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MICROWAVE ASSISTED SYNTHESIS OF PYRIDO [2,3,4-*KL*] ACRIDINES UNIT OF SOME MARINE ALKALOIDS

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ABSTRACT

Microwave irradiation is well known for its ability to reduce reaction times dramatically, to increase product yields, and to enhance product purities by reducing unwanted side reactions compared with conventional heating. Many marine alkaloids are known which have a common structural feature of tetracyclic pyrido [2,3,4-kl] acridine system. Most of these molecules exhibit very interesting biological properties like antitumor, cytotoxic and antiviral activities. This is due to their interesting biological activities and challenging structures. We report a simple and convenient synthesis of pyrido [2, 3, 4-kl] acridine utilizing microwave irradiated intramolecular nitrene insertion reaction as key step for generating the tetracyclic framework of marine alkaloids.

Keywords: *Microwave, Marine alkaloids, Nitrene insertion, Pyrido[2,3,4-kl] acridine.*

1. INTRODUCTION

Marine organisms have been the source of a wide variety of novel natural products. There is currently great interest in marine pyridoacridines due to their significant biological properties. Almost all have been reported as having significant cytotoxicity; however, several specific biological properties have also emerged for different compounds: inhibition of anti-HIV activity,[1-3] topoisomerase II,[4,5] Ca^{2+} release activity,[6] metal chelating properties,[7] and intercalation of DNA.[5,7] In addition, pyridoacridines have drawn attention because of their novel heterocyclic chemistry and an unprecedented distribution across several phyla of marine invertebrates.

The short reaction times and expanded reaction range that is offered by microwave assisted organic synthesis are suited to the increased demands in industry. The main reasons for the increase include the availability of commercial microwave equipment intended for organic chemistry and the development of the solvent-free technique, which has improved the safety aspects, but are mostly due to an increased interest in shorter reaction times. Microwave irradiation has gained popularity in the past decade as a powerful tool for rapid and efficient synthesis of a variety of compounds because of selective absorption of microwave energy by polar molecules[8]

The unique structural features, biological activities of pyrido [2,3,4-*kl*] acridine and related compounds have prompted us to undertake this work. They are generally prepared by the following methods; (a) refluxing for hours (b) using solvents. The disadvantage of these

methods includes drastic conditions, tedious work up, time consuming and use of solvents. Here, we report an alternative synthesis for pyrido [2,3,4-*kl*] acridine unit, along with their cytotoxicity profile against three different tumour cell lines.

2. MATERIALS AND METHODS

The melting points were determined using capillary tube and are uncorrected. The FTIR spectra were recorded on Spectrum One Perkin Elmer (US). The ^{1}H NMR spectra were recorded on a Bruker AVANCE (300 MHz) spectrometer (with TMS as internal reference). ^{13}C NMR spectra were recorded on Bruker AVANCE (75MHz) spectrometer. Mass spectra were recorded on API-3000 MD-series (US). Elemental analyses were carried out in EA 3000, Euro Vector, Italy. The purity of the compounds was checked by TLC on pre-coated SiO_2 gel (200mesh).

3. RESULTS AND DISCUSSION

The synthesis of this important pyridoacridines intermediate (19) (Figure-1) has been achieved in 7 steps with overall 76% yield. Our synthesis stared with the condensation of commercially available benzoylacetic ethyl ester and 2- methoxy -5-nitrobenzenamine which led to the formation of the amide (5) in 81% yield. Such a condensation reaction is carried out by heating for longer time in a Dean stark apparatus. Hence, we have to carry out in a microwave oven to decrease the time required for condensation. In the electromagnetic spectrum, the microwave radiation region is located between infrared radiation and radio-waves[9,10]. In order to avoid interference with these systems, the household and industrial microwave ovens operate at a fixed frequency of 2.45 GHz.[11-13] The energy of the quantum involved can be calculated by the Planck's law E = h ν and is found to be 0.3 cal mol^{-1}. In microwave heating, the substance must possess a dipole moment. A dipole is sensitive to external electric field and tries to align itself with the field by rotation. If submitted to an alternating current, the electric field is inversed at each alterance and therefore dipoles tend to move together to follow the inversed electric field. Such a characteristic induces rotation and friction of the molecules, which dissipates as internal homogeneous heating. The cyclization of the corresponding product with 80% H_2SO_4, which smoothly yielded the quinolinone (7) in 49% yield. After constructing the quinolinone ring successfully, we concentrated on building the last ring, utilizing the intramolecular nitrene insertion methodology. Conversion of quinolinone ring to the corresponding azide(10) was then carried out by the classical nitrous acid followed by sodium azide combination of reactions. The next step in the synthesis of pyrido [2,3,4-*kl*] acridine unit was the key intramolecular nitrene insertion reaction. The synthesis of the tetracyclic compound constitutes achievement of one of the major objectives, namely the synthesis of an advanced tetracyclic intermediate in which the B ring is functionalized. Analogues of natural products containing pyridoacridine units could be available from it by manipulation of the quinolinone ring. The key compound (19) was then easily prepared from (18) by conversion to the chloroquinoline in 83% yield.

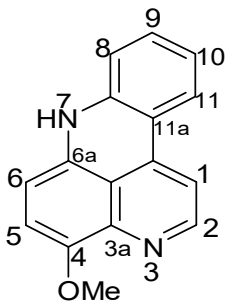

Fig. 1 : Pyridoacridines intermediate

3.1. Experimental procedure

3.1.1 *N-(2-methoxy-5-nitrophenyl)-3-oxo-3-phenylpropanamide (5)*

A mixture of 2-methoxy-5-nitroaniline (0.009mol) and benzoylacetic ethyl ester (0.009mol) was irradiated in a microwave oven at 100% intensity for 7 min. The reaction mixture was taken out of microwave oven and was allowed to cool. The yellow coloured solid was recrystallized from chloroform. Yield 3.56 g, 81%, yellow crystals, m p 183-185°C (lit.[14]).

MS: *m/z* 314 (M+). 1H NMR($CDCl_3$): 4.02 (s, 3H), 4.08 (s, 2H), 6.86 (d, *J* = 8.4 Hz,1H), 7.60 (s, 5H), 8.0 (m, 2H). Anal. calcd. for $C_{16}H_{14}N_2O_5$:C 61.14, H 4.49, N 8.91; found: C 60.98, H 4.53, N 8.78

8-methoxy-5-nitro-4-phenylquinolin-2(1*H*)-one was prepared as per procedure reported in literature[15].

3.1.2 *8-methoxy-5-nitro-4-phenylquinolin-2(1H)-one (7)*

N-(2-Methoxy-5-nitrophenyl)-3-oxo-3-phenylpropanamide (0.014mol) was stirred in H_2SO_4 (80%, 100 ml) at 75°C for 1 hour. The reaction mixture was cooled to room temperature and poured into ice water. The resulting solution was then neutralized with Na_2CO_3 and the Na_2SO_4 which separated was removed by filtration and the filtrate was extracted with CH_2Cl_2 (30X4 ml) and the combined extracts were dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The crude product obtained was recrystallized from toluene. to give yield: 2.030 g, 49%, orange solid, m.p. 155-158^{0}C.MS: *m/z* 296 (M+). **^{1}H NMR** (CDCl3): 9.853(s, 1H; H-1) ,7.643-7.692(m, 5H; Ar H- 2',3',4',5',6'),7.518-7.548(d,1H ; H-6), (J = 9 Hz),6.982 -6.952(d, 1H; H-7), (J = 9 Hz), 5.728 (s, 1H; H-3), 3.982 (s, 3H; -OCH_3).IR:3370(ν amide N-H),1624.93 (ν amide C=O),1605.65 (ν Ar C=C),1326.99 and 1514.48 (ν –NO2),1017.07 (ν C-O) **^{13}C-NMR**: 159.9(C-2, C=O), 158.8 (C-8), 139.7 (C-5), 139.0 (C-7), 137.1 (C-6),129.5 (C-4), 128.4(C-8a),128.2 (C-4a), 127.5 (C-3),127.3 (C-1'), 125.2(C-2'),123.4 (C-3'), 120.7 (C-5'),115.6 (C-6'),111.4 (C-4'),56.2(-OCH_3).

Anal. calcd. for $C_{16}H_{14}N_2O_5$:C 64.86, H 4.08, N 9.46; found: C 65.02, H 4.22, N 9.65

3.1.3 *5- amino-8-methoxy-4-phenylquinolin-2(1H)-one (10)*

5- Amino-8-methoxy-4-phenylquinolin-2(1*H*)-one was prepared from 8-methoxy-5-nitro-4-phenylquinolin-2(1*H*)-one (0.005mol), Zn dust (0.006 mol) in methanol (5ml) and 90% HCOOH (2.5ml) After completion of the reaction (monitored by TLC), the mixture was filtered off. The organic layer was evaporated and the residue dissolved in $CHCl_3$ and washed with saturated NaCl. The organic layer upon evaporation gave the yellow crystalline product of 5- amino-8-methoxy-4-phenylquinolin-2(1H)-one, (yield: 78%), m.p. 216-218°C (lit.[13]).

3.1.4 *5-azido-8-methoxy-4-phenylquinolin-2(1H)-one (14)*

5-azido-8-methoxy-4-phenylquinolin-2(1*H*)-one was prepared from 5-amino-8-methoxy-4-phenylquinolin-2(1*H*)-one (0.007 mol), concentrated H_2SO_4 (1.72 ml), sodium nitrite (0.001mol) and sodium azide (0.001mol), The mixture was stirred for 10 minutes at room temperature, then more water (3 ml) was added and the mixture was cooled to 0–5°C in an ice water bath. A solution of sodium nitrite (0.001mol) in water (5 ml) was carefully added drop wise and the mixture was stirred for 45 minutes. With constant stirring, a solution of sodium azide (0.001mol) in water (5 ml) was then added drop wise and stirring was continued for a further 40 min. The reaction mixture was poured into water (30 ml), and the resulting solution neutralized with Na_2CO_3 and extracted with CH_2Cl_2 (3 × 40 ml). The organic extracts were dried ($MgSO_4$), filtered, and the solvent was evaporated under reduced pressure. The yellow crystalline solid of 5-azido-8-methoxy-4-phenylquinolin-2(1H)-one was obtained, (yield: 90%), m.p. 261^{0}C.

3.1.5 ***4-methoxy -3H-pyrido [2,3,4-kl] acridin-2(7H)-one (16)***

5-Azido-8-methoxy-4-phenylquinolin-2(1*H*)-one (2 mmol) was refluxed in xylene for 1.5 hours. The reaction mixture was cooled to room temperature and left overnight. The solid, which separated, was collected by filtration and washed with petroleum ether (bp 40–60°C, 25 ml). The dark brown residue thus obtained was purified by column chromatography using silica gel adsorbent. Elution with mixture of ethyl acetate and ethanol (4:2) followed by distillation for recovery of solvent. Yield: 0.39 g ,75%, brown crystalline solid, m. p. 270 °C (lit[14]. m.p.270°C).

3.1.6 ***2-chloro-4-methoxy -7H-pyrido[2,3,4-kl] acridine (18)***

4-Methoxy -3-H-pyrido [2,3,4-kl] acridin-2(7H)-one (0.75mmol) was refluxed in $POCl_3$ (30 ml) for 1 hour. The reaction mixture was cooled to room temperature and poured into water (50 ml). The resulting solution was neutralized with solid Na_2CO_3 and the solid, which separated, was collected by filtration. Yield: 1.74g, 83%, red crystalline solid, m.p.202°C (lit[14]. m.p.202°C).

3.1.7 ***Formation of 4-methoxy -7H-pyrido [2,3,4-kl] acridine (19)***

A suspension of 2-chloro-4-methoxy -*7H*-pyrido [2,3,4-*kl*] acridine (0.002mol), Zn dust (0.006 mol) in methanol (5ml) was stirred with 90% HCOOH (2.5ml) at room temperature. After completion of the reaction (monitored by TLC), the mixture was filtered off. The organic layer was evaporated and the residue dissolved in $CHCl_3$ and washed with saturated NaCl. The organic layer upon evaporation gave the red crystalline product of **4-methoxy -7*H*-pyrido [2,3,4-*kl*] acridine.** Yield: 0.94 g ,76%), m.p. 224°C (lit.[14]).

3.2. BIOLOGICAL RESULTS

The cytotoxic activity of compounds **16**, 18 and **19** was tested in murine limphoma (P388D), human cell lung carcinoma (A549), human colon carcinoma (HT-29), and human melanoma (SK-MEL-28) cell lines and the results are detailed in **Table 1**. All those compounds present cytotoxic activity. A comparison of the activity of our tetracyclic compounds **16–19** with ascididemine **1;** shows a minor activity for **16 & 19**, although **18** has a similar potency. Finally, the tetracyclic compound **18** presented an excellent cytotoxic activity, especially against human cell lung carcinoma (A-549) and human melanoma (MEL-28).

Table 1: Cytotoxic activity IC50 (iM) of compounds 1, 16, 18 & 19

Compound	A549	P-388D	HT-29	SK-MEL-28
1[a]	0.02	0.33	0.35	0.003
16	4.32	4.32	21.37	4.16
18	0.004	0.03	0.16	0.009
19	4.25	1.08	4.31	4.31

[a] Tested on related HCT-116 line cells.[16]

4. CONCLUSION

We have successfully synthesized pyrido [2,3,4-kl] acridine unit. The route we present here involves two important key steps, cyclization to form the quinolinone moiety by utilization of microwave irradiation and intramolecular nitrene insertion to build a tetracyclic system. Microwave irradiation reduced reaction time dramatically, increases product yield and enhance product purities by reducing unwanted side reactions.

This new synthetic route to pyrido [2,3,4-kl] acridine unit has substantially reduced synthesis time and uses less reagents compared to the reported procedure.[56] Analogues of natural products containing pyridoacridine units could be available from it by manipulation of the quinolinone ring. Some of them are Arnoamine B, Arnoamine A, Norsegoline, Styelsamine D, Cystodytins–A, Methyl ether,

Cystodytin J, Amphimedine, Eupomatidine, Eilatiane. Above table result shown that compound 5 have selective cytotoxicity towards the carcinoma(A-549) and human melanoma (MEL-28).

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REFERENCES

1. Ma del Mar Blanco, Carmen A.,J. Carlos Menendez, *Tetrahedron*, **1999,** 55,12637-12646.
2. (a) M.H.G. Munro, R.T. Luibrand, and J.W. Blunt. In Bioorganic marine chemistry. Vol. 1. Edited by P.J. Scheuer. Springer, New York. 1987; (b) T. Ozturk. In The alkaloids.Vol. 49. Edited by G.A. Cordell. Academic Press, New York.1997; (c) C.J. Moody and R. Thomas. *Adv. Heterocycl. Nat.Prod. Synth.* 2, 377 (1992); (d) T.F. Molinski. *Chem. Rev*. 93,1825 (1993).
3. McDonald, L. A., Eldredge, G. S., Barrows, L. R., Ireland, C. M. *: J. Med.Chem*.1994, 37, 3819.
4. Schmitz, F. J., Deguzman, F. S., Hossain, M. B., Vanderhelm, D;*J. Org. Chem.*,1991, 56, 804.
5. Taraporewala, I. B., Cessac, J. W., Chanh, T. C., Delgado, A. V., Schinazi, R. F.; *J.Med. Chem.* 1992, 35, 2744.
6. Kobayashi, J., Cheng, J., Walchi, M. R., Nakamura, H., Hirata, Y., Sasaki, T., Ohizumi, Y.; *J. Org. Chem.* 1988, 53, 1800.
7. Gunawardana, G.P., Koehn, F.E., Lee, A.Y., Clardy, J., He, H.Y., Faulkner, D. J.;*J.Org.Chem*. 1992,57,1523-1526.
8. Kappe CO;*Angrew Chem. Int Edn*;2004,43,6256
9. Galema SA; Halstead BSJ; Mingos DMP; *Chem. Soc. Rev*.; 1998; 2; 213–232.
10. P.Lidstrom, et.al ;Tetrahedron,2001,57,9225-9283
11. Horikoshi S; Hamamura T; Kajitani M; Yoshizawa-Fujita M; Serpone N; *Org. Process Res. Dev.*; 2008; 12; 1089–1093.
12. Varma RS; *Green Chem*.; 1999; 1; 43–55.
13. Gedye RN; Smith FE; Westaway KC; *Can. J. Chem*.; 1988; 66; 17–26.
14. Turan, O., Alexander, Mc.; *Canadian Journal of chemistry* , 2000, 78(9), 1158-1164.
15. Delfourne, E., Roubin, C. and Bastide, J.; *J.Org. Chem*., 2000, 65, 5476-5479.
16. J. Kobayashi, J. Cheng, H. Nakamura, Y. Ohizumi, Y. Hirata, T. Sasaki, T. Ohta, S. Nozoe, *Tetrahedron Let*t. 1988, 29, 1177–1180.