Synthesis and Cytotoxic Activities of 2-(4-(2-heterocycloethoxy)phenyl)-1,2,4-triazolo[1,5-a] Pyridines

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Abstract: Nine 2-(4-(2-heterocycloethoxy)phenyl)-1,2,4-triazolo[1,5-a] pyridines have been synthesized. The structures of all products were confirmed by ^{1}H NMR and HRMS. The cytotoxic activities of these compounds were evaluated against human ovary cancer cell line (HO-8910) *in vitro* by MTT method. The preliminary results showed that compound **4e** (IC_{50} 0.11mM) and compound **4f** (IC_{50} 0.11mM) exhibited moderate activity against the cancer cell line when compared with Cisplatin.

Keywords: Synthesis, cytotoxic, 1,2,4-triazolo[1,5-a] pyridines.

2-Aryl-1,2,4-triazolo[1,5-a]pyridines have been found to have pregnancy interceptive activity [1]. The mechanism of pregnancy interceptive activity was cell apoptosis to cause luteolysis [2]. Because tumor cells grow vigorously like embryo cells, we had synthesized 2-(substituted) phenyl 1,2,4- triazolo[1,5-a] pyridines and their cytotoxic activities had been evaluated in the recent paper [3]. We founded that 2-(4-benzyloxyphenyl)-8- methyl-1, 2, 4-triazolo [1, 5-a] pyridine and 2-(4- benzyloxyphenyl)-5-methyl-1, 2, 4-triazolo [1, 5-a] pyridine exhibited stronger activity against the cancer cell line when compared with Cisplatin. However, they have very low solubility, which hinder them from clinic application.

Heterocycles, such as piperazine, piperidine and morpholine are frequently introduced to compounds to improve their hydrophilic character. Heterocycles also play an important role in the optimization of leading compound. For example, 5-Methyl-2-(morpholino-4-yl)-ethyloxy-phenyl-1,2,4- triazolo [1, 5-a] pyridine has growth inhibitory activities against fungi[4] and 5-methyl-2- (piperazin-1-yl)-ethyloxyphenyl-1,2,4- triazolo [1, 5-a] pyridine has cytotoxic activities against cancer cell lines[5]. Thus, we designed and synthesized 2-(4-(2-hetero cycloethoxy) phenyl)-1,2,4-triazolo [1,5-a] pyridines in order to increase their solubility and find new compounds which have stronger cytotoxic cell activity.

RESULTS AND DISCUSSION

Scheme **1** outline the synthetic sequences employed in our laboratories for preparation of **4a-4i**. Compounds **1** were prepared by the procedure of the previous paper in our laboratory [3]. Hydrogenation of **1** by 10% Pd/C afforded compounds **2**. **2** were coupled to 1,2-dibromo ethane to form compounds **3**, which on treatment with the various heterocycles gave target compounds **4a-4i**. Physical properties and HRMS [M^+] data of **4a-4i** were summarized in Table **1**.

The cytotoxic activities of **4a-4i** were evaluated against human ovary cancer cell line (HO-8910) *in vitro* by MTT method [6]. The results were summarized in Table **1**. The IC_{50} value represents the drug concentration (mM) required to inhibit the cell growth by 50%. The preliminary results showed that some synthetic compounds exhibited activities against human ovary cancer cell line (HO-8910) *in vitro*. The most potent compounds were 5-methyl-2-(morpholino-4-yl)ethyloxy phenyl-1, 2, 4-triazolo [1, 5-a] pyridine **4e** and 8-methyl-2-(morpholino-4-yl)ethyloxyphenyl-1,2, 4-triazolo [1, 5-a] pyridine **4f**. Their IC_{50} values were 0.11mM and 0.11mM respectively. The cytotoxic activities of compounds were affected by the sort of heterocycle (**4a**,**4c**,**4e**,**4h**), the compound with morpholine (**4e**) had more potent cytotoxic activity. 5-Methyl substituted compounds and 8-methyl substituted compounds almost had same cytotoxic activities (**4a** *vs* **4b**, **4c** *vs* **4d**, **4e** *vs* **4f**), 7-methyl substituted compound had less cytotoxic activity (**4g** *vs* **4e** and **4f**).

EXPERIMENTAL

Melting points were recorded on a BUCHI melting point B-540 apparatus and were uncorrected. ^{1}HNMR spectral were determined in $CDCl_3$ on a Bruker 400MHz spectrometer with $SiMe_4$ as the internal standard. J values are given in Hz. Mass spectral data were recorded on a Bruker Esquir 3000 plus instrument. HRMS data were obtained on a Bruker FT-ICR-MS Apex III apparutas.

General Procedure for the Synthesis of 2-hydroxyphenyl-1, 2, 4-triazolo [1, 5-a] Pyridines 2

2.80 g 2-(4-Benzyloxyphenyl)-1, 2, 4-triazolo [1, 5-a] pyridines **1**, 0.28 g 10% Pd/C and 30 ml DMF were added to a 50 ml round-bottomed flask. After three vacuum/hydrogen

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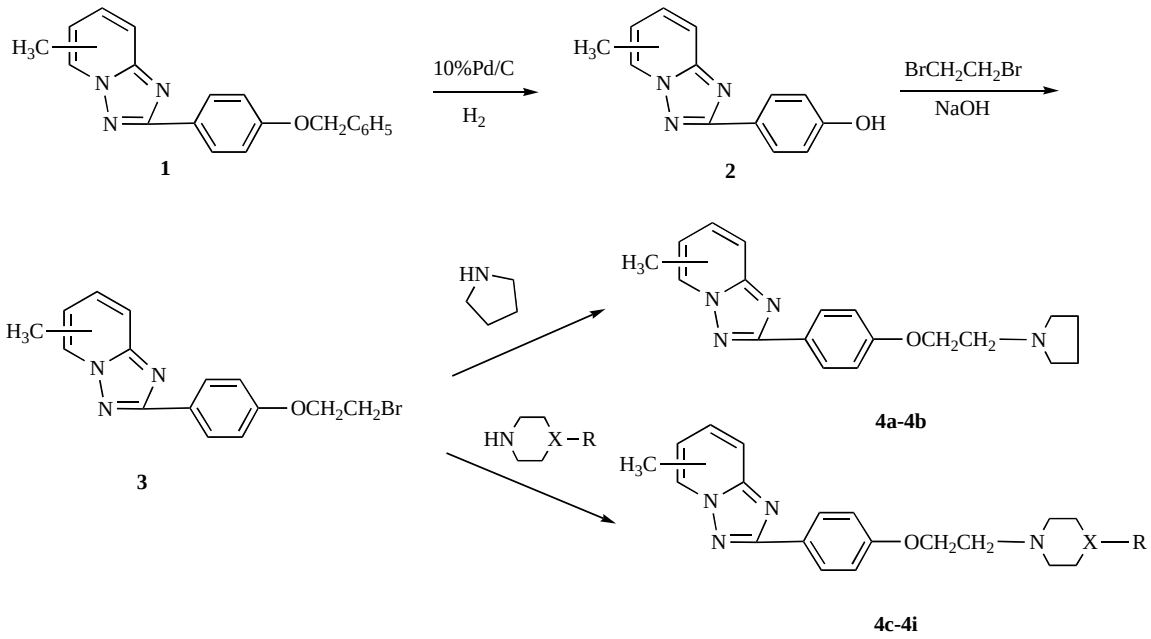
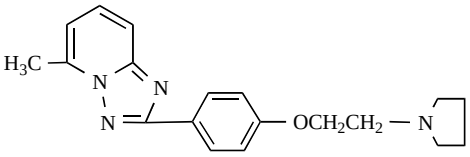
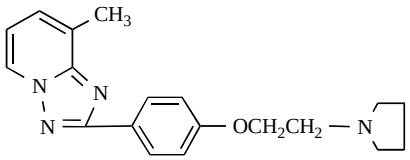
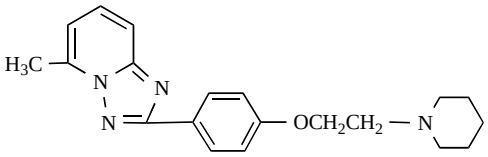
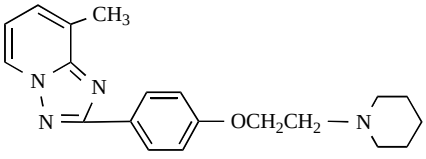
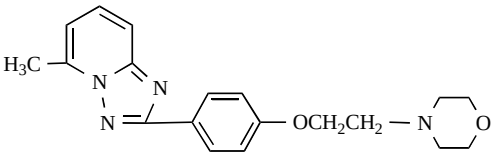
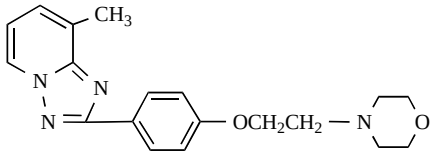
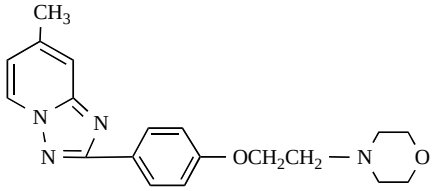
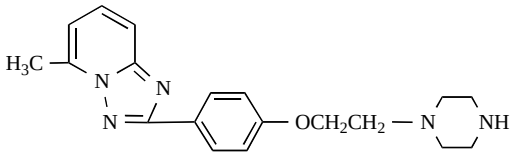
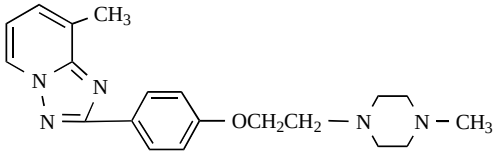


Scheme 1.

Table 1. Physical Analytical Data and IC_{50} of Compounds 4a-4i

Compound		Mp (°C)	Molecular Formula	HRMS [M^+] Calcd	Found	IC_{50} (mM)
4a		46-48	$C_{19}H_{22}N_4O$	322.1794	322.1797	*
4b		96-98	$C_{19}H_{22}N_4O$	322.1794	322.1792	*
4c		78-80	$C_{20}H_{24}N_4O$	336.1950	336.1952	0.18
4d		76-78	$C_{20}H_{24}N_4O$	336.1950	336.1951	0.18
4e		134-136[4]	$C_{19}H_{22}N_4O_2$	338.1743	338.1745	0.11

(Table 1). Contd…..

Compound		Mp (°C)	Molecular Formula	HRMS [M^+] Calcd	Found	IC_{50} (mM)
4f		80-82	$C_{19}H_{22}N_4O_2$	338.1743	338.1742	0.11
4g		102-104	$C_{19}H_{22}N_4O_2$	338.1743	338.1745	0.27
4h		92-94[5]	$C_{19}H_{23}N_5O$	337.1903	337.1902	0.47
4i		79-81	$C_{20}H_{25}N_5O$	351.2059	351.2057	0.37
cisplatin						0.04

*The IC_{50} values were more than 1.5mM.

cycles to remove air from the reaction flask, the reaction mixture was stirred at room temperature under hydrogen. When the reaction was deemed complete by TLC, the mixture was filtrated and the filtration was diluted with 100 ml water. The solid was collected by vacuum filtration, washed twice with 30 ml water and dried under vacuum. The product was carried on to the next step without further purification.

General Procedure for the Synthesis of 2-bromoethyloxy phenyl-1, 2, 4-triazolo [1, 5-a] Pyridines 3

1.50 g (6 mmol) 2-Hydroxyphenyl-1, 2, 4-triazolo [1, 5-a] pyridines **2**, 6.76 g (36 mmol) $BrCH_2CH_2Br$, 2.4 g NaOH, 3.6 ml water and 0.1 g TBAB were added to a 25 ml round-bottomed flask. The reaction mixture was stirred at reflux temperature. When the reaction was deemed complete by TLC, the mixture was cooled and diluted with 50 ml CH_2Cl_2 and 50 ml water. The CH_2Cl_2 layer was washed with water (3 × 30ml), dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was purified by column chromatography (ethyl acetate: petroleum ether = 1:2) to afford the target compound.

2-Bromoethyloxyphenyl-5-methyl-1,2,4- triazolo [1, 5-a] pyridine (3a)

This compound was obtained as a white solid. yield, 85.8%; mp 123~125°C; ^{1}H nmr: 2.84 (s, 3H), 3.68 (t, J=6.3Hz, 2H), 4.37 (t, J=6.3Hz, 2H), 6.83 (d, J=7.1Hz, 1H), 7.03 (d, J=8.8Hz , 2H), 7.45 (t, J=7.1Hz, J=8.8Hz, 1H), 7.64 (d, J=8.8Hz,1H), 8.28(d, J=8.8Hz , 2H); ms: m/z 331(M^+), 333 (M^++2).

2-Bromoethyloxyphenyl-7-methyl-1,2,4- triazolo [1, 5-a] pyridine (3b)

This compound was obtained as a white solid. yield, 87.9%; mp 126~128°C; ^{1}H nmr: 2.50 (s, 3H), 3.68 (t, J=6.3Hz, 2H), 4.37 (t, J=6.3Hz, 2H), 6.83 (dd, J=6.9Hz, J=1.1Hz, 1H), 7.02 (d, J=8.7Hz , 2H), 7.53 (s, 1H), 8.22 (d, J=8.7Hz, 2H), 8.44 (d, J=6.9Hz , 1H); ms: m/z 331(M^+), 333 (M^++2).

2-Bromoethyloxyphenyl-8-methyl-1, 2, 4-triazolo [1, 5-a] pyridine (3c)

This compound was obtained as a white solid. yield, 87.9%; mp 112~114°C; ^{1}H nmr: 2.69 (s, 3H), 3.68 (t, J=6.3Hz, 2H), 4.36 (t, J=6.3Hz, 2H), 6.89 (dd, J=6.9Hz, J=1.1Hz, 1H), 7.01 (d, J=8.7Hz , 2H), 7.25 (d, J=6.9Hz, 1H), 8.23 (d, J=8.7Hz, 2H), 8.42 (d, J=6.9Hz , 1H); ms: m/z 331(M^+), 333 (M^++2).ms: m/z 239 (M^+).

General Procedure for the Synthesis of 2-(4-(2-hetero cycloethoxy) phenyl-1,2,4- triazolo [1,5-a] Pyridines 4

0.25 g (0.75 mmol) 2-Bromoethyloxyphenyl-1, 2, 4-triazolo [1, 5-a] pyridines **3**, 5 mmol heterocycle and 5 ml $CHCl_3$ were added to a 25 ml round-bottomed flask. The reaction mixture was stirred at room temperature. When the reaction was deemed complete by TLC, the mixture was diluted with 50 ml CH_2Cl_2 and 50 ml water. The CH_2Cl_2 layer was washed with water (3 × 30ml), dried over

anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was purified by column chromatography (methanol: CH_2Cl_2 = 1:20) to afford the target compound.

5-Methyl-2-(pyrrolidin-1-yl)ethyloxyphenyl-1, 2, 4- triazolo [1, 5-a] pyridine (4a)

This compound was obtained as a pale yellow solid. yield, 99%; ^{1}H nmr: 1.87 (br, 4H), 2.74 (br, 4H), 2.83 (s, 3H), 3.00(t, J=5.7Hz, 2H), 4.23(t, J=5.7Hz, 2H), 6.81 (dd, J=0.6Hz, J=7.0Hz, 1H), 7.02 (d, J=8.6Hz, 2H), 7.42 (t, J=7.0Hz, J=8.6Hz, 1H), 7.60 (d, J=8.6Hz, 1H), 8.24(d, J=8.6Hz, 2H).

8-Methyl-2-(pyrrolidin-1-yl)ethyloxyphenyl-1, 2, 4- triazolo [1, 5-a] pyridine (4b)

This compound was obtained as a pale yellow solid. yield, 97%; mp 96~98°C; ^{1}H nmr: 1.84 (br, 4H), 2.68 (m, 7H),2.96 (t, J=5.9Hz, 2H), 4.20 (t, J=5.9Hz, 2H), 6.87 (t, J=6.9Hz, 1H), 7.02 (d, J=8.8Hz, 2H), 7.24 (d, J=6.9Hz, 1H), 8.21 (d, J=8.8Hz, 2H), 8.43 (d, 1H, J=6.9Hz).

5-Methyl-2-(piperidin-1-yl)-ethyloxyphenyl-1, 2, 4- triazolo [1, 5-a] pyridine (4c)

This compound was obtained as a pale yellow solid. yield, 99%; ^{1}H nmr: 1.49 (br, 2H), 1.67 (br, 4H), 2.60 (br, 4H), 2.83 (s, 3H), 2.86 (t, J=5.9Hz, 2H), 4.22 (t, J=5.9Hz, 2H), 6.80 (d, J=7.0Hz, 1H), 7.02 (d, J=8.8Hz, 2H), 7.41 (t, J=7.0Hz, J=8.8Hz, 1H), 7.60 (d, J=8.8Hz, 1H), 8.25 (d, 2H, J=8.8Hz).

8-Methyl-2-(piperidin-1-yl)-ethyloxyphenyl-1, 2, 4- triazolo [1, 5-a] pyridine (4d)

This compound was obtained as a pale yellow solid. yield, 98%; ^{1}H nmr: 1.47 (br, 2H), 1.64 (br, 4H), 2.56 (br, 4H), 2.68 (s, 3H), 2.83 (t, J=6.0Hz, 2H), 4.20 (t, J=6.0Hz, 2H), 6.86 (d, J=6.9Hz, 1H), 7.01 (d, 2H, J=8.7Hz), 7.25 (d, J=6.9Hz, 1H), 8.22 (d, J=8.7Hz, 2H), 8.42(d, J=6.9Hz, 1H).

5-Methyl-2-(morpholino-4-yl)-ethyloxyphenyl-1, 2, 4-triazolo [1, 5-a] pyridine (4e)

This compound was obtained as a pale yellow solid. yield, 97%; ^{1}H nmr: 2.62 (br, 4H), 2.83~2.86 (m, 5H), 3.76 (t, J=4.5Hz, 4H), 4.20 (t, J=5.6Hz, 2H), 6.81 (d, J=7.0Hz, 1H), 7.02 (d, J=8.7Hz, 2H), 7.42 (t, J=7.0Hz, J=8.8Hz, 1H), 7.61 (d, J=8.8Hz, 1H), 8.25 (d, J=8.8Hz, 2H).

8-Methyl-2-(morpholino-4-yl)-ethyloxyphenyl-1, 2, 4-triazolo [1, 5-a] pyridine (4f)

This compound was obtained as a pale yellow solid. yield, 98%; ^{1}H nmr: 2.64 (br, 4H), 2.71 (s, 3H), 2.87 (t, J=5.6Hz, 2H), 3.78 (t, J=4.5Hz, 4H), 4.21 (t, J=5.6Hz, 2H), 6.90 (d, J=6.9Hz, 1H), 7.03 (d, J=8.6Hz, 2H), 7.27 (d, J=6.9Hz, 1H), 8.24 (d, J=8.6Hz, 2H), 8.45(d, J=6.9Hz, 1H).

7-Methyl-2-(morpholino-4-yl)-ethyloxyphenyl-1, 2, 4-triazolo [1, 5-a] pyridine (4g)

This compound was obtained as a pale yellow solid. yield, 97%; ^{1}H nmr: 2.48 (s, 3H), 2.62 (br, 4H), 2.84 (t, J=5.6Hz, 2H), 3.75 (t, J=4.3Hz, 4H), 4.19 (t, J=5.6Hz, 2H), 6.80 (d, J=6.9Hz, 1H), 7.01 (d, J=8.4Hz, 2H), 7.47 (s, 1H), 8.19 (d, J=8.4Hz, 2H), 8.43 (d, J=6.9Hz, 1H).

5-Methyl-2-(piperazin-1-yl)-ethyloxyphenyl-1, 2, 4- triazolo [1, 5-a] pyridine (4h)

This compound was obtained as a pale yellow solid. yield, 97%; ^{1}H nmr: 2.64 (br, 4H), 2.84~2.87 (m, 5H), 2.98 (m, 4H), 3.10 (br, 1H), 4.20 (t, J=5.7Hz, 2H), 6.81 (d, J=7.0Hz, 1H), 7.02 (d, J=8.8Hz, 2H), 7.43 (t, J=7.0Hz, J=8.6Hz, 1H), 7.62 (d, J=8.8Hz, 1H), 8.27 (d, J=8.6Hz, 2H).

5-Methyl-2-(4-methylpiperazin-1-yl)-ethyloxyphenyl-1, 2, 4-triazolo [1, 5-a] pyridine (4i)

This compound was obtained as a pale yellow solid. yield, 98%; ^{1}H nmr: 2.31 (s, 3H), 2.54 (br, 8H), 2.67 (s, 3H), 2.86 (t, J=5.8Hz, 2H), 4.18 (t, J=5.8Hz, 2H), 6.87 (t, J=6.9Hz, 1H), 7.01 (d, J=8.8Hz, 2H), 7.24 (d, J=6.9Hz, 1H), 8.21(d, J=8.8Hz, 2H), 8.42 (d, J=6.9Hz, 1H).

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