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An improved synthesis of the alkaloid Luotonin-A employing ionic liquid and water as key solvents

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ABSTRACT

Luotonin A is among the first known natural product possessing the heteroaromatic pyrroloquinazoline ring system. Although many syntheses have been reported for this compound, but they all have one or the other draw back such as a large number of steps, use of hazardous reagents like sodium hydride, low temperature reactions, and lengthy reaction time. Herein we report the synthesis of luotonin A achieved in five steps in which, two of the steps involved green solvents such as an ionic liquid and water as reaction media. In particular, we have achieved the reaction steps 1 and 3 involving the green solvents in much shorter reaction time than hitherto reported.

1. Introduction

Heterocyclic compounds are of particular interest in medicinal chemistry. Among these, alkaloids that incorporate the pyrroloquinazoline chromophores have been isolated form natural sources [1,2] display a wide range of biological activities [3-5]. Luotonin A (Figure 1), the first known natural product to possess the heteroaromatic pyrroloquinazoline ring system, has been isolated by Nomura and co-workers in 1997 from the aerial parts of the *Peganum nigellastrum* (Chinese Bung) [6]. Luotonin A showed potent cytotoxic activity against mouse leukemia P-388 cells and has been the subject of several syntheses [6]. There are obvious similarities between a good lead anticancer agent, 20-(S)-campothecin [7-13] and luotonin A notably in identical rings ABC [14,15].

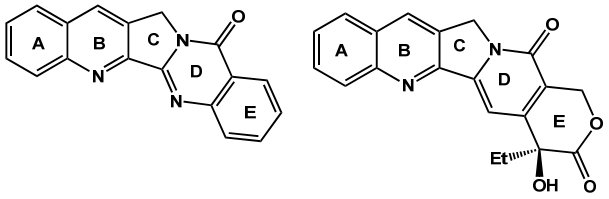

Figure 1. Structures of Luotonin A and 20-(s)-campothecin.

Interest in luotonin A has increased considerably following the report by Hecht and coworkers that it mediates topoisomerase-dependent cytotoxicity [16]. These observations are important because it has been thought until very recently that the E-ring hydroxylactone of campothecin was indispensable for anticancer activity [17-19]. However Luotonin is a good lead compound and Hecht [20,21], Dallavalle [22] and their coworkers recently reported the synthesis of an assortment of substituted E-ring analogues with a few A ring analogues.

In view of the importance of luotonin A and its derivatives in medicinal chemistry, a number of total syntheses have been reported in literature [23-38]. Although many syntheses have been reported, they all have the one or the other draw back of large number of steps, use of hazardous reagents like sodium hydride, low temperature reactions and lengthy reaction time. In many synthesis, the use of halogenated volatile organic compounds (VOC) like dichloromethane or solvents like benzene and dimethylformamide are detrimental to the environment which need to be recovered and recycled completely adding to the economics of the process. In continuation of our ongoing work on the synthesis of bioactive natural products, we herein report the formal synthesis of luotonin A which avoids many of the above mentioned drawbacks. We initiated this study with the goal of expanding the efficacy and efficiency of the methodologies developed by us using ionic liquids [39] for the synthesis of luotonin A, particularly for the first step and the involvement of water as solvent in another step.

2. Experimental

2.1. Instrumentation

Melting points were recorded in open capillary on Buchi melting point B-540 apparatus and are uncorrected. ^{1}H NMR

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spectra were recorded on Bruker AC-200 MHz, MSL-300 MHz and DRX-500 MHz spectrometers using tetramethylsilane (TMS) as an internal standard. Chemical shifts have been expressed in ppm units downfield from TMS. ^{13} C NMR spectra were recorded on Bruker AC-50 MHz, MSL-75 MHz and DRX-125 MHz spectrometers. Elemental analyses were obtained using a flash EA 1112 thermofinnigan instrument.

2.2. Synthesis of methyl 2-methyquinoline-3-carboxylate (3)

A mixture of 2-aminobenzaldehyde **1** (1.0 g, 8.2 mmol) and methyl acetoacetate **2** (1.1 g, 9.9 mmol) in the ionic liquid (IL), 1-*n*-butyl-imidazolium tertrafluoroborate ([Hbim]BF_4) (10 mL) was heated at 100 ^{o}C with stirring for 1.5 h. The progress of reaction was monitored by Thin layer chromatography (TLC). After completion of reaction, the reaction mixture was diluted with water (10 mL) and product was extracted using ethyl acetate (3 x 30 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to afford a crude product which was purified by column chromatography using petroleum ether: ethyl acetate (9:1) to obtain 1.21 g (73%) of the pure methyl 2-methylquinoline-3-carboxylate (**3**) as white solid. M.p.: 67-69 ^{o}C. IR (KBr, ν_{max}, cm^{-1}): 2951, 1726, 1621, 1565, 1489, 1438, 1278, 1200, 1127, 1064, 788. ^{1}H NMR (200 MHz, $CDCl_3$, δ, ppm): 3.01 (s, 3H, CH_3), 3.99 (s, 3H, OCH_3), 7.51-7.59 (m, 1H, ArH), 7.76-7.89 (m, 2H, ArH), 8.04-8.08 (d, *J* = 8.49 Hz, 1H, ArH), 8.76 (s, 1H, ArH). ^{13}C NMR (50 MHz, δ, ppm): 169.4, 25.5 (CH_3), 52.2 (C as O-CH_3), 123.3, 125.6, 126.2, 128.3, 131.6, 139.9, 148.48, 158.39 ($CH_{methine}$), 166.77 (C as C=O). Anal. Calcd. for $C_{12}H_{11}NO_2$ (%): C, 71.63; H, 5.51; N, 6.96. Found: C, 71.51; H, 5.67; N, 7.08.

2.3. Synthesis of methyl 2-formyl quionoline-3-carboxylate (4)

A mixture of methyl 2-methylquinoline-3-carboxylate **3** (0.8 g, 4 mmol) and selenium dioxide (0.046 g, 4.2 mmol) in xylene (25 mL) was refluxed for 8 h. The progress of reaction was monitored by TLC. After completion of reaction solvent was evaporated under reduced pressure to get a blackish residue and the product was extracted using ethyl acetate (3 x 30 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to afford a crude product which was purified by column chromatography using petroleum ether: ethyl acetate (8.5:1.5) to afford 0.590 g (69%) of pure methyl 2-formyl quionoline-3-carboxylate **4** as dark brown viscous liquid. IR (KBr, ν_{max}, cm^{-1}): 3020, 2954, 2853, 1770, 1727, 1567, 1439, 1216, 1051, 945, 756, 667. ^{1}H NMR (200 MHz, $CDCl_3$, δ, ppm): 4.02, (s, 3H, OCH_3), 7.71-7.79 (m, 1H, ArH), 7.87-7.99 (m, 2H, ArH), 8.28-8.32 (d, *J* = 8.49 Hz, 1H, ArH), 8.61 (s, 1H, ArH), 10.41 (s, 1H, CHO). ^{13}C NMR (50 MHz, δ, ppm): 53 (O-CH_3), 124.4, 127.8, 128.3, 129.7, 130.4, 132.1, 138.5, 147.9, 151.2 ($CH_{methine}$), 166.9 (C_{ester} as C=O), 191.5 ($C_{aldehyde}$ as C=O). Anal. Calcd. for $C_{12}H_9NO_3$ (%): C, 66.97; H, 4.22; N, 6.51. Found: C, 66.87; H, 4.35; N, 6.67.

2.4. Synthesis of methyl 2-(4-oxo-3,4-dihydroquinazolin-2-yl) quioline-3-carboxylate (6)

A mixture of methyl 2-formyl quionoline-3-carboxylate **4** (0.4 g, 1.86 mmol), anthranilamide **5** (0.265 g, 1.96 mmol), ferric chloride hexahydrate (1.05 g, 3.9 mmol) in water (20 mL) was refluxed for 4 h. The progress of reaction was monitored by TLC. After completion of reaction, the reaction mixture was extracted using ethyl acetate (3 x 30 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to afford a crude product which was purified by column chromatography using petroleum ether: ethyl acetate (8:2) to obtain 0.47 g (76%) of the pure methyl 2-(4-oxo-3,4-dihydroquinazolin-2-yl) quioline-3-carboxylate **6** as a white solid. M.p.: 226-227 ^{o}C. IR (KBr, ν_{max}, cm^{-1}): 3331, 119, 2926, 2854, 1731, 1681, 1608, 1469, 1406, 1215, 1051, 902, 757, 668. ^{1}H NMR (200 MHz, $CDCl_3$, δ, ppm): 4.04 (s 3H, OCH_3), 7.50-7.58 (m, 1H, ArH), 7.65-7.81 (m, 3H, ArH), 7.83-7.94 (m, 2H, ArH), 8.16-8.20 (d, *J* = 8.42 Hz, 1H, ArH), 8.35-8.38 (d, *J* = 6.58 Hz, 2H, ArH), 11.01 (bs, 1H, NH). ^{13}C NMR (50 MHz, δ, ppm): 52.8 (O-CH_3), 122.4, 126.5, 126.7, 127.5, 127.8, 127.9, 128.0, 129.1, 129.5, 131.6, 137.5, 136.9, 144.6, 146.4, 147.6, 148.3 ($CH_{methine}$), 161.2 (C as C=O), 168.88 (C_{ester} as C=O). Anal. Calcd. for $C_{19}H_{13}N_3O_3$ (%): C, 68.88; H, 3.95; N, 12.68. Found: C, 68.76; H, 3.81; N, 12.81.

2.5. Synthesis of 2-(3-(hydroxylmethyl)quinolin-2-yl) quinozoline-4(3H)-one (7)

To a well stirred solution of methyl 2-(4-oxo-3,4-dihydroquinazolin-2-yl)quioline-3-carboxylate **6** (0.165 g, 0.5 mmol) in ethanol (5 mL) was added $CaCl_2$ (0.273 g, 2.5 mmol) and the resulting solution was stirred for 15 min. Finally powdered sodium borohydride (0.094 g, 2.5 mmol) was then added slowly in three portions and the reaction mixture further stirred at room temperature for 2 h. The progress of reaction was monitored by TLC. After completion of reaction, the reaction mixture was quenched with aqueous ammonium chloride. The solvent was evaporated under vacuum to get residue. Water (10 mL) was added to the residue and the product was extracted using ethyl acetate (2 x 15 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to afford a crude product which was purified by column chromatography using petroleum ether : ethyl acetate (6.5:3.5) to afford 0.130 g (87%) of the pure 2-(3-(hydroxylmethyl)quinolin-2-yl)quinozoline-4(3*H*)-one **7** as white solid. M.p.: 209-211 ^{o}C. IR (KBr, ν_{max}, cm^{-1}): 3423, 3308, 3018, 2926, 2855, 1682, 1603, 1565, 1470, 1236, 1215, 1021, 951, 758, 668. ^{1}H NMR (200 MHz, $CDCl_3$, δ, ppm): 5.09 (s, 2H, CH_2), 6.37 (bs, 1H, OH), 7.59-7.71 (m, 2H, ArH), 7.81-7.92 (m 4H, ArH), 8.18-8.20 (d, *J* = 8.36 Hz, 1H, ArH), 8.31 (s, 1H, ArH), 8.40-8.42 (d, *J* = 8.02 Hz, 1H, ArH), 11.49 (bs, 1H, 2H). ^{13}C NMR (50 MHz, δ, ppm): 63.9 (CH_2), 122.4 126.9, 127.5, 127.6, 128.2, 128.9, 129.0, 129.3 130.8, 133.9, 134.9, 139.2, 145.8, 146.5, 147.8, 150.1 ($CH_{methine}$), 161.0 (C as C=O). Anal. Calcd. for $C_{18}H_{13}N_3O_2$ (%) C, 71.28; H, 4.32; N, 13.85. Found: C, 71.43; H, 4.28; N, 13.68.

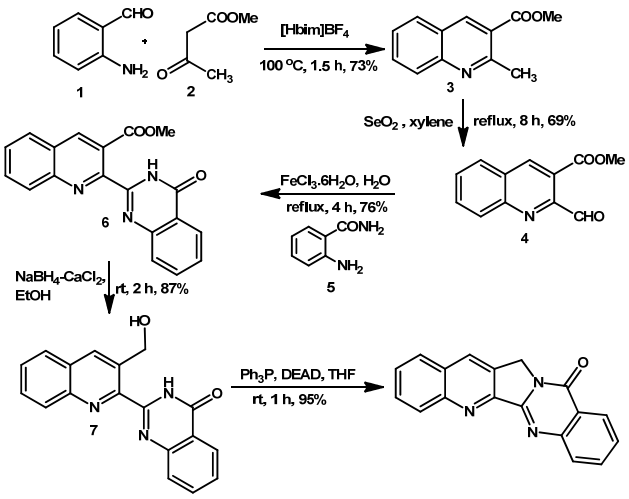
2.6. Quino[2',3':3,4]pyrrolo[2,1-b]quinazolin-11(13H)-one (Luotonin A)

To the solution of compound **7** (100 mg, 0.33 mmol) and TPP (112 mg, 0.43 mmol) in THF (5 mL) was added solution of DEAD (0.08 mL, 0.39 mmol) in Tetrahydrofuran (THF) (2 mL) dropwise over a period of 10 min at room temperature and further stirred for 1 h. The progress of the reaction was monitored by TLC. On completion the reaction mixture was concentrated in vacuo. The column chromatographic purification of the residue using ethyl acetate as an eluant furnished luotonin, 90 mg (95% yield). M.p.: 284-285 °C. IR (KBr, ν_{max}, cm^{-1}): 1670, 1630, 1605, 1466. ^{1}H NMR (200 MHz, $CDCl_3$, δ, ppm): 5.34 (s, 2H), 7.58 (t, *J* = 10 Hz, 1H), 7.68 (t, *J* = 10 Hz, 1H), 7.84 (t, *J* = 10 Hz, 1H), 7.86 (t, *J* = 10 Hz, 1H), 7.94 (d, *J* = 10 Hz, 1H), 8.12 (d, *J* = 10 Hz, 1H), 8.43 (d, *J* = 10 Hz, 1H), 8.44 (s, 1H), 8.47 (d, *J* = 10 Hz, 1H). ^{13}C NMR (50 MHz, δ, ppm): 47.3 (CH_2), 121.3, 126.4, 127.4, 127.9, 128.5, 128.8, 129.4, 130.68, 130.71, 131.5, 134.6, 149.35, 149.42, 151.2 ($CH_{methine}$), 152.5 (C as C= O), 160.7 (C as C=N). Anal. Calcd. for $C_{18}H_{11}N_3O$ (%): C, 75.78; H, 3.89; N, 14.73. Found: C, 75.929; H, 3.96; N, 14.68.

3. Results and discussion

The synthesis of luotonin A was initiated from 2-aminobenzaldehyde **1** (Scheme 1). Firstly, 2-aminobenzal-

dehyde was condensed with methyl acetoacetate **2** by Friedlander quinoline synthesis using IL, 1-*n*-butylimidazolium tetrafluoroborate ([Hbim]BF_4) as reaction medium as well as promoter to afford 2-methyl quinoline-3-carboxylic acid methyl ester in 73% yield in the absence of any additional catalyst as described in our previous report [39].



Scheme 1

The Bronsted acidic IL was responsible for promoting the reaction. The ^{1}H NMR spectrum of 3 shows peak at 3.01 ppm as singlet corresponding to the methyl at C-2 and peak at 3.99 ppm as singlet corresponding to the methyl of methylcarboxylate supporting its formation.

The product **3** was then oxidized using selenium dioxide in xylene to afford 2-formylquinoline-3-carboxylic acid methyl ester **4** in 69% yields. The formation of compound **4** was confirmed by ^{1}H NMR showing the peak at 10.4 ppm for the hydrogen of aldehyde and the disappearance of peak at 3.01 ppm corresponding to the methyl at C-2. The compound **4** was then condensed with anthranilamide **5** using ferric chloride ($FeCl_3.6H_2O$) in water to afford **6** via Schiff base formation and spontaneous cyclization the yield was 76%. The ^{13}C NMR of compound showed peaks at 52.8 (carbon of methyl ester), 161.2 (carbonyl carbon of amide), 168.8 (carbonyl carbon of ester), and 148.3 (C-2 of quinazolinone) confirming its formation. An important point to note is that is water has been employed as a green solvent which is cheap and abundantly available.

Compound **6** was then reduced by using sodium borohydride-calcium chloride in ethanol to furnish 2-(-quinoline-3-hydroxymethyl)-4(3*H*)-quinazoline **7** in 87%. This compound has been converted to luotonin A in the literature in one step by using standard Mitsunobu reaction conditions using triphenylphosphine, diethyl azodicarboxylate in tetrahydrofuran by a simple procedure [27]. We followed the method published in literature to complete the synthesis of Luotonin A The spectral data and melting point of luotonin A exactly matched the standard compound. In conclusion, the formal synthesis of luotonin A was achieved in five steps; the green solvents ionic liquid and water were used step 1 and 3 which have resulted in shortened reaction times. The overall yield is better than those reported so far. Also the synthesis of luotonin A in our laboratory was achieved at easily accessible reaction temperatures. The process is amenable to scale up and can be gainfully employed to synthesize a library of luotonin analogues.

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