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Design and synthesis of 3-aminophthalazine derivatives and structural analogues as PDE5 inhibitors: anti-allodynic effect against neuropathic pain in a mouse model

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Highlights

- Design of homologues, isosteres and structural analogues of MY 5445
- Evaluation for their inhibitory activity towards PDE 5
- *In vivo* proof of efficacy of **16h** and **41n** in neuropathic allodynia

Abstract: Neuropathic pain is a chronic pain caused by a lesion or disease affecting the somatosensory nervous system. To date, no specific treatment has been developed to cure this pain. Antidepressants and anticonvulsant drugs are used, but they do not demonstrate universal efficacy, and they often cause detrimental adverse effects. Some studies highlighted the efficacy of sildenafil, a well-known inhibitor of phosphodiesterase 5 (PDE5, (IC_{50}=3.3nM)), in models of pain. Based on these results, we focused our attention on MY 5445, another known PDE5 inhibitor. Homologues, isosteres and structural analogues of MY 5445 were designed and all synthesized compounds were evaluated for their inhibitory activity toward PDE5. Selectivity profiles towards other PDE1-4 isoenzymes, water solubility and stability in acidic medium of the most potent PDE5 inhibitors were determined and the aminophthalazine **16h** and its mimetic **41n** (3-aminoindazole) were evaluated in comparison to MY 5445 (**4b**) *in vivo* in a model of neuropathic pain induced by sciatic nerve cuffing in mice (3 and 0.5 mg/kg, *ip* twice a day). Both compounds showed the same efficacy on neuropathic allodynia as MY 5445, and thus produced a significant relief of mechanical hypersensitivity after 12 days of treatment.

Introduction

Cyclic AMP (cAMP) and cyclic GMP (cGMP) play major roles in normal and physio-pathological intracellular signalling. Levels of cAMP and cGMP are influenced by cyclic nucleotide phosphodiesterase (PDE) families that specifically and rapidly convert the cyclic nucleotide to their corresponding 5' nucleotide. Eleven PDE families (PDE1 to PDE11) have been characterized to date. They differ by their substrate specificities, kinetic properties, tissue and subcellular localizations, and drug and mediator sensitivities, representing fine tuned new therapeutic targets.[1] Among these families, the PDE5 family specifically hydrolyzes cGMP, and is characterized by two GAF allosteric domains.[2] We characterized the first selective PDE5 inhibitor, zaprinast **1**, by using purified PDE5 (also known as cGMP-PDE, or cGMP-binding-PDE) from vascular smooth muscle [3] and pulmonary smooth muscle.[4] Since, multiple other PDE5 inhibitors has been developed, like DMPPO **2** and sildenafil **3**.[1] PDE5 inhibitors constitute a new class of vasoactive drugs that have been developed for the treatment of erectile dysfunction in patients. Inhibition of PDE5 increases cyclic GMP (cGMP) levels,[5] and favors smooth muscle relaxation and penis erection. Sildenafil is also indicated clinically for treatment of pulmonary hypertension [6,7] and cardiac hypertrophy.[8] In addition, further investigations showed that vardenafil, another PDE5-inhibitor, could be effective against ischemia.[9] In the previous decade, studies in animal models also proposed a beneficial effect of PDE5 inhibitors on various pain conditions.[10–16].This pain-relieving action was observed in somatic [10,11] and in visceral [12,13] inflammatory pain models, as well as in models of neuropathic pain, either lesional [15] or metabolic.[16] Moreover, a case report proposed that sildenafil may improve neuropathic pain symptoms in patients with diabetic peripheral neuropathy. [17] Mechanistically, this analgesia mediated by PDE5 inhibitors has been related to their action on the nitric oxide (NO)/cGMP pathway.[10, 12, 14–16, 18–20]. While NO may

contribute to neuropathic pain symptoms *via* an up-regulation of nitric oxide synthases [21, 22] and an activation of the NO-cGMP-PKG pathway [23, 24] in the spinal cord and dorsal root ganglia, evidence accumulated showing that NO can also have anti-nociceptive properties. Indeed, the NO-cGMP pathway contributes to the analgesic effect of opioid mu receptor agonists [25] of gabapentinoïds [26, 27] as well as of PDE5 inhibitors [10, 12, 14–16, 18–20].

Zaprinast **1** [28] is the prototype of the most important series of PDE5 inhibitors deriving from pyrimidones-containing bicyclic compounds (Figure 1, cpds **1-3**). The well known sildenafil **3** (Viagra®) [29] still continued to provide novel safer derivatives and isosteres [30, 31] (better selectivity profiles towards other PDE isoforms, water-soluble analogues).[32] MY5445 [33] (cpd **4b**), a phthalazine-deriving PDE5-I showed significant difference between both scaffolds of **1** and **3**. In addition few SAR analyses published data dealing with MY5445 series could not be clearly correlated with other structural analogues **5** [35, 34] and **6** [36] depicted in Figure 1.

The *N*-benzyl phthalazine **5** could be regarded as a superior homologue of MY5445 (**4b**) [37]. However the respective beneficial effects combining both homology and substitutions effects at the phthalazine nucleus are not known. We selected in Figure 1 the *N*-benzyl quinazoline **6** as a putative structural equivalent (pyridazine vs pyrimidine), but with different substitution effects at both the benzo and *N*-benzyl fragments.

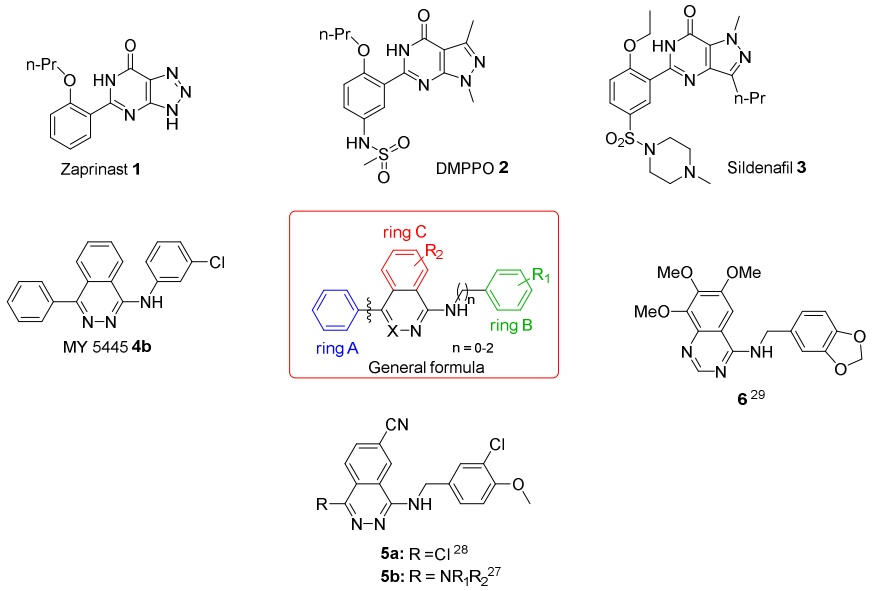


Figure 1. Zaprinast, structurally-related compounds (**2** and **3**), MY 5445 **4b**, and structurally-related compounds **5** and **6** as selected PDE5 inhibitors.

Our general objective was the first attempts to introduce a systematic SAR analysis of series of PDE-5 belonging to a common general formula in Figure 1. In particular, as depicting in Figure 2 and starting from MY5445, we evaluated the importance of 1) the benzo group C, 2) the possible substitution on both phenyl rings A and B, 3) the replacement of ring A, 4) the homologation, and 5) deletion of the imine function leading to benzamides derivatives which could be considered as seco derivatives of both **4b** and **6** subseries. Finally, starting from the benzamide structure, different semi-rigid derivatives were designed. This large topological exploration of both the scaffold and the different substitutions of critical fragments (rings A, B and C) should i) help to select the most potent PDE5-I, ii) determine their selectivity toward a panel of PDE isoforms, iii) characterize the *in vivo* efficacy of a couple of selected compounds in a neuropathic pain model in mice.

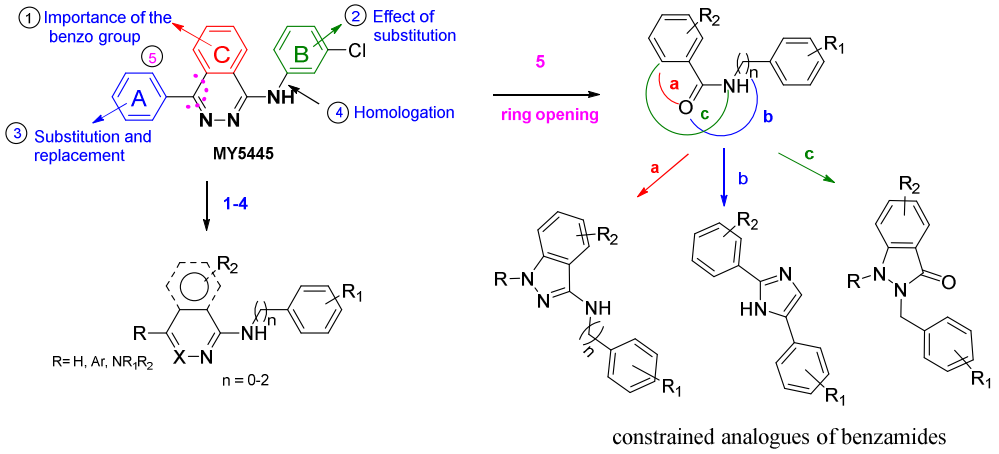
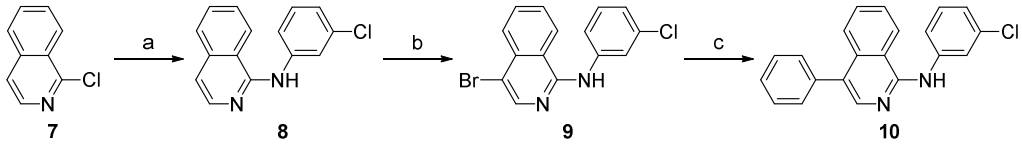


Figure 2. Topologic study on MY5445.

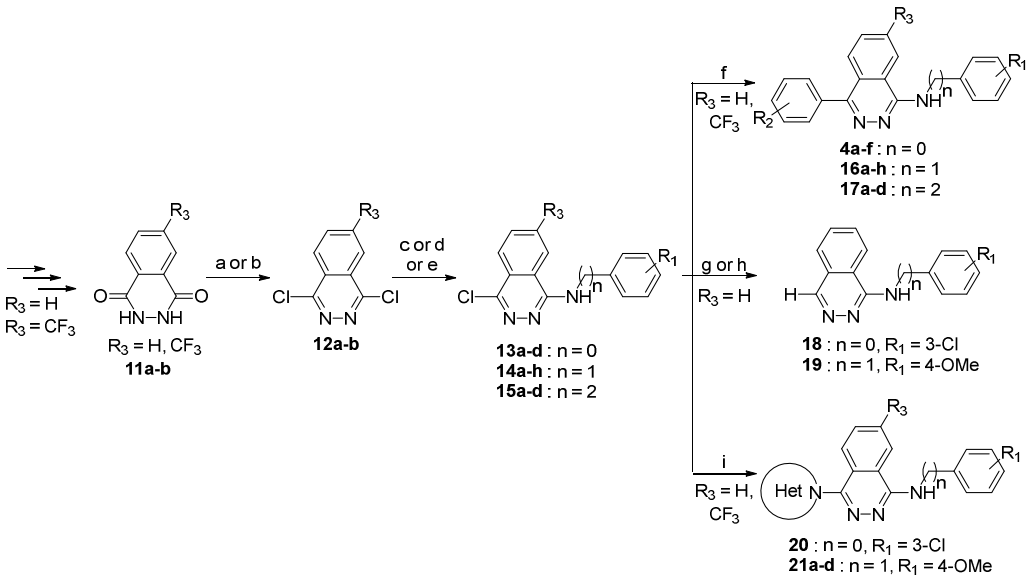
1-Chemistry

The 4-phenyl isoquinoline-amine **10** was synthesized in a three step sequence starting from the commercially available 2-chloroisoquinoline **7** (Scheme 1). The aniline intermediate **8** was prepared in 78% yield by heating **7** at 110 °C in presence of 3-chloroaniline in NMP.[38] Regioselective bromination was performed efficiently by treatment with phenyltrimethylammonium tribromide,[38] yielding the desired bromoisoquinoline **9** in 99% yield. Finally a Suzuki-Miyaura reaction of **9** with phenylboronic acid afforded the target product **10** in 52% yield.



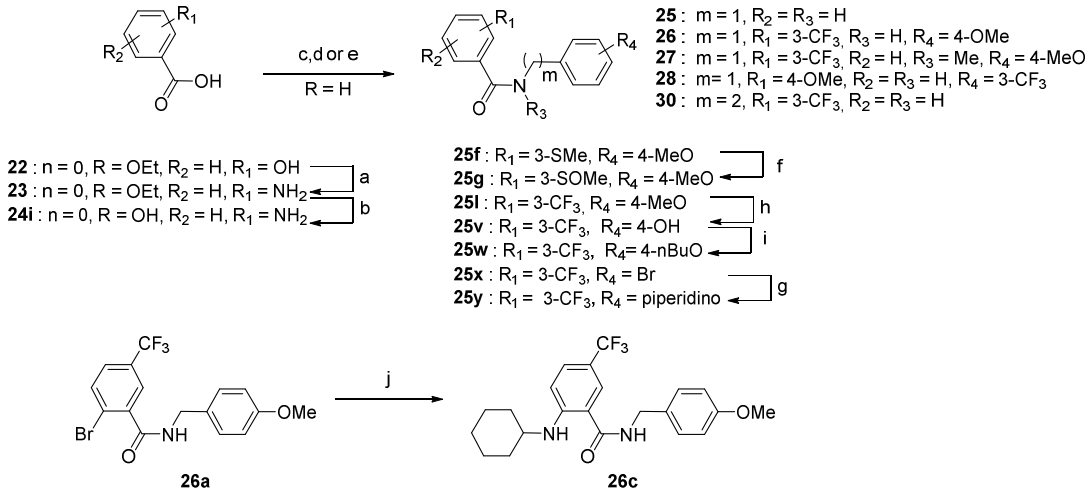
Scheme 1. Synthesis of isoquinoline **10**. (a) 3-chloroaniline (1.2 eq.), NMP, 110 °C, 15 h, 78%; (b) Me_3PhNBr_3 (0.90 eq.), THF, 0-25 °C, 16 h, 99%; (c) $PhB(OH)_2$ (1.2 eq.), $Pd(PPh_3)_4$ (5 mol%), Na_2CO_3 (3 eq.), Tol:EtOH:H_2O (5:1:1), 120 °C, 2 h, 52%.

The aminophthalazine derivatives **4a-f**, **16-21** were prepared using methodologies depicted in Scheme 2. The easily preparable 1,4-dichlorophthalazine derivatives **12a-b** were treated with various amines affording intermediates **13a-d, 14a-g and 15a-d** in good yields. Compounds **13-15** smoothly underwent a Suzuki-Miyaura cross-coupling reaction with the appropriate boronic acids enabling formation of final 6-aryl amino phthalazine analogues **4a-f, 16a-g** and **17a-d**. Subsequent condensation of **13b, 14e and 14h** with the appropriate cyclic amines afforded the diaminophthalazine derivatives **20** and **21a-c**. Finally analogues **18-19** were obtained via a standard dehalogenation of chloro compounds **13b** and **14e**. 6-Substituted trifluoromethyl-aminophthalazine derivatives **16h** and **21d** were prepared from the readily available trifluoromethyl 1,4-dichlorophthalazine **12b** [36] in a similar fashion (Scheme 2).



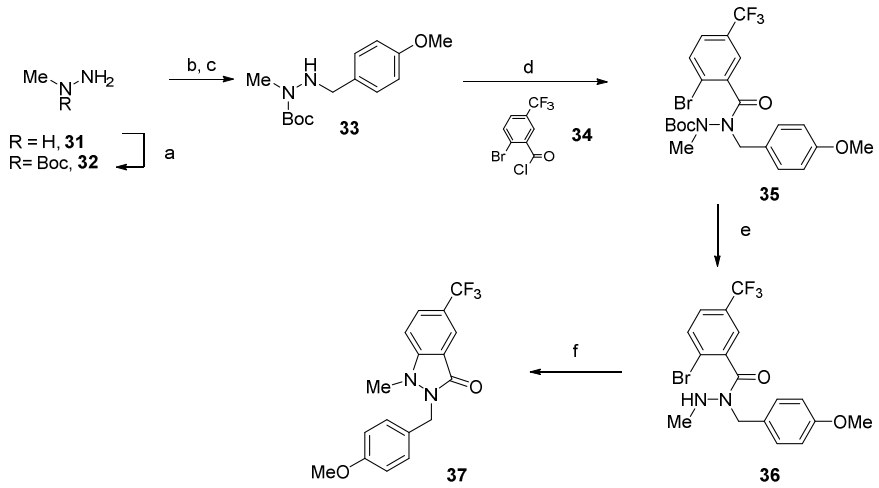
Scheme 2. Synthesis of phthalazines derivatives **4, 16-21**. (a) pyridine (2 eq.), $POCl_3$ (35 eq.), 110 °C, 1 h, 93%; (b) DIEA (1.1 eq.), $POCl_3$ (25 eq.), 120°C, 3h, 75%; (c) *for n=0 and R_3=H* : R_1PhNH_2 (1 eq.), *i*PrOH, 110 °C, 1 h, 18-100%; (d) *for n=1 or 2 and R_3=H* : $NH_2(CH_2)n$-R_1Ph (1.1 eq.), Na_2CO_3 (2eq.), DMF, 130 °C, 16 h, 57-96%; (e) *for n=1 and R_3=CF_3* : 4-methoxybenzylamine (1.2 eq.), dbu (2.5 eq.), NMP, rt, 2h, 51%; (f) $R_2PhB(OH)_2$ (1.1-1.5 eq.), $Pd(PPh_3)_4$ (5 mol%), Na_2CO_3 (3 eq.), Tol:EtOH:H_2O (5:1:1), 120 °C, 16 h, 17-100%; (g) *for n = 0 and R_1 = 3-Cl* : formic acid (1.0 eq.), $Pd(PPh_3)_4$ (4 mol%), NEt_3 (12.0 eq.), 110 °C, 25 min, µW, 77%; (h) *for n = 1 and R_1 = 4-OMe* : H_2 (60 psi), Pd/C (5 mol%), EtOH, rt, 16 h, 19%; (i) cyclic amine (5.18 eq.), DIPEA (5.14 eq.), NMP, 170 °C, 16 h, 26-79%.

The substituted benzamides **25-30** were carried out using standard coupling procedures starting from the corresponding benzoic acids, as illustrated in Scheme 3. If the benzoic acids were not commercially available, they were easily prepared by known experimental methods (see experimental part). Further structural modifications were performed on phenyl rings of some benzamide derivatives. As an illustration, the thioanisole derivative **25f** was conveniently converted into the corresponding sulfoxide **25g** at room temperature using sodium periodate as oxidant.[39] Demethylation of **25l** with BBr_3 under microwave irradiation led to the corresponding phenol **25v**. Alkylation of **25v** with *n*Bu-Br in DMF afforded **25w** in a satisfactory yield. Buchwald amination reaction of **25x** with the help of JohnPhos allowed the formation of **25y** in 63% yield. Finally, a ligand free Ullmann *N*-arylation was performed starting from **26a** to introduce the primary cyclohexylamine (cpd **26c**).



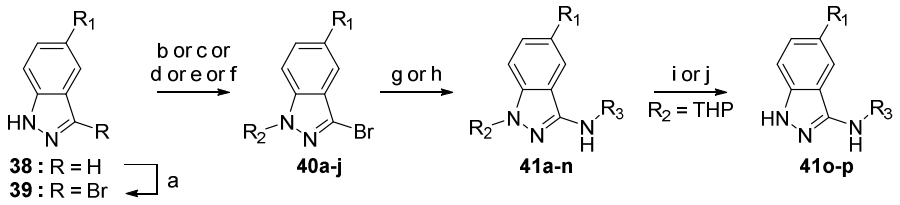
Scheme 3. Synthesis of benzamides **25-30.** (a) HOBt.NH_3 (1.5 eq.), EDC (1.2 eq.), DMF, rt, 17 h, 72%; (b) NaOH (5 eq.), MeOH:H_2O (1:1), rt, 15 h, 82%; (c) $SOCl_2$, 80 °C, 2 h; (d) $R_3NH(CH_2)$m-PhR_4 (1.2 eq.), NEt_3 (2 eq.), DCM, rt, 16 h, 37-76% over 2 steps; (e) $R_3NH(CH_2)$m-R_4Ph (2 eq.), 1-HOBT (1.1 eq.), EDC.HCl (1.5 eq.), DMF, rt, 16 h, 28-99%; (f) $NaIO_4$ (0.5M, 1 eq.), MeOH, 0-25 °C, 16 h, 90%; (g) piperidine (1 eq.), $Pd_2(dba)_3$ (10 mol%), JohnPhos (40 mol%), *t*BuONa (4 eq.), dioxane, 90 °C, 14 h, 63%; (h) BBr_3 (3 eq.), DCM, 100 °C, 5 mins, µW, 6 bars, 77%; (i) *n*BuBr (1 eq.), K_2CO_3 (1 eq.), DMF, 90 °C, 16 h, 65%; (j) $CyNH_2$ (2 eq.), CuCl (1 mol%), K_3PO_4 (2 eq.), DMF, 50 °C, 3 days, 76%.

The preparation of the indazolone **37** is outlined in Scheme 4. The requisite diprotected *N*-methylhydrazine **33** was prepared in two steps starting from commercially available *N*-Me hydrazine, following a standard literature procedure.[40] The corresponding hydrazide **35** was synthesized easily from 2-bromobenzoyl chloride **34** and **33**. Boc-deprotection with TFA in dichloromethane afforded the hydrazide **36**, which was cyclized into indazolone **37** by a copper-mediated cross-coupling reaction using a β-diketone as the ligand.



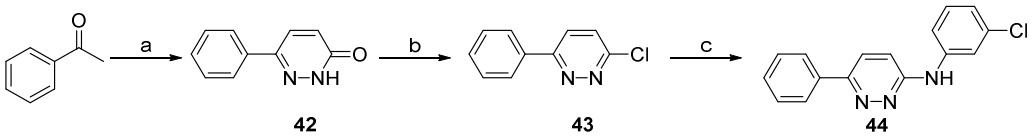
Scheme 4. Synthesis of indazolone **37**. (a) Boc_2O (0.75 eq.), MeOH, rt, 15h, quant.; (b) 4-methoxybenzaldehyde (1.1 eq.), *i*PrOH, 90°C, 2h, quant.; (c) $NaBH_3CN$ (1.5 eq.), AcOH (2 eq.), MeOH, rt, 39h, 63%; (d) 2-bromo-5-(trifluoromethyl)benzoyl chloride **34** (1 eq.), NEt_3 (2.9 eq.), DCM, rt, 21h, 86%; (e) TFA:DCM (1:10), rt, 1h, 99%; (f) CuI (5 mol%), 2-(2-methylpropanoyl)cyclohexan-1-one (10 mol%), Cs_2CO_3 (2 eq.), DMF, 90°C, 4.5h, 85%.

The functionalized aminoindazoles **41a-p** were readily prepared starting from the commercially available indazole derivatives **38** in four steps, as illustrated in Scheme 5. 3-Bromo indazoles **39** were generated from **38** after regioselective bromination by Br_2 in the presence of NaOH following a previously described method.[41] Protection of **39** by DHP afforded the *N1*-THP indazoles **40a-b**, whereas 3-alkylindazoles **40c-h** were easily obtained by *N*-alkylation with the appropriate alkylhalide at 50 °C in DMF. However, when starting from secondary alkylbromides, the reaction needed to be run at 90 °C in presence of TBAI. Likewise, *N1*-aryl or heteroaryl indazoles **40i-j** were successfully introduced via a copper-catalyzed *N*-arylation reaction using *trans*-cyclohexyldiamine as the ligand. Finally, the bromoindazole derivatives **40a-j** could be substituted at the C3 position by appropriate amines under Buchwald-Hartwig conditions with the help of JosiPhos or BrettPhos (cpds **41a-n**). Subsequent *N*-THP deprotection in TFA or HCl 1N yielded NH free indazole derivatives **41o-p** after inverse flash chromatography purification. Structures of these compounds were established and validated by 2D RMN experiments (see SI).



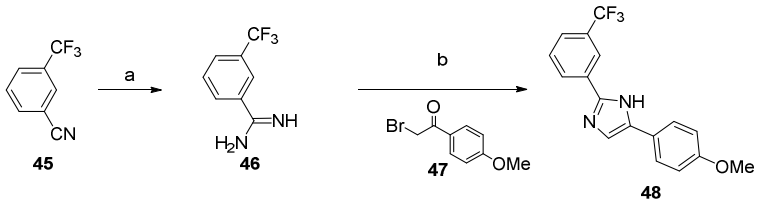
Scheme 5. Synthesis of indazoles **41**. (a) Br_2, 1.32 eq.), NaOH, DMF, rt, 16h, 68-98%; (b) *for R_2 = MeI* : MeI (1.5 eq.), Na_2CO_3 (2.5 eq.), DMF, 50°C, 12h, 66%; (c) *for R_2 = THP* : DHP (2 eq.), PTSA (5 mol%), AcOEt, 95°C, 16h, 72-83%; (d) *for R_2 = Bn* : BnBr (1.5 eq.), Na_2CO_3 (2.5 eq.), DMF, 55°C, 16h, 77%; (e) *R_2 = cPen or cHex* : cPenBr or cHexBr (1.3 eq.), TBAI (3 mol%), K_2CO_3 (4.5 eq.), DMF, 90°C, 16h, 73-89%; (f) *for R_2 = Ph or 3-pyridine* : R_2I (3 eq.), CuI (5 mol%), trans-*N,N'*- dimethylcyclohexane-1,2-diamine (20 mol%), K_2CO_3 (3 eq.), toluene, 130°C, 16h, 43-60%; (g) 4-methoxybenzylamine (1.2 eq.), $Pd(OAc)_2$ (5 mol%), JosiPhos (5 mol%), Cs_2CO_3 (1.5 eq.), dioxane, 120°C, 1h, 20-76%; (h) 4-methoxybenzylamine (1.2 eq.), $Pd(OAc)_2$ (5 mol%), BrettPhos (10 mol%), Cs_2CO_3 (1.5 eq.), dioxane, 120°C, 16h, 23-48%; (i) *for R_1 = H* : TFA:DCM (1:1), rt, 1.5h, 26%; (j) *for R_1 = 3-CF_3* : HCl 1M (2.9 eq.), THF, rt, 20h, 26%.

The target 3-amino pyridazine **44** was prepared as outlined in Scheme 6. Acetophenone was first reacted with glyoxylic acid and then the resulting mixture was treated with hydrazine to afford the desired pyridazinone **42** in 70% yield. Subsequently, the pyridazinone **42** was treated with phosphorous oxychloride to afford the key intermediate **43**. Finally, the 6-chloropyridazine **43** was submitted to a Buchwald-Hartwig cross coupling reaction with the help of $Pd(OAc)_2$ and JosiPhos to afford the desired compound.



Scheme 6. Synthesis of pyridazine **44** (a) glyoxylic acid hydrate (1.0 eq.), hydrazine hydrate (1.0 eq.), water, reflux, 4 h, 70%; (b) $POCl_3$, reflux, 2 h, 99%; (c) 3-chloroaniline (1.2 eq.), $Pd(OAc)_2$ (5 mol%), JosiPhos (5 mol%), Cs_2CO_3 (1.5 eq.), DMF, 50 °C, 24 h, 69%.

Condensation of the 3-trifluoromethyl benzamidine **46**, readily available from the corresponding nitrile **45** by treatment with LiHMDS,[42] was reacted with the α-bromoketone **47**, and gave access to the 2,4-disubstituted imidazoles **48** in a nearly quantitative yield (Scheme 7).



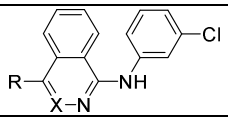
Scheme 7. Synthesis of imidazole **48**. (a) LiHMDS 1M (1.5 eq.), THF, rt, 15 h, quant.; (b) $NaHCO_3$ (4 eq.), THF:H_2O (4:1), 75 °C, 3 h, 95%.

2- Results and discussion

Structure-Activity Relationship and Structural Optimization

In our experimental conditions, MY 5445 (**4b**) showed an IC_{50} value in the 1-10 µM range (6.7 µM, determined at 1 µM cGMP concentration). This is in accordance with the original data published for MY5445, which reported an IC_{50} value of 0.6 µM determined at 0.4 µM cGMP concentration.[33] Under the same conditions, we obtained an IC_{50} value of 0.3 µM determined at 1 µM cGMP for the reference PDE5 inhibitor, zaprinast. Table 1 shows the importance for activity of i) the pyridazine nucleus (compare **4b** and **10**), ii) the presence of the benzo ring in the structure of **4b** (compare **4b** and the inactive pyridazine **44** in Fig. 3), iii) a fair beneficial substituent effect could be observed by introduction of a methoxy group in the para position (**4f**). However, compounds within this series had very low water solubilities.

Table 1. Importance of the scaffold and the 6-phenylring of MY 5445 [a,b]



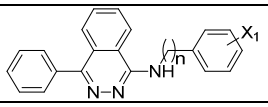
			PDE5	
Cpd	R	X	%inhib at 10µM	IC_{50} (µM)
4b (MY 5445)	Ph	N	42	6.7
10	Ph	CH	15	>50
18	H	N	33	>10
4e	4-ClPh	N	34	>10
4f	4-OMePh	N	76[c)]	3.0
20	*N*-piperidino	N	28	>10

a) Zaprinast as positive control PDE5-I (inhibition at 10µM: 90%, IC_{50} = 0.3 µM); b) Sildenafil as a second positive control PDE5-I (inhibition at 1µM: 98%, IC_{50} = 3.3 nM); c) Fairly soluble in DMSO/water mixture (1%)

The superior homologues of MY 5445 (n = 1, 2) were reported in Table 2. For each homologue series, we checked substitution effects on the *N*-aralkyl fragment ($X_1 \neq H$). A strong beneficial effect was found with specific aromatic substitutions (m-Cl, p-OMe) in the *N*-benzyl phthalazine series **16** (n=1, compare **16f** and **4b**). However this effect could not be clearly understood, as both the 3-Cl (**16b**) and 4-OMe (**16e**) derivatives did not show significantly improved

activity when compared to the unsubstituted derivative **16a**. As the p-methoxy benzyl derivative **16e** showed a similar potency when compared with the MY 5445, we conserved the same p-methoxy benzyl fragment in the other following sub-series**.**

Table 2. Substitution on the aniline moiety and homologues (n=0, 1, 2) [a, b]

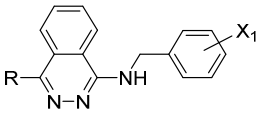


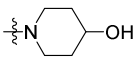
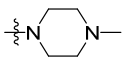
Cpd	n	X_1	PDE5 %inhib at 10µM	PDE5 IC_{50} (µM)
4b (MY 5445)	0	3-Cl	42	6.7
4a	0	H	5	nd
4c	0	4-Cl	31	38.1
4d	0	4-OMe	21	nd
16a	1	H	43	11.3
16b	1	3-Cl	14	nd
16c	1	2-OMe	25	>10
16d	1	3-OMe	41	11.4
16e	1	4-OMe	68	7.2
16f	1	3-Cl-4-OMe	86	0.41
16g	1	3,4-O-CH_2-O-	73	12.6
17a	2	H	47	≥10
17b	2	3-Cl	14	nd
17c	2	4-Cl	19	nd
17d	2	4-OMe	28	>10

a) Zaprinast as positive control PDE5-I (inhibition at 10µM: 90%, IC_{50} = 0.3 µM); b) Sildenafil as a second positive control PDE5-I (inhibition at1µM: 98%, IC_{50} = 3.3 nM); nd = not determined.

The phenyl ring in phthalazine **16e** might be important for activity (compare **16e** and **19**, Table 3). However, it could be replaced by a piperidine ring (compare **21a, b** and **16e**). Finally, introduction of a fully protonated basic nitrogen in *N*-methyl piperazino (**21c**) instead of a lipophilic substituent decreased the activity significantly.

Table 3. Importance of the phenyl ring in position 6 in the *N*-para-methoxybenzylaminophthalazine series [a, b]



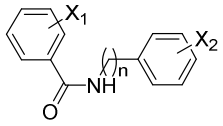
Cpd	R	X_1	PDE5 %inhib at 10µM	PDE5 IC_{50} (µM)
16e	Ph	4-OMe	68	7.2
19	H	4-OMe	35	>10
21a	*N*-piperidino	4-OMe	46	6.3
21b		4-OMe	60	5.3
21c		4-OMe	23	nd
4b (MY 5445)	Ph	3-Cl	42	6.7

a) Zaprinast as positive control PDE5-I (inhibition at 10µM: 90%, IC_{50} = 0.3 µM); b) Sildenafil as a second positive control PDE5-I (inhibition at1µM: 98%, IC_{50} = 3.3 nM); nd = not determined

We considered the benzamides **25**, **26** and **30** described in Table 4 (see also table A in SI) as seco analogues of phthalazines **16** after removal of a phenyl imine in their structure. The aim of studying this sub-series of easily available compounds was to identify some beneficial substitution effects on the phenyl ring ($X_1 \neq H$). Among the various substituents introduced mainly at position 3, we selected electron-withdrawing groups (CF_3, NO_2, $SOCH_3$, SO_2CH_3, CN),

as such substituents were already showing beneficial effects in the *N*-benzyl phthalazine series **5**.[35,36] When compared with the almost inactive substituted derivative **25a**, a fairly but significantly better activity was found with 3-NO_2 (**25i**), 3-CF_3 (**25l**) and the superior homologue (n = 2, **30c**). As observed in the phthalazine series (Table 2), the 3-chloro-4-methoxyphenyl phthalazine also presented a dramatical twenty fold increase in potency (compare **25t** and **25l**). However we did not observe a specific beneficial effect of the CF_3 group in the meta position (compare **25t** and **16f**). Based on these results, the benzamides **25** may not be considered as seco analogues of phthalazines **16**.

Table 4. Effects of aromatic substitution within the *N*-paramethoxybenzylbenzamide series[a, b]

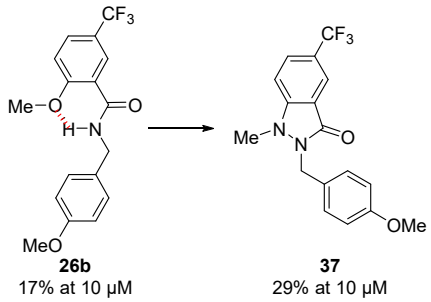


				PDE5	
Cpd	n	X_1	X_2	%inhib at 10µM	IC_{50} (µM)
25a	1	H	4-OMe	26	>50
25i	1	3-NO_2	4-OMe	44	19.0
25l	1	3-CF_3	4-OMe	32	23.7
25t	1	3-CF_3	3-Cl-4-OMe	77	0.94
30c	2	3-CF_3	3-Cl	51	19.2
4b (MY 5445)	-	-	-	42	6.7

a) Zaprinast as positive control PDE5-I(inhibition at 10µM: 90%, IC_{50} = 0.3 µM); b) Sildenafil as a second positive control PDE5-I (inhibitor (inhibition at1µM: 98%, IC_{50} = 3.3 nM).

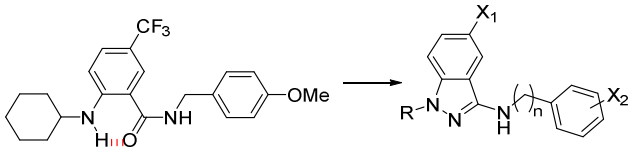
An additional substituent X_1 was introduced in benzamides **26b** and **26c**. An H bond accepting (6-OMe, **26b**), or donating group (6-NH-*c*Hex, **26c**) was introduced in the ortho position of the amide, establishing in both cases an internal H bond interaction, and leading to specific conformers, as outlined in Table 5. The benzamide **26c** was particularly active with an IC_{50} value less than 1 µM, whereas the other benzamide **26b** was inactive. These data support a specific active conformation for benzamides that may be mimicked by the already described phthalazines **4b**, **16**, **17**, but also novel heterocyclics such as indazoles.

Table 5. Mimetics of internal H bond interactions in selected benzamides **26b** and **26c** [a, b]



26b
17% at 10 µM

37
29% at 10 µM



26c
81% at 10µM
IC_{50} = 0.86 µM

41

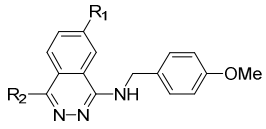
Compd	n	X_1	X_2	R	%inhib at 10µM	IC_{50} (µM)
41f	0	CF_3	3-Cl	*c*Pen	72	1.67
41c	1	CF_3	4-OMePh	Me	51	15.1
41d	1	H	4-OMePh	*c*Pen	20	>10
41e	1	Cl	4-OMePh	*c*Pen	57	10.6
41g	1	CF_3	4-OMePh	*cPen*	48	2.74
41h	1	CF_3	3-Cl-4-OMePh	*c*Pen	74	0.96
41i	1	CF_3	3-F-4-OMePh	*c*Pen	69	5.5
41k	1	CF_3	4-OMePh	*c*Hex	56	5.1
41l	1	CF_3	4-OMePh	Bn	69	1.44
41m	1	CF_3	4-OMePh	Ph	58	7.2
41n	1	CF_3	4-OMePh	Pyridin-3-yl	70	1.8
41o	1	H	4-OMePh	H	14	>10
41p	1	CF_3	4-OMePh	H	29	>10
4b (MY 5445)	-	-	-	-	42	6.7

a) Zaprinast as positive control PDE5-I (inhibition at 10µM: 90%, IC_{50} = 0.3 µM); b) Sildenafil as a second positive control PDE5-I (inhibition at1µM: 98%, IC_{50} = 3.3 nM).

As expected, the rigid indazolone **37** was found to be inactive. The 3-aminoindazoles **41** were particularly interesting with compounds showing IC_{50} values in the 1 µM range. The presence of a lipophilic substituent (*c*Hex) in the structure of the amide **26c** may contribute to its potency through an additional hydrophobic interaction, which is supported by data obtained within the indazole series **41**. The presence of a lipophilic group (R = *c*Pen, *c*Hex, Bn) increased their potency, whereas the lack (R = H, **41p**), or the presence of a small alkyl group (R = Me, **41c**) led to inactive compounds. Again in the indazole series, we observed a slight beneficial effect of a chlorine atom in the *N*-(p-methoxybenzyl) fragment (compare **41h** and **41g**). It is also noteworthy that i) indazoles that lack a CF_3 group on the benzo ring of indazoles are inactive (**41d** and **41o**), ii) the *N*-(3-chlorophenyl)aminoindazole **41f** was relatively potent and may be considered as a mimetic of MY 5445, as the cyclopentyl group could fully overlap the phenyl group in the structure of MY 5445.

Finally *N*-(p-methoxyphenylmethyl)phthalazines were revisited to investigate the beneficial effect of CF_3 on the benzo ring of the phthalazine scaffold (Table 6). This effect is clearly shown when comparing relative potencies of **16e** and **16h** (factor 30). The chlorine atom in **14h** could partially mimic lipophilicity of a phenyl ring (compare **16h** and **14h**). Moreover replacement of the phenyl ring (**16h**) by a piperidine (**21e**) is also well tolerated.

Table 6. Novel benzo-substituted phthalazines [a, b]



			PDE5	
Cpd	R_1	R_2	%inhib at 10µM	IC_{50} (µM)
16e	H	Ph	68	19.8
14h	CF_3	Cl	81	0.16
16h	CF_3	Ph	82	0.065
21d	CF_3	*N*-piperidino	79	0.35
4b (MY 5445)	-	-	42	6.7

a) Zaprinast as positive control PDE5-I (inhibition at 10µM: 90%, IC_{50} = 0.3 µM); b) Sildenafil as a second positive control PDE5-I (inhibition at1µM: 98%, IC_{50} = 3.3 nM).

Some inactive compounds are represented in Figure 3. They contribute to a better knowledge for pharmacophoric patterns for MY 5445 and other active structurally-related compounds. The lack of activity of *N*-methyl benzamide **27** and the “inverso” amides **29** and **28** support the critical need in the active compounds for both an H bond acceptor and a donor group for interaction within the pocket of PDE5.

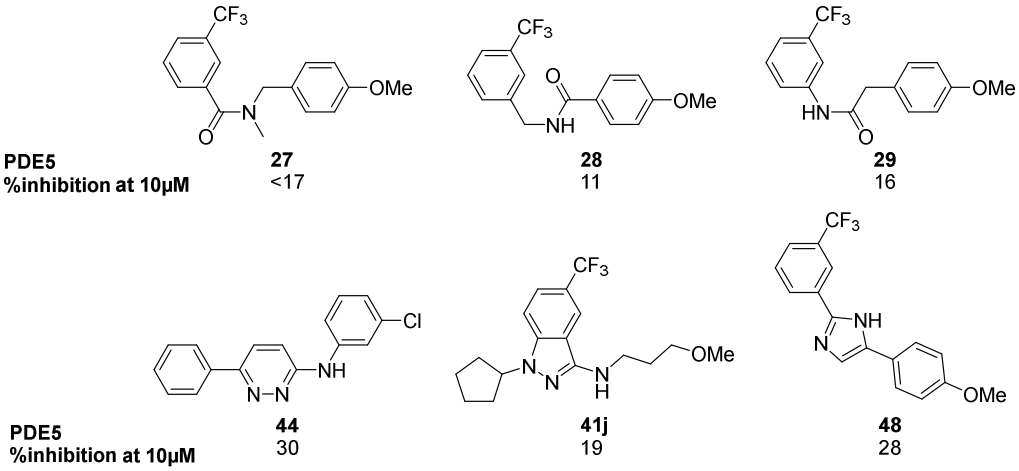


Figure 3. Other structurally-related compounds as inactive as PDE5 inhibitors.

PDE5 / PDE1-4 selectivity profiles

Sildenafil is a potent (IC_{50} close to 1nM) PDE5-I. However, it presents a moderate selectivity profile towards other PDE1-4 isoforms in particular PDE1 and PDE6 (ratios 120 and 7 respectively) [32, 37]. In general, preliminar evaluation of the selectivity profile towards other PDE isoenzymes (PDE 1, 2, 3 and 4) was satisfactory, as most of them did not show enzyme inhibition by more than 30% at 10 µM (see supporting information, raw data, table D). The selectivity profiles of the most potent PDE5 inhibitors in each chemical series are given in Table 7. We observed that MY 5445 is fairly selective towards other PDE1-PDE4 isoforms.The novel phthalazine **16h** and the amino indazole **41n** showed similar selectivity profiles when compared to MY 5445; all three compounds present a similar potency on PDE5, as well as PDE4. Unexpectedly the more flexible benzamide **25t** showed a 0.9 µM IC_{50} on PDE5, but presented no significant activity on other PDE's. Finally, MY 5445 and other selected PDE5-I congeners **16h** and **41n** were found fairly selective versus PDE4 inhibition (ratio close to 5). These disappointing results did not encourage us to further check their selectivity profiles.

Table 7. Selectivity towards other PDE 1 to 4 isoenzymes

	%inhib at 10µM (IC_{50} µM)[a]					
Compd	PDE1	PDE2	PDE3	PDE4	PDE5	PDE4/PDE5 ratio
4b (MY5445)	39 (>100)	25(>100)	14 (>100)	54 (37)	42 (6.7)[b]	5
16h	22	29	8	(0.32)	82 (0.065)	5
25t	27	26	10	24	77 (0.94)	-
41n	12	27	20	(4.4)	70 (1.8)	2

a) IC_{50} value determined with 1µM of cGMP. b IC_{50} = 0.6µM at 0.4µM of cGMP [33].

Physical properties of phthalazines and structurally-related compounds

Water solubility and comparative stabilities of phthalazines and indazoles in acidic medium of selected compounds

It is generally well accepted that a given lead under early stage development has to present a minimal water solubility of at least 0.1 mg/mL. Biological testing highlighted the very low water solubility of some of our compounds tested as PDE5 inhibitors. The water solubility of selected compounds representing phthalazines (MY 5445, **16e**, **16h**, **21e**), indazoles (**41f**, **41n**) and benzamides (**25l**, **26c**, **25g**) has been measured using a solubility test using a HPLC/UV method (see SI, table B). Phthalazines, indazoles and benzamides all have very low water solubility (0.01-0.06 mg/mL). Interestingly replacing the phenyl ring of phthalazines by a piperidine significantly (ten-fold) increased the water solubility (0.33 mg/mL), in agreement with criteria needed for further *in vivo* investigations.

The p-methoxy benzyl group (PMB) is a well-known protective group that can be easily removed in more or less acidic medium.[43] A rapid evaluation of the stability of both the phthalazines and indazoles series at pH 1 has been carried out at different reaction times (1-96 hours, see SI, Table C). The whole series of 3-aminophthalazines is stable in acidic medium, as about 100% of the starting products are still present in the acidic medium after 96 hours exposure. The stability of indazoles in acidic medium is moderate and strongly dependent on the nature of substituents R and R'. Most of them bearing a substituent R ≠ H or Me are not stable (about 30 – 50% of the compounds are *N*-deprotected at 96 hours). Surprisingly, the *N*-(3-pyridyl)indazole **41n** presents a good stability even after 4 days at pH 1, but no clear explanation could be advanced. Moreover, the role of the PMB is the main source of instability of these compounds, as supported by a completely restored chemical stability when replacing the PMB group by a substituted phenyl group (compare **41g** with **41f**).

We decided to test in an *in vivo* model of neuropathic pain our initial hit (MY 5445, **4b**) the most potent compound (based on activity toward PDE5, water solubility and acidic stability) of both scaffold phthalazine and indazole, corresponding respectively to **16h** and **41n**.

Anti-allodynic properties of selected PDE5-I (4b, 16h and 41n) in a neuropathic pain model in mice

As previously reported, cuff-implantation around the main branch of the right sciatic nerve induced an ispsilateral mechanical allodynia in mice, while sham surgery did not affect paw withdrawal thresholds [44-46] (Figure 3; surgery x paw x time interaction, $F_{8,128} = 1.8$, $p < 0.05$; post hoc: "Cuff Hydroxymethylcellulose right paw" < "Sham Hydroxymethylcellulose right paw" at $p < 0.01$ on treatment days 0 to 21 and "Cuff Hydroxymethylcellulose right paw" < "Cuff Hydroxymethylcellulose left paw" at $p < 0.01$ on treatment days 0 to 21). Two weeks after Cuff insertion, we started the treatments with **4b**, **16h**, **41n**, pregabalin or amitriptyline (3 and 0.5 mg/kg) or the control 0.1% hydroxymethylcellulose solution. The gabapentinoid pregabalin and the tricyclic antidepressant amitriptyline were chosen as reference treatments, as these drugs are clinically among the present first-line treaments for neuropathic pain. [47] Mice received two injections per day for around 3 weeks, and were tested on given days in the morning before drug injection. The **4b** treatment had no effect in sham mice, but it alleviated the cuff-induced allodynia after 15 days of treatment for the two tested doses (Figure 4; 3 mg/kg: paw x time interaction, $F_{8,64} = 4.5$, $p < 0.001$; post hoc: "Right paw" < "Left paw" at $p < 0.05$ on treatment days 0 to 12; 0.5 mg/kg: paw x time interaction, $F_{6,48} = 3.8$, $p < 0.01$; post hoc: "Right paw" < "Left paw" at $p < 0.01$ on treatment days 0 to 10). The same antiallodynic effect was also presented with **16h** (Figure 4; 3 mg/kg: group x time interaction, $F_{8,64} = 3.5$, $p < 0.01$; post hoc: "Right paw" < "Left paw" at $p < 0.05$ on treatment days 0 to 12; 0.5 mg/kg: paw x time interaction, $F_{6,48} = 3.1$, $p < 0.05$; post hoc: "Right paw" < "Left paw" at $p < 0.05$ on treatment days 0 to 10) and with **41n** (Figure 4; 3 mg/kg: paw x time interaction, $F_{8,64} = 2.8$, $p < 0.01$; post hoc: "Right paw" < "Left paw" at $p < 0.05$ on treatment days 0 to 12; 0.5 mg/kg: paw x time interaction, $F_{6,48} = 4.3$, $p < 0.01$; post hoc: "Right paw" < "Left paw" at $p < 0.01$ on treatment days 0 to 15). For the reference treatments pregabalin and amitriptyline, only the 3 mg/kg dose alleviated the cuff-induced allodynia, after 15 days of treatment for pregabalin (Figure 4; 3 mg/kg: paw x time interaction, $F_{7,70} = 6.8$, $p < 0.001$; post hoc: "Right paw" < "Left paw" at $p < 0.05$ on treatment days 0 to 12; 0.5 mg/kg: paw x time interaction, $F_{7,70} = 8.2$, $p < 0.01$; post hoc: "Right paw" < "Left paw" at $p < 0.001$ on treatment days 0 to 21) and at 21 days for amitriptyline (Figure 4; 3 mg/kg: paw x time interaction, $F_{7,70} = 3.5$, $p < 0.005$; post hoc: "Right paw" < "Left paw" at $p < 0.05$ on treatment days 0 to 18; 0.5 mg/kg: paw x time interaction, $F_{7,70} = 9.2$, $p < 0.001$; post hoc: "Right paw" < "Left paw" at $p < 0.001$ on treatment days 0 to 21). These results can be summarized by the area under the curve (AUC) histogram (Figure 4), showing that the positive controls pregabalin and amitriptyline were effective at 3 mg/kg only ($F_{1,48} = 6.9$, $p < 0.001$; post-hoc: "Cuff pregabalin 3 mg/kg" and "Cuff amitriptyline 3 mg/kg" > "Cuff hydroxymethylcellulose" at $p < 0.05$), while the 3 PDE5 inhibitors were effective at both doses ($F_{1,48} = 6.9$, $p < 0.001$; post-hoc: all groups > "Cuff hydroxymethylcellulose" at $p < 0.05$).

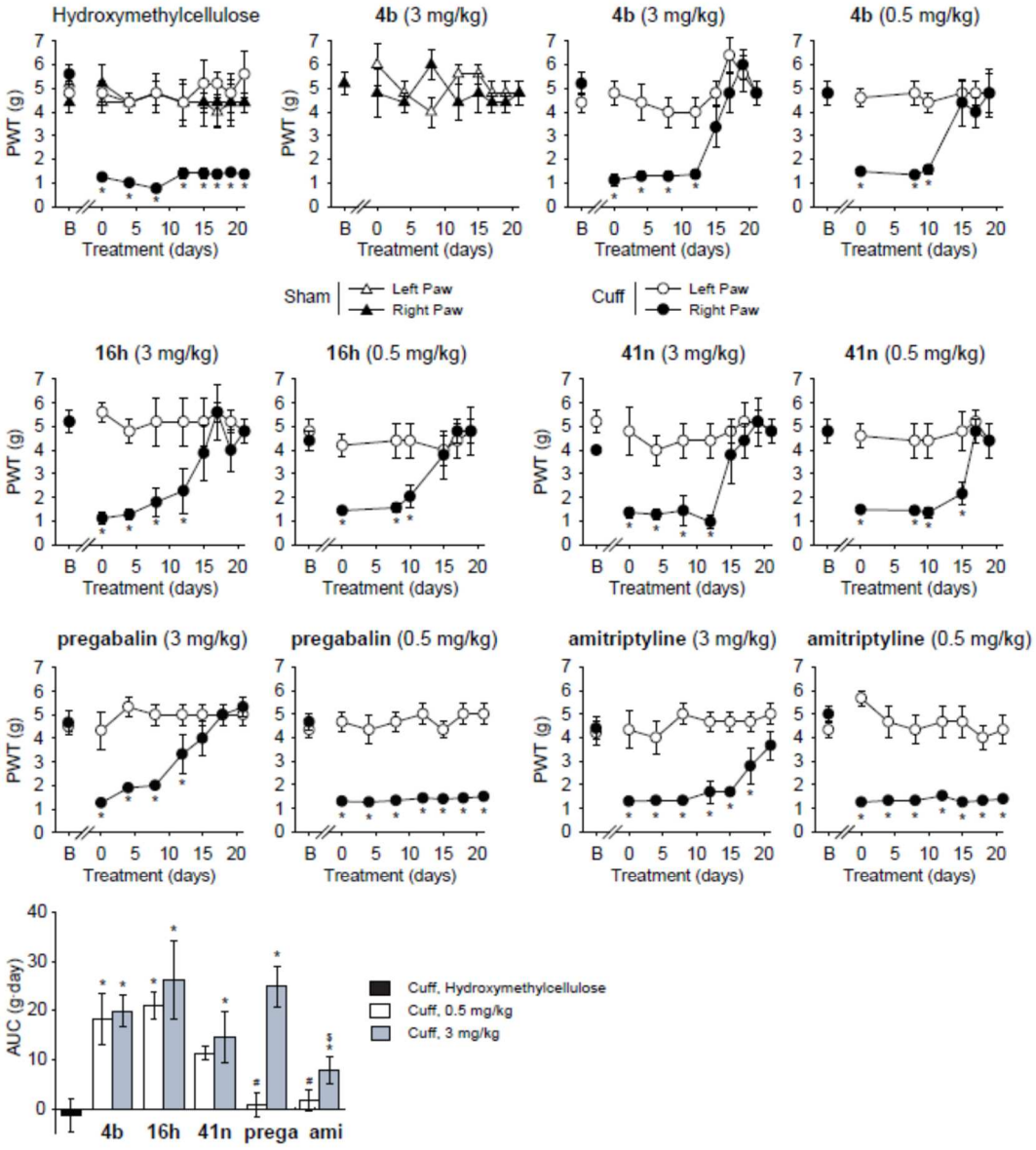


Figure 4. Anti-allodynic properties of MY 5445 **4b,** of the structurally-related compounds **16h** and **41n,** and of reference treatments pregabalin and amitriptyline, in a neuropathic pain model in mice. Two weeks after unilateral surgery on the right hindpaw, treatments started and were continued until 3 weeks. The mechanical threshold of hindpaw withdrawal was evaluated using von Frey filaments before surgery (B: baseline), and on given days during the chronic treatment. **4b**, **16h** and **41n** treatments (3 and 0.5 mg/kg, i.p., twice a day) reversed the mechanical allodynia in mice (n = 5 per group, $^{*}p < 0.05$ compared to contralateral left paw), without affecting thresholds of the controlateral paw (left paw). Pregabalin and amitriptyline treatments were effective at dose 3 mg/kg (i.p., twice a day, n = 6 per group, $^{*}p < 0.05$ compared to contralateral left paw) but not at dose 0.5 mg/kg (i.p., twice a day). The area under the curve (AUC) above pre-treatment values is calculated over 19 treatment days and is displayed in the last graph ($^{*}p < 0.05$ compared to Cuff hydroxymethylcellulose; $^{\#}p < 0.05$ compared to Cuff 4b, 16h and 41n at 0.5 mg/kg; $^{\$}p < 0.05$ compared to Cuff 16h and pregabalin at 3 mg/kg).

The above results show that a chronic treatment with these three PDE5 inhibitors relieved mechanical allodynia in an animal model of neuropathic pain, which is pain related to a lesion or disease of the somatosensory system.|48] The 2-week therapeutic delay accompanying this action is in agreement with therapeutic delays that are observed for various antidepressant and anticonvulsivant drugs [present data, 44, 47, 49-51] which are presently clinical first line treatments

for neuropathic pain. [44, 51] Moreover, present results suggest that these PDE5-I may be effective against neuropathic allodynia at relatively low doses, which is of therapeutic interest. In comparison, the 2 reference treatments required higher doses to relieve neuropathic allodynia in the animal model, and were ineffective at dose 0.5 mg/kg, whis is similar to previous report using another tricyclic antidepressant, nortriptyline [52]. Mechanistically, the action of the PDE5 inhibitors is likely related to the NO-cGMP pathway. While an increased NO production often accompanies neuropathic pain, and while general NO donors potentiate pain-related symptoms and NOS inhibitors display pain relieving action,[53–55] it has been evidenced that NO can have both pro- and antinociceptive functions.[56–58] In this regard, the literature converges to point out that the NO-cGMP pathway is in fact critical to the analgesic action of PDE5 inhibitors on pain. Indeed, previous studies have highlighted anti-hyperalgesic properties and/or neuroprotective properties of various PDE5 inhibitors, via NO and its downstream activation of the cGMP pathway, and through various subsequent downstream effectors [10-12, 14, 16, 18-20]. Those effectors contributing to pain relief include protein kinase G1 [11, 16], ATP-sensitive K^+ channels and Ca^{++}-activated K^+ channels [11-12, 20], and Ca^{++} channels [18]. These pathways mediate peripheral sildenafil and vardenafil analgesia [10,14,18] as well as the systemic action of sildenafil and of tadalafil in experimental hyperalgesia.[10,59] The action of PDE5 inhibitors on pain requires inhibitory neuronal transmission, in particular GABA receptors [15], and it has also been associated with an attenuation of oxidative stress and of inflammatory cytokines, such as IL-1β, IL-6, IL-18, IL-1rα and TNFα, [19,60-62] which is relevant in the context of neuropathic pain.[47, 53, 61, 63]

3-Conclusion

In conclusion, the proof of concept supporting the therapeutic use of PDE5 inhibitors for the treatment of neuropathic pain including sildenafil and other zaprinast structurally-related compounds has justified further work on different classes of PDE5-I, in particular the phthalazines (MY 5445, **5** and isosteric **6**). SAR data showed the pharmacophoric elements of 3-aminophthalazines and other deriving analogues (benzamides, aminopyrazoles). Novel compounds were demonstrated as acceptable mimetics of MY 5445 with similar potencies (versus PDE5) and selectivity profiles (PDE1-4). Moreover, a couple of PDE5-I (**16h**, **41n**) showed similar *in vivo* efficacy, when compared with MY 5445, in the neuropathic pain model in mice. In the animal model, this action was even present for doses lower than present reference treatments of neuropathic pain. In addition, the replacement of the phenyl ring in phthalazines (compare MY 5445 and **21d**) increased significantly their water solubility. The continued discovery of novel MY 5445 structurally related compounds will help to enhance the quality of novel leads with increased metabolic stability, water solubility and selectivity, in particular versus PDE4.

4-Experimental Section

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 were 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 0.040-0.063nm) or using Armen spot flash chromatography (normal phase column: Interchim 30 SHIP 25g; reverse phase column: AIT 50g 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 ^{1}H NMR or HPLC. ^{1}H, ^{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 x 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 to 1 min: 50 to 60% of B then t = 1 min to t = 10 min: 60 to 100% of B and t = 10 min to t = 15 min: 100% of B.
Microwave irradiation was performed with a Biotage Initiator EXP (external sensor type).

Pd-Catalyzed Suzuki-Miyaura cross-coupling using $Pd(PPh_3)_4$ in a mixture toluene:EtOH:H_2O: General method A for the preparation of **4a**, **4c-f**, **10**, **16a-h**, **17a-d**. A microvawe vial under argon was charged with the corresponding halogeno derivatives (1.0 equiv.), the corresponding arylboronic acid (1.1 to 1.5 equiv.), $Pd(PPh_3)_4$ (5 mol%), Na_2CO_3 (3.0 equiv.) and a mixture toluene:ethanol:water (5:1:1, 0.9 mmol/mL). The vial was capped properly, flushed with argon and heated to 120°C until complete conversion of the starting material. After cooling at room temperature, the reaction mixture was concentrated under vacuum. The crude residue was diluted with 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, using the appropriate heptanes: EtOAc mixture.

Nucleophilic substitution of dichlorophthalazine 12a with aniline derivatives: General method B for the preparation of **13a-e**. A mixture of 2,6-dichlorophthalazine **12a** (1.0 equiv.) and the corresponding aniline derivative (1.0 equiv.) in *i*PrOH (0.3 mmol/mL) was heated at 100°C for 1 hour. After cooling at room temperature, the reaction media was concentrated and purified by silica gel column chromatography using the appropriate heptanes: EtOAc mixture.

Nucleophilic substitution of dichlorophthalazine 12a with benzylamine and phenethylamine derivatives: **General method C** for the preparation of compounds **14a-g** and **15a-d**. A mixture of 2,6-dichlorophthalazine **12a** (1.0 equiv.), the appropriate benzylamine or phenethylamine derivative (1.1 equiv.) and Na_2CO_3 (2.0 equiv.) in DMF (0.3 mmol/mL) was heated overnight at 130°C. After cooling, the reaction media was diluted with water and the aqueous 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, using the appropriate heptanes: EtOAc mixture**.**

Nucleophilic substitution of chlorophthalazine with NH heterocycles: General method D for the preparation of compounds **20** and **21 a-d.** A mixture of **13** or **14** (1.0 equiv.), the appropriate NH heterocycles (5.2 equiv.) and DIEA (5.1 equiv.) in NMP (0.3 mmol/mL) was heated overnight at 170°C. After cooling, the reaction media was diluted with water and the aqueous 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 using the appropriate heptanes: EtOAc mixture**.**

Bromation of indazole derivatives 39: General method E for the preparation of compounds **39a-c**. A solution of the corresponding indazole derivative **38** (1.0 equiv.), in an aqueous solution of NaOH 5M (16.1 equiv.) and DMF (4.8 equiv.) was cooled at 0°C and Br_2 (1.3 equiv.) was added dropwise. The resulting mixture was stirred at rt for 8 hours. A saturated solution of $Na_2S_2O_3$ was added to neutralize the excess of Br_2. The aqueous phase was extracted 3 times with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel column chromatography, using the appropriate heptanes:EtOAc mixture.

Protection of indazole derivatives using a THP function: General method F for the preparation of compounds **40a-b.** A solution of the correspond indazole (1.0 equiv.), DHP (2.0 equiv.) and PTSA (5 mol%) in EtOAc (0.7 mmol/mL) was heated overnight at 95°C. After cooling, the solution was concentrated under vacuum. The residue was diluted with water and the aqueous phase was extracted 3 times with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel column chromatography, using the appropriate heptanes:EtOAc mixture.

N-Alkylation of indazole derivatives using secondary alkyl bromides: General method G for the preparation of compounds **40d-g.** A solution of the corresponding indazole (1.0 equiv.), TBAI (3 mol%) and K_2CO_3 (4.5 equiv.) in DMF (1.3 mmol/mL) was heated at 90°C and the corresponding bromoalkyl derivative (1.3 equiv.) was added dropwise. The resulting mixture was stirred overnight at 90°C. After cooling, the solution was diluted with water and the aqueous phase was extracted 3 times with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel column chromatography, using the appropriate heptanes:EtOAc mixture.

Cu-Catalyzed Ullmann cross-coupling: General method H for the preparation of compounds **40i-j.** A microvawe vial (ovendried and under argon) was charged with the corresponding indazole derivative (1.0 equiv.), the iodo compound (3.0 equiv.), CuI (5 mol%), trans-*N*,*N*'-dimethylcyclohexane-1,2-diamine (20 mol%), K_2CO_3 (3.0 equiv.) and anhydrous toluene (0.9 mmol/mL). The vial was capped properly, flushed with argon and heated to 130°C overnight. After cooling, the reaction mixture was concentrated under vacuum. The crude residue was diluted with 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 using the appropriate heptanes:EtOAc mixture.

Pd-Catalyzed Buchwald-Hartwig cross-coupling using $Pd(OAc)_2$ and JosiPhos: General method I for the preparation of compounds **41a-c**, **41g**, **41i-j**, **41l.** A microvawe vial (ovendried and under argon) was charged with the corresponding bromoindazole derivative (1.0 equiv.), the appropriate amine (1.2 equiv.), $Pd(OAc)_2$ (5 mol%), JosiPhos (5 mol%), Cs_2CO_3 (1.5 equiv.) and anhydrous dioxane (0.2 mmol/mL). The vial was capped properly, flushed with argon and heated to 120°C overnight. After cooling, the reaction mixture was concentrated under vacuum. The crude residue was diluted with water and the aqueous 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, using the appropriate heptanes:EtOAc mixture.

Pd-Catalyzed Buchwald-Hartwig cross-coupling using $Pd(OAc)_2$ and BrettPhos: General method J for the preparation of compounds **41d-f**, **41h-k**, **41m-n.** A microvawe vial (ovendried and under argon) was charged with the corresponding bromoindazole derivative (1.0 equiv.), the appropriate amine (1.2 equiv.), $Pd(OAc)_2$ (5 mol%), BrettPhos (10 mol%), Cs_2CO_3 (1.5 equiv.) and anhydrous dioxane (0.2 mmol/mL). The vial was capped properly, flushed with argon and heated to 120°C overnight. After cooling, the reaction mixture was concentrated under vacuum. The crude residue was diluted with water and the aqueous 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, using the appropriate heptanes:EtOAc mixture.

***N*,4-Diphenylphthalazin-1-amine (4a).** Following general method A and starting from **13a** (15 mg, 0.059 mmol) and phenylboronic acid (8 mg, 0.065 mmol), **4a** was obtained as a white solid (14 mg, 0.046 mmol, 78%). Purity ≥ 98 %; mp = 232-234 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.05 (t, 1H, J = 7.3 Hz), 7.38 (t, 2H, J = 7.8 Hz), 7.52-7.61 (m, 3H), 7.67 (dd, 2H, J = 1.8 Hz, J = 8.2 Hz), 7.88-7.97 (m, 4H), 8.03 (td, 1H, J = 0.9 Hz, J = 7.2 Hz), 8.67 (d, 1H, J = 8.2 Hz), 9.26 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 120.7, 122.0, 122.5, 125.5, 125.8, 128.1, 128.3, 129.4, 131.3, 131.9, 134.0, 136.5, 140.4, 151.6, 161.4.

***N*-(4-Chlorophenyl)-4-phenylphthalazin-1-amine (4c).** Following general method A and starting from **13c** (100 mg, 0.34 mmol) and phenylboronic acid (46 mg, 0.37 mmol), **4c** was obtained as a yellow solid (114 mg, 0.34 mmol, 100%). Purity = 95 %; mp = 202-206 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.42 (d, 2H, J = 8.9 Hz), 7.54-7.61 (m, 3H), 7.67 (dd, 2H, J = 1.4 Hz, J = 8.0 Hz), 7.89 (dd, 1H, J = 0.9 Hz, J = 8.2 Hz), 7.95 (td, 1H, J = 0.9 Hz, J = 8.2 Hz), 8.02-8.06 (m, 3H), 8.66 (d, 1H, J = 8.3 Hz), 9.39 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 118.4, 122.1, 122.6, 125.5, 125.7, 125.8, 128.2, 128.3, 128.5, 129.6, 131.6, 132.2, 136.6, 139.6, 151.6, 153.6.

***N*-(4-Methoxyphenyl)-4-phenylphthalazin-1-amine (4d).** Following general method A and starting from **13d** (100 mg, 0.35 mmol) and phenylboronic acid (51 mg, 0.52 mmol), **4d** was obtained as a yellow solid (102 mg, 0.31 mmol, 89%). Purity ≥ 98 %; mp = 132-134 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.83 (s, 3H), 7.13 (d, 2H, J = 8.3 Hz), 7.56 (d, 2H, J = 8.3 Hz), 7.62-7.71 (m, 5H), 7.98 (d, 1H, J = 7.8 Hz), 8.18 (t, 1H, J = 7.5 Hz), 8.26 (t, 1H, J = 7.5 Hz), 8.92 (d, 1H, J = 7.9 Hz), 11.14 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 55.9, 115.5, 121.5, 125.2, 127.4, 127.5, 128.4, 128.6, 129.4, 130.2, 130.7, 133.0, 134.6, 135.9, 152.32 152.8, 158.9.

***N*-(3-Chlorophenyl)-4-(4-chlorophenyl)phthalazin-1-amine (4e).** Following general method A and starting from **13b (**100 mg, 0.34 mmol) and 4-chlorophenylboronic acid (59 mg, 0.38 mmol), **4e** was obtained as a white solid (86 mg, 0.23 mmol, 68%). Purity ≥ 98 %; mp = 219-224°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.09 (dd, 1H, J = 1.9 Hz, J = 7.7 Hz), 7.40 (t, 1H, J = 8.2 Hz), 7.64 (d, 2H, J = 8.5 Hz), 7.72 (d, 2H, J = 8.5 Hz), 7.89 (d, 2H, J = 8.3 Hz), 7.97 (t, 1H, J = 7.7 Hz), 8.06 (t, 1H, J = 8.3 Hz), 8.27 (t, 1H, J = 1.9 Hz), 8.67 (d, 1H, J = 8.3 Hz), 9.47 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 118.4, 118.8, 119.8, 121.6, 122.6, 125.6, 125.6, 128.5, 123.0, 131.5, 131.8, 132.5, 132.8, 133.5, 135.4, 142.1, 151.7, 152.9.

***N*-(3-Chlorophenyl)-4-(4-methoxyphenyl)phthalazin-1-amine (4f).** Following general method A and starting from **13b** (100 mg, 0.34 mmol) and 4-methoxyphenylboronic acid (58 mg, 0.38 mmol), **4f** was obtained as a white solid (92 mg, 0.26 mmol, 74%). Purity ≥ 98 %; mp = 233-234°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.03 (s, 3H), 6.24 (dd, 1H, J = 1.3 Hz, J = 7.9 Hz), 6.31 (d, 2H, J = 8.7 Hz), 6.56 (t, 1H, J = 8.2 Hz), 6.79 (d, 2H, J = 8.7 Hz), 7.05 (dd, 1H, J = 1.3 Hz, J = 8.3 Hz), 7.10-7.15 (m, 2H), 7.19-7.23 (m, 1H), 7.49 (t, 1H, J = 2.0 Hz), 7.81 (d, 1H, J = 8.3 Hz), 8.57 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 55.2, 113.9, 118.6, 118.7, 119.6, 121.4, 122.5, 125.8, 126.0, 128.8, 130.0, 131.0, 131.6, 132.2, 132.7, 142.3, 151.3, 153.6, 159.6.

***N*-(3-Chlorophenyl)isoquinoline (8).** A mixture of 1-chloro-isoquinoline **7** (100 mg, 0.61 mmol, 1.0 equiv.) and 3-chloroaniline (78.0 µL, 0.73 mmol, 1.2 equiv.) in NMP (0.6 mL) was heated overnight at 110°C. After cooling, the reaction mixture was diluted with water and the aqueous phase was extracted twice with AcOEt. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel column chromatography (heptanes:EtOAc 9:1), to yield **8** as a white solid (121 mg, 0.48 mmol, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.02 (ddd, 1H, J = 1.0 Hz, J = 2.0 Hz, J = 8.2 Hz), 7.13 (s, 1H), 7.18 (d, 1H, J = 5.8 Hz), 7.26 (t, 1H, J = 8.2 Hz), 7.49 (ddd, 1H, J = 1.0 Hz, J = 2.0 Hz, J = 8.2 Hz), 7.56 (ddd, 1H, J = 1.0 Hz, J = 6.9 Hz, J = 8.2 Hz), 7.66 (ddd, 1H, J = 1.0 Hz, J = 6.9 Hz, J = 8.2 Hz), 7.77 (d, 1H, J = 8.2 Hz), 7.87 (t, 1H, J = 2.0 Hz), 7.91 (d, 1H, J = 8.2 Hz), 8.12 (d, 1H, J = 5.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 114.1, 117.9, 119.8, 121.2, 122.4, 126.7, 127.6, 129.9, 130.0, 134.6, 137.5, 140.8, 141.8.

4-Bromo-*N*-(3-chlorophenyl)isoquinolin-1-amine (9). A mixture of **8** (77 mg, 0.30 mmol, 1.0 equiv.) in THF (0.5 mL) was cooled at 0°C. A solution of phenyltrimethylammonium tribromide (103 mg, 0.27 mmol, 0.9 equiv.) in THF (1.0 mL) was added dropwise in the previous solution. The reaction media was stirred at rt for 6 hours and concentrated under vacuum. The residue was diluted with water and the aqueous phase was extracted twice with EtOAc. The combined organic layers were washed with a saturated solution of $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel column chromatography (heptanes:EtOAc 9:1), to yield **9** as an orange solid (101 mg, 0.30 mmol, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.05 (d, 1H, J = 8.0 Hz), 7.11 (s, 1H), 7.17 (t, 1H, J = 8.0 Hz), 7.47 (d, 1H, J = 8.4 Hz), 7.64 (t, 1H, J = 8.0 Hz), 7.79 (t, 1H, J = 8.4 Hz), 7.83 (s, 1H), 7.90 (d, 1H, J = 8.4 Hz), 8.15 (d, 1H, J = 8.4 Hz), 8.29 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 110.3, 118.1, 120.1, 121.5, 122.9, 127.1, 127.6, 129.9, 131.2, 134.6, 135.7, 141.2, 142.2, 151.1.

***N*-(3-Chlorophenyl)-4-phenylisoquinolin-1-amine (10).** Following general method A and starting from **9** (60 mg, 0.18 mmol) and phenylboronic acid (26 mg, 0.22 mmol), **10** was obtained as a white solid (54 mg, 0.16 mmol, 90%). Purity = 95 %; mp = 202-204°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.20 (d, 1H, J = 7.0 Hz), 7.45-7.55 (m, 6H), 7.76-7.83 (m, 5H), 8.03 (s, 1H), 8.69 (d, 1H, J = 7.7 Hz); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 118.8, 121.0, 122.0, 123.8, 124.8, 125.3, 126.0, 127.8, 128.2, 129.2, 130.4, 131.0, 132.5, 133.7, 135.9, 136.7, 152.0, 170.8; HRMS (M+H$^+$) 331.0992 (calcd for $C_{21}H_{15}ClN_2H$+ 331.0997).

1,4-Dichlorophthalazine (12a). A mixture of phthalhydrazide **11a** (2.50 g, 15.42 mmol, 1.0 equiv.) and pyridine (2.49 mL, 30.80 mmol, 2.0 equiv.) in $POCl_3$ (50.3 mL) was heated at reflux for 1 hour. After cooling, the reaction mixture was concentrated under vacuum. The residue was dissolved in cooled DCM and poured slowly into ice-cold water. The organic phase was separated and the aqueous phase was extracted twice with DCM. The combined organic layers were washed with a saturated solution of $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, concentrated under vacuum and purified by silica gel column chromatography (heptanes:EtOAc 3:2), to yield **12a** as a white solid (2.84 g, 14.27 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.05-8.07 (m, 2H), 8.30-8.32 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 126.2, 127.7, 134.8, 155.4.

1,4-Dichloro-6-(trifluoromethyl)phthalazine (12b). A mixture of **11b** (176 mg, 0.76 mmol, 1.0 equiv.) and pyridine (123.6 µL, 1.53 mmol, 2.0 equiv.) in $POCl_3$ (2.5 mL) was heated at reflux for 1 hour. After cooling, the reaction mixture was concentrated under vacuum. The residue was dissolved in cooled DCM and poured slowly into ice-cold wate. The organic phase was separated and the aqueous phase was extracted twice with DCM. The combined organic layers were washed with a saturated solution of $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, concentrated under vacuum and purified by silica gel column chromatography (heptanes:EtOAc 7:3 to yield **12b** as a white solid (186 mg, 0.70 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.26 (dd, 1H, J = 1.8 Hz, J = 8.7 Hz), 8.49 (dt, 1H, J = 0.8 Hz, J = 8.7 Hz), 8.61 (t, 1H, J = 0.8 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ ppm -63.1; ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 121.5, 123.9 (q, J = 4.4 Hz), 124.2, 127.2, 127.7, 128.9, 130.6 (q, J = 2.9 Hz), 136.5, 155.2 (d, J = 34.5 Hz).

4-Chloro-*N*-phenylphthalazin-1-amine (13a). Following general method B and starting from 1,4-dichlorophthalazine **12a** (150 mg, 0.75 mmol) and aniline (68.7 µL, 0.75 mmol), **13a** was obtained as a white solid (34 mg, 0.13 mmol, 18%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.06 (t, 1H, J = 7.4 Hz), 7.38 (t, 1H, J = 7.4 Hz), 7.87 (dd, 2H, J = 1.1 Hz, J = 8.4 Hz), 8.07-8.13 (m, 2H), 8.16-8.19 (m, 1H), 8.67 (dd, 1H, J = 2.0 Hz, J = 6.5 Hz), 9.34 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 121.0, 121.8, 123.2, 123.7, 125.2, 126.2, 128.9, 133.5, 133.8, 140.5, 146.9, 153.3.

4-Chloro-*N*-(3-chlorophenyl)phthalazin-1-amine (13b). Following general method B and starting from 1,4-dichlorophthalazine **12a** (100 mg, 0.50 mmol) and 3-chloroaniline (53.4 µL, 0.50 mmol), **13b** was obtained as a white solid (146 mg, 0.50 mmol, 100%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.11 (dd, 1H, J = 1.8 Hz, J = 7.8 Hz), 7.39 (t, 1H, J = 8.2 Hz), 7.82 (dd, 1H, J = 1.8 Hz, J = 7.8 Hz), 8.08-8.16 (m, 3H), 8.19 (dd, 1H, J = 2.0 Hz, J = 7.3 Hz), 8.67 (dd, 1H, J = 2.0 Hz, J = 7.3 Hz), 9.50 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 119.2, 120.1, 120.5, 122.1, 123.2, 124.8, 125.6, 130.0, 132.7, 133.1, 133.5, 141.5, 147.1, 152.6.

4-Chloro-*N*-(4-chlorophenyl)phthalazin-1-amine (13c). Following general method B and starting from 1,4-dichlorophthalazine **12a** (100 mg, 0.50 mmol) and 4-chloroaniline (64.1 mg, 0.50 mmol), **13c** was obtained as a white solid (142 mg, 0.49 mmol, 98%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.42 (d, 2H, J = 8.9 Hz), 7.93 (d, 2H, J = 8.9 Hz), 8.07-8.14 (m, 2H), 8.18 (dd, 1H, J = 2.1 Hz, J = 7.4 Hz), 8.65 (dd, 1H, J = 2.1 Hz, J = 7.4 Hz), 9.45 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 121.0, 123.1, 123.7, 125.3, 126.1, 126.7, 128.8, 133.6, 134.0, 139.5, 147.3, 153.1.

4-Chloro-*N*-(4-methoxyphenyl)phthalazin-1-amine (13d). Following general method B and starting from 1,4-dichlorophthalazine **12a** (100 mg, 0.50 mmol) and 4-methoxyaniline (58.4 µL, 0.50 mmol), **13d** was obtained as a yellow solid (126 mg, 0.44 mmol, 88%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.77 (s, 3H), 6.96 (d, 2H, J = 8.9 Hz), 7.72 (d, 2H, J = 8.9 Hz), 8.05-8.10 (m, 2H), 8.13 (dd, 1H, J = 2.1 Hz, J = 7.0 Hz), 8.62 (dd, 1H, J = 1.4 Hz, J = 7.2 Hz), 9.23 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 54.9, 113.4, 120.0, 122.8, 123.2, 124.4, 125.3, 132.5, 132.6, 132.9, 145.4, 152.6, 155.0.

***N*-Benzyl-4-chlorophthalazin-1-amine (14a).** Following general method C and starting from 1,4-dichlorophthalazine **12a** (100 mg, 0.50 mmol) and benzylamine (60.4 µL, 0.55 mmol), **14a** was obtained as a white solid (94 mg, 0.35 mmol, 70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.78 (d, 2H, J = 5.8 Hz), 7.22 (t, 1H, J = 7.2 Hz), 7.31 (t, 1H, J = 7.4 Hz), 7.39 (d, 2H, J = 7.4 Hz), 7.99-8.02 (m, 2H), 8.05-8.08 (m, 1H), 8.32 (t, 1H, J = 5.5 Hz), 8.42-8.45 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 44.1, 119.9, 122.8, 124.5, 125.2, 126.5, 127.2, 128.1, 132.5, 132.9, 139.6, 144.3, 153.8.

4-Chloro-*N*-[(3-chlorophenyl)methyl]phthalazin-1-amine (14b). Following general method C and starting from 1,4-dichlorophthalazine **12a** (100 mg, 0.50 mmol) and 3-chlorobenzylamine (68.1 µL, 0.55 mmol), **14b** was obtained as a white solid (90 mg, 0.30 mmol, 59%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.77 (d, 2H, J = 5.9 Hz), 7.27-7.30 (m, 1H), 7.32-7.37 (m, 2H), 7.44 (s, 1H), 8.00-8.05 (m, 2H), 8.06-8.10 (m, 1H), 8.36 (t, 1H, J = 5.9 Hz), 8.40-8.43 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 44.2, 120.4, 123.3, 125.1, 125.7, 126.4, 127.0, 127.5, 130.6, 133.2, 133.3, 133.5, 142.9, 145.1, 154.2.

4-Chloro-*N*-[(2-methoxyphenyl)methyl]phthalazin-1-amine (14c). Following general method C and starting from 1,4-dichlorophthalazine **12a** (100 mg, 0.50 mmol) and 2-methoxybenzylamine (72.2 µL, 0.55 mmol), **14c** was obtained as a yellow solid (96 mg, 0.32 mmol, 64%). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 3.85 (s, 3H), 4.74 (d, 2H, J = 5.6 Hz), 6.84 (td, 1H, J = 0.9 Hz, J = 7.4 Hz), 7.01 (d, 1H, J = 8.0 Hz), 7.18-7.24 (m, 2H), 8.00 (m, 2H), 8.07-8.09 (m, 1H), 8.13 (t, 1H, J = 5.8 Hz), 8.46-8.48 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 39.9, 55.8, 110.9, 120.4, 120.5, 123.4, 125.0, 125.8, 127.2, 127.7, 128.2, 133.1, 133.4, 144.7, 154.5, 157.3.

4-Chloro-*N*-[(3-methoxyphenyl)methyl]phthalazin-1-amine (14d). Following general method C and starting from 1,4-dichlorophthalazine **12a** (100 mg, 0.50 mmol) and 3-methoxybenzylamine (70.9 µL, 0.55 mmol), **14d** was obtained as a yellow solid (118 mg, 0.39 mmol, 79%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.71 (s, 3H), 4.75 (d, 2H, J = 5.9 Hz), 6.79 (dd, 1H, J = 2.4 Hz, J = 8.0 Hz), 6.95-6.97 (m, 2H), 7.22 (t, 1H, J = 8.0 Hz), 7.99-8.02 (m, 2H), 8.06-8.09 (m, 1H), 8.29 (t, 1H, J = 5.9 Hz), 8.42-8.45 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 44.6, 55.4, 112.3, 113.6, 119.9, 120.4, 123.3, 125.0, 125.8, 129.7, 133.1, 133.5, 141.8, 144.8, 154.4, 159.7.

4-Chloro-*N*-[(4-methoxyphenyl)methyl]phthalazin-1-amine (14e). Following general method C and starting from 1,4-dichlorophthalazine **12a** (100 mg, 0.50 mmol) and 4-methoxybenzylamine (72.2 µL, 0.55 mmol), **14e** was obtained as a white solid (128 mg, 0.43 mmol, 85%). Purity ≥ 98 %; mp = 154°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.71 (s, 3H), 4.70 (d, 2H, J = 5.8 Hz), 6.87 (d, 2H, J = 8.7 Hz), 7.32 (d, 2H, J = 8.7 Hz), 7.98-8.03 (m, 2H), 8.06-8.09 (m, 1H), 8.23 (t, 1H, J = 5.8 Hz), 8.40-8.43 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 43.6, 54.9, 113.5, 119.9, 122.8, 124.4, 125.2, 128.6, 131.4, 132.5, 132.8, 144.1, 153.7, 158.1.

4-Chloro-*N*-[(3-chloro-4-methoxyphenyl)methyl]phthalazin-1-amine (14f). Following general method C and starting from 1,4-dichlorophthalazine **12a** (100 mg, 0.50 mmol) and 3-chloro-4-methoxybenzylamine (80.4 µL, 0.55 mmol), **14f** was obtained as a yellow solid (104 mg, 0.31 mmol, 62%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.81 (s, 3H), 4.69 (d, 2H, J = 5.8 Hz), 7.08 (d, 1H, J = 8.4 Hz), 7.34 (dd, 1H, J = 2.1 Hz, J = 8.4 Hz), 7.45 (d, 1H, J = 2.1 Hz), 7.99-8.02 (m, 2H), 8.06-8.08 (m, 1H), 8.28 (t, 1H, J = 5.8 Hz), 8.38-8.41 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 43.2, 56.0, 112.5, 119.9, 120.5, 122.8, 124.5, 125.3, 127.4, 128.9, 132.7, 132.9, 133.0, 144.4, 153.3, 153.8.

***N*-(2H-1,3-Benzodioxol-5-ylmethyl)-4-chlorophthalazin-1-amine (14g).** Following general method C and starting from 1,4-dichlorophthalazine **12a** (100 mg, 0.50 mmol) and piperonylamine (69.0 µL, 0.55 mmol), **14g** was obtained as a white solid (151 mg, 0.48 mmol, 96%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.67 (d, 2H, J = 5.8 Hz), 5.96 (s, 2H), 6.84 (d, 1H, J = 7.9 Hz), 6.88 (dd, 1H, J = 1.4 Hz, J = 7.9 Hz), 6.97 (d, 1H, J = 1.4 Hz), 7.99-8.02 (m, 2H), 8.05-8.08 (m, 1H), 8.23 (t, 1H, J = 5.8 Hz), 8.39-8.42 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 43.9, 100.6, 107.8, 108.0, 119.8, 120.4, 122.7, 124.4, 125.1, 132.5, 132.8, 133.4, 144.2, 145.8, 147.0, 153.7.

4-Chloro-*N*-[(4-methoxyphenyl)methyl]-7-(trifluoromethyl)phthalazin-1-amine (14h). A mixture of **12b** (150 mg, 0.56 mmol, 1.0 equiv.), 4-methoxybenzylamine (88.1 µL, 0.67 mmol, 1.2 equiv.) and dbu (208.9 µL, 1.40 mmol, 2.5 equiv.) in NMP (1.2 mL) was stirred at rt for 2 hours. After cooling, the reaction mixture was diluted with water. The aqueous phase was extracted 3 times with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel column chromatography (heptanes:EtOAc 9:1 to 7:3), to yield **14h** as a yellow solid (105 mg, 0.29 mmol, 51%). Purity ≥ 95 %; mp = 138-142 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.77 (s, 3H), 4.79 (d, 1H, J = 5.0 Hz), 5.67 (s, 1H), 6.85 (d, 2H, J = 8.7 Hz), 7.36 (d, 2H, J = 8.7 Hz), 8.06 (dd, 1H, J = 1.3 Hz, J = 8.7 Hz), 8.11 (s, 1H), 8.31 (d, 1H, J = 8.7 Hz); ^{19}F NMR (376 MHz, CDCl3) δ ppm -62.7; ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 46.1, 55.5, 114.3, 119.2 (q, J = 3.7 Hz), 127.3, 128.5 (q, J = 3.7 Hz), 130.1, 130.2, 133.8, 134.1, 159.5; HRMS ($M+H^+$) 368.0769 (calcd for $C_{17}H_{13}F_3N_3OH^+$ 368.0772).

4-Chloro-*N*-(2-phenylethyl)phthalazin-1-amine (15a). Following general method C and starting from 1,4-dichlorophthalazine **12a** (80 mg, 0.40 mmol) and phenethylamine (55.7 µL, 0.44 mmol), **15a** was obtained as a white solid (65 mg, 0.23 mmol, 57%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.01 (t, 2H, J = 7.6 Hz),3.76 (q, 2H, J = 7.6 Hz), 7.19-7.23 (m, 1H), 7.27-7.32 (m, 4H), 7.84 (t, 1H, J = 5.2 Hz), 7.98-8.02 (m, 2H), 8.06-8.08 (m, 1H), 8.32-8.35 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 34.7, 43.3, 120.5, 123.2, 125.0, 125.7, 126.5, 128.8, 129.2, 133.0, 133.3, 140.3, 144.5, 154.4.

4-Chloro-*N*-[2-(3-chlorophenyl)ethyl]phthalazin-1-amine (15b). Following general method C and starting from 1,4-dichlorophthalazine **12a** (100 mg, 0.50 mmol) and 2-(3-chlorophenyl)ethylamine (76.9 µL, 0.55 mmol), **15b** was obtained as a white solid (125 mg, 0.39 mmol, 78%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.02 (t, 2H, J = 7.0 Hz), 3.77 (q, 2H, J = 7.0 Hz), 7.23-7.27 (m, 2H), 7.31 (d, 1H, J = 7.5 Hz), 7.36 (s, 1H), 7.83 (t, 1H, J = 5.4 Hz), 7.98-8.00 (m, 2H), 8.06-8.08 (m, 1H), 8.31-8.34 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 34.2, 42.9, 120.4, 123.2, 125.0, 125.7, 126.5, 128.0, 129.0, 130.6, 133.0, 133.4, 143.0, 144.6, 154.4.

4-Chloro-*N*-[2-(4-chlorophenyl)ethyl]phthalazin-1-amine (15c). Following general method C and starting from 1,4-dichlorophthalazine **12a** (200 mg, 1.00 mmol) and 2-(4-chlorophenyl)ethylamine (154.7 µL, 1.11 mmol), **15c** was obtained as a white solid (235 mg, 0.74 mmol, 73%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.01 (t, 2H, J = 7.2 Hz), 3.75 (q, 2H, J = 7.2 Hz), 7.31 (d, 2H, J = 8.4 Hz), 7.35 (d, 2H, J = 8.4 Hz), 7.81 (t, 1H, J = 5.3 Hz), 7.99-8.01 (m, 2H), 8.07-8.09 (m, 1H), 8.31-8.34 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 33.9, 43.0, 120.5, 123.2, 125.0, 125.7, 128.7, 131.1, 133.0, 133.4, 144.6, 154.5.

4-Chloro-*N*-[2-(4-methoxyphenyl)ethyl]phthalazin-1-amine (15d). Following general method C and starting from 1,4-dichlorophthalazine **12a** (100 mg, 0.50 mmol) and 2-(4-methoxyphenyl)ethylamine (81.1 µL, 0.55 mmol), **15d** was obtained as a white solid (134 mg, 0.43 mmol, 85%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.94 (t, 2H, J = 7.7 Hz), 3.68-3.73 (m, 2H), 3.71 (s, 3H), 6.85 (d, 2H, J = 8.7 Hz), 7.19 (d, 2H, J = 8.7 Hz), 7.80 (t, 1H, J = 5.3 Hz), 7.97-8.00 (m, 2H), 8.05-8.07 (m, 1H), 8.32-8.34 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 33.8, 43.5, 55.4, 114.2, 120.5, 123.2, 125.0, 125.7, 130.1, 132.1, 133.0, 133.3, 144.4, 154.5, 158.1.

***N*-Benzyl-4-phenylphthalazin-1-amine (16a).** Following general method A and starting from **14a** (75 mg, 0.28 mmol) and phenylboronic acid (41 mg, 0.33 mmol), **16a** was obtained as a white solid (55 mg, 0.18 mmol, 64%). Purity ≥ 98 %; mp = 218-220 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.87 (d, 2H, J = 5.9 Hz), 7.23 (t, 1H, J = 7.3 Hz), 7.33 (t, 2H, J = 7.3 Hz), 7.44 (d, 2H, J = 7.3 Hz), 7.48-7.57 (m, 3H), 7.61 (td, 2H, J = 1.4 Hz, J = 8.2 Hz), 7.81 (dd, 1H, J = 0.9 Hz, J = 8.2 Hz), 7.86 (td, 1H, J = 0.9 Hz, J = 8.2 Hz), 7.93 (td, 1H, J = 1.4 Hz, H = 8.0 Hz), 8.19 (t, 1H, J = 5.9 Hz), 8.45 (d, 1H, J = 8.2 Hz); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 44.5, 118.3, 122.8, 125.9, 126.0, 127.0, 127.7, 128.6, 128.7, 128.8, 130.0, 131.6, 132.2, 137.6, 140.8, 151.9, 153.3.

***N*-[(3-Chlorophenyl)methyl]-4-phenylphthalazin-1-amine (16b).** Following general method A and starting from **14b** (50 mg, 0.16 mmol) and phenylboronic acid (21 mg, 0.17 mmol), **16b** was obtained as a colorless oil (10 mg, 0.03 mmol, 17%). Purity ≥ 98 %; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.90 (s, 2H), 7.37-7.46 (m, 3H), 7.57 (s, 1H), 7.62-7.69 (m, 5H), 7.95 (d, 1H, J = 8.0 Hz), 8.14 (t, 1H, J = 8.0 Hz), 8.20 (t, 1H, J = 7.4 Hz), 8.76 (d, 1H, J = 8.0 Hz), 10.10 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 45.1, 121.1, 124.9, 126.7, 127.2, 127.9, 128.0, 128.5, 129.3, 130.2, 130.7, 130.9, 132.9, 133.7, 134.6, 135.8, 139.4, 152.3, 152.6; HRMS ($M+H^+$) 346.1099 (calcd for $C_{21}H_{13}ClN_3H^+$ 346.1106).

***N*-[(2-Methoxyphenyl)methyl]-4-phenylphthalazin-1-amine (16c).** Following general method A and starting from **14c** (83 mg, 0.28 mmol) and phenylboronic acid (41 mg, 0.33 mmol), **16c** was obtained as a white solid (21 mg, 0.06 mmol, 22%). Purity ≥ 98 %; mp = 161-162 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.90 (s, 3H), 4.86 (d, 2H, J = 5.6 Hz), 6.89 (t, 1H, J = 7.4 Hz), 7.04 (d, 1H, J = 8.0 Hz), 7.23-7.29 (m, 2H), 7.52-7.58 (m, 3H), 7.61-7.64 (m, 2H), 7.84 (d, 1H, J = 7.8 Hz), 7.89 (t, 1H, J = 7.4 Hz), 7.96 (t, 1H, J = 6.8 Hz), 6.03 (t, 1H, J = 5.6 Hz), 8.52 (d, 1H, J = 8.0 Hz); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 39.9, 55.8, 110.8, 118.4, 120.5, 122.9, 125.9, 126.0, 127.6, 127.8, 128.1, 128.8, 130.0, 131.6, 132.2, 134.5, 137.6, 151.8, 153.5, 157.3; HRMS ($M+H^+$) 342.1596 (calcd for $C_{22}H_{19}N_3OH^+$ 342.1601).

***N*-[(3-Methoxyphenyl)methyl]-4-phenylphthalazin-1-amine (16d).** Following general method A and starting from **14d** (98 mg, 0.33 mmol) and phenylboronic acid (48 mg, 0.39 mmol), **16d** was obtained as a colorless oil (55 mg, 0.16 mmol, 49%). Purity ≥ 98 %; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.72 (s, 3H), 4.83 (d, 2H, J = 5.9 Hz), 6.80 (dd, 1H, J= 2.5 Hz, J = 7.5 Hz), 7.00-7.02 (m, 2H), 7.23 (t, 1H, J = 7.9 Hz), 7.32 (t, 1H, J = 7.5 Hz), 7.50-7.56 (m, 3H), 7.59-7.61 (m, 2H), 7.77-7.82 (m, 2H), 7.86 (td, 1H, J = 1.2 Hz, J = 8.2 Hz), 7.93 (td, 1H, J = 1.2 Hz, J = 8.2 Hz), 8.02 (s, 1H), 8.17 (t, 1H, J = 5.9 Hz), 8.45 (d, 1H, J = 8.2 Hz); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 44.6, 55.4, 112.2, 113.6, 118.4, 119.9, 122.8, 125.9, 126.0, 128.7, 128.8, 129.7, 130.0, 131.6, 132.2, 134.5, 137.6, 142.4, 151.9, 153.3, 159.7; HRMS ($M+H^+$) 342.1599 (calcd for $C_{22}H_{19}N_3OH^+$ 342.1601).

***N*-[(4-Methoxyphenyl)methyl]-4-phenylphthalazin-1-amine (16e).** Following general method A and starting from **14e** (83 mg, 0.28 mmol) and phenylboronic acid (41 mg, 0.33 mmol), **16e** was obtained as a white solid (70 mg, 0.20 mmol, 74%). Purity ≥ 98 %; mp = 69-74 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.72 (s, 3H), 4.78 (d, 2H, J = 5.5 Hz), 6.89 (d, 2H, J = 8.6 Hz), 7.37 (d, 2H, J = 8.6 Hz), 7.48-7.57 (m, 3H), 7.60 (d, 2H, J = 8.0 Hz), 7.80 (d, 1H, J = 8.0 Hz), 7.85 (t, 1H, J = 8.2 Hz), 7.92 (t, 1H, J = 7.0 Hz), 8.11 (t, 1H, J = 5.5

Hz), 8.42 (d, 1H, J = 8.2 Hz); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 44.0, 55.5, 114.0, 118.3, 122.8, 125.9, 126.0, 128.7, 128.8, 129.1, 130.0, 131.6, 132.2, 132.5, 137.5, 151.8, 153.3, 158.5; HRMS (M+H$^+$) 342.1596 (calcd for $C_{22}H_{19}N_3OH^+$ 342.1601).

***N*-[(3-Chloro-4-methoxyphenyl)methyl]-4-phenylphthalazin-1-amine (16f).** Following general method A and starting from **14f** (80 mg, 0.24 mmol) and phenylboronic acid (31 mg, 0.25 mmol), **16f** was obtained as a white solid (60 mg, 0.16 mmol, 66%). Purity ≥ 98 %; mp = 97-99 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.86 (s, 3H), 4.85 (d, 2H, J = 4.5 Hz), 5.51 (s, 1H), 6.86 (d, 1H, J = 8.4 Hz), 7.35 (dd, 1H, J = 1.5 Hz, J = 8.4 Hz), 7.43-7.51 (m, 4H), 7.68 (d, 2H, J = 6.8 Hz), 7.72-7.77 (m, 2H), 7.84 (d, 1H, J = 7.3 Hz), 7.96 (d, 1H, J = 7.3 Hz); ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 45.3, 56.4, 112.3, 118.6, 120.8, 122.6, 126.5, 127.0, 128.1, 128.5, 128.7, 130.1, 130.3, 131.2, 131.5, 132.5, 137.2, 152.8, 153.6, 154.4; HRMS (M+H$^+$) 376.1207 (calcd for $C_{22}H_{18}ClN_3OH^+$ 376.1211).

***N*-(2H-1,3-Benzodioxol-5-ylmethyl)-4-phenylphthalazin-1-amine (16g).** Following general method A and starting from **14g** (70 mg, 0.22 mmol) and phenylboronic acid (33 mg, 0.27 mmol), **16g** was obtained as a white solid (53 mg, 0.15 mmol, 67%). Purity ≥ 98 %; mp = 170-172 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.81 (d, 2H, J = 4.1 Hz), 6.01 (s, 2H), 6.93 (d, 1H, J = 7.9 Hz), 7.00 (d, 1H, J = 7.9 Hz), 7.10 (s, 1H), 7.64-7.67 (m, 5H), 7.93 (d, 1H, J = 7.9 Hz), 8.10-8.19 (m, 2H), 8.79 (d, 1H, J = 7.9 Hz), 10.05 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 45.5, 101.5, 108.7, 108.9, 121.1, 121.6, 125.0, 127.1, 128.2, 129.3, 130.1, 130.5, 133.5, 134.2, 135.6, 147.1, 147.8, 151.7, 152.4, 157.0; HRMS (M+H$^+$) 356.1391 (calcd for $C_{22}H_{17}N_3O_2H^+$ 356.1394).

***N*-(4-methoxybenzyl)-4-phenyl-7-(trifluoromethyl)phthalazin-1-amine (16h).** Following general method A and starting from **14h** (62 mg, 0.17 mmol) and phenylboronic acid (23 mg, 0.19 mmol), **16h** was obtained as a yellow solid (44 mg, 0.11 mmol, 64%). Purity ≥ 95 %; mp = 93-97 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.77 (s, 3H), 4.86 (d, 2H, J = 5.0 Hz), 5.65 (t, 1H, J = 5.0 Hz), 6.86 (d, 2H, J = 8.7 Hz), 7.40 (d, 2H, J = 8.7 Hz), 7.48-7.52 (m, 3H), 7.65 (dd, 2H, J = 1.9 Hz, J = 8.0 Hz), 7.89 (dd, 1H, J = 1.3 Hz, J = 8.7 Hz), 8.08 (d, 1H, J = 8.7 Hz), 8.13 (s, 1H); ^{19}F NMR (376 MHz, $CDCl_3$) δ ppm -62.7; ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 46.0, 55.5, 114.3, 119.0 (q, J = 3.7 Hz), 124.9, 127.3 (q, J = 2.9 Hz), 127.9, 128.2, 128.7, 129.0, 130.1, 130.2, 130.8, 132.5, 132.8, 136.6, 152.8 (d, J = 3.7 Hz); HRMS (M+H$^+$) 410.1478 (calcd for $C_{23}H_{18}F_3N_3OH^+$ 410.1475).

4-Phenyl-*N*-(2-phenylethyl)phthalazin-1-amine (17a). Following general method A and starting from **15a** (57 mg, 0.20 mmol) and phenylboronic acid (29 mg, 0.24 mmol), **17a** was obtained as a white solid (41 mg, 0.13 mmol, 64%). Purity ≥ 98 %; mp = 132-134 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 3.10 (t, 2H, J = 8.0 Hz), 3.90 (q, 2H, J = 8.0 Hz), 7.24 (t, 1H, J = 7.2 Hz), 7.33 (t, 2H, J = 7.2 Hz), 7.37 (d, 2H, J = 7.2 Hz), 7.64-7.68 (m, 5H), 7.93 (d, 1H, J = 8.1 Hz), 8.14 (t, 1H, J = 7.2 Hz), 8.19 (t, 1H, J = 7.2 Hz), 9.76 (d, 1H, J = 8.1 Hz), 9.99 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 33.9, 44.1, 121.3, 125.1, 127.0, 127.1, 128.4, 128.9, 129.3, 129.4, 130.1, 130.5, 134.3, 136.0, 138.8, 152.4.

***N*-[2-(3-Chlorophenyl)ethyl-4-phenylphthalazin-1-amine (17b).** Following general method A and starting from **15b** (69 mg, 0.22 mmol) and phenylboronic acid (32 mg, 0.26 mmol), **17b** was obtained as a white solid (22 mg, 0.06 mmol, 28%). Purity ≥ 98 %; mp = 111-112 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.09 (t, 2H, J = 7.8 Hz), 3.90 (q, 2H, J = 7.8 Hz), 7.30-7.37 (m, 3H), 3.49 (t, 1H, J = 1.5 Hz), 7.64-7.68 (m, 5H), 7.94 (dd, 1H, J = 1.5 Hz, J = 8.2 Hz), 8.14 (td, 1H, J = 1.4 Hz, J = 8.2 Hz), 8.19 (td, J = 1.5 Hz, J = 8.2 Hz), 8.72 (d, 1H, J = 7.8 Hz), 9.95 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 33.5, 43.7, 121.3, 125.1, 127.0, 127.1, 128.2, 128.4, 129.3, 129.4, 130.1, 130.5, 130.7, 133.5, 134.3, 136.0, 141.4, 152.5; HRMS (M+H$^+$) 360.1261 (calcd for $C_{22}H_{18}ClN_3H^+$ 360.1262).

***N*-[2-(4-Chlorophenyl)ethyl-4-phenylphthalazin-1-amine (17c).** Following general method A and starting from **15c** (150 mg, 0.47 mmol) and phenylboronic acid (63 mg, 0.52 mmol), 17c was obtained as a white solid (113 mg, 0.24 mmol, 50%). Purity ≥ 98 %; mp = 131-133 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.09 (t, 2H, J = 7.0 Hz), 3.90 (q, 2H, J = 7.0 Hz), 7.37-7.40 (m, 4H), 7.60-7.69 (m, 5H), 7.92 (d, 1H, J = 7.7 Hz), 8.11-8.19 (m, 2H), 8.77 (d, 1H, J = 7.7 Hz), 10.07 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 33.2, 43.9, 121.4, 125.2, 127.1, 128.4, 128.8, 129.3, 130.1, 130.5, 131.3, 131.7, 133.6, 134.3, 136.0, 137.9, 151.2, 152.4; HRMS (M+H$^+$) 360.1265 (calcd for $C_{22}H_{18}ClN_3H^+$ 360.1262).

***N*-[2-(4-Methoxyphenyl)ethyl-4-phenylphthalazin-1-amine (17d).** Following general method A and starting from **15d** (75 mg, 0.24 mmol) and phenylboronic acid (35 mg, 0.29 mmol), **17d** was obtained as a white solid (67 mg, 0.19 mmol, 79%). Purity ≥ 98 %; mp = 139-142 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.99 (t, 2H, J = 7.6 Hz), 3.79 (q, 2H, J = 7.6 Hz), 6.87 (d, 2H, J = 8.5 Hz), 7.23 (d, 2H, J = 8.5 Hz), 7.51 (d, 1H, J = 7.0 Hz), 7.55 (t, 2H, J = 7.0 Hz), 7.62 (d, 2H, J = 7.0 Hz), 7.66 (t, 1H, J = 5.5 Hz), 7.79 (d, 1H, J = 8.2 Hz), 7.83 (t, 1H, J = 7.0 Hz), 7.89 (t, 1H, J = 7.0 Hz), 8.34 (d, 1H, J = 8.2 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 34.2, 43.5, 55.4, 114.3, 118.4, 122.8, 125.8, 125.9, 128.7, 128.8, 130.0, 130.1, 131.5, 132.1, 132.4, 137.7, 151.6, 153.5, 158.1; HRMS (M+H$^+$) 356.1752 (calcd for $C_{23}H_{21}N_3OH^+$ 356.1757).

***N*-(3-Chlorophenyl)phthalazin-1-amine (18).** A microvawe vial (oven-dried and under argon) was charged with a solution of 13b (100 mg, 0.34 mmol, 1.0 equiv) in DMF (2.3 mL), and NEt_3 (574.9 µL, 4.14 mmol, 12.0 equiv) and $Pd(PPh_3)_4$ (15.9 mg, 0.014 mmol, 4 mol%) were added. The vial was capped properly, flushed with argon and formic acid (13.0 µL, 0.34 mmol, 1.0 equiv) was added. The resulting mixture was microvawe heated to 110°C for 25 minutes. After it cooling, the reaction mixture was diluted with water and the aqueous 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 (heptanes:EtOAc 3:2), to yield **18** as a white solid (68 mg, 0.27 mmol, 77%). Purity ≥ 98 %; mp = 216-218°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.07 (dd, 1H, J = 2.0 Hz, J = 8.0 Hz), 7.38 (t, 1H, J = 8.0 Hz), 7.88 (dd, 1H, J = 2.0 Hz, J = 8.2 Hz), 7.96-8.07 (m, 3H), 8.25 (t, 1H, J = 2.0 Hz), 8.59 (d, 1H, J = 8.2 Hz), 9.19 (s, 1H), 9.33 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 118.7, 119.2, 120.2, 122.0, 122.6, 127.2, 127.7, 130.5, 132.6, 132.7, 133.3, 142.7, 146.4, 152.6.

***N*-[(4-Methoxyphenyl)methyl]phthalazin-1-amine (19).** In a hydrogenation flask, **14e** (91 mg, 0.30 mmol, 1.0 equiv.) was dissolved in ethanol (25.0 mL) then argon was bubble in through. Pd/C (1 mg, 0.013 mmol, 4 mol%) was added and the reaction media was stirred at rt for 24 hours under a dihydrogen pressure (64 psi). The reaction media was filtered through a pad of Celite® and washed with DCM. The filtrate was concentrated under vacuum and purified by silica gel column chromatography (heptanes:EtOAc 0:1), to yield **19** as a

yellow solid (15 mg, 0.06 mmol, 19%). Purity ≥ 98 %; mp = 176-177 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.71 (s, 3H), 4.73 (d, 2H, J = 5.8 Hz), 6.87 (d, 2H, J = 8.6 Hz), 7.33 (d, 2H, J = 8.6 Hz), 7.85-7.88 (m, 2H), 7.90-7.93 (m, 1H), 7.96 (t, 1H, J = 5.8 Hz), 8.32-8.34 (m, 1H), 8.89 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 44.0, 55.5, 114.0, 118.2, 122.3, 126.7, 127.4, 129.1, 131.7, 132.0, 132.6, 143.8, 153.8, 158.5.

***N*-(3-Chlorophenyl)-4-(piperidin-1-yl)phthalazin-1-amine (20).** Following general method D and starting from **13b** (200 mg, 0.62 mmol) and piperidine (317.4 µL, 3.21 mmol), **20** was obtained as a white solid (126 mg, 0.37 mmol, 60%). Purity ≥ 98 %; mp = 205-207 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.62 (br s, 2H), 1.76 (br s, 4H), 3.20 (br s, 4H), 6.99 (d, 1H, J = 7.8 Hz), 7.32 (t, 1H, J = 7.8 Hz), 7.79 (d, 1H, J = 7.8 Hz), 7.93-7.96 (m, 2H), 8.03 (d, 1H, J = 6.5 Hz), 8.21 (s, 1H), 8.50 (d, 1H, J = 6.5 Hz), 9.10 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 24.7, 26.2, 52.9, 118.4, 119.2, 121.1, 121.3, 123.1, 123.3, 125.0, 130.4, 131.8, 132.1, 133.3, 143.4, 150.4, 157.4; HRMS (M+H$^+$) 339.1360 (calcd for $C_{19}H_{19}ClN_4H^+$ 339.1371).

***N*-[(4-Methoxyphenyl)methyl]-4-(piperidin-1-yl)phthalazin-1-amine (21a).** Following general method D and starting from **14e** (80 mg, 0.27 mmol) and piperidine (136.5 µL, 1.38 mmol), **21a** was obtained as a white solid (67 mg, 0.19 mmol, 79%). Purity ≥ 98 %; mp = 89-91 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.66 (m, 2H), 1.79-1.84 (m, 4H), 3.28 (t, 2H, J = 5.0 Hz), 4.77 (d, 2H, J = 4.4 Hz), 4.92 (s, 1H), 6.90 (d, 2H, J = 8.7 Hz), 7.39 (d, 2H, J = 8.7 Hz), 7.69 (d, 2H, J = 3.6 Hz), 7.72-7.76 (m, 1H), 8.05 (d, 1H, J = 8.0 Hz); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 24.9, 26.5, 46.0, 53.0, 55.5, 114.3, 121.0, 121.4, 123.8, 125.5, 129.9, 130.9, 131.0, 131.6, 151.6, 157.0, 159.3; HRMS (M+H$^+$) 349.2019 (calcd for $C_{21}H_{24}N_4OH^+$ 349.2022).

1-(4-{[(4-Methoxyphenyl)methyl]amino}phtalazin-1-yl)piperidin-4-ol (21b). Following general method D and starting from **14e** (150 mg, 0.50 mmol) and 4-hydroxypiperidine (262.2 µL, 2.59 mmol), **21**b was obtained as a yellow solid (114 mg, 0.31 mmol, 63%). Purity ≥ 98 %; mp = 92-97 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.65-1.69 (m, 2H), 1.90-1.94 (m, 2H), 2.90 (t, 2H, J = 12.7 Hz), 3.18 (d, 1H, J = 4.9 Hz), 3.34-3.37 (m, 2H), 3.71 (s, 3H), 4.65 (d, 2H, J = 5.6 Hz), 4.72 (d, 1H, J = 3.9 Hz), 6.87 (d, 1H, J = 8.7 Hz), 7.32 (d, 2H, J = 8.7 Hz), 7.62 (t, 1H, J = 5.6 Hz), 7.83-7.86 (m, 2H), 7.94-7.97 (m, 1H), 8.29-8.31 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 35.1, 44.2, 49.7, 55.5, 66.8, 114.0, 120.7, 123.0, 123.1, 124.6, 129.0, 131.2, 131.6, 133.0, 152.0, 155.2, 158.5; HRMS (M+H$^+$) 365.1968 (calcd for $C_{21}H_{24}N_4O_2H^+$ 365.1972).

***N*-[(4-Methoxyphenyl)methyl]-4-(4-methylpiperazin-1-yl)phthalazin-1-amine (21c).** Following general method D and starting from **14e** (33 mg, 0.11 mmol) and 1-methylpiperazine (63.2 µL, 2.59 mmol), **21c** was obtained as an orange oil (11 mg, 0.03 mmol, 26%). Purity ≥ 98 %; mp = 92-97 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.27 (s, 3H), 2.55-2.58 (m, 4H), 3.12-3.15 (m, 4H), 3.71 (s, 3H), 4.64 (d, 2H, J = 5.8 Hz), 6.86 (d, 2H, J = 8.7 Hz), 7.31 (d, 2H, J = 8.7 Hz), 7.64 (t, 1H, J = 5.8 Hz), 7.83-7.86 (m, 2H), 7.95-7.98 (m, 1H), 8.28-8.31 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 44.1, 46.3, 51.5, 55.3, 55.5, 114.0, 120.6, 122.8, 123.1, 124.6, 129.0, 131.3, 131.6, 133.0, 152.1, 154.7; HRMS (M+H+) 364.2117 (calcd for $C_{21}H_{25}N_5OH^+$ 364.2132).

***N*-[(4-Methoxyphenyl)methyl]-4-(piperidin-1-yl)-7-(trifluoromethyl)phthalazin-1-amine (21d).** Following general method D and starting from **14h** (88 mg, 0.24 mmol) and piperidine (122.4µL, 1.24 mmol), **21d** was obtained as a yellow oil (42 mg, 0.10 mmol, 42%). Purity ≥ 98 %; ^{1}H NMR (400 MHz, CDCl3) δ ppm δ 1.58-1.62 (m, 2H), 1.71-1.77 (m, 4H), 3.10 (t, 4H, J = 5.1 Hz), 3.72 (s, 3H), 4.65 (d, 1H, J = 5.5 Hz), 6.88 (d, 2H, J = 8.5 Hz), 7.33 (d, 2H, J = 8.5 Hz), 7.95 (t, 1H, J = 5.5 Hz), 8.14 (s, 2H), 8.81 (s, 1H); ^{19}F NMR (376 MHz, CDCl3) δ ppm -61.0; ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 24.6, 26.2, 44.3, 52.9, 55.5, 114.0, 120.2, 121.5 (q, J = 3.7 Hz), 124.9, 126.4, 127.4 (q, J = 2.9 Hz), 129.3, 131.0 (q, J = 32.3 Hz), 132.5, 151.8, 155.1, 158.6; HRMS (M+H$^+$) 417.1895 (calcd for $C_{22}H_{23}F_3N_4OH^+$ 417.1897).

Methyl 3-carbamoylbenzoate (23). A solution of mono-methyl isophthalate **22** (150 mg, 0.83 mmol, 1.0 equiv.), HOBt.NH_3 (190 mg, 1.25 mmol, 1.5 equiv.) and EDC (192 mg, 1.00 mmol, 1.2 equiv.) in DMF (1.5 mL) was stirred at rt overnight. The reaction media was diluted with water and the aqueous phase was extracted 3 times with EtOAc. The combined organic layers were washed with a saturated solution of $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated under vacuum, to yield **23** as white solid (108 mg, 0.60 mmol, 72%). ^{1}H NMR (400 MHz, MeOD) δ ppm 3.94 (s, 3H), 7.59 (t, 1H, J = 7.8 Hz), 8.10 (ddd, 1H, J = 1.3 Hz, J = 1.9 Hz, J = 7.8 Hz), 8.17 (dt, 1H, J = 1.3 Hz, J = 7.8 Hz), 8.53 (t, 1H, J = 1.9 Hz); ^{13}C NMR (101 MHz, MeOD) δ ppm 51.4, 128.3, 128.5, 130.4, 131.7, 132.2, 134.2, 166.3, 169.8.

3-Carbamoylbenzoic acid (24i). NaOH (107 mg, 2.68 mmol, 5.0 equiv.), in water (1.5 mL) was added in a solution of **23** (96 mg, 0.54 mmol, 1.0 equiv.) in methanol (1.5 mL) and the resulting mixture was stirred at rt overnight. The methanol was evaporated and the aqueous phase was acidified until pH = 2 using a solution of HCl 1N. The solid was filtered, and **24i** was obtained as a white solid (72 mg, 0.44 mmol, 82%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.48 (s, 1H), 7.58 (t, 1H, J = 7.7 Hz), 8.09 (ddt, 2H, J = 1.6 Hz, J = 7.7 Hz, J = 12.9 Hz), 8.15-8.18 (m, 1H), 8.45 (t, 1H, J = 1.6 Hz), 13.18 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 128.9, 129.1, 131.4, 132.1, 132.3, 135.2, 167.4, 167.6.

3-Bromo-1H-indazole (39a). Following general method E and starting from indazole **38a** (800 mg, 6.77 mmol), **39a** was obtained as a white solid (912 mg, 4.63 mmol, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.25 (td, 1H, J = 1.0 Hz, J = 8.4 Hz), 7.47 (td, 1H, J = 1.0 Hz, J = 8.4 Hz), 7.57 (d, 1H, J = 8.4 Hz), 7.66 (d, 1H, J = 8.4 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 110.3, 120.2, 121.9, 122.9, 128.1, 141.2.

3-Bromo-5-chloro-1H-indazole (39b). Following general method E and starting from 5-chloro-1H-indazole **38b** (500 mg, 3.28 mmol), **39b** was obtained as a yellow solid (592 mg, 2.56 mmol, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.45 (dd, 1H, J = 1.9 Hz, J = 8.9 Hz), 7.62-7.64 (m, 2H), 13.62 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 113.2, 118.7, 120.1, 123.5, 126.4, 128.4, 140.0.

3-Bromo-5-(trifluoromethyl)-1H-indazole (39c). Following general method E and starting from 5-(trifluoromethyl)-1H-indazole **38c** (300 mg, 1.61 mmol), **39c** was obtained as a white solid (419 mg, 1.58 mmol, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.67-7.71 (m, 2H), 7.99 (s, 1H), 11.13 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 111.1, 118.7 (q, J = 4.4 Hz), 124.1, 124.8 (q, J = 2.9 Hz).

3-Bromo-1-(oxan-2-yl)-1H-indazole (40a). Following general method F and starting from **39a** (115 mg, 0.58 mmol), **40a** was obtained as a yellow solid (119 mg, 0.42 mmol, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.68-1.81 (m, 3H), 2.04-2.17 (m, 2H), 2.51-2.60 (m, 1H), 3.70-3.76 (m, 1H), 4.00-4.04 (m, 1H), 5.68 (dd, 1H, J = 2.6 Hz, J = 9.3 Hz), 7.23 (t, 1H, J = 7.7 Hz), 7.44 (t, 1H, J = 7.2 Hz), 7.56-7.62 (m, 2H); ^{13}C NMR (101 MHz, CDCl3) δ ppm 22.4, 25.0, 29.3, 67.4, 85.5, 110.4, 120.4, 122.0, 122.1, 124.5, 127.7, 140.6.

3-Bromo-1-(oxan-2-yl)-5-(trifluoromethyl)-1H-indazole (40b). Following general method F and starting from **39c** (215 mg, 0.81 mmol), **40b** was obtained as a white solid (235 mg, 0.67mmol, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.67-1.80 (m, 3H), 2.07-2.19 (m, 2H), 2.48-2.57 (m, 1H), 3.71-3.77 (m, 1H), 3.97-4.02 (m, 1H), 5.72 (dd, 1H, J = 2.9 Hz, J = 9.0 Hz), 7.65 (dd, 1H, J = 1.6 Hz, J = 8.9 Hz), 7.70 (d, 1H, J = 8.9 Hz), 7.93 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 22.1, 24.9, 29.3, 67.3, 85.9, 111.5, 118.7 (q, J = 4.4 Hz), 122.9, 123.9, 124.3 (q, J = 2.9 Hz).

3-Bromo-1-methyl-5-(trifluoromethyl)-1H-indazole (40c). MeI (105.7 µL, 1.70 mmol, 1.5 equiv.) was added dropwise to a solution of **39c** (300 mg, 1.13 mmol, 1.0 equiv.) and Na_2CO_3 (300 mg, 2.83 mmol, 2.5 equiv.) in DMF (1.1 mL) and the resulting mixture was heated at 50°C overnight. After cooling, the reaction mixture was diluted with water and the aqueous 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 (heptanes:EtOAc 9:1), to yield **40c** as a yellow solid (209 mg, 0.75 mmol, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.09 (s, 3H), 7.47 (d, 1H, J = 8.8 Hz), 7.65 (dd, 1H, J = 1.5 Hz, J = 8.8 Hz), 7.93 (d, 1H, J = 1.5 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 36.2, 110.0, 118.8 (q, J = 4.4 Hz), 121.3, 123.1, 123.8, 124.1 (q, J = 3.7 Hz).

3-Bromo-1-cyclopentyl-1H-indazole (40d). Following general method G and starting from **39a** (197 mg, 1.00 mmol) and bromocyclopentane (139.4 µL, 1.30 mmol), **40d** was obtained as a yellow oil (209 mg, 0.79 mmol, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.54-1.57 (m, 2H), 1.78-1.81 (m, 2H), 1.97-2.00 (m, 4H), 4.77 (quint., 1H, J = 7.4 Hz), 7.00 (ddd, 1H, J = 1.5 Hz, J = 6.1 Hz, J = 7.9 Hz), 7.20-7.26 (m, 2H), 7.42 (dt, 1H, J = 1.5 Hz, J = 7.9 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 24.7, 32.4, 80.3, 109.7, 119.8, 120.6, 121.3, 124.0, 127.2, 140.7.

3-Bromo-5-chloro-1-cyclopentyl-1H-indazole (40e). Following general method G and starting from **39b** (200 mg, 0.86 mmol) and bromocyclopentane (120.4 µL, 1.12 mmol), **40e** was obtained as an orange oil (202 mg, 0.67 mmol, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.62-1.70 (m, 2H), 1.86-1.95 (m, 2H), 2.05-2.11 (m, 4H), 4.82 (quint., 1H, J = 7.4 Hz), 7.27 (s, 2H), 7.50 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 24.5, 32.3, 60.4, 110.6, 118.8, 119.6, 124.6, 126.9, 127.8, 139.0.

3-Bromo-5-(trifluoromethyl)-1-cyclopentyl-1H-indazole (40f). Following general method G and starting from **39c** (300 mg, 1.13 mmol) and bromocyclopentane (157.8 µL, 1.47 mmol), **40f** was obtained as a colorless oil (337 mg, 1.01 mmol, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.72-1.79 (m, 2H), 1.97-2.01 (m, 2H), 2.15-2.21 (m, 4H), 4.97 (quint., 1H, J = 7.4 Hz), 7.52 (d, 1H, J = 8.9 Hz), 7.61 (dd, 1H, J = 1.6 Hz, J = 8.9 Hz), 7.92 (t, 1H, J = 1.6 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 24.5, 32.4, 60.4, 110.3, 118.8 (q, J = 4.4 Hz), 123.6 (q, J = 2.9 Hz), 141.3.

3-Bromo-1-cyclohexyl-5-(trifluoromethyl)-1H-indazole (40g). Following general method G and starting from 39c (80 mg, 0.30 mmol) and bromocycloheptane (48.1 µL, 0.39 mmol), **40g** was obtained as a colorless oil (77 mg, 0.22 mmol, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.35 (tt, 1H, J = 3.4 Hz, J = 12.7 Hz), 1.42-1.53 (m, 2H), 1.78 (dt, 1H, J = 3.4 Hz, J = 12.7 Hz), 1.86 (dt, 1H, J = 3.4 Hz, J = 13.6 Hz), 2.01-2.07 (m, 4H), 4.36-4.43 (m, 1H), 7.52 (d, 1H, J = 8.9 Hz), 7.61 (dd, 1H, J = 1.5 Hz, J = 8.9 Hz), 7.92 (d, 1H, J = 1.5 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 25.2, 25.7, 32.5, 59.1, 110.1, 118.9 (q, J = 4.4 Hz), 121.1, 123.5 (q, J = 2.9 Hz), 140.7.

1-Benzyl-3-bromo-5-(trifluoromethyl)-1H-indazole (40h). BnBr (67.7 µL, 0.57 mmol, 1.5 equiv.) was added dropwise to a solution of **39c** (100 mg, 0.38 mmol, 1.0 equiv.) and Na_2CO_3 (100 mg, 0.94 mmol, 2.5 equiv.) in DMF (0.4 mL) and the resulting mixture was heated at 50°C overnight. After cooling, the reaction mixture was diluted with water and the aqueous 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 (heptanes:EtOAc 9:1), to yield **40h** as a white solid (104 mg, 0.29 mmol, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 5.59 (s, 2H), 7.21-7.24 (m, 2H), 7.29-7.35 (m, 3H), 7.40 (d, 1H, J = 8.8 Hz), 7.58 (dd, 1H, J = 1.6 Hz, J = 8.8 Hz), 7.95 (t, 1H, J = 1.6 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 53.9, 110.4, 118.9 (q, J = 5.1 Hz), 124.2 (q, J = 2.9 Hz), 127.3, 128.3, 129.0, 135.6, 141.6.

3-Bromo-1-phenyl-5-(trifluoromethyl)-1H-indazole (40i). Following general method H and starting from **39c** (50 mg, 0.19 mmol) and iodobenzene (63.4 µL, 0.57 mmol), **40i** was obtained as a yellow oil (39 mg, 0.11 mmol, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.43 (tt, 1H, J = 1.1 Hz, J = 7.5 Hz), 7.57 (t, 2H, J = 7.5 Hz), 7.67-7.71 (m, 3H), 7.79 (d, 1H, J = 8.9 Hz), 8.02 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 111.5, 119.1 (q, J = 4.4 Hz), 123.0, 124.7, 124.9 (q, J = 3.7 Hz), 127.8, 129.7, 138.9.

3-Bromo-1-(pyridine-3-yl)-5-(trifluoromethyl)-1H-indazole (40j). Following general method H and starting from **39c** (100 mg, 0.38 mmol) and 3-iodopyridine (232 mg, 1.13 mmol), **40j** was obtained as a white solid (56 mg, 0.16 mmol, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.53 (dd, 1H, J = 4.6 Hz, J = 8.2 Hz), 7.75 (d, 1H, J = 8.9 Hz), 7.79 (d, 1H, J = 8.9 Hz), 7.91 (s, 1H), 8.05 (d, 1H, J = 8.2 Hz), 8.68 (d, 1H, J = 4.6 Hz), 9.02 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 97.3, 110.9, 120.6 (q, J = 4.4 Hz), 124.3, 125.5 (q, J = 2.9 Hz), 129.3, 130.3, 135.8, 140.5, 143.6, 148.8.

***N*-[(4-Methoxyphenyl)methyl]-1-(oxan-2-yl)-1H-indazol-3-amine (41a).** Following general method I and starting from **40a** (50 mg, 0.18 mmol) and 4-methoxybenzylamine (27.9 µL, 0.21 mmol), **41a** was obtained as a white solid (39 mg, 0.11 mmol, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.63-1.69 (m, 3H), 1.90-1.94 (m, 1H), 2.04-2.07 (m, 1H), 2.46-2.54 (m, 1H), 3.60-3.63 (m, 1H), 3.73 (s, 3H), 3.98-4.01 (m, 1H), 4.49 (d, 2H, J = 3.3 Hz), 5.45 (dd, 1H, J = 2.5 Hz, J = 9.8 Hz), 6.81 (d, 2H, J = 8.5 Hz), 6.93 (t, 1H, J = 8.0 Hz), 7.24-7.33 (m, 4H), 7.39 (d, 1H, J = 8.0 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 23.1, 25.2, 29.4, 29.8, 47.8, 55.3, 67.7, 84.9, 109.5, 113.9, 115.9, 119.2, 119.3, 127.1, 129.5, 131.9, 141.3, 149.7, 158.9.

***N*-[(4-Methoxyphenyl)methyl]-1-(oxan-2-yl)-5-(trifluoromethyl)-1H-indazol-3-amine (41b).** Following general method I and starting from **40b** (350 mg, 1.00 mmol) and 4-methoxybenzylamine (157.2 µL, 1.20 mmol), **41b** was obtained as a yellow solid (308 mg, 0.76 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.72-1.76 (m, 3H), 2.01 (dq, 1H, J = 2.9 Hz, J = 12.9 Hz), 2.12-2.17 (m, 1H), 2.50-2.60 (m, 1H), 3.72 (td, 1H, J = 2.9 Hz, J = 11.0 Hz), 3.81 (s, 3H), 4.06 (dd, 1H, J = 2.3 Hz, J = 11.0 Hz), 4.55 (d, 2H, J = 5.5 Hz), 5.55 (dd, 1H, J = 2.6 Hz, J = 9.7 Hz), 6.90 (d, 2H, J = 8.7 Hz), 7.37 (d, 2H, J = 8.7 Hz), 7.47 (d, 1H, J = 8.8 Hz), 7.54 (dd, 1H, J = 1.5 Hz, J = 8.8 Hz), 7.78 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 22.8, 25.1, 29.4, 47.7, 55.3, 67.7, 85.1, 110.1, 114.0, 115.1, 117.5 (q, J = 4.4 Hz), 123.7 (q, J = 2.9 Hz), 129.5, 131.5, 142.1, 150.0, 159.0.

***N*-[(4-Methoxyphenyl)methyl]-1-methyl-5-(trifluoromethyl)-1H-indazol-3-amine (41c).** Following general method I and starting from **40c** (195 mg, 0.70 mmol) and 4-methoxybenzylamine (109.6 µL, 0.83 mmol), **41c** was obtained as a yellow solid (164 mg, 0.49 mmol, 70%). Purity ≥ 98 %; mp = 92-93°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.81 (s, 3H), 3.90 (s, 3H), 4.53 (s, 2H), 6.90 (d, 2H, J = 8.5 Hz), 7.24 (d, 1H, J = 8.8 Hz), 7.37 (d, 2H, J = 8.5 Hz), 7.52 (dd, 1H, J = 1.4 Hz, J = 8.8 Hz), 7.79 (d, 1H, J = 1.4 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ ppm -60.5; ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 35.1, 47.9, 55.3, 108.8, 113.4, 114.0, 117.7 (q, J = 4.4 Hz), 123.5 (q, J = 3.7 Hz), 129.4, 131.3, 142.2, 149.7, 159.1; HRMS (M+H+) 336.1315 (calcd for $C_{17}H_{16}F_3N_3OH^+$ 336.1318).

1-Cyclopentyl-*N*-[(4-methoxyphenyl)methyl]-1H-indazol-3-amine (41d). Following general method J and starting from **40d** (150 mg, 0.56 mmol) and 4-methoxybenzylamine (88.7 µL, 0.68 mmol), **41d** was obtained as colorless oil (61 mg, 0.19 mmol, 33%). Purity ≥ 98 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.67-1.70 (m, 2H), 1.92-1.96 (m, 2H), 2.04-2.13 (m, 4H), 3.80 (s, 3H), 4.07 (s, 1H), 4.54 (s, 2H), 4.80 (quin., 1H, J = 7.5 Hz), 6.87 (d, 2H, J = 8.7 Hz), 6.93 (ddd, 1H, J = 1.6 Hz, J = 5.9 Hz, J = 7.8 Hz), 7.24-7.27 (m, 2H), 7.38 (d, 2H, J = 8.7 Hz), 7.46 (d, 1H, J = 8.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 25.1, 31.9, 48.5, 55.6, 59.2, 109.2, 114.1, 115.0, 118.3, 119.6, 126.6, 129.7, 132.6, 141.2, 148.8, 159.2; HRMS (M+H+) 322.1917 (calcd for $C_{20}H_{23}N_3OH^+$ 322.1914).

5-Chloro-1-cyclopentyl-*N*-[(4-methoxyphenyl)methyl]-1H-indazol-3-amine (41e). Following general method J and starting from **40e** (150 mg, 0.50 mmol) and 4-methoxybenzylamine (78.5 µL, 0.60 mmol), **41e** was obtained as a yellow oil (83 mg, 0.23 mmol, 47%). Purity ≥ 95 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.68-1.71 (m, 2H), 1.93-1.96 (m, 2H), 2.04-2.11 (m, 4H), 3.81 (s, 3H), 4.51 (s, 2H), 4.76 (quin., 1H, J = 7.3 Hz), 6.88 (d, 2H, J = 8.5 Hz), 7.18 (d, 1H, J = 8.9 Hz), 7.22 (dd, 1H, J = 1.8 Hz, J = 8.9 Hz), 7.37 (d, 2H, J = 8.5 Hz), 7.44 (d, 1H, J = 1.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 24.6, 31.7, 48.1, 55.3, 59.2, 109.9, 114.0, 115.3, 118.8, 123.3, 126.8, 129.4, 147.8, 158.9; HRMS ($M+H^+$) 356.1523 (calcd for $C_{20}H_{22}ClN_3OH^+$ 356.1524).

***N*-(3-Chlorophenyl)-1-cyclopentyl-5-(trifluoromethyl)-1H-indazol-3-amine (41f).** Following general method J and starting from **40f** (83 mg, 0.25 mmol) and 3-chloroaniline (31.8 µL, 0.30 mmol), **41f** was obtained as a white solid (34 mg, 0.09 mmol, 36%). Purity ≥ 98 %; mp = 153-154 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.70-1.73 (m, 2H), 1.91-2.03 (m, 4H), 2.08-2.15 (m, 2H), 5.12-5.16 (m, 1H), 6.88 (d, 1H, J = 8.0 Hz), 7.31 (t, 1H, J = 8.0 Hz), 7.51 (d, 1H, J = 8.0 Hz), 7.63 (d, 1H, J = 8.9 Hz), 7.75 (d, 1H, J = 8.9 Hz), 7.99 (s, 1H), 8.48 (s, 1H), 9.46 (s, 1H); ^{19}F NMR (376 MHz, DMSO-d_6) δ ppm -58.8; ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 24.9, 32.3, 58.6, 110.7, 114.3, 114.9, 115.7, 119.2 (q, J = 4.4 Hz), 119.3, 119.6 (q, J = 31.5 Hz), 123.5 (q, J = 2.9 Hz), 125.6 (q, J = 270.7 Hz), 130.8, 133.8, 140.6, 144.1, 144.8; HRMS ($M+H^+$) 380.1118 (calcd for $C_{19}H_{17}ClF_3N_3H^+$ 380.1136).

1-Cyclopentyl-*N*-[(4-methoxyphenyl)methyl]-5-(trifluoromethyl)-1H-indazol-3-amine (41g). Following general method I and starting from **40f** (104 mg, 0.31 mmol) and 4-methoxybenzylamine (48.9 µL, 0.37 mmol), **41g** was obtained as a yellow oil (24 mg, 0.06 mmol, 20%). Purity ≥ 98 %; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.62-1.65 (m, 2H), 1.83-1.99 (m, 6H), 3.72 (s, 3H), 4.37 (d, 2H, J = 6.9 Hz), 4.95 (quint., 1H, J = 7.2 Hz), 6.78 (t, 1H, J = 6.0 Hz), 6.87 (d, 2H, J = 8.7 Hz), 7.35 (d, 2H, J = 8.7 Hz), 7.49 (dd, 1H, J = 1.5 Hz, J = 8.9 Hz), 7.55 (d, 1H, J = 8.9 Hz), 8.23 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 24.7, 31.7, 46.6, 55.5, 58.3, 110.0, 113.6, 113.9, 118.2 (q, J = 30.9 Hz), 119.5 (q, J = 4.5 Hz), 122.9 (q, J = 3.6 Hz), 129.7, 132.6, 141.8, 150.2, 158.6; HRMS ($M+H^+$) 390.1785 (calcd for $C_{21}H_{22}F_3N_3OH^+$ 390.1788).

***N*-[(3-Chloro-4-methoxyphenyl)methyl]-1-cyclopentyl-5-(trifluoromethyl)-1H-indazol-3-amine (41h).** Following general method J and starting from **40f** (83 mg, 0.25 mmol) and 3-chloro-4-methoxybenzylamine (43.5 µL, 0.30 mmol), **41h** was obtained as a yellow oil (47 mg, 0.11 mmol, 44%). Purity ≥ 95 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.69-1.72 (m, 2H), 1.94-1.97 (m, 2H), 2.05-2.12 (m, 4H), 3.89 (s, 3H), 4.52 (s, 2H), 4.81 (quint., 1H, J = 6.7 Hz), 6.89 (d, 1H, J = 8.4 Hz), 7.30 (dd, 2H, J = 2.3 Hz, J = 8.4 Hz), 7.48 (d, 1H, J = 8.4 Hz), 7.51 (d, 1H, J = 2.3 Hz), 7.78 (s, 1H); ^{19}F NMR (376 MHz, $CDCl_3$) δ ppm -60.5; ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 24.7, 31.8, 47.4, 56.2, 59.1, 109.2, 112.0, 113.6, 117.6 (q, J = 4.5 Hz), 122.3, 123.0 (q, J = 2.7 Hz), 127.5, 130.3, 133.0, 141.5, 148.8, 154.2; HRMS ($M+H^+$) 424.1387 (calcd for $C_{21}H_{21}Cl_2F_3N_3OH^+$ 424.1398).

***N*-[(3-Fluoro-4-methoxyphenyl)methyl]-1-cyclopentyl-5-(trifluoromethyl)-1H-indazol-3-amine (41i).** Following general method J and starting from **40f** (83 mg, 0.25 mmol) and 3-fluoro-4-methoxybenzylamine (41.2 µL, 0.30 mmol), **41i** was obtained as a colorless oil (38 mg, 0.09 mmol, 38%). Purity ≥ 98 %; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.69-1.72 (m, 2H), 1.94-1.96 (m, 2H), 2.05-2.10 (m, 4H), 3.88 (s, 3H), 4.23 (t, 1H, J = 5.8 Hz), 4.52 (d, 2H, J = 5.8 Hz), 4.81 (quint., 1H, J = 7.3 Hz), 6.92 (t, 1H, J = 8.4 Hz), 7.15 (d, 1H, J = 8.4 Hz), 7.21 (dd, 1H, J = 2.1 Hz, J = 12.1 Hz), 7.31 (d, 1H, J = 8.9 Hz), 7.48 (dd, 1H, J = 2.1 Hz, J = 8.9 Hz), 7.77 (s, 1H); ^{19}F NMR (376 MHz, $CDCl_3$) δ ppm -60.5, -135.2; ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 24.7, 31.8, 47.5, 56.4, 59.1, 109.2, 113.3 (d, J = 2.7 Hz), 113.6, 116.1 (d, J = 19.1 Hz), 117.6 (q, J = 4.5 Hz), 120.1 (q, J = 31.8 Hz), 123.0 (q, J = 3.6 Hz), 123.8 (d, J = 3.6 Hz), 125.0 (q, J = 270.7 Hz), 132.9 (d, J = 5.4 Hz), 141.5, 146.8 (d, J = 10.9 Hz), 148.9, 152.3 (d, J = 246.1 Hz); HRMS ($M+H^+$) 408.1687 (calcd for $C_{21}H_{21}F_4N_3OH^+$ 408.1694).

1-Cyclopentyl-*N*-(3-methoxypropyl)-5-(trifluoromethyl)-1H-indazol-3-amine (41j). Following general method J and starting from **40f** (83 mg, 0.25 mmol) and 3-methoxypropylamine (30.6 µL, 0.30 mmol), *41j* was obtained as an orange oil (19 mg, 0.06 mmol, 23%). Purity ≥ 98 %; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.62-1.65 (m, 2H), 1.82-1.98 (m, 4H), 1.92-1.98 (m, 4H), 3.24 (s, 3H), 3.29 (q, 2H, J = 6.4 Hz), 3.44 (t, 2H, J = 6.4 Hz), 4.94 (quint., 1H, J = 6.9 Hz), 6.34 (t, 1H, J = 5.5 Hz), 7.49 (dd, 1H, J = 1.8 Hz, J = 8.9 Hz), 7.55 (d, 1H, J = 8.9 Hz), 8.19 (s, 1H); ^{19}F NMR (376 MHz, DMSO) δ ppm -58.6; ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 24.7, 29.4, 31.7, 58.3,

70.4, 109.9, 113.7, 118.1 (q, J = 31.5 Hz), 119.5 (q, J = 4.4 Hz), 122.9 (q, J = 2.9 Hz), 141.7, 150.4; HRMS (M+H$^+$) 342.1787 (calcd for $C_{17}H_{22}F_3N_3OH^+$ 342.1788).

1-Cyclohexyl-*N*-[(4-methoxyphenyl)methyl]-5-(trifluoromethyl)-1H-indazol-3-amine (41k). Following general method J and starting from **40g** (62 mg, 0.18 mmol) and 4-methoxybenzylamine (28.0 µL, 0.21 mmol), **41k** was obtained as yellow oil (20 mg, 0.05 mmol, 27%). Purity = 95 %; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.24 (q, 1H, J = 9.4 Hz), 1.45 (q, 2H, J = 12.8 Hz), 1.67 (d, 1H, J = 12.2 Hz), 1.82-1.90 (m, 7H), 3.72 (s, 3H), 4.36 (br s, 1H), 4.38 (d, 2H, J = 5.9 Hz), 6.71 (t, 1H, J = 5.9 Hz), 6.88 (d, 2H, J = 8.5 Hz), 7.35 (d, 2H, J = 8.5 Hz), 7.48 (d, 1H, J = 8.9 Hz), 7.58 (d, 1H, J = 8.9 Hz), 8.24 (s, 1H); ^{19}F NMR (376 MHz, DMSO-d_6) δ ppm -58.6; ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 24.8, 31.6, 45.9, 54.8, 55.9, 109.2, 113.3, 118.9 (q, J = 2.9 Hz), 122.1 (q, J = 4.4 Hz), 129.0, 149.4; HRMS (M+H$^+$) 404.1938 (calcd for $C_{22}H_{24}F_3N_3OH^+$ 404.1944).

1-Benzyl-*N*-[(4-methoxyphenyl)methyl]-5-(trifluoromethyl)-1H-indazol-3-amine (41l). Following general method I and starting from **40h** (102 mg, 0.29 mmol) and 4-methoxybenzylamine (45.0 µL, 0.34 mmol), **41l** was obtained as colorless oil (59 mg, 0.14 mmol, 50%). Purity ≥ 98 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.73 (s, 3H), 4.48 (s, 2H), 5.35 (s, 2H), 6.80 (d, 2H, J = 8.7 Hz), 7.09-7.14 (m, 3H), 7.17-7.22 (m, 2H), 7.28 (d, 2H, J = 8.7 Hz), 7.40 (dd, 1H, J = 1.6 Hz, J = 8.9 Hz), 7.74 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 48.0, 52.4, 55.3, 109.3, 114.0, 114.1, 117.9 (q, J = 4.4 Hz), 123.9 (q, J = 2.9 Hz), 127.1, 127.7, 128.7, 129.5, 131.2, 136.9, 142.0, 149.6, 159.1; HRMS (M+H$^+$) 412.1626 (calcd for $C_{23}H_{20}F_3N_3OH^+$ 412.1631).

***N*-[(4-Methoxyphenyl)methyl]-1-phenyl-5-(trifluoromethyl)-1H-indazol-3-amine (41m).** Following general method J and starting from **40i** (68 mg, 0.20 mmol) and 4-methoxybenzylamine (31.2 µL, 0.24 mmol), **41m** was obtained as a yellow oil (38 mg, 0.10 mmol, 48%). Purity ≥ 98 %; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.82 (s, 3H), 4.62 (s, 2H), 6.92 (d, 2H, J = 8.6 Hz), 7.29 (t, 1H, J = 7.5 Hz), 7.41 (d, 2H, J = 8.6 Hz), 7.51 (t, 1H, J = 7.5 Hz), 7.58 (dd, 1H, J = 1.7 Hz, J = 9.0 Hz), 7.68-7.72 (m, 3.0 Hz), 7.86 (s, 1H); ^{19}F NMR (376 MHz, $CDCl_3$) δ ppm -60.7; ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 47.6, 55.3, 110.5, 114.1, 115.9, 117.7 (q, J = 4.5 Hz), 121.7, 121.8 (q, J = 32.7 Hz), 124.4 (q, J = 3.6 Hz), 124.6 (q, J = 270.7 Hz), 125.6, 127.9, 129.4, 129.5, 131.3, 140.2, 140.7, 151.0, 159.1; HRMS (M+H$^+$) 398.1462 (calcd for $C_{22}H_{18}F_3N_3OH^+$ 398.1475).

***N*-[(4-Methoxyphenyl)methyl]-1-(pyridine-3-yl)-5-(trifluoromethyl)-1H-indazol-3-amine (41n).** Following general method J and starting from **40j** (44 mg, 0.13 mmol) and 4-methoxybenzylamine (20.2 µL, 0.15 mmol), **41n** was obtained as an orange solid (23 mg, 0.06 mmol, 45%). Purity ≥ 95 %; mp = 113-114 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.82 (s, 3H), 4.48 (t, 1H, J = 5.2 Hz), 4.62 (d, 1H, J = 5.2 Hz), 6.92 (d, 2H, J = 8.5 Hz), 7.41 (d, 2H, J = 8.5 Hz), 7.44 (dd, 1H, J = 4.7 Hz, J = 8.2 Hz), 7.64 (dd, 1H, J = 1.5 Hz, J = 8.9 Hz), 7.72 (d, 1H, J = 8.9 Hz), 7.87 (s, 1H), 8.02 (ddd, 1H, J = 1.5 Hz, J = 2.4 Hz, J = 8.2 Hz), 8.51 (dd, 1H, J = 0.9 Hz, J = 4.7 Hz), 9.04 (d, 1H, J = 2.4 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ ppm -60.9; ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 47.5, 55.3, 110.3, 114.1, 116.5, 117.8 (q, J = 3.6 Hz), 122.5 (q, J = 32.7 Hz), 124.0, 124.5 (q, J = 271.6 Hz), 125.0 (q, J = 3.6 Hz), 128.4, 129.5, 131.0, 137.1, 140.8, 142.5, 146.3, 151.6, 159.2; HRMS (M+H$^+$) 399.1414 (calcd for $C_{21}H_{17}F_3N_4OH^+$ 399.1427).

***N*-[(4-Methoxyphenyl)methyl]-1H-indazol-3-amine (41o).** A solution of **41a** (70 mg, 0.21 mmol, 1.0 equiv.) in a mixture TFA:DCM (1:1 mL) was stirred at rt for 1 hour. The solution was concentrated under vacuum and purified by reverse chromatography (MeOH/H2O+0.05%TFA), to yield **41o** as a yellow oil (14 mg, 0.05 mmol, 26%). Purity ≥ 98 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.76 (s, 3H), 5.22 (s, 2H), 6.81 (d, 2H, J = 8.5 Hz), 7.16-7.22 (m, 3H), 7.29 (d, 1H, J = 8.0 Hz), 7.58 (t, 1H, J = 8.0 Hz), 7.67 (d, 1H, J = 8.0 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 51.9, 55.3, 110.3, 114.3, 121.5, 121.6, 129.5, 132.3; HRMS (M+H$^+$) 254.1285 (calcd for $C_{15}H_{15}N_3OH^+$ 254.1288).

***N*-[(4-Methoxyphenyl)methyl]-5-(trifluoromethyl)-1H-indazol-3-amine (41p).** HCl 1N (0.73 mL) was added to a solution of **41b** (101 mg, 0.25 mmol, 1.0 equiv.) in THF (0.4 mL) and the resulting mixture was stirred overnight at rt. The solution was basified until pH = 7 using a saturated solution of $NaHCO_3$. The aqueous 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 (heptanes:EtOAc), to yield **41p** as a white solid (21 mg, 0.07 mmol, 26%). Purity ≥ 98 %; mp = 109-112°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.80 (s, 3H), 4.58 (s, 2H), 6.88 (d, 2H, J = 8.0 Hz), 7.34-7.38 (m, 3H), 7.55 (d, 1H, J = 8.4 Hz), 7.84 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 47.7, 55.3, 110.5 (q, J = 3.7 Hz), 114.1, 124.6 (q, J = 4.4 Hz), 129.2, 130.8, 159.1; HRMS (M+H$^+$) 322.1156 (calcd for $C_{16}H_{14}F_3N_3OH^+$ 322.1162).

6-Phenyl-2,3-dihydropyridazin-3-one (42). A mixture of acetophenone (19.0 mL, 163.0 mmol, 3.0 equiv.) and glyoxylic acid hydrate (5.0 g, 54.3 mmol, 1.0 equiv.) were heated at 100 °C for 2 hours. After it was cooled at 40 °C, water (20 mL) and ammonia (4 mL) were added. The aqueous phase was extracted 3 times with DCM. Hydrazine hydrate (2.6 mL, 54.3 mmol, 1.0 equiv.) was added in the aqueous phase and the resulting solution was heated at reflux four 4 hours. After cooling, the solid was filtered and washed with water, to afford **42** as white solid (6.5 g, 37.5 mmol, 70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.00 (d, 1H, J = 9.6 Hz), 7.45-7.52 (m, 3H), 7.87 (d, 2H, J = 7.2 Hz), 8.04 (d, 1H, J = 9.6 Hz), 13.2 (s, 1H).

2-Chloro-4-phenylpyridazine (43). A mixture of 42 (5.6 g, 32.4 mmol, 1.0 equiv.), $POCl_3$ (30 mL) was heated at reflux for 2 hours. After cooling, the reaction mixture was concentrated under vacuum. The residue was dissolved in cooled DCM and added slowly in ice-cooled water. The organic phase was separated and the aqueous phase was extracted twice with DCM. The combined organic layers were washed with a saturated solution of $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, concentrated under vacuum and purified by silica gel column chromatography (heptanes:EtOAc 2:1), to afford 43 as a white solid (6.1 g, 32.0 mmol, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.51-7.54 (m, 3H), 7.56 (d, 1H, J = 8.9 Hz), 7.83 (d, 1H, J = 8.9 Hz), 8.03-8.07 (m, 2H).

***N*-(3-Chlorophenyl)-6-phenylpyridazin-3-amine (44).** A microvawe vial (ovendried and under argon) was charged with 3-chloro-6-phenylpyridazine **43** (50 mg, 0.26 mmol, 1.0 equiv.), 3-chloroaniline (33.5 µL, 0.31 mmol, 1.2 equiv.), $Pd(OAc)_2$ (3 mg, 0.01 mmol, 5 mol%), JosiPhos (7 mg, 0.01 mmol, 5 mol%), Cs_2CO_3 (128 mg, 0.39 mmol, 1.5 equiv.) and anhydrous DMF (0.5 mL) and heated

overnight at 50°C. After cooling, the reaction mixture was diluted with water and the aqueous 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 (heptanes:EtOAc 7:3), to afford **44** as a brown solid (51 mg, 0.18 mmol, 69%). Purity ≥ 98 %; mp = 199-200 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.02 (ddd, 1H, J = 0.6 Hz, J = 2.0 Hz, J = 7.9 Hz), 7.24 (d, 1H, J = 9.3 Hz), 7.35 (t, 1H, J = 8.2 Hz), 7.44-7.54 (m, 3H), 7.58 (ddd, 1H, J = 0.6 Hz, J = 2.0 Hz, J = 8.3 Hz), 8.05-8.08 (m, 3H), 8.20 (t, 1H, J = 2.0 Hz), 9.62 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 116.8, 117.4, 117.6, 120.7, 125.6, 125.8, 128.8, 129.0, 130.3, 133.15 136.3, 142.2, 152.0, 156.1.

3-(Trifluoromethyl)benzene-1-carboximidamide (46). A microvawe vial (ovendried and under argon) was charged with 3-cyanobenzotrifluoride **45** (117.2 µL, 0.88 mmol, 1.0 equiv.) and anhydrous THF (2.2 mL). A solution of LiHMDS 1M in toluene (1.32 mL, 1.32 mmol, 1.5 equiv.) was added dropwise and the resulting mixture was stirred overnight at rt. The solution was acidified until pH = 1 using a solution of HCl 1N and then concentrated under vacuum. The residue was basified until pH =12 using a solution of NaOH. The aqueous phase was extracted 3 times with DCM. The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered and concentrated, to yield **46** as an orange oil (168 mg, 0.88 mmol, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 5.19 (s, 3H), 7.55 (t, 1H, J = 7.8 Hz), 7.71 (d, 1H, J = 7.8 Hz), 7.80 (d, 1H, J = 7.8 Hz), 7.87 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 123.1 (q, J = 3.7 Hz), 125.0, 127.1 (q, J = 3.7 Hz), 129.4, 129.5, 137.3, 164.2.

5-(4-Methoxyphenyl)-2-[3-(trifluoromethyl)phenyl]-1H-imidazole (48). 46 (145 mg, 0.77 mmol, 1.0 equiv.) and $NaHCO_3$ (259 mg, 3.08 mmol, 4.0 equiv.) were dissolved in a mixture THF:H_2O (3.88:0.97 mL) and the resulting mixture was heated at reflux for 20 mins. After it was cooled, a solution of 2-bromo-4'-methoxyacetophenone 47 (176 mg, 0.77 mmol, 1.0 equiv.) in THF (300 µL) was added dropwise and the solution was heated at reflux for 3 hours. The solution was concentrated under vacuum and the residue was diluted with water. The aqueous phase was extracted 3 times with EtOAc. The organic layers were combined, washed with brine, dried over Na2SO4, filtered, concentrated and purified by silica gel column chromatography (heptane:EtOAc 7:3), to afford 48 as a yellow oil (232 mg, 0.73 mmol, 95%). Purity ≥ 98 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.82 (s, 3H), 6.91 (d, 2H, J = 8.8 Hz), 7.31 (s, 1H), 7.39 (t, 1H, J = 7.8 Hz), 7.50 (d, 1H, J = 7.8 Hz), 7.65 (d, 2H, J = 8.8 Hz), 7.96 (d, 1H, J = 7.8 Hz), 8.03 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 55.3, 114.3, 122.1 (q, J = 3.7 Hz), 125.0 (q, J = 3.7 Hz), 125.2, 126.4, 128.5, 129.3, 130.9, 131.2 (q, J = 33.0 Hz), 145.5, 159.1; HRMS ($M+H^+$) 319.1045 (calcd for $C_{17}H_{13}F_3N_2OH^+$ 319.1053).

Biological methods

Cyclic nucleotide phosphodiesterase activity assay

PDE1, PDE3, PDE4 and PDE5 were isolated by anion exchange chromatography from bovine aortic smooth muscle cytosolic fraction according to the literature.[3] PDE2 was isolated from human platelets following the methods indicated in the literature.[2, 3] Purified PDEs were stored until use at -80°C in small aliquots (200 µL).
PDE activities were measured by a radioenzymatic assay as previously described in detail [64] at a substrate concentration of 1 µM cAMP or 1 µM cGMP in the presence of 10,000 cpm [^{3}H]-cAMP or [^{3}H]-cGMP as tracers. The buffer solution was of the following composition: 50 mM Tris-HCl (pH 7.5), 2 mM magnesium acetate, 50 mg BSA. PDE1 was assayed at 1 µM cGMP in calmodulin activated state (18 nM calmodulin with 10 µM $CaCl_2$). PDE2 was evaluated at 1 µM cAMP in activated state (in presence of 5 µM cGMP). PDE3 and PDE4 were assayed at 1 µM cAMP + 1 mM EGTA. To prevent the influence of reciprocal cross-contamination between PDE3 and PDE4, the studies were always carried out in the presence of 50 µM rolipram (a generous gift of Schering, Berlin. Germany), for PDE3 and in presence of 50 µM cGMP for PDE4. PDE5 activity was measured at 1 µM cGMP in the presence of 1 mM of EGTA. The compounds were dissolved in DMSO; the final concentration of DMSO in the assay (1%) did not affect PDE activity. The concentration of compounds that produced 50% inhibition of substrate hydrolysis (IC_{50}) was calculated by non-linear regression analysis (GraphPad Prism 5.00.288 software, San Diego, Ca) from concentration-response curves including 5 different concentrations of inhibitors. Maximal drug concentration used was limited to 10 µM and therefore the percentage of inhibition obtained was given.

In vivo studies.

Experiments were performed using C57BL/6J male mice (Charles River, L'Arbresle, France) between 8 and 10 weeks old at surgery time. Mice were group-housed five per cage and kept under a 12 hour light/dark cycle with food and water ad libitum. A total of 57 C57BL/6J mice were used for the experiments. All animals received proper care in agreement with European guidelines (EU 2010/63). At the end of the experiments, mice were killed by CO_2 inhalation (CO_2 Euthanasia programmer 6.5 version, TEMSEGA, Pessac, France) followed by cervical dislocation, according to the institutional ethical guidelines. The animal facilities Chronobiotron UMS3415 are registered for animal experimentation under the Animal House Agreement A67-2018-38. All protocols were approved by the "Comité d'Ethique en Matière d'Expérimentation Animale de Strasbourg" (CREMEAS, CEEA35).
Neuropathic pain was induced by cuffing the right sciatic nerve.[44, 45] Surgeries were performed under ketamine (68 mg/kg) / xylazine (10 mg/kg) intraperitoneal (i.p.) anesthesia (Centravet, Tadden, France). The main branch of the right

sciatic nerve was exposed and a cuff of PE-20 polyethylene tubing (Harvard Apparatus, Les Ulis, France) of standardized length (2 mm) was unilaterally inserted around it (Cuff group). The shaved skin was closed using suture. Sham-operated mice underwent the same surgical procedure without implantation of the cuff (Sham group). All mice were allowed to recover from surgery for at least two weeks before starting treatments.

Mechanical allodynia was tested using von Frey hairs and results were expressed in grams. Tests were done during the morning, starting at least 2 hours after lights on. Mice were placed in clear Plexiglas boxes (7 cm x 9 cm x 7 cm) on an elevated mesh screen. Calibrated 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.[45, 65] The person who conducted the tests was blinded to the treatments.

Treatments began fifteen days after the neuropathy was induced, and were maintained at least three weeks. During the treatment, the mice received two intraperitoneal injections per day (morning and evening) of **4b**, **16h**, **41n**, **pregabalin** (Lyrica, Pfizer) or **amitriptyline hydrochloride** (Sigma-Aldrich) (3 or 0.5 mg/kg, 5 mL/kg). Drugs were prepared in 0.1% hydroxymethylcellulose 0.9% NaCl solution that was also used for control injections.

Data were expressed as mean +/- SEM, and statistical analyses were performed using STATISTICA 7.1 (Statsoft, Tulsa, OK, USA), with ANOVA for multiple comparisons and the Duncan test for posthoc analyses**.**

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Supporting information available

General procedures and experimental data for compounds **25-30**, **42-44** and **46-48**. Noesy NMR of **41d**. Selectivity towards other PDE 1 to 4 isoenzymes for compounds **4c**, **4f, 16e**, **25e**, **25g**, **25h**, **25l**, and **45**. Experimental details and results for water solubility and chemical stability of phthalazines and indazoles derivatives. These materials are available free of charge via the Internet at http: //pubs.acs.org.

Keywords: PDE-5 inhibitors, MY5445, Structure activity relationship (SAR) studies, aminophthalazine derivatives, neuropathic pain.

References:

[1] T. Keravis, C. Lugnier, Cyclic nucleotide phosphodiesterase (PDE) isozymes as targets of the intracellular signalling network: benefits of PDE inhibitors in various diseases and perspectives for future therapeutic developments, Br. J. Pharmacol. 165 (2012) 1288–1305. doi:10.1111/j.1476-5381.2011.01729.x.

[2] J.F.K. Tcheudji, L. Lebeau, N. Virmaux, C.G. Maftei, R.H. Cote, C. Lugnier, P. Schultz, Molecular organization of bovine rod cGMP-phosphodiesterase 6, J. Mol. Biol. 310 (2001) 781–791. doi:10.1006/jmbi.2001.4813.

[3] C. Lugnier, P. Schoeffter, A. Le Bec, E. Strouthou, J.C. Stoclet, Selective inhibition of cyclic nucleotide phosphodiesterases of human, bovine and rat aorta, Biochem. Pharmacol. 35 (1986) 1743–1751. doi:10.1016/0006-2952(86)90333-3.

(4) R.H. Francis, T.M. Lincoln, J.D. Corbin, Characterization of a novel cGMP binding protein from rat lung., J. Biol. Chem. 255 (1980) 620–626.

(5) J.E. Souness, R. Brazdil, B.K. Diocee, R. Jordan, Role of selective cyclic GMP phosphodiesterase inhibition in the myorelaxant actions of M&B 22,948, MY-5445, vinpocetine and 1-methyl-3-isobutyl-8-(methylamino)xanthine, Br. J. Pharmacol. 98 (1989) 725–734. doi:10.1111/j.1476-5381.1989.tb14599.x.

(6) N. Galiè, H.A. Ghofrani, A. Torbicki, R.J. Barst, L.J. Rubin, D. Badesch, T. Fleming, T. Parpia, G. Burgess, A. Branzi, F. Grimminger, M. Kurzyna, G. Simonneau, Sildenafil Citrate Therapy for Pulmonary Arterial Hypertension, N. Engl. J. Med. 353 (2005) 2148–2157. doi:10.1056/NEJMoa050010.

(7) S. Korkmaz, T. Radovits, E. Barnucz, P. Neugebauer, R. Arif, K. Hirschberg, S. Loganathan, B. Seidel, M. Karck, G. Szabó, Dose-dependent effects of a selective phosphodiesterase-5-inhibitor on endothelial dysfunction induced by peroxynitrite in rat aorta, Eur. J. Pharmacol. 615 (2009) 155–162. doi:10.1016/j.ejphar.2009.05.020.

(8) E. Takimoto, H.C. Champion, M. Li, D. Belardi, S. Ren, E.R. Rodriguez, D. Bedja, K.L. Gabrielson, Y. Wang, D.A. Kass, Chronic inhibition of cyclic GMP phosphodiesterase 5A prevents and reverses cardiac hypertrophy, Nat. Med. 11 (2005) 214–222. doi:10.1038/nm1175.

(9) R. Zhang, Y. Wang, L. Zhang, Z. Zhang, W. Tsang, M. Lu, L. Zhang, M. Chopp, Sildenafil (Viagra) Induces Neurogenesis and Promotes Functional Recovery After Stroke in Rats, Stroke. 33 (2002) 2675–2680. doi:10.1161/01.STR.0000034399.95249.59.

(10) N.K. Jain, C.S. Patil, A. Singh, S.K. Kulkarni, Sildenafil-induced peripheral analgesia and activation of the nitric oxide–cyclic GMP pathway, Brain Res. 909 (2001) 170–178. doi:10.1016/S0006-8993(01)02673-7.

(11) C.I. Araiza-Saldaña, G. Reyes-García, D.Y. Bermúdez-Ocaña, F. Pérez-Severiano, V. Granados-Soto, Effect of diabetes on the mechanisms of intrathecal antinociception of sildenafil in rats, Eur. J. Pharmacol. 527 (2005) 60–70. doi:10.1016/j.ejphar.2005.10.016.

(12) M.L. Vale, D.E. Rolim, I.F. Cavalcante, R.A. Ribeiro, M.H.L.P. Souza, Role of NO/cGMP/KATP pathway in antinociceptive effect of sildenafil in zymosan writhing response in mice, Inflamm. Res. 56 (2007) 83–88. doi:10.1007/s00011-006-6109-8.

(13) M.M. Bezerra, V. Lima, V.C.C. Girao, R.C. Teixeira, J.R.V. Graca, Antinociceptive activity of sildenafil and adrenergic agents in the writhing test in mice, Pharmacol. Rep. 60 (2008) 339–344.

(14) C.S. Patil, N.K. Jain, V.P. Singh, S.K. Kulkarni, Cholinergic-NO-cGMP mediation of sildenafil-induced antinociception, Indian J. Exp. Biol. 42 (2004) 361–367.
(15) L.J. Huang, M.H. Yoon, J.I. Choi, W.M. Kim, H.G. Lee, Y.O. Kim, Effect of Sildenafil on Neuropathic Pain and Hemodynamics in Rats, Yonsei Med. J. 51 (2010) 82–87. doi:10.3349/ymj.2010.51.1.82..
(16) L. Wang, M. Chopp, A. Szalad, Z. Liu, M. Bolz, F.M. Ãlvarez, M. Lu, L. Zhang, Y. Cui, R.L. Zhang, Z.G. Zhang, Phosphodiesterase-5 is a therapeutic target for peripheral neuropathy in diabetic mice, Neuroscience. 193 (2011) 399–410. doi:10.1016/j.neuroscience.2011.07.039.
(17) G. Hackett, PDE5 inhibitors in diabetic peripheral neuropathy, Int. J. Clin. Pract. 60 (2006) 1123–1126. doi:10.1111/j.1742-1241.2006.01087.x..
(18) E.I. Gediz, C. Nacitarhan, E. Minareci, G. Sadan, Antinociceptive effect of vardenafil on carrageenan-induced hyperalgesia in rat: involvement of nitric oxide/cyclic guanosine monophosphate/calcium channels pathway, Iran. J. Pharm. Res. 14 (2015) 1137–1143.
(19) F. Rocha, F. Silva Jr, A. Leite, A. Leite, V. Girão, R. Castro, F. Cunha, Tadalafil analgesia in experimental arthritis involves suppression of intra-articular TNF release, Br. J. Pharmacol. 164 (2011) 828–835. doi:10.1111/j.1476-5381.2011.01469.x.
(20) M. Ambriz-Tututi, D.A. Velázquez-Zamora, H. Urquiza-Marín, V. Granados-Soto, Analysis of the mechanism underlying the peripheral antinociceptive action of sildenafil in the formalin test, Eur. J. Pharmacol. 512 (2005) 121–127. doi:10.1016/j.ejphar.2005.01.055.
(21) D. Levy, A. Höke, D. W. Zochone, Local expression of inducible nitric oxide synthase in an animal model of neuropathic pain, Neurosci. Lett.260 (1999) 207-209. doi:10.1016/s0304-3940(98)00982-3.
(22) A. Hervera, R. Negrete, S. Leánez, J. M. Martín-Campos, O. Pol, The spinal cord expression of neuronal and inducible nitric oxide synthases and their contribution in the maintenance of neuropathic pain in mice. PLoS ONE, 5 (2010), e14321. doi:10.1371/journal.pone.0014321.
(23) Y. Guan, M. Yaster, S. N. Raja, Y. X. Tao, Genetic Knockout and Pharmacologic Inhibition of Neuronal Nitric Oxide Synthase Attenuate Nerve Injury-Induced Mechanical Hypersensitivity in Mice. Mol. Pain, 3 (2007), 1744–8069–3–29. doi:10.1186/1744-8069-3-29.
(24) M. Tanabe, Y. Nagatani, K. Saitoh, K.Takasu, H. Ono, Pharmacological assessments of nitric oxide synthase isoforms and downstream diversity of NO signaling in the maintenance of thermal and mechanical hypersensitivity after peripheral nerve injury in mice. Neuropharmacology, 56 (2009), 702–708. doi:10.1016/j.neuropharm.2008.12.003.
(25) V. Granados-Soto, M. de O. Rufino, L. D. Gomes Lopes, S. H. Ferreira, Evidence for the involvement of the nitric oxide–cGMP pathway in the antinociception of morphine in the formalin test. Eur J Clin Pharmacol., 340 (1997), 177–180. doi:10.1016/s0014-2999(97)01399-x.
(26) K. Takasu, M. Honda, H. Ono, M. Tanabe, Spinal α2-adrenergic and muscarinic receptors and the NO release cascade mediate supraspinally produced effectiveness of gabapentin at decreasing mechanical hypersensitivity in mice after partial nerve injury. Br J Pharmacol., 148 (2006), 233–244. doi:10.1038/sj.bjp.0706731.
(27) T. Mixcoatl-Zecuatl, F. J. Flores-Murrieta, V. Granados-Soto, The nitric oxide-cyclic GMP-protein kinase G-K+ channel pathway participates in the antiallodynic effect of spinal gabapentin. Eur. J. Pharmacol., 531 (2006), 87–95. doi:10.1016/j.ejphar.2005.12.006.
(28) A. Gibson, Phosphodiesterase 5 inhibitors and nitrergic transmission—from zaprinast to sildenafil, Eur. J. Pharmacol. 411 (2001) 1–10. doi:10.1016/S0014-2999(00)00824-4.
(29) A.M. Martel, A. Graul, X. Rabasseda, R. Castaner, Sildenafil. Treatment of erectile dysfunction, Phosphodiesterase V inhibitor, Drug Future. 22 (1997) 138–143.
(30) D. Ormrod, S.E. Easthope, D.P. Figgitt, Vardenafil, Drugs Aging. 19 (2002) 217–227. doi:10.2165/00002512-200219030-00005.
(31) B. Dumaître, N. Dodic, Synthesis and Cyclic GMP Phosphodiesterase Inhibitory Activity of a Series of 6-Phenylpyrazolo[3,4- d]pyrimidones. J. Med. Chem. 39, (1996) 1635–1644. doi:org/10.1021/jm950812j.
(32) C.M.N. Allerton, C.G. Barber, K.C. Beaumont, D.G. Brown, S.M. Cole, D. Ellis, C.A.L. Lane, G.N. Maw, N.M. Mount, D.J. Rawson, C.M. Robinson, S.D.A. Street, N.W. Summerhill,. A Novel Series of Potent and Selective PDE5 Inhibitors with Potential for High and Dose-Independent Oral Bioavailability. J. Med. Chem. 49 (2006) 3581–3594. doi.:org/10.1021/jm060113e.
(33) M. Hagiwara, T. Endo, T. Kanayama, H. Hidaka, Effect of 1 -(3-Chloroanilino)--4-Phenylphthalazine (MY-5445), a Specific Inhibitor of Cyclic GMP Phosphodiesterase, on Human Platelet Aggregation, J. Pharmacol. Exp. Ther. 228 (1984) 467–471.
(34) N. Watanabe, H. Adachi, Y. Takase, H. Ozaki, M. Matsukura, K. Miyazaki, K. Ishibashi, H. Ishihara, K. Kodama, M. Nishino, M. Kakiki, Y. Kabasawa, 4-(3-Chloro-4-methoxybenzyl)aminophthalazines: Synthesis and Inhibitory Activity toward Phosphodiesterase 5, J. Med. Chem. 43 (2000) 2523–2529. doi:10.1021/jm9905054.
(35) M.A.J. Duncton, E.L. Piatnitski Chekler, R. Katoch-Rouse, D. Sherman, W.C. Wong, L.M. Smith II, J.K. Kawakami, A.S. Kiselyov, D.L. Milligan, C. Balagtas, Y.R. Hadari, Y. Wang, S.N. Patel, R.L. Rolster, J.R. Tonra, D. Surguladze, S. Mitelman, P. Kussie, P. Bohlen, J.F. Doody, Arylphthalazines as potent, and orally bioavailable inhibitors of VEGFR-2, Bioorg. Med. Chem. 17 (2009) 731–740. doi:10.1016/j.bmc.2008.11.049.
(36) Y. Takase, T. Saeki, M. Fujimoto, I. Saito, Cyclic GMP phosphodiesterase inhibitors. 1. The discovery of a novel potent inhibitor, 4-[[3,4-(methylenedioxy)benzyl]amino]-6,7,8-trimethoxyquinazoline, J. Med. Chem. 36 (1993) 3765–3770. doi: 10.1021/jm00076a003.
(37) G. Yu, H.J. Mason, X. Wu, J. Wang, S. Chong, G. Dorough, A. Henwood, R Pongrac, L. Seliger, B., He, D. Normandin, L Adam, J. Krupinski, J.E. Macor, Substituted Pyrazolopyridines as Potent and Selective PDE5 Inhibitors: Potential Agents for Treatment of Erectile Dysfunction. J. Med. Chem. 44 (2001) 1025–1027. doi:org/10.1021/jm0155042.
(38) N. Anand, S. Brown, Z. Tesfai, C. Zaharia, Phthalazine Derivatives as Jak1 Inhibitors, WO2012037132 (A1), 2012. https://worldwide.espacenet.com/publicationDetails/biblio?FT=D&date=20120322&DB=EPODOC&locale=en_EP&CC=WO&NR=2012037132A1&KC=A1&ND=5 (accessed March 7, 2017).
(39) L.T. Spada, J.G. Shiah, P.M. Hughes, T.C. Malone, G.W. Devries, J.L. Edelman, J.A. Wurster, Kinase Inhibitors, WO2010078393 (A1), 2010.https://worldwide.espacenet.com/publicationDetails/biblio?FT=D&date=20100708&DB=EPODOC&locale=en_EP&CC=WO&NR=2010078393A1&KC=A1&ND=4 (accessed March 7, 2017).
(40) H. Dyker, J. Scherkenbeck, D. Gondol, A. Goehrt, A. Harder, Azadepsipeptides: Synthesis and Evaluation of a Novel Class of Peptidomimetics, J. Org. Chem. 66 (2001) 3760–3766. doi:10.1021/jo001749v.
(41) E. Lohou, V. Collot, S. Stiebing, S. Rault, Direct Access to 3-Aminoindazoles by Buchwald-Hartwig C-N Coupling Reaction, Synthesis. 2011 (2011) 2651–2663. doi:10.1055/s-0030-1260110.
(42) Y. Mao, G. Tian, Z. Liu, J. Shen, J. Shen, An Improved Synthetic Route for Preparative Process of Vardenafil, Org. Process Res. Dev. 13 (2009) 1206–1208. doi:10.1021/op900235p.
(43) J.B. Smaill, A.J. Gonzales, J.A. Spicer, H. Lee, J.E. Reed, K. Sexton, I.W. Althaus, T. Zhu, S.L. Black, A. Blaser, W.A. Denny, P.A. Ellis, S. Fakhoury, P.J. Harvey, K. Hook, F.O.J. McCarthy, B.D. Palmer, F. Rivault, K. Schlosser, T. Ellis, A.M. Thompson, E. Trachet, R.T. Winters, H. Tecle, A. Bridges, Tyrosine Kinase Inhibitors. 20. Optimization of Substituted Quinazoline and Pyrido[3,4-d]pyrimidine Derivatives as Orally Active, Irreversible Inhibitors of the Epidermal Growth Factor Receptor Family, J. Med. Chem. 59 (2016) 8103–8124. doi:10.1021/acs.jmedchem.6b00883.
(44) M. Benbouzid, V. Pallage, M. Rajalu, E. Waltisperger, S. Doridot, P. Poisbeau, M.J. Freund-Mercier, M. Barrot, Sciatic nerve cuffing in mice: A model of sustained neuropathic pain, Eur. J. Pain. 12 (2008) 591–599. doi:10.1016/j.ejpain.2007.10.002.
(45) I. Yalcin, E. Waltisperger, M. Barrot, S. Megat, F. Barthas, M. Kremer, E. Salvat, The sciatic nerve cuffing model of neuropathic pain in mice, J. Vis. Exp. JoVE. 89 (2014) e51608.doi:10.3791/51608
(46) M. Bollenbach, E. Salvat, F.Daubeuf, P. Wagner,.I.Yalcin, M. Humo, M. Schmitt, Phenylpyridine-2-ylguanidines and rigid mimetics as novel inhibitors of TNFα overproduction: Beneficial action in models of neuropathic pain and of acute lung inflammation. Eur. J. Med. Chem. 147 (2018), 163–182. doi:10.1016/j.ejmech.2018.01.049.
(47) M. Kremer, E. Salvat, A. Muller, I. Yalcin, M. Barrot, Antidepressants and gabapentinoids in neuropathic pain: Mechanistic insights, Neuroscience. 338 (2016) 183–206. doi:10.1016/j.neuroscience.2016.06.057.

(48) R.-D. Treede, T.S. Jensen, J.N. Campbell, G. Cruccu, J.O. Dostrovsky, J.W. Griffin, P. Hansson, R. Hughes, T. Nurmikko, J. Serra, Neuropathic pain Redefinition and a grading system for clinical and research purposes, Neurology. 70 (2008) 1630–1635. doi:10.1212/01.wnl.0000282763.29778.59.

(49) Y. Bohren, L.-H. Tessier, S. Megat, H. Petitjean, S. Hugel, D. Daniel, M. Kremer, S. Fournel, L. Hein, R. Schlichter, M.-J. Freund-Mercier, I. Yalcin, M. Barrot, Antidepressants suppress neuropathic pain by a peripheral β2-adrenoceptor mediated anti-TNFα mechanism, Neurobiol. Dis. 60 (2013) 39–50. doi:10.1016/j.nbd.2013.08.012.

(50) I. Yalcin, L.-H. Tessier, N. Petit-Demoulière, S. Doridot, L. Hein, M.-J. Freund-Mercier, M. Barrot, β2-adrenoceptors are essential for desipramine, venlafaxine or reboxetine action in neuropathic pain, Neurobiol. Dis. 33 (2009) 386–394. doi:10.1016/j.nbd.2008.11.003.

(51) M. Kremer, I. Yalcin, Y. Goumont, X. Wurtz, L. Nexon, D. Daniel, S. Megat, R. A. Ceredig, C. Ernst, G. Turecki, V. Chavant, J-F.Theroux, A. Lacaud, L-E. Joganah, V. Lelivre, D. Massotte, P6E. Lutz, R. Glisbach, E. Salvat, M. Barrot, A Dual Noradrenergic Mechanism for the Relief of Neuropathic Allodynia by the Antidepressant Drugs Duloxetine and Amitriptyline . J. Neurosci. 38 (2018). 9934-9954. doi:10.1523/jneurosci.1004-18.2018.

(52) M. Benbouzid, N. Choucair-Jaafar, I. Yalcin, E. Waltisperger, A. Muller, M. J. Freund-Mercier, M. Barrot, Chronic, but not acute, tricyclic antidepressant treatment alleviates neuropathic allodynia after sciatic nerve cuffing in mice. European Journal of Pain, 12 (2008), 1008–1017. doi:10.1016/j.ejpain.2008.01.010.

(53) A.K. Naik, S.K. Tandan, D. Kumar, S.P. Dudhgaonkar, Nitric oxide and its modulators in chronic constriction injury-induced neuropathic pain in rats, Eur. J. Pharmacol. 530 (2006) 59–69. doi:10.1016/j.ejphar.2005.11.029.

(54) D. Levy, D.W. Zochodne, NO Pain: Potential Roles of Nitric Oxide in Neuropathic Pain, Pain Pract. 4 (2004) 11–18. doi:10.1111/j.1533-2500.2004.04002.x.

(55) A. Hervera, R. Negrete, S. Leánez, J.M. Martín-Campos, O. Pol, The Spinal Cord Expression of Neuronal and Inducible Nitric Oxide Synthases and Their Contribution in the Maintenance of Neuropathic Pain in Mice, PLoS ONE. 5 (2010) e14321. doi:10.1371/journal.pone.0014321.

(56) I.D.G. Duarte, S.H. Ferreira, The molecular mechanism of central analgesia induced by morphine or carbachol and the L-arginine-nitric oxide-cGMP pathway, Eur. J. Pharmacol. 221 (1992) 171–174. doi:10.1016/0014-2999(92)90789-7.

(57) G. Petho, P.W. Reeh, Sensory and Signaling Mechanisms of Bradykinin, Eicosanoids, Platelet-Activating Factor, and Nitric Oxide in Peripheral Nociceptors, Physiol. Rev. 92 (2012) 1699–1775. doi:10.1152/physrev.00048.2010.

(58) A. Schmidtko, Nitric Oxide-Mediated Pain Processing in the Spinal Cord, in: H.-G. Schaible (Ed.), Pain Control, Springer Berlin Heidelberg, Berlin, Heidelberg, 2015: pp. 103–117. doi:10.1007/978-3-662-46450-2_6..

(59) K.V. Otari, C.D. Upasani, Involvement of NO–cGMP pathway in anti-hyperalgesic effect of PDE5 inhibitor tadalafil in experimental hyperalgesia, Inflammopharmacology. 23 (2015) 187–194. doi:10.1007/s10787-015-0240-5.

(60) L. Garcia, S. Hlaing, R. Gutierrez, M. Sanchez, I. Kovanecz, J. Artaza, M. Ferrini, Sildenafil Attenuates Inflammation and Oxidative Stress in Pelvic Ganglia Neurons after Bilateral Cavernosal Nerve Damage, Int. J. Mol. Sci. 15 (2014) 17204–17220. doi:10.3390/ijms151017204.

(61) P.J. Austin, G. Moalem-Taylor, The neuro-immune balance in neuropathic pain: Involvement of inflammatory immune cells, immune-like glial cells and cytokines, J. Neuroimmunol. 229 (2010) 26–50. doi:10.1016/j.jneuroim.2010.08.013.

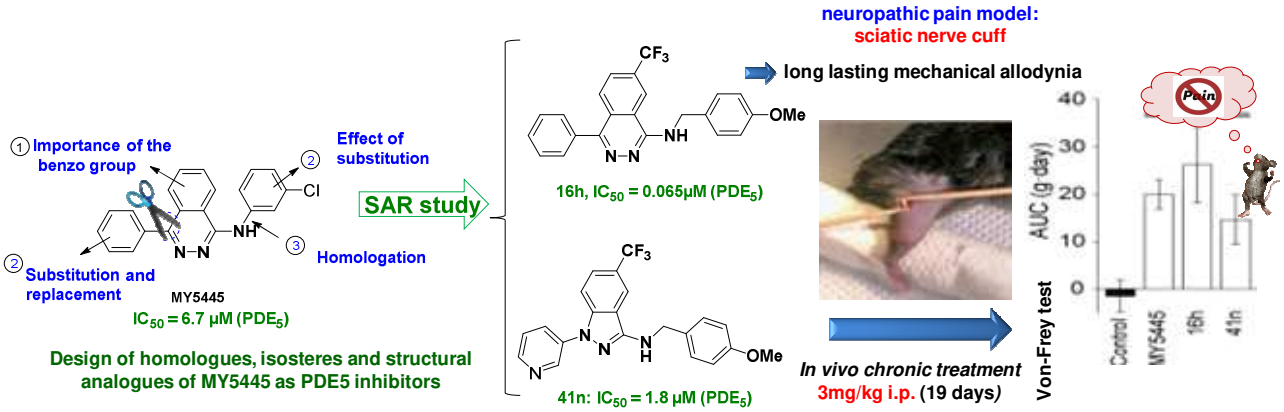
(62) E., Rojewska, A. Piotrowska, A. Jurga, W. Makuch, J. Mika, Zaprinast diminished pain and enhanced opioid analgesia in a rat neuropathic pain model. Eur. J. Pharmacol. 839 (2018) 21–32. doi: 10.1016/j.ejphar.2018.09.001.

(63) A. Singh Jaggi, N. Singh, Therapeutic Targets for the Management of Peripheral Nerve Injury- Induced Neuropathic Pain, CNS Neurol. Disord. - Drug Targets. 10 (2011) 589–609. doi:10.2174/187152711796235041.

(64) T. Keravis, R. Thaseldar-Roumié, C. Lugnier, Assessment of Phosphodiesterase Isozyme Contribution in Cell and Tissue Extracts, in: Phosphodiesterase Methods Protoc., Humana Press, New Jersey, 2005: pp. 063–074. doi:10.1385/1-59259-839-0:063.

(65) M. Barrot, Tests and models of nociception and pain in rodents, Neuroscience. 211 (2012) 39–50. doi:10.1016/j.neuroscience.2011.12.041.

Entry for the Table of Contents



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