

**Design, synthesis and biological evaluation of hybrid nitroxide-based non-steroidal anti-inflammatory drugs**

Thomas, Komba; Moody, Terry W.; Jensen, Robert T.; Tong, Jason; Rayner, Cassie L.; Barnett, Nigel L.; Fairfull-Smith, Kathryn E.; Ridnour, Lisa A.; Wink, David A.; Bottle, Steven E.

*Published in:*  
European Journal of Medicinal Chemistry

*DOI:*  
[10.1016/j.ejmech.2018.01.077](https://doi.org/10.1016/j.ejmech.2018.01.077)

*Licence:*  
CC BY-NC-ND

[Link to output in Bond University research repository.](#)

*Recommended citation(APA):*

Thomas, K., Moody, T. W., Jensen, R. T., Tong, J., Rayner, C. L., Barnett, N. L., Fairfull-Smith, K. E., Ridnour, L. A., Wink, D. A., & Bottle, S. E. (2018). Design, synthesis and biological evaluation of hybrid nitroxide-based non-steroidal anti-inflammatory drugs. *European Journal of Medicinal Chemistry*, 147, 34-47. <https://doi.org/10.1016/j.ejmech.2018.01.077>

**General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

For more information, or if you believe that this document breaches copyright, please contact the Bond University research repository coordinator.

# Design, Synthesis and Biological Evaluation of Hybrid Nitroxide-Based Non-Steroidal Anti-inflammatory Drugs

*Komba Thomas<sup>1</sup>, Terry W. Moody<sup>2</sup>, Robert T. Jensen<sup>2</sup>, Jason Tong<sup>3</sup>, Cassie L Rayner<sup>3</sup>, Nigel L Barnett<sup>3,4</sup>, Kathryn E. Fairfull-Smith<sup>1</sup>, Lisa A. Ridnour<sup>2</sup>, David A. Wink<sup>2</sup> and Steven E. Bottle<sup>1</sup>*

<sup>1</sup>School of Chemistry, Physics and Mechanical Engineering, Queensland University of Technology, (QUT) GPO Box 2434, Brisbane, QLD 4001, Australia

<sup>2</sup>Center for Cancer Research, National Cancer Institute, Cancer and Inflammation Program, Frederick, MD 21702-1201, USA

<sup>3</sup>Queensland Eye Institute, South Brisbane, Queensland, Australia

<sup>4</sup>The University of Queensland, UQ Centre for Clinical Research, Herston, Queensland, Australia

**Keywords:** Antioxidants/ Inflammation/ Cyclooxygenase/ Dual-Action/ Nitroxides/ A549 Non-Small Cell Lung Cancer/ NSAID/ Oxidative Stress/ Retina/ Photoreceptors/ Pharmacophore Hybridization/ Reactive Oxygen Species

**ABSTRACT:** Dual-acting hybrid anti-oxidant/anti-inflammatory agents were developed employing the principle of pharmacophore hybridization. Hybrid agents were synthesised by combining stable anti-oxidant nitroxides with conventional non-steroidal anti-inflammatory drugs (NSAIDs). Several of the hybrid nitroxide-NSAID conjugates displayed promising anti-oxidant and anti-inflammatory effects on two Non-Small Cell Lung Cancer (NSCLC) cells (A549 and NCI-H1299) and in ameliorating oxidative stress induced in 661W retinal cells. One ester-linked nitroxide-aspirin analogue (**27**) delivered better anti-inflammatory effects (cyclooxygenase inhibition) than the parent compound (aspirin), and also showed similar reactive oxygen scavenging activity to the anti-oxidant, Tempol. In addition, a nitroxide linked to the anti-inflammatory drug indomethacin (**39**) significantly ameliorated the effects of oxidative stress on 661W retinal neurons at efficacies greater or equal to the anti-oxidant Lutein. Other examples of the hybrid conjugates displayed promising anti-cancer activity, as demonstrated by their inhibitory effects on the proliferation of A549 NSCLC cells.

## **INTRODUCTION**

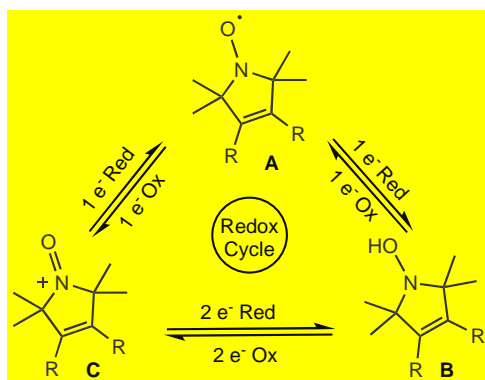
Chronic inflammation is a key contributing factor in the mechanisms underlying the pathogenesis of numerous inflammatory and neurodegenerative diseases such as rheumatoid arthritis, atherosclerosis, Parkinson's disease, and some cancers [1-10]. Chronic inflammation is mainly characterized by oxidative stress and prolonged and excessive inflammation that may translate into an irreparable damage to host tissues and, in severe situations, organ malfunction or death [11, 12]. In general, non-steroidal anti-inflammatory drugs (NSAIDs) are the most common therapeutic agents used to manage inflammatory symptoms [9, 13-15]. NSAIDs are a structurally diverse group of drugs with a similar mode of action. They exert their therapeutic action (as anti-pyretics, anti-inflammatories and analgesics) mainly by

inhibiting the *cyclooxygenase* (COX) enzyme [6, 14-24]. COX has two main isoforms: COX-1 is the constitutively expressed isoform that, under physiological conditions, is involved in basic cytoprotective functions such as maintaining the gastrointestinal mucosal integrity. COX-2 is inducibly expressed, mainly in response to inflammatory stimuli from infections or injuries [6,9,13].

Most traditional NSAIDs, such as indomethacin and aspirin, inhibit both COX-1 and COX-2 enzymes. The non-selectivity of conventional NSAID therapy can lead to adverse side effects, notably gastrointestinal ulceration and bleeding, platelet dysfunction and renal complications, as a result of decreased levels of cytoprotective prostaglandins [25]. Notably, oxidative stress is recognized as a major contributor to NSAID-induced gastric mucosa ulceration [26].

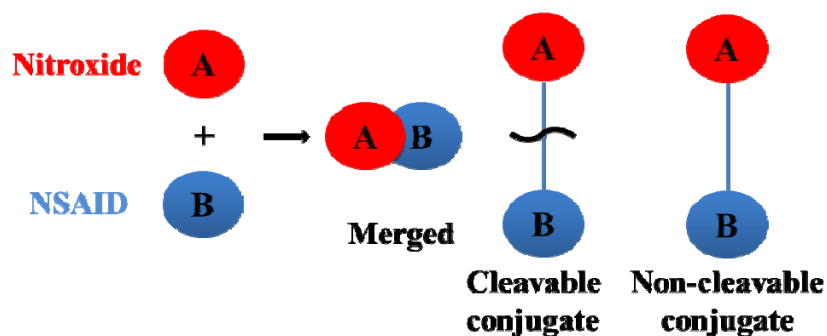
Thus, to effectively manage chronic inflammatory diseases and limit the associated NSAID-induced damage, there is a clear need for an effective anti-oxidant intervention. Our approach to this [27] was to exploit the anti-oxidant capacity of stable nitroxide compounds - which is mainly attributed to the redox cycle that involves the nitroxide (**A**), and its hydroxylamine (**B**) and oxoammonium ion (**C**) derivatives (**Scheme 1**). This redox cycle enables nitroxides to protect biological tissues against oxidative stress, potentially via superoxide dismutase-mimetic activity, via direct scavenging of radicals and reaction with reactive oxygen species (ROS), and/or via the inhibition of lipid peroxidation processes and enzymes that produce ROS such as myeloperoxidase [1, 28, 29].

**Scheme 1.** Reversible redox cycle of nitroxides.



Our aim in this work was to employ the pharmacophore hybridization strategy [31, 31] to synthetically combine anti-oxidant nitroxides with a series of NSAIDs to produce novel hybrid dual-acting, nitroxide-based NSAID agents. The hybrid agents were constructed by either merging the two structural subunits or via cleavable (ester and amide bonds) and non-cleavable (amine bond) linkages (**Scheme 2**). We anticipated that the hybrid agents would retain the anti-inflammatory therapeutic benefits of the parent templates (anti-oxidant and anti-inflammatory effects) and at the same time, the presence of the nitroxide unit would minimize the drug-induced oxidative stress-related side effects. To this end, we report herein the synthesis and some properties of NSAID pharmacophores (32 examples including aspirin, salicylic acid, indomethacin, 5-aminosalicylic acid 5-ASA and 2-hydroxy-5-[2-(4-trifluoromethylphenyl)-ethylaminobenzoic acid) linked with various nitroxide compounds and the therapeutic evaluation of representative lead compounds on 3 well studied cell lines linked to oxidative stress.

**Scheme 2.** The design of novel nitroxide-NSAID agents employing pharmacophore hybridization strategies “

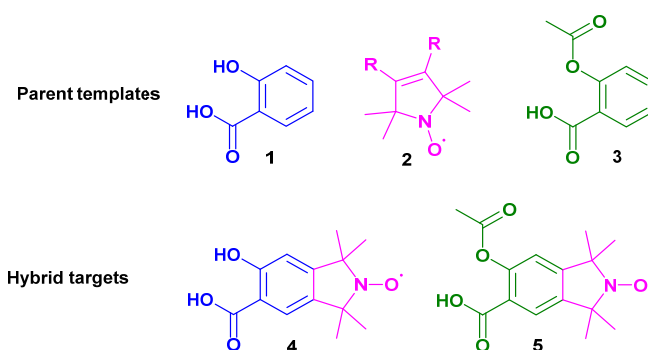


<sup>a</sup>Nitroxides (A) and NSAIDs (B) are chemically combined to produce merged, cleavable and non-cleavable hybrid agents.

## RESULTS AND DISCUSSION

**Chemistry.** The salicylate class of NSAIDs was first incorporated with anti-oxidant nitroxides by taking advantage of the structural similarities of the parent templates. Specifically, pyrroline nitroxide **2** was merged with salicylic acid **1** and acetylsalicylic acid **3** to produce new hybrid nitroxide-salicylate molecules 5-carboxy-6-hydroxy-1,1,3,3-tetramethylisoin-dolin-2-yloxy **4** (salicylic acid-TMIO) and 5-carboxy-6-acetoxy-1,1,3,3-tetramethylisoin-2-yloxy **5** (aspirin-TMIO) as shown in **Figure 1**.

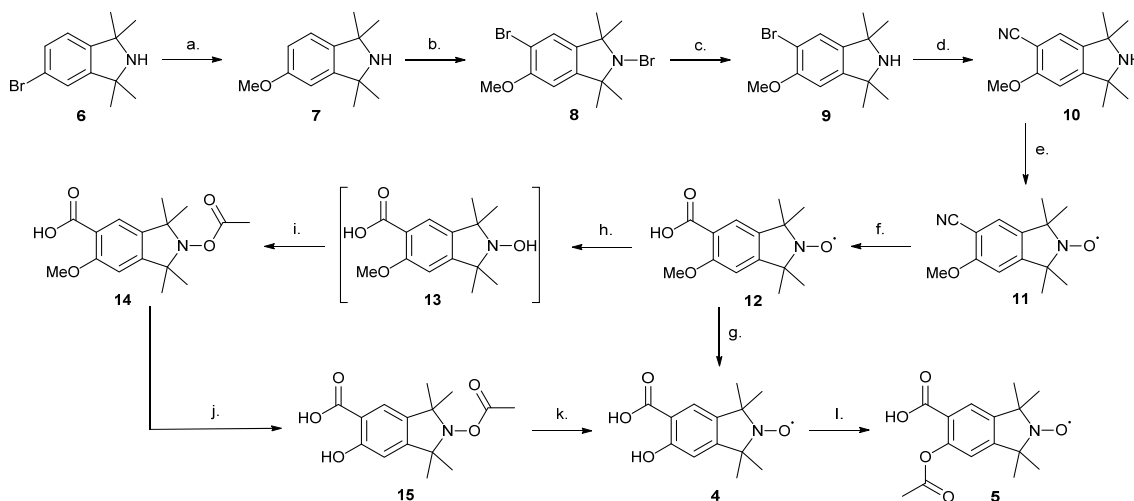
**Figure 1.** Parent templates and merged-hybrid nitroxide-salicylate target compounds



The synthesis of the merged-hybrid target compounds **4** and **5** is outlined in **Scheme 3**. The 5-bromo-1,1,3,3-tetramethylisoin-doline precursor **6** was synthesized in three steps from

commercially available phthalic anhydride by following previous established literature procedures [32].

**Scheme 3.** Synthesis of merged salicylic acid TMIO **4** and aspirin TMIO **5**<sup>a</sup>

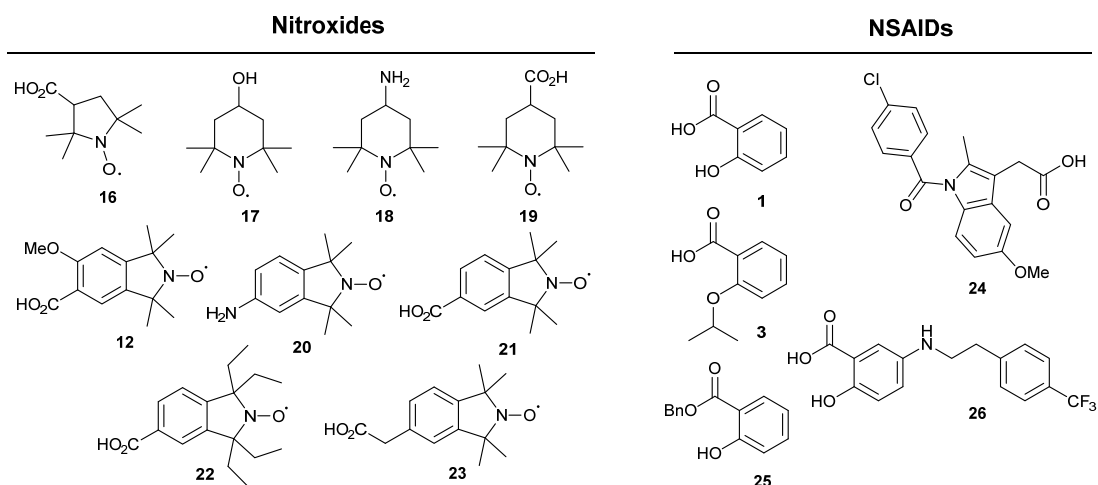


<sup>a</sup>Reagents and conditions: a. NaOMe/ MeOH, DMF, reflux, Ar, 85%; b. Br<sub>2</sub>, AlCl<sub>3</sub>, DCM, 0 °C, 1 h, Ar, 87%; c. H<sub>2</sub>O<sub>2</sub>, NaHCO<sub>3</sub>, DCM/MeOH, 5 min, 98%; d. K<sub>4</sub>[Fe(CN)<sub>6</sub>], *n*Bulmi, CuI, *o*-xylene, 180 °C, 3 d, 78%; e. *m*CPBA, DCM, 0 °C, 90%; f. NaOH, EtOH/H<sub>2</sub>O reflux, 16 h, 89%; g. BBr<sub>3</sub>, DCM, -78 °C-RT, 18 h, 40%; h. H<sub>2</sub>, Pd/C, THF, RT, Ar, 15 min, then i. AcCl, TEA, 0 °C, 1.5 h, 96%; j. BBr<sub>3</sub>, DCM, -78 °C-RT, 18 h, 85%; k. LiOH, H<sub>2</sub>O/MeOH, 0 °C, RT 20 min, PbO<sub>2</sub>, 92%; l. TEA, AcCl, THF, 0 °C-RT, 3 h, Ar, 91%.

When bromoamine **6** was subjected to a copper (I) catalyzed methanolysis, in the presence of dimethylformamide as co-solvent, the 5-methoxyamine derivative **7** was obtained in 88% yield. Selective ring mono-bromination of **7**, achieved with bromine in the presence of anhydrous aluminium chloride, yielded the 2,5-dibromo compound **8** in good yield (78%) which was subsequently reduced to the corresponding secondary amine **9** in excellent yield (98%). The cyanation of **9** was achieved using potassium hexacyanoferrate(II) (K<sub>4</sub>[Fe(CN)<sub>6</sub>]), as the cyanide source. In this case, the aminonitrile **10** was obtained in high yield by heating a reaction mixture of **9** and K<sub>4</sub>[Fe(CN)<sub>6</sub>] at reflux in the presence of catalytic CuI with *N*-butylimidazole as a co-solvent. The amino nitrile **10** was oxidized to the corresponding

nitroxide derivative **11** (94%) under mild oxidation conditions using *m*-chloroperoxybenzoic acid (*m*CPBA). Basic hydrolysis of **11** furnished the corresponding carboxylic acid **12** in 89% yield. The target salicylic acid TMIO **4** was obtained initially by direct de-methylation of compound **12** using boron tribromide. Using this reagent however only gave compound **4** in a modest yield (40%). The low yield was attributed to the potential formation of a complex between BBr<sub>3</sub> and the nitroxide moiety. Such nitroxide-BBr<sub>3</sub> complex formation could initiate multiple degradation pathways for both the starting material and the desired nitroxide targets. Alternatively, the nitroxide moiety of **12** was first protected with an acetyl protecting group prior to the de-methylation. This was achieved by first reducing compound **12** to its corresponding hydroxylamine **13** via palladium-catalyzed hydrogenation. The *in situ* generated hydroxylamine **13** was then allowed to react with acetyl chloride in the presence triethylamine to give the *N*-acetyl protected compound **14**. Compound **14** was then de-methylated using boron tribromide to afford *N*-acetoxy salicylic acid **15** which was subsequently hydrolyzed to the desired salicylic acid TMIO **4** in 75% overall yield over the three steps from **12**. The target aspirin-TMIO **5** was obtained almost quantitatively by acetylating salicylic acid-TMIO **4** with acetyl chloride in the presence of triethylamine.

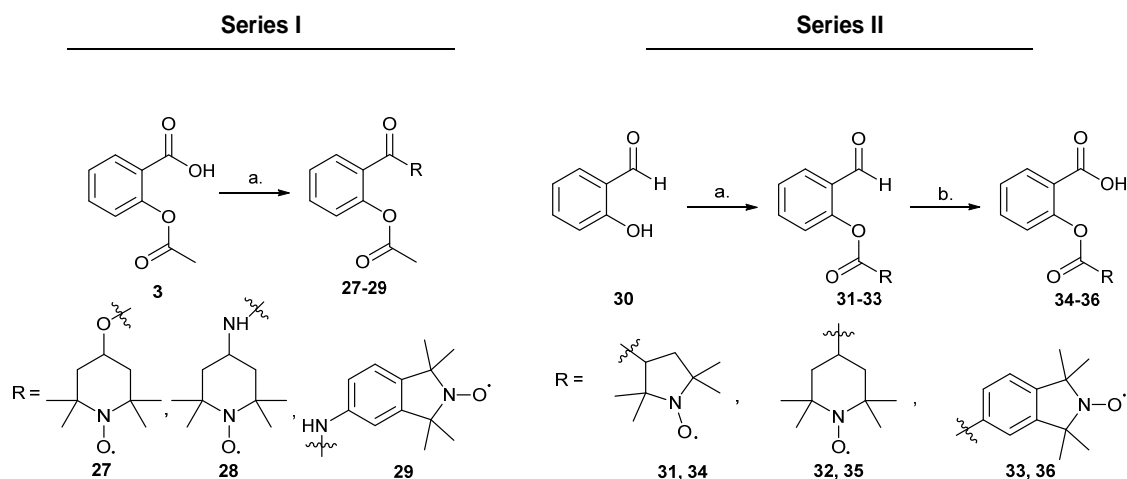
**Figure 2.** Chemical structures of parent nitroxides and NSAIDs.





**Figure 2** shows the structures of the parent templates (stable nitroxides and NSAIDs) used for the synthesis of the cleavable and non-cleavable nitroxide-NSAID hybrid conjugates. The ester and amide-linked nitroxide-aspirin conjugates (**27-29**) were synthesized by reacting aspirin with the respective nitroxide precursors (**17**, **18** and **20**) under carbodiimide coupling conditions (**Scheme 4**, Series I).

**Scheme 4.** Synthesis of two series of salicylate-nitroxide conjugates <sup>a,b</sup>

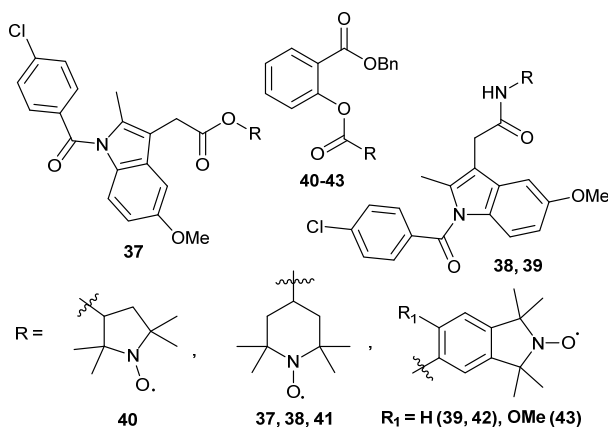


<sup>a</sup> Series I: Reagents and conditions: a. Nitroxide (**17**, **18** or **20**), EDC, DMAP, DCM, RT, 1 d, 53-90%.

<sup>b</sup> Series II: Reagents and conditions: a. Nitroxide (**16**, **19** or **21**), EDC, DMAP, DCM, RT, overnight, 83-91%; b. NaClO<sub>2</sub>, NaH<sub>2</sub>PO<sub>4</sub>, H<sub>2</sub>O<sub>2</sub>, MeCN, 0 °C-RT, 80-88%.

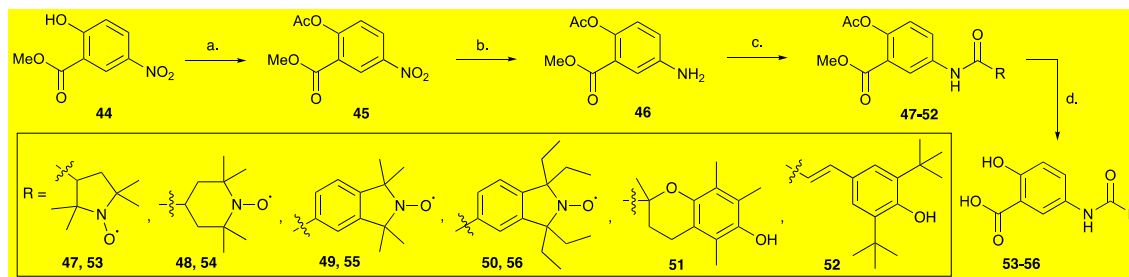
With the next set of target nitroxide-salicylate conjugates (**34-36**), the *o*-formyl phenyl ester nitroxide intermediates (**31-33**) were obtained following the carbodiimide coupling of carboxylic acid nitroxides (**16**, **19** and **21**) with salicylaldehyde **30** (**Scheme 4**, Series II). The *o*-formyl derivatives (**31-33**) were then oxidized to the corresponding salicylates (**34-36**) in high yields (80-88%) under Pinnick oxidation conditions. Similar structural modifications were carried out with indomethacin **24** and benzyl 2-hydroxybenzoate **25** to give novel indomethacin and benzyl salicylate-nitroxide cleavable conjugates (**37-43**, **Figure 3**).

**Figure 3.** Hybrid indomethacin and benzyl salicylate-nitroxide cleavable conjugates



In addition to the salicylate and indomethacin hybrids, a number of stable nitroxide compounds were incorporated into the 5-ASA framework as depicted in **Scheme 5**. Methyl 2-hydroxy-5-nitrobenzoate **44** was first protected with an acetyl group to furnish the nitro-diester **45** which was reduced to the amine derivative **46** under Pd/C hydrogenolysis. The amino ester **46** was then coupled to various carboxylic acid nitroxides (**16**, **19**, **21** and **22**) to give the amide derivatives (**47-50**). A mild basic hydrolysis of the amide-diester (**47-50**) afforded the amide salicylates (**53-56**) in high yields (83-91%). To compare the therapeutic efficacy of the novel nitroxides conjugates to known pharmacophores, the 5-ASA conjugates of known anti-oxidants Trolox and cinnamic acid were also prepared (**51** and **52**).

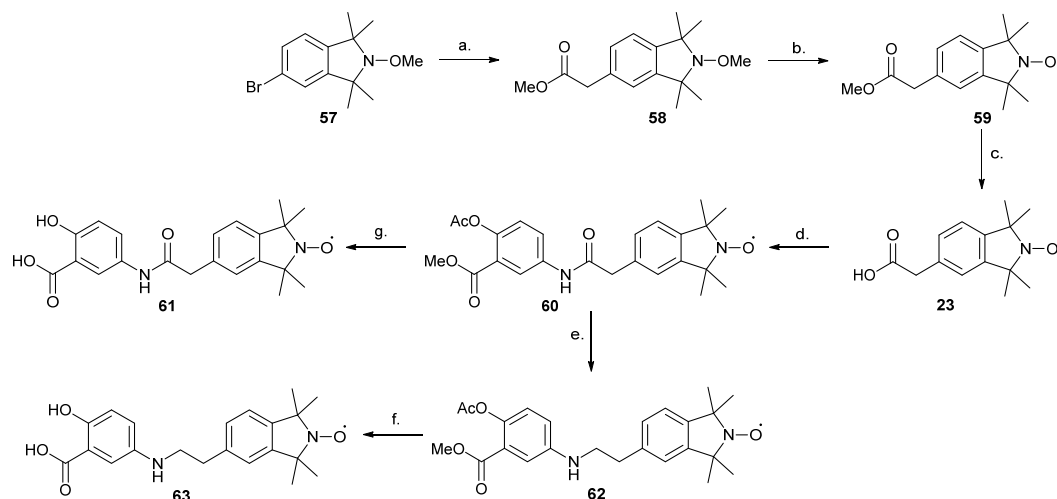
**Scheme 5.** Synthesis of amide-linked nitroxide-5-ASA conjugates<sup>a</sup>



<sup>a</sup>Reagents and conditions: a. AcCl, TEA, 0 °C, 30 min. to RT, 1 h, 91%; b. H<sub>2</sub>, Pd/C, EtOAc, 50 psi, 5 h, 90%; c. Appropriate carboxylic acid, EDC, DMAP, DCM, RT, 1 d, 83-91%; d. 1 M NaOH/MeOH, overnight, 83-91%.

In addition to the 5-ASA amide conjugates, the ethylamino-linked nitroxide-5-ASA non-cleavable conjugate **63** was also synthesized (**Scheme 6**). The bromomethoxyamine precursor **57** was generated in two steps from bromoamine **6** following literature procedures [33]. The methyl ester **58** was obtained following an oxidative decarboxylative coupling protocol that involved refluxing a degassed reaction mixture of bromomethoxyamine **57** and potassium malonate in the presence of catalytic amounts of BINAP, and DMAP [34]. Methyl ester **58** was readily converted to the nitroxide **59** using *m*CPBA and the carboxylic acid derivative **23** was obtained in almost quantitative yield following basic hydrolysis of methyl ester **59**. EDC-mediated coupling of carboxylic acid nitroxide **23** with the amino ester **46** furnished the amide derivative **60**. Subsequent hydrolysis of compound **60** afforded the amide-linked salicylic acid nitroxide conjugate **61**. Selective reduction of the amide group of **60** to furnish the corresponding amine **62** was achieved using a pre-formed solution of sodium acyloxyborohydride in refluxing dioxane. Final hydrolysis of **62** afforded the amine-linked salicylic acid nitroxide **63** in 72% yield.

**Scheme 6.** Synthesis of amine-linked nitroxide-5-ASA conjugate<sup>a</sup>



<sup>a</sup>Reagents and conditions: a. Methyl potassium malonate, [Pd(Allyl)Cl]<sub>2</sub>, BINAP, DMAP, mesitylene, reflux, Ar, 1d, 54%; b. *m*CPBA, DCM, 0 °C, 3 h, 89%; c. MeOH/NaOH, 60 °C, 2 h, 94%; d. **46**, EDC, DMAP, DCM, RT, 1 d, 95%; e. NaBH<sub>4</sub>, AcOH, dioxane, reflux, Ar, 30 min, 68%; f. 0.5 M NaOH/MeOH, overnight, 72%; g. 1 M NaOH/MeOH, overnight, 72%.

**Biological evaluation.** A range of novel nitroxide-NSAID hybrids were investigated for their *in vitro* anti-oxidant, anti-inflammatory and anti-cancer effects. The efficacy of two lead compounds (**27** and **39**) on ROS generation was tested on three different ROS-sensitive cell types, two Non-Small Cell Lung Cancer (NSCLC) cell lines, A549 and NIH-H1299, as well as a mouse photoreceptor cone cell line (661W retinal photoreceptor cells). The A549 NSCLC cells are a type of epithelial lung cancer that is relatively insensitive to chemotherapy and radiation therapy, and which accounts for over 80% of lung cancers [35]. The 661W photoreceptor cells are also highly valuable for investigating ROS injury, in this case, derived from the high flux of oxygen in the retina that is linked to dysfunction and eventual loss of vision.

***In vitro* anti-oxidant action.** The anti-oxidant capacity of the nitroxide-NSAID conjugates was determined by evaluating their ability to scavenge ROS generated in A549 NSCLC cells via the addition of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Noting the limitations of the methodology, an

indication of the H<sub>2</sub>O<sub>2</sub>-induced ROS produced by A549 cells was obtained through fluorescence generated from 2,7-dichlorofluorescein diacetate (DCFH-DA) [36]. Since the radical scavenging effect of the new hybrid compounds would be expected to arise primarily from the nitroxide moiety, the studies were carried out by comparing Tempol, probably the most widely studied anti-oxidant nitroxide, to the structurally-analogous hybrid compound **27** (**Table 1**). Both Tempol and the conjugate drug **27** lowered the increase in ROS caused by H<sub>2</sub>O<sub>2</sub>, but had no effect on basal ROS levels. Notably, only 10 μM of the hybrid compound **27** was needed to generate similar ROS scavenging delivered by Tempol used at 10-times the concentration (100 μM).

**Table 1.** ROS scavenging action of nitroxide-NSAID-conjugates on NSCLC A549 cells

Compound	Concentration (μM)	Relative fluorescence (%)
None		100 ± 5 <sup>aa</sup>
H <sub>2</sub> O <sub>2</sub>		194 ± 13 <sup>**</sup>
H <sub>2</sub> O <sub>2</sub> + <b>27</b>	10	144 ± 12 <sup>*a</sup>
H <sub>2</sub> O <sub>2</sub> + Tempol	100	155 ± 15 <sup>*a</sup>
Tempol	100	103 ± 6 <sup>aa</sup>
<b>27</b>	10	98 ± 4 <sup>aa</sup>

\* The mean value ± S.D. of 8 determinations is indicated; \*, p < 0.05; \*\* p < 0.01 relative to control; a p < 0.05; aa p < 0.01 using the Student's t-test (relative to H<sub>2</sub>O<sub>2</sub>). This experiment is representative of 2 others.

***In vitro* anti-inflammatory and anti-cancer effects.** Epidermal growth factor receptor (EGFR) was exploited as the target protein for evaluating the anti-inflammatory capacity of the novel nitroxide-NSAID hybrids. As a common NSCLC drug target, the EGFR signaling pathway is responsible for COX-2 prostaglandin (PGs) production [37-40]. The COX-induced prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) produced by A549 cells was quantified using the enzyme-linked immunosorbent assay. As shown in **Table 2**, the ester-linked conjugates (**27**, **34**, **41** and **43**) displayed strong inhibition of the COX-induced PGE<sub>2</sub> production. In contrast, the amide-linked conjugates (**28**, **29** and **39**) showed moderate inhibitory action.

**Table 2.** Inhibitory effects of nitroxide-NSAID conjugates on COX-induced PGE<sub>2</sub> in NSCLC A549 cells

Compound	Concentration (μM)	PGE <sub>2</sub> , pg.mL
None		57 ± 4
<b>4</b>	30	50 ± 5
<b>5</b>	30	52 ± 5
<b>27</b>	30	31 ± 4*
<b>28</b>	30	54 ± 6
<b>29</b>	30	45 ± 9
<b>34</b>	30	34 ± 6*
<b>39</b>	30	58 ± 3
<b>41</b>	30	23 ± 5*

42

30

21 ± 6\*

The mean value ± S.D. or 3 determinations each repeated in duplicate is indicated using A549 cells; p < 0.05, \*, p < 0.01, \*\* using the Student's t-test.

Further COX inhibition experiments were conducted at lower concentrations (4 μM) of selected conjugates (**27** and **28**) along with Tempol and the parent aspirin (**Table 3**). The ester-linked conjugate **27** significantly inhibited PGE<sub>2</sub> productions even at the lower concentration of 4 μM. The amide-linked conjugate **28** on the other hand showed only moderate inhibition at 4 μM. The most interesting result was the inhibitory action of the conjugates in comparison to the aspirin parent. Notably, compound **27** was approximately an order of magnitude more effective at inhibiting COX-induced PGE<sub>2</sub> production in NSCLC cells than aspirin.

**Table 3.** Comparison of individual components with nitroxide-NSAID conjugates on inhibiting of COX-induced PGE<sub>2</sub> in NSCLC A549 cells

Compound	Concentration (μM)	PGE <sub>2</sub> , pg.mL
None		126 ± 17
Aspirin	90	79 ± 11
Tempol	90	105 ± 11
Aspirin + Tempol	90	75 ± 14*
<b>27</b>	4	87 ± 9*
<b>28</b>	4	99 ± 13

The mean value  $\pm$ S.D. of 3 determinations each repeated in duplicate is indicated using A549 cells;  $p < 0.05$ , \*;  $p < 0.01$ , \*\* using the Student's t-test.

The nitroxide-NSAID conjugates were further tested for their inhibitory action on NSCLC proliferation using the MMT assay. The ester-linked conjugates (**27**, **41** and **42**) inhibited A549 cell proliferation with  $IC_{50}$  values in the range of 118-151  $\mu$ M (**Table 4**). In contrast, their amide-linked counterparts (**28**, **29** and **37**) displayed moderate cell inhibitory potency with  $IC_{50}$  values  $\geq 300$   $\mu$ M).

**Table 4.** Inhibitory effects of nitroxide-NSAID conjugates on NSCLC A549 cell growth.

Compound	$IC_{50}$ ( $\mu$ M)
<b>27</b>	$130 \pm 23$
<b>28</b>	$\geq 300$
<b>29</b>	$\geq 300$
<b>37</b>	$151 \pm 8$
<b>41</b>	$121 \pm 12$
<b>42</b>	$118 \pm 16$

The mean value  $\pm$ S.D. of 3 experiments each repeated in duplicate is shown using NSCLC A549 cells;  $p < 0.05$ , \* using the Student's t-test.

Further cell growth inhibitory studies using NCI-H1299 cells were conducted with compound **27**, along with Tempol and aspirin, at different concentrations (**Table 5**). At a concentration of 180  $\mu$ M, compound **27** displayed strong inhibitory capacity against the



growth of these cells. However, no significant inhibition was observed for compound **27** at a lower concentration (18  $\mu\text{M}$ ). In contrast, Tempol at 90  $\mu\text{M}$  or 900  $\mu\text{M}$  concentrations had little effect on NCI-H1299 proliferation. Only a moderate inhibitory action was observed for aspirin. However, this was only observed at higher aspirin concentrations (900  $\mu\text{M}$ ).

**Table 5.** Inhibitory effects of nitroxide-NSAID conjugates on NCI-H1299 cell growth.

Compound	Concentration ( $\mu\text{M}$ )	% Growth
Aspirin	90	103 $\pm$ 4
Aspirin	900	49 $\pm$ 2*
Tempol	90	100 $\pm$ 4
Tempol	900	98 $\pm$ 5
Aspirin + Tempol	90	102 $\pm$ 6
Aspirin + Tempol	900	53 $\pm$ 4*
<b>27</b>	18	97 $\pm$ 3
<b>27</b>	180	13 $\pm$ 1*

The mean value  $\pm$  S.D. of 3 experiments each repeated in duplicate is shown using NCI-H1299 cells;  $p < 0.05$ , \* using the Student's t-test.

### *Cell Culture of Retinal Cells*

The 661W retinal photoreceptor cell line was established in T75 tissue culture flasks in Dulbecco Modified-Eagles Medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 50 U/ml penicillin, 50 µg/ml streptomycin. Cells were harvested for experimental use upon reaching 80-85% confluence.

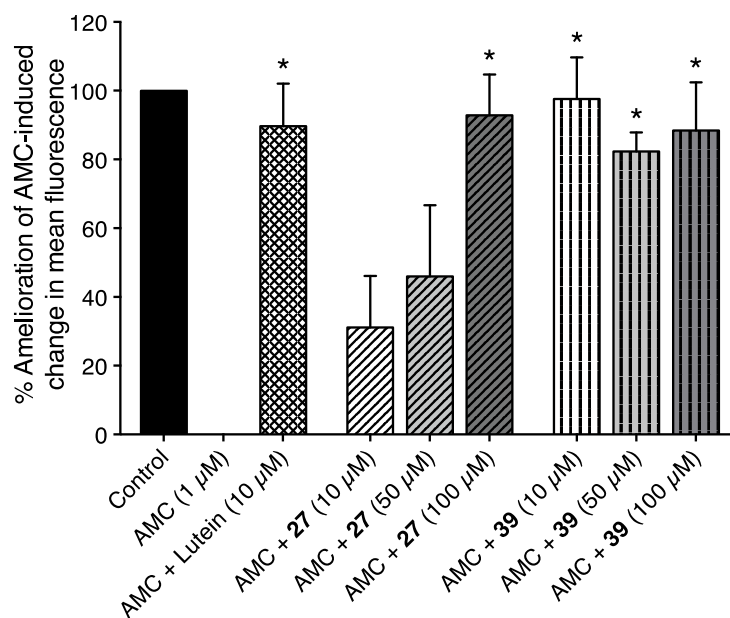
The prospects for therapeutic intervention to reduce or prevent cellular damage induced by oxidative stress was evaluated by a fluorescence-based response for cell populations using flow cytometry and exploiting a fluorescent probe that responds to changes in the cellular redox environment under both pro- and anti-oxidant conditions [41]. Flow cytometry provides a convenient and rapid screening method to measure the biological efficacy of novel anti-oxidant compounds and we have previously exploited it to monitor the overall changes to the cellular redox environment of cells with varying metabolic activity [42].

Two representative dual-acting anti-inflammatory, anti-oxidant compounds, the aspirin-nitroxide hybrid **27** and the indomethacin-nitroxide **39**, were tested for their efficacy for alleviating antimycin (AMC) derived fluorescence response with lutein used as a comparison. Lutein is an effective, widely adopted anti-oxidant and free radical scavenger that displays anti-inflammatory properties, and affords cellular protection, particularly to retinal neurons, from oxidative injury [43, 44].

Stimulation of mitochondrial ROS production in 661W retinal cells with AMC (1 µM) resulted in a 50% reduction in mean fluorescence intensity using a fluorescent probe that responds to cellular oxidative status [40]. Anti-oxidant data were normalized to the maximal effect of AMC and expressed as the % amelioration of the AMC-induced change in mean fluorescence i.e.  $(100 * (\text{AMC} + \text{antioxidant} - \text{AMC}) / (\text{Control} - \text{AMC}))$ . Lutein significantly reduced the effects of AMC on probe fluorescence by  $89.7 \pm 12.4\%$ ;  $p = 0.0002$  (NB. Larger % represents better response). The indomethacin-nitroxide hybrid **39** significantly

ameliorated the effects of AMC on probe fluorescence at each concentration tested; ( $97.6 \pm 12.1\%$  for  $10 \mu\text{M}$ ;  $p = 0.0003$ ;  $82.3 \pm 5.5\%$  for  $50 \mu\text{M}$ ;  $p = 0.0015$ ;  $88.4 \pm 14.0\%$  for  $100 \mu\text{M}$ ;  $p = 0.0007$ ), while compound **27** also produced a dose dependent effect, it significantly lowered the impact of AMC only at  $100 \mu\text{M}$  ( $31.1 \pm 15.0\%$  for  $10 \mu\text{M}$ ;  $p = 0.4318$ ;  $46.0 \pm 20.7\%$  for  $50 \mu\text{M}$ ;  $p = 0.1099$ ;  $92.8 \pm 11.9\%$  for  $100 \mu\text{M}$ ;  $p = 0.0004$ ) (**Figure 4**). Notably the indomethacin-nitroxide hybrid **39** provided greater protection against AMC at  $10 \mu\text{M}$ , than that provided by the same concentration of lutein, suggesting, especially at lower concentrations, this hybrid compound may provide greater protection against oxidative stress than current state-of-the-art anti-oxidants.

**Figure 4.** Hybrid indomethacin and aspirin nitroxides and their impact on antimycin-induced mitochondrial ROS production<sup>a</sup>



<sup>a</sup>Data were obtained from 3-5 independent experiments and expressed as the mean  $\pm$  SEM. Statistical comparisons were made between the effects of each experimental compound and the effect of AMC alone using a one-way ANOVA with Dunnett's multiple comparison test.  $p < 0.05$  was taken to be statistically significant for this assay.

## CONCLUSION

A pharmacophore hybridization strategy was successfully employed to design and synthesise a series of 30 novel potential dual-acting (anti-oxidant and anti-inflammatory) nitroxide-NSAID conjugates. Selected novel hybrids were evaluated for their antioxidant, anti-inflammatory and anticancer effects on A549 Non-Small Cell Lung Cancer cells. Several nitroxide conjugates displayed significant antioxidant effects by inhibiting ROS generated by A549 cells. While the ester-linked conjugates inhibited of the COX enzyme, the amide-linked counterparts delivered only moderate inhibition. Notably, the nitroxide conjugate **27** provides better inhibition of the COX enzyme than parent aspirin. Another nitroxide hybrid (**39**), a structural combination with the anti-inflammatory drug indomethacin, significantly ameliorated the effects of oxidative stress on 661W retinal neurons at efficacies greater or equal to the recognized anti-oxidant Lutein. Importantly, the hybrid conjugates also possess promising anticancer effects in inhibiting the proliferation of NIH-H1299 NSCLC cells. This work demonstrates that merged/cleavable/non-cleavable hybrid agents can deliver enhanced therapeutic efficacy with multiple modes of action over the individual parent species.

Ó Û Ü Ý à í õ È Î γ

**Supporting Information.** Characterization data for final and representative compounds in each series.

## AUTHOR INFORMATION

### Corresponding Author

\*(S.E.B.) Email: s.bottle@qut.edu.au.

## ACKNOWLEDGEMENT

This work was supported by the Australian Research Council Centre of Excellence for Free Radical Chemistry and Biotechnology (CE 0561607) and Queensland University of Technology (KT, KEF, SEB). The research was also supported by the Intramural Research Program of the NIH, Cancer and Inflammation Program (TWM, LAR, DAW).

### **ABBREVIATIONS USED**

AcCl acetyl chloride; AMC antimycin; ASA amino salicylic acid; BINAP ( $\pm$ )-2,2'-Bis(diphenylphosphino)-1,1'-binaphthalene; br broad; COX cyclooxygenase; DCM dichloromethane; dd doublet of doublet; DMAP 4-Dimethylaminopyridine; EDC *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride; EtOAc ethyl acetate; EtOH ethanol; FTIR fourier transform infrared spectroscopy; MeCN acetonitrile; MeOH methanol; MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide]; *n*Bulmi *N*-butylimidazole; O/N overnight; PG, PGE<sub>2</sub> Prostaglandins E<sub>2</sub>; QTOF LC-MS Quadrupole Time of Flight Mass Spectrometer Liquid Chromatography-Mass Chromatography; RT room temperature; TEA trimethylamine; TEMPO 2,2,6,6-tetramethylpiperidine-1-yloxy; Tempol 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-yloxy; THF tetrahydrofuran; TMIO 1,1,3-tetramethylisoindolin-2-yloxy; w weak.

### **EXPERIMENTAL SECTION**

**General Procedures.** All air-sensitive reactions were carried out under ultra-high pure argon. Diethyl ether and toluene were dried by storing with sodium wire. All other solvents were dried using the Pure Solv Micro 4 L solvent purification system (PSM-13-672). Solvents used for extractions and silica gel column chromatography were AR grade. Crystalline K<sub>4</sub>[Fe(CN)<sub>6</sub>]·3H<sub>2</sub>O was ground to a fine powder and then dried at 80 °C at 0.5 Torr for 10 h. All other reagents were purchased from commercial suppliers and used without

further purification  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded with Bruker Avance 600 MHz, 400 MHz or Varian 400 MHz spectrometers and referenced to the relevant solvent peak. HPLC was performed with a HP Agilent 1100 HPLC instrument. HRMS was performed with an Agilent accurate Quadrupole Time of Flight Mass Spectrometer Liquid Chromatography-Mass Chromatography (QTOF LC-MS) mass spectrometer. Formulations were calculated by elemental analysis using a Mass Lynx 4.0 or Micromass Opus 3.6 instrument. FTIR spectra were recorded with a Nicolet 870 Nexus Fourier Transform Infrared Spectrometer equipped with a DTGS TEC detector and an ATR objective. All melting points were measured on a Buchi Melting Point M-565 apparatus. The EPR spectra were recorded on a Magnettech MiniScope MS400 spectrometer. Whereas the pyrrolidine and piperidines nitroxides (**16-19**) are commercially available, the isoindoline nitroxides (**20-22**) were synthesized by following previously published literature procedures [45-50]. An earlier report on the synthesis of compound **28** provided only limited characterisation data [51] and we have previously described the synthesis of **27** and **37** [52]. Others have also reported further data on these compounds [53, 54].

**5-Methoxy-1,1,3,3-tetramethylisoindoline 7.** CuI (56.25 mg, 0.2 equiv.) was added to a solution of 5-bromo-1,1,3,3-tetramethylisoindoline **6** (500 mg, 1.967 mmol, 1 equiv.) in DMF (3 mL) and NaOMe (5 M in MeOH, 12 mL) under Ar. The reaction mixture was heated at reflux for 15 h and allowed to return to RT. It was then diluted with H<sub>2</sub>O and extracted with Et<sub>2</sub>O. The combined Et<sub>2</sub>O (4 x 40 mL) extracts were washed with brine (50 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The resulting solid residue was purified by silica column chromatography (CHCl<sub>3</sub>/EtOH, 10:0.5) to give 5-methoxy-1,1,3,3-tetramethylisoindoline **7** as a pale white semi-solid (343.3 mg, 85%). Mp. 57-58 °C. HPLC purity (>95%).  $^1\text{H}$  NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  = 1.45 (s, 6 H, CH<sub>3</sub>), 1.47 (s, 6 H, CH<sub>3</sub>), 3.87 (s, 3 H, OCH<sub>3</sub>), 6.65-7.39 (m, 3 H, Ar-H).  $^{13}\text{C}$  NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  = 31.9 (C-CH<sub>3</sub>),

32.0 (C-CH<sub>3</sub>), 55.4 (OCH<sub>3</sub>), 62.5 (CCH<sub>3</sub>), 62.8 (CCH<sub>3</sub>), 112.8 (Ar-C), 123.3 (Ar-C), 125.7 (Ar-C), 140.0 (Ar-C), 147.2 (Ar-C), 157.4 (Ar-C). HRMS (ES): *m/z* (%) = calcd. for C<sub>13</sub>H<sub>20</sub>NO [M + H]<sup>+</sup> 206.1539; found 206.1574. ATR-FTIR:  $\nu_{\max}$  = 3415 (s, N-H), 1154 (s, C-N), 1042 (C-O) cm<sup>-1</sup>.

**2,5-Dibromo-6-methoxy-1,1,3,3-tetramethylisoindoline 8.** A solution of 5-methoxy-1,1,3,3-tetramethylisoindoline **7** (1.50 g, 731  $\mu$ mol, 1 equiv.) in DCM (25 mL) under Ar was cooled to 0 °C. A solution of bromine (942  $\mu$ L, 1.83 mmol, 2.5 equiv.) in DCM (10 mL) was added dropwise followed by addition of anhydrous aluminium trichloride (3.48 g, 2.56 mmol, 3.5 equiv.). The reaction mixture was stirred for 1 hr at 0 °C, then poured onto ice (40 mL) and stirred vigorously for further 20 min. The solution was then basified to or above pH 12 with aqueous NaOH (10 M) solution and stirred for 10 min. The mixture was extracted with DCM (4 x 50 mL), the combined DCM extracts were washed with brine (50 mL) and the solvent removed under reduced pressure to give light yellow oil. The oil was triturated with methanol to give 2,5-dibromo-6-methoxy-1,1,3,3-tetramethylisoindoline **8** as a yellow solid (2.307 g, 87%). Mp. 97-98 °C. HPLC purity (93%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  = 1.41 (s, 6 H, CH<sub>3</sub>), 1.44 (s, 6 H, CH<sub>3</sub>), 3.91 (s, 3 H, OCH<sub>3</sub>), 6.66 (s, 1 H, Ar-H), 7.3 (s, 1 H, Ar-H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  = 28.1 (C-CH<sub>3</sub>), 28.3 (C-CH<sub>3</sub>), 56.5 (OCH<sub>3</sub>), 69.2 (C-CH<sub>3</sub>), 69.7 (C-CH<sub>3</sub>), 105.4 (Ar-C), 110.6 (Ar-C), 126.6 (Ar-C), 137.7 (Ar-C), 144.8 (Ar-C), 155.3 (Ar-C). ATR-FTIR:  $\nu_{\max}$  = 3000 (m, Ar C-H), 1232 (s, C-N), 1034 (C-O) cm<sup>-1</sup>.

**5-Bromo-6-methoxy-1,1,3,3-tetramethylisoindoline 9.** To a suspension of 2,5-dibromo-6-methoxy-1,1,3,3-tetramethylisoindoline **8** (900 mg, 2.479 mmol, 1 equiv.) and NaHCO<sub>3</sub> (208 mg, 2.479 mmol, 1 equiv.) in MeOH/DCM (10:5 mL) was added dropwise aqueous H<sub>2</sub>O<sub>2</sub> (30%) until the observed effervescence ceased. The reaction mixture was stirred for 5 min followed by the addition of NaOH (5 M). The resulting solution was extracted with DCM (4 x 40 mL) and the combined DCM extracts were washed with brine (50 mL), dried

over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure to give 5-bromo-6-methoxy-1,1,3,3-tetramethylisoindoline **9** as a beige solid (688 mg, 98%). Mp. 59-60 °C. HPLC purity (>99%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz): δ = 1.42 (s, 6 H, CH<sub>3</sub>), 1.45 (s, 6 H, CH<sub>3</sub>), 3.91 (s, 3 H, OCH<sub>3</sub>), 6.62 (s, 1 H, Ar-H), 7.26 (s, 1 H, Ar-H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ = 31.8 (C-CH<sub>3</sub>), 32.0 (C-CH<sub>3</sub>), 56.4 (OCH<sub>3</sub>), 62.4 (C-CH<sub>3</sub>), 62.8 (C-CH<sub>3</sub>), 105.1 (Ar-C), 110.5 (Ar-C), 126.2 (Ar-C), 142.3 (Ar-C), 149.6 (Ar-C), 155.3 (Ar-C). HRMS (ES): *m/z* (%) = calcd. for C<sub>13</sub>H<sub>19</sub>BrNO [M + H]<sup>+</sup> 284.0645; found 284.0723. ATR-FTIR: ν<sub>max</sub> = 3307 (w, N-H), 2961 (m, Ar C-H), 1307 (s, C-N), 1038 (C-O) 699 (s, C-Br) cm<sup>-1</sup>.

**5-Cyano-6-methoxy-1,1,3,3-tetramethylisoindoline 10.** A Schlenk vessel that contained a mixture of 5-bromo-6-methoxy-1,1,3,3-tetramethylisoindoline **9** (2.76 g, 9.72 mmol, 1 equiv.), K<sub>4</sub>[Fe(CN)<sub>6</sub>] (837 mg, 1.94 mmol, 0.2 equiv.), CuI (223 mg, 1.17 mmol, 0.12 equiv.) *N*-butylimidazole (2.5 mL, 19.45 mmol, 2 equiv.) in *o*-xylene (20 mL) was degassed and then heated at reflux at 180 °C for 3 d. The resulting mixture was allowed to return to RT before it was diluted with water and then extracted with Et<sub>2</sub>O (4 x 60 mL). The combined Et<sub>2</sub>O extracts were washed with brine (50 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (EtOAc) and recrystallized from cyclohexane to give 5-cyano-6-methoxy-1,1,3,3-tetramethylisoindoline **10** as an off-white solid (1.75 g, 78%). Mp. 138-139 °C. HPLC purity (>99%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ = 1.42 (s, 6 H, CH<sub>3</sub>), 1.45 (s, 6 H, CH<sub>3</sub>), 1.73 (s, 1 H, N-H), 3.94 (s, 3 H, OCH<sub>3</sub>), 6.66 (s, 1 H, Ar-H), 7.27 (s, 1 H, Ar-H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ = 31.6 (C-CH<sub>3</sub>), 31.9 (C-CH<sub>3</sub>), 56.2 (OCH<sub>3</sub>), 62.4 (C-CH<sub>3</sub>), 63.2 (C-CH<sub>3</sub>), 100.8 (C=N), 104.5 (Ar-C), 117.0 (Ar-C), 126.8 (Ar-C), 141.4 (Ar-C), 156.2 (Ar-C), 161.5 (Ar-C). HRMS (ES): *m/z* (%) = calcd. for C<sub>14</sub>H<sub>19</sub>N<sub>2</sub>O [M + H]<sup>+</sup> 231.1492; found 231.1560. ATR-



FTIR:  $\nu_{\max}$  = 3326 (w, N-H), 2968 (m, Ar C-H), 2221 (m, C=N), 1155 (s, C-N), 1042 (C-O)  $\text{cm}^{-1}$ .

**5-Cyano-6-methoxy-1,1,3,3-tetramethylisoindolin-2-yloxyl 11.** *m*-Chloroperoxybenzoic acid (1.78 g, 6.43 mmol, 1.3 equiv.) was added to a solution of 5-cyano-6-methoxy-1,1,3,3-tetramethylisoindoline **10** (1.14 g, 4.95 mmol, 1 equiv.) in DCM (100 mL) at 0 °C. The cooling bath was removed after 30 min and the reaction stirred at RT for a further 1.5 h. The DCM layer was washed with HCl (2 M), NaOH (5 M), and brine solutions (50 mL) and before being dried over anhydrous  $\text{Na}_2\text{SO}_4$ . The DCM was removed under reduced pressure and the solid residue obtained was recrystallized from EtOH to give bright yellow needles of 5-cyano-6-methoxy-1,1,3,3-tetramethylisoindolin-2-yloxyl **11** (1.09 g, 90%). Mp. 200-201 °C. HPLC purity (>99%). HRMS (ES):  $m/z$  (%) = calcd. for  $\text{C}_{14}\text{H}_{17}\text{N}_2\text{NaO}_2^+$  [M + Na]<sup>+</sup> 268.1182; found 268.1230. ATR-FTIR:  $\nu_{\max}$  = 3048 (w, Ar C-H), 2231 (m, C=N), 1472 (N-O), 1161 (s, C-N), 1041 (C-O)  $\text{cm}^{-1}$ ; EPR:  $g = 2.0009$ ,  $a_N = 1.81$  mT.

**5-Carboxy-6-methoxy-1,1,3,3-tetramethylisoindolin-2-yloxyl 12.** A suspension of 5-cyano-6-methoxy-1,1,3,3-tetramethylisoindolin-2-yloxyl **11** (760 mg, 3.1 mmol, 1.00 equiv.) in NaOH (5 M, 10 mL)/EtOH (5 mL) was heated at reflux for 16 h. The reaction mixture was cooled to RT, then diluted with  $\text{H}_2\text{O}$  and washed with  $\text{Et}_2\text{O}$  (2 x 40 mL). The  $\text{Et}_2\text{O}$  layer was discarded. The aqueous layer was cooled in ice bath and acidified with HCl (2 M) before it was extracted with  $\text{Et}_2\text{O}$  (50 mL x 4). The combined  $\text{Et}_2\text{O}$  extracts were washed with brine (50 mL) and dried over anhydrous  $\text{Na}_2\text{SO}_4$  and evaporated under reduced pressure. The residue was recrystallized from  $\text{H}_2\text{O}/\text{EtOH}$  to give 5-carboxy-6-methoxy-1,1,3,3-tetramethylisoindolin-2-yloxyl **12** as yellow solid (729 mg, 89%). Mp. 244-245 °C (dec.). HPLC purity (>99%). HRMS (ES):  $m/z$  (%) = calcd. for  $\text{C}_{14}\text{H}_{18}\text{NNaO}_4^+$  [M + Na]<sup>+</sup> 287.1128;

found 287.1714. ATR-FTIR:  $\nu_{\max}$  = 3400-2450 (m, br, OH), 2973 (m, Ar C-H), 1675 (s, C=O), 1360 (s, C-N), 1202 (C-O)  $\text{cm}^{-1}$ ; EPR:  $g = 2.0009$ ,  $a_{\text{N}} = 1.83$  mT.

**5-Carboxy-6-hydroxy-1,1,3,3-tetramethylisoindolin-2-yloxyl 4** from **12**.  $\text{BBr}_3$  (1.9 mL, 1.89 mmol, 1 M solution in DCM, 2.5 equiv.) was added dropwise to a solution of 5-carboxy-6-methoxy-1,1,3,3-tetramethylisoindolin-2-yloxyl **12** (200 mg, 757  $\mu\text{mol}$ , 1 equiv.) in DCM (15 mL) at  $-78$   $^{\circ}\text{C}$  under Ar atmosphere. The reaction was allowed to return to RT and left to stir for 18 h.  $\text{H}_2\text{O}$  was added to the resulting mixture to quench excess any  $\text{BBr}_3$  reagent. The crude product was extracted with EtOAc (50 mL x 4) and the combined EtOAc extracts were washed with brine (50 mL), dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (DCM/MeOH, 6:0.4) and recrystallized from  $\text{H}_2\text{O}/\text{EtOH}$  to give 5-carboxy-6-hydroxy-1,1,3,3-tetramethylisoindolin-2-yloxyl **4** as yellow crystals (76 mg, 40%). Mp. 207-208  $^{\circ}\text{C}$  (dec.). HPLC purity (>99%). HRMS (ES):  $m/z$  (%) = calcd. for  $\text{C}_{13}\text{H}_{18}\text{NO}_4^+$   $[\text{M} + 2\text{H}]^+$  252.1230; found 252.1186. ATR-FTIR:  $\nu_{\max}$  = 3400-2500 (m, br, OH), 2972 (m, Ar C-H), 1674 (s, C=O), 1201 (C-O)  $\text{cm}^{-1}$ ; EPR:  $g = 2.0017$ ,  $a_{\text{N}} = 1.80$  mT.

**2-Acetoxy-5-carboxy-6-methoxy-1,1,3,3-tetramethylisoindoline 14**. A reaction mixture of 5-carboxy-6-methoxy-1,1,3,3-tetramethylisoindolin-2-yloxyl **12** (660 mg, 2.5 mmol, 1 equiv.) and Pd/C (66 mg, 10%, 62.5  $\mu\text{mol}$ , 0.025 equiv.) in THF (15 mL) was flushed with Ar for 10 min. Then, a balloon of  $\text{H}_2$  was connected and the reaction mixture stirred for 15 min and then cooled in ice/ $\text{H}_2\text{O}$  bath. TEA (697  $\mu\text{L}$ , 5 mmol, 2 equiv.) and AcCl (355  $\mu\text{L}$ , 5 mmol, 2 equiv.) were added dropwise and the resulting mixture was stirred for 30 min. The cooling bath was removed and stirring was continued for a further 1 h. The reaction mixture was filtered through Celite and concentrated *in vacuo*. The crude residue was stirred in aqueous MeOH (10 mL, 2 mL  $\text{H}_2\text{O}$ ) for 1 h, then diluted with  $\text{H}_2\text{O}$ , and extracted with EtOAc (4 x 50 mL). The EtOAc extracts were washed with brine (40 mL), dried over

anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated *in vacuo* to give 2-acetoxy-5-carboxy-6-methoxy-1,1,3,3-tetramethylisoindoline **14** as a clear solid (738 mg, 96%). Although compound **14** was pure enough to be used subsequent step, it was further purified by silica gel flash column chromatography (EtOAc/CHCl<sub>3</sub>, 2:1) and recrystallized from cyclohexane. Mp. 149-150 °C. HPLC purity (>99%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ = 1.42 (d, 6 H, CH<sub>3</sub>), 1.48 (d, 6 H, CH<sub>3</sub>), 2.2 (s, 3 H, C=OCH<sub>3</sub>), 4.1 (s, 3 H, OCH<sub>3</sub>), 6.78 (s, 1 H, Ar-H), 7.98 (s, 1 H, Ar-H), 10.81 (s, br, 1 H, CO<sub>2</sub>H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ = 19.2 (CO-CH<sub>3</sub>) 25.0, 25.2 (C-CH<sub>3</sub>), 28.6, 28.8 (C-CH<sub>3</sub>), 57.0 (OCH<sub>3</sub>), 67.9 (C-CH<sub>3</sub>), 68.4 (C-CH<sub>3</sub>), 105.0 (Ar-C), 117.4 (Ar-C), 127.5 (Ar-C), 138.1 (Ar-C), 151.7 (Ar-C), 158.3 (Ar-C), 165.2 (CO<sub>2</sub>H), 171.2 (C=OCH<sub>3</sub>). HRMS (ES): *m/z* (%) = calcd. for C<sub>16</sub>H<sub>21</sub>NNaO<sub>5</sub> [M + Na]<sup>+</sup> 330.1312; found 330.1401. ATR-FTIR: ν<sub>max</sub>= 3267 (m, br, OH), 2973 (m, Ar C-H), 1772 (s, Ac C=O), 1709 (carboxylic acid C=O) 1194 (C-O) cm<sup>-1</sup>.

**2-Acetoxy-5-carboxy-6-hydroxy-1,1,3,3-tetramethylisoindoline 15.** BBr<sub>3</sub> (1.4 mL, 1.4 mmol, 1 M solution in DCM, 2.5 equiv.) was added dropwise to a solution of 2-acetoxy-5-carboxy-6-methoxy-1,1,3,3-tetramethylisoindoline **14** (170 mg, 559 μmol, 1 equiv.) in DCM (10 mL) at -78 °C under an Ar atmosphere. The reaction was allowed to return to RT and left to stir for 18 h. H<sub>2</sub>O was added to the resulting mixture to quench any excess BBr<sub>3</sub> reagent. The crude product was extracted with EtOAc (4 x 20 mL) and the combined EtOAc extracts were washed with brine (20 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (DCM/MeOH, 6:0.4) and recrystallized from cyclohexane to give 2-acetoxy-5-carboxy-6-hydroxy-1,1,3,3-tetramethylisoindoline **15** as a white solid (153 mg, 85%). Mp. 168-169 °C. HPLC purity (>98%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ = 1.4 (s, 6 H, CH<sub>3</sub>), 1.48 (s, 6 H, CH<sub>3</sub>), 2.2 (s, 3 H, C=OCH<sub>3</sub>), 6.77 (s, 1 H, Ar-H), 7.64 (s, 1 H, Ar-H), 10.6 (s, br, 1 H, CO<sub>2</sub>H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ = 19.2 (CO-CH<sub>3</sub>) 25, 25.2 (C-CH<sub>3</sub>), 28.6, 28.8 (C-CH<sub>3</sub>), 57.0

(OCH<sub>3</sub>), 67.9 (C-CH<sub>3</sub>), 68.4 (C-CH<sub>3</sub>), 105.0 (Ar-C), 117.4 (Ar-C), 127.5 (Ar-C), 138.07 (Ar-C), 151.7 (Ar-C), 158.3 (Ar-C), 165.2 (CO<sub>2</sub>H), 171.2 (C=OCH<sub>3</sub>). HRMS (ES):  $m/z$  (%) = calcd. for C<sub>15</sub>H<sub>20</sub>NO<sub>5</sub> [M + H]<sup>+</sup> 294.1336; found 294.2269. ATR-FTIR:  $\nu_{\max}$  = 3095 (m, br, OH), 2973 (m, Ar C-H), 1737 (s, Ac C=O), 1677 (carboxylic acid C=O), 1160 (C-O) cm<sup>-1</sup>.

**5-Carboxy-6-hydroxy-1,1,3,3-tetramethylisoindolin-2-yloxyl 4 from 15.** A suspension of 2-acetoxy-5-carboxy-6-hydroxy-1,1,3,3-tetramethylisoindoline **15** (136 mg, 464  $\mu$ mol, 1 equiv.) in H<sub>2</sub>O/MeOH (2 mL/2 mL) was cooled to 0 °C. LiOH (56 mg, 2.3 mmol, 5 equiv.) was added and the reaction mixture stirred overnight while allowed to warm to RT. The resulting solution was washed with Et<sub>2</sub>O and the Et<sub>2</sub>O layer discarded. The aqueous layer was cooled in ice bath, acidified with HCl (2 M) and then extracted with Et<sub>2</sub>O (4 x 30 mL). The combined Et<sub>2</sub>O extracts were stirred over PbO<sub>2</sub> (28 mg, 116  $\mu$ mol, 0.25 equiv.) for 20 min, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated *in vacuo*. The crude residue was recrystallized from H<sub>2</sub>O/EtOH to give 5-carboxy-6-hydroxy-1,1,3,3-tetramethylisoindolin-2-yloxyl **4** as yellow crystals (107 mg, 92%). Mp. 207-208 °C (dec.). HPLC purity (>99%). HRMS (ES):  $m/z$  (%) = calcd. for C<sub>13</sub>H<sub>18</sub>NO<sub>4</sub><sup>+</sup> [M + 2H]<sup>+</sup> 252.1230; found 252.1186. ATR-FTIR:  $\nu_{\max}$  = 3400-2500 (m, br, OH), 2972 (m, Ar C-H), 1674 (s, C=O), 1201 (C-O) cm<sup>-1</sup>; EPR:  $g = 2.0017$ ,  $a_N = 1.80$  mT.

**6-Acetoxy-5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl 5.** A solution of 5-carboxy-6-hydroxy-1,1,3,3-tetramethylisoindolin-2-yloxyl **4** (89 mg, 354.4  $\mu$ mol, 1 equiv.) in THF under Ar was cooled to 0 °C. TEA (99  $\mu$ L, 709  $\mu$ mol, 2 equiv.) and AcCl (50.4  $\mu$ L, 709  $\mu$ mol, 2 equiv.) were added dropwise and the resulting mixture stirred for 3 h while allowing to return to RT. Water was added to the reaction mixture and it was then extracted with DCM (3 x 30 mL). The DCM extracts were washed with HCl (1 M, 15 mL), brine (20 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The combined DCM was removed under reduced pressure and the crude residue obtained was recrystallized from H<sub>2</sub>O/EtOH to give 6-acetoxy-5-carboxy-

1,1,3,3-tetramethylisoindolin-2-ylloxyl **5** as a yellow solid (94 mg, 91%). Mp. 206-207 °C. HPLC purity (>99%). HRMS (ES):  $m/z$  (%) = calcd. for  $C_{15}H_{19}NO_5^+$   $[M + H]^+$  293.1258; found 293.1221. ATR-FTIR:  $\nu_{max}$  = 3400-2600 (m, br, OH), 2972 (m, Ar C-H), 1765 (s, Ac C=O), 1695 (carboxylic acid C=O) 1184 (C-O)  $cm^{-1}$ ; EPR:  $g = 2.0016$ ,  $a_N = 1.80$  mT.

**Benzyl 2-hydroxybenzoate 25.**  $NaHCO_3$  (1.46 g, 17.4 mmol, 1.2 equiv.) was added to a solution salicylic acid **1** (2 g, 14.5 mmol, 1 equiv.) in DMF (20 mL) and the resulting mixture was stirred at 70 °C for 10 min. The temperature was reduced to 50 °C followed by the addition of benzylbromide (1.81 mL, 15.2 mmol, 1.05 equiv.). The reaction mixture was stirred for 4 h and then allowed to cool to RT.  $H_2O$  (50 mL) was added and the crude product was extracted with EtOAc (4 x 60 mL). The combined EtOAc extracts were washed with brine (50 mL), dried over anhydrous  $Na_2SO_4$  and evaporated under reduced pressure. Purification of the crude residue by silica gel chromatography (Hexane/EtOAc, 5:0.2) afforded compound **25** as clear oil (3.11 g, 94%). HPLC purity (>99%).  $^1H$  NMR ( $CDCl_3$ , 400 MHz):  $\delta = 5.42$  (s, 2 H,  $CH_2$ ), 6.9 (m, 1 H, Ar- $H$ ), 7.4 (m, 6 H, Ar- $H$ ), 7.91 (q, 1 H, Ar- $H$ ), 10.78 (s, 1 H, O- $H$ ).  $^{13}C$  NMR ( $CDCl_3$ , 100 MHz):  $\delta = 67.0$  ( $CH_2$ ), 112.4 (Ar- $C$ ), 117.6 (Ar- $C$ ), 119.2 (Ar- $C$ ), 128.3 (Ar- $C$ ), 128.6 (Ar- $C$ ), 128.7 (Ar- $C$ ), 130.0 (Ar- $C$ ), 135.3 (Ar- $C$ ), 135.8 (Ar- $C$ ), 161.8 (Ar- $C$ ), 170.0 (C=O). ATR-FTIR:  $\nu_{max}$  = 3188 (m, br, O-H), 3000 (m, Ar C-H), 1670 (s, C=O), 1086 and 1133 (s, C-O)  $cm^{-1}$ .

**General procedure for the synthesis of salicylic acid derivatives 27-29.** A solution of aspirin **3** (150 mg, 833  $\mu$ mol, 1 equiv.), appropriate nitroxide **17**, **18** or **20** (1 mmol, 1.2 equiv.), EDC (191.5 mg, 1 mmol, 1.2 equiv.), and DMAP (13 mg, 104  $\mu$ mol, 0.125 equiv.) in DCM (10 mL) was stirred under Ar for 1 d. The resulting reaction mixture was diluted (DCM, 150 mL), washed HCl (2 M, 30 mL) and brine (30 mL) solutions, dried over anhydrous  $Na_2SO_4$  and evaporated under reduced pressure. The crude residue was purified by

silica gel column chromatography ( $\text{CHCl}_3$ ) and then recrystallization from cyclohexane/EtOAc (except **28**) to give the corresponding salicylate-nitroxide.

**2,2,6,6-tetramethylpiperidin-1-yloxy-4-yl 2-acetoxybenzoate 27.** Reddish brown solid (251 mg, 90%). Mp. 52-53 °C. HPLC purity (>95%). HRMS (ES):  $m/z$  (%) = calcd. for  $\text{C}_{18}\text{H}_{25}\text{NO}_5^+$   $[\text{M} + \text{H}]^+$  335.1727; found 335.1688. ATR-FTIR:  $\nu_{\text{max}}$  = 2977 (w, ArC-H), 1724 (s, C=O), 1255 (s, C-O)  $\text{cm}^{-1}$ ; EPR:  $g = 2.0012$ ,  $a_{\text{N}} = 1.97$  mT.

**2-((2,2,6,6-tetramethylpiperidin-1-yloxy-4-yl)carbamoyl)phenyl acetate 28.** Reddish brown solid (153 mg, 55%). Mp. 50-52 °C. HPLC purity (>95%). HRMS (ES):  $m/z$  (%) = calcd. for  $\text{C}_{18}\text{H}_{26}\text{N}_2\text{O}_4^+$   $[\text{M} + \text{H}]^+$  334.1887; found 334.1850. ATR-FTIR:  $\nu_{\text{max}}$  = 3314 (m, N-H), 2976 (w, ArC-H), 1640 (s, C=O), 1546 (s, C=O), 1229 (s, C-O)  $\text{cm}^{-1}$ ;  $g = 2.0012$ ,  $a_{\text{N}} = 1.96$  mT.

**2-((1,1,3,3-tetramethylisoindolin-2-yloxy-5-yl)carbamoyl)phenyl acetate 29.** Yellow solid (162 mg, 53%). Mp. 101-102 °C. HPLC purity (>95%). HRMS (ES):  $m/z$  (%) = calcd. for  $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_4^+$   $[\text{M} + \text{H}]^+$  368.1731; found 368.1689. ATR-FTIR:  $\nu_{\text{max}}$  = 3340 (m, N-H), 2971 (w, ArC-H), 1681 (s, C=O), 1543 (s, C=O), 1255 (s, C-O)  $\text{cm}^{-1}$ ;  $g = 2.0010$ ,  $a_{\text{N}} = 1.80$  mT.

**General procedure for the synthesis of formyl-nitroxides 31-33.** Following similar procedure as for **27-29**, compounds **31-33** were obtained from **30** (1.31 mmol, 1.05 equiv.) and the appropriate carboxy-nitroxide **16**, **19** and **21** (1.25 mmol, 1 equiv.).

**2-Formylphenyl 2,2,5,5-tetramethylpyrrolidin-1-yloxy-3-carboxylate 31.** Yellow crystalline solid (319 mg, 88%). HPLC purity (>95%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 600 MHz):  $\delta = 7.19$  (s, br, 1 H, Ar-H), 7.47 (d, br, 1 H, Ar-H), 7.7 (s, br, 1 H, Ar-H), 7.9 (s, br, 1 H, Ar-H), 10.1 (s, br, HC=O). HRMS (ES):  $m/z$  (%) = calcd. for  $\text{C}_{16}\text{H}_{21}\text{NO}_4^+$   $[\text{M} + \text{H}]^+$  291.1465; found 291.1428. ATR-FTIR:  $\nu_{\text{max}}$  = 3350-2400 (m, br, O-H), 1734 (s, C=O), 1688 (s, C=O), 1288 (s, C-N), 1196 (s, C-O)  $\text{cm}^{-1}$ ;  $g = 2.0009$ ,  $a_{\text{N}} = 1.84$  mT.

**2-Formylphenyl 2,2,6,6-tetramethylpiperidin-1-yloxy-4-carboxylate 32.** Light reddish-brown, fluffy crystals (327 mg, 86%). Mp. 94-95 °C. HPLC purity (>95%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz): δ = 7.18 (s, br, 1 H, Ar-H), 7.47 (d, br, 1 H, Ar-H), 7.69 (s, br, 1 H, Ar-H), 7.91 (d, br, 1 H, *J* = 7.2 Hz, Ar-H), 10.1 (s, br, HC=O). HRMS (ES): *m/z* (%) = calcd. for C<sub>17</sub>H<sub>22</sub>NNaO<sub>4</sub><sup>+</sup> [M + Na]<sup>+</sup> 327.1441; found 327.1442. ATR-FTIR: ν<sub>max</sub> = 3079 (m, ArC-H), 1745 (s, C=O), 1699 (s, C=O), 1230 (s, C-N), 1154 (s, C-O) cm<sup>-1</sup>; g = 2.0011, a<sub>N</sub> = 1.97 mT.

**2-Formylphenyl 1,1,3,3-tetramethylisoindolin-2-yloxy-5-carboxylate 33.** Yellow crystalline solid (372 mg, 88%). Mp. 151-152 °C. HPLC purity (>95%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz): δ = 7.34 (d, br, 1 H, *J* = 7.2 Hz, Ar-H), 7.49 (t, br, 1 H, *J* = 7.2 Hz, Ar-H), 7.72 (t, br, 1 H, Ar-H), 7.98 (d, br, 1 H, *J* = 7.2 Hz, Ar-H), 10.21 (s, br, HC=O). HRMS (ES): *m/z* (%) = calcd. for C<sub>20</sub>H<sub>20</sub>NNaO<sub>4</sub><sup>+</sup> [M + Na]<sup>+</sup> 361.1285; found 361.1292. ATR-FTIR: ν<sub>max</sub> = 2978 (m, ArC-H), 1749 (s, C=O), 1708 (s, C=O), 1234 (s, C-N), 1197 (s, C-O) cm<sup>-1</sup>; g = 2.0010, a<sub>N</sub> = 1.83 mT.

**General procedure for the synthesis of carboxylic acids 34-36.** KH<sub>2</sub>PO<sub>4</sub> (48.3 mg, 355 μmol, 2 equiv. in 0.5 mL H<sub>2</sub>O) and H<sub>2</sub>O<sub>2</sub> (30 μL, 355 μmol, 1.5 equiv. 30% in H<sub>2</sub>O) were added to a solution of appropriate formyl nitroxide **31-33** (177.3 μmol, 1 equiv.) in MeCN (5 mL) at 0 °C. NaClO<sub>2</sub> (40.3 mg, 356 μmol, 2 equiv. in 0.5 mL H<sub>2</sub>O) was then added dropwise and the resulting solution stirred for 2 h. The reaction mixture was diluted with H<sub>2</sub>O and the aqueous layer extracted with DCM (3 x 30 mL). The DCM extract was washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography (EtOAc/0.01%AcOH) and then recrystallization from H<sub>2</sub>O/MeOH to give the corresponding carboxylic acid nitroxide (**34-36**).

**2-((2,2,5,5-Tetramethylpyrrolidin-1-yloxy-3-carbonyl)oxy)benzoic acid 34.** Yellow crystalline solid (319 mg, 88%). Mp. 161-162 °C. HPLC purity (>95%). <sup>1</sup>H NMR (CDCl<sub>3</sub>,

600 MHz):  $\delta$  = 7.18 (s, br, 1 H, Ar-*H*), 7.74 (br, s, 1 H, Ar-*H*), 7.66 (s, br, 1 H, Ar-*H*), 8.1 (d, br, 1 H, Ar-*H*). HRMS (ES):  $m/z$  (%) = calcd. for C<sub>16</sub>H<sub>21</sub>NO<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup> 307.1414; found 307.1377. ATR-FTIR:  $\nu_{\max}$  = 3500-2400 (m, br, O-H), 2976 (w, ArC-H), 1766 (s, C=O), 1713 (s, C=O), 1204 (s, C-N), 1126 (s, C-O) cm<sup>-1</sup>;  $g$  = 2.0009,  $a_N$  = 1.84 mT.

**2-((2,2,6,6-Tetramethylpiperidin-1-yloxy-4-carbonyloxy)benzoic acid 35.** Light reddish-brown, fluffy crystals (327 mg, 86%). Mp. 149 °C (dec.). HPLC purity (>95%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz):  $\delta$  = 7.17 (s, br, 1 H, Ar-*H*), 7.4 (s, br, 1 H, Ar-*H*), 7.67 (s, br, 1 H, Ar-*H*), 8.1 (s, br, 1 H, Ar-*H*). HRMS (ES):  $m/z$  (%) = calcd. for C<sub>17</sub>H<sub>23</sub>NO<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup> 321.1571; found 321.1532. ATR-FTIR:  $\nu_{\max}$  = 3500-2400 (m, br, O-H), 1755 (s, C=O), 1716 (s, C=O), 1241 (s, C-N), 1145 (s, C-O) cm<sup>-1</sup>;  $g$  = 2.009,  $a_N$  = 1.84 mT.

**2-((1,3,3-Tetramethylisindolin-2-yloxy-5-carbonyloxy)benzoic acid 36.** Yellow crystalline solid (372 mg, 88%). Mp. 193 °C (dec.). HPLC purity (>95%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz):  $\delta$  = 7.27 (s, br, 1 H, Ar-*H*), 7.41 (s, br, 1 H, Ar-*H*), 7.68 (s, br, 1 H, Ar-*H*), 8.12 (s, br, 1 H, Ar-*H*). HRMS (ES):  $m/z$  (%) = (30); calcd. for C<sub>20</sub>H<sub>22</sub>NO<sub>5</sub><sup>+</sup> [M + 2H]<sup>+</sup> 356.1492; found 356.1409. ATR-FTIR:  $\nu_{\max}$  = 3350-2400 (m, br, O-H), 1734 (s, C=O), 1688 (s, C=O), 1288 (s, C-N), 1196 (s, C-O) cm<sup>-1</sup>;  $g$  = 2.0009,  $a_N$  = 1.80 mT.

Indomethacin-nitroxide derivatives **37-39** were obtained from benzyl indomethacin (453  $\mu$ mol, 1 equiv.) and the appropriate carboxy-nitroxide **17-19** (498  $\mu$ mol, 1.1 equiv.) following similar procedure as for **27-29** (silica gel column chromatography: Hexane/EtOAc, 3:1).

**2-(1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)-N-(2,2,6,6-tetramethylpiperidin-1-yloxy-yl)acetate 37.** Pale orange solid (97%). Mp. 71-72 °C. HPLC purity (>95%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  = 2.44 (br, s, 3 H, CH<sub>3</sub>), 3.70 (br, s, 2 H, CH<sub>2</sub>), 3.86 (br, s, 3 H, OCH<sub>3</sub>), 6.69 (br, d, 1 H,  $J$  = 7.8 Hz, Ar-*H*), 6.87 (br, d, 1 H,  $J$  = 8.4 Hz, Ar-*H*), 6.99 (br, s, 1 H, Ar-*H*), 7.49 (br, d, 2 H,  $J$  = 6 Hz, Ar-*H*), 7.67 (br, d, 2 H,  $J$  = 6.6



Hz, Ar-*H*). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ = 21.0 (CH<sub>3</sub>), 30.9 (CH<sub>2</sub>), 52.2 (OCH<sub>3</sub>), 117.3 (Ar-C), 117.31 (Ar-C), 120.0 (Ar-C), 123.3 (Ar-C), 124.4 (Ar-C), 142.5 (Ar-C), 144.3 (Ar-C), 165.1 (Ar-C), 170.4 (C=O), 207.0 (C=O). HRMS (ES): *m/z* (%) = calcd. for C<sub>28</sub>H<sub>33</sub>ClN<sub>2</sub>O<sub>5</sub>· [M + H]<sup>+</sup> 512.2073; found 512.1929. ATR-FTIR: ν<sub>max</sub>= 2973 (m, Ar C-H), 1732 (s, C=O), 1680 (C=O), 1314 (C-N), 1141 (C-O) cm<sup>-1</sup>; g = 2.0012, a<sub>N</sub> = 1.94 mT.

**2-(1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)-*N*-(2,2,6,6-tetramethylpiperidin-1-yloxy-4-yl)acetamide 38.** Reddish brown crystals (87%). Mp. 199-201 °C. HPLC purity (>95%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ = 2.46 (br, s, 3 H, CH<sub>3</sub>), 3.7 (br, s, 2 H, CH<sub>2</sub>), 3.91 (br, s, 3 H, OCH<sub>3</sub>), 5.28 (br, s, 2 H, Ar-*H*), 6.69 (br, d, 2 H, Ar-*H*), 7.28 (br, s, 2 H, Ar-), 7.6 (br, d, 2 H, Ar-). HRMS (ES): *m/z* (%) = calcd. for C<sub>28</sub>H<sub>34</sub>ClN<sub>3</sub>O<sub>4</sub>· [M + H]<sup>+</sup> 511.2232; found 511.2199. ATR-FTIR: ν<sub>max</sub>= 1079 (w, N-H), 2973 (m, Ar C-H), 1636 (s, C=O), 1555 (C=O), 1350 (C-N), 1111 (C-O) cm<sup>-1</sup>; g = 2.0012, a<sub>N</sub> = 1.95 mT.

**2-(1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)-*N*-(1,1,3,3-tetramethylisoindolin-2-yloxy-5-yl)acetamide 39.** Light yellow crystals (92%). Mp. 164-166 °C. HPLC purity (>95%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ = 2.49 (br, s, 3 H, CH<sub>3</sub>), 3.83 (br, s, 3 H, OCH<sub>3</sub>), 3.88 (br, s, 2 H, CH<sub>2</sub>), 6.74 (br, d, 1 H, *J* = 8.4 Hz, Ar-*H*), 6.9 (br, d, 1 H, *J* = 8.4 Hz, Ar-*H*), 6.96 (br, s, 1 H, Ar-*H*), 7.51 (br, d, 2 H, *J* = 6 Hz, Ar-*H*), 7.7 (br, d, 2 H, *J* = 6 Hz, Ar-*H*). HRMS (ES): *m/z* (%) = calcd. for C<sub>31</sub>H<sub>31</sub>ClN<sub>3</sub>NaO<sub>4</sub>· [M + Na]<sup>+</sup> 567.1901; found 567.1942. ATR-FTIR: ν<sub>max</sub>= 3299 (w, N-H), 2973 (m, Ar C-H), 1677 (s, C=O), 1599 (C=O), 1313 (C-N), 1223 (C-O) cm<sup>-1</sup>; g = 2.0010, a<sub>N</sub> = 1.79 mT.

Benzyl benzoate-nitroxide derivatives **40-43** were obtained from benzyl salicylate **25** (1.31 mmol, 1.05 equiv.) and the appropriate carboxy-nitroxide **12**, **16**, **19** and **21** (1.25 mmol, 1 equiv.) following similar procedure as for **27-29** (silica gel column chromatography: Hexane/EtOAc, 5:1).

**2-(Benzyloxy)carbonylphenyl-2,2,5,5-tetramethylpyrrolidin-1-yloxy-3-carboxylate**

**40.** Yellow oil (78%). HPLC purity (>95%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz): δ = 5.33 (s, br, 2 H, CH<sub>2</sub>), 6.9 (s, br, 1 H, Ar-H), 7.4 (br, m, 7 H, Ar-H), 7.62 (s, br, 1 H, Ar-H), 8.1 (d, br, 1 H, J = 7.6 Hz, Ar-H). HRMS (ES): *m/z* (%) = calcd. for C<sub>23</sub>H<sub>26</sub>NNaO<sub>5</sub><sup>+</sup> [M + Na]<sup>+</sup> 419.1703; found 419.1699. ATR-FTIR: ν<sub>max</sub> = 2975 (w, ArC-H), 1761 (s, C=O), 1719 (s, C=O), 1251 (s, C-N), 1134 and 1075 (s, C-O) cm<sup>-1</sup>; g = 2.0009, a<sub>N</sub> = 1.84 mT.

**2-(Benzyloxycarbonyl)phenyl-2,2,6,6-tetramethylpiperidin-1-yloxy-4-carboxylate 41.**

Light reddish-brown fluffy solid (71%). Mp. 112-113 °C. HPLC purity (>95%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz): δ = 5.34 (s, br, 2 H, CH<sub>2</sub>), 7.1 (d, br, 1 H, Ar-H), 7.34 (br, m, 7 H, Ar-H), 7.6 (s, br, 1 H, Ar-H), 8.1 (d, br, 1 H, J = 7.2 Hz, Ar-H). HRMS (ES): *m/z* (%) = calcd. for C<sub>24</sub>H<sub>30</sub>NO<sub>5</sub><sup>+</sup> [M + 2H]<sup>+</sup> 412.2118; found 412.2126. ATR-FTIR: ν<sub>max</sub> = 2981 (w, ArC-H), 1745 (s, C=O), 1722 (s, C=O), 1257 (s, C-N), 1085 (s, C-O) cm<sup>-1</sup>; g = 2.0012, a<sub>N</sub> = 1.96 mT.

**2-(Benzyloxycarbonyl)phenyl-1,1,3,3-tetramethylisoindolin-2-yloxy-5-carboxylate 42.**

Yellow crystalline solid (72%). Mp. 97-98 °C. HPLC purity (>95%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz): δ = 5.25 (s, br, 2 H, CH<sub>2</sub>), 7.26 (s, br, 7 H, Ar-H), 7.42 (br, s, 1 H, Ar-H), 7.66 (s, br, 1 H, Ar-H), 8.17 (d, br, 1 H, J = 7.2 Hz, Ar-H). HRMS (ES): *m/z* (%) = calcd. for C<sub>27</sub>H<sub>28</sub>NO<sub>5</sub><sup>+</sup> [M + 2H]<sup>+</sup> 444.1962; found 446.1980. ATR-FTIR: ν<sub>max</sub> = 3038 (w, ArC-H), 1744 (s, C=O), 1705 (s, C=O), 1290 (s, C-N), 1192 (s, C-O) cm<sup>-1</sup>; g = 2.0010, a<sub>N</sub> = 1.80 mT.

**2-(Benzyloxycarbonyl)phenyl-6-methoxy-1,1,3,3-tetramethylisoindolin-2-yloxy-5-carboxylate 43.**

Yellow solid (73%). Mp. 142-143 °C. HPLC purity (>95%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz): δ = 4.21 (br, s, 3 H, OCH<sub>3</sub>), 5.27 (s, br, 2 H, CH<sub>2</sub>), 7.27 (s, br, 2 H, Ar-H), 7.4 (br, m, 2 H, Ar-H), 7.62 (s, br, 1 H, Ar-H), 8.11 (d, br, 1 H, J = 7.8 Hz, Ar-H). HRMS (ES): *m/z* (%) = calcd. for C<sub>28</sub>H<sub>28</sub>NNaO<sub>6</sub><sup>+</sup> [M + Na]<sup>+</sup> 497.1809; found 497.01806. ATR-FTIR: ν<sub>max</sub> = 2979 (w, ArC-H), 1748 (s, C=O), 1718 (s, C=O), 1294 (s, C-N), 1192 (s, C-O) cm<sup>-1</sup>; g = 2.0010, a<sub>N</sub> = 1.79 mT.

**Methyl 2-acetoxy-5-nitrobenzoate 45.** A suspension of methyl 2-hydroxy-5-nitrobenzoate **44** (650 mg, 3.3 mmol, 1 equiv.) in THF (15 mL) under Ar was cooled to 0 °C. TEA (1.15 mL, 8.24 mmol, 2.5 equiv.) and AcCl (459  $\mu$ L, 6.59 mmol, 2 equiv.) were added dropwise and the resulting mixture stirred for 2 h. The reaction mixture was diluted with H<sub>2</sub>O and the aqueous layer extracted with DCM (4 x 60 mL). The combined DCM extracts were washed brine (50 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (CHCl<sub>3</sub>/Hexane, 1:1) and then recrystallization from cyclohexane to give **45** as a white crystalline solid (91%). Mp. 75-76 °C (Lit.,<sup>47</sup> 73-74 °C). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  = 2.4 (s, 3 H, C=OCH<sub>3</sub>), 3.94 (s, 3 H, OCH<sub>3</sub>), 7.3 (d, 1 H, *J* = 8.4 Hz, Ar-*H*), 8.42 (dd, 1 H, *J* = 8.4 Hz, 2.8 Hz, Ar-*H*), 8.9 (d, 1 H, *J* = 2.8 Hz, Ar-*H*). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  = 20.9 (C=OCH<sub>3</sub>), 52.8 (OCH<sub>3</sub>), 124.4 (Ar-C), 125.3 (Ar-C), 127.4 (Ar-C), 128.5 (Ar-C), 145.3 (Ar-C), 155.3 (Ar-C), 163.0 (C=O), 168.8 (C=O). ATR-FTIR:  $\nu_{\text{max}}$  = 3102 (w, Ar C-H), 1759 (s, C=O), 1727 (s, C=O), 1529 (s, NO<sub>2</sub>), 1190 (s, C-O) cm<sup>-1</sup>.

**Methyl 2-acetoxy-5-aminobenzoate 46.** A solution of methyl 2-acetoxy-5-nitrobenzoate **45** (500 mg, 2.09 mmol, 1 equiv.) in EtOAc (20 mL) was hydrogenated at 50 psi over 10% Pd/C (50 mg) for 4 h in a Parr Hydrogenator. The resulting solution was filtered through Celite and the filtrate was concentrated under reduced pressure. Compound **46** was obtained as a light brown solid (394 mg, 90%) and was used in the next step without further purification. It could be recrystallised from cyclohexane/EtOAc. Mp. 107 °C (dec.), (Lit., [51] 103-105 °C). HPLC purity (>95%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  = 2.31 (s, 3 H, C=OCH<sub>3</sub>), 3.74 (s, 2 H, NH<sub>2</sub>), 3.84 (s, 3 H, OCH<sub>3</sub>), 6.82 (dd, 1 H, *J* = 8.4 Hz, 3 Hz, Ar-*H*), 6.88 (d, 1 H, *J* = 8.4 Hz, Ar-*H*), 7.28 (d, 1 H, *J* = 3 Hz, Ar-*H*). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  = 21.0 (C=OCH<sub>3</sub>), 52.2 (OCH<sub>3</sub>), 117.5 (Ar-C), 120.0 (Ar-C), 123.3 (Ar-C), 124.4 (Ar-C), 142.5 (Ar-C), 144.4 (Ar-C), 165.1 (C=O), 170.4 (C=O).

Methyl benzoate amide-nitroxides **47-52** were obtained from **46** (124 mg, 591  $\mu\text{mol}$ , 1.1 equiv.) and the appropriate carboxylic acid **16**, **19**, **21**, **23**, cinnamic acid, and trolox (537  $\mu\text{mol}$ , 1 equiv.) following similar procedure as for **27-29** (silica gel column chromatography: Hexane/EtOAc, 3:1).

**Methyl 2-acetoxy-5-(2,2,5,5-tetramethylpyrrolidin-1-yloxy-3-carboxamido)benzoate 47.** Yellow solid (170 mg, 84%). Mp. 69-70 °C. HPLC purity (>95%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 600 MHz):  $\delta$  = 2.37 (s, 3 H,  $\text{C}=\text{OCH}_3$ ), 3.89 (s, 3 H,  $\text{OCH}_3$ ), 7.12 (br, s, 1 H, Ar-H), 7.84 (br, s, 1 H, Ar-H), 8.04 (br, s, 1 H, Ar-H). HRMS (ES):  $m/z$  (%) = calcd. for  $\text{C}_{19}\text{H}_{26}\text{N}_2\text{O}_6$   $[\text{M} + \text{H}]^+$  378.1785; found 378.1744. ATR-FTIR:  $\nu_{\text{max}}$  = 3330 (m, N-H), 2975 (m, ArC-H), 1768 (s, C=O), 1726 (s, C=O), 1540 (s, C=O), 1366 (s, C-N), 1183 (s, C-O)  $\text{cm}^{-1}$ ;  $g$  = 2.0008,  $a_{\text{N}}$  = 1.83 mT.

**Methyl 2-acetoxy-5-(2,2,6,6-tetramethylpiperidin-1-yloxy-4-carboxamido)benzoate 48.** Light reddish-brown solid (156 mg, 74%). Mp. 205 °C (dec.). HPLC purity (>95%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 600 MHz):  $\delta$  = 2.39 (s, 3 H,  $\text{C}=\text{OCH}_3$ ), 3.85 (s, 3 H,  $\text{OCH}_3$ ), 7.28 (br, dd, 1 H, Ar-H), 7.73 (br, d, 1 H, Ar-H), 8.14 (br, d, 1 H, Ar-H). HRMS (ES):  $m/z$  (%) = calcd. for  $\text{C}_{20}\text{H}_{27}\text{N}_2\text{NaO}_6$   $[\text{M} + \text{Na}]^+$  414.1761; found 414.1760. ATR-FTIR:  $\nu_{\text{max}}$  = 3339 (m, N-H), 2977 (m, ArC-H), 1765, 1727 (s, C=O), 1691 (s, C=O), 1548 (s, C=O), 1176 (s, C-N), 1076 (s, C-O)  $\text{cm}^{-1}$ ;  $g$  = 2.0011,  $a_{\text{N}}$  = 1.95 mT.

**Methyl 2-acetoxy-5-(1,1,3,3-tetramethylisoinolin-2-yloxy-5-carboxamido)benzoate 49.** Yellow solid (190 mg, 83%). Mp. 122-124 °C. HPLC purity (>95%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 600 MHz):  $\delta$  = 2.30 (s, 3 H,  $\text{C}=\text{OCH}_3$ ), 3.79 (s, 3 H,  $\text{OCH}_3$ ), 7.05 (br, s, 1 H, Ar-H), 7.92 (br, s, 1 H, Ar-H), 8.09 (br, s, 1 H, Ar-H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  = 21.3 ( $\text{C}=\text{OCH}_3$ ), 52.6 ( $\text{OCH}_3$ ), 123.52 (Ar-C), 124.7 (Ar-C), 135.5 (Ar-C), 147.2 (Ar-C), 164.8 (C=O), 170.1 (C=O). HRMS (ES):  $m/z$  (%) = calcd. for  $\text{C}_{23}\text{H}_{26}\text{N}_2\text{O}_6$   $[\text{M} + \text{H}]^+$  426.1785; found 426.1794.

ATR-FTIR:  $\nu_{\max}$  = 3376 (m, N-H), 2980 (m, ArC-H), 1735, 1725 (s, C=O), 1664 (s, C=O), 1522 (s, C=O), 1271 (s, C-N), 1226 and 1189 (s, C-O)  $\text{cm}^{-1}$ ;  $g = 2.0009$ ,  $a_{\text{N}} = 1.80$  mT.

**Methyl 2-acetoxy-5-(1,1,3,3-tetraethylisoinolin-2-yloxy-5-carboxamido)benzoate 50.**

Yellow solid (209.5 mg, 81%). Mp. 122-124 °C. HPLC purity (>95%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 600 MHz):  $\delta = 2.41$  (s, 3 H,  $\text{C}=\text{OCH}_3$ ), 3.91 (s, 3 H,  $\text{OCH}_3$ ), 7.17 (br, s, 1 H, Ar-H), 8.05 (br, s, 1 H, Ar-H), 8.22 (br, s, 1 H, Ar-H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta = 21.15$  ( $\text{C}=\text{OCH}_3$ ), 52.49 ( $\text{OCH}_3$ ), 123.5 (Ar-C), 124 (Ar-C), 135.67 (Ar-C), 147.13 (Ar-C), 164.48 ( $\text{C}=\text{O}$ ), 170.07 ( $\text{C}=\text{O}$ ). HRMS (ES):  $m/z$  (%) = calcd. for  $\text{C}_{27}\text{H}_{34}\text{N}_2\text{O}_6$   $[\text{M} + \text{H}]^+$  482.2411; found 482.2407. ATR-FTIR:  $\nu_{\max}$  = 3369 (m, N-H), 2971 (m, ArC-H), 1743, 1719 (s, C=O), 1675 (s, C=O), 1534 (s, C=O), 1298 (s, C-N), 1219 and 1188 (s, C-O)  $\text{cm}^{-1}$ ;  $g = 2.0010$ ,  $a_{\text{N}} = 1.76$  mT.

**Methyl 2-acetoxy-5-(6-hydroxy-2,5,7,8-tetraethylchromane-2-carboxamido)benzoate**

**51.** Beige solid (206.3 mg, 87%). Mp. 123-124 °C. HPLC purity (>95%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 600 MHz):  $\delta = 1.58$  (s, 3 H,  $\text{CH}_3$ ), 1.96 (m, 1 H,  $\text{CH}_2$ ), 2.21 (s, 3 H,  $\text{CH}_3$ ), 2.27 (s, 3 H,  $\text{CH}_3$ ), 2.33 (s, 3 H,  $\text{CH}_3$ ), 2.4 (s, 3 H,  $\text{CH}_3$ ), 2.42 (m, 1 H,  $\text{CH}_2$ ), 2.65 (m, 2 H,  $\text{CH}_2$ ), 3.86 (s, 3 H,  $\text{OCH}_3$ ), 4.32 (s, 1 H, NH), 7.05 (d, 1 H,  $J = 9$  Hz, Ar-H), 7.75 (dd, 1 H,  $J = 9$  Hz, 3 Hz, Ar-H), 8.08 (d, 1 H,  $J = 3$  Hz, Ar-H), 8.38 (s, 1 H, OH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta = 11.3$  ( $\text{CH}_3$ ), 12.1 ( $\text{CH}_3$ ), 12.3 ( $\text{CH}_3$ ), 20.4 ( $\text{CH}_3$ ), 20.9 ( $\text{CH}_3$ ), 24.2 ( $\text{CH}_2$ ), 29.5 ( $\text{CH}_2$ ), 52.4 ( $\text{OCH}_3$ ), 118.0 (Ar-C), 119.1 (Ar-C), 121.6 (Ar-C), 121.9 (Ar-C), 122.4 (Ar-C), 123.5 (Ar-C), 124.4 (Ar-C), 124.8 (Ar-C), 135.3 (Ar-C), 143.9 (Ar-C), 146.0 (Ar-C), 146.8 (Ar-C), 164.5 ( $\text{C}=\text{O}$ ), 169.8 ( $\text{C}=\text{O}$ ), 172.9 ( $\text{C}=\text{O}$ ). HRMS (ES):  $m/z$  (%) = calcd. for  $\text{C}_{24}\text{H}_{28}\text{NO}_7$   $[\text{M} + \text{H}]^+$  442.1860; found 442.1799. ATR-FTIR:  $\nu_{\max}$  = 3478 (m, O-H), 3364 (m, N-H), 2929 (m, ArC-H), 1762, 1728 (s, C=O), 1671 (s, C=O), 1531 (s, C=O), 1184 and 1078 (s, C-O)  $\text{cm}^{-1}$ .

**Methyl (E)-2-acetoxy-5-(3-(3,5-di-tert-butyl-4-hydroxyphenyl)acrylamido)benzoate**

**52.** Beige solid (233.5 mg, 93%). Mp. 181-182 °C. HPLC purity (>95%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta = 1.48$  (s, 18 H, 2 x  $\text{C}(\text{CH}_3)_3$ ), 2.36 (s, 3 H,  $\text{CH}_3$ ), 3.86 (s, 3 H,  $\text{OCH}_3$ ), 5.52 (s, 1

H, *NH*), 6.35 (d, 1 H,  $J = 15.6$  Hz, *CH=CH*), 7.07 (d, 1 H,  $J = 7.8$  Hz, *Ar-H*), 7.41 (d, 3 H, *Ar-H*), 7.72 (d, 1 H,  $J = 15.6$  Hz, *CH=CH*), 7.96 (d, 1 H,  $J = 7.8$  Hz, *Ar-H*), 8.13 (s, 1 H, *OH*).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta = 21$  ( $\text{CH}_3$ ), 30.12 ( $\text{C}(\text{CH}_3)_3$ ), 34.36 ( $\text{C}(\text{CH}_3)_3$ ), 52.3 ( $\text{OCH}_3$ ), 116.83 (*Ar-C*), 122.65 (*Ar-C*), 123.2 (*Ar-C*), 124.3 (*Ar-C*), 125.16 (*Ar-C*), 125.41 (*CH=CH*), 125.8 (*Ar-C*), 136.28 (*Ar-C*), 136.38 (*CH=CH*), 144.02 (*Ar-C*), 146.5 (*Ar-C*), 156.07 (*Ar-C*), 164.5 ( $\text{C}=\text{O}$ ), 164.57 ( $\text{C}=\text{O}$ ), 170.23 ( $\text{C}=\text{O}$ ). HRMS (ES):  $m/z$  (%) = calcd. for  $\text{C}_{27}\text{H}_{34}\text{NO}_6$  [ $\text{M} + \text{H}$ ] $^+$  468.2381; found 468.2379. ATR-FTIR:  $\nu_{\text{max}} = 3623$  (m, br, O-H), 3305 (m, br, N-H), 2954 (m, ArC-H), 1726 (s,  $\text{C}=\text{O}$ ), 1621 (s,  $\text{C}=\text{O}$ ), 1540 (s,  $\text{C}=\text{O}$ ), 1185 (s, C-O)  $\text{cm}^{-1}$ .

**General procedure for the synthesis of 5-ASA nitroxides 53-56.** A solution of NaOH (3 mL, 1 M) was added to a solution of appropriate amide (**47-50**) in THF (5 mL) and the reaction mixture was stirred at RT overnight. THF was removed under pressure and the aqueous layer washed with DCM, then cooled in ice/ $\text{H}_2\text{O}$  bath and acidified (to pH 1) with HCl (2 M). The precipitate formed was isolated by filtration and purified by recrystallization from  $\text{H}_2\text{O}/\text{MeOH}$  to give the corresponding salicylic acid derivative (**53-56**).

**2-Hydroxy-5-(2,2,5,5-tetramethylpyrrolidin-1-yloxy)-3-carboxamido)benzoic acid 53.** Yellow solid (170 mg, 84%). Mp. 171 °C (dec.). HPLC purity (>95%). HRMS (ES):  $m/z$  (%) = calcd. for  $\text{C}_{16}\text{H}_{23}\text{N}_2\text{O}_5$  [ $\text{M} + 2\text{H}$ ] $^+$  323.1601; found 323.1590. ATR-FTIR:  $\nu_{\text{max}} = 3200$  (m, br, O-H), 3921 (m, ArC-H), 1657 (s,  $\text{C}=\text{O}$ ), 1547 (s,  $\text{C}=\text{O}$ ), 1225 (s, C-O)  $\text{cm}^{-1}$ ;  $g = 2.0008$ ,  $a_{\text{N}} = 1.84$  mT.

**2-Hydroxy-5-(2,2,6,6-tetramethylpiperidin-1-yloxy)-4-carboxamido)benzoic acid 54.** Light reddish-brown solid (156 mg, 74%). HPLC purity (>95%). HRMS (ES):  $m/z$  (%) = calcd. for  $\text{C}_{17}\text{H}_{25}\text{N}_2\text{O}_5$  [ $\text{M} + 2\text{H}$ ] $^+$  337.1758; found 337.1753. ATR-FTIR:  $\nu_{\text{max}} = 3200$  (m, br, O-H), 3921 (m, ArC-H), 1657 (s,  $\text{C}=\text{O}$ ), 1547 (s,  $\text{C}=\text{O}$ ), 1225 (s, C-O)  $\text{cm}^{-1}$ ;  $g = 2.0012$ ,  $a_{\text{N}} = 1.94$  mT.

**2-Hydroxy-5-(1,1,3,3-tetramethylisoindolin-2-yloxy-5-carboxamido)benzoic acid 55.**

Yellow solid (190 mg, 83%). Mp. 207 °C (dec.). HPLC purity (>95%). HRMS (ES):  $m/z$  (%) = calcd. for  $C_{20}H_{21}N_2NaO_5^+$   $[M + Na]^+$  392.1343; found 392.3146. ATR-FTIR:  $\nu_{max}$  = 3100 (m, br, O-H), 2954 (m, ArC-H), 1676, 1644 (s, C=O), 1565 (s, C=O), 1187 (s, C-O)  $cm^{-1}$ ;  $g = 2.0009$ ,  $a_N = 1.78$  mT.

**2-Hydroxy-5-(1,1,3,3-tetraethylisoindolin-2-yloxy-5-carboxamido)benzoic acid 56.**

Yellow solid (209.5 mg, 81%). Mp. 208 °C (dec.). HPLC purity (>95%). HRMS (ES):  $m/z$  (%) = calcd. for  $C_{24}H_{30}N_2O_5^+$   $[M + H]^+$  426.2110; found 425.2109. ATR-FTIR:  $\nu_{max}$  = 3295 (m, br, O-H), 2968 (m, ArC-H), 1686, 1637 (s, C=O), 1536 (s, C=O), 1167 (s, C-O)  $cm^{-1}$ ;  $g = 2.0010$ ,  $a_N = 1.75$  mT.

**2-Methoxy-5-methoxycarbonylmethyl-1,1,3,3-tetramethylisoindoline 58.** A Schlenk tube containing 5-bromo-2-methoxy-1,1,3,3-tetramethylisoindoline **57** (316 mg, 1.11 mmol, 1 equiv.), potassium methyl malonate (434.13 mg, 2.78 mmol, 2.5 equiv.), allylpalladium (II) chloride dimer (8.14 mg, 22  $\mu$ mol, 0.02 equiv.), BINAP (41.54 mg, 67  $\mu$ mol, 0.06 equiv.), and DMAP (13.6 mg, 11  $\mu$ ol, 0.01 equiv.) in mesitylene (10 mL) was degassed and then heated for 1 d at 140 °C. The resulting mixture was concentrated under reduced pressure. The crude residue obtained was purified by silica gel column chromatography (Hexane/EtOAc, 5:0.2) to give 2-methoxy-5-methoxycarbonylmethyl-1,1,3,3-tetramethylisoindoline **58** as a white solid (164 mg, 54%). Mp. 79-80 °C. HPLC purity (>95%).  $^1H$  NMR ( $CDCl_3$ , 400 MHz):  $\delta$  = 1.42 (br, s, 12 H,  $CH_3$ ), 3.61 (s, 2 H,  $CH_2$ ), 3.69 (s, 3 H,  $OCH_3$ ), 3.77 (s, 3 H,  $OCH_3$ ), 7.00 (s, 1 H, Ar-H), 7.05 (d, 1 H,  $J = 8$  Hz, Ar-H), 7.14 (d, 1 H,  $J = 8$  Hz, Ar-H).  $^{13}C$  NMR ( $CDCl_3$ , 100 MHz):  $\delta$  = 25.3 (w, br,  $CH_3$ ), 29.7 (w, br,  $CH_3$ ), 41.2 ( $CH_2$ ), 52.1 ( $OCH_3$ ), 65.5 ( $OCH_3$ ), 66.9 ( $C-CH_3$ ), 67.1 ( $C-CH_3$ ), 121.7 (Ar-C), 122.4 (Ar-C), 128.2 (Ar-C), 132.9 (Ar-C), 144.1 (Ar-C), 145.6 (Ar-C), 172.2 ( $C=O$ ). HRMS (ES):  $m/z$  (%) = calcd. for

$C_{16}H_{24}NO_3$   $[M + H]^+$  278.1715; found 278.1680. ATR-FTIR:  $\nu_{max}$  = 2975 (m, Ar C-H), 1736 (s, C=O), 1206 and 1143 (s, C-O)  $cm^{-1}$ .

**5-Methoxycarbonylmethyl-1,1,3,3-tetramethylisoindolin-2-yloxy 59.** *m*-Chloroperoxybenzoic acid (471 mg, 1.62 mmol, 1.5 equiv., 77%) was added to a solution of methyl 2-methoxy-5-methoxycarbonylmethyl-1,1,3,3-tetramethylisoindoline **58** (300 mg, 1.08 mmol, 1 equiv.) in DCM (150 mL) at 0 °C. The reaction mixture was stirred for 30 min and then at RT for further 3.5 h. The resulting solution was washed with saturated  $NaHCO_3$  (2 x 30 mL) and brine solutions and dried over anhydrous  $Na_2SO_4$ . The filtrate was concentrated *in vacuo* and the crude residue purified by flash column chromatography (Hexane/EtOAc, 4:1) to give **59** as bright yellow solid (253 mg, 89%). Mp. 96-96.5 °C. HPLC purity (>95%). HRMS (ES):  $m/z$  (%) = calcd. for  $C_{15}H_{21}NO_3^+$   $[M + H]^+$  263.1516; found 263.1508. ATR-FTIR:  $\nu_{max}$  = 2976 (m, Ar C-H), 1737 (s, C=O), 1155 (s, C-O)  $cm^{-1}$ ;  $g = 2.0009$ ,  $a_N = 1.84$  mT.

**5-Carboxymethyl-1,1,3,3-tetramethylisoindolin-2-yloxy 23.** NaOH (4 mL, 2 M) was added to a solution of methyl ester **59** (250 mg, 953  $\mu$ mol, 1 equiv.) in MeOH (6 mL) and the resulting reaction mixture was stirred for 2 h at 60 °C. The reaction mixture was cooled to RT and diluted with  $H_2O$  (30 mL). The aqueous layer was washed with  $Et_2O$  (30 mL) and acidified (pH 1) with HCl (2 M). The aqueous layer was extracted with  $Et_2O$  (3 x 60 mL) and the combined organic extracts were washed with brine (40 mL), dried over anhydrous  $Na_2SO_4$  and concentrated *in vacuo* to give the carboxylic acid **23** as yellow solid (222 mg, 94%). Mp. 123-124 °C. HPLC purity (>95%). HRMS (ES):  $m/z$  (%) = calcd. for  $C_{14}H_{18}NNaO_3^+$   $[M + Na]^+$  271.1179; found 271.1174. ATR-FTIR:  $\nu_{max}$  = 3000 (s, br, O-H), 2977 (m, Ar C-H), 1729 (s, C=O), 1144 (s, C-O)  $cm^{-1}$   $g = 2.0009$ ,  $a_N = 1.83$  mT.

**Methyl 2-acetoxy-5-(2-(1,1,3,3-tetramethylisoindolin-2-yloxy-5-yl)acetamido) benzoate 60** was obtained from **46** (124 mg, 591  $\mu$ mol, 1.1 equiv.), **59** (133.3 mg, 537  $\mu$ mol, 1 equiv.),



EDC (123.5 mg, 599  $\mu\text{mol}$ , 1.2 equiv.), and DMAP (8.2 mg, 67  $\mu\text{mol}$ , 0.125 equiv.) in DCM (10 mL) by following similar coupling conditions as described for **27-29** (silica gel column chromatography: Hexane/EtOAc, 3:1). Yellow solid (224.2 mg, 95%). Mp. 215 °C (dec.). HPLC purity (>95%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 600 MHz):  $\delta$  = 2.34 (s, 3 H,  $\text{C}=\text{OCH}_3$ ), 3.85 (s, 3 H,  $\text{OCH}_3$ ), 4.21 (br, s, 2 H,  $\text{CH}_2$ ), 7.09 (br, s, 1 H, Ar-H), 7.86 (br, s, 1 H, Ar-H), 7.95 (br, s, 1 H, Ar-H). HRMS (ES):  $m/z$  (%) = calcd. for  $\text{C}_{24}\text{H}_{28}\text{N}_2\text{O}_6$   $[\text{M} + \text{H}]^+$  440.1942; found 440.1939. ATR-FTIR:  $\nu_{\text{max}}$  = 3273 (m, N-H), 2976 (m, ArC-H), 1760, 1729 (s, C=O), 1696 (s, C=O), 1548 (s, C=O), 1297 (s, C-N), 1191 and 1079 (s, C-O)  $\text{cm}^{-1}$ ;  $g$  = 2.0009,  $a_{\text{N}}$  = 1.82 mT.

**2-Hydroxy-5-(2-(1,1,3,3-tetramethylisoindolin-2-yloxy-5-yl)acetamido)benzoic acid 61** was obtained from **60** by following similar conditions as described for **53-56**. Yellow solid (95%). Mp. 115 °C (dec.). HPLC purity (>95%). HRMS (ES):  $m/z$  (%) = calcd. for  $\text{C}_{21}\text{H}_{25}\text{N}_2\text{O}_5$   $[\text{M} + 2\text{H}]^+$  385.1758; found 385.1763. ATR-FTIR:  $\nu_{\text{max}}$  = 3263 (m, br, O-H), 2923 (m, ArC-H), 1674, 1647 (s, C=O), 1561 (s, C=O), 1208 (s, C-O)  $\text{cm}^{-1}$ ;  $g$  = 2.0009,  $a_{\text{N}}$  = 1.82 mT.

**Methyl 2-acetoxy-5-((2-(1,1,3,3-tetramethyl isoindolin-2-yloxy-5-yl)amino)benzoate 62.** Anhydrous acetic acid (42  $\mu\text{L}$ , 728  $\mu\text{mol}$ , 5 equiv.) was added dropwise over 10 min to a suspension of  $\text{NaBH}_4$  (28 mg, 735  $\mu\text{mol}$ , 5.05 equiv.) and methyl benzoate **60** (64 mg, 146  $\mu\text{mol}$ , 1 equiv.) in dry dioxane (3 mL). The resulting reaction mixture was refluxed for 30 min and then allowed to return to RT.  $\text{H}_2\text{O}$  was added and the resulting aqueous solution was extracted with EtOAc (4 x 30 mL). The combined organic layer was washed with brine (30 mL), dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated *in vacuo*. The crude product was purified silica gel column chromatography (Hexane/EtOAc, 3:1) to give methyl 2-acetoxy-5-((2-(1,1,3,3-tetramethylisoindolin-2-yloxy-5-yl)amino)benzoate **62** as yellow solid (42 mg, 68%). Mp. 85-87 °C. HPLC purity (>95%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 600 MHz):  $\delta$  = 2.31 (s, 3 H,  $\text{C}=\text{OCH}_3$ ), 3.45 (br, s, 2 H,  $\text{CH}_2$ ), 3.85 (s, 3 H,  $\text{OCH}_3$ ), 6.76 (br, s, 1 H, Ar-H), 6.92 (br, s, 1

H, Ar-H), 7.08 (br, s, 1 H, Ar-H). HRMS (ES):  $m/z$  (%) = calcd. for  $C_{24}H_{30}N_2O_5^+$  [M + H]<sup>+</sup> 426.2149; found 426.2151. ATR-FTIR:  $\nu_{max}$  = 3371 (m, N-H), 2972 (w, Ar C-H), 1746 (s, C=O), 1715 (s, C=O), 1195 (s, C-O)  $cm^{-1}$ ;  $g = 2.0009$ ,  $a_N = 1.81$  mT.

**2-Hydroxy-5-((2-(1,1,3,3-tetramethylisoindolin-2-yl)oxyethyl)amino)benzoic acid** **63** was obtained from **62** by following similar conditions as described for **53-56**. Brownish yellow solid (72%). Mp. 200 °C (dec.). HRMS (ES):  $m/z$  (%) = calcd. for  $C_{21}H_{26}N_2O_4^+$  [M + H]<sup>+</sup> 370.1887; found 370.1895. ATR-FTIR:  $\nu_{max}$  = 3500-2500 (m, br, O-H), 2975 (m, Ar C-H), 1681 (s, C=O), 1215 (s, C-O)  $cm^{-1}$ ;  $g = 2.0009$ ,  $a_N = 1.81$  mT.

## REFERENCES

- (1) Ferencik, M.; Novak, M.; Rovensky, J.; Rybar, I. Alzheimer's disease, inflammation and non-steroidal anti-inflammatory drugs. *Bratisl Lek Listy* **2001**, *102*, 123-132.
- (2) But, P.G.; Murav'ev, R.A.; Fomina, V.A.; Rogovin, V.V. Antimicrobial activity of myeloperoxidase from neutrophilic peroxisome. *Biol Bull* **2002**, *29*, 212-215.
- (3) Floyd, R.A. Antioxidants, oxidative stress, and degenerative neurological disorders. *Proc Soc Exp Biol Med* **1999**, *222*, 236-245.
- (4) Tantry, U.S.; Mahla, E.; Gurbel, P.A. Aspirin resistance. *Prog Cardiovasc Dis* **2009**, *52*, 141-152.
- (5) Soule, B.P.; Hyodo, F.; Matsumoto, K.-I.; Simone, N.L.; Cook, J.A.; Krishna, M.C.; Mitchell, J.B. The chemistry and biology of nitroxide compounds. *Free Radical Biol Med* **2007**, *42*, 1632-1650.
- (6) Helmersson, J.; Vessby, B.; Larsson, A.; Basu, S. Cyclooxygenase-mediated prostaglandin F<sub>2</sub> $\alpha$  is decreased in an elderly population treated with low-dose aspirin. *Prostaglandins, Leukotrienes Essent Fatty Acids* **2005**, *72*, 227-233.
- (7) Ong, C.K.S.; Lirk, P.; Tan, C.H.; Seymour, R.A. An evidence-based update on nonsteroidal anti-inflammatory drugs. *Clin Med Res* **2007**, *5*, 19-34.
- (8) Supinski, G.S.; Callahan, L.A. Free radical-mediated skeletal muscle dysfunction in inflammatory conditions. *J Appl Physiol* **2007**, *102*, 2056-2063.
- (9) Vane, J.; Botting, R. Inflammation and the mechanism of action of anti-inflammatory drugs. *FASEB J* **1987**, *1*, 89-96.
- (10) Mutlu, L.K.; Woiciechowsky, C.; Bechmann, I. Inflammatory response after neurosurgery. *Best Pract Res, Clin Anaesthesiol* **2004**, *18*, 407-424.
- (11) Halliwell, B.; Hoult, J.R.; Blake, D.R. Oxidants, inflammation, and anti-inflammatory drugs. *FASEB J* **1988**, *2*, 2867-2873.

- (12) Dal-Pizzol, F.; Ritter, C.; Cassol, O. J., Jr.; Rezin, G. T.; Petronilho, F.; Zugno, A. I.; Quevedo, J.; Streck, E. L. Oxidative Mechanisms of Brain Dysfunction During Sepsis. *Neurochem Res* **2010**, *35*, 1-12.
- (13) Rao, P.P.N.; Kabir, S.N.; Mohamed, T. Nonsteroidal anti-inflammatory drugs (NSAIDs): progress in small molecule drug development. *Pharmaceuticals* **2010**, *3*, 1530-1549.
- (14) Rainsford, K.D.; Whitehouse, M.W. Paracetamol [acetaminophen]-induced gastrotoxicity: revealed by induced hyperacidity in combination with acute or chronic inflammation. *Inflammopharmacology* **2006**, *14*, 150-154.
- (15) Robak, P.; Smolewski, P.; Robak, T. The role of non-steroidal anti-inflammatory drugs in the risk of development and treatment of hematologic malignancies. *Leuk Lymphoma* **2008**, *49*, 1452-1462.
- (16) Closa, D.; Folch-Puy, E. Oxygen Free Radicals and the Systemic Inflammatory Response. *IUBMB Life* **2004**, *56*, 185-191.
- (17) Arnhold, J. Properties, Functions, and Secretion of Human Myeloperoxidase. *Biochemistry (Moscow, Russ Fed)* **2004**, *69*, 4-9.
- (18) Grisham, M.B. A radical approach to treating inflammation. *Trends Pharmacol Sci* **2000**, *21*, 119-120.
- (19) Pattison, D.I.; Davies, M.J. Reactions of myeloperoxidase-derived oxidants with biological substrates: gaining chemical insight into human inflammatory diseases. *Curr Med Chem* **2006**, *13*, 3271-3290.
- (20) Jaeschke, H. Reactive oxygen and mechanisms of inflammatory liver injury. *J Gastroenterol Hepatol* **2000**, *15*, 718-724.
- (21) Chapple, I.L.C. Reactive oxygen species and antioxidants in inflammatory diseases. *J Clin Periodontol* **1997**, *24*, 287-296.
- (22) Liew, F.Y.; McInnes, I.B. The role of innate mediators in inflammatory response. *Mol Immunol* **2002**, *38*, 887-890.
- (23) Molina, D.M.; Wetterholm, A.; Kohl, A.; McCarthy, A.A.; Niegowski, D.; Ohlson, E.; Hammarberg, T.; Eshaghi, S.; Haeggstroem, J.Z.; Nordlund, P. Structural basis for synthesis of inflammatory mediators by human leukotriene C4 synthase. *Nature (London, U. K.)* **2007**, *448*, 613-616.
- (24) Botting, R. COX-1 and COX-3 inhibitors. *Thromb Res* **2003**, *110*, 269-272.
- (25) Peskar, B.M. Role of cyclooxygenase isoforms in gastric mucosal defense and ulcer healing. *Inflammopharmacology* **2005**, *13*, 15-26.
- (26) Deguchi, H.; Yasukawa, K.; Yamasaki, T.; Mito, F.; Kinoshita, Y.; Naganuma, T.; Sato, S.; Yamato, M.; Ichikawa, K.; Sakai, K.; Utsumi, H.; Yamada, K. Nitroxides prevent exacerbation of indomethacin-induced gastric damage in adjuvant arthritis rats. *Free Radic Biol Med* **2011**, *51*, 1799-1805.

- (27) Flores-Santana, W.; Moody, T.; Chen, W.; Gorczynski, M.J.; Shoman, M.E.; Velázquez, C.; Thetford, A.; Mitchel, J.B.; Cherukuril, M.K.; King, S.B.; Wink, D.A. Nitroxide derivatives of non-steroidal anti-inflammatory drugs exert anti-inflammatory and superoxide dismutase scavenging properties in A459 cells. *Br J Pharmacol.* **2012**, *165*, 1058-1067
- (28) Trnka, J.; Blaikie, F.H.; Logan, A.; Smith, R.A.; Murphy, M.P. Antioxidant properties of MitoTEMPO and its hydroxylamine. *Free Radic Res* **2009**, *43*, 4-12.
- (29) Soule, B.P.; Hyodo, F.; Matsumoto, K.-I.; Simone, N.L.; Cook, J.A.; Krishna, M.C.; Mitchell, J.B. Therapeutic and clinical applications of nitroxide compounds. *Antioxid Redox Signaling* **2007**, *9*, 1731-1743.
- (30) Decker, M. Design of Hybrid Molecules for Drug Development, ISBN: 9780081010112, **2017**, Elsevier Publishers, 352 pages.
- (31) Lewandowski, M.; Gwozdziński, K. Nitroxides as Antioxidants and Anticancer Drugs, *Int. J. Mol. Sci.* **2017**, *18*(11), 2490-2515
- (32) Micallef, A.S.; Bott, R.C.; Bottle, S.E.; Smith, G.; White, J.M.; Matsuda, K.; Iwamura, H. Brominated isoindolines: precursors to functionalized nitroxides. *J Chem Soc, Perkin Trans. 2* **1999**, 65-72.
- (33) Fairfull-Smith, K.E.; Bottle, S.E. The synthesis and physical properties of novel polyaromatic profluorescent isoindoline nitroxide probes. *Eur J Org Chem* **2008**, *32*, 5391-5400.
- (34) Shang, R.; Ji, D.S.; Chu, L.; Fu, Y.; Liu, L. Synthesis of  $\alpha$ -Aryl Nitriles through Palladium-Catalyzed Decarboxylative Coupling of Cyanoacetate Salts with Aryl Halides and Triflates. *Angew Chem* **2011**, *50*(19), 4470-4474.
- (35) Ettinger, D.S.; Akerley, W.; Bepler, G.; Blum, M.G.; Chang, A.; Cheney, R.T.; Chirieac, L.R.; D'Amico, T.A.; Demmy, T.L.; Ganti, A.K.P. Non-small cell lung cancer: clinical practice guidelines in oncology. *J Natl Compr Cancer Network* **2010**, *8*, 740-801.
- (36) Kalyanaraman, B.; Darley-Usmar, V.; Davies, K.J.A.; Dennery, P.A.; Forman, H.J.; Grisham, M.B.; Mann, G.E.; Moore, K.; Roberts, II, L.J. Ischiropoulos, H. Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations, *Free Radic Biol Med.* **2012**; *52*(1): 1-6.
- (37) Lippman, S.M.; Gibson, N.; Subbaramaiah, K.; Dannenberg, A.J. Combined targeting of the epidermal growth factor receptor and cyclooxygenase-2 pathways. *Clin Cancer Res* **2005**, *11*, 6097-6099.
- (38) Buchanan, F.G.; Wang, D.; Bargiacchi, F.; DuBois, R.N. Prostaglandin E2 regulates cell migration via the intracellular activation of epidermal growth factor receptor. *J Biol Chem* **2003**, *278*, 35451-35457.
- (39) Dannenberg, A.J.; Subbaramaiah, K. Targeting cyclooxygenase-2 in human neoplasia: Rationale and promise. *Cancer Cell* **2003**, *4*, 431-6.

- (40) Pai, R.; Soreghan, B.; Szabo, I.L.; Pavelka, M.; Baatar, D.; Tarnawski, A.S. Prostaglandin E2 transactivates EGF receptor: A novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. *Nat Med* **2002**, *8*, 289-93.
- (41) Rayner, C.L.; Bottle, S.E.; Gole, G.A.; Ward, M.S.; Barnett, N.L. Real-time quantification of oxidative stress and the protective effect of nitroxide antioxidants. *Neurochem Int* **2016**; *92*, 1-12.
- (42) Morrow, B.J.; Keddie, D.J.; Gueven, N.; Lavin, M.F.; Bottle, S.E. A novel profluorescent nitroxide as a sensitive probe for the cellular redox environment. *Free Radic Biol Med* **2010**; *49(1)*, 67-76.
- (43) Li, S.-Y.; Fung, F.K.C.; Fu, Z.J.; Wong, D.; Chan, H.H.L.; Lo, A.C.Y. Anti-inflammatory effects of lutein in retinal ischemic/hypoxic injury: in vivo and in vitro studies. *Investig Ophthalmol Vis. Sci.* **2012**, *53*, 5976-5984.
- (44) Rayner, C.L.; Gole, G.A.; Bottle, S.E.; Barnett, N.L. Dynamic, in vivo, real-time detection of retinal oxidative status in a model of elevated intraocular pressure using a novel, reversibly responsive, profluorescent nitroxide probe. *Exp Eye Research.* **2014**; *129*, 48-56.
- (45) Griffiths, P.G.; Moad, G.; Rizzardo, E. Synthesis of the radical scavenger 1,1,3,3-tetramethylisoindolin-2-yloxy. *Aust J Chem* **1983**, *36*, 397-401.
- (46) Bolton, R.; Gillies, D.G.; Sutcliffe, L.H.; Wu, X. An EPR and NMR study of some tetramethylisoindolin-2-yloxy free radicals. *J Chem Soc Perkin Trans 2* **1993**, 2049-2052.
- (47) Fairfull-Smith, K. E.; Brackmann, F.; Bottle, S. E. The Synthesis of Novel Isoindoline Nitroxides Bearing Water-Solubilising Functionality. *Eur J Org Chem* **2009**, *12*, 1902-1915.
- (48) Thomas, K.; Chalmers, B.A.; Fairfull-Smith, K.E.; Bottle, S.E. Approaches to the Synthesis of a Water-Soluble Carboxy Nitroxide. *Eur J Org Chem* **2013**, *5*, 853-857.
- (49) Guo, W.; Li, J.; Fan, N.; Wu, W.; Zhou, P.; Xia, C. A simple and effective method for chemoselective esterification of phenolic acids. *Synth Commun* **2005**, *35*, 145-152.
- (50) Csipo, I.; Szabo, G.; Sztaricskai, F. 5-Aminosalicylic acid and its glucopyranosyl derivatives. *Magy Kem Foly* **1991**, *97*, 143-148.
- (51) Akdogan, Y.; Emrullahoglu, M.; Tatlidil, D.; Ucuncu, M.; Cakan-Akdogan, G., EPR studies of intermolecular interactions and competitive binding of drugs in a drug-BSA binding model, *Phys Chem Chem Phys* **2016**, *18(32)*, 22531-22539.
- (52) Wink, D.A.; Flores-Santana, W.; King, S.B.; Cherukuri, M.K.; Mitchell, J.B. Nitroxide modified non-steroidal anti-inflammatory compounds and their preparation and use for the treatment and prevention of diseases, *U.S. Pat. Appl.* **2012**, US 20120263650 A1 20121018
- (53) Hauenschild, T.; Reichenwallner, J.; Enkelmann, V.; Hinderberger, D. Characterizing Active Pharmaceutical Ingredient Binding to Human Serum Albumin by Spin-Labeling and EPR Spectroscopy, *Chemistry - A European Journal* **2016**, *22(36)*, 12825-12838.

(54) Wood, M.E.; Winyard, P.G.; Ferguson, D.C.; Whiteman, M. Combinations of a photosensitizer with a hydrogen sulfide donor, thioredoxin inhibitor or nitroxide for use in photodynamic therapy, *PCT Int. Appl.* **2015**, WO 2015185918 A1 20151210

For Table of Contents Only

