Synthesis and Biological Evaluation of Pyridooxazine–Tetrahydroisoquinoline Derivatives as MDR Modulators

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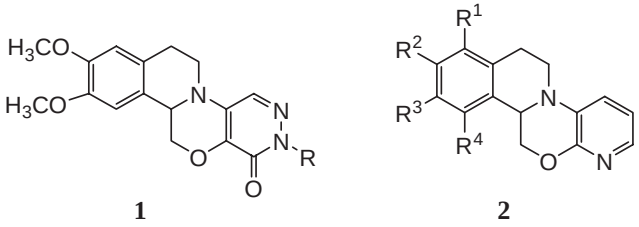
Pyridooxazine–tetrahydroisoquinoline derivatives were designed and synthesized for MDR modulating activity. Pyridooxazin-2-one scaffolds were constructed in a one-pot annulation of *N*-substituted-2-chloroacetamides with 2-bromo-3-hydroxy pyridine via Smiles rearrangement. The Pictet–Spengler cyclization to form tetrahydroisoquinoline ring afforded target compounds in 17–37% overall yields. Some of these compounds exhibited multidrug resistance (MDR) reversing activity.

Tetrahydroisoquinoline alkaloids are important because of their occurrence in nature and their physiological properties.[1] They possess diverse type of biological activities and are extensively studied over past hundred years. Some tetrahydroisoquinoline derivatives such as higenamine, coclaurine, and pyrimidoisoquinoline exhibit good biological activities.[2]

Recently, there has been a growing interest in developing general and versatile synthetic methods[3] for these heterocyclic systems. As part of our program to the development of novel inhibitors of multidrug resistance (MDR) pumps,[4] we recently introduced a very efficient entry to the synthesis of tetrahydroisoquinolines containing pyridazinone and oxazine moieties **1**.[5] Herein, we report the synthesis and biological evaluation of tetrahydroisoquinolines containing pyridine and oxazine moieties **2** (Figure 1).

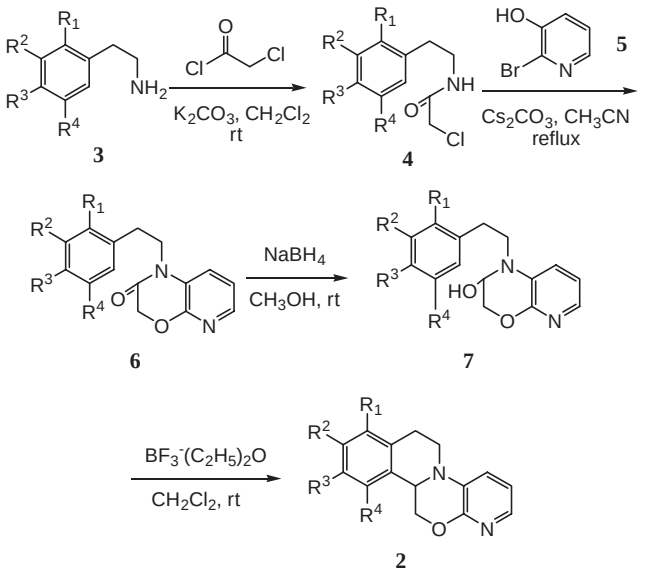


1 **2**

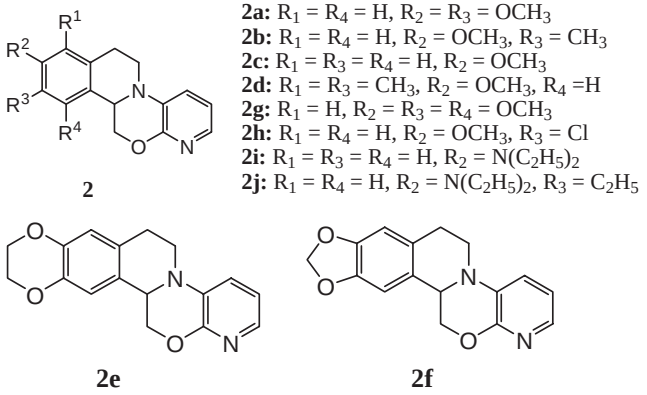
Figure 1.

The synthesis of target compounds **2** was showed in Scheme 1. By inference, substituted phenylethylamines **3** were defined as the starting materials. Reaction of substituted phenylethylamines **3** with 2-chloroacetyl chlorides in dichloromethane in the presence of potassium carbonate afforded 2-chloroacetamides **4** in excellent yields. The reactions occurred at room temperature for 12 h or at reflux for 2 h. Then, reaction of 2-chloroacetamides **4** with 2-bromo-3-hydropyridine **5** furnished pyridooxazin-2-ones **6**. The cyclization occurred smoothly in the presence of cesium carbonate at refluxing acetonitrile via Smiles rearrangement.[6]

Reduction of pyridooxazin-2-ones **6** in the presence of sodium borohydride in methanol at room temperature afforded



Scheme 1.



2a: $R_1 = R_4 = H$, $R_2 = R_3 = OCH_3$
2b: $R_1 = R_4 = H$, $R_2 = OCH_3$, $R_3 = CH_3$
2c: $R_1 = R_3 = R_4 = H$, $R_2 = OCH_3$
2d: $R_1 = R_3 = CH_3$, $R_2 = OCH_3$, $R_4 = H$
2g: $R_1 = H$, $R_2 = R_3 = R_4 = OCH_3$
2h: $R_1 = R_4 = H$, $R_2 = OCH_3$, $R_3 = Cl$
2i: $R_1 = R_3 = R_4 = H$, $R_2 = N(C_2H_5)_2$
2j: $R_1 = R_4 = H$, $R_2 = N(C_2H_5)_2$, $R_3 = C_2H_5$

2e **2f**

Figure 2.

the corresponding reducing products **7** in good yield. Towards the end of the subjection, the treatment of **7** with Lewis acids such as boron trifluoride–ether in dichloromethane by Pictet–Spengler cyclization afforded the desired target compounds **2** in 17–37% overall yields (Figure 2). The structures of the compounds **2** were confirmed by IR, ^{1}H NMR, ^{13}C NMR, and elemental analyses.

The appearance of tumor cell resistant to range of cytotoxic drugs is a serious problem in cancer chemotherapy. This phenomenon is called MDR. Recently, Robert has reviewed a

Table 1. The effect of compounds (**2a**–**2j**) on the cellular accumulation of VCR in multidrug-resistant MCF7-ADR human breast cancer cells

Compound	VCR accumulation[a] with a compound concentration of	
	1 μg/mL	10 μg/mL
2a	104	238
2b	135	192
2c	157	146
2d	136	179
2e	115	362
2f	73	334
2g	141	381
2h	95	125
2i	118	101
2j	97	92
Verapamil	103	353

[a]The amounts of VCR accumulated in multidrug-resistant MCF7-ADR human breast cancer cells were examined in the presence of 1 and 10 μg/mL of pyridooxazine–tetrahydroisoquinolines. The values expressed as the relative amounts of VCR accumulated in cancer cells as compared with the control experiment.

number of MDR reversing agents for clinical trials.[7] However, the development of new MDR modulators is still in its infancy. Previously, we reported the inhibitory activity of compounds **1**.[5] Herein, we observed promising activity of compounds **2** in reversing MDR.

The effect of compounds (**2a**–**2j**) on the cellular accumulation of vincristine (VCR) in multidrug-resistant MCF7-ADR human breast cancer cells was examined and the results were showed in Table 1. Verapamil at 1 and 10 μg/mL increased the VCR accumulation in a dose dependent manner. Among these compounds, **2a**, **2e**, **2f**, and **2g** increased the VCR accumulation as potent as verapamil. Compounds **2b**, **2d**, and **2h** increased moderately the accumulation, while compounds **2c**, **2i**, and **2j** decreased the VCR accumulation in MCF7-ADR human breast cancer cells. The results showed that potent compounds possess polyalkoxy substituents on phenyl ring.

Cytotoxic activity of compounds **2a**–**2j** against leukemia HL_{60} cells and human epidermoid carcinoma KB cells was showed in Table 2. Some structure–activity relationships of these compounds could be discerned. The compounds **2g** and **2h** showed very potent cytotoxicity while compounds **2e**, **2f**, **2i**, and **2j** did not show such cytotoxicity. Replacement of methoxy substituent by alkyl substituent seemed to have a negative effect (**2b** and **2d**), while the presence of more methoxy substituents appeared to obviously enhance the activity (**2g**). The compound **2h** (replacement of a methoxy substituent by chlorine) exhibited the most effective activity among these compounds.

Table 2. Cytotoxic activity of compounds (**2a**–**2j**) against leukemia HL_{60} cells and human epidermoid carcinoma KB cells

Compound	HL_{60} IC_{50} (μg/mL)[a]	KB IC_{50} (μg/mL)[a]
2a	8.5	3.1
2b	>20	14.2
2c	>20	>20
2d	13.4	7.6
2e	>20	>20
2f	>20	>20
2g	4.3	1.9
2h	2.5	1.2
2i	>20	>20
2j	>20	>20

[a]All the IC_{50} values in this text represent the mean of a minimum of three experiments.

In conclusion, the first total synthesis of pyridooxazine–tetrahydroisoquinolines proceeded efficiently in high overall yields via Smiles rearrangement and Pictet–Spengler cyclization. These compounds were evaluated for the discovery of new multidrug resistance reversal agents. Some compounds exhibited good activity as modifiers of multidrug resistance. The results suggested that the introduction of polyalkoxy groups on phenyl ring was effective for overcoming multidrug resistance. In particular, the compound **2g** with trimethoxy groups on phenyl ring showed the high MDR-modulating activity and cytotoxic activity, indicating a new drug candidate for MDR cancer chemotherapy.

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