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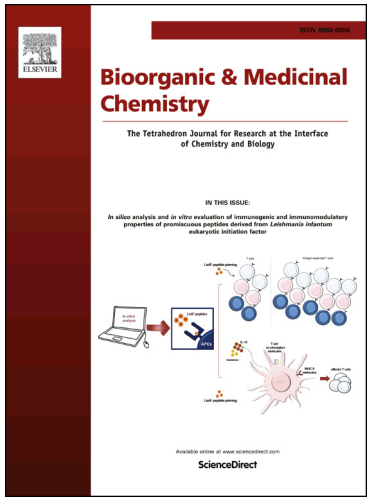

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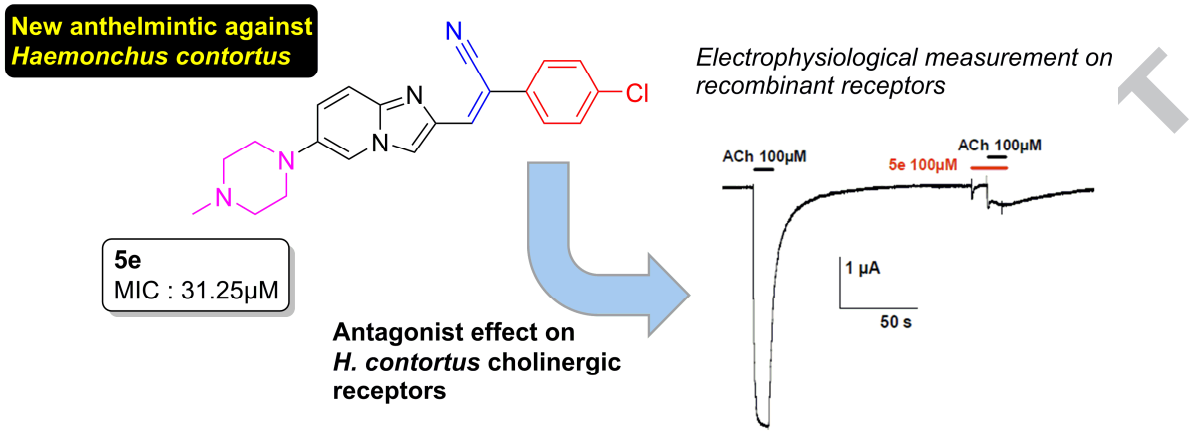

Discovery of imidazo[1,2-*a*]pyridine-based anthelmintic targeting cholinergic receptors of *Haemonchus Contortus*

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Abstract

We report the synthesis of a series of imidazo[1,2-*a*]pyridine-based molecules as anthelmintic against the livestock parasite *Haemonchus contortus*. The molecules were tested by using Larval Paralysis Test (LPT), in order to target ionic channels, as most of the prominent marketed anthelminthics present such mechanism of action. The most active compound (**5e**) displayed paralysis on *H. contortus* stage 3 larvae until 31.25µM. Effect of **5e** on *H. contortus* cholinergic receptors (L-AChR1 and 2) was characterized *via* electrophysiological measurement and a rare antagonist mode of action was unveiled.

Keywords: imidazo[1,2-*a*]pyridine, anthelmintic, *Haemonchus contortus*, cholinergic receptors, antagonist effect.

1. Introduction

Helminth infections of livestock in the world are clearly of considerable importance, and cause major financial losses.[1] In this regard, the barber's pole worm, *Haemonchus contortus*, is one of the most important parasites of small ruminants and causes important morbidity and mortality.[2,3] Since the introduction of piperazine in 1954 as veterinary anthelmintic, only a limited number of major compound classes have been described (Figure 1), such as benzimidazoles (*e.g.* Thiabendazole, 1960), nicotinic agonists (*e.g.* Levamisole, 1968) or macrocyclic lactones (*e.g.* Ivermectin, 1981).[4]

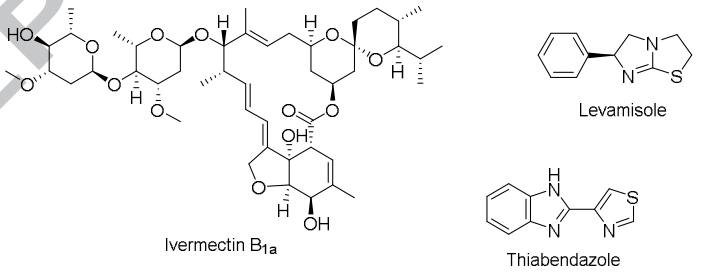


Figure 1: Principal classes of currently used anthelmintics

Despite the success of these different classes of anthelmintic compounds, resistance to all three groups has been recorded, and increasing population of parasite with multiple resistance have been observed.[5,6] In spite of the obvious need for new anthelmintic, only three new classes of compounds became commercially available over the past two decades (Figure 2): Emodepside (cyclooctadepsipeptide), Monepantel (amino acetonitrile derivatives AADs) and Derquantel (spiroindol).[7]

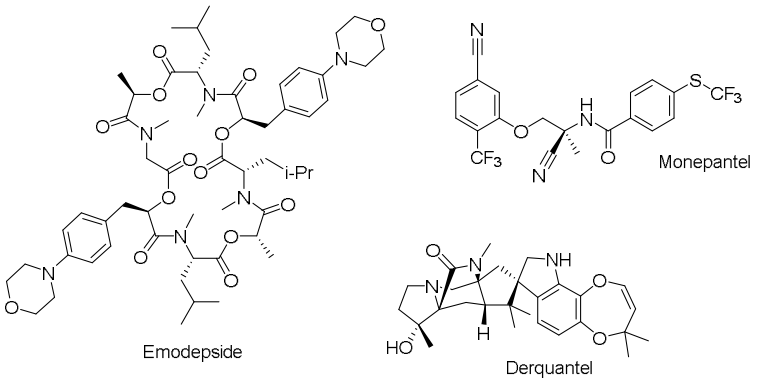


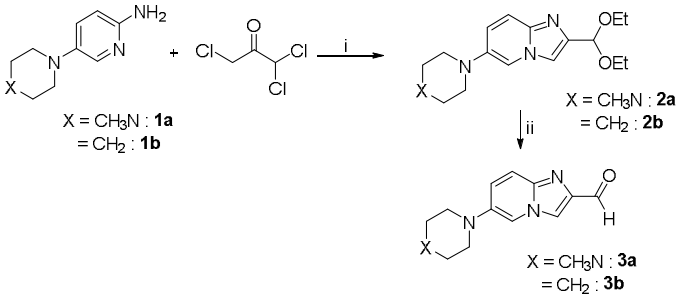
Figure 2: New classes of anthelmintic commercially available

In order to overcome the inevitable appearance of resistance, there is an urgent need for new anthelmintic, especially new chemical entities.[8] To this end we described here a series of imidazo[1,2-*a*]pyridine-based small molecule active against *H. contortus*. In the present study, we aimed to seek molecules active on nicotinic acetylcholine receptors (nAChRs). Among the nAChRs, the levamisole-sensitive nAChR subtypes (L-nAChRs) are the targets of a wide range of currently used anthelmintic compounds (imidazothiazoles, tetrahydropyrimidines).[9] In most cases, cholinergic agonists affect the L-nAChRs at the nematode neuromuscular junction, causing spastic paralysis. Therefore the L-nAChRs are concentrating a lot of attention and interest, due to their diverse and fascinating pharmacologies, as well as the development of novel compounds such as tribendimidine and derquantel.[10]

2. Results and Discussion

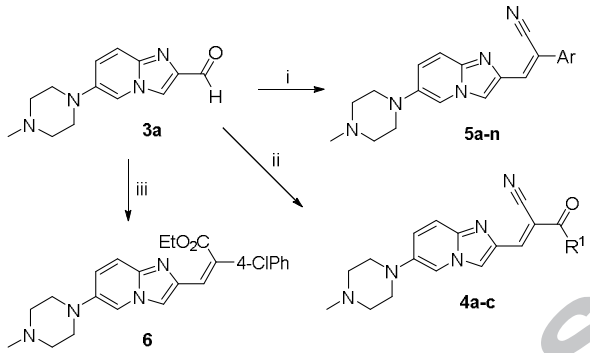
2.1. Chemistry

Imidazo[1,2-*a*]pyridine has been widely exploited in various pharmacological areas[11] and our laboratory has been interested on imidazo[1,2-*a*]pyridine chemistry and biological activity for the past decades.[12] This heterocycle scaffold was used as the core of anthelminthic compounds long time ago,[13] and has recently regain interest with the preparation of imidazo[1,2-*a*]pyridine with chalcone side chain active against *Haemonchus contortus* by Ouattara's group.[14] Acrylonitrile has also been described very recently as a valuable scaffold for anthelmintic purpose,[15] we thus decided to prepare hybrid molecules bearing imidazo[1,2-*a*]pyridine core and acrylonitrile side chain, as a starting point to discover new chemical entities targeting cholinergic receptors of *Haemonchus contortus*. The synthesis started by the preparation of common aldehyde intermediate **3a-b** (scheme 1).



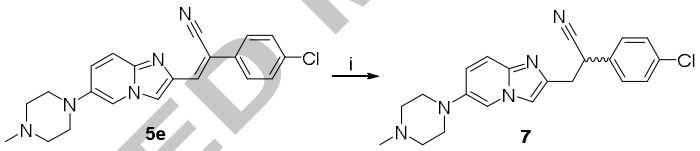
Scheme 1: Synthesis of **3a-b**. Reagents and conditions: (i) DME, r.t., 5h, then EtOH, reflux, 2h. 59%. (ii) HCl, CH_3CN:H_2O 3:1, 100°C, 3h. 83-91%.

First, the imidazo[1,2-*a*]pyridine ring formation between commercially available **1a** or **1b** and 1,1,3-trichloroacetone, at room temperature in DME and then in refluxing ethanol afforded acetals **2a-b** in 59% yield. The acetals **2a-b** were further converted into aldehydes **3a-b** in 83% and 91% yield, respectively.[16] Compound **3a** was then used in Knoevenagel condensation with acetonitriles derivatives in order to furnish acrylonitriles **4a-c** and **5a-n** (scheme 2).



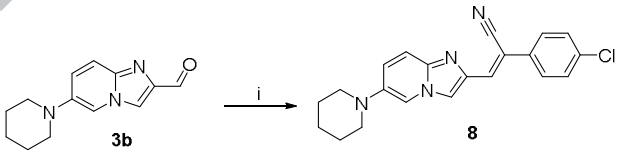
Scheme 2: Synthesis of **4a-c**, **5a-n** and **6**. Reagents and conditions: (i) $ArCH_2CN$, MeONa 5%, MeOH, reflux, 1,5h. 38-99% (ii) R^1COCH_2CN, piperidine 10%, EtOH, reflux, 3h. 97-99% (iii) LDA, 4-$ClPhCH_2CO_2Et$, THF, -10°C→r.t., 4h. 7%.

Depending on the acetonitrile derivative, two conditions were used: MeONa 5% in refluxing methanol for acetonitriles with aromatic substituents and piperidine 10% in refluxing ethanol for carbonyl susbstituted acetonitriles. Other compounds with related structures were prepared as well, like ethylacrylate **6**, from ethyl 4-chlorophenylacetate (scheme 2) and saturated analog **7**, by reduction of double bond using $NaBH_4$ (scheme 3).



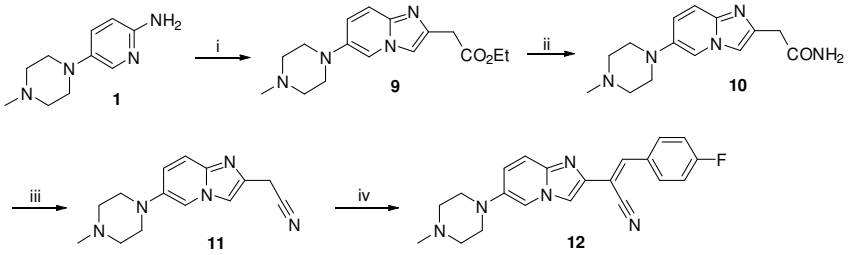
Scheme 3: synthesis of **7**. Reagents and conditions: (i) $NaBH_4$, EtOH, r.t. 77%.

Compound **8** with piperidine ring instead of *N*-methylpiperazine in 6-position was prepared using the same sequence as **5a-m** but starting from **3b** (scheme 4).



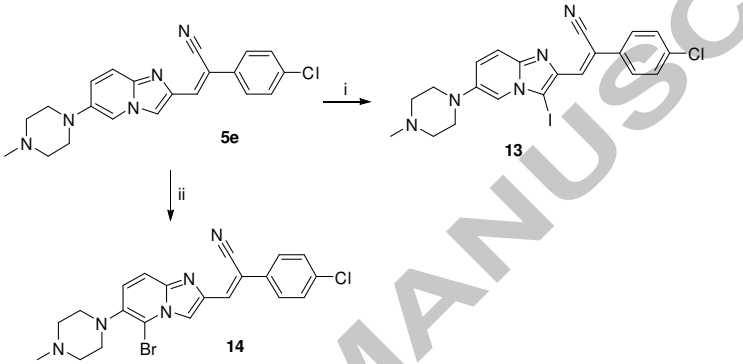
Scheme 4: Synthesis of **8**. Reagents and conditions: (i) $ArCH_2CN$, MeONa 5%, MeOH, reflux, 1,5h. 76%.

Derivative with reverse acrylonitrile **12** was prepared according to the following sequence: 1) heterocyclization from **1** and ethyl 4-chloroacetoacetate gave ester **9** (scheme 5). 2) Compound **9** was further converted into amide **10** by action of aqueous ammonia. 3) **10** was then transformed into nitrile **11** by using $POCl_3$. 4) Ultimately, 2-acetonitrile-imidazo[1,2-*a*]pyridine compound **11** was subjected to Knoevenagel condensation with 4-fluoro-benzaldehyde to yield product **12**.



Scheme 5: Synthesis of **12**. Reagents and conditions: (i) $ClCH_2COCH_2COOEt$, DME, r.t., 4h, then EtOH, reflux, 5h. 35% (ii) NH_3 aq, H_2O:THF 3:1, r.t., overnight. 84% (iii) $POCl_3$, 2h, reflux. 60% (iv) 4-F-benzaldehyde, MeONa 5%, MeOH, reflux, 1,5h. 67%.

We then tried to explore the possibility to functionalize the 3-position. To this purpose we used classic halogenating agent, ICl and NBS. (scheme 6).



Scheme 6: Synthesis of **13** and **14**. Reagents and conditions: (i) ICl, $CHCl_3$, r.t., 45min. (ii) NBS, CH_3CN, r.t., 30min.

If the use of ICl afforded the expected 3-iodinated compound **13** as the sole isomer, surprisingly NBS formed selectively 5-brominated compound **14**. The structure of both products was confirmed by NMR analysis and X-ray crystal structure analysis for compound **14** (figure 3).[17] This regioselective halogenation pave the way for functionalization of both 3- and 5-position.

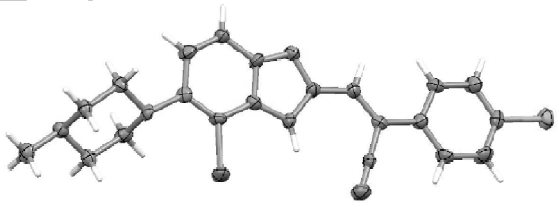
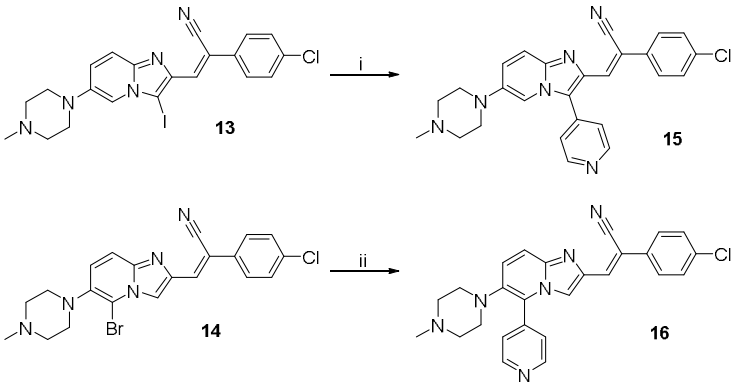


Figure 3: ORTEP diagram for **14**.

With **13** and **14** in hands, we prepared compounds **15** and **16** by Suzuki-Miyaura cross-coupling with 4-pyridylB$(OH)_2$ (scheme 7).



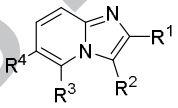
Scheme 7: Synthesis of **15** and **16**. Reagents and conditions: (i) 4-PyrB(OH)$_2$, Pd(PPh$_3$)$_4$, Na$_2$CO$_3$, dioxane:H$_2$O 2:1, 100°C (MW), 1h. (ii) 4-PyrB(OH)$_2$, Pd(PPh$_3$)$_4$, Na$_2$CO$_3$, DME:H$_2$O 2:1, 100°C (MW), 1h.

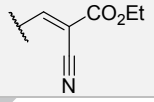
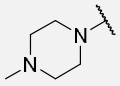
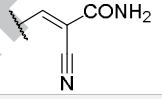
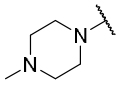
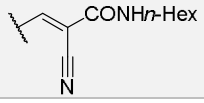
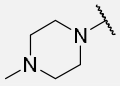
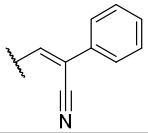
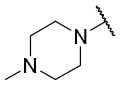
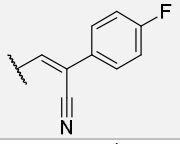
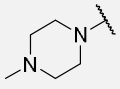
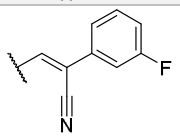
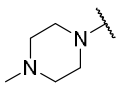
2.2. Pharmacology

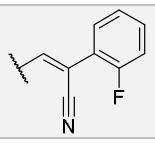
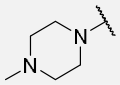
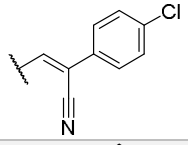
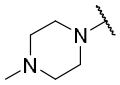
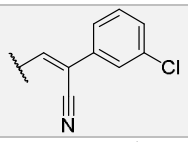
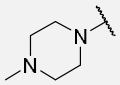
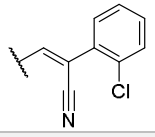
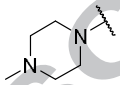
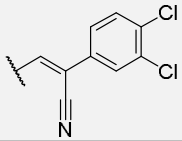
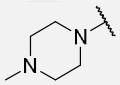
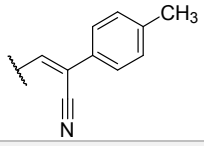
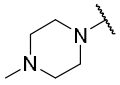
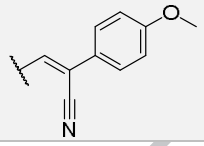
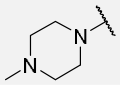
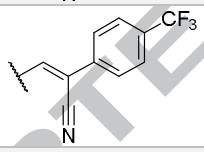
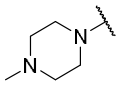
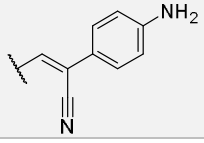
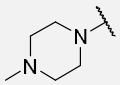
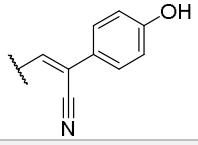
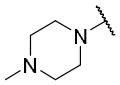
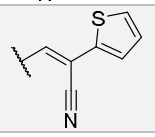
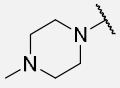
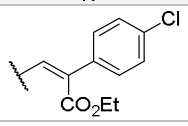
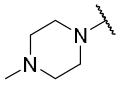
2.2.1. Larval Paralysis Test (LPT)

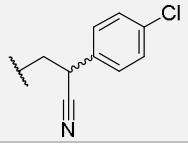
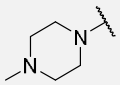
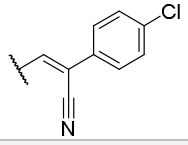
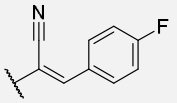
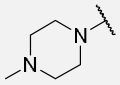
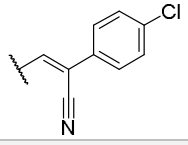
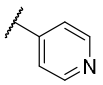
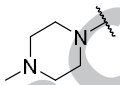
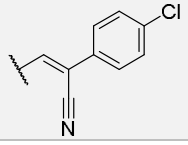
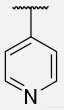
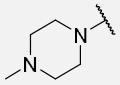
In order to test our molecules and to evaluate their potential action on neuromuscular cholinergic receptors we choose the Larval Paralysis Test (LPT)[18] as preliminary screening method, to directly observe the effect of our molecules on stage 3 larvae (L3) motility, with serial dilutions from 500µM to 31.25µM. Minimum Inhibitory Concentrations (MIC) for all our compounds are presented in Table 1.

Table 1: Structures and biological activities of compounds **4a-c**, **5a-n**, **6**, **7**, **8**, **13**, **12** and **15** against *H. contortus* on Larval Paralysis Test.



Compound[a]	R^1	R^2	R^3	R^4	MIC (µM)[b]
4a		H	H		250
4b		H	H		> 500
4c		H	H		> 500
5a		H	H		250
5b		H	H		62.5
5c		H	H		125

5d		H	H		250
5e		H	H		31.25
5f		H	H		62.5
5g		H	H		250
5h		H	H		62.5
5i		H	H		62.5
5j		H	H		62.5
5k		H	H		125
5l		H	H		62.5
5m		H	H		62.5
5n		H	H		> 500
6		H	H		> 500

7		H	H		250
8		H	H		> 500
12		H	H		> 500
15			H		> 500
16		H			> 500

a) all compounds were tested as tartrate salt. b) Lowest concentration for a minimum of 80% paralyzed larvae.

The three compounds with carbonyl-substituted acrylonitrile moieties (**4a-c**) gave only no or low activity. The aryl-substituted acrylonitrile compounds (**5a-5n**) presented higher activities. If the phenyl derivative had a moderate activity (**5a**, 250µM), introducing a fluorine at the para position (**5b**) increased dramatically the activity (62.5µM). Comparison between **5b**, **5c** and **5d** clearly indicated a relation between the position of the fluorine atom on the phenyl ring and the activity, *i.e.* para > meta > ortho, with MIC of 62.5, 125 and 250µM, respectively. Changing fluorine to chlorine (**5e**) increased even more the activity, with 31.25µM MIC (to compare with 10µM for levamisole). Position of chlorine on the aromatic ring followed the same logic than for fluorine (**5e**, **5f**, **5g**) with MIC of 46.5, 62.5 and 250µM, respectively. 3,4-dichloro **5h** didn't improve the activity, neither than different para substituents (**5i-m**). The only heteroaryl (2-thienyl) substituted analog **5n** showed no activity. To probe SAR associated with **5e**, some changes around the structure were undertaken. Changing nitrile group to ethyl ester (**6**) rendered this analog completely inactive, as well as changing *N*-methylpiperazine to piperidine (**9**). For the piperidine-substituted compound the drop of activity might be due to a decreased solubility. Reducing the double bond furnished the saturated analog **7**, showing a reduced activity (250µM). The isomer **12** also exhibited a total loss of activity. Ultimately, both 3- and 5-(4-pyridyl) substituted analogs **16** and **17** were inactive. Once again the loss of activity for this two compounds may be partially attributed to a drop of solubility.[19] All the SAR information are outlined in figure 4.

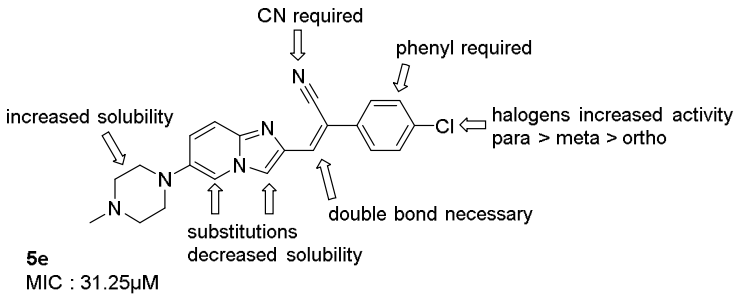


Figure 4: Structure Activity Relationship associated with **5e**

2.2.2. Actions of the compounds on H. contortus receptors expressed in Xenopus laevis oocytes

To complement the LPT approach we then assessed our hypothesis that the observed paralysis could be related to an action on the L-nAChRs by performing electrophysiological studies as previously described.[20] Two different subtypes of *H. contortus* levamisole receptors, Hco-L-AChR1 and Hco-L-AChR2, were expressed in *Xenopus laevis* oocytes, and currents were monitored to follow the activation (agonist effect inducing a current *via* channel opening) or the inhibition (antagonist effect avoiding a current *via* channel blocking) of the channels. **5e** was tested on both receptors and no agonist action was observed (Figure 5A and 5B) as no current was recorded after perfusion of 100µM solution of **5e** on either Hco-L-AChR1 or Hco-L-AChR2. However, the perfusion of oocytes expressing the L-nAChRs for 10 s with **5e** (100µM) prior to an application of 100µM acetylcholine in the continued presence of **5e** resulted in a strong reduction in the current amplitude recorded from both receptors (Figure 5C and 5D). Indeed, perfusing **5e** prior to natural neuromediator acetylcholine, almost completely annihilated the elicited currents, in a reversible way. This striking antagonist effect is of considerable interest, since most of the drugs targeting L-nAChRs are cholinergic agonists. To date, only one marketed molecule has been described as L-nAChR antagonist (Derquantel).[21] Noteworthy, inactive compound **4b** has been tested as well on Hco-L-AChR1, and neither agonist nor antagonist effect was observed.[22] These result proves the pertinence of LPT test when aiming to target nAChR. Developing new anthelmintics with rare mechanism of action is highly desirable, especially in the context of increased population of resistant parasites.

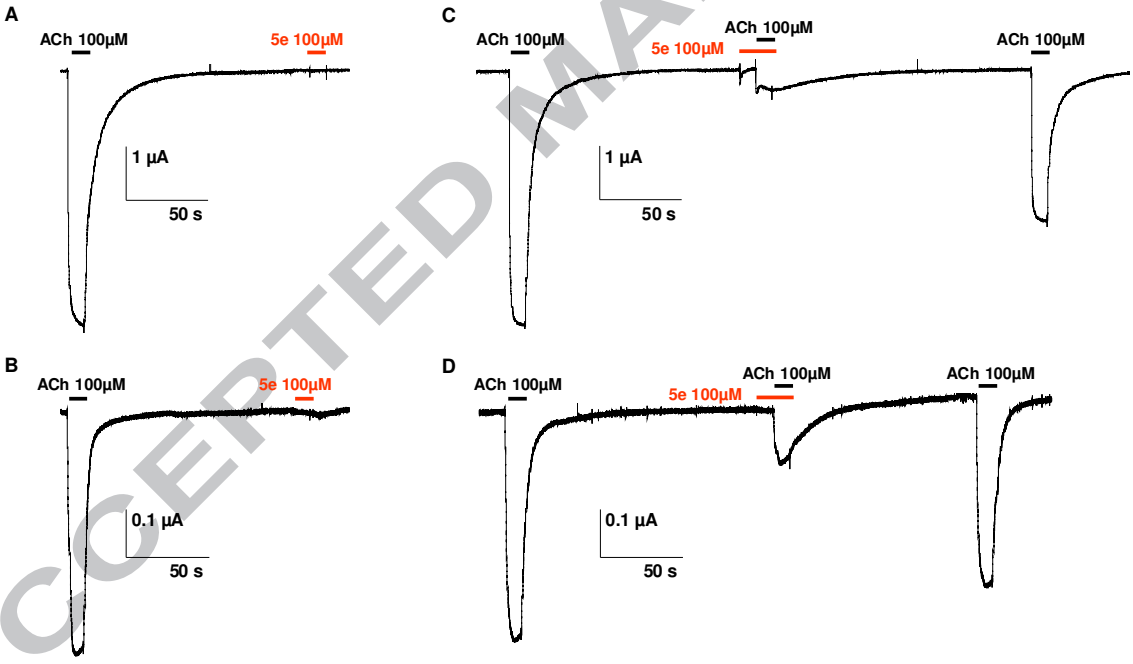


Figure 5: Effect of **5e** on *H. contortus* L-AChRs expressed in *Xenopus* oocytes: (A) The *H. contortus* L-AChR1 and (B) L-AChR2 were challenged with 100µM of acetylcholine (ACh) and 100µM **5e**. (C) The inward currents from oocytes expressing the *H. contortus* L-AChR1 and (D) L-AChR2 mediated by 100µM ACh can be efficiently blocked by 100µM **5e** revealing an antagonist effect. The bars indicate the time period of the compound (acetylcholine or **5e**) application.

3. Conclusions

We described here a novel series of imidazo[1,2-*a*]pyridine-based anthelmintics, showing action on the strongyle parasite nematode *Haemonchus contortus*. Interestingly, the most potent compound **5e** inhibited the motility of the worms, until 31.25µM. Furthermore, an original mode of action has

been unveiled for **5e**, since the observed paralysis was then correlated to antagonist effect on both Levamisole–nAChR1 and 2 subtypes. Further investigations, including experiments on receptors from different species of helminths and deeper pharmacological studies may help the design of novel anthelmintic agents.

4. Experimental section

4.1. Chemistry

4.1.1. General considerations

All reagents were used directly as obtained commercially. Thin-layer chromatography (TLC) were performed using Merk® silica gel $60F_{254}$ plates. Column chromatography were performed using Merck Geduran® Si 60 (40-63µm) silica. Melting points were determined on a capillary apparatus (Stuart, Staffordshire, United Kingdom) and are uncorrected. Microwave heating was performed using CEM® Explorer SP 12 S class apparatus (max power 300W). NMR experiments were performed at 300 mHz (^{1}H) and 75 mHz (^{13}C) on a Bruker-Avance 300 MHz spectrometer. Assignment of carbons noted C* may be interchanged. Mass spectra were determined on a Hewlett Packard 5988A spectrometer or on a Shimadzu QP 2010 spectrometer by direct inlet at 70 eV.

*4.1.2. Synthesis of compounds **2**-**17***

General method for acetal synthesis (**2a-b**)

To a stirred solution of **1a-b** (*e.g.* 8.5g, 44.3mmol, 1eq) in DME (100mL) was added 1,1,2-trichloroacetone (6.8mL, 63.5mmol, 1.4eq) dropwise at room temperature. After stirring at room temperature for 5h, the formed solid was filtered, and washed with thrice 25mL of DME. The off-white solid was transfered to a second round-bottom flask, ethanol (1L) was added, and the suspension was heated under reflux for 2h. After cooling, ethanol was evaporated, the crude was alkalinized with 100mL of Na_2CO_3 aqueous saturated solution. The aqueous phase was extracted with thrice with 100mL of CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and evaporated to dryness. The crude solid (8.3g, 59%) was used directly for the next step without any purification.

General method for aldehyde synthesis

To a stirred solution of **2a-b** (*e.g.* 27mmol, 1eq) in CH_3CN (100mL) and H_2O (33mL) was added concentrated HCl (3mL, 35.1mmol, 1.3eq) dropwise at room temperature. The reaction mixture was heated at 100°C for 3h. After cooling the reaction mixture was alkalinized with Na_2CO_3 (*ca.* 3-4g). The aqueous phase was extracted with thrice 60mL of CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and evaporated to dryness to afford **3a-b**.

6-(4-Methylpiperazin-1-yl)imidazo[1,2-*a*]pyridine-2-carbaldehyde (**3a**)

Brown solid, 5.5g, 83%. ^{1}H NMR (300MHz, $CDCl_3$) δ: 10.06 (s, 1H, CHO), 8.04 (s, 1H, H-3), 7.52 (d, 1H, *J* = 9.9Hz, H-8), 7.48 (d, 1H, *J* = 1.8Hz, H-5), 7.16 (dd, 1H, J = 9.9, 2.1Hz, H-7), 3.14-3.11 (m, 4H, 2 CH_2 piperazine), 2.63-2.59 (m, 4H, 2 CH_2 piperazine), 2.37 (s, 3H, CH_3). ^{13}C NMR (75MHz, $CDCl_3$) δ: 187.9 (CHO), 143.7 (C-2), 143.0 (C-8a), 141.3 (C-6), 123.9 (C-7), 118.9 (C-8), 116.1 (C-3), 110.8 (C-5), 54.8 (2 CH_2 piperazine), 49.8 (2 CH_2 piperazine), 46.1 (NCH_3). m.p.: 170-174°C. HRMS (ESI): *m/z* calc. for $C_{13}H_{16}N_4O$ $[M+H]^+$: 245.13969, found: 245.13859.

6-(Piperidin-1-yl)imidazo[1,2-*a*]pyridine-2-carbaldehyde (**3b**)

Brown solid, 1.5g, 91%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 10.04 (s, 1H, C<u>H</u>O), 8.02 (s, 1H, H-3), 7.48 (d, 1H, *J* = 9.9Hz, H-8), 7.45 (d, 1H, *J* = 1.8Hz, H-5), 7.16 (dd, 1H, *J* = 9.9, 2.2Hz, H-7), 3.06-2.98 (m, 4H,

piperidine), 1.76-1.68 (m, 4H, piperidine), 1.61-1.53 (m, 2H, piperidine). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 187.9 (CHO), 143.5 (C-2), 142.9 (C-8a), 142.3 (C-6), 124.8 (C-7), 118.6 (C-8), 116.0 (C-3), 110.7 (C-5), 51.3 (piperidine, 2 CH_2), 25.7 (piperidine, 2 CH_2), 23.9 (piperidine, CH_2). m.p.: 121-125°C. HRMS (ESI): *m/z* calc. for $C_{13}H_{15}N_3O$ $[M+H]^+$: 230.12879, found: 230.12798.

General method for Knoevenagel condensation

Method A

To a stirred solution of MeONa in MeOH (5%, 1.5mL) was added **3a** (*ca.* 122mg, 0.5mmol, 1eq), immediately followed by cyano derivative (1.1eq) at room temperature. The reaction mixture was heated under reflux for 1.5h. After cooling for 1h in ice bath, the solid was filtered and washed with a minimum of cold MeOH. If not pure, crude product was purified by column chromatography on silica gel with CH_2Cl_2/MeOH: 99:1→90:10 as eluent.

Method B

To a stirred solution of 3 (*ca.* 122mg, 0.5mmol, 1eq) in EtOH (1.5mL) was added cyano derivative (1.1eq), followed by piperidine (10%, 5µL). The reaction mixture was heated under reflux for 3h. After cooling for 1h in ice bath, the solid was filtered and washed with a minimum of cold MeOH. If not pure, crude product was purified by column chromatography on silica gel with CH_2Cl_2/MeOH: 99:1→90:10 as eluent.

Ethyl (*E*)-2-cyano-3-(6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylate (**4a**)

Method B, yellow solid, 164mg, 97%. ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.53 (s, 1H, H-3), 8.41 (s, 1H, CH=C), 7.49-7.46 (m, 2H, H-5 & H-8), 7.15 (s, 1H, dd, *J* = 9.9, 2.1Hz, H-7), 4.37 (q, 2H, *J* = 7.2Hz, OCH_2CH_3), 3.14-3.11 (m, 4H, 2 CH_2 piperazine), 2.63-2.60 (m, 4H, 2 CH_2 piperazine), 2.49 (s, 3H, NCH_3), 1.38 (t, 3H, *J* = 7.2Hz, OCH_2CH_3). ^{13}C NMR (75MHz, $CDCl_3$) δ: 162.6 (CO), 149.2 (CH=C), 143.0 (C-8a*), 140.9 (C-6), 138.0 (C-2*), 123.8 (C-7), 118.5 (C-8), 117.8 (C-3), 116.3 (CN), 110.9 (C-5), 101.8 (CH=C), 62.6 (OCH_2CH_3), 54.5 (2 CH_2 piperazine), 49.4 (2 CH_2 piperazine), 45.6 (NCH_3), 14.3 (OCH_2CH_3). m.p.: 150-154°C. HRMS (ESI): *m/z* calc. for $C_{18}H_{21}N_5O_2$ $[M+H]^+$: 340.17680, found: 340.17542.

(*E*)-2-Cyano-3-(6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylamide (**4b**)

Method B, yellow solid, 95mg, 98%. ^{1}H NMR (300MHz, DMSO-d_6) δ: 8.51 (s, 1H, H-3), 8.15 (s, 1H, CH=C), 8.11 (d, 1H, *J* = 2.2 Hz, H-5), 7.85 (bs, 1H, NH), 7.68 (bs, 1H, NH), 7.50 (d, 1H, *J* = 9.9 Hz, H-8), 7.39 (dd, 1H, *J* = 10.0, 2.2 Hz, H-7), 3.07 (dd, 4H, *J* = 6.3, 3.5 Hz, 2 CH_2 piperazine), 2,55-2,45 (m, 4H, 2 CH_2 piperazine), 2.23 (s, 3H, NCH_3). ^{13}C NMR (75MHz, DMSO-d_6) δ: 163.0 (CO), 143.6 (CH=C), 142.2 (C-8a*), 140.1 (C-6*), 137.3 (C-2), 123.6 (C-7), 118.2 (C-3), 117.0 (C-8), 116.7 (CN), 111.1 (C-5), 103.9 (CH=C), 54.3 (2 CH_2 piperazine), 48.9 (2 CH_2 piperazine), 45.7 (NCH_3). m.p.: 187-191°C. HRMS (ESI): *m/z* calc. for $C_{16}H_{18}N_6O$ $[M+H]^+$: 311.16149, found: 311.16019.

(*E*)-2-Cyano-*N*-hexyl-3-(6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylamide (**4c**)

Method B, brown solid, 195mg, 99%. ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.37 (s, 1H, CH=C), 8.20 (s, 1H, H-3), 7.54 (d, 1H, *J* = 10.2Hz, H-8), 7.52 (d, 1H, *J* = 1,5Hz, H-5), 7.13 (dd, 1H, *J* = 9.8, 2.3Hz, H-7), 6.34 (t, 1H, *J* = 5.7Hz, NH), 3.41 (td, 2H, *J* = 7.3, 5.7Hz, CH_2 *n*-Hex), 3.26 (t, 4H, *J* = 5.1 Hz, 2 CH_2 piperazine), 2.81 (t, 4H, *J* = 5.1Hz, 2 CH_2 piperazine), 2.51 (s, 3H, NCH_3), 1.68-1.50 (m, 2H, CH_2 *n*-Hex), 1.42-1.23 (m, 6H, 3 CH_2 *n*-Hex), 0.95-0.81 (m, 3H, CH_3 *n*-Hex). ^{13}C NMR (75MHz, $CDCl_3$) δ: 160.5 (CO), 145.6 (CH=C), 143.2 (C-8a), 140.9 (C-6), 138.4 (C-2), 123.7 (C-7), 118.5 (C-8), 117.7 (C-3), 117.5 (CN), 110.5 (C-5), 102.9 (CH=C), 54.7 (2 CH_2 piperazine), 49.8 (2 CH_2 piperazine), 46.0 (NCH_3), 40.7 (CH_2 *n*-Hex), 31.5

(CH_2 *n*-Hex), 29.5 (CH_2 *n*-Hex), 26.7 (CH_2 *n*-Hex), 22.6 (CH_2 *n*-Hex), 14.1 (CH_3 *n*-Hex). m.p.: 155-159°C. HRMS (ESI): *m/z* calc. for $C_{22}H_{30}N_6O$ $[M+H]^+$: 395.25539, found: 395.25395.

(*Z*)-3-(6-(4-Methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)-2-phenylacrylonitrile (**5a**)

Method A, yellow solid, 121mg, 70%. ^1H NMR (300MHz, $CDCl_3$) δ: 8.48 (s, 1H, H-3), 7.77 (s, 1H, CH=C), 7.67 (m, 2H, Ph-2,6), 7.53 (d, 1H, *J* = 1.8Hz, H-5), 7.64-7.32 (m, 4H, Ph-3,4,5 & H-8), 7.11 (dd, 1H, *J* = 9.6, 2.1Hz, H-7), 3.13 (m, 4H, 2 CH_2 piperazine), 2.63 (m, 4H, 2 CH_2 piperazine), 2.38 (s, 3H, NCH_3). ^{13}C NMR (75MHz, $CDCl_3$) δ: 142.2 (C-8a), 140.7 (C-6), 139.9 (C-2), 136.2 (CH=C), 133.8 (Ph-1), 129.2 (Ph-3,5), 129.1 (Ph-4), 125.7 (Ph-2,6), 122.9 (C-7), 118.7 (CN), 117.7 (C-8), 114.2 (C-3), 110.9 (C-5), 110.2 (CH=C), 54.8 (2 CH_2 piperazine), 50.0 (2 CH_2 piperazine), 46.1 (NCH_3). m.p.: 167-171°C. HRMS (ESI): *m/z* calc. for $C_{21}H_{21}N_5$ $[M+H]^+$: 344.18697, found: 344.18554.

(*Z*)-2-(4-Fluorophenyl)-3-(6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylonitrile (**5b**)

Method A, yellow solid, 179mg, 99%. ^1H NMR (300MHz, $CDCl_3$) δ: 8.46 (s, 1H, H-3), 7.70 (s, 1H, CH=C), 7.68-7.62 (m, 2H, Ph-2,6), 7.54 (d, 1H, *J* = 1.6 Hz, H-5), 7.46 (d, 1H, *J* = 9.8 Hz, H-8), 7.17-7.14 (m, 1H, H-7), 7.13-7.08 (m, 2H, Ph-3,5), 3.18-3.08 (m, 4H, 2 CH_2 piperazine), 2.68-2.59 (m, 4H, 2 CH_2 piperazine), 2.37 (s, 3H, NCH_3). ^{13}C NMR (75MHz, $CDCl_3$) δ: 163.2 (d, *J* = 248.2Hz, F-Ph-4), 142.3 (C-8a*), 140.8 (C-6*) , 139.7 (C-2), 136.2 (d, *J* = 1.5 Hz CH=C), 130.0 (d, *J* = 3.7Hz, F-Ph-1), 127.6 (d, *J* = 8.2Hz, F-Ph-2,6), 123.0 (C-7), 118.6 (CN), 117.7 (C-8), 116.3 (d, *J* = 21,9Hz, F-Ph-3,5), 114.2 (C-3), 110.8 (C-5), 109.1 (CH=C), 54.9 (2 CH_2 piperazine), 50.1 (2 CH_2 piperazine), 46.2 (NCH_3). ^{19}F $\{^1H\}$ NMR (282MHz, $CDCl_3$) δ: -106.7. m.p.: 187-191°C. HRMS (ESI): *m/z* calc. for $C_{21}H_{20}FN_5$ $[M+H]^+$: 362.17755, found: 362.17587.

(*Z*)-2-(3-Fluorophenyl)-3-(6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylonitrile (**5c**)

Method B, eluent : CH_2Cl_2/MeOH : 99:1→90:10. Green solid, 60mg, 40%. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.48 (s, 1H, H-3), 7.78 (s, 1H, CH=C), 7.54 (d, 1H, *J* = 1.6Hz, H-5), 7.47 (m, 2H, H-8 et F-Ph-6), 7.43-7.35 (m, 2H, F-Ph-2,5), 7.14 (dd, 1H, *J* = 9.9, 2.2Hz, H-7), 7.07 (tdd, 1H, *J* = 8.2, 2.4, 1.0Hz, F-Ph-4), 3.17-3.12 (m, 4H, 2 CH_2 piperazine), 2.67-2.61 (m, 4H, 2 CH_2 piperazine), 2.39 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.3 (d, *J* = 203.2Hz, F-Ph-3), 142.4 (C-8a*), 140.9 (C-6*), 139.5 (C-2*), 137.3 (CH=C), 130.8 (d, *J* = 8.2Hz, F-Ph-5), 122.7 (C-7), 121.4 (d, *J* = 3.0Hz, F-Ph-6), 118.3 (CN), 117.8 (C-8), 116.0 (d, *J* = 21.7Hz, F-Ph-4), 114.6 (C-3), 112.7 (d, *J* = 23.2Hz, F-Ph-2), 110.8 (C-5), 109.0 (CH=C), 54.9 (2 CH_2 piperazine), 50.1 (2 CH_2 piperazine), 46.1 (NCH_3). One carbon is missing. ^{19}F $\{^1H\}$ NMR (282MHz, $CDCl_3$) δ: -111.7. m.p.: 152-156°C. HRMS (ESI): *m/z* calc. for $C_{21}H_{20}FN_5$ $[M+H]^+$: 362.17755, found: 362.17621.

(*Z*)-2-(2-Fluorophenyl)-3-(6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylonitrile (**5d**)

Method B, yellow solid, 100mg, 67%. ^1H NMR (300MHz, $CDCl_3$) δ: 8.50 (s, 1H, H-3), 7.85 (s, 1H, CH=C), 7.64-7.57 (m, 1H, F-Ph-6), 7.56 (d, 1H, *J* = 1.8Hz, H-5), 7.48 (d, 1H, *J* = 9.8Hz, H-8), 7.39-7.31 (m, 1H, F-Ph-3), 7.24 (dd, 1H, *J* = 7.7, 1.2Hz, F-Ph-4), 7.18 (dd, 1H, *J* = 9.5, 1.2Hz, H-7), 7.15-7.10 (m, 1H, F-Ph-5), 3.25-3.10 (m, 4H, 2 CH_2 piperazine), 2.73-2.63 (m, 4H, 2 CH_2 piperazine), 2.43 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.0 (d, *J* = 250,5Hz, F-Ph-2), 142.3 (C-8a), 141.2 (d, *J* = 9.0Hz CH=C), 140.8 (C-6), 139.7 (C-2), 130.5 (d, *J* = 8,2Hz, F-Ph-4), 129.6 (d, *J* = 2,2Hz, F-Ph-5), 124.8 (d, *J* = 3,7Hz, F-Ph-6), 123.1 (C-7), 122.4 (d, *J* = 10.8 Hz, F-Ph-1), 118.4 (CN), 117.8 (C-8), 116.7 (d, *J* = 22.1Hz, F-Ph-3), 114.6 (C-3), 110.8 (C-5), 104.9 (d, *J* = 1.5Hz, CH=C), 54.9 (2 CH_2 piperazine), 50.1 (2 CH_2 piperazine), 46.2 (NCH_3). ^{19}F $\{^1H\}$ NMR (282MHz, $CDCl_3$) δ: -113.7. m.p.: 155-159°C. HRMS (ESI): *m/z* calc. for $C_{21}H_{20}FN_5$ $[M+H]^+$: 362.17755, found: 362.17618.

(*Z*)-2-(4-Chlorophenyl)-3-(6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylonitrile (**5e**)

Method A, yellow solid, 92mg, 60%. ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.48 (s, 1H, H-3), 7.75 (s, 1H, CH=C), 7.61 (d, 2H, J = 8.6Hz, Ph-2,6), 7.54 (d, 1H, J = 2.2Hz, H-5), 7.47 (d, 1H, J = 9.8Hz, H-8), 7.41 (d, 2H, J = 8.6Hz, Ph-3,5), 7.14 (dd, 1H, J = 9.9, 2.1Hz, H-7), 3.19-3.08 (m, 4H, 2 CH_2 piperazine), 2.68-2.56 (m, 4H, 2 CH_2 piperazine), 2.38 (s, 3H, NCH_3). ^{13}C NMR (75MHz, $CDCl_3$) δ: 142.3 (C-8a), 140.9 (C-6), 139.6 (C-2), 136.7 (CH=C), 135.1 (Cl-Ph-4), 132.4 (Cl-Ph-1), 129.5 (Cl-Ph-3,5), 126.9 (Cl-Ph-2,6), 123.1 (C-7), 118.4 (CN), 117.7 (C-8), 114.4 (C-3), 110.8 (C-5), 109.0 (CH=C), 54.9 (2 CH_2 piperazine), 50.1 (2 CH_2 piperazine), 46.2 (NCH_3). m.p.: 200-204°C. HRMS (ESI): *m/z* calc. for $C_{21}H_{20}ClN_5$ $[M+H]^+$: 378.14800, found: 378.14679.

(*Z*)-2-(3-Chlorophenyl)-3-(6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylonitrile (**5f**)

Method B, yellow solid, 125mg, 80%. ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.47 (s, 1H, H-3), 7.78 (s, 1H, CH=C), 7.67 (m, 1H, Cl-Ph-2), 7.58-7.54 (m, 2H, H-5 & Cl-Ph-6), 7.47 (d, 1H, J = 9.8Hz, H-8), 7.40-7.33 (m, 2H, Cl-Ph-4,5), 7.14 (dd, 1H, J = 9.9, 2.2Hz, H-7), 3.18-3.11 (m, 4H, 2 CH_2 piperazine), 2.66-2.61 (m, 4H, 2 CH_2 piperazine), 2.39 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 142.4 (C-8a) 140.9 (C-6), 139.5(C-2), 137.4 (CH=C), 135.7 (Cl-Ph-3), 135.4(Cl-Ph-1), 130.5(Cl-Ph-5*), 129.1 (Cl-Ph-4*), 125.9 (Cl-Ph-2), 123.8 (Cl-Ph-6), 123.2 (C-7), 118.3 (CN), 117.8 (C-8), 114.6 (C-3), 110.8(C-5), 108.8 (CH=C), 54.9 (2 CH_2 piperazine), 50.1 (2 CH_2 piperazine), 46.1 (NCH_3). m.p.: 68-72°C. HRMS (ESI): *m/z* calc. for $C_{21}H_{20}ClN_5$ $[M+H]^+$: 378.14800, found: 378.14666.

(*Z*)-2-(2-Chlorophenyl)-3-(6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylonitrile (**5g**)

Method B, green oil, 149mg, 96%. ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.52 (s, 1H, H-3), 7.55 (d, 1H, J = 1.9Hz, H-5), 7.53 (s, 1H, CH=C), 7.49-7.44 (m, 3H, H-8 & Cl-Ph-3,6), 7.36-7.32 (m, 2H, Cl-Ph-4,5), 7.14 (dd, 1H, J = 9.9, 2.2Hz, H-7), 3.18-3.03 (m, 4H, 2 CH_2 piperazine), 2.66-2.55 (m, 4H, 2 CH_2 piperazine), 2.37 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 142.7 (CH=C), 142.3 (C-6), 140.9 (C-8a), 139.3 (C-2), 133.8 (Cl-Ph-2), 133.1 (Cl-Ph-1), 130.8 (Cl-Ph-5*), 130.6 (Cl-Ph-6*), 130.3 (Cl-Ph-3*), 127.5 (Cl-Ph-4*), 123.1 (C-7), 118.1 (CN), 117.8 (C-8), 114.5 (C-3), 110.8 (C-5), 107.9 (CH=C), 54.9 (2 CH_2 piperazine), 50.1 (2 CH_2 piperazine), 46.2 (NCH_3). HRMS (ESI): *m/z* calc. for $C_{21}H_{20}ClN_5$ $[M+H]^+$: 378.14800, found: 378.14689.

(*Z*)-2-(3,4-Dichlorophenyl)-3-(6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylonitrile (**5h**)

Method B, brown solid, 152mg, 89%. ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.45 (s, 1H, H-3), 7.75 (s, 1H, CH=C), 7.74 (s, 1H, Cl_2-Ph-2), 7.52 (d, 1H, J = 1.7Hz, H-5), 7.50-7.48 (m, 2H; Cl_2-Ph-5,6), 7.46 (d, 1H, J = 9.9Hz, H-8), 7.14 (dd, 1H, J = 9.9, 2.1Hz, H-7), 3.18-3.07 (m, 4H, 2 CH_2 piperazine), 2.65-2.56 (m, 4H, 2 CH_2 piperazine), 2.37 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 142.4 (C-8a), 141.0 (C-6), 139.3 (C-2), 137.5 (CH=C), 133.9 (Cl_2-Ph-4*), 133.6 (Cl_2-Ph-3*), 133.1 (Cl_2-Ph-1*), 131.2 (Cl_2-Ph-5), 127.5 (Cl_2-Ph-2), 124.7 (Cl_2-Ph-6), 123.2 (C-7), 118 (CN), 117.8 (C-8), 114.7 (C-3), 110.7 (C-5), 107.7 (CH=C), 54.9 (2 CH_2 piperazine), 50.1(2 CH_2 piperazine), 46.2 (NCH_3). m.p.: 195-199°C. HRMS (ESI): *m/z* calc. for $C_{21}H_{19}Cl_2N_5$ $[M+H]^+$: 412.10903, found: 412.10791.

(*Z*)-3-(6-(4-Methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)-2-(*p*-tolyl)acrylonitrile (**5i**)

Method A, green solid, 80mg, 54%. ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.47 (s, 1H, H-3), 7.74 (s, 1H, CH=C), 7.57 (d, 2H, J = 8.2Hz, *p*-Tolyl-2,6), 7.54 (d, 1H, J = 1.7Hz, H-5), 7.46 (d, 1H, J = 9.8Hz, H-8), 7.23 (d, 2H, J = 8.4Hz, *p*-Tolyl-3,5), 7.12 (dd, 1H, J = 9.8, 2.1Hz, H-7), 3.18-3.07 (m, 4H, 2 CH_2 piperazine), 2.69-2.56 (m, 4H, 2 CH_2 piperazine), 2.38 (s, 3H, CH_3-Ph), 2.37 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 142.2 (C-8a), 140.7 (C-6*), 140.0 (*p*-Tolyl-4*), 139.3 (C-2), 135.3 (CH=C), 131.0 (*p*-Tolyl-1), 129.9 (*p*-Tolyl-3,5), 125.6 (*p*-Tolyl-2,6), 122.9 (C-7), 118.8 (CN), 117.6 (C-8), 114.0 (C-3), 110.9 (C-5), 110.2 (CH=C), 54.9 (2 CH_2 piperazine), 50.2 (2 CH_2 piperazine), 46.2 (NCH_3), 21.3 (CH_3-Ph). m.p.: 217-221°C. HRMS (ESI): *m/z* calc. for $C_{22}H_{23}N_5$ $[M+H]^+$: 358.20262, found: 358.20125.

(*Z*)-2-(4-Methoxyphenyl)-3-(6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylonitrile (**5j**)

Method A, pale green solid, 115mg, 75%. ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.44 (s, 1H, H-3), 7.65 (s, 1H, CH=C), 7.61 (d, 2H, *J* = 8.8Hz, MeO-Ph-2,6), 7.54 (d, 1H, *J* = 1.8Hz, H-5), 7.45 (d, 1H, *J* = 9.8Hz, H-8), 7.11 (dd, 1H, *J* = 9.8, 2.1Hz, H-7), 6.95 (d, 2H, *J* = 8.9Hz, MeO-Ph-3,5), 3.84 (s, 3H, OCH_3), 3.18-3.08 (m, 4H, 2 CH_2 piperazine), 2.66-2.55 (m, 4H, 2 CH_2 piperazine), 2.38 (s, 3H, NCH_3). ^{13}C NMR (75MHz, $CDCl_3$) δ: 160.4 (MeO-Ph-4), 142.2 (C-8a), 140.7 (C-6), 140.1 (C-2), 134.2 (CH=C), 127.1 (CH_3O-Ph-2,6), 126.4 (MeO-Ph-1), 122.8 (C-7), 118.8 (CN), 117.6 (C-8), 114.6 (MeO-Ph-3,5), 113.7 (C-3), 110.9 (C-5), 109.9 (CH=C), 55.6 (OCH_3), 54.9 (2 CH_2 piperazine), 50.2 (2 CH_2 piperazine), 46.2 (NCH_3). m.p.: 190-194°C. HRMS (ESI): *m/z* calc. for $C_{22}H_{23}N_5O$ $[M+H]^+$: 374.19754, found: 374.19610.

(*Z*)-3-(6-(4-Methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)-2-(4-(trifluoromethyl)phenyl)acrylonitrile (**5k**)

Method A, pale green solid, 65mg, 38%. ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.51 (s, 1H, H-3), 7.87 (s, 1H, CH=C), 7.80 (d, 2H, *J* = 8.3Hz, CF_3-Ph-2,6), 7.70 (d, 2H, *J* = 8.3Hz, CF_3-Ph-3,5), 7.56 (d, 1H, *J* = 2.1Hz, H-5), 7.49 (d, 1H, *J* = 9.8Hz, H-8), 7.15 (dd, 1H, *J* = 9.9, 2.2Hz, H-7), 3.17 (t, 4H, *J* = 5.0Hz, 2 CH_2 piperazine), 2.76-2.59 (m, 4H, 2 CH_2 piperazine), 2.42 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 142.4 (C-8a), 141.0 (C-6), 139.3 (C-2), 138.2 (CH=C), 137.3 (CF_3-Ph-1), 131.0 (q, *J* = 33.0Hz, CF_3-Ph-4) , 126.2 (q, *J* = 3.7Hz, CF_3-Ph-3,5), 126.0 (CF_3-Ph-2,6), 124.0 (q, *J* = 270.7Hz, CF_3), 123.3 (C-7), 118.2 (CN), 117.8 (C-8), 114.8 (C-3), 110.7 (C-5), 108.6 (CH=C), 54.8 (2 CH_2 piperazine), 50.0 (2 CH_2 piperazine), 46.1 (NCH_3). ^{19}F $\{^{1}H\}$ NMR (282MHz, $CDCl_3$) δ: -62.7. m.p.: 228-232°C. HRMS (ESI): *m/z* calc. for $C_{22}H_{20}F_3N_5$ $[M+H]^+$: 412.17436, found: 412.17255.

(*Z*)-2-(4-Aminophenyl)-3-(6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylonitrile (**5l**)

Method A, yellow solid, 82mg, 56%. ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.43 (s, 1H, H-3), 7.59 (s, 1H, CH=C), 7.53-7.43 (m, 4H, H-5, H-8 & H_2N-Ph-2,6), 7.10 (dd, 1H, *J* = 9.6, 1.5Hz, H-7), 6.71 (d, 2H, *J* = 8.4Hz, H_2N-Ph-3,5), 3.89 (bs, 2H, NH_2), 3.20-3.03 (m, 4H, 2 CH_2 piperazine), 2.64-2.58 (m, 4H, 2 CH_2 piperazine), 2.37 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 147.5 (H_2N-Ph-4)), 142.1 (C-8a), 140.6 (C-6*), 140.4 (C-2*), 132.4 (CH=C), 127.0 (H_2N-Ph-2,6), 123.9 (H_2N-Ph-1), 122.7 (C-7), 119.0 (CN), 117.5 (C-8), 115.3 (H_2N-Ph-3,5), 113.4 (C-3), 110.9 (C-5), 110.5 (CH=C), 54.9 (2 CH_2 piperazine), 50.2 (2 CH_2 piperazine), 46.2 (NCH_3). m.p.: 196-200°C. HRMS (ESI): *m/z* calc. for $C_{21}H_{22}N_6$ $[M+H]^+$: 359.19787, found: 359.19673.

(*Z*)-2-(4-Hydroxyphenyl)-3-(6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylonitrile (**5m**)

Method A, yellow solid, 108mg, 73%. ^{1}H NMR (300MHz, DMSO-d_6) δ: 8.36 (s, 1H, H-3), 8.12 (s, 1H, H-5), 7.72 (s, 1H, CH=C), 7.56 (d, 2H, *J* = 8.7Hz, HO-Ph-2,6), 7.45 (d, 1H, *J* = 9.9Hz, H-8), 7.32 (dd, 1H, *J* = 9.9, 1.9Hz, H-7), 6.85 (d, 2H, *J* = 8.7Hz, HO-Ph-3,5), 3.10-3,00 (m, 4H, 2 CH_2 piperazine), 2,55-2,45 (m, 4H, 2 CH_2 piperazine), 2.23 (s, 3H, NCH_3). ^{13}C NMR (75MHz, DMSO-d_6) δ: 158.6 (HO-Ph-4), 141.6 (C-8a*), 139.8 (C-6*), 139.4 (C-2), 132.4 (CH=C), 126.9 (HO-Ph-2,6), 124.4 (HO-Ph-1), 122.7 (C-7), 118.3 (CN), 116.5 (C-8), 116.0 (HO-Ph-3,5), 114.8 (C-3), 111.3 (C-5), 108.2 (CH=C), 54.4 (2 CH_2 piperazine), 49.2 (2 CH_2 piperazine), 45.7 (NCH_3). m.p.: 274-278°C. HRMS (ESI): *m/z* calc. for $C_{21}H_{21}N_5O$ $[M+H]^+$: 360.18189, found: 360.18063.

(*E*)-3-(6-(4-Methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)-2-(thiophen-2-yl)acrylonitrile (**5n**)

Method A, green solid, 69mg, 48%. ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.39 (s, 1H, H-3), 7.59 (s, 1H, CH=C), 7.53 (s, 1H, H-5), 7.46 (d, 1H, *J* = 9.6Hz, H-8), 7.34-7.29 (m, 2H, Thienyl-3,5), 7.13-7.06 (m, 2H, H-7 & Thienyl-4), 3.14 (bs, 4H, 2 CH_2 piperazine), 2.64 (bs, 4H, 2 CH_2 piperazine), 2.39 (s, 3H, NCH_3). ^{13}C NMR (75MHz, $CDCl_3$) δ: 142.4 (C-8a), 140.8 (C-6), 139.6 (C-2*), 138.9 (Thienyl-1*), 134.1 (CH=C), 128.2

(Thienyl-4), 126.8 (Thienyl-3*), 126.3 (Thienyl-5*), 123.0 (C-7), 117.7 (C-8), 117.5 (CN), 114.0 (C-3), 110.9 (C-5), 105.1 (CH=C), 54.9 (2 CH_2 piperazine), 50.1 (2 CH_2 piperazine), 46.1 (NCH_3). m.p.: 196-200°C. HRMS (ESI): *m/z* calc. for $C_{19}H_{19}N_5S$ $[M+H]^+$: 350.14339, found: 350.14207.

Ethyl (*Z*)-2-(4-chlorophenyl)-3-(6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylate (**6**)

To a stirred solution of DIPA (211µL, 1.5mmol, 1.5eq) in THF (500µL) was added *n*-BuLi (1mL, 1.5mmol, 1.5eq) dropwise at -10°C. Ethyl 2-(4-chlorophenyl)acetate (199mg, 1mmol, 1eq) was then added, in solution in THF (400µL) at -10°C. **3a** (244mg, 1mmol, 1eq) was then added, in solution in THF (800µL). The reaction was warmed to room temperature and stirred for 4h. The reaction was then quenched with NH_4Cl aqueous saturated solution (5mL) and the aqueous phase was extracted thrice with 5mL of AcOEt. The combined organic phases were dried over $MgSO_4$ and evaporated to dryness. The crude mixture was purified by column chromatography on alumina with a mixture of petroleum ether and AcOEt (5:5→3:7) as eluent. **6** was obtained as a brown oil (32mg, 7%).

^{1}H NMR (300MHz, $CDCl_3$) *δ*: 8.02 (s, 1H, CH=C), 7.45 (m, 2H, Ph-2,6), 7.40 (d, 1H, *J* = 9.8 Hz, H-8), 7.28-7.23 (m, 3H, H-5 & Ph-3,5), 7.03 (dd, 1H, *J* = 9.8, 2.2Hz, H-7), 6.49 (s, 1H, H-3), 4.25 (q, 2H, *J* = 7.1Hz, OCH_2CH_3), 3.12-3.05 (m, 4H, 2 CH_2 piperazine), 2.66 – 2.59 (m, 4H, 2 CH_2 piperazine), 2.39 (s, 3H, NCH_3), 1.30 (t, 3H, *J* = 7.1Hz, OCH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 167.0 (C=O), 141.9 (C-8a), 140.34 (C-2*), 140.29 (C-6*), 135.5 (CH=C), 135.4 (Cl-Ph-4), 134.1 (Cl-Ph-1), 131.0 (Cl-Ph-2,6), 130.9 (CH=C), 129.4 (Cl-Ph-3,5), 122.3 (C-7), 117.6 (C-8), 113.9 (C-3), 110.9 (C-5), 61.3 (OCH_2CH_3), 54.7 (2 CH_2 piperazine), 50.0 (2 CH_2 piperazine), 45.9 (NCH_3), 14.3 (OCH_2CH_3). HRMS (ESI): *m/z* calc. for $C_{23}H_{25}ClN_4O_2$ $[M+H]^+$: 425.17388, found: 425.17222.

2-(4-Chlorophenyl)-3-(6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)propanenitrile (**7**)

To a stirred suspension of **5e** (100mg, 0.265mmol, 1eq) in EtOH (10mL) was added $NaBH_4$ (20mg, 0.529mmol, 2eq) in one portion at room temperature. After stirring 1h, $NaBH_4$ (50mg, 1.32mmol, 5eq) was added. After 1h and to insure completion (followed by TLC) $NaBH_4$ (50mg, 1.32mmol, 5eq) was added.The reaction mixture was then partitioned between CH_2Cl_2 and water (30:30mL), and the aqueous phase was extracted twice with 30mL of CH_2Cl_2. The crude mixture was purified by column chromatography on silica gel with a mixture of CH_2Cl_2 and MeOH (99:1→92:8) as eluent. **7** was obtained as a green solid (78mg, 77%).

^{1}H NMR (300MHz, $CDCl_3$) *δ*: 7.51 (s, 1H, H-3), 7.43 (d, 1H, *J* = 9.6Hz, H-8), 7.35-7.33 (m, 5H, H-5 & Cl-Ph-2,3,5,6), 7.06 (d, 1H, *J* = 9.7Hz, H-7), 4.40 (t, 1H, *J* = 7.4Hz, CH_2CHCN), 3.34-3.19 (m, 6H, CH_2CHCN & 2 CH_2 piperazine), 2.78 (m, 4H, 2 CH_2 piperazine), 2.49 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 142.4 (C-8a*), 141.1 (C-6*), 139.8 (C-2*), 134.13 (Cl-Ph-4), 134.11 (Cl-Ph-1), 129.2 (Cl-Ph-3,5), 128.8 (Cl-Ph-2,6), 121.9 (C-7), 120.5 (CN), 116.4 (C-5), 111.43 (C-3*), 111.37 (C-8*), 54.8 (2 CH_2 piperazine), 50.3 (2 CH_2 piperazine), 46.0 (NCH_3), 37.5 (CH_2CHCN), 35.7 (CH_2CHCN). m.p: 171-175°C. HRMS (ESI): *m/z* calc. for $C_{21}H_{22}ClN_5$ $[M+H]^+$: 380.16365, found: 380.16209.

(*Z*)-2-(4-Chlorophenyl)-3-(6-(piperidin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylonitrile (**8**)

Method A, green solid, 605mg, 76%. ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 8.45 (s, 1H, H-3), 7.74 (s, 1H, CH=C), 7.60 (d, 2H, *J* = 8.7Hz, Cl-Ph-2,6), 7.50 (d, 1H, *J* = 1.7Hz, H-5), 7,41 (t, 2H, *J* = 10.0Hz, Cl-Ph-3,5), 7.39 (d, 1H, *J* = 9Hz, H-8), 7,13 (dd, 1H, *J* = 9.9, 2.2Hz, H-7), 3.08-3.00 (m, 4H, 2 CH_2 piperidine), 1.79-1.71 (m, 4H, 2 CH_2 piperidine), 1.64-1.56 (m, 2H, CH_2 piperidine). ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 142.3 (C-6), 141.8 (C-8a), 139.4 (C-2), 136.8 (CH=C), 135.0 (Cl-Ph-4), 132.4 (Cl-Ph-1), 129.4 (Cl-Ph-3,5), 126.9 (Cl-Ph-2,6), 124.0 (C-7), 118.4 (CN); 117.4 (C-8), 114.3 (C-3), 110.8 (C-5), 108.7 (CH=C), 51.6 (2 CH_2

piperidine), 25.8 (2 CH_2 piperidine), 24.0 (CH_2 piperidine). m.p.: 178-182°C. HRMS (ESI): *m/z* calc. for $C_{21}H_{19}ClN_4$ $[M+H]^+$: 363.13710, found: 363.13567.

Ethyl 2-(6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acetate (**9**)

To a stirred solution of **1** (2.5g, 13mmol, 1eq) in EtOH (100mL) was added ethyl 4-chloroacetoacetate (3mL, 18.4mmol, 1.4eq) dropwise. The reaction mixture was heated under reflux for 4h. After cooling ethanol was evaporated, the crude was alkalinized with 100mL of Na_2CO_3 aqueous saturated solution. The aqueous phase was extracted thrice with 100mL of CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and evaporated to dryness. The crude mixture was purified by chromatography on neutral alumina column with EtOAc:MeOH = 100:0→99:1 as eluent to furnish 9 as a brown solid (1.38g, 35%).

^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 7.50-7.47 (m, 2H, H-3 & H-5), 7.41 (d, 1H, *J* = 9.7Hz, H-8), 7.02 (dd, 1H, *J* = 9.8, 2.2Hz, H-7), 4.18 (q, 2H, *J* = 7.1Hz, O$\underline{CH_2}$$CH_3$), 3.81 (s, 2H, $\underline{CH_2}$COOEt), 3.11-3.03 (m, 4H, piperazine), 2.66-2.56 (m, 4H, piperazine), 2.37 (s, 3H, NCH_3), 1.26 (t, 3H, *J* = 7.1Hz, $OCH_2\underline{CH_3}$). ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 171.1 (COOEt), 142.2 (C-8a), 139.8 (C-6), 139.4 (C-2), 121.6 (C-7), 117.2 (C-8), 111,5 (C-5), 111.5 (C-3), 61.1 (O$\underline{CH_2}$$CH_3$), 54.9 (2 CH_2 piperazine), 50.5 (2 CH_2 piperazine), 46.1 (NCH_3), 35.3 ($\underline{CH_2}$COOEt), 14.3 ($OCH_2\underline{CH_3}$). m.p.: 92-96°C. HRMS (ESI): *m/z* calc. for $C_{16}H_{22}N_4O_2$ $[M+H]^+$: 303.18155, found: 303.18036.

2-(6-(4-Methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acetamide (**10**)

To a stirred solution of **9** (630mg, 2.08mmol, 1eq) in THF (4mL) was added 30% aqueous ammonia solution (12mL). After stirring overnight at room temperature, the volatiles were evaporated under vacuum to yield **10** as a brown solid (480mg, 84%).

^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 7.49 (d, 1H, *J* = 2.0Hz, H-5), 7.41 (d, 1H, *J* = 9.9Hz, H-8), 7.39 (s, 1H, H-3), 7,30 (bs, 1H, NH), 7.07 (dd, 1H, *J* = 9.7, 2.1Hz, H-7), 5.72 (bs, 1H, NH), 3.67 (s, 2H, $\underline{CH_2}CONH_2$), 3.12-3.05 (m, 4H, 2 CH_2 piperazine), 2.64 – 2.57 (m, 4H, 2 CH_2 piperazine), 2.35 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 172.6 ($\underline{C}ONH_2$), 142.6 (C-8a), 140.5 (C-6*), 140.2 (C-2*), 122.1 (C-7), 116.9 (C-8), 111.5 (C-3*), 111.4 (C-5*), 55.0 (2 CH_2 piperazine), 50.5 (2 CH_2 piperazine), 46.2 (NCH_3), 36.3 ($\underline{CH_2}CONH_2$). m.p.: 137-141°C. HRMS (ESI): *m/z* calc. for $C_{14}H_{19}N_5O$ $[M+H]^+$: 274.16624, found: 274.16582.

2-(6-(4-Methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acetonitrile (**11**)

10 (150mg, 0.55mmol) was charged into a round-bottom flask. $POCl_3$ (1.4mL) was added carefully, and the reaction mixture was heated at 125°C for 1h. The $POCl_3$ was then evaporated under vacuum and the crude solid was carefully alkalinized with a Na_2CO_3 saturated aqueous solution (*ca*. 20-30mL). The aqueous phase was extracted thrice with 15mL of CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and evaporated to dryness to yield **11** as a black oil, without further purification (84mg, 60%).

^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 7.52 (s, 1H, H-3), 7.48 (s, 1H, H-5), 7.41 (d, 1H, *J* = 9.8Hz, H-8), 7.09 (dd, 1H, *J* = 9.8, 1.8Hz, H-7), 3.87 (s, 2H, $\underline{CH_2}CN$), 3.10-3.07 (m, 4H, 2 CH_2 piperazine), 2.62-2.55 (m, 4H, 2 CH_2 piperazine), 2.36 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 142.6 (C-8a), 140.3 (C-6), 135.9 (C-2), 122.4 (C-7), 117.4 (CN), 117.2 (C-8), 111.3 (C-5*), 110.9 (C-3*), 54.9 (2 CH_2 piperazine), 50.4 (2 CH_2 piperazine), 46.1 (NCH_3), 18.5 ($\underline{CH_2}CN$). HRMS (ESI): *m/z* calc. for $C_{14}H_{17}N_5$ $[M+H]^+$: 256.15567, found: 256.15455.

(*Z*)-3-(4-Fluorophenyl)-2-(6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylonitrile (**12**)

Method A, green solid, 75mg, 67%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.08 (s, 1H, CH=C), 7.93 (dd, 2H, J = 8.7, 5.4Hz, F-Ph-2,6), 7.77 (s, 1H, H-3), 7.51 (s, 1H, H-5), 7.45 (d, 1H, J = 9.8Hz, H-8), 7,17-7,11 (m, 3H, F-Ph-3,5 & H-7), 3.17-3.08 (m, 4H, 2 CH_2 piperazine), 2.67-2.58 (m, 4H, 2 CH_2 piperazine), 2.38 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163,8 (d, J = 250,5Hz, F-Ph-4), 143.2 (C-2), 140.7 (C-6*), 140.4 (C-8a*), 139.2 (CH=C), 131.5 (d, J = 8,25Hz, F-Ph-2,6), 130.3 (d, J = 3.0Hz, F-Ph-1), 123.4 (C-7), 117.9 (CN), 117.1 (C-8), 116.3 (d, J = 21,7Hz, F-Ph-3,5), 112.0 (C-3), 111.2 (C-5), 103.8 (CH=C), 54.9 (2 CH_2 piperazine), 50.3 (2 CH_2 piperazine), 46.2 (NCH_3). ^{19}F {^{1}H} NMR (282MHz, $CDCl_3$) δ: -108.6. m.p.: 228-232°C. HRMS (ESI): *m/z* calc. for $C_{21}H_{20}FN_5$ $[M+H]^+$: 362.17755, found: 362.17629.

(*Z*)-2-(4-Chlorophenyl)-3-(3-iodo-6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylonitrile (**13**)

To a stirred solution of **5e** (189mg, 0.5mmol, 1eq) in $CHCl_3$ (6.5mL) was added ICl (244mg, 1.5mmol, 3eq) dropwise, in solution in $CHCl_3$ (3mL) at room temperature. The reaction mixture was stirred at room temperature for 45 minutes. The reaction mixture was then alkalinized with 25mL of Na_2CO_3 aqueous saturated solution, and extracted twice with 20mL of CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and evaporated to dryness. The crude solid was purified by column chromatography on silica gel (eluent: CH_2Cl_2:MeOH, 98:2→94:6) to yield **13** as a yellow solid (148mg, 58%).

^{1}H NMR (300MHz, $CDCl_3$) δ: 7.68 (d, 2H, J = 8.6Hz, Cl-Ph-2,6), 7.57 (d, 1H, J = 9.8Hz, H-8), 7.45 (s, 1H, CH=C), 7.44-7.38 (m, 3H, Cl-Ph-3,5 & H-5), 7.20 (dd, 1H, J = 9.8, 2.2Hz, H-7), 3.22-3.16 (m, 4H, 2 CH_2 piperazine), 2.66 – 2.61 (m, 4H, 2 CH_2 piperazine), 2.38 (s, 3H, NCH_3). ^{13}C NMR (75MHz, $CDCl_3$) δ: 145.6 (C-8a), 142.7 (C-2*), 141.4 (C-6*), 135.2 (Cl-Ph-4), 133.3 (Cl-Ph-1), 131.7 (CH=C), 129.3 (Cl-Ph-3,5), 127.5 (Cl-Ph-2,6), 123.6 (C-7), 118.6 (C-8), 117.6 (CN), 111.0 (C-5), 70.4 (C-3), 54.9 (2 CH_2 piperazine), 49.9 (2 CH_2 piperazine), 46.2 (NCH_3). One carbon is missing. m.p.: 184-188°C. HRMS (ESI): *m/z* calc. for $C_{21}H_{19}ICIN_5$ $[M+H]^+$: 504.04464, found: 504.04312.

(*Z*)-3-(5-Bromo-6-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl)-2-(4-chlorophenyl)acrylonitrile (**14**)

To a stirred solution of **5e** (189mg, 0.5mmol, 1eq) in CH_3CN (3.5mL) was added NBS (89mg, 0.5mmol, 1eq) in one portion at room temperature. The reaction mixture was stirred at room temperature for 30 minutes. The reaction mixture was then partitioned between CH_2Cl_2 (10mL) and water (10mL). The aqueous phase was extracted twice with 10mL of CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and evaporated to dryness. The crude solid was purified by column chromatography on silica gel (eluent: CH_2Cl_2:MeOH, 97:3→90:10) to yield **14** as a yellow solid (156mg, 68%).

^{1}H NMR (300MHz, $CDCl_3$) δ: 8.73 (s, 1H, H-3), 7.74 (s, 1H, CH=C), 7.64-7.58 (m, 2H, Ph-2,6), 7.59 (d, 1H, J = 9.5Hz, H-8), 7.42 (d, 2H, J = 9.0Hz, Ph-3,5), 7.30 (d, 1H, J = 9.8Hz, H-7), 3.13 (m, 4H, 2 CH_2 piperazine), 2.77 (bs, 4H, 2 CH_2 piperazine), 2.48 (s, 3H, NCH_3). ^{13}C NMR (75MHz, $CDCl_3$) δ: 143.7 (C-8a), 139.9 (C-2*), 139.4 (C-6*), 135.6 (CH=C), 135.4 (Cl-Ph-4), 132.2 (Cl-Ph-1), 129.4 (Cl-Ph-3,5), 127.1 (Cl-Ph-2,6), 122.2 (C-8), 117.9 (CN), 116.5 (C-7), 115.5 (C-3), 112.5 (C-5), 110.6 (CH=C), 55.4 (2 CH_2 piperazine), 51.7 (2 CH_2 piperazine), 46.1 (NCH_3). m.p.: 221-225°C. HRMS (ESI): *m/z* calc. for $C_{21}H_{19}BrClN_5$ $[M+H]^+$: 456.05851, found: 456.05725.

(*Z*)-2-(4-Chlorophenyl)-3-(6-(4-methylpiperazin-1-yl)-3-(pyridin-4-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylonitrile (**15**)

A stirred solution of **13** (100mg, 0.2mmol, 1eq), 4-PyrB(OH)$_2$ (27mg, 0.22mmol, 1.1eq), Na_2CO_3 (42mg, 0.4mmol, 2eq), and $Pd(PPh_3)_4$ (11mg, 0.01mmol, 5%) in dioxane:H_2O (800µL:400µL) was heated using microwaves irradiation at 100°C for 1h. After cooling the reaction mixture was partitioned between CH_2Cl_2 and water (10mL of each) and the aqueous phase was extracted twice with 10mL of CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and evaporated to dryness. The crude solid was purified by column chromatography on silica gel (eluent: CH_2Cl_2:MeOH, 99:1→92:8) to yield **15** as a yellow solid (58mg, 64%).

^{1}H NMR (300MHz, $CDCl_3$) *δ*: 8.87 (d, 2H, *J* = 5.6Hz, Pyr-2,6), 7.70 (d, 1H, *J* = 9.9Hz, H-8), 7.59 (d, 2H, *J* = 8.7Hz, Cl-Ph-2,6), 7.52 (s, 1H, H-5), 7.46 (m, 2H, Pyr-3,5), 7.43-7.37 (m, 2H, Cl-Ph-3,5), 7.36 (s, 1H, C<u>H</u>=C), 7.24 (dd, 1H, *J* = 9.9, 1.9Hz, H-7), 3.18 (bs, 4H, 2 CH_2 piperazine), 2.76 (bs, 4H, 2 CH_2 piperazine), 2.48 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.3 (Pyr-2,6), 151.1 (C-3), 143.3 (C-8a*), 141.1 (C-6*), 138.5 (C-2), 136.3 (Pyr-1), 135.3 (Ph-4), 133.3 (Ph-1), 131.2 (<u>C</u>H=C), 129.4 (Cl-Ph-3,5), 127.4 (Cl-Ph-2,6), 123.9 (Pyr-3,5), 123.6 (C-7), 118.9 (C-8), 117.7 (CN), 111.7 (CH=<u>C</u>) 107.6 (C-5), 54.7 (2 CH_2 piperazine), 49.8 (2 CH_2 piperazine), 46.0 (NCH_3). m.p.: 251-255°C. HRMS (ESI): *m/z* calc. for $C_{26}H_{23}ClN_6$ $[M+H]^+$: 455.17455, found: 455.17321.

(*Z*)-2-(4-chlorophenyl)-3-(6-(4-methylpiperazin-1-yl)-5-(pyridin-4-yl)imidazo[1,2-*a*]pyridin-2-yl)acrylonitrile (**16**)

A stirred solution of **14** (80mg, 0.15mmol, 1eq), 4-PyrB(OH)$_2$ (21.5mg, 0.175mmol, 1.2eq), Na_2CO_3 (32mg, 0.30mmol, 2eq), and $Pd(PPh_3)_4$ (8.6mg, 0.0075mmol, 5%) in DME:H_2O (1mL:0.5mL) was heated using microwaves irradiation at 100°C for 1h. After cooling the reaction mixture was partitioned between CH_2Cl_2 and water (10mL of each) and the aqueous phase was extracted with twice 10mL of CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and evaporated to dryness. The crude solid was purified by column chromatography on silica gel (eluent: CH_2Cl_2:MeOH, 100:0→90:10) to yield **16** as a yellow solid (52mg, 78%).

^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 8.89 (d, 2H, *J* = 4.5Hz, Pyr-3,5), 8.21 (s, 1H, H-3), 7.68-7.64 (m, 2H, H-8 & C<u>H</u>=C), 7.60-7.53 (m, 4H, Cl-Ph-2,6 & Pyr-2,6), 7.41-7.36 (m, 3H, Cl-Ph-3,5 & H-7), 2.94-2.90 (m, 4H, 2 CH_2 piperazine), 2.36-2.40 (m, 4H, 2 CH_2 piperazine), 2.31 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 151.1 (Pyr-3,5), 143.9 (C-2), 140.4 (C-5*), 140.1 (C-8a*), 138.2 (C-6*), 135.5 (<u>C</u>H=C), 135.4 (Cl-Ph-4), 132.2 (Cl-Ph-1), 129.5 (Cl-Ph-3,5), 127.1 (Cl-Ph-2,6*), 124.2 (Pyr-2,6*), 122.7 (C-7), 118.4 (C-8), 117.9 (CN), 112.7 (C-3), 110.6 (CH=<u>C</u>), 55.0 (2 CH_2 piperazine), 51.7 (2 CH_2 piperazine), 45.9 (NCH_3). One carbon is missing. m.p.: 242-246°C. HRMS (ESI): *m/z* calc. for $C_{26}H_{23}ClN_6$ $[M+H]^+$: 455.17455, found: 455.17335.

4.2. Pharmacology

4.2.1. Larval Paralysis Test (LPT)

LPT was realized on infective larvae (L3s). Stock solutions of all compounds as tartrate salts were prepared in DMSO at 20mM. Serial dilutions in water were then prepared to test compounds at 500µM, 250µM, 125µM, 62.5µM, 31.25µM and 15.625µM. 100µL of a solution of L3s (*ca.* 5000 L3s/mL) and 100µL of solution of compounds were mixed into the wells of a 96-well microtitre plate. After 5 minutes of contact, wells were observed by using binocular lens. Estimation of larvae paralysis was made at t = 5min, 15min, 30min, 1h and 2h. MIC were defined as the lowest concentration with a minimum of 80% estimated larvae paralysis. Levamisole (50µM) and DMSO (2,5%) were used as positive and negative controls.

4.2.2. *Electrophysiological studies in Xenopus laevis oocytes*[20]

Defolliculated *Xenopus laevis* oocytes (Ecocyte Bioscience) were injected with ~36 nL of a cRNA mix containing 50 ng/µL of each cRNA mix encoding either the Hco-L-AChR1 or Hco-L-AChR2 channels using the Nanoject II microinjector (Drummond) as described previously.[23] The oocytes were voltage-clamped at -60mV in a recording chamber 4-5 days after injection and continuously superfused in recording buffer (100 mM NaCl, 2.5 mM KCl, 1 mM $CaCl_2$.2H2O, 5 mM HEPES, pH 7.3) at room temperature. Prior to recording, the oocytes were incubated ~4 h with 100µM of the calcium chelator BAPTA-AM (Sigma, St. Louis, MO, USA) to prevent activation of endogenous calcium-activated chloride channels. Acetylcholine 100 µM was applied first to the oocytes in order to check for the presence of the L-nAChRs. Data were collected and analysed using the pCLAMP 10.4 package (Molecular Devices).

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