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Novel Analogues of α-Terthienyl, Thienyl 1, 3, 4-Thia(oxa)diazoles as Potential Photoactivated Insecticides: Synthesis and Bioactivity

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Abstract: Novel 1, 3, 4-thia(oxa)diazoles containing thienyl groups were synthesized. Analogous to the naturally-occurring compound, α-terthienyl, they can be photosensitized by ultraviolet light and showed phototoxicities against the 2nd instar larvae of southern armyworm (*Pseudaletia separata* Walker), they also showed photocleavage action to pBR322 DNA.

Keywords: Thiadiazoles, oxadiazoles, α-terthienyl, phototoxic.

α-Terthienyl (αT) is a phototoxic compound originated from the marigold *Tagetes eretra*[1]. The αT and its analogues have been widely used for photodynamic killing of insects, in photodynamic therapy for dermatophytes, and in human immunodeficiency virus (HIV)[2]. The phototoxicity of αT and its derivatives are attributed to their triplet states that easily generate a reactive oxygen species (ROS). Many of the biomolecules and enzymes are targets of these ROS, such as DNA *in vitro* and *in vivo*, plasma membrane and membrane proteins and enzymes. αT and its derivatives possess all the desirable properties of a good insecticide/pesticide. In contrast with conventional insecticides, αT is fast acting, non-toxic, economic and has a property of easy degradation to make it more useful and safe[3]. This kind of compounds might be a new-generation eco-friendly green pesticides.

Here, we designed and synthesized a series of analogues of αT, 1, 3, 4-thiadiazoles and 1, 3, 4-oxadiazoles containing thienyl groups, and expected that they would show photobiologic activities. However, as unnatural analogues of αT, these compounds have never been researched as photoactivated insecticides or DNA photocleavers, although three of them were known and used as optic brightener[4] or electron/hole transporting material[5]. Their phototoxicities were tested against the second instar larvae of *Pseudaletia separata* Walker and pBR322 DNA, respectively.

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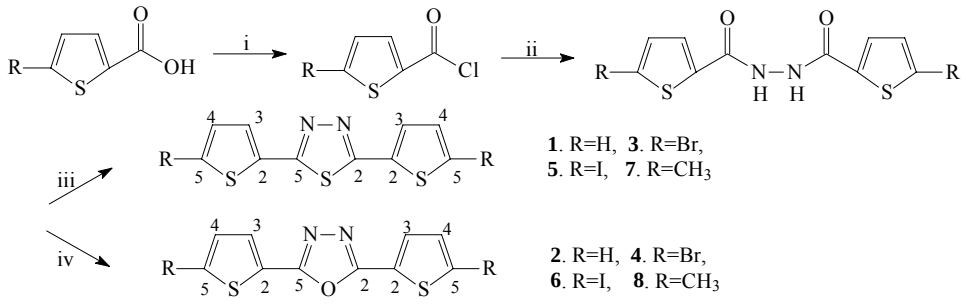
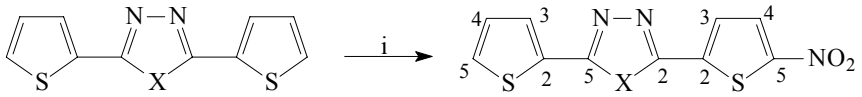
Scheme 1 Synthetic pattern for the preparation of symmetrical compound **1-8**



Conditions: i) $SOCl_2$, reflux, 2 h; ii) $NH_2NH_2 \cdot H_2O$/dioxane, r.t., 1 h; iii) P_2S_5, pyridine, reflux, 5 h; iv) $POCl_3$, reflux, 2 h

Scheme 2 Synthetic pattern for the preparation of asymmetrical compound **9** and **10**



1. X=S; **2**. X=O **9**. X=S; **10**. X=O

Conditions: i) fuming nitric acid, glacial acetic acid, acetic anhydride, methylene chloride, r.t., 8 h

Scheme 1 outlines the synthetic methods for the preparation of symmetrical 1, 3, 4-thia(oxa)diazoles, compound **1-8**, starting from commercial thiophene-2-carboxylic acid and its derivatives. The acids can easily be transformed into the corresponding thiadiazoles or oxadiazoles by the following pattern: acid → acid chloride → carbonyl hydrazine → thia(oxa)diazole through steps i to iv. Nitration of compound **1** and **2** gave asymmetrical compound **9** and **10**, respectively, which is shown in **Scheme 2**.

The starting materials, thiophene-2-carboxylic acid and its derivatives are available in commerce. The relative acid chlorides and carbonyl hydrazine were prepared according to the corresponding literature[6]. The symmetrical thienyl 1, 3, 4-thiadiazoles **1**[4], **3**, **5** and **7** were prepared by treating carbonyl hydrazine with phosphorus pentasulfide in pyridine under refluxing for 5 h. While, the mixture of carbonyl hydrazine and phosphorus oxychloride was refluxed for 2 h to give symmetrical thienyl 1, 3, 4-oxadiazole **2**[7], **4**[8], **6** and **8**. Nitration of compound **1**and **2** with fuming nitric acid/glacial acetic acid in acetic anhydride/methylene chloride at r.t. for 8 h afforded the corresponding asymmetrical 1, 3, 4-thiadiazole **9** and 1, 3, 4-oxadiazole **10**, respectively.

At individual level, the compounds **1**, **2**, **3**, **4**, **5**, **6**, **9** and **10** were tested against the second instar larvae of *Pseudaletia separata* Walker. The test solution was made by

Table 1 The percent mortality of larvae and duration of time

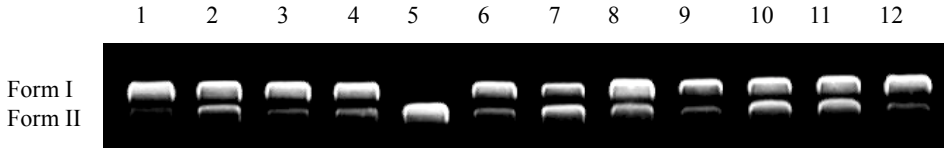
Compound	**1**	**2**	**3**	**4**	**5**	**6**	**9**	**10**
72 hr (%)	17.14	13.69	6.67	13.33	26.67	20.00	3.33	55.12
120 hr (%)	47.64	27.50	19.44	7.36	63.75	15.42	19.44	67.77

dissolving the compound in a small amount of DMF, then adding a few drops of surfactant, finally adding water to a proper volume. A moderate mealie leaves treated with the above test solution (final concentration 1000 ppm) were placed on moistened filter paper in three broad bottles. Each bottle was infested with 10 second-instar larvae of the southern armyworm. The treated mealie leaves were added everyday. After culturing the larvae in dark for 24 hours, the percent mortality was determined by the number and size of live larvae in the treated bottles, one set of which was irradiated under fluorescent lamps (intensity of 23 microwatts cm^{-2} at 365 nm) and the other set of which was in dark, relative to the untreated controls in 5 days. All the treatments were maintained between 25~28°C with the relative humidity of 70%. The results of killing larvae are presented in **Table 1**. Although these compounds showed a few toxicities to the larvae in dark just analogous to IGR (Insect growth-regulator)[9], they mainly displayed strong phototoxicities in the presence of ultraviolet light. The phototoxicities increased with the duration of time.

At molecular level, the photocleavage activity of all these compounds were tested by treating with supercoiled pBR322 DNA under irradiation of ultraviolet light. The electrophoretic analysis showed that nicking of DNA took place after irradiation. The mechanism by which pBR322 DNA was damage was not clear though. The analysis of the cleavage of supercoiled pBR322 DNA is shown in **Figure 1**. The analysis results obtained by Gel-Pro software are presented in **Table 2**. According to the analysis results, the most active sensitizer is compound **4**, which nicks supercoiled DNA (form I) to produce relaxed open circular DNA (form II) with about 80% conversion. Compound **1**, **6**, **7**, **9** and **10** showed moderate activity with about 10-35% conversion, while the rest compound **2**, **3**, **5** and **8** showed weak activity, with less than 10% conversion.

According to the above results, the phototoxicities of these compounds against the second instar larvae of *Pseudaletia separata* Walker *in vivo* and supercoiled pBR322 DNA *in vitro* are uncoincident. Considering the process *in vivo* is complicated, associated with digestion, transportation, distribution, metabolism and excretion of these

Figure 1 Cleavage of supercoiled pBR322 DNA by compounds **1-10**



Lane 1: DNA alone (without irradiation); lane 2-11: DNA and sensitizer **1-10** respectively, 50 μmol/L, 3 h irradiation (density 950 $\mu W/cm^{-2}$); lane 12: DNA alone, 3 h irradiation (density 950 $\mu W/cm^{-2}$).

Table 2 Analysis results of photocleavage of supercoiled pBR322 DNA with Gel-Pro software

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Conversion to Form II (%)	12.24	1.78	8.11	82.74	7.57	33.98	9.57	6.66	23.20	21.00

compounds. So the uncoincidence of the data *in vivo* and *in vitro* is reasonable. In summary, these compounds displayed more or less phototoxicities against second-instar larvae of armyworm (*Pseudaletia separata* Walker) and pBR322 DNA, and are of potential value in the investigation of photoactivated insecticides or DNA photocleavers.

Acknowledgments

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10. Data of 2, 5-bis(5-bromothiophen-2-yl)-1, 3, 4-thiadiazole **3**: mp 229-231°C; MS *m/z* 406 (M^+); ^{1}H NMR ($CDCl_3$, δppm) 7.28 (d, 1H, J=3.79Hz, H-4), 7.11 (d, 1H, J=3.79Hz, H-3). Data of 2, 5-bis(5-iodothiophen-2-yl)-1, 3, 4-thiadiazole **5**: mp 252-254°C; MS *m/z* 502 (M^+); ^{1}H NMR ($CDCl_3$, δppm) 7.29 (d, 1H, J=3.94Hz, H-4), 7.11 (d, 1H, J=3.94Hz, H-3). Data of 2, 5-bis(5-iodothiophen-2-yl)-1, 3, 4-oxadiazole **6**: mp 193-194°C; MS *m/z* 486 (M^+); ^{1}H NMR ($CDCl_3$, δppm) 7.45 (d, 1H, J=3.82Hz, H-4), 7.34 (d, 1H, J=3.82Hz, H-3). Data of 2, 5-bis(5-methylthiophen-2-yl)-1, 3, 4-thiadiazole **7**: mp 179-180°C ; MS *m/z* 278 (M^+); ^{1}H NMR (Acetone-d_6, δppm) 7.52 (d, 1H, J=3.57Hz, H-3), 6.94 (d, 1H, J=3.57Hz, H-4), 2.56 (s, 3H, CH_3). Data of 2, 5-bis(5-methylthiophen-2-yl)-1, 3, 4-oxadiazole **8**: mp 131-132°C; MS *m/z* 262 (M^+); ^{1}H NMR (Acetone-d_6, δppm) 7.65 (d, 1H, J=3.57Hz, H-3), 6.98 (d, 1H, J=3.57Hz, H-4), 2.57 (s, 3H, CH_3). Data of 2-(5-nitrothiophen- 2-yl)-5-(thiophen-2-yl)-1, 3, 4-thiadiazole **9**: mp 262-264°C; MS *m/z* 295 (M^+); ^{1}H NMR ($CDCl_3$, δppm) 7.94 (d, 1H, J=4.29Hz, H-4), 7.64 (d, 1H, J=3.64Hz, H-5), 7.58 (d, 1H, J=4.29Hz, H-3), 7.45 (d, 1H, J=4.25Hz, H-3), 7.18 (t, 1H, J=3.64Hz, J=4.25Hz, H-4). Data of 2-(5-nitro thiophen-2-yl)-5-(thiophen-2-yl)-1, 3, 4-oxadiazole **10**: mp 227-229°C; MS *m/z* 279 (M^+); ^{1}H NMR (Acetone-d_6, δppm) 7.98 (d, 1H, J=4.4Hz, H-4), 7.64 (dd, 1H, J=3.57Hz, J=1.1Hz, H-5), 7.58 (d, 1H, J=4.4Hz, H-3), 7.45 (dd, 1H, J=4.95Hz, J=1.1Hz, H-3), 7.18 (t, 1H, J=3.57Hz, J=4.95Hz, H-4).

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