

## ABSTRACT

Hand, foot and mouth disease (HFMD), a highly contagious disease in children, is caused by human enteroviruses, including Enterovirus 71 (EV71), Coxsakievirus A16 (CVA16) and Coxsakievirus A6 (CVA6). Although HFMD is usually mild and self-limiting, EV71 infection occasionally leads to fatal neurological disorders. Currently, no commercial antiviral drugs for HFMD treatment are available. Here, numerous sulfonated azo dyes, widely used as food additives, were identified to have potent antiviral activities against human enteroviruses. Among them, brilliant black BN (E151) was able to inhibit all tested EV71, CVA16 and CVA6 strains. In rhabdomyosarcoma cells, 50% inhibitory concentration of the dye E151 for various strains of EV71 ranged from 2.39  $\mu$ M to 28.12  $\mu$ M whereas its 50% cytotoxic concentration was 1870  $\mu$ M. Food azo dyes, including E151, interacted with the vertex of the 5-fold axis of EV71 and prevented viral entry. Their efficacy on viral inhibition was regulated by amino acids at VP1-98, VP1-145 and/or VP1-246. Dye E151 not only prevented EV71 attachment but also displaced attached viruses in a concentration dependent manner. Moreover, E151 inhibited the interaction between EV71 and its cellular uncoating factor cyclophilin A. *In vivo* studies demonstrated that E151 at a dose of 200 mg/kg/day given at the initial four days of challenge protected AG129 mice challenged with 10 of 50% lethal dose of wild type EV71 isolates. Taken together, these data highlight E151 as a promising antiviral agent against EV71 infection.