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Original article

The synthesis and photoactivated cytotoxicity of novel 5-phenyl-3-(2,2′:5′,2″-terthien-5-yl)-4,5-dihydro-1*H*-pyrazolines

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ABSTRACT

A series of novel 5-phenyl-3-(2,2′:5′,2″-terthien-5-yl)-4,5-dihydro-1*H*-pyrazolines were synthesized in this report. Their photoactivated cytotoxicities on the *Spodoptera litura* (SL) cell line were evaluated using the MTT method. It was noticed that the inhibitory activities of all the conjugates was enhanced when irradiated with UV-A light. Compounds **4**, **6** and **8** were found to be most effective with inhibition rates of 88.1%, 88.0%, and 90.5%, respectively. For compound **5**, the inhibition rate value was only slightly enhanced under the irradiation treatment (78.3%) compared to the dark treatment (74.8%). The relationship analysis between structure and activity showed that the middle thiophene ring played an important role on the inhibitory activities. It was shown that these compounds could be the potential candidates for new photoactivated pesticides.

1. Introduction

Among the various classes of nitrogen-containing heterocyclic compounds, pyrazolines display a broad spectrum of biological activity and play an important role as a basic skeleton for the design of a number of antibiotics, such as echinomycin, actinomycin, and leromycin [1–3]. It was reported that these compounds inhibit the growth of gram-positive bacteria and exhibit the anticancer activity [4]. Moreover, the pyrazoline ring also exhibits pharmacological utility as antidepressant, antipyretic, analgesic, and anti-inflammatory agents because of their antimicrobial properties, especially antibacterial and antifungal activities [5,6].

In recent years, thiophene derivatives such as α-terthienyl (α-T) have stimulated much interest because of their phototoxic activity, easy degradation, short half-life, safe to the microorganismsin in soils and rapid elimination by a polysubstrate monooxygenase mediated mechanism [7–9]. The phototoxicity of α-T and its derivatives has been attributed to their triplet states that easily generate a reactive oxygen species (ROS). A number of biomolecules are the targets of these ROS, such as DNA, plasma or membrane proteins and enzymes. Therefore, α-T and its derivatives possess all the desirable properties of a good insecticide/pesticide. In contrast with conventional insecticides, α-T is fast acting, non-toxic, economic and has a property of easy degradation to make it more useful and safer [10,11].

In this study, a series of novel pyrazolines were firstly designed and synthesized with 5-formyl-2,2′:5′,2″-terthiophene as the starting material. Their photoactivated cytotoxicities on *Spodoptera litura* (SL) cells were also evaluated by performing the MTT assessment. The results showed that the inhibitory activities of all the conjugates were enhanced under the irradiation conditions compared to that in the dark.

2. Experimental

2.1. Reagents and instrumental analysis

All melting point assessments were carried out on a WRR digital melting point apparatus. IR spectra were obtained on a FT-IR360 (USA, Nicolet) spectrometer (KBr Pellets). The ^{1}H NMR spectra were recorded on a Bruker AV-600 instrument, where chemical shifts are reported in ppm using tetramethylsilane as secondary reference standard and coupling constant in Hz. Cytotoxicity experiments were performed on a enzyme labeled instrument (Bio-Rad, location) and flow cytometer (FACS Calibur, Becton Dickinson, NJ).

2.2. Synthesis of the compounds

The synthetic pathways are shown in Scheme 1. Ni(dppp)Cl_2 was purchased from Sigma–Aldrich Chemical Co., USA. α-T was synthesized by Ni(dppp)Cl_2 catalyzed cross-coupling of 2-thienylmagnesium bromide with 2,5-dibromothiophene in diethyl ether [12]. Compound 5-formyl-2,2′:5′,2″-terthiophene (**1**) was

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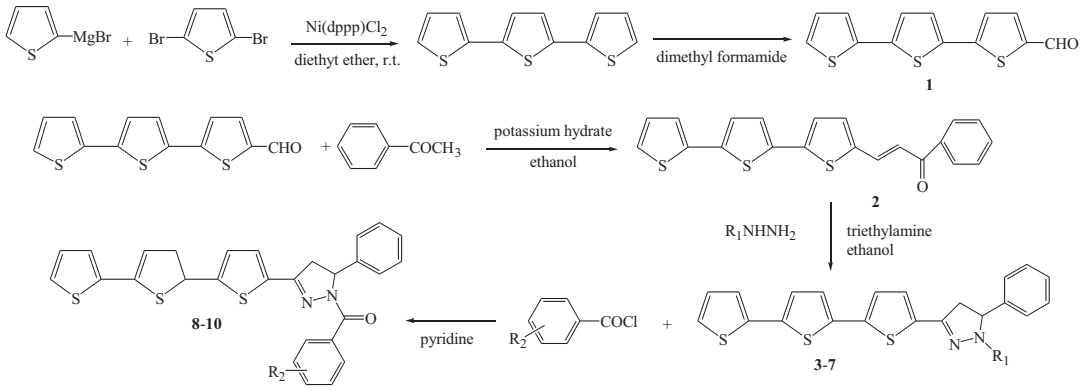

Scheme 1. The synthesis route of the compounds.

prepared according to literatures [13]. 1-phenyl-3-(2,2′:5′,2″-terthien-5-yl)prop-2-en-1-one (**2**) was synthesized by treating 5-formyl-2,2′:5′,2″-terthiophene with acetophenone in the presence of ethanol and potassium hydrate solution [14]. The prepared chalcones were treated with compounds containing the hydrazine moiety (R^1NHNH_2) to give 5-phenyl-3-(2,2′:5′,2″-terthien-5-yl)-4,5-dihydro-1*H*-pyrazolines compounds (**3**–**7**). The target product (**3**) was further treated with substituted benzoyl chloride in presence of pyridine to give 1-benzoyl-5-phenyl-3-(2,2′:5′,2″-terthien-5-yl)-4,5-dihydro-1*H*-pyrazolines (**8**–**10**) [15,16]. All products were purified by column chromatography on silica gel.

2.3. Biological assays

Cell culture: *Spodoptera litura* (SL) cells were obtained from the Key Laboratory of Pesticide and Chemical Biology of Ministry of Education, Central China Normal University, China. The SL cells were further cultured with Grace's insect cell culture medium (Gibco, America) containing 9% new born calf serum at 27.5 °C. Cells in logarithmic phase of growth were used in all experiments. The stock solutions of all compounds were prepared in advance in dimethylsulfoxide (DMSO). They were further diluted with culture medium to the desired concentrations on the day of the experiments. The concentration of DMSO was kept 0.5% in treated groups. Control cultures were performed in the presence of DMSO under the same culture conditions.

Effect of the compounds on SL cell activity: Cytotoxicity of the compounds (**1**–**10**) on SL cells was confirmed by MTT assay as previously reported [17]. Cells were incubated in 96-well cell culture plates dish at 10,000 cells/well in the absence (control), or the presence of the compounds. After 30 min of incubation, the cultures were either irradiated with light (3 min, 40 W, UV-A), or not. Two ultraviolet lights from Philips were used as light source, which give a light intensity of 2600 μW/cm^2 at the spectrum peak of 365 nm as measured with a Huandi UV-A radiometer (Photoelectric Instrument Factory of Beijing Normal University, China), then cultured in the incubator for 24 h. The medium was replaced by a solution of MTT (0.5 mg/mL), and the cells were incubated at 37 °C for 4 h in a 5% CO_2 atmosphere. The absorbance was measured on a Bio-Rad ELISA reader at 570 nm. The inhibition rate was calculated by the absorbance. Each experiment was performed in triplicate.

Statistical analysis: Values were expressed as the means ± SE. To evaluate the inhibition rate differences between the data of two chosen groups, the statistical analysis was carried out by one-way analysis of variance (Duncan's multiple range test) using DPS software. The level of significance was set at $p < 0.05$.

3. Results and discussion

3.1. Characterization of the synthesized compounds

5-Formyl-2,2′:5′,2″-terthiophene (**1**): Yellow solid, yield 80%, mp 135–136 °C (lit. 136–138 °C); IR (KBr, cm^{-1}): 1650 (C=O); MS: *m*/*z* 276 (M+); ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.94 (s, lH, CHO), 7.94 (d, 1H, *J* = 3.6 Hz, thiophene-H), 7.49–7.52 (m, 3H, thiophene-H), 7.39 (dd, 1H, *J* = 3.6 Hz, 1.2 Hz, thiophene-H), 7.32 (dd, lH, *J* = 4.2 Hz, 1.2 Hz, thiophene-H), 7.13 (dd, lH, *J* = 3.6 Hz, 4.2 Hz, thiophene-H); exact mass calcd. for $C_{13}H_8OS_3$: 275.9737; found: 275.9737.

1-Phenyl-3-(2,2′:5′,2″-terthien-5-yl)prop-2-en-1-one (**2**): Yellow powder, yield 67%, mp 162–164 °C; IR (KBr, cm^{-1}): 1649 (C=O), 1585 (C=C); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.01 (d, 2H, *J* = 6.0 Hz, Ar–H), 7.90 (d, 1H, *J* = 15 Hz, thiophene-H), 7.57–7.60 (m, 3H, Ar–H), 7.51 (t, 2H, *J* = 6.6 Hz, Ar–H), 7.30 (d, 1H, *J* = 3.6 Hz, thiophene-H), 7.27 (d, 1H, *J* = 3.6 Hz, thiophene-H), 7.25 (d, 1H, *J* = 3.6 Hz, thiophene-H), 7.21 (d, 1H, *J* = 3.6 Hz, =CH–), 7.18 (dd, 1H, *J* = 3.6 Hz, 1.2 Hz, thiophene-H), 7.15 (dd, 1H, *J* = 4.2 Hz, 1.2 Hz, thiophene-H), 7.12 (dd, 1H, *J* = 3.6 Hz, 4.2 Hz, thiophene-H), 7.04 (d, 1H, *J* = 3.6 Hz, =CH–); anal. calcd. for $C_{21}H_{14}OS_3$: C 66.63, H 3.73; found: C 66.61, H 3.70.

5-Phenyl-3-(2,2′:5′,2″-terthien-5-yl)-4,5-dihydro-1*H*-pyrazoline (**3**): Yellow powder, IR (KBr, cm^{-1}): 3280 (N–H), 1589 (C=N), 1319 (C–N); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.71–7.73 (m, 2H, Ar–H), 7.43 (d, 1H, *J* = 4.8 Hz, thiophene-H), 7.40 (m, 3H, Ar–H), 7.29 (d, 1H, *J* = 3.6 Hz, thiophene-H), 7.20 (d, 1H, *J* = 3.6 Hz, thiophene-H), 7.18 (d, 1H, *J* = 4.2 Hz, thiophene-H), 7.15 (dd, 1H, *J* = 3.6 Hz, 1.2 Hz, thiophene-H), 7.09 (dd, 1H, *J* = 4.2 Hz, 1.2 Hz, thiophene-H), 7.04 (dd, 1H, *J* = 3.6 Hz, 4.2 Hz, thiophene-H), 5.21 (q, 1H, *J* = 3.0 Hz, –CH), 3.55 (dd, 1H, *J* = 4.8 Hz, 1.2 Hz, –CH_2), 3.05 (dd, 1H, *J* = 4.8 Hz, 1.2 Hz, –CH_2); anal. calcd. for $C_{21}H_{16}N_2S_3$: C 64.25, H 4.11, N 7.14; found: C 64.20, H 4.13, N 7.11.

1-Acetyl-5-phenyl-3-(2,2′:5′,2″-terthien-5-yl)-4,5-dihydro-1*H*-pyrazoline (**4**): Yellow powder, IR (KBr, cm^{-1}): 1647 (C=O), 1595 (C=N), 1334 (C–N); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.87–7.88 (m, 2H, Ar–H), 7.48–7.50 (m, 3H, Ar–H), 7.43 (d, 1H, *J* = 4.8 Hz, thiophene-H), 7.28 (d, 1H, *J* = 3.6 Hz, thiophene-H), 7.29 (d, 1H, *J* = 3.6 Hz, thiophene-H), 7.16 (d, 1H, *J* = 4.2 Hz, thiophene-H), 7.11 (dd, 1H, *J* = 3.6 Hz, 1.2 Hz, thiophene-H), 7.08 (dd, 1H, *J* = 4.8 Hz, 1.2 Hz, thiophene-H), 7.04 (dd, 1H, *J* = 3.6 Hz, 1.2 Hz, thiophene-H), 5.91 (q, 1H, *J* = 3.6 Hz, –CH), 3.95 (dd, 1H, *J* = 4.8 Hz, 2.4 Hz, –CH_2), 3.50 (dd, 1H, *J* = 4.8 Hz, 2.4 Hz, –CH_2), 2.32 (s, 3H, –CH_3); anal. calcd. for $C_{23}H_{18}N_2OS_3$: C 63.56, H 4.17, N 6.45; found: C 63.51, H 4.14, N 6.43.

5-Phenyl-3-(2,2′:5′,2″-terthien-5-yl)-4,5-dihydro-1*H*-pyrazoline-1-carbothioamide (**5**): Yellow powder, IR (KBr, cm^{-1}): 3261