Flupirtine Analogues: Explorative Synthesis and Influence of Chemical Structure on Kᵥ7.2/Kᵥ7.3 Channel Opening Activity

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Dedicated to Bernd Clement on the occasion of his 70th birthday and retirement as university professor

Neuronal voltage-gated potassium channels Kᵥ7.2/Kᵥ7.3 are sensitive to small-molecule drugs such as flupirtine, even though physiological response occurs in the absence of ligands. Clinically, prolonged use of flupirtine as a pain medication is associated with rare cases of drug-induced liver injury. Thus, safety concerns prevent a broader use of this non-opioid and non-steroidal analgesic in therapeutic areas with unmet medical needs such as hyperactive bladder or neonatal seizures. With the goal of studying influences of chemical structure on activity and toxicity of flupirtine, we explored modifications of the benzylamino bridge and the substitution pattern in both rings of flupirtine. Among twelve derivatives, four novel thioether derivatives showed the desired activity in cellular assays and may serve as leads for safer Kᵥ channel openers.

Flupirtine (1) is a non-opioid and non-steroidal, centrally acting analgesic with a unique mode of action. The analgesic effect of 1 is believed to be associated with the opening of heterotetrameric voltage-gated potassium channels, consisting of the subunits Kᵥ7.2 and Kᵥ7.3, which are encoded by KCNQ2 and KCNQ3, leading to membrane potential stabilization and decreased excitability.[1] The Kᵥ7.2/Kᵥ7.3 channel generates M-currents that control the subthreshold excitability of the cell membrane, therefore, drugs that stabilize the open state of Kᵥ7.2/Kᵥ7.3 channels could be used in a broad range of CNS diseases that are characterized by neuronal over-activity, including pain, stress, anxiety and epilepsy. While 1 is regarded safe in short-term use for acute pain, a recent clinical study provided indirect evidence that 1 can be oxidized in healthy volunteers to unstable ortho- or para-azaquinone diimines 3a and/or b (Figure 1) as reactive metabolites.[2] While neither compound 3a nor 3b could be identified directly, their formation was deduced from presence of cysteine metabolites in biological fluids,[3] formed by reactions of the reactive intermediates with glutathione to yield adducts such as 4. While it is not known whether reactive electrophiles 3a or 3b are the causative agent for drug-induced liver injury (DILI), safety issues lead to a termination of a clinical study with 1 in hyperactive bladder in 2013.[3]

Although 1 was approved for use as a centrally acting analgesic in a number of European countries until 2018, it was not approved as an anticonvulsant. Two recent animal studies however have demonstrated that 1 terminates seizures in neonatal mammals effectively, and that a combination of 1 and diazepam is superior to diazepam alone.[4] Because the closely-

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related \( K_v \) channel opener retigabine (2) had been used as antiepileptic, until it was also withdrawn from the market in 2017, this additional indication for 1 would seem a logical endeavor. To date, treatment options for severe cases of status epilepticus are scarce.

Follow-up substance 2 marketed as Trobalt® has been taken of the market by GSK, last year. One of the main reasons was a blueish tissue discoloration.[1] While the exact structure of the blue dye could not be resolved, it is most likely that the formation of polymers of 2 is the cause for this adverse effect. Based on earlier findings that 1 and its metabolites can only be traced to a limited extent in excretes, we speculate that diimines such as 3a and b could form insoluble polymers in vivo, as well. This hypothesis is supported by the report, that DILI caused by 1 is not influenced by typical polymorphisms of metabolic enzymes but associated to Human leucocyte antigen (HLA) genotype.[2] It seems possible, that polymerization in vivo triggers an immune response that could lead to the observed hepatotoxicity. Consequently, the search for flupirtine analogues with a better safety profile seems worthwhile.

The tendency of 1 to form 3a or 3b or polymers may be attributed to electronic features such as oxidation potentials. By calculating the molecular orbitals of flupirtine (Figure 2) one finds that the highest occupied molecular orbital (HOMO) and HOMO-1 are localized around the pyridine ring. This explains the oxidation susceptibility of the heterocyclic ring to form azaquinone diimine metabolites. The same calculation for deazathio-flupirtine 5 showed that the HOMO orbital will shift from pyridine to the sulfur atom. This flupirtine analogue 5 is equally active as 1 but non-toxic in vitro and was thus selected as a starting point for the synthesis of more potent flupirtine analogues.[3] Our experiments on the oxidation of thio-analogues to sulfoxides with a stoichiometric equivalent of \( m \)-chloroperbenzoic acid also confirm this computational result. In order to alter these unfavorable electronic features of 1, we synthesized flupirtine analogues with modifications that should avoid the formation of azaquinone diimines and polymers in vivo.

To plan the modifications of 1, we divided its chemical structure into four regions; the 4-fluorobenzyl moiety, the secondary amine linker, the primary amine substituent, and the carbamate group. All analogues reported here have a sulfur containing functional group in place of the secondary amine linker. As shown in the HOMO calculation, the placement of this sulfur atom is envisioned to hinder the possible formation of azaquinone metabolites like 3a. Compounds 9b–9g and compounds 10b–10c have other rings or different substitution patterns in place of the 4-fluorobenzyl group (Table 1). Compounds 9g–9h and 10d, on the other hand, have a methyl or methyl amine moiety instead of a primary amine. This modification, owning to the absence of primary amine, may result in analogues with little tendency to form metabolites like 3b. In addition, the ethyl carbamate of 1 was replaced with fluoro-substituted phenyl or benzyl groups to give compounds 9a, 9g and 10a, respectively. The rational for this bulky, lipophilic modification comes from the recent patent literature on this class of compounds.[4]

In an initial test set of systematically alkylated flupirtine derivatives reported earlier, EC\textsubscript{50} values for their \( K_v \) channel opening activity correlated with oxidation potentials.[5] In order to evaluate this proposed connection, we followed a similar approach to increase stability towards oxidation. By replacement of the secondary amino group connecting the two aromatic moieties in 1 by a thioether or thioster group in 9a–h, we aimed to alter the oxidation pathway of the molecule while retaining or even improving biological activity.

The synthesis of most of the compounds was commenced with the amination of 2,6-dichloro-3-nitropyridine yielding intermediates 6a or b (Scheme 1). The only exceptions were the syntheses of compounds 7c, 8h, 9h and 10d, which instead started with 6-bromo-2-methyl-3-nitropyridine (6c). The halogen substituents in 6a–c were unambiguously replaced by a thiol group. Subsequently, thioether analogues were synthesized by straightforward nucleophilic attack of the thiol group on reactants with different cyclic structures in \( \beta \)-position. Following the thioether synthesis, the 3-nitro group in 8a–h was reduced to a primary amine with various classical reducing agents and then acylated to give the corresponding carbamate.

![Figure 2. A) HOMO of flupirtine (1); B) HOMO of equally active but putatively non-toxic lead 5.](image)

| Table 1. Residues R\textsubscript{1}–R\textsubscript{2} in intermediates 8a–h, thioethers 9a–d,f–h and thioesters 9e, and sulfoxides 10a–d |
|-----------------|--------------|-----------------|-----------------|
| Entry | R\textsubscript{1} | R\textsubscript{2} | Yield\textsuperscript{[a]} [%] |
| 8a | NH\textsubscript{2} | 4-Fluorobenzyl | 85 |
| 8b | NH\textsubscript{2} | 4-Phenylenbenzyl | 90 |
| 8c | NH\textsubscript{2} | 3,5-Dimethoxybenzyl | 76 |
| 8d | NH\textsubscript{2} | 2-Pyrrolidinomethyl | 88 |
| 8e | NH\textsubscript{2} | 4-Fluorobenzyl | 82 |
| 8f | NH\textsubscript{2} | Piperidylethyl | 87 |
| 8g | NHCH\textsubscript{2} | Benzyl | 89 |
| 8h | CH\textsubscript{2} | 4-Fluorobenzyl | 66 |
| 9a | NH\textsubscript{2} | 4-Fluorobenzyl | 3,4-Difluorophenyl | 35 |
| 9b | NH\textsubscript{2} | 4-Phenylenbenzyl | Ethoxy | 38 |
| 9c | NH\textsubscript{2} | 3,5-Dimethoxybenzyl | Ethoxy | 52 |
| 9d | NH\textsubscript{2} | 2-Pyrrolidinomethyl | Ethoxy | 30 |
| 9e | NH\textsubscript{2} | 4-Fluorobenzyl | Ethoxy | 28 |
| 9f | NH\textsubscript{2} | Piperidylethyl | Ethoxy | 32 |
| 9g | NHCH\textsubscript{2} | Benzyl | 3,5-Difluorobenzyl | 8 |
| 9h | CH\textsubscript{2} | 4-Fluorobenzyl | Ethoxy | 84 |
| 10a | NH\textsubscript{2} | 4-Fluorobenzyl | 3,4-Difluorobenzyl | 30 |
| 10b | NH\textsubscript{2} | 4-Phenylenbenzyl | Ethoxy | 88 |
| 10c | NH\textsubscript{2} | 3,5-Dimethoxybenzyl | Ethoxy | 18 |
| 10d | CH\textsubscript{2} | 4-Fluorobenzyl | Ethoxy | 25 |

\textsuperscript{[a]} Isolated yield.
The closely related deazathio-flupirtine analogues 9a and b are as active as the marketed drug 1. The less similar compound 9g is even markedly more potent and effective (Figure 3).

Thioester 9e was inactive in the tested concentration range, which shows that a sulfide bridge is the much better biososteric surrogate for the amino bridge than a thioester in its thionoester form. The products of the chemical oxidation experiments demonstrated that formation of reactive diimines does not occur but instead the oxidative reactivity is shifted towards relatively benign S-oxidation, at least in the sulfide series, as was anticipated. Oxidation is generally hampered in comparison to 1, as the anodic peak potentials (Epa) are higher. The resulting oxidation products, namely sulfones and sulfones, are putative metabolites that could also form from metabolic oxidation. Therefore, their cell toxicity is of interest and selected derivatives (10a-d) were thus evaluated for *in vitro* hepatotoxicity in hepatocellular models with that use the TAMH and HEP-G2 cell lines.[13]

Except for compounds 1, 9a, c, and d, LD50 values after 24 h could not be determined due to a lack of aqueous solubility. To gain another quantitative indicator of toxicity, LD50 values after 48 h, which could be determined at lower concentrations where water solubility was not an issue, were determined for the most promising compounds. The LD50 for highly potent and effective 9g is 6 ± 3 and 4 ± 4 μM in the TAMH and HEP-G2 cell lines, respectively, and thus considerably lower than for 1 (Table 2). However, this increase in cell toxicity is more than compensated by the superior K7,2/3 channel opening activity of 9g compared to 1, yielding better safety indices of 400 versus 112 and 267 versus 81 in the TAMH and HEP-G2 cell lines, respectively. Because 9g has the highest logD24 within this series of compounds, lipophilicity but not oxidizability or resulting reactivity might be important for the underlying mechanisms of action and toxicity.

Based on these findings, we conclude that the development of thio-analogues of known drugs 1 and 2 may result in K7,2/3 channel openers with more favorable therapeutic indices than flupirtine. Because they do not form reactive oxidation products in vitro they could even help to separate structure-activity from structure-toxicity relationships.
N-(6-Benzothiazlo)-2-(methylamino)pyridin-3-yl]-2-(3,5-difluorophenyl)acetamide (9g)

Compounds 8g (2.8 mmol, 771 mg), iron powder (28 mmol, 1.57 g) and ammonium chloride (28 mmol, 1.5 g) were suspended in 15 mL ethanol 20%. The suspension was stirred at 100 °C for 2 hours, filtered over diatomaceous earth, and the filter washed with ethyl acetate. The filtrate was poured into water. The collected precipitate was washed with ethyl acetate. 2,6-Dichlorophenyl acetic acid = DMSO-d6 found: 398.1157. For synthetic details of other compounds see supporting information.

| Entry | E0 \( \text{mV} \) | log\( \text{K} \) | EC50 \( \text{mM} \) | E40% \( \text{mM} \) | LD50 \( \text{mM} \) | LD50 \( \text{mM} \) | Toxic/Act. | LD50 \( \text{mM} \) | LD50 \( \text{mM} \) | Toxic/Act. |
|-------|----------------|-----------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 1     | 350            | 2.96      | 0.918 ± 0.099  | 100            | 487 ± 51       | 103 ± 47       | 112            | 547 ± 111      | 74 ± 40        | 81             |
| 9a    | 442            | 3.90      | 0.26 ± 0.082   | 100 ± 23       | > 30           | 13 ± 04        | 50             | 8 ± 1          | 4 ± 1          | 15             |
| 9b    | 452            | 4.34      | 0.253 ± 0.042  | 69 ± 9         | > 63           | 14 ± 13        | 55             | > 250          | n.d.           | n.d.           |
| 9c    | 450            | 3.61      | > 10           | > 250          | n.d.           | n.d.           | 134 ± 22       | n.d.           | n.d.           | n.d.           |
| 9d    | 499            | 2.62      | > 10           | > 100          | n.d.           | n.d.           | 831 ± 149      | n.d.           | n.d.           | n.d.           |
| 9e    | 573            | 3.00      | > 10           | > 125          | n.d.           | n.d.           | > 250          | n.d.           | n.d.           | n.d.           |
| 9f    | 573            | 2.04      | > 10           | > 500          | n.d.           | n.d.           | > 500          | n.d.           | n.d.           | n.d.           |
| 9g    | 631            | 4.27      | 0.015 ± 0.002  | 147 ± 9        | > 7.5          | 6 ± 03         | 400 ± 10       | > 4 ± 1        | 267            |
| 9h    | 855            | 3.85      | 0.269 ± 0.031  | 129 ± 3        | > 10           | > 10           | -              | 25 ± 16        | 93             |
| 10a   | 628            | 3.24      | > 10           | > 100          | n.d.           | n.d.           | > 125          | n.d.           | n.d.           | n.d.           |
| 10b   | 654            | 3.72      | > 10           | > 100          | n.d.           | n.d.           | > 63           | n.d.           | n.d.           | n.d.           |
| 10c   | 442            | 2.81      | > 10           | > 500          | n.d.           | n.d.           | > 500          | n.d.           | n.d.           | n.d.           |
| 10d   | n.d.           | 3.02      | > 10           | > 500          | n.d.           | n.d.           | > 500          | n.d.           | n.d.           | n.d.           |

[a] Determined with 1.0 mM compound in 100 mM TRIS-buffer (pH 7.4); [b] EC50 and LD50 values are means and standard deviations of 4–5 independent determinations; [c] determined using TAMH cells; [d] determined using HEP-G2 cells; [e] flupiridine maleate salt was used; [f] not determined; [g] non-oxidizable.

Experimental Section

Keywords: medicinal chemistry · ion channels · oxidation · structure-activity relationships · sulfides

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Conflict of Interest

The authors declare no conflict of interest.