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Review article

Phenylpyridine-2-ylguanidines and rigid mimetics as novel inhibitors of TNFα overproduction: Beneficial action in models of neuropathic pain and of acute lung inflammation

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ABSTRACT

4-phenylpyridin-2-yl-guanidine (5b): a new inhibitor of the overproduction of pro-inflammatory cytokines (TNFα and Il1β) was identified from a high-throughput screening of a chemical library on human peripheral blood mononuclear cells (PBMCs) after LPS stimulation. Derivatives, homologues and rigid mimetics of **5b** were designed and synthesized, and their cytotoxicity and ability to inhibit TNFα overproduction were evaluated. Among them, compound **5b** and its mimetic **12** (2-aminodihydroquinazoline) showed similar inhibitory activities, and were evaluated *in vivo* in models of lung inflammation and neuropathic pain in mice. In particular, compound **12** proved to be active (5 mg/kg, ip) in both models.

1. Introduction

Tumor necrosis factor-alpha (TNFα) is a cytokine that is overproduced in inflammatory disease states, mimicked in animal models by LPS (lipopolysaccharide) administration, for instance in the airways [1–3]. The clinical efficacy and FDA (Food and Drug Administration) approval of anti-TNFα biologics such as etarnercept, infliximab, adalimumab, certolizumab pegol and golimumab confirm its critical role in inflammatory diseases [4–8].

Proof of concept with anti-TNFα biologics was also obtained in animal models for the treatment of neuropathic pain [9–12], which arises from a lesion or disease affecting the somatosensory system. The contribution of cytokines originating from immune and/or glial cells, including TNFα, to the pathophysiology of neuropathic pain is supported by experimental and clinical evidence [9,12–19]. Some of the first line treatments of neuropathic pain, namely antidepressant drugs targeting aminergic uptake sites (such as nortriptyline and venlafaxine) and gabapentinoids targeting calcium channels (such as pregabalin), suppress the TNFα overexpression observed in experimental neuropathic pain [12,20]. While existing immunotherapies could be an option to block TNFα in these diseases, their potent action in blocking TNFα is associated with major adverse effects. This limitation might be overcome by the development of new chemicals exerting a milder action on TNFα.

Small molecules inhibiting TNFα production would be expected to be effective disease-modifying treatments for inflammation that recruits this cytokine. For example, thalidomide (and its derivatives), xanthine derivatives (such as pentoxifylline), the antidepressant bupropion and the natural compound curcumin have been shown to induce the inhibition of TNFα expression [21–24].

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During the last decade our laboratory has built a chemical library comprising about 10,000 compounds with large chemical diversity. This library is constituted of new chemical entities (NCE) resulting from specific organic chemistry methodologies and various medicinal chemistry programs, including mainly heterocyclics derivatives, but also short peptides (1–3 aminoacid residues) and natural compounds from plants. The main characteristic they share in common is their drug-like character. Known pharmacological agents were added as reference compounds and served to evaluate the predictivity of the screening assay. In particular, we introduced rolipram and other type 4 phosphodiesterase inhibitors developed in recent years [25–28], which are known TNFα production inhibitors [29] but are limited to topical administration due to adverse effects.

The screening assay used a cell-based approach rather than target-based molecular approaches. We used human peripheral blood mononuclear cells (hPBMCs) from healthy volunteers (Centre de transfusion sanguine de Strasbourg, France), originating from pooled blood pockets in order to minimize the standard deviation between two experiments. The PCBIS (Plateforme de chimie biologique Intégrative de Strasbourg) performed the screening using fully automated robotic workstations into 96-well microplates. hPBMCs were stimulated with LPS inducing TNFα and IL1β production. Cells were incubated in the presence of the screening compounds at 10 μM during 24 h, then TNFα and IL1β productions were measured. At the same time cell survival was evaluated in order to check toxicity of the compounds. Hits were selected on the basis of their capacity to inhibit TNFα overproduction (≥50% at 10 μM concentration) and to be non-cytotoxic (>70% of cell survival at 10 μM). We obtained a hit rate close to 2%. Active molecules were then validated for reproducibility by determining the dose-reponse effect. After removal of the control hits (PDE4 inhibitors), about 20 molecules were selected based on their structural properties (low molecular weight with relatively few decorations, pharmacophore specificity). Finally, a 1-(4- phenylpyridin-2-yl) guanidine derivative (compound **5b**, Ar = 4-Ph) was chosen as a valuable hit for further structural optimization (Chart 1).

Careful analysis of literature describing aryl and heteroaryl guanidines as specific chemotypes highlighted several pharmacological agents listed in Chart 2 [30–35]. Specific target proteins were selected based on keywords associated with pain/hyperalgesia, inflammation and cytokines/TNFα. Several serotonergic drugs acting on specific receptor subtypes (5HT5, 5HT7 and 5HT3 [31,36,37]) have shown anti-hyperalgesic effects. Pyridinyl 2-guanidines have been designed as α_2-adrenoreceptor antagonists [38,39]. Small arginine-containing peptides can act as antagonists of the GPCR NPFF-R [32] and NPY-R [33]. More recently, peptidomimetic heterocyclic compounds (quinolin or quinazolin guanidines) that retained the critical moiety (cation and set of H bond acceptor-donor system surrounded by an aromatic ring) were also shown to display NPFF-R antagonist properties [32]. More interestingly, NPFF-R antagonists were shown to be anti-hyperalgesic in chronic pain situations [40], which might be related to their regulation of the NF-κB pathway and TNFα production [41,42]. Other systems including purinergic (P2X4, P2X7) [34] and glutamatergic [35,43] antagonists may also be relevant. Other target proteins have also been selected in Chart 2, as potentially involved in pain and inflammation. The tissue-type and urokinase-type plasminogen activators (tPA and uPA) are induced in dorsal root ganglia (DRG) neurons and tPA plays a role in inducing neuropathic pain [30,44].

Based on this literature, our first objective was to perform a preliminary study with the starting hit **5b**. First, we performed a survey of the potential beneficial effect of a phenyl ring at positions 3 to 6 along the pyridine nucleus. Secondly, further substitution effects on ortho, meta or para position by means of prototypical substituents (OMe, Cl, CF_3) were considered to potentially improve the potency accounting for geometric, electronic or lipophilic parameters. Homologues and isosteres of **5b** have also been prepared and tested. Finally, the second part of this work was dedicated to the replacement of the pyridine nucleus by a bicyclic scaffold as a valuable rigid frame able to mimic a highly favored internal H bond interaction within the structure of **5** (see the favored internal H bond interaction in the structure of I and their rigid mimetics II in Table 2). Different homologues were considered, and derivatives of amino-dihydroquinazolines (**12**, **17**, **24**), benzimidazoles **28**, and dihydro 1,3-benzodiazepines **34** were synthesized for this purpose.

2. Chemistry

Chemical methodologies used are described in Schemes 1–8. The synthesis of HCl or TFA salts of the target 2-guanidinopyridines **5a-w** was based on two different pathways (Scheme 1). The first one (Method 1) involved a Suzuki-Miyaura cross-coupling reaction with the corresponding halogeno-aminopyridines **1** followed by a guanylation reaction with SMe-isothiourea in presence of $HgCl_2$. Finally a deprotection of the Boc group allowed the formation of the HCl or TFA salts of target products **5a-w**. Two optimized conditions (a or b) were developed for the Suzuki-Miyaura cross coupling reaction. In particular, amino pyridines **1c** and **1d** were coupled under standard methods using $Pd(PPh_3)_4$ in a mixture of toluene:ethanol:water (5:1:1). However, starting from the relatively more basic halogeno-aminopyridines **1a** and **1b**, no reaction was observed under these conditions.

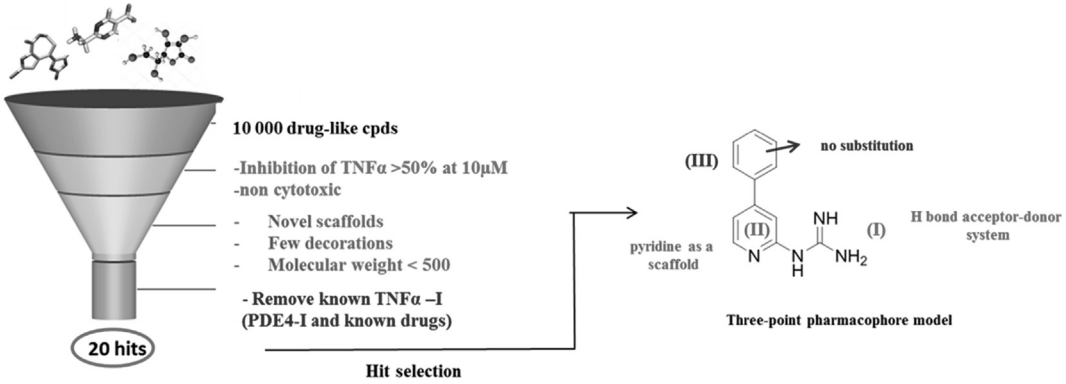


Chart 1. Workflow from cell-based assay using hPBMC's to identification of 1-(4-phenyl-pyridin-2-yl)guanidine as an inhibitor of TNFα overproduction.

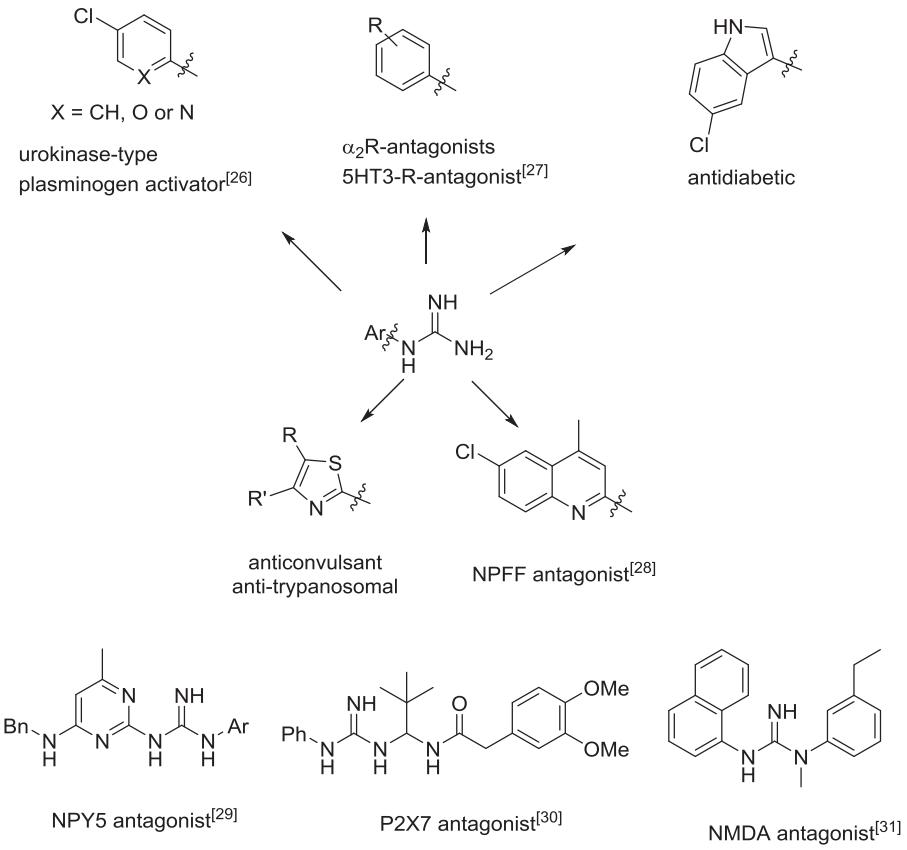
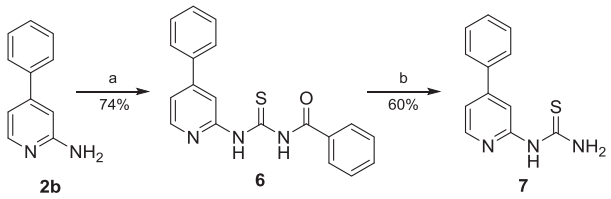


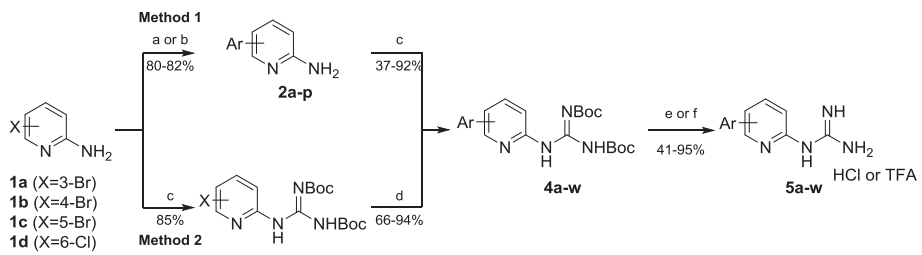
Chart 2. Aryl and heteroaryl guanidines as known pharmacological agents.

Instead, the use of S-Phos in the presence of $Pd(OAc)_2$ [45] proceeded with good yields. As an example, the reaction starting from 2-amino-4-bromopyridine **1b** and 4-methoxyphenylboronic acid afforded **2d** in 72% yield after purification. Unfortunately, under the same conditions, the reaction using 4-chlorophenylboronic acid was unsuccessful (data not shown). So, we developed a second method (Method 2), in which we reversed the reaction order (guanylation followed by a Suzuki-Miyaura reaction). In that case, the Suzuki-Miyaura cross-coupling reaction was performed on the corresponding *N*-diBoc pyridyl guanidine derivatives **3** in the presence of $Pd(OAc)_2$ and X-Phos at 50 °C [46]. These mild conditions avoided the removal of the Boc protecting groups during the cross-coupling reaction.

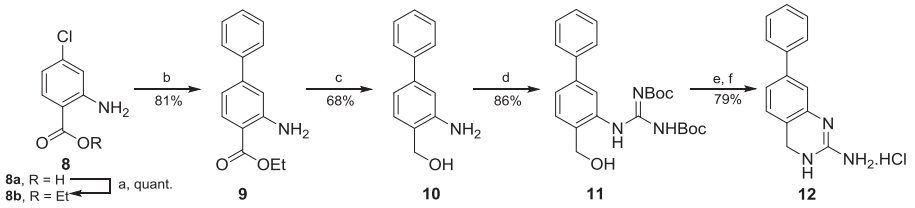
The reaction of the 2-amino-4-phenylpyridine **2b** with benzoyl isothiocyanate afforded the corresponding *N*-heteroaryl-*N*-benzoylthiourea **6**, which was hydrolyzed under basic conditions to give **7** with moderate yield (Scheme 2). Schemes 3–6 outline the different synthetic routes for the preparation of the 2-aminodihydroquinazolines **12**, **17a**, **17b** and **24.** Different methodologies leading to 2-aminodihydroquinazolines derivatives are described in the literature, but among them, there is no generalized method for their construction. So, our synthetic strategies were based on i)



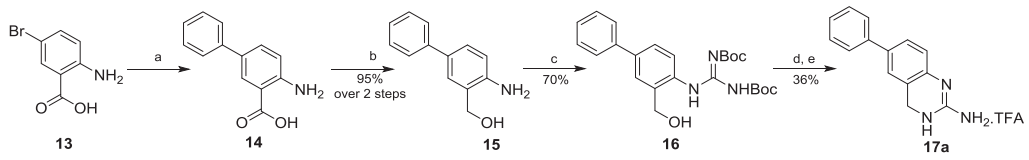
Scheme 2. Synthesis of 2-thioureapyridine **7**. (a) Benzoyl isothiocyanate, THF, 70 °C, 14 h; (b) NaOH, EtOH:H_2O (1:1), 80 °C, 1.5 h.



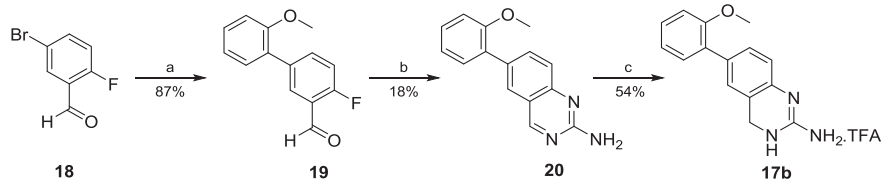
Scheme 1. General synthesis of 2-guanidinopyridines **5**. (a) $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, Tol:EtOH:H_2O (5:1:1), 120 °C, 2–16 h; (b) $ArB(OH)_2$, $Pd(OAc)_2$, S-Phos, K_2CO_3, MeCN:H_2O (2:1), 105 °C, 3 h. (c) *N,N′*-diBoc-*S*-methylisothiourea, $HgCl_2$, NEt_3, DCM, rt, 16 h; (d) $ArB(OH)_2$, $Pd(OAc)_2$, X-Phos, Cs_2CO_3, *n*BuOH:H_2O (4:1), 50 °C, 16 h; (e) TFA:DCM (1:1), rt, 1 h; (f) HCl, Diethyl ether, rt, 5 h; *Yields over 2 steps.



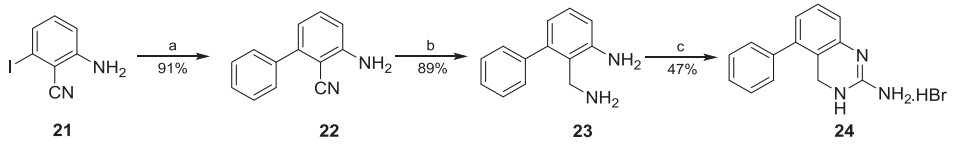
Scheme 3. Synthesis of 2-amino-7-phenyldihydroquinazoline **12**. (a) H_2SO_4, EtOH, 90 °C, 4 h; (b) $PhB(OH)_2$, $Pd(OAc)_2$, X-Phos, Cs_2CO_3, *n*BuOH:H_2O (4:1), 50 °C, 16 h; (c) $LiBH_4$, THF, 70 °C, 5 h; (d) *N*,*N*′-diBoc-*S*-methylisothiourea, $HgCl_2$, NEt_3, DCM, rt, 16 h; (e) $SOCl_2$, DCM, 50 °C, 2.5 h; (f) HCl in dioxane, rt, 12 h.



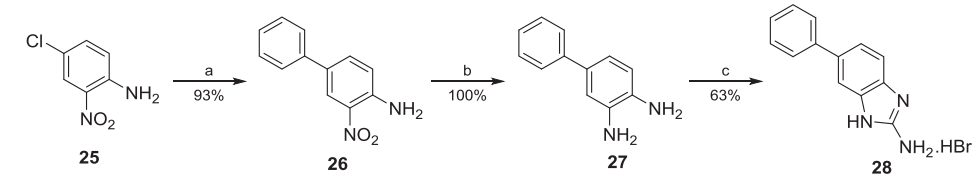
Scheme 4. Synthesis of 2-amino-6-phenyldihydroquinazoline **17**. (a) $PhB(OH)_2$, $Pd(OAc)_2$, Na_2CO_3, H_2O, 95 °C, 16 h; (b) LAH, THF, rt, 12 h; (c) *N*,*N*′-diBoc-*S*-methylisothiourea, $HgCl_2$, NEt_3, DCM, rt, 16 h; (d) $SOCl_2$, DCM, 50 °C, 2.5 h; (e) TFA:DCM (1:1), rt, 1 h.



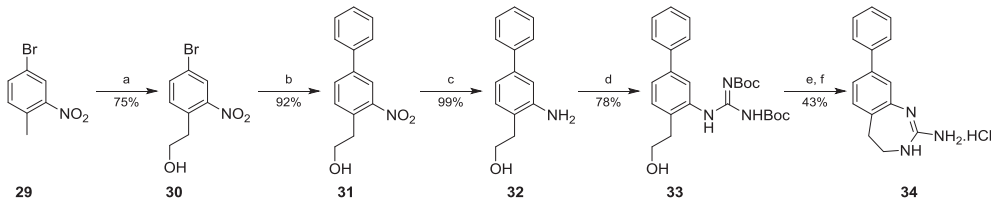
Scheme 5. Synthesis of 2-amino-6-(2-methoxyphenyl)dihydroquinazoline **21**. (a) $PhB(OH)_2$, $Pd(OAc)_2$, S-Phos, K_2CO_3, MeCN:H_2O (2:1), 105 °C, 3 h; (b) guanidine carbonate, DIEA, NMM, 150 °C, 16 h; (c) Et_3SiH, TFA, DCM, rt, 15 h.



Scheme 6. Synthesis of 2-amino-5-phenyldihydroquinazoline **25**. (a) $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, Tol:EtOH:H_2O (5:1:1), 120 °C, 2 h; (b) $BH_3.SMe_2$, THF, 75 °C, 2 h; (c) BrCN, Toluene, 110 °C, 4 h.



Scheme 7. Synthesis of 2-amino-5-phenylbenzimidazole **29**. (a) $PhB(OH)_2$, $Pd(OAc)_2$, S-Phos, K_2CO_3, MeCN:H_2O (2:1), 105 °C, 3 h; (b) Sn, HCl, EtOH, 0–80 °C, 1 h; (c) BrCN, Toluene, 110 °C, 4 h.



Scheme 8. Synthesis of 2-amino-8-phenyldihydrobenzodiazepine **34**. (a) HO-$(CH_2O)_n$H, Triton B, DMSO, 90 °C, 16 h; (b) $PhB(OH)_2$, $Pd(OAc)_2$, TBAB, K_2CO_3, H_2O, 70 °C, 3 h; (c) Pd/C, H_2 (60 psi), MeOH, rt, 3 days; (d) *N*,*N*′-diBoc-*S*-methylisothiourea, $HgCl_2$, NEt_3, DCM, rt, 16 h; (e) DIAD (2.0 equiv.), PPh_3 (2.0 equiv.), THF, rt, 1.5 h; (f) HCl in dioxane, rt, 12 h.

the nature and position of the substituent on the aromatic ring; and ii) the availability of the starting materials.

2-Amino-4-chlorobenzoic acid **8a** served as a suitable starting material for the preparation of the 7-phenyl dihydroquinazoline **12** (Scheme 3). After an esterification step under acidic conditions, a Suzuki-Miyaura cross-coupling reaction was performed on the ester **8b** using $Pd(OAc)_2$ and X-Phos as the catalytic system. The reduction of the ester group by means of $LiBH_4$, followed by a guanylation reaction resulted in the key alcohol intermediate **11**. Finally an intramolecular cyclization was achieved using $SOCl_2$ followed by deprotection of the remaining Boc residue with HCl to yield the target derivative **12**. The 2-amino-6-phenyl-dihydroquinazoline **17a** was synthesized using a similar strategy starting from the 4-bromoanthranilic acid **13** (Scheme 4). A ligand-free Suzuki-Miyaura reaction was performed directly on **13** in presence of $Pd(OAc)_2$ in water [47]. Reduction of the carboxylic acid using $LiAlH_4$, followed by a guanylation reaction, intramolecular cyclization of the intermediate **16,** and finally an acidic treatment, yielded the desired compound **17a.** Unfortunately, due to the steric hindrance, the Suzuki-Miyaura reaction was unsuccessful, when using 2-methoxyphenylboronic acid. An alternative strategy was developed starting from 2-fluoro-5-bromobenzaldehyde **18** (Scheme 5).

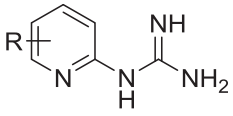
The key steps of the sequence involved first the Suzuki-Miyaura reaction in presence of $Pd(PPh_3)_4$ and the formation of the quinazoline derivative **20** with the help of guanidine carbonate via a nucleophilic aromatic substitution [48]. Finally, a partial hydrogenation step using Et_3SiH in presence of TFA allowed the formation of the expected dihydroquinazoline **17b** [49].

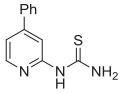
The 2-amino-5-phenyldihydroquinazoline **24** [50] was synthesized in a three-step sequence starting from 2-amino-6-iodobenzonitrile **21** via a Suzuki-Miyaura reaction (Scheme 6). Reduction of the nitrile group by means of $BH_3.SMe_2$ yielded the corresponding diamino intermediate **23** [51]. A final cyclization using cyanogen bromide [52] allowed the formation of the target compound **24**.

The lower homologue 2-aminobenzimidazole **28** was synthesized starting from 4-chloro-2-nitroaniline **25** through a Suzuki-Miyaura/reduction/cyanogen bromide sequence (Scheme 7). Reduction of the resulting 2-nitrolaniline **26** to the corresponding 4-phenylbenzene-1,2-diamine **27** was accomplished using tin in presence of HCl.

Finally, the superior homologue of **17a**, the 2-amino-dihydrobenzodiazepine **34** was obtained in 6 steps starting from 4-bromo-2-nitrotoluene **29** (Scheme 8). Hydroxymethylation of the methyl group of **29** was carried out with paraformaldehyde in the presence of triton B. Introduction of the C-8 phenyl via a Suzuki Miyaura reaction in water in the presence of a phase transfer catalyst (TBAB) yielded the intermediate **31** [53]. After a catalytic hydrogenation of the nitro group, a guanylation reaction was performed in presence of $HgCl_2$ to form the key intermediate **33**. An intramolecular cyclization using a Mitsunobu reaction followed by acidic treatment gave the expected dihydrobenzodiazepine **34**.

Table 1
Inhibition of cytokine production by pyridyl-2-guanidines.[a,b]



Entry	Cmpd	R	TNFα		IL1β		Cell viability
			%inhib at 10 μM[a]	IC_{50} (μM)	%inhib at 10 μM	IC_{50} (μM)	% at 10 μM
1	**5a**	3-Ph	11	nd	1	nd	108
2	**5b**	4-Ph	67	1.2 ± 0.1	91	5.8 ± 0.4	72
3	**5c**	4-(2-ClPh)	65	nd	23	nd	42
4	**5d**	4-(2-OMePh)	10	nd	−1	nd	99
5	**5e**	4-(3-ClPh)	64	nd	53	nd	38
6	**5f**	4-(3-OMePh)	56	nd	29	nd	66
7	**5g**	4-(4-ClPh)	69	nd	97	nd	24
8	**5h**	4-(4-OMePh)	69	nd	86	nd	34
9	**5i**	4-(4-CF_3Ph)	71	nd	98	nd	24
10	**5j**	5-Ph	−9	nd	−1	nd	97
11	**5k**	5-(2-ClPh)	82	nd	96	nd	55
12	**5l**	5-(2-OMePh)	56	7.7 ± 0.9	4	>30	78
13	**5m**	5-(3-ClPh)	52	5.5 ± 0.7	8	17.5 ± 0.6	94
14	**5n**	5-(3-OMePh)	25	nd	4	nd	116
15	**5o**	5-(4-ClPh)	72	nd	97	nd	23
16	**5p**	5-(4-OMePh)	58	nd	48	nd	34
17	**5q**	6-Ph	18	18.6 ± 0.9	3	>30	100
18	**5r**	6-(2-ClPh)	47	19.2 ± 1.9	9	>30	74
19	**5s**	6-(2-OMePh)	7	nd	1	nd	106
20	**5t**	6-(3-ClPh)	74	nd	80	nd	40
21	**5u**	6-(3-OMePh)	7	nd	2	nd	104
22	**5v**	6-(4-ClPh)	72	nd	77	nd	35
23	**5w**	6-(4-OMePh)	27	nd	−6	nd	94
24	**7**		4	nd	1	nd	86

[a] Rolipram as positive control, **TNFα**: (inhibition at 10 μM: 79%, $IC_{50} = 0.13 \pm 0.03$ μM, standard deviation (n = 2)), IL1β: nd; cell viability at 10 μM: 104%; nd = not determined (low potency or cytotoxicity).
[b] Standard deviation (n = 2).

3. Results and discussion

A first evaluation of the cell viability in the presence of the tested compounds eliminated strongly cytotoxic compounds (% viability less than 70% at 10 μM). The TNFα/IL1β inhibitory effects of derivatives of the firstly identified hit **5b**, its congeners (**5a-w**) and their structural analogues (compounds **12**, **17**, **24**, **28** and **34**) were evaluated in parallel. All the results are summarized in Tables 1 and 2. Shifting the phenyl ring to the position 3 (**5a**), or 5 (**5j**), or 6 (**5q**) yielded inactive compounds (compare entries 2 with 1, 10 and 17 in Table 1). A set of substituted 4-aryl 2-pyridylguanidines **5c-5i** were tested, but were found to be inactive or cytotoxic. A similar set of 5-aryl (**5j-5p**) and 6-aryl (**5q-5w**) pyridyl-2-guanidines were tested, but again were found to be inactive or cytotoxic. However, some activity could be recovered in the 5-substituted pyridine sub-series through beneficial effects of aromatic substitution (in ortho position) with a methoxy group (**5l**, entry 12) and a chlorine in meta position (compound **5m**, entry 13). Finally, replacement of the guanidine moiety in **5b** by its isosteric thiourea **7** yielded an inactive compound. The results clearly emphasized the critical role played by a phenyl ring specifically at position 4 (para position of the pyridine nitrogen).

The different aryl bicyclic structural analogues of **5** (n = 0, 1 or 2 in structure II) are listed in Table 2. This series appeared less cytotoxic. Among them, the 7-phenyl 2-amino dihydropyrimidine **12** was particularly interesting, as it confirmed our working medicinal chemistry hypothesis. Compound **12** appeared as an efficient mimetic of **5b** (with the same localization of the phenyl ring in both structures). Finally, we selected a monocyclic (**5b**) and a bicyclic (**12**) compound for further pharmacological studies (Fig. 1), first for *in vivo* evaluation.

3.1. In vivo experiments in an acute lung inflammation model

Evaluation of the anti-inflammatory effect of compounds *in vivo* was performed in an acute lung inflammation model in C57BL/6 mice, in response to intranasal LPS. We compared the effect of compounds **5b** and **12** at a dose of 5 mg/kg (i.p., *BID*). Analysis of airway inflammation in BAL fluid (BALF) shows that compounds **5b** and **12** have no effect on the number of inflammatory cells recovered in BAL fluid as compared to solvent. LPS challenge increases the total number of cells recovered in BAL fluid. This effect is related to a significant influx of neutrophils and lymphocytes (###, $p < 0.001$ *vs* control; Fig. 2). Compounds **5b** and **12** (5 mg/kg, i.p.) induced a statistically reliable inhibition of the recruitment of neutrophils (39% and 62%, respectively) and lymphocytes (52% and 63%, respectively) in the airways.

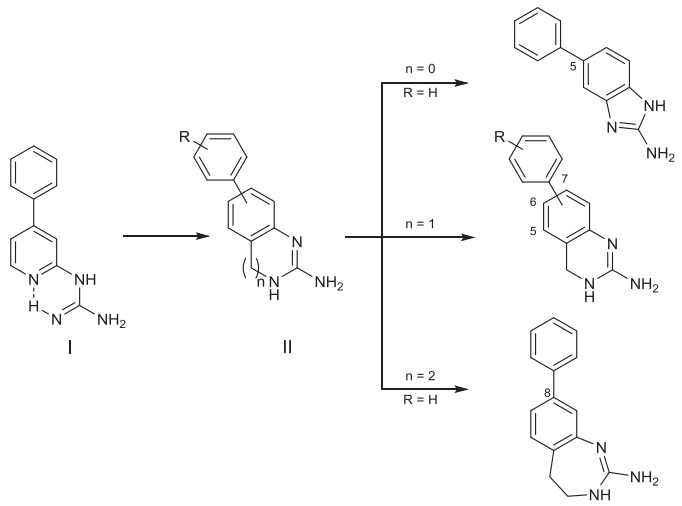
The release of TNFα in BALF was significantly increased by LPS challenge (#, $p < 0.05$ *vs* control). Compound **12** induced a statistically reliable decrease in TNFα release in BALF (49%).

3.2. In vivo experiments in a neuropathic pain model

Neuropathic pain was induced in mice by inserting a standardized polyethylene cuff around the main branch of the right sciatic nerve [54,55]. In this model, heat hyperalgesia only lasts 2–3 weeks [54,55] while mechanical allodynia, which is clinically more relevant [56], remains stable over 2 months [54,55]. Moreover, the mechanical allodynia in this model is sensitive to clinical treatments of neuropathic pain, such as tricyclic antidepressants and gabapentinoids [20,54,55,57], and its consistency allows reducing the number of animals per group.

Implantation of the cuff around the sciatic nerve induced a long

Table 2
Rigid 2-pyridylguanidines as TNFα/IL1β inhibitors.[a,b]



Entry	Compd	n	Position	R	TNFα		IL1β		Cell viability
					%inhib at 10 μM	IC_{50} (μM)[a]	%inhib at 10 μM	IC_{50} (μM)[a]	% at 10 μM
1	**28**	0	5	/	15	nd	3	nd	114
2	**24**	1	5	H	7	nd	0	nd	111
3	**17a**	1	6	H	22	nd		nd	90
4	**17b**	1	6	2-OMe	13	nd	1	nd	126
5	**12**	1	7	H	76	1.0 ± 0.1	93	3.7 ± 0.2	81
6	**34**	2	8	/	−7	nd	−4	nd	100

[a] Rolipram as positive control, **TNFα**: (inhibition at 10 μM: 79%, $IC_{50} = 0.13 \pm 0.03$ μM, standard deviation(n = 2)), IL1β: nd; cell viability at 10 μM: 104%; nd = not determined (low potency or cytotoxicity.
[b] Standard deviation (n = 2).

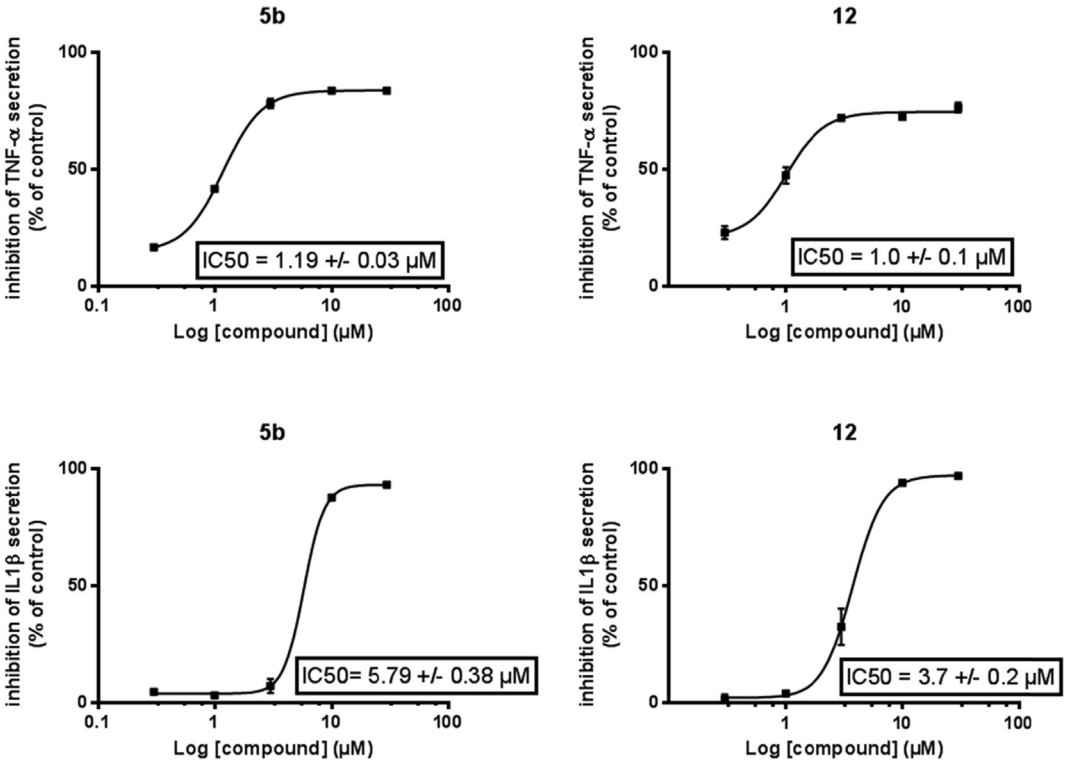


Fig. 1. IC_{50} curves of inhibition of TNFα and IL1β of compounds **5b** and **12**.

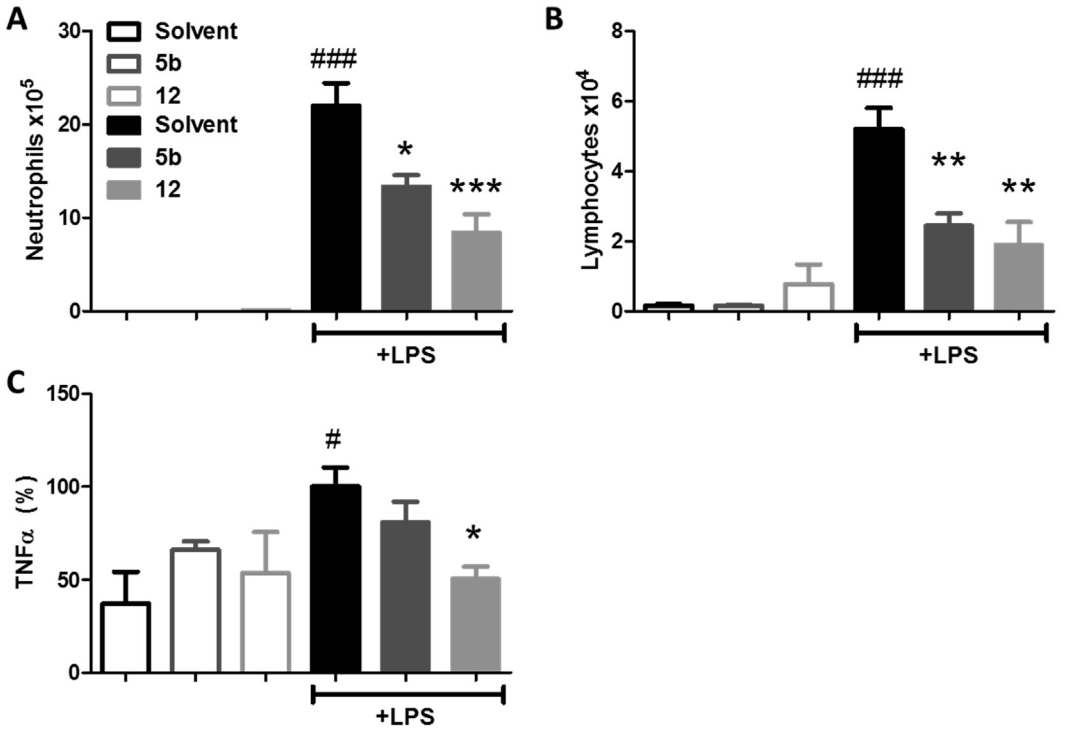


Fig. 2. Anti-inflammatory activity of compounds 5b and 12 in an acute lung inflammation model in C57BL/6 mice. Compounds 5b and 12 were administered by IP injection (5 mg/kg) 1 h before and 10 h after LPS or saline challenge. Absolute numbers of neutophils (**A**) lymphocytes (**B**) in BAL are shown. TNFα levels in BAL fluid (**C**) quantified by ELISA with R&d systems kit are expressed as % of the LPS group. Blocks are means and bars are SEM values (n = 3 to 9 per group). $^{\#}p \leq 0.05$, $^{\#\#\#}p \leq 0.001$, vs control group and $^{*}p \leq 0.05$, $^{**}p \leq 0.001$, $^{***}p \leq 0.001$ vs LPS group.

lasting ipsilateral mechanical allodynia, while sham surgery did not affect the paw withdrawal thresholds (Fig. 3a; surgery x paw × time interaction, $F8,64 = 2.24$, $p = 0.035$, post hoc: "Cuff postsurgery, right paw"< "Sham", "Cuff, baseline right paw" and "Cuff, left paw" at $p < 0.001$ on pretreatment and treatment days 4–17). Two to three weeks after cuff insertion, treatments started with saline,

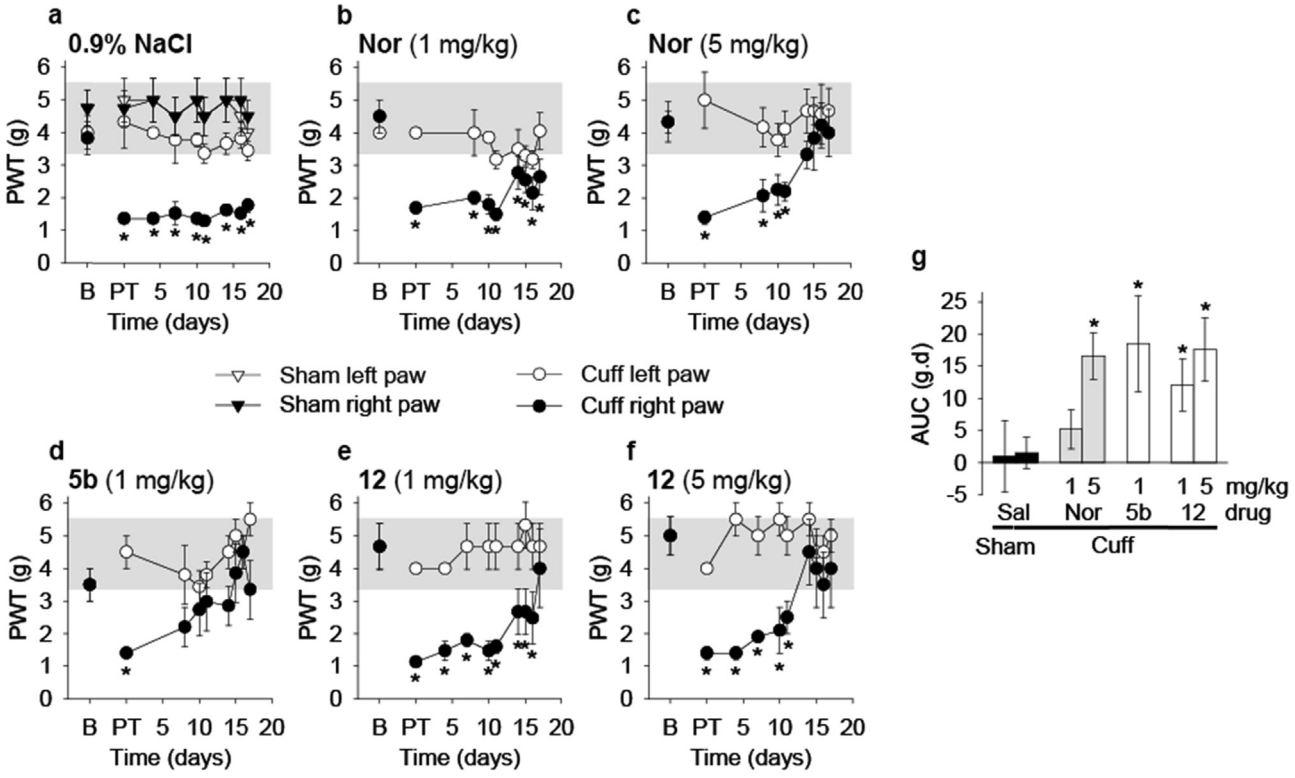


Fig. 3. Chronic treatments with **5b** and **12** alleviated mechanical allodynia in mice. Two to three weeks after sham surgery (n = 4) or unilateral cuff implantation on the right sciatic nerve (n = 3–6 per group), treatments started and were continued for 17 days. The mechanical hindpaw withdrawal threshold (PWT) was evaluated using von Frey filaments before surgery (B: baseline), before starting treatments (PT: pre-treatment) and on given days during the chronic treatment. Nortriptyline (Nor, 1 and 5 mg/kg, i.p., twice a day) was used as positive control (**b,c**). **5b** treatment (1 mg/kg, i.p., twice a day) (**d**) and **12** treatments (1 and 5 mg/kg, i.p., twice a day) (**e,f**) reversed the mechanical allodynia in mice (*p < 0.05 compared to the baseline right paw). The shaded area represents the mean±std of the 31 mice at baseline, thus providing a visual reference on the graphs for the absence of mechanical allodynia. The area under the curve (AUC) above pre-treatment values is calculated for the whole treatment period and is displayed in graph **g**. It shows that nortriptyline 5 mg/kg, **5b** 1 mg/kg and **12** 1 and 5 mg/kg have an antiallodynic action that significantly differs from saline treatment (*p < 0.05 compared to Cuff/saline data).

with the tricyclic antidepressant nortriptyline as a positive control, or with the compounds **5b** and **12**, at doses of 1 and 5 mg/kg. Area under the curve (AUC) analysis for allodynia relief is presented in Fig. 3g, and time-courses in Fig. 3a and f. Saline treatment had no impact *per se* (Fig. 3a). Nortriptyline at a dose of 1 mg/kg was only partially effective and did not lead to full recovery of baseline values (Fig. 3b, paw × time interaction, $F_{8,25} = 3.75$, $p = 0.005$; post hoc: "Right paw"< "Baseline right paw" at $p < 0.05$ on treatment days 0–17), while (as previously reported) [12,57] chronic nortriptyline alleviated neuropathic allodynia at a dose of 5 mg/kg (Fig. 3c, paw × time interaction, $F_{8,48} = 2.45$, $p = 0.025$; post hoc: "Right paw"< "Baseline right paw" at $p < 0.02$ on treatment days 0–11). **5b** at a dose of 5 mg/kg was the only condition that led to a 5% decrease in body weight, with an important induration at injection sites that developed after a few injections; behavioral data at this dose were thus not considered. **5b** at a dose of 1 mg/kg was not accompanied by these adverse effects, and it alleviated the cuff-induced allodynia (Fig. 3d, paw × time interaction, $F_{8,24} = 1.96$, $p = 0.09$; post hoc: "Right paw"< "Baseline right paw" at $p < 0.02$ on pretreatment day). **12** at doses of 1 and 5 mg/kg alleviated the cuff-induced allodynia after 16 and 11 days, respectively (Figure 3e, 1 mg/kg, paw × time interaction, $F_{9,18} = 3.14$, $p = 0.018$; post hoc: "Right paw"< "Baseline right paw" at $p < 0.01$ on treatment days 0–16) (Figure 3f, 5 mg/kg, paw × time interaction, $F_{9,27} = 7.96$, $p = 0.000012$; post hoc: "Right paw"< "Baseline right paw" at $p < 0.05$ on treatment days 0–11). The AUC analysis confirmed the lack of effect of saline treatment, the mild but non-significant improvement with nortriptyline 1 mg/kg ($p = 0.3$ vs. Cuff/saline), and the significant antiallodynic action of chronic treatments with nortriptyline at 5 mg/kg ($p = 0.005$), **5b** at 1 mg/kg ($p = 0.034$), **12** at 1 mg/kg ($p = 0.046$) and **12** at 5 mg/kg ($p = 0.010$).

This work evaluates the pharmacologic activity of **5b,** a hit recently discovered as an inhibitor of TNFα overproduction by human PBMCs upon exposure to LPS. This non-cytotoxic hit was selected with an IC_{50} value in the μM range. However various derivatives (24 compounds) and structural analogues (6 compounds) of **5b** showed few active compounds with similar *in vitro* potencies (**5f**, **5l**, **5m**). Interestingly, the novel bicyclic sub-series based on a **5b** mimetic highlighted the dihydroquinazoline **12** as a particularly interesting molecule (TNFα inhibition: $IC_{50} = 1.0 \pm 0.1$ μM). Pharmacophoric critical substitutions of **5b**, in particular the presence and location of the phenyl ring in position 4, was referred to the newly formed covalent bond of II replacing the internal H bond interaction in I (I -> II, Table 2). The optimal homologue (n = 1) was the rigidified compound of **5b**, whereas other homologues (n = 0 and n = 2) were found inactive. The active conformer with the phenyl ring is correctly located at position 7 is strongly active. As observed with the inactive phenyl pyridyl guanidine isomers (**5a**, **5j, 5q**, to be compared with the active **5b**), a SAR analysis of the location of the phenyl group was similar (inactive phenyl isomers of aminodihydroquinazolines **24** and **17a**, to be compared with the active compound **12**). Among the novel series of aminodihydroquinazolines, we focused on compound **12**, which presented TNFα inhibiting properties with a 1 μM IC_{50} value. The selected TNFα inhibitors **5b** and **12** were analyzed in *in vivo* experiments (acute lung inflammation and neuropathic pain), which confirmed their efficacies in both selected animal models. Indeed, the 2-amino dihydroquinazoline **12** was found effective in a

neuropathic pain model in mice at 1 and 5 mg/kg *i.p.* (Fig. 3). However the compound **5b** presented some *in vivo* toxicity with repeated treatment at dose 5 mg/kg twice a day. This adverse effect was absent at a dose of 1 mg/kg, which was effective in relieving neuropathic allodynia. We used the tricyclic antidepressant nortriptyline as reference control for these experiments. Tricyclic antidepressants are among the first line treatments of neuropathic pain [58,59], and we previously showed that nortriptyline inhibits TNFα overproduction through the noradrenergic recruitment of β_2-adrenoceptors in the dorsal root ganglia of a mouse model of neuropathic pain [12]. Validation of our strategy using a human PBMCS screening assay highlighted valuable hits to be tested *in vivo*. The novel 2-aminodihydroquinazoline (2-ADHQ, **12** as representative) compared to 2-ADHQ derivatives presented similar *in vitro* and *in vivo* pharmacological properties (Chart 2) [37,60,61], as shown in Chart 2. A particularly interesting set of pharmacological agents containing 2-ADHQ derivatives were very similar to mimetic pharmacological compounds in Chart 2 (5HT-R subtypes, α2-R subtypes). Interestingly, a similar working hypothesis of the use of a specific bicyclic scaffold able to mimic an internal H bond interaction [62] (I to II in Table 2) was already applied with success by Rozas et al. for the design of novel noradrenergic ligands [38].

Based on our *in vivo* data, we onfirm the rationale of the working hypothesis, i.e. the anti-inflammatory potential of our compounds evaluated in a LPS-induced pulmonary inflammation, releasing TNFα as previously reported [1–3]. Compound **12** was found to be efficient in the neuropathic pain model in mice at doses of 1 and 5 mg/kg, *i.p.*, with no apparent sign of behavioral toxicity. This result is in line with previous data showing that the two first-line clinical treatments of neuropathic pain, antidepressants and gabapentinoids, indirectly lead to decrease in the nerve injury-induced TNFα overproduction in the dorsal root ganglia of a model of neuropathic pain [12,20], and with data showing an efficacy of anti-TNFα biotherapies in animal models of neuropathic pain [9–12]. Moreover, other pharmacological agents known to inhibit the production of TNFα, such as type 4 phosphodiesterase (PDE4) inhibitors or less selective PDE/PDE4 inhibitors, such as rolipram, theophylline at high dose, or pentoxifylline, have been found active in *in vivo* neuropathic pain models(unpublished data and [63–66]), which fully supported our working hypothesis of an interest for small molecules to oppose the overproduction of TNFα observed in specific diseases, including neuropathic pain. The major concern for the discovery of novel hits from a phenotypic screening is the lack of knowledge of the target protein(s) accounting for both the cellular and the *in vivo* effects of a given molecule. The compound **12** may be potent enough ($IC_{50} \approx 1$ μM in PBMCs) to be a valuable tool for target fishing (affinity column chromatography), or for target identification by means of chemoproteomics and chemoinformatic approaches. So far in the literature, $5HT_3$ [67], α2 receptors [68], P_{2X7} purinergic receptors [69,70] ligands have been associated with neuropathic pain treatments for both aryl/heteroaryl guanidines and 2-ADHQ (represented in Chart 1 and Chart 3).

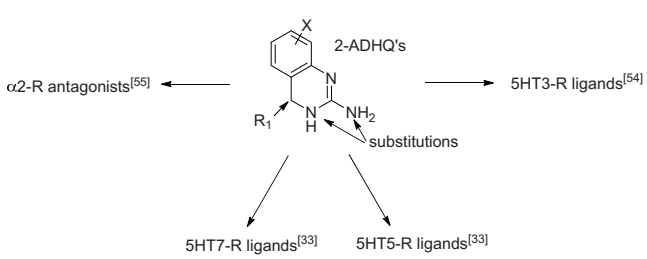


Chart 3. Pharmacological properties of selected 2-ADHQ's.

4. Conclusion

LPS-stimulated hPBMCs-based assay and primary screen of about 10,000 drug-like compounds led to the selection of an initial hit, the 1-(4-phenylpyridin-2yl) guanidine (**5b**) as a TNFα overproduction inhibitor (IC_{50} ~1 μM). SAR analysis highlighted the beneficial effect of a phenyl ring at position 4 (**5b**). Design of a mimetic drug leading to a novel scaffold (2-amino dihydroquinazoline **12**) showed a similar μM IC_{50} value. Both compounds were found active in mice models of acute lung inflammation and neuropathic pain. This shows a successful proof of concept for the search of small molecules as inhibitors of TNFα production for use as novel treatments of neuropathic pain and acute inflammation. An optimization program for the further development of a valuable successor of **12** (with a gain of magnitude from 1 μM → 0.1 μM) is now in progress, which will allow the study of its precise mechanism of action.

5. Experimental section

5.1. Chemistry

General Methods for Chemistry. All reactions were carried out under usual atmosphere unless otherwise stated. Chemicals and solvents were purchased from Sigma-Aldrich and were used without further purification. Analytical TLC was performed using silica gel plates Merck 60F254 and plates were visualized by exposure to ultraviolet light. Compounds were purified on silica gel Merck 60 (particle size 0.040–0.063 nm) or using Armen spot flash chromatography (normal phase column: Interchim 30 SHIP 25 g; reverse phase column: AIT 50 g C18). Microwave irradiation was performed with a Biotage Initiator EXP (external sensor type). Yields refer to isolated compounds, estimated to be >95% pure as determined by ^1H NMR or HPLC. ^1H, ^{19}F and ^{13}C NMR spectra were recorded on Bruker Avance Spectrometer operating at 400 or 500 MHz, 376 MHz and 100 or 125 MHz, respectively. All chemical shift values δ and coupling constants J are quoted in ppm and in Hz, respectively, multiplicity (s = singulet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad). Melting points were realized using a Büchi Melting point B-540. Analytical RP-HPLC-MS was performed using a LC-MSD 1200SL Agilent with a Thermo Hypersilgold® column (C18, 30 mm × 1 mm; 1.9 μm) using the following parameters: 1) The solvent system: A (acetonitrile) and B (0.05% TFA in H_2O); 2) A linear gradient: t = 0 min, 98%B; t = 5 min, 5%B; t = 6 min, 5%B; t = 7 min, 98%B; t = 9 min, 98%B; 3) Flow rate of 0.3 mL/min; 4) Column temperature: 50 °C; 5) The ratio of products was determinate by integration of spectra recorded at 210 nm or 254 nm; 6) Ionization mode: MM-ES+APCI. HPLC were performed using a Dionex UltiMate 300 using the following parameters: Flow rate of 0.5 mL/min, column temperature: 30 °C, solvent system: A (MeOH) and B (0.05% of THA in H_2O), t = 0 min–1 min: 50–60% of B then t = 1 min to t = 10 min: 60–100% of B and t = 10 min to t = 15 min: 100% of B.

Pd-Catalyzed Suzuki-Miyaura cross-coupling using $Pd(PPh_3)_4$: General method A. A microvave vial under argon was charged with the corresponding halogeno derivatives (1.0 equiv.), the corresponding phenylboronic acid (1.2 equiv.), $Pd(PPh_3)_4$ (5 mol %), Na_2CO_3 (3.0 equiv.) and a mixture toluene:ethanol:water (5:1:1). The vial was capped properly, flushed with argon and heated to 120 °C until complete conversion of the starting material. After cooling, the reaction mixture was concentrated under vacuum. The crude residue was diluted in water. The organic phase was extracted 3 times with EtOAc. The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel column chromatography, eluting with the

appropriate *n*heptane:EtOAc mixture.

Pd-Catalyzed Suzuki-Miyaura cross-coupling using $Pd(OAc)_2$ and S-Phos: General method B. A microvave vial under argon was charged with the corresponding halogeno derivatives (1.0 equiv.), the corresponding phenylboronic acid (1.2 equiv.), $Pd(OAc)_2$ (2–10 mol%), S-Phos (4–20 mol%), Na_2CO_3 (3.0 equiv.) and a mixture CH_3CN:H_2O (1:1). The vial was capped properly, flushed with argon and heated to 100 °C until complete conversion of the starting material. After cooling, the reaction mixture was concentrated under vacuum. The crude residue was diluted in water. The organic phase was extracted 3 times with EtOAc. The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel column chromatography, eluting with the appropriate *n*heptane:EtOAc mixture.

Pd-Catalyzed Suzuki-Miyaura cross-coupling using $Pd(OAc)_2$ and X-Phos: General method C. A microvave vial under argon was charged with the corresponding halogeno derivatives (1.0 equiv.), the corresponding phenylboronic acid (1.2 equiv.), $Pd(OAc)_2$ (6 mol %), S-Phos (7 mol%), Cs_2CO_3 (2.5 equiv.) and a mixture *n*BuOH:H_2O (4:1). The vial was capped properly, flushed with argon and heated to 50 °C until complete conversion of the starting material. After cooling, the reaction mixture was concentrated under vacuum. The crude residue was diluted in water. The organic phase was extracted 3 times with EtOAc. The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel column chromatography, eluting with the appropriate *n*heptane:EtOAc mixture.

Preparation of Boc-Protected guanidines derivatives: General method D. A solution of starting amine (1.0 equiv.), *N,N′-bis-*(*tert*-butoxycarbonyl)-*S*-methylisothiourea (0.95 equiv.), triethylamine (4.4 equiv.) and mercuric chloride (1.1 equiv.) in CH_2Cl_2 were stirred at rt overnight. After completion of the reaction, the reaction mixture was filtered through a pad of Celite® and washed with CH_2Cl_2. The filtrate was concentrated under vacuum and purified by silica gel column chromatography, eluting with the appropriate *n*heptane:EtOAc mixture.

Formation of guanidine hydrochloride salts: General method E. The protected guanidine (1.0 equiv.) was dissolved in ether. The reaction mixture was cooled at 0 °C and a solution of HCl 37% (18 equiv.) was added dropwise. The mixture was stirred at room temperature for 6 h. The solution was concentrated under vacuum and purified by reverse chromatography, eluting with the appropriate MeOH/H_2O+0.05%HCl mixture.

Formation of guanidine trifluoroacetate salts: General method F. The protected guanidine (1 equiv.) was dissolved in a mixture of TFA:DCM (1:1). The solution was stirred at room temperature for 2 h. The solution was concentrated under vacuum and purified by reverse chromatography, eluting with the appropriate MeOH/H_2O+0.05%TFA mixture.

3-Phenylpyridin-2-amine (2a). Following general method B and starting from 2-amino-3-bromopyridine **1a** (200 mg, 1.16 mmol) and phenylboronic acid (169 mg, 1.39 mmol), **2a** was obtained as a white solid (160 mg, 0.95 mmol, 82%). Data are consistent with literature values [71] ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.54 (s, 2H), 6.67 (dd, 1H, J = 5.0 Hz, J = 7.4 Hz), 7.28 (dd, 2H, J = 1.9 Hz, J = 7.3 Hz), 7.37–7.39 (m, 4H), 8.00 (dd, 1H, J = 1.9 Hz, J = 5.0 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 114.5, 121.9, 127.8, 128.7, 129.1, 137.8, 138.1, 147.2, 155.9.

4-Phenylpyridin-2-amine (2b). Following general method B and starting from 2-amino-4-bromopyridine **1b** (700 mg, 4.05 mmol) and phenylboronic acid (592 mg, 4.86 mmol), **2b** was obtained as a yellow solid (552 mg, 3.24 mmol, 80%). Data are consistent with literature values [72]. mp = 164–165 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.52 (br s, 2H), 6.71 (d, 1H, J = 0.8 Hz), 6.89 (dd; 1H, J = 1.5 Hz, J = 5.4 Hz), 7.38–7.47 (m, 3H), 7.58 (dd, 2H, J = 1.2 Hz, J = 8.4 Hz), 8.12 (d, 1H, J = 5.4 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 106.4, 112.9, 127.0, 128.9, 129.0, 138.8, 148.5, 150.5, 159.0.

4-(2-Methoxyphenyl)pyridin-2-amine (2c). Following general method B and starting from 2-amino-4-bromopyridine **1b** (100 mg, 0.58 mmol) and 2-methoxyphenylboronic acid (105 mg, 0.69 mmol), **2c** was obtained as a yellow oil (75 mg, 0.37 mmol, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.75 (s, 3H), 4.41 (s, 2H), 6.60 (s, 1H), 6.75 (dd, 1H, J = 1.9 Hz, J = 3.9 Hz), 6.91 (d, 1H, J = 8.7 Hz), 6.95 (td, 1H, J = 1.1 Hz, J = 7.5 Hz), 7.22 (dd, J = 1.8 Hz, J = 7.5 Hz), 7.28 (td, 1H, J = 1.9 Hz, J = 7.5 Hz), 8.00 (d, 1H, J = 5.3 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 55.6, 109.0, 111.4, 115.4, 120.9, 128.4, 129.8, 130.3, 147.5, 148.2, 156.5, 158.4.

4-(3-Methoxyphenyl)pyridin-2-amine (2d). Following general method B and starting from 2-amino-4-bromopyridine **1b** (200 mg, 1.16 mmol) and 3-methoxyphenylboronic acid (211 mg, 1.39 mmol), **2d** was obtained as a white solid (168 mg, 0.84 mmol, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.79 (s, 3H), 4.48 (s, 2H), 6.62 (d, 1H, J = 1.5 Hz), 6.80 (dd, 1H, J = 1.5 Hz, J = 5.4 Hz), 6.88 (dd, 1H, J = 2.6 Hz, J = 8.0 Hz), 7.02 (t, J = 2.5 Hz), 7.08 (dd, 1H, J = 1.5 Hz, J = 7.8 Hz), 7.28 (t, 1H, J = 7.8 Hz), 8.03 (d, 1H, J = 5.3 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 55.4, 106.5, 112.7, 112.8, 114.2, 119.4, 129.9, 140.2, 148.1, 150.5, 158.8, 160.0.

4-(4-Methoxyphenyl)pyridin-2-amine (2e). Following general method B and starting from 2-amino-4-bromopyridine **1b** (150 mg, 0.87 mmol) and 4-methoxyphenylboronic acid (158 mg, 1.04 mmol), **2e** was obtained as a white solid (125 mg, 0.62 mmol, 72%). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 3.80 (s, 3H), 5.90 (br s, 2H), 6.66 (d, 1H, J = 1.8 Hz), 6.74 (dd, 1H, J = 1.8 Hz, J = 5.4 Hz), 7.03 (d, 2H, J = 8.8 Hz), 7.59 (d, 2H, J = 8.8 Hz), 7.92 (d, 1H, J = 5.4 Hz); ^{13}C NMR (101 MHz, DMSO) δ ppm 55.7, 104.8, 110.2, 114.9, 128.1, 131.0, 148.1, 148.8, 160.3, 160.9.

4-(4-Trifluoromethylphenyl)pyridin-2-amine (2f). Following general method B and starting from 2-amino-4-bromopyridine **1b** (200 mg, 1.16 mmol) and 4-trifluoromethylphenylboronic acid (264 mg, 1.39 mmol), **2f** was obtained as a white solid (186 mg, 0.78 mmol, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.52 (s, 2H), 6.62 (d, 1H, J = 1.6 Hz), 6.80 (dd, 1H, J = 1.6 Hz, J = 5.4 Hz), 7.59–7.65 (m, 4H), 8.00 (d, 1H, J = 5.4 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 106.4, 112.7, 125.9 (q, J = 4.4 Hz), 127.3, 130.9, 142.4, 148.8, 148.9, 159.0.

5-Phenylpyridin-2-amine (2g). Following general method A and starting from 2-amino-5-bromopyridine **1c** (100 mg, 0.58 mmol) and phenylboronic acid (85 mg, 0.69 mmol), **2g** was obtained as a white solid (89 mg, 0.52 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.78 (br s, 2H), 6.59 (d, 1H, J = 8.4 Hz), 7.33 (t, 1H, J = 7.4 Hz), 7.40–7.45 (m, 2H), 7.51 (d, 2H, J = 7.4 Hz), 7.69 (d, 1H, J = 8.4 Hz), 8.40 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 109.1, 126.3, 127.0, 127.3, 129.0, 136.9, 138.1, 145.5, 157.4.

5-(2-Chlorophenyl)pyridin-2-amine (2h). Following general method A and starting from 2-amino-5-bromopyridine **1c** (200 mg, 1.16 mmol) and 2-chlorophenylboronic acid (217 mg, 1.39 mmol), **2h** was obtained as a white solid (177 mg, 0.87 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.51 (s, 2H), 6.48 (d, 1H, J = 8.4 Hz), 7.16–7.125 (m, 3H), 7.38 (d, 1H, J = 8.0 Hz), 7.51 (dd, 1H, J = 2.4 Hz, J = 8.4 Hz), 8.07 (d, 1H, J = 2.4 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 107.6, 125.6, 127.0, 128.5, 130.0, 131.1, 132.8, 137.5, 138.9, 148.3, 157.6.

5-(2-Methoxyphenyl)pyridin-2-amine (2i). Following general method A and starting from 2-amino-5-bromopyridine **1c** (200 mg, 1.16 mmol) and 2-methoxyphenylboronic acid (211 mg, 1.39 mmol), **2i** was obtained as a colorless oil (232 mg, 1.16 mmol, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.81 (s, 3H), 4.53 (s, 2H), 6.54 (dd, 1H, J = 1.1 Hz, J = 8.5 Hz), 6.97 (d, 1H, J = 8.5 Hz), 7.01 (td, 1H, J = 1.1 Hz, J = 7.5 Hz), 7.27–7.31 (m, 2H), 7.66 (dd, 1H, J = 2.4 Hz, J = 8.5 Hz), 8.24 (d, 1H, J = 2.4 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 55.5, 107.8, 111.3, 121.0, 124.6, 127.6, 128.5, 130.2, 139.0, 148.1, 156.6, 157.1.

5-(3-Chlorophenyl)pyridin-2-amine (2j). Following general method A and starting from 2-amino-5-bromopyridine **1c** (200 mg, 1.16 mmol) and 3-chlorophenylboronic acid (217 mg, 1.39 mmol), **2j** was obtained as a white solid (180 mg, 0.88 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.57 (s, 2H), 6.49 (d, 1H, $J = 8.5$ Hz), 7.20 (dt, 1H, $J = 1.9$ Hz, $J = 7.7$ Hz), 7.26 (t, 1H, $J = 7.7$ Hz), 7.30 (dt, 1H, $J = 1.9$ Hz, $J = 7.7$ Hz), 7.40 (t, 1H, $J = 1.9$ Hz), 7.55 (dd, 1H, $J = 2.5$ Hz, $J = 8.5$ Hz), 8.22 (d, 1H, $J = 2.5$ Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 108.6, 124.3, 125.9, 126.3, 126.9, 130.1, 134.8, 136.5, 140.2, 146.3, 158.0.

5-(3-Methoxyphenyl)pyridin-2-amine (2k). Following general method A and starting from 2-amino-5-bromopyridine **1c** (200 mg, 1.16 mmol) and 3-methoxyphenylboronic acid (211 mg, 1.39 mmol), **2k** was obtained as a colorless oil (168 mg, 0.84 mmol, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.85 (s, 3H), 4.58 (s, 2H), 6.56 (d, 1H, $J = 8.5$ Hz), 6.86 (ddd, 1H, $J = 0.6$ Hz, $J = 2.5$ Hz, $J = 8.5$ Hz), 7.03 (t, 1H, $J = 2.5$ Hz), 7.09 (dq, 1H, $J = 0.6$ Hz, $J = 7.9$ Hz), 7.33 (t, 1H, $J = 7.9$ Hz), 7.66 (dd, 1H, $J = 2.5$ Hz, $J = 8.5$ Hz), 8.32 (d, 1H, $J = 2.5$ Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 55.3, 108.5, 112.1, 112.2, 118.8, 127.2, 129.9, 136.6, 139.8, 146.4, 157.8, 160.1.

5-(4-Chlorophenyl)pyridin-2-amine (2l). Following general method A and starting from 2-amino-5-bromopyridine **1c** (100 mg, 0.58 mmol) and 4-chlorophenylboronic acid (109 mg, 0.69 mmol), **2l** was obtained as a white solid (81 mg, 0.40 mmol, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.65 (br s, 2H), 6.57 (d, 1H, $J = 8.5$ Hz), 7.37 (d, 2H, $J = 8.5$ Hz), 7.42 (d, 2H, $J = 8.5$ Hz), 7.62 (d, 1H, $J = 8.5$ Hz), 8.29 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 108.7, 126.2, 127.5, 129.1, 133.0, 136.5, 136.7, 146.0, 157.7.

5-(4-Methoxyphenyl)pyridin-2-amine (2m). Following general method A and starting from 2-amino-5-bromopyridine **1c** (100 mg, 0.58 mmol) and 4-methoxyphenylboronic acid (105 mg, 0.69 mmol), **2m** was obtained as a yellow solid (90 mg, 0.45 mmol, 77%). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 3.76 (s, 3H), 5.96 (s, 2H), 6.50 (d, 1H, $J = 8.5$ Hz), 6.96 (d, 2H, $J = 8.7$ Hz), 7.47 (d, 2H, $J = 8.7$ Hz), 7.62 (d, 1H, $J = 8.5$ Hz), 8.17 (s, 1H); ^{13}C NMR (101 MHz, DMSO) δ ppm 55.6, 108.4, 114.8, 124.3, 127.0, 131.1, 135.5, 145.6, 158.5, 159.2.

6-Phenylpyridin-2-amine (2n). Following general method A and starting from 2-amino-6-chloropyridine **1d** (200 mg, 1.56 mmol) and phenylboronic acid (228 mg, 1.87 mmol), **2n** was obtained as a yellow oil (242 mg, 1.42 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.54 (s, 2H), 6.45 (d, 1H, $J = 7.8$ Hz), 7.10 (d, 1H, $J = 7.8$ Hz), 7.38 (t, 1H, $J = 7.7$ Hz), 7.44 (t, 2H, $J = 7.7$ Hz), 7.50 (t, 1H, $J = 7.8$ Hz), 7.94 (d, 2H, $J = 7.7$ Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 107.1, 111.0, 126.8, 128.5, 128.6, 138.4, 139.7, 156.2, 158.3.

6-(2-Chlorophenyl)pyridin-2-amine (2o). Following general method A and starting from 2-amino-6-chloropyridine **1d** (300 mg, 2.33 mmol) and 2-chlorophenylboronic acid (438 mg, 2.80 mmol), **2o** was obtained as a white solid (283 mg, 1.38 mmol, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.59 (s, 2H), 6.50 (d, 1H, $J = 7.8$ Hz), 6.94 (d, 1H, $J = 7.8$ Hz), 7.31 (td, 2H, $J = 1.6$ Hz, $J = 7.4$ Hz), 7.44 (dd, 1H, $J = 1.6$ Hz, $J = 7.4$ Hz), 7.48–7.53 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 107.5, 115.0, 126.8, 129.2, 130.0, 131.2, 137.7, 139.4, 155.3, 158.1.

6-(2-Methoxyphenyl)pyridin-2-amine (2p). Following general method A and starting from 2-amino-6-chloropyridine **1d** (150 mg, 1.17 mmol) and 2-methoxyphenylboronic acid (213 mg, 1.40 mmol), **2p** was obtained as a yellow solid (201 mg, 1.01 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.76 (s, 3H), 4.40 (s, 2H), 6.36 (dd, 1H, $J = 0.8$ Hz, $J = 8.3$ Hz), 6.89 (dd, 1H, $J = 0.8$ Hz, $J = 8.3$ Hz), 6.97 (td, 1H, $J = 0.8$ Hz, $J = 7.4$ Hz), 7.06 (dd, 1H, $J = 0.8$ Hz, $J = 7.4$ Hz), 7.25 (ddd, 1H, $J = 1.9$ Hz, $J = 7.4$ Hz, $J = 8.3$ Hz), 7.39 (t, 1H, $J = 7.9$ Hz), 7.61 (dd, 1H, $J = 1.9$ Hz, $J = 7.9$ Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 55.7, 106.7, 111.5, 115.5, 120.9, 129.4, 129.5, 130.9, 137.5, 154.6, 157.0, 158.1.

4-Bromo-1-[2,3-di(tert-butoxycarbonyl)guanidino]pyridine (3a). Following general method D and starting from 2-amino-4-bromopyridine **1d** (100 mg, 0.58 mmol), **3a** was obtained as a white solid (193 mg, 0.47 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.56 (s, 18H), 7.20 (d, 1H, $J = 5.4$ Hz), 8.13 (d, 1H, $J = 5.4$ Hz), 8.64 (br s, 1H), 10.99 (br s, 1H), 11.52 (br s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 28.0, 28.1, 119.0, 123.2, 134.1, 148.5, 151.4, 152.8.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-6-chloropyridine (3b). Following general method D and starting from 2-amino-6-chloropyridine **1d** (1.00 g, 7.78 mmol), **3b** was obtained as a white solid (2.36 g, 6.37 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.53 (s, 18H), 7.03 (d, 1H, $J = 7.9$ Hz), 7.65 (t, 1H, $J = 7.9$ Hz), 8.36 (d, 1H, $J = 7.9$ Hz), 10.85 (s, 1H), 11.50 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 28.0, 28.1, 80.2, 84.2, 114.2, 119.8, 140.6, 153.0.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-3-phenylpyridine (4a). Following general method D and starting from **2a** (143 mg, 0.84 mmol)**, 4a** was obtained as a colorless oil (95 mg, 0.23 mmol, 37%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.38 (s, 18H), 7.07 (t, 1H, $J = 5.6$ Hz), 7.28–7.40 (m, 5H), 7.60 (d, 1H, $J = 7.2$ Hz), 8.28 (s, 1H), 11.01 (s, 1H), 11.99 (s, 1H).

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-4-phenylpyridine (4b). Following general method D and starting from **2b** (350 mg, 2.06 mmol)**, 4b** was obtained as a white solid (597 mg, 1.45 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.54 (s, 18H), 7.26 (d, 1H, $J = 5.3$ Hz), 7.40–7.50 (m, 3H), 7.71 (d, 2H, $J = 7.2$ Hz), 8.33 (d, 1H, $J = 5.3$ Hz), 8.72 (br s, 1H), 10.93 (br s, 1H), 11.48 (br s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 28.1, 117.9, 127.2, 128.9, 129.0, 150.4.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-4-(2-chlorophenyl)pyridine (4c). Following general method C and starting from **3a** (200 mg, 0.48 mmol) and 2-chlorophenylboronic acid (90 mg, 0.58 mmol), **4c** was obtained and used directly in the *next step* without further purification.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-4-(2-methoxyphenyl) pyridine (4d). Following general method D and starting from **2c** (66 mg, 0.33 mmol)**, 4d** was obtained as a yellow solid (91 mg, 0.21 mmol, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.41 (s, 18H), 3.81 (s, 3H), 4.41 (s, 2H), 6.67 (s, 1H), 6.93–7.00 (m, 2H), 6.75 (dd, 1H, $J = 5.3$ Hz, 1.4 Hz), 7.29–7.36 (m, 2H), 8.23 (d, 1H, $J = 5.3$ Hz), 10.86 (s, 1H), 11.45 (s, 1H).

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-4-(3-chlorophenyl)pyridine (4e). Following general method C and starting from **3a** (200 mg, 0.48 mmol) and 3-chlorophenylboronic acid (90 mg, 0.58 mmol), **4e** was obtained and used directly in the *next step* without further purification.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-4-(3-methoxyphenyl) pyridine (4f). Following general method D and starting from **2d** (156 mg, 0.78 mmol)**, 4f** was obtained as a white solid (265 mg, 0.60 mmol, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.46 (s, 18H), 3.81 (s, 3H), 6.90 (dd, 1H, $J = 2.5$ Hz, $J = 8.0$ Hz), 7.17–7.22 (m, 3H), 7.31 (t, 1H, $J = 8.0$ Hz), 8.25 (d, 1H, $J = 5.3$ Hz), 8.55 (s, 1H), 10.94 (s, 1H), 11.40 (s, 1H).

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-4-(4-chlorophenyl)pyridine (4g). Following general method C and starting from **3a** (50 mg, 0.12 mmol) and 4-chlorophenylboronic acid (23 mg, 0.14 mmol), **4g** was obtained as a white solid (35 mg, 0.08 mmol, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.54 (s, 9H), 7.21 (d, 1H, $J = 5.3$ Hz), 7.44 (d, 2H, $J = 8.4$ Hz), 7.64 (d, 2H, $J = 8.4$ Hz), 6.33 (d, 1H, $J = 5.3$ Hz), 8.71 (br s, 1H), 10.93 (br s, 1H), 11.47 (br s, 1H).

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-4-(4-methoxyphenyl) pyridine (4h). Following general method D and starting from **2e** (110 mg, 0.55 mmol)**, 4h** was obtained as a white solid (184 mg, 0.41 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.54 (s, 18H), 3.86 (s, 3H), 6.99 (d, 2H, $J = 8.8$ Hz), 7.22 (dd, 1H, $J = 1.5$ Hz, $J = 5.3$ Hz), 7.66 (d, 2H, $J = 8.8$ Hz), 8.29 (d, 1H, $J = 5.3$ Hz), 8.64 (br s,

1H), 10.92 (br s, 1H), 11.44 (br s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 28.1, 55.4, 114.3, 117.3, 128.3, 149.8, 160.5.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-4-(4-trifluoromethylphenyl) pyridine (4i). Following general method D and starting from **2f** (175 mg, 0.73 mmol)**, 4i** was obtained as a white solid (232 mg, 0.48 mmol, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.47 (s, 18H), 7.18 (s, 1H), 7.66 (d, 1H, J = 8.3 Hz), 7.74 (d, 1H, J = 8.3 Hz), 8.31 (d, 1H, J = 5.1 Hz), 8.67 (s, 1H), 10.93 (s, 1H), 11.40 (s, 1H).

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-5-phenylpyridine (4j). Following general method D and starting from **2g** (73 mg, 0.43 mmol)**, 4j** was obtained as a white solid (111 mg, 0.27 mmol, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.35 (s, 18H), 7.18 (t, 1H, J = 7.7 Hz), 7.26 (t, 2H, J = 7.7 Hz), 7.36 (d, 2H, J = 7.7 Hz), 7.72 (d, 1H, J = 8.4 Hz), 8.27 (br s, 1H), 8.33 (s, 1H), 10.76 (br s, 1H), 11.35 (br s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 28.1, 28.2, 115.9, 126.8, 127.7, 129.0, 132.9, 136.6, 137.6, 146.2, 149.8, 153.1.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-5-(2-chlorophenyl)pyridine (4k). Following general method D and starting from **2h** (90 mg, 0.44 mmol)**, 4k** was obtained as a colorless oil (147 mg, 0.33 mmol, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.54 (s, 9H), 1.55 (s, 9H), 7.34 (dt, 1H, J = 1.8 Hz, J = 7.7 Hz), 7.38 (t, 1H, J = 7.7 Hz), 7.43 (dt, 1H, J = 1.8 Hz, J = 7.7 Hz), 7.53 (s, 1H), 7.89 (dd, 1H, J = 2.5 Hz, J = 8.7 Hz), 8.48 (s, 1H), 8.50 (d, 1H, J = 2.5 Hz), 10.97 (s, 1H), 11.55 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 28.0, 28.2, 60.4, 80.1, 84.1, 115.9, 125.0, 126.9, 127.7, 130.3, 131.5, 135.0, 136.5, 139.4, 146.2, 150.3, 153.0.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-5-(2-methoxyphenyl) pyridine (4l). Following general method D and starting from **2i** (132 mg, 0.66 mmol)**, 4l** was obtained as a colorless oil (194 mg, 0.44 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.47 (s, 18H), 3.74 (s, 3H), 6.92 (d, 1H, J = 8.5 Hz), 6.97 (td, 1H, J = 0.9 Hz, J = 7.5 Hz), 7.23 (dd, 1H, J = 1.8 Hz, J = 7.5 Hz), 7.27 (td, 1H, J = 1.8 Hz, J = 7.5 Hz), 7.83 (dd, 1H, J = 1.8 Hz, J = 8.5 Hz), 8.33 (s, 1H), 8.39 (d, 1H, J = 1.9 Hz), 10.86 (s, 1H), 11.49 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 28.1, 28.2, 55.5, 111.4, 115.2, 121.0, 126.9, 129.2, 130.4, 139.1, 148.3, 149.1, 153.1, 156.7, 163.3.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-5-(3-chlorophenyl)pyridine (4m). Following general method D and starting from **2j** (90 mg, 0.44 mmol)**, 4m** was obtained as a white solid (143 mg, 0.32 mmol, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.54 (s, 9H), 1.55 (s, 9H), 7.30–7.34 (m, 3H), 7.48 (dt, 1H, J = 2.3 Hz, J = 8.5 Hz), 7.83 (dd, 1H, J = 2.3 Hz, J = 8.5 Hz), 8.38 (d, 1H, J = 2.3 Hz), 8.45 (br s, 1H), 10.98 (s, 1H), 11.56 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 28.0, 28.2, 80.0, 84.0, 115.2, 127.1, 129.1, 130.2, 131.2, 132.9, 136.8, 139.1, 148.2, 149.8, 153.1, 163.2.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-5-(3-methoxyphenyl) pyridine (4n). Following general method D and starting from **2k** (79 mg, 0.40 mmol)**, 4n** was obtained as a white solid (114 mg, 0.26 mmol, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.54 (s, 18H), 3.87 (s, 3H), 6.92 (dd, 1H, J = 2.0 Hz, J = 8.0 Hz), 7.08 (t, 1H, J = 2.0 Hz), 7.14 (d, 1H, J = 8.0 Hz), 7.37 (t, 1H, J = 8.0 Hz), 7.91 (dd, 1H, J = 2.4 Hz, J = 8.7 Hz), 8.45 (br, s), 8.52 (d, 1H, J = 2.4 Hz), 10.96 (s, 1H), 11.55 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 28.1, 28.2, 55.3, 80.0, 84.0, 112.7, 113.0, 115.8, 119.3, 130.1, 132.7, 136.6, 139.0, 146.2, 149.9, 153.1, 160.1, 163.2.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-5-(4-chlorophenyl)pyridine (4o). Following general method D and starting from **2l** (100 mg, 0.49 mmol)**, 4o** was obtained as a white solid (160 mg, 0.36 mmol, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.47 (s, 18H), 7.19 (s, 1H), 7.35 (d, 2H, J = 8.4 Hz), 7.41 (d, 2H, J = 8.4 Hz), 7.81 (dd, 1H, J = 2.8 Hz, J = 8.4 Hz), 8.40 (br s, 1H), 8.42 (d, 1H, J = 2.8 Hz), 10.91 (br s, 1H), 11.47 (br s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 28.1, 31.3, 128.0, 129.2, 133.9, 136.0, 136.4.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-5-(4-methoxyphenyl) pyridine (4p). Following general method D and starting from **2m** (80 mg, 0.40 mmol)**, 4p** was obtained as a white solid (125 mg, 0.28 mmol, 75%) and used directly for the Boc deprotection step without further purification.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-6-phenylpyridine (4q). Following general method D and starting from **2n** (496 mg, 2.91 mmol)**, 4q** was obtained as a white solid (944 mg, 2.29 mmol, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.55 (s, 9H), 1.56 (s, 9H), 7.39–7.48 (m, 4H), 7.77 (t, 1H, J = 7.7 Hz), 8.01 (d, 2H, J = 6.0 Hz), 8.26 (br s, 1H), 10.87 (s, 1H), 11.58 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 28.0, 28.2, 116.3, 126.8, 128.7, 129.1, 138.5, 129.0.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-6-(2-chlorophenyl)pyridine (4r). Following general method D and starting from **2o** (174 mg, 0.85 mmol)**, 4r** was obtained as a yellow solid (332 mg, 0.74 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.53 (s, 18H), 7.30–7.36 (m, 3H), 7.45 (dd, 1H, J = 2.0 Hz, J = 7.2 Hz), 7.57 (dd, 1H, J = 2.0 Hz, J = 7.2 Hz), 7.77 (t, 1H, J = 7.9 Hz), 8.35 (br s, 1H), 10.82 (s, 1H), 11.56 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 28.1, 126.9, 129.5, 130.1, 131.6, 138.0.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-6-(2-methoxyphenyl) pyridine (4s). Following general method D and starting from **2p** (184 mg, 0.92 mmol)**, 4s** was obtained as a white solid (310 mg, 0.70 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.53 (s, 18H), 3.85 (s, 3H), 6.99 (d, 1H, J = 8.2 Hz), 7.06 (t, 1H, J = 7.4 Hz), 7.35 (td, 1H, J = 1.8 Hz, J = 7.4 Hz), 7.61 (d, 1H, J = 7.4 Hz), 7.72 (t, 1H, J = 8.2 Hz), 7.83 (br s, 1H), 8.28 (br s, 1H), 10.80 (s, 1H), 11.57 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 28.2, 55.6, 111.5, 121.0, 130.0, 131.2, 137.8, 157.2.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-6-(3-chlorophenyl)pyridine (4t). Following general method C and starting from **3b** (50 mg, 0.13 mmol) and 3-chlorophenylboronic acid (25 mg, 0.16 mmol), **4t** was obtained as a white solid (48 mg, 0.11 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.55 (s, 18H), 7.36–7.39 (m, 2H), 7.45 (d, 1H, J = 7.8 Hz), 7.78 (t, 1H, J = 7.8 Hz), 7.88 (br s, 1H), 8.01 (s, 1H), 8.38 (br s, 1H), 10.88 (s, 1H), 11.58 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 28.1, 116.3, 124.9, 127.0, 129.0, 129.9, 134.7, 139.1, 155.0.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-6-(3-methoxyphenyl) pyridine (4u). Following general method C and starting from **3b** (150 mg, 0.40 mmol) and 3-methoxyphenylboronic acid (74 mg, 0.49 mmol), **4u** was obtained as a white solid (75 mg, 0.17 mmol, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.55 (s, 18H), 3.89 (s, 3H), 6.95 (dd, 1H, J = 2.0 Hz, J = 7.9 Hz), 7.36 (t, 1H, J = 7.9 Hz), 7.46 (d, 1H, J = 7.2 Hz), 7.57 (d, 1H, J = 7.2 Hz), 7.76 (t, 1H, J = 7.9 Hz), 8.36 (br s, 1H), 10.87 (s, 1H), 11.59 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 28.1, 28.2, 55.4, 112.5, 114.6, 116.4, 119.3, 129.6, 138.9, 160.0.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-6-(4-chlorophenyl)pyridine (4v). Following general method C and starting from **3b** (100 mg, 0.27 mmol) and 4-chlorophenylboronic acid (42 mg, 0.27 mmol), **4v** was obtained and used directly for the Boc deprotection step without further purification.

1-[2,3-Di(tert-butoxycarbonyl)guanidino]-6-(4-methoxyphenyl) pyridine (4w). Following general method C and starting from **3b** (200 mg, 0.54 mmol) and 4-methoxyphenylboronic acid (98 mg, 0.65 mmol), **4w** was obtained as a white solid (224 mg, 0.51 mmol, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.54 (s, 18H), 3.86 (s, 3H), 6.97 (d, 2H, J = 8.9 Hz), 7.41 (d, 1H, J = 7.8 Hz), 7.23 (t, 1H, J = 7.8 Hz), 7.96 (br s, 2H), 8.29 (br s, 1H), 10.84 (s, 1H), 11.58 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 28.2, 55.4, 114.1, 115.4, 128.1, 138.9, 160.5.

1-(3-Phenylpyridin-2-yl)guanidine Hydrochloride (5a). Following general method E and starting from **4a** (75 mg, 0.18 mmol), **5a** was obtained as a white solid (35 mg, 0.14 mmol, 77%). Purity ≥98%; mp > 410 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 7.34 (dd, 1H, J = 5.0 Hz, J = 7.5 Hz), 7.48 (d, 2H, J = 8.2 Hz),

7.52–7.58 (m, 3H), 7.78 (dd, 1H, $J = 1.5$ Hz, $J = 7.5$ Hz), 8.38 (dd, 1H, $J = 1.5$ Hz, $J = 5.0$ Hz); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 120.7, 127.5, 129.2, 129.7, 135.4, 141.1, 146.4, 148.6, 156.1; HRMS ($M+H^+$) 213.1118 (calcd for $C_{12}H_{12}N_4H^+$ 213.1135).

1-(4-Phenylpyridin-2-yl)guanidine Hydrochloride (5b). Following general method E and starting from **4b** (100 mg, 0.24 mmol), **5b** was obtained as a white solid (46 mg, 0.19 mmol, 76%). Purity ≥98%; mp = 206–208 °C; ^{1}H NMR (400 MHz, MeOD) δ ppm 7.31 (d, 1H, $J = 1.5$ Hz), 7.47 (dd, 1H, $J = 1.5$ Hz, $J = 5.5$ Hz), 7.50–7.56 (m, 3H), 7.74 (dd, 2H, $J = 1.5$ Hz, $J = 7.7$ Hz), 8.39 (d, 1H, $J = 5.5$ Hz); ^{13}C NMR (101 MHz, MeOD) δ ppm 110.2, 117.5, 126.6, 129.0, 129.5, 136.9, 147.0, 151.7, 152.6, 155.8.

1-[4-(2-Chlorophenyl)pyridin-2-yl]guanidine Hydochloride (5c). Following general method E and starting from **4c** (144 mg, 0.32 mmol), **5c** was obtained as a white solid (26 mg, 0.09 mmol, 29% over 2 steps). Purity ≥98%; mp = 189–191 °C; ^{1}H NMR (400 MHz, MeOD) δ ppm 7.16 (s, 1H), 7.30 (dd, 1H, $J = 1.3$ Hz, $J = 5.3$ Hz), 7.44–7.50 (m, 3H), 7.57–7.61 (m, 1H), 8.44 (d, 1H, $J = 5.3$ Hz); ^{13}C NMR (101 MHz, MeOD) δ ppm 113.3, 120.2, 127.3, 130.0, 130.2, 130.6, 131.5, 136.9, 146.4, 150.5, 152.0, 155.8; HRMS ($M+H^+$) 247.0737 (calcd for $C_{12}H_{11}ClN_4H^+$ 247.0745).

1-[4-(2-Methoxyphenyl)pyridin-2-yl]guanidine Trifluoroacetate (5d). Following general method F and starting from **4d** (94 mg, 0.21 mmol), **5d** was obtained as a white solid (47 mg, 0.13 mmol, 61%). Purity ≥98%; mp = 137–138 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 3.82 (s, 3H), 7.10 (t, 1H, $J = 7.4$ Hz), 7.19 (d, 1H, $J = 8.2$ Hz), 7.25 (s, 1H), 7.32 (dd, 1H, $J = 1.4$ Hz, $J = 5.4$ Hz), 7.41 (dd, 1H, $J = 1.7$ Hz, $J = 7.6$ Hz), 7.47 (td, 1H, $J = 1.8$ Hz, $J = 7.8$ Hz), 8.34 (d, 1H, $J = 5.4$ Hz), 8.44 (s, 4H), 11.22 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 56.1, 112.6, 113.7, 120.3, 121.3, 126.4, 130.5, 131.4, 146.8, 149.3, 152.5, 155.8, 156.8; HRMS ($M+H^+$) 243.1238 (calcd for $C_{13}H_{14}N_4OH^+$ 243.1240).

1-[4-(3-Chlorophenyl)pyridin-2-yl]guanidine Hydrochloride (5e). Following general method E and starting from **4e** (144 mg, 0.32 mmol), **5e** was obtained as a white solid (26 mg, 0.09 mmol, 29% over 2 steps). Purity ≥98%; mp = 102–105 °C; ^{1}H NMR (400 MHz, MeOD) δ ppm 7.33 (s, 1H), 7.48 (d, 1H, $J = 5.4$ Hz), 7.52–7.55 (m, 2H), 7.69 (d, 1H, $J = 6.5$ Hz), 7.76 (s, 1H), 8.42 (d, 1H, $J = 5.4$ Hz); ^{13}C NMR (101 MHz, MeOD) δ ppm 110.5, 117.6, 125.2, 126.7, 129.4, 130.6, 134.9, 138.9, 147.2, 150.3, 152.6, 155.8; HRMS ($M+H^+$) 247.0742 (calcd for $C_{12}H_{11}ClN_4H^+$ 247.0745).

1-[4-(3-Methoxyphenyl)pyridin-2-yl]guanidine Hydrochloride (5f). Following general method E and starting from **4f** (238 mg, 0.54 mmol), **5f** was obtained as a white solid (89 mg, 0.32 mmol, 59%). Purity ≥98%; mp = 107–110 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.73 (s, 3H), 6.82 (dd, 1H, $J = 2.4$ Hz, $J = 8.3$ Hz), 6.94 (t, 1H, $J = 2.4$ Hz), 7.00–7.03 (m, 2H), 7.13 (s, 1H), 7.22 (t, 1H, $J = 7.9$ Hz), 8.05 (d, 1H, $J = 5.3$ Hz), 8.30 (s, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 55.4, 111.5, 112.6, 114.7, 117.0, 119.3, 130.2, 138.6, 146.6, 151.1, 153.8, 156.5, 160.0; HRMS ($M+H^+$) 243.1243 (calcd for $C_{13}H_{14}N_4OH^+$ 243.1240).

1-[4-(4-Chlorophenyl)pyridin-2-yl]guanidine Hydrochloride (5g). Following general method E and starting from **4g** (30 mg, 0.07 mmol), **5g** was obtained as a white solid (13 mg, 0.05 mmol, 68%). Purity ≥98%; mp = 255–256 °C; ^{1}H NMR (400 MHz, MeOD) δ ppm 7.29 (d, 1H, $J = 1.4$ Hz), 7.50 (dd, 1H, $J = 1.4$ Hz, $J = 5.4$ Hz), 7.58 (d, 2H, $J = 8.5$ Hz), 7.76 (d, 2H, $J = 8.5$ Hz), 8.42 (d, 1H, $J = 5.4$ Hz); ^{13}C NMR (101 MHz, MeOD) δ ppm 110.1, 117.4, 128.3, 129.1, 147.2; HRMS ($M+H^+$) 247.0742 (calcd for $C_{12}H_{11}ClN_4H^+$ 247.0745).

1-[4-(4-Methoxyphenyl)pyridin-2-yl]guanidine Hydrochloride (5h). Following general method E and starting from **4h** (140 mg, 0.32 mmol), **5h** was obtained as a white solid (55 mg, 0.20 mmol, 63%). Purity ≥98%; mp = 283–284 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 3.82 (s, 3H), 7.10 (d, 2H, $J = 8.5$ Hz), 7.26 (s, 1H), 7.47 (d, 1H, $J = 5.4$ Hz), 7.71 (d, 2H, $J = 8.5$ Hz), 8.31 (d, 1H, $J = 5.4$ Hz), 8.39 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 55.9, 109.6, 115.3, 117.1, 128.6, 128.9, 147.7, 150.2, 153.1, 155.8, 161.2; HRMS ($M+H^+$) 243.1237 (calcd for $C_{13}H_{14}N_4OH^+$ 243.1240).

1-[4-(4-Trifluoromethylphenyl)pyridin-2-yl]guanidine Hydrochloride (5i). Following general method E and starting from **4i** (220 mg, 0.46 mmol), **5i** was obtained as a white solid (60 mg, 0.19 mmol, 41%). Purity ≥98%; mp = 255–256 °C; ^{1}H NMR (400 MHz, MeOD) δ ppm 7.24 (s, 1H), 7.41 (d, 1H, $J = 5.4$ Hz), 7.72 (d, 1H, $J = 8.3$ Hz), 7.82 (d, 1H, $J = 8.3$ Hz), 8.33 (d, 1H, $J = 5.4$ Hz); ^{19}F NMR (376 MHz, DMSO-d6) δ ppm −61.2; ^{13}C NMR (101 MHz, DMSO) δ ppm 111.0, 117.8, 124.5 (q, J = 272.2 Hz), 126.6 (q, J = 3.7 Hz), 126.3, 130.3 (q, J = 32.3 Hz), 141.1, 148.1, 149.2, 153.2, 155.9; HRMS ($M+H^+$) 281.1017 (calcd for $C_{13}H_{11}F_3N_4H^+$ 281.1009).

1-(5-Phenylpyridin-2-yl)guanidine Trifluoroacetate (5j). Following general method F and starting from **4j** (180 mg, 0.44 mmol), **5j** was obtained as a white solid (130 mg, 0.40 mmol, 91%). Purity ≥98%; mp = 189–191 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 7.17 (d, 1H, $J = 8.4$ Hz), 7.41 (t, 1H, $J = 7.4$ Hz), 7.50 (t, 2H, $J = 7.4$ Hz), 7.70 (d, 2H, $J = 7.4$ Hz), 8.20 (d, 1H, $J = 8.54$ Hz), 8.55 (br s, 4H), 8.62 (s, 1H), 11.5 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 113.8, 127.0, 128.5, 129.6, 131.7, 136.6, 138.1, 144.7, 151.7, 155.7, 160.2 (q, J = 32.3 Hz); HRMS ($M+H^+$) 213.1126 (calcd for $C_{12}H_{12}N_4H^+$ 213.1135).

1-[5-(2-Chlorophenyl)pyridin-2-yl]guanidine Trifluoroacetate (5k). Following general method F and starting from **4k** (105 mg, 0.24 mmol), **5k** was obtained as a white solid (64 mg, 0.18 mmol, 75%). Purity ≥98%; mp = 170–171 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 7.18 (d, 1H, $J = 8.5$ Hz), 7.44–7.49 (m, 3H), 7.59–7.63 (m, 1H), 8.00 (dd, 1H, $J = 2.4$ Hz, $J = 8.5$ Hz), 8.39 (d, 1H, $J = 2.4$ Hz), 8.56 (br s, 4H), 11.55 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 113.1, 128.3, 130.3, 130.4, 132.0, 132.1, 136.1, 140.8, 146.8, 151.9, 155.8, 160.1 (q, J = 32 Hz); HRMS ($M+H^+$) 247.0744 (calcd for $C_{12}H_{11}ClN_4H^+$ 247.0745).

1-[5-(2-Methoxyphenyl)pyridin-2-yl]guanidine Trifluoroacetate (5l). Following general method F and starting from **4l** (92 mg, 0.21 mmol), **5l** was obtained as a white solid (70 mg, 0.20 mmol, 95%). Purity ≥98%; mp = 128–130 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 3.79 (s, 3H), 7.06 (t, 1H, $J = 7.4$ Hz), 7.4 (t, 2H, $J = 8.5$ Hz), 7.35 (d, 1H, $J = 7.4$ Hz), 7.40 (t, 1H, $J = 7.4$ Hz), 8.01 (dd, 1H, $J = 2.3$ Hz, $J = 8.5$ Hz), 8.48 (br s, 4H), 11.35 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 56.1, 112.3, 113.1, 125.9, 129.8, 130.2, 130.6, 140.6, 146.7, 151.1, 155.7, 156.7, 159.7; HRMS ($M+H^+$) 243.1232 (calcd for $C_{13}H_{14}N_4OH^+$ 243.1240).

1-[5-(3-Chlorophenyl)pyridin-2-yl]guanidine Trifluoroacetate (5m). Following general method F and starting from **4m** (87 mg, 0.19 mmol), **5m** was obtained as a white solid (59 mg, 0.16 mmol, 84%). Purity ≥98%; mp = 187–188 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 7.17 (d, 1H, $J = 8.4$ Hz), 7.47 (d, 1H, $J = 7.5$ Hz), 7.53 (t, 1H, $J = 7.5$ Hz), 7.68 (d, 1H, $J = 7.5$ Hz), 7.79 (s, 1H), 8.24 (d, 1H, $J = 8.5$ Hz), 8.57 (br s, 4H), 8.66 (s, 1H), 11.54 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 113.8, 125.7, 126.7, 128.2, 130.2, 131.4, 134.4, 138.3, 138.8, 145.1, 152.2, 155.7, 160.2 (q, J = 32 Hz); HRMS ($M+H^+$) 247.0739 (calcd for $C_{12}H_{11}ClN_4H^+$ 247.0745).

1-[5-(3-Methoxyphenyl)pyridin-2-yl]guanidine Trifluoroacetate (5n). Following general method F and starting from **4n** (76 mg, 0.16 mmol), **5n** was obtained as a white solid (50 mg, 0.14 mmol, 86%). Purity ≥98%; mp = 171–172 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 3.83 (s, 3H), 6.98 (dd, 1H, $J = 2.3$ Hz, $J = 7.9$ Hz), 7.16 (d, 1H, $J = 8.5$ Hz), 7.22–7.26 (m, 2H), 7.41 (t, 1H, $J = 7.9$ Hz), 8.20 (dd, 1H, $J = 2.3$ Hz, $J = 8.5$ Hz), 8.56 (br s, 4H), 8.63 (d, 1H, $J = 2.3$ Hz), 11.51 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 55.7, 112.5, 113.7, 114.0, 119.2, 130.7, 131.6, 138.1, 138.2, 144.8, 151.8, 155.7, 160.4; HRMS ($M+H^+$) 243.1243 (calcd for $C_{13}H_{14}N_4OH^+$ 243.1240).

1-[5-(4-Chlorophenyl)pyridin-2-yl]guanidine

Trifluoroacetate (5o). Following general method F and starting from **4o** (134 mg, 0.30 mmol), **5o** was obtained as a white solid (72 mg, 0.20 mmol, 66%). Purity ≥98%; mp = 216–218 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 7.16 (d, 1H, *J* = 8.4 Hz), 7.56 (d, 2H, *J* = 8.4 Hz), 7.74 (d, 2H, *J* = 8.4 Hz), 8.21 (d, 1H, *J* = 8.4 Hz), 8.44 (br s, 3H), 8.64 (s, 1H), 11.29 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 113.4, 128.3, 129.1, 130.0, 132.9, 135.0, 137.6, 144.3, 151.4, 155.1; HRMS (M+H$^+$) 247.0745 (calcd for $C_{12}H_{11}ClN_4H^+$ 247.0745).

1-[5-(4-Methoxyphenyl)pyridin-2-yl]guanidine Trifluoroacetate (5p). Following general method F and starting from **4p** (125 mg, 0.28 mmol), **5p** was obtained as a white solid (66 mg, 0.19 mmol, 65%). Purity ≥98%; mp = 246–249 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 3.80 (s, 3H), 7.06 (d, 2H, *J* = 8.8 Hz), 7.13 (d, 1H, *J* = 8.4 Hz), 7.64 (d, 2H, *J* = 8.8 Hz), 8.15 (d, 1H, *J* = 8.4 Hz), 8.41 (br s, 3H), 8.58 (s, 1H), 11.20 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 55.2, 113.4, 114.6, 127.7, 128.4, 131.1, 137.1, 143.7, 150.5, 155.1, 159.3; HRMS (M+H$^+$) 243.1242 (calcd for $C_{13}H_{14}N_4OH^+$ 243.1240).

1-(6-Phenylpyridin-2-yl)guanidine Hydrochloride (5q). Following general method E and starting from **4q** (150 mg, 0.36 mmol), **5q** was obtained as a white solid (73 mg, 0.29 mmol, 80%). Purity ≥98%; mp = 238–239 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 7.06 (d, 1H, *J* = 7.9 Hz), 7.48–7.55 (m, 3H), 7.72 (d, 1H, *J* = 7.9 Hz), 7.93–7.99 (m, 3H), 8.42 (br s, 4H), 11.65 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d6) δ ppm 112.5, 116.6, 127.1, 129.5, 130.1, 138.0, 141.0, 152.4, 154.7, 155.9; HRMS (M+H$^+$) 213.1125 (calcd for $C_{12}H_{12}N_4H^+$ 213.1135).

1-[6-(2-Chlorophenyl)pyridin-2-yl]guanidine Hydrochloride (5r). Following general method E and starting from **4r** (100 mg, 0.22 mmol), **5r** was obtained as a white solid (46 mg, 0.16 mmol, 73%). Purity ≥98%; mp = 206–207 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 7.12 (d, 1H, *J* = 8.2 Hz), 7.43 (d, 1H, *J* = 7.5 Hz), 7.48–7.51 (m, 2H), 7.61–7.63 (m, 2H), 8.01 (t, 1H, *J* = 8.2 Hz), 8.37 (br s, 4H), 11.74 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 112.8, 120.4, 128.2, 130.8, 131.1, 131.5, 131.9, 137.8, 140.6, 151.9, 153.9, 155.8; HRMS (M+H$^+$) 247.0739 (calcd for $C_{12}H_{11}ClN_4H^+$ 247.0745).

1-[6-(2-Methoxyphenyl)pyridin-2-yl]guanidine Trifluoroacetate (5s). Following general method F and starting from **4s** (172 mg, 0.39 mmol), **5s** was obtained as a white solid (115 mg, 0.32 mmol, 83%). Purity ≥98%; mp = 183–184 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 3.83 (s, 3H), 7.01 (d, 1H, *J* = 8.0 Hz), 7.09 (t, 1H, *J* = 7.7 Hz), 7.20 (d, 1H, *J* = 8.0 Hz), 7.46 (td, 1H, *J* = 1.8 Hz, *J* = 7.7 Hz), 7.53 (d, 1H, *J* = 7.7 Hz), 7.59 (dd, 1H, *J* = 1.8 Hz, *J* = 7.7 Hz), 7.93 (t, 1H, *J* = 8.0 Hz), 8.59 (br s, 4H), 11.34 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 56.1, 111.8, 112.8, 120.0, 121.4, 127.4, 130.7, 131.2, 140.2, 151.8, 153.6, 155.9, 157.2, 160.0 (q, *J* = 32.3 Hz); HRMS (M+H$^+$) 243.1239 (calcd for $C_{13}H_{14}N_4OH^+$ 243.1240).

1-[6-(3-Chlorophenyl)pyridin-2-yl]guanidine Hydrochloride (5t). Following general method E and starting from **4t** (28 mg, 0.063 mmol), **5t** was obtained as a white solid (7 mg, 0.026 mmol, 42%). Purity ≥98%; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 7.09 (d, 1H, *J* = 8.2 Hz), 7.55–7.56 (m, 2H), 7.77 (d, 1H, *J* = 7.7 Hz), 7.90–7.93 (m, 1H), 7.97 (s, 1H), 7.99 (t, 1H, *J* = 7.7 Hz), 8.53 (br s, 4H), 11.41 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 113.4, 117.1, 125.8, 126.9, 129.9, 131.4, 134.4, 140.2, 141.2, 152.5, 153.2, 155.7; HRMS (M+H$^+$) 247.0738 (calcd for $C_{12}H_{11}ClN_4H^+$ 247.0745).

1-[6-(3-Methoxyphenyl)pyridin-2-yl]guanidine Trifluoroacetate (5u). Following general method F and starting from **4u** (43 mg, 0.10 mmol), **5u** was obtained as a white solid (34 mg, 0.09 mmol, 99%). Purity ≥98%; mp = 138–139 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 3.84 (s, 3H), 7.05–7.08 (m, 2H), 7.43–7.52 (m, 3H), 7.73 (d, 1H, *J* = 7.8 Hz), 7.97 (t, 1H, *J* = 7.8 Hz), 8.51 (br s, 4H), 11.25 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 55.7, 112.5, 112.8, 115.7, 116.9, 119.4, 130.7, 139.6, 141.0, 152.4, 154.5, 155.8, 159.3 (q, *J* = 32.7 Hz), 160.3; HRMS (M+H$^+$) 243.1234 (calcd for $C_{13}H_{14}N_4OH^+$ 243.1240).

1-[6-(4-Chlorophenyl)pyridin-2-yl]guanidine Trifluoroacetate (5v). Following general method F and starting from the crude **4v**, **5v** was obtained as a white solid (32 mg, 0.09 mmol, 33% over 2 steps). Purity ≥98%; mp = 253–254 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 7.07 (d, 1H, *J* = 7.9 Hz), 7.58 (d, 2H, *J* = 8.5 Hz), 7.73 (d, 1H, *J* = 7.9 Hz), 7.95–8.00 (m, 3H), 8.53 (br s, 4H), 11.41 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 113.0, 116.7, 129.0, 129.5, 134.9, 136.9, 141.1, 152.5, 153.5, 155.8; HRMS (M+H$^+$) 247.0729 (calcd for $C_{12}H_{11}ClN_4H^+$ 247.0745).

1-[6-(4-Methoxyphenyl)pyridin-2-yl]guanidine Trifluoroacetate (5w). Following general method F and starting from **4w** (100 mg, 0.23 mmol), **5w** was obtained as a white solid (64 mg, 0.18 mmol, 79%). Purity ≥98%; mp = 198–199 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 3.82 (s, 3H), 6.98 (d, 1H, *J* = 7.9 Hz), 7.07 (d, 2H, *J* = 8.8 Hz), 7.65 (d, 1H, *J* = 7.9 Hz), 7.89 (d, 2H, *J* = 8.8 Hz), 7.92 (t, 1H, *J* = 7.9 Hz), 8.59 (br s, 4H), 11.39 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 55.8, 111.7, 114.9, 115.8, 128.5, 130.4, 140.9, 152.4, 154.5, 155.9, 161.0; HRMS (M+H$^+$) 243.1232 (calcd for $C_{13}H_{14}N_4OH^+$ 243.1240).

1-Benzoyl-3-(4-phenylpyridin-2-yl)thiourea (6). 2b (100 mg, 0.59 mmol, 1 equiv.) and benzoyl isothiocyanate (87 μL, 0.65 mmol, 1.1 equiv.) were dissolved in THF (4.8 mL). The resulting mixture was heated at reflux for 3 h. After cooling, the reaction mixture was concentrated under vacuum and purified by silica gel column chromatography (n-heptane:EtOAc 8:2), yielding to **6** as a yellow solid (144 mg, 0.43 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.40 (dd, 1H, *J* = 1.6 Hz, *J* = 5.1 Hz), 7.43–7.57 (m, 5H), 7.66 (tt, 1H, *J* = 2.0 Hz, *J* = 7.4 Hz), 7.72 (d, 2H, *J* = 6.9 Hz), 7.92 (d, 2H, *J* = 7.3 Hz), 8.48 (d, 1H, *J* = 5.1 Hz), 9.08 (s, 1H), 9.16 (s, 1H), 13.19 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 114.0, 119.5, 127.2, 127.6, 129.1, 129.2, 129.3, 131.7, 133.8, 138.0, 149.0, 150.3, 151.9, 166.4, 177.1.

(4-Phenylpyridin-2-yl)thiourea (7). 6 (80 mg, 0.24 mmol, 1 equiv.) was dissolved in ethanol (1.8 mL). NaOH (29 mg, 0.72 mmol, 3 equiv.) was dissolved in water (1.8 mL) and added in the previous solution. The resulting solution was heated at reflux for 1 h. After cooling, the reaction mixture was concentrated under vacuum. The crude residue was diluted in water. The organic phase was extracted 3 times with EtOAc. The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel column chromatography (n-heptane:EtOAc 4:1), yielding to **7** as a white solid (33 mg, 0.14 mmol, 60%). Purity ≥98%; mp = 207–209 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 7.37 (dd, 1H, *J* = 1.5 Hz, *J* = 5.4 Hz), 7.47–7.56 (m, 4H), 7.69 (d, 2H, *J* = 7.0 Hz), 8.30 (d, 1H, *J* = 5.4 Hz), 8.93 (s, 1H), 10.54 (s, 1H), 10.59 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 110.3, 116.3, 127.2, 129.8, 130.0, 137.4, 147.2, 150.2, 154.8, 181.2; HRMS (M+H$^+$) 230.0736 (calcd for $C_{12}H_{11}N_3SH^+$ 230.0746).

Ethyl 2-amino-4-chlorobenzoate (8b). The 2-amino-4-chlorobenzoic acid **8a** (1.50 g, 8.74 mmol, 1 equiv.) was dissolved in ethanol (53.0 mL) and H_2SO_4 was added (8 mL). The reaction media was heated at reflux for 21 h. After it was cooled, the solution was concentrated under vacuum. K_2CO_3 was added in the residue by small portions to reach a pH of 8. The residue was diluted with water and filtered. The aqueous phase was extracted 3 times with AcOEt. The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered and concentrated, yielding to **8b** as an orange oil (1.75 g, 8.74 mmol, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.37 (t, 3H, *J* = 7.2 Hz), 4.32 (q, 2H, *J* = 7.2 Hz), 5.81 (s, 2H), 6.59 (dd, 1H, *J* = 2.0 Hz, *J* = 8.7 Hz), 6.65 (d, 1H, *J* = 2.0 Hz), 7.79 (d, 1H, *J* = 8.7 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 14.3, 60.5, 109.6, 115.9, 116.6, 132.6, 139.9, 151.2, 167.6.

Ethyl 2-amino-4-phenylbenzoate (9). Following general method C and starting from **8b** (625 mg, 3.13 mmol) and phenylboronic acid (458 mg, 3.75 mmol), **9** was obtained as a brown solid

(747 mg, 3.10 mmol, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.41 (t, 3H, $J = 7.2$ Hz), 4.36 (q, 2H, $J = 7.2$ Hz), 5.81 (s, 2H), 6.87 (d, 1H, $J = 1.6$ Hz), 6.89 (dd, 1H, $J = 1.6$ Hz, $J = 8.2$ Hz), 7.37 (t, 1H, $J = 7.0$ Hz), 7.44 (t, 2H, $J = 7.0$ Hz), 7.58 (d, 2H, $J = 7.0$ Hz), 7.95 (d, 1H, $J = 8.2$ Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 14.4, 60.3, 110.1, 114.9, 115.6, 127.1, 128.0, 128.7, 131.8, 140.3, 146.7, 150.7, 168.1.

(2-Amino-4-phenylphenyl)methanol (10). In an ovendried one-neck round-bottom flasks, **9** (320 mg, 1.41 mmol, 1 equiv.) was dissolved in anhydrous THF (8.7 mL). $LiBH_4$ (123 mg, 4 equiv.) was added by small portion at 0 °C. The resulting mixture was heated at reflux for 7 h. The reaction media was cooled at 0 °C and methanol was added dropwise. The solution was concentrated under vacuum and diluted with water. The aqueous phase was extracted 3 times with AcOEt. The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel column chromatography (n-heptane:EtOAc 1:1), to yield **10** as an orange solid (224 mg, 1.13 mmol, 80%). ^{1}H NMR (400 MHz, MeOD) δ ppm 4.65 (s, 2H), 6.96 (dd, 1H, $J = 1.8$ Hz, $J = 7.8$ Hz), 7.05 (d, 1H, $J = 1.8$ Hz), 7.19 (d, 1H, $J = 7.7$ Hz), 7.32 (t, 1H, $J = 7.4$ Hz), 7.41 (t, 2H, $J = 7.4$ Hz), 7.59 (d, 2H, $J = 7.4$ Hz); ^{13}C NMR (101 MHz, MeOD) δ ppm 61.9, 114.3, 116.4, 124.7, 126.4, 126.7, 128.3, 128.9, 141.2, 141.6, 146.2.

{2-[2,3-Di(tert-butoxycarbonyl)guanidino]-4-phenylphenyl} methanol (11). Following general method D and starting from **10** (479 mg, 2.40 mmol)**, 11** was obtained as a white solid (870 mg, 1.97 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.45 (s, 9H), 1.56 (s, 9H), 4.55 (s, 2H), 7.35 (t, 1H, $J = 7.3$ Hz), 7.43 (t, 2H, $J = 7.3$ Hz), 7.48–7.59 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 28.1, 61.7, 80.1, 84.2, 124.5, 126.2, 127.1, 127.6, 128.8, 132.1, 134.3, 135.6, 140.0, 141.8, 153.2, 155.7, 162.6.

7-Phenyl-3,4-dihydroquinazolin-2-amine Hydrochloride (12). 11 (100 mg, 0.23 mmol, 1 equiv.) was dissolved in DCM (2.9 mL), and $SOCl_2$ (0.66 mL, 9.06 mmol, 40 equiv.) was added. The resulting mixture was heated at reflux for 6 h. After cooling, the solution was concentrated under vacuum. The residue was diluted in ether (1.0 mL) and HCl 37% was added (1.0 mL). The reaction mixture was stirred at rt for 6 h. The solution was concentrated under vacuum and purified by reverse chromatography (MeOH/H_2O+0.05%HCl), yielding to **12** as a white solid (47 mg, 0.18 mmol, 79%). Purity ≥98%; mp = 200–203 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 4.53 (s, 2H), 7.22 (s, 1H), 7.27 (d, 1H, $J = 8.0$ Hz), 7.36–7.41 (m, 2H), 7.48 (t, 2H, $J = 7.7$ Hz), 7.60 (d, 2H, $J = 7.7$ Hz), 7.78 (s, 2H), 8.68 (s, 1H), 11.08 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 41.0, 113.6, 117.6, 122.8, 127.0, 127.4, 128.3, 129.5, 134.2, 139.7, 140.9, 153.3; HRMS ($M+H^+$) 224.1178 (calcd for $C_{14}H_{13}N_3H^+$ 224.1182).

2-Amino-5-phenylbenzoic acid (14). A microvave vial under argon was charged with 5-bromoanthranilic acid **13** (500 mg, 2.31 mmol, 1 equiv.), phenylboronic acid (310 mg, 2.55 mmol, 1.1 equiv.), $Pd(OAc)_2$ (52 mg, 0.23 mmol, 10 mol%) and Na_2CO_3 (736 mg, 6.94 mmol, 3 equiv.) in water (1.0 mL). The vial was capped properly, flushed with argon and heated to 90 °C for 16 h. After cooling, the reaction mixture was filtered through a pad of Celite®, washed with AcOEt and concentrated under vacuum, yielding to **14** which was used for the next step without further purification. The crude material was purified by silica gel chromatography (0–15% EtOAc/Hexanes gradient column) to give an oil. Which was triturated with ether and petroleum ether yielding **14** as a white solid which was collected by filtration (0.485 g, 98%).Data are consistent with literature values [73]. mp = 204–205 °C, ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 6.85 (d, 1H, J = 7.3 Hz), 7.24 (d, 1H, J = 7.3 Hz), 7.35–7.39 (m, 2H), 7.54–7.60 (m, 3H), 7.95 (bs, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 110.3, 117.5, 125.8, 126.6, 126.9, 129.2, 129.3, 132.6, 140.2, 151.4.

(2-Amino-5-phenylphenyl)methanol (15). In a two-neck round-bottom flask (ovendried and under argon) LAH (220 mg, 5.79 mmol, 2.5 equiv.) was dissolved in anhydrous THF (10 mL). A solution of crude **14** in anhydrous THF (5 mL) was added slowly in the previous solution. The reaction mixture was stirred overnight at rt. Water was slowly added at 0 °C to neutralized the excess of LAH and the aqueous phase was extracted twice with AcOEt. The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered and concentrated, yielding to **15** as a yellow solid (440 mg, 2.21 mmol, 95% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.27 (br s, 2H), 4.77 (s, 2H), 6.80 (d, 1H, $J = 7.6$ Hz), 7.28–7.32 (m, 1H), 7.36–7.49 (m, 5H), 7.55 (d, 2H, $J = 6.8$ Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 62.4, 108.3, 124.6, 163.7, 127.2, 127.9, 129.8, 130.1, 131.4, 144.8, 148.9.

{2-[2,3-Di(tert-butoxycarbonyl)guanidino]-5-phenylphenyl} methanol (16). Following general method A and starting from **15** (440 mg, 2.21 mmol), **16** was obtained as a white solid (680 mg, 1.54 mmol, 70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.47 (s, 9H), 1.57 (s, 9H), 4.29 (s, 1H), 4.60 (s, 2H), 7.35–7.70 (m, 8H), 10.21 (s, 1H), 11.63 (s, 1H).

6-Phenyl-3,4-dihydroquinazolin-2-amine Trifluoroacetate (17a). 16 (662 mg, 1.50 mmol, 1 equiv.) was dissolved in DCM (20.0 mL), and $SOCl_2$ (4.38 mL, 59.97 mmol, 40 equiv.) was added. The resulting mixture was heated at reflux for 3 h. After cooling, the solution was concentrated under vacuum. The residue was diluted in a mixture DCM:TFA (1:1). The reaction mixture was stirred at rt for 2 h. The solution was concentrated under vacuum and purified by reverse chromatography (MeOH/H_2O+0.05%TFA), yielding to **17** as a white solid (140 mg, 0.54 mmol, 36%). Purity ≥98%; mp = 249–250 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ ppm 4.55 (s, 2H), 7.04 (d, 1H, $J = 8.4$ Hz), 7.33 (t, 1H, $J = 6.9$ Hz), 7.44 (t, 2H, $J = 7.5$ Hz), 7.51–7.63 (m, 4H), 7.86 (s, 2H), 8.59 (s, 1H), 11.07 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 40.9, 115.6, 118.6, 124.5, 126.2, 126.6, 127.3, 128.9, 132.7, 135.9, 139.2, 152.6; HRMS ($M+H^+$) 224.1187 (calcd for $C_{14}H_{13}N_3H^+$ 224.1182).

2-Fluoro-5-(2-methoxyphenyl)benzaldehyde (19). Following general method B and starting from 5-bromo-2-fluorobenzaldehyde **18** (200 mg, 0.99 mmol) and 2-methoxyphenylboronic acid (180 mg, 1.18 mmol), **19** was obtained as a colorless oil (197 mg, 0.86 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.82 (s, 3H), 6.99 (d, 1H, $J = 8.3$ Hz), 7.04 (t, 1H, $J = 7.5$ Hz), 7.20 (t, 1H, $J = 8.3$ Hz), 7.30 (d, 1H, $J = 7.5$ Hz), 7.36 (t, 1H, $J = 7.5$ Hz), 7.77–7.81 (m, 1H), 8.01 (d, 1H, $J = 8.3$ Hz) 10.40 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 55.6, 111.3, 116.0 (d, $J = 20$ Hz), 121.0, 128.3, 129.4, 129.6 (d, $J = 2$ Hz), 130.6, 135.4, 137.5 (d, $J = 9$ Hz), 156.3, 162.5, 165.0, 187.3 (d, $J = 6$ Hz).

6-(2-Methoxyphenyl)quinazolin-2-amine (20). 19 (184 mg, 0.80 mmol, 1 equiv.), DIEA (349.8 μL, 2.00 mmol, 2.5 equiv.) and guanidine carbonate (180 mg, 1.00 mmol, 1.25 equiv.) were dissolved in NMP (3.4 mL). The resulting mixture was heated at 150 °C for 16 h. After cooling at room temperature, the solution was poured in water (15 mL) and brine (1.6 mL) was added. The brown solid was filtered, purified by silica gel column chromatography (n-heptane:EtOAc 1:1 to 1:4), yielding to **19** as an orange solid (36 mg, 0.14 mmol, 18%). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 3.79 (s, 3H), 6.84 (s, 2H), 7.05 (t, 1H, $J = 7.4$ Hz), 7.13 (d, 1H, $J = 8.7$ Hz), 7.36 (t, 2H, $J = 7.4$ Hz), 7.43 (d, 2H, $J = 8.7$ Hz), 7.82 (d, 1H, $J = 8.7$ Hz), 7.86 (s, 1H), 9.13 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 56.0, 112.3, 119.8, 119.9, 121.3, 124.3, 128.1, 129.4, 129.5, 130.8, 132.2, 136.2, 151.4, 156.7, 161.4, 162.9.

6-(2-Methoxyphenyl)-3,4-dihydroquinazolin-2-amine Trifluoroacetate (17b). 20 (15 mg, 0.06 mmol, 1 equiv.) was dissolved in a mixture DCM (0.2 mL) and TFA (21.3 μL, 0.29 mmol, 4.96 equiv.). Et_3SiH (22.8 μL, 0.14 mmol, 2.45 equiv.) was added dropwise. The resulting mixture was stirred at room temperature for 15 h. The reaction media was concentrated under vacuum and purified by reverse chromatography (MeOH/H_2O+0.05%TFA), to yield **21** as a white solid (11 mg, 0.03 mmol, 54%). Purity ≥98%;

mp = 124–126 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 3.75 (s, 3H), 4.52 (s, 2H), 7.01 (t, 2H, *J* = 8.3 Hz), 7.10 (d, 1H, *J* = 8.3 Hz), 7.25 (dd, 1H, *J* = 1.6 Hz, *J* = 7.5 Hz), 7.29 (d, 1H, *J* = 1.6 Hz), 7.31–7.38 (m, 2H), 7.77 (s, 2H), 8.53 (s, 1H), 10.92 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 41.4, 56.0, 112.3, 115.3, 118.1, 121.3, 127.5, 129.3, 129.4, 129.8, 130.6, 132.6, 134.5, 153.1, 156.6; HRMS (M+H$^+$) 254.1294 (calcd for $C_{15}H_{15}N_3OH^+$ 254.1288).

2-Amino-6-phenylbenzonitrile (22). Following general method A and starting from 2-amino-6-iodobenzonitrile **21** (300 mg, 1.23 mmol) and phenylboronic acid (180 mg, 1.47 mmol), **22** was obtained as a yellow solid (218 mg, 1.12 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.53 (s, 2H), 6.73 (dd, 1H, *J* = 0.8 Hz, *J* = 7.9 Hz), 6.79 (dd, 1H, *J* = 0.8 Hz, *J* = 7.9 Hz), 7.35 (t, 1H, *J* = 7.9 Hz), 7.40–7.49 (m, 3H), 7.53–7.56 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 113.6, 117.4, 119.0, 128.5, 128.6, 133.4, 138.9, 145.9, 150.6.

2-(Aminomethyl)-3-phenylaniline (23). In a one-neck round-bottom flask under argon, **22** (200 mg, 1.03 mmol, 1 equiv.) was dissolved in THF (14.3 mL) and $BH_3.SMe_2$ (195.3 μL, 2.06 mmol, 2 equiv.) was added dropwise. The resulting solution was heated at reflux for 2 h. After cooling, HCl 2N was added and the solution was heated at 90 °C for 1 h. The solution was then concentrated under vacuum and purified by reverse chromatography (MeOH/H_2O+0.05%TFA). The desired salt was solubilized in water and a saturated solution of $NaHCO_3$ was added to reach a pH of 8. The aqueous phase was extracted twice with AcOEt. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated, yielding to **23** as a white solid (181 mg, 0.91 mmol, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.81 (br s, 4H), 3.71 (s, 2H), 6.57 (d, 1H, *J* = 7.9 Hz), 6.62 (d, 1H, *J* = 7.9 Hz), 7.02 (t, 1H, *J* = 7.9 Hz), 7.17–7.31 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 41.6, 120.1, 123.4, 126.8, 127.5, 128.0, 129.1, 142.0, 142.9, 147.2.

5-Phenyl-3,4-dihydroquinazolin-2-amine Hydrobromide (24). 23 (53 mg, 0.27 mmol, 1 equiv.) was dissolved in toluene (0.7 mL). A solution of BrCN (42 mg, 0.40 mmol, 1.5 equiv.) in toluene (0.4 mL) was added dropwise. The resulting solution was heated at 110 °C for 4 h. After cooling, the solution was concentrated under vacuum and purified by reverse chromatography (MeOH/H_2O+0.05%HBr), yielding to **24** as an orange solid (38 mg, 0.13 mmol, 47%). Purity ≥98%; mp = 252–253 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 4.37 (s, 2H), 7.05 (d, 1H, *J* = 7.4 Hz), 7.32–7.38 (m, 3H), 7.40–7.50 (m, 3H), 7.58 (s, 2H), 8.12 (s, 1H), 10.65 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 115.2, 116.4, 125.9, 128.3, 128.9, 129.0, 129.1, 134.2, 138.9, 140.1, 153.1; HRMS (M+H$^+$) 224.1171 (calcd for $C_{14}H_{13}N_3H^+$ 224.1182).

2-Nitro-4-phenylaniline (26). Following general method B and starting from 4-chloro-2-nitroaniline **25** (250 mg, 1.45 mmol) and phenylboronic acid (212 mg, 1.74 mmol), **26** was obtained as a red solid (288 mg, 1.35 mmol, 93%). Data are consistent with literature values [74]. mp = 124–126 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 6.12 (s, 2H), 6.90 (d, 1H, *J* = 8.7 Hz), 7.34 (t, 1H, *J* = 7.3 Hz), 7.43 (t, 2H, *J* = 7.3 Hz), 7.55 (d, 2H, *J* = 7.3 Hz), 7.65 (dd, 1H, *J* = 2.3 Hz, *J* = 8.7 Hz), 8.37 (d, 1H, *J* = 2.3 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 119.3, 123.9, 126.3, 127.3, 128.9, 130.4, 134.5, 138.8, 143.7.

4-Phenylbenzene-1,2-diamine (27). In a one-neck round-bottom flask, **26** (200 mg, 0.93 mmol, 1 equiv.) and Sn (708 mg, 3.73 mmol, 2.63 equiv.) were dissolved in ethanol (4.0 mL). The reaction media was cooled at 0 °C and HCl 37% (2.8 mL, 36 equiv.) was added slowly. The resulting solution was heated at reflux for 1 h. After cooling, the reaction mixture was filtered through a pad of Celite® and washed with ethanol. The filtrate was concentrated under vacuum. The residue was dissolved in a saturated solution of $NaHCO_3$. The aqueous phase was extracted twice with AcOEt. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated, yielding to **27** as a white solid (139 mg, 0.75 mmol, 100%). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 5.88 (br s, 4H), 6.73 (d, 1H, *J* = 8.0 Hz), 6.87 (dd, 1H, *J* = 2.1 Hz, *J* = 8.0 Hz), 7.00 (d, 1H, *J* = 2.1 Hz), 7.23 (t, 1H, *J* = 7.5 Hz), 7.37 (t, 2H, *J* = 7.5 Hz), 7.49 (d, 2H, *J* = 7.5 Hz); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 114.9, 117.1, 117.9, 126.1, 126.5, 129.2, 131.3, 133.8, 134.0, 141.2.

6-phenyl-1H-benzo[d]imidazol-2-amine hydrobromide (28). 27 (80 mg, 0.43 mmol, 1 equiv.) was dissolved in toluene (1.1 mL). A solution of BrCN (69 mg, 0.65 mmol, 1.5 equiv.) in toluene (0.7 mL) was added dropwise. The resulting solution was heated at 110 °C for 4 h. After cooling, the solution was concentrated under vacuum and purified by reverse chromatography (MeOH/H_2O+0.05%HBr), yielding to **28** as a white solid (79 mg, 0.27 mmol, 63%). Purity ≥98%; mp = 226–227 °C; ^{1}H NMR (400 MHz, DMSO) δ ppm 7.37 (t, 1H, J = 7.4 Hz), 7.43–7.52 (m, 4H), 7.58 (s, 1H), 7.63 (d, 2H, J = 7.5 Hz), 8.50 (s, 2H), 12.47 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 109.9, 112.2, 122.6, 127.3, 127.8, 129.5, 129.7, 130.9, 136.2, 140.5, 151.3.

(4-Bromo-2-nitrophenyl)ethanol (30). Paraformaldehyde (114 mg, 3.80 mmol, 1.0 equiv.), 4-bromo-2-nitrotoluene **29** (2.00 g, 9.26 mmol, 2.44 equiv.) and Triton-B (114 μL, 40% in methanol) were dissolved in DMSO (2.0 mL). The resulting mixture was heated 2 h at 90 °C. After cooling, the reaction mixture was diluted with a saturated solution of NH_4Cl. The aqueous phase was extracted twice with AcOEt. The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel column chromatography (*n*heptane:EtOAc 1:1 to 1:2), yielding to **30** as a yellow solid (700 mg, 2.84 mmol, 75%). Data are consistent with literature values [75]. m.p 75–76 °C.^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.15 (t, 2H, *J* = 6.3 Hz), 3.96 (t, 2H, *J* = 6.3 Hz), 7.35 (d, 1H, *J* = 8.3 Hz), 7.69 (dd, 1H, *J* = 1.9 Hz, *J* = 8.3 Hz), 8.09 (d, 1H, *J* = 1.9 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 35.8, 62.4, 120.5, 127.7, 132.8, 134.4, 136.1, 150.2.

(2-Nitro-4-phenylphenyl)ethanol (31). A microvawe vial under argon was charged with **30** (246 mg, 1.00 mmol, 1.0 equiv.), phenylboronic acid (134 mg, 1.10 mmol, 1.1 equiv.), $Pd(OAc)_2$ (4 mg, 0.02 mmol, 20 mol%), K_2CO_3 (345 mg, 2.50 mmol, 2.5 equiv.) and TBAB (322 mg, 1.00 mmol, 1.0 equiv.) in water (1.1 mL). The vial was capped properly, flushed with argon and heated to 70 °C for 3 h. After cooling, the reaction mixture was diluted with water and the aqueous phase was extracted twice with AcOEt. The organic phase was dried over Na_2SO_4, filtered, concentrated under vacuum and purified by silica gel column chromatography (n-heptane:EtOAc 2:1), yielding to **31** a colorless oil (197 mg, 0.92 mmol, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 3.22 (t, 2H, *J* = 6.3 Hz), 4.01 (t, 2H, *J* = 6.3 Hz), 7.42–7.54 (m, 4H), 7.61 (d, 2H, *J* = 6.6 Hz), 7.78 (d, 1H, *J* = 7.8 Hz), 8.17 (s, 1H).

(2-Amino-4-phenylphenyl)ethanol (32). In a hydrogenation flask, **31** (197 mg, 0.81 mmol, 1 equiv.) was dissolved in ethanol (10.0 mL) then argon was bubble in through. Pd/C (25 mg, 10 mol%) was added and the reaction media was stirred at rt for 24 h under a dihydrogen pressure (70 psi). The reaction media was filtered through a pad of Celite® and washed with ethanol. The filtrate was concentrated, yielding to **32** as a white solid (171 mg, 0.80 mmol, 99%). mp = 99–100 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.84 (br s, 1H), 2.87 (t, 2H, J = 6.0 Hz), 3.98 (t, 2H, J = 6.0 Hz), 3.97 (bs, 2H), 6.97 (d, 1H, J = 2 Hz), 7.02 (dd, 1H, J = 7.6 Hz, J = 2.0 Hz), 7.15 (d, 1H, J = 7.6 Hz), 7.35 (t, 1H, J = 7.2 Hz), 7.44 (t, 2H, J = 7.2 Hz), 7.59 (d, 2H, J = 7.2 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 145.4, 141.1, 140.8, 131.0, 128.6, 127.1, 126.9, 123.4, 118.1, 114.9, 63.1, 34.5.

{2-[2,3-Di(tert-butoxycarbonyl)guanidino]-4-phenylphenyl} ethanol (33). Following general method A and starting from **32** (171 mg, 0.80 mmol), **33** was obtained as a white solid (285 mg, 0.63 mmol, 78%). mp = 107–109 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.46 (s, 9H), 1.56 (s, 9H), 2.93 (t, 2H, *J* = 6.0 Hz), 3.95 (q, 2H, *J* = 6.0 Hz), 7.34–7.47 (m, 4H), 7.62 (d, 2H, *J* = 7.2 Hz), 7.88 (s, 1H), 10.19 (s, 1H), 11.65 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 28.0,

28.2, 34.4, 62.5, 125.2, 125.3, 127.1, 127.3, 128.7, 130.5, 132.5, 135.5, 140,1, 140.4, 154,9.

8-phenyl-4,5-dihydro-1H-benzo[d] [1,3]diazepin-2-amine Hydrochloride (34). 33 (285 mg, 0.63 mmol, 1 equiv.) was dissolved in THF (10.0 mL), PPh_3 (328 mg, 1.25 mmol, 2 equiv.) and DIAD (243.0 μL, 1.25 mmol, 2 equiv.) were added. The resulting mixture was stirred at rt for 3 h. The solution was concentrated under vacuum and purified by silica gel column chromatography (n-heptane:EtOAc 2:1). The intermediate was diluted in a solution of HCl (4.0 N) in dioxane. The reaction mixture was stirred overnight at rt. The solution was concentrated under vacuum, yielding to **34** as a white solid (72 mg, 0.26 mmol, 43%). Purity ≥98%; mp = 236–237 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 3.18 (t, 1H, J = 8.0 Hz), 4.09 (t, 1H, J = 8.0 Hz), 7.37–7.43 (m, 3H), 7.47 (t, J = 7.5 Hz, 2H), 7.53 (s, 1H), 7.69 (d, 2H, J = 7.5 Hz), 8.06 (br s, 4H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 27.5, 51.4, 113.5, 123.4, 126.5, 127.4, 128.1, 129.4, 133.1, 140.2, 140.6, 141.4, 154.6; HRMS ($M+H^+$) 238.1336 (calcd for $C_{15}H_{15}N_3H^+$ 238.1339).

5.2. Biological methods

5.2.1. In vitro anti-inflammatory activity

Peripheral blood mononuclear cells (PBMCs) isolation and culture PBMCs were prepared from the peripheral blood of healthy donors (Etablissement Français du Sang) according to Kümmerle et al. [29] with modifications [76]. Briefly PBMCs were isolated using Histopaque gradient (Sigma), washed in Hanks' balanced salt solution (Sigma) and were then cultured in RPMI-1640 medium (Sigma) supplemented with 10% (v/v) fetal bovine serum (FBS) (PAA), 100 U/mL penicillin and 100 μg/mL streptomycin (PAA). PBMCs were seeded into 24-well plates (5×10^5 cells/well) and stimulated with 5 μg/mL LPS (Sigma), into a total volume of 1 mL per well. Compounds were re-suspended in DMSO and tested at different concentrations (Final DMSO concentration was adjusted to 1% maximum for each well). Cells without LPS stimulation were considered as bio-inactive control (basal level of cytokine). Cells incubation was realized at 37 °C (5% CO2).

5.3. Characterization of cytokine secretion

Quantitative evaluation of TNFα and IL1β secreted by PBMCs was achieved by ELISA using conditions as previously described [29] or HTRF technology (Cisbio bioassays) according to supplier recommendations as previously described [76].

Briefly, detection of TNFα and IL1β was done on culture supernatant after 24 h. ELISAs were performed using antibodies from Antibody Solutions (Half Moon Bay, CA, USA); HTRF assays were performed using (Cisbio 62TNFPEC assay for TNFα and 62IL1PEB for IL1β) according to supplier recommendations. Reading was performed after 2 h and 30 min of incubation with HTRF tools.

5.4. Cell viability assay

Cell viability was measured using the WST-1 (Ozyme) assay according to the manufacturer protocol and Houël et al. [76], after supernatant transfer for cytokine determination, WST-1-containing medium was added to cells and cell viability was determined by measuring absorbance at 450 nm using the Victor^3 reader (PerkinElmer) after 2 h incubation at 37 °C.

Each measurement was performed in triplicate with three independent experiments. The results were expressed as means of three independent experiments.

5.4.1. In vivo assay on LPS-induced lung inflammation

Nine-week-old male C57BL/6 mice were purchased from Janvier Laboratories. Animal experimentation was conducted with the ministerial approval through the Regional Ethics Committee for animal research at the Strasbourg University (APAFIS Authorization number 20150603112018276v2). Mice were treated by intraperitoneal (i.p.) injection (12 mL/kg) of the selected compounds or solvent, 1 h before and 10 h after LPS (lipopolysaccharide E.coli O55:B5, Sigma-Aldrich, L2880) or saline challenge by intranasal route (i.n., 25 μL). LPS was used at the dose of 1 μg/mouse. BAL was performed 24 h after LPS challenge as described [77]. Mice were anaesthetized i.p. (ketamine 150 mg/kg – xylazine 10 mg/kg). After semi-excision of the trachea, a plastic cannula was inserted, and airspace washed with 0.5 mL of 0.9% NaCl injected with a 1 mL syringe. This procedure was performed 10 times. The initial concentrated supernatant of the 2 first washes (volume = 2 × 0.5 mL administered, ~0.5 mL recovered) was collected for cytokine measurements. The remaining BAL fluid was centrifuged (300 g for 5 min, 4 °C), and cell pellets pooled. The cell pellet was suspended in 500 μL of PBS-EDTA 3 mM. BAL cells were counted on a Muse® Cell Analyser (Millipore) and differential cell counts were assessed by flow cytometry (LSRII® cytometer, BD Bioscience). BAL cells were added with FCblock (0.5 μL, 553142, BD Bioscience) in a black microplate, incubated for 20 min at room temperature. Then, marker antibodies were added: CD11c-FITC (557400, BD bioscience), Gr-1-Pe-eFluor610 (61-5931-82, eBioscience), CD11b-APC-Cy7 (557657, BD bioscience), CD45-AlexaFluor700 (103128, BioLegend), CD3-BV605 (564009, BD bioscience), CD19-PE-Cy7 (552854, BD bioscience). Antibodies were incubated with BAL cells for 30 min at room temperature before DAPI (5 μL, BD bioscience) addition, and flow cytometry was performed immediately. Live leukocytes were identified as $CD45^+DAPI^-$ cells then differentiated into T cells ($CD11b^{low}CD19^-CD3^+$), B cells ($CD11b^{low}CD19^+CD3^-$), eosinophils ($CD11b^+CD11c^-GR1^-$), neutrophils ($CD11b^+CD11c^-GR1^+$) and macrophages ($CD11b^+GR1^-CD11c^+$).

5.5. Measurement of cytokines

TNFα ELISA was obtained from R&D systems and performed according to the manufacturer's instructions.

5.5.1. In vivo essay on a neuropathic pain model

Experiments were done using 35C57BL/6J male mice (Charles River, L'Arbresle, France), 8–10 weeks old at surgery time. They were housed 2 to 5 per cage, under a 12 h light/dark cycle, with food and water *ad libitum*. The animal facilities (Chronobiotron UMS3415) are registered for animal experimentation (agreement #A67-2018-38) and protocols were approved by the institutional ethical committee (CREMEAS, CEEA35).

Neuropathic pain was induced by placing a cuff around the right sciatic nerve [54,55]. Surgeries were performed under intraperitoneal (i.p.) ketamine (68 mg/kg) and xylazine (10 mg/kg) anesthesia (Centravet, Tadden, France). The main branch of the right sciatic nerve was exposed and a PE-20 polyethylene cuff (Harvard Apparatus, Les Ulis, France) of standard length (2 mm) was unilaterally inserted around it (Cuff group). The skin was closed using suture. Sham-operated mice underwent the same procedure without implantation of the cuff (Sham group). Mice were allowed to recover from surgery for at 2–3 weeks before starting treatments.

Mechanical allodynia (hypersensitivity) was assessed using von Frey hairs. Mice were placed in Plexiglas boxes (7 cm × 9 cm x 7 cm) on an elevated mesh screen. The von Frey filaments (Bioseb, Vitrolles, France) were applied to the plantar surface of each hindpaw until they just bent, in a series of ascending forces up to the mechanical threshold. Filaments were tested five times per paw

and the paw withdrawal threshold (PWT) was defined as the lower of two consecutive filaments for which three or more withdrawals out of the five trials were observed [55,78]. Results were expressed in grams.

Treatments began 2–3 weeks after the surgery and lasted for 17 consecutive days. During the treatment, mice received two i.p. injections per day (morning and evening) of either 0.9% NaCl, nortriptyline hydrochloride (Sigma-Aldrich) (1 or 5 mg/kg, 5 mL/kg), **5b** (1 or 5 mg/kg, 5 mL/kg), or **12** (1 or 5 mg/kg, 5 mL/kg). The 4 mice treated with **5b** 5 mg/kg were discarded from the analyses due to adverse effects of the chronic treatment (see results). All drugs were prepared in 0.9% NaCl solution.

Data were expressed as mean ± SEM, and statistical analyses were performed using STATISTICA 7.1 (Statsoft, Tulsa, OK, USA). ANOVA for multiple comparisons and the Duncan test for posthoc analyses were used for time-course analyses.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2018.01.049.

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