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Synthesis and methemoglobinemia-inducing properties of analogues of *para*-aminopropiophenone designed as humane rodenticides

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ABSTRACT

A number of structural analogues of the known toxicant *para*-aminopropiophenone (PAPP) have been prepared and evaluated for their capacity to induce methemoglobinemia—with a view to their possible application as humane pest control agents. It was found that an optimal lipophilicity for the formation of methemoglobin (metHb) in vitro existed for alkyl analogues of PAPP (aminophenones **1**–**20**; compound **6** metHb% = 74.1 ± 2). Besides lipophilicity, this structural sub-class suggested there were certain structural requirements for activity, with both branched (**10**–**16**) and cyclic (**17**–**20**) alkyl analogues exhibiting inferior in vitro metHb induction. Of the four candidates (compounds **4**, **6**, **13** and **23**) evaluated in vivo, **4** exhibited the greatest toxicity. In parallel, aminophenone bioisosteres, including oximes **30**–**32**, sulfoxide **33**, sulfone **34** and sulfonamides **35**–**36**, were found to be inferior metHb inducers to lead ketone **4**. Closer examination of Hammett substituent constants suggests that a particular combination of the field and resonance parameters may be significant with respect to the redox mechanisms behind PAPPs metHb toxicity.

Rats can be counted amongst man's most serious competitors causing substantial damage to agricultural interests worldwide. The World Health Organization estimates that 20% of all human food is destroyed or contaminated by rodents each year,[1] while one US government report claims that each rat damages up to $10 worth of food and stored grains per annum, and contaminates 5–10 times that amount.[2] More recently, the Food and Agriculture Organization of the United Nations reported that, globally, rats ruined more than 42 million tons of food, worth an estimated US$30 billion p.a.[3] On a domestic level, property damage can also be estimated in millions of dollars annually, often the product of building fires attributed to rodents chewing through lead gas pipes or stripping insulation from electrical wires.[3] In addition to their substantial contribution to economic loss, rats constitute a major threat to health, primarily as a consequence of the diseases they harbour. Acting as vectors for both viral and bacterial diseases, rats are responsible for transmitting more than 35 types of disease to humans including cholera, dysentery, salmonella and the bubonic plague.[2] In the past century alone, more than 10 million people have died from rodent-borne diseases.[3] Furthermore, as an invasive species second only to humans, rats are responsible for a loss of biodiversity through predation and habitat destruction.[4]

In New Zealand alone, almost NZ$200 million is spent on vertebrate pest control products annually.[5] Most of these, however, share common disadvantages such as secondary non-target poisoning, risks of environmental contamination, and accumulation/persistence of residues in the food chain. Moreover, most of these agents lack humaneness. In the 1980s, the United States Fish and Wildlife Services investigated *para*-aminopropiophenone **2** (PAPP) (Fig. 1) for the control of coyotes (*Canis latrans*), demonstrating it to be highly toxic to canids *per se*.[6] Subsequently, PAPP has been evaluated in both New Zealand and Australia for the humane control of introduced predators such as feral cats and stoats.[7–9] In 2011 PAPP was successfully registered for the control of these pests in New Zealand, and has since been marketed under the trade name Predastop®.[10,11] In addition to its intrinsic toxicity against such problem species, the use of PAPP as a pest control agent offers a number of complimentary benefits—one being its comparatively humane mode of action.[7] PAPP induces its lethal effect by reducing oxygen supply to the brain, leading to symptoms such as progressive lethargy, drowsiness, stupor and ultimately unconsciousness prior to death. From a welfare perspective, this mechanism of toxicity

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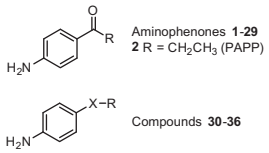


Figure 1. Aminophenones **1**–**29**, including PAPP **2**, and compounds **30**–**36** (aminophenone bioisosteres); see Tables 1 and 3.

could be viewed as advantageous in minimizing animal distress.[7] Moreover, the time to death is relatively short, usually within 1–2 h following ingestion.[12,13] Having induced its toxic effect, PAPP is readily converted to comparatively innocuous metabolites, and as a consequence is unlikely to be present in carcasses at levels that will cause secondary poisoning to non-target species, nor should it persist in the food chain.[6] Indeed, should the accidental poisoning of non-target species such as pets or farm dogs occur, a simple and relatively inexpensive antidote, methylene blue, is available.[14] Further to this, PAPP is relatively non-toxic to most bird species (LD_{50} >100 mg/kg),[6,7,13,15] so from an avian conservation perspective this agent is particularly attractive.

Mechanism of action—The toxic effects of PAPP can be attributed to its capacity to induce the formation of methemoglobin (metHb),[16] leading to a physiological phenomenon known as methemoglobinemia, an impairment of the red blood cells' (erythrocytes) capacity to transport oxygen—resulting in cerebral hypoxia, central nervous system depression and ultimately death.[15] Upon systemic absorption, PAPP is metabolized by hepatic mixed function oxidases to *para*-hydroxylaminopropiophenone **2a** (PHAPP).[17] Once taken up into the erythrocyte, and in the presence of oxygen, PHAPP undergoes a coupled, or two-stage, redox reaction with hemoglobin ($HbFe^{2+}$) to form methemoglobin ($HbFe^{3+}$, metHb), the toxic endpoint (Fig. 2). The first stage involves the oxidation of PHAPP to *para*-nitrosopropiophenone **2b** (PNPP), and the complementary reduction of molecular oxygen to hydrogen peroxide. Consequently, the hydrogen peroxide reacts in situ to oxidize hemoglobin to methemoglobin.[18] Catalytic methemoglobin induction is an NADPH-dependent process involving reductive enzymes present in erythrocytes such as diaphorase and NADP flavin reductase, as well as the antioxidant glutathione. These enzymes work to drive the catalytic cycle of metHb formation via the back-reduction of PNPP to PHAPP, the net result being a small quantity of PAPP having the capacity to turnover a large number of hemoglobin equivalents to methemoglobin.[17,19] To regulate this process NADH-cytochrome *b* reductase oxidase, also referred to as methemoglobin reductase, is known to reduce methemoglobin back to hemoglobin.[20,21] In addition to PAPP's well documented properties as a toxicant, Pan et al.[22] demonstrated that for a homologous series of related aminophenones, acute oral toxicity in rats was elevated for *p*-aminobutyrophenone **3** and *p*-aminovalerophenone **4**, when directly compared to *p*-aminoacetophenone **1**, *p*-aminocaprophenone **5** and PAPP itself (Table 3). Although investigated no further, Pan et al attributed such observations to be the result of high levels of methemoglobineamia. On the basis of such findings it was now our intention to prepare a series of PAPP-related analogues to further investigate the structural features and physicochemical properties responsible for, and the in vitro biological markers indicative of, the in vivo toxicity of aminophenones in rats.

Synthesis—Aminophenones **3**, **5**, **10**–**12**, **14**–**20** and **22** were prepared over two steps, via their corresponding bromophenones, by Friedel–Crafts acylation[23] of bromobenzene (**37**) with the appropriate acid chloride, employing anhydrous aluminum chloride under solvent-free conditions. Subsequent copper(I)-catalyzed amination,[24] using aqueous ammonia in the presence of copper(I)

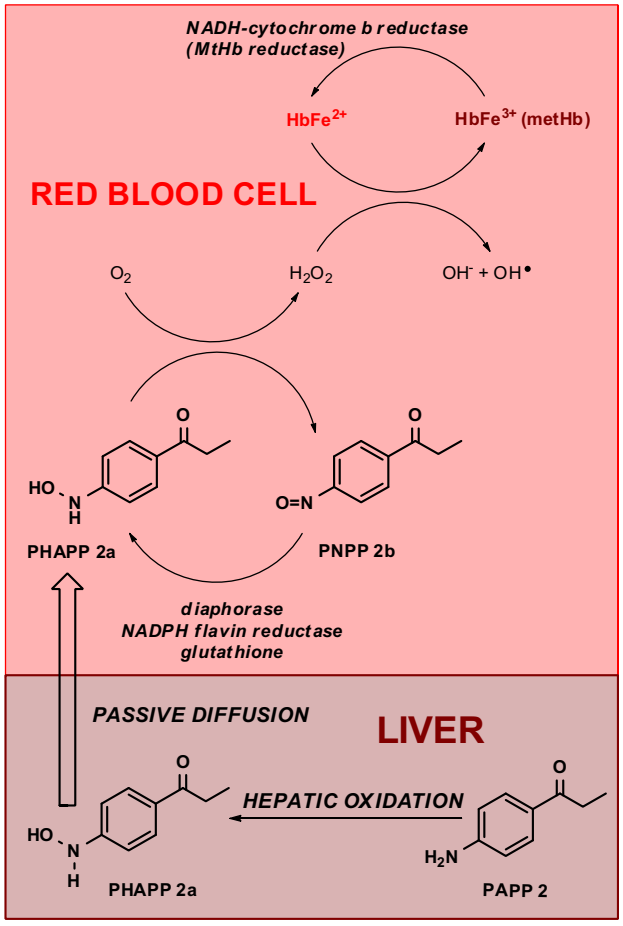


Figure 2. Postulated mechanism for the in vivo formation of metHb by PAPP.

oxide, afforded the corresponding aminophenones (Scheme 1). Aminoacetophenone **1** was subject to a one-step Ru(II)-catalyzed α-alkylation[25] with the appropriate alcohol or aldehyde, in the presence of dichlorotris(triphenylphosphine)ruthenium(II) and potassium hydroxide at elevated temperature, to afford aminophenones **6**–**9**, **23** and **25**–**29** (Scheme 2). As a consequence of our failure to access pivaloyl aminophenone **13** using the above Friedel–Crafts acylation route,[23] **13** was synthesized through a Cu(I)-promoted acylation[26] of *p*-bromobenzoyl chloride (**38**) with *tert*-butyllithium in the presence of copper(I) bromide dimethyl sulfide complex, again progressing via a bromophenone intermediate. Subsequent amination afforded pivaloyl aminophenone **13** over two steps. Aminophenone **21** was prepared from *p*-bromobenzoyl chloride using Friedel–Crafts chemistry (Scheme 3).

Treatment of ketone **2** with *O*-methyl hydroxylamine hydrochloride and sodium carbonate at elevated temperature[27] directly afforded *O*-methyl ketoxime **30** in 70% yield, exclusively as the *E* isomer (Scheme 4). The synthesis of *O*-methyl α-chloroaldoxime **31** commenced with the condensation[28] of *p*-nitrobenzaldehyde (**39**) and hydroxylamine hydrochloride to afford aldoxime **40** in 90% yield. Alkylation[29] of **40** with methyl iodide led to *O*-methyl aldoxime **41** in 59% yield. Treatment of **41** with *N*-chlorosuccinimide[28] afforded *O*-methyl α-chloroaldoxime **42** in 39% yield, with subsequent reduction[30] using iron and hydrochloric acid furnishing the desired *O*-methyl α-chloroaldoxime **31** in 35% yield, exclusively as the *Z* isomer (Scheme 5). The preparation of *O*-methyl α-cyanoaldoxime **32** began with the chlorination of aldoxime **40**, as previously described,[28] to give **43**. The cyano group was installed through treatment of α-chloroaldoxime **43** with

Table 1
Physicochemical properties and in vitro methemoglobin formation for compounds **1–29** (see Fig. 1)

No.	R	log$P_{exp.}$[a]	δ ^{13}C–NH_2[b]	% metHb[c]
1	CH_3	1.34	151.1	4.5 ± 0.9
2	CH_2CH_3	1.65	151.0	15.2 ± 3.1
3	$(CH_2)_2CH_3$	2.06	150.9	40.8 ± 2.2
4	$(CH_2)_3CH_3$	2.48	151.0	58.1 ± 3.8
5	$(CH_2)_4CH_3$	3.01	150.8	70.9 ± 0.7
6	$(CH_2)_5CH_3$	3.49	150.8	74.1 ± 2.8
7	$(CH_2)_6CH_3$	3.91	150.8	54.2 ± 1.0
8	$(CH_2)_8CH_3$	4.58	150.8	29.0 ± 1.6
9	$(CH_2)_{10}CH_3$	5.15	150.9	13.3 ± 1.0
10	$CH(CH_3)_2$	2.02	150.9	19.7 ± 2.8
11	$CH(CH_3)CH_2CH_3$	2.44	150.8	31.1 ± 1.2
12	$CH_2CH(CH_3)_2$	2.46	151.0	36.9 ± 1.7
13	$C(CH_3)_3$	2.46	149.8	54.2 ± 1.6
14	$CH(CH_3)CH_2CH_2CH_3$	2.84	151.1	49.0 ± 1.0
15	$CH_2CH(CH_3)CH_2CH_3$	2.85	151.2	49.5 ± 0.7
16	$CH_2CH_2CH(CH_3)_2$	2.95	150.8	52.7 ± 1.6
17	$CHc(CH_2)_2$	1.82	150.9	23.7 ± 2.1
18	$CHc(CH_2)_3$	2.29	150.8	29.3 ± 3.3
19	$CHc(CH_2)_4$	2.71	150.7	33.5 ± 1.1
20	$CHc(CH_2)_5$	3.07	150.8	40.5 ± 1.6
21	Ph	2.42	150.9	38.0 ± 3.6
22	CH_2Ph	2.58	151.2	47.0 ± 1.9
23	CH_2CH_2Ph	3.07	151.3	57.3 ± 1.5
24	CH=CHPh	3.20	151.1	29.6 ± 1.4
25	CH_2CH_2-(2-pyridyl)	1.84	151.3	31.0 ± 1.1
26	CH_2CH_2-(2-furanyl)	2.58	151.1	45.5 ± 1.1
27	CH_2CH_2-(2-thienyl)	2.92	151.1	52.3 ± 1.3
28	$CH_2CH_2CHc(CH_2)_5$	3.81	150.9	51.1 ± 0.3
29	$CH_2CH_2CH_2Ph$	3.37	150.9	62.4 ± 0.4

[a] Log$P_{exp.}$ determined by RP-HPLC methods using a standard curve.
[b] Chemical shift for ^{13}C–NH_2 as measured by ^{13}C NMR (in ppm).
[c] Percentage methemoglobin formation in vitro at a compound concentration of 10 μM (37 °C, 1 h, n = 3). Data represented as mean ± SD.[50]

Table 2
In vitro metabolism and in vitro erythrocytic uptake for compounds **1–6** (see Fig. 1)

No.	% Microsomal metabolism[a]	% Erythrocytic uptake[b]
1	7.63 ± 1.1	46.6 ± 3.0
2	11.0 ± 1.8	59.8 ± 2.2
3	16.7 ± 2.8	70.4 ± 1.3
4	15.3 ± 2.8	78.9 ± 0.8
5	14.9 ± 1.3	88.5 ± 2.3
6	17.8 ± 2.5	94.0 ± 0.5

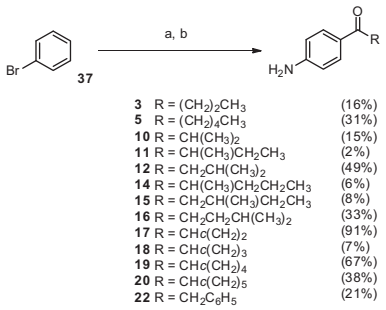
[a] Percentage loss of parent compound post incubation with rat liver microsomes and NADPH (37 °C, 0.5 h, n = 4). Data represented as mean ± SE.[44]
[b] Percentage compound extracted from rat erythrocytes following incubation (37 °C, 0.5 h, n = 4). Data represented as mean ± SE.[44]

Table 3
Physicochemical properties and in vitro methemoglobin formation for compounds **30–36** (see Fig. 1)

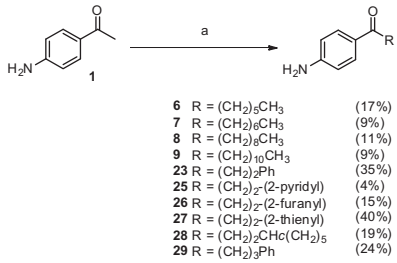
No.	X	R	log$P_{exp.}$[a]	δ ^{13}C–NH_2[b]	% metHb[c]
30	N=OCH_3	CH_2CH_3	2.30	147.3	6.4 ± 0.8
31	N=OCH_3	Cl	2.42	148.7	8.2 ± 0.9
32	N=OCH_3	CN	2.44	149.3	8.4 ± 1.9
33	S=O	$(CH_2)_4CH_3$	2.26	149.4	13.2 ± 0.8
34	SO_2	$(CH_2)_4CH_3$	2.53	151.3	33.5 ± 2.9
35	SO_2NH	$(CH_2)_3CH_3$	2.05	150.5	5.1 ± 0.4
36	SO_2NCH_3	$(CH_2)_3CH_3$	2.73	150.4	22.0 ± 0.5

[a] Log$P_{exp.}$ determined by RP-HPLC methods using a standard curve.
[b] Chemical shift for ^{13}C–NH_2 as measured by ^{13}C NMR (in ppm).
[c] Percentage methemoglobin formation in vitro at a compound concentration of 10 μM (37 °C, 1 h, n = 3). Data represented as mean ± SD.[50]

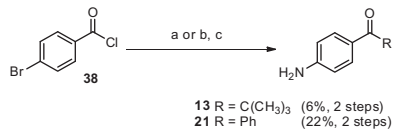
sodium cyanide in the presence of triethylamine,[30] affording α-cyanoaldoxime **44** in 19% yield. Alkylation[30] of **44** using methyl iodide, in the presence of tetra-*n*-butylammonium bromide and



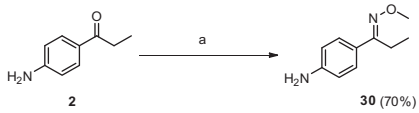
Scheme 1. Reagents and conditions: (a) RCOCl, $AlCl_3$, rt, 3–6 h; (b) aq NH_3, CuO, DMSO, 90 °C (sealed-tube), 16 h.



Scheme 2. Reagents and conditions: (a) RCH_2OH (for **6–9**, **29**) or RCHO (for **23**, **25–29**), $RuCl_2(PPh_3)_3$, KOH, 1,4-dioxane, 90 °C (sealed-tube), 42 h.



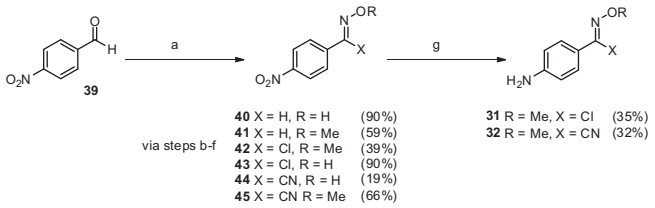
Scheme 3. Reagents and conditions: (a) *t*BuLi, $CuBr{\cdot}CH_3SCH_3$, THF, −78 °C, 4 h (for **13**); (b) $AlCl_3$, C_6H_6, reflux, 36 h (for **21**); (c) aq NH_3, CuO, DMSO, 90 °C (sealed-tube), 16 h.



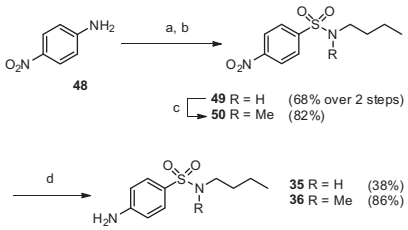
Scheme 4. Reagents and conditions: (a) $NH_2OMe{\cdot}HCl$, Na_2CO_3, EtOH, H_2O, reflux, 36 h.

sodium hydroxide at elevated temperature, afforded *O*-methyl α-cyanoaldoximes **45** in 66% yield. Ultimately, **45** was reduced as described previously[30] to access the desired *O*-methyl α-cyanoaldoxime **32** in 32% yield, exclusively as the *Z* isomer (Scheme 5). Note that the geometrical assignment of (*Z*)-oximes **31**, **32**, **41–45** was made through comparison of their ^{1}H NMR data to that of related literature examples.[30]

Aminophenyl alkyl sulfoxide **33** and aminophenyl alkyl sulfone **34** were prepared from the common nitrophenyl alkyl sulfide intermediate **47**, which in turn was obtained by thiolation[31] of *p*-chloronitrobenzene (**46**), using *n*-pentanethiol in the presence of potassium hydroxide at elevated temperature. Reduction[32] of nitrophenyl alkyl sulfide **47** with tin(II) chloride afforded an aminophenyl alkyl sulfide intermediate, which was subsequently subjected to mild oxidation conditions[33] using hydrogen peroxide to afford the desired aminophenyl alkyl sulfoxide **33** in low yield over two steps. Oxidation[31] of nitrophenyl alkyl sulfide **47** using *m*-chloroperoxybenzoic acid yielded a nitrophenyl alkyl sulfone intermediate. Selective nitro group reduction[31] using tin(II)



Scheme 5. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, NaOH, H_2O, EtOH, rt, 3 h (to give **40**); (b) (i) **40**, NaH, DMF, rt, 0.25 h, (ii) MeI, DMF, rt, 0.5 h (**41**); (c) **41**, NCS, DMF, 0 °C → rt, 48 h (**42**); (d) **40**, NCS, DMF, 0 °C → rt, 24 h (**43**); (e) **43**, NaCN, NEt_3, *i*PrOH, DCE, H_2O, 0 °C, 5 h (**44**); (f) **44**, MeI, TBAB, NaOH, THF, H_2O, 70 °C, 3 h (**45**); (g) **42**, Fe, aq 10 M HCl, EtOH, H_2O, 70 °C, 3 h (**31**) or **45**, Fe, aq 10 M HCl, EtOH, H_2O, 70 °C, 3 h (**32**).

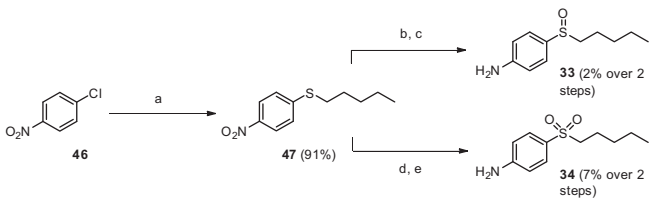


Scheme 7. Reagents and conditions: (a) (i) $NaNO_2$, aq 10 M HCl, H_2O, 0 °C, 0.25 h, (ii) $SOCl_2$, CuCl, H_2O, 0 °C → rt, 1.25 h; (b) $CH_3(CH_2)_3NH_2$, THF, rt, 0.5 h; (c) NaH, MeI, 0 °C → reflux, 18 h; (d) **49**, Fe, NH_4Cl, MeOH, H_2O, rt → 70 °C, 2.5 h (to give **35**) or **50**, Fe, NH_4Cl, MeOH, H_2O, rt → 70 °C, 2.5 h (**36**).

chloride furnished aminophenyl alkyl sulfone **34**, again in low yield, over two steps (Scheme 6).

The first step towards the synthesis of aminophenyl alkyl sulfonamide **35** involved diazotization[34] of 4-nitroaniline (**48**), with subsequent treatment of the resulting diazonium salt with thionyl chloride yielding a sulfonyl chloride intermediate. Subsequent amination[35] with butylamine afforded nitrophenyl alkyl sulfonamide **49** in 68% yield over two steps. Reduction[36] of the nitro group of **49** using iron and ammonium chloride at elevated temperature furnished the desired aminophenyl alkyl sulfonamide **35** in 38% yield. Methylation[37] of nitrophenyl alkyl sulfonamide **49** using sodium hydride and methyl iodide afforded *N*-methyl nitrophenyl alkyl sulfonamide **50** in 82% yield, which underwent reduction of the nitro group as described previously[36] to afford aminophenyl alkyl *N*-methyl sulfonamide **36** in 86% yield (Scheme 7). Aminophenones **1**, **2**, and **4** were obtained from commercial sources.[38,39] All new compounds were fully characterized spectroscopically (see Supplementary data).

Biological activity—Structural and electronic considerations aside, the aforementioned trend in acute oral toxicity reported by Pan et al.,[22] presumed to be a consequence of methemoglobinemia in vivo, suggests that there is a potential correlation between the lipophilic parameter (π) and the metHb inducing properties of these compounds. *Alkyl aminophenones*—In accordance with general partition coefficient theory,[40] for each extension of the alkyl chain by a single carbon unit within such a series, it is generally accepted that there is a corresponding stepwise increase in the overall lipophilicity of the molecule. Evaluation of the partition coefficient of aminophenones **1**–**20** using RP-HPLC methods confirmed that their lipophilic properties increased in a stepwise fashion, by approximately 0.4 log *P* units per carbon atom, as the length of the alkyl chain increased (Table 1, Fig. 1). Straight chain aminophenones **1**–**9** exhibited a steady increase in their capacity to induce the formation of metHb in vitro until the alkyl chain reached 6 carbons in length (compound **6** metHb 74.1 ± 2.8%), after which further extension caused the level of metHb induction to decline. Building on this established relationship, further structural exploration through aminophenones **10**–**16** was undertaken to



Scheme 6. Reagents and conditions: (a) $CH_3(CH_2)_4SH$, KOH, DMF, 100 °C, 6 h; (b) $SnCl_2$, EtOH, 70 °C, 5 h; (c) H_2O_2, H_2O, 70 °C, 1 h; (d) *m*-CPBA, CH_2Cl_2, 0 °C → rt, 4 h; (e) $SnCl_2$, EtOH, 70 °C, 4 h.

determine the effect of alkyl chain branching on in vitro metHb induction. Despite possessing similar partition coefficients, branched aminophenones **11** (metHb 31.1 ± 1.2%) and **12** (metHb 36.9 ± 1.7%) were demonstrated to induce lower levels of metHb when compared to straight chain aminophenone **4** (metHb 58.1 ± 1.5%). This pattern was further reinforced on comparing aminophenones **14** (metHb 49.0 ± 1.0%), **15** (metHb 49.5 ± 0.7%) and **16** (metHb 52.7 ± 1.6%) to aminophenone **5** (metHb 70.9 ± 0.7%) (Table 1, Figs. 1 and 3).

Contrary to the trend, pivaloyl aminophenone **13** (metHb 54.2 ± 1.6%), constituting the greatest degree of alkyl chain branching, induced the highest levels of metHb formation within the branched chain series. This anomalous result may have been influenced by the slightly lower electron-withdrawing nature of the pivaloyl substituent, relative to other alkyl aminophenones within the series; as evidenced by the upfield chemical shift of its ^{13}C–NH_2 signal (δ 149.8 ppm), taken as an approximate measure of electron-richness of the aryl amine ring, when compared to the same carbon in PAPP (δ 151.0 ppm). Such observations were largely in agreement with the literature, with the reported Hammett substituent constant for the pivaloyl substituent (σ_{para} (CO*t*Bu) +0.32) being appreciably lower than that of the propionyl group (σ_{para} (COEt) +0.48).[41] One plausible explanation for this anomaly has been proposed by Bowden et al.,[42] who suggested that the electronic contribution of the pivaloyl group is less than that of its branched counterparts due to its steric bulk interacting with the aromatic ring, resulting in significant twisting and subsequent deconjugation of the carbonyl group relative to the plane of the π-system. To complete the series a selection of cyclic alkyl chain aminophenones, namely **17**–**20**, were prepared. In vitro evaluation of aminophenones **17** (metHb 23.7 ± 2.1%), **18** (metHb 29.3 ± 3.3%), **19** (metHb 33.5 ± 1.6%) and **20** (metHb 40.5 ± 1.1%) suggested that metHb induction was again strongly influenced by the lipophilic parameter, though once again the levels of metHb formation in vitro were found to be lower when compared to those of the unbranched alkyl aminophenone series (Table 1, Figs. 1 and 3).

Aryl aminophenones—In a parallel study, attention focused on the preparation and evaluation of a series of aryl aminophenones. Despite sharing similar lipophilic profiles, aminobenzophenone **21** (metHb 39.1 ± 2.1%) displayed markedly lower levels of in vitro metHb induction when compared to alkyl aminophenone **4** (metHb 58.1 ± 3.8). The introduction of a methylene spacer between the carbonyl group and the aryl ring of **21** afforded aminophenone **22** (metHb 47.0 ± 1.9%), and a moderate gain in activity, while further extending the length of the spacer led to a stepwise increase in metHb formation, as demonstrated in aminophenones **23** (metHb 57.3 ± 1.5%) and **29** (metHb 62.4 ± 0.4%). Conversely, replacement of the saturated ethylene spacer of dihydrochalcone **23** with an olefin bond, generating an α,β-unsaturated ketone, resulted in a notable decrease in metHb induction, as demonstrated by aminochalcone **24** (metHb 29.6 ± 1.4%) (Table 1, Fig. 1). Given that the observed ^{13}C–NH_2 chemical shifts of aryl aminophenones **21**–**24**

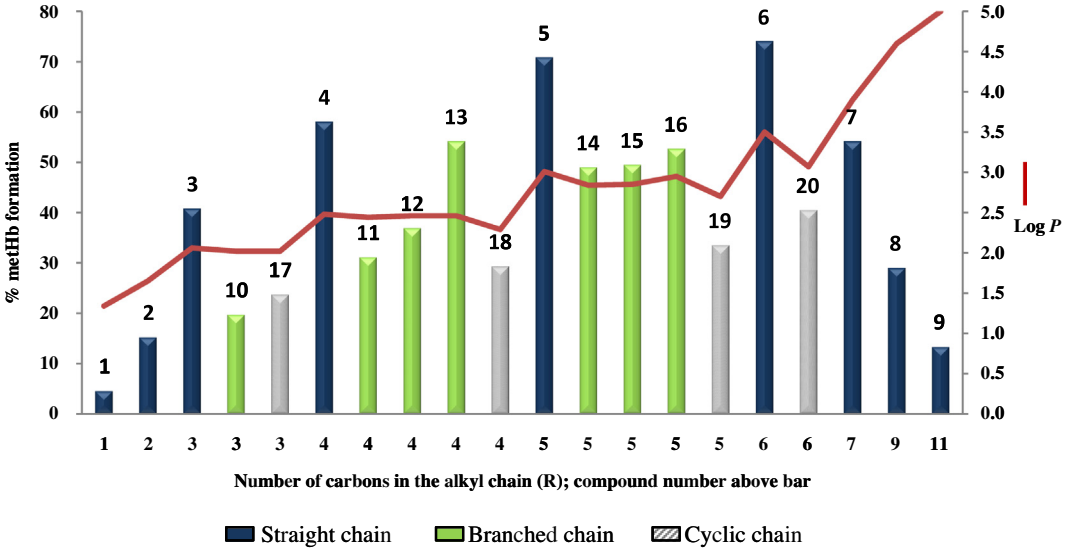


Figure 3. Percentage methemoglobin formation in vitro for aminophenones **1**–**20** (37 °C, 1 h, $n = 3$). Data represented as mean ± SD.[50]

and **29** were closely related to that of PAPP (δ 151.0 ppm) this again suggests that the electronic contribution of the side chain to the aryl amine ring has a minimal effect on the observed activity, even in the case of aminobenzophenone **21** (δ 150.9 ppm) and aminochalcone **24** (δ 151.1 ppm), where conjugation was anticipated to be a factor. Such observations were again generally in agreement with the literature, for example, the experimentally determined Hammett substituent constants for the propionyl (σ_{para} (COEt) +0.48) and benzoyl (σ_{para} COPh +0.43) groups, as featuring in PAPP and aminobenzophenone **21**, respectively, were likewise found to be comparable.[41] Given that both the partition coefficients and electronic properties of aminochalcone **24** and aminodihydrochalcone **23** (δ 151.3 ppm) were largely analogous, it is hypothesized that a loss in conformational flexibility may be responsible for the observed fall in metHb induction in the former. In summary, aryl aminophenone homologues **21**–**23** and **29** exhibited a similar trend to that observed within the parallel alkyl aminophenone series, again predominantly influenced by the partition coefficient.

Finally, substitution of the phenyl group of compound **23** with a selection of heteroaromatic ring equivalents completed our aryl aminophenone series. As anticipated, the partition coefficients of aminophenones **25**–**27** were largely influenced by the contribution of the heterocycle. All heterocyclic bioisosteres **25** (metHb 34.5 ± 1.1%), **26** (metHb 50.6 ± 1.1%), **27** (metHb 58.2 ± 1.3%) explored exhibited decreased in vitro metHb induction compared to aminodihydrochalcone **23** (metHb 63.9 ± 0.6%). This was particularly evident in the case of the more polar examples, whereas non-aromatic aminophenone **28** (metHb 56.9 ± 0.3%) appeared to be of comparable efficacy; once again such observations largely correlated to the lipophilic parameter.

In vitro summary (aminophenones)—From these preliminary studies, it became apparent that metHb induction in vitro, as observed in vivo, was strongly influenced by the lipophilic parameter of the analogue. One hypothesis for the aforementioned observations is that in vitro, lead aminophenones **5** and **6** are more readily metabolized to their corresponding *N*-hydroxyl metabolites.[43] However, when measuring the loss of parent compound following incubation in the presence of rat liver microsomes and NADPH, collaborative research by Yang[44] demonstrated that while compounds **1** and **2** appeared less susceptible to metabolism, no significant difference was observed between aminophenones **3**–**6** (Table 2, Fig. 4). A second explanation could relate to the uptake of these *N*-hydroxyl metabolites into the erythrocyte. Uncharged molecules

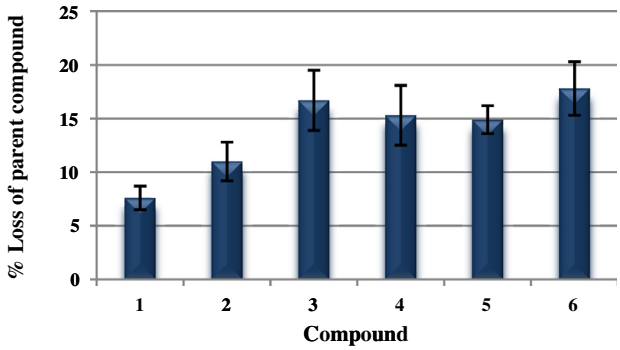


Figure 4. Effect of increasing alkyl chain length on the metabolism of aminophenones **1**–**6** following incubation in the presence of rat liver microsomes and NADPH (37 °C, 0.5 h, $n = 4$). Data represented as mean ± SE.[44]

are predominantly transported into erythrocytes via passive diffusion down a concentration gradient, thus the greater levels of in vitro metHb induction observed for aminophenones **5** and **6** may be a result of the enhanced penetration of their corresponding *N*-hydroxyl metabolites across the red blood cell membrane.[45] Indeed, further studies by Yang[44] would suggest that there is a relationship between the lipophilicity of the aminophenone and its uptake into erythrocytes (Table 2, Fig. 5); note that these experiments were conducted using the parent aminophenones and not their *N*-hydroxyl metabolites—in silico generated partition coefficients would suggest the log *P* values of *N*-hydroxyaminophenones to be 0.5–1 log*P* units below that of the corresponding aminophenones. A third rationalization for the observed tail off in metHb induction at log *P* values greater than 3.5 may be that these compounds simply begin to lose their solubility under assay conditions, leading to precipitation and consequently reduced metabolism/uptake.

In vivo evaluation (aminophenones)—Interestingly, the degree of lipophilicity required for maximum in vitro metHb induction did not appear to correlate with the aforementioned observations of Pan et al.,[22] with respect to in vivo toxicity, in rats. To validate this discrepancy, four candidates were nominated for appraisal in vivo, namely aminophenones **4**, **6**, **13** and aminodihydrochalcone **23**. Aminophenone **4** was found to be the most toxic compound in vivo, again, presumably a consequence of methemoglobinemia,

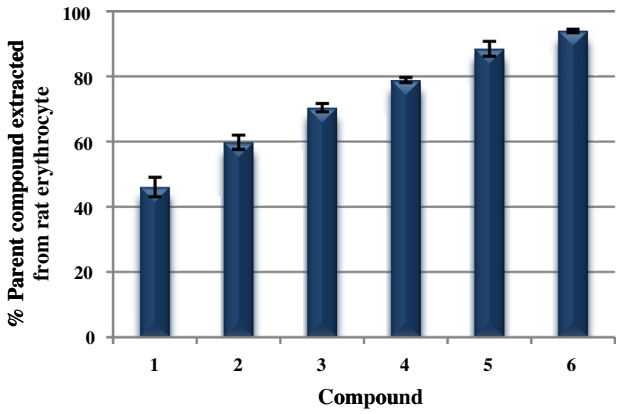


Figure 5. Effect of increasing alkyl chain length on the uptake of aminophenones **1**–**6** into rat erythrocytes post incubation (37 °C, 0.5 h, $n = 4$). Data represented as mean ± SE.[44]

while compounds **6** and **23**, both potent metHb inducers in vitro, were shown to be considerably less toxic. In both cases, rats exhibited symptoms characteristic of sub-lethal methemoglobinemia (blue paws, tail and nose, lethargy and ataxia), and in one example in each treatment group death eventuated. Surprisingly, compound **13** was found to be largely devoid of toxicity at the administered dose (Table 4). One possible explanation for these in vitro/in vivo discrepancies could relate to aminophenones **3** and **4** exhibiting greater bioavailability than aminophenones **5** and **6**. With these additional complicating factors in mind, synthesis of metHb inducers from this point forward focused on the design of bioisosteric analogues possessing a log *P* value similar to that of ketone **4**, an established potent in vivo metHb inducer,[22] despite aminophenone **6** exhibiting superior in vitro metHb induction.

Carbonyl isosteres—Attention next turned to investigating the role of the ketone itself. Taking into consideration the aforementioned biochemical mechanism behind metHb induction, it was postulated that the electronic contribution of the ketone to the aryl amine ring was fundamental to PAPP's toxicity. To further probe this theory a number of carbonyl bioisosteres were explored, starting with the commonly employed oxime moiety.[46] With guidance from in silico partition coefficient calculations, *O*-methyl ketoxime **30** was proposed, incorporating an *O*-alkyl chain tailored to achieve a lipophilic profile comparable to that of lead methemoglobinemia inducing agent aminophenone **4** (our most potent in vivo metHb inducer).[47] As predicted, the experimental partition coefficient of ketoxime **30** was comparable to that of **4**. The $^{13}C–NH_2$ chemical shift of compound **30** (δ 147.3 ppm), however, was found to be appreciably upfield of that of PAPP (δ 151.0 ppm), suggesting the *O*-alkyl ketoxime moiety to be less electron-withdrawing than the parent ketone (Table 3, Fig. 1).

In a study employing oxime ethers as ester surrogates, Bromidge et al.[48] demonstrated that the pK_a of azabicyclic muscarinic agonists could be manipulated through the introduction of an electron-withdrawing group into the oxime function. In light of this *O*-methyl α-chloroaldoxime **31** and *O*-methyl α-cyanoaldoxime **32** were prepared and subsequently demonstrated to be of comparable lipophilicity to both ketoxime **30** and ketone **4**.[47] As anticipated, the electronic trend within this series was of increasing $^{13}C–NH_2$ chemical shift relative to the increasing electron-withdrawing potential of the oxime derivative, as evidenced by compounds **30** (δ 147.3 ppm), **31** (δ 148.7 ppm) and **32** (δ 149.3 ppm). Disappointingly, ketoxime **30** (metHb 6.4 ± 0.8%) induced only low levels of metHb in vitro, which was postulated to be a consequence of the relatively weak electron-withdrawing nature of the oxime ether moiety compared with that of the parent ketone. However, contrary to expectation, α-chloroaldoxime **31** (metHb 8.4 ± 0.9%) and α-cyanoaldoxime **32** (metHb 8.2 ± 1.9%) were likewise found to induce similarly low levels of metHb, thus providing no clear relationship between the electronic parameter and metHb induction within this series. In summary, these results would suggest that the derivatized oxime moieties were insufficiently electron-withdrawing to provide an optimal electronic contribution to the aryl amine ring. Structural requirements should also be considered with respect to in vitro metHb activity, as derivatized ketoxime moieties within this series may not be as well tolerated as the ketone (Table 3, Fig. 1).

In an effort to further extend our study on the role of electronics in metHb toxicity, an emphasis was now placed on carbonyl isosteric replacements possessing stronger electron-withdrawing properties; as evidenced by their literature Hammett substituent constants[41]—including bioisosteres based on the sulfoxide (σ_{para} (SOMe) +0.49) and sulfone (σ_{para} (SO_2Me) +0.72) groups.[41,46] Traditionally a bioisostere for the carboxyl moiety,[49] the sulfonamide (σ_{para} (SO_2NMe_2) +0.65) group was also considered, due to its evidently suitable electronic properties. With guidance from in silico log *P* calculations, aminophenyl sulfoxide **33**, sulfone **34**, and sulfonamides **35** and **36** were proposed, again each incorporating an alkyl chain tailored to achieve a lipophilic profile comparable to that of lead aminophenone **4**.[47]

Sulfone **34** (metHb 33.5 ± 2.9%), bearing the largest positive Hammett substituent constant within the series, demonstrated the greatest level of activity, while sulfoxide **33** (metHb 13.2 ± 0.8%), possessing a σ_{para} value virtually analogous to that of the ketone group, was found to be a poor inducer of metHb in vitro. Again, despite its perceived favorable electronics, sulfonamide **35** (metHb 5.1 ± 0.4%) exhibited the weakest in vitro metHb induction within the study. The low level of activity observed for compound **35** was loosely attributed to the presence of the N–H hydrogen bond donor function of the sulfonamide, and its presumed detrimental impact on the mechanisms behind metHb induction. Gratifyingly, N-alkylation of sulfonamide **35** to reveal *N*-methyl sulfonamide **36** (metHb 22.0 ± 0.5%) led to a significant

Table 4
In vivo evaluation of compounds **1**–**6**, **13** and **23**, in rats (p.o.)

No.	LD_{50} (mg/Kg)[22]	Dose (mg/Kg)	No. of deaths observed	Symptoms recorded
1	381 ± 22	–	–	–
2	221 ± 26	–	–	–
3	85 ± 31	–	–	–
4	85 ± 31	60	5/7	Blue paws, tail and nose; lethargy; ataxia; prone[a]
5	216 ± 43	–	–	–
6	–	120	1/4	Blue paws, tail and nose; lethargy; ataxia[a]
13	–	120	0/2	Blue paws; lethargy[a]
23	–	120	1/4	Blue paws, tail and nose; lethargy; ataxia[a]

[a] Visual signs consistent with methemoglobinemia.

Table 5
Hammett substituent constants[41]

R	σ_{para}	F	R
COEt	+0.48	+0.34	+0.14
CO*t*Bu	+0.32	+0.26	+0.06
SOMe	+0.49	+0.52	−0.03
SO_2NMe_2	+0.65	+0.44	+0.21
SO_2Me	+0.72	+0.53	+0.19

recovery in activity. Largely in disagreement with their literature Hammett substituent constants, the $^{13}C–NH_2$ chemical shift of sulfone **34** (δ 151.3 ppm) was found to closely resemble that of ketone **2** (δ 151.0 ppm), while that of sulfoxide **33** (δ 149.4 ppm), and to a lesser extent sulfonamide **36** (δ 150.4 ppm), were revealed to be less comparable (Table 3, Fig. 1).

One explanation for such anomalies, or at least in the example of sulfoxide **33**, may relate to the 'component specific' nature of each substituent's electronic contribution. The Hammett substituent constant is composed of two key parts, a *field* (F) component, and a *resonance* (R) component—the former being dictated by the electronegativity of the atoms or groups involved, and the latter derived from the delocalization of the electrons in a π-system. On the surface, evaluation of the Hammett substituent constants for the propionyl and sulfoxide groups, as featured in PAPP and sulfoxide **33**, respectively, would suggest these substituents to be relatively similar in terms of their electronic nature. Upon closer inspection of each substituents corresponding F and R components, however, the resonance (R) parameter is seen to afford a larger contribution to the overall σ_{para} constant of that found in PAPP, compared with that found for sulfoxide **33** (Table 5). It could be postulated that such variations in F/R composition influence the critical electronic factors behind metHb toxicity, namely N-hydroxylation and the accompanying coupled redox cycle.

In summary, in an endeavour to further develop the known methemoglobinemia-inducing agent PAPP, a series of related aminophenones were prepared and evaluated in vitro. Of these, compounds **5** and **6** were found to induce the formation of metHb to a greater extent than their straight chain aminophenone homologues, including PAPP itself. Likewise, as a subclass, straight chain aminophenones were generally found to be more active than both their branched and cyclic chain counterparts. Subsequent evaluation revealed that aminophenones **5** and **6** were absorbed by erythrocytes to a greater degree, offering one potential explanation for their enhanced activity. Disappointingly such trends in in vitro activity did not translate into toxicity in vivo, in rats, potentially a consequence of poor pharmacokinetics.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.10.046.

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50. Standard metHb assay conditions similar to those of Coleman et al. (*Pharm. Pharmacol.* **1991**, *43*, 779–784) were adopted: washed erthyrocytes (100 μL) were incubated (37 °C, 1 h, $n = 3$) with compound (10 mM) in DMSO (1% v/v), microsomes (1 mg/mL) and NADPH (1 mM), with a final volume of 200 μL, diluted with PBS (0.1 M, pH 7.4), in an Eppendorf Thermomixer Compact. PAPP was used as a standard to calibrate the assay. Subsequent to incubation, samples were immediately put on ice and a 35 μL aliquot of sample assayed for CO-oximetry parameters using an ABL700 series blood-gas analyzer, 100–240 V, 50–60 Hz, 90 W.