



Reversible Small Molecule Inhibitors of MAO A and MAO B with Anilide Motifs

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Background: Ligands consisting of two aryl moieties connected via a short spacer were shown to be potent inhibitors of monoamine oxidases (MAO) A and B, which are known as suitable targets in treatment of neurological diseases. Based on this general blueprint, we synthesized a series of 66 small aromatic amide derivatives as novel MAO A/B inhibitors.

Methods: The compounds were synthesized, purified and structurally confirmed by spectroscopic methods. Fluorimetric enzymological assays were performed to determine MAO A/B inhibition properties. Mode and reversibility of inhibition was determined for the most potent MAO B inhibitor. Docking poses and pharmacophore models were generated to confirm the in vitro results.

Results: *N*-(2,4-Dinitrophenyl)benzo[d][1,3]dioxole-5-carboxamide (**55**, ST-2043) was found to be a reversible competitive moderately selective MAO B inhibitor ($IC_{50} = 56$ nM, $K_i = 6.3$ nM), while *N*-(2,4-dinitrophenyl)benzamide (**7**, ST-2023) showed higher preference for MAO A ($IC_{50} = 126$ nM). Computational analysis confirmed in vitro binding properties, where the anilides examined possessed high surface complementarity to MAO A/B active sites.

Conclusion: The small molecule anilides with different substitution patterns were identified as potent MAO A/B inhibitors, which were active in nanomolar concentrations ranges. These small and easily accessible molecules are promising motifs, especially for newly designed multitargeted ligands taking advantage of these fragments.

Keywords: salicylic acid derivatives, molecular modeling, Parkinson's disease, enzyme inhibitor, pharmacophore, structure-activity relationships

Plain Language Summary

Monoamine oxidases (MAO) A and B are neurotransmitter-catabolizing enzymes, which play a role in the pathophysiology of neurological diseases such as Parkinson's disease, depression or schizophrenia. Small molecules consisting of two aryl moieties connected via a short spacer were shown to be potent MAO A/B inhibitors. In this study, aromatic amide derivatives with different structural variations and substitution pattern were demonstrated to have MAO A/B inhibition properties in a nanomolar concentration range. Compound **55** was found to be a reversible competitive MAO B preferring inhibitor ($IC_{50} = 56$ nM, $K_i = 6.3$ nM), while compound **7** showed a higher preference for MAO A ($IC_{50} = 126$ nM). Computational analysis confirmed in vitro binding properties as the respective anilides possessed high surface complementarity to MAO A/B active sites. These results suggest that the herein described anilides are small and easily accessible molecules, which may serve as promising precursors for the design of selective or multitargeting MAO A/B inhibitors.

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Introduction

The neurotransmitter-catabolizing monoamine oxidases (MAO) are localized in the outer mitochondrial membrane and are classified into the A and B isoforms. MAO A is mainly involved in the degradation of serotonin, melatonin, norepinephrine, and epinephrine, and is expressed nearly ubiquitously in the human body. MAO B breaks down phenethylamine and benzylamine and it is highly expressed in the central nervous system (CNS). Dopamine, tyramine and tryptamine can be metabolized by both isoforms but with individual metabolic activity for each substrate. Consequently, inhibitors of both enzymes are established in the pharmacotherapy for neurological diseases.^{1–3} Moclobemide was the first marketed MAO inhibitor and entered the Swedish market in 1989.⁴ Slowing down the degradation of neurotransmitters like dopamine and norepinephrine via MAO A inhibition, this drug exhibits mood-lifting properties facilitating its use as an antidepressant.⁴ Since then the irreversible nonselective MAO inhibitor tranylcypromine as well as the irreversible MAO B inhibitors rasagiline and selegiline were approved for the treatment of depression and Parkinson's disease (PD), respectively. Recently, safinamide as first reversible MAO B inhibitor in PD therapy was approved.^{5–7} As the imminent therapeutic benefits of MAO inhibitors are nowadays clear, multiple efforts have been made to develop new reversible and irreversible inhibitors. Due to long-term enzyme inactivation by irreversible MAO inhibitors, these can be associated with serious side effects or drug–drug interactions, eg leading to a serotonin syndrome.⁸ Reversible MAO inhibitors have a more favorable side effect profile; however, they can be displaced from the active site in case of high levels of the endogenous ligands. To maintain pharmacological efficacy, they are aimed to show a high affinity towards the MAOs and/or tight binding behavior.⁹ The design of such reversible and selective inhibitors of MAO A and B became a strongly researched area to identify suitable drug candidates for the treatment of neurological diseases, particularly of PD.

Having in mind that such diseases usually derive from multifactorial disorders, the development of sophisticated multitargeted ligands (MTLs) became of major exploration to treat these CNS disorders.^{10,11} The combination of different biological active moieties in one molecule is challenging since the affinity for each target needs to be preserved together with maintaining drug-likeness properties. Identification of small chemical entities with a promising target affinity are of great value in MTL drug design, as they might be easily combined

or fused with other pharmacophores with minor influences on molecular size.

Accordingly, we synthesized a series of 66 anilides with diverse substitution patterns. The presented anilides are small-sized and have low molecular weights. The ligands show structural similarities to previously described potent MAO inhibitors assuring potential MAO inhibition activities. Multiple publications identified two aromatic moieties linked by a short bridging element as mutual MAO scaffold.^{8,12–16} Due to their high stability under physiological conditions, amides were used as linkers in our series. Aromatic moieties bearing different substituents were chosen to elucidate electronic and steric effects on the binding affinities towards both MAO isoforms. The presented ligands were characterized *in vitro*. For a better understanding of the binding modes, computational docking experiments were performed and a ligand-based pharmacophore-model was established.

Materials and Methods

Reagents and Instrumentation

Reagents and solvents for synthesis were purchased from Sigma-Aldrich, VWR Chemicals, Fisher Scientific, Panreac AppliChem, Alfa Aesar and Chemsolute and were used without further purifications (unless stated otherwise). ¹H NMR and ¹³C NMR were recorded on a Bruker AMX spectrometer (Bruker, Germany) at 300 and 75 MHz respectively, where either CDCl₃ or DMSO-d₆ was used as a solvent. Tetramethylsilane was used as standard and chemical shifts are reported in parts per million (ppm). Elementary analyses (C, H, N) were measured on a CHN-Rapid (Heraeus, Germany) and were within 0.4% of the theoretical values for final compounds. LC-MS analysis was performed on a Bruker Elute SP; Column: Intensity Solo C₁₈ RP; column dimensions: 100 x 2.1 mm; eluent: acetonitrile (1–40%) in water, 0.1% formic acid; flow: 0.3 mL/min) combined with mass spectrometric detection (Bruker amazon speed with ESI; detection: ion-trap; ion-polarity: positive; scan: 100–600 m/z). Data are given as retention time (*t_R*), mass number ([M + H]⁺), and normalized peak area (%) as approximated purity. Melting points (m.p., uncorrected) were determined on a M-564 Büchi melting point apparatus (Büchi, Germany). Thin-layer chromatography (TLC) was carried out using pre-coated silica gel 60 with fluorescence indicator at UV 254 nm (Macherey-Nagel, Germany). The structure and purity of each compound were confirmed

using ^1H NMR, ^{13}C NMR, LC-MS and/or elemental analysis.

Human recombinant monoamine oxidase A (MAO A, E.C. 1.4.3.4), human recombinant monoamine oxidase B (MAO B, E.C. 1.4.3.4), kynuramine dihydrobromide (KYN) and dimethylsulfoxide (DMSO) were purchased from Sigma Aldrich. For biological evaluation tests, compounds were dissolved in 100% DMSO (max. 10^{-3} M). Fluorescence intensity measurements were performed with an infinite M1000 Pro multimode reader (Tecan, Switzerland). Assay pipetting was partly automated using a Freedom EVO pipetting robot (Tecan, Switzerland).

Experimental Procedures

N-Phenylbenzamid (1)³⁴

Using Method A the title compound was isolated as a white amorphous powder. Yield: 53%

^1H NMR (300 MHz, DMSO- d_6) δ 10.25 (s, 1H), 8.00–7.93 (m, 2H), 7.82–7.75 (m, 2H), 7.65–7.49 (m, 3H), 7.41–7.29 (m, 2H), 7.15–7.06 (m, 1H). ^{13}C NMR (75 MHz, DMSO) δ 165.50, 139.13, 134.95, 131.49, 128.55, 128.33, 127.60, 123.60, 120.31.

Elemental Analysis: calc C 79.17%, H 5.62%, N 7.10%; found C 78.91%, H 5.53%, N 6.89%.

m.p.: 163°C (lit. 163–164°C).³⁵

N-(Pyridin-2-yl)benzamide (2)³⁶

Using Method C the title compound was isolated as a white amorphous powder. Yield: 83%

^1H NMR (300 MHz, DMSO- d_6) δ 11.95 (s, 1H), 8.51 (dt, J = 5.6, 1.3 Hz, 1H), 8.28–8.24 (m, 2H), 8.20–8.12 (m, 2H), 7.73–7.64 (m, 1H), 7.62–7.54 (m, 2H), 7.47 (td, J = 5.7, 2.7 Hz, 1H).

^{13}C NMR (75 MHz, DMSO- d_6) δ 166.89, 149.65, 143.30, 142.29, 132.94, 132.55, 128.60, 128.32, 120.34, 116.28.

LC-MS t_R = 16.8 min; $[\text{M}+\text{H}]^+$ = 198.9; 100%.

m.p.: 78°C (lit. 75–80°C).

N-(4-Cyanophenyl)benzamide (3)³⁷

Using Method A the title compound was isolated as a white solid. Yield: 57%

^1H NMR (300 MHz, DMSO- d_6) δ 10.65 (s, 1H), 8.06–7.92 (m, 4H), 7.87–7.78 (m, 2H), 7.68–7.49 (m, 3H).

^{13}C NMR (75 MHz, DMSO) δ 166.15, 143.46, 134.34, 133.07, 131.99, 128.44, 127.80, 120.12, 119.04, 105.30.

Elemental Analysis: calc C 75.60%, H 4.54%, N 12.60%; found C 75.44%, H 4.24%, N 12.33%.

m.p.: 165°C (lit. 165–166°C).³⁸

N-(4-(Trifluoromethoxy)phenyl)benzamide (4)³⁹

Using Method A the title compound was isolated as a white solid. Yield: 62%

^1H NMR (300 MHz, DMSO- d_6) δ 10.44 (s, 1H), 8.14–7.79 (m, 4H), 7.57 (ddd, J = 14.4, 7.8, 6.0 Hz, 3H), 7.37 (d, J = 8.6 Hz, 2H).

^{13}C NMR (75 MHz, DMSO) δ 165.66, 143.82, 143.79, 138.36, 134.61, 131.68, 128.37, 127.65, 121.60, 121.41.

Elemental Analysis: calc C 59.79%, H 3.58%, N 4.98%; found C 59.76%, H 3.42%, N 4.90%.

m.p.: 179°C (lit. 176–178°C).⁴⁰

N-(4-Amino-2-methylphenyl)benzamide (5)⁴¹

Using Method D the title compound was isolated as a white solid. Yield 89%

^1H NMR (300 MHz, DMSO- d_6) δ 9.57 (s, 1H), 8.06–7.86 (m, 2H), 7.62–7.43 (m, 3H), 6.91 (d, J = 8.2 Hz, 1H), 6.47 (d, J = 2.5 Hz, 1H), 6.42 (dd, J = 8.3, 2.6 Hz, 1H), 2.08 (s, 3H).

^{13}C NMR (75 MHz, DMSO) δ 165.29, 146.96, 134.81, 134.59, 131.13, 128.26, 127.78, 127.40, 124.87, 115.19, 111.43, 17.99.

Elemental Analysis: calc C 74.31%, H 6.05%, N 12.38%; found C 74.22%, H 6.05%, N 12.08%.

m.p.: 201°C (lit. 199–201°C).⁴²

N-(2-Methyl-4-nitrophenyl)benzamide (6)⁴¹

Using Method A the title compound was isolated as a faint yellow solid. 63%

^1H NMR (300 MHz, DMSO- d_6) δ 10.11 (s, 1H), 8.19 (dd, J = 2.6, 0.9 Hz, 1H), 8.12 (dd, J = 8.8, 2.8 Hz, 1H), 8.05–7.98 (m, 2H), 7.84 (d, J = 8.8 Hz, 1H), 7.68–7.52 (m, 3H), 2.41 (s, 3H).

^{13}C NMR (75 MHz, DMSO) δ 165.59, 144.14, 143.00, 133.99, 133.88, 131.99, 128.47, 127.86, 125.57, 125.35, 121.49, 17.90.

Elemental Analysis: calc C 65.62%, H 4.72%, N 10.93%; found C 65.38%, H 4.42%, N 10.74%.

m.p.: 186°C (lit. 181–186°C).⁴²

N-(2,4-Dinitrophenyl)benzamide (7)⁴³

Using Method A the title compound was isolated as a yellow solid. Yield 23%

^1H NMR (300 MHz, DMSO- d_6) δ 11.23 (s, 1H), 8.76 (d, J = 2.6 Hz, 1H), 8.59 (dd, J = 9.1, 2.7 Hz, 1H), 8.15 (d, J = 9.1 Hz, 1H), 8.05–7.91 (m, 2H), 7.78–7.53 (m, 3H).

^{13}C NMR (75 MHz, DMSO) δ 164.49, 156.60, 141.19, 139.12, 137.12, 134.71, 131.32, 129.31, 123.56, 121.58, 119.84, 117.83, 116.95.

Elemental Analysis: calc C 54.36%, H 3.16%, N 14.63%; found C 54.21%, H 3.48%, N 14.80%.

m.p.: 199°C (lit: 199–201°C).⁴⁴

***N*-(4-Amino-2-methoxyphenyl)benzamide (8)⁴⁵**

Using Method D the title compound was isolated as an off-white solid. Yield 90%

^1H NMR (300 MHz, DMSO- d_6) δ 9.17 (s, 1H), 8.01–7.88 (m, 2H), 7.63–7.40 (m, 3H), 7.18 (d, J = 8.3 Hz, 1H), 6.31 (d, J = 2.3 Hz, 1H), 6.15 (dd, J = 8.4, 2.3 Hz, 1H), 5.08 (s, 2H), 3.71 (s, 3H).

^{13}C NMR (75 MHz, DMSO) δ 153.40, 147.70, 134.82, 131.12, 128.28, 127.29, 126.68, 115.16, 105.13, 97.61, 55.12.

Elemental Analysis: calc C 69.41%, H 5.81%, N 11.56%; found C 69.49%, H 5.82%, N 11.38%.

m.p.: 115°C (lit: 112–113°C).

***N*-(2-Methoxy-4-nitrophenyl)benzamide (9)⁴⁵**

Using Method A the title compound was isolated as a yellow solid. Yield 60%

^1H NMR (300 MHz, DMSO- d_6) δ 9.63 (s, 1H), 8.31 (d, J = 8.9 Hz, 1H), 8.03–7.88 (m, 3H), 7.85 (d, J = 2.5 Hz, 1H), 7.69–7.49 (m, 3H), 4.00 (s, 3H).

^{13}C NMR (75 MHz, DMSO) δ 165.32, 149.81, 143.47, 133.76, 132.16, 128.57, 127.62, 121.16, 116.53, 105.97, 56.55.

Elemental Analysis: calc C 61.76%, H 4.44%, N 10.29%; found C 61.71%, H 4.53%, N 10.33%.

m.p.: 149°C (lit: 148–151°C).

***N*-(2-Chloro-4-nitrophenyl)benzamide (10)⁴⁶**

Using Method A the title compound was isolated as a pale-yellow solid. Yield 59%

^1H NMR (300 MHz, DMSO- d_6) δ 10.29 (s, 1H), 8.41 (d, J = 2.6 Hz, 1H), 8.27 (dd, J = 9.0, 2.6 Hz, 1H), 8.09 (d, J = 9.0 Hz, 1H), 8.05–7.95 (m, 2H), 7.73–7.50 (m, 3H).

^{13}C NMR (75 MHz, DMSO) δ 165.49, 144.45, 141.31, 133.37, 132.36, 128.59, 128.01, 127.89, 126.58, 124.85, 122.89.

Elemental Analysis: calc C 56.44%, H 3.28%, N 10.13%; found C 56.14%, H 3.38%, N 10.18%.

m.p.: 161°C (lit: 160°C).

***N*-(4-Bromophenyl)picolinamide (11)⁴⁷**

Using Method B the title compound was isolated as a white solid. Yield 75%

^1H NMR (300 MHz, DMSO- d_6) δ 10.80 (s, 1H), 8.75 (ddd, J = 4.8, 1.7, 0.9 Hz, 1H), 8.17 (dt, J = 7.8, 1.2 Hz, 1H), 8.07 (td, J = 7.7, 1.7 Hz, 1H), 7.96–7.88 (m, 2H), 7.69 (ddd, J = 7.5, 4.8, 1.4 Hz, 1H), 7.59–7.52 (m, 2H).

^{13}C NMR (75 MHz, DMSO- d_6) δ 162.65, 149.66, 148.41, 138.12, 137.77, 131.41, 127.00, 122.46, 122.24, 115.64.

Elemental Analysis: calc C 52.01%, H 3.27%, N 10.11%; found C 51.74%, H 3.06%, N 10.10%.

m.p.: 148°C (lit: 147–148°C).⁴⁸

2-Hydroxy-*N*-phenylbenzamide (12)⁴⁹

Using Method A the title compound was isolated as a white solid. Yield 61%

^1H NMR (300 MHz, DMSO- d_6) δ 11.83 (s, 1H), 10.40 (s, 1H), 7.99 (dd, J = 7.9, 1.7 Hz, 1H), 7.75–7.69 (m, 2H), 7.51–7.33 (m, 3H), 7.18–7.09 (m, 1H), 7.03–6.93 (m, 2H).

^{13}C NMR (75 MHz, DMSO) δ 166.57, 158.48, 138.10, 133.62, 128.99, 128.69, 124.15, 120.96, 118.98, 117.42, 117.19.

Elemental Analysis: calc C 73.23%, H 5.20%, N 6.57%; found C 73.13%, H 5.21%, N 6.46%.

m.p.: 132°C (lit: 127–129°C).⁵⁰

***N*-(4-Cyanophenyl)-2-hydroxybenzamide (13)⁵¹**

Using Method A the title compound was isolated as a white solid. Yield 47%

^1H NMR (300 MHz, DMSO- d_6) δ 11.41 (s, 1H), 10.66 (s, 1H), 7.94 (d, J = 8.8 Hz, 2H), 7.88 (dd, J = 7.9, 1.7 Hz, 1H), 7.83 (d, J = 8.8 Hz, 2H), 7.45 (ddd, J = 8.2, 7.2, 1.7 Hz, 1H), 7.05–6.92 (m, 2H).

^{13}C NMR (75 MHz, DMSO) δ 166.45, 157.49, 142.68, 133.67, 133.15, 129.48, 120.40, 119.18, 118.96, 118.47, 117.05, 105.57.

Elemental Analysis: calc C 70.58%, H 4.23%, N 11.76%; found C 70.40%, H 4.11%, N 11.67%.

m.p.: 176°C (lit: 175–176.5°C).⁵²

***N*-(2,4-Dinitrophenyl)-2-hydroxybenzamide (14)⁵³**

Using Method A the title compound was isolated as a yellow solid. Yield 22%

^1H NMR (300 MHz, DMSO- d_6) δ 12.41 (s, 1H), 11.96 (s, 1H), 9.01 (d, J = 9.4 Hz, 1H), 8.88 (d, J = 2.7 Hz, 1H), 8.59 (dd, J = 9.4, 2.8 Hz, 1H), 8.01 (dd, J = 8.0, 1.8 Hz, 1H), 7.50 (ddd, J = 8.6, 7.2, 1.8 Hz, 1H), 7.11–6.97 (m, 2H).

^{13}C NMR (75 MHz, DMSO) δ 165.50, 142.73, 137.31, 132.90, 132.80, 128.81, 128.61, 127.91, 125.49, 121.16.

Elemental Analysis: calc C 51.49%, H 2.99%, N 13.86%; found C 51.40%, H 2.77%, N 13.97%.

m.p.: 212°C (lit: 213–214°C).⁵⁴

2-Hydroxy-*N*-(2-methoxy-4-nitrophenyl)benzamide (15)⁵⁵
Using Method A the title compound was isolated as a yellow solid. Yield 45%

¹H NMR (300 MHz, DMSO-*d*₆) δ 11.86 (s, 1H), 11.28 (s, 1H), 8.73 (d, *J* = 9.0 Hz, 1H), 8.03 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.95 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.85 (d, *J* = 2.5 Hz, 1H), 7.45 (ddd, *J* = 8.2, 7.2, 1.8 Hz, 1H), 7.09–6.96 (m, 2H), 4.04 (s, 3H).

¹³C NMR (75 MHz, DMSO) δ 163.54, 156.01, 147.85, 142.27, 134.60, 133.98, 131.09, 119.89, 118.33, 118.17, 117.34, 116.95, 105.64, 56.78.

Elemental Analysis: calc C 58.33%, H 4.20%, N 9.72%; found C 58.15%, H 4.05%, N 9.54%.

m.p.: 207°C (lit: 205–206°C).

***N*-(2-Chloro-4-nitrophenyl)-2-hydroxybenzamide (16)**⁵⁶
Using Method A the title compound was isolated as a pale-yellow solid. Yield 55%

¹H NMR (300 MHz, DMSO-*d*₆) δ 12.18 (s, 1H), 11.42 (s, 1H), 8.89 (d, *J* = 9.3 Hz, 1H), 8.44 (d, *J* = 2.6 Hz, 1H), 8.31 (dd, *J* = 9.3, 2.7 Hz, 1H), 8.09 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.53 (ddd, *J* = 8.2, 7.2, 1.8 Hz, 1H), 7.16–7.02 (m, 2H).

¹³C NMR (75 MHz, DMSO) δ 163.74, 156.15, 142.21, 141.45, 134.41, 131.14, 124.67, 123.78, 122.09, 120.45, 120.03, 117.82, 116.98.

Elemental Analysis: calc C 53.35%, H 3.10%, N 9.57%; found C 53.05%, H 3.32%, N 9.27%.

m.p.: 219°C (lit: 218–220°C).

4-Methyl-*N*-(4-nitro-2-(trifluoromethyl)phenyl)benzamide (17)

Using Method A the title compound was isolated as a paleyellow solid. Yield 12%

¹H NMR (300 MHz, DMSO-*d*₆) δ 10.31 (s, 1H), 8.56 (dd, *J* = 8.7, 2.7 Hz, 1H), 8.52 (d, *J* = 2.6 Hz, 1H), 7.96 (d, *J* = 8.8 Hz, 1H), 7.92–7.86 (m, 2H), 7.38 (d, *J* = 7.9 Hz, 2H), 2.40 (s, 3H).

¹³C NMR (75 MHz, DMSO) δ 166.12, 145.09, 142.45, 141.88, 141.86, 141.84, 131.48, 130.41, 129.09, 127.87, 127.80, 126.04, 125.63, 124.29, 122.32, 122.25, 120.66, 20.99.

Elemental Analysis: calc C 55.56%, H 3.42%, N 8.64%; found C 55.46%, H 3.25%, N 8.39%.

m.p.: 144°C.

4-Chloro-*N*-phenylbenzamide (18)⁵⁷

Using Method A the title compound was isolated as a white solid. Yield 63%

¹H NMR (300 MHz, DMSO-*d*₆) δ 10.32 (s, 1H), 8.04–7.95 (m, 2H), 7.83–7.74 (m, 2H), 7.65–7.55 (m, 2H), 7.36 (dd, *J* = 8.5, 7.3 Hz, 2H), 7.16–7.07 (m, 1H).

¹³C NMR (75 MHz, DMSO) δ 164.38, 138.92, 136.34, 133.61, 129.57, 128.58, 128.40, 123.77, 120.38.

Elemental Analysis: calc C 67.40%, H 4.35%, N 6.05%; found C 67.37%, H 4.21%, N 5.93%.

m.p.: 198°C (lit: 200–201°C).

4-Chloro-*N*-(pyridin-2-yl)benzamide (19)⁵⁸

Using Method C the title compound was isolated as a white solid. Yield 78%

¹H NMR (300 MHz, DMSO-*d*₆) δ 12.13 (s, 1H), 8.51 (ddd, *J* = 5.7, 1.7, 0.9 Hz, 1H), 8.36–8.14 (m, 4H), 7.73–7.60 (m, 2H), 7.47 (ddd, *J* = 7.1, 5.6, 1.5 Hz, 1H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 165.86, 149.58, 143.24, 142.44, 137.80, 131.38, 130.33, 128.66, 120.45, 116.30.

LC-MS *t*_R = 22.3 min; [M+H]⁺ = 232.9; 100%.

m.p.: 138°C (lit: 137–139°C).⁵⁹

4-Chloro-*N*-(4-cyanophenyl)benzamide (20)

Using Method A the title compound was isolated as a white solid. Yield 49%

¹H NMR (300 MHz, DMSO-*d*₆) δ 10.67 (s, 1H), 8.00 (d, *J* = 1.6 Hz, 2H), 7.97 (d, *J* = 1.8 Hz, 2H), 7.81 (d, *J* = 8.8 Hz, 2H), 7.61 (d, *J* = 8.6 Hz, 2H).

¹³C NMR (75 MHz, DMSO) δ 164.97, 143.25, 136.87, 133.04, 132.97, 129.75, 128.49, 120.18, 118.98, 105.48.

Elemental Analysis: calc C 65.51%, H 3.53%, N 10.91%; found C 65.62%, H 3.51%, N 10.95%.

m.p.: 208°C.

4-Chloro-*N*-(2-methyl-4-nitrophenyl)benzamide (21)⁶⁰

Using Method A the title compound was isolated as a yellow solid. Yield 52%

¹H NMR (300 MHz, DMSO-*d*₆) δ 10.18 (s, 1H), 8.19 (d, *J* = 2.7 Hz, 1H), 8.11 (dd, *J* = 8.8, 2.7 Hz, 1H), 8.02 (d, *J* = 8.6 Hz, 2H), 7.81 (d, *J* = 8.8 Hz, 1H), 7.63 (d, *J* = 8.6 Hz, 2H), 2.40 (s, 3H).

¹³C NMR (75 MHz, DMSO) δ 164.57, 144.26, 142.76, 136.83, 134.00, 132.70, 129.81, 128.53, 125.72, 125.36, 121.48, 17.89.

Elemental Analysis: calc C 57.84%, H 3.81%, N 9.64%; found C 58.03%, H 3.99%, N 9.64%.

m.p.: 193°C.

4-Chloro-*N*-(2-methoxy-4-nitrophenyl)benzamide (**22**)

Using Method A the title compound was isolated as a yellow solid. Yield 54%

¹H NMR (300 MHz, DMSO-*d*₆) δ 9.83 (s, 1H), 8.24 (d, *J* = 8.9 Hz, 1H), 8.03–7.91 (m, 3H), 7.88 (d, *J* = 2.5 Hz, 1H), 7.67–7.58 (m, 2H), 4.00 (s, 3H).

¹³C NMR (75 MHz, DMSO) δ 164.51, 150.30, 143.83, 136.97, 133.55, 132.59, 129.72, 128.60, 122.03, 116.45, 106.18, 56.56.

Elemental Analysis: calc C 54.83%, H 3.62%, N 9.13%; found C 54.67%, H 3.57%, N 8.86%.

m.p.: 200°C (decomposition).

4-Chloro-*N*-(2-chloro-4-nitrophenyl)benzamide (**23**)

Using Method A the title compound was isolated as a yellow solid. Yield 17%

¹H NMR (300 MHz, DMSO-*d*₆) δ 10.42 (s, 1H), 8.43 (d, *J* = 2.6 Hz, 1H), 8.27 (dd, *J* = 8.9, 2.6 Hz, 1H), 8.09–7.98 (m, 3H), 7.66 (d, *J* = 8.6 Hz, 2H).

¹³C NMR (75 MHz, DMSO) δ 164.56, 144.69, 141.12, 137.19, 132.13, 129.87, 128.69, 128.32, 127.00, 124.90, 122.89.

Elemental Analysis: calc C 50.19%, H 2.59%, N 9.00%; found C 50.44%, H 2.30%, N 8.96%.

m.p.: 219°C.

4-Bromo-*N*-(2-chloro-4-nitrophenyl)benzamide (**24**)

Using Method A the title compound was isolated as an off-white solid. Yield 20%

¹H NMR (300 MHz, DMSO-*d*₆) δ 10.41 (s, 1H), 8.41 (d, *J* = 2.6 Hz, 1H), 8.27 (dd, *J* = 9.0, 2.6 Hz, 1H), 8.04 (d, *J* = 9.0 Hz, 1H), 7.99–7.92 (m, 2H), 7.83–7.76 (m, 2H).

¹³C NMR (75 MHz, DMSO) δ 164.70, 144.65, 141.10, 132.49, 131.62, 130.00, 128.25, 126.92, 126.19, 124.88, 122.88.

Elemental Analysis: calc C 43.91%, H 2.27%, N 7.88%; found C 43.65%, H 1.90%, N 7.53%.

m.p.: 205°C.

5-Amino-2-hydroxy-*N*-phenylbenzamide (**25**)⁶¹

Using Method D the title compound was isolated as an off-white solid. Yield 90%

¹H NMR (300 MHz, DMSO-*d*₆) δ 10.47 (s, 1H), 7.69 (dd, *J* = 8.3, 1.3 Hz, 2H), 7.36 (t, *J* = 7.9 Hz, 2H), 7.21–7.15 (m, 1H), 7.12 (d, *J* = 7.4 Hz, 1H), 6.73 (d, *J* = 2.0 Hz, 2H), 4.72 (s, 2H).

¹³C NMR (75 MHz, DMSO) δ 165.96, 148.64, 140.98, 138.50, 128.72, 123.71, 120.37, 120.29, 118.02, 117.50, 113.68.

Elemental Analysis: calc C 68.41%, H 5.30%, N 12.27%; found C 68.01%, H 4.96%, N 12.38%.

m.p.: 176°C (lit: 174–176°C).⁶²

2-Hydroxy-5-nitro-*N*-phenylbenzamide (**26**)⁶¹

Using Method A the title compound was isolated as a white solid. Yield 61%

¹H NMR (300 MHz, DMSO-*d*₆) δ 12.78 (s, 1H), 10.57 (s, 1H), 8.79 (d, *J* = 2.9 Hz, 1H), 8.29 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.39 (t, *J* = 7.7 Hz, 2H), 7.16 (dd, *J* = 8.4, 5.5 Hz, 2H).

¹³C NMR (75 MHz, DMSO) δ 164.08, 163.27, 139.34, 137.89, 128.75, 128.33, 125.71, 124.39, 120.77, 119.33, 117.97.

Elemental Analysis: calc C 60.47%, H 3.90%, N 10.85%; found C 60.20%, H 3.85%, N 10.65%.

m.p.: 223°C (lit: 219–221°C).

5-Chloro-2-hydroxy-*N*-phenylbenzamide (**27**)⁵⁶

Using Method A the title compound was isolated as a white solid. Yield 62%

¹H NMR (300 MHz, DMSO-*d*₆) δ 11.87 (s, 1H), 10.41 (s, 1H), 7.98 (d, *J* = 2.7 Hz, 1H), 7.77–7.64 (m, 2H), 7.47 (dd, *J* = 8.8, 2.7 Hz, 1H), 7.43–7.32 (m, 2H), 7.21–7.10 (m, 1H), 7.02 (d, *J* = 8.8 Hz, 1H).

¹³C NMR (75 MHz, DMSO) δ 164.96, 156.87, 137.95, 132.99, 128.72, 128.32, 124.29, 122.68, 120.81, 119.45, 119.04.

Elemental Analysis: calc C 63.04%, H 4.07%, N 5.66%; found C 62.83%, H 4.17%, N 5.55%.

m.p.: 209°C (lit: 211–212°C).⁶³

5-Chloro-*N*-(2-chlorophenyl)-2-hydroxybenzamide (**28**)⁶⁴

Using Method A the title compound was isolated as a pale-yellow solid. Yield 46%

¹H NMR (300 MHz, DMSO-*d*₆) δ 12.26 (s, 1H), 10.88 (s, 1H), 8.40 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.99 (d, *J* = 2.8 Hz, 1H), 7.54 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.48 (dd, *J* = 8.7, 2.8 Hz, 1H), 7.43–7.34 (m, 1H), 7.17 (ddd, *J* = 8.0, 7.4, 1.6 Hz, 1H), 7.07 (d, *J* = 8.8 Hz, 1H).

^{13}C NMR (75 MHz, DMSO- d_6) δ 162.59, 155.35, 134.98, 133.33, 129.72, 129.29, 127.78, 125.32, 123.46, 122.76, 119.58, 118.99.

Elemental Analysis: calc C 55.35%, H 3.22%, N 4.96%; found C 55.35%, H 3.31%, N 4.75%.

m.p.: 189°C (lit: 184–186°C).⁶²

5-Chloro-*N*-(3-chlorophenyl)-2-hydroxybenzamide (**29**)⁵⁶

Using Method A the title compound was isolated as a pale-yellow solid. Yield 41%

^1H NMR (300 MHz, DMSO- d_6) δ 11.65 (s, 1H), 10.49 (s, 1H), 7.92 (t, J = 2.1 Hz, 1H), 7.90 (d, J = 2.7 Hz, 1H), 7.61 (ddd, J = 8.3, 2.1, 1.0 Hz, 1H), 7.47 (dd, J = 8.8, 2.7 Hz, 1H), 7.40 (t, J = 8.1 Hz, 1H), 7.20 (ddd, J = 8.0, 2.1, 1.0 Hz, 1H), 7.02 (d, J = 8.8 Hz, 1H).

^{13}C NMR (75 MHz, DMSO- d_6) δ 164.96, 156.41, 139.55, 133.01, 130.39, 128.44, 123.87, 122.72, 120.05, 119.94, 118.99.

Elemental Analysis: calc C 55.35%, H 3.22%, N 4.96%; found C 55.12%, H 3.25%, N 4.86%.

m.p.: 219°C (lit: 217–218°C).⁶²

Ethyl 4-(5-chloro-2-hydroxybenzamido)benzoate (**30**)⁶⁵

Using Method A the title compound was isolated as a pale-yellow solid. Yield 37%

^1H NMR (300 MHz, DMSO- d_6) δ 11.63 (s, 1H), 10.65 (s, 1H), 8.03–7.93 (m, 2H), 7.92–7.83 (m, 3H), 7.47 (dd, J = 8.8, 2.7 Hz, 1H), 7.03 (d, J = 8.8 Hz, 1H), 4.30 (q, J = 7.1 Hz, 2H), 1.32 (t, J = 7.1 Hz, 3H).

^{13}C NMR (75 MHz, DMSO) δ 165.22, 164.87, 156.25, 142.53, 132.99, 130.11, 128.61, 125.00, 122.74, 120.28, 119.80, 118.96, 60.49, 14.17.

Elemental Analysis: calc C 60.10%, H 4.41%, N 4.38%; found C 60.46%, H 4.38%, N 4.32%.

m.p.: 214°C (lit: 212–214°C).

5-Chloro-2-hydroxy-*N*-(4-(trifluoromethyl)phenyl)benzamide (**31**)⁶⁶

Using Method A the title compound was isolated as a pale-yellow solid. Yield 62%

^1H NMR (300 MHz, DMSO- d_6) δ 11.65 (s, 1H), 10.65 (s, 1H), 7.94 (d, J = 8.5 Hz, 2H), 7.90 (d, J = 2.7 Hz, 1H), 7.72 (dd, J = 8.7, 0.9 Hz, 2H), 7.46 (dd, J = 8.8, 2.7 Hz, 1H), 7.03 (d, J = 8.8 Hz, 1H).

^{13}C NMR (75 MHz, DMSO) δ 165.02, 156.30, 141.75, 141.73, 133.02, 128.57, 125.97, 125.92, 122.78, 120.39, 120.07, 118.95.

Elemental Analysis: calc C 53.27%, H 2.87%, N 4.44%; found C 53.09%, H 2.63%, N 4.38%.

m.p.: 221°C (lit: 222–223°C).⁶³

5-Chloro-2-hydroxy-*N*-(4-nitrophenyl)benzamide (**32**)⁶⁵

Using Method A the title compound was isolated as a yellow solid. Yield 9%

^1H NMR (300 MHz, DMSO- d_6) δ 11.46 (s, 1H), 10.84 (s, 1H), 8.28 (d, J = 9.2 Hz, 2H), 7.99 (d, J = 9.2 Hz, 2H), 7.84 (d, J = 2.8 Hz, 1H), 7.48 (dd, J = 8.8, 2.8 Hz, 1H), 7.04 (d, J = 8.8 Hz, 1H).

^{13}C NMR (75 MHz, DMSO) δ 164.98, 155.83, 144.50, 142.70, 133.00, 128.73, 124.85, 122.77, 120.88, 120.01, 118.90.

Elemental Analysis: calc C 53.35%, H 3.10%, N 9.57%; found C 53.37%, H 3.02%, N 9.36%.

m.p.: 259°C (li: 260–262°C).⁶²

5-Chloro-2-hydroxy-*N*-(4-(trifluoromethoxy)phenyl)benzamide (**33**)⁶³

Using Method A the title compound was isolated as a white solid. Yield 60%

^1H NMR (300 MHz, DMSO- d_6) δ 11.76 (s, 1H), 10.54 (s, 1H), 7.94 (d, J = 2.7 Hz, 1H), 7.83 (d, J = 9.0 Hz, 2H), 7.46 (dd, J = 8.8, 2.7 Hz, 1H), 7.41–7.30 (m, 2H), 7.02 (d, J = 8.8 Hz, 1H).

^{13}C NMR (75 MHz, DMSO) δ 165.04, 156.73, 144.25 (m), 137.21, 133.02, 128.36, 122.71, 122.12, 121.46, 119.51, 118.99.

Elemental Analysis: calc C 50.70%, H 2.74%, N 4.22%; found C 50.30%, H 2.65%, N 4.06%.

m.p.: 200°C (lit: 200–202°C).

N-(4-Bromophenyl)-5-chloro-2-hydroxybenzamide (**34**)⁶⁷

Using Method A the title compound was isolated as a yellow solid. Yield 55%

^1H NMR (300 MHz, DMSO- d_6) δ 11.75 (s, 1H), 7.93 (d, J = 2.7 Hz, 1H), 7.76–7.65 (m, 2H), 7.59–7.50 (m, 2H), 7.45 (dd, J = 8.8, 2.7 Hz, 1H), 7.01 (d, J = 8.8 Hz, 1H).

^{13}C NMR (75 MHz, DMSO) δ 164.92, 156.63, 137.43, 133.00, 131.52, 128.38, 122.71, 122.57, 119.64, 119.00, 115.97.

Elemental Analysis: calc C 47.81%, H 2.78%, N 4.29%; found C 47.49%, H 2.47%, N 3.94%.

m.p.: 237°C (lit: 240–241.5°C).⁶⁸

5-Chloro-2-hydroxy-*N*-(2-methyl-4-nitrophenyl)benzamide (**35**)⁶⁹

Using Method A the title compound was isolated as a yellow solid. Yield 16%

^1H NMR (300 MHz, DMSO- d_6) δ 12.33 (s, 1H), 10.73 (s, 1H), 8.52 (d, J = 9.0 Hz, 1H), 8.25–8.11 (m, 2H), 7.98 (d, J = 2.8 Hz, 1H), 7.53 (dd, J = 8.7, 2.8 Hz, 1H), 7.10 (d, J = 8.7 Hz, 1H), 2.45 (s, 3H).

^{13}C NMR not performed due to poor solubility.

Elemental Analysis: calc C 54.83%, H 3.62%, N 9.13%; found C 54.78%, H 3.32%, N 8.96%.

m.p.: 229°C (lit: 225–227°).

N-(2,4-Bis(trifluoromethyl)phenyl)-5-chloro-2-hydroxybenzamide (**36**)⁷⁰

Using Method A the title compound was isolated as an off-white solid. Yield 51%

^1H NMR (300 MHz, DMSO- d_6) δ 11.42 (s, 1H), 10.76 (s, 1H), 8.36 (d, J = 1.8 Hz, 2H), 7.91–7.63 (m, 2H), 7.39 (dd, J = 8.8, 2.7 Hz, 1H), 6.96 (d, J = 8.8 Hz, 1H).

^{13}C NMR (75 MHz, DMSO) δ 165.46, 156.39, 140.14, 133.21, 130.64 (q, J = 32.9 Hz), 128.47, 124.94, 122.73, 121.33, 120.22, 119.74, 118.97, 116.70 (q, J = 7.8, 4.0 Hz).

Elemental Analysis: calc C 46.96%, H 2.10%, N 3.65%; found C 46.73%, H 1.70%, N 3.61%.

m.p.: 171°C.

5-Chloro-2-hydroxy-*N*-(4-nitro-2-(trifluoromethyl)phenyl)benzamide (**37**)

Using Method A the title compound was isolated as a pale-yellow solid. Yield 63%

^1H NMR (300 MHz, DMSO- d_6) δ 12.51 (s, 1H), 11.24 (s, 1H), 8.77 (d, J = 9.2 Hz, 1H), 8.53 (dd, J = 9.2, 2.7 Hz, 1H), 8.43 (d, J = 2.7 Hz, 1H), 7.91 (d, J = 2.8 Hz, 1H), 7.51 (dd, J = 8.8, 2.8 Hz, 1H), 7.06 (d, J = 8.8 Hz, 1H).

^{13}C NMR (75 MHz, DMSO) δ 162.50, 155.11, 142.28, 141.10, 134.09, 130.06, 128.68, 123.81, 123.74, 122.11, 122.02, 118.98, 118.95, 118.26.

Elemental Analysis: calc C 46.62%, H 2.24%, N 7.77%; found C 46.32%, H 1.94%, N 7.64%.

m.p.: 191°C.

5-Chloro-2-hydroxy-*N*-(2-methoxy-4-nitrophenyl)benzamide (**38**)⁷¹

Using Method A the title compound was isolated as an off-white solid. Yield 13%

^1H NMR (300 MHz, DMSO- d_6) δ 12.25 (s, 1H), 11.24 (s, 1H), 8.69 (d, J = 9.0 Hz, 1H), 8.04–7.82 (m, 3H), 7.50 (dd, J = 8.7, 2.9 Hz, 1H), 7.08 (d, J = 8.8 Hz, 1H), 4.04 (s, 3H).

^{13}C NMR (75 MHz, DMSO) δ 162.21, 154.92, 147.93, 142.54, 134.19, 133.54, 130.00, 123.62, 119.85, 119.04, 118.34, 117.31, 105.72, 56.84.

Elemental Analysis: calc C 52.10%, H 3.28%, N 8.68%; found C 52.10%, H 3.28%, N 8.65%.

m.p.: 230°C (lit: 233–235°C).⁷²

Dimethyl 2-(5-chloro-2-hydroxybenzamido)terephthalate (**39**)

Using Method A the title compound was isolated as a pale-yellow solid. Yield 57%

^1H NMR (300 MHz, DMSO- d_6) δ 12.05 (s, 1H), 11.89 (s, 1H), 9.23 (d, J = 1.6 Hz, 1H), 8.09 (d, J = 8.3 Hz, 1H), 7.92 (d, J = 2.8 Hz, 1H), 7.77 (dd, J = 8.2, 1.7 Hz, 1H), 7.49 (dd, J = 8.8, 2.8 Hz, 1H), 7.06 (d, J = 8.8 Hz, 1H), 3.91 (s, 3H), 3.90 (s, 3H).

^{13}C NMR (75 MHz, DMSO) δ 166.13, 165.35, 163.21, 155.43, 139.41, 133.74, 133.34, 131.12, 129.96, 123.60, 123.07, 122.67, 121.54, 120.19, 118.83, 52.78, 52.58.

Elemental Analysis: calc C 56.13%, H 3.88%, N 3.85%; found C 56.53%, H 3.64%, N 4.00%.

m.p.: 193°C.

5-Chloro-2-hydroxy-*N*-(5-fluoro-2-nitrophenyl)benzamide (**40**)

Using Method A the title compound was isolated as a yellow solid. Yield 11%

^1H NMR (300 MHz, DMSO- d_6) δ 12.24 (s, 1H), 12.20 (s, 1H), 8.58 (dd, J = 11.9, 2.9 Hz, 1H), 8.30 (dd, J = 9.2, 5.8 Hz, 1H), 7.90 (d, J = 3.0 Hz, 1H), 7.49 (dd, J = 8.6, 2.9 Hz, 1H), 7.20 (ddd, J = 9.8, 7.3, 3.0 Hz, 1H), 7.05 (d, J = 8.7 Hz, 1H).

^{13}C NMR (75 MHz, DMSO- d_6) δ 164.87 (d, J = 252.5 Hz), 163.08, 155.34, 136.01 (d, J = 13.7 Hz), 134.52 (d, J = 2.7 Hz), 133.83, 130.12, 128.75 (d, J = 11.4 Hz), 123.36, 119.48, 118.92, 111.09 (d, J = 24.2 Hz), 109.40 (d, J = 29.8 Hz).

Elemental Analysis: calc C 50.26%, H 2.60%, N 9.02%; found C 50.19%, H 2.73%, N 9.02%.

m.p.: 178°C.

5-Chloro-2-hydroxy-*N*-(4-methyl-3-nitrophenyl)benzamide (**41**)

Using Method A the title compound was isolated as a brown solid. Yield 37%

^1H NMR (300 MHz, DMSO- d_6) δ 11.61 (s, 1H), 10.65 (s, 1H), 8.50 (d, J = 2.2 Hz, 1H), 7.89 (dd, J = 8.1, 2.5 Hz, 2H), 7.58–7.44 (m, 2H), 7.03 (d, J = 8.8 Hz, 1H), 3.33 (s, 3H).

^{13}C NMR (75 MHz, DMSO) δ 165.18, 156.50, 148.51, 137.04, 133.10, 133.04, 128.39, 128.15, 125.13, 122.69, 119.80, 119.02, 115.79, 19.19.

Elemental Analysis: calc C 54.83%, H 3.62%, N 9.13%; found C 55.21%, H 3.50%, N 8.73%.

m.p.: 278°C.

5-Chloro-2-hydroxy-*N*-(3,4-dimethoxyphenyl)benzamide (42)⁷³

Using Method A the title compound was isolated as a yellow solid. Yield 29%

^1H NMR (300 MHz, DMSO- d_6) δ 12.04 (s, 1H), 10.30 (s, 1H), 8.01 (d, J = 2.7 Hz, 1H), 7.46 (dd, J = 8.8, 2.7 Hz, 1H), 7.39 (d, J = 2.4 Hz, 1H), 7.25 (dd, J = 8.7, 2.4 Hz, 1H), 7.01 (d, J = 8.8 Hz, 1H), 6.95 (d, J = 8.7 Hz, 1H), 3.77 (s, 3H), 3.75 (s, 3H).

^{13}C NMR (75 MHz, DMSO) δ 165.02, 157.36, 148.46, 145.70, 133.03, 131.21, 127.99, 122.58, 119.10, 118.81, 113.05, 111.77, 106.00, 55.63, 55.42.

Elemental Analysis: calc C 58.55%, H 4.59%, N 4.55%; found C 58.53%, H 4.68%, N 4.46%.

m.p.: 185°C.

5-Chloro-2-hydroxy-*N*-(3,5-dichlorophenyl)benzamide (43)⁶³

Using Method A the title compound was isolated as a pale-yellow solid. Yield 56%

^1H NMR (300 MHz, DMSO- d_6) δ 11.75–11.27 (s, 1H), 10.56 (s, 1H), 7.90–7.76 (m, 2H), 7.47 (dd, J = 8.8, 2.7 Hz, 1H), 7.34 (t, J = 1.9 Hz, 1H), 7.03 (d, J = 8.8 Hz, 1H).

^{13}C NMR (75 MHz, DMSO) δ 164.99, 156.11, 140.56, 133.99, 133.05, 128.53, 123.23, 122.74, 120.21, 118.93, 118.54.

Elemental Analysis: calc C 49.32%, H 2.55%, N 4.42%; found C 49.70%, H 2.20%, N 4.36%.

m.p.: 247 °C (lit: 247–249 °C).

2-Chloro-4-nitro-*N*-(2-chlorophenyl)benzamide (44)

Using Method A the title compound was isolated as an off-white solid. Yield 50%

^1H NMR (300 MHz, DMSO- d_6) δ 10.52 (s, 1H), 8.42 (d, J = 2.2 Hz, 1H), 8.32 (dd, J = 8.4, 2.2 Hz, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.74 (dd, J = 8.0, 1.7 Hz, 1H), 7.58 (dd, J = 8.0, 1.5 Hz, 1H), 7.43 (td, J = 7.6, 1.6 Hz, 1H), 7.32 (td, J = 7.7, 1.7 Hz, 1H).

^{13}C NMR (75 MHz, DMSO- d_6) δ 163.90, 148.38, 141.98, 133.87, 131.20, 130.11, 129.72, 128.35, 127.69, 127.56, 124.57, 122.40.

Elemental Analysis: calc C 50.19%, H 2.59%, N 9.00%; found C 50.11%, H 2.70%, N 8.81%.

m.p.: 171°C.

2-Chloro-4-nitro-*N*-(3-chlorophenyl)benzamide (45)

Using Method A the title compound was isolated as an off-white solid. Yield 51%

^1H NMR (300 MHz, DMSO- d_6) δ 10.95 (s, 1H), 8.44 (d, J = 2.2 Hz, 1H), 8.32 (dd, J = 8.4, 2.2 Hz, 1H), 7.99–7.87 (m, 2H), 7.58 (ddd, J = 8.3, 2.1, 1.0 Hz, 1H), 7.42 (t, J = 8.1 Hz, 1H), 7.22 (ddd, J = 8.0, 2.1, 1.0 Hz, 1H).

^{13}C NMR (75 MHz, DMSO- d_6) δ 163.57, 148.44, 141.85, 139.78, 133.18, 131.09, 130.62, 130.06, 124.63, 123.98, 122.49, 119.14, 118.09.

Elemental Analysis: calc C 50.19%, H 2.59%, N 9.00%; found C 50.26%, H 2.64%, N 8.92%.

m.p.: 163°C.

2-Chloro-4-nitro-*N*-(4-nitrophenyl)benzamide (46)⁷⁴

Using Method A the title compound was isolated as a pale-yellow solid. Yield 39%

^1H NMR (300 MHz, DMSO- d_6) δ 11.36 (s, 1H), 8.45 (d, J = 2.2 Hz, 1H), 8.36–8.25 (m, 3H), 7.97 (dd, J = 8.7, 5.3 Hz, 3H).

^{13}C NMR (75 MHz, DMSO- d_6) δ 164.04, 148.58, 144.35, 142.96, 141.45, 131.09, 130.15, 125.03, 124.67, 122.54, 119.54.

Elemental Analysis: calc C 48.54%, H 2.51%, N 13.06%; found C 48.16%, H 2.63%, N 12.81%.

m.p.: 197°C (lit: 196–198°C).

2-Chloro-4-nitro-*N*-(5-chloro-2-hydroxyphenyl)benzamide (47)

Using Method B the title compound was isolated as a yellow solid. Yield 20%

^1H NMR (300 MHz, DMSO- d_6) δ 10.26 (s, 1H), 10.11 (s, 1H), 8.38 (d, J = 2.2 Hz, 1H), 8.27 (dd, J = 8.4, 2.2 Hz, 1H), 8.01 (d, J = 2.6 Hz, 1H), 7.85 (d, J = 8.4 Hz, 1H), 7.08 (dd, J = 8.6, 2.6 Hz, 1H), 6.92 (d, J = 8.6 Hz, 1H).

^{13}C NMR (75 MHz, DMSO- d_6) δ 163.88, 148.22, 147.47, 142.16, 131.16, 130.13, 126.51, 124.96, 124.35, 122.24, 121.98, 116.59.

Elemental Analysis: calc C 47.79%, H 2.47%, N 8.56%; found C 47.68%, H 2.48%, N 8.51%.

m.p.: 223°C.

3,4-Diethoxy-*N*-(4-(trifluoromethoxy)phenyl)benzamide (48)

Using Method A the title compound was isolated as a white solid. Yield 66%

^1H NMR (300 MHz, DMSO- d_6) δ 10.22 (s, 1H), 7.95–7.81 (m, 2H), 7.60 (dd, J = 8.4, 2.1 Hz, 1H), 7.53 (d, J = 2.1 Hz, 1H), 7.41–7.30 (m, 2H), 7.08 (d, J = 8.5 Hz, 1H), 4.11 (qd, J = 7.0, 1.7 Hz, 4H), 1.36 (td, J = 6.9, 1.1 Hz, 6H).

^{13}C NMR (75 MHz, DMSO) δ 165.01, 151.23, 147.59, 143.67, 143.64, 138.50, 126.46, 121.63, 121.35, 121.16, 112.52, 111.99, 63.96, 63.82, 14.67, 14.55.

Elemental Analysis: calc C 58.54%, H 4.91%, N 3.79%; found C 58.64%, H 5.16%, N 3.66%.

m.p.: 173°C.

3,4-Diethoxy-*N*-(4-bromophenyl)benzamide (49)

Using Method A the title compound was isolated as a white solid. Yield 57%

^1H NMR (300 MHz, DMSO- d_6) δ 10.16 (s, 1H), 7.79–7.71 (m, 2H), 7.59 (dd, J = 8.4, 2.1 Hz, 1H), 7.56–7.49 (m, 3H), 7.07 (d, J = 8.5 Hz, 1H), 4.11 (qd, J = 7.0, 1.3 Hz, 4H), 1.36 (td, J = 7.0, 1.1 Hz, 6H).

^{13}C NMR (75 MHz, DMSO) δ 164.96, 151.19, 147.57, 138.67, 131.31, 126.53, 122.22, 121.14, 115.03, 112.48, 111.99, 63.96, 63.82, 14.69, 14.57.

Elemental Analysis: calc C 56.06%, H 4.98%, N 3.85%; found C 56.19%, H 5.26%, N 3.77%.

m.p.: 198°C.

3,4-Diethoxy-*N*-(4-nitro-2-(trifluoromethyl)phenyl)benzamide (50)

Using Method A the title compound was isolated as a white solid. Yield 64%

^1H NMR (300 MHz, DMSO- d_6) δ 10.20 (s, 1H), 8.56 (dd, J = 8.7, 2.7 Hz, 1H), 8.52 (d, J = 2.7 Hz, 1H), 7.93 (d, J = 8.8 Hz, 1H), 7.60 (dd, J = 8.4, 2.1 Hz, 1H), 7.53 (d, J = 2.1 Hz, 1H), 7.12 (d, J = 8.5 Hz, 1H), 4.24–4.01 (m, 4H), 1.37 (t, J = 6.9 Hz, 6H).

^{13}C NMR (75 MHz, DMSO) δ 165.66, 151.64, 147.68, 145.02, 142.03, 131.43, 127.87, 125.97, 125.56, 125.10, 122.36, 121.35, 112.38, 112.13, 63.92, 63.88, 14.63, 14.53.

Elemental Analysis: calc C 54.27%, H 4.30%, N 7.03%; found C 54.18%, H 4.22%, N 6.96%.

m.p.: 158°C.

3,4-Diethoxy-*N*-(2-chloro-4-nitrophenyl)benzamide (51)

Using Method A the title compound was isolated as an off-white solid. Yield 65%

^1H NMR (300 MHz, DMSO- d_6) δ 10.08 (s, 1H), 8.41 (d, J = 2.6 Hz, 1H), 8.26 (dd, J = 9.0, 2.6 Hz, 1H), 8.04 (d,

J = 9.0 Hz, 1H), 7.64 (dd, J = 8.4, 2.1 Hz, 1H), 7.56 (d, J = 2.1 Hz, 1H), 7.11 (d, J = 8.5 Hz, 1H), 4.12 (qd, J = 6.9, 5.4 Hz, 4H), 1.37 (t, J = 6.9 Hz, 6H).

^{13}C NMR (75 MHz, DMSO) δ 164.82, 151.71, 147.70, 144.28, 141.56, 127.89, 126.54, 125.22, 124.84, 122.86, 121.51, 112.43, 112.10, 63.94, 63.89, 14.65, 14.54.

Elemental Analysis: calc C 55.98%, H 4.70%, N 7.68%; found C 56.00%, H 4.69%, N 7.54%.

m.p.: 168°C.

N-Phenylbenzo[d][1,3]dioxole-5-carboxamide (52)⁷⁵

Using Method A the title compound was isolated as a white solid. Yield 64%

^1H NMR (300 MHz, DMSO- d_6) δ 10.06 (s, 1H), 7.83–7.72 (m, 2H), 7.59 (dd, J = 8.2, 1.8 Hz, 1H), 7.53 (d, J = 1.8 Hz, 1H), 7.34 (dd, J = 8.5, 7.3 Hz, 2H), 7.15–7.02 (m, 2H), 6.14 (s, 2H).

^{13}C NMR (75 MHz, DMSO) δ 164.44, 149.98, 147.32, 139.18, 128.70, 128.50, 123.46, 122.79, 120.31, 107.87, 107.68, 101.76.

Elemental Analysis: calc C 69.70%, H 4.60%, N 5.81%; found C 69.54%, H 4.65%, N 5.77%.

m.p.: 142°C (lit: 138–139°C).⁷⁶

N-(4-Cyanophenyl)benzo[d][1,3]dioxole-5-carboxamide (53)

Using Method A the title compound was isolated as a white solid. Yield 48%

^1H NMR (300 MHz, DMSO- d_6) δ 10.44 (s, 1H), 7.97 (d, J = 8.8 Hz, 2H), 7.80 (d, J = 8.7 Hz, 2H), 7.59 (dd, J = 8.1, 1.8 Hz, 1H), 7.52 (d, J = 1.8 Hz, 1H), 7.08 (d, J = 8.1 Hz, 1H), 6.15 (s, 2H).

^{13}C NMR (75 MHz, DMSO) δ 165.02, 150.42, 147.41, 143.54, 133.02, 128.01, 123.21, 120.06, 119.06, 107.96, 107.80, 105.10, 101.91.

Elemental Analysis: calc C 67.67%, H 3.79%, N 10.52%; found C 67.62%, H 3.87%, N 10.67%.

m.p.: 193°C.

N-(2-Methyl-4-nitrophenyl)benzo[d][1,3]dioxole-5-carboxamide (54)

Using Method A the title compound was isolated as an off-white solid. Yield 18%

^1H NMR (300 MHz, DMSO- d_6) δ 9.92 (s, 1H), 8.19 (d, J = 2.7 Hz, 1H), 8.10 (dd, J = 8.8, 2.8 Hz, 1H), 7.78 (d, J = 8.8 Hz, 1H), 7.61 (dd, J = 8.1, 1.8 Hz, 1H), 7.53 (d, J = 1.8 Hz, 1H), 7.09 (d, J = 8.2 Hz, 1H), 6.15 (s, 2H), 2.39 (s, 3H).

^{13}C NMR (75 MHz, DMSO) δ 164.56, 150.40, 147.43, 144.05, 143.11, 133.85, 127.74, 125.59, 125.33, 123.20, 121.47, 108.02, 107.83, 101.88, 17.89.

Elemental Analysis: calc C 60.00%, H 4.03%, N 9.33%; found C 59.85%, H 3.76%, N 9.22%.

m.p.: 230°C.

***N*-(2,4-Dinitrophenyl)benzo[d][1,3]dioxole-5-carboxamide (55)**

Using Method A the title compound was isolated as a yellow solid. Yield 10%

^1H NMR (300 MHz, DMSO- d_6) δ 11.04 (s, 1H), 8.74 (d, J = 2.6 Hz, 1H), 8.57 (dd, J = 9.0, 2.7 Hz, 1H), 8.12 (d, J = 9.1 Hz, 1H), 7.59 (dd, J = 8.2, 1.8 Hz, 1H), 7.47 (d, J = 1.8 Hz, 1H), 7.12 (d, J = 8.2 Hz, 1H), 6.17 (s, 2H).

^{13}C NMR (75 MHz, DMSO) δ 164.49, 151.20, 147.73, 142.52, 140.60, 137.48, 128.56, 126.54, 125.30, 123.62, 121.14, 108.26, 107.68, 102.18.

Elemental Analysis: calc C 50.77%, H 2.74%, N 12.69%; found C 50.84%, H 2.46%, N 12.68%.

m.p.: 200°C.

***N*-(2-Methoxy-4-nitrophenyl)benzo[d][1,3]dioxole-5-carboxamide (56)**

Using Method A the title compound was isolated as a yellow solid. Yield 33%

^1H NMR (300 MHz, DMSO- d_6) δ 9.53 (s, 1H), 8.24 (d, J = 8.9 Hz, 1H), 7.94 (dd, J = 8.9, 2.5 Hz, 1H), 7.87 (d, J = 2.5 Hz, 1H), 7.59 (dd, J = 8.2, 1.8 Hz, 1H), 7.52 (d, J = 1.8 Hz, 1H), 7.08 (d, J = 8.1 Hz, 1H), 6.15 (s, 2H), 4.01 (s, 3H).

^{13}C NMR (75 MHz, DMSO) δ 164.40, 150.58, 150.01, 147.55, 143.50, 133.87, 127.62, 123.09, 121.56, 116.53, 108.09, 107.67, 106.07, 101.96, 56.57.

Elemental Analysis: calc C 56.97%, H 3.82%, N 8.86%; found C 56.67%, H 3.71%, N 8.64%.

m.p.: 237°C.

***N*-(2-Chloro-4-nitrophenyl)benzo[d][1,3]dioxole-5-carboxamide (57)**

Using Method A the title compound was isolated as a pale-yellow solid. Yield 53%

^1H NMR (300 MHz, DMSO- d_6) δ 10.11 (s, 1H), 8.41 (d, J = 2.6 Hz, 1H), 8.26 (dd, J = 9.0, 2.6 Hz, 1H), 8.04 (d, J = 9.0 Hz, 1H), 7.63 (dd, J = 8.2, 1.8 Hz, 1H), 7.53 (d, J = 1.8 Hz, 1H), 7.10 (d, J = 8.2 Hz, 1H), 6.16 (s, 2H).

^{13}C NMR (75 MHz, DMSO) δ 164.49, 150.72, 147.55, 144.38, 141.41, 127.99, 127.12, 126.65, 124.85, 123.39, 122.86, 108.12, 107.78, 101.99.

Elemental Analysis: calc C 52.44%, H 2.83%, N 8.74%; found C 52.20%, H 2.85%, N 8.65%.

m.p.: 227°C.

***N*-(2-Chloro-4-nitrophenyl)-2-naphthamide (58)**

Using Method A the title compound was isolated as a pale-yellow solid. Yield 52%

^1H NMR (300 MHz, Chloroform- d) δ 8.86 (d, J = 9.2 Hz, 1H), 8.79 (s, 1H), 8.43–8.37 (m, 1H), 8.31 (d, J = 2.5 Hz, 1H), 8.19 (ddd, J = 9.3, 2.6, 0.5 Hz, 1H), 7.99–7.82 (m, 4H), 7.63–7.49 (m, 2H).

^{13}C NMR (75 MHz, CDCl_3) δ 165.44, 143.07, 140.51, 135.31, 132.57, 130.76, 129.30, 129.24, 128.64, 128.32, 127.92, 127.37, 124.76, 123.83, 123.06, 122.59, 120.24.

Elemental Analysis: calc C 62.49%, H 3.39%, N 8.57%; found C 62.72%, H 3.09%, N 8.71%.

m.p.: 225°C.

***1*-Hydroxy-*N*-(2-chloro-4-nitrophenyl)-2-naphthamide (59)⁵⁶**

Using Method A the title compound was isolated as a yellow solid. Yield 10%

^1H NMR (300 MHz, DMSO- d_6) δ 12.21 (s, 1H), 11.64 (s, 1H), 8.91 (d, J = 9.2 Hz, 1H), 8.75 (s, 1H), 8.46 (d, J = 2.6 Hz, 1H), 8.34 (dd, J = 9.2, 2.7 Hz, 1H), 8.03 (d, J = 8.2 Hz, 1H), 7.81 (dd, J = 8.5, 1.2 Hz, 1H), 7.56 (ddd, J = 8.2, 6.9, 1.3 Hz, 1H), 7.46–7.35 (m, 2H).

^{13}C NMR (75 MHz, DMSO) δ 163.56, 152.25, 142.46, 141.41, 136.28, 133.41, 129.23, 128.86, 127.21, 125.73, 124.81, 124.14, 123.93, 122.35, 120.73, 120.10, 110.95.

Elemental Analysis: calc C 59.58%, H 3.24%, N 8.17%; found C 58.97%, H 3.20%, N 7.67%.

m.p.: 230°C (lit: 233°C).⁷⁷

***3*-Hydroxy-*N*-(4-(trifluoromethyl)phenyl)-2-naphthamide (60)⁷⁸**

Using Method A the title compound was isolated as an off-white solid. Yield 26%

^1H NMR (300 MHz, DMSO- d_6) δ 11.20 (s, 1H), 10.89 (s, 1H), 8.48 (s, 1H), 8.01 (d, J = 8.4 Hz, 2H), 7.94 (d, J = 8.2 Hz, 1H), 7.76 (dd, J = 8.7, 4.6 Hz, 3H), 7.51 (ddd, J = 8.3, 6.9, 1.3 Hz, 1H), 7.42–7.31 (m, 2H).

^{13}C NMR (75 MHz, DMSO) δ 165.84, 153.25, 142.22, 135.71, 130.63, 128.66, 128.11, 126.83, 126.12, 126.06, 126.01, 125.75, 123.72, 122.51, 120.11, 110.47.

Elemental Analysis: calc C 65.26%, H 3.65%, N 4.23%; found C 64.43%, H 3.69%, N 3.93%.

m.p.: 280°C (lit: 276–278°C).

3-Hydroxy-*N*-(4-bromophenyl)-2-naphthamide (61)⁷⁸

Using Method A the title compound was isolated as an off-white solid. Yield 29%

¹H NMR (300 MHz, DMSO-*d*₆) δ 11.22 (s, 1H), 10.68 (s, 1H), 8.47 (s, 1H), 7.93 (d, *J* = 8.2 Hz, 1H), 7.83–7.71 (m, 4H), 7.63–7.45 (m, 4H), 7.42–7.30 (m, 2H).

¹³C NMR (75 MHz, DMSO) δ 165.64, 153.46, 137.91, 135.70, 131.57, 130.44, 128.65, 128.09, 126.82, 125.75, 123.72, 122.26, 122.12, 115.62, 110.48.

Elemental Analysis: calc C 59.67%, H 3.53%, N 4.09%; found C 59.37%, H 3.56%, N 3.98%.

m.p.: 250°C (lit: 248–250°C).

3-Hydroxy-*N*-(2-chloro-4-nitrophenyl)-2-naphthamide (62)⁷⁹

Using Method A the title compound was isolated as a yellow solid. Yield 13%

¹H NMR (300 MHz, DMSO-*d*₆) δ 13.06 (s, 1H), 11.92–11.53 (m, 1H), 8.50–8.42 (m, 2H), 8.38 (d, *J* = 8.2 Hz, 1H), 8.31 (dd, *J* = 9.1, 2.6 Hz, 1H), 8.07 (d, *J* = 8.8 Hz, 1H), 7.92 (d, *J* = 8.1 Hz, 1H), 7.72–7.54 (m, 2H), 7.48 (d, *J* = 8.8 Hz, 1H).

¹³C NMR (75 MHz, DMSO) δ 167.42, 143.79, 141.30, 136.40, 129.02, 127.62, 126.20, 125.80, 124.85, 124.75, 124.52, 123.42, 123.35, 109.88.

Elemental Analysis: calc C 59.58%, H 3.24%, N 8.17%; found C 59.36%, H 3.25%, N 8.05%.

m.p.: 238°C.

***N*-(2-Chloro-4-nitrophenyl)-3-methyl-1*H*-indene-2-carboxamide (63)**

Using Method A the title compound was isolated as a pale-yellow solid. Yield 47%

¹H NMR (300 MHz, Chloroform-*d*) δ 8.90 (d, *J* = 9.2 Hz, 1H), 8.46–8.29 (m, 2H), 8.21 (dd, *J* = 9.3, 2.6 Hz, 1H), 7.57 (ddt, *J* = 5.3, 3.8, 1.9 Hz, 2H), 7.50–7.39 (m, 2H), 3.80 (q, *J* = 2.4 Hz, 2H), 2.69 (t, *J* = 2.4 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 163.71, 152.04, 145.10, 142.64, 141.96, 140.74, 130.78, 128.36, 127.23, 124.68, 124.04, 123.76, 122.02, 121.40, 119.77, 38.03, 12.76.

Elemental Analysis: calc C 62.11%, H 3.99%, N 8.54%; found C 62.23%, H 3.59%, N 8.29%.

m.p.: 216°C.

***N*-(2,4-Dinitrophenyl)benzofuran-2-carboxamide (64)**

Using Method A the title compound was isolated as a yellow solid. Yield 13%

¹H NMR (300 MHz, DMSO-*d*₆) δ 11.58 (s, 1H), 8.82 (d, *J* = 2.6 Hz, 1H), 8.63 (dd, *J* = 9.1, 2.7 Hz, 1H), 8.37 (d, *J* = 9.1 Hz, 1H), 7.94–7.84 (m, 2H), 7.78 (dd, *J* = 8.4, 1.0 Hz, 1H), 7.58 (ddd, *J* = 8.4, 7.2, 1.3 Hz, 1H), 7.42 (td, *J* = 7.6, 1.0 Hz, 1H).

¹³C NMR not performed due to poor solubility.

Elemental Analysis: calc C 55.05%, H 2.77%, N 12.84%; found C 54.77%, H 2.67%, N 12.85%.

m.p.: 237°C.

***N*-(2-Chloro-4-nitrophenyl)benzofuran-2-carboxamide (65)**

Using Method A the title compound was isolated as a yellow solid. Yield 54%

¹H NMR (300 MHz, Chloroform-*d*) δ 9.13 (s, 1H), 8.79 (d, *J* = 9.2 Hz, 1H), 8.29 (d, *J* = 2.6 Hz, 1H), 8.15 (ddd, *J* = 9.2, 2.6, 0.6 Hz, 1H), 7.71–7.65 (m, 1H), 7.62 (d, *J* = 1.0 Hz, 1H), 7.54 (dq, *J* = 8.5, 1.0 Hz, 1H), 7.44 (ddd, *J* = 8.4, 7.2, 1.3 Hz, 1H), 7.29 (ddd, *J* = 8.0, 7.2, 1.1 Hz, 1H).

¹³C NMR (75 MHz, CDCl₃) δ 156.56, 155.05, 147.26, 143.26, 139.86, 128.12, 127.41, 124.84, 124.33, 123.71, 123.13, 122.73, 120.23, 113.21, 112.13.

Elemental Analysis: calc C 56.89%, H 2.86%, N 8.85%; found C 56.99%, H 2.78%, N 8.64%.

m.p.: 203°C.

***N*-(2-Chloro-4-nitrophenyl)benzo[*b*]thiophene-2-carboxamide (66)⁸⁰**

Using Method A the title compound was isolated as an off-white solid. Yield 21%

¹H NMR (300 MHz, DMSO-*d*₆) δ 10.59 (s, 1H), 8.49–8.39 (m, 2H), 8.28 (dd, *J* = 8.9, 2.6 Hz, 1H), 8.13–7.99 (m, 3H), 7.58–7.45 (m, 2H).

¹³C NMR (75 MHz, DMSO) δ 160.53, 144.74, 140.72, 140.63, 138.90, 138.05, 128.21, 127.35, 127.03, 126.94, 125.69, 125.20, 124.97, 122.93, 122.90.

Elemental Analysis: calc C 54.14%, H 2.73%, N 8.42%, S 9.63%; found C 54.34%, H 2.46%, N 8.37%, S 9.49%.

m.p.: 228°C.

Chemistry**General Synthesis Procedures****Method A**

The aniline derivative (6 mmol, 1 eq) and the aromatic acid derivative (6 mmol, 1 eq) were suspended in toluene (10 mL). After heating to reflux and dissolving of the precipitate, phosphor trichloride (2.4 mmol, 0.4 eq) was

added in a dropwise manner. The reaction was monitored using TLC (ethyl acetate:n-hexane 1:4). After reaction completion, the hot mixture was filtered and the organic solution was stored at 5 °C. The formed precipitate was filtered off, washed with ice-cold toluene and recrystallized from toluene.¹⁷

Method B

1,1'-Carbonyldiimidazole (CDI) (9 mmol, 1.5 eq) was dissolved in THF (10 mL). To this solution the respective aromatic acid (9 mmol, 1.5 eq) was added dissolved in THF (10 mL). After 10 min stirring at room temperature, a solution of the aniline derivative (6 mmol, 1 eq) in THF (10 mL) was added. The reaction mixture was stirred for 10 min at room temperature and then refluxed overnight. After cooling to room temperature, the solvent was evaporated under reduced pressure and the residue dissolved in ethylacetate. The organic phase was then washed with saturated aqueous sodium hydrogen carbonate solution and brine. After drying over magnesium sulfate, the solvent was evaporated under reduced pressure and the residue was recrystallized from toluene.¹⁸

Method C

The respective pyridine derivative (6 mmol, 1 eq) and the benzoyl chloride derivative (6 mmol, 1 eq) were sealed in a glass vial. The reaction mixture was stirred for 4 min at 160°C under microwave irradiation. The resulting mixture was recrystallized from ethanol.¹⁹

Method D

The respective nitro derivative (**6**, **9**, **26**) (6 mmol, 1 eq) was dissolved in dried ethanol (20 mL). After adding Pd/C (0.6 mmol, 0.1 eq) and hydrazine monohydrate (60 mmol, 10 eq), the mixture was refluxed for 2 h and afterwards stirred for another 45 min at room temperature. Filtration via a short Celite column and evaporation of the solvent afforded the desired compounds (**5**, **8**, **25**).²⁰

Enzymology

Monoamine oxidase A/B inhibition assays were performed as described previously using the discontinuous fluorimetric method with kynuramine as MAO substrate.²¹ For the screening (test concentration: 10^{-6} M) and IC_{50} determinations (ten suitable concentrations ranging from 10^{-11} to 10^{-5} M), the test compounds were pre-mixed with the substrate ($2 \times K_M$ final concentration; K_M of 20 μ M and

30 μ M for MAO A and B, respectively). MAO A (1.25 ng mL^{-1} , 900 units mL^{-1}) or MAO B (1.67 ng mL^{-1} , 375 units mL^{-1}), respectively, was added to start the reactions. The reaction mixtures were incubated for 20 min incubation time at 37°C. All enzyme assays were conducted in pre-warmed potassium phosphate buffer (50 mM, pH = 7.4) and a final assay volume of 100 μ L. Reactions were stopped by adding 35 μ L sodium hydroxide solution (2 N) to the assay mixture. The enzyme activity was determined by measuring the 4-hydroxyquinoline formed during incubation time (expressed as RFU; $\lambda_{Em} = 405 \pm 20 \text{ nm}$ and $\lambda_{Ex} = 320 \pm 20 \text{ nm}$). Compounds showing a MAO B inhibition >90% at 10^{-6} M were evaluated for their IC_{50} -values. The IC_{50} curves were fitted (via nonlinear regression) either to the respective bottom plateau (graphically defined by RFUs for the at least two highest concentrations used, see [Figure S12](#) in [supplemental material](#)) or set to zero in case when the bottom plateau was not reached with highest concentration tested (leading to “ IC_{50} estimates”). The highest concentrations to be tested had to be identified considering each compound's solubility and potential interfering fluorescence properties under assay conditions.

Reversibility of inhibition was confirmed via preincubation of inhibitor ($10 \times IC_{50}$ in preincubation setting) with MAO B ($10 \text{ ng } \mu\text{L}^{-1}$ in preincubation setting) for 0, 30, 60 and 90 min (37 °C), followed by $50\times$ dilution in buffer and assayed with an excess of substrate ($10 \times K_M$ final concentration) as described above.²¹ Data were calculated as percentage of vehicle control (DMSO; set to 100% enzyme activity remained) for each time point.

Mode of MAO B inhibition was determined by substrate-dependent (seven concentrations, 5 to 400 μ M kynuramine) Michaelis-Menten kinetic analysis without inhibitor and in the presence of five different inhibitor concentrations ($0.25\times$, $0.5\times$, $0.75\times$, $1\times$ and $2 \times IC_{50}$) and assayed as described above. Data were fitted using nonlinear “Competitive inhibition” fit and transformed into double reciprocal (Lineweaver–Burk) plots. The slopes of respective Lineweaver–Burk linearization were plotted against inhibitor concentration for additional K_i determination.²²

All data were analyzed with GraphPad Prism 6.

Molecular Modelling

The crystal structure 2Z5X²³ was used as the representative complex structure of MAO A and the crystal structure 6FVZ⁹ for MAO B. Both crystal structures were chosen due to the lipophilicity of the crystalized

ligands (HRM for MAO A and E8Z for MAO B) and the presence of a peptide bond of the MAO B ligand. The enzyme structures were prepared using the Quick Prep tool in MOE 2018.0101 (Chemical Computing Group Inc., Canada) including protonation and energy minimization. Poses were generated using the Dock tool in MOE 2018.0101. A general docking was conducted. In case of MAO A, solvent was ignored. The placement method triangle matcher with the scoring function London dG was chosen. The refinement method rigid receptor with the scoring function Alpha HB was chosen.

Pharmacophore Modeling

The pharmacophore hypotheses of the MAO A and B inhibitors were constructed using the HipHop module of Discovery studio software 2.5.5. A subset of 25 representative compounds was selected and classified as active, moderately active, and inactive (Table S1 in the [supplemental material](#)). In total 16 HipHop runs were separately conducted for MAO A and MAO B ligands that ultimately generate 160 models (10 from each run) for each target by varying the types and ranges of pharmacophoric features and the number of features (Table S2 in the [supplemental material](#)). The generated models were allowed to compete in Receiver Operating Characteristic (ROC) curve analysis to assess their abilities to selectively capture diverse MAO A or MAO B inhibitors from a large list of decoys. The decoy list was prepared as described by Verdonk et al.²⁴ The testing sets include structurally diverse active inhibitors of 14 MAO A and 18 MAO B retrieved from literature.^{14,25,26} 36 decoys were selected for each active compound in the testing set retrieved from ZINC-database. The ROC testing set was screened by each pharmacophore for ROC analysis employing the “Best rigid search” option, while the conformational spaces of the compounds were generated employing the “CAESAR conformation generation option”. Compounds missing one or more features were discarded from hit lists. The validity of a particular pharmacophore is assessed by the area under the curve (AUC) of the corresponding ROC curve, as well as accuracy, specificity, true positive rate, and false negative rate of the pharmacophore. Table S3 and Figure S10 in the [supplemental material](#) show the ROC performance of the best pharmacophore models generated for MAO A and MAO B. The active/

inactive classification accuracy of these models is good with ROC-AUC values of 0.779 and 0.750 for MAO A and MAO B models, respectively. Further theoretical and experimental methodology of ROC analyses are described in [supplemental material](#).

Results and Discussion

Chemistry

In 2018 the European Medicines Agency (EMA) recommended 22 biological active small molecules for market authorization.²⁷ All newly approved small molecules exhibit rather complex structures with the need for multistep synthesis, often including stereoselective synthesis steps. Using a one-step synthesis followed by easy purification procedures, we generated a series of promising MAO A/B inhibitors. These small entities might be of interest for the development of selective or multitargeting MAO A/B inhibitors. The substituted aromatic amides were synthesized in low to good yields via activation of the carboxylic acid using either PCl_3 or CDI. CDI was used as a coupling agent for compound 47 to prevent condensation to a substituted benzoxazole, which was the major product using PCl_3 method. For compounds 2 and 19, solvent free acylation using benzoyl chloride derivatives was performed under microwave irradiation (Figure 1). The aromatic amines (5, 8, 25) were obtained in excellent yields from reduction of the respective nitro derivatives (6, 9, 26). Due to their simple structures, many of our compounds have been described previously, but to our knowledge none of them were determined as potent inhibitors for MAO A or B so far.

Biological Activity

MAO A and B Inhibition

An enzyme inhibition screening at a concentration of 1 μM revealed that the synthesized anilides showed the presumed MAO inhibition potency. The majority of derivatives (32 compounds) were found to be MAO B-preferring inhibitors, while 24 compounds were not selective for either of the isoforms and only ten compounds exhibited MAO A preference (Table 1 and see Figure 2 for all compound structures). The most potent MAO inhibitors were chosen for further IC_{50} evaluation. Six compounds (31, 33, 34, 39, 55, and 65) showed >90% inhibition of MAO B. The most potent MAO A-preferring inhibitor (with MAO B inhibition <50%), compound 7 was also considered for additional investigations.

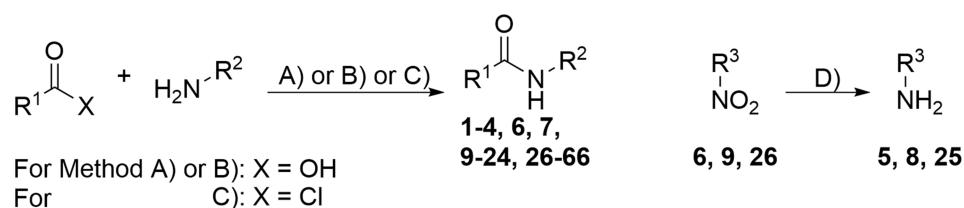


Figure 1 Synthetic route to the amide analogs **1–4**, **6**, **7**, **9–24**, **26–66**. Reagents and conditions: (A) PCl_3 , toluene, reflux. (B) CDI, THF, reflux. (C) mw, 160 °C. For R^1 , R^2 , and R^3 see Table 1.

Compounds **31**, **33**, **34**, **55**, and **65** showed the expected preference for MAO B with IC_{50} values in nanomolar concentration ranges (IC_{50} values between 55 nM and 139 nM; Table 2).

A preference for MAO A could be confirmed for compound **7** (**ST-2023**) (MAO A, IC_{50} = 126 nM) with an IC_{50} > 1000 nM for MAO B; selectivity index, SI = 0.1). The potent MAO

Table 1 MAO A and B Inhibition Percentages Measured at One-Point Screening of Anilides (Screening Concentration 1 μM)

n	% Inhibition at 1 μM		n	% Inhibition at 1 μM	
	MAO A	MAO B		MAO A	MAO B
1	12±8	5±3	15	9±3	7±5
2	11±5	2±4	16	20±12	14±10
3	29±3	27±3	17	10±5	69±28
4	24±20	29±3	18	18±5	24±9
5	1±2	−2±3	19	13±2	11±7
6	16±7	7±5	20	72±2	61±4
7	82±2	46±4	21	13±1	10±7
8	10±4	1±3	22	2±1	14±3
9	14±7	14±5	23	23±3	25±4
10	28±8	25±3	24	27±4	48±5
11	48±3	85±1	25	25±2	11±4
12	28±8	5±3	26	11±2	2±3
13	50±4	32±10	27	20±6	44±23
14	49±5	18±11	28	8±4	5±2
29	15±1	87±5	41	8±4	85±3
30	7±3	38±7	42	5±3	30±5
31	82±1	104±2	43	4±8	0±5
32	45±5	66±5	44	34±3	6±2
33	74±2	96±2	45	13±2	36±4
34	71±5	96±1	46	13±3	68±2
35	8±1	5±10	47	35±3	36±4

(Continued)

Table 1 (Continued).

n	% Inhibition at 1 μ M		n	% Inhibition at 1 μ M	
	MAO A	MAO B		MAO A	MAO B
36	6 \pm 4	84 \pm 12	48	5 \pm 5	1 \pm 2
37	13 \pm 4	30 \pm 10	49	3 \pm 3	13 \pm 6
38	9 \pm 2	-2 \pm 2	50	6 \pm 5	62 \pm 2
39	20 \pm 8	91\pm1	51	3 \pm 4	34 \pm 2
40	8 \pm 4	6 \pm 2	52	19 \pm 4	80 \pm 4
53	38 \pm 6	87 \pm 1	60	-12 \pm 18	67 \pm 2
54	7 \pm 9	24 \pm 26	61	-3 \pm 5	72 \pm 2
55	44 \pm 4	91\pm2	62	-2 \pm 20	48 \pm 5
56	1 \pm 2	47 \pm 5	63	-3 \pm 19	44 \pm 23
57	9 \pm 9	62 \pm 7	64	84 \pm 2	80 \pm 4
58	11 \pm 7	58 \pm 1	65	52 \pm 10	92\pm4
59	-4 \pm 10	28 \pm 2	66	11 \pm 8	40 \pm 31

Notes: ^aData represent mean values \pm standard deviation of at least two independent experiments each performed in duplicates (global fit). Percentage values were calculated relative to control (set to 100% remained activity). Bold values represent inhibition rates >90 % MAO B and highest MAO A inhibitions.

B inhibition capacity of **39**, presumed depending on screening results, could not be verified in IC₅₀ determinations (MAO B, IC₅₀ >1000 nM), where assay limitations did not allow the determination of the bottom plateau due to low solubility and fluorescence crosstalk. Assuming that the real but nondetectable bottom plateau might be \gg 0, the previous determined percent inhibition value in one-point screening would have been overestimated (as calculated with bottom = 0). The most potent compound **55** (ST-2043) showed high inhibition of MAO B (IC₅₀ = 56 nM), which is in a similar range as described for the marketed drug safinamide (IC₅₀ = 53 nM; Table 1). Compared to safinamide (MAO A/B SI >940), **55** demonstrated only an about five-fold lower inhibition of isoform A, thus being a rather balanced MAO inhibitor. The highest MAO B preference within this series was shown for compound **31** with an SI >10 (MAO B, IC₅₀ = 92 nM).

The docking experiments revealed a good match of all compounds in the lipophilic binding pockets of the MAO A and B with exception of compound **39**, where no suitable docking pose into the MAO A binding pocket could be identified (see Figures S1-S9 in the supplemental material). According to the result of the docking experiments, surface complementarities influence the binding properties of the anilides nearly exclusively, whereas direct interactions are not

involved in the binding of the presented ligands. Compounds **31**, **33**, and **34** showed close structural similarities and comparable inhibition properties. All of these ligands are substituted with a hydroxyl group at the 2-position of the benzylic site and a chloro substituent at the 5-position. The aniline moiety in all cases is para-substituted with a highly lipophilic residue, ie a bromo-substituent (**34**), a trifluoromethoxy group (**33**), and a trifluoromethyl group (**31**) in the para-position. The interaction of these hydrophobic residues with the lipophilic cavity of the MAO B binding pocket, including the nonpolar amino acids Pro102 and Leu164, might be responsible for their potent MAO B inhibition properties. The carbonyl function of the linking amide and the hydroxyl groups in 2-position are directed towards Cys172 and interact via polar aprotic forces (see Figures S5-S7 in the supplemental material). A similar polar system is present in pyrrolo-pyridinyl derivatives synthesized by Tzvetkov et al indicating that a central hydrogen bond donor/acceptor complex is favorable for MAO B binding.²⁸ The docking pose of the MAO A preferring inhibitor **7** (ST-2023) is inverted by 180° in the binding pocket of the MAO B model, where the polar nitro-group is interacted with Cys172 (Figure 3). This would enlarge the distance to the FAD and could be responsible for the low MAO B activity compared with MAO A. This reversed pose was also observed in

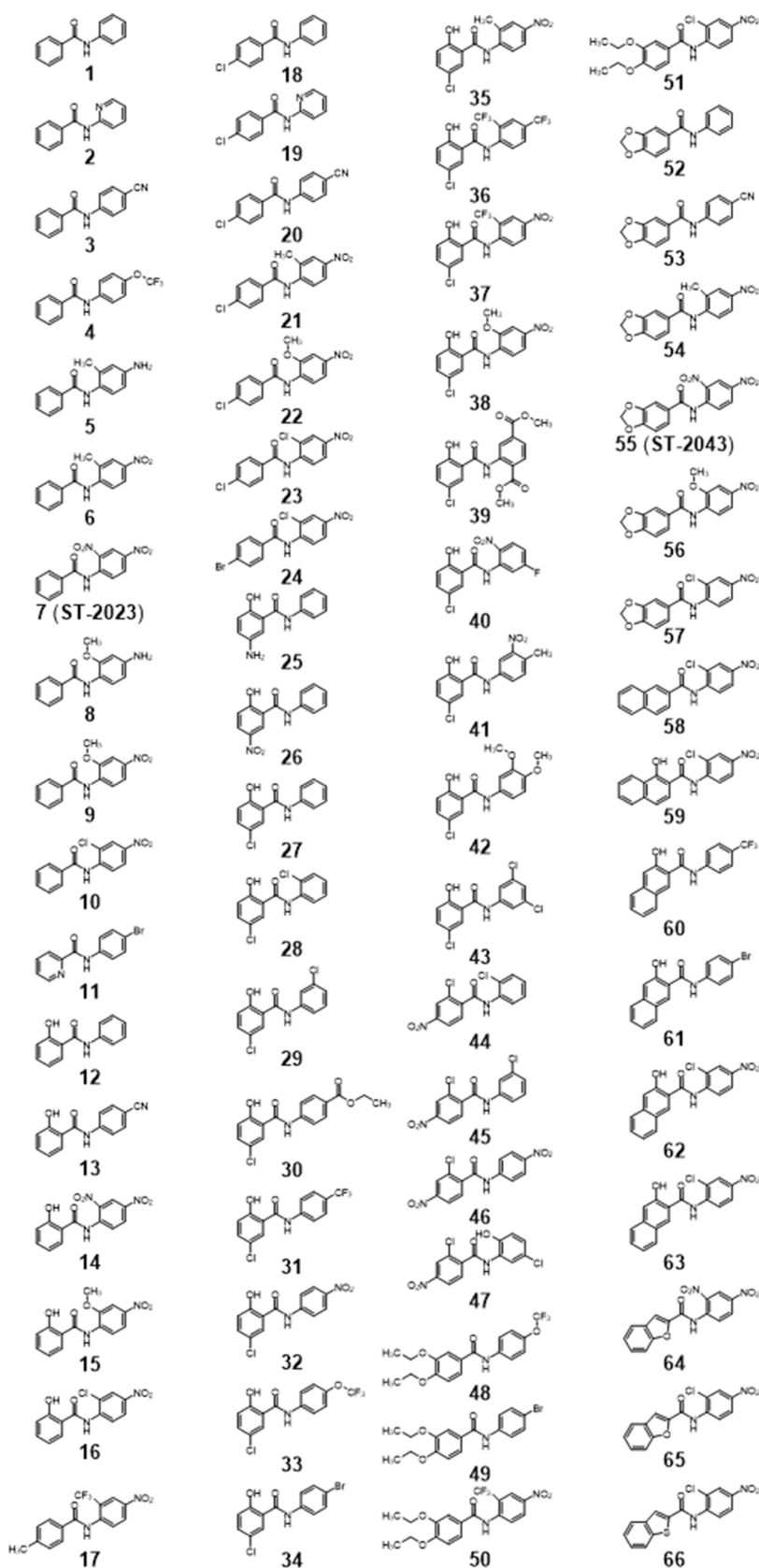


Figure 2 Compounds with anilide motifs taken to MAO A and B screening.

Table 2 MAO A and B Inhibition Activities of the Most Potent Compounds Expressed as IC₅₀ Values Within the 95%CI

No.	IC ₅₀ [nM] [95%CI] (N) ^a		MAO SI ^c
	hMAO B	hMAO A	
7 (ST-2023)	1052 [759; 1459] (3)	126 [101; 158] (4)	0.1
31	92.3 [70.3; 121] (3)	>1000 ^b	>10.8
33	128 [54.0; 305] (3)	>1000 ^b	>7.8
34	139 [64.7; 300] (3)	>1000 ^b	>7.2
39	>1000 ^b	>5000 ^b	
55 (ST-2043)	55.5 [31.9; 96.7] (5)	289 [193; 433] (5)	5.2
65	123 [87.6; 172] (5)	293 [203; 424] (3)	2.4
Safinamide ^d	53 [20; 141] (4)	>50000 (4)	>940

Notes: ^aData represent mean values within the 95%CI of multiple independent experiments (n) each performed in duplicates. ^bCompounds did not reach bottom plateau at highest tested concentrations; Data are estimates extrapolated with bottom was set to zero. ^cSelectivity index (SI) = IC₅₀ MAO A/IC₅₀ MAO B. ^dValues taken from Affini et al.²¹

compounds **39** and **65**. In case of inhibitor **39**, the salicylic moiety is shifted towards the lipophilic cavity at the entrance of the binding pocket, which might be responsible for its rather poor inhibition properties (see [Figures S8](#) in the [supplemental material](#)). In contrast, inhibitor **65** maintained the lipophilic interaction with its benzofuran residue resulting in an orientation of the polar nitro group towards the FAD (see [Figures S9](#) in the [supplemental material](#)). Carboxamides synthesized by Tzvetkov et al showed the same orientation resulting from

a polar interaction with a bridging water molecule and a tyrosine close to the FAD.²⁹ The most potent inhibitor **55 (ST-2043)** in this series is oriented with its piperonylic acid moiety towards the FAD ([Figure 3](#)). Using the same moiety, Vishnu et al designed MTLs where the piperonylic acid aligned within the hydrophobic region opposite to the FAD, and the high inhibition capacities resulted from irreversible binding via a propargyl amine residue.³⁰

Due to their small size and molecular weight, the presented structures are useful starting skeletons to design MTLs with MAO B activity. The docking experiments suggest using the aniline residue of **55 (ST-2043)** as an attachment point to implement pharmacophores of additional targets. Zhang et al showed that larger substituents at this position are tolerated, where a bulky fluorobenzyl group is located further outside of the enzymes binding pocket than the here presented aniline groups.³¹ The hypothesis that substitution at this position might be suitable for designing MTLs is coherent with the linear structures synthesized by Pisani et al that combine MAO B inhibition with nitric oxide releasing precursors and acetylcholine esterase (AChE) inhibition moieties.³²

In the MAO A binding pocket, the polar salicylic group of compounds **31**, **33**, **34**, and **39** did not match the surrounding hydrophobic cavity, which might explain the loss of inhibition properties for MAO A (see [Figures S1-S3](#) in the [supplemental material](#)). The three compounds, missing the salicylic moiety (**7**, **55**, **65**), are aligned well into the MAO

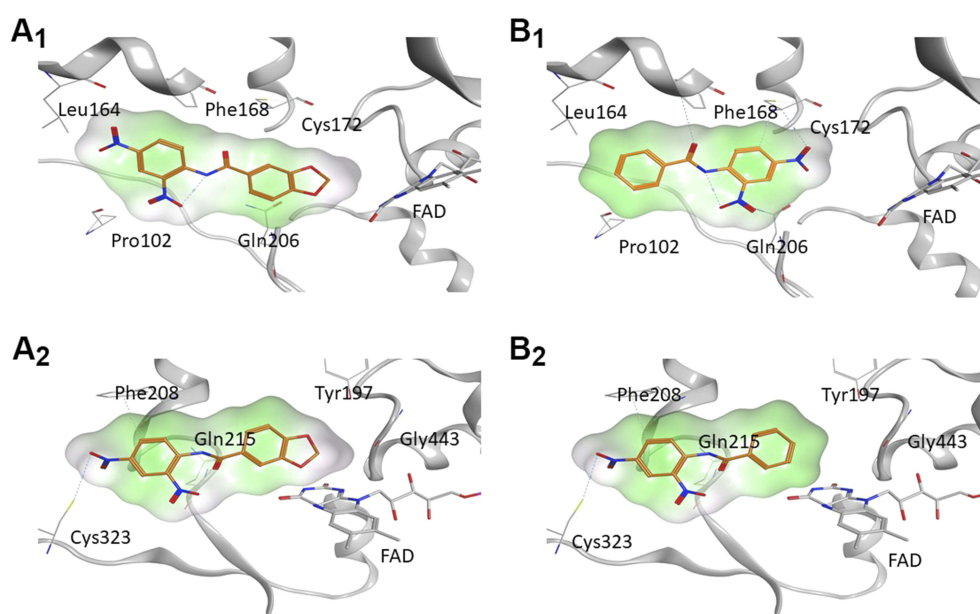


Figure 3 Visualization of **55 (ST-2043, A₁, A₂)** and **7 (ST-2023, B₁, B₂)** in the binding pockets of MAO A and B. Compound **55 (ST-2043)** binding to the crystal structure of **A₁** hMAO B (PDB: 6FVZ) and **A₂** hMAO A (PDB: 2Z5X). Compound **7 (ST-2023)** binding to the crystal structure of **B₁** hMAO B (PDB: 6FVZ) and **B₂** hMAO A (PDB: 2Z5X). Surface coloring: white: neutral, green: lipophilic; magenta: hydrophilic.

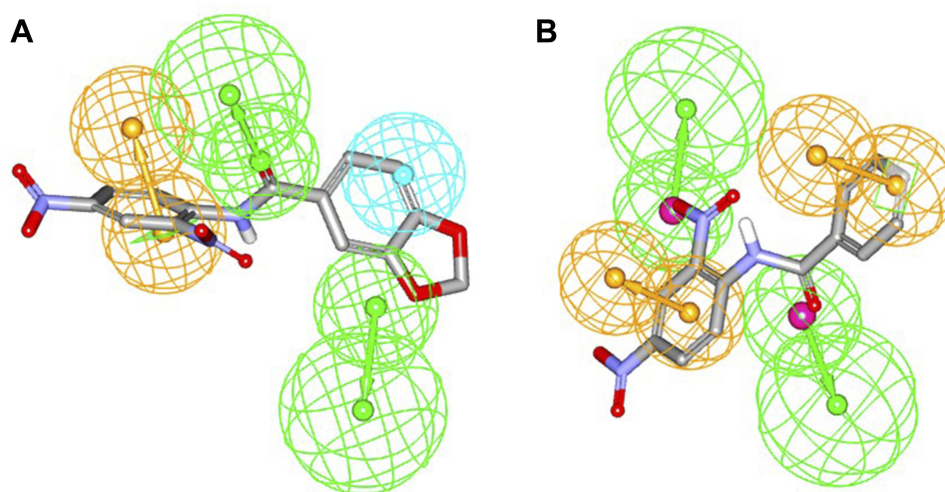


Figure 4 ROC-selected pharmacophores of MAO A and MAO B. **(A)** Overlay of compound **55 (ST-2043)** and MAO B pharmacophore model. **(B)** Overlay of compound **7 (ST-2013)** and MAO A pharmacophore model. Color code: green vectored sphere: hydrogen bond acceptor; orange vectored sphere: aromatic feature; blue sphere: hydrophobic feature.

A active site with respect to surface complementarity, showing that small alteration in the molecular structure can have a major impact on the modulation of the MAO isoform selectivity. The modeling results are further supported by ROC-selected pharmacophore models based on inhibitors presented here (see [Figure 4](#) and [supplemental material](#)). The essential predicted characteristics for MAO A and MAO B binding are a central hydrogen bond acceptor flanked by aromatic or hydrophobic features. This is in good accordance with the docking poses, with the limitation that the molecular docking showed no direct hydrogen bond interactions between the ligands and the amino acids of the binding pocket.

The most potent MAO B inhibitor **55 (ST-2043)** was further investigated to determine mode and type of inhibition. Reversibility studies with excess of substrate verified the expected reversible mode of inhibition. Compared to the suicide inhibitor *L*-deprenyl, inhibition by compound **55 (ST-2043)** (10-fold IC_{50} concentrations) preincubated with MAO B for 30, 60 or even 90 min was completely reversed after 50-fold dilution in assays mixture ([Figure 5](#)). It could be assumed, that compound **55** was readily displaced from the MAO B active side, while a more tight-binding interaction, e. g. as shown for safinamide, is considered to be more favorable in terms of pharmacological activity.⁹

Inhibition studies using different concentrations of **55 (ST-2043)** with seven appropriate substrate concentrations suggest a competitive inhibition type as demonstrated by Lineweaver–Burk plots ([Figure 6](#)). Compound **55 (ST-2043)** showed promising K_i values of 6.3 nM with 95%

CI = [5.0; 7.5] (Michaelis Menten fit “competitive inhibition”) and 9.5 nM (slopes from Lineweaver–Burk plots vs inhibitor concentration) for MAO B, respectively.

Cholinesterase Inhibition

Cholinesterase enzymes are of potential interest in MTL drug design for the treatment of neurodegenerative diseases.³³ Therefore, compound **55 (ST-2043)** was screened for inhibition of acetyl- and butyrylcholine esterases (AChE/BuChE). At a concentration of 1 μ M, **55 (ST-2043)** showed only moderate inhibition capacity with $24 \pm 1.8\%$ and $57 \pm 4.5\%$ for AChE and BuChE, respectively (see [Table S4](#) and [Figure S11](#) in [supplemental material](#)). Thus, no further characterization in this direction has been performed.

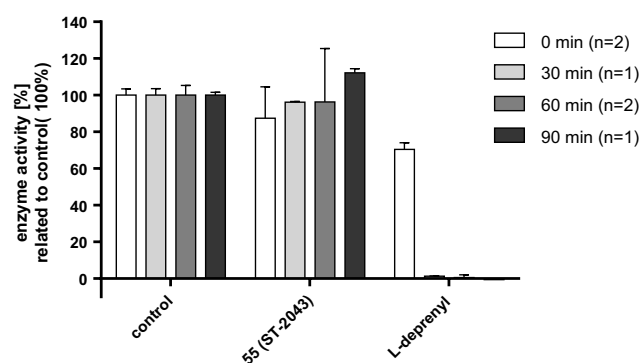


Figure 5 Reversibility of inhibition after preincubation of **55 (ST-2043)** and *L*-deprenyl with MAO B. Inhibitors ($10 \times IC_{50}$) were preincubated with enzyme for 0, 30, 60 or 90 min prior to 50-fold dilution in assays mixture containing kynuramine as substrate ($10 \times K_M$). Data represent mean values \pm SD of *n* independent experiments, each performed in duplicates.

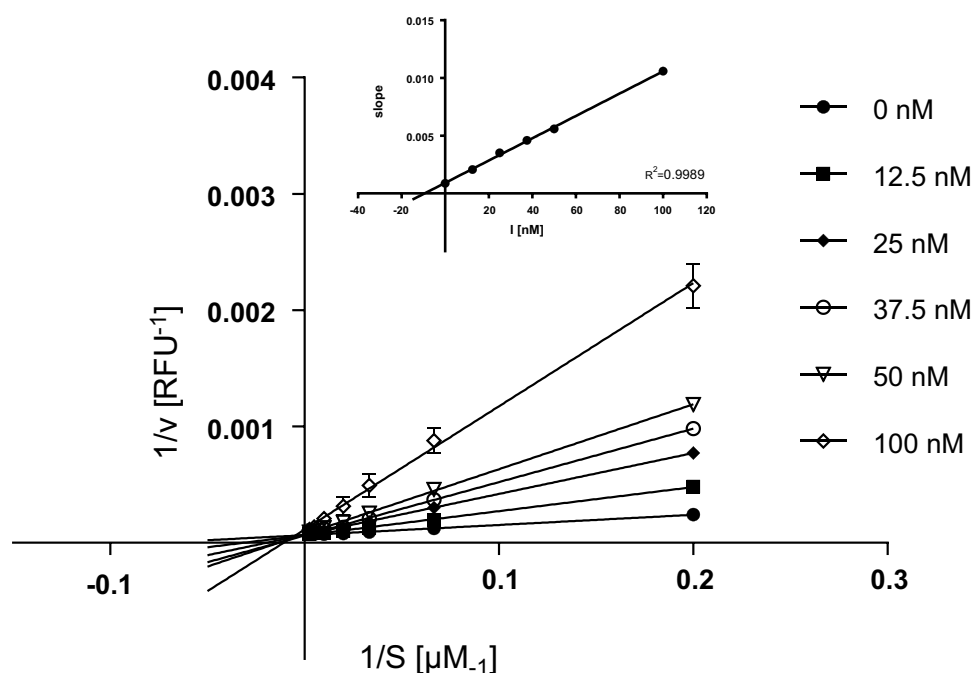


Figure 6 Lineweaver–Burk plots of compound **55** (ST-2043) (12.5 to 100 nM) with seven suitable concentrations of kynuramine. Data represent mean values \pm SD of one representative experiment performed in duplicates.

Conclusion

This study presented small anilides as MAO A and B inhibitors. Out of 66 screened compounds seven showed promising inhibition properties at a concentration of 1 μ M (>90% or >80% inhibition for MAO B and A, respectively). Evaluation of their IC_{50} values revealed six MAO B preferring (**31**, **33**, **34**, **55** (ST-2043), **65**) inhibitors and one MAO A preferring (**7** (ST-2023)) inhibitor with activities in low nanomolar concentration ranges. The MAO B preferring inhibitors showed IC_{50} values ranging from 56 nM to 128 nM with selectivity indices between 2.4 and >10. Computational analysis confirmed in vitro binding properties by demonstrating good surface complementarity of the inhibitors with the binding-pockets of the two MAO isoforms. The highest affinity for MAO A was found for ST-2023 (**7**, IC_{50} = 126 nM) with 8.3-fold lower affinity towards MAO B. ST-2043 (**55**) was identified as most potent MAO B inhibitor (IC_{50} = 56 nM, K_i = 6.3 nM) within this series, which demonstrated a similar inhibition capacity as the reference reversible MAO B inhibitor safinamide, but with rather low MAO selectivity (SI = 5.2). Further characterization suggested a competitive mode of MAO B inhibition for **55**, however, with a readily reversible binding rather than tight binding behavior as anticipated for pharmacological efficacy. Nevertheless, these small-sized ligands might be a promising starting point

for the design of new selective or multitargeting MAO inhibitors.

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Disclosure

The authors report no conflicts of interest in this work.

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