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Synthesis and Biological Evaluation of Derivatives of Indoline as Highly Potent Antioxidant and Anti-inflammatory Agents

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ABSTRACT: We describe the preparation and evaluation of novel indoline derivatives with potent antioxidant and anti-inflammatory activities for the treatment of pathological conditions associated with chronic inflammation. The indolines are substituted at position 1 with chains carrying amino, ester**,** amide or alcohol groups and some have additional substituents, Cl, MeO, Me, F, HO or BnO on the benzo ring. Concentrations of 1pM-1 nM of several compounds protected RAW264.7 macrophages against H_2O_2 induced cytotoxicity and LPS induced elevation of NO, TNF-α and IL-6. Several derivatives had anti-inflammatory activity at 1/100th of the concentration of unsubstituted indoline. Four compounds with ester, amine, amide or alcohol side chains injected subcutaneously in mice at a dose of 1 μmole/kg or less, like dexamethasone (5.6 μmoles/kg) prevented LPS-induced cytokine elevation in the brain and peripheral tissues. Subcutaneous injection of 100 μmoles/kg of these compounds caused no noticeable adverse effects in mice during 3 days observation.

INTRODUCTION

Inflammation is a normal response to infection and injury and involves the recruitment of the immune system to neutralize invading pathogens, repair injured tissues and promote wound healing.[1] However, during chronic or excessive activation of the immune system, nitric oxide synthase (iNOS) is stimulated by which nitric oxide (NO) and pro-inflammatory cytokines e.g. tumour necrosis factor-α (TNF-α), interleukin 6 (IL-6), interleukin 1β (IL-1β) are released.[2] In diverse pathological conditions, an increase also occurs in reactive oxygen species (ROS) including H_2O_2.[3] H_2O_2 is a freely diffusible component that can elevate TNF-α, and interleukins in macrophages.[4,5] Conversely, chronic infection or the addition of the bacterial pathogen to macrophages is responsible for the formation of ROS, induced by lipopolysaccharide (LPS).[6] Together, these have been implicated in the etiology of ulcerative colitis,[7] Crohn's disease[8] rheumatoid arthritis,[9] diabetes,[10] atherosclerosis,[11] neuropathic pain,[12] psoriasis[13] and cancer.[14] ROS and proinflammatory cytokines may also play a role in the etiology of Parkinson's and Alzheimer's disease (AD)[15-17] and contribute to ischemic stroke,[18] amyotrophic lateral sclerosis,[19] and depression.[20]

The most effective TNF-α inhibitors in clinical use are monoclonal antibodies that are given by injection. However they can cause serious adverse effects including lymphomas, infections (especially reactivation of latent tuberculosis), congestive heart failure and demyelinating disease. Also, as this antibody treatment is given chronically, it is inherently immunogenic and there have been reports of allergic reactions, serum sickness and thromboebolic events[21,22] as well the induction of anti-drug antibodies that can reduce treatment efficacy. [23-25] While the adverse events may result from blockade

of the actions of TNF-α and the immune system, the allergic reactions and formation of blocking antibodies are due to the protein nature of the TNF-α inhibitors. Therefore, there is a need for safer, small, non-protein molecules that do not block the essential activity of TNF-α but inhibit the excessive release occuring in conditions like rheumatoid arthritis and ulcerative colitis. Several such compounds have recently been described with thiazole or pyrazolidine cores that inhibit LPS induced TNF-α release in macrophages in the micromolar range.[26,27]

Our previously reported carbamate derivatives of indoline-3-propionic acid esters and indoline-3-(3-aminopropyl) have the advantage over the corresponding indoles of being sufficiently basic to form water soluble acid addition salts. Surprisingly, the indolines were found to display about a 100 fold higher antioxidant activity than the indoles, and in addition to inhibiting acetylcholinesterase (AChE) and butyryl cholinesterase they also showed cytoprotective activity against cytotoxicity induced by ROS.[28] Subsequently, indoline carbamates were also found to reduce the release of TNF-α and IL-6 from LPS-stimulated macrophages at concentrations of 1 nM or less.[29] Their mechanism of action includes inhibition of phosphorylation of the mitogen activated protein kinase (MAPK) and p38 and reduction of the nuclear translocation of the transcription factors activator protein 1 (AP1) and nuclear factor κB (NF-κB), consequent to toll-4 receptor stimulation by LPS.[30]

AChE inhibitors reduce the release of cytokines from LPS-activated macrophages in the presence of a choline ester by indirect stimulation of α7 nicotinic acetylcholine receptors, [31-34] which activates the Jak2/STAT3 pathway.[35] By prolonging the action of acetylcholine, AChE inhibitors also decrease pro-inflammatory cytokines in various

animal models of inflammation.[32,36,37] Therefore we reasoned that the most potent AChE inhibitor among the indoline carbamates, 3-(3-aminopropyl)-indolin-4-ylethyl (methyl) carbamate (**1**), may combat inflammation *in vivo* by a combination of these two mechanisms. However, surprisingly we found that **1** was less effective than **2**, (methyl 3-(6-((ethyl(methyl)carbamoyl)oxy)indolin-3-yl)propanoate which did not inhibit AChE at the dose administered, in preventing inflammation and colon damage in a mouse model of colitis,[38] although the compounds had similar anti-inflammatory activity *in vitro*. Therefore, we concluded that the carbamate moiety did not improve the antioxidant and anti-inflammatory activity and may even increase the potential for adverse cholinergic effects.

Derivatives **1** and **2** that contain a chiral carbon were tested as racemates (Scheme 1).[28] In the current study we prepared indoline derivatives lacking both a carbamate moiety to reduce possible cholinergic side effects, and chiral centers to avoid the need for enantiomeric separations. Therefore, we synthesized the isomeric analogues having the side chain linked to the *N*-1 of the indoline ring. Switching from structure **3** to structure **7a** a double isosteric change took place, in which the NH moiety was replaced by a CH_2 group, and the CH-CH_2, by an N-CH_2 group. Although, isosteric replacements yield compounds that are not identical having structural and electronic differences, nevertheless, if the isosters being considered have similar structural, geometrical, and/or electronic features they are commonly prepared and compared under the assumption that the structural changes still enable possible binding of both isosters to the same receptor. Thus, the novel achiral compounds studied herein all have side chains linked to position 1, based on the isosteric models of compounds **3** and **7a** (Figure 1A) and their respective

electrostatic potential calculations (Figure 1B) that are found to be virtually superimposable.

Scheme 1 and Figure 1

The synthesized indoline derivatives (Table 1) contain side chains substituted with amino (**13** and **15**), amido (**18**), ester (**7**), acid (**7b'**) or alcohol (**9**) groups attached to *N*-1 of indoline ring. The antioxidant and anti-inflammatory activities of compounds **3** and **23** with methyl propionate or 3-aminopropyl side chains attached to position 3, respectively, were compared to those in which the analogous side chains were attached to position 1 (**7** and **13**). Like the indoline derivatives **1** and **3**, two of the compounds described below, **7b** and **15a** also reduced phosphorylation of p38 MAP kinase and prevented nuclear translocation of AP1.[39]

Table 1

■ RESULTS AND DISCUSSION

Chemistry. Indolines substituted with a methyl propionyl chain at position 1 were prepared from the corresponding indoles **4a-d**,**f** upon treatment with methyl acrylate/DBU. Reduction of the *N*-alkylated indoles **5a-d**,**f** with $NaBH_3CN/AcOH$[40] or $NaBH_4/TFA$[28] or Et_3SiH/TFA[41] gave the corresponding indolines **6a-d**, **f**. Alternatively, **6a** was obtained in one step from indoline **8a**. The final products were isolated as hydrochlorides **7a-c** or *p*-toluenesulfonate salts (*p*-TSA) **7d**, **f**. Compound **7a** did not undergo hydrolysis at room temperature, in aqueous media for at least 72 h, as determined by its stability in a D_2O solution prepared for NMR determination. Under more strenuous acidic conditions, 3N HCl at room temperature for 48 h, ester **7b** was

converted into the free acid **7b'**. In addition, ester reduction[42] of **6a**, **b** followed by acidification gave the alcohols **9a**, **b** (Scheme 2).

Scheme 2

The salts of the *N*-(3-aminopropyl) derivatives **13a**, **g** were prepared from the corresponding indoles **4a**, **g** upon *N*-alkylation with acrylonitrile,[43] followed by initial reduction of the indolic to the indoline system[28,40] and subsequent reduction of the CN[44] to the corresponding aminomethyl group. The final products were isolated as *p*-TSA salts **13a**, **g** (Scheme 3).

Scheme 3

An unexpected reaction took place when an attempt was made to debenzylate **12g** under catalytic hydrogenation conditions in a Parr apparatus. Apparently, the hydrogenator contained traces of acetone, which condensed with the amine to give an intermediate imine that underwent further reduction and acidification to give *N*-(3-isopropylamino)propyl derivative **15i** (Scheme 4).

Scheme 4

Other *N*-(3-isopropylamino)propyl indolines **15**, as well as the corresponding *N*-(3-isopropylpropanamide)indolines **18**, were prepared. *N*-Alkylation of indoles with *N*-isopropylacrylamide[45] gave amides **16**. Reduction of indoles to indolines[28,40] and subsequent acidification led to amides **18.** *N*-(3-Isopropylamino)propyl indolines **15** were obtained from **16** by a sequence of reactions involving reduction of the amides to the amines[42] **19**, followed by indole to indoline reduction[28,40] to give **14** and finally, acidification to give salts **15** (Scheme 5).

Scheme 5

For comparison with compound **13a**, substituted at position 1 with a 3-aminopropyl group, the analog **23** was prepared substituted at position 3 with the same 3-aminopropyl group. Amidation of IPA[46] gave **20**, which underwent two reductive[42] steps, followed by acidification to finally give **23** (Scheme 6).

Scheme 6

In addition to altering the side chains attached to positions 1 or 3 of the indoline system, modifications were also made on the substituents attached to the benzo ring. Following the well-established "Operation Scheme" developed by Topliss,[47] the unsubstituted benzo ring (compounds **7a**, **9a**, **13a**, **18a** and **23**), was initially substituted with an electron withdrawing chloro (**7d**, **18d** and **18h**) or fluoro (**18e**) groups. Subsequent replacement of the electron withdrawing groups by electron donating ones gave the MeO (**7b**, **7b'**, **9b** and **15b**), BnO (**7f** and **13g**), HO (**15i**) or Me (**7c**) derivatives. The final compounds evaluated for their pharmacological activity are listed in Table 1. A detailed discussion on SAR of the various analogs is presented below in the pharmacology section.

Biology.

Effect of indoline derivatives on cell viability. None of the compounds tested at concentrations ranging from 1 pM to 100 nM had any significant effect on cell viability (Table 2).

Table 2

Influence of side chain position and that of other substituents on protection against oxidative stress. H_2O_2 (100 μM) reduced cell viability in these

experiments to 65-72% of that in the vehicle control group. Quercetin, used as positive control, restored viability to that of vehicle from a concentration of 1 pM (Figure 2). Prevention by the indoline derivatives of cytotoxicity induced by H_2O_2 depended on the nature of the substituent and its position. Compound **13a**, with a 3-aminopropyl group at position 1, restored viability only at a concentration of 10 nM, but this was reduced to 1 pM when the group was at position 3 (**23**). In contrast, indoline-*N*-methyl propionate (**7a**) restored viability of cells at a concentration 10 fold lower than its analog indoline 3-methyl propionate (**3**).

We are unaware of any studies that investigated the potential antioxidant effect of unsubstituted indoline (**8a)**. Accordingly, we compared its effect to that of four derivatives having side chains containing a terminal amino (**13a**), ester (**7a**), amide (**18a**) or alcohol (**9a**) group in position 1. Indoline completely protected cells against cytotoxicity at a concentration of 10 nM but not at lower concentrations (Figure 2). Compounds **13a** or **7a** did not differ in their cytoprotective activity from that of indoline but 3-(indolin-1-yl)-*N*-isopropylpropanamide (**18a**) was much more potent and caused complete protection at a concentration of 1 pM. On the other hand, the introduction of *N*-(3-hydroxypropyl) (**9a**) decreased the protective activity of **8a** a 10,000-fold (Figure 2).

Figure 2

Table 3 shows the effect on cytoprotective activity of additional substituents at position 1 on the benzo ring of indoline with 3-aminopropyl, methyl propionate, 3-(*N*-isopropylpropanamido), or 3-hydroxypropyl groups which depended both on the nature of the *N*-side chain and that of the benzo-substituent. A 7-BnO group in indoline-N-(3-aminopropyl (**13g**) reduced protective activity from 1 nM to greater than 100 nM, in

comparison to **13a**. Replacement of the primary NH_2 (**13a**) with a secondary NH-*i*-Pr (**15a**) increased potency 1000-fold, completely inhibiting cytotoxicity at a concentration of 1 pM. However, addition of 7-HO (**15i**), or 5-MeO (**15b**) reduced protective activity to less than that in the primary amine, **13a**. By contrast 5-MeO-substitution in the propyl alcohol **9a** or propionic ester (**7a**), which are much less effective protective agents than **15a,** increased protective activity 10,000 fold (Figure 3). A 5-Cl (**7d**) substituent in **7a**, was equally effective in increasing activity, while 6-Me (**7c**) had no effect (Fig. 3 Table 3). Hydrolysis of ester **7b** to the acid **7b'**, reduced protective activity to that of the unsubstituted propionate. Addition of 6-F (**18e**) to the highly active *N*-isopropylpropanamide **18a** reduced the activity 10,000-fold (Table 3).

Figure 3 and Table 3

Summary: (a) The most potent compounds are those that are substituted with 5-MeO electron donating group, i.e. compounds **7b** and in particular, **9b**, which appears to be much more potent than unsubstituted **9a**. The exception includes compounds that carry an NH-*i*-Pr group, **15a** (-CH_2NH-*i*-Pr) or **18a** (-CONH-*i*-Pr) at the end of the chain and do not have any substituents on the benzo ring. (b) Addition of substituents to positions 6 or 7, as in compounds **18e** (6-F), **15i** (7-HO), **13g** (7-BnO), decreases cytoprotective potency. (c) The presence of a terminal secondary amino group, NH-*i*-Pr (**15a**) markedly improves potency in comparison to a primary NH_2 group (**13a**). (d) The least potent compounds, **9a**, **13a**, and **7b'** are those with the most polar OH, NH_2 and COOH groups respectively, at the terminal end of the side chain or 6-F (**18e**) in the *N*-isopropylpropanamide, **18a**.

Other measures indicating protection against oxidative stress: lactic acid dehydrogenase release and caspase 3 activation. The maximal reduction in lactic acid dehydrogenase (LDH) release induced by any compound did not exceed 16%. However, 1 pM of **7b**, **15a** and **18a** significantly reduced LDH release, indicating that the compounds were able to improve the integrity of the cell membrane in the presence of oxidative stress (Figure 4A).

Oxidative stress can cause apoptosis, mediated by a cascade of proteolytic enzymes called caspases (cysteinyl aspartate-specific proteases). Caspase 3 is one of a number of effector caspases involved in this cascade. Compounds **7a**, **7b** and **15a** all reduced caspase 3 by about 60% at a concentration of 1 pM. Compounds **13a** and **13g**, which were among the least effective compounds against cytotoxicity in the MTT assay, were also significantly less active in reducing caspase 3 (Figure 4B).

Figure 4

Influence of the side chain position and of other substituents on reduction of nitric oxide and cytokines in LPS activated RAW 246.7 macrophages. The steroid, budesonide was used as a positive control for these experiments. Because of the large number of compounds that were tested at six different concentrations, it was necessary to perform many separate experiments. The increase in NO and cytokines achieved in response to LPS ranged for NO, 1.3-12.0 μM; TNF-α, 1.4-13.2 nM; IL-6, 3-70 nM. Therefore, we expressed the effect of each concentration of compound as a % of the LPS + medium value obtained in each experiment. Irrespective of the initial values found for LPS, all the compounds showed a similar reduction in NO to that of budesonide at concentrations of 1 pM to 1 nM, but budesonide caused a larger

reduction in TNF-α and IL-6 at higher concentrations. The greater efficacy of the steroid results from its ability to prevent AP-1 and NF-κB from inducing gene transcription, whereas the indoline derivatives appear to act further upstream by reducing phosphorylation of MAPK p38.[30] Maximal reductions in NO, TNF-α and IL-6 in LPS-activated macrophages by indoline derivatives with 3-aminopropyl or methyl propionate side chains in position 1 (**13a** and **7a**), or 3 (**23** and **3**), were achieved at a concentration of 100 pM or 1 nM (Figure 5). There were no significant differences among the compounds.

Figure 5

3-(Indolin-1-yl)-*N*-isopropylpropanamide, **18a**, was 1000-fold more potent than indoline as an antioxidant and was also the most potent anti-inflammatory agent, reducing NO and IL-6 by 50% at a concentration of 100 pM. Compounds **13a** and **7a** did not differ from indoline in their anti-oxidant activity but caused significantly greater reductions in NO and TNF-α at all concentrations. 3-Hydroxypropyl derivative **9a**, did not reduce NO more than indoline **8a** at any concentration, but was more effective in decreasing TNF-α and IL-6 at three of the higher concentrations (Figure 6).

Figure 6

The anti-inflammatory activity of compounds substituted at position 1 with 3-aminopropyl, methyl propionate, 3-(*N*-isopropylpropanamido) and 3-hydroxypropyl groups, and having additional substituents on the benzo ring, depended on: a) their anti-oxidant activity; b) the measure, cytokine or NO; c) the nature of the *N*-side chain; and d) the substituent on the benzo ring. All the 3-aminopropyl derivatives except **15i**, showed similar dose-related reductions in NO and TNF-α. **15i** was less active at higher

concentrations than the other amines in reducing NO and TNF-α. This is consistent with its weaker anti-oxidant effect (Figure 7).

Introduction of 5-Cl (**7d**) or 5-MeO (**7b**) in **7a** did not affect the reduction of NO but **7d** caused a greater reduction in TNF-α at all concentrations tested (Figure 8). A 6-Me group in (**7c**) decreased the anti-inflammatory activity of **7a**, as did hydrolysis of the ester to give **7b'**, in keeping with its lower protective activity against oxidative stress. In contrast to the effect on methyl propionate, addition of 5-Cl (**18d**) or 6-F (**18e**) markedly reduced anti-inflammatory activity of **18a** at almost all concentrations. Introduction of 5-MeO (**9b**) in the alcohol derivative **9a** also increased anti-inflammatory (Figure 9).

Figures 7-9

Summary of *in vitro* studies: The most potent anti-inflammatory compounds are also more effective protectants against cytotoxicity induced by oxidative stress and include **15a**, **15b**, **18a**, **9b**, **7a**, **7b** and **7d.** In response to LPS stimulation these compounds may act by inhibiting the activation of IL-1R-associated kinase-1 (IRAK-1) and IRAK-4 thereby preventing nuclear translocation of NF-κB, like other antioxidants[48] and natural compounds or their derivatives that have antioxidant activity, although the latter are only active in the μM range.[49] Indoline derivatives can also reduce nuclear translocation of AP-1 by inhibiting the phosphorylation of MAPK p38.[30] Introduction of 5-Cl (**18d**) or 6-F (**18e**) in the most potent antioxidant and anti-inflammatory compound, **18a** reduced both these activities, as did addition of 7-HO (**15i**) to **15a** or 7-BnO (**13g**) to **13a**. However, the introduction of 5-Cl (**7d**) to the weaker antioxidant, methyl propionate **7a** increased both activities.

***In vivo* studies. Prevention of cytokine elevation induced in mice by LPS.** Representative indolines having a side chain carrying an ester (**7a**), isopropylamine (**15a**), *N*-isopropylamido (**18a**) or *N*-isopropyl alcohol (**9b**), that showed the highest anti-inflammatory and antioxidant activity *in vitro,* were selected for *in vivo* studies in mice. Of these, **15a** injected sc in doses of 0.1-10 μmoles/kg produced the most consistent dose-related reduction in TNF-α and IL-6 after their elevation in plasma (Figure 10) and tissues (Figures 11 and 12). In keeping with the low concentration that reduced cytokines in cells, **18a** caused a maximal decrease in cytokines in plasma and brain at a dose of 0.1 μmoles/kg. Higher doses were not more, or were less effective. **18a** had no effect on IL-6 in the liver, suggesting that it may be metabolized to an inactive compound in that organ. All four compounds significantly reduced IL-6 in the brain even at the lowest dose indicating that they may have potential therapeutic benefit in pathological brain conditions involving oxidative stress and chronic cytokine elevation.

Figures 10-12

Acute toxicity of compounds in mice. Compounds **7a**, **15a** and **18a** (100 μmoles/kg) caused no change in food or water intake, body weight or activity of mice during a period of 72 h observation and could not be distinguished from those given saline.

■ CONCLUSIONS

We have described the synthesis, antioxidant and anti-inflammatory activity *in vitro* and in *vivo* of indoline and its derivatives substituted at position 1 with 3-aminopropyl [$CH_2CH_2CH_2NH_2$]; (3-isopropylamino)propyl [$CH_2CH_2CH_2NH$-*i*-Pr]; methyl propionate

[CH_2CH_2COOMe]; 3-(*N*-isopropylpropanamido) [CH_2CH_2CONH-*i*-Pr]; or 3-hydroxy propyl [$CH_2CH_2CH_2OH$] groups. Some of the compounds have one of the following additional substituents on the benzo ring: 5-Cl, 6-Cl, 5-MeO, 6-Me, 6-F, 7-HO or 6-BnO, 7-BnO. Indoline completely protected RAW 264.7 macrophages against cytotoxicity induced by H_2O_2 at a concentration of 1 nM, but reduced NO, TNF-α and IL-6 in LPS activated macrophages by no more than 20% at concentrations of 100 pM-100 nM. The esters **7b** and **7d**; the *N*-isopropylamide derivatives **18a** and **18d** and the alcohol **9b**, are more potent antioxidants than indoline. Whether they have similar (**15a** and **15b**), or greater (**7b**, **7d**, **18a**, **18d**, and **9b**), anti-oxidant activity than **8a**, the compounds are much more potent at reducing NO and/or cytokines by 50% at concentrations ranging from 10 pM (**18a** and **7d)**, 100 pM (**9b**) and 1 nM (**15a** and 1**5b**). Representative compounds bearing either *N*-isopropylamide (**18a**) (0.1 μmole/kg), isopropylamine (**15a**) methyl propionate (**7a**), or propyl alcohol (**9b**) (1 μmole/kg) substituents reduce the elevation of TNF-α and/or IL-6 in plasma, spleen and brain induced by LPS (10 mg/kg) to a similar extent to dexamethasone (5.6 μmoles/kg). Compounds **7a**, **15a** and **9b**, but not **18a**, also reduce IL-6 in the liver. **18a** may be metabolized to inactive compound(s) in that organ.

The data support our previous findings of the prevention by **7b** and **15a** (1 μmole/kg) of liver damage and increase in TNF-α and/or IL-6 after injection of galactosamine and LPS in mice[39]. Representative indolines were shown to decrease cytokine release in LPS activated macrophages [28,29] and in the liver after injection of LPS/galactosamine[39] by inhibiting nuclear translocation of AP-1 and NF-κB. Although there are no clinical data on the indoline derivatives to compare with that of TNF-α monoclonal antibodies, several of them reduced inflammation, colon damage, colon levels of TNF-α and IL-6 in rat[50]

and mouse models[38] of ulcerative colitis and in LPS induced lung injury.[30] We suggest that the indoline derivatives, in particular **7a**, **15a** and **18a**, may have therapeutic benefit in a variety of pathological conditions in which there is an excessive increase in ROS and pro-inflammatory cytokines in the peripheral and central nervous systems. Because they are not proteins and do not block the normal actions of cytokines, but only reduce their excessive release, they are less likely to cause the adverse immune effects of TNF-α antagonists. The compounds do not appear to cause acute toxicity in mice at 100 times the active dose. However, it remains to be seen whether they also display a low incidence of adverse effects after chronic administration in suitable animal disease models or in humans.

■ EXPERIMENTAL SECTION

General Chemistry Methods. ^{1}H-NMR, ^{13}C-NMR spectra were obtained on Bruker Avance-DPX-300, Avance-400, Avance-DMX-600 and Avance-III-700 spectrometers. Chemical shifts are expressed in ppm downfield from Me_4Si (TMS), used as internal standard, and values are given in δ scale. The "t" is indicative of a multiplet similar to a triplet with second order characteristics. HRMS and Electron Spray Ionization (ESI) were obtained on Synapt ESI-Q-TOF, Waters company-UK or Agilent 6545 Q-TOF LC-MS. Progress of the reactions was monitored by TLC on silica gel (Merck, Art. 5554). Flash chromatographic procedures were carried out on silica gel. Moisture sensitive reactions were carried out in flame-dried vessels. Melting points were determined on a Fisher-Johns apparatus. Commercially available compounds were used without further purification. The nomenclature of the compounds was given according to

ChemDraw Ultra v. 16 (CambridgeSoft). 3-(3-Aminopropyl)-indolin-4-yl ethyl(methyl)carbamate dihydrochloride (**1**),[29] methyl 3-(6-((ethyl(methyl)carbamoyl)oxy)indolin-3-yl)propanoate *p*-toluenesulfonate (**2**),[28] and methyl 3-(indolin-3-yl)propanoate hydrochloride (**3**), have been reported earlier.[51] The analytical methods used to determine purity were based on ^{1}H and ^{13}C NMR, HRMS, and elemental analysis for those compounds that were not highly hygroscopic or were obtained as non-crystalline oils. The final compounds which were tested biologically displayed ≥95% purity. The structural model of compounds **3** and **7a** were created using Discovery Studio 4.1. The Electrostatic potentials calculations were carried out with Gaussian 09 using B3LYP functional and 6-31G(d) basis set. Avogadro software (1.2.0 version) was used to display electrostatic potential.

Methyl 3-(Indolin-1-yl)propanoate Hydrochloride, (7a). The hydrochloride salt **7a** prepared by addition of HCl (g) to **6a** in dry diethyl ether was isolated as a hygroscopic orange solid in approximately quantitative yield. ^{1}H-NMR (300 MHz, CD_3OD) ppm δ 7.63-7.60 (m, 1H), 7.54-7.47 (m, 3H), 4.00 (t, *J* = 7.5 Hz, 2H), 3.86 (t, *J* = 6.9 Hz, 2H), 3.74 (s, 3H), 3.37 (t, *J* = 7.5 Hz, 2H), 2.97 (t, *J* = 6.9 Hz, 2H; ^{13}C-NMR (75 MHz, CD_3OD) ppm δ 172.10, 141.41, 136.65, 131.77, 129.94, 127.68, 119.88, 55.54, 53.33, 52.84, 30.25, 28.92; Anal. Calcd for $C_{12}H_{16}ClNO_2$ (MW 241.72 g/mol): C, 59.63; H, 6.67; N, 5.71. Found C, 59.85; H 6.71;1 N 5.83. HRMS: calcd. for $C_{12}H_{15}NO_2$ ($M^{+\cdot}$; ESI^{+}) 205.1097, found 205.1101.

Methyl 3-(5-Methoxyindolin-1-yl)propanoate Hydrochloride, (7b). Compound **7b** prepared by addition of HCl (g) to **6b** in dry diethyl ether was isolated as a dark red oil in approximately quantitative yield. ^{1}H-NMR **(**300 MHz, CD_3OD) ppm δ 7.52

(d, *J* = 8.7 Hz, 1H, 7.06-6.98 (m, 2H), 4.02 (t, *J* = 7.5 Hz, 2H), 3.84 (s, 3H), 3.81 (t, *J* = 7.2 Hz, 2H), 3.73 (s, 3H), 3.34 (t, *J* = 7.5 Hz, 2H), 2.97 (t, *J* = 7.2 Hz, 2H); ^{13}C-NMR (75 MHz, CD_3OD) ppm δ 172.01, 163.20, 138.55, 133.45, 121.00, 115.89, 112.13, 56.48, 56.05, 53.64, 52.87, 30.20, 29.11. Anal. Calcd for $C_{13}H_{18}ClNO_3$ (MW 271.74 g/mol): C, 57.46; H, 6.68; N, 5.15. Found C, 57.63; H 6.92;1 N 5.38. MS (TOF MS ES$^+$) *m/z* 236; HRMS: calcd. for $C_{13}H_{17}NO_3$ (MH^+, ESI^+) 236.12812, found 236.12731.

3-(5-Methoxyindolin-1-yl)propanoic Acid Hydrochloride, (7b'). A solution of **7b** in 3N HCl was stirred for 48 h at room temperature and evaporated. The precipitated **7b'** was isolated as a white hygroscopic solid in approximately quantitative yield. ^{1}H-NMR (300 MHz, CD_3OD) ppm δ 7.52 (d, *J* = 8.7 Hz, 1H), 7.06 (s, 1H), 7.00 (d, *J* =8.7 Hz, 1H), 4.02 (t, *J* = 7.2 Hz, 2H), 3.84 (s, 3H), 3.78 (t, *J* = 7.2 Hz, 2H), 3.34 (t, *J* = 6.9 Hz, 2H), 2.93 (t, *J* = 6.9 Hz, 2H); ^{13}C-NMR (75 MHz, CD_3OD) ppm δ 173.08, 163.05, 138.47, 133.47, 120.93, 115.82, 112.09, 56.47, 55.94, 53.74, 30.14, 29.07. HRMS: calcd. for $C_{12}H_{15}NO_3$ $(M-H)^-$ 220.09792, found 220.09767.

Methyl 3-(6-Methylindolin-1-yl)propanoate Hydrochloride, (7c). Compound **7c** prepared by addition of HCl (g) to **6c** in dry diethyl ether was isolated as a yellow oil in approximately quantitative yield. ^{1}H-NMR **(**300 MHz, CD_3OD) ppm δ 7.46 (s, 1H), 7.38-7.30 (m, 2H), 4.00 (t, *J* = 7.5 Hz, 2H), 3.84 (t, *J* = 7.2 Hz, 2H), 3.70 (s, 3H), 3.30 (t, *J* = 7.5 Hz, 2H), 3.00 (t, *J* = 6.9 Hz, 2H), 2.40 (s, 3H); ^{13}C-NMR (75 MHz, CD_3OD) ppm δ 171.75, 140.93, 140.55, 133.50, 132.68, 127.12, 120.34, 55.53, 53.19, 52.79, 30.16, 28.52, 21.34; HRMS: calcd. for $C_{13}H_{17}NO_2$ (MH^+, ESI^+) 220.13321, found 220.1327.

Methyl 3-(5-Chloroindolin-1-yl)propanoate *p*-Toluenesulfonate, (7d). Compound **7d** prepared by addition of *p*-TSA to a solution of **6d** in *tert*-butyl methyl

ether. The ethereal solution was evaporated to give **7d** as a brown oil. ^{1}H-NMR (300 MHz, CD_3OD) ppm δ 7.62 (d, *J* = 8.4 Hz, 4H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.35-7.30 (m, 2H), 3.98 (t, *J* = 7.5 Hz, 2H), 3.79 (t, *J* = 7.2 Hz, 2H), 3.65 (s, 3H), 3.24 (t, *J* = 7.5 Hz, 2H), 2.91 (t, *J* = 7.2 Hz, 2H), 2.29 (s, 6H); ^{13}C-NMR (75 MHz, CD_3OD) ppm δ 171.53, 142.60, 141.73, 139.59, 138.86, 137.05, 129.76, 129.62, 127.39, 126.60, 121.47, 55.77, 53.27, 52.72, 30.03, 28.65, 27.14, 21.32; MS (ES^+) *m/z* 240 (MH^+); HRMS: calcd. for $C_{12}H_{14}ClNO_2$ ($M^{+\cdot}$; ESI^+) 239.07076, found 239.07129.

Methyl 3-(6-(Benzyloxy)indolin-1-yl)propanoate *p*-Toluenesulfonate, (7f). Compound **7f** prepared by addition of *p*-TSA (1 eq) to **6f** (1 eq) in *tert*-butyl methyl ether (10 mL) was isolated as a hygroscopic white solid. An H-->D exchange took place when **7f** was dissolved in CD_3OD in an NMR tube. This exchange was detected by NMR already after 5 min, and was shown to take place primarily at position 5**,** and partly at both positions 5 and 7. ^{1}H-NMR **(**600 MHz, CD_3OD) ppm δ 7.66 (d, *J* = 8.4 Hz, 4.5H), 7.43 (d, *J* = 7.2 Hz, 2H), 7.36 (t, *J* = 7.2 Hz, 2H), 7.33 (s, 1H), 7.32-7.29 (m, 1H), 7.21 (d, *J* = 8.4 Hz, 4.5H), 7.15 (s, 0.4H), 5.10 (s, 2H), 3.93 (t, *J* = 8.2 Hz, 2H), 3.77 (t, *J* = 8.2 Hz, 2H), 3.71 (s, 3H), 3.20 (t, *J* = 8.2 Hz, 2H), 2.86 (t, *J* = 8.2 Hz, 2H), 2.35 (s, 7H); ^{13}C-NMR (150 MHz, CD_3OD) ppm δ 172.30, 160.69, 143.50, 143.01, 141.75, 138.00, 129.84, 129.62, 129.15, 128.76, 127.88, 127.82, 126.97, 105.67, 71.70, 56.26, 52.91, 52.82, 30.40, 28.28, 21.33; MS (non deuterated product **7f**). (EI^+) *m/z* 311.152 ($M^{+\cdot}$), 312.155 (MH^+); HRMS: calcd. for $C_{19}H_{21}NO_3$ ($M^{+\cdot}$; EI^+) 311.1521, found 311.1524 and calcd. for $C_{19}H_{21}NO_3Na$ (MNa^+, ESI^+) 334.1419, found 334.1422.

3-(Indolin-1-yl)propan-1-ol Hydrochloride, (9a). Compound **9a** prepared by addition of HCl (g) to **9a free base** in dry diethyl ether was isolated as a greyish

hygroscopic solid in approximately quantitative yield. ^{1}H-NMR (600 MHz, CD_3OD) ppm δ 7.61 (d, *J* = 7.8 Hz, 1H), 7.53-7.52 (m, 2H), 7.51-7.48 (m, 1H), 4.01 (bt, 2H), 3.74 (t, *J* = 6.0 Hz, 2H), 3.67 (bt, 2H), 3.37 (t, *J* = 7.8 Hz, 2H), 2.02 (quint, *J* = 7.2 Hz, 2H); ^{13}C-NMR (150 MHz, CD_3OD) ppm δ 141.66, 136.68, 131.79, 129.96, 127.71, 119.97, 60.00, 56.17, 55.50, 29.00, 28.48; MS (ES^+) *m/z* 178.2 (MH^+); HRMS: calcd. for $C_{11}H_{15}NO$ (MH^+, ESI^+) 178.12264, found 178.12231.

3-(5-Methoxyindolin-1-yl)propan-1-ol Hydrochloride, (9b). Compound **9b** prepared by addition of HCl (g) to **9b free base** in dry diethyl ether was isolated as a brown hygroscopic solid in approximately quantitative yield. ^{1}H-NMR (700 MHz, CD_3OD, at 275 ^{o}K) ppm δ 7.53 (d, *J* = 9.1 Hz, 1H), 7.05 (d, *J* = 2.1 Hz, 1H), 6.97 (dd, *J* = 9.1, 2.8 Hz, 1H), 4.16 (br, 1H), 3.9-3.8 (br, 2H), 3.82 (s, 3H), 3.71 (t, *J* = 6.3 Hz, 2H), 3.45 (br, 1H), 3.36 (br, 1H), 3.27 (br, 1H), 2.07 (br, 1H), 1.98 (br, 1H); ^{13}C-NMR (176 MHz, CD_3OD, 275 ^{o}K) ppm δ 162.66, 138.35, 133.53, 120.92, 115.542, 111.80, 59.58, 56.37, 55.73, 55.45, 29.03, 28.33. Anal. Calcd for $C_{12}H_{18}ClNO_2.0.5H_2O$ (MW 252.11 g/mol): C, 57.03; H, 7.58; N, 5.54. Found C, 57.16; H 7.39; N 5.65. MS (ES^+) *m/z* 208 (MH^+); HRMS: calcd. for $C_{12}H_{17}NO_2$ (MH^+, ESI^+) 208.13321, found 208.13375.

3-(Indolin-1-yl)propan-1-amine Hydrochloride, (13a).[52] Compound **13a** prepared by addition of HCl to **12a** (2 g, 11 mmol) in ether (60 mL), was crystallized from DCM-ether and was isolated as a hygroscopic off-white solid. ^{1}H-NMR (400 MHz, CD_3OD) ppm δ 7.06-7.025 (m, 2H), 6.65 (t, *J* = 7.6 Hz, 1H), 6.58 (d, *J* = 7.6 Hz, 1H), 3.31 (t, *J* = 8.2 Hz, 2H), 3.17 (t, *J* = 6.8 Hz, 2H), 3.07 (t, *J* = 7.6 Hz, 2H), 2.93 (t, *J* = 8.0 Hz, 2H), 1.99 (quint, *J* = 7.6 Hz, 2H); ^{13}C-NMR (100 MHz, CD_3OD) ppm δ 153.57, 131.45, 128.23, 125.38, 119.55, 108.80, 54.48, 48.27, 39.17, 29.41, 26.61. MS (EI^+) *m/z*

176.137 ($M^{+\cdot}$), 177.142 (MH^+). Anal. Calcd for $C_{11}H_{17}ClN_2$ (MW 212.11 g/mol): C, 62.11; H, 8.06; N, 13.17. Found C, 62.05; H 7.94; N 13.22. MS (EI^+) *m/z* 176.137 ($M^{+\cdot}$), 177.142 (MH^+); HRMS: calcd. for $C_{11}H_{16}N_2$ ($M^{+\cdot}$; EI^+) 176.1313, found 176.1370.

3-(7-(Benzyloxy)indolin-1-yl)propan-1-amine di-*p*-Toluenesulfonate, (13g). Compound **13g** prepared by addition of *p*-TSA to a solution of **12g** in *tert*-butyl methyl ether, was crystallized from DCM-ether and was isolated as a hygroscopic white solid in approximately quantitative yield. ^{1}H-NMR (300 MHz, CD_3OD) ppm δ 7.69-7.65 (m, 4H), 7.52-7.33 (m, 6H), 7.20-7.14 (m, 5H), 7.03 (dd, *J* = 7.5, 0.6 Hz, 1H), 4.01 (t, *J* = 7.5 Hz, 2H), 3.60 ("t", *J* = 7.2 Hz, 2H), 3.35-3.30 (m, 2H), 2.96 (t, *J* = 7.5 Hz, 2H), 2.35 (s, 6H), 2.12-2.06 (m, 2H); ^{13}C-NMR (75 MHz, CD_3OD) ppm δ 151.32, 143.28, 141.85, 138.83, 137.13, 133.91, 129.90, 129.82, 129.55, 129.08, 127.98, 126.86, 119.13, 113.67, 72.12, 66.84, 55.44, 54.01, 37.80, 29.32, 23.78, 21.31, 15.44; HRMS: calcd. for $C_{18}H_{22}N_2ONa$ ($M^{+\cdot}$; ESI^+) 305.1630, found 305.1626.

3-(Indolin-1-yl)-*N*-isopropylpropan-1-amine Dihydrochloride, (15a). Compound **15a** prepared by addition of HCl to a solution of **14a** in ether was isolated as a hygroscopic brown solid. ^{1}H-NMR (400 MHz, CD_3OD) ppm δ 7.67-7.65 (m, 1H), 7.54-7.48 (m, 3H), 4.06 (t, *J* = 7.6 Hz, 2H), 3.74 ("t", *J* = 8.0 Hz, 2H), 3.46-3.36 (m, 3H), 3.20 (t, *J* = 7.6 Hz, 2H), 2.27 (quint, *J* = 8 Hz, 2H); ^{13}C-NMR (100 MHz, CD_3OD) ppm δ 141.23, 136.63, 131.75, 129.94, 127.69, 119.97, 55.26, 54.39, 52.32, 43.00, 29.05, 23.15, 19.23, 13.19. Anal. Calcd for $C_{14}H_{24}Cl_2N_2$ (MW 291.26 g/mol): C, 57.73; H, 8.32; N, 9.62. Found C, 57.50; H 8.31; N 9.60. HRMS: calcd. for $C_{14}H_{22}N_2$ (MH^+, ESI^+) 219.18558, found 219.18599.

***N*-Isopropyl-3-(5-methoxyindolin-1-yl)propan-1-amine Dihydrochloride, (15b).** Compound **15b** prepared by addition of HCl (g) to **14b** in dry diethyl ether was isolated as a hygroscopic yellow solid in approximately quantitative yield. ^{1}H-NMR (300 MHz, $CDCl_3$) ppm δ 7.08-7.05 (m, 1H), 6.91-6.90 (m, 1H), 6.85-6.82 (m, 1H), 3.77 (s, 3H), 3.67 (t, *J* = 7.8, 2H), 3.42-3.34 (m, 3H), 3.19-3.11 (m, 4H), 2.12 (quint, *J* = 7.5 Hz, 2H), 1.35 (d, *J* = 6.6 Hz, 6H); ^{13}C-NMR (75 MHz, $CDCl_3$) ppm δ 159.54, 139.55, 136.10, 116.08, 114.58, 112.34, 56.41, 55.43, 52.41, 52.08, 43.53, 29.41, 24.07, 19.25; MS (ES^+) *m/z* 249.2 (MH^+); HRMS: calcd. for $C_{15}H_{24}N_2O$ (MH^+, ESI^+) 249.19614, found 249.19606.

1-(3-(Isopropylamino)propyl)indolin-7-ol di-*p*-Toluenesulfonate, (15i). Compound **15i** prepared by addition of *p*-TSA (2 eq) to **14i** (1 eq) in *tert*-butyl methyl ether, was crystallized from DCM-ether and was isolated as a hygroscopic solid in quantitative yield. ^{1}H-NMR (700 MHz, CD_3OD+D_2O) ppm δ 7.69 (d, *J* = 8.4 Hz, 4H, H-20), 7.32 (t, *J* = 7.7 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 4H), 6.94 (dd, *J* = 7.7, 0.7 Hz, 1H), 6.91 (d, *J* = 7.7 Hz, 1H), 3.95 (t, *J* = 7.7 Hz, 2H), 3.66 ("t", *J* = 7.7 Hz, 2H), 3.37 (septet, *J* = 6.3 Hz, 1H), 3.34 (t, *J* = 7.7 Hz, 2H), 3.13 (t, *J* = 7.7 Hz, 2H), 2.37 (s, 6H), 2.16 (quint, *J* = 7.7 Hz, 2H), 1.33 (d, *J* = 6.3 Hz, 6H); ^{13}C-NMR (176 MHz, CD_3OD+D_2O) ppm δ 149.98, 142.95, 141.99, 138.18, 132.90, 129.92, 127.00, 126.82, 117.74, 116.41, 55.22, 53.35, 52.19, 43.02, 29.31, 23.06, 21.34, 19.20; HRMS: calcd. for $C_{14}H_{22}N_2O$ ($M^{+\cdot}$; EI^+) 234.1732, found 234.1736.

3-(Indolin-1-yl)-*N*-isopropylpropanamide Hydrochloride, (18a). Compound **18a** prepared by addition of 3N HCl to **17a** in ethyl acetate was crystalized from MeOH/ether and was isolated as a white-brown solid, in approximately quantitative

yield. Mp 92-93 °C; ^{1}H-NMR (300 MHz, CD_3OD) ppm δ 7.64 (d, *J* = 7.2 Hz, 1H), 7.53-7.46 (m, 3H), 4.06 (t, *J* = 7.2 Hz, 2H), 3.89 (septet, *J* = 6.6 Hz, 1H), 3.87 (t, *J* = 6.9 Hz, 2H), 3.39 (t, *J* = 6.9 Hz, 2H), 2.87 (t, *J* = 6.6 Hz, 2H), 1.14 (d, *J* = 6.6 Hz, 6H); ^{13}C-NMR (75 MHz, CD_3OD) ppm δ 169.96, 140.59, 136.64, 131.70, 129.66, 127.47, 120.18, 54.96, 53.65, 42.72, 31.22, 28.91, 22.44; MS (ES^+) *m/z* 233 (MH^+), 255 (MNa^+); HRMS: calcd. for $C_{14}H_{20}N_2O$ (MH^+, ESI^+) 233.1648, found 233.1647.

***N*-Isopropyl-3-(5-methoxyindolin-1-yl)propanamide Hydrochloride, (18b).** Compound **18b** prepared by addition of 3N HCl to **17b** in ethyl acetate was isolated as an orange oil in approximately quantitative yield. ^{1}H-NMR (400 MHz, $CDCl_3$) ppm δ 6.94-6.91 (m, 1H), 6.71 (d, *J* = 2.4 Hz, 1H), 6.59 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.44 (d, *J* = 8.4 Hz, 1H), 4.05 (septet, *J* = 6.8 Hz, 1H), 3.62 (s, 3H), 3.30-3.20 (m, 4H), 2.90-2.80 (m, 2H), 2.43 (t, *J* = 6.8 Hz, 2H), 1.10 (d, *J* = 6.8 Hz, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) ppm δ 200.88, 152.91, 145.94, 131.40, 111.59, 107.70, 55.65, 53.57, 46.89, 40.86; HRMS: calcd. for $C_{14}H_{22}N_2O_2$ (MNa^+, ESI^+) 285.15735, found 285.15824.

3-(5-Chloro-1*H*-indol-1-yl)-*N*-isopropylpropanamide Hydrochloride, (18d). Compound **18d** prepared by addition of 3N HCl to **17d** in EtOAc was isolated as a dark yellow oil in approximately quantitative yield. ^{1}H-NMR (300 MHz, CD_3OD) ppm δ 7.66-7.63 (m, 1H), 7.56-7.50 (m, 2H), 4.08 (t, *J* = 7.5 Hz, 2H), 3.90 (septet, *J* = 6.6 Hz, 1H), 3.86 (t, *J* = 6.9 Hz, 2H), 3.41 (t, *J* = 7.5 Hz, 2H), 2.83 (t, *J* = 6.9 Hz, 2H), 1.14 (d, *J* = 6.6 Hz, 6H); ^{13}C-NMR (75 MHz, CD_3OD) ppm δ 170.14, 139.73, 139.25, 137.35, 129.91, 127.685, 121.73, 55.57, 53.94, 42.82, 31.22, 29.03, 22.50; MS (ES^+) *m/z* 267 (MH^+), 289 (MNa^+); HRMS: calcd. for $C_{14}H_{19}ClN_2O$ (MH^+, ESI^+) 267.12587, found 267.12535.

3-(6-Fluoroindolin-1-yl)-*N*-isopropylpropanamide Hydrochloride, (18e). Compound **18e** prepared by addition of HCl (g) to **17e** in dry diethyl ether was isolated as a hygroscopic white-pink solid, in approximately quantitative yield. ^{1}H-NMR (400 MHz, CD_3OD) ppm δ 7.52 (dd, *J* = 8.4, 5.4 Hz, 1H), 7.46 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.26 (td, *J* = 8.8, 2.4 Hz, 1H), 4.06 (t, *J* = 7.6 Hz, 2H), 3.98 (septet, *J* = 6.4 Hz, 1H), 3.84 (t, *J* = 6.8 Hz, 2H), 3.33 (t, *J* = 7.6 Hz, 2H), 2.78 (t, *J* = 6.8 Hz, 2H), 1.15 (d, *J* = 6.8 Hz, 6H); ^{13}C-NMR (100 MHz, CD_3OD) ppm δ 170.48, 165.03+162.58, 142.71, 132.56, 128.89+128.80, 118.66+118.44, 108.09+107.81, 56.24, 53.90, 42.85, 31.17, 28.49, 22.54; MS (ES^+) *m/z* 251 (MH^+), 273 (MNa^+); HRMS: calcd. for $C_{14}H_{19}FN_2O$ (MH^+, ESI^+) 251.15542, found 251.15495.

3-(6-Chloroindolin-1-yl)-*N*-isopropylpropanamide *p*-Toluenesulfonate, (18h). Compound **18h,** prepared by addition of *p*-TSA (1 eq) to **17h** (1 eq) in *tert*-butyl methyl ether was isolated as a brown oil in 57% yield. ^{1}H-NMR (300 MHz, CD_3OD) ppm δ 7.67 (d, *J* = 8.1 Hz, 2H), 7.58-7.54 (m, 1H), 7.42-7.39 (m, 2H), 7.22 (d, *J* = 8.1 Hz, 2H), 4.00-3.93 (m, 1H), 3.96 (t, *J* = 7.5 Hz, 2H), 3.76 (t, *J* = 6.6 Hz, 2H), 3.26 (t, *J* = 7.5 Hz, 2H), 2.72 (t, *J* = 6.9 Hz, 2H), 2.36 (s, 3H), 1.13 (d, *J* = 6.6 Hz, 2H); ^{13}C-NMR (75 MHz, CD_3OD) ppm δ 170.66, 143.69, 143.22, 141.84, 135.15, 134.98, 130.58, 129.87, 128.48, 126.86, 119.37, 55.81, 53.47, 55.81, 53.47, 42.76, 31.33, 28.61, 22.49, 21.32; MS (ES^+) *m/z* 267 (MH^+), 289 (MNa^+); HRMS: calcd. for $C_{14}H_{19}ClN_2O$ (MH^+, ESI^+) 267.12587, found 267.12713.

3-(Indolin-3-yl)propan-1-amine di-*p*-Toluenesulfonate, (23). Compound **23,** prepared by addition of *p*-TSA to a solution of **22** in *tert*-butyl methyl ether was isolated as a red-brown oil, in approximately quantitative yield. ^{1}H-NMR (300 MHz, $CDCl_3$) ppm

δ 7.05-7.03 (m, 2H), 6.70 (t, *J* = 7.5 Hz, 1H), 6.60 (d, *J* = 7.8Hz, 1H), 6.74 (s, 1H), 2.77 (bs, 2H), 2.64 (t, *J* = 7.2 Hz, 2H), 2.56 (t, *J* = 7.2 Hz, 2H), 1.69 (quint, *J* = 7.2 Hz, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) ppm δ 136.27, 127.11, 121.43, 121.16, 118.40, 118.38, 114.86, 111.08, 61.45, 41.40, 33.36, 29.61, 22.14, 13.86.

PHARMACOLOGY.

Compounds **7a**, **7b**, **7b'**, **7c, 7d**, **9a**, **9b**, **13a**, **13g**, **15a**, **15b**, **15d**, **15e, 15i**, **18a**, **18d**, **18e**, and **23**, were tested as mono- or dihydrochlorides, mono- or di-*p*-toluenesulfonate salts. All concentrations are expressed as that of the respective salts.

Cell line. The mouse macrophage RAW 264.7 cell line was cultured in Dulbecco's Modified Eagle's Medium (DMEM), 4500 mg/L D-glucose with 10% fetal calf serum (FCS), 10000 U/mL penicillin, 100 mg/mL streptomycin and 25 µg/mL Amphotericin B at 37 °C with 95% air and 5% CO_2.

Cell viability. Cell viability was determined using the MTT colorimetric assay. The assay is based on the ability of mitochondrial dehydrogenases to reduce yellow MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to purple insoluble formazan crystals. RAW 264.7 cells were seeded at a density of $1x10^4$ cells/mL in a 96-well plate and incubated for 24 h. MTT stock solution prepared in FCS free medium was added to each well to a final concentration of 0.5 mg/mL and incubated for 1 h at 37 °C. Media was removed and the cells were lysed with 100% DMSO to allow the crystals to fully solubilize. Optical density at 570 nm was determined using a Labsystems plate-reader. Viability of cells treated with different compounds was compared to cells treated with medium alone.

Protective activity against oxidative stress. H_2O_2, a precursor of various ROS that freely penetrates cell membranes, was chosen as the oxidant reagent for the following experiments. Compounds were tested for their potential to protect against cell death induced by H_2O_2. RAW 264.7 cells were seeded in 96 well-plates at a density of 1 x 10^4 cells/mL and incubated with different indoline derivatives in concentrations ranging from 1 pM to 100 nM, for 2 h prior to addition of H_2O_2 (100 μM). This H_2O_2 concentration reduced cell viability by 25-35% relative to that in vehicle treated cells. All concentrations of each compound were tested in 2-3 experiments with a minimum of 6 wells per concentration in each experiment.

LDH activity. An increase in lactate dehydrogenase (LDH) activity is indicative of cell membrane damage. LDH is an oxidoreductase enzyme that catalyzes the interconversion of pyruvate and lactate. LDH reduces NAD to NADH, which was detected using a colorimetric assay (Sigma Aldrich Ltd. Mo USA). Cells were seeded and incubated with H_2O_2 (100 μM) as described above. Experiments were performed in serum-free medium. Culture media were collected at the end of the experiment and centrifuged. Each 50 μL aliquot was incubated with 50 μL mixed reaction solutions, at room temperature, for 30 min. Absorbance was measured at 490 nm using a 96-well plate reader. Color intensity is proportional to LDH activity.

Caspase 3 activity. The activity of caspase 3 was measured by means of a luminescent assay (Caspase-Glo 3/7 Assay Promega Ltd). Macrophages were seeded and pretreated as described above with concentrations of 1 pM and 1 nM of several of the compounds, 4 h before the addition of H_2O_2 (100 μM). The medium was aspirated 90 min later, 100 μL of fresh DMEM were added to each well and the plates were incubated

overnight. Caspase-GloR Reagent (100 μL) was added to each well and the contents were gently mixed using a plate shaker at 300–500 rpm for 30 sec. The plates were left at room temperature for 30 minutes and then luminescence of each sample was measured in a plate-reading luminometer (Cytation 3).

Anti-inflammatory activity *in vitro*. RAW 264.7 cells were seeded at a density of $1x10^5$ cells per well in 48-well culture plates and grown overnight in DMEM supplemented as indicated above. The wells were washed with PBS (pH 7.4) and the compounds, prepared in sterile double distilled water, were added to give final concentrations ranging from 1 pM to 100 nM in medium containing 2% FCS. The cells were incubated for 2 h at 37 ^oC prior to stimulation with LPS, 2.5 μg/mL from Escherichia coli (Sigma-Aldrich serotype 0111:B4). Supernatants were harvested after 8 h for measurement of TNF-α and after 24 h for IL-6 and nitric oxide (NO) measurement. NO production was detected by a colorimetric method using Griess reagent (0.2% naphthylenediamine dihydrochloride, and 2% sulfanilamide in 5% phosphoric acid), which measures the concentration of nitrite, a stable metabolite produced from NO. TNF-α and IL-6 protein were detected by a commercial ELISA kit (Biolegend, San Diego, CA, USA) according to the manufacturer's instructions. Each concentration of each compound was tested in two-three separate experiments in 6 wells per concentration.

Measurement of cytokines in mouse tissues. Male Balb/C mice (7-8 weeks old) were injected sc with saline or compounds **7a**, **9b**, **15a** and **18a**, (0.1, 1 and 10 μmoles/kg) LPS (10 mg/kg) was injected ip 15 min later. Dexamethasone was used in the current study as a positive control at a dose of 3 mg/kg (5.6 μmoles/kg). This dose was shown to cause maximal reduction in lethality and cytokine levels in mice injected with

LPS. [53] Mice were sacrificed 4 h later for measurements of cytokines in plasma and spleen as previously described.[30] The tissues were rapidly collected, snap frozen in liquid nitrogen and stored at (-80 °C) until use. For cytokine measurements, spleens were weighed and homogenized in PBS solution containing NaCl, (0.8%), NaH_2PO_4 (0.144%), KH_2PO_4 (0.024%) and protease inhibitor cocktail (1%) at a speed of 24,000 rpm and centrifuged at 14,000 g at 4 ºC for 15 min. Cytokine detection was performed in the collected supernatants by ELISA as described above. Protein concentrations were determined by means of a BCA protein assay kit. Under the conditions of the experiment it was only possible to measure IL-6 in the brain and liver.

Acute toxicity of compounds in mice. Compounds **7a**, **15a** and **18a** (100 μmoles/kg) or saline (1 mL/100 g) were injected sc in groups of 3 Balb/C male mice weighing 24-28 g. The mice were allowed free access to food and water and were observed for 72 h.

Statistical analyses. The statistical evaluation of the data was performed using Statistical Package for the Social Science (SPSS) 22. Comparisons were performed by one-way ANOVA followed by Duncan's *post hoc* test. Differences between groups were considered statistically significant at a level of $p<0.05$.

■ ASSOCIATED CONTENT

Supporting information

The Supporting Information is available free of charge on the ACS Publications website at _____.

The supporting information contains experimental procedures for the starting materials and intermediates, which were not tested biologically. It also contains molecular formula strings and some data (CVS)

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Notes

The authors declare no competing financial interest

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■ ABBREVIATIONS USED

AD, Alzheimer's disease; AChE, acetylcholine esterase; AP1, activator protein 1; CDI, carbonyldiimidazole; CSF Colony Stimulating Factor; Dex, dexamethasone; DMEM, Dulbecco's modification of Eagle's medium; FCS, Fetal Calf Serum; HBSS, Hank's Balance Salt Solution; iNOS, inducible nitric oxide synthase; IL-1β, interleukin 1β; IL-6, interleukin 6; IPA, indole propionic acid; LDH, lactic acid dehydrogenase; LPS, lipopolysaccharide; MAPK, mitogen activated protein kinase; MTT, 3-(4,5-dimethyl-1,3-

thiazol-2-yl)-2,5-diphenyl-2H-tetrazole-3-ium bromide; NF-κB, nuclear factor κB; TNF-α, tumor necrosis factor; *p*-TSA, *para*-toluene sulfonic acid.

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Legends for Figures

Figure 1. A. Model of overlap of 1- and 3-alkylated indolines. **B.** Electrostatic potentials surface.

Figure 2. **A**. Effect of indoline derivatives on viability of macrophages after their exposure to H_2O_2. Data represent the mean ± SEM from 8-14 replicates per concentration of each compound as measured by MTT. Concentrations lower than 1 nM of indoline, **3**, **7a** and **13a** and 10 nM of **9a** did not significantly increase viability compared to H_2O_2 alone. Viability not different from that of vehicle *, viability not different from H_2O_2 #.

Figure 3. Effect of indoline derivatives with an amine, ester and amide or alcohol substituent in position 1 on viability of macrophages exposed to H_2O_2. Viability not different from that of vehicle *, viability not different from H_2O_2, #. Significantly different from same concentration of **13a**, [b], $p<0.01$.

Figure 4. **A**. Reduction by compounds of LDH induced in macrophages by exposure to H_2O_2. Data represent the mean ± SEM from 8-10 replicates per concentration of each compound. Significantly different from H_2O_2 alone, * $p<0.05$; ** $p<0.01$. **B**. Reduction by compounds of caspase 3 activity induced in macrophages by exposure to H_2O_2. Data represent the mean ± SD from 4-6 replicates per concentration of each compound. Significantly different from same concentration of the other compounds, * $p<0.01$.

Figure 5. Effect of side chain position on reduction by indoline derivatives of

cytokines and NO in LPS-activated macrophages. There were no significant differences in the reduction of NO or TNF between compounds with side chain in position 1 and 3 and only a small difference in the reduction of IL-6. Significantly different from value obtained with the same concentration of the compound with the respective side chain in position 1, [a] $p<0.05$; [b] $p<0.01$.

Figure 6. Effect of an additional substituent, amine, ester, amide or alcohol in position 1 of indoline on the reduction NO, TNF-α and IL-6 in LPS activated macrophages. Data represent the mean ± SEM from 9-18 replicates per concentration of each compound. ANOVA for NO, $F_{30,476} = 25.4$, $p<0.0001$; TNF-α, $F_{30,425} = 20.8$, $p<0.0001$; Il-6, $F_{30,376} = 43.0$, $p<0.0001$. Significantly different from same concentration of indoline, * $p<0.05$, ** $p<0.01$.

Figure 7. Reduction of NO, TNF-α and IL-6 in LPS activated macrophages, by indoline derivatives substituted at position 1 with chains carrying terminal primary or secondary amines. Data represent the mean ± SEM from 9-18 replicates per concentration of each compound. ANOVA for NO, $F_{30,568} = 30.8$, $p<0.0001$; TNF-α, $F_{30,444}$ 24.2, $p<0.0001$; Il-6, $F_{30,444} = 27.0$, $p<0.0001$. Significantly different from same concentration of **13a**, [a] $p<0.05$; [b] $p<0.01$. Significantly different from same concentration of **15a**, [c] $p<0.05$; [d] $p<0.01$.

Figure 8. Reduction of NO, TNF-α and IL-6 in LPS activated macrophages, by indoline derivatives substituted at position 1 with chains carrying terminal ester or acid

groups. Data represent the mean ± SEM from 9-18 replicates per concentration of each compound. ANOVA for NO, $F_{30,620} = 52.3$, $p<0.0001$; TNF-α, $F_{30,480} = 85.7$, $p<0.0001$; Il-6, $F_{30,480} = 39.5$, $p<0.0001$. Significantly different from same concentration of **7a**, [a] $p<0.05$; [b] $p<0.01$. Significantly different from same concentration of **7b**, [c] $p<0.05$; [d] $p<0.01$.

Figure 9. Reduction of NO, TNF-α and IL-6 in LPS activated macrophages, by indoline derivatives substituted at position 1 with chains carrying terminal amide or alcohol groups. Reduction by compounds with an amido or alcohol group in position 1 of indoline of NO, TNF-α and IL-6 in LPS activated macrophages. Data represent the mean ± SEM from 12-16 replicates per concentration of each compound. Only **18a** reduced NO and cytokines by 50% at concentration of 100 pM or less. Significantly different from same concentration of **18a**, [a] $p<0.05$; [b] $p<0.01$. Significantly different from same concentration of **9a**, [c] $p<0.05$; [d] $p<0.01$.

Figure 10. Effect of four indoline derivatives on levels of TNF-α and IL-6 in plasma of mice injected with LPS. Data represent mean ± SEM of 8-10 mice per treatment. Dex = dexamethasone (5.8 μmoles/kg). Significantly different from LPS alone, * $p<0.05$; ** $p<0.01$. Significantly different from values obtained with the preceding dose # $p<0.05$.

Figure 11. Effect of four indoline derivatives on levels of TNF-α and IL-6 in spleen of mice injected with LPS. Legend as in Figure 10.

Figure 12. Effect of four indoline derivatives on levels of IL-6 in brain and liver of mice injected with LPS. **A**. Brain; **B**. Liver. Legend as in Figure 10.

Table of Contents Graphic

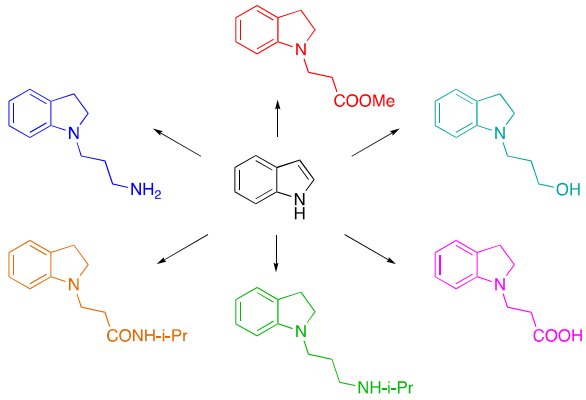
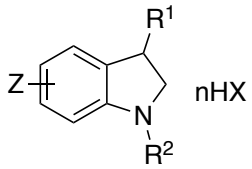


Table 1. Biologically Tested Indoline Derivatives



compd	Z	R^1	R^2	n	HX
1	4-(Et,Me)NCO_2	$CH_2CH_2CH_2NH_2$	H	2	HCl
2	6-(Et,Me)NCO_2	CH_2CH_2COOMe	H	1	HCl
3	H	CH_2CH_2COOMe	H	1	HCl
7a	H	H	CH_2CH_2COOMe	1	HCl
7b	5-MeO	H	CH_2CH_2COOMe	1	HCl
7b'	5-MeO	H	CH_2CH_2COOH	1	HCl
7c	6-Me	H	CH_2CH_2COOMe	1	HCl
7d	5-Cl	H	CH_2CH_2COOMe	1	*p*-TSA
7f	6-BnO	H	CH_2CH_2COOMe	1	*p*-TSA
9a	H	H	$CH_2CH_2CH_2OH$	1	HCl
9b	5-MeO	H	$CH_2CH_2CH_2OH$	1	HCl
13a	H	H	$CH_2CH_2CH_2NH_2$	1	HCl
13g	7-BnO	H	$CH_2CH_2CH_2NH_2$	2	*p*-TSA
15a	H	H	$CH_2CH_2CH_2NH$-*i*-Pr	2	HCl
15b	5-MeO	H	$CH_2CH_2CH_2NH$-*i*-Pr	2	HCl
15i	7-HO	H	$CH_2CH_2CH_2NH$-*i*-Pr	2	*p*-TSA
18a	H	H	CH_2CH_2CONH-*i*-Pr	1	HCl
18b	5-MeO	H	CH_2CH_2CONH-*i*-Pr	1	HCl
18d	5-Cl	H	CH_2CH_2CONH-*i*-Pr	1	HCl
18e	6-F	H	CH_2CH_2CONH-*i*-Pr	1	HCl
18h	6-Cl	H	CH_2CH_2CONH-*i*-Pr	1	HCl
23	H	$CH_2CH_2CH_2NH_2$	H	2	*p*-TSA

Table 2. Effect of Selected Compounds on Cell Viability[a]

	Concentration (M)					
compd	10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}
3	99.5 ± 3.9	93.2 ± 3.2	96.6 ± 3.4	97.1 ± 3.9	94.5 ± 2.4	92.9 ± 4.3
18d	95.6 ± 3.4	96.3 ± 3.3	98.2 ± 4.1	97.9 ± 4.9	104 ± 7	103 ± 5
7a	95.0 ± 3.6	91.5 ± 3.3	93.3 ± 3.9	92.6 ± 4.6	91.8 ± 5.1	90.8 ± 4.9
15a	93.3 ± 2.2	102 ± 2	99.2 ± 2.6	92.1 ± 2.9	90.9 ± 3.3	91.9 ± 5.9
9a	89.5 ± 1.0	89.9 ± 2.1	91.6 ± 3.6	95.1 ± 2.8	92.3 ± 2.7	94.4 ± 3.2
9b	91.9 ± 2.9	93.4 ± 3.2	93.9 ± 3.7	95.3 ± 2.5	91.4 ± 2.9	91.9 ± 3.0
7d	108 ± 5	106 ± 5	104 ± 4	104 ± 3	107 ± 6	105 ± 5

[a]Data represent percent viability as mean ± SEM of 6-9 replicates per concentration

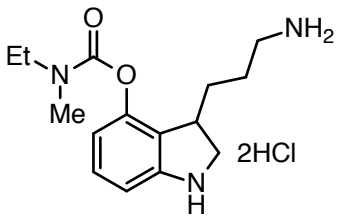
Table 3. Lowest Concentration at which Compounds Reach Cell Viability of that in Vehicle Treated Controls

		% of Vehicle			
compd	concentration	1 pM	1 nM	10 nM	100 nM
Vehicle	100.2 ± 1.8				
Vehicle + H_2O_2	71.2 ± 1.3				
8a				90.4 ± 3.1	
Amines					
13a			91.9 ± 2.7		
13g					79.3 ± 1.6*
15a		98.5 ± 3.5			
15h					89.7 ± 5.1
15b				87.1 ± 1.8	
Esters and acid					
Vehicle	100.7 ± 1.8				
Vehicle + H_2O_2	68.0 ± 0.8				
7a				85.9 ± 2.0	
7d		89.7 ± 1.3			
7c				87.2 ± 3.7	
7b		91.0 ± 6.8			
7b'					87.1 ± 1.8
Amides					
Vehicle	100.1 ± 1.3				
Vehicle + H_2O_2	70.7 ± 1.0				
18a		93.7 ± 2.8			
18d		95.7 ± 2.0			
18e					86.9 ± 3.5
Alcohols					
Vehicle	100.6 ± 3.1				
Vehicle + H_2O_2	72.8 ± 1.7				
9a					89.8 ± 6.8
9b		95.6 ± 2.3			

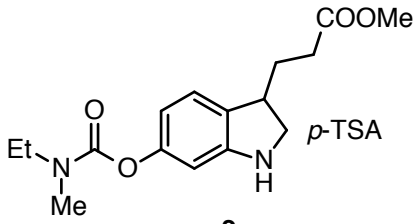
Significantly different from H_2O_2 and vehicle, *$p<0.05$.

ANOVA: Amines, $F_{31,493} = 17.51$, $p<0.0001$; Esters and acid, $F_{31,508} = 11.59$, $p<0.0001$; Amides, $F_{19,353} = 21.95$, $p<0.0001$; Alcohols, $F_{13,195} = 10.65$, $p<0.0001$.

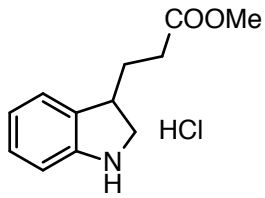
Scheme 1. Indolines Substituted at Position 3



1

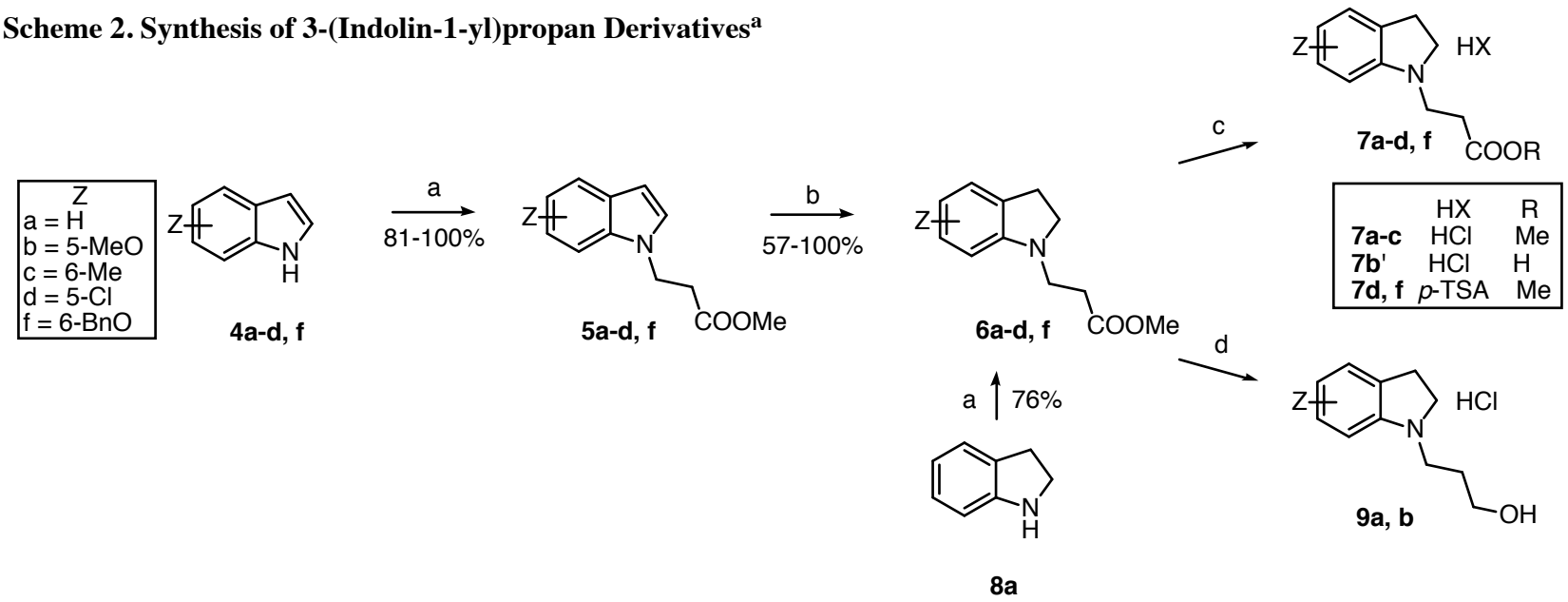


2



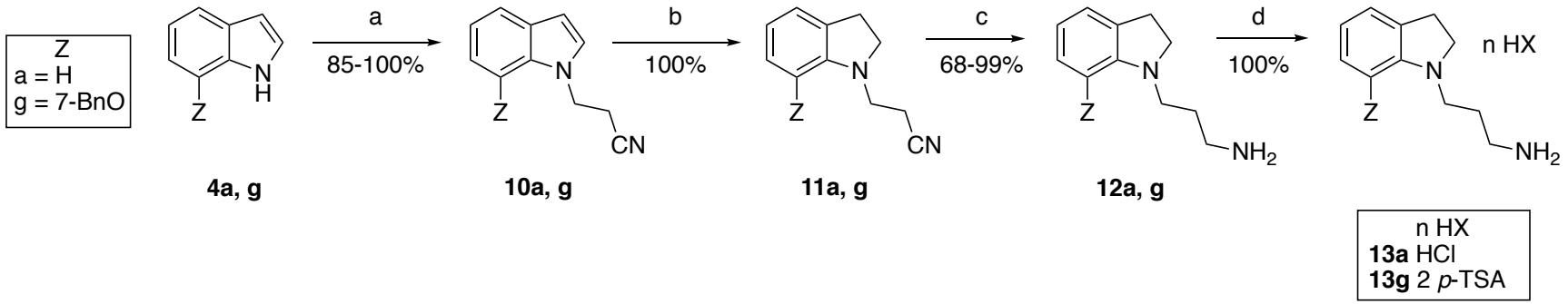
3

Scheme 2. Synthesis of 3-(Indolin-1-yl)propan Derivatives[a]



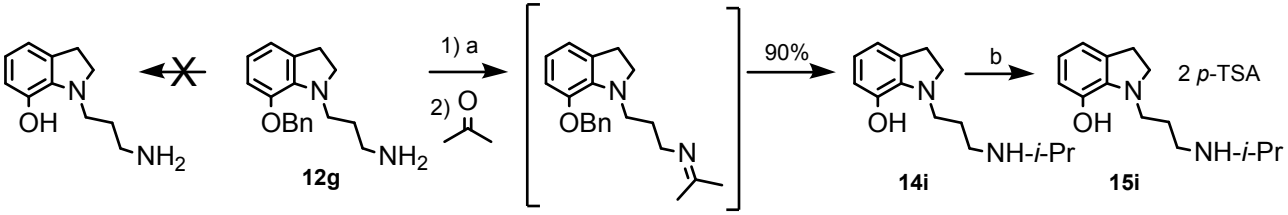
[a]Reagents and conditions: a) CH_2=CHCOOMe/MeCN/DBU/50 °C; b) $NaCNBH_3$/AcOH or Et_3SiH/TFA or $NaBH_4$/TFA; c) HCl(g) or *p*-TSA/*tert*-butyl methyl ether; d) LAH/THF/HCl

Scheme 3. Synthesis of 3-(Indolin-1-yl)propan-1-amines[a]



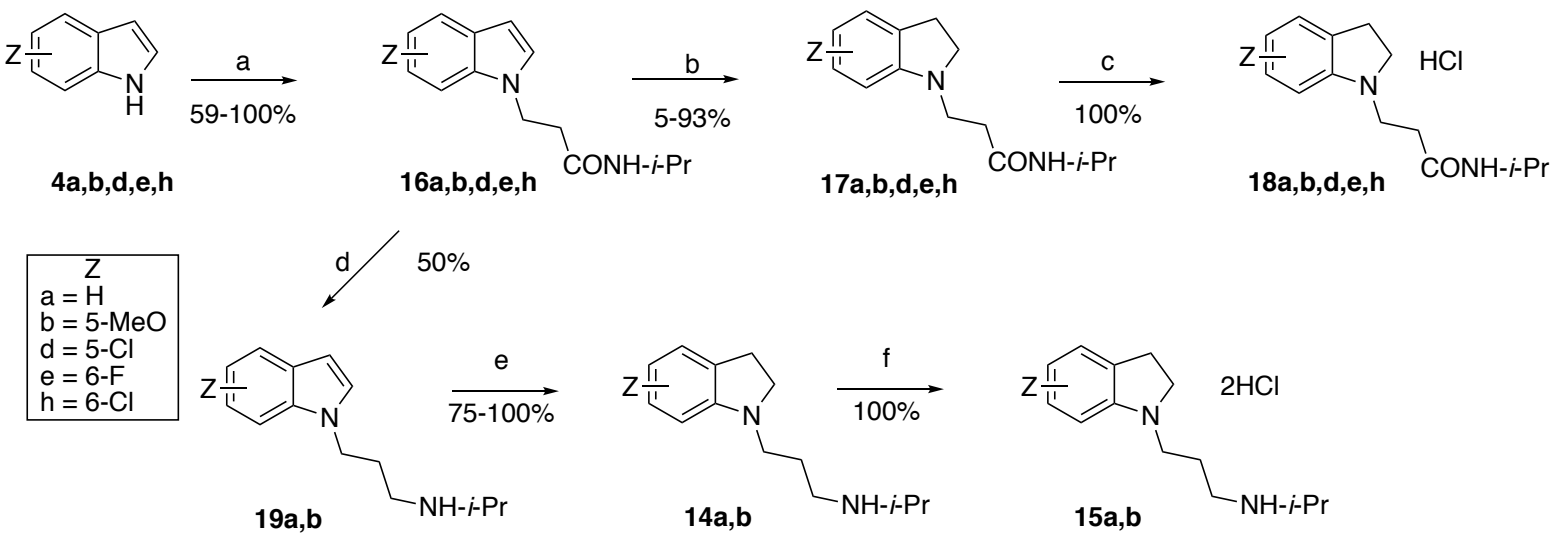
[a]Reagents and conditions: (a) CH_2=CHCN/Triton B/dioxane, 0 °C to rt; (b) $NaCNBH_3$/AcOH, rt; (c) LAH/diethyl ether; (d) *p*-TSA/*tert*-butyl methyl ether or HCl/ether

Scheme 4. Synthesis of 1-(3-Isopropylamino)propyl)indolines[a]



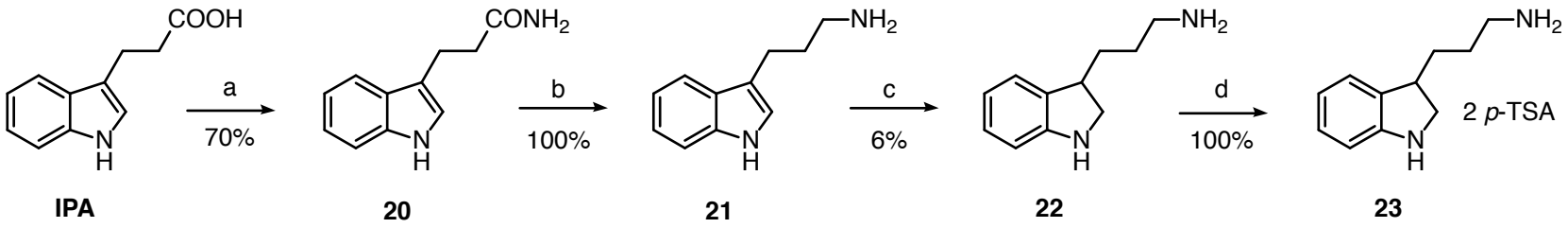
[a]Reagents and conditions: (a) H_2/10% Pd/C/MeOH, 4 atm (and traces of acetone in the hydrogenator); (b) *p*-TSA/*tert*-butyl methyl ether

Scheme 5. Synthesis of 3-(Indolin-1-yl)-*N*-isopropylpropanamides 18 and 3-(Indolin-1-yl)-*N*-isopropylpropan-1-amines 15[a]

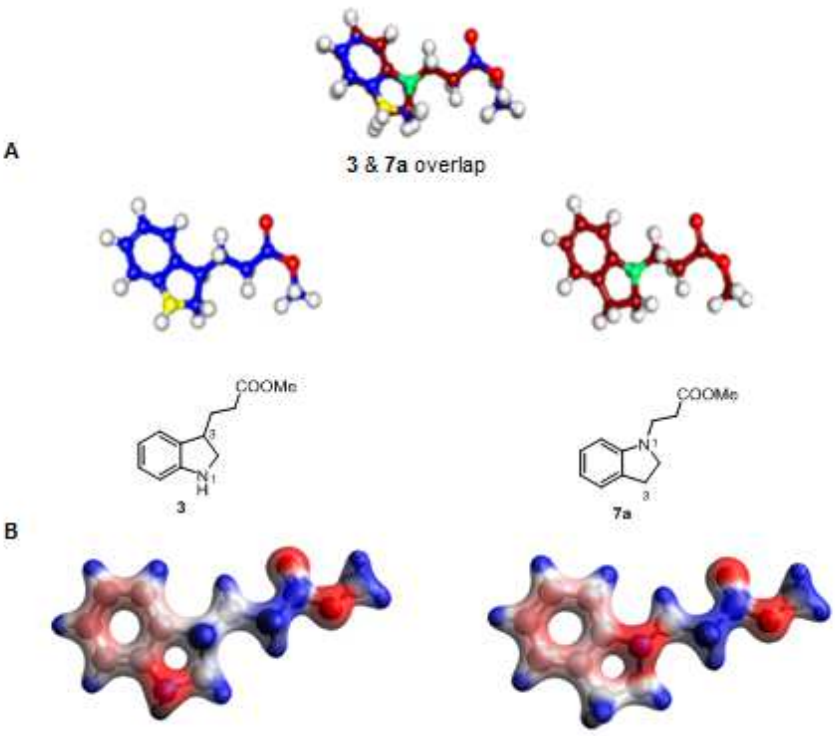


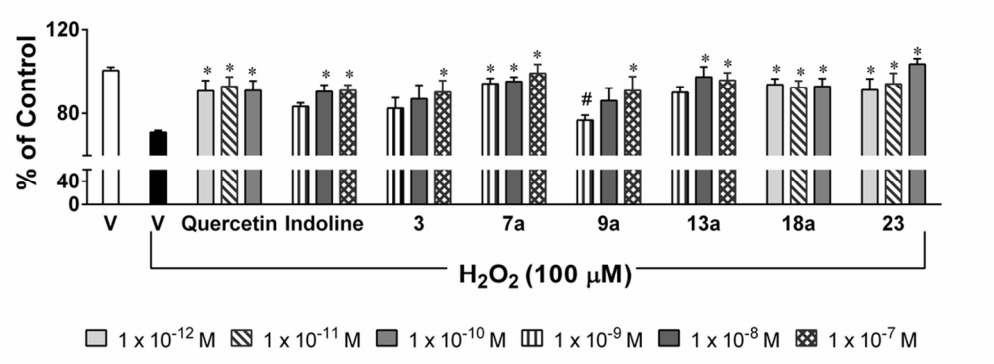
[a]Reagents and conditions: (a) CH_2=CH-CONH-*i*-Pr/KOH/Dioxane; (b) $NaBH_4$/TFA; (c) HCl (g)/diethyl ether or HCl 3N/EtOAc; (d) LAH/THF; (e) $NaCNBH_3$/AcOH or $NaBH_4$/TFA; (f) HCl (g)/diethyl ether

Scheme 6. Synthesis of 3-(Indolin-3-yl)propan-1-amidine di-p-Toluenesulfonate, 23[a]



[a]Reagents and conditions: a) CDI/NH_4OH; b) LAH/THF; c) Et_3SiH/TFA; d) *p*-TSA


A
3 & 7a overlap
COOMe
N1
H
3
COOMe
N1
3
7a
B



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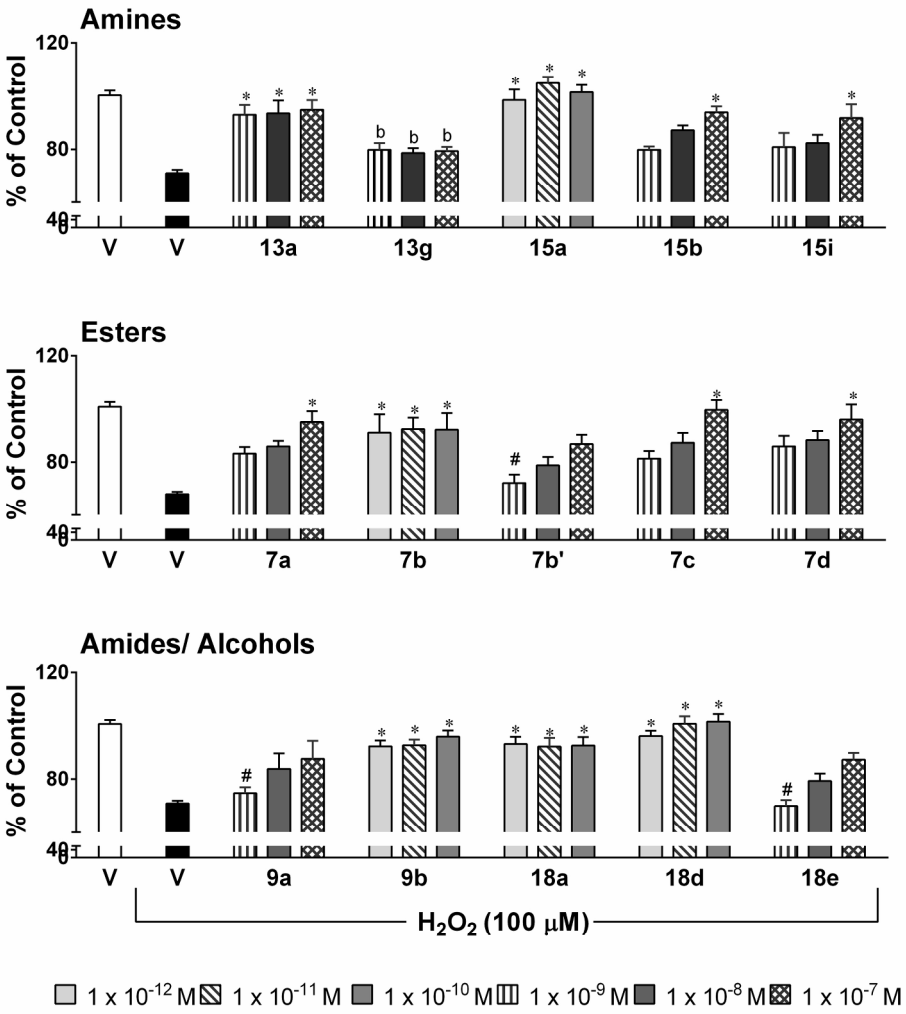
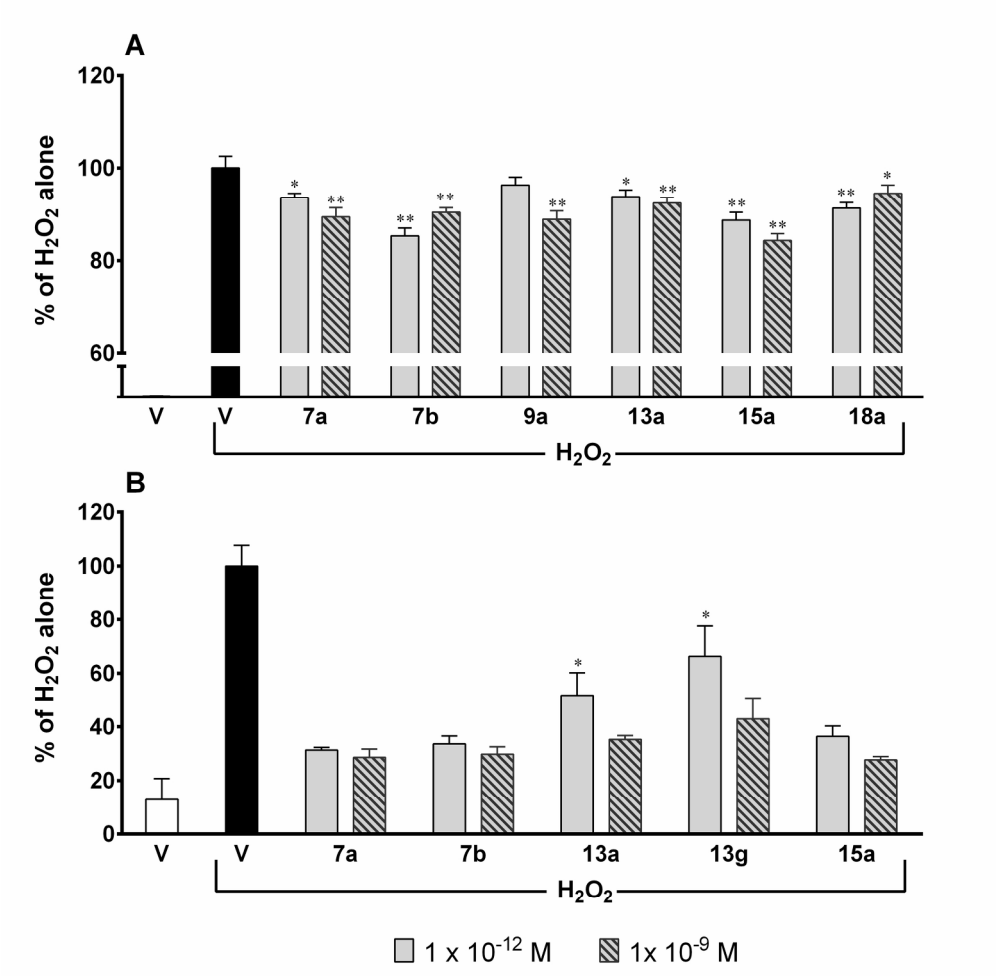
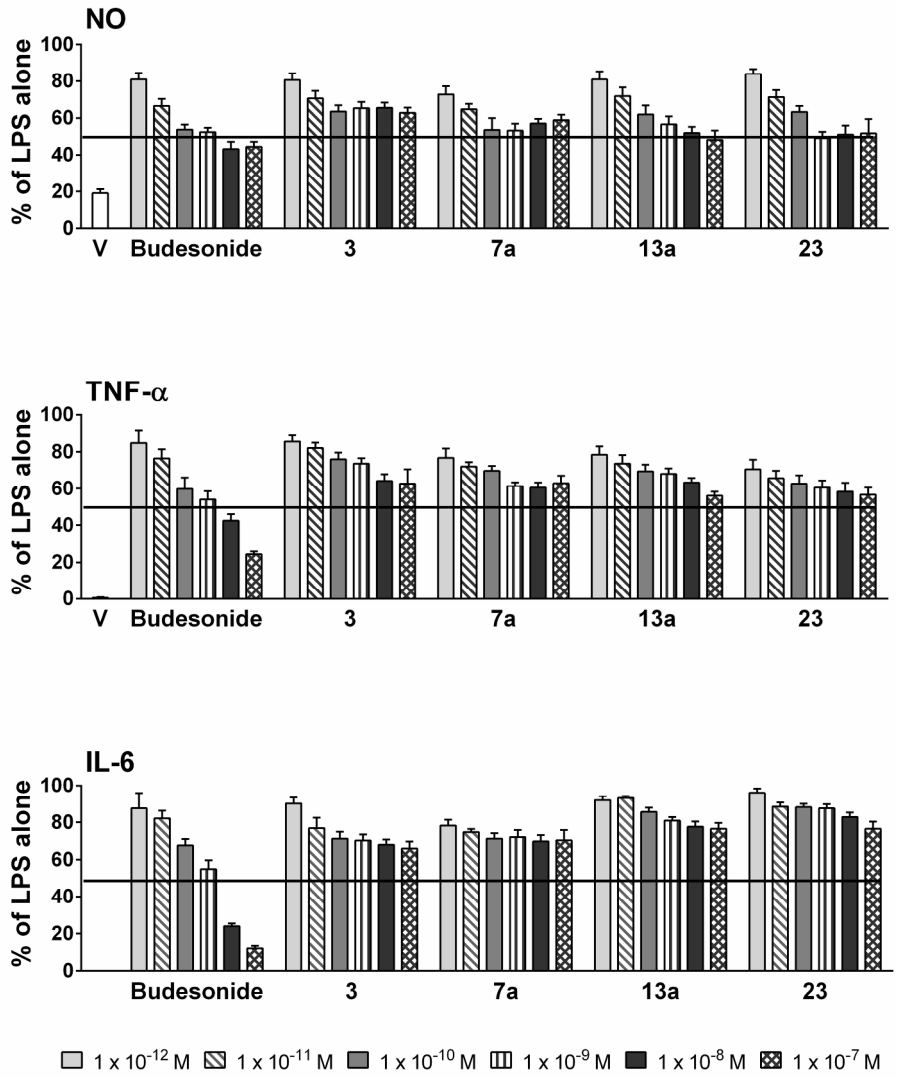


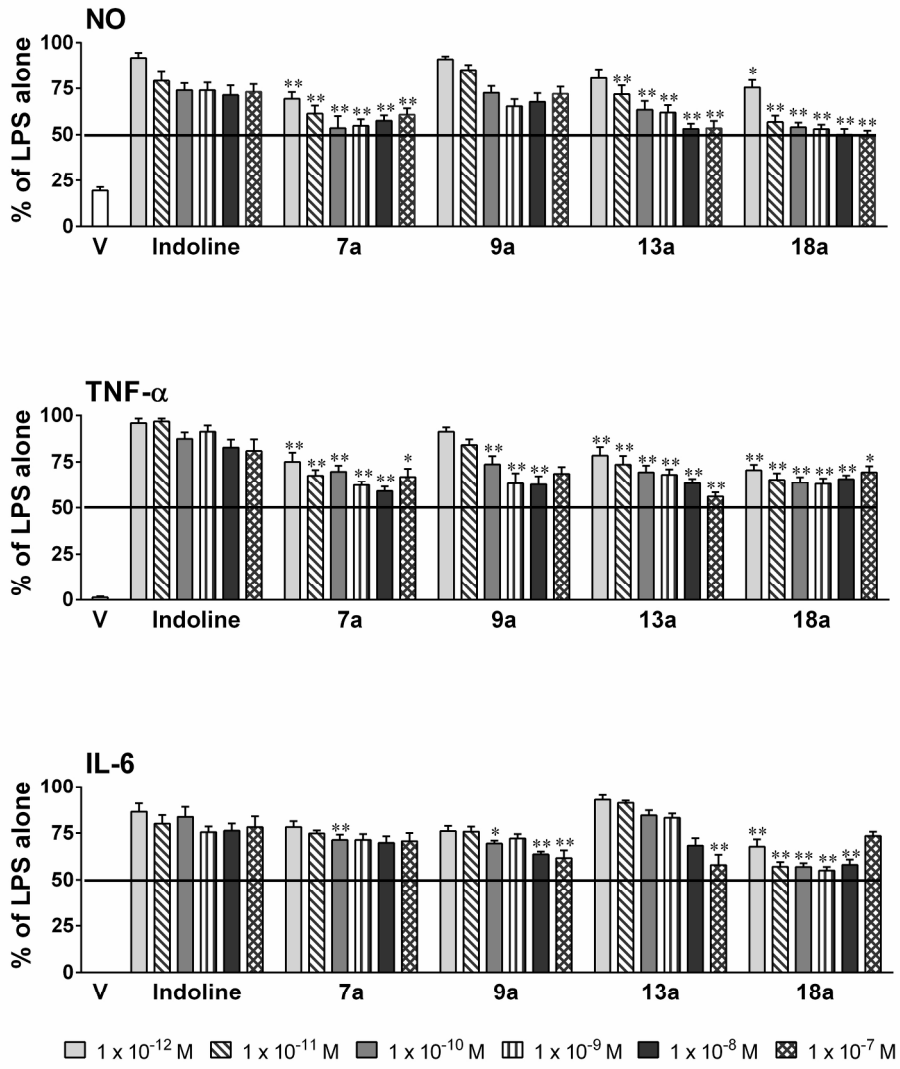
216x230mm (300 x 300 DPI)



204x203mm (300 x 300 DPI)



229x259mm (300 x 300 DPI)



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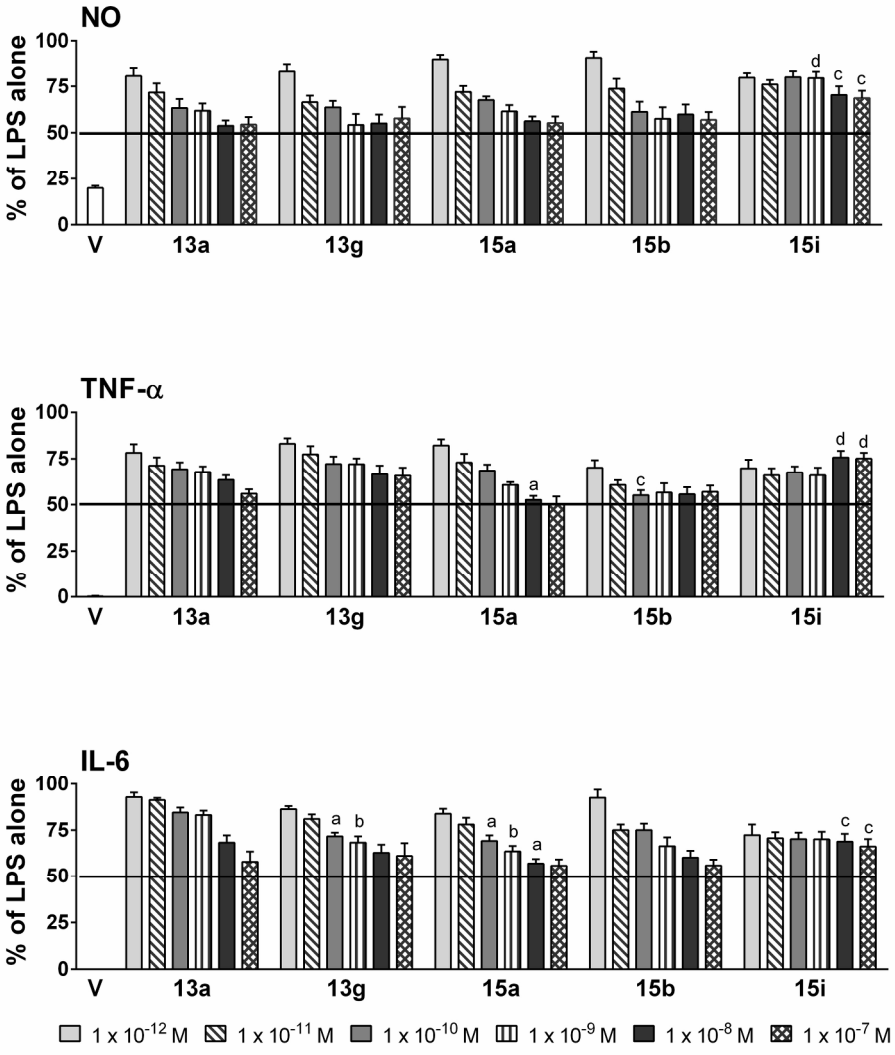
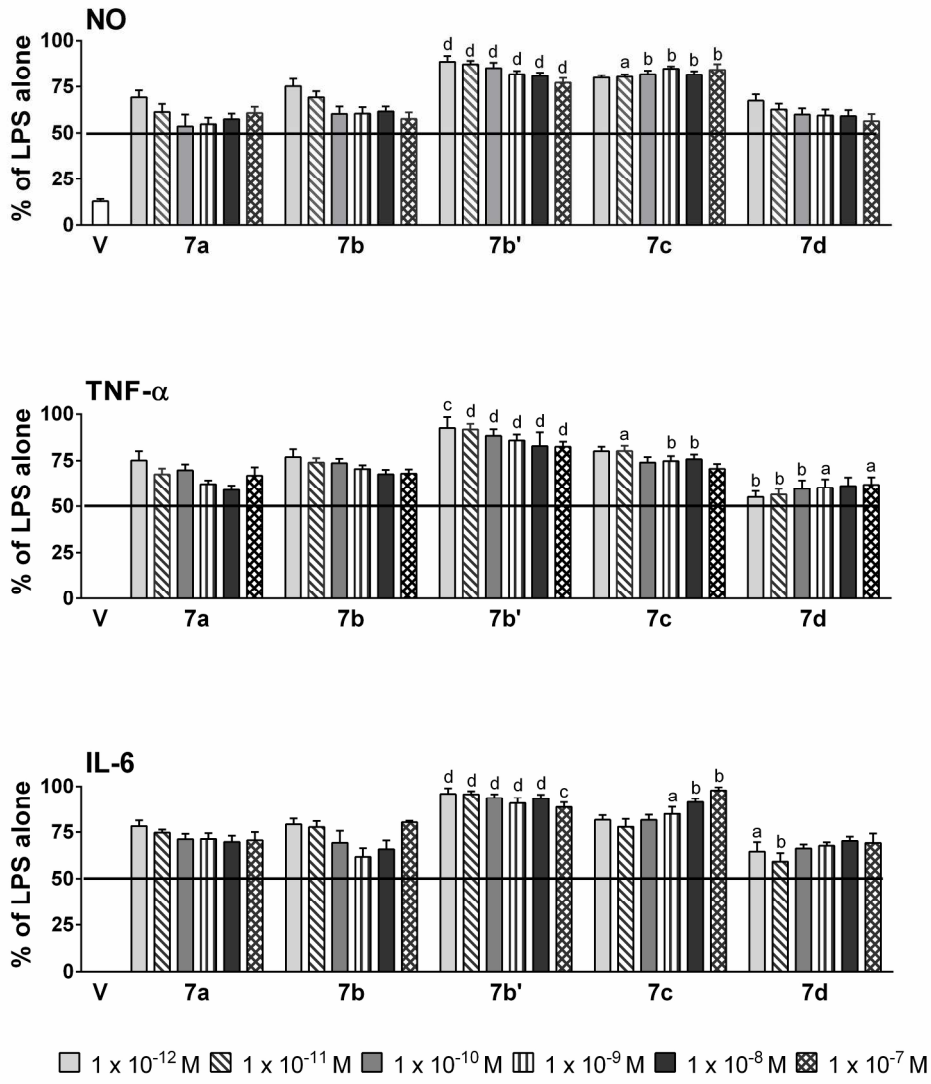
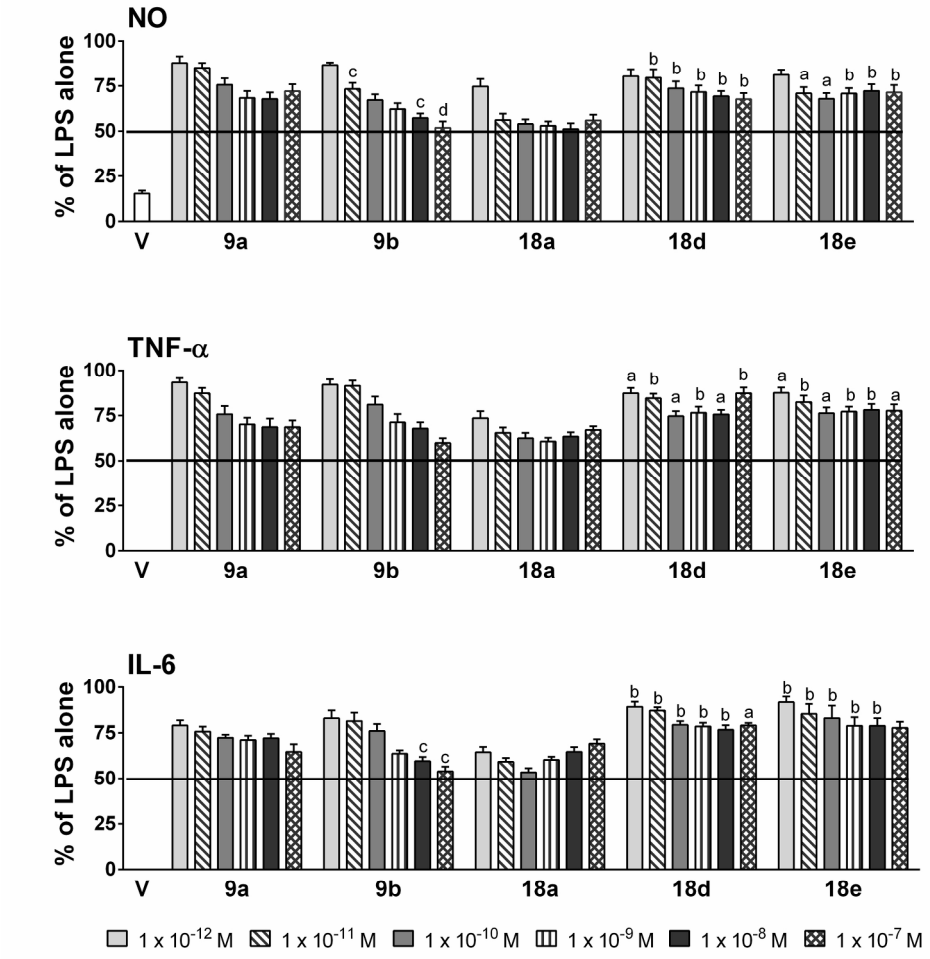


224x248mm (300 x 300 DPI)



230x261mm (300 x 300 DPI)



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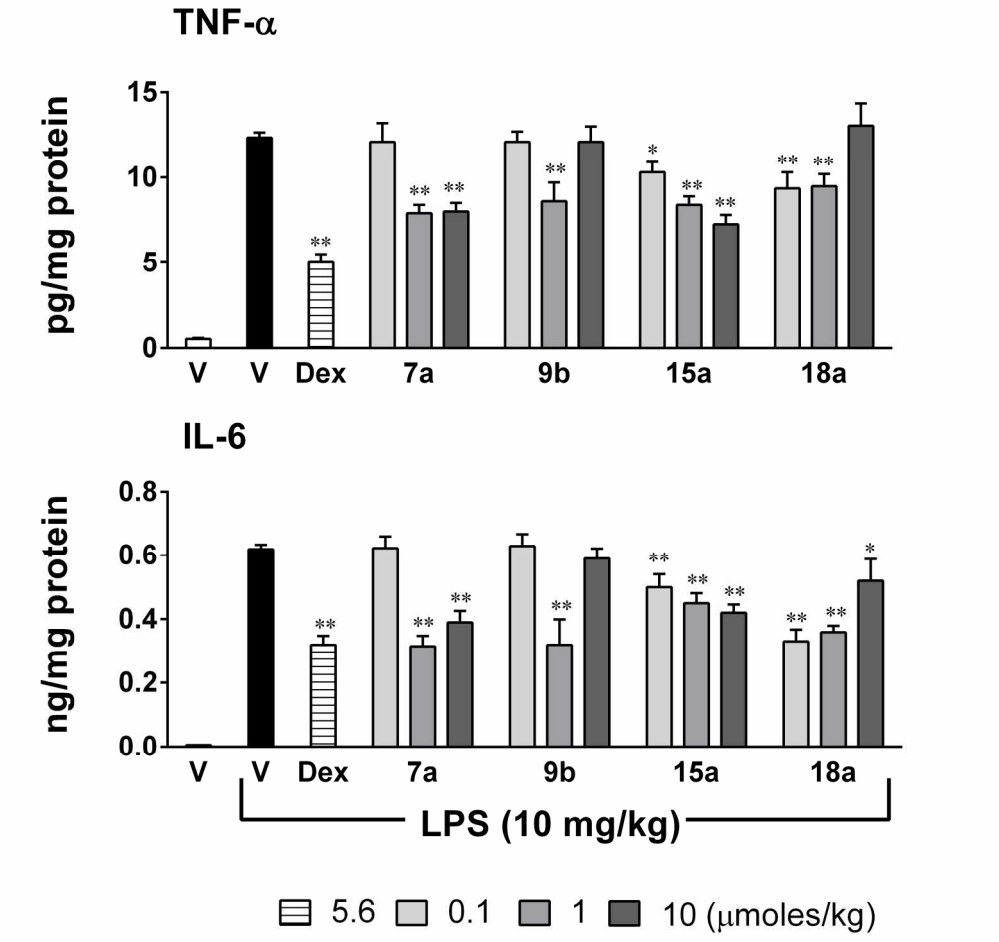

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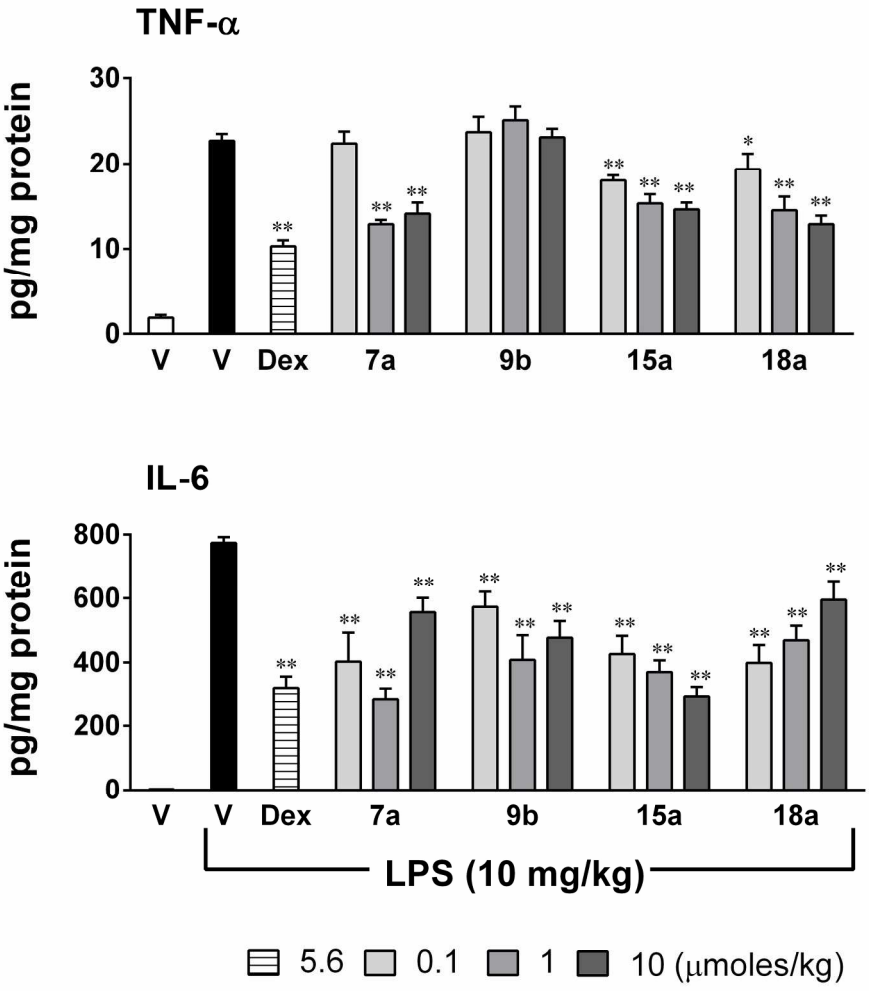
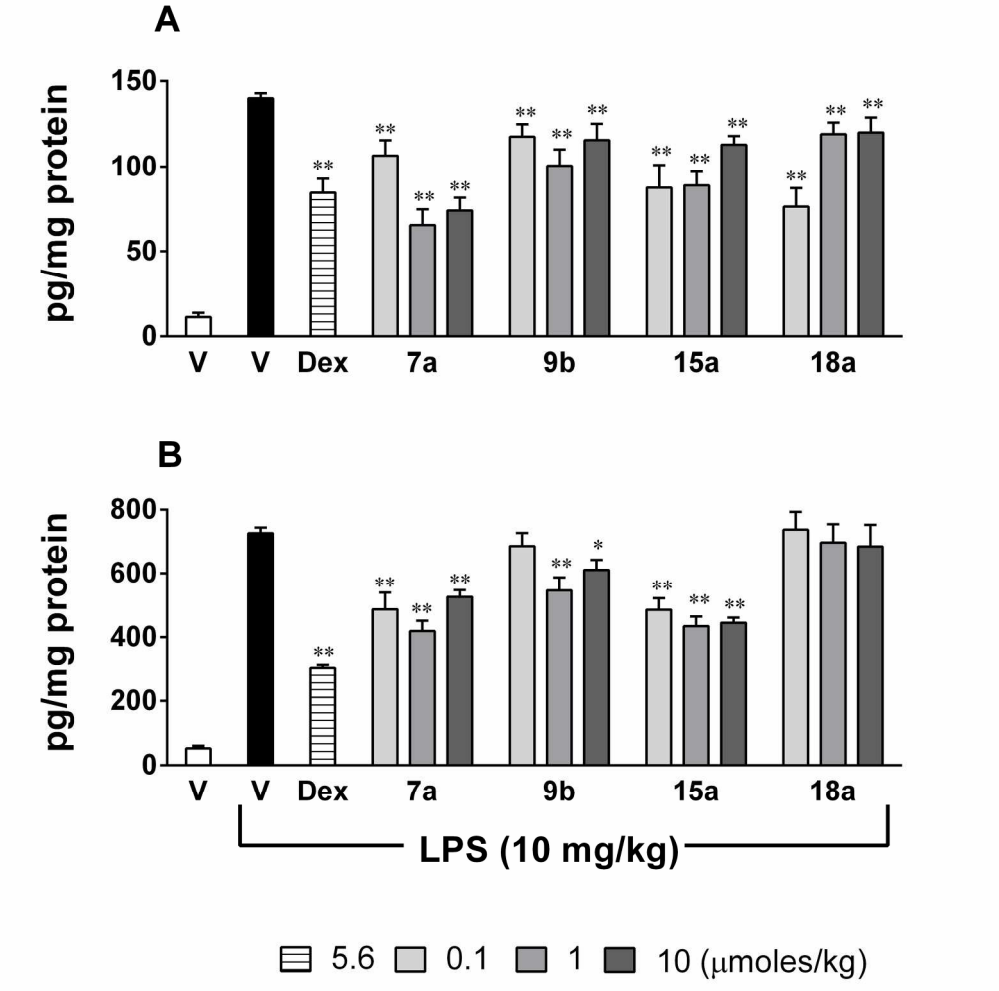
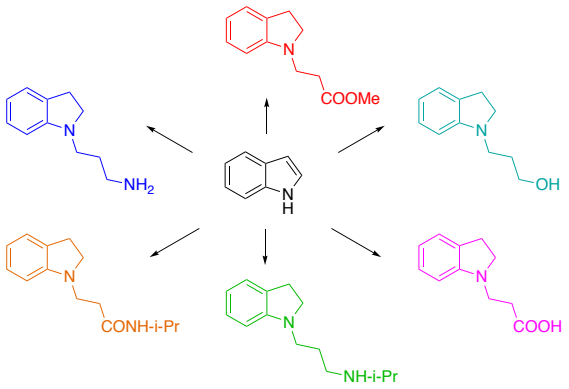


206x214mm (300 x 300 DPI)



209x216mm (300 x 300 DPI)


COOMe
NH2
OH
CONH-i-Pr
COOH
NH-i-Pr