One-Pot Synthesis and Evaluation for 15-Lipoxygenase Inhibition of 1-Ethoxy-4-cyano-5-ethoxycarbonyl-3*H*-azuleno[1,2-c]pyran-3-imine

Bing Bing Lin,[a,b,c,d]* and Yun-Shan Lin[a]

[a]Graduate Institute of Chemistry, Tamkang University, Tamsui, Taipei (251), Taiwan
[b]Department of Industrial and Business Management, Chang Gung University, Tao Yuan (333), Taiwan
[c]Department of Health Care Management, Chang Gung University, Tao Yuan (333), Taiwan
[d]Center of General Education, Chang Gung University, Tao Yuan (333), Taiwan
*E-mail: a332199@gmail.com

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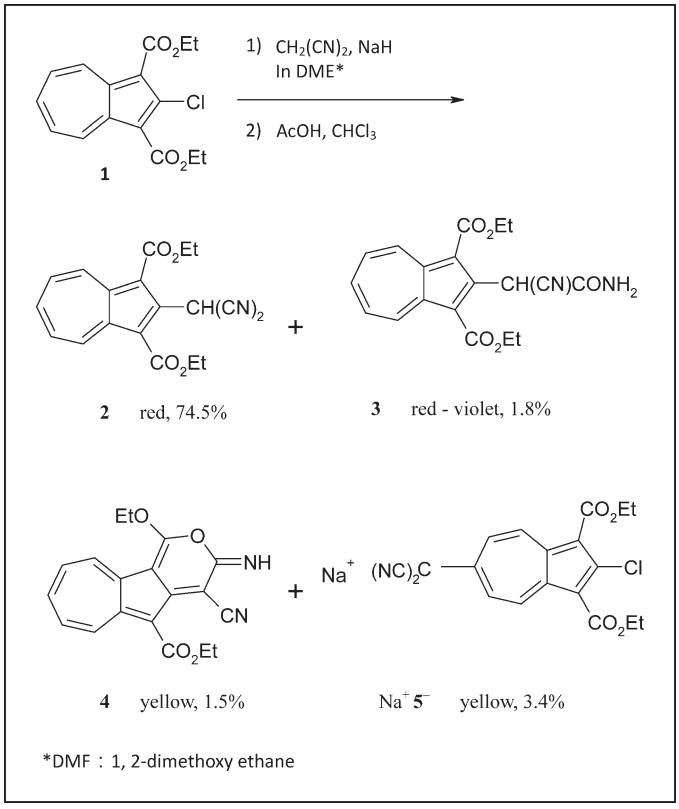

A method at room temperature, with one pot of 24 h reaction, to synthesize 1-ethoxy-4-cyano-5-ethoxycarbonyl-3*H*-azuleno[1,2-c]pyran-3-imine which showed inhibitory effect on 15-lipoxygenase at $IC_{50} = 23.2 \pm 1.3$ m*M*.

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INTRODUCTION

Lipoxygenase (LOX) is a biological target for many diseases, such as asthma, atherosclerosis, and cancer [1,2]. LOXs are classified with respect to their positional specificity of arachidonic acid oxygenation, in particular, the reticulocyte-type 15-LOX and the human 5-LOX, are well characterized with respect to their structural and functional properties [3,4]. Some natural azulene derivatives (chamazulene, guaiazulene) and synthetic azulene derivatives showed antioxidant activity and TXA2/prostaglandin endoperoxide receptor antagonist [5,6]. Furthermore, synthetic azulene analogues, such as 3-alkyl or 3-(hydroxy)alkylazulene-1-carboxylic acids and esters showed their effects on inhibition of soybean lipoxygenase by 100% at 1 m*M* [7].

Diazoquinones (diazoxides, quinone diazides) are important synthetic intermediates because of their high reactivity, photochemically and thermochemically [8]. Among the 22 possible isomers of diazoazulenequinone (diazo-dihydro-oxoazulenes, diazoazulenequinones), the compounds of 2-D-2,6-AQ(2-diazo-2,6-azulenequinone) type are stable to be isolated, whereas 6-D-2,6-AQ(6-

diazo-2,6-azulenequinone) type are unstable to be isolated [9]. We had reported the facile synthesis of 2-diazo-1-3-dicyano-6-oxo-2,6-dihydroazulene, the diazotization of diethyl 6-amino-2-hydroxyazulene-1,3-dicarboxylate [10,11], and the facile synthesis of 1-ethoxy-4-cyano-5-ethoxycarbonyl-3*H*-azuleno[1,2-c]pyran-3-one and its selective inhibition activity on 15-lipoxygenase [12]. In this study, we report a method at room temperature, with one pot of 24 h reaction, to synthesize the compound 1-ethoxy-4-cyano-5-ethoxycarbonyl-3*H*-azuleno [1,2-c]pyran-3-imine (**4**) from tropolone [9], via the corresponding diethyl 2-chloroazulene-1,3-dicarboxylate (**1**). The compound (**4**) showed inhibitory effect on 15-lipoxygenase (soybean source) at $IC_{50} = 23.2 \pm 1.3$ m*M*.

RESULT AND DISCUSSION

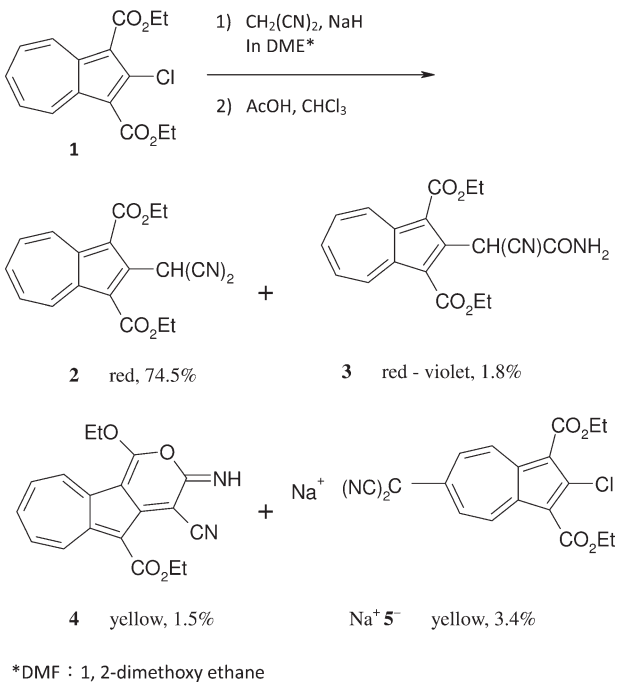
One-pot synthesis at room temperature for the title compound. The reaction of diethyl 2-chloroazulene-1,3-dicarboxylate (**1**), with (918 mg, 3 mmol) malonitrile (218 was stirred at 25°C for 24 h to produce red color sodium salts precipitates. The mixture was then acidified mg, 3.3 mmol), in the presence of anhydrous 1,2-dimethoxy ethane (8 mL) and sodium hydride 168 mg, by anhydrous acetic acid, then extracted with water/chloroform. The organic layer was neutralized with sodium bicarbonate and then worked up. The residue was chromatographed on silica-gel with successive elution (500 mL each) of benzene, chloroform, and ethyl acetate to obtain four different products (Scheme 1): diethyl 2-dicyanomethylazulene-1,3-dicarboxylate (**2**) (in a yield of 74.5%), diethyl 2-cyano-carbamoylazulene-1,3-dicarboxylate (**3**) (in a yield of 1.8%), 1-ethoxy-4-cyano-5-ethoxycarbonyl-3*H*-azuleno[1,2-c]pyran-3-imine (**4**) (in a yield of 1.5%), and with sodium salt of diethyl 2-chloro-6-dicyanomethylazulene-1,3-dicarboxylate (**5**) (in a yield of 3.4%).

Title compound (**4**), containing the 1,2-azulenoquinone dimethide structure, showed yellow color, which is different from the red-violet color or blue color of ordinary azulene compounds without 1,2-dimethide structure. By 2D NMR H–H COSY, it revealed that the five hydrogens on the seven-member ring were not replaced.

Evaluation for 15-lipoxygenase inhibition of title compound (4). 1-Ethoxy-4-cyano-5-ethoxycarbonyl-3*H*-azuleno[1,2-c]pyran-3-imine (**4**) showed inhibitory effect on 15-lipoxygenase at $IC_{50} = 23.2 \pm 1.3$ m*M* (phenidone was used as a reference compound in this 15-lipoxygenase inhibition assay and showed $IC_{50} = 2.8 \pm 0.3$ m*M*). The biological effects and the related *in vitro* effects of compound (**4**) may merit further study.

The inhibitory effect of the title compound (**4**) seems to act as an antioxidant, interfering with the redox cycle of 15-lipoxygenase. This might be similar to other 15-lipoxygenase inhibitors, such as the heterocyclic compounds: pyrimido[4,5-b][1,4]benzothiazine derivatives [13].

Scheme 1



EXPERIMENTAL

General. All melting points are uncorrected. IR spectra were recorded on a Perkin–Elmer IR-983G spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a Bruker AC-300 spectrometer. Tetramethylsilane (TMS) was used as an internal standard for ^{1}H NMR. Chemical shifts (δ/ppm) and coupling constants (Hz) were measured with respect to TMS. Mass spectra were recorded on a Finnigan TSQ-46C spectrometer at 70eV ionizing irradiation. Higher-resolution mass spectra (HRMS) were recorded on a JEOL JMS-HX 110spectrometer. Ultraviolet-visible spectra were recorded on Shimadzu UV-202 and UV-160 spectrophotometer.

The procedure for the preparation of title compound (4). A mixture of diethyl 2-chloroazulene-1,3-dicarboxylate (**1**), (918 mg, 3 mmol) malonitrile (218 mg, 3.3 mmol), anhydrous 1,2-dimethoxy ethane (8 mL), and sodium hydride 168 mg was stirred at 25°C for 24 h to produce red color sodium salts precipitates. The mixture was then acidified by anhydrous acetic acid, and extracted with water/chloroform. The organic layer was neutralized with sodium bicarbonate and then worked up. The residue was chromatographed on silica-gel with successive elution (500 mL each) of benzene, chloroform, and ethyl acetate to obtain four different products: diethyl 2-dicyanomethylazulene-1,3-dicarboxylate (**2**) (in a yield of 74.5%), diethyl 2-cyanocarbamoylazulene-1,3-dicarboxylate (**3**) (in a yield of 1.8%), 1-ethoxy-4-cyano-5-ethoxycarbonyl-

3*H*-azuleno[1,2-c]pyran-3-imine (**4**) (in a yield of 1.5%), and with sodium salt of diethyl 2-chloro-6-dicyanomethylazulene-1,3-dicarboxylate (**5**) (in a yield of 3.4%).

Diethyl 2-chloroazulene-1,3-dicarboxylate (1). The starting material (**1**) was prepared according to the literature method [14]. Red prisms (from EtOH), mp 77–78°C, yield 78%, UV: λmax in MeOH nm (logε): 298 (4.61), 308 (4.69), 326 (3.75), 353 (3.78), 367 (4.26), 370 (3.61), 504 (2.67), 525 (2.66). ^{1}H NMR ($CDCl_3$, 100 MHz): δ 1.47 (6H, t, J = 7.0 Hz, $CO_2CH_2CH_3$ × 2), 4.48 (4H,q, J = 7.0 Hz, $CO_2CH_2CH_3$ × 2), 7.40–7.93 (3H, m, H-5,6,7), 9.38–9.75 (2H, m, H-4.8).

2-Dicyanomethylazulene-1,3-dicarboxylate (2). Red prisms (from benzene), mp 139–140°C, yield 74.5%, UV: λmax nm (log ε, chloroform): 278 (4.60), 293^{sh} (4.68), 302 (4.76), 332 (4.00), 370 (4.08), 520 (2.91).

Diethyl 2-cyanocarbamoylazulene-1,3-dicarboxylate (3). Reddish violet needles (from benzene), mp 184–185°C, yield 1.8%, UV: λmax nm (log ε, MeOH): 237 (4.48), 278 (4.44), 294^{sh} (4.59), 304 (4.62), 366 (3.83), 510 (2.83). λmax in MeOH-aq. NaOH:226 (4.27), 380 (4.40), 494 (4.13). IR (KBr, cm^{-1}): 3460, 3195, 2242(w), 1704, 1684, 1431, 1198. ^{1}H NMR (60 MHz, DMSO-d_6): δ 1.56 (6H, t, J = 7.0 Hz, CH_3), 4.61 (4H, q, J = 7.0 Hz, CH_2), 6.46 (s, CH), 8.00 (3H, m, H-5,6,7), 9.81 (2H, d, J = 11 Hz, H-4.8). Element analysis found: C, 64.68%; H, 5.444%, N, 7.91%; Calculated for $C_{19}H_{18}N_2O_5$: C, 64.40; H, 5.12; N, 7.91%.

1-Ethoxy-4-cyano-5-ethoxycarbonyl-3H-azuleno[1,2-c]pyran-3-imine (4). Orange yellow needles (from EtOH), mp 229–230°C, yield 1.5%, UV: λmax nm (log ε, MeOH): 205.2 (4.5), 226.8 (4.39), 260.8 (4.43), 332.8 (4.57), 358^{sh} (4.26), 395^{sh} (3.71), 415 (3.73), 438.5 (3.68), 490^{sh} (3.38), 535^{sh} (2.95), 580^{sh} (2.72). UV: λmax nm (log ε, $CHCl_3$): 233.6 (4.45), 239.2 (4.52), 254.8 (4.50), 292.8 (4.19), 332.6 (4.64), 360^{sh} (4.39), 390.5 (3.68), 415.5 (3.66), 438.5 (3.61), 490^{sh} (3.14), 530^{sh} (2.99), 580^{sh} (2.53). ^{1}H NMR (300 MHz, DMSO-d_6) (δ): 1.39 (3H, t, J = 7.0 Hz, —OCH_2CH_3), 1.54 (3H, t, J = 7.0 Hz, —OCH_2CH_3), 4.41 (2H,q, J = 7.0 Hz, —OCH_2CH_3), 4.70 (2H,q, J = 7.0 Hz, —OCH_2CH_3),7.83 (3H, m, H-7,8,9), 8.39(1H, br, NH), 9.04 (1H, d, J = 10 Hz, H-6), 9.19 (1H, d, J = 9.0 Hz, H-10). ^{1}H NMR (400 MHz, Acetone-d_6) (δ): 1.45 (3H, t, J = 7.1 Hz, —OCH_2CH_3), 1.57 (3H, t, J = 7.1 Hz, —OCH_2CH_3), 4.51 (2H,q, J = 7.1 Hz, —OCH_2CH_3), 4.72(2H,q, J = 7.1 Hz, —OCH_2CH_3),7.82 (1H, ddd, J = 10.5, 9.0, 1 Hz, H-7), 7.84 (1H, ddd, J = 10, 9.0, 1 Hz, H-8), 7.92 (1H, ddd, J = 10.5, 9.0, 1 Hz, H-9), 9.22 (1H, ddd, J = 10.8, 1.0, 1 Hz, H-6), 9.35 (1H, d, J = 9.0, 1 Hz, H-10). ^{13}C NMR (δ): 13.94, 14.24, 59.49, 63.14, 75.89, 105.03, 110.22, 116.27, 132.10, 132.72, 134.02, 134.97, 137.86, 138.78, 146.73, 148.20, 161.67, 164.37, 167.08. IR (KBr, cm^{-1}): 3453, 2213, 1707, 1618, 1202, 1034. DEPT(distortionless enhancement by polarization transfer) found: there are 2 primary —CH_3, 2 secondary —CH_2, 5 tertiary —CH and 10 quarterly C. EIMS(20eV)(m/z, %): 336 (M^+, 3), 209 (3), 185 (9), 166 (15), 135 (13.6), 110 (9), 85 (65), 83 (100), 28 (71). HRMS: found M^+ 336.1114 (calculated for $C_{19}H_{16}N_2O_4$: M^+, 336.1104.

Diethyl 2-chloro-6-dicyanomethylazulene-1,3-dicarboxylate (5). Red needles (from benzene-cyclohexane), mp 163–164°C, yield 3.4%, UV: λmax nm (log ε, chloroform): 270 (4.19), 303 (4.66), 313 (4.74), 356 (3.96), 480 (2.39), 420^{sh} (2.13). UV: λmax nm (log ε, MeOH): 224 (4.38), 274 (4.37), 349 (4.34), 468 (4.68). ^{1}H NMR (300 MHz, $CDCl_3$) (δ): 1.67 (6H, t, J = 7.1 Hz, CH_3), 4.08 (s, CH), 4.49(4H,q, J = 7.1 Hz, CH_2), 8.51 (2H, d, J = 11.3 Hz, H-5,7), 9.54 (2H, d, J = 11.4 Hz, H-4,8). Analysis found: C, 61.41; H, 4.13; N, 7.44%, Calculated for $C_{19}H_{15}N_2O_4Cl$: C,61.55; H, 4.08; N, 7.55%.

Na-salt of (5). Yellow prisms (from acetone-ethyl acetate), mp over 300°C, UV: λmax nm (log ε, MeOH): 224^{sh}, 274, 347, 468. IR (KBr, cm^{-1}): 2198 (s), 1667, 1642, 1439, 1340, 1244, 1205, 1028, 896, 832. ^{1}H NMR (60 MHz, DMSO-d_6) (δ): 1.45 (6H, t, J = 7.0 Hz, CH_3), 4.45 (4H, q, J = 7.0 Hz, CH_2), 7.94 (2H, d, J = 11.0 Hz, H-5,7), 9.58 (2H, d, J = 11.0 Hz, H-4, 8). Analysis found: C, 58.22; H, 3.65; N, 7.02%; Calculated for $[C_{19}H_{14}N_2O_4Cl]Na$: C, 58.10; H, 3.60; N, 7.15%

General procedure for the assay of 15-lipoxygenase inhibition. Assay of 15-lipoxygenase (soybean source) inhibition was run using the enzyme preincubated with test compound for 4 min. The buffer condition used for the incubation was a 0.1*M* phosphate buffer, pH 7.4, and 0.26 m*M* linoleic acid was used as substrate. The reaction was initiated upon the addition of substrate (0.26 m*M* linoleic acid), run for 10 min, pH 7.4, at 25°C, and then terminated by the addition of NaOH. The formation of 15-HETE was determined by measuring absorbance at 234 nm [15–19].

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