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Substituted 2-Styrylquinazoline Derivatives: Preparation and Their Biological Activities

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Abstract: In this study, a series of five ring-substituted 2-styrylquinazolin-4(3*H*)-one and five ring-substituted 4-chloro-2-styrylquinazoline derivatives were prepared. The procedures for synthesis of the compounds are presented. The compounds were analyzed using RP-HPLC to determine lipophilicity. They were tested for their activity related to inhibition of photosynthetic electron transport (PET) in spinach (*Spinacia oleracea* L.) chloroplasts. Primary *in vitro* screening of the synthesized compounds was also performed against four mycobacterial strains. Several compounds showed biological activity comparable with or higher than the standard isoniazid. For all the compounds, the relationships between the lipophilicity and the chemical structure of the studied compounds are discussed, as well as their structure-activity relationships (SAR).

Keywords: Styrylquinazoline derivatives; Lipophilicity; PET inhibition; Spinach chloroplasts; *In vitro* antimycobacterial activity; Structure-activity relationships.

INTRODUCTION

A quinoline moiety is present in many classes of biologically-active compounds. A number of them have been clinically used as antifungal, antibacterial and antiprotozoic drugs [1,2] as well as antituberculotic agents [3-6]. Some quinoline-based compounds also showed antineoplastics, antiasthmatic and antiplatelet activity [7-12]. A series of compounds derived from 8-hydroxyquinoline and styrylquinoline derivatives as potential HIV-1 integrase inhibitors were recently synthesized [13-16]. These compounds showed a significant similarity to some novel antifungal agents, namely homoallylamines. [17]. Our previous study dealing with 8-hydroxyquinoline and styrylquinoline derivatives showed that they could also possess strong antifungal activity [18,19]. According to the results reported recently, some new hydroxyquinoline derivatives also possess interesting herbicidal activities [18,20-22]. In addition, some of the quinoline derivatives investigated also showed antineoplastic activity [20,23].

Tuberculosis is a worldwide pandemic. About 1/3 of the world's population is infected with *Mycobacterium tuberculosis*, and every year almost 2 million people die as a result [24]. The *Mycobacterium* genus is composed of the *M. tuberculosis* complex and other species known as nontuberculous mycobacteria (NTM). In recent decades, the decrease in the prevalence of tuberculosis in developed countries has resulted in an increase in the proportion of diseases caused by NTM [25]. Among these species, the *M. avium* complex (MAC) has emerged as a major human pathogen, being a common cause of disseminated disease and death in patients with HIV/AIDS [26]. Chronic pulmonary disease is the most common clinical manifestation among the diseases caused by NTM, and the most common pathogens are the species belonging to the MAC, followed by *M. kansasii*. The clinical characteristics of NTM-related pulmonary disease are in many cases very similar to those of tuberculosis. Other clinical manifestations are caused principally by *M. fortuitum*, *M. smegmatis* and *M. abscessus* due to peritoneal infection as a result of catheterization or postsurgical infections [27]. The above mentioned nontuberculous strains are sometimes resistant to commonly used drugs (isoniazid, rifampicin, pyrazinamide) and other antituberculous drugs [24], therefore systematic development of new effective compounds is necessary.

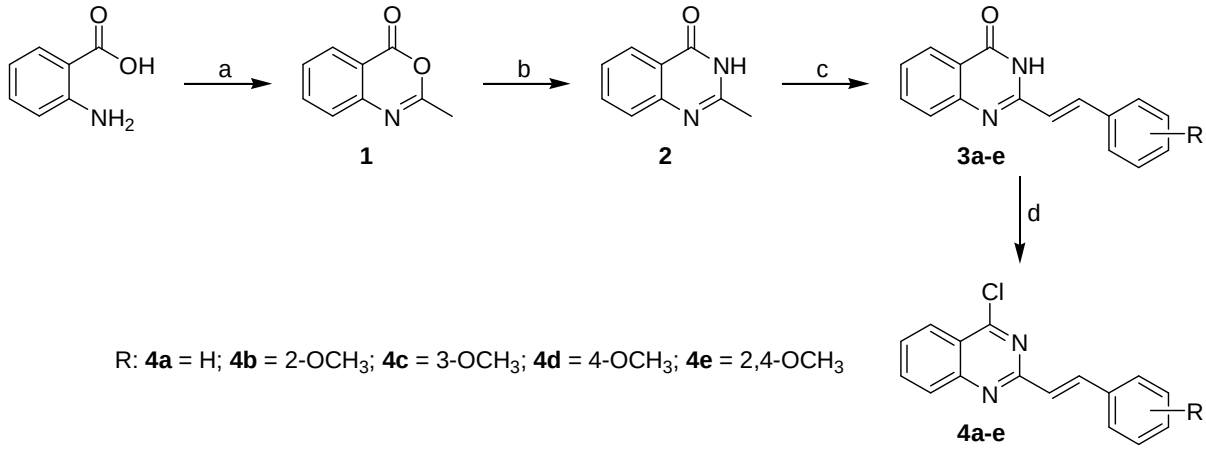
Over 50% of commercially available herbicides act by reversibly binding to photosystem II (PS II), a membrane-protein complex in the thylakoid membranes which catalyses the oxidation of water and the reduction of plastoquinone [28] and thereby inhibit photosynthesis [29-31]. Some organic compounds, e.g. substituted benzanilides [32] or substituted anilides of 2,6-disubstituted pyridine-4-thiocarboxamides [33] or pyrazine-2-carboxylic acids [34] were found to interact with tyrosine radicals Tyr_Z and Tyr_D which are situated in D_1 and D_2 proteins on the donor side of PS II and due to this interaction interruption of the photosynthetic electron transport occurred.

This is a follow-up paper to our previous articles [6,13-16,18-23] dealing with synthesis and biological activities of ring-substituted quinazolinone derivatives. Primary *in vitro* screening of the synthesized compounds was performed against four mycobacterial strains. The compounds were also tested for their photosynthesis-inhibiting activity (the inhibition of photosynthetic electron transport in spinach chloroplasts (*Spinacia oleracea* L.). Lipophilicity (log *k*) of the compounds was determined using RP-HPLC. The procedure was performed under isocratic conditions with methanol as an organic modifier in the mobile phase using end-capped non-polar C_{18} stationary RP column. Relationships among the structure and *in vitro* antimicrobial activities or/and inhibitory activity related to inhibition of photosynthetic electron transport (PET) in spinach chloroplasts of the new compounds are discussed.

RESULTS AND DISCUSSION

All studied compounds were prepared according to Scheme 1. Microwave-assisted synthesis facilitated the process of obtaining quinazoline-related structures. 2-Methyl-4*H*-benzo[*d*][1,3]oxazin-4-one (**1**) was synthesized from anthranilic acids and acetic anhydride. A further reaction with ammonia afforded 2-methylquinazolin-4(3*H*)-one (**2**). Compounds **3a-e** were obtained from appropriate aldehydes using neat microwave-assisted synthesis [35]. Further aromatization with $POCl_3$ yielded 4-chloro-2-styrylquinazoline derivatives (**4a-e**).

Scheme 1. Synthesis of quinazoline derivatives **1**-**4**: (a) Ac_2O, microwave irradiation; (b) $NH_{3\ aq}$, microwave irradiation; (c) aldehyde, microwave irradiation; (d) $POCl_3$.



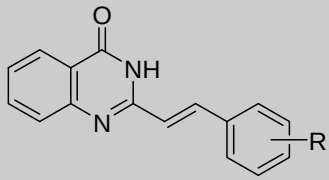
R: **4a** = H; **4b** = 2-OCH_3; **4c** = 3-OCH_3; **4d** = 4-OCH_3; **4e** = 2,4-OCH_3

Hydrophobicities (log *P*/Clog *P*) of compounds **3a-e** and **4a-e** were calculated using two commercially available programs (ChemDraw Ultra 10.0 and ACD/LogP) and also measured by means of the RP-HPLC determination of capacity factors *k* with subsequent calculation of log *k*. The procedure was performed under isocratic conditions with methanol as an organic modifier in the mobile phase using an end-capped non-polar C_{18} stationary RP column. The program ChemDraw did not resolve the varying lipophilicity values of individual positional isomers, in that the same log *P*/Clog *P* data were calculated for **3b-d** and **4b-d**. The results are shown in Table 1 and illustrated in Figure 1.

The results obtained with respect to all compounds show that the experimentally determined lipophilicities (log *k* values) are lower than those indicated by the calculated log *P*/Clog *P* as shown in Figure 1. The results show that experimentally determined log *k* values correlate relatively poorly with the calculated log *P*/Clog *P*. Surprisingly, in RP-HPLC measurements series **4** showed much lower lipophilicity than expected on the basis of calculated log *P*/Clog *P* data. Series **4** possessed lower hydrophobicity than series **3** in contrast to all calculated data.

Compounds **3e** and **4e** showed the highest lipophilicity, while compounds **3a** and **4a** possessed the lowest hydrophobicity within individual series of compounds. Compounds **3a** and **4a** showed lower hydrophobicity in comparison with the lipophilicity results calculated by the software. If the lipophilicity data log *k* of three position isomers **3b-d**, **4b-d** are compared, it can be stated that 3-methoxy derivative **3c**/**4c** possessed higher hydrophobicity than 2-methoxy derivative **3b**/**4b** and 4-methoxy derivative **3d**/**4d** showed the lowest lipophilicity. Due to the facts discussed above, it can be assumed that lipophilicity of individual compounds within both series is strongly influenced by intramolecular interactions. It can be assumed, that the determined log *k* data specify lipophilicity within the individual series of compounds (H < 4-OCH_3 < 2-OCH_3 < 3-OCH_3 < 2,4-OCH_3), see Figure 1.

Table 1. Comparison of the calculated lipophilicities (log *P*/Clog *P*) with the determined log *k* values of compounds **3a-e**, **4a-e**, electronic Hammett's parameters σ and bulk parameters MR (volume of substituents) [36].



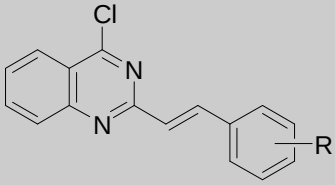
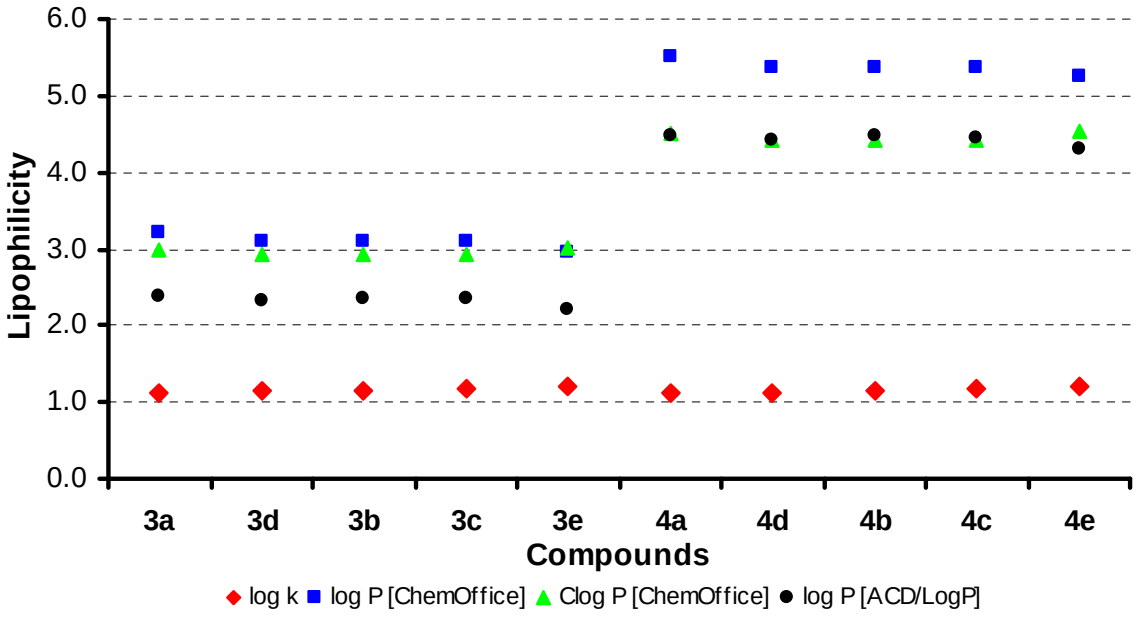
Comp.	R	log *k*	log *P*/Clog *P* ChemOffice	log *P* ACD/LogP	σ [36]	MR [36]
3a	H	1.1148	3.21 / 2.997	2.37 ± 0.61	0.0	0.0
3b	2-OCH_3	1.1509	3.09 / 2.916	2.35 ± 0.62	-0.390 [37]	6.5
3c	3-OCH_3	1.1837	3.09 / 2.916	2.35 ± 0.62	0.115	6.5
3d	4-OCH_3	1.1351	3.09 / 2.916	2.32 ± 0.62	-0.268	6.5
3e	2,4-OCH_3	1.1982	2.96 / 3.005	2.20 ± 0.62	-0.658	13.0
						
4a	H	1.1088	5.50 / 4.51522	4.47 ± 0.56	0.0	0.0
4b	2-OCH_3	1.1497	5.38 / 4.43422	4.47 ± 0.57	-0.390 [37]	6.5
4c	3-OCH_3	1.1827	5.38 / 4.43422	4.44 ± 0.57	0.115	6.5
4d	4-OCH_3	1.1337	5.38 / 4.43422	4.41 ± 0.57	-0.268	6.5
4e	2,4-OCH_3	1.1953	5.25 / 4.52322	4.30 ± 0.57	-0.658	13.0

Figure 1. Comparison of the log *P*/Clog *P* values computed using two the programs with the calculated log *k* values. Compounds **3a-4e** and **4a-e** are ordered according to the increase in log *k* values.



The evaluated quinazoline derivatives showed relatively low activity related to inhibition of photosynthetic electron transport (PET) in spinach chloroplasts, see Table 2. Compounds **4a** and **4c** expressed the highest PET-inhibiting activity (IC_{50}: 285 and 303 µmol/L, respectively). PET inhibition by several compounds (**3a-c** and **4b**) could not be determined due to precipitation of the compounds during the experiment. The PET-inhibiting activity was expressed by negative logarithm of IC_{50} value (compound concentration in mol/L causing 50% inhibition of PET). Despite the relatively low inhibitory activity of the studied compounds as well as the relative scarcity of compounds for which PET-inhibiting activity could be determined, correlations between log ($1/IC_{50}$) and log *k* or Hammett's parameters (σ) of the R substituent for compounds **4a-d** were performed. σ values [36,37] mentioned in Table 1 were used for calculations. The σ value for R: 2,4-OCH_3 was calculated from the sum of corresponding σ values for R: 2-OCH_3 and 4-OCH_3.

Table 2. IC_{50} [µmol/L] values related to PET inhibition in spinach chloroplasts and antimycobacterial activity MIC/IC_{90} [µg/mL] of compounds **3a-e**, **4a-e** in comparison with standards 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), pyrazinamide (PZA) and isoniazid (INH).

Comp.	PET inhibition IC_{50} [µmol/L]	MIC/IC_{90} [µg/mL]			
		M. smegmatis	*M. absessus*	*M. kansasii*	*M. avium* complex
3a	[a]	>300	>300	>300	>300
3b	[a]	>100	>100	>100	>100
3c	[a]	>100	>100	>100	>100
3d	693	>100	>100	>100	>100
3e	391	>100	80	20	80
4a	285	>100	>100	>100	>100
4b	[a]	>100	80	60	80
4c	303	>100	>100	60	>100
4d	390	>100	>100	>100	>100
4e	508	>100	>100	>100	>100
DCMU	1.9	–	–	–	–
PZA	–	>100	>100	>100	>100
INH	–	39	>100	<10	<10

[a] precipitation during the experiment.

The inhibitory activity of compounds **4a-e** depended predominantly on the Hammett's constants (σ) of R substituents:

$\log (1/IC_{50}) = 3.507 (\pm 0.020) + 0.324 (\pm 0.056)\ \sigma$

$r = 0.971$, $s = 0.033$, $F = 33.24$, $n = 4$

The introduction of the lipophilicity of the whole molecule (expressed by log *k*) in the above equation for compounds **4a-e** partially improved the results of statistical analysis:

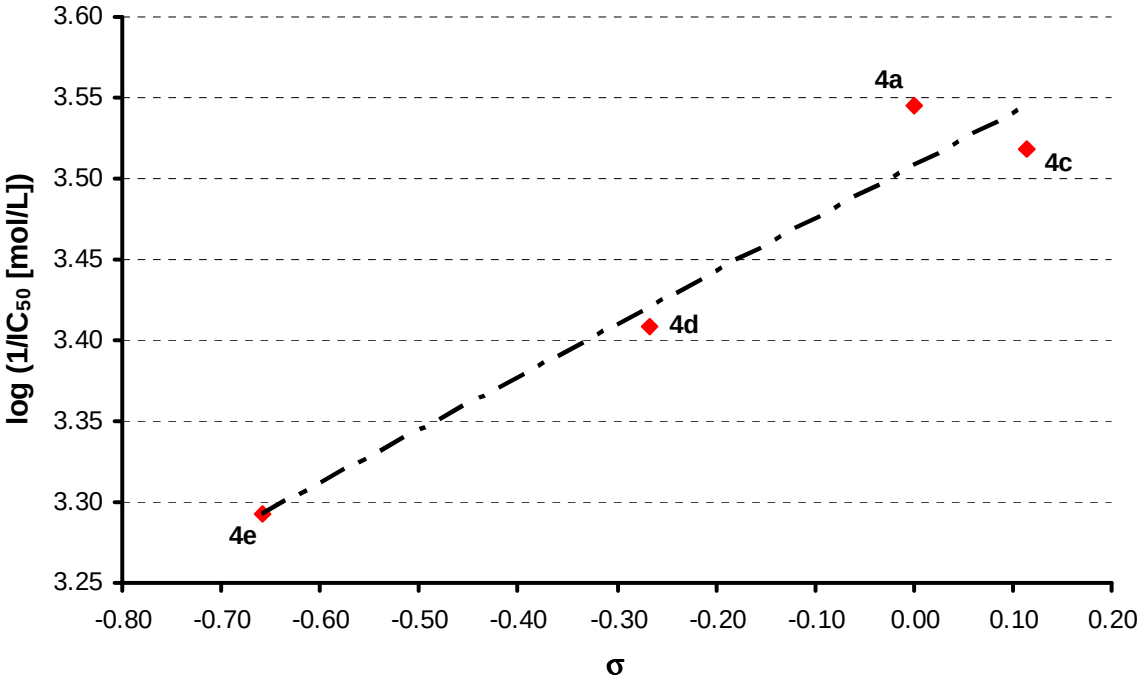
$\log (1/IC_{50}) = 4.122 (\pm 0.579) - 0.537 (\pm 0.506) \log k + 0.298 (\pm 0.060)\ \sigma$

$r = 0.987$, $s = 0.032$, $F = 18.24$, $n = 4$

Thus, it could be concluded that the electronic properties of the R substituent were decisive for photosynthesis-inhibiting activity. The dependences of PET-inhibiting activity on the Hammett's constants (σ) of the R substituent of the studied compounds are shown in Figure 2. There the PET-inhibiting activity was expressed by negative logarithm of IC_{50} value.

IC_{50} values related to PET-inhibition determined for both compounds **3d** and **3e** do not enable us to make conclusions about structure-activity relationships.

Figure 2. The relationships between the PET-inhibiting activity log ($1/IC_{50}$) [mol/L] in spinach chloroplasts and the electronic Hammett's constants (σ) of the R substituent of the studied compounds **4a-e**.



All compounds were evaluated for their *in vitro* antimycobacterial activity against four mycobacterial strains and the results are shown in Table 2. All the evaluated compounds except for **3a** (H) showed biological activity comparable with or higher than the standard pyrazinamide (PZA). Nevertheless it can be stated that compounds **3b** (2-OCH_3), **3c** (3-OCH_3), **3d** (4-OCH_3), **4a** (H), **4d** (3-OCH_3) and **4e** (2,4-OCH_3) did not exhibit any significant antimycobacterial activity.

Compound **4b** (2-OCH_3) had an interesting MIC especially against *M. absessus*, *M. kansasii* and *M. avium* complex and likewise compound **4c** (3-OCH_3) against *M. kansasii*. Both compounds were either more active than or comparable with the standard pyrazinamide (PZA) in all cases. Compound **4b** was more active in case of *M. absessus* as its activity was higher than the standard isoniazid (INH). 2-[(*E*)-2-(2,4-Dimethoxyphenyl)vinyl]quinazolin-4(3*H*)-one (**3e**) expressed the highest activity against *M. kansasii*, *M. avium* complex and *M. absessus*. This compound is more active than PZA and in the case of *M. absessus* it is more active than INH.

Due to the medium and/or moderate activity of the compounds **3a-e** and **4a-e**, it is difficult to determine simple structure-activity relationships. However some observations seem to be interesting. The position and the number of substitutions on the benzylidene part of the molecule is important for antimycobacterial activity; for example, if one compares the activity of compounds **3a**/**3e** (the unsubstituted compound without any activity / the disubstituted compound with the highest activity) or **4b**/**4a**,**4d**,**4e** (2-OCH_3 isomer showed the highest activity within this series). Bulk parameters (volume/number of the substituents on the benzylidene part) MR [36] are also very important for activity, especially again for series **3**. According to Tables 1 and 2 it can be assumed that compounds with bulky substituents showed higher antimycobacterial activity. Unsubstituted compound **3a** (H) possessed less activity than methoxy monosubstituted compounds **3b**-**3d**. Disubstituted compound **3e** (2,4-OCH_3) exhibited the highest activity.

EXPERIMENTAL

General

All reagents were purchased from Aldrich. Kieselgel 60, 0.040-0.063 mm (Merck, Darmstadt, Germany) was used for column chromatography. TLC experiments were performed on alumina-backed silica gel 40 F_{254} plates (Merck, Darmstadt, Germany). The plates were illuminated under UV (254 nm) and evaluated in iodine vapour. The melting points were determined on a Boetius PHMK 05 instrument (VEB Kombinat Nagema, Radebeul, Germany) and are uncorrected. The purity of the final compounds was checked by HPLC. A detection wavelength of 210 nm was chosen. The peaks in the chromatogram of the solvent (blank) were deducted from the peaks in the chromatogram of the sample solution. The purity of individual compounds was determined from the area peaks in the chromatogram of the sample solution. UV spectra (λ, nm) were determined on a Waters Photodiode Array Detector 2996 (Waters Corp., Milford, MA, U.S.A.) in methanolic solution (*ca.* 6×10^{-4} mol) and log ε (the logarithm of molar absorption coefficient ε) was calculated for the absolute maximum λ_{max} of individual target compounds. Infrared spectra were recorded using KBr pellets on the FT-IR spectrometer Nicolet 6700 (Nicolet - Thermo Scientific, U.S.A.). All ^{1}H NMR spectra were recorded on a Bruker AM-500 (499.95 MHz for ^{1}H), Bruker BioSpin Corp., Germany. Chemical shifts are reported in ppm (δ) against the internal standard, $Si(CH_3)_4$. Easily exchangeable signals were omitted when diffuse. Syntheses were performed on Plazmatronika RM-800PC microwave reactor with monomode cavity, magnetic stirrer and external IR temperature measurements. Microwave power was automatically adjusted to achieve the desired temperature unless specified otherwise.

Synthesis

General method for synthesis of styryl-compounds **3a-e**: A mixture of compound **2** (0.01 mol) and the appropriate aldehyde (0.02 mol) was mixed thoroughly and irradiated in monomode cavity of microwave reactor using pulse sequence (3×5 minutes with 30 sec. intervals) at 250 W. During irradiation, the temperature was controlled between the range 150-180 °C. After the reaction, the mixture was cooled and washed with boiling ether. The product was crystallized from acetic acid.

2-(E)-Styrylquinazolin-4(3H)-one (**3a**). [38] Yield 50% of a white crystalline compound. Mp 253-255 °C (Mp 252 °C [39]). HPLC purity: 97.95%. UV (nm), λ_{max}/log ε: 321.3/3.53. ^{1}H NMR (DMSO-d_6, 500 MHz), δ: 7.00 (d, *J*=16.23 Hz, 1H, C=C-H), 7.41 (t, 1H, Ar-H), 7.42-7.49 (m, 3H, Ar-H), 7.65-7.68 (m, 3H, Ar-H), 7.80 (t, 1H, Ar-H), 7.95 (d, *J*=16.16 Hz, 1H, C=C-H), 8.10 (d, 1H, Ar-H), 12.35 (s, 1H, N-H).

2-[(E)-2-(2-Methoxyphenyl)vinyl]quinazolin-4(3H)-one (**3b**). [38] Yield 76% of a white crystalline compound. Mp 234-236 °C (Mp 234-236 °C [39]). HPLC purity: 94.04%. UV (nm), λ_{max}/log ε: 343.4/3.62. ^{1}H NMR (DMSO-d_6, 500 MHz), δ: 3.90 (s, 3H, OCH_3), 7.02 (t, 1H, Ar-H), 7.07 (d, *J*=16.24 Hz, 1H, C=C-H), 7.11 (d, 1H, Ar-H), 7.39 (t, 1H, Ar-H), 7.45 (t, 1H, Ar-H), 7.60 (d, 1H, Ar-H), 7.67 (d, 1H, Ar-H), 7.79 (t, 1H, Ar-H), 8.09 (d, 1H, Ar-H), 8.15 (d, *J*=16.12 Hz, 1H, C=C-H), 12.36 (s, 1H, N-H).

2-[(E)-2-(3-Methoxyphenyl)vinyl]quinazolin-4(3H)-one (**3c**). [38] Yield 68% of a white crystalline compound. Mp 239-241 °C. HPLC purity: 96.82%. UV (nm), λ_{max}/log ε: 326.4/3.58. ^{1}H NMR (DMSO-d_6, 500 MHz), δ: 3.81 (s, 3H, OCH_3), 6.98 (d, 1H, Ar-H), 7.01 (d, *J*=16.81 Hz, 1H, C=C-H), 7.22 (s, 1H, Ar-H), 7.23 (d, 1H, Ar-H), 7.37 (t, 1H, Ar-H), 7.47 (t, 1H, Ar-H), 7.66 (d, 1H, Ar-H), 7.80 (t, 1H, Ar-H), 7.91 (d, *J*=16.14 Hz, 1H, C=C-H), 8.10 (d, 1H, Ar-H), 12.31 (s, 1H, NH).

2-[(E)-2-(4-Methoxyphenyl)vinyl]quinazolin-4(3H)-one (**3d**). [38] Yield 33% of a white crystalline compound. Mp 280-281 °C (Mp 284-285 °C [39]). HPLC purity: 94.36%. UV (nm), λ_{max}/log ε: 322.7/3.59. ^{1}H NMR (DMSO-d_6, 500 MHz), δ: 3.80 (s, 3H, OCH_3), 6.84 (d, *J*=16.23 Hz, 1H, C=C-H), 7.01 (d, 2H, Ar-H), 7.45 (t, 1H, Ar-H), 7.60 (d, 2H, Ar-H), 7.64 (d, 1H, Ar-H), 7.78 (t, 1H, Ar-H), 7.90 (d, *J*=16.08 Hz, 1H, C=C-H), 8.08 (d, 1H, Ar-H), 12,25 (s, 1H, N-H).

2-[(E)-2-(2,4-Dimethoxyphenyl)vinyl]quinazolin-4(3H)-one (**3e**). [38] Yield 57% of a white crystalline compound. Mp 228-230 °C (Mp 228-230 °C [39]). HPLC purity: 96.27%. UV (nm), λ_{max}/log ε: 350.1/3.67. ^{1}H NMR (DMSO-d_6, 500 MHz), δ: 3.82 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.63 (d, 1H, Ar-H), 6.64 (s, 1H, Ar-H), 6.94 (d, *J*=16.15 Hz, 1H, C=C-H), 7.43 (t, 1H, Ar-H), 7.53 (d, 1H, Ar-H), 7.64 (d, 1H, Ar-H), 7.77 (t, 1H, Ar-H), 8.07 (d, *J*=15.21 Hz, 1H, C=C-H), 8.08 (d, 1H, Ar-H), 12.26 (s, 1H, N-H).

General method for synthesis of compounds **4a-e**: A mixture of styrylquinazolinone derivatives **3** (0.01 mol), *N*,*N*-dimethylaniline (0.02 mol) and phosphorus oxychloride (0.015 mol) in dry benzene (50 mL) was stirred and heated under reflux for 3 h. The reaction mixture was then cooled and filtered. The filtrate was diluted with benzene (30 mL) and the solution washed with water (50 mL), twice with 20% aqueous NaOH (50 mL) and finally twice with water. After drying with $MgSO_4$, the organic solvent was evaporated and the product obtained was crystallized from heptane.

4-Chloro-2-(E)-styrylquinazoline (**4a**). [38] Yield 81% of an orange crystalline compound. Mp 104 °C, (Mp 100-101 °C [40]). HPLC purity: 99.34%. UV (nm), λ_{max}/log ε: 314.9/3.67. ^{1}H NMR (DMSO-d_6, 500 MHz), δ: 7.34 (d, *J*=15.91 Hz, 1H, C=C-H), 7.41 (t, 1H, Ar-H), 7.47 (t, 2H, Ar-H), 7.77-7.80 (m, 3H, Ar-H), 8.02 (d, 1H, Ar-H), 8.06 (t, 1H, Ar-H), 8.12 (d, *J*=16.01 Hz, 1H, C=C-H), 8.26 (d, 1H, Ar-H).

4-Chloro-2-[(E)-2-(2-methoxyphenyl)vinyl]quinazoline (**4b**). [38] Yield 86% of a light yellow crystalline compound. Mp 153 °C. HPLC purity: 99.95%. UV (nm), λ_{max}/log ε: 345.1/3.67. ^{1}H NMR (DMSO-d_6, 500 MHz), δ: 3.98 (s, 3H, OCH_3), 7.04 (t, 1H, Ar-H), 7.12 (d, 1H, Ar-H), 7.37 (d, *J*=16.15 Hz, 1H, C=C-H), 7.40 (t, 1H, Ar-H), 7.78 (t, 1H, Ar-H), 7.82 (d, 1H, Ar-H), 8.01 (d, 1H, Ar-H), 8.06 (t, 1H, Ar-H), 8.27 (d, 1H, Ar-H), 8.47 (d, *J*=16.15 Hz, 1H, C=C-H).

4-Chloro-2-[(E)-2-(3-methoxyphenyl)vinyl]quinazoline (**4c**). [38] Yield 81% of a light yellow crystalline compound. Mp 137 °C. HPLC purity: 98.62%. UV (nm), λ_{max}/log ε: 342.9/3.64. ^{1}H NMR (DMSO-d_6, 500 MHz), δ: 3.90 (s, 3H, OCH_3), 6.98 (d, 1H, Ar-H), 7.35 (d, *J*=15.75 Hz, 1H, C=C-H), 7.35-7.39 (m, 2H, Ar-H), 7.36 (s, 1H, Ar-H), 7.80 (t, 1H, Ar-H), 8.02 (d, 1H, Ar-H), 8.06 (d, 1H, Ar-H), 8.10 (d, *J*=15.82 Hz, 1H, C=C-H), 8.28 (d, 1H, Ar-H).

4-Chloro-2-[(E)-2-(4-methoxyphenyl)vinyl]quinazoline (**4d**). [38] Yield 51% of a yellow crystalline compound. Mp 130-131 °C, (Mp 130-131 °C [40]). HPLC purity: 97.43%. UV (nm), λ_{max}/log ε: 339.9/3.64. ^{1}H NMR (DMSO-d_6, 500 MHz), δ: 3.87 (s, 3H, OCH_3), 7.03 (d, 2H, Ar-H), 7.20 (d, *J*=15.87 Hz, 1H, C=C-H), 7.75 (d, 2H, Ar-H), 7.76 (t, 1H, Ar-H), 7.99 (d, 1H, Ar-H), 8.04 (t, 1H, Ar-H), 8.08 (d, *J*=15.89 Hz, 1H, C=C-H), 8.25 (d, 1H, Ar-H).

4-Chloro-2-[(E)-2-(2,4-dimethoxyphenyl)vinyl]quinazoline (**4e**). [38] Yield 48% of a yellow crystalline compound. Mp 172 °C. HPLC purity: 98.57%. UV (nm), λ_{max}/log ε: 355.0/3.67. IR (KBr, cm^{-1}): 2980, 2938, 1607, 1556, 1504, 1477, 1450, 1384, 1329, 959, 768, 756.

^{1}H NMR ($(CD_3)_2CO$, 500 MHz), δ: 3.88 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 6.64 (d, 1H, Ar-H), 6.66 (s, 1H, Ar-H), 7.27 (d, *J*=16.13 Hz, 1H, C=C-H), 7.75 (d, 1H, Ar-H), 7.60 (t, 1H, Ar-H), 7.98 (d, 1H, Ar-H), 8.02 (t, 1H, Ar-H), 8.25 (d, 1H, Ar-H), 8.40 (d, *J*=16.13 Hz, 1H, C=C-H).

Lipophilicity HPLC determination (capacity factor k / calculated log k)

The HPLC separation module Waters Alliance 2695 XE and Waters Photodiode Array Detector 2996 (Waters Corp., Milford, MA, U.S.A.) were used. The chromatographic column Symmetry® C_{18} 5 μm, 4.6×250 mm, Part No. WAT054275, (Waters Corp., Milford, MA, U.S.A.) was used. The HPLC separation process was monitored by the Millennium32® Chromatography Manager Software, Waters 2004 (Waters Corp., Milford, MA, U.S.A.). The mixture of MeOH p.a. (55.0%) and H_2O-HPLC – Mili-Q Grade (45.0%) was used as a mobile phase. The total flow of the column was 0.9 mL/min, injection 30 μL, column temperature 30 °C and sample temperature 10 °C. The detection wavelength of 210 nm was chosen. The KI methanolic solution was used for the dead time (t_D) determination. Retention times (t_R) were measured in minutes.

The capacity factors *k* were calculated using the Millennium32® Chromatography Manager Software according to the formula $k = (t_R - t_D) / t_D$, where t_R is the retention time of the solute, whereas t_D denotes the dead time obtained via an unretained analyte. Log *k*, calculated from the capacity factor *k*, is used as the lipophilicity index converted to the log *P* scale. The log *k* values of the individual compounds are shown in Table 1.

Lipophilicity calculations

Log *P*, *i.e.* the logarithm of the partition coefficient for *n*-octanol/water, was calculated using the programmes CS ChemOffice Ultra ver. 10.0 (CambridgeSoft, Cambridge, MA, U.S.A.) and ACD/LogP ver. 1.0 (Advanced Chemistry Development Inc., Toronto, Canada). Clog *P* values (the logarithm of *n*-octanol/water partition coefficient based on established chemical interactions) were generated by means of the CS ChemOffice Ultra ver. 10.0 (CambridgeSoft, Cambridge, MA, U.S.A.) software. The results are shown in Table 1.

Study of inhibition photosynthetic electron transport (PET) in spinach chloroplasts

Chloroplasts were prepared from spinach (*Spinacia oleracea* L.) according to Masarovicova and Kralova [41]. The inhibition of oxygen evolution rate (inhibition of photosynthetic electron transport, PET) in spinach chloroplasts was determined spectrophotometrically (Genesys 6, Thermo Scientific, U.S.A.) using the artificial electron acceptor 2,6-dichlorophenol-indophenol (DCIPP) according to Kralova et al. [42] and the rate of photosynthetic electron transport was monitored as a photoreduction of DCPIP. The measurements were carried out in phosphate buffer (0.02 mol/L, pH 7.2) containing sucrose (0.4 mol/L), $MgCl_2$ (0.005 mol/L) and NaCl (0.015 mol/L). The chlorophyll content was 30 mg/L in these experiments and the samples were irradiated (~100 W/m^2) from 10 cm distance with a halogen lamp (250 W) using a 4 cm water filter to prevent warming of the samples (suspension temperature 22 °C). The studied compounds were dissolved in DMSO due to their limited water solubility. The applied DMSO concentration (up to 4%) did not affect the photochemical activity in spinach chloroplasts. The inhibitory efficiency of the studied compounds was expressed by IC_{50} values, *i.e.* by molar concentration of the compounds causing 50% decrease in the oxygen evolution rate relative to the untreated control. The comparable IC_{50} value for a selective herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea, DCMU (Diurone®) was about 1.9 μmol/L [43]. The results are summarized in Table 2.

In vitro antimycobacterial evaluation

Clinical isolates of *Mycobacterium avium* complex CIT19/06, *M. kansasii* CIT11/06, *M. absessus* CIT21/06 and strain *M. smegmatis* MC2155 were grown in Middlebrook broth (MB), supplemented with OADC supplement (Oleic, Albumin, Dextrose, Catalase, Becton Dickinson, U.K.). Identification of these isolates was performed using biochemical and molecular protocols. At log phase growth, the 10 mL culture was centrifuged at 15,000 RPM for 20 minutes using a bench top centrifuge (Model CR 4-12 Jouan Inc U.K). Following the removal of the supernatant, the pellet was washed in fresh Middlebrook 7H9GC broth and re-suspended in 10 ml of fresh supplemented MB. The turbidity was adjusted to match McFarland standard No. 1 (3×10^8 CFU) with MB broth. A further 1:20 dilution of the culture was then performed in MB broth.

The antimicrobial susceptibility of all four mycobacteria was investigated in 96 well plate format. Here, 300 µL of sterile deionised water was added to all outer-perimeter wells of the plates to minimize evaporation of the medium in the test wells during incubation. 300 µL of each dilution was incubated with 300 µL of each of the mycobacterial species. Dilutions of each compound were prepared in duplicate. For all synthesized compounds, final concentrations ranged from 300 µg/mL to 10 µg/mL. All compounds were prepared in DMSO and subsequent dilutions were made in supplemented Middlebrook broth. The plates were sealed with parafilm and were incubated at 37 °C overnight in the case of *M. smegmatis* and *M. absessus* and for 5 days in the case of *M. kansasii* and *M. avium* complex. Following incubation, a 10% addition of alamarBlue (AbD Serotec) was mixed into each well and readings at 570 nm and 600 nm were taken, initially for background subtraction and subsequently after 24 hour re-incubation. The background subtraction is necessary with strongly coloured compounds which may interfere with the interpretation of any colour change. In non-interfering compounds, a blue colour in the well was interpreted as an absence of growth, and a pink colour was scored as growth. The MIC was initially defined as the lowest concentration which prevented a visual colour change from blue to pink. The results are shown in Table 2.

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REFERENCES

1. Roth, H.J.; Fenner, H. *Arzneistoffe* 3rd Ed.; Deutscher Apotheker Verlag: Stuttgart, Germany, 2000; pp. 51–114.
2. Harris, C.R.; Thorarensen, A. *Curr. Med. Chem.* **2004**, *11*, 2213.
3. Andries, K.; Verhasselt, P.; Guillemont, J.; Gohlmann, H.W.; Neefs, J.M.; Winkler, H.; Van Gestel, J.; Timmerman, P.; Zhu, M.; Lee, E.; Williams, P.; de Chaffoy, D.; Huitric, E.; Hoffner, S.; Cambau, E.; Truffot-Pernot, C.; Lounis, N.; Jarlier, V. *Science* **2005**, *307*, 223.
4. Vangapandu, S.; Jain, M.; Jain, R.; Kaur, S.; Singh, P.P. *Bioorg. Med. Chem.* **2004**, *12*, 2501.
5. Carta, A.; Piras, S.; Palomba, M.; Jabes, D.; Molicotti, P.; Zanetti, S. *Anti-Infective Agents Med. Chem.* **2008**, *7*, 134.

6. Jampilek, J.; Musiol, R.; Carroll, J.; O'Mahony, J.; Coffey, A.; Finster, J.; Tabak, D.; Niedbala, H.; Dohnal, J.; Polanski, J. Book of Abstracts: *The 6th Joint Meeting on Medicinal Chemistry 2009*, Budapest, Hungary, June 24-27, 2009, p. 73
7. Sissi, C.; Palumbo, M. *Curr. Med. Chem. Anti-Canc. Agents* **2003**, *3*, 439.
8. Bossu, E.; Agliano, A.M.; Desideri, N.; Sestili, I.; Porra, R.; Grandilone, M.; Quaglia, M.G. *J. Pharm. Biomed. Anal.* **1999**, *19*, 539.
9. Ko, T.C.; Hour, M.J.; Lien, J.C.; Teng, C.M.; Lee, K. H.; Kuo, S.C.; Huang, L.J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 279.
10. Jampilek, J.; Dolezal, M.; Kunes, J.; Vichova, P.; Jun, D.; Raich, I.; O´Connor, R.; Clynes, M. *J. Pharm. Pharmacol.* **2004**, *56*, 783.
11. Jampilek, J.; Dolezal, M.; Kunes, J.; Vichova, P.; Jun, D.; Raich, I.; O´Connor, R.; Clynes, M. *Curr. Org. Chem.* **2004**, *8*, 1235.
12. Jampilek, J.; Dolezal, M.; Opletalova, V.; Hartl. J. *Curr. Med. Chem.* **2006**, *13*, 117.
13. Polanski, J.; Zouhiri, F.; Jeanson, L.; Desmaele, D.; d'Angelo, J.; Mouscadet, J.-F.; Gieleciak, R.; Gasteiger, J.; Le Bret. M. *J. Med. Chem.* **2002**, *45*, 4647.
14. Polanski, J.; Niedbala, H.; Musiol, R.; Tabak, D.; Podeszwa, B.; Gieleciak, R.; Bak, A.; Palka, A.; Magdziarz, T. *Acta Poloniae Pharm. Drug Res*. **2004**, *61*, 3.
15. Polanski, J.; Niedbala, H.; Musiol, R.; Podeszwa, B.; Tabak, D.; Palka, A.; Mencel, A.; Finster, J.; Mouscadet, J.F.; Le Bret, M. *Lett. Drugs Des. Disc.* **2006**, *3*, 175.
16. Polanski, J.; Niedbala, H.; Musiol, R.; Podeszwa, B.; Tabak, D.; Palka, A.; Mencel, A.; Mouscadet, J.F.; Le Bret, M. *Lett. Drugs Des. Disc*. **2007**, *4*, 99.
17. Vargas, L.Y.; Castelli, M.V.; Kouznetsov, V.V.; Urbina, J.M.; Lopez, S.N.; Sortino, M.; Enriz, R.D.; Ribas, J.C.; Zacchino, S. *Bioorg. Med. Chem.* **2003**, *11*, 1531.
18. Jampilek, J.; Dolezal, M.; Kunes, J.; Buchta, V.; Kralova, K. *Med. Chem.* **2005**, *1*, 591.
19. Musiol, R.; Jampilek, J.; Buchta, V.; Niedbala, H.; Podeszwa, B.; Palka, A.; Majerz-Maniecka, K.; Oleksyn, B.; Polanski, J. *Bioorg. Med. Chem.* **2006**, *14*, 3592.
20. Musiol, R.; Jampilek, J.; Kralova, K.; Richardson, D.R.; Kalinowski, D.; Podeszwa, B.; Finster, J.; Niedbala, H.; Palka, A.; Polanski, J. *Bioorg. Med. Chem.* **2007**, *15*, 1280.
21. Musiol, R.; Tabak, D.; Niedbala, H.; Podeszwa, B.; Jampilek, J.; Kralova, K.; Dohnal, J.; Finster, J.; Mencel, A.; Polanski, J. *Bioorg. Med. Chem.* **2008**, *16*, 4490.
22. Jampilek, J.; Musiol, R.; Pesko, M.; Kralova, K.; Vejsova, M.; Carroll, J.; Coffey, A.; Finster, J.; Tabak, D.; Niedbala, H.; Kozik, V.; Polanski, J.; Csollei, J.; Dohnal, J. *Molecules* **2009**, *14*, 1145.
23. Podeszwa, B.; Niedbala, H.; Polanski, J.; Musiol, R.; Tabak, D.; Finster, J.; Serafin, K.; Wietrzyk, J.; Boryczka, S.; Mol, W.; Jampilek, J.; Dohnal, J.; Kalinowski, D.; Richardson, D.R. *Bioorg. Med. Chem. Lett.* **2007**, 17, 6138.
24. http://www.who.int/tb/publications/global_report/2008/summary/en/index.html (September 2009)
25. Field, S.K.; Cowie, R.L. *Chest* **2006**, *129*, 1653.
26. Wagner, D.; Young, L.S. *Infection* **2004**, *32*, 257.
27. Morrone, N.; Cruvinel, M.C.; Morrone, N. Jr.; Freire, J.A.; Oliveira, L.M.; Gonçalves, C. *J. Pneumol.* **2003**, *29*, 341.
28. Draber, W.; Tietjen, K.; Kluth, J.F.; Trebst, A. *Angewandte Chemie* **1991,** *3*, 1621.
29. Tischer, W.; Strotmann, H. *Biochim. Biophys. Acta* **1977**, *460*, 113.
30. Trebst, A.; Draber, W. *Structure activity correlations of recent herbicides in photosynthetic reactions*. In *Advances in Pesticide Science*; Greissbuehler H. Ed.; Pergamon Press: Oxford, 1979; pp. 223–234.
31. Bowyer, J.R.; Camilleri, P.; Vermaas, W.F.J. In *Herbicides, Topics in Photosynthesis*, vol. 10. Baker N.R., Percival M.P. Eds.; Elsevier: Amsterdam, The Netherlands, 1991; pp. 27–85.

32. Kralova, K.; Sersen, F.; Kubicova, L.; Waisser, K. *J. Trace Microprobe Techn.* **2000**, *18*, 251-256.
33. Kralova, K.; Sersen, F.; Miletin, M., Dolezal, M. Chem. Pap. **2002**, *56*, 214.
34. Dolezal, M.; Miletin, M.; Kunes, J.; Kralova, K. *Molecules* **2002**, *7*, 363.
35. Musiol, R.; Podeszwa, B.; Finster, J.; Niedbala, H.; Polanski; J. *Monatsh. Chem.* **2006**, *137*, 1211.
36. Norrington, F.E.; Hyde, R.M.; Williams, S.G.; Wotton, R. *J. Med. Chem.* **1975**, *18*, 604.
37. Takahata, Y.; Chong, D.P. *Int. J. Quantum Chem.* **2005**, *103*, 509.
38. Finster, J.; Kalinowski, D.; Musiol, R.; Mrozek, A.; Szurko, A.; Serafin, A.; Kamalapuram, S.K.; Kovacevic, Z.; Jampilek, J.; Ratuszna, A.; Rezeszowska-Wolny, J.; Richardson, D.R.; Polanski, J. *Bioorg. Med. Chem.* **2009**, submitted.
39. Kovalenko, S.; Belenichev, I.; Nikitin, V.; Karpenko, A. *Acta Pol. Pharm. Drug Design* **2003**, *60*, 275.
40. Botros, S.; Shaban, M. *Pharmazie* **1978**, *33*, 646.
41. Masarovicova, E.; Kralova, K. *Approaches to measuring plant photosynthesis activity*. In *Handbook of Photosynthesis* 2nd Ed.; Pessarakli M. Eds.; Taylor & Francis Group: Boca Raton, London-New York-Singapore, 2005; pp. 617–656.
42. Kralova, K.; Sersen, F.; Sidoova, E. *Chem. Pap.* **1992**, *46*, 348.
43. Fedke, C. *Biochemistry and Physiology of Herbicide Action*. Springer Verlag: Berlin-Heidelberg-New York, **1982**.