SYNTHESIS AND BIOLOGICAL PROPERTIES OF SOME ISOQUINOLINE DERIVATIVES.

C. O. Usifoh

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Nigeria.

ABSTRACT

The reaction of 3-phenyl-isoquinolin-1-(2H)-one **3** with bromoalkylphthalimides in the presence of potassium carbonate in a refluxing mixture of acetonitrile-dimethylacetamide produced the alkylated derivatives of 3-phenyl-isoquinolin-1-(2H)-one. The compounds 5a-c were characterized spectroscopically and screened for possible anticonvulsant and antimicrobial activities.

KEYWORDS: Isoquinolones, Anticonvulsant, 3-Phenyl-isoquinolin-1-(2H)-ones,

INTRODUCTION

The need to design novel, non-toxic, receptor-specific drugs is a continuing synthetic and neuropharmacologic priority. ã-Amino butyric acid (GABA) is a major central neurohormonal inhibitory modulator in the forebrain. Its role in the aetiology and control of epilepsy is important[1,2]. Drugs that have antiepileptic activity by exerting their action by different mechanisms, which include influence on the ion transport across cell membranes, or inhibitory or excitatory neurotransmitter systems have been synthesized[3,4]. Recently the synthesis and anticonvulsant activity of some 2-N-(phthalimido)-1-alkylesters have been reported[5,6]. A series of isoquinolin-1-ones and quinazolin-4-ones and related derivatives were prepared and evaluated for their ability to inhibit tumor necrosis factor á (TNFá) production in human peripheral blood monocysts[7]. It was therefore rationalized that a synthetic combination of alkylphthalimides and isoquinolines should produce compounds with interesting biological activities.

EXPERIMENTAL

Melting points were determined with a Kofler hot stage microscope and were uncorrected. The reaction and purity of the products were monitored by thin layer chromatography (tlc) using pre-coated silica gel plates (Merck $60F_{254}$). Silica gel Merck 60 (70-230 mesh) was used for column chromatography. NMR (^1H and ^{13}C) were recorded on a Varian Gemini 200 (TMS), IR were measured on a Perkin-Elmer type 457 and the MS were determined using Varian MAT 44S, EL: 70 ev.

Synthesis of N-methyl-o-toluamide 2.

To o-toluic acid (10.2g, 0.075mol) in a 250ml flask was added thionyl chloride (15.0g, 0.125mol) and refluxed for 2h. After distilling off excess thionyl chloride, 60ml of diethyl ether was added and the mixture gradually poured into a stirring ice-cooled 30ml of 40% methylamine. The precipitate formed was filtered and the mother liquor was concentrated in vacuo to afford some more of the precipitate. Crystallization of the precipitate from ethanol-water gave 2 as a crystalline solid 8.5g (76%); m.p 76-77°C (78-79°C[5]); ^1H-NMR ($CDCl_3$): =2.37 (s, 3H, CH_3), 2.88-2.90 (d, J = 4.8Hz, 3H, NCH_3), 6.14 (brs, 1H, NH), 7.10-7.17 (m, 2H, Ar-H), 7.23-7.28 (m, 2H, Ar-H); ^{13}C-NMR ($CDCl_3$): = 20.3, 27.1, 126.2, 127.2, 130.5, 131.5, 136.5, 137.0, 171.5; MS: m/z: 149 [M^+].

Synthesis of 3-phenyl-isoquinoline 3.

To N-methyl-o-toluamide (7.46g, 50mmol) in 300ml of dry tetrahydrofuran under nitrogen in a 500ml flask cooled in an ice-salt bath, was added slowly n-butyllithium (80ml, 135mmol) such that the internal temperature never exceeded 15°C. The resulting orange-red reaction mixture was stirred at 0°C for 1.5h, cooled to -63°C and the benzonitrile (62mmol) in 40ml tetrahydrofuran was added via a syringe as quickly as possible. The cooling bath was removed and the reaction mixture allowed to warm up to room temperature. Saturated ammonium chloride solution (150ml) was carefully added to reaction mixture until the resulting phases separated. The organic phase was washed with water (2 x 30ml) and dried over anhydrous sodium sulphate. Subsequent filteration and removal of organic solvent in vacuo gave a crude product, which was recrystallised from ethanol and was obtained as needles 2.1g (91%); m.p 199-200°C; (198-199°C[8,9]); ^1H-NMR ($CDCl_3$): =6.77 (s, 1H, 4-H), 7.24-7.48 (m, 4H, Ar-H), 7.49 (d, J = 7.8Hz, 1H, Ar-H), 7.52-7.54 (t, J = 7.8Hz, 1H, Ar-H), 7.73-7.78 (m, 2H, Ar-H), 8.38 (d, J = 8.0Hz, 1H, Ar-H); ^{13}C-NMR ($CDCl_3$) = 104.2, 124.9, 126.2, 126.5, 126.6, 127.4, 129.1, 129.5, 132.8, 134.3, 138.3, 139.6, 164.0; MS m/z = 222 (18%)[M^+].

General synthetic procedure for Isoquinolines 3a-c

The isoquinoline **3** (6.02mmol), bromoalkylphthalimide (6.02mmol) and potassium carbonate (12.05mmol) were refluxed in acetonitrile-dimethylacetamide (9:1) until tlc indicated absence of the starting materials. Acetonitrile was removed in vacuo and the crude mixture was poured into 50ml of ice water. The mixture was stirred for 30 minutes, filtered and dried. Subsequent column chromatography using dichloromethane gave the respective isoquinoline 5a-c which were either recrystallised from hexane or dichloromethane-hexane mixture.

1-[2-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)-N-ethoxy]-3-phenylisoquinoline 5a

This compound was obtained after refluxing for 36h according to the general procedure and was recrystallised from dichloromethane/hexane mp 146-147°C; IR (KBr): 1760 (C=O), 1630, 1600cm^{-1}; ^1H NMR ($CDCl_3$): ä =4.25-4.31 (t, J = 5.4 Hz,

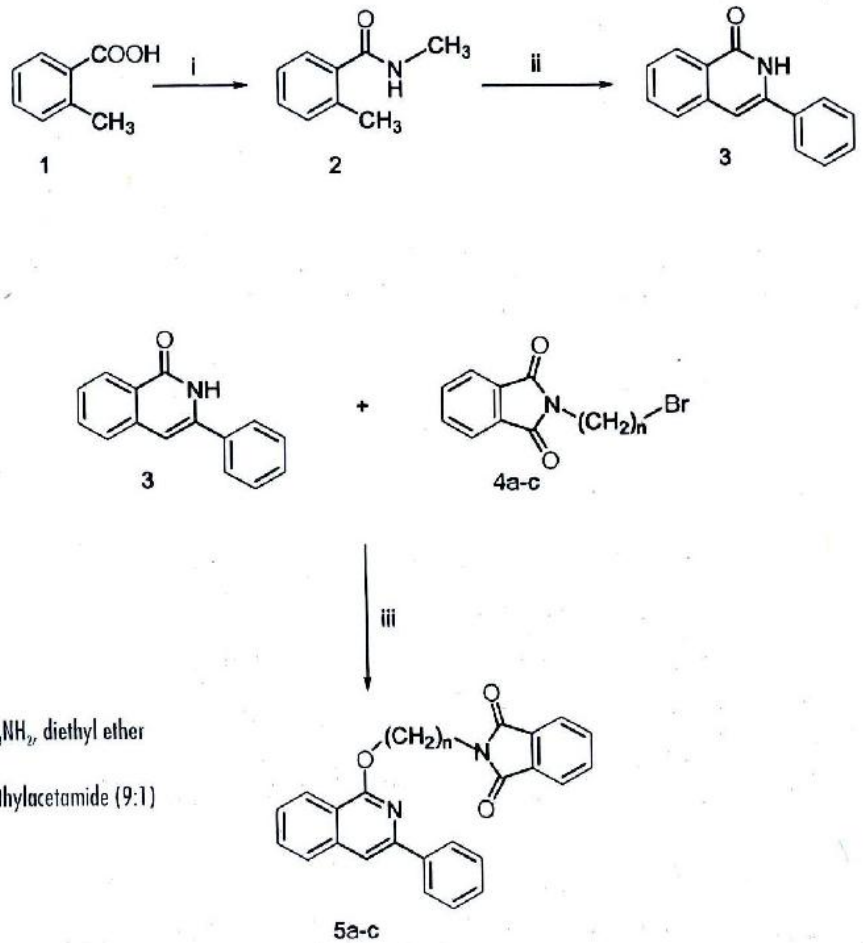
2H, 2'-H), 4.86-4.91 (t, J = 5.4 Hz, 2H, 1'H), 7.42-7.80 (m, 11H, Ar-H), 8.08 (d, J = 7.0 Hz, 2H, Ar-H), 8.20 (d, J=7.8 Hz, 1H, Ar-H); ^{13}C NMR ($CDCl_3$): ä =37.9, 68.8, 111.1, 119.1, 123.7, 124.7, 126.9, 127.0, 128.8, 129.0, 131.0, 132.5, 134.3, 139.2, 148.0, 160.0, 168.7; MS:m/z =394 [M$^+$] (20), 234(10), 221(100), 204(6), 193(4), 175(4), 174(60), 147(25), 130(17), 89(3). $C_{25}H_{18}N_2O_3$ (394.41) 1.43 g (80%); Analysis calculated C 76.13 H 4.60 N 7.10 Found C 76.09 H 4.40 N 7.08.

1-[3-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)-N-propoxy]-3-phenylisoquinoline 5b

After refluxing for 48h and subsequent column chromatography **5b** was recrystallised from dichloromethane/hexane as colorless needles 1.67g (98%); mp 122-123°C; IR (KBr): 1765, 1630, 1600cm^{-1}; ^{1}H NMR ($CDCl_3$): ä=2.29-2.42 (quint, J=6.5Hz, 2H, 2'-H), 3.96-4.04 (t, J=6.9Hz, 2H, 3'-H), 4.70-4.76 (t, 6.4Hz, 2H, 1'-H), 7.34-7.80 (m, 11H, Ar-H), 8.08 (d, J=7.0Hz, 2H, Ar-H), 8.15 (d, J=8.0Hz, 1H, Ar-H); ^{13}C NMR ($CDCl_3$) ä=28.5, 36.2, 64.1, 110.9, 119.2, 123.6, 124.5, 126.8, 127.0, 128.7, 129.0, 130.9, 132.6, 134.8, 139.2, 139.8, 148.1, 160.2, 168.8; MS: m/z = 409[M$^+$+1] (6), 408 [M$^+$] (21), 378 (8), 222(18), 221 (90), 189(13), 188(100), 160(62), 130(10), 115(3), 89(6); $C_{26}H_{20}N_2O_3$ (408.44) Analysis calculated C 76.46 H 4.94 N 6.86. Found C 76.42 H 4.82 N 6.84.

1-[4-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)-N-butoxy]-3-phenylisoquinoline 5c

This compound was obtained after refluxing for 72h according to general procedure and was recrystallised from hexane. 1.44g (75%); mp 103-105°C; IR (KBr): 1760, 1640, 1600cm^{-1}; ^{1}H NMR ($CDCl_3$): ä=1.97-2.06 (m, 4H, 2'-H, 3'-H), 3.78-3.82 (t, J=6.3Hz, 2H, 4'-H), 4.62-4.67 (t, J=6.4Hz, 2H, 1'-H), 7.42-7.50 (m, 4-H, Ar-H), 7.61-7.82 (m, 11H, Ar-H), 8.10 (d, J=7.1Hz, 2H, Ar-H), 8.20 (d, J-8.0Hz, 1H, Ar-H); ^{13}C NMR ($CDCl_3$): ä =26.1, 27.0, 38.3, 65.8, 110.7, 119.3, 123.6, 124.6, 126.8, 127.0, 128.7, 129.0, 130.9, 132.5, 139.2, 139.8, 148.2, 160.9, 168.8; MS: m/z= 423 [M++1 (8), 422 [M+], (28), 408 (5), 392 (15), 378(10), 364 (15), 232(20), 221(100), 202(24), 188(14), 160(75), 133(12), 118(3), 77(4); $C_{27}H_{22}N_2O_3$ (422.48). Analysis calculated C 76.76 H 5.25 N 6.63. Found C 76.84 H 5.26 N 6.60.



i: $SOCl_2$, 2H, reflux, 40%CH_3NH_2, diethyl ether
ii: n-Buli, THF, benzonitrile
iii: K_2CO_3, acetonitrile/dimethylacetamide (9:1)
a: n=2; b: n=3; c: n=4

Anticonvulsant Assays

The Antiepileptic Drug Development Programme, Epilepsy Branch, National Institute of Neurological and Communicative Disorder and Stroke, National institute of Health, Bethseda, MD, USA, executed anticonvulsant activity and neurological toxicity assays according to standard procedures[5]. The compounds 5a-c were injected intraperitoneally into mice as suspensions in 0.5% aqueous methylcellulose at three dosage levels (30, 100 and 300mg/kg). A qualitative evaluation which utilized small groups of animals (1-8) included three tests: maximal electroshock seizure (MES), subcutaneous pentyleneteterazole (Sc Met) and rotorod test for neurological toxicity (Tox) with anticonvulsant activity and neurotoxicity noted 30 minutes and 4h after administration.

Antimicrobial Assay

Molten nutrient agar (25ml) were aseptically poured into each of the two sterile petri-dishes and this was allowed to solidify. The cultured organism Escherichia coli (NCTC 101418) and Staphylococcus aureus (NCTC 6571) were used to flood each of the two nutrient agar plate and the excess poured away after some time. Using a sterile cork borer, four wells were made on each of the nutrient agar plate. Into each of these wells, 0.1ml molten nutrient agar was poured in order to seal the base. Using a pipette, 0.2ml (200µg) of 1mg/ml in dimethylsulphoxide (DMSO) of compounds 5a, 5b, 5c, ampicillin and 0.2ml of DMSO were aseptically introduced into each of the wells respectively. It was allowed to stand on the bench for 30 minutes and incubated at 37°C for 24 hours. The corresponding zones of inhibition were recorded to nearest millimeter accordingly.

RESULTS AND DISCUSSION

Chemistry.

The isoquinoline **3** was synthesized and characterized as recorded in the literature[8,9]. The mixture of isoquinoline 3 and bromoalkylphthalimides in acetonitrile was refluxed for over 72 hours and no traceable product on thin layer chromatography (tlc) was observed. When acetonitrile-dimethylacetamide mixture was used as the refluxing solvent, tlc revealed the formation of a product which turned out to be the expected product after structural elucidation by spectroscopic analyses. The optimal ratio for the mixture was found to be 9:1 (acetonitrile:dimethylacetamide).

The reaction products were predominantly O-alkylated and not N-alkylated. This was probably due to steric hinderance as a result of the phenyl group at position 3. It was observed that as the alkyl chain increases, the reaction time for the formation of the product also increases. However, the melting point decreased correspondingly.

The yields were generally high (>70%). The structures of the compounds were characterized on the basis of elemental and spectroscopic analyses (IR, ^{1}H and ^{13}C NMR, MS).

Pharmacology

The anticonvulsant properties were evaluated by the Antiepileptic Drug Development (ADD) Programme. Phase 1 of the evaluation included three tests maximal electroshock (MES), subcutaneous pentylenetetrazole (sc Met), and the rotorod test for neurological toxicity (Tox).

The results of the anticonvulsant assays for the compounds 5a-c are summarized in table 1. The MES assay has predictive value for agents of potential therapeutic value in the management of grand mal epilepsy, whereas Sc Met test is for agents likely to be effective against petit mal epilepsy[5].

Table 1: Anticonvulsant screening project (ASP).
Phase 1: Test results in mice.

Compound	Dose (mg/kg)	Test						ASP[d] Class
		MES[a]		Sc Met[b]		Tox[c]		
		0.5h	4h	0.5h	4h	0.5h	4h	
5a	3	-	0/4	-	-	-	0/4	1
	10	-	0/4	-	-	-	0/4	
	30	0/1	1/1	0/1	0/1	0/4	0/2	
	100	0/3	2/3	0/1	0/1	2/8	1/4	
	300	0/1	1/1	0/1	0/1	2/4	1/2	
5b	30	0/1	0/1	0/1	0/1	0/4	0/2	3
	100	0/3	0/3	0/1	0/1	0/8	0/4	
	300	0/1	0/1	0/1	0/1	0/4	0/2	
5c	30	0/1	0/1	0/1	0/1	0/4	0/4	3
	100	0/3	0/3	0/1	0/1	0/8	0/8	
	300	0/1	0/1	0/1	0/1	0/1	1/4	

A: Number of animals protected /Number of animals tested in the MES test

b: Number of animals protected/Number of animals tested in the Sc Met test

c: Number of animals protected/Number of animals tested in the rotorod test

d: The classification is as follows:

1 = anticonvulsant activity ay 100mg/kg

2 = anticonvulsant activity at doses greater than 100mg/kg

3 = compound inactive at 300mg/kg.

Table 2: Anticonvulsant Screening: Test results in rats (doses 15mg/kg p.o)

Compound	Test	Time (h)				
		0.25	0.50	1.00	2.00	4.00
5a	MES	1/4	0/4	1/4	0/4	0/4
	TOX	0/4	0/4	0/4	0/4	0/4

Compounds 5b and 5c had no significant anticonvulsant activity as shown in table 1. At doses up to 300mg/kg, the animals were not protected either at the MES or Sc Met screening. The compounds 5b and 5c were consequently classified as class 3 in the Anticonvulsant Screening Project (ASP). Compound 5a at the MES however possesses anticonvulsant activity at 100mg/kg. When the dose was reduced to 30mg/kg, the corresponding activity displayed was evident after 4h. This probably indicates a slow onset of action of the compound. There was however no activity at Sc Met even at a high dose of 300mg/kg. The compound was not neurotoxic at 30mg/kg but 25% of the animals screened were neurotoxic at 100mg/kg and 50% at 300mg/kg.

A further anticonvulsant screening in rats at 15mg/kg at MES revealed 25% protection after 15 minutes and 1h for compound 5a. From the above results, it was observed that elongation of the methylene group (>2 carbons) probably caused reduction of observed anticonvulsant activity.

Table 3. Quantitative anticonvulsant (TD_{50}, ED_{50}, MES, and PI) activity of **5a** and standard anticonvulsants after intraperitoneal administration to mice. The values are expressed in mg/kg. 95% confidence intervals are listed in brackets, while the time of test is listed in square brackets.

Compounds	Td_{50} (Rotorod)	ED_{50} (MES)	PI (TD_{50}/ED_{50})
5a	94.2 (74.4-110.8) [2h]	44.0 (28.4-70.6) [2h]	2.1
Phenytoin	42.8 (36.4-47.5) [2h]	6.48 (5.65-7.24) [2h]	6.6
Valporate	483 (412-517) [1/4h]	287 (237-359) [1/4h]	1.7

Antimicrobial Activity

The antibacterial activity was performed on the compounds using cultured organisms like Staphylococcus aureus and Escherichia coli and the results showed that there was no appreciable anti-microbial activity against gram positive and gram negative microorganisms used.

Table 4: Staphylococcus aureus (NCTC 6571)

Compounds used (Dose/Concentration = 200ig)	Zone of inhibition (After 24hrs)
5a	None
5b	None
5c	None
Ampicillin	18mm
Dimethylsuphoxide (0.2ml)	None

Table 5: Escherichia coli (NCTC 10418)

Compounds used (Dose/Concentration = 200ig)	Zone of inhibition (After 24hrs)
5a	None
5b	None
5c	None
Ampicillin	21mm
Dimethylsuphoxide (0.2ml)	None

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