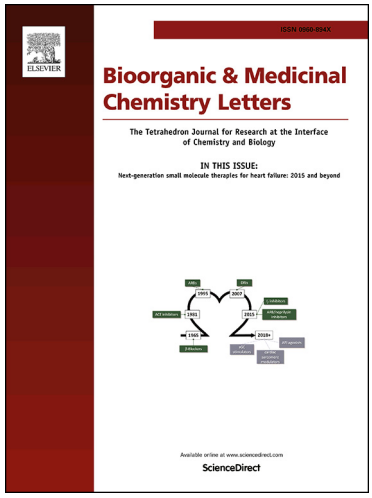

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PII: S0960-894X(19)30033-2
DOI: https://doi.org/10.1016/j.bmcl.2019.01.024
Reference: BMCL 26261

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 23 October 2018
Revised Date: 12 January 2019
Accepted Date: 17 January 2019

Please cite this article as: Lesuisse, D., Malanda, A., Peyronel, J-F., Evanno, Y., Lardenois, P., De-Peretti, D., Abécassis, P-Y., Barnéoud, P., Brunel, P., Burgevin, M-C., Cegarra, C., Auger, F., Dommergue, A., Lafon, C., Even, L., Tsi, J., Luc, T.P.H., Almario, A., Olivier, A., Castel, M-N., Taupin, V., Rooney, T., Vigé, X., Development of a novel NURR1/NOT agonist from hit to lead and candidate for the potential treatment of Parkinson's disease, *Bioorganic & Medicinal Chemistry Letters* (2019), doi: https://doi.org/10.1016/j.bmcl.2019.01.024

Development of a novel NURR1/NOT agonist from hit to lead and candidate for the potential treatment of Parkinson's disease

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Development of a novel NURR1/NOT agonist from hit to lead and candidate for the potential treatment of Parkinson's disease
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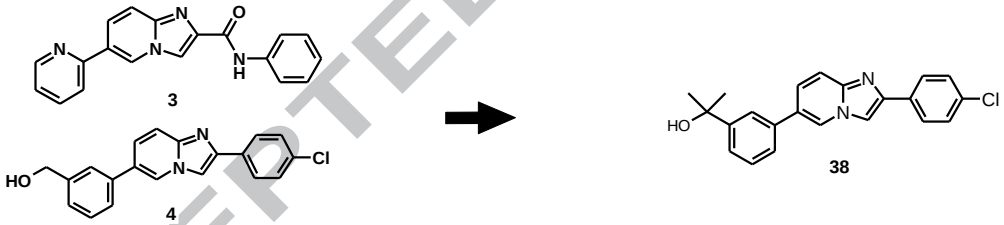
In the course of a programme aimed at identifying Nurr1/NOT agonists for potential treatment of Parkinson's disease, a few hits from high throughput screening were identified and developed into initial leads 3 and 4. A comprehensive program of optimization led to a potent and safe candidate drug 38 displaying neuroprotective and anti-inflammatory activity in several in vitro and in vivo models.



Abstract: In the course of a programme aimed at identifying Nurr1/NOT agonists for potential treatment of Parkinson's disease, a few hits from high throughput screening were identified and characterized . A combined optimization pointed to a very narrow and stringent structure activity relationship. A comprehensive program of optimization led to a potent and safe candidate drug displaying neuroprotective and anti-inflammatory activity in several *in vitro* and *in vivo* models.

Parkinson's disease is a devastating neurodegenerative disease that will affect 1 person out of 100 at 60 years (7 to 10 million worldwide)[1]. Dopaminergic neurons are mostly affected resulting in less dopamine and less activation of motor neurons in the central nervous system. The nuclear receptor Nurr1 has been shown to be essential to the development and survival of dopaminergic neurons in the substantia nigra and VTA[2]. Nurr1 KO mice display increased vulnerability to MPTP[3] and age-dependent dopaminergic dysfunction[4]. In addition, NOT (human gene) polymorphisms have been reported in Parkinson's disease[5]. Nurr1 is an orphan

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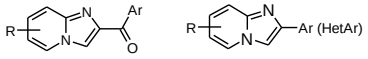
receptor, no endogenous ligand has been identified to-date and it has even been reported as a class of ligand-independent nuclear receptors[6]. This paper describes a combined medicinal chemistry effort that led to the identification of a potent imidazopyridine NOT agonist and allowed us to uncover some of its neuroprotective potential.

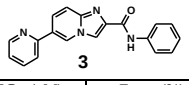
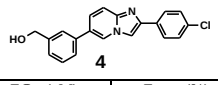
Screening was performed on a CHO cell line overexpressing a fusion protein of full length NOT with a Gal4 DNA binding domain and a Gal4 response element coupled with luciferase and, a neuronal N2A cell line containing endogenous mouse Nurr1 stably transfected with NBRE coupled to luciferase reporter gene. Strikingly, the screening on both cell lines led to very similar hits belonging to the bicyclic imidazopyridines series of general structures **1** and **2**.



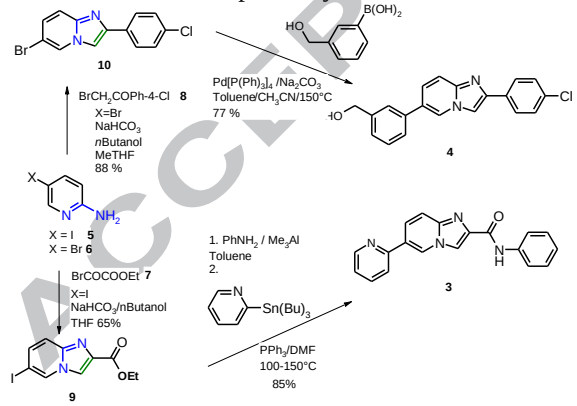
1 **2**

From there, optimization was conducted to give the two potent Nurr1/NOT agonists **3**[7] and **4**[8] (Table 1).

Table 1 : *In vitro* Nurr1/NOT activities of compounds **3** and **4**

	 **3**		 **4**	
	EC_{50} (nM)	Emax (%)	EC_{50} (nM)	Emax (%)
N2A cells	1	204	1	240
CHO cells	140	203	75	239

The synthesis of the two compounds is straightforward using classical chemistry reported for synthesis of imidazo[1,2a]pyridines[9] (**scheme 1**). The 4-iodo- or 4-bromoaminopyridines **5** and **6** were condensed with the bromooxalate and bromoketones **7** and **8** to produce the bicyclic imidazopyridines **9**[10] and **10**, respectively. Anilide formation and pyridine stannane coupling on **9** and hydroxymethylphenylboronic acid Susuki coupling on **10** led to **3** and **4**, respectively.



Scheme 1

Several assays were developed in order to assess the potential of these new compounds on both neuroprotection and inflammation in models related to Parkinson's disease. A rat mesencephalic primary neuronal model was used to evaluate the impact of the agonists on tyrosine hydroxylase (TH) expression. In vitro neuroprotection was evaluated in a co-culture of mouse primary neurons and microglia treated with LPS.

In parallel, the effects of Nurr1/NOT agonists on the production of the pro-inflammatory cytokine IL-1β were assessed in LPS-treated MG7 mouse microglia cell line. Results are summarized in **table 2**.

Table 2: In vitro cellular activities of compounds **3** and **4.**

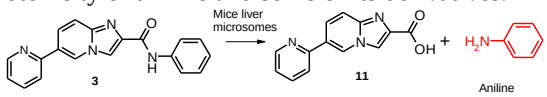
Assays	**3**	**4**
TH expression (% increase)	174-216 @3 µM	188-313 @3 µM
IL-1β secretion in MG7 (%inhibition)	Not done	58 % @ 3 µM
LPS-Induced Neurotoxicity (% of neuroprotection)	Not done	37% @ 3µM 65% @10 µM

Before evaluating the activity in *in vivo* pharmacological models, a full ADMET and PK exploration of these two leads was undertaken. Both displayed very poor solubilities and high clearance. In addition, hErg channel activation at low concentration was recorded for compound **4** (Table 3).

Table 3 : ADMET and PK properties of compounds **3** and **4**

Property	Parameter	**3**	**4**
Solubility	HPLC	<1 µM	<1µM
Plasma stability	Human/mouse	Stable/unstable	Stable/stable
Lipophilicity	LogD/PSA	3.65/59	2.95/38
pKa		3.6	5.16
Permeability	Caco2 (Papp)	High	High
Metabolism	%(mouse/human)	21/20	8/22
Clearance	Human hepatocytes ($mL.hr^{-1}.10^{-6}$ cells)	Mod/High (0.128)	High (0.42)
BBB	Brain/Plasma AUC	1.6	2.9
CYP inhibition	3A4/2D6/2C9 (IC_{50} µM)	>10/>10/8.6	>10/>10/8.6
CYP induction	% of Emax	None (<20%)	None (<20%)
Cardiotoxicity	hErg (EC_{50} µM)	>10	0.9
Genotoxicity	Ames, MNT, Fetax	Negative	Negative

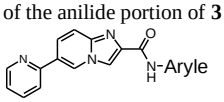
When analyzing the specific metabolism of **3**, even though it was not metabolized in human plasma or liver microsomes, the finding that it gave rise to amide hydrolysis with aniline release in mice microsomes (**scheme 2**) was of major concern in view of the known genotoxicity of aniline and some of its derivatives.



Scheme 2

As it has been reported that some substituted anilines are devoid of mutagenicity[11], we synthesized a series of analogs of **3** varying on one hand by the substitution of the aryl group. Surprisingly the activity on the receptor was lost in almost all analogs in the CHO cell line while activation (Emax) was very weak in the N2A cell line (**Table 4**). Only the N-(6-fluoropyridin-2-yl) derivative **20** kept some activity in both screening cell lines; however it turned out inactive in the TH assay.

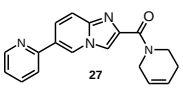
Table 4: Modifications of the anilide portion of **3**

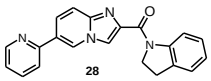


Cpd	Aryle	EC_{50} N2A (nM)	Emax N2A (%)	EC_{50} CHO (nM)	Emax CHO (%)
3	Ph	1	204	140	203
12	Ph(3,5-F2)	2,5	85	>1000	nd
13	Ph(2-Cl)	26,5	68	>1000	nd
14	Ph(3-F)	1	103	>1000	nd

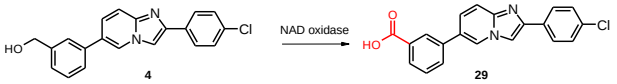
15	Ph(2-F)	1	104	>1000	nd
16	Ph(2,5-F2)	1	68	>1000	nd
17	Ph(2,3-F2)	1,2	64	>1000	nd
18	Ph(2-CN)	292	52	>1000	nd
19	Py(2)	2	87	>1000	nd
20	Py(2)(6-F)	0,6	86	25	138
21	Thienyl(3)	1	85	>1000	nd
22	Thiazole(2)	0,8	72	>1000	nd
23	Isoxazole(3)	20	62	>1000	nd
24	Thiadiazole(2)	67,6	70	>1000	nd
25	Pyrrazole(3)	21	61	>1000	nd
26	Isoxazole(4)	55	85	>1000	nd

Replacements of the anilide by heteroaryls such as tetrahydropyridine or indoline in **27** and **28** gave rise also to inactive compounds. Therefore this series was stopped.





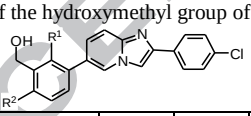
The second lead 4, even though not metabolized in liver microsomes, displayed a non-CYP450-mediated oxidation (most probably NAD oxidase-dependent) when profiled in human hepatocytes, leading to fast formation of the inactive carboxylic acid metabolite **29** (**scheme 3**).



Scheme 3

Classical medicinal chemistry on lead 4 did not allow correcting of these liabilities. Hindering the primary alcohol by introducing substitution on the aryl group was undertaken (**Table 5**). Addition of a fluorine atom in ortho positions of the hydroxymethylene permitted to keep the activity (CHO assay), however the hepatic clearance remained unacceptable (cpds **30** & **31**). Strikingly, one of the ortho positions was not tolerated with a single fluorine atom (cpd **32**) while disubstitution permitted to keep the activity (cpd **31**). We have no explanation for this result. By contrast, activity was totally lost when a methyl group was introduced in both ortho positions (cpds **33** &**34**).

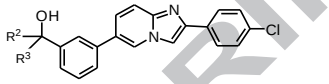
Table 5: Hindering of the hydroxymethyl group of **4**



Cpd	EC_{50} CHO (nM)	R^1	R^2	HEP clearance ($mL.hr^{-1}.10^{-6}$ cells)
4	45 ± 14	H	H	0.42
30	14 ± 2	F	H	0.188
31	38 ± 20	F	F	0.13675
32	>1000	H	F	
33	>1000	H	Me	
34	>1000	Me	H	

Replacements for the hydroxymethyl by a variety of alcoxy- or alkylamino-methyl, amides (with the exception of primary methyl amide) or cyano groups led to total loss of activity showing a stringent need for a hydrogen bond donor group at this position (**Supplement Table 1**). We therefore focused on modifications while keeping the hydroxyl group in place. Here the space was again strikingly narrow as only the methyl group was tolerated as a substituent (**Table 6**). Addition of one methyl on the hydroxymethyl group led to comparable activity in the CHO Gal4 assay (cpd **35**). The enantiomers were separated (cpds **36** & **37**) and the most active (cpd **36**) again showed a high hepatocyte clearance not predicted by microsomal metabolism. Any substitution other than methyle (ethyle, butyle, pentyle, hexyle, *i*Butyle, cyclopentyle, phenyle) abolished the activity (**Supplement Table 2**). Addition of a second methyl group led to compound **38** with acceptable activity and with a solved clearance issue. With the exception of oxetanyl **39** all other disubstitutions gave rise to inactive compounds (cpds **40**-**41**) (**Table 6**).

Table 6: Modifications on the hydroxymethyl group of **4**



Cpd	EC_{50} CHO	R^2	R^3	HEP clearance	Comment
4	45 ± 14	H	H	0.42	
35	99 ± 24	Me	H		rac
36	75 ± 45	Me	H	0.45	(+)
37	219 ± 174	Me	H		(-)
38	218 ± 38	Me	Me	0.051	
39	323 ± 86	CH2OCH2			
40	>1000	(CH2)2O(CH2)2			
41	>1000	Me	CH2OH		

The initial lead **4** displayed hErg inhibition (IC_{50}=0.9 µM) not compatible with chronic long term treatment. Therefore, throughout the above optimization this parameter was also closely monitored. Unfortunately, the optimized lead **38** still displayed hErg inhibition (IC_{50}=1.5 µM). As the difluorinated analog **31** showed reduced hErg activity, compound **42**, combining substitutions of **38** and **31**, was synthesized. However, this compound did not show reduced hErg activity and lost its activity on the target (**Figure 1**).

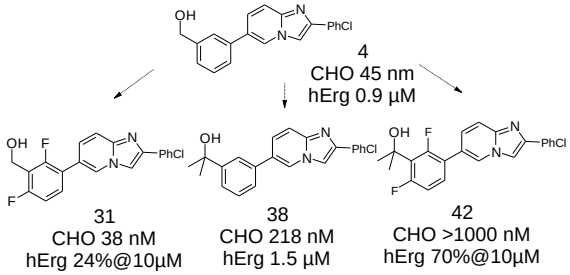


Figure 1

Since the discovery of a link between drug-induced hERG inhibition and cardiac safety related to Torsade de Pointes (TdP)[12], this parameter has become one of the essential counter screening parameters leading to elimination of hERG inhibitors. However, for some medicinal chemistry targets such as kinases where the binding site combines lipophilicity, flatness and the need for basic nitrogens, totally eliminating hErg activity might become a real challenge. Any attempts we made to modulate the pKa of lead **4** scaffold by removing or adding nitrogen atoms such as in compounds **43**-**46** totally abolished the activity (CHO assay) (**Figure 2**).

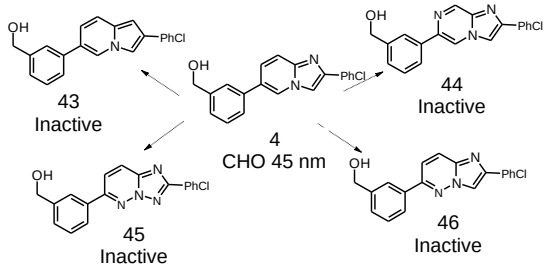


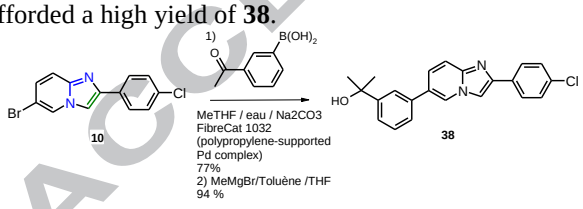
Figure 2

However, it has been shown that hErg is not always predictive of proarrhythmic risk. For example, it has been shown that Clomiphene, a potent hErg inhibitor, does not display a significant QT effect in the perfused heart of guinea pig[13]. Discrepancies have been proposed to be related to different binding kinetics in the hErg channel.[14] As compound **38** displayed a good brain PK profile (**Table 7**) along with a safe profile on other parameters, such as CYP (inhibition and induction) and selectivity vs a panel of GPCR and nuclear receptors, it was decided to further investigate its potential cardiac liabilities. Importantly, compound **38** showed no significant hemodynamic, electrocardiographic or electrophysiological effect after single 5-min iv administration in anaesthetized guinea pigs and dogs despite high heart exposure in both species (up to 17 and 40 µM total concentrations in guinea pig and dog respectively (supporting information).

Table 7: Pharmacokinetic parameters following single IV (3 mg/kg) and oral (10 mg/kg) administration of **38** to female C57BL/6 mice.

Route	Matrix	Dose	C_0 or C_{max}	t_{max}	AUC	CL	V_{ss}	$t_{1/2z}$	Brain /plasma AUC ratio
		(mg/kg)	(µg/mL)	(day)	(µg.h/mL)	(L/(h.kg))	(mL/kg)	(day)	(%)
iv	Plasma	3	4.87	-	20.6	0.1	0.9	4.1	
iv	Brain	3	2.63		15.0			3.8	
po	Plasma	10	4.17	1	70.3			8.1	0.7
po	Brain	10	3.08	8	55.6			8.2	0.8

The synthesis of **38** was straightforward (**scheme 4**). From 10, Suzuki coupling followed by Grignard reaction afforded a high yield of **38**.



Scheme 4

Compound 38 displayed good neuroprotective and anti-inflammatory activity *in vitro* in several assays and increased the expression of TH in rat mesencephalic dopaminergic neurons (**Table 8**).

Table 8: In vitro cellular activities of compound **38** at 3µM.

TH expression (% increase)	188-284
IL-1β secretion in MG7 (% inhibition)	28-46
LPS-induced neurotoxicity (% of neuroprotection)	86-99

Compound **38** demonstrated *in vivo* anti-inflammatory activity in a LPS-induced TNF-α release model, with a 47% inhibition of serum TNF-α at 30mg/kg (supporting information). It was also shown to induce dopamine neuroprotection and to reduce brain gliosis and IL-6 levels in a Parkinson's Disease lesion model induced by inflammatory stimulant Poly(I:C)[15].

A few hits from an HTS were characterized as agonists of the NURR1/NOT receptor. Combined optimization pointed to a very narrow and stringent structure activity relationship and led to a potent candidate displaying neuroprotective and anti-inflammatory activities in several *in vitro* and *in vivo* models. During this work, we prioritized compounds that were active on both CHO and N2A cell lines. CHO cell line overexpressing NOT as a fusion protein coupled to Gal4 only selects agonists of homodimeric receptor, while N2A cell line relying on endogenous expression of mouse Nurr1 has the potential to unveil agonists of both homodimeric or heterodimeric receptors. Recent evidence suggests that the dimeric transcription factor Nurr1/retinoid X receptor (RXR) is a key player in anti-inflammatory and neuroprotective activities[16]. Some of the compounds reported here could also be dual agonists but this was not looked at. A few reports describing NURR1 or NOT agonists can be found in the literature[17,18] however it is not clear whether any of these compounds are actively developed. Unlike some of the reported agonists of these receptors, our series offer the advantage of having no polar function preventing access to the brain.

Acknowledgements

We are grateful to the analytical department for compound analyses and enantiomers separation.

References and Notes

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