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Synthesis of Some Potential Antiangiogenic 1,3-Dihydro-1,3-dioxo-2*H*-isoindole Derivatives

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Abstract: Based on the structure-activity relationships of RGD-containing peptides, a series of 1,3-dihydro-1,3-dioxo-2*H*-isoindole derivatives were synthesized. All of them were first reported. Their structures were confirmed by spectral data and elemental analysis. Their ability to inhibit angiogenesis were evaluated in the chick embryo chorioallantoic membrane assay at 10^{-5} mol/L. Compounds **5b** and **5e** displayed obviously antiangiogenic activity.

Keywords: RGD sequence, $\alpha_v\beta_3$ receptor, 1,3-dihydro-1,3-dioxo-2*H*-isoindole derivatives, synthesis, antiangiogenic activity.

The proliferation, invasion and metastasis of malignant tumors are closely related with angiogenesis induced by tumor cells. Blocking tumor-induced angiogenesis is an efficient strategy to prevent and cure cancer[1]. Recent studies indicated that the angiogenic process depends on vascular endothelial cell migration and invasion, which regulated by $\alpha_v\beta_3$ receptor[2]. The receptor and extracellular matrix can recognize each other through Arg-Gly-Asp (RGD) consensus sequence found in matrix ligands[3]. Importantly, express of $\alpha_v\beta_3$ receptor is minimal on resting or normal blood vessels, but is significantly up-regulated on vascular cells in response to growth factors *in vitro* and *in vivo* and within human tumor[2]. Brooks *et al.* showed that RGD-containing peptides antagonists of $\alpha_v\beta_3$ inhibited tumor-induced angiogenesis and tumor growth[4]. But the RGD-containing peptides have very short half-lives in circulation, thus decreasing their therapeutic and biological potentials *in vivo*. The investigations of cyclic RGD-containing peptides indicated that the guanidinium of arginine and the carboxylate of aspartate are necessary for RGD recognition by integrin. The turn type is not necessary for binding, but simply serves as scaffolding for positioning the essential functional groups in proper juxtaposition[5]. Guided by these cyclic peptide studies, we have reported that the RGD peptidomimetics containing a 1,3-dihydro-1,3-dioxo-2*H*-isoindole core have been shown to effectively inhibit angiogenesis in the chick embryo chorioallantoic membrane (CAM) assay[6,7]. In an extension of this work, the present

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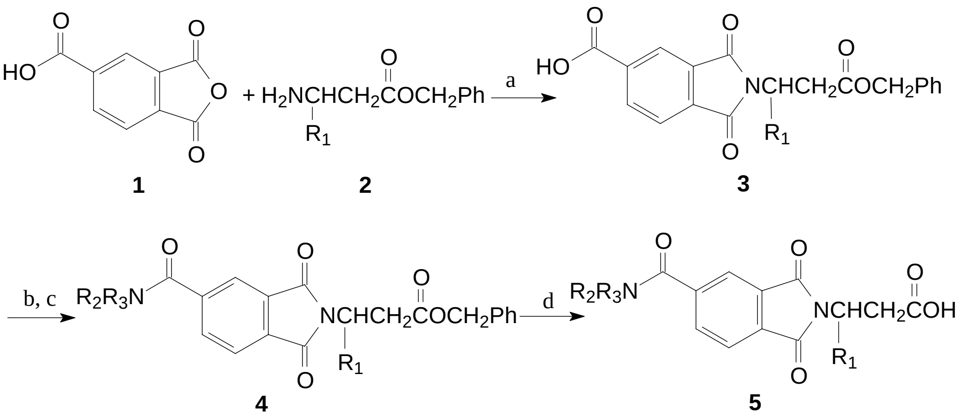
paper describes the synthesis and biological evaluation of a series of novel 1,3-dihydro-1,3-dioxo-2*H*-isoindole derivatives, which were considered as interesting leading compounds for exploring potential antioangiogenic agents.

Compounds prepared in this study and their synthetic routes are outlined in **Scheme 1**. The starting material were 1,3-dihydro-1,3-dioxo-5-isobenzofurancarboxylic acid **1** and (±)-3-amino-3-arylpropanoic acid benzyl ester **2**, which condensed in toluene in the presence of triethylamine gave (±)-5-carboxy-1,3-dihydro-1,3-dioxo-2*H*-isoindole-2-(3-arylpropanoic acid) benzyl ester **3**. The benzyl ester **3** was converted to an activated carboxylic acid intermediate use DCC/HOBT or $SOCl_2$, and the intermediate subsequently reacted with an appropriate amine to afford the corresponding amide **4**. In our hands, the DCC/HOBT method gave low yields and suffered from stringent reaction conditions and tedious work-up. Therefore we activated carboxylic acid with $SOCl_2$. Compound **4** on further treatment with 5% Pd/C in methanol gave the target compounds **5** in excellent yield. Compounds **4a-e** and **5a-e** were not previously reported in the literature. Their structures were confirmed by elemental analysis, IR, ^{1}HNMR spectra[8].

General procedures for preparation of compounds **4a-e**: A mixture of (±)-5-carboxy-1,3-dihydro-1,3-dioxo-2*H*-isoindole-2-(3-arylpropanoic acid) benzyl ester **3** (0.01 mol) and 2.92 mL thionyl chloride (0.04 mol) was refluxed for 3.5 hours. The excess thionyl chloride was evaporated at 40°C under reduced pressure to yield crude product as off-brown oil. It was dissolved in 50 mL dry dichloromethane and the solution dropwise added to a stirred solution of amine (0.015 mol), triethylamine(6.2 mL)

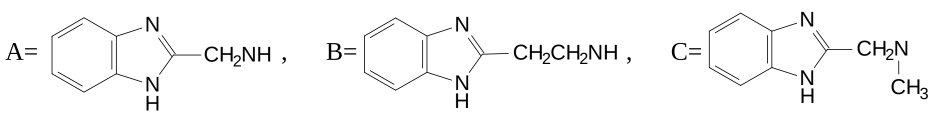
Scheme 1



Reagents and conditions: a) Et_3N, toluene, reflux, (85~90%); b) $SOCl_2$, reflux; c) R_2R_3NH, pyridine, Et_3N, 0~5 °C, (72~83%); d) 5%Pd/C, MeOH, r.t., (75~82%).

4a,**5a**: $R_1= C_6H_5$, $R_2R_3N=$ A **4b**,**5b**: $R_1= C_6H_5$, $R_2R_3N=$ B **4c**,**5c**: $R_1= C_6H_5$, $R_2R_3N=$ C

4d,**5d**: $R_1=$ 4-$CH_3OC_6H_5$, $R_2R_3N=$ A **4e**,**5e**: $R_1=$4-$CH_3OC_6H_5$, $R_2R_3N=$ B



and pyridine (10 mL) in 70 mL dry dichloromethane at 0~5°C over a period of 60 min. The reaction mixture was allowed to warm up to room temperature and stand for 12 hours. It then was washed with 10% Na_2CO_3, brine, dried, filtrated and concentrated. The residue crude product was recrystallized from acetone/ether (1:1) to afford **4a-e** as white powder.

The preparation of compounds **5a-e:** A mixture of substituted arylpropanoic acid benzyl ester **4**(0.01 mol) and 5% Pd/C(0.8 g) in methanol(80 mL)was treated with H_2 at atmospheric pressure for 6 hours. The mixture was filtrated through celite, and the excess of solvent was then removed under reduced pressure. The solid obtained was recrystallized from ethanol to yield **5a-e** as white powder.

All target compounds were evaluated *in vitro* for their ability to inhibit angiogenesis by use the chick embryo chorioallantoic membrane (CAM) assay described by Brooks *et al.*[4]. Preliminary bioassay indicated that compounds **5b** and **5e** were found siginificant angiogenesis inhibiting in CAM induced by basic fibroblast growth factor and the angiogenesis inhibition index was 68±8.2% and 35±5.7%, respectively at the concentration of 10^{-5} mol/L. Rest of the target compounds were inactive against angiogenesis in CAM.

References and Notes

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8. Elemental analysis, data of C, H, N were all within ±0.3% of the corresponding theoretical values. ^{1}HNMR(400MHz, DMSO-d_6 δ_{ppm}) and IR(KBr) data of the compounds **4a~e** and **5a~e**:
   4a: 12.35(brs,1H, imidazole-NH), 9.59(t,1H,J=5.30Hz,$NHCH_2$), 8.38(s,1H,Ar-H_4), 8.34(d,1H, J=8.2Hz,Ar-H_6), 7.98(d,1H,J=8.2Hz,Ar-H_7), 7.46(m,2H, benzimidazole-$H_{4,7}$), 7.35(m,5H,Ph), 7.22(s,5H,CH_2Ph), 7.15(m,2H, benzimidazole-$H_{5,6}$) 5.75(dd,1H, J_1=6.6Hz,J_2=9.6Hz, NCH), 5.05(s,2H,CH_2Ph), 4.74(d,2H,J=5.30Hz,CH_2NH), 3.68(dd,1H,J_1=9.6Hz,J_2=16.4Hz,CH_2COO), 3.45(dd,1H,J_1=6.6Hz,J_2=16.4Hz,CH_2COO); IR (KBr): 3371, 1774, 1715, 1648, 1544, 1429, 1360, 1270, 1171, 735, 699 cm^{-1}.
   4b: 12.35(s,1H, imidazole-NH), 9.07(t,1H,J=5.1Hz,$NHCH_2$), 8.25(m,H,Ar-H_6,Ar-H_4), 7.92(d, 1H,J=7.6Hz,Ar-H_7), 7.42(m,2H,benzimidazole-$H_{4,7}$), 7.36(m,5H,Ph), 7.19(s,5H,CH_2Ph), 7.14 (m, 2H,benzimidazole-$H_{5,6}$), 5.74(dd,1H,J_1=6.4Hz,J_2=9.4Hz,CH), 5.03(s,2H,CH_2Ph), 3.75 (m,2H, $NHCH_2$), 3.65(dd,1H,J_1=9.4Hz,J_2=16.4Hz,CH_2COO), 3.46(dd,1H,J_1=6.4Hz,J_2= 16.4Hz, CH_2-COO), 3.12(t,2H,J=6.70Hz,CH_2); IR (KBr): 3414, 3304, 1773, 1713, 1641, 1542, 1428, 1360, 1271, 1172, 732, 699 cm^{-1}.
   4c: 12.44(brs,1H, imidazole-NH), 7.98(d,1H,J=7.6Hz, Ar-H_6), 7.94(s,1H,Ar-H_4), 7.86(d,1H, J=7.6Hz,Ar-H_7), 7.54(m,2H,benzimidazole-$H_{4,7}$), 7.33(m,5H,Ph), 7.21(s,5H,CH_2Ph), 7.17 (m,2H, benzimidazole-$H_{5,6}$), 5.72(dd,1H,J_1= 6.4Hz,J_2=9.8Hz,NCH), 5.03(d,2H,J= 8.4Hz, CH_2Ph), 4.92 (s,1H,CH_2NCH_3), 4.63(s,1H,CH_2NCH_3), 3.64(dd,1H,J_1=9.8Hz,J_2= 16.4Hz, CH_2COO), 3.39 (dd,1H,J_1=6.4Hz,J_2=16.4Hz,CH_2COO), 3.02(s,3/2H,CH_2NCH_3), 2.96 (s,3/2H, CH_2NCH_3); IR (KBr): 3368, 1774, 1713, 1636, 1497, 1358, 1270, 1170, 743, 698 cm^{-1}.
   4d: 12.35(brs,1H, imidazole-NH), 9.59(t,1H,J=5.20Hz,$NHCH_2$), 8.37(s,1H,Ar-H_4), 8.34(d,1H, J=8.0Hz,Ar-H_6), 7.96(d,1H,J=8.0Hz,Ar-H_7), 7.49(m,2H,benzimidazole-$H_{4,7}$), 7.36(d,2H,J=8.42

Hz, $PhOCH_3$), 7.21(s, 5H, CH_2Ph), 7.16(m, 2H, benzimidazole-$H_{5,6}$), 6.89(d, 2H, J=8.42Hz, $PhOCH_3$), 5.69(dd,1H, J_1=7.0Hz,J_2=9.2Hz,NCH), 5.04(s,2H, CH_2Ph), 4.74(d,2H, J=5.20Hz, $NHCH_2$), 3.72(s,3H, OCH_3), 3.68(dd,1H, J_1=9.2Hz,J_2=16.6Hz,CH_2COO), 3.42(dd,1H, J_1=7.0Hz, J_2=16.6Hz,CH_2COO); IR (KBr): 3329, 1774, 1713, 1645, 1547, 1357, 1250, 1174, 743 cm^{-1}.

4e: 12.31(brs,1H, imidazole-NH), 9.07(t,1H, J=4.90Hz,$NHCH_2$), 8.27(d,1H, J=7.60Hz,Ar-H_6), 8.23(s,1H, Ar-H_4), 7.91(d,1H, J=7.60Hz,Ar-H_7), 7.48(m,2H, benzimidazole-$H_{4,7}$), 7.34(d,2H, J=8.5Hz,$PhOCH_3$), 7.19(s,5H, CH_2Ph), 7.10(m,2H, benzimidazole-$H_{5,6}$), 6.88(d,2H, J=8.50Hz, $PhOCH_3$), 5.67(dd,1H, J_1=6.7Hz,J_2=9.3Hz,NCH), 5.03(s,2H, CH_2Ph), 3.77(m,2H, $NHCH_2$), 3.71(s,3H, OCH_3), 3.63(dd,1H, J_1=9.3Hz,J_2=16.3Hz,CH_2COO), 3.42(dd,1H, J_1=6.7Hz, J_2=16.3Hz,CH_2COO), 3.12(t,2H, J=6.90Hz,CH_2CH_2NH); IR (KBr): 3319, 1772, 1712, 1639, 1547, 1514, 1359, 1270, 1175, 740, 698 cm^{-1}.

5a: 12.31(brs,2H, COOH, imidazole-NH), 9.59(t,1H, J=5.20Hz,CH_2NH), 8.41(s,1H, Ar-H_4), 8.36(d,1H, J=7.50Hz,Ar-H_6), 8.00(d,1H,J=7.50Hz,Ar-H_7), 7.45(m,2H,benzimidazole-$H_{4,7}$), 7.35 (m,5H, Ph), 7.16(m,2H, benzimidazole-$H_{5,6}$), 5.71(t,1H, J=7.40Hz,NCH), 4.73(d,2H, J=5.20Hz, CH_2NH), 3.48(m,2H, CH_2COOH); IR (KBr): 3430, 1769, 1709, 1644, 1543, 1456, 1377, 1337, 737, 700, 632 cm^{-1}.

5b: 12.36(brs,1H, imidazole-NH), 9.09(t,1H, J=5.1Hz,$NHCH_2$), 8.28(s,1H, Ar-H_4), 8.26(d,1H, J=8.16Hz,Ar-H_6), 7.95(d,1H, J=8.16Hz,Ar-H_7), 7.46(m,2H, benzimidazole-$H_{4,7}$), 7.37 (m,5H,Ph), 7.12(m,2H, benzimidazole-$H_{5,6}$), 5.70(t,1H, J=7.18Hz,CH), 3.73(q,2H, $NHCH_2$), 3.42(m,2H, CH_2COOH), 3.11(t,2H,J=7.0Hz,CH_2); IR (KBr): 3426, 1773, 1711, 1646, 1545, 1362, 1219, 731, 700, 613 cm^{-1}.

5c: 12.42(brs,2H, COOH, imidazole-NH), 8.01(s,1H, Ar-H_4), 7.98(d,1H,J=7.60Hz, Ar-H_6), 7.92(d,1H, J=7.6Hz, Ar-H_7), 7.55(m,2H, benzimidazole-$H_{4,7}$), 7.36(m,5H, Ph), 7.19(m,2H, benzimidazole-$H_{5,6}$), 5.70(t,1H, J=7.90Hz,NCH), 4.93(s,1H, CH_2NCH_3), 4.63(s,1H, CH_2NCH_3), 3.43(m,2H, CH_2COOH), 3.02(s,3/2H, CH_2NCH_3), 2.97(s,3/2H, CH_2NCH_3); IR (KBr): 3439, 1773, 1713, 1632, 1500, 1360, 1273, 1184, 744, 700, 621 cm^{-1}.

5d: 12.32(brs, 2H, COOH, imidazole-NH), 9.59(t,1H, J=5.20Hz, CH_2NH), 8,40(s,1H, Ar-H_4), 8.35(d,1H, J=7.80Hz, Ar-H_6), 7.99(d,1H, J=7.80Hz, Ar-H_7), 7.48(m,2H, benzimidazole-$H_{4,7}$), 7.36(d,2H, J=8.40Hz, $PhOCH_3$), 7.14(m,2H, benzimidazole-$H_{5,6}$), 6.90(d,2H, J=8.40Hz, PhO-CH_3), 5.65(t,1H,J=7.32Hz,NCH), 4.73(d,2H,J=5.20Hz,CH_2NH), 3.72(s,3H,OCH_3), 3.42(m, 2H, CH_2COOH); IR (KBr): 3428, 1770, 1709, 1646, 1545, 1515, 1380, 1257, 1180, 742, 627 cm^{-1}.

5e: 12.38(brs,2H, COOH, imidazole-NH), 9.08(t,1H, J=5.0Hz, $NHCH_2$), 8.28(s,1H, Ar-H_4), 8.26(d,1H, J=8.0Hz, Ar-H_6), 7.94(d,1H, J=8.0Hz, Ar-H_7), 7.47(m,2H, benzimidazole-$H_{4,7}$), 7.34 (d,2H, J=8.4Hz, $PhOCH_3$), 7.11(m,2H, benzimidazole-$H_{5,6}$), 6.88(d,2H, J=8.40Hz, $PhOCH_3$), 5.63(t,1H, J=7.40Hz, NCH_2), 3.76(m,2H, CH_2NH), 3.71(s,3H, OCH_3), 3.33(m,2H, CH_2COOH), 3.11(t,2H, J=7.0Hz,CH_2CH_2NH); IR (KBr): 3426, 1772, 1710, 1646, 1546, 1514, 1363, 1253, 1181, 733, 617 cm^{-1}.

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