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Letter to the Editor

Comment on "Cysteine-Targeted Insecticides against *A. gambiae* Acetylcholinesterase Are Neither Selective nor Reversible Inhibitors"

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In the article by Gorecki et al. (*ACS Med. Chem. Lett.* **2020**, *11*, 65−71), the authors reported that maleimides irreversibly inhibited both the African malaria mosquito (*Anopheles gambiae*) Ace-paralogous acetylcholinesterase (*aka* agAP-AChE[1]) bearing a free Cys and the human counterpart devoid of the Cys and concluded that "Cys-targeted insecticides against *A. gambiae* acetylcholinesterase are neither selective nor reversible inhibitors". This conclusion is problematic for at least six reasons.

(1) A prerequisite to claim that maleimide PM20[2] does not conjugate with the agAP-AChE Cys is to confirm that the Cys is not oxidized. However, in the Gorecki article, no evidence was provided to show that the Cys in their agAP-AChE was active for conjugation.

(2) In the Gorecki article, their agAP-AChE was claimed to be "in very good correlation" with the reported agAP-AChE[3] that was used in ref 2. However, only K_M was reported for their agAP-AChE. Their omission of k_{cat}, which is also needed for enzyme comparison, raises concerns with their specific enzyme activity. Their enzyme could be reasonably active (like crude extracts) and suitable for certain inhibitor characterizations but unsuitable for the kinetic characterization of PM20 described in ref 2.

(3) In the Gorecki article, 10 μM **PM20** and 10 μM paraoxon (rather than 1 nM **PM20** and 200 nM paraoxon in ref 2) showed no agAP-AChE activity recovery after dialysis. Using higher inhibitor concentrations here would prompt for determining the specific enzyme activity at the time when the assay was performed. However, no specific enzyme activity was reported in the Gorecki article. This substantiates the activity concerns above.

(4) Potential irreversible inhibitors can be identified by their *progressive* inhibition from reversible inhibitors that exhibit *constant* inhibition in a time-course experiment. This requires controls at proper concentrations to define a period of time over which the *progressive* inhibition can be observed. Using high inhibitor concentrations can make the *progressive* inhibition period too short to practically measure the enzyme activity over the period. In ref 2, 6 nM **PM20**, 300 nM **PMS20**, and 700 nM paraoxon showed 20−30% agAP-AChE inhibition after 3 min, and then **PM20** and paraoxon showed *progressive* agAP-AChE inhibition over the 3−15 min period, whereas **PMS20** showed *constant* 20% agAP-AChE inhibition over the same period, wherein **PMS20** (a maleimide-free analog of **PM20**) and paraoxon (known to conjugate with the catalytic Ser of cholinesterases) were controls to show respective *constant* and *progressive* inhibitions. In the Gorecki article, 800 nM **PM20** exhibited nearly *constant* agAP-AChE inhibition over the 3−15 min period in their experiment without using known inhibitors (e.g., **PMS20** and paraoxon) to show that their experimental conditions are suitable to observe *constant* and *progressive* inhibitions. The use of a high **PM20** concentration and the lack of controls raise concerns about their study design.

(5) The conclusion of the Gorecki article regarding inhibitor selectivity is IC_{50}-based and contradicts the textbook[4] on irreversible inhibitor evaluation using k_{inact}/K_I: "An important point to realize here is that attempts to quantify the relative potency of irreversible enzyme inactivators by more traditional parameters, such as IC_{50} values, are entirely inappropriate because these values will vary with time, in different ways for different compounds. Hence the SAR derived from IC_{50} values, determined at a fixed time point in the reaction progress curve, is meaningless and can be misleading in terms of compound optimization."

(6) Maleimides are nonselective alkylating agents that have never been used as insecticides. **PM20** was intended only for mass spectrometric evidence for Cys conjugation with agAP-AChE.[2] Equating maleimides with "Cys-targeted insecticides" in the Gorecki article is improper and misleading.

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