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# Synthesis and antileukemic activity of new 3-(5-methylisoxazol-3-yl) and 3-(pyrimidin-2-yl)-2-styrylquinazolin-4(3*H*)-ones

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**Abstract**

3-(3-Methylisoxazol-5-yl) and 3-(pyrimidin-2-yl)-2-styrylquinazolin-4(3*H*)-ones **8a–l** and **9a,c–e,h–l** were synthesized by refluxing in acetic acid the corresponding 2-methylquinazolinones **6** and **8** with the opportune benzoic aldehyde for 12 h. The synthesized styrylquinazolinones **8a–l** and **9a,c–e,h–l** were tested in vitro for their antileukemic activity against L-1210 (murine leukemia), K-562 (human chronic myelogenous leukemia) and HL-60 (human leukemia) cell lines showing in some cases good activity.


*Keywords:* 3-(3-Methylisoxazol-5-yl)-2-styrylquinazolin-4(3*H*)-ones; 3-(Pyrimidin-2-yl)-2-styrylquinazolin-4(3*H*)-ones; Antileukemic activity

## 1. Introduction

Quinazolinones are very interesting drugs with antiproliferative activity known to bind to tubulin and interfering with its polymerization [1,2]; the 2-styrylquinazolinones are the best example of this class of antimitotic agents [3].

Owing to the antitumoral activity described for the styrylquinazolinones, our group has been recently engaged in the synthesis of some new 6-*R*-3-(1-phenyl-3-methyl-pyrazol-5-yl)-2-styrylquinazolin-4(3*H*)-ones **2** and **3** [4] (Fig. 1).

The synthesized styrylquinazolinones **2** and **3** were tested in vitro for their antileukemic activity against L-1210 (murine leukemia), K-562 (human chronic myelogenous leukemia) and HL-60 (human leukemia) cell lines showing in some cases good activity ($IC_{50} \sim 2$ μM).

Pursuing our research in this field, in order to further explore the structure–activity relationships of this class of antileukemic agents, we refer now the synthesis and the biological activity of new analogs of styrylquinazolinones **2** and **3**.

In particular, in the new synthesized 2-styrylquinazolinones **8** and **9**, we substituted *N*-3 pyrazole with the 5-methyl-isoxazol-2-yl and with the pyrimidin-2-yl moieties.

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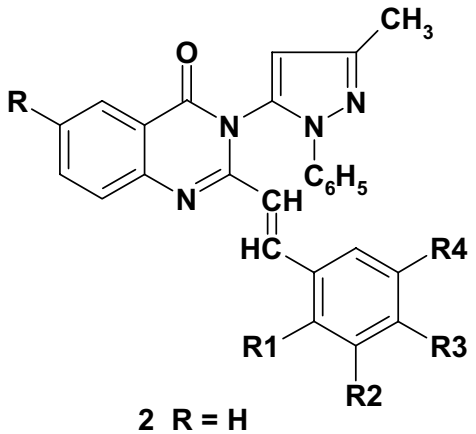
## 2. Chemistry

6-Cl-3-(5-methylisoxazol-5-yl)-2-styrylquinazolinones **8a–l** and 6-Cl-3-(pyrimidin-2-yl)-2-styrylquinazolinones **9a,c–e,h–l** were obtained starting from the corresponding 2-methylquinazolinones **6** and **7** by condensation with the opportune benzaldehyde (Scheme 1); the reaction was performed by refluxing equimolar amounts of the opportune 2-methylquinazolinone **6,7** and benzaldehyde in glacial acetic acid [5].

The structures of new compounds were elucidated by analytical as well as spectroscopic measurements. In particular, as reported for derivatives **2** and **3** [4], the $^1$H NMR spectra of compounds **8** and **9** are consistent with a E-olefinic structure: β-olefinic protons appeared as doublets at δ 6.48–6.88 ($J$ = 15.3–15.9 Hz) for compounds **8** and at δ 5.92–6.38 ($J$ = 14.9–15.7 Hz) for compounds **9** as requested for a E structure [6] while the α-olefinic hydrogens were found along with aromatic multiplet because of the deshielding of two quinazolinone nitrogens; only for compound **8i** the α-olefinic hydrogens were found at δ 8.41.

6-Cl-3-(5-methylisoxazol-5-yl)-2-methylquinazolinone **6** [7] and 6-Cl-3-(pyrimidin-2-yl)-2-methylquinazolinones **7** [8] were known and were obtained, as reported, by fusion of the 6-chloro-2-methyl-benzoxazin-4(3*H*)-one **5** [9] with the opportune amine according with the Scheme 1.




2 R = H
3 R = Cl

| | a | b | c | d | e | f | g | h | i | l | m | n | o | p | q |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **R1** | H | $OCH_3$ | H | H | $OCH_3$ | $OCH_3$ | H | H | $OCH_3$ | $CH_3$ | H | H | $NO_2$ | H | H |
| **R2** | H | H | $OCH_3$ | H | H | $OCH_3$ | $OCH_3$ | $OCH_3$ | $OCH_3$ | H | $CH_3$ | H | H | H | OH |
| **R3** | H | H | H | $OCH_3$ | $OCH_3$ | H | $OCH_3$ | $OCH_3$ | $OCH_3$ | H | H | $CH_3$ | H | Cl | $OCH_3$ |
| **R4** | H | H | H | H | H | H | H | $OCH_3$ | H | H | H | H | H | H | H |

Fig. 1. Structural formulas of compounds **2** and **3**

## 3. Biological results and discussion

The synthesized styrylquinazolinones **8a–l** and **9a,c–e, h–l** were tested in vitro for their antileukemic activity against L-1210 (murine leukemia), K-562 (human chronic myelogenous leukemia) and HL-60 (human leukemia) cell lines. Colchicine **10**, whose antileukemic activity is well known, was used as reference compound. The results as percent of growth inhibition at 1 μg/ml concentration are reported in Table 1. In Table 2 the $IC_{50}$ values for compounds that exhibited at least 50% of growth inhibition at screening concentration are reported.

Among the tested new 2-styrylquinazolinones **8** and **9**, several compounds were active at the microgram level even if their antileukemic activity (Table 1) was lower than the reference compound (colchicine **10**).

A comparison between the antileukemic activity of both **2,3** [4] and **8,9** (Table 1) series, showed that there are no substantial difference among these groups of compounds; compounds **2,3** and **8,9** were characterized by the same degree of activity against L-1210, K-562 and HL-60 cell lines.

In particular, the antileukemic activity was not positively affected by substituting the *N*-3 pyrazole (compounds **2,3**) with the 5-methyl-isoxazol-2-yl (compounds **8**) and the pyrimidin-2-yl (compounds **9**) moieties.

The most active compounds **8d, 8e** and **9l** (Table 2) were characterized by the presence of two methoxyl groups (**8d,e**) and a chlorine atom (**9l**) in the styryl moiety.

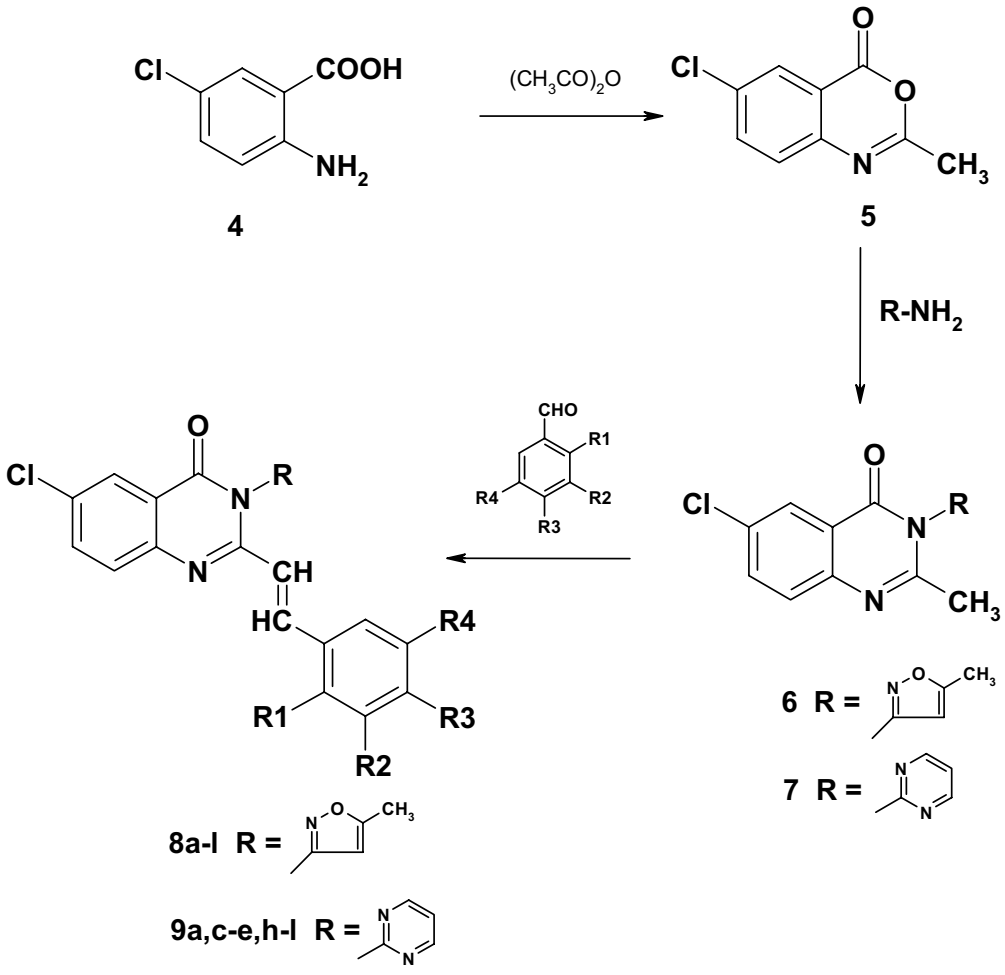
Table 1
Percent growth inhibiti on recorded on K-562, HL-60 and L-1210 cell lines at 1 μg/ml concentration of **8a–l** and **9a,c–e,h–l** compounds

| Compounds | K-562 | HL-60 | L-1210 |
|---|---|---|---|
| **8a** | n.s. | n.s. | 30.0 |
| **8b** | n.s. | n.s. | 20.4 |
| **8c** | n.t. | 34.0 | n.s. |
| **8d** | 50.0 | 22.0 | 88.0 |
| **8e** | 58.0 | n.s. | 20.0 |
| **8f** | 37.0 | n.s. | 27.0 |
| **8g** | n.s. | n.s. | 18.2 |
| **8h** | 44 | n.s. | 21.0 |
| **8i** | n.s. | n.s. | n.s. |
| **8l** | 36.5 | n.s. | 29.0 |
| **9a** | 26.1 | n.s. | 25.0 |
| **9c** | n.s. | 36.8 | n.s. |
| **9d** | n.s. | 20.6 | n.s. |
| **9e** | n.t. | 19.4 | n.s. |
| **9h** | n.s. | 19.4 | 16.4 |
| **9i** | 39.0 | 36.8 | 28.4 |
| **9l** | 63.0 | 40.0 | 39.4 |
| **Colchicine** | 84.7 | 84.8 | 79.0 |

Values are the means of at least three independent determinations; coefficient of variation was less than 15%.
n.s.: not significant, % inhibition <10%; n.t.: not tested.

Table 2
$IC_{50}$ recorded on K-562 and L-1210 cell lines of compounds **8d,e** and **9l**

| | Compounds | $IC_{50}$ (μM) |
|---|---|---|
| L-1210 | **8d** | 0.20 ± 0.03 |
| | **Colchicine** | <0.025 |
| K-562 | **8d** | 2.50 ± 0.22 |
| | **8e** | 1.61 ± 0.13 |
| | **9l** | 1.21 ± 0.18 |
| | **Colchicine** | <0.025 |




| | a | b | c | d | e | f | g | h | i | l |
|---|---|---|---|---|---|---|---|---|---|---|
| **R1** | $OCH_3$ | H | H | $OCH_3$ | $OCH_3$ | H | H | $OCH_3$ | $NO_2$ | H |
| **R2** | H | $OCH_3$ | H | H | $OCH_3$ | $OCH_3$ | $OCH_3$ | $OCH_3$ | H | H |
| **R3** | H | H | $OCH_3$ | $OCH_3$ | H | $OCH_3$ | $OCH_3$ | $OCH_3$ | H | Cl |
| **R4** | H | H | H | H | H | H | $OCH_3$ | H | H | H |

Scheme 1. General synthetic route to compounds **8a-l** and **9a, c-e, h-l**

## 4. Conclusion

A series of 2-styrylquinazolinone derivatives have been synthesized and evaluated for their antileukemic activity.

Although most of the new synthesized compounds exhibited moderate antileukemic activity they were less active than the reference compound (colchicine **10**) and, on the basis of the obtained results, it can be concluded that there are no advantages in replacing the *N*-3 pyrazole with the 5-methylisoxazol-2-yl and the pyrimidin-2-yl moieties.

## 5. Experimental section

### *5.1. Chemistry*

All melting points were determined on a Büchi 530 capillary melting point apparatus and are uncorrected; IR spectra were recorded with a Jasco IR-810 spectrophotometer as Nujol Mull supported on NaCl disks; $^1$H NMR spectra were obtained using a Bruker AC-E 250 MHz spectrometer (tetramethylsilane as internal standard). Microanalyses (C, H, N) performed in the laboratories of the Dipartimento di Scienze Farmaceutiche—Università di Catania, were within ±0.4% of the theoretical values.

6-Cl-3-(5-methylisoxazol-5-yl)-2-styrylquinazolinones **8a–l** and 6-Cl-3-(pyrimidin-2-yl)-2-styrylquinazolinones **9a,c–e,h–l**.

Equimolar amounts (10 mmol) of 6-Cl-3-(5-methylisoxazol-5-yl)-2-methylquinazolinone **6** and 6-Cl-3-(pyrimidin-2-yl)-2-methylquinazolinone **7** and the opportune benzoic aldehyde were reacted in acetic acid (10 ml) under reflux for 12 h.

The solid product, which separated, was filtered and crystallized to give pure **8a–l** and **9a,c–e,h–l**. Yield 22–96%.

Table 3
Physical data for compounds **8a–l** and **9a,c–e,h–l**

| Compounds | Melting point (°C) | Crystallization solvent | Analyses | Formula | Yield (%) |
|---|---|---|---|---|---|
| **8a** | 210–212 | Ethyl acetate | C, H, N | $C_{21}H_{16}N_3O_3Cl$ | 73 |
| **8b** | 213–216 | Dioxane | C, H, N | $C_{21}H_{16}N_3O_3Cl$ | 80 |
| **8c** | 225–228 | Ethyl acetate | C, H, N | $C_{21}H_{16}N_3O_3Cl$ | 41 |
| **8d** | 214–215 | Dioxane | C, H, N | $C_{22}H_{18}N_3O_4Cl$ | 38 |
| **8e** | 248–249 | Dioxane | C, H, N | $C_{22}H_{18}N_3O_4Cl$ | 41 |
| **8f** | 233–235 | Dioxane | C, H, N | $C_{22}H_{18}N_3O_4Cl$ | 59 |
| **8g** | 245–247 | Dioxane | C, H, N | $C_{23}H_{20}N_3O_5Cl$ | 52 |
| **8h** | 202–203 | Dioxane | C, H, N | $C_{23}H_{20}N_3O_5Cl$ | 37 |
| **8i** | >250 | Dioxane | C, H, N | $C_{20}H_{13}N_4O_4Cl$ | 29 |
| **8l** | 247–249 | Dioxane | C, H, N | $C_{20}H_{13}N_3O_2Cl_2$ | 24 |
| **9a** | 212–214 | Dioxane | C, H, N | $C_{21}H_{15}N_4O_2Cl$ | 44 |
| **9c** | 220–222 | Dioxane | C, H, N | $C_{21}H_{15}N_4O_2Cl$ | 15 |
| **9d** | 208–209 | Ethanol | C, H, N | $C_{22}H_{17}N_4O_3Cl$ | 12 |
| **9e** | 205–208 | Dioxane | C, H, N | $C_{22}H_{17}N_4O_3Cl$ | 12 |
| **9h** | 179–180 | Ethanol | C, H, N | $C_{23}H_{19}N_4O_4Cl$ | 44 |
| **9i** | 218–220 | Dioxane | C, H, N | $C_{20}H_{12}N_5O_3Cl$ | 15 |
| **9l** | >250 | Dioxane | C, H, N | $C_{20}H_{12}N_4OCl_2$ | 41 |

Compounds **8a–l** and **9a,c–e,h–l** are listed in Table 3.

**8a**: IR ($cm^{-1}$): 1694 (CO). $^1H$ NMR ($CDCl_3$) (δ): 2.59 (3H, s, $CH_3$); 3.85 (3H, s, $OCH_3$); 6.24 (1H, s, isoxazole H-4); 6.76 (1H, d, CH, *J* = 15.7 Hz); 6.88–8.28 (8H, a set of signals, aromatic protons and CH). **8b**: IR ($cm^{-1}$): 1702 (CO). $^1H$ NMR ($CDCl_3$) (δ): 2.60 (3H, s, $CH_3$); 3.86 (3H, s, $OCH_3$); 6.25 (1H, s, isoxazole H-4); 6.56 (1H, d, CH, *J* = 15.4 Hz); 6.88–8.22 (8H, a set of signals, aromatic protons and CH). **8c**: IR ($cm^{-1}$): 1696 (CO). $^1H$ NMR ($CDCl_3$) (δ): 2.60 (3H, s, $CH_3$); 3.83 (3H, s, $OCH_3$); 6.24 (1H, s, isoxazole H-4); 6.43 (1H, d, CH, *J* = 15.3 Hz); 6.86–8.21 (8H, a set of signals, aromatic protons and CH). **8d**: IR ($cm^{-1}$): 1693 (CO). $^1H$ NMR ($CDCl_3$) (δ): 2.58 (3H, s, $CH_3$); 3.80 (3H, s, $OCH_3$); 3.82 (3H, s, $OCH_3$); 6.24 (1H, s, isoxazole H-4); 6.40–8.18 (8H, a set of signals, aromatic protons and 2XCH). **8e**: IR ($cm^{-1}$): 1688 (CO). $^1H$ NMR ($CDCl_3$) (δ): 2.58 (3H, s, $CH_3$); 3.82 (3H, s, $OCH_3$); 3.87 (3H, s, $OCH_3$); 6.25 (1H, s, isoxazole H-4); 6.80 (1H, d, CH, *J* = 15.9 Hz); 6.89–8.22 (7H, a set of signals, aromatic protons and CH). **8f**: IR ($cm^{-1}$): 1687 (CO). $^1H$ NMR ($CDCl_3$) (δ): 2.59 (3H, s, $CH_3$); 3.88 (3H, s, $OCH_3$); 3.90 (3H, s, $OCH_3$); 6.26 (1H, s, isoxazole H-4); 6.42 (1H, d, CH, *J* = 15.9 Hz); 6.82–8.20 (7H, a set of signals, aromatic protons and CH). **8g**: IR ($cm^{-1}$): 1695 (CO). $^1H$ NMR ($CDCl_3$) (δ): 2.59 (3H, s, $CH_3$); 3.87 (9H, s, $3XOCH_3$); 6.26 (1H, s, isoxazole H-4); 6.48 (1H, d, CH, *J* = 15.6 Hz); 6.67–8.23 (6H, a set of signals, aromatic protons and CH). **8h**: IR ($cm^{-1}$): 1697 (CO). $^1H$ NMR ($CDCl_3$) (δ): 2.58 (3H, s, $CH_3$); 3.85 (3H, s, $OCH_3$); 3.86 (3H, s, $OCH_3$); 3.88 (3H, s, $OCH_3$); 6.26 (1H, s, isoxazole H-4); 6.64–8.20 (7H, a set of signals, aromatic protons and 2XCH). **8i**: IR ($cm^{-1}$): 1698 (CO). $^1H$ NMR ($CDCl_3$) (δ): 2.57 (3H, s, $CH_3$); 6.28 (1H, s, isoxazole H-4); 6.56 (1H, d, CH, *J* = 15.3 Hz); 7.48–8.44 (8H, a set of signals, aromatic protons and CH); 8.41 (1H, d, CH, *J* = 15.3 Hz). **8l**: IR ($cm^{-1}$): 1703 (CO). $^1H$ NMR ($CDCl_3$) (δ): 2.60 (3H, s, $CH_3$); 6.27 (1H, s, isoxazole H-4); 6.54 (1H, d, CH, *J* = 15.5 Hz); 7.34–8.22 (8H, a set of signals, aromatic protons and CH). **9a**: IR ($cm^{-1}$): 1691, 1677 (multiple bands, CO). $^1H$ NMR ($CDCl_3$) (δ): 3.74 (3H, s, $OCH_3$); 6.28 (1H, d, CH, *J* = 15.1 Hz); 6.82–9.02 (11H, a set of signals, aromatic protons and CH). **9c**: IR ($cm^{-1}$): 1672 (CO). $^1H$ NMR (DMSO-$d_6$) (*δ*): 3.77 (3H, s, $OCH_3$); 5.96 (1H, d, CH, *J* = 15.2 Hz); 6.88–9.18 (11H, a set of signals, aromatic protons and CH). **9d**: IR ($cm^{-1}$): 1684 (CO). $^1H$ NMR ($CDCl_3$) (δ): 3.71 (3H, s, $OCH_3$); 3.80 (3H, s, $OCH_3$); 6.17 (1H, d, CH, *J* = 15.4 Hz); 6.37–9.04 (10H, a set of signals, aromatic protons and CH). **9e**: IR ($cm^{-1}$): 1685 (CO). $^1H$ NMR ($CDCl_3$) (*δ*): 3.73 (3H, s, $OCH_3$); 3.85 (3H, s, $OCH_3$); 6.38 (1H, d, CH, *J* = 15.7 Hz); 6.88–9.02 (10H, a set of signals, aromatic protons and CH). **9h**: IR ($cm^{-1}$): 1681 (CO). $^1H$ NMR ($CDCl_3$) (*δ*): 3.73 (3H, s, $OCH_3$); 3.81 (3H, s, $OCH_3$); 3.84 (3H, s, $OCH_3$); 6.24 (1H, d, CH, *J* = 15.5 Hz); 6.57–9.01 (9H, a set of signals, aromatic protons and CH). **9i**: IR ($cm^{-1}$): 1685 (CO). $^1H$ NMR (DMSO-$d_6$) (δ): 6.22 (1H, d, CH, *J* = 15.2 Hz); 7.57–9.15 (11H, a set of signals, aromatic protons and CH). **9l**: IR ($cm^{-1}$): 1672 (CO). $^1H$ NMR (DMSO-$d_6$) (δ): 6.21 (1H, d, CH, *J* = 15.6 Hz); 7.22–9.18 (11H, a set of signals, aromatic protons and CH).

### *5.2. Biology*

#### *5.2.1. Antiproliferative activity in vitro*

Compounds **8a–l** and **9a,c–e,h–l** were tested in vitro for antileukemic activity against L-1210 (murine leukemia), K-562 (human chronic myelogenous leukemia) and HL-60 (human leukemia) cell lines. These cell lines were grown at 37 °C in a humidified atmosphere containing 5% $CO_2$, in RPMI-1640 medium (Biochrom KG) supplemented with 10% fetal calf serum and antibiotics.

Cells were suspended at a density of $1 \times 10^5$ (L-1210 and K-562) or $2\ 10^5$ (HL-60) cells per ml in growth medium, transferred to 24-well plate (1 ml per well), cultured with or without (control wells) screening concentration of compounds and incubated at 37 °C for 48 h (HL-60, K-562) or

72 h (L-1210). Control wells were added with DMSO used to dissolve our compounds to exclude a solvent activity.

Numbers of viable cells were determined by counting in a hematocytometer after dye exclusion with trypan blue [10]. We determined $IC_{50}$ values (test agent concentration at which the cell proliferation was inhibited to 50% of the untreated growth control) for compounds that exhibited the best activity at screening concentration.

## Acknowledgements

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