

RESEARCH ARTICLE

4-Methyl-2H-Benzo[h]Chromen-2-one as Focal Intermediates for the Synthesis of 4-Methyl-Naphtho [1,2-b] Pyridin-2-one-1-(2',3'-Diphenyl Isoquinolinyl [1,5-c] Azoles

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4-Methyl-2H-Benzo[h]Chromen-2-one as Focal Intermediates for the Synthesis of 4-Methyl-Naphtho [1,2-*b*] Pyridin-2-one-1-(2',3'-Diphenyl Isoquinolinyl [1,5-*c*] Azoles

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Abstract

A series of 4-methyl-naphtho [1,2-*b*] pyridin-2-one-1-(2',3'-diphenyl isoquinolinyl) [1,5-*c*] azoles were synthesized using 4-methyl-naphtho [1,2-*b*] pyran-2-one/4-methyl 2H-benzo[h]chromen-2-one **1** as key intermediate. 4-Methyl-naphtho [1,2-*b*] pyran-2-one **1** were rehabilitated to 4-methyl-naphtho [1,2-*b*] pyridine-2-one-1-phenyl-4'-carboxylate **2**. Compound **2** was further cyclized to 4-methyl-naphtho [1,2-*b*] pyridine-2-one-1-(3',4'-diphenyl-isocoumarin) **3** using benzoin. Finally, four compounds **4**, **5**, **6** and **7** were obtained using different diamines. The target compounds were evaluated for their antiviral activity against two animal viruses viz. *Japanese encephalitis virus* (JEV) (strain P20778) and *Herpes simplex virus-1* (HSV-1) (strain 753166) on vero cells *in vitro*.

Keywords: Pyridin-2-one; isoquinoline; azoles; antiviral activity; mean lethal dose; fetal bovine serum.

1. Introduction

Pyridine-2-one is nitrogen containing synthetically designed scaffold with a broad spectrum of biological activities [1, 2]. This moiety frequently found in a variety of interesting compounds has received remarkable attention due to its promising feature as a key scaffold and in privilege building blocks. The pyridine motif is also found in a wide range of biologically active compounds including pyridoxal, niacin, and stimulant nicotine [3]. Many substituted pyridines are used in pharmaceuticals very common one being isoniazid for tuberculosis [4]. Further, condensed derivatives of pyridine have received significance owing to their potential antimicrobial effects [5]. Some of the naphthyl pyridine derivatives have shown activity comparable to Ampicillin® as reference drug [6]. N-substituted pyrid-2-ones have fascinated the researchers which is due in part of their chemical reactivity, photochemical behavior as well as biological activities. However, synthetic examples of N-alkyl pyridones are scarce [7].

As a part of our endeavor aimed at the development of novel, simple and efficient procedure for the synthesis of biologically active heterocyclic nitrogenous molecules we thought it worthwhile to introduce a series of 4-methyl-naphtho [1,2-*b*] pyridin-2-one-1-(2',3'-diphenyl isoquinolinyl [1,5-*c*] azoles that seem promising for further chemical transformation and biological evaluation studies.

2. Methods

2.1 Syntheses

Melting points were determined in open capillaries using a Toshniwal melting point apparatus (Japan) and the values recorded, are therefore uncorrected. IR spectra in KBr were recorded in the 4000-400cm⁻¹ range using KBr discs on FTR IR 8201 VC Perkin Elmer Spectrophotometer model 337 (USA). The NMR spectra were recorded on a Varian 60 D instrument (200 MHz) (USA) using MeOH/DMSO-d₆. TMS was used as internal standard (δ in ppm). Mass spectra of compounds were run on a Hitachi-Elmer RMV-7 spectrometer at 70 eV and FAB mass spectra were recorded on JEOL SX 102/DA-600 Mass Spectrometer\Data System using Argon/Xenon (6 KV, 10 mA) as the FAB gas. Elemental analyses were performed on Carlo-Erba-1108 instrument or Elementar's Vario EL III microanalyser. C, H, and N values were calculated as per the atomic weight of C= 12.01, H= 1.008, N= 14.007, O= 15.999, F= Cl= 35.453, Br= 79.90. The values obtained for each element were expressed as percentage of total molecular weight

of the compound and agreed with the calculated ones. Purity of the compounds was checked by TLC on silica gel plates. All reagents were purchased from Merck and Ranbaxy. Synthesis of polyphosphoric acid [8, 9] was accomplished according to the previously reported procedures.

4-Methyl-naphtho [1,2-*b*] pyran-2-one (1)

To a mixture of α -naphthol (0.1 mole) and ethyl acetoacetate (0.12 mole) was added H_2SO_4 (84%, 50 mL) during 30 min at 0°C . The reaction mixture was stirred for 3 h and poured into ice-cooled water (~200 mL). The precipitate formed was filtered off, washed repeatedly with water (3x20 mL) and extracted with warm methyl alcohol (3x200 mL). Solvent was removed by distillation to give an orange coloured product, m.p. 170°C , which was recrystallized from methanol (charcoal) as colourless needles. It melted at $172\text{-}174^\circ\text{C}$ [10], yield 66%. Anal. Calcd. for $\text{C}_{14}\text{H}_{10}\text{O}_2$ (%): C 80.0; H 4.76. Found: C 79.68; H 4.80. Solution of the compound in conc. H_2SO_4 produced a green fluorescence.

4-Methyl-naphtho [1,2-*b*] pyridine-2-one-1-phenyl-4'-carboxylate (2)

4-Methyl-naphtho [1,2-*b*] pyran-2-one **1** and *p*-amino benzoic acid (0.05 mole each) were dissolved in anhydrous pyridine (20 mL) and refluxed for 6 h. The contents were cooled to room temperature and poured into ice-cooled water (100 mL) acidified with HCl (10 mL). The solid thus precipitated was filtered, washed with cold water (3x20 mL) and dried in vacuum. It was recrystallized from ethanol as colourless needles, m.p. $158\text{-}159^\circ\text{C}$, yield 50%. Anal. Calcd. for $\text{C}_{21}\text{H}_{15}\text{NO}_3$ (%): C 76.58; H 4.59; N 4.25 Found: C 76.50; H 4.66; N 4.13. Mass: (FAB+) (m/z) ($\text{M}^+ + 1$): 330. IR (KBr, cm^{-1}): 1665 (C=O), 1700 (C=O acid), 3540 (OH). ^1H NMR (CDCl_3 , δ): 2.42 (s, 3H, CH_3), 6.30 (s, 1H, CH), 7.22 (s, 1H, ArH), 7.52-8.04 (m, 9H, Ar-H), 11.0 (s, 1H, OH).

4-Methyl-naphtho [1,2-*b*] pyridine-2-one-1-(3',4'-diphenyl-isocoumarin) (3)

A mixture of 4-methyl-naphtho [1,2-*b*] pyridine-2-one-1-phenyl-4'-carboxylate **2** (0.02 mole) and benzoin (desyl alcohol) (0.02 mole) in freshly prepared polyphosphoric acid (P.P.A.) (30 mL) was refluxed for 4 h under anhydrous reaction conditions. The reaction mixture was cooled to room temperature and poured into ice-cooled water (100 mL). The solid obtained was filtered, washed repeatedly with cold water (3x20 mL) and dried *in vacuo*. Recrystallization from ethanol ensued shiny green crystals, m.p. $160\text{-}161^\circ\text{C}$, yield 57%. Anal. Calcd. for $\text{C}_{35}\text{H}_{23}\text{NO}_3$ (%): C 83.15; H 4.59; N 2.77 Found: C 83.10; H 4.66; N 2.76. Mass: (FAB+) (m/z) ($\text{M}^+ + 1$): 505. IR (KBr, cm^{-1}): 1730 (C=O), 1152 (C-O-C), 1645 (C=O). ^1H NMR (CDCl_3 , δ): 2.46 (s, 3H, CH_3), 6.32 (s, 1H, CH), 7.25- 8.02 (m, 18H, Ar-H), 8.18 (s, 1H, ArH).

4-Methyl-naphtho [1,2-*b*] pyridine-2-one-1-(2',3'-diphenyl isoquinolinyl) [1,5-*c*] imidazole (4)

To a solution of 4-methyl-naphtho [1,2-*b*] pyridine-2-one-1-(3',4'-diphenyl isocoumarin) **3** (0.01 mole) in anhydrous pyridine (15 mL) was added ethylene diamine (0.01 mole). The resulting mixture was refluxed for 6 h and cooled to room temperature. The addition of ice-cooled water (100 mL) acidified with HCl (10 mL), ensued a solid which was filtered, washed with cold water (4x20 mL) and dried in vacuum. Recrystallization from ethanol afforded light brown crystals, m.p. $>300^\circ\text{C}$, yield 46%, Anal. Calcd. for $\text{C}_{37}\text{H}_{27}\text{N}_3\text{O}$ (%): C 83.91; H 5.14; N 7.93. Found: C 83.89; H 5.16; N 7.90. Mass: (FAB+) (m/z) ($\text{M}^+ + 1$): 529. IR (KBr, cm^{-1}): 1665 (C=O), 1642 (C=N). ^1H NMR (CDCl_3 , δ): 2.42 (s, 3H, CH_3), 4.83 (m, 2H, CH_2), 5.97 (m, 2H, CH_2), 6.35 (s, 1H, CH), 6.84 (s, 1H, ArH), 7.22- 8.52 (m, 18H, Ar-H).

4-Methyl-naphtho [1,2-*b*] pyridine-2-one-1-(2',3'-diphenylisoquinolinyl) [1,5-*c*] benzimidazoles (5)

A mixture of 4-methyl naphtho [1,2-*b*] pyridine-2-one-1-(3',4'-diphenyl isocoumarin) **3** (0.01 mole) and *o*-phenylene diamine (0.01 mole) in anhydrous pyridine (20 mL) was refluxed for 6 h. It was cooled to room temperature and poured into ice-cooled water (100 mL) acidified with HCl (10 mL). The solid precipitated out was filtered and successively washed with cold water (3x20 mL). The crude product was dried in vacuum and recrystallized from ethanol as brown crystals, m.p. $189\text{-}190^\circ\text{C}$, yield 50%. Anal. Calcd. for $\text{C}_{41}\text{H}_{27}\text{N}_3\text{O}$ (%): C 85.25; H 4.71; N 7.27 Found: C 85.21; H 4.76; N 7.25. Mass: (FAB+) (m/z) ($\text{M}^+ + 1$): 577. IR (KBr, cm^{-1}): 1665 (C=O), 1643 (C=N). ^1H NMR (CDCl_3 , δ): 2.42 (s, 3H, CH_3), 6.35 (s, 1H, CH), 6.84 (s, 1H, ArH), 7.22- 8.52 (m, 22H, Ar-H).

4-Methyl-naphtho [1,2-*b*] pyridine-2-one-1-(2',3'-diphenyl isoquinolinyl) [1,5-*c*] thiaziazole (6)

A solution of 4-methyl-naphtho [1,2-*b*] pyridine-2-one-1-(3',4'-diphenyl isocoumarin) **3** (0.01 mole) and thiosemicarbazide (0.01 mole) in ethanol (20 mL) was refluxed for 4h. Solvent was distilled off and the crude product

obtained was washed with cold water, filtered, dried and recrystallized from methanol as light brown crystals, m.p. 150-152°C, yield 50%, Anal. Calcd. for C₃₆H₂₄N₄OS (%): C 77.12; H 4.31; N 9.99 Found: C 77.08; H 4.34; N 9.96. Mass: (FAB+) (m/z) (M⁺+1) 560. IR (KBr, cm⁻¹): 1085 (C=S), 1665 (C=O), 1643 (C=N), 2560 (SH), 3308 (NH). ¹H NMR (CDCl₃, δ): 2.42 (s, 3H, CH₃), 4.40 (brs, 1H, CSNH), 6.30 (s, 1H, CH), 6.84 (s, 1H, ArH), 7.24- 8.22 (m, 18H, Ar-H).

4-Methyl-naphtho [1,2-*b*] pyridine-2-one-1-(2',3'-diphenyl isoquinolinyl) [1,5-*c*] oxatriazole (7)

To a mixture of 4-methyl-naphtho [1,2-*b*] pyridine-2-one-1-(3',4'-diphenyl isocoumarin) **3** (0.01 mole) and semicarbazide hydrochloride (0.01 mole) was added ethanol (20 mL) at room temperature. The reaction mixture was allowed to stand for 24 h, then refluxed for 4 h, cooled and poured into crushed ice. The separated solid material was filtered washed with cold water (3x25 mL) and dried. Recrystallization from methanol afforded colourless crystals, m.p. 140-141°C yield 48%, Anal. Calcd. for C₃₆H₂₄N₄O₂ (%):C 79.39; H 4.44; N 10.29 Found: C 79.35; H 4.48; N 10.25. Mass: (FAB+) (m/z) (M⁺+1) 545. IR (KBr, cm⁻¹): 1667 (C=O), 1642 (C=N), 3315 (NH). ¹H NMR (CDCl₃, δ): 2.42 (s, 3H, CH₃), 4.60 (brs, 1H, CONH), 6.35 (s, 1H, CH), 6.84 (s, 1H, ArH), 7.22- 8.52 (m, 19H, Ar-H).

2.2 Pharmacological activity

Antiviral activity

All the four targeted compounds were subjected for their assay against two animal viruses viz. *Japanese encephalitis virus* (JEV) (strain P20778), an RNA virus of high pathogenicity, and *Herpes simplex virus-1* (HSV-1) (strain 753166), a DNA virus, originally obtained from National Institute of Virology, Pune (India).

Maintenance of Japanese encephalitis virus (JEV). It was properly maintained by intra-cerebral passages in 1-3 day(s) old suckling albino Swiss mice. The brains of the infected mice with specific paralytic symptoms were triturated and a 10% homogenate (m/V) was made in phosphate buffered saline (PBS) of pH 7.2. The mean lethal dose (LD₅₀) of the virus in mice was calculated before each experiment.

Maintenance of Herpes simplex virus-1 (HSV-1). It was maintained in 5-6 g albino Swiss mice following the same route as for JEV; a 10% virus homogenate (m/V) was prepared and LD₅₀ was calculated as for JEV.

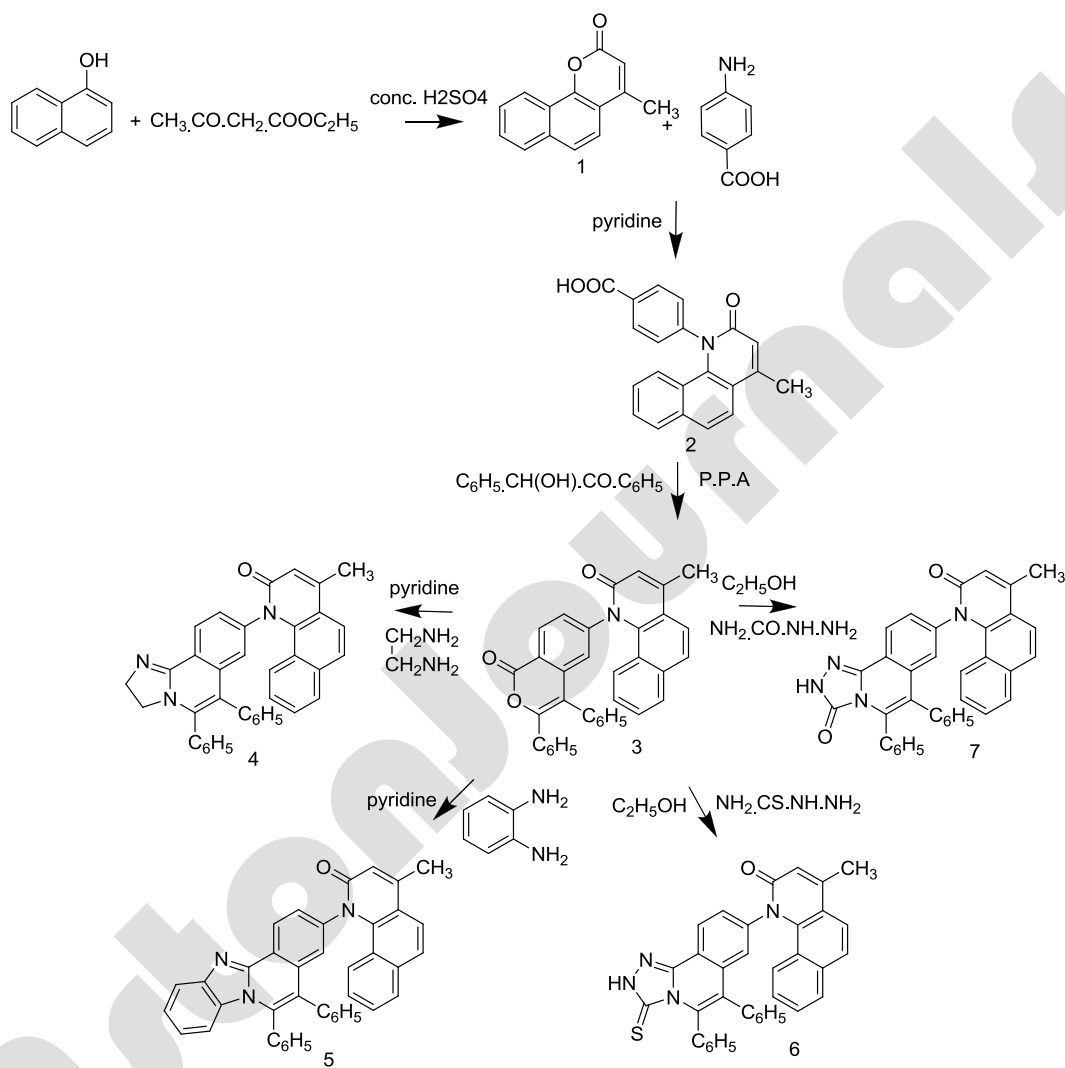
Maintenance of cells. Vero cells were maintained in minimum essential medium (MEM) (Sigma, USA) with 10% foetal bovine serum (FBS) (Gibco, USA); 100 units of penicillin, 100 µg of streptomycin and 40 µg of gentamycin were added per mL of the medium.

Cytotoxicity test and antiviral assay in vitro. Cytotoxicity and antiviral assays of the compounds were performed by the standard method [11]. The experiments were performed in 96 well tissue culture plates. Equal volumes of maintenance medium and compound solution were poured into each well; concentration of 500 µg mL⁻¹ of the compound tested was applied into the first well. Successive dilution by factor 2 was performed in further wells: the compound concentration in the 8th well was 1.9µg mL⁻¹. The treated cultures were incubated for a period of 24 h at 37°C and then observed microscopically for evidence of cytotoxicity, such as distortion, swelling and sloughing of cells [12-15]. For the antiviral activity, 0.1 mL of the virus (10TC ID₅₀ mL⁻¹, i.e. the dilution previous to 1TC ID₅₀, which is the virus dilution that shows 50% cytopathic effect, where TC ID₅₀ is 50% tissue culture infectious dose) was allowed to adsorb onto cell monolayers for 90 min at 37°C [16]. The unadsorbed virus was removed by washing with 0.1 mL of MEM and then 0.1 mL of MEM with 2.5% FBS was filled into each well. Non-toxic concentration of the compound tested, ranging from 3.6 to 125µg mL⁻¹ of the compound, was added into each well. Each dilution was tested in duplicate, keeping separate the virus control and cell control (containing only MEM with 2.5% serum). The culture plates were incubated at 37°C for 72 h and examined microscopically for evidence of cytopathogenicity caused by the virus and its inhibition by the examined compound.

3. Results and Discussion

Formation of 4-methyl-naphtho [1,2-*b*] pyran-2-one **1** takes place via cyclization reaction of α-naphthol and ethylacetoacetate in presence of sulphuric acid. Compound **1** interacts with *p*-amino benzoic acid to form 4-methyl-naphtho [1,2-*b*] pyridine-2-one-1-phenyl-4'-carboxylate **2**. The reaction proceeds by nucleophilic attack of amine nitrogen on carbonyl carbon atom of **1**. Conversion of **2** to 4-methyl-naphtho [1,2-*b*] pyridine-2-one-1-(3',4-

diphenyl-isocoumarin) **3** takes place most conveniently and effectively in presence of PPA as shown in the scheme. Reaction of **3** with ethylene diamine, *o*-phenylenediamine, thiosemicarbazide and semicarbazide hydrochloride afforded 4-methyl-naphtho [1,2-*b*] pyridine-2-one-1-(2',3'-diphenylisoquinolinyl) [1,5-*c*] azoles **4**, **5**, **6** and **7** respectively. These compounds were characterized by their elemental analysis, IR, ¹HNMR, and mass spectral data. The compounds were also subjected to bio-evaluation upon *Japanese encephalitis virus* and *Herpes simplex virus-1*. The antiviral activity data has been incorporated in the Table.



Scheme

Table: Antiviral activity of 4-methyl-naphtho [1,2-*b*] pyridin-2-one-1-(2',3'-diphenyl isoquinolinyl) [1,5-*c*] azoles.

Compd. No.	Anti-JEV in vitro				Anti-HSV-1 in vitro		
	CT ₅₀ (µg/mL)	EC ₅₀ (µg/mL)	TI	Inhibition (%)	EC ₅₀ (µg/mL)	TI	Inhibition (%)
4	500	-	-	-	125	04	15
5	250	-	-	-	125	02	23
6	250	7.8	32	30	125	02	23
7	500	-	-	-	-	-	-

CT₅₀ = 50% Cytotoxic concentration; EC₅₀ = 50% Effective concentration; TI = Therapeutic index.

4. Conclusion

Much significant antiviral activity against JEV and HSV-I for compounds **4**, **5**, **6** and **7** was not observed as is evident from the antiviral activity data incorporated in the Table. Only compound **6** of this category displayed anti-JEV activity to the order of 30%, while other three compounds *viz.* **4**, **5** and **7** did not show any noticeable anti-JEV activity at the same concentration. Three compounds of this series were found to inhibit the multiplication of HSV-1 virus mildly. Thus, compounds **4**, **5** and **6** showed 15%, 23% and 23% anti-HSV-activity, respectively. It is interesting that compound **6** containing a $-C=S$ in the ring was active to the magnitude of 30% against JEV and 23% against HSV-I, while compound **7** containing a $-C=O$ in the ring was inactive upon both the viruses. Thus, such a minor change in the molecular architecture seems largely responsible for the difference in their antiviral activity. Although, azoles like imidazoles, benzimidazoles, triazoles etc., have been widely used as antiviral agents in certain cases of viral infections, it seems that unless a large number of such compounds with greater number of such variations are synthesized, their potentialities cannot be predicted with great certainty.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

ZT and ST carried out the synthesis, MNJ carried out the bioassays and VKP planned the work and drafted the manuscript.

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