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Research paper

Discovery of antitubercular 2,4-diphenyl-1*H*-imidazoles from chemical library repositioning and rational design

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ABSTRACT

TB, caused by *Mycobacterium tuberculosis*, is one of the deadliest infections worldwide. The co-infection with HIV and the emergence of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) strains have further increased the burden for this disease. In the attempt to respond to the constant need of novel therapeutic options, we herein report the discovery of 2,4-diphenyl-1*H*-imidazoles as effective antitubercular agents, with MIC in the low micromolar range against actively replicating and persistent *M. tuberculosis* strains. The good activity, along with the lack of toxicity and the feasible synthesis, underscore their value as novel scaffolds for the development of new anti-TB drugs.

1. Introduction

Tuberculosis (TB) is a lung infection caused by *Mycobacterium tuberculosis* (*Mtb*). After decades of oblivion, in which it was thought to be under control, this dreadful disease is sparkling again the interest of the researchers, as nowadays it is considered one of the biggest threat for public health [1]. In 2011, there were an estimated 8.7 million new cases of TB (13% of which co-infected with HIV) and 1.4 million people died from the disease; moreover, nearly one-third of the world's population is estimated to be latently infected by *Mtb* [2–4]. India and China have been heavily struck by TB, accounting together for almost 40% of the world's TB new cases [5], whereas in the African regions there are the highest rates of cases and deaths per capita [6]. These facts may lead to consider TB as a plague regarding the developing countries; however, the increasing migration flux from regions where TB is endemic are making the TB scourge a concern also for the developed countries. Moreover, the uncertain economic situation worldwide, and the increasing stress to which people are steadily exposed, may affect the immune system and lead to the exacerbation of the latent infection [7]. The current recommended therapeutic strategy, termed DOTS (Directly Observed Therapy, Short-course) [8], is based on the co-administration of the so called first-line drugs isoniazid (INH), rifampin (RMP), ethambutol (EMB), and pyrazinamide (PZA) for the first two months, followed by a prolonged treatment with INH and RMP for additional 4–7 months. The peculiar ability of *Mtb* to modify his metabolism in such a way to slow down replication, therefore surviving in a dormant state (NRP-TB, non-replicating persistent TB) and withstanding the therapy, is the cause of the long-lasting period of treatment [9]. In addition, the poor patient compliance contributes to the emergence of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB), that further jeopardize the positive outcome [4]. The treatment of resistant strains requires a prolongation of the therapy, needs more toxic drugs, and increases the financial burden, thus making TB a vicious cycle [10,11].

Bedaquiline [12] is the only new anti-TB chemotherapeutic marketed over the last half century since RMP and it is the first

Abbreviations: DMF, *N*,*N*-dimethyl formamide; DOTS, directly observed therapy short-course; INH, isoniazid; LORA, low oxygen recovery assay; MABA, microplate Alamar blue assay; MIC, minimum inhibitory concentration; MDR-TB, multidrug-resistant tuberculosis; MOX, moxifloxacin; *Mtb*, *Mycobacterium tuberculosis*; NRP-TB, non-replicating persistent tuberculosis; R-TB, replicating tuberculosis; RMP, rifampin; TB, tuberculosis; XDR-TB, extensively drug-resistant tuberculosis.

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molecule expressively studied to target *Mtb*. However, recent findings about the cardiotoxicity of bedaquiline, probably due to its promiscuous mechanism of action, have chilled the enthusiasm about this drug, that is currently recommended as last resort treatment for resistant infections [13]. These facts highlight the need to keep pursuing the research on novel antituberculars, so as to feed the anti-TB pipeline with compounds that, possibly, are able to kill *Mtb* both in the replicating and dormant state, active toward resistant strains, and endowed with a certain degree of chemical feasibility [14]. In our continuous pursuing the synthesis of novel anti-TB chemotypes [15–24], we herein report a series of 2,4-diphenyl-1*H*-imidazoles with activity in the submicromolar range toward actively replicating and non-replicating persistent *Mtb* strains.

Over the years, drug repositioning is widely emerging as an extremely fruitful strategy to inspire drug discovery [25], and both big pharmaceutical companies and academia institutions screen in house chemical libraries of compounds for purposes other than those for which they were initially conceived. We have previously reported a series of substituted 2-aminothiazoles endowed with good antitubercular activity and selectivity index, for which a thorough investigation of the Structure-Activity Relationship (SAR) was carried out [16]. The main features of these molecules (Fig. 1) were the presence of two aromatic rings, suitably substituted, connected by a 5-terms heterocycle, in this case a 2-aminothiazole. The presence of such a pattern in an in house library of diarylimidazoles, originally designed as sodium channel blockers [26–32], prompted us to test some representatives of this series in a whole-cell phenotypic assay against *Mtb*. The encouraging preliminary results inspired a wiser selection of further compounds to be tested, along with the synthesis of structurally related analogues. This iterative work led to the discovery of compound **26**, able to inhibit the growth of actively replicating *Mtb* at low micromolar concentration (MIC = 1.7 μM), setting the scene for the study of this novel chemotype for the treatment of TB. The synthesis of novel derivatives and a preliminary SAR for these novel imidazyl-based antituberculars are herein reported.

2. Chemistry

The majority of the compounds were already reported and the synthesis of the novel derivatives has relied on the established synthetic protocol [33]. Briefly, the reaction is carried out from the suitable substituted phenylglyoxal and aldehyde, using ammonium acetate as an ammonia source and methanol at room temperature, with yields ranging from 30 to 51% (Scheme 1). For the synthesis of compound **31**, [3-(trifluoromethyl)phenyl]-1,2-propanedione was used in place of the phenylglyoxal, in the same overall conditions. However, likely due to the presence of a ketone in place of the glioxal moiety, that decreases the reactivity of the functional group, a remarkable drop in the yield was noticed (9%). Compound **32** was prepared treating **26** with methyl iodide in the presence of excess sodium hydride, in DMF at room temperature, giving the desired methylated product in 85% yield.

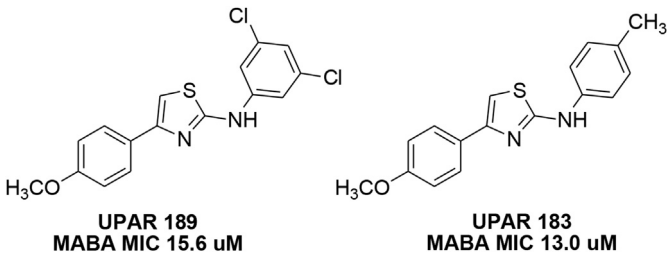
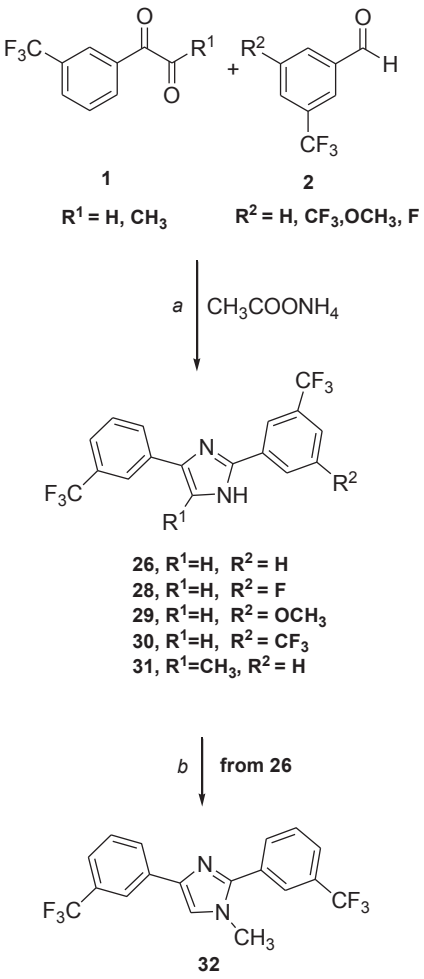


Fig. 1. Antitubercular aminothiazoles.



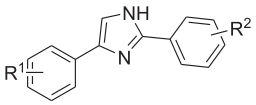
Scheme 1. Synthesis of the 2,4(1H)-diarylimidazoles **28**–**32**. *a*) Reagents and conditions: 1 equiv of **1** in 3.8 mL CH_3OH, 1 equiv of **2** and 4 equiv CH_3COONH_4 in 3.5 mL CH_3OH, Overnight at rt; *b*) from **26** (0.10 mmol), NaH (0.29 mmol) in dry DMF (2 mL) at 0 °C, then MeI (0.20 mmol), rt, overnight.

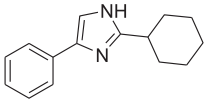
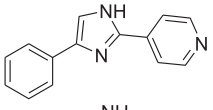
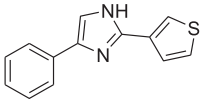
3. Results and discussion

Since the numerous compounds available in the library, in order to save time and money avoiding redundant assays, a batch of carefully selected substituted imidazoles was sent for the preliminary biological evaluation (Table 1).

The representative 2,4-diphenyl-1*H*-imidazole **3** was selected, along with its derivatives in which the 4-phenyl moiety and the 2-phenyl moiety were, on turn, kept unadorned (Table 1). In this first round of evaluation, small functional groups, different in electronic nature and physicochemical characteristics, were used to decorate the phenyl rings at various positions. Regarding the 4-phenyl moiety, a compound bearing a nitro group was selected (**4**), as it is a common substituent in some of the most advanced antituberculars in the pipeline (PA-824 [34] and TBA-354 [35]). The methoxy group (**5**, **6**) was selected as in the parental series previously reported (Fig. 1) it had yielded the most active compounds when in the *para* position of the phenyl ring. Halogens and halogenated groups, attached at different positions of the phenyl rings, were first selected for their capability to enhance lipophilicity, a characteristic likely to be important in the penetration through the tick

Table 1
Anti-TB activity of compounds 3–24 against *M. tuberculosis* strain H37Rv.



Comp	R^1	R^2	MABA[a] MIC (μM)	Comp	R^1	R^2	MABA MIC (μM)
3	H	H	127.6	**14**	H	*p*-CH_3	124.0
4	*p*-NO_2	H	>128	**15**	H	p-OH	>128
5	*p*-OCH_3	H	82.4	**16**	H	*p*-Cl	59.8
6	*m*-OCH_3	H	88.4	**17**	H	*m*-Cl	60.4
7	*p*-Cl	H	59.5	**18**	H	*p*-CF_3	48.9
8	*m*-Cl	H	60.3	**19**	H	*m*-CF_3	30.6
9	*p*-CF_3	H	>128	**20**	H	*m*-NO_2	60.6
10	*m*-CF_3	H	37	**21**	H	*m*-OCF_3	35.5
11	H	*o*-OCH_3	>128	**22**			124.8
12	H	*p*-OCH_3	119.3	**23**			>128
13	H	*m*-OCH_3	73.7	**24**			107.3
INH			0.10	**RMP**			0.05

[a] MIC values determined by MABA are the mean of replicated experiments (SD < 15%).

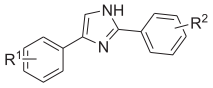
and greasy *Mtb* cell wall (**7**–**10**). The same rationale applied for the selection of the substituents at the 2-phenyl moiety (**11**–**21**). To add further variability, also compounds bearing cycloaliphatic moieties or heterocycles attached at position C-2 of the imidazole (**22**–**24**) were selected.

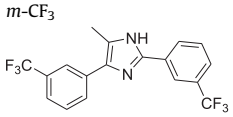
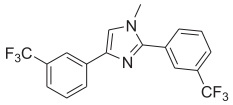
We were pleased to notice that the majority of the compounds showed low-to-moderate activity toward the inhibition of mycobacterial growth, thereby corroborating our initial hypothesis (Table 1).

Also, a preliminary SAR could be outlined. The unadorned compound (**3**, MIC = 127.6 μM), as well as the *p*-nitro derivative (**4**, MIC = >128 μM) at the 4-phenyl ring, were inactive. At the same ring, the methoxy group, either at the *para* (**5**, MIC = 82.4 μM) or the *meta* (**6**, MIC = 88.4 μM) position, contributed only slightly in ameliorating the activity of the compounds.

At the same positions, electron-withdrawing groups with a more lipophilic character led in general to an improvement of the activity over compound **3** (**7**, MIC = 59.5 μM, **8**, MIC = 60.3 μM, **9**, MIC = >128 μM, **10**, MIC = 37.0 μM). Surprisingly, the CF_3 moiety at the *para* position failed to show any activity, whereas in the *meta* position led to around a 4-fold improvement in potency over **3**. Based on these results, we can roughly conclude that, regarding the

Table 2
Anti-TB activity of compounds **25**–**32** against *M. tuberculosis* strain H37Rv.



Comp	R^1	R^2	MABA[a] MIC (μM)	LORA[b] MIC (μM)
25	*m*-CF_3	*p*-Cl	29.8	nd[c]
26	*m*-CF_3	*m*-CF_3	1.7	25.4
27	H	*o*-OCH_3, *m*-OCH_3	55.2	nd
28	*m*-CF_3	*m*-CF_3, *m*-F	6.0	17.6
29	*m*-CF_3	*m*-CF_3, *m*-OCH_3	7.3	63.7
30	*m*-CF_3	*m*-CF_3, *m*-CF_3	7.7	11.3
31			14.0	nd
32			7.9	50.7
RMP			0.05	0.21
INH			0.10	

[a] MIC values determined by MABA are the mean of replicated experiments (SD < 15%).
[b] LORA MIC values represent single measurements.
[c] nd = not determined.

4-phenyl ring of imidazole, lipophilic electron-withdrawing substituents are well tolerated, although not in the *para* position (see **10** *vs* **4** and **9**). Concerning the substituents at the 2-phenyl ring, also in this case the methoxy group, moved around the ring at the *para*, *meta* and *ortho* positions, failed to give any appreciable inhibitory activity (**11**, MIC = >128 μM, **12**, MIC = 119.3 μM, **13**, MIC = 73.7 μM). Also another electron-donating group, such as the methyl (**14**, MIC = 124 μM) was found detrimental for the activity, as well as the hydroxyl moiety (**15**, MIC = >128 μM). The result for the latter was somehow expected, since the high polarity of the hydroxyl group might have hampered the cellular penetration. Again, halogens and halogenated substituents gave the best results, with activities improved over the unsubstituted compound **3** up to 4-times (**16**, MIC = 59.8 μM, **17**, MIC = 60.4 μM, **18**, MIC = 48.9 μM and **19**, MIC = 30.6 μM). As commonly noticed, it might be thought that the higher activity is strictly correlated with the raise of the lipophilicity of the compounds, even though the lack of activity of compound **14** does not fully sustain this hypothesis. Rather, it seems that the electron-withdrawing properties of the substituent and its position on the phenyl ring account for the improved activity, as furtherly corroborated by the activity of compounds **20** and **21**, bearing an *m*-NO_2 and an *m*-OCF_3, respectively. In particular compound **21** was found to show an MIC comparable to that of **19**. Five- and six-terms heterocycles (**23**, MIC = >128 μM, **24**, MIC = 107.3 μM) or the cycloaliphatic ring (**22**, MIC = 214.8 μM) did not give any positive feedback.

In summary, also for what concerns the 4-phenyl ring, halogens and halogenated groups granted good activity, with *m*-CF_3 resulting the best substituent.

Reasoning on the above mentioned hints, we deemed of interest the test of few more compounds, either from the available pool, and newly synthesized (Table 2).

First we investigated whether the combination of the best structural features for the two phenyl rings, that is the *m*-CF_3 moiety at the 4-phenyl ring and the substitution with halogens or halogenated groups at the 2-phenyl ring, would have been beneficial. Compounds **25** and **26** were tested and we were pleased to notice that, not only they possessed good activity (**25**, MIC = 29.8 μM, **26**, MIC = 1.7 μM), but in particular compound **26**, bearing an *m*-CF_3 substitution at both the phenyl rings attached to the imidazole, resulted around 18-fold more active than the monosubstituted derivatives (**26** *vs* **10** and **19**) and around 75-fold more active than the unsubstituted parent compound (**3** *vs* **26**).

These encouraging results prompted us to synthesize novel imidazole derivatives in which the favorable characteristics for the activity, that is the CF_3 group at the *meta* positions of the 4 and 2-phenyl ring were maintained, and another substituent in the meta position was introduced in the 2-phenyl ring. Another CF_3 moiety was chosen since it had given the best activity so far; the fluorine atom is still an electron-withdrawing group, but smaller and less lipophilic in character, enhancing the drug-likeness; the methoxy group was chosen to provide a more polar group and enhance the drug-likeness. Although it might be objected that the methoxy group is detrimental for the activity, its effect could be mitigated by the presence of an activity-enhancing groups such as the *m*-CF_3. Although less active than **26**, all of the newly prepared derivatives maintained an MIC lower than 10 μM (**28**, MIC = 6.0 μM, **29**, MIC = 7.3 μM, **30**, MIC = 7.7 μM), confirming the reliability of the design.

Finally, to give additional hints of SAR, we wanted to investigate whether a small substituent at the C-5 of the imidazole or the alkylation of the imidazole nitrogen would have hampered the activity. Both the compounds prepared (**31**, MIC = 14.0 μM and **32**, MIC = 7.9 μM) were found to be significantly active, opening the way to further chemical manipulation. In particular compound **32**, one of the most active compound of the series, led to speculate that, for the interaction with the target, as yet unknown, only the H-bond acceptor, and not the H-bond donor, properties of the imidazole were important.

Some of the compounds were also tested in LORA [36], a plausible surrogate for NRP-TB. In the majority of the cases, the LORA MIC values are reported to be several fold higher than those of MABA MIC. Although this applies to compound **26** (MABA MIC 1.7 μM, LORA MIC 25.4 μM), for compounds **28** and **30** the activity against the persistent *Mtb* strains was very similar to that on the actively replicating ones (**28**, MABA MIC 6.0 μM, LORA MIC 17.6 μM; **30**, MABA MIC 7.7 μM, LORA MIC 11.3 μM). Among the current TB drugs, only RMP and PZA have been reported to show good activity toward the dormant bacteria, and it generally accepted that targeting the NRP-TB plays a crucial role in shortening the TB treatment.

4. Conclusions

Starting from a plethora of rational considerations, we have repositioned a library of 2,4-diphenyl-1*H*-imidazoles originally prepared as sodium channel blockers to inhibitors of *Mtb* growth. Moreover, based on the preliminary antitubercular activity, we have rationally synthesized a few more compounds, all of them showing inhibitory activities toward the replicating *Mtb* in the low micromolar range. Some of them, tested in a LORA assay, showed good activity also against the non-replicating persistent *Mtb* phenotype. The newly synthesized molecules were also more active than **UPAR-183** and **UPAR-189**, by which this study was inspired. Moreover, the previous investigation about this series of derivatives has demonstrated the lack of cellular toxicity and their suitability for *in vivo* administration [30,37]. In fact, animals (mice and rats) treated with some representatives of this series, were monitored for overt signs of impaired neurological or muscular function through the rotarod procedure, highlighting the lack of toxicity at the dose tested (100–300 mg/kg) [38].

The target protein of these compounds, and whether it is the same of the 2-aminothiazoles already reported, is a matter of investigation that is currently underway in our laboratories, along with the study of the activity toward resistant strains. Overall, these preliminary data establish these 2,4-diphenyl-1*H*-imidazole derivatives as promising novel class of compounds in the pursuit of highly effective anti-TB agents.

5. Experimental section

5.1. Chemistry

All products were characterized by ^{1}H NMR. The ^{1}H NMR spectra were recorded on a Bruker 300 Avance spectrometer (300 MHz), on a Bruker 400 Avance spectrometer (400 MHz) and on a Agilent 600 Advance spectrometer (600 MHz); Chemical shifts (δ scale) are reported in parts per million (ppm) relative to the central peak of the solvent. ^{1}H NMR Spectra are reported in order: multiplicity and number of protons; signals were characterized as s (singlet), d (doublet) dd (doublet of doublet), t (triplet), m (multiplet), bs (broad signal) as (apparent signal). HRMS experiments were performed using an LTQ ORBITRAP XL Thermo by Thermo-scientific instrument coupled to HPLC endowed with a column Alltima C18 5 μ 150 mm*4.6 mm, Alltech Italia Srl. Reactions were monitored by TLC, on Kieselgel 60 F 254 (DC-Alufolien, Merck). All the final compounds were more than 95% pure by analytical HPLC.

5.2. Biology

The MICs were determined using *Mtb* $H_{37}Rv$ ATCC 27294 in MABA and LORA assays according to published procedures. [36] [39], The reported MICs are an average value from 2 to 3 individual experiments. For a brief description of the biological assays see the supporting information.

5.2.1. General procedure for the synthesis of substituted 2,4-diphenyl-1H-imidazoles

To a solution of the suitably substituted benzaldehyde (1 equiv) and ammonium acetate (5 equiv) in methanol (3 mL/mmol) was added, over a period of 10 min, a solution of the substituted phenylglyoxal monohydrate or 1-(3-trifluoromethylphenyl)-1,2-propanedione (1 equiv) in methanol. The reaction mixture was stirred overnight at room temperature, then the solvent was evaporated, and the residue was partitioned between saturated aqueous $NaHCO_3$ solution and methylene chloride. The organic phase was dried over Na_2SO_4, and the solvent was removed *in vacuo*. The hydrochloride salt was prepared by treating the free base with a 5% w/w ethanolic HCl solution. The products were then crystallized from absolute ethanol/dry diethyl ether.

5.2.2. 2,4-bis(3-(trifluoromethyl)phenyl)-1-methyl-1H-imidazole (32)

Compound **26** (35 mg, 0.10 mmol) was added to a suspension of NaH (60% in mineral oil, 12 mg, 0.29 mmol) in dry DMF (2 mL) at 0 °C. After stirring for 15 min, methyl iodide (27 mg, 0.20 mmol) was added and the reaction mixture was stirred at rt overnight. The mixture was then cautiously poured into ice water (10 mL), extracted with EtOAc (3 × 10 mL) and the organic layers were washed with brine and dried (Na_2SO_4). After filtration, the solvent was removed *in vacuo* and the yellow oil obtained was purified by flash column chromatography (EtOAc–petroleum ether 20:80) to give **32** as a yellowish oil (31 mg, 85%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.84 (s, 3H), 7.39 (s, 1H), 7.50–7.54 (m, 2H), 7.63–7.75 (m, 2H), 7.91 (d, J = 8.8 Hz, 1H), 7.98–8.11 (m, 4H). HRMS (ESI) calculated for $C_{18}H_{12}F_6N_2$ $[M+H]^+$ 371.0905, found: 371.0909.

5.2.3. 2-(3-Fluoro-5-(trifluoromethyl)phenyl)-4-(3-(trifluoromethyl)phenyl)-1H-imidazole (28)

Hydrochloride salt. White powder, 29% yield. ^{1}H NMR (300 MHz, MeOD): δ = 7.72–7.92 (m, 3H), 8.10–8.16 (m, 2H), 8.27 (as, 2H), 8.31 (bs, 1H). HRMS (ESI) calculated for $C_{17}H_9F_7N_2$ $[M+H]^+$ 375.0654, found: 375.0666.

5.2.4. 2-(3-(trifluoromethyl)-5-methoxyphenyl)-4-(3-(trifluoromethyl)phenyl)-1H-imidazole (29)

Hydrochloride salt. White powder, 51% yield. ^{1}H NMR (300 MHz, d_6-DMSO): δ = 4.00 (s, 3H), 7.46 (bs, 1H), 7.70–7.80 (m, 2H), 8.15–8.20 (m, 2H), 8.27–8.43 (m, 3H). HRMS (ESI) calculated for $C_{18}H_{12}F_6N_2O$ $[M+H]^+$ 387.0854, found: 387.0852.

5.2.5. 2-(3,5-bis(trifluoromethyl)phenyl)-4-(3-(trifluoromethyl)phenyl)-1H-imidazole (30)

Hydrochloride salt. White powder, 47% yield. ^{1}H NMR (400 MHz, MeOD): δ = 7.75–7.88 (m, 2H), 8.10–8.17 (m, 1H), 8.25–8.30 (m, 2H), 8.36 (bs, 1H), 8.73 (bs, 1H). HRMS (ESI) calculated for $C_{18}H_9F_9N_2$ $[M+H]^+$ 425.0622, found: 425.0632.

5.2.6. 2,4-bis(3-(trifluoromethyl)phenyl)-5-methyl-1H-imidazole (31)

Hydrochloride salt. White powder, 9% yield. ^{1}H NMR (400 MHz, d_6-DMSO): δ = 2.55 (s, 3H), 7.78–8.00 (m, 4H), 8.01–8.07 (m, 1H), 8.11 (bs, 1H), 8.40–8.53 (m, 2H). HRMS (ESI) calculated for $C_{18}H_{12}F_6N_2$ $[M+H]^+$ 371.0905, found: 371.0925.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.05.048.

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