SYNTHESIS AND EVALUATION OF PYRROLIDINONE-BEARING BENZENESULFONAMIDES AS HUMAN CARBONIC ANHYDRASE INHIBITORS

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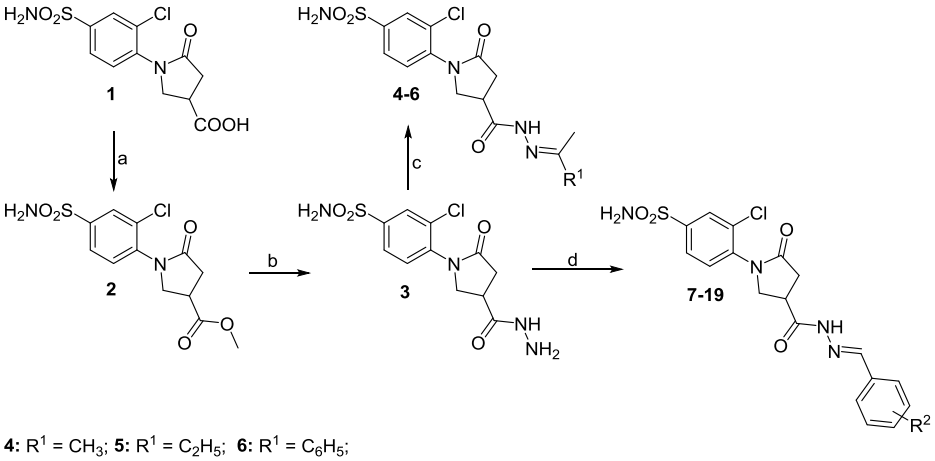
There are twelve alpha carbonic anhydrase (CA) isozymes in human that catalyze reversible hydration of CO_2 to protons and bicarbonate, a vital reaction for the respiration and transport of CO_2 between tissues, in pH regulation and homeostasis [1]. Increased expression levels of several CA isozymes are associated with numerous diseases. Currently CAs are established therapeutic targets of cancer (CA IX and CA XII), glaucoma (CA II, CA IV, CA XII), high altitude sickness (CA II) and obesity (CA VA and CA VB). Many recent studies have focused on designing and developing of inhibitors against CA IX that show potential for treating solid tumors [2,3].

In this study we synthesized pyrrolidinone-based chlorinated benzenesulfonamide derivatives (Fig. 1) and evaluated their binding toward all 12 human catalytically active CA isozymes. Ester **2** was synthesized by an esterification reaction of the acid **1** with an excess of methanol at reflux temperature in the presence of sulfuric acid as a catalyst. Reaction of ester **5** with hydrazine hydrate in propan-2-ol at reflux temperature gave hydrazide **3**.



4: $R^1 = CH_3$; **5:** $R^1 = C_2H_5$; **6:** $R^1 = C_6H_5$;
7: R^2 = H; **8:** R^2 = 2-OH; **9:** R^2 = 3-OH; **10:** R^2 = 4-OH; **11:** R^2 = 2-Cl; **12:** R^2 = 3-Cl; **13:** R^2 = 4-Cl; **14:** R^2 = 4-F; **15:** R^2 = 4-OCH_3; **16:** R^2 = 2-NO_2; **17:** R^2 = 3-NO_2; **18:** R^2 = 4-NO_2; **19:** R^2 = 4-COOH;
Reaction conditions: (a) MeOH, conc. H_2SO_4, reflux, 8 h; (b) $N_2H_4 \cdot H_2O$, propan-2-ol, reflux, 5 h;
(c) corresponding ketone, propan-2-ol, reflux, 2-4 h; (d) corresponding aldehyde, propan-2-ol, reflux, 1-2 h;

Fig. 1. Synthesis of pyrrolidinone-bearing benzenesulfonamides

Hydrazide **3** reactions with corresponding ketones in propan-2-ol at reflux temperature yielded hydrazones **4**–**6**, whereas the ones with corresponding benzaldehydes provided corresponding hydrazones **7**–**19**. The structures of all synthesized compounds have been confirmed by the data of IR, ^{1}H and ^{13}C NMR spectroscopy as well as mass spectrometry data.

The binding affinity of synthesized compounds was determined by the thermal shift assay and stop-flow CO_2 hydration assay. Most of the compounds showed nanomolar binding affinities towards CA II, CA IX and CA XIV. The compounds have a potential for further development of CA inhibitors with higher selectivity for a particular CA isozyme.

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