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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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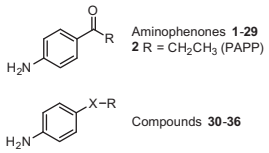

Figure 1. Aminophenones **1**–**29**, including PAPP **2**, and compounds **30**–**36** (aminophenone bioisosteres); see Tables 1 and 3.

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)

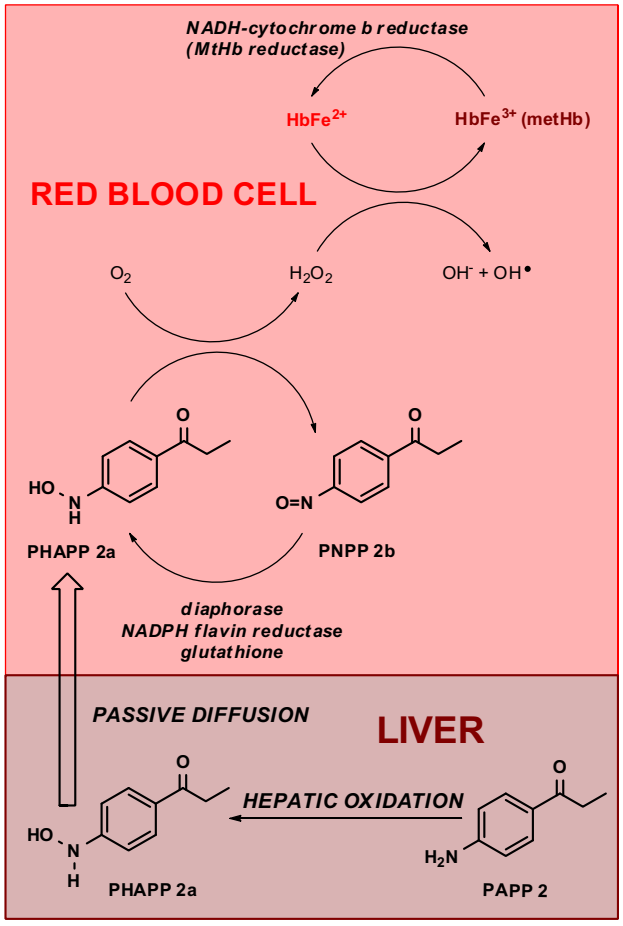

Figure 2. Postulated mechanism for the in vivo formation of metHb by PAPP.

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