Triazolophthalazines: Easily Accessible Compounds with Potent Antitubercular Activity

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Tuberculosis (TB) remains one of the major causes of death worldwide, in particular because of the emergence of multidrug-resistant TB. Herein we explored the potential of an alternative class of molecules as anti-TB agents. Thus, a series of novel 3-substituted triazolophthalazines was quickly and easily prepared from commercial hydralazine hydrochloride as starting material and were further evaluated for their antimycobacterial activities and cytotoxicities. Four of the synthesized compounds were found to effectively inhibit the *Mycobacterium tuberculosis* (*M.tb*) H_{37}Rv strain with minimum inhibitory concentration (MIC) values $< 10\ \mu g\,mL^{-1}$, whereas no compounds displayed cytotoxicity against HCT116 human cell lines ($IC_{50} > 100\ \mu M$). More remarkably, the most potent compounds proved to be active to a similar extent against various multidrug-resistant *M.tb* strains, thus uncovering a mode of action distinct from that of standard antitubercular agents. Overall, their ease of preparation, combined with their attractive antimycobacterial activities, make such triazolophthalazine-based derivatives promising leads for further development.

Introduction

Mycobacterium tuberculosis (*M.tb*), the causative agent of tuberculosis (TB), represents an enduring, deadly infectious disease for all mankind. According to the World Health Organization (WHO), one-third of the world's population is infected, currently making TB the second leading cause of mortality worldwide.[1] It is estimated that there were about 9 million cases of TB and 1.5 million deaths in 2014. In recent years, the significance of the disease has increased dramatically, as TB is also the major cause of death among patients co-infected with HIV. In a concerted effort to control and eradicate TB, an impressive range of anti-TB drugs has been discovered.[2] Nevertheless, treatment still remains lengthy and, most importantly, new drug-resistant strains have emerged. In fact, nearly half a million new cases of multidrug- and extremely drug-resistant TB appear every year. In addition, one of the most potent TB drugs, rifampicin, inactivates several antiviral drugs, making it difficult to use for the treatment of TB–HIV co-infected patients

(Figure 1).[3] Due to an urgent need for the development of novel drugs, the TB drug pipeline is filled with several new therapeutic compounds for the first time in decades, many of them in phase II or III clinical trials (Figure 1).[4] Whatever the eventual outcome of these drugs currently in the TB pipeline, continuous efforts should still be made in order to find new compounds active against novel essential targets or compounds with different modes of action against known targets.

Cinnamic acid and derivatives also represent a relevant class of compounds having a century-old history as anti-TB agents.[5] First reported to be used alone as early as 1894,[6] the structur-

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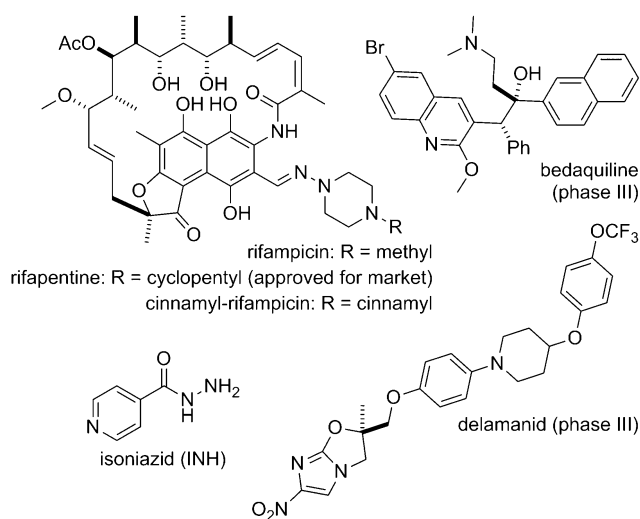Supporting information for this article can be found under http://dx.doi.org/10.1002/cmdc.201600085.

Figure 1. Examples of anti-TB drugs: the commonly used rifampicin and isoniazid, as well as some drugs currently in the TB pipeline.

ally simple *trans*-cinnamic acid was later demonstrated to exert a synergistic effect in combination with known anti-TB drugs, such as rifampicin and isoniazid (INH)[7] (Figure 1). Also remarkable is that the anti-TB profile of rifampicin could be improved by replacing the methyl group of its piperazinyl moiety with a cinnamyl group (i.e., cinnamyl-rifampicin, depicted in Figure 1).[8]

In that context, and due to our interest in bioactive compounds bearing the cinnamic scaffold,[5,9] we recently described the synthesis of derivatives exhibiting promising anti-TB activities.[10] Amongst all derivatives investigated so far, 4-alkoxy cinnamoyl derivatives featuring both a styryl and triazolophthalazine moiety were found to be the most promising (Scheme 1).[11] Encouraged by these preliminary results, we
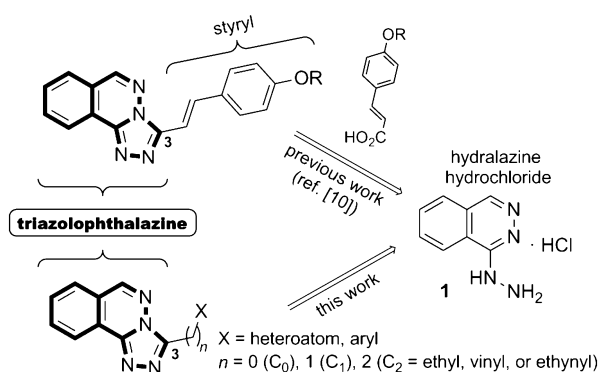
Scheme 1. Triazolophthalazine derivatives as potential *M.tb* inhibitors: from previously reported 3-styrylated triazolophthalazines[10] to the derivatives investigated in this study.

report herein the synthesis of novel 3-substituted triazolophthalazine derivatives, along with their biological evaluation as anti-TB agents (Scheme 1). This will allow us to determine whether the triazolophthalazine core could be regarded as a novel and relevant pharmacophore in the TB context. Indeed, although triazolophthalazines have been reported several times for exhibiting other bioactivities,[9b, 12] to the best of our knowledge, they have received no attention as potential anti-TB agents.[13] Our initial results with 35 3-substituted triazolophthalazine derivatives are presented below.

Results and Discussion

Chemistry

As outlined in Scheme 1, we envisioned the preparation of three series of triazolophthalazine derivatives (i.e., C_{0-2}; with reference to the number of carbon atoms between position 3 of the triazolophthalazine nucleus and the terminal moiety, X Scheme 1). Of note, the synthesis of all derivatives was envisaged in a few steps using commercially available hydralazine hydrochloride **1** as the starting material, regardless of the series.

Synthesis of C_0 series derivatives

We began with the synthesis of unmodified triazolophthalazine **2**, which was obtained by condensation of hydralazine hydrochloride **1** with trimethyl orthoformate (Scheme 2). The reaction was carried out either in pure trimethyl orthoformate or with trimethyl orthoformate in dichloromethane as solvent in
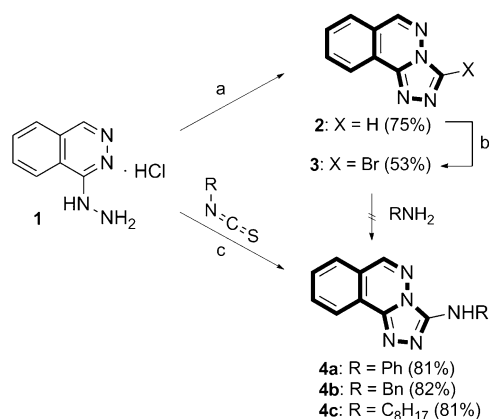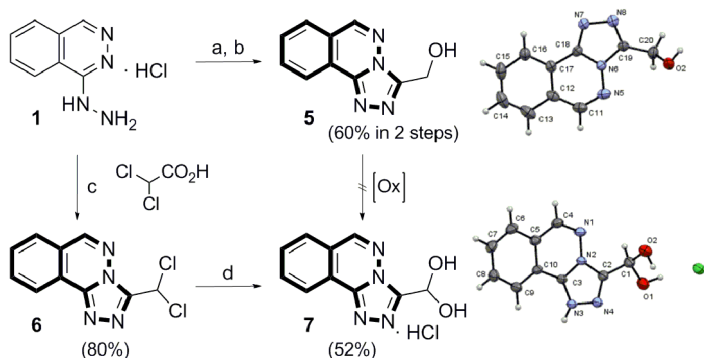

Scheme 2. Synthesis of C_0 series derivatives. *Reagents and conditions*: a) **1** (1.0 equiv), HC(OMe)$_3$ (1.0 equiv), Et$_3$N (1.6 equiv), CH$_2$Cl$_2$, RT→40 °C, 15 h; b) **1** (1.0 equiv), Br$_2$ (excess), AcOH, reflux, 5 h; c) **1** (1.0 equiv), RN=C=S (1.1 equiv), EDC (1.1 equiv), Na$_2$CO$_3$ (3 equiv), DMF, 80 °C, 4.5 h.

the presence of triethylamine to yield compound **2** in 75 % yield. Triazolophthalazine **2** was then selectively brominated at position 3 with *N*-bromosuccinimide in acetic acid to furnish 3-bromotriazolophthalazine **3** in 53 % yield (Scheme 2). Product **3** was easily identified by ^{1}H NMR by disappearance of the corresponding singlet at 9 ppm.

Attempts to synthesize 3-amino derivatives **4a–c** first started from **3**, but the expected nucleophilic aromatic substitution of the bromine atom with amines proved unsuccessful in methanol under either conventional or microwave heating (Scheme 2). The starting compound **3** was fully recovered in pure form, thus indicating that the bromine atom of **3** was much less labile than previously postulated.[14] An alternative approach, taking advantage of the fact that isothiocyanates are known building blocks for the production of heterocycles, was then investigated for the elaboration of 3-amino derivatives **4a–c**. In that respect, we took advantage of a well-established cyclization/desulfurization process but, rather than using DCC as originally reported,[15] we opted for EDC as the activating agent. Under these slightly modified conditions, 3-amino derivatives **4a–c** were obtained in good yields after purification by flash chromatography.

Synthesis of C_1 series derivatives

In that series, we first prepared hydroxymethylated triazolophthalazine **5** by reaction of triazolophthalazine **2** with an aqueous solution of formaldehyde in a small amount of dioxane, as previously described for another triazolo system (Scheme 3).[16]

Scheme 3. Synthesis of C_1 series derivatives. *Reagents and conditions*: a) **1** (1.0 equiv), HC(OMe)$_3$ (1.0 equiv), Et$_3$N (1.6 equiv), CH$_2$Cl$_2$, RT→40 °C, 15 h, 75 %; b) **2**, 37 % aqueous formaldehyde (excess), dioxane, 15 h, 81 %; c) **1** (1.0 equiv), Et$_3$N (8.0 equiv), CH$_2$Cl$_2$, RT, 0.5 h, then CHCl$_2$CO$_2$H (excess), 60→110 °C, 2.5 h; d) **6**, morpholine (excess), 100 °C, 5 h, then 25 % aqueous HCl, RT, 1 h.

Product **5** was isolated in good yield, and its structure was further confirmed by its X-ray crystallography.

Oxidation of **5** to aldehyde and/or to its acid derivative was then attempted but without success. Upon exposure of **5** to strong oxidizers (such as KMnO$_4$ or CrO$_3$[17]), only compound **2** was obtained, due to easy decarboxylation of the resulting acid (Scheme 2).[18] We also tried to use milder oxidation conditions, such as the IBX/oxone system[19] in acetonitrile/water, but still without success. Other oxidation methods that selectively lead to the formation of aldehyde were then examined. Reactions using IBX or Dess–Martin periodinane did not afford selective oxidation and delivered a complex mixture of products. Similar results were observed using manganese(IV) oxide as the oxidant in dichloromethane. Taking into account these difficulties for oxidizing **5**, we opted for an alternative two-step approach involving 3-dichloromethylated triazolophthalazine **6** as an intermediate. The reaction of **1** in boiling dichloroacetic acid for 2 h allowed easy formation of **6**, which was isolated in good yield (80 %). Treatment of **6** with two equivalents of morpholine, followed by hydrolysis (25 % hydrochloric acid), afforded gem-diol **7** in its hydrochloride form with 52 % yield. It is noteworthy that **7** was predominant in methanol or DMSO in its gem-diol form, whereas the aldehyde form was observed in chloroform. This result was confirmed by NMR spectroscopy, mass spectrometry, and X-ray analysis.

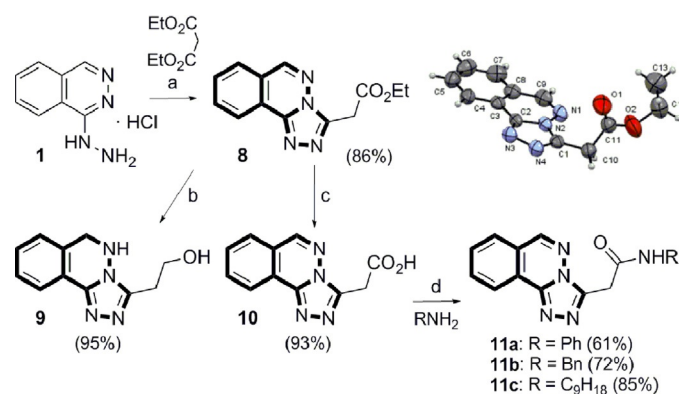### Synthesis of C_2 series derivatives

Beginning from diethyl malonate

The synthesis of compounds **8**–**11** was based on the condensation of **1** with diethyl malonate and further modifications of the resulting compound **8** (Scheme 4). The condensation was carried out in an excess of diethyl malonate with subsequent purification using short-path vacuum distillation to afford ethyl ester **8** in 86 % yield. The reduction and saponification of **8** proved efficient under standard conditions, furnishing primary alcohol **9** and carboxylic acid **10**, respectively, in excellent yields. Acid **10** could be readily converted into amides **11a**–

c using HOBt and EDC as coupling reagents in the presence of *N,N*-diisopropylethylamine (DIPEA).

Beginning from cinnamic derivatives

As mentioned in the introduction (Scheme 1), we previously reported the synthesis of 3-styrylated triazolophthalazines, starting from *trans*-4-hydroxycinnamic acid (i.e., *trans*-4-coumaric acid).[10] To further evaluate the anti-TB potential of these derivatives, we aimed toward building a small, focused library of styryltriazolophthalazines. The reported four-step sequence nevertheless suffered from some drawbacks, such as the number of steps, the need for long reaction times, and its convergent approach (Scheme 5, *route a*). According to *route b* (Scheme 5), we thus

Scheme 4. Synthesis of C_2 series derivatives from diethyl malonate. *Reagents and conditions*: a) **1** (1.0 equiv), Et$_3$N (8.0 equiv), CH$_2$Cl$_2$, RT, 0.5 h, then CH$_2$(CO$_2$Et)$_2$ (excess), 110 °C, 5 h; b) **8** (1.0 equiv), NaBH$_4$ (excess), EtOH, 80 °C, 5 h; c) **8**, 5 M aqueous NaOH/EtOH (1:2), 10 h; d) **10** (1.0 equiv), HOBt (1.1 equiv), EDC (1.1 equiv), DMF, 0.5 h, then RNH$_2$ (1.1 equiv), DIPEA (2.1 equiv), 10 h.
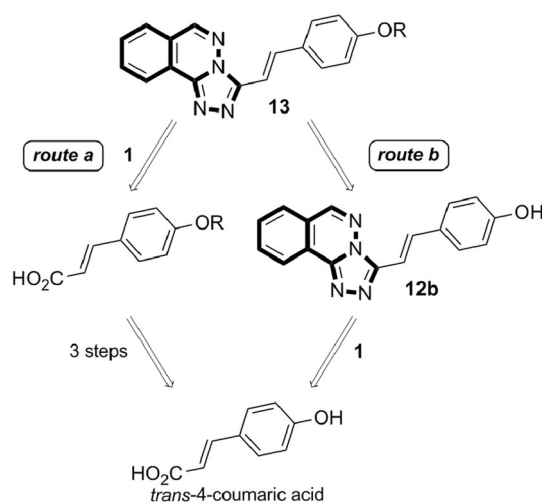
Scheme 5. Retrosynthetic analyses of alkoxylated triazolophthalazine derivatives **13**: *route a*: previously reported four-step and convergent route; *route b*: two-step and divergent route proposed herein.

envisioned that alkoxylated derivatives of type **13** could be prepared in a more straightforward and divergent manner through O-alkylation of phenolic precursor **12 b**, whose synthesis was envisaged in one pot from **1** and *trans*-4-coumaric acid as condensation partners.

Accordingly, we first focused our attention on finding adequate conditions for the one-pot synthesis of *trans*-styryltriazolophthalazines (Scheme 6). Using **1** and cinnamic acid as model partners, we examined the effect of microwave irradiation on the condensation process, furnishing **12 a** as the de-
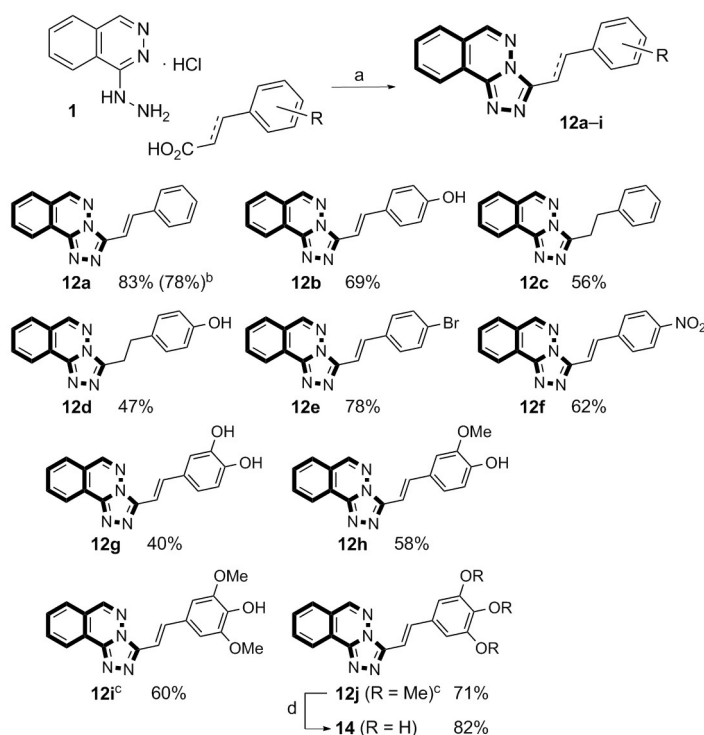
Scheme 6. One-pot synthesis of C$_2$ series derivatives from cinnamic derivatives. *Reagents and conditions*: a) reactions run with cinnamic acid (1.0 equiv), **1** (1.5 equiv), HOBt (1.1 equiv), EDC (1.1 equiv), Et$_3$N (4 equiv), CH$_3$CN, microwave 102 °C, 1 h, unless otherwise stated; b) reaction run under conventional heating at 95 °C in a sealed tube; c) DIPEA was used instead of Et$_3$N; d) reaction run with **12 k** (1.0 equiv), BBr$_3$ (9 equiv), CH$_2$Cl$_2$, −78 °C, 48 h.

sired triazolophthalazine adduct. To our delight, microwaving was found to highly activate the condensation, with complete conversion achieved in only 1 h under microwave irradiation, while 48 h was required under conventional heating. With microwave irradiation in acetonitrile as solvent and in the presence of triethylamine as base, derivative **12 a** was isolated in pure form and good yield (83%). The same condensation conditions proved further successful for preparing the expected phenolic derivative, **12 b**. In that case, the time needed for complete conversion was similar (1 h), and **12 b** was obtained in 69% yield after a simple filtration–recrystallization sequence. Due to their efficiency, we decided to further exploit these reaction conditions for the synthesis of other triazolophthalazine derivatives (Scheme 6). We submitted various commercial cinnamic acids (dihydrogenated and/or 4-substituted) to the con-

densation process and were thus able to prepare derivatives **12 c–j** in modest to satisfactory yields, ranging from 40 to 83%. As an additional derivative, we prepared trihydroxylated compound **14** through demethylation of **12 j** using standard conditions (excess BBr$_3$ in dichloromethane at −78 °C).

Using a similar approach, we also planned the synthesis of another C$_2$ derivative, 3-ethynyltriazolophthalazine **15** (Scheme 7), but our attempts to obtain this compound directly through microwave-mediated condensation of **1** and 3-trimethylsilyl propiolic acid failed. A very complex mixture of products was obtained, thus revealing the instability of the propiolic acid under our microwave conditions. Slight modification of the conditions nevertheless enabled the synthesis of **15**. Construction of the triazolophthalazine scaffold indeed proved effective when performed in two steps rather than in a one-pot manner. Trimethylsilyl propiolic acid was first coupled with **1** under milder temperature conditions to afford acyclic compound **16**, which was isolated and then cyclized to silylated intermediate **17** under microwave irradiation in almost quantitative yield. Final deprotection of **17** under standard conditions (i.e., K$_2$CO$_3$ in MeOH) furnished desired derivative **15** in 31% overall yield over three steps.

With phenolic derivative **12 b** in hand, this opened a route to access previously mentioned derivatives **13** (Scheme 5, *route b*). In our hands, the phenolic functionality of **12 b** proved easy to alkylate under standard reaction conditions, thus allowing the preparation of six additional alkoxylated derivatives, **13 a–f**, with yields ranging from 46 to 85% (Scheme 8). Of note, the alkylating agents used were chosen based on their chain lengths as well as their unsaturation/branching profiles.

Finally, because stereochemical configurations of double bonds were demonstrated several times to have crucial effects on biological activities,[20] we also envisioned preparation of some *cis*-styryltriazolophthalazines from their *trans* isomers. In the TB context, an illustrative example is given by cinnamic acid itself, the *cis* isomer of which was recently
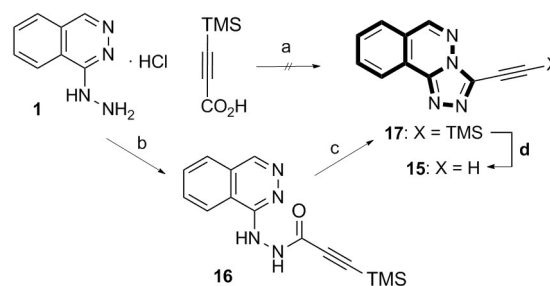
Scheme 7. Synthesis of 3-ethynylated derivative **15**. *Reagents and conditions*: a) 3-(trimethylsilyl)propynoic acid (1.0 equiv), **1** (1.5 equiv), HOBt (1.1 equiv), EDC (1.1 equiv), Et$_3$N (4 equiv), CH$_3$CN, microwave 102 °C, 1 h; b) 3-(trimethylsilyl)propynoic acid (1.0 equiv), **1** (1.5 equiv), HOBt (1.1 equiv), EDC (1.1 equiv), Et$_3$N (4 equiv), CH$_3$CN, 0 °C→RT, 3 h; c) **16**, CH$_3$CN, microwave 102 °C, 1 h; d) **17** (1.0 equiv), K$_2$CO$_3$ (3.2 equiv), MeOH, 12 h, 31% (steps b–d).
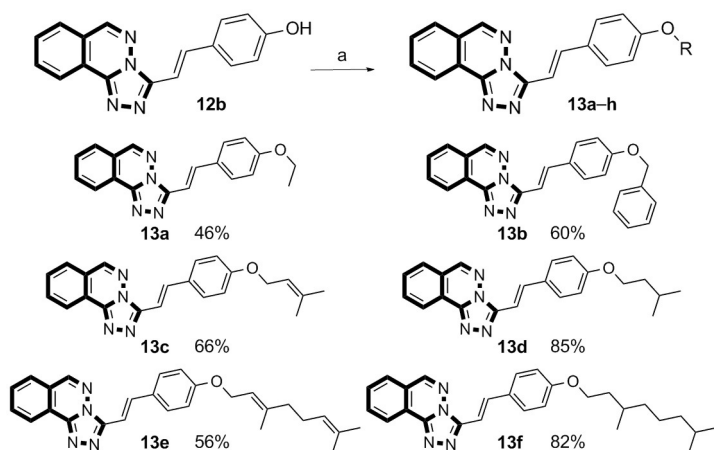
Scheme 8. Synthesis of alkoxylated *trans*-styryltriazolophthalazine derivatives **13**. *Reagents and conditions*: a) **12 b** (1.0 equiv), R—Br (1.2–1.5 equiv), KI (1.5 equiv), K_2CO_3 (1.5 equiv), DMF, RT→60 °C, 12 h.

found to be 120-fold more active against *M.tb* than the *trans* isomer.[21] We then selected five *trans* derivatives and submitted them to photoisomerization conditions, similar to conditions reported elsewhere for promoting the *trans–cis* isomerization of stilbenes (Scheme 9).[22] While photoirradiation of **12 a**, **12 e**, and **13 a** promoted expected *trans–cis* conversion in a clean manner,[23] the formation of complex mixtures of compounds was observed in the cases of **13 c** and **13 e**. Good to excellent conversions were attained after 50 h irradiation in acetonitrile, and the resulting mixture of diastereomers was purified by column chromatography to give **18 a–c**.
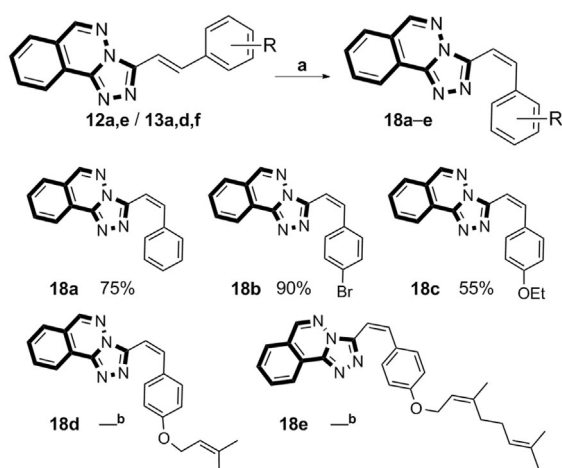
Scheme 9. Synthesis of *cis*-styryltriazolophthalazine derivatives **18** from their *trans* isomers. *Reagents and conditions*: a) reactions run with *trans* derivative at 0.015 M in CH_3CN, *hν*, 50 h; b) the formation of a complex mixture of photoproducts was observed.

Biology

Antimycobacterial activities

The newly synthesized compounds were evaluated for their inhibitory activity against *M.tb* (H$_{37}$Rv strain) using a dilution

method. Minimum inhibitory concentrations (MICs) are reported in Table 1 using INH and ethambutol (ETH) as controls. For clarity, compounds are separated in the table according to the series to which they belong (i.e., C$_{0–2}$).

Compounds of the C$_0$ and C$_1$ series (Schemes 2 and 3) all proved to be poor inhibitors, with **4 a** (MIC 33 μM) and **7** (MIC 43 μM) being the most active (Table 1). More gratifyingly, some compounds of the C$_2$ series displayed good to excellent activities against the *M.tb* H$_{37}$Rv strain. Within the amide series (compounds **11 a–d**, Scheme 4), inhibitory activity appeared to be closely related to the chain length. Indeed the longer the R chain, the higher the potency (i.e., MIC: **11 a** > **11 b** > **11 c**). Of note, a similar trend was found in diverse triazole series that we recently developed.[24–26]

Table 1. Inhibitory activity of triazolophthalazines against the *M.tb* H$_{37}$Rv strain.

Compd	MIC [μg mL^{-1} (μM)][a]	Compd	MIC [μg mL^{-1} (μM)][a]
	C$_0$ compounds		*C$_2$ compounds*
2	> 10 (> 59)	12 e	4 (11.4)
3	> 10 (> 40)	12 f	> 4 (> 12.6)
4 a	10 (33)	12 g	> 4 (> 13.9)
4 b	> 10 (> 36)	12 h	> 4 (> 12.6)
4 c	> 10 (> 44)	12 i	> 4 (> 11.5)
	C$_1$ compounds	12 j	> 5 (> 14.4)
5	> 10 (> 50)	13 a	6 (19.0)
6	> 10 (> 40)	13 b	> 4 (> 10.5)
7	10 (43)	13 c	1 (2.8)
	C$_2$ compounds	13 d	> 4 (> 11.2)
8	> 10 (> 39)	13 e	0.5 (1.2)
9	> 10 (> 40)	13 f	> 4 (> 9.3)
10	> 10 (> 44)	14	> 5 (> 15.6)
11 a	> 10 (> 33)	15	2.25 (12.9)
11 b	10 (32)	18 a	> 4 (> 14.7)
11 c	2.5 (7)	18 b	8 (22.8)
12 a	> 4 (> 14.7)	18 c	> 10 (> 31)
12 b	> 4 (> 13.9)	INH[b]	0.05 (0.36)
12 c	> 4 (> 14.7)	ETH[b]	1 (4.90)
12 d	> 4 (> 13.8)		

[a] Values are the mean ± 0.025 μg L^{-1} (*n* = 3). [b] INH (isoniazid) and ETH (ethambutol) were used as controls.

In sharp contrast, a wide disparity in MIC values was observed for the C$_2$ series of cinnamic derivatives (Schemes 6 and 8). The substituent at the *para* position of the aryl group was clearly found to be crucial for inhibitory activity. Amongst all of the cinnamic-derived compounds, compound **12 e**, bearing a bromine atom, and compounds **13 c** and **e**, bearing either an isopentenyl or a geranyl side chain, proved to be the best inhibitors with regard to their MIC values.[27] Compounds **13 d** and **f**, corresponding to saturated analogues **13 c** and **e**, were found to be inactive, thus revealing the key role played by unsaturation on inhibitory potential. With regard to the C2 unit linking the triazolophthalazine and the aromatic groups, neither its oxidation state nor its stereochemistry appeared to be

critical for the targeted anti-TB activity. Indeed, shifting from the C=C to its reduced C−C form (**12c** vs. **12a** and **12d** vs. **12b**) or from the *E* to its diastereomeric *Z* forms (**18a** vs. **12a**, **18b** vs. **12e**, and **18c** vs. **13a**) did not cause any profitable effect. It is also noteworthy that all of the prepared phenolic derivatives, from monophenolic **12b**, **i**, and **j** to catechol- and pyrogallol-based derivatives **12h** and **14**, unfortunately proved ineffective as anti-TB agents. Finally, and to our delight, compound **15**, the shortest C_2 derivative in our series, showed good anti-TB activity with a MIC value of ∼10 μM.

Antimycobacterial activities against M.tb-resistant strains and cytotoxicities

To further explore the potential of our compounds, a number of active triazolophthalazine derivatives were screened against drug-resistant strains and cytotoxicity against a human colon cancer cell line (HCT116) as a model cell line (Table 2). Although most of the synthesized compounds were tested for

Table 2. Inhibitory activity against *M.tb*-resistant strains and cytotoxic activity of selected triazolophthalazines.

Compd	MIC [μg mL^{-1} (μM)][a,b]			IC$_{50}$ [μM][c]
	CI1	CI2	CI3	
2	nd[d]	nd[d]	nd[d]	> 100
12b	nd[d]	nd[d]	nd[d]	> 100
12c	nd[d]	nd[d]	nd[d]	> 100
12e	4 (11.4)	4 (11.4)	4 (11.4)	> 100
12i	nd[d]	nd[d]	nd[d]	> 100
13d	nd[d]	nd[d]	nd[d]	53
13e	0.5 (1.2)	0.5 (1.2)	0.5 (1.2)	> 100
15	2.25 (12.9)	2.25 (12.9)	2.25 (12.9)	> 100
INH[e]	> 0.2 (1.5)	> 0.2 (1.5)	> 0.2 (1.5)	nd[d]

[a] Drug resistance profiles of *M.tb* clinical isolates (CI): CI1: resistant to streptomycin, INH, rifampicin, and ETH; CI2: resistant to streptomycin, INH, rifampicin, ETH, pyrazinamide, ethionamide, and capreomicin; CI3: resistant to streptomycin, INH, rifampicin, ETH, pyrazinamide, and ethionamide. [b] Values are the mean ± 0.025 μg L^{-1} ($n = 3$). [c] Cytotoxicity IC$_{50}$ values in the HCT116 human colon cancer cell line. [d] Not determined. [e] INH (isoniazid) was used as a control.

cytotoxicity, only a selection of data, characteristic of the general cytotoxic profiles we observed for triazolophthalazine derivatives, is given in Table 2. With the exception of compound **13d**, which exhibited moderate cytotoxicity toward HCT116 (i.e., IC$_{50}$ = 53 μM), no tested triazolophthalazine derivatives demonstrated cytotoxicity against this human cell line (i.e., IC$_{50}$ > 100 μM).

In light of their relevant inhibitory efficiencies against the *M.tb* H$_{37}$Rv strain, compounds **12e**, **13e**, and **15** were selected and further evaluated for their inhibitory potential against three multidrug-resistant *M.tb* clinical isolates (i.e., CI1–3 in Table 2). The results showed that the level of efficiency of each compound was preserved regardless of the nature of the strain, thus constituting another valuable asset over standard anti-TB drugs, such as INH and ETH.

Conclusions

A series of 35 3-substituted triazolophthalazine derivatives was prepared from a single commercially available precursor (hydralazine hydrochloride) using straightforward synthetic sequences (one- to three-step syntheses). These easy-to-prepare derivatives did not manifest cytotoxicity toward HCT116 human cells, and among these, four compounds (**11c**, **12e**, **13e**, and **15**) exhibited attractive inhibitory activities against *M.tb* H$_{37}$Rv strain. More significantly, these compounds presented similar activities against multidrug-resistant *M.tb* strains, thus revealing an alternative mode of action for these triazolophthalazine-based compounds compared with standard anti-TB drugs. Further work is now underway to tackle the identification of the protein targets of this class of potent anti-TB compounds. The synthesis of other triazolophthalazine-based heterocyclic systems will be envisaged as soon as the target is clearly identified.

Experimental Section

Chemistry

General: All starting materials were purchased from commercial sources and were used as received. Anhydrous solvents were freshly distilled before use or were obtained from the M. Braun solvent purification system (MB-SPS-800). The reactions were monitored by thin-layer chromatography carried out on silica plates (silica gel 60 F$_{254}$, Merck) using a UV light for visualization. Column chromatographies were performed 1) on silica gel 60 (0.040–0.063 mm, Merck) using mixtures of ethyl acetate (or diethyl ether) and cyclohexane as eluents, or 2) with a puriFlash 430 system using puriFlash columns from Interchim. Evaporation of solvents was conducted under reduced pressure at temperatures below 30 °C unless otherwise noted. Melting points (mp) were measured with a Stuart SMP30 apparatus in open capillary tubes or with a Mettler Toledo MP50 melting point system and are uncorrected. IR spectra were obtained from the Service Commun de Spectroscopie Infrarouge et Raman of the Plateforme Technique, Institut de Chimie de Toulouse. Values are reported in cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 spectrometer at 300 and 75 MHz, respectively. Chemical shifts (δ) and coupling constants (J) are given in ppm and Hz, respectively. Chemical shifts are reported relative to residual solvent as an internal standard (CDCl$_3$: 7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C; CD$_3$OD: 3.31 ppm for ^{1}H and 49.0 ppm for ^{13}C; [D$_6$]DMSO: 2.50 ppm for ^{1}H NMR and 39.5 ppm for ^{13}C NMR). ^{1}H multiplicities are designated by the following abbreviations: s = singlet, d = doublet, t = triplet, m = multiplet, b = broad. Carbon multiplicities were determined by DEPT135 or J_{mod} experiments. Electrospray (ESI) and desorption chemical ionization (DCI) low-/high-resolution mass spectra were obtained from the Service Commun de Spectroscopie de Masse of the Plateforme Technique, Institut de Chimie de Toulouse. Crystallographic data for compounds **5**, **7**, and **8**[28] were collected on a Bruker-AXS SMART APEX II diffractometer (**8**) and a Bruker-AXS Quazar APEX II diffractometer using a 30 W air-cooled microfocus source (ImS) with focusing multilayer optics (**5** and **7**) at a temperature of 193(2) K, with Mo$_{Ka}$ radiation (λ: 0.71073 Å) using Φ and Ω scans. The data were integrated with SAINT,[29] and an empirical absorption correction with SADABS[30] was applied. The structures were solved by

direct methods, using SHELXS-97, and refined using the least-squares method on F2.[31]

Synthesis of [1,2,4]triazolo[3,4-a]phthalazine (2): 1-Hydrazinyl-phthalazine hydrochloride **1** (500 mg, 2.5 mmol) and Et$_3$N (0.55 mL, 4 mmol) in dry CH$_2$Cl$_2$ (10 mL) were stirred for 30 min at room temperature, then trimethyl orthoformate (0.43 mL, 4 mmol) was added. The resulting mixture was stirred for 15 h at 40 °C, filtered, and recrystallized from CH$_2$Cl$_2$/MeOH (12:1) to furnish the title compound **2** in pure form as a white solid (yield: 320 mg, 75%): mp: 185 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 9.00 (s, 1H), 8.61 (s, 1H), 8.59 (m, 1H), 7.94–7.91 (m, 2H), 7.78 ppm (td, J = 7.1 Hz, 1.1 Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 150.8, 149.6, 142.5, 135.1, 132.5, 129.8, 123.8, 123.2, 122.1 ppm; FTIR-ATR (neat): $\tilde{v}$ = 3100, 3070, 2990, 2960, 1610, 1520, 1450, 1318 cm^{-1}; HRMS (ESI$^+$) m/z [M+H]$^+$ calcd for C$_9$H$_7$N$_4$: 171.0671, found: 171.0665.

Synthesis of 3-bromo[1,2,4]triazolo[3,4-a]phthalazine (3): An excess of bromine (2 mL) was added to a solution of compound **2** (200 mg, 1 mmol) in pure acetic acid (5 mL). After stirring at reflux for 5 h, the mixture was cooled to room temperature, quenched with aqueous NaHCO$_3$ until pH~10 was reached, and then stirred at room temperature for an additional 8 h. The resulting precipitate was filtered to furnish the title compound **3** in pure form as a white solid (yield: 160 mg, 53%): mp: 202 °C; ^{1}H NMR (300 MHz, CD$_3$OD): δ = 9.01 (s, 1H), 8.57 (bd, J = 7.9 Hz, 1H), 8.21 (bd, J = 7.9 Hz, 1H), 8.21 (td, J = 7.9, 1.2 Hz, 1H), 7.98 ppm (td, J = 7.9, 1.2 Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 149.3, 144.2, 134.6, 131.5, 129.2, 125.9, 123.2, 122.2, 121.8 ppm; FTIR-ATR (neat): $\tilde{v}$ = 3050, 3023, 2981, 1526, 1458, 1414, 1208 cm^{-1}; HRMS (ESI$^+$) m/z [M+H]$^+$ calcd for C$_9$H$_6$BrN$_4$: 248.9796/250.9755, found: 248.9788/250.9764.

General procedure for the synthesis of 3-amino[1,2,4]triazolo[3,4-a]phthalazines 4a–c: A DMF (10 mL) mixture of 1-hydrazinylphthalazine hydrochloride **1** (100 mg, 0.5 mmol, 1 equiv), Na$_2$CO$_3$ (150 mg, 1.5 mmol, 3 equiv), and the corresponding isothiocyanate (0.55 mmol, 1.1 equiv) was stirred at 80 °C for 30 min. EDC·HCl (110 mg, 0.55 mmol, 1.1 equiv) was then added, and the resulting mixture was stirred for additional 4 h at 80 °C. After cooling to room temperature and adding water (20 mL), the resulting mixture was extracted with EtOAc (3×20 mL), and the combined EtOAc layers were dried over Na$_2$SO$_4$, filtered, and evaporated. Purification of the residue by flash chromatography, eluting with EtOAc/MeOH (9:1), gave title compounds **4a–c** in pure forms.

N-Phenyl[1,2,4]triazolo[3,4-a]phthalazin-3-amine (4a): Compound **4a** was obtained as a yellow powder (yield: 110 mg, 81%): mp: 219–220 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.53 (d, J = 8.1 Hz, 1H), 8.44 (s, 1H), 7.89–7.77 (m, 4H), 7.73–7.68 (m, 1H), 7.40–7.35 (m, 2H), 7.7 (br s, 1H), 7.06–7.00 ppm (m, 1H); ^{13}C NMR (75 MHz, CDCl$_3$): δ = 146.7, 146.3, 139.2, 138.9, 133.9, 130.3, 129.2, 128.2, 123.9, 122.9, 121.9, 117.0 ppm; FTIR-ATR (neat): $\tilde{v}$ = 3454, 3275, 3057, 2919, 1616, 1577, 1452, 1246 cm^{-1}; HRMS (ESI$^+$) m/z [M+H]$^+$ calcd for C$_{15}$H$_{12}$N$_5$: 262.1093, found: 262.1092.

N-Benzyl[1,2,4]triazolo[3,4-a]phthalazin-3-amine (4b): Compound **4b** was obtained as a yellow powder (yield: 110 mg, 82%): mp: 114 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.49 (d, J = 7.9 Hz, 1H), 8.33 (s, 1H), 7.83 (td, J = 8.3 Hz, 1.1 Hz, 1H), 7.78 (d, J = 7.6 Hz, 1H), 7.67 (td, J = 8.1 Hz, 0.9 Hz, 1H), 7.48–7.45 (m, 2H), 7.37–7.27 (m, 3H), 5.08 (t, J = 5.7 Hz, 1H), 4.81 ppm (d, J = 6.0 Hz, 2H); ^{13}C NMR (75 MHz, CDCl$_3$): δ = 150.7, 145.9, 139.7, 138.4, 133.7, 129.9, 128.6, 128.1, 128.0, 127.6, 124.1, 122.72, 122.67, 47.3 ppm; FTIR-ATR (neat): $\tilde{v}$ = 3391, 3255, 3084, 3030, 2866, 1608, 1538, 1356 cm^{-1};

HRMS (ESI$^+$) m/z [M+H]$^+$ calcd for C$_{16}$H$_{14}$N$_5$: 276.1249, found: 276.1239.

N-Octyl[1,2,4]triazolo[3,4-a]phthalazin-3-amine (4c): Compound **4c** was obtained as a yellow powder (yield: 120 mg, 81%): mp: 109–110 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.48 (d, J = 7.9 Hz, 1H), 8.35 (s, 1H), 7.82 (td, J = 7.1 Hz, 1.2 Hz, 1H), 7.78 (d, J = 7.1 Hz, 1H), 7.67 (td, J = 7.1 Hz, 1.1 Hz, 1H), 4.74 (t, J = 5.8 Hz, 1H), 3.65–3.58 (m, 2H), 1.79–1.69 (m, 2H), 1.43–1.21 (m, 10H), 0.86 ppm (t, J = 6.4 Hz, 3H); ^{13}C NMR (75 MHz, CDCl$_3$): δ = 150.9, 145.8, 139.5, 133.7, 129.8, 128.0, 124.2, 122.74, 122.69, 43.4, 31.8, 29.8, 29.3, 29.2, 26.8, 22.6, 14.1 ppm; FTIR-ATR (neat): $\tilde{v}$ = 3378, 3265, 3047, 3014, 2924, 2855, 1607, 1581, 1535, 1377 cm^{-1}; HRMS (ESI$^+$) m/z [M+H]$^+$ calcd for C$_{17}$H$_{24}$N$_5$: 298.2032, found: 298.2026.

Synthesis of [1,2,4]triazolo[3,4-a]phthalazin-3-ylmethanol (5): Triazolophthalazine **2** (200 mg, 1 mmol) was heated at 100 °C in a mixture of 37% aqueous formaldehyde solution (5 mL) and dioxane (2 mL). After complete conversion of the starting material (TLC monitoring), the reaction mixture was evaporated under reduced pressure to furnish title compound **5** in pure form as a white solid (yield: 210 mg, 81%): mp: 244–246 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO) δ = 9.23 (s, 1H), 8.60 (d, J = 7.9 Hz, 1H), 8.29 (d, J = 7.8 Hz, 1H), 8.12 (td, J = 7.6 Hz, 1.1 Hz, 1H), 8.01 (td, J = 7.5 Hz, 1.1 Hz, 1H), 6.60 (bs, 1H), 4.99 ppm (s, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 150.3, 149.2, 142.0, 134.7, 132.0, 129.3, 123.4, 122.7, 121.7, 52.6 ppm; FTIR-ATR (neat): $\tilde{v}$ = 3264 (OH), 3042, 2843, 2580, 2551, 1639, 1593, 1460, 1218 cm^{-1}; HRMS (ESI$^+$) m/z [M+H]$^+$ calcd for C$_{10}$H$_9$N$_4$O: 201.0776, found: 201.0781.

Synthesis of 3-(dichloromethyl)[1,2,4]triazolo[3,4-a]phthalazine (6): 1-Hydrazinylphthalazine hydrochloride **1** (100 mg, 0.5 mmol) and Et$_3$N (0.55 mL, 4 mmol) in dry CH$_2$Cl$_2$ (10 mL) were stirred for 30 min at room temperature, then dichloroacetic acid (10 mL) was carefully added. After stirring for an additional 30 min at 60 °C until complete evaporation of CH$_2$Cl$_2$, the reaction mixture was heated at 110 °C for 2 h. After cooling to room temperature, the mixture was diluted with H$_2$O (40 mL), and the resulting precipitate was filtered and finally recrystallized from MeOH to furnish title compound **6** in pure form as a yellow powder (yield: 120 mg, 80%): mp: 225 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.27 (s, 1H), 8.57 (d, J = 7.9 Hz, 1H), 8.28 (d, J = 7.6 Hz, 1H), 8.12 (td, J = 7.6 Hz, 1.2 Hz, 1H), 8.09 (s, 1H), 8.01 ppm (td, J = 7.6 Hz, 1.2 Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 149.3, 147.1, 143.8, 134.8, 131.9, 129.3, 123.2, 122.4, 122.2, 60.1 ppm; FTIR-ATR (neat): $\tilde{v}$ = 3057, 3018, 1526, 1456, 1216 cm^{-1}; HRMS (ESI$^+$) m/z [M+H]$^+$ calcd for C$_{10}$H$_7$Cl$_2$N$_4$: 253.0048, found: 253.0037.

Synthesis of [1,2,4]triazolo[3,4-a]phthalazine-3-ylmethanediol (7): A solution of phthalazine **6** (100 mg, 0.4 mmol) in morpholine (5 mL) was heated for 5 h at 100 °C. The resulting red solution was then cooled to room temperature, concentrated to dryness, and treated with a 25% HCl solution (5 mL). After stirring for 1 h, the resulting precipitate was filtered and finally recrystallized from MeOH to furnish title compound **7** in pure form as an off-white powder (yield: 50 mg, 52%): mp: 228–230 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.25 (s, 1H), 8.56 (d, J = 7.9 Hz, 1H), 8.27 (d, J = 7.9, 1.1 Hz, 1H), 8.11 (td, J = 7.6, 0.9 Hz, 1H), 8.07 (s, 1H), 8.00 ppm (d, J = 7.6 Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 149.4, 147.1, 143.9, 134.9, 132.0, 129.4, 123.2, 122.5, 121.2, 60.1 ppm; HRMS (ESI$^+$) m/z [M+H]$^+$ calcd for C$_{10}$H$_7$N$_4$O: 199.0620, found: 199.0621.

Synthesis of ethyl [1,2,4]triazolo[3,4-a]phthalazin-3-ylacetate (8): 1-Hydrazinylphthalazine hydrochloride **1** (100 mg, 0.5 mmol) and Et$_3$N (0.55 mL, 4 mmol) in dry CH$_2$Cl$_2$ (10 mL) were stirred for 30 min at room temperature, then diethyl malonate (10 mL) was

carefully added. After heating for 5 h at 110 °C, the reaction mixture was evaporated and further distilled under vacuum. Purification of the residue by flash chromatography, eluting with CH_2Cl_2/MeOH (9:1), gave title compound 8 in pure form as a yellow powder (yield: 110 mg, 86%): mp: 135 °C; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 8.63–8.60$ (m, 1H), 8.60 (s, 1H), 7.95–7.89 (m, 2H), 7.78 (td, $J = 7.3$ Hz, 1.0 Hz, 1H), 4.30 (s, 2H), 4.19 (q, $J = 7.2$ Hz, 2H), 1.23 ppm (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 167.6$, 147.6, 145.1, 142.9, 134.0, 130.8, 128.0, 123.4, 123.1, 123.0, 61.6, 30.8, 14.1 ppm; FTIR-ATR (neat): $\tilde{v} = 3446$, 3024, 2984, 2880, 1731, 1527, 1458, 1323, 1200 cm^{-1}; HRMS (ESI$^+$) m/z $[M+H]^+$ calcd for $C_{13}H_{13}N_4O_2$: 257.1039, found: 257.1039.

Synthesis of 2-(5,6-dihydro[1,2,4]triazolo[3,4-a]phthalazin-3-yle-thanol (9): An EtOH (10 mL) solution of phthalazine 8 (100 mg, 0.4 mmol) and $NaBH_4$ (300 mg) was heated for 5 h at 80 °C. After cooling to room temperature, the resulting mixture was extracted with EtOAc (3×20 mL), and the combined EtOAc layers were dried over Na_2SO_4, filtered, and evaporated, giving title compound 9 in pure form as a white solid (yield: 90 mg, 95%): mp: 200 °C; ^1H NMR (300 MHz, CD_3OD): $\delta = 7.98–7.96$ (m, 1H), 7.54–7.40 (m, 3H), 4.33 (s, 2H), 3.95 (t, $J = 6.7$ Hz, 2H), 3.09 ppm (t, $J = 6.7$ Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 152.7$, 149.9, 134.9, 132.1, 129.4, 127.2, 124.6, 123.4, 60.3, 50.2, 28.5 ppm. FTIR-ATR (neat): $\tilde{v} = 3464$, 3268, 2923, 2328, 1524, 1462, 1367 cm^{-1}; HRMS (ESI$^+$) m/z $[M+H]^+$ calcd for $C_{11}H_{13}N_4O$: 217.1089, found: 217.1084.

Synthesis of [1,2,4]triazolo[3,4-a]phthalazin-3-ylacetic acid (10): An EtOH (10 mL) solution of phthalazine 8 (100 mg, 0.4 mmol) and NaOH (5 M, 5 mL) was stirred at room temperature for 10 h. The reaction mixture was then acidified with concentrated HCl to pH∼4, and the resulting precipitate was filtered, giving title compound 10 in pure form as a white solid (yield: 85 mg, 93%): mp: 175 °C; ^1H NMR (300 MHz, [D$_6$]DMSO): $\delta = 12.93$ (s, 1H), 9.10 (s, 1H), 8.51 (d, $J = 7.9$ Hz, 1H), 8.23 (d, $J = 7.7$ Hz, 1H), 8.08 (td, $J = 7.7$ Hz, 0.9 Hz, 1H), 7.95 (td, $J = 7.8$ Hz, 0.9 Hz, 1H), 4.24 ppm (s, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 169.3$, 148.3, 145.6, 142.2, 134.4, 131.1, 129.2, 122.8, 122.5, 122.1, 30.5 ppm; FTIR-ATR (neat): $\tilde{v} = 3070$, 3038, 2979, 2771, 2434, 1712, 1530, 1469, 1272, 1159 cm^{-1}; HRMS (ESI$^+$) m/z $[M+H]^+$ calcd for $C_{11}H_9N_4O_2$: 229.0726, found: 229.0729.

General procedure for the synthesis of amides 11 a–c: A DMF (10 mL) mixture of phthalazine 10 (100 mg, 0.4 mmol, 1.0 equiv), EDC·HCl (92 mg, 1.1 equiv), and HOBt (64 mg, 1.1 equiv) was stirred for 30 min at room temperature. DIPEA (120 mg, 2.1 equiv) and the corresponding amine (1.1 equiv) was then added to the mixture. After stirring for 10 h at room temperature, the resulting mixture was diluted with water (20 mL) and extracted with EtOAc (3× 20 mL). The combined EtOAc layers were dried over Na_2SO_4, filtered, and evaporated. Purification of the residue by flash chromatography, eluting with CH_2Cl_2/MeOH (9:1), gave title compounds 11 a–c in pure forms.

N-Phenyl-2-([1,2,4]triazolo[3,4-a]phthalazin-3-yl)acetamide (11a): Compound 11 a was obtained as a white powder (yield: 75 mg, 61%): mp: 226 °C; ^1H NMR (300 MHz, [D$_6$]DMSO): $\delta = 9.71$ (s, 1H), 8.70 (s, 1H), 8.66 (d, $J = 7.7$ Hz, 1H), 8.02–7.96 (m, 2H), 7.85 (td, $J = 7.5$ Hz, 1.1 Hz, 1H), 7.62–7.59 (m, 2H), 7.32–7.27 (m, 2H), 7.11–7.06 (m, 1H), 4.43 ppm (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 163.7$, 154.3, 148.2, 137.7, 134.4, 131.3, 128.9, 128.3, 124.5, 123.31, 123.27, 123.25, 120.1, 115.0, 32.8 ppm; FTIR-ATR (neat): $\tilde{v} = 3298$, 3054, 3031, 2924, 1661, 1527, 1447, 1233 cm^{-1}; HRMS (ESI$^+$) m/z $[M+H]^+$ calcd for $C_{17}H_{14}N_5O$: 304.1198, found: 304.1207.

N-Benzyl-2-([1,2,4]triazolo[3,4-a]phthlazin-3-yl)acetamide (11 b): Compound 11 b was obtained as a white powder (yield: 92 mg, 72%): mp: 194 °C; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 8.64$ (s, 1H), 8.58 (d, $J = 7.5$ Hz, 1H), 7.97–7.79 (m, 4H), 7.30–7.24 (m, 5H), 4.50 (d, $J = 5.8$ Hz, 2H), 4.27 ppm (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 165.8$, 147.9, 146.2, 143.0, 137.9, 134.2, 131.1, 128.6, 128.2, 127.7, 127.4, 123.22, 123.16, 123.1, 43.8, 31.8 ppm; FTIR-ATR (neat): $\tilde{v} = 3203$, 3025, 2966, 2901, 1646, 1560, 1461, 1272 cm^{-1}; HRMS (ESI$^+$) m/z $[M+H]^+$ calcd for $C_{18}H_{16}N_5O$: 318.1355, found: 318.1357.

N-Nonyl-2-([1,2,4]triazolo[3,4-a]phthalazin-3-yl)acetamide (11 c): Compound 11 c was obtained as a yellow powder (yield: 120 mg, 85%): mp: 173 °C; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 8.65$ (s, 1H), 8.63 (d, $J = 8.0$ Hz, 1H), 7.99–7.93 (m, 2H), 7.82 (td, $J = 7.5$ Hz, 1.1 Hz, 1H), 7.46 (bs, 1H), 4.22 (s, 2H), 3.32–3.25 (m, 2H), 1.54–1.49 (m, 2H), 1.31–1.22 (m, 12H), 0.85 ppm (t, $J = 7.0$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 165.7$, 148.0, 146.5, 143.04, 134.2, 131.2, 128.3, 123.4, 123.26, 123.24, 40.0, 31.93, 31.89, 29.5, 29.4, 29.32, 29.29, 27.0, 22.7, 14.2 ppm; FTIR-ATR (neat): $\tilde{v} = 3263$, 3081, 3030, 2921, 2853, 2394, 1664, 1569, 1457, 1230 cm^{-1}; HRMS (ESI$^+$) m/z $[M+H]^+$ calcd for $C_{20}H_{28}N_5O$: 354.2294, found: 354.2290.

General procedure for the synthesis of 3-styrylated derivatives 12 a–k and 14: 1-Hydrazinylphthalazine hydrochloride 1 (1.5 equiv), HOBt (1.1 equiv), EDC·HCl (1.5 equiv), and Et$_3$N (4 equiv) were successively added to a CH_3CN solution of the required (dehydro)cinnamic acid (1.0 equiv) in a 5 mL microwave reactor. After microwave irradiation for 40 to 60 min (∼100 °C, 260 W), the resulting mixture was cooled to room temperature and evaporated under reduced pressure. The thus-obtained crude materials were diluted with CH_2Cl_2, and the resulting organic layer was washed with saturated aqueous NH_4Cl solution, water, and brine, dried over Na_2SO_4, filtered, and evaporated. Purification of the residue by flash chromatography, eluting with the appropriate eluent, gave title compounds 12 a–k and 14 in pure forms.

E-3-Styryl-2-([1,2,4]triazolo[3,4-a]phthalazine (12 a): Compound 12 a was obtained as a yellow powder (yield: 83%): mp: 196 °C; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 8.76$ (d, $J = 8.0$ Hz, 1H), 8.72 (s, 1H), 8.20 (d, $J = 16.6$ Hz, 1H), 8.02–7.96 (m, 2H), 7.85 (td, $J = 7.5$ Hz, 1.1 Hz, 1H), 7.69–7.67 (m, 2H), 7.52 (d, $J = 16.6$ Hz, 1H), 7.45–7.34 ppm (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 148.5$, 147.4, 142.7, 135.90, 135.89, 133.9, 130.7, 129.0, 128.7, 128.0, 127.2, 123.3, 123.1, 122.9, 110.5 ppm; FTIR-ATR (neat): $\tilde{v} = 3041$, 3012, 2983, 2958, 2886, 2360, 2317, 2157, 1980, 1640, 1627, 1518, 1504, 1468, 1450, 1392 cm^{-1}; HRMS (ESI$^+$) m/z $[M+H]^+$ calcd for $C_{17}H_{13}N_4$: 273.1140, found: 273.1141.

E-4-(2-([1,2,4]Triazolo[3,4-a]phthalazin-3-yl)vinyl)phenol (12 b): Compound 12 b was obtained as a yellow powder (yield: 69%): mp > 250 °C; ^1H NMR (300 MHz, [D$_6$]DMSO): $\delta = 9.34$ (s, 1H), 8.57 (bd, $J = 7.7$ Hz, 1H), 8.33 (bd, $J = 7.5$ Hz, 1H), 8.12 (td, $J = 7.5$ Hz, 1.1 Hz, 1H), 8.06–8.00 (m, 2H), 7.67–7.64 (m, 2H), 7.35 (d, $J = 16.6$ Hz, 1H), 6.88–6.85 ppm (m, 2H); ^{13}C NMR (75 MHz, 5% [D$_1$]TFA in [D$_6$]DMSO): $\delta = 159.2$, 148.3, 148.2, 143.9, 142.6, 136.4, 134.3, 131.2, 131.1, 130.9, 129.3, 128.9, 127.7, 126.6, 125.8, 124.3, 123.0, 122.7, 122.1, 117.1, 115.6 ppm; FTIR-ATR (neat): $\tilde{v} = 3325$, 3063, 3022, 2959, 2888, 2766, 2695, 2359, 2332, 1734, 1607, 1590, 1517, 1500, 1454, 1391, 1360, 1289 cm^{-1}; HRMS (ESI$^+$) m/z $[M+H]^+$ calcd for $C_{17}H_{13}N_4O$: 289.1089, found: 289.1089.

E-3-Phenethyl-[1,2,4]triazolo[3,4-a]phthalazine (12 c): Compound 12 c was obtained as a yellow powder (yield: 56%): mp: 145 °C; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 8.65$ (dd, $J = 8.0$, 0.6 Hz, 1H), 8.59 (s, 1H), 7.97–7.90 (m, 2H), 7.80 (td, $J = 7.5$ Hz, 1.1 Hz, 1H), 7.32–7.19 (m, 5H), 3.56–3.50 (m, 2H), 3.30–3.24 ppm (m, 2H); ^{13}C NMR

(75 MHz, CDCl$_3$): δ = 150.6, 147.3, 142.6, 140.5, 133.9, 130.7, 128.5, 128.4, 128.0, 126.3, 123.5, 123.2, 123.0, 33.0, 26.3 ppm; FTIR-ATR (neat): $\tilde{v}$ = 3485, 3432, 3242, 3054, 3025, 2929, 2356, 2329, 1625, 1603, 1526, 1497, 1354, 1318 cm^{-1}; HRMS (ESI$^+$) m/z [M + H]$^+$ calcd for C$_{17}$H$_{15}$N$_4$: 275.1297, found: 275.1294.

E-4-[1,2,4]Triazolo[3,4-a]phthalazin-3-yl)phenol (12d): Compound **12d** was obtained as a yellow powder (yield: 47%): mp: 235 °C; ^{1}H NMR (300 MHz, 5% [D$_1$]TFA in CD$_3$CN): δ = 9.25 (s, 1H), 8.59–8.56 (m, 1H), 8.37–8.34 (m, 1H), 8.29–8.17 (m, 2H), 7.11–7.08 (m, 2H), 6.75–6.72 (m, 2H), 3.61 (t, J = 7.5 Hz, 2H), 3.18 ppm (t, J = 7.5 Hz, 2H); ^{13}C NMR (75 MHz, CDCl$_3$): δ = 153.9, 151.7, 151.1, 141.4, 136.3, 135.1, 130.5, 129.5, 124.8, 124.2, 116.6, 115.7, 112.8, 31.1, 25.7 ppm; FTIR-ATR (neat): $\tilde{v}$ = 3317, 3032, 2792, 2587, 1736, 1605, 1583, 1532, 1461, 1372, 1280, 1225 cm^{-1}; HRMS (ESI$^+$) m/z [M + H]$^+$ calcd for C$_{17}$H$_{15}$N$_4$O: 291.1246, found: 291.1247.

E-3-(4-Bromostyryl)-[1,2,4]triazolo[3,4-a]phthalazine (12e): Compound **12e** was obtained as a white powder (yield: 78%): mp: 242 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.73–8.70 (m, 2H), 8.10 (d, J = 16.6 Hz, 1H), 8.01–7.96 (m, 2H), 7.84 (td, J = 7.5 Hz, 1.1 Hz, 1H), 7.57–7.48 ppm (m, 5H); ^{13}C NMR (75 MHz, CDCl$_3$): δ = 148.4, 147.6, 147.5, 142.9, 135.0, 134.7, 134.2, 132.0, 130.9, 128.7, 128.1, 123.5, 123.4, 123.1, 111.3 ppm; FTIR-ATR (neat): $\tilde{v}$ = 3063, 3020, 2925, 1762, 1725, 1615, 1532, 1450, 1389, 1268, 1232 cm^{-1}; HRMS (ESI$^+$) m/z [M + H]$^+$ calcd for C$_{17}$H$_{12}$N$_4$^{79}Br: 351.0245, found: 351.0235.

E-3-(4-Nitrostyryl)-[1,2,4]triazolo[3,4-a]phthalazine (12f): Compound **12f** was obtained as a yellow powder (yield: 62%): mp: 190 °C; ^{1}H NMR (300 MHz, 5% [D$_1$]TFA in CDCl$_3$): δ = 9.16 (s, 1H), 8.78 (bd, J = 7.8 Hz, 1H), 8.43–8.23 (m, 6H), 7.90–7.87 (m, 2H), 7.67 ppm (d, J = 16.7 Hz, 1H); ^{13}C NMR (75 MHz, 5% [D$_1$]TFA in CDCl$_3$): δ = 150.7, 148.9, 146.9, 141.8, 141.1, 140.0, 136.7, 135.3, 129.5, 128.9, 125.1, 124.5, 124.4, 119.0, 109.6 ppm; FTIR-ATR (neat): $\tilde{v}$ = 3041, 2913, 1750, 1732, 1501, 1464, 1425, 1333, 1264, 1207 cm^{-1}; HRMS (ESI$^+$) m/z [M + H]$^+$ calcd for C$_{17}$H$_{12}$N$_5$O$_2$: 318.0991, found: 318.1001.

E-4-(2-[1,2,4]Triazolo[3,4-a]phthalazin-3-yl)vinyl)benzene-1,2-diol (12g): Compound **12g** was obtained as a yellow powder (yield: 40%): mp: 196 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.13 (s, 1H), 8.53 (bd, J = 7.9 Hz, 1H), 8.24 (bd, J = 7.5 Hz, 1H), 8.07 (bt, J = 7.5 Hz, 1H), 7.95–7.81 (m, 2H), 7.23–7.03 (m, 3H), 6.80 ppm (d, J = 8.0 Hz, 1H); ^{13}C NMR (75 MHz, 5% [D$_1$]TFA in [D$_6$]DMSO): δ = 148.5, 148.1, 147.3, 145.7, 142.2, 135.7, 134.5, 131.1, 129.1, 127.1, 123.1, 122.7, 122.2, 120.0, 115.9, 113.7, 106.9 ppm; FTIR-ATR (neat): $\tilde{v}$ = 3437, 3318, 3020, 2602, 1618, 1601, 1520, 1445, 1350, 1304, 1268, 1236 cm^{-1}; HRMS (ESI$^+$) m/z [M + H]$^+$ calcd for C$_{17}$H$_{13}$N$_4$O$_2$: 305.1039, found: 305.1036.

E-4-(2-[1,2,4]Triazolo[3,4-a]phthalazin-3-yl)vinyl)-2-methoxyphenol (12h): Compound **12h** was obtained as a yellow powder (yield: 58%): mp: 240 °C; ^{1}H NMR (300 MHz, 5% [D$_1$]TFA in CDCl$_3$) δ = 9.05 (s, 1H), 8.67 (bd, J = 7.6 Hz, 1H), 8.32 (d, J = 16.5 Hz, 1H), 8.24–8.13 (m, 3H), 7.34–7.27 (m, 2H), 7.20 (d, J = 1.7 Hz, 1H), 7.01 (d, J = 8.2 Hz, 1H), 4.00 ppm (s, 3H); ^{13}C NMR (75 MHz, 5% [D$_1$]TFA in CDCl$_3$): δ = 152.0, 148.9, 147.4, 147.2, 146.4, 142.1, 136.3, 134.8, 129.6, 126.8, 124.4, 124.3, 124.1, 120.1, 115.1, 109.9, 100.8, 56.0 ppm; FTIR-ATR (neat): $\tilde{v}$ = 3084, 2359, 2332, 1636, 1594, 1526, 1498, 1468, 1452, 1423, 1381, 1303, 1286, 1244 cm^{-1}; HRMS (ESI$^+$) m/z [M + H]$^+$ calcd for C$_{18}$H$_{15}$N$_4$O$_2$: 319.1195, found: 319.1199.

E-4-(2-[1,2,4]Triazolo[3,4-a]phthalazin-3-yl)vinyl)-2,6-dimethoxyphenol (12i): Compound **12i** was obtained as a yellow powder (yield: 60%): mp: 236 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.70–8.68 (m, 2H), 8.07 (d, J = 16.5 Hz, 1H), 7.99–7.93 (m, 2H), 7.82 (td, J =

7.4, 1.1 Hz, 1H), 7.35 (d, J = 16.5 Hz, 1H), 6.89 (s, 2H), 3.97 ppm (s, 6H); ^{13}C NMR (75 MHz, CDCl$_3$): δ = 148.8, 147.4, 147.3, 142.8, 136.2, 136.1, 134.1, 130.7, 128.0, 127.6, 123.7, 123.3, 123.0, 108.8, 104.2, 56.4 ppm; FTIR-ATR (neat): $\tilde{v}$ = 3090, 3025, 2986, 2932, 2851, 2831, 1639, 1598, 1517, 1453, 1328, 1260, 1218, 1121 cm^{-1}; HRMS (ESI$^+$) m/z [M + H]$^+$ calcd for C$_{19}$H$_{17}$N$_4$O$_3$: 349.1301, found: 349.1303.

E-3-(2-(3,4,5-Trimethoxyphenyl)ethenyl[1,2,4]triazolo[3,4-a]phthalazine (12j): Compound **12j** was obtained as a yellow powder (yield: 71%): mp: 200 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.64 (s, 1H), 8.61 (bd, J = 7.5 Hz, 1H), 8.01 (d, J = 16.5 Hz, 1H), 7.93–7.88 (m, 2H), 7.76 (td, J = 7.5, 1.1 Hz, 1H), 7.33 (d, J = 16.5 Hz, 1H), 6.82 (s, 2H), 3.91 (s, 6H), 3.87 ppm (s, 3H); ^{13}C NMR (75 MHz, CDCl$_3$): δ = 153.4, 148.4, 147.4, 142.8, 139.0, 135.7, 134.0, 131.6, 130.7, 128.0, 123.4, 123.1, 122.9, 110.1, 104.2, 60.9, 56.1 ppm; FTIR-ATR (neat): $\tilde{v}$ = 3264, 3065, 3032, 3003, 2968, 2939, 2837, 1643, 1585, 1524, 1511, 1450, 1333, 1251, 1123 cm^{-1}; HRMS (ESI$^+$) m/z [M + H]$^+$ calcd for C$_{20}$H$_{19}$N$_4$O$_3$: 363.1457, found: 363.1472.

General procedure for the synthesis of 3-styrylated derivatives 13a–f: K$_2$CO$_3$ (1.5 equiv) and KI (1.5 equiv) were added to a DMF solution of **12b**. After stirring the suspension at room temperature for 15 min, the corresponding alkylating agent (1.2 equiv) was added. After heating overnight at 60 °C, the mixture was cooled to room temperature and filtered. The resulting filtrate was evaporated and diluted with EtOAc, and the organic layer was then washed with saturated aqueous NH$_4$Cl solution, water, and brine, dried over Na$_2$SO$_4$, filtered, and evaporated. Purification of the residue by flash chromatography, eluting with the appropriate eluent, gave title compounds **13a–f**.

E-3-(4-Ethoxystyryl)-[1,2,4]triazolo[3,4-a]phthalazine (13a): Compound **13a** was obtained as a yellow powder (yield: 46%): mp: 195 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.69 (bd, J = 7.9 Hz, 1H), 8.66 (s, 1H), 8.10 (d, J = 16.6 Hz, 1H), 7.98–7.93 (m, 2H), 7.81 (td, J = 7.5, 1.1 Hz, 1H), 7.62–7.58 (m, 2H), 7.37 (d, J = 16.6 Hz, 1H), 6.96–6.91 (m, 2H), 4.08 (q, J = 6.0 Hz, 2H), 1.44 ppm (t, J = 6.0 Hz, 3H); ^{13}C NMR (75 MHz, CDCl$_3$): δ = 159.8, 148.9, 147.2, 142.6, 135.5, 133.9, 130.6, 128.6, 127.9, 123.6, 123.1, 122.9, 114.7, 108.2, 63.5, 14.7 ppm; FTIR-ATR (neat): $\tilde{v}$ = 3060, 3027, 2974, 2849, 1623, 1604, 1515, 1452, 1392, 1347, 1301, 1250, 1217 cm^{-1}; MS (ESI$^+$) m/z [M + H]$^+$ 317.14.

E-3-(4-Benzyloxystyryl)-[1,2,4]triazolo[3,4-a]phthalazine (13b): Compound **13b** was obtained as a yellow powder (yield: 60%): mp: 210 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.69 (bd, J = 7.7 Hz, 1H), 8.67 (s, 1H), 8.10 (d, J = 16.5 Hz, 1H), 7.98–7.92 (m, 2H), 7.80 (td, J = 7.5, 1.0 Hz, 1H), 7.63–7.59 (m, 2H), 7.47–7.32 (m, 6H), 7.04–6.99 (m, 2H), 5.12 ppm (s, 2H); ^{13}C NMR (75 MHz, CDCl$_3$): δ = 159.6, 148.9, 147.3, 142.7, 136.6, 135.4, 133.9, 130.6, 129.1, 128.7, 128.6, 128.0, 127.9, 127.4, 123.7, 123.2, 122.9, 115.2, 108.6, 70.0 ppm; FTIR-ATR (neat): $\tilde{v}$ = 3324, 3048, 2953, 2853, 2360, 2333, 1638, 1604, 1574, 1520, 1460, 1389, 1346, 1210 cm^{-1}; HRMS (ESI$^+$) m/z [M + H]$^+$ calcd for C$_{24}$H$_{19}$N$_4$O: 379.1559, found: 379.1555.

E-3-(4-((3-Methylbut-2-en-1-yl)oxy)styryl)-[1,2,4]triazolo[3,4-a]phthalazine (13c): Compound **13c** was obtained as a yellow powder (yield: 66%): mp: 170 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.69 (bd, J = 8.1 Hz, 1H), 8.67 (s, 1H), 8.10 (d, J = 16.6 Hz, 1H), 7.98–7.93 (m, 2H), 7.80 (td, J = 7.5, 1.0 Hz, 1H), 7.62–7.59 (m, 2H), 7.38 (d, J = 16.6 Hz, 1H), 6.97–6.94 (m, 2H), 5.54–5.49 (m, 1H), 4.56 (d, J = 6.7 Hz, 2H), 1.77 (s, 3H), 1.62 ppm (s, 3H); ^{13}C NMR (75 MHz, CDCl$_3$): δ = 159.7, 149.0, 147.2, 142.7, 138.4, 135.6, 133.9, 130.6, 128.7, 128.6, 128.0, 123.6, 123.1, 123.0, 119.4, 114.9, 108.3, 64.8, 25.8, 18.2 ppm; FTIR-ATR (neat): $\tilde{v}$ = 3035, 3023, 2961, 2877, 1609,

1602, 1573, 1511, 1449, 1346, 1305, 1258, 1244 cm^{-1}; MS (ESI$^+$) m/z [M+H]$^+$ 357.17.

E-3-(4-Isopentyloxy)styryl)-[1,2,4]triazolo[3,4-a]phthalazine (13 d): Compound **13 d** was obtained as a yellow powder (yield: 85%): mp 161 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.69 (bd, J = 8.0 Hz, 1H), 8.66 (s, 1H), 8.10 (d, J = 16.6 Hz, 1H), 7.98–7.93 (m, 2H), 7.80 (td, J = 7.5 Hz, 1.1 Hz, 1H), 7.62–7.58 (m, 2H), 7.37 (d, J = 16.6 Hz, 1H), 6.96–6.91 (m, 2H), 4.04 (t, J = 6.6 Hz, 2H), 1.90–1.81 (m, 1H), 1.78 (q, J = 6.9 Hz, 2H), 0.98 ppm (d, J = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, CDCl$_3$): δ = 160.0, 148.9, 147.2, 142.6, 135.5, 133.9, 130.5, 128.6, 127.9, 123.6, 123.1, 122.9, 114.7, 108.2, 66.4, 37.9, 25.0, 22.5 ppm; FTIR-ATR (neat): $\tilde{\nu}$ = 3072, 3031, 2957, 2872, 2359, 1743, 1631, 1601, 1570, 1515, 1455, 1387, 1310, 1252 cm^{-1}; HRMS (ESI$^+$) m/z [M+H]$^+$ calcd for C$_{22}$H$_{23}$N$_4$O: 359.1872, found: 359.1864.

E-3-(4-((E-3,7-Dimethylocta-2,6-dien-1-yl)oxy)styryl)-[1,2,4]triazolo[3,4-a]phthalazine (13 e): Compound **13 e** was obtained as a yellow powder (yield: 56%): mp: 150 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.69 (bd, J = 7.9 Hz, 1H), 8.66 (s, 1H), 8.10 (d, J = 16.6 Hz, 1H), 7.98–7.92 (m, 2H), 7.80 (td, J = 7.5 Hz, 1.2 Hz, 1H), 7.61–7.59 (m, 2H), 7.37 (d, J = 16.6 Hz, 1H), 6.97–6.93 (m, 2H), 5.52–5.48 (m, 1H), 5.12–5.09 (m, 1H), 4.59 (d, J = 6.5 Hz, 2H), 2.15–2.11 (m, 4H), 1.76 (s, 3H), 1.68 (s, 3H), 1.61 ppm (s, 3H); ^{13}C NMR (75 MHz, CDCl$_3$): δ = 159.7, 148.9, 147.2, 142.6, 141.4, 135.5, 133.9, 131.8, 130.6, 128.7, 128.6, 127.9, 123.7, 123.6, 123.1, 123.0, 119.2, 115.0, 108.3, 64.9, 39.5, 26.2, 25.7, 17.7, 16.7 ppm; FTIR-ATR (neat): $\tilde{\nu}$ = 3239, 3063, 2965, 2926, 2362, 2338, 1711, 1633, 1603, 1514, 1452, 1375, 1348, 1243 cm^{-1}; HRMS (ESI$^+$) m/z [M+H]$^+$ calcd for C$_{27}$H$_{29}$N$_4$O: 425.2341 found: 425.2344.

E-3-(4-(3,7-Dimethyloctyl)oxy)styryl)-[1,2,4]triazolo[3,4-a]phthalazine (13 f): Compound **13 f** was obtained as a yellow powder (yield: 82%): mp: 124 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.75 (bd, J = 7.7 Hz, 1H), 8.71 (s, 1H), 8.18 (d, J = 16.5 Hz, 1H), 8.01–7.95 (m, 2H), 7.84 (td, J = 7.5 Hz, 1.1 Hz, 1H), 7.63–7.59 (m, 2H), 7.37 (d, J = 16.5 Hz, 1H), 6.95–6.92 (m, 2H), 4.07–4.02 (m, 2H), 1.89–1.20 (m, 10H), 0.96 (d, J = 6.4 Hz, 3H), 0.88 ppm (d, J = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, CDCl$_3$): δ = 160.0, 148.9, 147.3, 142.6, 135.8, 133.9, 130.7, 128.7, 128.5, 128.0, 123.6, 123.2, 123.0, 114.8, 108.1, 66.4, 39.2, 37.3, 36.1, 29.8, 27.9, 24.6, 22.7, 22.6, 19.6 ppm; FTIR-ATR (neat): $\tilde{\nu}$ = 3060, 3027, 2951, 2918, 2836, 1636, 1602, 1575, 1522, 1468, 1449, 1383, 1363, 1348, 1295, 1242, 1225, 1210 cm^{-1}; HRMS (ESI$^+$) m/z [M+H]$^+$ calcd for C$_{27}$H$_{33}$N$_4$O: 429.2654 found: 429.2657.

Synthesis of E-4-(2-[1,2,4]triazolo[3,4-a]phthalazin-3-yl)vinyl)benzene-1,2,3-triol (14): BBr$_3$ (1 M in CH$_2$Cl$_2$, 3 mL, 9 equiv) was added dropwise to a suspension of **12 j** (120 mg, 1.0 equiv) at −78 °C. The mixture was allowed to warm to room temperature, and after stirring for 2 days, the mixture was quenched with water (10 mL) and treated 30 min later with CH$_2$Cl$_2$ (30 mL). After evaporation of the aqueous layer, title compound **14** was precipitated from CH$_2$Cl$_2$ as a brown powder in 82% yield (90 mg): mp: 299 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.16 (s, 1H), 8.52 (d, J = 7.8 Hz, 1H), 8.25 (d, J = 7.7 Hz, 1H), 8.08 (t, J = 7.4 Hz, 1H), 7.95 (t, J = 7.8 Hz, 1H), 7.75 (d, J = 16.4 Hz, 1H), 7.14 (d, J = 16.4 Hz, 1H), 6.68 ppm (s, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 148.7, 148.0, 146.3, 142.2, 136.2, 135.3, 134.5, 131.3, 129.2, 126.0, 123.2, 122.6, 122.2, 106.7, 106.5 ppm; FTIR-ATR (neat): $\tilde{\nu}$ = 3093, 3060, 3030, 2931, 2851, 1631, 1605, 1535, 1462, 1321, 1228, 1185 cm^{-1}; HRMS (ESI$^+$) m/z [M+H]$^+$ calcd for C$_{17}$H$_{13}$N$_4$O$_3$: 321.0988, found: 321.0988.

Synthesis of 3-ethynyl-[1,2,4]triazolo[3,4-a]phthalazine (15): Et$_3$N (0.4 mL, 4 equiv) was added to a CH$_2$Cl$_2$ solution (3 mL) of 3-(trimethylsilyl)propynoic acid (100 mg, 0.7 mmol, 1 equiv), 1-hydrazinylphthalazine hydrochloride **1** (207 mg, 1.5 equiv), HOBt (104 mg,

1.1 equiv) and EDC·HCl (148 mg, 1.1 equiv) at 0 °C. The mixture was stirred for 30 min at 0 °C and then stirred for additional 2 h at room temperature. The resulting precipitate was filtered, washed with saturated aqueous NH$_4$Cl solution (10 mL), water (2×10 mL) and Et$_2$O (3×10 mL), thus giving the crude product **16**, which was used without further purification in the next step: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.51 (bs, 1H), 10.45 (bs, 1H), 8.20–7.91 (m, 2H), 7.77–7.60 (m, 3H), 0.26 ppm (s, 9H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 152.4, 151.9, 145.5, 136.4, 135.1, 129.2, 127.8, 124.2, 96.5, 94.7, 59.8, −0.9 ppm; MS (DCI$^+$) m/z [M+H]$^+$ 285.1. After microwave irradiation for 60 min (~100 °C, 260 W) of a CH$_3$CN solution (3 mL) of crude **16** (10 mg, 0.035 mmol), the resulting mixture was cooled to room temperature and evaporated under reduced pressure. The thus-obtained crude product was diluted with EtOAc (20 mL), and the resulting organic layer was washed with water (2×10 mL) and brine (2×10 mL), dried over Na$_2$SO$_4$, filtered, and evaporated. The resulting crude product **17** was directly submitted to deprotection conditions. Compound **17** and K$_2$CO$_3$ (15 mg, 3.2 equiv) were suspended in MeOH, and this mixture was stirred for 12 h at room temperature. The mixture was then diluted with EtOAc (20 mL), and the resulting organic layer was washed with saturated aqueous NH$_4$Cl solution (2×10 mL), water (2×10 mL), brine (2×10 mL), dried over Na$_2$SO$_4$, filtered, and evaporated. Purification of the residue by flash chromatography, eluting with EtOAc/petroleum ether (1:1), gave title compound **15** in pure form (6 mg, 31% in 3 steps): mp: 224 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.76 (s, 1H), 8.73 (bd, J = 7.7 Hz, 1H), 8.05–7.98 (m, 2H), 7.88 (td, J = 7.3 Hz, 1.2 Hz, 1H), 3.80 ppm (s, 1H); ^{13}C NMR (75 MHz, CDCl$_3$): δ = 150.0, 142.3, 135.4, 133.0, 128.8, 124.2, 123.8, 121.5, 113.1, 89.5, 66.8 ppm; FTIR-ATR (neat): $\tilde{\nu}$ = 3626, 3344, 3179, 3079, 3046, 2536, 2121, 1968, 1673, 1640, 1623, 1553, 1521, 1470, 1446, 1378, 1314, 1264, 1233 cm^{-1}; HRMS (ESI$^+$) m/z [M+H]$^+$ calcd for C$_{11}$H$_7$N$_4$: 195.0671, found: 195.0668.

General procedure for the photoisomerization of (E) to (Z) derivatives 18 a–c: A 0.02 M CH$_3$CN solution of E-styrylated triazolophthalazine **12 a**, **12 e**, or **13 a** was irradiated by a 350 W halogen lamp for 50 h. A solution of K$_2$CO$_3$ (1.5 equiv) and KI (1.5 equiv) was added. After cooling to room temperature, the mixture was evaporated and the residue was purified by flash chromatography, eluting with the appropriate eluent, to furnish title compounds **18 a–c** in pure forms.

Z-3-Styryl-2-([1,2,4]triazolo[3,4-a]phthalazine (18a): Compound **18 a** was obtained as a yellow oil (yield: 75%): ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.75 (bd, J = 8.0 Hz, 1H), 8.63 (s, 1H), 8.01–7.93 (m, 4H), 7.83 (td, J = 7.5 Hz, 1.1 Hz, 1H), 7.39–7.33 (m, 3H), 7.12 (d, J = 13.0 Hz, 1H), 6.92 ppm (d, J = 16.6 Hz, 1H); ^{13}C NMR (75 MHz, CDCl$_3$): δ = 147.6, 147.3, 142.1, 137.9, 135.5, 134.0, 130.9, 130.0, 128.9, 128.2, 128.0, 123.5, 123.4, 123.1, 110.0 ppm; FTIR-ATR (neat): $\tilde{\nu}$ = 3041, 3012, 2983, 2958, 2886, 2360, 2317, 2157, 1980, 1640, 1627, 1518, 1504, 1468, 1450, 1392 cm^{-1}; HRMS (ESI$^+$) m/z [M+H]$^+$ calcd for C$_{17}$H$_{13}$N$_4$: 273.1140, found: 273.1141.

Z-3-(4-bromostyryl)-[1,2,4]triazolo[3,4-a]phthalazine (18b): Compound **18 b** was obtained as a colorless oil (yield: 90%): ^{1}H NMR (300 MHz, 5% [D$_1$]TFA in CDCl$_3$): δ = 8.70 (bd, J = 7.8 Hz, 1H), 8.64 (s, 1H), 8.00–7.93 (m, 4H), 7.84 (td, J = 7.5 Hz, 1.0 Hz, 1H), 7.51–7.48 (m, 2H), 7.01 (d, J = 13.1 Hz, 1H), 6.96 ppm (d, J = 13.1 Hz, 1H); ^{13}C NMR (75 MHz, CDCl$_3$): δ = 147.6, 147.3, 142.1, 136.4, 134.3, 134.2, 131.7, 131.3, 131.1, 128.1, 123.5, 123.24, 123.20, 123.1, 110.3 ppm; FTIR-ATR (neat): $\tilde{\nu}$ = 3063, 3020, 2925, 1762, 1725, 1615, 1532, 1450, 1389, 1268, 1232 cm^{-1}; HRMS (ESI$^+$) m/z [M+H]$^+$ calcd for C$_{17}$H$_{12}$N$_4$^{79}Br: 351.0185, found: 351.0235.

Biology

Growth conditions of *M.tb* strains and determination of minimum inhibitory concentrations (MICs): *M.tb* H37Rv was used as the reference strain, and clinical isolates were grown at 37 °C in Middlebrook 7H9 broth (Difco), supplemented with 0.05 % Tween 80, or on solid Middlebrook 7H11 medium (Difco) supplemented with oleic acid–albumin–dextrose–catalase (OADC). MIC values for the new compounds were determined by means of the micro-broth dilution method. Dilutions of *M.tb* culture ($\sim 10^5$–10^6 cfu mL^{-1}) were streaked onto 7H11 solid medium containing a range of drug concentrations (0.25 µg mL^{-1} to 40 µg mL^{-1}). Plates were incubated at 37 °C for about 21 days, and the growth was visually evaluated. The lowest drug dilution at which visible growth failed to occur was taken as the MIC value. Results were expressed as the average of at least three independent determinations.

M.tb clinical isolates and drug susceptibility testing:[26] Three *M.tb* multidrug-resistant isolates were collected at the Sondalo Division of the Valtellina and Valchiavenna, Italy, hospital authority in 2012. Their resistance profile is shown in Table 2. All clinical isolates were grown in BACTEC MGIT 960 and Lowenstein–Jensen slants. Drug susceptibility testing for all first-line antitubercular drugs was performed with the BACTEC MGIT 960 System (BectoneDickinson Diagnostic Systems, Sparks, Maryland) for isoniazid (0.1 µg mL^{-1}; 0.4 µg mL^{-1}), rifampicin (1 µg mL^{-1}), streptomycin (1 µg mL^{-1}; 4 µg mL^{-1}), ethambutol (5 µg mL^{-1}), and pyrazinamide (100 µg mL^{-1}), in accordance with the manufacturer's instructions. MIC value determination for second-line drugs (cycloserine, 50 µg mL^{-1}; amikacin, 1–5 µg mL^{-1}; ciprofloxacin, 2 µg mL^{-1}; ethionamide, 5 or 10 µg mL^{-1}; *para*-aminosalicylic acid, 4 or 8 µg mL^{-1}; ofloxacin, 10 µg mL^{-1}) was also performed using the MGIT 960 System.

Cytotoxicity: HCT116 human colon cancer cells (ATCC) were cultured in DMEM supplemented with 10 % fetal calf serum. For cytotoxicity evaluation, 3000 cells were seeded per well in 96-well plates and, 24 h later, were treated with test compounds at concentrations ranging from 50 nM to 100 µM (eight replicates for each). After 4 days of treatment, cells were incubated with the cell proliferation reagent WST-1 (Roche) for 3–4 h, and the absorbance was then measured with a scanning multiwell spectrophotometer. The measured absorbance directly correlates to the number of viable cells. Data were analyzed using Excel (Microsoft) and Prism (GraphPad) software.

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