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Synthesis and Antitumor Activity of 3-Arylisoquinoline Derivatives

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In order to study the structure-activity relationship of 7,8-dimethoxy-2-methyl-3-(4,5-methylenedioxy-2-vinylphenyl)isoquinoline-1(*2H*)-one (**2**), which has exhibited significant antitumor activity, chemical modifications of **2** were performed to yield the corresponding products (**3-7**). Further systematic uses of an efficient procedure for the synthesis of 3-arylisoquinoline derivatives produced the substituted compounds (**9a-9g**), which were tested for *in vitro* antitumor activity against five different human cancer cell lines.

Key words : Antitumor activity, 3-Arylisoquinoline derivatives

INTRODUCTION

In a previous paper, we reported the significant antitumor activity of 7,8-dimethoxy-2-methyl-3-(4,5-methylenedioxy-2-vinylphenyl)isoquinoline-1(*2H*)-one (ED_{50}= 0.001 μg/ml: SK-MEL) (Cho *et al.*, 1996). The serendipitous finding of this compound attracted us to explore the structure-activity relationship (SAR) of 3-arylisoquinoline to learn how to enhance its activity as well as synthesize its derivatives more effectively. The styrene compound (**2**) is thought to be a bioisostere of benzo[*c*]phenanthridine which has been shown to exhibit strong antitumor activity by inhibiting topoisomerase I or II, as shown in scheme 1 (Fang *et al.*, 1993, Janin *et al.*, 1993).

benzo[*c*]phenanthridine 3-arylisoquinoline

Scheme 1.

The disconnection of the C ring of benzo[*c*]phenanthridine skeleton gives the styrene compound, 3-arylisoquinoline, in what is considered to be an isosteric interchange (Thornber *et al.*, 1979). Therefore, we decided to study the primary SAR using the styrene (**2**) as a lead compound for defining the essential functional group exhibiting the activity. In order to modify the functional group of styrene (**2**), the amide moiety was reduced to dihydro- isoquinoline, a double bond was hydrogenated and the demethylation of the aromatic methoxy group was performed. However, a limitation of the diverse modification of **2** made us develop an alternative to the biomimetic transformation method of synthesizing 3-arylisoquinoline derivatives, which not only needs a preparation of protoberberine, but also restricts the introduction of substituents on the aromatic ring due to the difficulty of synthesis (Hanaoka *et al.*, 1991). For this reason we used the known procedure, taking *N*-methyltoluamide (**7**) and benzonitrile (**8**) as starting materials in the presence of a base (Poindexter *et al.*, 1982). The advantages of this method are: (1) the various substituted starting materials are commercially available or easily prepared, and (2) the one pot procedure saves time in synthesizing the desired molecules. By this method, several types of substituents were systematically introduced to the 3-phenyl ring and tested for *in vitro* antitumor activity. In this paper a modification of styrene (**2**) and the synthesis of 3-arylisoquinoline derivatives are described with the result of anti-tumor activities against five different tumor cell lines.

MATERIALS AND METHODS

Melting points were determined on a Electrothermal IA9200 melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra (^{1}H-NMR) were recorded on a Bruker AC 80 instrument, using TMS as the internal standard. Chemical shifts are re-

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ported in parts per million (δ) and signals are quoted as a s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). IR spectra were recorded on a Perkin-Elmer 783 spectrometer and Nicolet instrument using KBr pellets. Solvents were routinely distilled prior to use. Anhydrous tetrahydrofuran (THF) was distilled from sodium-benzophenon ketyl. Column chromatography was performed on Merck silica gel 60 (230-400 mesh). TLC was carried out using plates coated with silicagel 60F 254 purchased from Merck Co. The organic extract was dried with sodium sulphate.

Synthesis of compounds

(2-Ethyl-4,5-methylenedioxyphenyl)-7,8-Dimethoxy-2-methyl-3-isoquinoline-1(*2H*)-one (4): A solution of **2** (300 mg, 0.82 mmole) in MeOH (30 ml) was stirred with 5% Pd-C (120 mg) under hydrogen (1 atm) at room temperature overnight. The reaction mixture was filtered and the solvent was evaporated off. The residue was taken up in CH_2Cl_2. The organic layer was washed with brine, dried, and concentrated to dryness which was column chromatographed on silica gel with methylene chloride/methanol (100:3) to afford **4** (290 mg, 96%) as a solid. mp: 164-165°C, IR-$^{neat}cm^{-1}$: 1647 (amide), ^{1}H-NMR ($CDCl_3$) δ: 7.34, 7.19 (each 1H, AB-q, *J*=8.7 Hz, C_6-H and C_5-H), 6.81 (1H, s, $C_{6'}$-H), 6.67 (1H, s, $C_{3'}$-H), 6.27 (1H, s, C_4-H), 6.00 (2H, s, OCH_2O), 4.03, 3.95 (each 3H, each s, OMe x 2), 3.24 (3H, s, NMe), 2.40 (2H, q, *J*=7.2 Hz, benzylic H), 1.09 (3H, t, *J*=7.2 Hz, CH_2CH_3)

(2-Ethyl-4,5-methylenedioxyphenyl)-8-Hydroxy-7-methoxy-2-methyl-3-isoquinoline-1(*2H*)-one (6): A solution of **4** (380 mg, 1.03 mmole) and *c*-HCl (10 ml) in MeOH (30 ml) was heated to reflux for 12 h. 10%-NaOH solution (15 ml) was then added to the reaction mixture which was extracted with CH_2Cl_2. The extract was washed with water and brine, dried, and concentrated to dryness which was purified by column chromatography with methylene chloride/methanol (100:4) to afford **6** (360 mg, 98%) as a solid. mp: 143-144°C, IR$^{neat}cm^{-1}$: 1647 (amide), ^{1}H-NMR ($CDCl_3$) δ: 13.18 (1H, s, OH), 7.29, 6.88 (each1H, AB-q, *J*=10.0 Hz, C_6-H and C_5-H), 6.82 (1H, s, $C_{6'}$-H), 6.66(1H, s, $C_{3'}$-H), 6.38 (1H, s, C_4-H), 6.01 (2H, s, OCH_2O), 3.70 (3H, s, OMe), 3.25 (3H, s, NMe), 2.40 (2H, q, *J*=7.4 Hz, benzylic H), 1.18 (3H, t, J=7.4 Hz, CH_2CH_3).

(2-Ethyl-4,5-methylenedioxyphenyl)-1,2-Dihydro-7,8-dimethoxy-2-methyl-3-isoquinoline (5): $LiAlH_4$ (309 mg, 8.13 mmole) was added portionwise to a stirred solution of **4** (300 mg, 0.81 mmole) in dry THF (40 ml) at 0°C under nitrogen atmosphere. The reaction mixture was stirred for 1 h at room temperature under nitrogen atmosphere, then water was added at 0°C and the whole was filtered. The filtrate was concentrated and the residue was taken up in CH_2Cl_2. The solution was washed with water and brine, dried, and concentrated to dryness which was column chromatographed on silical gel with methylene chloride/methanol (100:4) to give **5** (250 mg, 87%). ^{1}H-NMR ($CDCl_3$) δ: 6.72, 6.55 (each 1H, AB-q, *J*=8.6 Hz, C_6-H and C_5-H), 6.73 (1H, s, $C_{6'}$-H), 6.66 (1H, s, $C_{3'}$-H), 6.00 (1H, s, C_4-H), 5.93 (2H, s, OCH_2O), 4.51 (2H, s, C_1-H), 3.83 (6H, s, OMe x 2), 2.50 (3H, s, NMe), 2.59 (2H, q, *J*= 7.6 Hz, benzylic H), 1.17 (3H, t, *J*=7.6 Hz, CH_2CH_3).

General procedure of the synthesis of 3-arylisoquinoline-1(*2H*)-one: *n*-BuLi (2.3 equivalent) was slowly added to stirred solution of *N*-methyl-o-toluamide (1 equivalent) in dry THF at ice-salt bath under nitrogen maintaining the reaction temperature never exceeded 20°C. After the addition was completed, the orange-red solution was stirred for 1 h at 0°C under nitrogen and the cooled to -50°C. A solution of benzonitrile (1 equivalent) in dry THF was quickly added to the reaction mixture. The cooling bath was removed and the reaction mixture allowed to warm to room temperature. The reaction mixture was quenched with water at room temperature and organic layer was separated, washed with brine, dried, and concentrated to dryness to give the crude yellow solid which was recrystallized from EtOH. The results for the preparation of 3-arylisoquinoline-1(*2H*)-one derivatives are summarized in Table I. The spectral and physical data obtained from the reaction are shown in Table III-1.

3-Phenylisoquinoline (12): $LiAlH_4$ (515 mg, 13.56 mmole) was added portionwise to a stirred solution of **9** (300 mg, 1.36 mmole) in dry THF (30 ml) at 0°C under nitrogen atmosphere. The reaction mixture was heated to reflux under nitrogen atmosphere, then water was added at 0°C and the whole was filtered through celite. The filtrate was washed brine, dried, and concentrated to dryness which was purified by column chromatography on silicagel with Hexane/EtOAC (5:1) to give **12** (110 mg, 39%) as a white solid. mp: 96-97°C. IR$^{neat}cm^{-1}$: 1640 (imine). H-NMR ($CDCl_3$) δ: 9.34 (1H, s, HC=N), 8.14-7.26 (10H, m, aromatic H).

General procedure of the synthesis of 1-chloro-3-

Table I. Substituents and Yield of Compounds

No	X	Yield	No	X	Yield
9a	H	43	10e	2-Me	93
9b	4-Br	27	10f	3-Me	86
9c	4-OMe	53	10g	4-Me	99
9d	4-Cl	37	11a	H	95
9e	2-Me	29	11b	4-Br	89
9f	3-Me	42	11c	4-OMe	85
9g	4-Me	57	11d	4-Cl	49
10a	H	86	11e	2-Me	95
10b	4-Br	94	11f	3-Me	64
10c	4-OMe	97	11g	4-Me	71
10d	4-Cl	69			

arylisoquinoline: Amide (**9**) (1.36 mmole) and phosphorous oxychloride (10 ml) were stirred at 50°C overnight. The phosphorous oxychloride was removed by vacuum distillation. The residue was taken up in EtOAC. The solution was washed with saturated $NaHCO_3$ solution, water, and brine, dried, and concentrated to dryness to give **10** as a solid. The results for the preparation of 1-chloro-3-arylisoquinoline-1(*2H*)-one derivatives are summarized in Table I. The spectral and physical data obtained from this reaction are shown in Table III-1.

General procedure of the synthesis of 1-(*N*-methyl) piperazinyl-3-arylisoquinolines: A mixture of iminechloride (**10**) (1.19 mmole), N-methylpiperazine (1.2 equivalent), and potassium carbonate (3 equivalent) in DMF (8 ml) was heated to reflux for 6 h. The reaction mixture was cooled to room temperature, diluted with water, and extracted with CH_2Cl_2. The combined organic extracts were washed with water, dried, and concentrated. The residue was purified by column chromatography on silicagel with Hexane/EtOAC (50:1 to 10:1) and then CH_2Cl_2:MeOH (100:1 to 100:4) to give the product (**11**) as a solid. The results for the preparation of 1-piperazinyl-3-arylisoquinoline derivatives are summarized in Table I. The spectral and physical data obtained from the reaction are shown in Table III-2.

Acetyl chloride (1.45 ml), (19.78 mmole) was slowly added to methanol (0.79 ml), (19.78 mmole) at 0°C. The solution was added all at once to the flask charged with **11** (300 mg), (0.99 mmole). The solvent was evaporated off to give hydrochloride salt of **11**.

Table II. *In vitro* antitumor activities of 3-arylisoquinolines

	ED_{50} (Log1/C)				
No.	A 549	SKOV-3	SK-MEL-2	XF 498	HCT 15
9a	5.24	5.61	5.59	5.23	5.86
9b	4.21	4.46	4.87	4.40	4.55
9c	3.81	3.03	4.52	3.98	4.20
9d	nd[a]	3.94	4.14	nd	4.14
9e	4.90	5.00	5.23	4.70	5.35
9f	4.39	4.61	nd	nd	4.62
9g	4.52	4.86	5.30	4.37	5.14
2	8.05	6.31	9.70	8.01	8.01
7	5.72	5.46	6.96	5.71	5.92
4	6.84	6.34	8.09	6.58	6.90
6	4.93	4.80	5.98	4.85	5.03
5	5.89	5.87	6.91	5.82	6.19
3b	6.49	5.57	6.75	5.90	5.55
11a	4.60	4.65	4.93	4.43	4.73
11b	4.82	4.77	5.32	4.79	5.18
11c	4.32	4.33	4.79	4.23	4.60
11d	4.68	4.72	5.03	4.80	4.75
11e	4.66	4.64	4.71	4.60	4.70
11f	4.67	4.65	5.00	4.72	4.75
11g	4.68	4.70	5.68	4.62	5.00

a: nd means not determined exact activity due to week activity (ED_{50}<30 μg/ml), b: The synthesis of this compound is already reported (Cho *et al.*, 1996). A 549: human lung, SK-OV-3: ovarian carcinoma, SK-MEL-2: melanoma, XF 498: CNS, HCT 15: colon

Antitumor test *in vitro*

The cytotoxicity experiment was performed using the five different human tumor cell lines, A-549 (human nonsmall cell lung), SKOV-3 (ovarian carcinoma), HCT-15 (colon), XF-498 (CNS), SK-MEL-2 (melanoma) which were purchased from National Cancer Institute (NCI) in U. S. A.

The cells were grown at 37°C in RPMI 1640 medium supplemented with 10% FBS and separated using PBS containing 0.25% trypsin and 3 mM EDTA. 5×10^3-2×10^4 cells were added to each well of 96 well plate and incubated at 37°C for 24 hrs. Each compounds was dissolved in DMSO and diluted with the above medium

Table III-1. Analytical and Physical Data

No	IR (Cm^{-1})	^{1}H-NMR ($CDCl_3$) δ	MP(°C)
9a	1650	10.45 (1H, s, NH), 8.40 (1H, d, J=7.6Hz, C8-H), 8.11-7.15 (8H, m, aromatic H), 6.89 (1H, s, C4-H)	202-203
9b	1651	11.50 (1H, s, NH), 8.22 (1H, d, J=7.5Hz, C8-H), 7.98-7.20 (7H, m, aromatic H), 6.93 (1H, s, C4-H),	266-267
9c	1647	11.34 (1H, s, NH), 8.19 (1H, d, J=7.9Hz, C8-H), 8.02-6.94 (7H, m, aromatic H), 6.80 (1H, s, C4-H), 3.83 (3H, s, OMe)	239-240
9d	1658	11.49 (1H, s, NH), 8.21 (1H, d, J=7.9Hz, C8-H), 7.87-7.17 (7H, m, aromatic H), 6.91 (1H, s, C4-H)	268-269
9e	1639	9.64 (1H, s, NH), 8.42 (1H, d, J=7.4Hz, C8-H), 8.10-7.10 (7H, m, aromatic H), 6.58 (1H, s, C4-H), 2.42 (3H, s, Me)	181-182
9f	1655	10.48 (1H, s, NH), 8.41 (1H, d, J=7.6Hz, C8-H), 8.11-7.14 (7H, m, aromatic H), 6.88 (1H, s, C4-H), 2.46 (3H, s, Me)	197-198
9g	1651	10.35 (1H, s, NH), 8.40 (1H, d, J=7.8Hz, C8-H), 8.10-7.00 (7H, m, aromatic H), 6.85 (1H, s, C4-H)	234-235
10a	-	8.40-7.25 (10H, m, aromatic H)	83-84
10b	-	8.31-7.45 (9H, m, aromatic H)	107-108
10c	-	8.37-6.92, (9H, m, aromatic H), 3.88 (3H, s, OMe)	93-94
10d	-	8.40-7.25 (9H, m, aromatic H)	116-117
10e	-	8.43-7.28 (9H, m, aromatic H), 2.43 (3H, s, Me)	65-67
10f	-	8.32-7.21 (9H, m, aromatic H), 2.45 (3H, s, Me)	62-63
10g	-	8.36-7.23 (9H, m, aromatic H), 2.41 (3H, s, Me)	69-70

Table III-2. Analytical and Physical Data

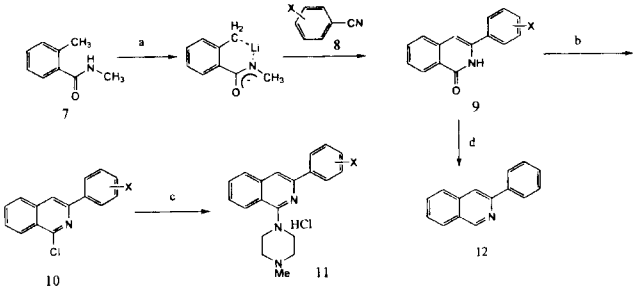
No	^{1}H-NMR ($CDCl_3$) δ	MP(°C)
11a	8.22-7.27 (10H, m, aromatic H), 3.67 (4H, t, J=4.7Hz, C2'-H and C6'-H), 2.93 (4H, t, J=4.7Hz, C3'-H and C5'-H), 2.48 (3H, s, NMe)	198-200
11b	8.02-7.51 (9H, m, aromatic H), 3.81 (4H, t, J=4.7Hz, C2'-H and C6'-H), 3.03 (4H, t, J=4.7Hz, C3'-H and C5'-H), 2.63 (3H, s, NMe)	154-155
11c	8.15-6.90 (9H, m, aromatic H), 3.86 (3H, s, OMe), 3.64 (4H, t, J=4.6Hz, C2'-H and C6'-H), 2.80 (4H, t, J=4.6Hz, C3'-H and C5'-H), 2.48 (3H, s, NMe)	138-140
11d	8.39-7.11 (9H, m, aromatic H), 3.65 (4H, t, J=4.7Hz, C2'-H and C6'-H), 2.82 (4H, t, J=4.7Hz, C3'-H and C5'-H), 2.48 (3H, s, NMe)	147-149
11e	8.15-7.10 (9H, m, aromatic H), 3.64 (4H, t, J=4.6Hz, C2'-H and C6'-H), 2.85 (4H, t, J=4.6Hz, C3'-H and C5'-H), 2.49 (3H, s, NMe), 2.46 (3H, s, Me)	133-134
11f	8.01-7.12 (9H, m, aromatic H), 3.53 (4H, s, C2'-H and C6'-H), 2.66 (4H, s, C3'-H and C5'-H), 2.41 (3H, s, NMe), 2.35 (3H, s, Me)	133-134
11g	8.37-7.22 (9H, m, aromatic H), 3.67 (4H, t, J=4.7Hz, C2'-H and C6'-H), 2.82 (4H, t, J=4.7Hz, C3'-H and C5'-H), 2.48 (3H, s, NMe), 2.41 (3H, s, Me)	158-160

at different concentrations with the range of 0.001-30 μg/ml. The DMSO concentration was set to be below 0.5% and filtrated using 0.22 mg filter. After removing the well medium by aspiration, a portion 200 ml of the solution was added to above well plates which were placed in 5% CO_2 incubator for 48 hrs. The protein stain assay was performed according to SRB method (Skehan *et al.*, 1990, Rubinstein *et al.*, 1990).

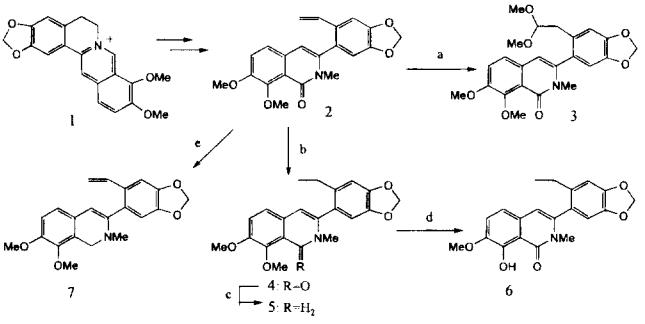
RESULTS AND DISCUSSION

Chemistry

The hydrogenation of the styrene moiety in compound **2** was attained by treatment with 5% Pd-C under hydrogen atmosphere (1atm) in methanol at room temperature, to produce the ethyl isoquinoline derivative (**4**) in 96% yield. The structure was apparent from the signals caused by the ethyl moiety at 2.40 and 1.09 (J=7.2 Hz) as the quartet and triplet of its ^{1}H-NMR spectrum. The methoxy group at position C8 in compound **4** was selectively demethylated with *c*-HCl by refluxing in methanol to afford **6** in 98% yield. This selectivity could be explained that the hydrogen bonding between amide ketone and oxygen atom of 7-methoxy group effected the cleavage in acidic reaction condition. The sequential reduction of **4** with $LiAlH_4$ in dry THF, and with $NaBH_4$ in methanol at room temperature produced the dihydrocompound (**5**) in 87% yield. 3-Arylisoquinoline derivatives were prepared using a reported procedure (G. S. Poindexer, 1984) as follows : a dry THF solution of *N*-methyl-*o*-toluamide was treated with 2.1 equivalents of *n*-butyllithum (*n*-BuLi) at 0°C to result in the formation of dianion as evidenced by its orange-red color; after cooling to -50°C, the solution was treated with 1.25 equivalents of substituted benzonitriles to afford the corresponding amide in 27-57% yield. These compounds (**9a-9g**) seem to exist predominantly in the amide tautomeric form rather than the enol tautomeric form on the basis of the presence of strong carbonyl stretching absorption at 1650 cm^{-1} in their IR spectra, and the broad peak of NH proton in their NMR spectra. The 3-phenylisoquinolinone derivatives (**9a-9g**) were converted to the 1-chloroisoquinoline derivatives (**10a-10g**) by treatment with phosphorus oxychloride, giving in 69-99% yield. The reaction of the 1-chloroisoquinolines (**10a-10g**) with *N*-methylpiperazine in the presence of potassium carbonate in refluxing dimethyl formamide (DMF) afforded the amines (**11a-11g**), which were transformed to HCl salt



Scheme 2. a; *n*-BuLi/THF b; $POCl_3$, c: N-methylpiperazine/DMF, K_2CO_3, then AcCl/MeOH d; $LiAlH_4$/THF 39%.



Scheme 3. a; $Tl(NO_3)_3$/MeOH 80% b; 5% Pd-C, H_2/MeOH 96% c; $LiAlH_4$/THF 87% d; *c*-HCl/MeOH 98% e; $LiAlH_4$/THF, $NaBH_4$/MeOH 53%.

by treatment with acetyl chloride and methanol in good yield (Behrens, 1990).

Biological activity

When the amide moiety of styrene (**2**) was transformed to dihydrocompound (**7**), the activity was significantly decreased. However, a hydrogenation of the styrene moiety of **2** into a saturated alkyl group (ethyl) showed a relatively weak influence on the activity. This means the amide ketone is essential for exhibiting the antitumor activity as expected electrostatic interaction (*i.e.* hydrogen bonding) between the substance and a receptor active site. The demethylation of the C8 position in **4** to the hydroxyl group (**6**) also decreased the activity. The systematic introduction of substituents on the 3-phenyl ring (**9a-9g**) gave some information about further modifications of the aromatic ring. As a result, none of the substituents (halogen, methoxy, alkyl) on the phenyl ring contributed to increase the activity over the non-substituent compound (**9a**). An electrostatic effect on the aromatic ring could not be detected. Interestingly, 3-phenylisoquinoline (**12**) did not exhibit any anti-tumor activity, which demonstrates that the amide group is crucial for show the activity. The poor solvent solubility of amides (**9a-9g**) made us replace the amide group to another bioisostere, retaining the hydrogen bonding ability. A N-methylpiperazinyl group was introduced to produce hydrochloride salts which were easily dissolved in water without losing their activity. The study of the structure-activity relationship of the A ring is in progress.

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