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# Reaction between quinone and thiazolidine. A study on the formation mechanism of new antiproliferative quinolindiones

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**Abstract**—Reaction between quinolinquinone and thiazolidine in basic medium was investigated. 2-Arylthiazolidine-4-carboxylic acid ethyl esters undergo two different cleavages in basic medium, yielding the 1-aryl-2-azadiene and a thiolic species. In the presence of quinolinquinone, the isomeric 1-aryl-3-ethoxycarbonyl-pyridoisoquinolin-5,10-diones and 3-amino-3-ethoxycarbonyl-dihydrothienoquinolin-4,9-diones are formed by a hetero-Diels–Alder reaction and 1,4-Michael addition reaction, respectively. A mechanism for the formation of the reaction products is presented.


## 1. Introduction

The quinone planar structure is common to numerous antitumor drugs and plays an important role in the DNA intercalating process, which is associated with antiproliferative activity.[1] The ability of quinones to accept electrons which form the corresponding semiquinone radicals is the first step of a redox chain which generates reactive species involved in enzymatic blocking and reading errors during the replication process.[2] Numerous studies on the activity of heterocyclic quinones containing nitrogen have shown that the number and position of nitrogens are considerably important for cytotoxicity,[3] while the optimum number of the rings in the polyannulated system ranges between 3 and 4. Significant examples of these DNA damaging agents are actinomycin D, doxorubicin, mitomycin, streptonigrin, and pyridophenoxazinones.[4] Therefore, it was of interest to study the activity of novel quinones related to 5*H*-pyrido[3,2-*a*]phenoxazin-5-one (PPH, Fig. 1), a potent anticancer agent previously described.[4] It is of note that the benzo-fused ring A of PPH seems to play an important role for π–π stacking interactions with the DNA base pairs and that the pyridine nitrogen is crucial for its antiproliferative activity.[4,5]

In pursuing our research in this field, we have designed a series of substituted pyridoquinolin-5,10-diones (PQDs),[6] which fulfill the requirements for intercalative binding between adjacent DNA base pairs and exhibit an anticancer activity at submicromolar concentrations on a large panel of limphoblastoid and solid-tumor derived cells. A topoisomerase I superhelix unwinding assay demonstrated the ability of PQDs to intercalate into double stranded DNA. UV–vis and $^1$H NMR spectroscopic investigation on the complex PIQD/[d(GAAGCTTC)]$_2$ also provided evidence that intercalation occurs and alters the base sequence GC.

In this paper, we report the synthesis and formation mechanism of the 1-phenyl-3-ethoxycarbonyl-pyrido [3,2-*g*]isoquinolin-9,10-dione (**1a**), the lead compound of PQD series. Compound **1a** was prepared by a cycloaddition reaction, using quinolinquinone (**QQ**)

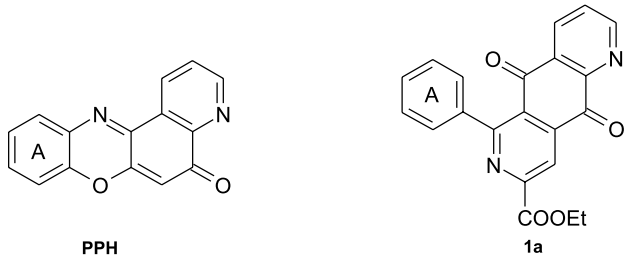


**Figure 1.**

*Keywords*: Quinone; Thiazolidine; Azadiene.
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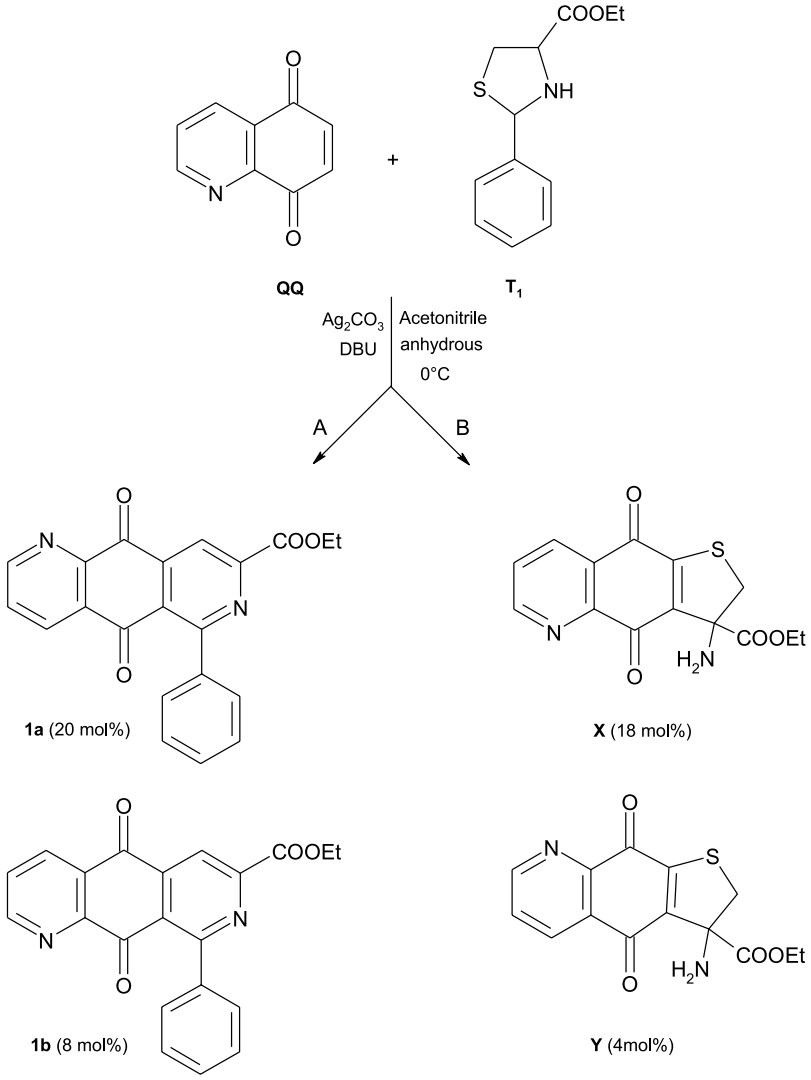
and 2-phenylthiazolidine-4-carboxylic acid ethyl ester ($\mathbf{T_1}$) in basic medium (Scheme 1) according to the general procedure reported in Section 2. It was previously reported that thiazolidines in basic medium yield the corresponding azadienes by cleavage of the heterocyclic ring.[7] $\mathbf{T_1}$ was prepared according to the reported procedures from L-cysteine ethyl ester and benzaldheyde.[8,9] **QQ** was prepared by hydroxylation followed by oxidation of 5-hydroxyquinoline.[10]

Together with **1a**, the products 1-phenyl-3-ethoxycarbonyl-pyrido[2,3-*g*]isoquinolin-5,10-dione (**1b**), 3-amino-3-ethoxy-carbonyl-2,3-dihydrothieno[2,3-*g*]quinolin-4,9-dione (**X**) and 3-amino-3-ethoxycarbonyl-2,3-dihydrothieno[3,2-*g*]quino-lin-4,9-dione (**Y**) were recovered from the reaction mixture.

Section 2 reports the general synthetic procedures and the characterization of all new compounds on the basis of the spectral data and the elemental analysis. Structural assign-ment of series **a** and **b** and of **X** and **Y** was accomplished through extensive 2D NMR spectroscopy, HMQC and HMBC. Figure 2 summarizes the HMBC correlations for compounds **1a**, **1b** (prototypes of series **a** and **b**), **X**, **Y** and **X′**. In the HMBC spectrum of **1a**, the double–doublet assigned to the H9 proton at $\delta_H$ 8.64 shows a very significant strong correlation $^3J_{C-H}$ with the singlet at $\delta_C$ 180.9 (C10, carbonyl group) and the H4 singlet at $\delta_H$ 8.82 with the singlet at $\delta_C$ 181.7 (C5, carbonyl group). The HMBC spectrum of **1b**, shows a very significant strong correlation $^3J_{C-H}$ of the singlet at $\delta_C$ 180.5 (C5, carbonyl group) with the double–doublet assigned to the H6 proton at $\delta_H$ 8.52 and the singlet at $\delta_H$ 8.92 assigned to the H4 proton. In the HMBC spectrum of **X**, the double–doublet assigned to the H8 proton at $\delta_H$ 8.40 shows a very significant, strong correlation $^3J_{C-H}$ with the singlet at $\delta_C$ 180.7 (C9, carbonyl group) and two weak interactions $^4J_{C-H}$ with the signals at $\delta_C$ 179.9 (C4, carbonyl group) and $\delta_C$ 154.9 (C9a). In the corresponding spectrum of **Y**, the double–doublet assigned to the H5 proton at $\delta_H$ 8.38 shows a very significant, strong correlation with the singlet at $\delta_C$ 180.0 (C4, carbonyl group) and two weak interactions $^4J_{C-H}$ with the signals at $\delta_C$ 180.1 (C9, carbonyl group) and $\delta_C$ 142.2 (C3a). The $^4J_{C-H}$ coupling of H8 proton with C9a, which is deshielded by the endocyclic sulfur effect, determinates the structure of **X**. Conversely, the $^4J_{C-H}$ coupling of H5 proton with the higher field C3a, determinates the structure of **Y**. To further




**Scheme 1.**

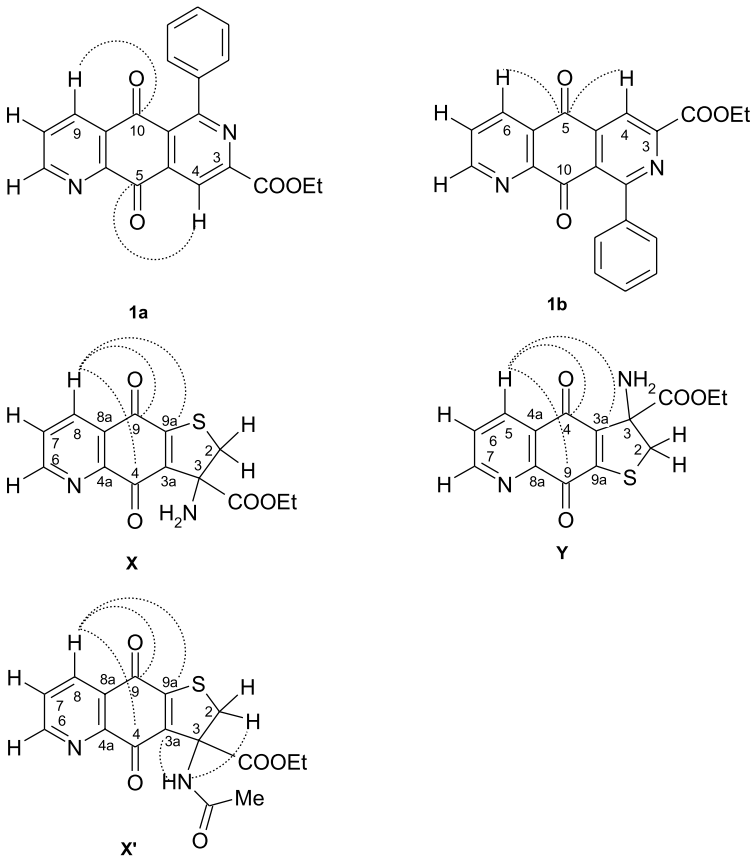


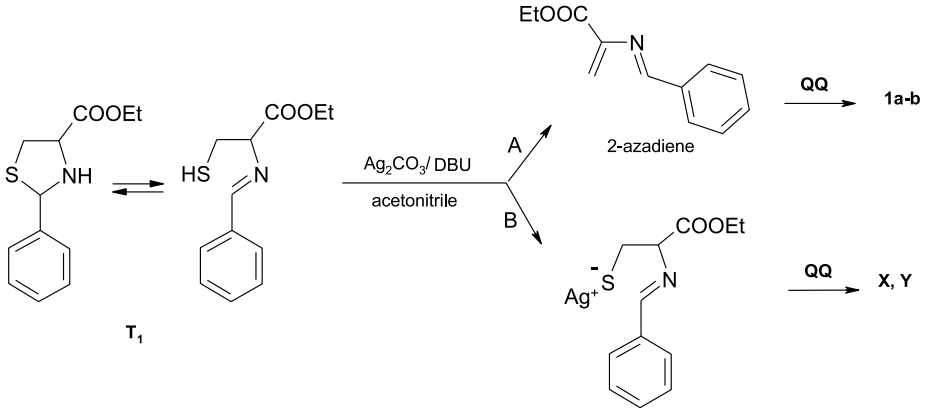

**Figure 2.** HMBC correlations for compounds **1a**, **1b**, **X**, **Y** and **X′**.

support the reported assignments, the N-acetyl derivative of **X** was prepared and analyzed by HMBC. The singlet assigned to the NH proton at $\delta_H$ 7.58 shows a strong $^3J_{C-H}$ correlation with the singlet at $\delta_C$ 142.0 (C3a) and with the triplet at $\delta_C$ 41.6 (C2), supporting the previous structure assignment.

In accordance with the widely reported reactivity of the quinone system toward the enophiles in the Diels–Alder reaction and toward the nucleophiles in the 1,4-Michael addition, the compounds **1a**-**b**, **X** and **Y** seem to be formed by the competitive attack of different species arising from the thiazolidine demolition[8,9] on **QQ** through the two different pathways A and B (Scheme 1). Scheme 2 depicts the species arising from the thiazolidine ring cleavage, according to the current literature.

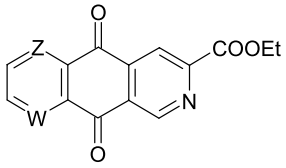
As a specific example, **1a**-**b** both seem to arise from a Diels–Alder reaction between the quinone acting as dienophile, and the enophile phenyl-2-aza-3-ethoxycarbonyl-1,3-butadiene, an azadiene which may be formed in situ by basic breakdown of $\mathbf{T_1}$[8,9] as described in Scheme 2. The total reaction yield was 50%, and the ratios **1a/1b**, **X/Y** were 20/8 and 18/4, respectively (Table 1). This result suggests that a preferential bond takes place between the 6,7 position of **QQ** and the 1,4 position of azadiene.

To further investigate this occurrence, the effect of



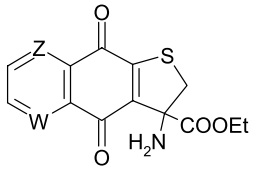

**Scheme 2.** Thiazolidine cleavage in basic medium (path A and B).

**Table 1.** Yields of products arising from the reaction between **QQ** and thiazolidines $\mathbf{T_1}$–$\mathbf{T_5}$




**a** W=CH, Z=N

**b** W=N, Z=CH




**X** W=N, Z=CH

**Y** W=CH, Z=N

| Compound | R | Yield (%) | Yield **a/b** | Yield **X/Y** |
|---|---|---|---|---|
| **1** | $C_6H_5$ | 50 | 20/8 | 18/4 |
| **2** | 4-Me–$C_6H_4$ | 33 | 8/8 | 10/7 |
| **3** | 4-Cl–$C_6H_4$ | 40 | 21/11 | 6/2 |
| **4** | 4-$NO_2$–$C_6H_4$ | 51 | 22/12 | 9/8 |
| **5** | H | 20 | — | 14/6 |

thiazolidine substituents on the reaction yields was examined. 2-(4-Methylphenyl)-thiazolidin-4-carboxylic acid ethyl ester ($\mathbf{T_2}$), 2-(4-chlorophenyl)-thiazolidin-4-carboxylic acid ethyl ester ($\mathbf{T_3}$), 2-(4-nitrophenyl)-thiazolidin-4-carboxylic acid ethyl ester ($\mathbf{T_4}$) and thiazolidin-4-carboxylic acid ethyl ester ($\mathbf{T_5}$) were synthesized and used to produce the corresponding azadienes.

On the basis of the results reported in Table 1, the 4-electron-withdrawing substituents on the phenyl group of the thiazolidine increase the yields of compounds **a**-**b** to the detriment of **X**, **Y**. Every attempt to synthesize 3-ethoxycarbonyl-pyrido[*g*]isoquinolin-5,10-diones under the reported conditions by using unsubstituted thiazolidine as a starting compound was unsuccessful and only **X**, **Y** were obtained.

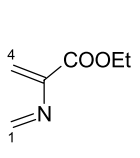
The bonds which develop between dienophile and enophile determining the product distribution in a Diels–Alder reaction can be predicted on the basis of the energy and orbital coefficients of the frontier orbitals (HOMO and LUMO).[11] Table 2 reports the corresponding data of the substituted 2-azadienes (diene) and of the quinolin-5,8-dione (dienophile) involved in the formation of the examined compounds. All the computations have been performed using the PBE0 hybrid density functional theory[12] implemented by one of the authors in the Gaussian03 package[13] with the 6-31G(d) (energies) and STO-3G (coefficients)[14] basis sets. The reliability of this computational level has been corroborated in several studies.[15]

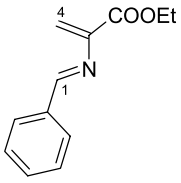
In agreement with our experimental results, the HOMO–LUMO gap between **QQ** and 2-phenylazadiene favours the formation of **1a**. Furthermore, the experimental trends (Table 1) are reproduced by the computations. In particular, the aromatic substituent reduces the gap between the azadiene HOMO and the **QQ** LUMO, thus favouring the Diels–Alder compounds and pushing the reaction to form **1a**-**b**, rather than **X**,**Y**. Unsubstituted thiazolidine, which gives rise to a higher energy gap, does not produce Diels–Alder adducts under our conditions, according to our experimental results.

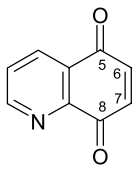
As regards compounds **X** and **Y**, (Scheme 1), they seem to be formed by a 1,4-Michael nucleophilic attack of the thiol group, arising from the opening of thiazolidines $\mathbf{T_{1-5}}$, on the 6 and 7 positions of the α–β unsaturated system of **QQ**.

Schemes 3 and 4, reporting a possible formation mechanism of the compounds **X** and **Y**, show that the silver ions play a determinant role in their formation. The thiazolidine ring, opened in basic medium (DBU), attacks the quinolin-5,8-dione through the thiolate group. A carbanion, arising from

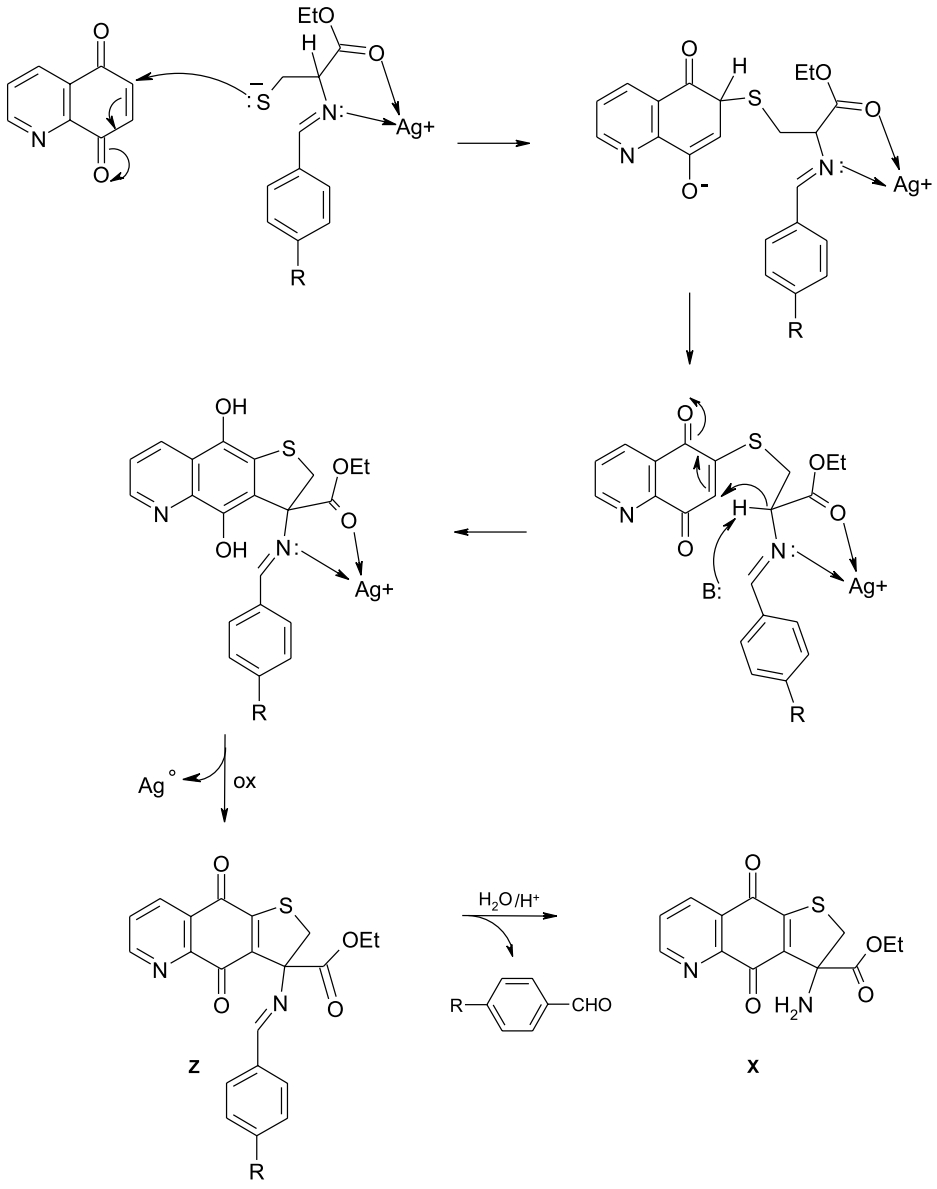
**Table 2.** Energies (eV) and orbital coefficients of the frontier orbitals of 2-aza-3-carbethoxybutadiene, phenyl-2-aza-3-carbethoxy-1,3-butadiene and quinolin-5,8-dione obtained by PBE0 computations (see text for details)




| *E* (eV) | HOMO | LUMO | HOMO | LUMO | | HOMO | LUMO |
|---|---|---|---|---|---|---|---|
| | −0.2674 | −0.0629 | −0.2406 | −0.0708 | | −0.2816 | −0.1241 |
| | | | Coefficients | | | | |
| $C_1$ | −0.3922 | 0.5193 | −0.2899 | 0.4757 | $C_5$ | −0.15153 | −0.30222 |
| N | −0.2823 | −0.3299 | −0.3812 | −0.4119 | $C_8$ | −0.15438 | 0.27806 |
| $C_3$ | 0.4385 | −0.4491 | 0.2376 | −0−3047 | $C_6$ | 0.21778 | −0.45891 |
| $C_4$ | 0.5316 | 0.6735 | 0.3770 | 0.5262 | $C_7$ | 0.21501 | 0.32492 |




**Scheme 3.** Formation mechanism of **X** involving 1,4-Michael addition (path B).

an acid–base reaction of a complex of silver–azomethine nitrogen–carbonyl oxygen, can attack the α,β-unsaturated system. Reduction, followed by re-oxidation, re-forms the quinone system. The unstable azomethine intermediates **Z** and **Z′** by acid treatment furnish **X** and **Y**, respectively. In accordance with the proposed mechanism, Ag° and benzaldehyde were recovered from the reaction mixture.

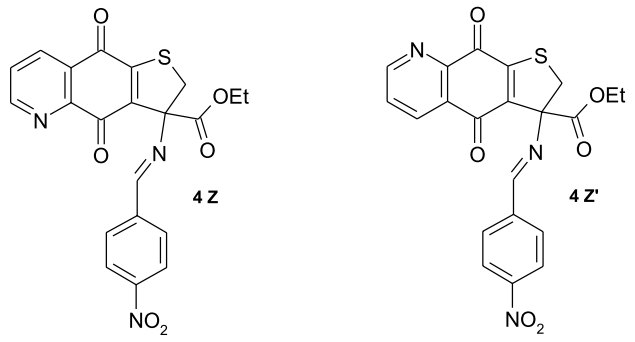
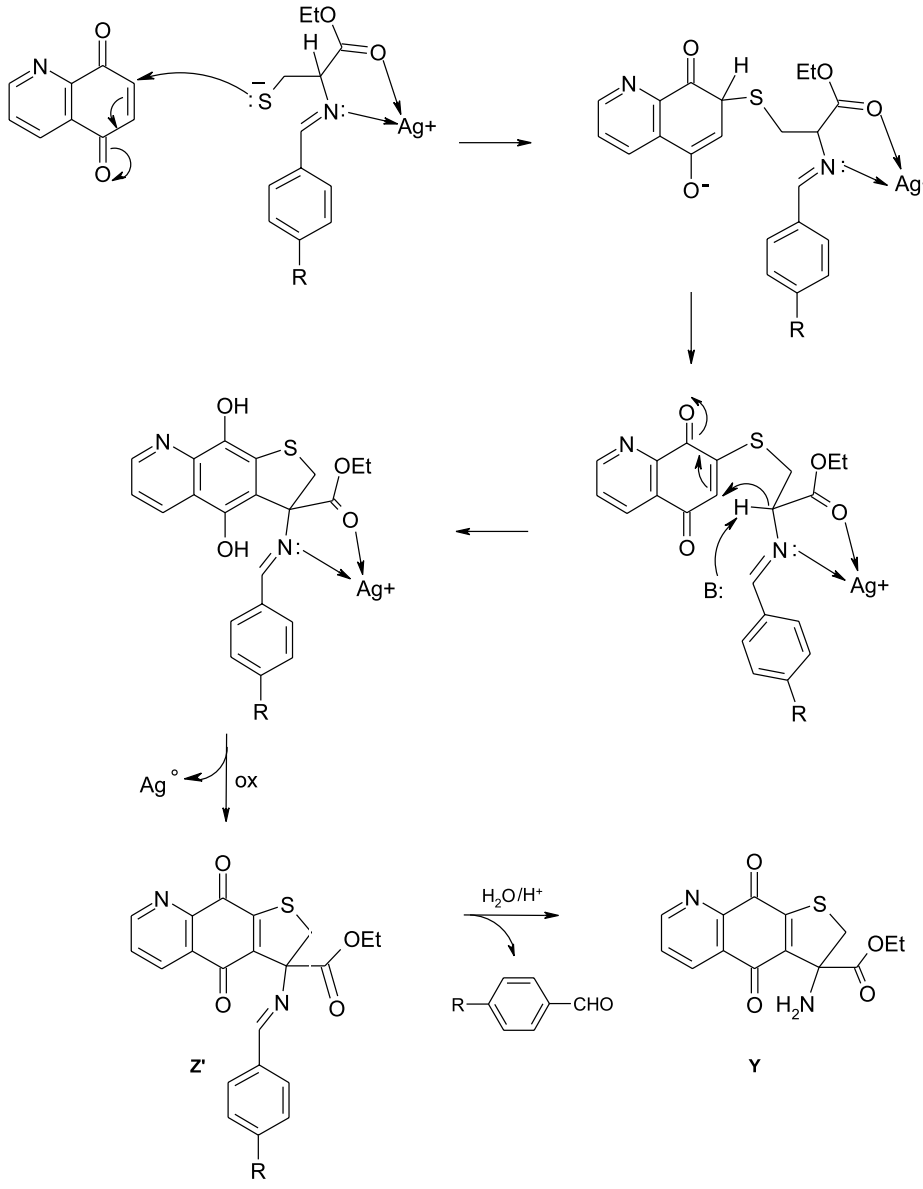



**Figure 3.** Azomethine intermediates **4Z** and **4Z′** isolated in the reaction mixture of **QQ** and $\mathbf{T_4}$.

In the exclusive case of the reaction performed with **QQ** and thiazolidine $\mathbf{T_4}$, the intermediates **4Z** and **4Z′** were isolated and characterized (Fig. 3). These compounds, present in small amount, support the formation mechanism of **X** and **Y**.

Summing up, 2-arylthiazolidine-4-carboxylic acid ethyl ester in basic medium seems to undergo two different cleavages: the first one produces an aryl-azadiene, the second one a thiolic species. In the presence of quinolinquinone, Diels–Alder product PQDs are formed together with those arising from an 1,4-addition reaction. No Diels–Alder compound is observed by using unsubstituted thiazolidine, which shows that the aryl substituents play a role in that reaction.




**Scheme 4.** Formation mechanism of **Y** involving 1,4-Michael addition (path B).

## 2. Experimental

### 2.1. General procedure

Melting points were determined by a Kofler apparatus and are uncorrected. Electron impact (EI) mass spectra were obtained at 70 eV on a ZAB 2F spectrometer. The purity of compounds was checked by ascending TLC on Merck's silica gel plates (0.25 mm) with fluorescent baking. UV spectra were recorded with Perkin–Elmer 550 S spectrophotometer. IR spectra were taken on Perkin–Elmer 399 spectrophotometer in KBr. NMR measurements (data reported in $\delta$) were performed on a Bruker AMX-500 spectrometer equipped with a Bruker X-32 computer, using the UXNMR software package. NMR spectra were measured at 500 MHz ($^{1}$H) and 125 MHz ($^{13}$C). The chemical shifts are referenced to $^{13}CDCl_3$ and $CDCl_3$ solvent signals at 77.0 and 7.26 ppm, respectively. Standard pulses sequences were employed for magnitude COSY. HMQC and HMBC experiments were optimized for $^{1}J_{C-H}=135$ Hz, $^{2,3}J_{C-H}=10$ Hz, respectively. $Me_4Si$ was used as internal reference.

### 2.2. General procedure for the synthesis of 1-aryl-3-ethoxycarbonyl-pyrido[2,3-*g*]isoquinolin-5,10-diones (1a-4a), 1-aryl-3-ethoxycarbonyl-pyrido [3,2-*g*]isoquinolin-5,10-diones (1b-4b), 3-amino-3-ethoxycarbonyl-dihydrothieno[2,3-*g*]quinolin-4,9-dione (X) and 3-amino-3-ethoxycarbonyl-dihydrothieno [3,2-*g*]quinolin-4,9-dione (Y)

A mixture of 2-aryl-1,3-thiazolidine ethyl esters (1 mmol) and silver carbonate (1 mmol), in anhydrous acetonitrile (50 mL), at −20 °C, was added to a solution of quinolin-5,8-dione (1 mmol) in anhydrous acetonitrile (50 mL). After 15 min, 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU, 1 mmol) was added. To the reaction mixture, stirred for 2 h at −20 °C and kept at

room temperature over night, diethyl ether was added (50 mL) and the solid residue containing silver was taken away. Diethyl ether $HCl_{sat}$ (10 mL), was added to the organic layer and the mixture was stirred for 10 min. Two fractions were recovered, an organic layer and a brown precipitate.

The organic layer, washed with 10% $NaHCO_3$ solution and dried with $Na_2SO_4$ anhydrous afforded crude Diels Alder products **1-4 a**, **b**. The isomeric **a** and **b** products were separated chromatographically on silica gel plates using a mixture of carbon tetrachloride/ethyl acetate (1:1; v:v) as eluent. Aldehydes arising from **X** and **Y** formation were also recovered.

The brown precipitate dissolved in water, neutralized with 10% $NaHCO_3$ solution was extracted with chloroform yielding the dihydrothienoquinolindiones **X** and **Y** as free bases. The racemic mixtures of **X** and **Y** were separated by flash chromatography using diethyl ether/$CHCl_3$ (3:7; v:v).

**2.2.1. 1-Phenyl-3-ethoxycarbonyl-pyrido[2,3-*g*]isoquinolin-5,10-dione (1a).** Pale-yellow solid (yield 80.6 mg, 20 mol%), mp 205–206 °C; [found: C, 70.41; H, 3.93; N, 7.84. $C_{21}H_{14}N_2O_4$ requires C, 70.39; H, 3.94; N, 7.82%]; $R_f$ (5% $Et_2O/CHCl_3$) 0.50; UV–vis ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) nm 358 (2.6); $\nu_{max}$ (KBr) 3154–2975 (br), 1695, 1590, 1499 $cm^{-1}$; $\delta_H$ (500 MHz $CDCl_3$) 9.13 (1H, dd; $J$=4.8, 1.2 Hz), 8.82 (1H, s), 8.64 (1H, dd; $J$=7.8, 1.2 Hz), 7.79 (1H, dd; $J$=7.8, 4.8 Hz), 7.59 (2H, d; $J$=7.0 Hz), 7.47 (3H, d+t; $J$=7.0 Hz), 4.53 (2H, q; $J$=7.2 Hz), 1.50 (3H, t; $J$=7.2 Hz); $\delta_C$ (125 MHz, $CDCl_3$) 181.7 (s, C5), 180.9 (s, C10), 163.6 (s, COO), 162.5 (s, C1), 156.1 (d, C7), 151.8 (s, C3), 149.7 (s, C5a), 141.3 (s, C10a), 138.9 (d, C1′), 135.2 (d, C9), 128.8 (d, C8), 128.7 (s, C9a), 128.5 (2C d, C2′6′), 128.0 (2C d, C3′5′), 126.9 (d, C4a), 126.8 (d, C4′), 119.3 (d, C4), 62.6 (t, *$CH_2$*–$CH_3$), 14.2 (q, $CH_2$–*$CH_3$*). MS (EI) *m/z* 358 (M+).

**2.2.2. 1-(4-Methylphenyl)-3-ethoxycarbonyl-pyrido[2,3-*g*]isoquinolin-5,10-diones (2a).** Pale-yellow solid (yield 29.6 mg, 8 mol%), mp 203–4 °C; [found: C, 70.92; H, 4.32; N, 7.53. $C_{21}H_{14}N_2O_4$ requires $C_{22}H_{16}N_2O_4$: C, 70.96; H, 4.33; N, 7.52%]; $R_f$ (5% $Et_2O/CHCl_3$) 0.45; UV–vis ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) nm 389 (3.3); $\nu_{max}$ (KBr) 3096–2930 (br), 1703, 1560, 1460 $cm^{-1}$; $\delta_H$ (500 MHz $CDCl_3$) 9.15 (1H, dd; $J$=4.8, 1.2 Hz), 8.89 (1H, s), 8.53 (1H, dd; $J$=7.8, 1.2 Hz), 7.76 (1H, dd; $J$=7.8, 4.8 Hz), 7.46 (2H, d; $J$=8.6 Hz), 7.30 (2H, d; $J$=8.6 Hz), 4.53 (2H, q; $J$=7.2 Hz), 2.42 (3H, s), 1.47 (3H, t; $J$=7.2 Hz). $\delta_C$ (125 MHz, $CDCl_3$) 181.7 (s, C5), 180.5 (s, C10), 163.7 (s, COO), 162.4 (s, C1), 156.0 (d, C7), 151.5 (s, C3), 149.5 (s, C5a), 141.3 (s, C10a), 139.4 (s, C1′), 136.0 (s, C4′), 135.1 (d, C9), 131.6 (s, C9a), 129.1 (2C d, C2′6′), 128.8 (2C d, C3′5′), 127.9 (d, C8), 125.6 (s, C4a), 118.9 (d, C4), 62.5 (t, *$CH_2$*–$CH_3$), 21.4 (q, $CH_3$), 14.1 (q, $CH_2$–*$CH_3$*). MS (EI) *m/z* 372 (M+).

**2.2.3. 1-(4-Chlorophenyl)-3-ethoxycarbonyl-pyrido[2,3-*g*]isoquinolin-5,10-diones (3a).** Pale-yellow solid (yield 82.3 mg, 21 mol%), mp 208–9 °C; [found: C, 64.30; H, 3.32; N, 7.14. $C_{21}H_{13}N_2O_4Cl$ requires C, 64.21; H, 3.34; N, 7.13%]; $R_f$ (5% $Et_2O/CHCl_3$) 0.55; UV–vis ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) nm 369 (2.8); $\nu_{max}$ (KBr) 3094–2937 (br), 1710, 1665, 1537, 1409, 1110 $cm^{-1}$; $\delta_H$ (500 MHz $CDCl_3$) 9.16 (1H, dd; $J$=4.8, 1.2 Hz), 8.84 (1H, s), 8.65 (1H, dd; $J$=7.8, 1.2 Hz), 7.79 (1H, dd; $J$=7.8, 4.8 Hz), 7.55 (2H, d; $J$=8.6 Hz), 7.45 (2H, d; $J$=8.6 Hz), 4.54 (2H, q; $J$=7.2 Hz), 1.48 (3H, t; $J$=7.2 Hz). $\delta_C$ (125 MHz, $CDCl_3$) 181.7 (s, C10), 181.1 (s, C5), 163.7 (s, COO), 161.4 (s, C1), 156.4 (d, C7), 152.0 (s, C3), 149.5 (s, C5a), 141.6 (s, C10a), 130.5 (2C d, C2′6′), 137.6 (s, C4′), 135.9 (d, C9), 135.5 (d, C9a), 129.6 (s, C1′), 128.7 (2C d, C3′5′), 128.4 (d, C8), 126.8 (s, C4a), 119.99 (d, C4), 62.5 (t, *$CH_2$*–$CH_3$), 14.1 (q, $CH_2$–*$CH_3$*). MS *m/z* 392 (M+), 394 (M+2, 32% M+).

**2.2.4. 1-(4-Nitrophenyl)-3-ethoxycarbonyl-pyrido[2,3-*g*]isoquinolin-5,10-diones (4a).** Yellow solid (yield 96.1 mg, 22 mol%), mp 308–9 °C; [found: C, 62.63; H, 3.26; N, 10.40. $C_{21}H_{13}N_3O_6$ requires C, 62.53; H, 3.25; N, 10.42%]; $R_f$ (5% $Et_2O/CHCl_3$) 0.40; UV–vis ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) nm 369 (2.8); $\nu_{max}$ (KBr) 3109–2975 (br), 2260, 1733, 1688, 1560, 1409 $cm^{-1}$; $\delta_H$ (500 MHz $CDCl_3$) 9.20 (1H, dd; $J$=4.8, 1.2 Hz), 9.02 (1H, s), 8.52 (1H, dd; $J$=7.8, 1.2 Hz), 8.37 (2H, d; $J$=8.6 Hz), 7.81 (1H, dd; $J$=7.8, 4.8 Hz), 7.69 (2H, d; $J$=8.6 Hz), 4.56 (2H, q; $J$=7.2 Hz), 1.48 (3H, t; $J$=7.2 Hz); $\delta_C$ (125 MHz, $CDCl_3$) 181.5 (s, C5), 180.5 (s, C10), 163.2 (s, COO), 161.8 (s, C1), 156.4 (d, C7), 151.7 (s, C3), 148.2 (s, C5a), 142.4 (s, C10a), 138.9 (s, C1′), 136.7 (d, C4′), 135.4 (d, C9), 132.2 (s, C9a), 130.1 (2C d, C3′5′), 129.0 (d, C8), 123.2 (2C d, C2′6′), 126.1 (s, C4a), 120.7 (d, C4), 62.7 (t, *$CH_2$*–$CH_3$), 14.2 (q, $CH_2$–*$CH_3$*). MS (EI) *m/z* 403 (M+).

**2.2.5. 1-Phenyl-3-ethoxycarbonyl-pyrido[3,2-*g*]isoquinolin-5,10-dione (1b).** Pale-yellow solid (yield 32.2 mg, 8 mol%), mp 209–10 °C; [found: C, 70.35; H, 3.95; N, 7.83. $C_{21}H_{14}N_2O_4$ requires C, 70.39; H, 3.94; N, 7.82%; $R_f$ (5% $Et_2O/CHCl_3$) 0.50; UV–vis ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) nm 360 (2.9); $\nu_{max}$ (KBr) 3050–2960 (br), 1710, 1655, 1575, 1447 $cm^{-1}$; $\delta_H$ (500 MHz, $CDCl_3$) 9.15 (1H, dd; $J$=4.8, 1.2 Hz), 8.92 (1H, s), 8.52 (1H, dd; $J$=7.8, 1.2 Hz), 7.77 (1H, dd; $J$=7.8, 4.8 Hz), 7.54 (2H, d; $J$=7.0 Hz), 7.51 (2H, t; $J$=7.2 Hz), 7.47 (1H, t; $J$=7.0 Hz), 4.53 (2H, q; $J$=7.2 Hz), 1.47 (3H, t; $J$=7.2 Hz). $\delta_C$ (125 MHz, $CDCl_3$) 181.6 (s, C10), 180.5 (s, C5), 163.5 (s, COO), 162.2 (s, C1), 155.4 (d, C8), 151.6 (s, C3), 147.7 (s, C9a), 141.7 (s, C10a), 139.4 (s, C1′), 135.8 (d, C6), 131.5 (s, C5a), 129.2 (2C d, C2′6′), 129.0 (2C d, C3′5′), 128.7 (d, C4′), 128.5 (d, C7), 125.5 (s, C4a), 120.0 (d, C4), 62.6 (t, *$CH_2$*–$CH_3$), 14.2 (q, $CH_2$–*$CH_3$*). MS *m/z* 358 (M+).

**2.2.6. 1-(4-Methylphenyl)-3-ethoxycarbonyl-pyrido[3,2-*g*]isoquinolin-5,10-diones (2b).** Pale-yellow solid (yield 29.6 mg, 8 mol%), mp 208–9 °C; [found: C, 70.96; H, 4.33; N, 7.50. $C_{22}H_{16}N_2O_4$ requires C, 70.96; H, 4.33; N, 7.52%]; $R_f$ (5% $Et_2O/CHCl_3$) 0.40; UV–vis ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) nm: 391 (3.5); $\nu_{max}$ (KBr) 3109–2930 (br), 1733, 1665, 1499 $cm^{-1}$; $\delta_H$ (500 MHz, $CDCl_3$) 9.16 (1H, dd; $J$=4.8, 1.2 Hz), 8.78 (1H, s), 8.64 (1H, dd; $J$=7.8, 1.2 Hz), 7.80 (1H, dd; $J$=7.8, 4.8 Hz), 7.51 (2H, d; $J$=8.6 Hz), 7.27 (2H, d; $J$=8.6 Hz), 4.53 (2H, q; $J$=7.2 Hz), 2.45 (3H, s), 1.46 (3H, t; $J$=7.2 Hz). $\delta_C$ (125 MHz, $CDCl_3$) 181.8 (s, C10), 181.1 (s, C5), 163.6 (s, COO), 162.3 (s, C1), 155.3 (d, C8), 151.8 (s, C3), 147.7 (s, C9a), 141.7 (s, C10a), 139.5 (s, C1′), 136.5 (s, C4′), 136.5 (d, C6), 129.9 (s, C5a), 129.3 (2C d, C2′6′), 128.9 (2C d, C3′,5′), 128.7 (d, C7), 126.9 (s, C4a),

119.7 (d, C4), 62.5 (t, *CH*$_2$–CH$_3$), 21.3 (q, CH$_3$), 14.2 (q, CH$_2$–*CH*$_3$). MS *m/z* 372 (M+).

**2.2.7. 1-(4-Chlorophenyl)-3-ethoxycarbonyl-pyrido[3,2-*g*] isoquinolin-5,10-diones (3b).** Pale-yellow solid (yield 43.1 mg, 11 mol%), mp 217–8 °C; [found: C, 64.41; H, 3.35; N, 7.15. $C_{21}H_{13}N_2O_4Cl$ requires C, 64.21; H, 3.34; N, 7.13%]; $R_f$ (5% Et$_2$O/CHCl$_3$) 0.45;); UV–vis (CHCl$_3$) $\lambda_{max}$ (log $\varepsilon$) nm: 370 (2.9); $\nu_{max}$ (KBr) 3070–2980 (br), 1675, 1575, 1409, 1110 cm$^{-1}$; $\delta_H$ (500 MHz, CDCl$_3$) 9.17 (1H, dd; *J*=4.8, 1.2 Hz), 8.94 (1H, s), 8.54 (1H, dd; *J*=7.8, 1.2 Hz), 7.82 (1H, dd; *J*=7.8, 4.8 Hz), 7.50 (2H, d; *J*=8.6 Hz), 7.47 (2H, d; *J*=8.6 Hz), 4.55 (2H, q; *J*=7.2 Hz), 1.46 (3H, t; *J*=7.2 Hz). $\delta_C$ (125 MHz, CDCl$_3$) 181.6 (s, C10), 180.2 (s, C5), 163.4 (s, COO), 160.9 (s, C1), 155.5 (d, C7), 152.3 (s, C3), 147.7 (s, C9a), 141.3 (s, C10a), 137.1 (2C d, C2′6′), 135.8 (d, C6), 135.6 (s, C4′), 130.4. (s, C1′), 129.4 (s, C5a), 128.6 (d, C7), 128.5 (2C d, C3′5′), 125.7 (s, C4a), 120.3 (d, C4), 62.7 (t, *CH*$_2$–CH$_3$), 14.1 (q, CH$_2$–*CH*$_3$). MS (EI) *m/z* 392 (M+), 394 (M+2, 32% M+).

**2.2.8. 1-(4-Nitrophenyl)-3-ethoxycarbonyl-pyrido[3,2-*g*] isoquinolin-5,10-diones (4b).** Yellow solid (yield 52.4 mg, 12 mol%), mp 311–2 °C; [found: C, 62.58; H, 3.26; N, 10.41. $C_{21}H_{13}N_3O_6$ requires C, 62.53; H, 3.25; N, 10.42%]; $R_f$ (5% Et$_2$O/CHCl$_3$) 0.55; UV–vis (CHCl$_3$) $\lambda_{max}$ (log $\varepsilon$) nm 371 (3.0); $\nu_{max}$ (KBr) 3190–2975 (br), 1733, 1655, 1590, 1432 cm$^{-1}$; $\delta_H$ (500 MHz, CDCl$_3$) 9.17 (1H, dd; *J*=4.8, 1.2 Hz), 8.93 (1H, s), 8.68 (1H, dd; *J*=7.8, 1.2 Hz), 8.34 (2H, d; *J*=8.6 Hz), 7.82 (1H, dd; *J*=7.8, 4.8 Hz), 7.73 (2H, d; *J*=8.6 Hz), 4.56 (2H, q; *J*=7.2 Hz), 1.48 (3H, t; *J*=7.2 Hz); $\delta_C$ (125 MHz, CDCl$_3$) 182.1 (s, C10), 181.9 (s, C5), 163.3 (s, COO), 162.5 (s, C1), 155.9 (d, C8), 152.1 (s, C3), 148.9 (s, C9a), 142.9 (s, C10a), 139.3 (s, C1′), 136.5 (d, C4′), 135.9 (d, C6), 130.0 (2C d, C3′5′), 129.5 (d, C5a), 128.4 (d, C7), 125.5 (s, C4a), 123.2 (2C d, C2′6′), 121.3 (d, C4), 63.0 (t, *CH*$_2$–CH$_3$), 14.1 (q, CH$_2$–*CH*$_3$). MS *m/z* 403 (M+).

**2.2.9. 3-Amino-3-ethoxycarbonyl-dihydrothieno[2,3-*g*] quinolin-4,9-dione (X).** Orange solid (yields from the reaction between **QQ** and **T$_1$** (54.7 mg, 18 mol%), **T$_2$** (30.4 mg, 10 mol%), **T$_3$** (18.2 mg, 6 mol%), **T$_4$** (27.3 mg, 9 mol%), and **T$_5$** (39.3 mg, 14 mol%), mp >200 °C dec.; $\nu_{max}$ (KBr) 3216–2945 (br), 1733, 1635, 1567, 1437 cm$^{-1}$; UV–vis (CHCl$_3$) $\lambda_{max}$ (log $\varepsilon$) nm 418 (3.6); $R_f$ (5% MeOH/CHCl$_3$) 0.55; $\delta_H$ (500 MHz, CDCl$_3$); 9.01 (1H, dd; *J*=4.3, 1.6 Hz), 8.40 (1H, dd; *J*=8.2, 1.6 Hz), 7.64 (1H, dd; *J*=8.2, 4.3 Hz), 4.27 (2H, q; *J*=7.2 Hz), 3.87 (1H, d; *J*=12.4 Hz), 3.34 (1H, d; *J*=12.4 Hz), 2.37 (2H, bs; NH$_2$), 1.27 (3H, t; *J*=7.2 Hz); $\delta_C$ (125 MHz, CDCl$_3$) 180.7 (s, C9), 179.9 (s, C4), 172.5 (s, COO), 154.9 (s, C9a), 154.3 (d, C6), 146.7 (s, C4a), 142.0 (s, C3a), 136.7 (d, C8), 129.1 (s, C8a), 128.2 (d, C7), 73.3 (s, C3), 62.6 (t, *CH*$_2$–CH$_3$), 42.4 (t, C2), 14.0 (q, CH$_2$–*CH*$_3$). MS (EI) *m/z* 304 (M+), 306 (M+2, 11% M+), 308 (M+4, 4% M+).

**2.2.10. 3-Acetylamino-3-ethoxycarbonyl-dihydrothieno [2,3-*g*]quinolin-4,9-dione (X′).** Compound **X** (30 mg), dissolved in dichloromethane (25 mL), treated with acetyl chloride (10% excess) was stirred for 2 h. The reaction mixture washed with 10% NaHCO$_3$ solution and dried with Na$_2$SO$_4$ anhydrous afforded crude the acetyl derivative **X′** (yield 31 mg, 90%), which was crystallized from ethanol. Yellow solid, mp 208–9 °C; [found: C, 55.36; H, 3.98; N, 9.23. $C_{16}H_{14}N_2O_5S$ requires C, 55.26; H, 3.97; N, 9.21]; UV–vis (CHCl$_3$) $\lambda_{max}$ (log $\varepsilon$) nm 401 (3.0); $\nu_{max}$ (KBr) 3065–2975 (br), 2267, 1718, 1635, 1605 cm$^{-1}$; $R_f$ (3% MeOH/CHCl$_3$) 0.65; $\delta_H$ (500 MHz, CDCl$_3$) 9.01 (1H, dd; *J*=4.3, 1.6 Hz), 8.36 (1H, dd; *J*=8.2, 1.6 Hz), 7.67 (1H, dd; *J*=8.2, 4.3 Hz), 7.58 (1H, s; NH), 4.32 (2H, q; *J*=7.2 Hz), 4.03 (1H, d; *J*=12.6 Hz), 3.90 (1H, d; *J*=12.6 Hz), 2.18 (3H, s), 1.26 (3H, t; *J*=7.2 Hz); $\delta_C$ (125 MHz, CDCl$_3$) 179.5 (s, C9), 178.0 (s, C5), 170.2 (s, COO), 169.5 (CONH), 155.2 (d, C6), 154.9 (s, C9a), 147.6 (s, C4a), 142.0 (s, C3a), 129.0 (s, C8a), 135.1 (d, C8), 125.9 (d, C7), 73.1 (s, C3), 62.4 (t, *CH*$_2$–CH$_3$), 41.6 (t, C2), 19.9 (q, CH$_3$), 14.0 (q, CH$_2$–*CH*$_3$). MS (EI) *m/z* 346 (M+), 348 (M+2, 121% M+), 350 (M+4, 4% M+).

**2.2.11. 3-Amino-3-ethoxycarbonyl-dihydrothieno[3,2-*g*] quinolin-4,9-dione (Y).** Orange solid (yields from the reaction between **QQ** and **T$_1$** (12.2 mg, 4 mol%), **T$_2$** (21.2 mg, 7 mol%), **T$_3$** (6.1 mg, 2 mol%), **T$_4$** (24.3 mg, 8 mol%), and **T$_5$** (18.2 mg, 6 mol%), mp >200 °C dec.; [found: C, 55.28; H, 3.97; N, 9.23. $C_{16}H_{14}N_2O_5S$ requires C, 55.26; H, 3.97; N, 9.21%]; $R_f$ (5% MeOH/CHCl$_3$) 0.40; UV–vis (CHCl$_3$) $\lambda_{max}$ (log $\varepsilon$) nm 413 (3.2); $\nu_{max}$ (KBr) 3336–2937 (br), 1733, 1642, 1575, 1432 cm$^{-1}$; $\delta_H$ (500 MHz, CDCl$_3$) 9.00 (1H, dd; *J*=4.3, 1.6 Hz), 8.38 (1H, dd; *J*=8.2, 1.6 Hz), 7.62 (1H, dd; *J*=8.2, 4.3 Hz), 4.27 (2H, q; *J*=7.2 Hz), 3.85 (1H, d; *J*=12.4 Hz), 3.36 (1H, d; *J*=12.4 Hz), 2.28 (2H, bs; NH$_2$), 1.27 (3H, t; *J*=7.2 Hz); $\delta_C$ (125 MHz, CDCl$_3$) 180.1 (s, C9), 180.0 (s, C4), 171.9 (s, COO), 155.5 (d, C7), 154.7 (s, C9a), 146.8 (s, C8a), 142.2 (s, C3a), 135.8 (d, C5), 128.9 (s, C4a), 128.5 (d, C6), 72.0 (s, C3), 62.5 (t, *CH*$_2$–CH$_3$), 43.1 (t, C2), 14.1 (q, CH$_2$–*CH*$_3$). MS (EI) *m/z* 304 (M+), 306 (M+2, 11% M+), 308 (M+4, 4% M+).

**2.2.12. 3-(4′-NO$_2$-Benzyliden)amino-3-ethoxycarbonyl-dihydrothieno[2,3-*g*]quinolin-4,9-dione (4Z).** Orange oil (yields 3.5 mg, 0.8 mol%); [found: C, 57.69; H, 3.44; N, 9.64. $C_{16}H_{14}N_2O_5S$ requires C, 57.66; H, 3.46; N, 9.61%]; $R_f$ (5% MeOH/CHCl$_3$) 0.60; UV–vis (CHCl$_3$) $\lambda_{max}$ (log $\varepsilon$) nm 421 (3.0); $\nu_{max}$ (KBr) 3099, 2996, 2606, 1703, 1665, 1620, 1590, 1510, 1432 cm$^{-1}$; $\delta_H$ (500 MHz, CDCl$_3$) 9.05 (1H, dd; *J*=4.8, 1.8 Hz), 8.54 (1H, s), 8.43 (1H, dd; *J*=7.2, 1.8 Hz), 8.24 (2H, d; *J*=8.4 Hz), 7.94 (2H, d; *J*=8.4 Hz), 7.66 (1H, dd; *J*=7.2, 4.8 Hz), 4.30 (2H, q; *J*=7.2 Hz), 4.13 (1H, d; *J*=11.8 Hz), 3.80 (1H, d; *J*=11.8 Hz), 1.26 (3H, t; *J*=7.2 Hz); $\delta_C$ (125 MHz, CDCl$_3$) 13.9 (q, *CH*$_2$–CH$_3$), 44.0 (t, S–*CH*$_2$), 61.8 (*CH*$_2$–CH$_3$), 72.6 (s, N–*C*–CO), 124.1 (d, 2C3′), 127.1 (d, C6), 128.7 (s, C4a), 128.8 (d, 2C2′), 134.5 (d, C5), 142.7 (s, C9a), 143.7 (s, C1′), 150.3 (s, C4′), 151.1 (s, C3a), 155.1 (s, C8a), 156.2 (d, C7), 162.1 (d, N=*CH*), 169.9 (s, C4) 177.4 (s, C9), 178.3 (s, CO ester). MS (EI) *m/z* 437 (M+), 439 (M+2, 11% M+), 441 (M+4, 4% M+).

**2.2.13. 3-(4′-NO$_2$-Benzyliden)amino-3-ethoxycarbonyl-dihydrothieno[3,2-*g*]quinolin-4,9-dione (4Z′).** Orange oil (yields 2,5 mg, 0.5 mol%); [found: C, 57.71; H, 3.47; N, 9.64. $C_{16}H_{14}N_2O_5S$ requires C, 57.66; H, 3.46; N, 9.61%]; $R_f$ (3% MeOH/CHCl$_3$) 0.50; UV–vis (CHCl$_3$) $\lambda_{max}$ (log $\varepsilon$) nm 423 (3.0); $\nu_{max}$ (KBr) 3096, 2965, 2614, 1703,

1620, 1447 $cm^{-1}$; $\delta_H$ (500 MHz $CDCl_3$) 9.03 (1H, dd; $J$= 4.8, 1.8 Hz), 8.54 (1H, s), 8.40 (1H, dd; $J$=7.2, 1.8 Hz), 8.21 (2H, d; $J$=8.4 Hz), 7.93 (2H, d; $J$=8.4 Hz), 7.64 (1H, dd; $J$=7.2, 4.8 Hz), 4.32 (2H, q; $J$=7.2 Hz), 4.14 (1H, d; $J$=11.8 Hz), 3.81 (1H, d; $J$=11.8 Hz), 1.28 (3H, t; $J$= 7.2 Hz); $\delta_C$ (125 MHz, $CDCl_3$) 14.1 (q, $CH_2$–$CH_3$), 40.2 (t, S–$CH_2$), 62.1 ($CH_2$–$CH_3$), 72.5 (s, N–*C*–CO), 124.3 (d, 2C3′), 127.3 (d, C6), 128.9 (s+d, C4a+2C2′), 134.5 (d, C5), 141.8 (s, C9a), 142.7 (s, C1′), 151.2 (s, C4′), 151.4 (s, C3a), 155.1 (s, C8a), 156.4 (d, C7), 162.1 (d, N=*CH*), 170.9 (s, C4) 177.1 (s, C9), 178.3 (s, CO ester). MS (EI) *m/z* 437 (M+), 439 (M+2, 11% M+), 441 (M+4, 4% M+).

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## References and notes

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