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In vitro studies on the inhibition of colon cancer by amino acid derivatives of bromothiazole

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ABSTRACT

The investment in cancer research is critical to find more and better treatments, but essentially to save lives. Here, we describe the synthesis and characterization on new bromothiazole derivatives with amino acids and with core of nitazoxanide, an FDA-approved antiprotozoal drug. Using a human adenocarcinoma-derived cell line (the Caco-2 cell line), we then investigated the antiproliferative (^{3}H-thymidine incorporation) and cytotoxic (extracellular lactate dehydrogenase activity) effect of these derivatives. All the derivatives caused a concentration–dependent decrease in cell proliferation and viability. At their highest concentration, all compounds were able to reduce ^{3}H-thymidine incorporation by more than 80%, corresponding to a more marked antiproliferative effect than butyrate. As to their cytotoxic effect, it was comparable to that of butyrate. The ability of bromo substituent in thiazole ring with new sequences of amino acids in inducing cell death and apoptosis in Caco-2 cells (and other cell lines) is now being studied.

Colorectal cancer (CRC) results from an accumulation of genetic and epigenetic changes in colon epithelial cells, which transforms them into adenocarcinomas, and remains the second leading cause of cancer deaths in the United States, with an estimated 129,700 new cases expected for 2017.[1] New strategies for drug discovery are therefore urgently needed because this process has become costly and only a few highly effective drugs reach the market yearly.[2] Recently, nitazoxanide (**1**) combined with the cytotoxic drug irinotecan was identified as a potential candidate for treatment of CRC.[3] Nitazoxanide, an FDA-approved anthelmintic drug, is a pro-drug that is deacetylated in the gastrointestinal tract to its active metabolite tizoxanide (**2**, Scheme 1).[4] RM-5038, a new thiazolide derivative in which the nitro group on the 5-position of the thiazole ring of its chemical structure was replaced by chloride, is better absorbed from the gastro-intestinal tract than nitazoxanide and well tolerated in animal toxicology studies when administered to rats and dogs for 48 consecutive days.[5–7] Also, the bromo-thiazole RM4819 has been previously shown to induce apoptosis in different colorectal tumor cell lines, i.e., Caco-2, HT29 and LSi74T.[8,9] Furthermore, glutathione-S-transferase P1 was found to physically interact with RM4819 and to be required for RM4819-induced cell death.[8]

To further explore and optimize the structure-activity relationship containing thiazole moiety, we have designed and synthesisized new derivatives with amino acids (**3** and **4**, Scheme 2) and 2-amino-5-bromothiazole (**5**), as starting material.[10] Amino acids as promoieties perhaps offer the most structural diversity and expanse of physicochemical properties.[11,12] The α-amino acids used differed in the nature of the side-chain (R group) attached to their α-carbon, which can vary in size: from one hydrogen atom in Gly to a large heterocyclic group in Phe (**3.1**–**3.9**). Also, the α-amino acids used, except for Gly, have a chiral α-carbon and can exist in two optical isomers, L- and D-form. The L-form of amino acids occurs naturally and prodrugs utilizing these amino acids are generally activated by naturally occurring enzymes. The L- and D-amino acid prodrugs tend to have very similar physicochemical properties but the latter is generally more stable to hydrolysis by naturally occurring enzymes.[13,14] In addition to the natural amino acids and their D-forms (only compound **3.9** is D-Phe), dipeptide of **5** was also developed (**4**). Thiazole condensation was based on the *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) coupling reagent usually employed in peptide synthesis.[10] N^{α}-Boc-protected amino acids were coupled

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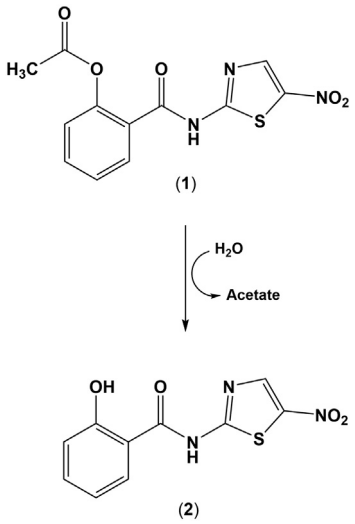
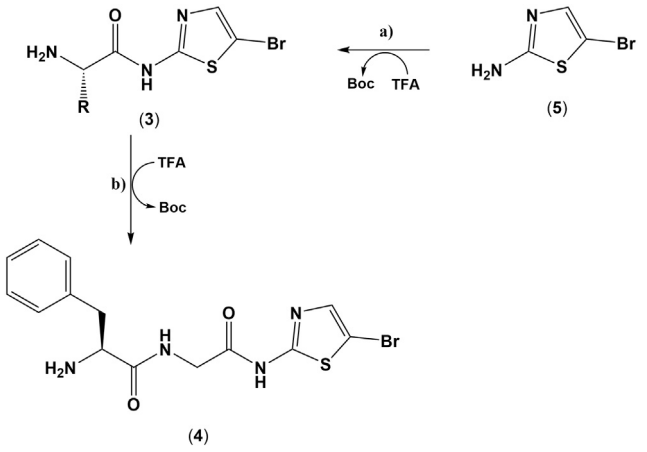
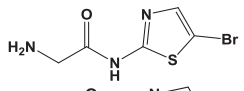
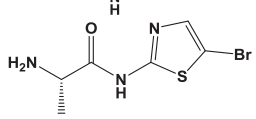
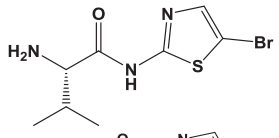
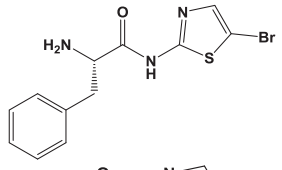
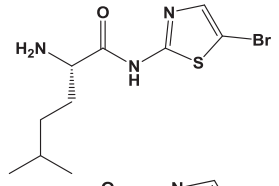
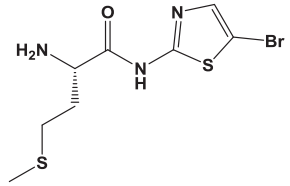
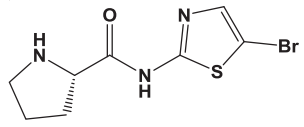


Scheme 1. Deacetylation of nitazoxanide (**1**) into tizoxanide (**2**), which is believed to be the active form.



Scheme 2. Synthesis of new derivatives of bromothiazole **5**. Reagents: (a) and (b) Boc-AA-OH, DIEA, TBTU; After purification, DCM/TFA 70%, Na_2CO_3 30%. Compound/R[1]: **3.1**/H, **3.2**/CH_3, **3.3**/$CH(CH_3)_2$, **3.4**/CH_2Ph, **3.5**/$CH_2CH(CH_3)_2$, **3.6**/$(CH_2)_2SCH_3$, **3.7**/Pro, **3.8**/$(CH_2)_4NH_2$, **3.9**/D-Phe, **4**/Phe-Gly.

Table 1
In-silico predicted physicochemical parameters[a] of the designed compounds (**3.1**–**3.9**, and **4**), nitazoxanide and effect of a 48 h-exposure to increasing concentrations of compounds **3.1**–**3.9**, **4** upon Caco-2 cell proliferation (A) and viability (B). Cellular proliferation was quantified by determination of [^{3}H]-thymidine incorporation, and cellular viability was determined by quantification of extracellular LDH activity, as described in Methods.[14] Butyrate (BT; 5 mM) was used as positive control. Results are presented as arithmetic means ± SEM (n = 6–16). *Significantly different from control ($p < 0.05$).

Compd	Structure	M_{wt}[b]	pKa[c] (NH_2)	Log P[d]	Log S[e]	[^{3}H]-thymidine incorporation	LDH
						IC_{50} (μM)	EC_{50} (μM)
3.1		236.09	6.749	0.29	−0.64	200.6	14.2
3.2		250.11	6.882	0.78	−1.05	338.3	48.0
3.3		278.17	6.906	1.67	−1.58	205.4	27.1
3.4		326.21	6.621	2.46	−2.69	441.8	52.1
3.5		306.22	6.916	2.43	−2.57	327.3	19.9
3.6		310.23	6.759	1.12	−2.03	172.9	2376
3.7		276.15	6.516	1.28	−1.56	220.3	321.9