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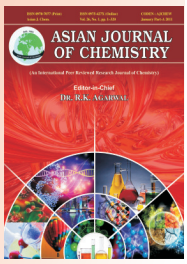

Synthesis and Biological Evaluation of Novel Tetrahydroisoquinoline Derivatives as Antitumor Agents

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Fifteen novel tetrahydroisoquinoline derivatives have been synthesized *via* a mild synthetic route. The structures of all the compounds were confirmed by ^{1}H NMR spectra, ^{13}C NMR spectra and mass spectra. Their antitumor activities were evaluated in four human tumor cell lines, including human colon carcinoma (HCT116), non-small cell lung cancer (H1975), human lung adenocarcinoma (A549) and human pancreatic cancer (BxPC-3). Among them, compounds **4i** and **4j** exhibited remarkable growth inhibitory activity against the subtotal tested subpanel tumor cell lines particularly H1975 with GI values of 59.1 and 63.0 %, respectively.

Keywords: Synthesis, Tetrahydroisoquinoline derivatives, Antitumour activity.

INTRODUCTION

Inspite of obtaining much progress in our understanding of the biological processes associated with cancer, there is still a tremendous need for new and effective agents to treat this disease well. Therefore, the design of new lead structures for the screen of antitumor agents is significant in contemporary medicinal chemistry. Among the kinds of pharmacologically active heterocycles, isoquinoline and related derivatives have attracted great attention over the past years. A considerable variety of natural products containing an isoquinoline skeleton exhibit anticancer activity[1,2], and more isoquinoline derivatives derived from the parent bicyclic system have caused great interest, especially tetrahydroisoquinoline derivatives[3,4]. With the increasing attention of tetrahydroisoquinoline family, the development of efficient synthetic approaches for tetrahydroisoquinoline and related derivatives can be considered of great importance.

Recently, 1-methylpiperidin-4-one derivatives have been effectively used as a base for design of new lead molecules and pharmacologically active compounds[5]. Among them, tetrahydroisoquinoline structural motif is particularly interesting with the construction of the benzene ring from a heterocyclic precursor[6]. Although the above methods offered several synthetic approaches to this class of important compounds, there is enough space to lower the reaction temperature. In our investigation, we would like to report a novel mild procedure for the synthesis of tetrahydroisoquinoline derivatives using 1-methylpiperidin-4-one as a starting material. Their *in-vitro* antitumor activity was evaluated according to the National Cancer Institute (NCI) *in-vitro* disease-oriented human cells screening panel assay.

EXPERIMENTAL

All aromatic aldehydes and 1-methylpiperidin-4-one were obtained from Kelong Chemical Reagent Co., Ltd. (Chengdu, China) and used as supplied. Unless otherwise indicated, other chemicals were purchased from commercial sources and used without further purification. Column chromatography was performed on silica gel (200-300 mesh, Qingdao Marine Chemical Ltd., Qingdao, China). Thin layer chromatography (TLC) was carried out on TLC silica gel 60 F254 plates. Melting points were determined on YRT-3 melting point measuring apparatus (Precision Instrument Plant, Tianjin University). The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM400 NMR spectrometer and the chemical shifts in ppm were reported relative to residual solvent peaks or internal tetramethylsilane (TMS). Mass spectrometry (ESI-MS) data were measured using a Bruker Daltonics amaZon SL mass spectrometer. All products were recrystallized from 95 % ethanol.

General procedure for the aldol condensation reaction of 1-methylpiperidin-4-one with aromatic aldehydes: A solution of 1-methyl-4-piperidone (565.8 mg, 5 mmol) and tetrahydro pyrrole (0.5 mL, 6 mmol) in dichloromethane (10 mL) was stirred at room temperature, to which aromatic aldehydes (5 mmol) was added. Subsequently, the resulting mixture was reacted at 40 °C for 4 h to obtain α, β-unsaturated ketones. The solvent was concentrated under reduced pressure and the product was isolated by column chromatography on

silica gel (200-300 mesh) using petroleum ether/ethyl acetate/ triethylamine (16:8:1, v/v/v).

General procedure for the reaction of α,β-unsaturated ketones with malononitrile: A solution of α,β-unsaturated ketones (1 mmol), malononitrile (132.1 mg, 2 mmol), DBU (304.5 mg, 2 mmol) in dichloromethane (4 mL) was stirred at room temperature for 6 h. After the completion of the reaction, dichloromethane was removed under reduced pressure. Further purification of the desired product was accomplished by column chromatography on silica gel, eluting with petroleum ether/ ethyl acetate/ triethylamine (16:8:1, v/v/v).

6-Amino-2-methyl-8-[4-(trifluoromethyl)phenyl]-1,2,3,4-tetrahydroisoquinoline-5,7-dicarbonitrile (4a): Yellowish green solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.33 (3H, s, NCH_3), 2.68 (2H, t, CH_2), 3.04 (2H, s, CH_2), 3.11 (2H, t, CH_2), 5.13 (2H, s, NH_2), 7.38 (2H, d, ArH), 7.77 (2H, d, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.7, 45.9, 51.3, 55.7, 96.2, 97.0, 114.8, 115.1, 123.9, 126.0, 126.1, 128.7, 131.6, 139.5, 144.7, 146.7, 150.1. MS (ESI): *m/z* = 357.1 $[M + H]^+$.

6-Amino-2-methyl-8-(naphthalen-2-yl)-1,2,3,4-tetrahydroisoquinoline-5,7-dicarbonitrile (4b): Pale yellowish green solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.28 (3H, s, NCH_3), 2.69 (2H, t, CH_2), 3.12 (4H, t, $2CH_2$), 5.10 (2H, s, NH_2), 7.32 (1H, dd, ArH), 7.57 (2H, m, ArH), 7.73 (1H, s, ArH), 7.90 (2H, td, ArH), 7.97 (1H, d, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.7, 45.9, 51.4, 55.9, 96.4, 96.8, 115.1, 115.5, 124.5, 125.4, 126.9, 127.1, 127.7, 127.9, 128.4, 128.9, 133.1, 133.3, 144.3, 148.4, 150.1. MS (ESI): *m/z* = 339.2 $[M + H]^+$.

6-Amino-8-(2,4-dichlorophenyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline-5,7-dicarbonitrile (4c): Green solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.33 (3H, s, NCH_3), 2.69 (2H, m, CH_2), 3.00 (2H, m, CH_2), 3.10 (2H, t, CH_2), 5.10 (2H, s, NH_2), 7.13 (1H, d, ArH), 7.40 (1H, dd, ArH), 7.57 (1H, d, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.6, 45.8, 51.2, 55.0, 96.5, 97.4, 114.7, 114.8, 124.6, 128.0, 130.3, 130.6, 133.2, 133.4, 136.2, 144.3, 144.7, 150.0. MS (ESI): *m/z* = 357.0 $[M + H]^+$.

6-Amino-2-methyl-8-(thiophen-2-yl)-1,2,3,4-tetrahydroisoquinoline-5,7-dicarbonitrile (4d): Yellowish brown solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.37 (3H, s, NCH_3), 2.68 (2H, t, CH_2), 3.08 (2H, t, CH_2), 3.25 (2H, s, CH_2), 5.10 (2H, s, NH_2), 7.09 (1H, m, ArH), 7.17 (1H, dd, ArH), 7.52 (1H, m, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.7, 45.9, 51.3, 55.9, 97.1, 97.7, 114.9, 115.3, 125.7, 127.6, 128.0, 128.8, 135.0, 141.1, 144.2, 150.0. MS (ESI): *m/z* = 295.2 $[M + H]^+$.

6-Amino-2-methyl-8-*m*-tolyl-1,2,3,4-tetrahydroisoquinoline-5,7-dicarbonitrile (4e): Pale yellowish green solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.32 (3H, s, NCH_3), 2.41 (3H, s, CH_3), 2.67 (2H, t, CH_2), 3.09 (4H, m, $2CH_2$), 5.07 (2H, s, NH_2), 7.02 (2H, m, ArH), 7.27 (1H, m, ArH), 7.37 (1H, m, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.5, 29.6, 45.9, 51.4, 55.8, 96.2, 96.7, 115.1, 115.5, 124.2, 125.1, 128.6, 128.8, 129.9, 135.8, 138.7, 144.1, 148.8, 150.0. MS (ESI): *m/z* = 303.2 $[M + H]^+$.

6-Amino-2-methyl-8-[3-(trifluoromethyl)phenyl]-1,2,3,4-tetrahydroisoquinoline-5,7-dicarbonitrile (4f): Pale yellowish green solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.33 (3H, s, NCH_3), 2.69 (2H, m, CH_2), 3.04 (2H, s, CH_2), 3.10 (2H, t, CH_2), 5.12 (2H, s, NH_2), 7.45 (1H, d, ArH), 7.51 (1H, s, ArH), 7.65 (1H, t, ArH), 7.76 (1H, d, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.7, 45.9, 51.3, 55.7, 96.4, 97.0, 114.8, 115.0, 124.1, 125.0, 125.1, 126.1, 126.2, 129.7, 131.6, 136.6, 144.8, 146.5, 150.1. MS (ESI): *m/z* = 357.1 $[M + H]^+$.

6-Amino-8-(3-bromophenyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline-5,7-dicarbonitrile (4g): Flavescens solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.34 (3H, s, NCH_3), 2.68 (2H, m, CH_2), 3.08 (4H, m, $2CH_2$), 5.12 (2H, s, NH_2), 7.18 (1H, m, ArH), 7.38 (2H, m, ArH), 7.62 (1H, m, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.6, 45.9, 51.3, 55.7, 96.4, 96.8, 114.9, 115.1, 123.0, 124.0, 126.8, 130.6, 131.0, 132.4, 137.8, 144.6, 146.6, 150.1. MS (ESI): *m/z* = 367.0 $[M + H]^+$.

6-Amino-8-(2-bromophenyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline-5,7-dicarbonitrile (4h): Yellowish green solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.32 (3H, s, NCH_3), 2.69 (2H, qt, CH_2), 3.01 (2H, s, CH_2), 3.10 (2H, m, CH_2), 5.09 (2H, s, NH_2), 7.18 (1H, dd, ArH), 7.36 (1H, td, ArH), 7.46 (1H, td, ArH), 7.73 (1H, dd, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.6, 45.8, 51.2, 55.0, 96.6, 97.1, 114.8, 114.9, 122.1, 124.5, 128.1, 129.6, 130.9, 133.4, 136.8, 144.5, 147.0, 150.0. MS (ESI): *m/z* = 367.1 $[M + H]^+$.

6-Amino-8-(3-methoxyphenyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline-5,7-dicarbonitrile (4i): Flavescens solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.33 (3H, s, NCH_3), 2.67 (2H, t, CH_2), 3.09 (4H, m, $2CH_2$), 3.84 (3H, s, OCH_3), 5.08 (2H, s, NH_2), 6.74 (1H, s, ArH), 6.80 (1H, d, ArH), 7.00 (1H, dd, ArH), 7.40 (1H, t, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.7, 45.9, 51.4, 55.4, 55.7, 96.3, 96.6, 113.8, 114.6, 115.1, 115.4, 120.3, 124.2, 130.1, 137.1, 144.2, 148.3, 150.0, 159.7. MS (ESI): *m/z* = 319.1 $[M + H]^+$.

6-Amino-8-(3,5-difluorophenyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline-5,7-dicarbonitrile (4j): Green solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.35 (3H, s, NCH_3), 2.68 (2H, t, CH_2), 3.09 (4H, m, $2CH_2$), 5.12 (2H, s, NH_2), 6.79 (2H, m, ArH), 6.94 (1H, tt, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.7, 45.9, 51.3, 55.5, 96.1, 97.2, 105.0, 111.5, 111.7, 111.8, 114.7, 114.8, 123.9, 144.8, 145.5, 150.0, 161.8, 164.3. MS (ESI): *m/z* = 325.1 $[M + H]^+$.

6-Amino-2-methyl-8-(4-nitrophenyl)-1,2,3,4-tetrahydroisoquinoline-5,7-dicarbonitrile (4k): Yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.32 (3H, s, NCH_3), 2.69 (2H, t, CH_2), 3.03 (2H, s, CH_2), 3.11 (2H, t, CH_2), 5.16 (2H, s, NH_2), 7.46 (2H, d, ArH), 8.38 (2H, d, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.7, 45.9, 51.2, 55.7, 95.8, 97.4, 114.6, 114.9, 123.7, 124.3, 129.5, 142.4, 145.0, 145.7, 148.4, 150.1. MS (ESI): *m/z* = 334.1 $[M + H]^+$.

6-Amino-8-(4-isopropylphenyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline-5,7-dicarbonitrile (4l): Green solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.31 (6H, d, $2CH_3$), 2.33 (3H, s, NCH_3), 2.67 (2H, t, CH_2), 2.98 (1H, m, CH), 3.09 (4H, m, $2CH_2$), 5.06 (2H, s, NH_2), 7.15 (2H, d, ArH), 7.33 (2H, d, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.9, 29.6, 33.9, 45.9, 51.4, 55.9, 96.1, 96.7, 115.2, 115.6, 124.3, 126.9, 128.1, 133.1, 144.0, 148.8, 149.8, 150.2. MS (ESI): *m/z* = 331.2 $[M + H]^+$.

6-Amino-8-(4-*tert*-butylphenyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline-5,7-dicarbonitrile (4m): Green solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.37 (9H, s, $3CH_3$), 2.34 (3H, s, NCH_3), 2.68 (2H, t, CH_2), 3.09 (4H, m, $2CH_2$), 5.07 (2H, s,

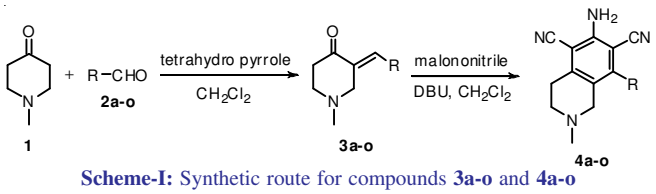
NH_2), 7.16 (2H, m, ArH), 7.48 (2H, m, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.6, 31.3, 34.8, 45.9, 51.5, 55.9, 96.1, 96.7, 115.2, 115.6, 124.3, 125.8, 127.8, 132.8, 144.0, 148.8, 150.2, 152.2. MS (ESI): *m/z* = 345.3 $[M + H]^+$.

6-Amino-2-methyl-8-(pyridin-3-yl)-1,2,3,4-tetrahydroisoquinoline-5,7-dicarbonitrile (4n): Yellowish green solid. ^1H NMR (400 MHz, $CDCl_3$): δ = 2.33 (3H, s, NCH_3), 2.69 (2H, m, CH_2), 3.08 (4H, m, $2CH_2$), 5.15 (2H, s, NH_2), 7.47 (1H, dd, ArH), 7.62 (1H, dt, ArH), 8.53 (1H, d, ArH), 8.75 (1H, dd, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.7, 45.9, 51.2, 55.9, 96.6, 97.2, 114.8, 115.1, 123.7, 124.4, 131.9, 135.9, 144.4, 144.8, 148.7, 150.2, 150.5. MS (ESI): *m/z* = 290.2 $[M + H]^+$.

6-Amino-8-(4-bromo-2-fluorophenyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline-5,7-dicarbonitrile (4o): Yellowish brown solid. ^1H NMR (400 MHz, $CDCl_3$): δ = 2.34 (3H, s, NCH_3), 2.69 (2H, m, CH_2), 3.08 (4H, m, $2CH_2$), 5.11 (2H, s, NH_2), 7.10 (1H, m, ArH), 7.44 (2H, m, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.6, 45.8, 51.2, 55.1, 96.7, 97.5, 114.7, 114.9, 120.1, 122.3, 124.3, 124.9, 128.3, 131.3, 140.9, 144.7, 150.0, 159.9. MS (ESI): *m/z* = 384.9 $[M + H]^+$.

RESULTS AND DISCUSSION

To explore conditions of the synthesis of tetrahydroisoquinoline derivatives, various reaction conditions were investigated. When 1-methylpiperidin-4-one was converted to corresponding α,β-unsaturated ketones, the reaction time should not exceed 4 h to avoid the formation of by-products. In the second step, the reaction of α,β-unsaturated ketones and malononitrile was examined using different strong bases, including NaOH, EtONa, DBU. However, the reaction occurred suiting our needs perfectly only when DBU was used as strong base. Therefore, DBU was chosen as the most suitable strong base for all further reactions. The progress of the reaction was monitored by TLC and 6 h was enough. The optimized reaction route was shown in **Scheme-I**.



Scheme-I: Synthetic route for compounds **3a-o** and **4a-o**

Based on these optimized conditions, a series of tetrahydroisoquinoline derivatives were synthesized. The results (Table-1, entries 1-15) indicated that for aromatic aldehydes bearing different functional groups such as trifluoromethyl, chloro, bromo, fluoro, nitro, methoxyl, methyl, isopropyl or tert-butyl the reaction proceeded smoothly in all cases.

The compounds were screened for their *in vitro* antitumor activity in the full NCI 60 cell panel, including human colon carcinoma (HCT116), non-small cell lung cancer (H1975), human lung adenocarcinoma (A549) and human pancreatic cancer (BxPC-3). In the protocol, all compounds were tested initially at three high doses, *i.e.*, 10, 20 and 40 μM. After the primary biological tests, only compounds which had better antitumor activities were chosen to carry out the five-dose screen. The data are reported as a mean graph of the percent growth

TABLE-1
SYNTHETIC RESULTS OF TETRAHYDRO-ISOQUINOLINE DERIVATIVES

Entry	R	Product	Yield (%)	m.p. (°C)
1	4-$CF_3C_6H_4$	**4a**	51	180-183
2	2-Naphthyl	**4b**	44	226-228
3	2,4-$Cl_2C_6H_3$	**4c**	41	211-214
4	2-Thienyl	**4d**	40	229-232
5	3-$CH_3C_6H_4$	**4e**	52	187-190
6	3-$CF_3C_6H_4$	**4f**	43	183-184
7	3-BrC_6H_4	**4g**	68	176-177
8	2-BrC_6H_4	**4h**	59	175-178
9	3-$CH_3OC_6H_4$	**4i**	61	215-218
10	3,5-$F_2C_6H_3$	**4j**	40	220-223
11	4-$NO_2C_6H_4$	**4k**	43	218-221
12	4-*i*PrC_6H_4	**4l**	45	184-187
13	4-*tert*-Butylphenyl	**4m**	42	220-222
14	3-Pyridyl	**4n**	77	235-238
15	2-F-4-BrC_6H_3	**4o**	43	226-228

of treated cells and presented as percentage growth inhibition (GI %) caused by 20 μM of the test compounds (Table-2). The obtained data revealed that compounds **4i** and **4j** exhibited noticeable growth inhibitory activity against the subtotal tested subpanel tumor cell lines particularly H1975 with GI values of 59.1 and 63.0 %, respectively. Other compounds also showed moderate activity against H1975, especially compound **4f** (GI 46.8 %). These heterocycles could be used as new lead structures for future derivatization or modification to obtain more potent and selective antitumor agents.

TABLE-2
in-vitro PERCENTAGE GROWTH INHIBITION (GI %)
CAUSED BY THE TEST COMPOUNDS AT DOSE OF 20 μM

Compound	Subpanel tumor cell lines (% growth inhibitory activity)			
	HCT116	H1975	A549	BxPC-3
4a	23.7	12.9	17.5	3.9
4b	21.2	28.4	12.0	3.5
4c	13.3	22.2	3.4	3.9
4d	5.6	24.2	19.1	18.8
4e	2.2	8.5	9.8	15.4
4f	0.5	46.8	17.5	22.7
4g	15.2	30.6	20.7	16.2
4h	5.4	13.6	10.8	2.7
4i	4.8	59.1	41.4	40.9
4j	39.7	63.0	39.3	10.7
4k	20.0	18.1	10.3	10.6
4l	8.5	11.6	2.9	7.6
4m	0.8	20.7	5.8	6.8
4n	3.2	6.4	1.8	21.0
4o	3.2	25.4	18.7	0.9

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