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New ultra-short acting hypnotic: Synthesis, biological evaluation, and metabolic profile of ethyl 8-oxo-5,6,7,8-tetrahydro-thiazolo[3,2-*a*][1,3]diazepin-3-carboxylate (HIE-124)☆

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Abstract—Ethyl 8-oxo-5,6,7,8-tetrahydro-thiazolo[3,2-*a*][1,3]diazepin-3-carboxylate (HIE-124, **4**) is a member of a new generation of ultra-short acting hypnotics. HIE-124 (**4**) exhibited potent in vivo activity with a rapid onset of action and a short duration of action, with no acute tolerance or noticeable side effects. The metabolic profile of **4** is also performed. HIE-124 (**4**) has the potential use as a preanesthetic medication, anesthesia inducer, and could be used with thiopental sodium to maintain anesthesia for longer duration.
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Surgical procedures require the administration of several intravenous drugs to ensure hypnosis, analgesia, relaxation, and control of visceral reflex responses. The use of intravenous drugs adds flexibility and permits the administration of lower doses of inhalational anesthetic agents. General anesthesia most often is initiated by an injection of thiopental, an ultra-short acting barbiturate, to induce sleep prior to administration of the agents that are necessary for maintaining anesthesia during the surgical procedure. Ultra-short acting barbiturates and benzodiazepines have an important place in the practice of anesthesiology. Thiopental sodium remains the standard for comparison with new agents.

Single intravenous anesthetic dose of thiopental produces unconsciousness within 10–20 s. The depth of anesthesia may increase for up to 40 s and then decreases progressively until consciousness returns in 20–30 min. However, recovery may require many hours if a large dose of thiopental sodium is administered.[2] Thiopental is metabolized slowly in the liver, which together with other factors may influence the depth of anesthesia, time of recovery, and duration of action.[3] The concentration employed of thiopental should not exceed 2.5% in aqueous solution. When greater concentration is injected extravascularly, severe pain and tissue necrosis may occur. Intra-arterial injection of a concentrated solution of thiopental may result in damage to the arterial endothelium, followed by endarteritis, often with thrombosis exacerbated by subsequent arteriolar spasm. Vascular ischemia and even gangrene may result.[4] Thiopental produces a long lasting hangover.[5] Patients using a large initial dose of thiopental will awake despite plasma concentrations that normally would cause sleep. The nature of this acute tolerance is not known. For this reason, thiopental cannot be used to maintain surgical anesthesia, but only as an induction agent. Thiopental produces a dose-related depression of respiration that

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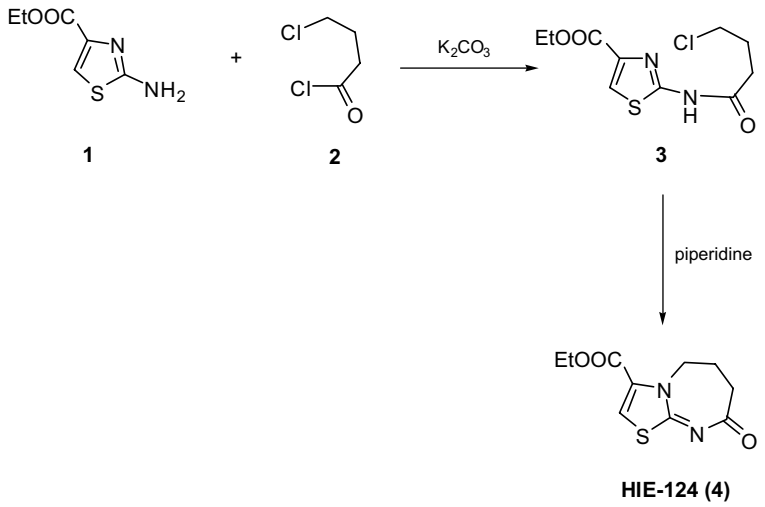
can be profound.[6–8] Larger doses of thiopental sodium cause more profound changes and respiration is maintained only by movements of the diaphragm.

Benzodiazepines were first introduced for the treatment of anxiety, then later on, they have been used as sedatives, anticonvulsants, and muscle relaxants. Hypnosis and unconsciousness may be produced with large doses. Benzodiazepines are not analgesics, nor can they produce a state of surgical anesthesia when used alone. It is necessary to combine several drugs to achieve surgical levels of anesthesia with a balance of sedation, analgesia, amnesia, relaxation, and freedom from reflex stimulation. Benzodiazepines are useful as the sole agent for procedures that do not require analgesia, such as endoscopy, cardiodepression, cardiac catheterization and a spectrum of radiodiagnostic procedures. For induction of anesthesia, the benzodiazepines are given intravenously, to minimize the burning sensation and avoid venous thrombosis that might accompany the iv administration.[9]

In the present investigation, we would like to report the ultra-short acting activity of ethyl 8-oxo-5,6,7,8-tetrahydro-thiazolo[3,2-*a*][1,3]diazepin-3-carboxylate (HIE-124, **4**), a member of a novel class which might overcome many of the disadvantages and problems, such as hangover and tolerance, that are usually associated with the use of thiopental or benzodiazepines as intravenous anesthetic agents. HIE-124 (**4**) has been shown to not only induce anesthesia but also maintain the anesthetic state during the surgical procedure. HIE-124 (**4**) exhibited a very rapid onset of action and a shorter duration of action with no acute tolerance or noticeable side effects such as salivation, dizziness, muscle twitches, and convulsions, when compared with thiopental sodium. This new finding granted the issuance of a German patent.[1]

The thiazolo[3,2-*a*][1,3]diazepine nucleus can be obtained by the use of published methods.[10] Ethyl 8-oxo-5,6,7,8-tetrahydro-thiazolo[3,2-*a*][1,3]-diazepin-3-carboxylate (HIE-124, **4**) was synthesized according to an inventive method (Scheme 1). Ethyl 2-amino-thiazole-4-carboxylate (**1**) was prepared using reported procedure,[11] and then alkylated with 4-chloro-butyryl chloride (**2**), in presence of anhydrous potassium carbonate to afford ethyl 2-(4-chloro-butanamido)thiazole-4-carboxylate (**3**). Compound **3** was cyclized using piperidine as a base to produce HIE-124 (**4**), which was purified using silica gel column chromatography. Structure elucidation of compounds **3** and **4** was obtained based on analysis of the ^{1}H, ^{13}C, DEPT, COSY, HMBC, and HMQC NMR spectra, in addition to mass spectrometry for each compound.

Intravenous administration of ethyl 8-oxo-5,6,7,8-tetrahydro-thiazolo[3,2-*a*][1,3]diazepin-3-carboxylate (HIE-124, **4**), at dose levels of 50 and 100 mg/kg, induced hypnosis, in all mice within few seconds measured by using the loss of righting reflex method (Table 1). Loss of righting reflex was defined as the inability of a mouse to right itself within 30 s. Return of the righting response was defined as the ability of a mouse to right itself twice in 1 min. Upon loss of righting reflex, mice were placed on their backs, and the time to regain righting reflex was measured. Sleeping time or duration of loss of righting reflex was defined as the time between loss and return of the righting response, while onset of action is the time between injection of the compound and loss of righting reflex.[12a] Tachypnea was observed in three mice out of 10 that received 100 mg/kg and dyspnea was also observed in one mouse out of 10 mice that received 100 mg/kg. Both doses of HIE-124 (**4**) potently potentiated the hypnotic effect produced by 50 mg/kg iv thiopental sodium (Table 1). Intraperitoneal administration of HIE-124 (**4**) at dose levels of 100 and 200 mg/kg induced hypnosis in all mice with onset of action less than 1 min (Table 2). Tachypnea was observed in two mice out of 10 that received 100 mg/kg, and ataxia and tachypnea were also observed in three mice out of 10 mice that received 200 mg/kg. The tachypnea, observed in 30% of animals received the high dose of HIE-124 (4) compound, may indicate that high doses of the com-



Scheme 1.

Table 1. Hypnotic effect of ethyl 8-oxo-5,6,7,8-tetrahydro-thiazolo[3,2-*a*][1,3]diazepin-3-carboxylate (HIE-124, **4**), 50, and 100 mg/kg iv, and its effect on the thiopental sodium, 50 mg/kg iv,-induced hypnosis in mice

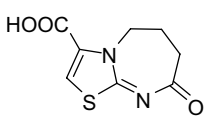
Treatment	Dose (mg/kg iv)	Sleeping time (min)
Thiopental sodium	50	4.90 ± 1.13
HIE-124 (**4**)	50	0.75 ± 0.01[a]
HIE-124 (**4**)	100	1.48 ± 0.21[a]
HIE-124 (**4**) + Thiopental	50 + 50	19.90 ± 5.21[a]
HIE-124 (**4**) + Thiopental	100 + 50	25.70 ± 5.11[a]

Data are expressed as means ± SE. $n = 10$.
[a] Significantly different as compared to thiopental, using Student's *t*-test, $P < 0.05$.

Table 2. Hypnotic effect of ethyl 8-oxo-5,6,7,8-tetrahydro-thiazolo[3,2-*a*][1,3]diazepin-3-carboxylate (HIE-124, **4**),100 and 200 mg/kg ip, and its effect on the thiopental sodium, 65 mg/kg ip, induced hypnosis in mice

Treatment	Dose (mg/kg ip)	Sleeping time (min)
Thiopental sodium	65	15.2 ± 1.40
HIE-124 (**4**)	100	2.4 ± 0.83[a]
HIE-124 (**4**)	200	9.1 ± 1.23[a]
HIE-124 (**4**) + Thiopental	100 + 65	49.2 ± 3.71[a]
HIE-124 (**4**) + Thiopental	200 + 65	155.7 ± 16.35[a]

Data are expressed as means ± SE. $n = 10$.
[a] Significantly different as compared to thiopental, using Student's *t*-test, $P < 0.05$.



5

Figure 1. Structure of the major metabolite **5**.

pound could have undesirable effects on respiratory system. Both doses of the compound, 100 and 200 mg/kg, potently potentiated the hypnotic effect produced by 65 mg/kg ip thiopental sodium[12b] (Table 2).

Acute toxicity test was performed according to the method described by Litchfield and Wilcoxon.[13] The calculated LD_{50} HIE-124 (**4**) was found to be 681.4 mg/kg with 95% confidence limits of 482.8–876.4. Acute tolerance test was conducted following the ip administration of 200 mg/kg of HIE-124 (**4**), three times/day for three consecutive days. There was no significant difference among the sleeping times after the first and the last administration of the compound. The sleeping time after the first administration of **4** was 10.3 ± 1.4 min, while after the ninth administration, the sleeping time was 8.7 ± 2.1 min.

Initial ESI LC–MS analysis of HIE-124 (**4**) (Fig. 1) dissolved in 60:40 $CH_3CN:H_2O$ showed the compound eluting at ~17 min with a protonated molecular ion peak [M+1]+ at *m/z* 241 in the positive ion mode (Fig. 2). Gradient elution was tailored such that HIE-124 (**4**) elutes at a relatively late analysis time to allow for sufficient time window for the elution of other metabolites, which are expected to be polar and hence elute at an earlier time. Screening for potential metabolites was performed by searching for ions with molecular weights corresponding to metabolites generated through one of the most common phase I and phase II metabolic transformations. For HIE-124 (**4**), these transformations were expected to be ester hydrolysis, aromatic hydroxylation, and glucuronic acid conjugation. The primary strategy for metabolite identification was thorough analysis of total ion current (TIC) chromatograms obtained from various LC–MS analyses. Blank TIC and UV chromatograms were subtracted from those containing compound **4** in either a urine sample or an in vitro incubation.

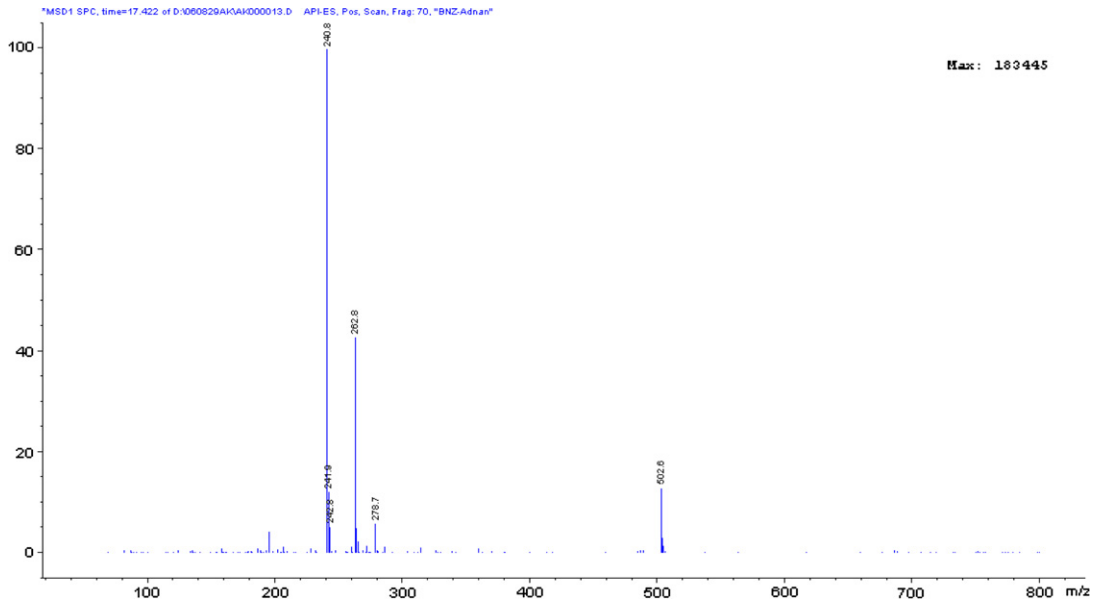


Figure 2. ESI mass spectrum of HIE-124 (**4**) dissolved in 60:40 $CH_3CN:H_2O$.

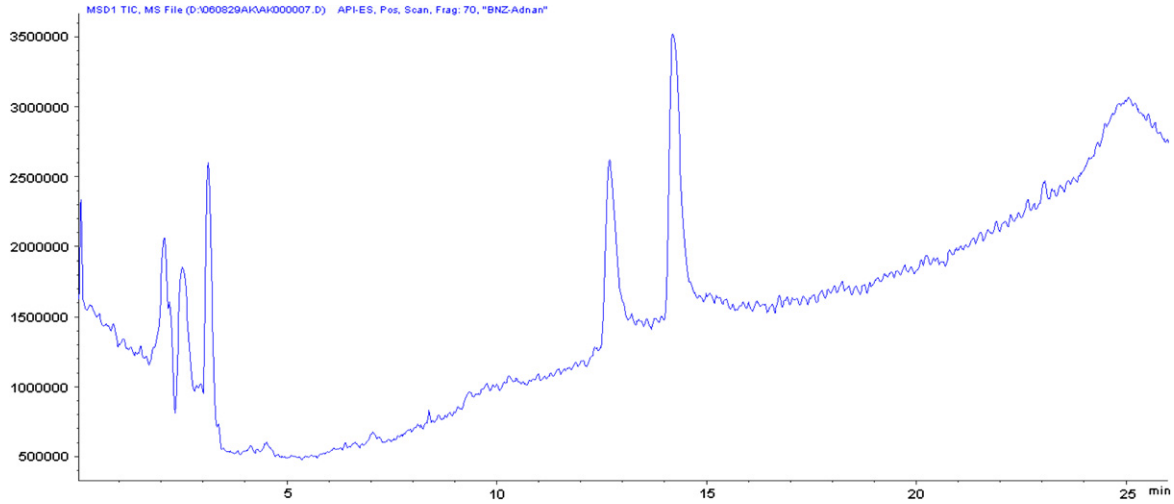


Figure 3. TIC chromatogram of blank hepatocyte incubation.

Screening of TIC chromatograms of pooled mice urine samples showed a prominent peak eluting at ~11 min, with a protonated molecular ion peak at *m/z* 213, with the absence of the peak at *m/z* 241 corresponding to the protonated **4** (Fig. 2). The peak was proposed to be the de-ethylated metabolite **5** $[M-28]^+$, resulting from hydrolysis of the ethyl ester of **4**. It is a well-documented route of biotransformation.[14–17] Careful screening of the TIC chromatogram showed the absence of any other peak corresponding to one of the expected metabolites resulting from either phase I reactions (e.g. *m/z* 229 resulting from hydroxylation) or phase II reactions (e.g. *m/z* 389 resulting from glucuronidation).

In vitro drug metabolism studies are widely regarded as reliable and accurate models for the prediction of drug metabolism in a variety of mammalian species. Among the different techniques to study in vitro metabolism are hepatic microsomal models and hepatocyte preparations.[18,19] This study attempted to utilize such techniques to gain more information about the metabolic profile of HIE-124 (**4**). Incubation of compound **4** with rat liver microsomes (RLMs) was straightforward and results were consistent with those obtained from in vivo experiments. The various incubation conditions were designed to obtain more details about the possible enzymatic processes involved in the in vitro metabolism of **4**. Incubations with NADPH alone with no cells were performed to determine the presence of non-enzymatic hydrolysis of the ester. Incubation with cells but no NADPH was designed to determine involvement of non-CYP450 isoforms in the metabolism of **4** since CYP450 isoforms need NADPH for the catalysis. Incubation with glutathione (GSH) was designed to determine the presence of GSH adducts, frequently formed with carboxylic acids.[20,21] Troglitazone (TGZ), known to form GSH adducts,[22] was used as a positive control.

LC–MS analysis of thein vitro studies gave similar and consistent results to the in vivo experiments. In RLM studies, the only metabolite detected for HIE-124 (**4**) was the de-ethylated product **5** $[M-28]^+$ at *m/z* 213 (Fig. 3). The chromatogram showed no evidence of the parent compound after the conclusion of the incubation time (Fig. 4). No other drug-related peaks were detected in the run compared to a blank TIC chromatogram.

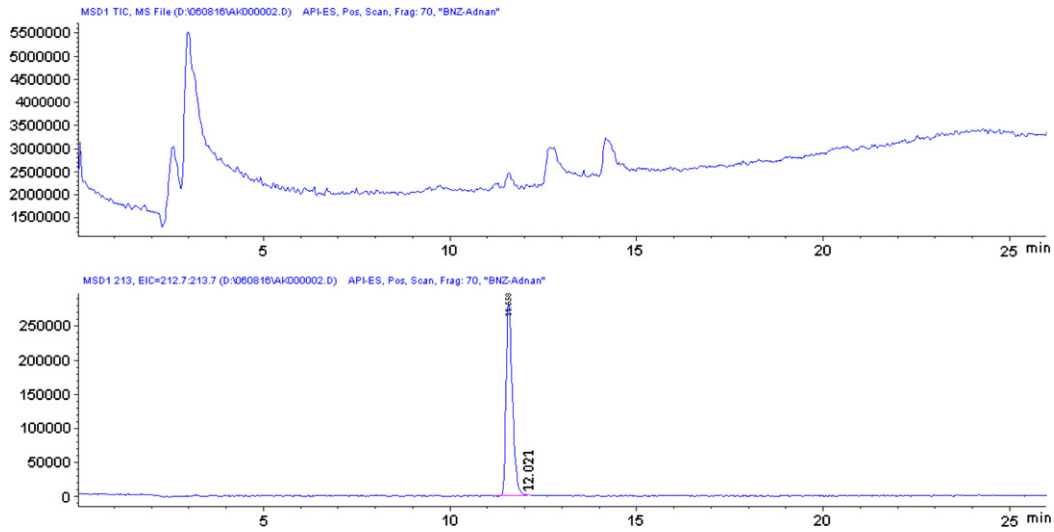


Figure 4. TIC and SIM (*m/z* 213) chromatograms of 30-min RLM incubation with HIE-124 (**4**).

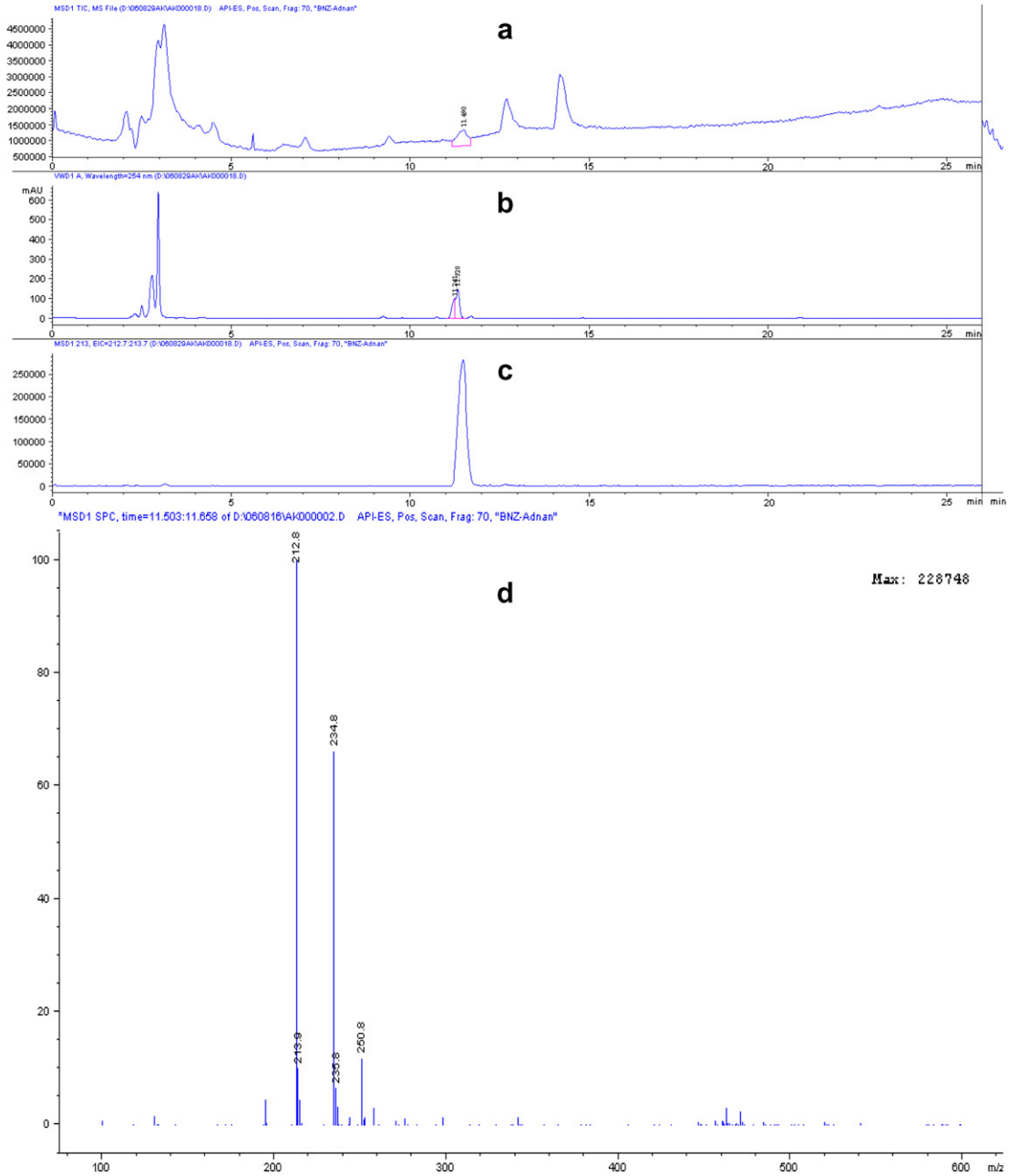


Figure 5. Chromatograms of hepatocyte incubation with HIE-124 (**4**) after 120 min. (a) TIC; (b) UV; (c) SIM (*m/z* 213); and (d) ESI mass spectrum of **5**.

Compound **5** was also the only metabolic product observed for **4** after hepatocyte incubations (Fig. 5). The compound appears to be the product of enzymatic hydrolysis by the action of liver esterases rather than a simple chemical hydrolysis as confirmed by the absence of any significant amounts of the hydrolyzed product, as in the case of RLM and hepatocyte studies. Furthermore, the involvement of esterases in the hydrolysis is supported in part by the presence of the hydrolyzed product in RLM incubations, which does not contain NADPH, the necessary co-factor for cytochrome P450-mediated metabolism but not for esterase-mediated hydrolysis. The presence of carboxyesterase activity in freshly thawed hepatocytes was confirmed by the carboxyfluorescein diacetate assay, frequently used to easily determine cell-derived enzymatic esterification.[23–27] HIE-124 (**4**) appeared to undergo rapid hydrolysis to the corresponding carboxylic acid derivative, with undetected levels of the parent compound remaining in aliquots untreated with CH_3CN taken at 0 min hepatocyte[28–30] incubation time. RLM incubations of **4** in the presence of GSH did not result in the formation of GSH adducts based on positive ion ESI LC–MS results.

The biological evaluation of HIE-124 (**4**) revealed that the compound is a very short acting hypnotic. The onset of action of this compound is very short compared to thiopental sodium. In all animals used, the onset of ac-

tion was less than 60 s. The duration of action when given iv or ip is significantly shorter than that of thiopental sodium. In addition, the investigated compound did not show any sign of acute tolerance reported with the second (maintenance) dose of thiopental sodium. Therefore, compound HIE-124 (**4**) has the potential use not only as a preanesthetic medication and induction of anesthesia but also has the potential to be used with thiopental sodium to maintain anesthesia for longer duration than using thiopental sodium alone. Compound **4** appears to undergo enzymatic hydrolysis by the action of liver esterases. Further metabolic studies are needed to characterize the metabolism of this compound from other mammalian systems.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.11.011.

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