancer cell line. [d] A549: human lung cancer cell line. [e] IMR32: human neuroblastoma cell line

and these results are close to that of standard drug Etoposide. Remaining compounds have shown moderate to zero activity against selected cell lines.

Conclusion

In conclusion, we herein extended the application of Sharpless Cu(I) catalyzed [3+2] cycloaddition methodology to synthesize some new isoxazole-piperidine-1,2,3-triazoles as in vitro anticancer agents. Among all, the compounds **4c** and **4f** exhibited promising activity against MCF-7, HeLa, A549 and IMR32 cell lines as compared to etoposide, while remaining compounds displayed good to zero activity against on selected cell liens when compared with the positive control. In specific, the compound 4c showed superior activity against IMR32 with IC_{50} value in μM = 3.2±0.3.

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