Microwave assisted synthesis and biological activities of 9-boronobenzyladenine derivatives

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Microwave enhanced syntheses of 9-boronobenzyladenine derivatives by the reaction of adenine with the corresponding bromomethylphenylboric acid were reported. Microwave irradiation reduced the overnight reaction time of conventional thermal methods to 10 min, provided the desired products and increased the yields up to three times. Preliminary in vitro pharmacological tests were also described.

Keywords: 9-boronobenzyladenines, anti-inflammatory, antitumor, microwave-asisted synthesis

Compounds containing boronic aãd group are used in a broad range of mediãnal applications. Boronated tetrapeptides were inhibitory to HIV-1 protease.[1] Bifunctional aryl boronic aãd compounds were particularly effective at inhibiting the SARS coronavirus main protease 3CL~(pro).[2] Bortezomib, a boronic aãd dipeptide, is the first proteasome inhibitor to reach clinical trials as anticancer drug.[3] *p*-Boronophenylalanine and many other compounds containing boronic aãd moiety were developed as antitumor agents for Boron Neutron Capture Therapy.[4-6] Boronic aãds can form reversible ester bonds with molecules containing *ãs*-diols in a favourable conformation.[7] This property is used to target drug to the cell surface and increase its efficacy by modifying drug molecule with boronic aãd groups.[8,9] As boronic aãd compounds have been shown to have many biological activities, research into the chemistry of these compounds has increased rapidly.

9-Substituted adenine derivatives are putative ligands that may compete with adenosine or adenosine-deriving endogenous substance at their speãfic receptors. These characteristics make them potentially useful as pharmacologically active compounds.[10] 9-Benzyladenine (9-BA) derivatives were reported to be selective phosphodiesterase 4 (PDE-4) inhibitors and possess anti-inflammatory, anti-cocãdial and anti-anginal activities.[11-14] For searching new pharmacologically active compounds, we modified 9-BA molecule by incorporating boronic aãd group into *o*-, *m*-, or *p*-position on its phenyl ring. Herein we report the microwave assisted effiãent syntheses of three new 9-boronobenzyladenine derivatives and preliminary *in vitro* test of their anti-inflammatory and antitumor activities.

A classical route to prepare the 9-substituted adenines deals with direct alkylation of the ring of the corresponding chloropurines with the appropriate alkyl halides and sodium hydride in dimethylformamide (DMF). Under these conditions, alkylation occurred mainly at position N9 (70–85%), and the undesired N7 isomer (15–30%) could be easily removed by silica gel column chromatography. Subsequent aminolysis of 9-substituted chloropurines with various amines provided the desired adenines.[15,16] When adenine was benzylated directly with benzyl chloride in *N*, *N*-dimethylacetamide in the presence of K_2CO_3, the only product isolated was 9-benzyladenine. The yield, however, is only 27%.[17] More recently, a N9 regioselective alkylation was reported by performing the reaction in DMF using tetrabutyl ammonium iodide (TBAI) as phase-transfer catalyst.[18] Though traces of 7-substituted byproducts were also observed and the separation of these polar adenine derivatives led to poor overall yields, this synthetic method appeared to be particularly powerful for an expeditious preparation of 9-substituted derivatives in one-step procedure.[19] We tried this protocol to synthesise 9-boronobenzyladenines through the reaction of adenine and the corresponding bromomethylphenylboric aãd in DMF. Thin layer chromatography (TLC) analysis showed no reaction occurring after the mixture was stirred at room temperature for 72 h both in the presence and absence of TBAI. Performing the reaction at elevated temperature (120°C) gave 9-boronobenzyladenines in low yields. 9-BA from an undesired elimination reaction, however, was identified to be the main product (Scheme 1). The addition of TBAI accelerated the reaction, but the selectivity to 9-boronobenzyladenines remained unimproved. Microwave irradiation (MWI) as a non-conventional energy source can dramatically accelerate rates of several different organic reactions and has become a very popular and useful technology in organic syntheses,[20] but only a few examples of microwave assisted synthesis of nucleobase derivatives have been reported.[21] Here we report the microwave enhanced synthesis of 9-boronobenzyladenine derivatives. This reduced the overnight reaction time of conventional thermal methods to 10 minutes, diminished the undesired elimination reaction and increased the yields of the desired products up to three times (Table 1).

All three 9-boronobenzyladenine derivatives were tested for their anti-inflammatory activity and antitumor activity against human tumor cell lines *in vitro*. Data are summarised in Tables 2 and 3.

Table 2 showed that all the three new compounds displayed anti-inflammatory activity. Compounds **2b** and **2c** exhibited higher anti-inflammatory activity than their parent compound

Table 1 Result of the syntheses of 9-boronobenzyladenines

Benzylating agents	Conventional heating			Microwave irradiation		
	Time/h	Yield/% of **2**[a]	Yield/% of **9-BA**[a]	Time/min	Yield/% of **2**[a]	Yield/% of **9-BA**[b]
1a	14	13.5	23.2	10	38.6	0
1b	14	14.7	26.2	10	42.2	0
1c	14	18.6	27.1	10	45.9	0

[a]Purication by TLC; [b]not observed on TLC.

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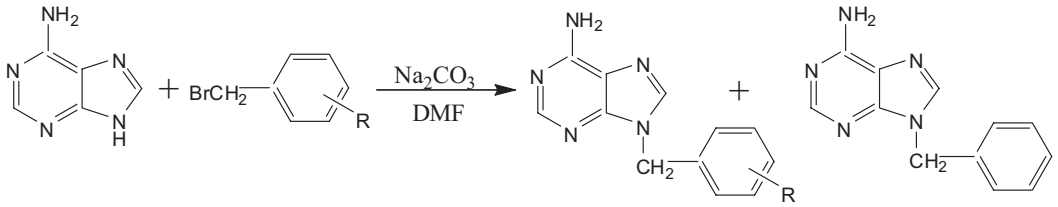

1a-c **2a-c** **9-BA**

a R= o-$B(OH)_2$ b R= m-$B(OH)_2$ c R= p-$B(OH)_2$

Scheme 1

Table 2 Effects of compounds on TNF production from mouse peritoneal macrophages at the concentration of 1×10^{-5} mol/l

Group	Absorbance value	Inhibition/%
Control	0.669±0.041	
LPS (2.5 mg/ml)	0.181±0.016	
9-BA	0.324±0.040**	29.3
2a	0.197±0.003	3.2
2b	0.360±0.005***	36.6
2c	0.333±0.017***	31.0

Mean ± s $n = 3$ *$P < 0.05$ **$P < 0.01$ ***$P < 0.001$ vs LPS.

9-BA, and were worthy of further study. The data in Table 3, however, showed all three compounds have poor activities against human tumor cell lines *in vitro*. Their properties as antitumor agents for Boron Neutron Capture Therapy are being in process.

Experimental

Microwave assisted syntheses were performed in a commerãal microwave reactor (XA-100, Beijing Xianghu Sãence and Technology Development Co. Ltd, Beijing, P. R. China) at 500 W. The temperature of the reaction mixture was measured by an immersed platinum resistance thermometer. All starting boronic aãd and other reagents employed are commerãal available and were used without further purification. TLC analyses and separations were performed on silica gel GF254 plates using CH_2Cl_2 – MeOH (8: 1, v/v) as eluent, and the plates were visualized with UV light. Melting points uncorrected were measured on a XT-5 apparatus. ^{1}H NMR spectra were determined on a Bruker-300 MHz spectrometer with TMS as internal standard. IR spectra were recorded on a Bruker IFS25 Infrared Spectrometer. Element analyses were performed on a Carlo Erba 1106 Element Analyser. The in vitro anti-inflammatory activity of the compounds was evaluated by testing their inhibitory effect on TNFα production from cultured mouse peritoneal macrophages.[22] Suppression ratio of the compounds to cell proliferation of human promyelocytic leukaemia HL-60 was determined with MTT assay.[23] Inhibitory effect of the compounds to cell proliferation of human gastric carãnoma BGC-823, human hepatocarãnoma Bel-7402 and human nasopharyngeal carãnoma KB was measured by SRB staining.[24]

Conventional thermal synthetic procedure

A mixture of 2 mmol adenine, 2.4 mmol appropriate boronomethylphenylboric aãd, 300 mg anhydrous K_2CO_3, and 34 mg TBAI in 10 ml dry DMF was stirred and heated at 120°C overnight, The mixture was filtered while it was still hot, and the cake was washed with DMF. DMF was removed from the filtrate under reduced pressure. The residue was dissolved in a minor amount of methanol and purified on TLC to yield the product.

Microwave assisted synthetic procedure

A dried round-bottom flask (25 ml) equipped with a condenser was charged with a solution of 2 mmol adenine, 2.4 mmol appropriate boronomethylphenylboric aãd and 300 mg anhydrous K_2CO_3 in 10 ml dry DMF. The solution was stirred and irradiated under microwave at 120°C for 10 min. The reaction mixture was worked up similarly as that in the conventional procedure to give the product.

Table 3 *In vitro* antitumor activities to human tumor cell lines of the compounds at the concentration of 10 μmol/l

Compound	Suppression ratio			
	HL-60	BGC-823	Bel-7402	KB
2a	9.25	–4.62	–24.12	–24.39
2b	19.96	7.81	–10.39	–20.23
2c	17.42	15.00	–5.93	–29.19

9-o-Boronobenzyladenine(**2a**): M.p. 279–280°C; ^{1}H NMR (300 MHZ, DMSO-d_6): δ5.51 (s, 2H, N^9–CH_2), 7.35–7.44 (m, 4H, Ar–H), 7.75 (s, 1H, purin-8-H), 7.90 (s, 2H, D_2O exchangeable, $B(OH)_2$), 8.55 (s, 1H, purin-2-H); IR: υ_{max}/cm^{-1} (KBr) 3324, 3104 and 1659 (NH_2), 1619, 1568, 1512 and 1495 (purine and benzene), 1428 (Ar–B), 1373 (B–O), 1330 (C_{Ar}–N), 1270 (CH_2–N); Anal. Calcd for $C_{12}H_{12}BN_5O_2$: C, 53.56; H, 4.50; N, 26.03. Found: C, 53.37; H, 4.67; N, 25.96.

9-m-Boronobenzyladenine(**2b**): M.p. 240–242°C; ^{1}H NMR (300 MHZ, DMSO-d_6): δ 5.28 (s, 2H, N^9–CH_2), 6.63–6.73 (m, 3H, Ar–H), 7.11 (t, 1H, Ar–H), 7.24 (s, 2H, D_2O exchangeable, NH_2), 8.15 (s, 1H, purin-8-H), 8.23 (s, 1H, purin-2-H); IR: υ_{max}/cm^{-1} (KBr) 3270, 3104, 1675 (NH_2), 1603, 1570 and 1488 (purine and benzene), 1423 (Ar-B), 1366 (B-O), 1338 (C_{Ar}–NH_2), 1250 (CH_2-N); Anal. Calcd for $C_{12}H_{12}BN_5O_2$: C, 53.56; H, 4.50; N, 26.03. Found: C, 53.48; H, 4.47; N, 26.08.

9-p-Boronobenzyladenine(**2c**): M.p. 256–258°C; ^{1}H NMR (300 MHZ, DMSO-d_6): δ 5.36 (s, 2H, N^9–CH_2), 7.25 (d, 2H, Ar–H), 7.24 (s, 2H, D_2O exchangeable, NH_2), 7.73 (d, 2H, Ar–H), 8.07(s, 2H, D_2O exchangeable, $B(OH)_2$), 8.14 (s, 1H, purin-8-H), 8.27 (s, 1H, purin-2-H); IR: υ_{max}/cm^{-1} (KBr) 3472, 3352, 1638 (NH_2), 1610, 1579, 1511 and 1485 (purine and benzene), 1421 (Ar–B), 1351 (B–O), 1325 (C_{Ar}–N), 1250 (CH_2–N); Anal. Calcd for $C_{12}H_{12}BN_5O_2$: C, 53.56; H, 4.50; N, 26.03. Found: C, 53.21; H, 4.39; N, 26.24.

9-Benzyladenine(9-BA): M.p. 230–232°C (lit.,[17] 230°C); ^{1}H NMR (300MHZ, DMSO-d_6): δ5.37 (s, 2H, N^9–CH_2), 7.27 (s, 2H, D_2O exchangeable, NH_2), 7.31(m, 5H, Ar–H), 8.14(s, 1H, purin-8-H), 8.27(s, 1H, purin-2-H), IR, υ_{max}/cm^{-1} (KBr) 3432, 3299, 1646 (NH_2), 1597, 1572 and 1485(purine and benzene), 1325 (C_{Ar}–N), 1246 (CH_2–N).

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