Intraperitoneal challenge with 150 µg/kg LPS caused an initial hypothermia in guinea pigs, which reversed over 3 hours post-LPS. At that point, they peaked to almost 20% below baseline, pre-LPS levels. SPP2020 did not prevent the change in QTc levels, suggesting that, like IL1β, it may not be the main driver of QTc prolongation in guinea pigs.

Methods
All experimentation was conducted in accordance with the guidelines on laboratory animal use of the Canadian Council against Animal Cruelty (CCAC).

Purpose of the study:
This research program aimed at quantifying the relationship between inflammatory cytokines and QTc prolongation through the use of LPS and Kdo2-Lipid A-interventions and plasma cytokine measurements, in the presence and absence of anti-inflammatory drugs and new innovative molecules.

Test system:
Male adult guinea pigs (250-400, Charles River) were used in these studies. The same program was started with rats in 2014. It was found that, while rats exhibited the anticipated response to the pro-inflammatory challenge, their electrocardiographic response was more variable and difficult to read, by virtue of exhibiting a much smaller 1-second ECG waveform. Since the guinea pig’s ECG and particularly ECG animal model, the experiments described below were rapidly modified to be applied to the guinea pig.

Experimentation: LPS and Kdo2-Lipid A were used to induce cytokine release in guinea pigs-concomitant ECG monitoring and blood draws, followed by Q-ELISA measurement of cytokine production. Male adult guinea pigs previously instrumented with subcutaneous thermometers on a single day (i.e. injection of 150 µg/kg LPS at time 0). The animals were lightly anesthetized with a mixture of 1.5% isoflurane USP in 95% O2 and 5% CO2, and ECG leads were placed on the animals after careful preparation of the skin. Blood was collected into iced tubes, spun immediately, and the plasma was frozen at -20°C until ready to be used.

ECG intervals were read manually using electronic cursors, or using pattern-recognition software. The QT, QTc, Tp–Tc, and Tp–Tp intervals were measured using the plasma collected as described above. The enzyme-linked immunosorbent assay (ELISA) is a specific and highly sensitive method for quantification of cytokines and other analytes in solution. The assay involved a guinea pig-specific monoclonal antibody (mAb) raised against each cytokine, which was used to coat a 96-well plate. A secondary mAb, used for detection, was labeled with horseradish peroxidase, which produced an intense red colored signal that was directly proportional to the amount of cytokine bound. The concentration of cytokine in the sample was determined by comparison with a standard curve of known cytokine concentrations.

Discussion
Anti-inflammatory compounds are seldom associated with drug-induced QTc prolongation, at least nowhere to the extent that other classes of drugs have been implicated with changes in QTc. In parallel, inflammatory processes have been associated with drug-induced QTc prolongation, particularly via cytokines, prostanoids, and other pro-inflammatory conditions frequently exhibit prolonged QTc intervals, which correlate with increases in tumor necrosis factor alpha (TNFα) levels. Studies in experimental models have shown these cytokines act through stimulation of reactive oxygen species.

The mechanistic identification of reactive oxygen species was beyond the scope of this project. In LPS-induced inflammatory reactions in guinea pigs, TNFα, IL6 and IL1β are increased significantly, if transiently, and this change correlated with the peak of QTc prolongation. The transient nature of the peak in cytokine levels, the so-called "storm", makes it difficult to assess cytokine levels in long-term studies: the levels increase and return to baseline within 4-6 hours, roughly. The sudden increase in levels, however, gives rise to reactive oxygen species and changes in ceramide levels which are thought to change the fluidity of lipid-rich and inter canal channel activation, proinflammatory regulatory mechanisms. The correlation between cytokine levels suggest that the initial rise in plasma levels increases QTc duration, and is followed by cytokine independent processes such as--for instance, not limited to, ceramide accumulation and the ensuing changes in ion channel kinetics.

By preventing the peak in TNFα--but not IL1β or IL6--SPP2020 completely abolished cytokine QTc prolongation in this model of LPS-induced cytokine storm in guinea pigs, the data suggests that an early increase in cytokine levels, up to 1 hour for IL1β and IL6 and 4 hour for IL6, is associated with prolonged QTc intervals. Beyond this, the relationship loses its causative character.

LPS-induced cytokine storm occurs QTc prolongation which can be prevented by an anti-inflammatory compound. 

References

Figure 2. QTc prolongation in LPS-induced cytokine storm

QTc prolongation in LPS-induced cytokine storm

QTc prolongation in LPS-induced cytokine storm

Figure 3. Plasma levels of TNFα in guinea pig challenged with LPS. Sample size: n = 5

Figure 4. Plasma levels of IL1β in guinea pig challenged with LPS. Sample size: n = 5

Figure 5. IL6 in guinea pig challenged with LPS. Sample size: n = 5

Figure 6. Correlation between cytokine levels and QTc prolongation. Sample size: n = 5