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Original article

Chemical synthesis and biological evaluation of triazole derivatives as inhibitors of InhA and antituberculosis agents

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ABSTRACT

A series of triazoles have been prepared and evaluated as inhibitors of InhA as well as inhibitors of *Mycobacterium tuberculosis* $H_{37}R_v$. Several of these new compounds possess a good activity against InhA, particularly compounds **17** and **18** for which molecular docking has been performed. Concerning their activities against *M. tuberculosis* $H_{37}R_V$ strain, two of them, **3** and **12**, were found to be good inhibitors with MIC values of 0.50 and 0.25 μg/mL, respectively. Particularly, compound **12** presenting the best MIC value of all compounds tested (0.6 μM) is totally inactive against InhA.

1. Introduction

Tuberculosis caused by *Mycobacterium tuberculosis* is responsible for more than two million deaths worldwide every year. The World Health Organization (WHO) has estimated than one-third of the world population is infected by *M. tuberculosis* [1]. Multidrug-resistant *M. tuberculosis* (MDR-MT) [2,3] and extensively drug-resistant MT (XDR-MT) [4] have become resistant at an alarming rate [5] to existing antibiotics: isoniazid, rifampicin (for MDR-MT) and fluoroquinolone kanamicyn, amikacin, and capreomycin (for XDR-MT). Currently there is an urgent need of new chemotherapeutics to combat the spread of this disease. Different families of compounds have been reported with antitubercular activities such as cinnamic derivatives [6], hydrazones [7], nitroimidazoles [8]. In recent years, triazoles have received much attention as central core in medicinal chemistry and especially in the synthesis of antitubercular agents [9]. Our own efforts on the development of potential inhibitors of the 2-trans-enoyl-ACP (acyl carrier protein) reductase enzyme (InhA or ENR, EC number: 1.3.1.9) from *M. tuberculosis* have focused on the synthesis of triazole derivatives to mimic the phenol central core of triclosan analogues as shown in Fig. 1 [10].

Among the different molecules conceived, synthetized and tested on InhA and *M. tuberculosis* $H_{37}R_v$ strain, compound **3** (Fig. 1) has been proven to be a good inhibitor of *M. tuberculosis* strain but a weak inhibitor of InhA [10].

In this study, we report the synthesis and the evaluation of a series of potential InhA inhibitors bearing different substituents around the triazole core as shown in Fig. 1. We assessed: 1) the effect of the nature of the substituent on the phenyl group; 2) the effect of the alkyl chain length and 3) the effect of the linking positions on the triazole fragment on InhA and *M. tuberculosis* activities.

2. Results and discussion

2.1. Chemistry

The triazole moiety was synthesized by copper catalyzed Huisgen 1,3 dipolar cycloaddition reaction between azides and alkynes. This cycloaddition remains very attractive to generate numerous applications and especially in medicinal chemistry. Also, they are

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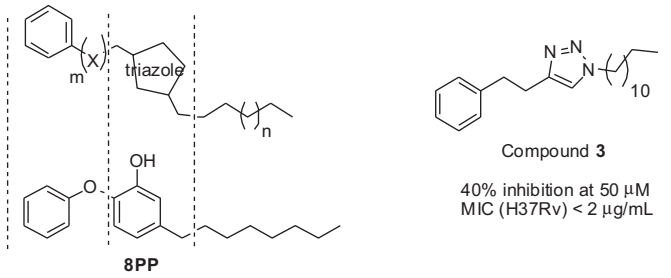


Fig. 1. Analogies between triclosan analogue **8PP** (also called **8PS**) [11] and compounds in this publication (left); inhibitor **3** [10] (right).

characterized by mild reaction conditions, high regioselectivities and high yields [12,13].

A series of 1,2,3-triazoles bearing alkyl and arylalkyl chains attached at either the carbon or the nitrogen atom of the triazole frame, were synthesized by click chemistry from the corresponding azide and commercially available alkynes.

Aryl ether derivatives were obtained through two different manners. First, the ether analogue **4** of compound **3** (Fig. 1) was synthesized in 79% from the corresponding azide and alkyne (R = PhO, $n = 1$), easily prepared from phenol and propargyl bromide [14] (Scheme 1).

Halogenated compound **5** was used for the elaboration of the other aryl ether derivatives. Compounds **6** and **7** were synthesized in 94% and 66% yield respectively, by condensing the bromide derivative **5**, synthetized through click chemistry between propargyl bromide and dodecyl azide, with the corresponding phenols in the presence of potassium carbonate (Scheme 2).

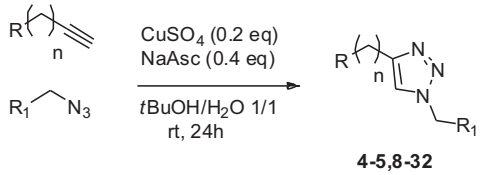
Finally, two other molecules, **33** and **34**, were synthesized as isomers of compounds **3** and **9**. To gain access to these 1,5-disubstituted triazoles, the corresponding azides and alkynes were combined and heated under reflux in toluene for 35 h. A small amount was purified enough to perform the biological test. Structural identification of compounds **33** and **34** was based upon comparing their spectroscopic (^{1}H and ^{13}C NMR) and thin layer chromatography data with those of 1,4-regioisomers **3**, **9** respectively, obtained through classical copper-induced click reaction (Scheme 3).

2.2. Biology

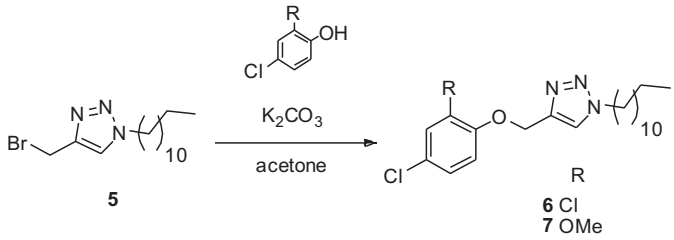
2.2.1. InhA inhibition assay

Recombinant *M. tuberculosis* InhA was expressed in *E. coli* and subsequently purified according to a previous reported procedure [10]. The synthetic compounds were evaluated *in vitro* for the inhibition of InhA from *M. tuberculosis* at 50 μM by applying a commonly used method [10]. Triclosan showed a complete inhibition of InhA at 10 μM. The results are shown in Table 2.

In analysing the results more closely, two families of compounds may be considered. The first one when the aromatic system is connected to the carbon atom of the triazole core; the second one when it is connected to the nitrogen atom of the triazole frame.



Scheme 1. Synthesis of triazoles **4**,**5**, **8**–**32**.



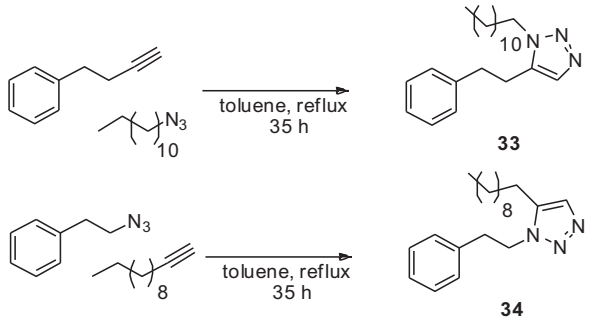
Scheme 2. Synthesis of triazoles **6** and **7**.

Concerning the first family, when two carbon atoms separate the aromatic ring from the triazole core, fair inhibition activities were obtained for compounds **1** and **3** and a much better one for compound **2** possessing 9-carbon alkyl chain. It is interesting to point out that when oxygen atom replaces the methylene group, a better activity was observed (54% *vs* 40% for compounds **4** and **3** respectively). 2,4-Dichloro substitutions on the aromatic ring are deleterious for the activity which is restored by replacement of the 2-chloro by the 2-OMe group (5% *vs* 35% for compounds **6** and **7** respectively).

While compounds **31** and **32**, bearing an aromatic ring directly attached to the triazole core are weak or no inhibitors at all, compounds with one methylene group between these two frames present much better activities. In fact, triazole derivatives **17**, **18** and **19** showed 73%, 75% and 58% inhibition activities at 50 μM in comparison to 46%, 58% and 40% for compounds **1**, **2** and **3** respectively. These inhibitors, even though they are relatively similar by their structure to triclosan analogue **8PS** [11], still present a much lower activity than the latter compound ($IC_{50} = 5$ nM). A hypothetical placement of compound **18** obtained by molecular docking calculations in the active site of InhA (PDB code 2B37) shown in Fig. 2. Both compounds **8PS** and **18** can easily be superimposed. However, the nanomolar activity of compound **8PS** could be attributed to the hydroxyl-substituted ring of triclosan that forms a hydrogen bond to Tyr158 and the 2′-hydroxy group of NAD^+, thus inducing a most favourable π-stacking interaction between the phenol and the NAD^+ rings [11].

Considering the second family of compounds, when two carbon atoms separate the aromatic and triazole frame, fair inhibition activities were obtained for compounds **9** and **10**. No major changes were observed when introducing a 2-OMe or a 2-Me substituent on the aromatic ring (compounds **13**, **14** and **15**, **16** respectively). An −OMe substitution at another positions of the aromatic ring are deleterious for the activities (compounds **11** and **12**). When one methylenic group separates the two frames, the compounds tested showed comparable (**9**/**22**; **20**/**23**; **13**/**26**) or less (**14**/**26**; **13**/**25**) activities against InhA.

Finally, compounds **33** and **34** α-substituted on the triazole core do not present any worth considering activity.



Scheme 3. Synthesis of 1,5-triazoles **33** and **34**.

Table 1
Synthesis of triazole derivatives.

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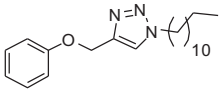
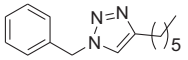
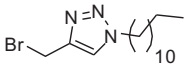
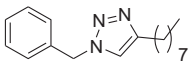
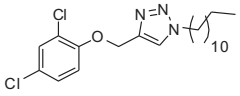
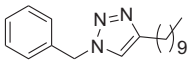
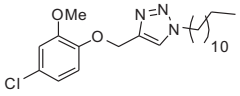
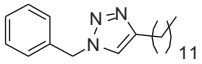
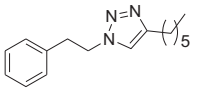
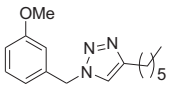
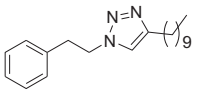
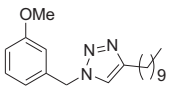
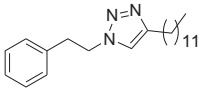
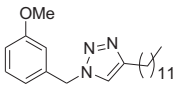
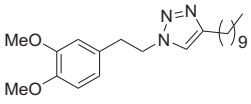
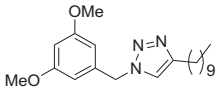
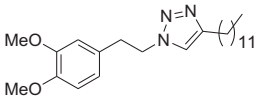
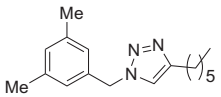
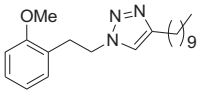
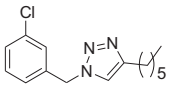
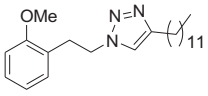
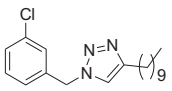
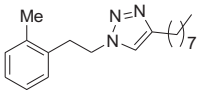
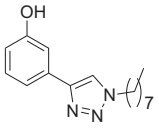
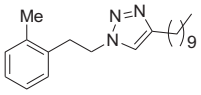
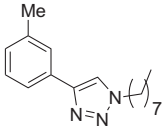
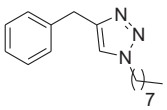
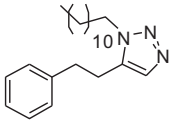
4		79	**20**		76
5		30	**21**		80
6		94	**22**		79
7		66	**23**		82
8		99	**24**		78
9		89	**25**		76
10		76	**26**		90
11		90	**27**		70
12		83	**28**		81
13		89	**29**		58
14		86	**30**		61
15		78	**31**		68
16		69	**32**		98
17		78	**33**		ND[a]

(*continued on next page*)

Table 1 (*continued*)

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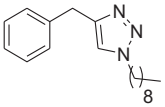
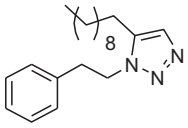
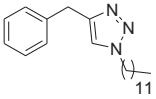
18		60	**34**		ND[a]
19		73			

[a] ND for Not determined.

2.2.2. Bacterial growth inhibition experiments

All of the synthesized compounds were evaluated by determining the minimal inhibitory concentration (MIC) on *M. tuberculosis* $H_{37}R_v$ strain (Table 3). Triclosan was used for comparison.

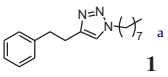
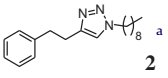
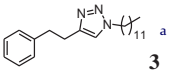
Among all compounds tested, those possessing on methylenic group between the aromatic group and the triazole frame do not present any notable activities against growth of *M. tuberculosis* $H_{37}R_v$ strain. In fact, all values are superior or equal to 4 μg/mL. The better results were obtained for compounds **26**, **29** and **30** with MIC values of 4 μg/mL (11.2, 14.4 and 12.0 μM respectively). It has to be noted that the length of the alkyl chain attached to the triazole core does not influence sharply the activities.

The most active compounds are to be found among those possessing two atoms between the two frames (aromatic and triazolo). Thus 8 compounds present activities against growth of *M. tuberculosis* $H_{37}R_v$ strain with values inferior or equal to 4 μg/mL (compounds **3**, **4**, **11**–**16**) and this independently of the linking positions of the aromatic or alkyl chain on the triazole core.

In general, the length of the alkyl chain is of importance. In fact the best results are obtained for the 12-carbon chain derivatives **3** and **12** that possess MIC values of 1.6 μM and 0.6 μM respectively and are 8–10 times more active than compounds **2** and **11** bearing respectively 9 and 10-carbon chain. It is also noteworthy to point out that compound **12** showing the best MIC value among all compounds tested does not inhibit at all the InhA enzyme. Compound **11**, possessing the same substitution pattern on the aromatic ring as compound **12**, presents also a good MIC value (5.3 μM) while it is a very weak InhA inhibitor. In contrast, the other six active compounds of the series (**3**, **4**, **13**–**16**) showed also fairly good inhibitions of InhA.

Finally, the 1,5-disubstitued triazole regioisomers are not well recognized by comparison with the 1,4-disubstituted isomers (**3** *versus* **33** and **9** *versus* **34**).

Table 2
Enzyme inhibition values. Results are expressed as a percentage of InhA inhbition.

Compd.	% Inhibition at 50 μM	Compd.	% Inhibition at 50 μM
Triclosan	>99	**18**	75
 **1** [a]	46	**19**	58
 **2** [a]	58	**20**	21
 **3** [a]	40	**21**	34
4	54	**22**	44
6	< 5	**23**	52
7	35	**24**	NI[b]
8	10	**25**	20
9	43	**26**	46
10	67	**27**	24
11	21	**28**	19
12	NI[b]	**29**	NI[b]
13	48	**30**	42
14	61	**31**	NI[b]
15	37	**32**	33
16	58	**33**	9
17	73	**34**	33

[a] See reference [10].
[b] NI for no inhibition at the given concentration.

3. Conclusion

To increase the chance of effective compounds coming from the research that can become available for TB care, we feel that the number and the structural variety of the compounds that might be conceived to enter the pipeline should be sufficiently large. In that respect, among the families of compounds developed in our group (cinnamic, triazolophtalazine ...) the triazolo ones become part as valuable scaffolds for that purpose.

Concerning the triazolo derivatives presented here, a series of alkyl triazoles were synthesized in which the A ring has been substituted with a variety of groups. Several of these new compounds possess a good activity against InhA. In particular, compounds **17** and **18**, possessing one methylene group between the triazolo and the aromatic rings present the best values among all compounds tested. Concerning their activities against *M. tuberculosis* $H_{37}R_V$ strain, two of them, **3** and **12**, were found to be good inhibitors with MIC values of 0.50 and 0.25 μg/mL, respectively. Particularly, compound **12** presenting the best MIC value of all compounds tested (0.6 μM) is totally inactive against InhA.

In conclusion, the results presented here may highlight not only compounds active against well established target (InhA), but also potentially active compounds with unknown target. In addition, these molecules bearing a relatively simple scaffold, could be structurally improved to afford better inhibitors. Concerning compound **12**, it should be interesting to search for its biological target. Studies are underway to improve their efficiency against InhA and *M. tuberculosis*.

4. Experimental

4.1. Material

Kinetic studies were perfomed on a Cary Bio 100. All chemicals were obtained from Aldrich or Acros Organics and used without further purification. Nuclear magnetic resonance spectra were recorded on a Bruker AC 300 spectrometer (^1H and ^{13}C NMR), and mass spectra were measured on a LCT Premier Waters spectrometer.

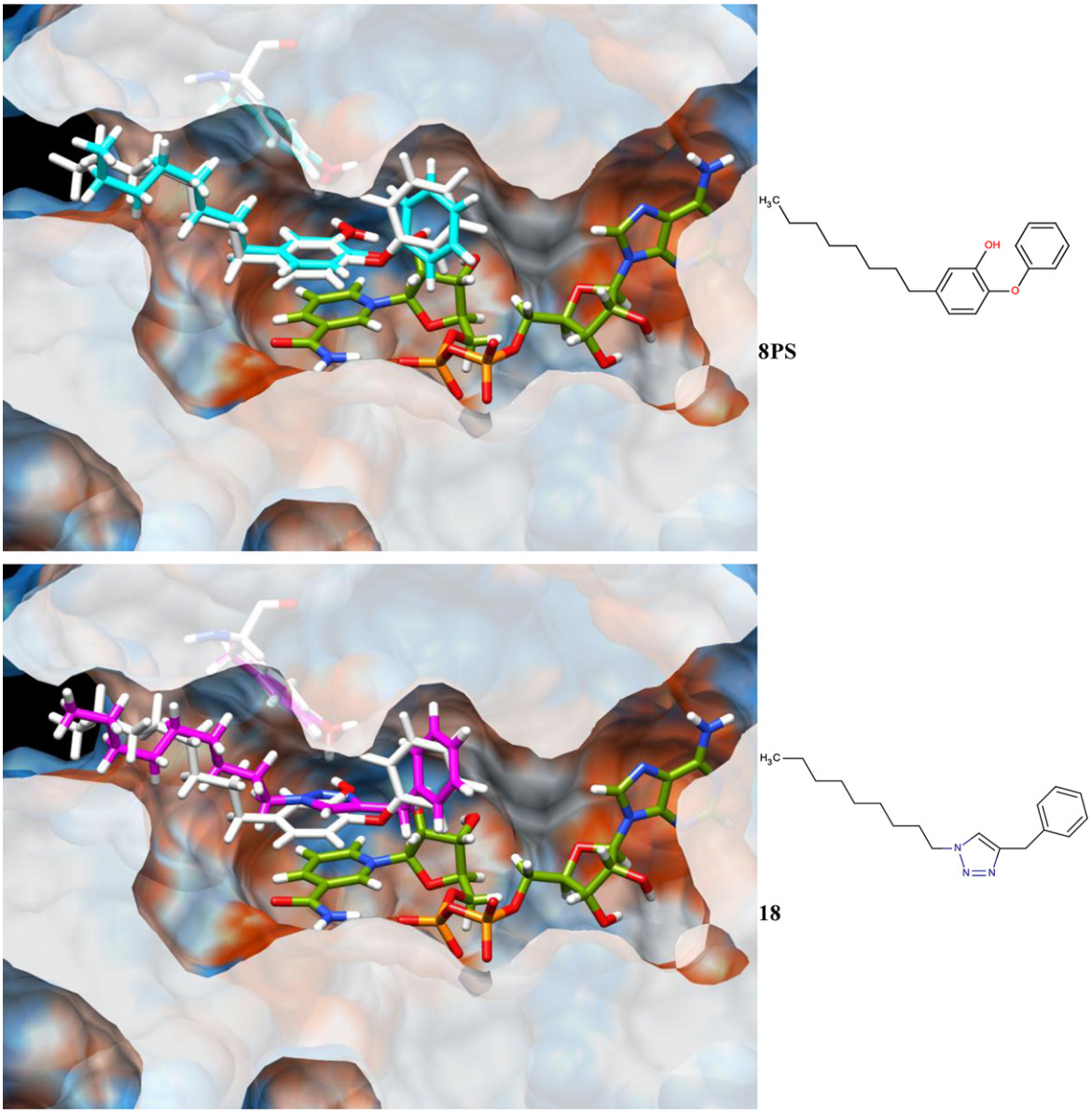


Fig. 2. Hypothetical binding mode of compounds **8PS** (up, cyan) and **18** (down, magenta) in the active site of InhA enzyme (PDB ID 2B37) obtained by molecular docking. Co-crystallised cofactor NAD^+ and **8PS** are coloured in green and white respectively; the molecular surface of protein is coloured using a hydrophobic scale and rendered using UCSF Chimera [15]. Tyr158 residue is coloured (white for 2B37 native state) in a final conformation obtained after docking. The same calculation protocol was used in both cases and the docking of 8PS shows an agreement in reproducing crystallography (RMSD = 0.94 Å) without displacing Tyr158. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.2. Chemistry

4.2.1. Synthesis of azide derivatives

To a DMF solution (3 mL) of bromide derivative, were added sodium iodide (0.05 eq) and sodium azide (1.2 eq) at 40 °C. The reaction mixture was stirred for overnight. Et_2O (20 mL) was added to the mixture then the organic layer was washed with water (3 × 20 mL), dried over $MgSO_4$ and concentrated under reduced pressure to give the azide derivative. Spectroscopic data of compounds are identical to those previously reported [10].

4.2.2. Preparation of compounds ***4,8–32***

A typical experimental procedure for the preparation of these compounds from the corresponding commercially available alkynes is described below. To a solution of alkyne (1.0 eq) with azide (1.2 eq) in *t*BuOH/H_2O (1/1), were added $CuSO_4$ (0.2 eq), AscNa (0.4 eq) at room temperature. The reaction mixture was stirred at rt for 24 h, then H_2O was added and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, concentrated under reduced pressure. The desired product was purified by flash chromatography (EtOAc/petroleum ether).

*4.2.2.1. 1-Dodecyl-4-(phenoxymethyl)-1H-1,2,3-triazole (**4**).* (The alkyne, (prop-2-ynyloxy)benzene, was synthesized following a known procedure [14].). White powder. Mp 75 °C. Yield 79%. ^1H NMR ($CDCl_3$) δ 7.58 (s, 1H); 7.27 (m, 2H); 6.96 (m, 3H); 5.19 (s, 2H); 4.32 (t, J = 7.2 Hz, 2H); 1.88 (m, 2H); 1.25 (m, 18H); 0.87 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 158.1; 144.1; 129.4; 122.4; 121.1; 114.6; 61.9; 50.3; 31.8; 30.1; 29.5; 29.4; 29.2; 29.2; 28.9; 26.3; 22.6; 14.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{21}H_{34}N_3O$: 344.2702. Found: 344.2711.

Table 3
Compounds tested as inhibitory agents of *M. tuberculosis* growth.

1,2,3-triazoles	MIC (μg/mL)/(μM)	1,2,3-triazoles	MIC (μg/mL)/(μM)
Triclosan	10/34.5	**18**	25/87.6
1	10/35	**19**	25/76.3
2	5/16.7	**20**	25/102.8
3	**0.5/1.6**	**21**	> 4/ > 14.7
4	2/5.8	**22**	> 4/ > 13.4
6	4/9.7	**23**	> 4/ > 12.2
7	> 4/ > 9.8	**24**	> 4/ > 14.6
8	10/38.9	**25**	> 4/ > 12.1
9	4/12.8	**26**	4/11.2
10	4/11.7	**27**	> 4/ > 11.1
11	2/5.3	**28**	ND
12	**0.25/0.6**	**29**	4/14.4
13	2/5.8	**30**	4/12.0
14	2/5.4	**31**	ND
15	2/6.7	**32**	> 4/ > 14.7
16	2/6.1	**33**	> 4/ > 11.7
17	25/92.1	**34**	> 4/ > 12.8

*4.2.2.2. 4-Hexyl-1-phenethyl-1H-1,2,3-triazole (**8**).* White powder. Mp 40 °C. Yield 99%. ^{1}H NMR ($CDCl_3$) δ 7.14 (m, 3H); 6.97 (dd, J = 7.9 Hz, 1.8 Hz, 2H); 6.96 (s, 1H); 4.42 (t, J = 7.1 Hz, 2H); 3.07 (t, J = 7.3 Hz, 2H); 2.54 (t, J = 7.4 Hz, 2H); 1.50 (m, 2H); 1.18 (m, 6H); 0.77 (t, J = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 147.5; 136.9; 128.3; 128.3; 126.5; 120.7; 51.0; 36.4; 31.2; 29.0; 28.4; 25.2; 22.1; 13.7; HRMS: (DCI/CH_4, m/z) calc. for $C_{16}H_{24}N_3$: 258.1970. Found: 258.1982.

*4.2.2.3. 4-Decyl-1-phenethyl-1H-1,2,3-triazole (**9**).* White powder. Mp 70 °C. Yield 90%. ^{1}H NMR ($CDCl_3$) δ 7.25 (m, 3H); 7.08 (dd, J = 7.9, 1.7 Hz, 2H); 6.98 (s, 1H); 4.52 (t, J = 7.2 Hz, 2H); 3.17 (t, J = 7.3 Hz, 2H); 2.64 (t, J = 7.5 Hz, 2H); 1.59 (m, 2H); 1.25 (m, 14H); 0.87 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 148.0; 137.2; 128.6; 128.6; 126.9; 120.9; 51.4; 36.8; 31.8; 29.5; 29.4; 29.3; 29.2; 29.1; 25.5; 22.6; 14.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{20}H_{32}N_3$: 314.2596. Found: 314.2610.

*4.2.2.4. 4-Dodecyl-1-phenethyl-1H-1,2,3-triazole (**10**).* White powder. Mp 76 °C. Yield: 76%. ^{1}H NMR ($CDCl_3$) δ 7.24 (m, 3H); 7.07 (dd, J = 7.9 Hz, 1.8 Hz, 2H); 6.98 (s, 1H); 4.52 (t, J = 7.2 Hz, 2H); 3.16 (t, J = 7.3 Hz, 2H); 2.64 (t, J = 7.5 Hz, 2H); 1.59 (m, 2H); 1.24 (m, 18H); 0.86 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 148.0; 137.1; 128.6; 128.6; 126.9; 120.9; 51.4; 36.7; 31.8; 29.6; 29.5; 29.5; 29.4; 29.3; 29.2; 29.0; 25.5; 22.6; 14.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{22}H_{36}N_3$: 342.2909. Found: 342.2914.

*4.2.2.5. 1-(3,4-Dimethoxyphenethyl)-4-decyl-1H-1,2,3-triazole (**11**).* White powder. Mp 89 °C. Yield 90%. ^{1}H NMR ($CDCl_3$) δ 6.98 (s, 1H); 6.76 (d, J = 8.2 Hz, 1H); 6.63 (dd, J = 8.1 Hz, 2.0 Hz, 1H); 6.51 (d, J = 1.9 Hz, 1H); 4.49 (t, J = 7.1 Hz, 2H); 3.84 (s, 3H); 3.78 (s, 3H); 3.11 (t, J = 7.1 Hz, 2H); 2.64 (t, J = 7.6 Hz, 2H); 1.59 (m, 2H); 1.24 (m, 14H); 0.86 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 148.9; 148.0; 147.9; 129.7; 121.0; 120.6; 111.7; 111.3; 55.8; 55.7; 51.7; 36.4; 31.8; 29.5; 29.5; 29.3; 29.2; 29.1; 25.5; 22.6; 14.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{22}H_{36}N_3O_2$: 374.2808. Found: 374.2805.

*4.2.2.6. 1-(3,4-Dimethoxyphenethyl)-4-dodecyl-1H-1,2,3-triazole (**12**).* White powder. Mp 94 °C. Yield 83%. ^{1}H NMR ($CDCl_3$) δ 6.97 (s, 1H); 6.74 (d, J = 8.2 Hz, 1H); 6.60 (dd, J = 8.1 Hz, 1.9 Hz, 1H); 6.49 (d, J = 1.9 Hz, 1H); 4.47 (t, J = 7.1 Hz, 2H); 3.81 (s, 3H); 3.76 (s, 3H); 3.08 (t, J = 7.1 Hz, 2H); 2.61 (t, J = 7.5 Hz, 2H); 1.57 (m, 2H); 1.21 (m, 18H); 0.84 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 148.9; 147.8; 129.6; 121.0; 120.5; 111.7; 111.2; 55.7; 55.6; 51.6; 36.3; 31.8; 29.5; 29.5; 29.4; 29.4; 29.2; 29.2; 29.1; 25.5; 22.5; 14.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{24}H_{40}N_3O_2$: 402.3121. Found: 402.3139.

*4.2.2.7. 1-(2-Methoxyphenethyl)-4-decyl-1H-1,2,3-triazole (**13**).* White powder. Mp 56 °C. Yield 89%. ^{1}H NMR ($CDCl_3$) δ 7.20 (td, J = 7.8 Hz, 1.8 Hz, 1H); 7.00 (s, 1H); 6.92 (dd, J = 7.4 Hz, 1.7 Hz, 1H); 6.83 (t, J = 8.5 Hz, 1H); 6.79 (dd, J = 8.4 Hz, 0.9 Hz, 1H); 4.51 (t, J = 7.1 Hz, 2H); 3.81 (s, 3H); 3.16 (t, J = 7.3 Hz, 2H); 2.63 (t, J = 7.5 Hz, 2H); 1.58 (m, 2H); 1.24 (m, 14H); 0.86 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 157.3; 147.9; 130.5; 128.3; 125.3; 120.7; 120.4; 110.1; 55.1; 49.6; 32.0; 31.8; 29.5; 29.4; 29.4; 29.3; 29.2; 29.0; 25.5; 22.5; 14.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{21}H_{34}N_3O$: 344.2702. Found: 344.2701.

*4.2.2.8. 1-(2-Methoxyphenethyl)-4-dodecyl-1H-1,2,3-triazole (**14**).* White powder. Mp 60 °C. Yield 86%. ^{1}H NMR ($CDCl_3$) δ 7.22 (td, J = 7.8 Hz, 1.8 Hz, 1H); 7.00 (s, 1H); 6.93 (dd, J = 7.4 Hz, 1.7 Hz, 1H); 6.85 (t, J = 7.8 Hz, 1H); 6.81 (dd, J = 7.4 Hz, 1.0 Hz, 1H); 4.52 (t, J = 7.1 Hz, 2H); 3.83 (s, 3H); 3.16 (t, J = 7.2 Hz, 2H); 2.64 (t, J = 7.6 Hz, 2H); 1.59 (m, 2H); 1.25 (m, 18H); 0.87 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 157.4; 147.9; 130.6; 128.3; 125.4; 120.7; 120.5; 110.2; 55.2; 49.7; 32.1; 31.8; 29.6; 29.6; 29.5; 29.5; 29.4; 29.3; 29.3; 29.1; 25.6; 22.6; 14.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{23}H_{38}N_3O$: 372.3015. Found: 372.3026.

*4.2.2.9. 1-(2-Methylphenethyl)-4-octyl-1H-1,2,3-triazole (**15**).* White powder. Mp 58 °C. Yield 98%. ^{1}H NMR ($CDCl_3$) δ 7.14 (m, 3H); 7.01 (d, J = 7.2 Hz, 1H); 6.95 (s, 1H); 4.50 (t, J = 7.2 Hz, 2H); 3.18 (t, J = 7.5 Hz, 2H); 2.66 (t, J = 7.5 Hz, 2H); 2.22 (s, 3H); 1.60 (m, 2H); 1.27 (m, 10H); 0.88 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 148.2; 136.2; 135.4; 130.5; 129.3; 127.1; 126.3; 120.8; 50.4; 34.1; 31.8; 29.4; 29.3; 29.2; 29.1; 25.5; 22.6; 19.1; 14.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{19}H_{30}N_3$: 300.2440. Found: 300.2442.

*4.2.2.10. 1-(2-Methylphenethyl)-4-decyl-1H-1,2,3-triazole (**16**).* White powder. Mp 64 °C Yield 69%. ^{1}H NMR ($CDCl_3$) δ 7.14 (m, 3H); 7.01 (d, J = 7.3 Hz, 1H); 6.96 (s, 1H); 4.50 (t, J = 7.2 Hz, 2H); 3.18 (t, J = 7.5 Hz, 2H); 2.65 (t, J = 7.5 Hz, 2H); 2.22 (s, 3H); 1.60 (m, 2H); 1.26 (m, 14H); 0.87 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 148.2; 136.2; 135.4; 129.3; 127.1; 126.3; 120.8; 50.4; 34.1; 31.8; 29.5; 29.5; 29.4; 29.3; 29.3; 29.1; 25.5; 22.6; 19.1; 14.1; HRMS: (DCI/CH_4, m/z) calc. for $C_{21}H_{34}N_3$: 328.2753. Found: 328.2753.

*4.2.2.11. 4-Benzyl-1-octyl-1H-1,2,3-triazole (**17**).* White powder. Mp 53 °C. Yield 78%. ^{1}H NMR ($CDCl_3$) δ 7.15–7.29 (m, 5H); 7.13 (s, 1H); 4.20 (t, J = 7.3 Hz, 2H); 4.03 (s, 2H); 1.80 (m, 2H); 1.23 (m, 10H); 0.84 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 147.1; 138.9; 128.4; 128.3; 126.1; 121.0; 49.9; 32.0; 31.4; 30.0; 28.7; 28.6; 26.2; 22.3; 13.8; HRMS: (DCI/CH_4, m/z) calc. for $C_{17}H_{26}N_3$: 272.2127. Found: 272.2134.

*4.2.2.12. 4-Benzyl-1-nonyl-1H-1,2,3-triazole (**18**).* White powder. Mp 58 °C. Yield 60%. ^{1}H NMR ($CDCl_3$) δ 7.27 (m, 5H); 7.13 (s, 1H); 4.26 (t, J = 7.3 Hz, 2H); 4.09 (s, 2H); 1.85 (m, 2H); 1.24 (m, 12 H); 0.87 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 147.5; 139.1; 128.6; 128.5; 126.4; 121.1; 50.2; 32.2; 31.7; 30.2; 29.2; 29.0; 28.9; 26.4; 22.5; 14.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{18}H_{28}N_3$: 286.2283. Found: 286.2292.

*4.2.2.13. 4-Benzyl-1-dodecyl-1H-1,2,3-triazole (**19**).* White powder. Mp 61 °C. Yield 73%. ^{1}H NMR ($CDCl_3$) δ 7.26 (m, 5H); 7.15 (s, 1H); 4.28 (t, J = 7.3 Hz, 2H); 4.1 (s, 2H); 1.86 (m, 2H); 1.25 (m, 18H); 0.89 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 147.6; 139.1; 128.6; 128.5; 126.3; 121.1; 50.2; 32.2; 31.8; 30.2; 29.5; 29.4; 29.3; 29.2; 28.9; 26.4; 22.6; 14.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{21}H_{34}N_3$: 328.2753. Found: 328.2757.

*4.2.2.14. 1-Benzyl-4-hexyl-1H-1,2,3-triazole (**20**).* White powder. Mp 57 °C. Yield 76%. ^{1}H NMR ($CDCl_3$) δ 7.22–7.35 (m, 6H); 5.56 (s, 2H); 2.64 (t, J = 7.7 Hz, 2H); 1.67 (m, 2H); 1.31 (m, 6H); 0.87 (t,

$J = 6.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 148.9; 135.1; 129.0; 128.6; 127.9; 120.6; 53.9; 31.5; 29.4; 28.9; 25.7; 22.5; 14.1; HRMS: (DCI/CH_4, m/z) calc. for $C_{15}H_{22}N_3$: 244.1814. Found: 244.1818.

*4.2.2.15. 1-Benzyl-4-octyl-1H-1,2,3-triazole (**21**).* White powder. Mp 72 °C. Yield 80%. ^{1}H NMR ($CDCl_3$) δ 7.31 (m, 3H); 7.24 (m, 2H); 7.20 (s, 1H); 5.46 (s, 2H); 2.66 (t, $J = 7.5$ Hz, 2H); 1.61 (m, 2H); 1.23 (m, 10H); 0.85 (t, $J = 6.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 148.8; 134.9; 128.9; 128.4; 127.8; 120.4; 53.8; 31.7; 29.3; 29.2; 29.1; 29.1; 25.6; 22.5; 14.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{17}H_{26}N_3$: 272.2127. Found: 272.2127.

*4.2.2.16. 1-Benzyl-4-decyl-1H-1,2,3-triazole (**22**).* White powder. Mp 81 °C. Yield 79%. White powder. Mp °C. Yield %. ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 3H); 7.26 (m, 2H); 7.18 (s, 1H); 5.48 (s, 2H); 2.67 (t, $J = 7.5$ Hz, 2H); 1.63 (m, 2H); 1.24 (m, 14H); 0.87 (t, $J = 6.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 148.9; 135.0; 129.0; 128.5; 127.9; 120.4; 53.9; 31.8; 29.5; 29.5; 29.4; 29.3; 29.3; 29.2; 25.7; 22.6; 14.1; HRMS: (DCI/CH_4, m/z) calc. for $C_{19}H_{30}N_3$: 300.2440. Found: 300.2443.

*4.2.2.17. 1-Benzyl-4-dodecyl-1H-1,2,3-triazole (**23**).* White powder. Mp 85 °C. Yield 82%. ^{1}H NMR ($CDCl_3$) δ 7.39 (m, 3H); 7.23 (m, 2H); 7.18 (s, 1H); 5.48 (s, 2H); 2.67 (t, $J = 7.7$ Hz, 2H); 1.62 (m, 2H); 1.24 (m, 18H); 0.87 (t, $J = 6.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 148.9; 135.0; 129.0; 128.5; 127.9; 120.4; 55.9; 31.8; 29.6; 29.6; 29.5; 29.5; 29.3; 29.2; 25.7; 22.6; 14.1; HRMS: (DCI/CH_4, m/z) calc. for $C_{21}H_{34}N_3$: 328.2753. Found: 328.2756.

*4.2.2.18. 1-(3-Methoxybenzyl)-4-hexyl-1H-1,2,3-triazole (**24**).* White powder. Mp 44 °C. Yield 78%. ^{1}H NMR ($CDCl_3$) δ 7.28 (t, $J = 7.9$ Hz, 1H); 7.18 (s, 1H); 6.87 (dd, $J = 8.3$ Hz, 2.6 Hz, 1H); 6.83 (d, $J = 7.5$ Hz, 1H); 6.77 (t, $J = 1.9$ Hz, 1H); 5.46 (s, 2H); 3.78 (s, 3H); 2.68 t, $J = 7.7$ Hz, 2H; 1.63 (m, 2H); 1.29 (m, 6H); 0.86 (t, $J = 7.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 160.1; 149.0; 136.4; 130.1; 120.5; 120.1; 114.1; 113.5; 55.3; 53.9; 31.5; 29.3; 29.0; 25.7; 22.5; 14.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{16}H_{24}N_3O$: 274.1919. Found: 274.1927.

*4.2.2.19. 1-(3-Methoxybenzyl)-4-decyl-1H-1,2,3-triazole (**25**).* White powder. Mp 59 °C. Yield 76%. ^{1}H NMR ($CDCl_3$) δ 7.26 (t, $J = 7.9$ Hz, 1H); 7.18 (s, 1H); 6.83 (m, 2H); 6.75 (t, $J = 1.9$ Hz, 1H); 5.44 (s, 2H); 3.76 (s, 3H); 2.66 (t, $J = 7.6$ Hz, 2H); 1.62 (m, 2H); 1.23 (m, 14H); 0.86 (t, $J = 6.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 160.0; 148.9; 136.4; 130.0; 120.4; 120.0; 114.0; 113.4; 55.2; 53.8; 31.8; 29.5; 29.5; 29.3; 29.3; 29.2; 29.2; 25.7; 22.6; 14.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{20}H_{32}N_3O$: 330.2545. Found: 330.2560.

*4.2.2.20. 1-(3-Methoxybenzyl)-4-dodecyl-1H-1,2,3-triazole (**26**).* White powder. Mp 65 °C. Yield 90%. ^{1}H NMR ($CDCl_3$) δ 7.28 (t, $J = 7.9$ Hz, 1H); 7.19 (s, 1H); 6.87 (dd, $J = 8.3$ Hz, 2.5 Hz, 1H); 6.83 (d, $J = 7.5$ Hz, 1H); 6.76 (t, $J = 1.9$ Hz, 1H); 5.46 (s, 2H); 3.77 (s, 3H); 2.68 (t, $J = 7.5$ Hz, 2H); 1.63 (m, 2H); 1.24 (m, 18H); 0.87 (t, $J = 6.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 160.0; 136.4; 130.1; 120.1; 114.1; 113.5; 55.3; 31.9; 29.6; 29.6; 29.5; 29.3; 29.2; 25.7; 22.7; 14.1; HRMS: (DCI/CH_4, m/z) calc. for $C_{22}H_{36}N_3O$: 358.2858. Found: 358.2868.

*4.2.2.21. 1-(3,5-Dimethoxybenzyl)-4-decyl-1H-1,2,3-triazole (**27**).* White powder. Mp 77 °C. Yield 70%. ^{1}H NMR ($CDCl_3$) δ 7.19 (s, 1H); 6.37 (m, 3H); 5.38 (s, 2H); 3.73 (s, 6H); 2.66 (t, $J = 7.6$ Hz, 2H); 1.62 (m, 2H); 1.23 (m, 14H); 0.85 (t, $J = 6.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 161.2; 148.9; 137.1; 120.5; 105.8; 100.2; 55.3; 53.9; 31.8; 29.5; 29.5; 29.3; 29.3; 29.2; 29.2; 25.6; 22.6; 14.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{21}H_{34}N_3O_2$: 360.2651. Found: 360.2663.

*4.2.2.22. 1-(3,5-Dimethylbenzyl)-4-hexyl-1H-1,2,3-triazole (**28**).* White powder. Mp 75 °C. Yield 81%. ^{1}H NMR ($CDCl_3$) δ 7.17 (s, 1H); 6.96 (s, 1H); 6.85 (s, 2H); 5.39 (s, 2H); 2.67 (t, $J = 7.6$ Hz, 2H); 2.28 (s, 6H); 1.63 (m, 2H); 1.29 (m, 6H); 0.85 (t, $J = 6.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 148.8; 138.6; 134.8; 130.1; 125.7; 120.4; 53.9; 31.5; 29.3; 28.8; 25.7; 22.5; 21.1; 14.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{17}H_{26}N_3$: 272.2127. Found: 272.2139.

*4.2.2.23. 1-(3-Chlorobenzyl)-4-hexyl-1H-1,2,3-triazole (**29**).* White powder. Mp 63 °C. Yield 58%. ^{1}H NMR ($CDCl_3$) δ 7.28 (m, 4H); 7.11 (m, 1H); 5.44 (s, 2H); 2.67 (t, $J = 7.6$ Hz, 2H); 1.62 (m, 2H); 1.27 (m, 6H); 0.84 (t, $J = 6.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 149.1; 136.9; 134.8; 130.2; 128.7; 127.8; 125.8; 120.5; 53.1; 31.4; 29.2; 28.8; 25.6; 22.4; 13.9; HRMS: (DCI/CH_4, m/z) calc. for $C_{15}H_{21}N_3Cl$: 278.1424. Found: 278.1435.

*4.2.2.24. 1-(3-Chlorobenzyl)-4-decyl-1H-1,2,3-triazole (**30**).* White powder. Mp 67 °C. Yield 61%. ^{1}H NMR ($CDCl_3$) δ 7.29 (m, 2H); 7.21 (m, 2H); 7.11 (m, 1H); 5.45 (s, 2H); 2.68 (t, $J = 7.5$ Hz, 2H); 1.63 (m, 2H); 1.23 (m, 14H); 0.86 (t, $J = 6.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 149.1; 136.9; 134.9; 130.3; 128.7; 127.9; 125.9; 120.5; 53.2; 31.8; 29.5; 29.5; 29.3; 29.3; 29.2; 29.2; 25.6; 22.6; 14.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{19}H_{29}N_3Cl$: 334.2050. Found: 334.2047.

*4.2.2.25. 3-Octyl-1H-1,2,3-triazol-4-yl)phenol (**31**).* White powder. Mp 69 °C. Yield 68%. ^{1}H NMR ($CDCl_3$) δ 7.68 (s, 1H); 7.64 (m, 1H); 7.24 (m, 2H); 6.90 (m, 1H); 4.36 (t, $J = 7.2$ Hz, 2H); 1.91 (m, 2H); 1.28 (m, 10H); 0.86 (t, $J = 6.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 157.0; 147.5; 131.4; 130.1; 119.9; 117.5; 115.8; 113.0; 50.6; 31.7; 30.2; 29.0; 28.9; 26.4; 22.6; 14.0; HRMS : (DCI/CH_4, m/z) calc. for $C_{16}H_{24}N_3O$: 274.1919. Found: 274.1917.

*4.2.2.26. 1-Octyl-4-m-tolyl-1H-1,2,3-triazole (**32**).* White powder. Mp 75 °C. Yield 98%. ^{1}H NMR ($CDCl_3$) δ 7.73 (s, 1H); 7.68 (s, 1H); 7.58 (d, $J = 7.7$ Hz, 1H); 7.27 (t, $J = 7.6$ Hz, 1H); 7.10 (d, $J = 7.6$ Hz, 1H); 4.32 (t, $J = 7.2$ Hz, 2H); 2.36 (s, 3H); 1.88 (m, 2H); 1.27 (m, 10H); 0.85 (t, $J = 6.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 147.5; 138.2; 130.5; 128.6; 128.5; 126.1; 122.6; 119.3; 50.2; 31.5; 30.1; 28.9; 28.8; 26.3; 22.4; 21.2; 13.9; HRMS : (DCI/CH_4, m/z) calc. for $C_{17}H_{26}N_3$: 272.2127. Found: 272.2126.

*4.2.3. Synthesis of compounds **6** and **7***

Compound **5** was synthesized as described for compounds **8**–**32**. Compounds **6** and **7** were synthesized according to the protocol bellow. To a solution of triazole **5** (1.0 eq) and phenol (1.0) in acetone, was added K_2CO_3 at room temperature. The reaction was stirred at reflux for overnight. Then the solution was concentrated, diluted with AcOEt. The organic layer was washed with water and brine, dried over MgSO4 and concentrated under reduced pressure. The desired compound was purified by flash chromatography (petroleum ether/EtOAc : 9/1).

*4.2.3.1. 4-((2,4-Dichlorophenoxy)methyl)-1-dodecyl-1H-1,2,3-triazole (**6**).* White powder. Mp 50 °C. Yield 94%. ^{1}H NMR ($CDCl_3$) δ 7.62 (m, 1H); 7.35 (d, $J = 2.5$ Hz, 1H); 7.17 (dd, $J = 8.8$ Hz, 2.5 Hz, 1H); 7.05 (d, $J = 8.8$ Hz, 1H); 5.27 (s, 2H); 4.34 (t, $J = 7.2$ Hz, 2H); 1.89 (m, 2H); 1.24 (m, 18H); 0.87 (t, $J = 6.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 152.5; 143.4; 130.0; 127.6; 126.4; 123.9; 122.6; 115.1; 63.6; 50.5; 31.8; 30.2; 29.5; 29.5; 29.4; 29.3; 29.3; 28.9; 26.4; 22.6; 14.1; HRMS: (DCI/CH_4, m/z) calc. for $C_{21}H_{32}N_3OCl_2$: 412.1922. Found: 412.1918.

*4.2.3.2. 4-((4-Chloro-2-methoxyphenoxy)methyl)-1-dodecyl-1H-1,2,3-triazole (**7**).* White powder. Mp 61 °C. Yield 66%. ^{1}H NMR ($CDCl_3$) δ 7.59 (s, 1H); 6.95 (d, $J = 8.7$ Hz, 1H); 6.84 (s, 1H); 6.82 (dd, $J = 8.6$ Hz, 2.4 Hz, 1H); 5.24 (s, 2H); 4.31 (t, $J = 7.3$ Hz, 2H); 3.93 (s, 3H); 1.87 (m, 2H); 1.23 (m, 18H); 0.86 (t, $J = 6.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 150.2; 146.4; 143.9; 126.6; 122.7; 120.4; 115.2; 112.5;

63.4; 56.1; 50.5; 30.3; 29.6; 29.5; 29.4; 29.4; 29.3; 29.3; 29.0; 26.5; 22.7; 14.1; HRMS: (DCI/CH_4, m/z) calc. for $C_{22}H_{35}N_3O_2Cl$: 408.2418. Found: 408.2424.

*4.2.4. Synthesis of compounds **33** and **34***

A solution of azide and alkyne was heated to reflux in toluene for 35 h. After cooling to room temperature, the reaction mixture was diluted with EtOAc, washed with water, dried over MgSO4, filtered and concentrated over reduced pressure. The mixture was purified by flash chromatography using a linear gradient 100% petroleum ether (solvent A) to 5/5 solvent A/solvent B (CH_2Cl_2/AcOEt 5/5) over 15 min.

*4.2.4.1. 1-Dodecyl-5-phenethyl-1H-1,2,3-triazole (**33**).* White powder. Mp 50 °C. ^{1}H NMR ($CDCl_3$) δ 7.48 (s, 1H); 7.28 (m, 3H); 7.15 (d, J = 7.8 Hz, 2H); 4.10 (t, J = 7.4 Hz, 2H); 2.95 (m, 4H); 1.78 (m, 2H); 1.24 (m, 18H); 0.87 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 139.8; 128.7; 128.3; 126.6; 47.7; 34.6; 31.8; 29.9; 29.5; 29.4; 29.3; 29.3; 29.0; 26.5; 25.3; 22.6; 14.1; HRMS: (DCI/CH_4, m/z) calc. for $C_{22}H_{36}N_3$: 342.2909. Found: 342.2916.

*4.2.4.2. 5-Decyl-1-phenethyl-1H-1,2,3-triazole (**34**).* Colourless oil. ^{1}H NMR ($CDCl_3$) δ 7.36 (s, 1H); 7.23 (m, 3H); 7.02 (m, 2H); 4.41 (t, J = 7.2 Hz, 2H); 3.19 (t, J = 7.2 Hz, 2H); 2.20 (t, J = 7.5 Hz, 2H); 1.43 (m, 2H); 1.24 (m, 14H); 0.87 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 137.7; 137.4; 131.7; 128.8; 128.7; 127.0; 49.0; 36.9; 31.9; 29.5; 29.5; 29.3; 29.2; 29.1; 27.9; 22.8; 22.7; 14.1; HRMS: (DCI/CH_4, m/z) calc. for $C_{20}H_{32}N_3$: 314.2596. Found: 314.2595.

4.3. Biology

4.3.1. InhA expression and purification

The production and purification were performed as described in reference 10.

4.3.2. Inhibition Kinetics

Stock solutions of all compounds were prepared in DMSO such that the final concentration of this co-solvent was constant at 5% v/v in a final volume 1 mL for all kinetic reactions. Kinetic assays using *trans*-2-dodecenoyl-Coenzyme A (DD-CoA) and wild-type InhA were performed as described in reference 10. Reactions were initiated by addition of InhA (100 nM final) to solutions containing DD-CoA (50 μM final), inhibitor, and NADH (250 μM final) in 30 mM PIPES, 150 mM NaCl, pH 6.8, buffer. Control reactions were carried out with the same conditions as described above but without inhibitor. The inhibitory activity of each derivative was expressed as the percentage inhibition of InhA activity (initial velocity of the reaction) with respect to the control reaction without inhibitor. All activity assays were performed in triplicate.

4.3.3. Growth conditions

M. tuberculosis H37Rv strain was grown either in Middlebrook 7H9 broth (Difco) supplemented with 0.05% Tween 80, or on Middlebrook 7H11 agar (Difco) supplemented with 0.5% glycerol, both supplemented with 10% (vol/vol) OADC. All compounds were dissolved in dimethyl sulfoxide (DMSO). Mycobacterial cultures were usually grown at 37 °C without shaking.

4.3.4. MIC determinations

A single colony of *M. tuberculosis* strain was used to inoculate complete Middlebrook 7H9. The cultures were incubated at 37 °C until exponential growth phase ($\sim 10^8$ CFU/mL) was reached, corresponding to an OD_{600nm} ranging from 0.8 to 1.0. Cultures were diluted to the final concentration of about 10^7 CFU/mL; 1 μl of the diluted cultures was then streaked onto plates containing two-fold serial dilutions of appropriate compound. MIC values were scored as the lowest drug concentrations inhibiting bacterial growth. All assays were repeated three times.

4.3.5. Molecular docking studies

The conformation active site of InhA is known to be flexible with states corresponding to a major or a minor portal opening and loop reordering (residues 195–210) [16–18]. The best active ligand (compound **18**) and some products of the library (cf. Table 1) are closed in shape by comparison with 5-octyl-2-phenoxy-phenol known as **8PS** hetero compound ID in Protein Data Bank (PDB) [19]. Consequently the corresponding structure of InhA cocrystallized with 8PS and NAD^+ (2B37 PDB ID, chain C) was used for docking studies [11].

Molegro Virtual Docker (MVD) 5.0 software (Molegro ApS, Aarhus, Denmark) was used [10,20]. The structures (protein, NAD^+, ligand) were imported in MVD. The cavity detection algorithm implemented in MVD was used to optimize the definition of a 15 Å (radius) potential binding site. The corresponding crystallographic NAD^+ molecule was used as cofactor using the MVD features. The water molecules were not taken in account. The side chains around the cofactor (30 residues around and upper the NAD^+) were set as flexible with a tolerance of 0.99 and strength of 0.91 (MVD units). A calculation scheme combining Moldock Score [GRID] and Moldock optimizer was used with a population size of 50, maximum iteration of 2000, scaling factor of 0.50, crossover rate of 0.90 and a variation-based termination scheme. After calculation minimization steps (global, lateral chain, ligands), optimization of hydrogen bonds was performed using MVD default features. For each ligand and a given calculation scheme, 20 runs (a set) were carried out using Tabu clustering. If the best values of Moldock Score and Rerank Score were found for the same pose, the corresponding conformation was retained as the best pose.

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Appendix A. Supplementary material

Supporting information related to this article can be found online at doi:10.1016/j.ejmech.2012.03.029.

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