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The family of N^9-adenine: New entry for adenine–benzamide conjugates linked via versatile spacers

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Abstract. We have prepared 4-nitrobenzamide-adenine conjugates (**8**, **13** and **14**) linked with versatile spacer such as triethylene glycol (TEG), aminocaproic acid and ethyl chains which were eventually reduced to obtain the corresponding 4-aminobenzamide-adenine conjugates (**1**–**3**) in good yields. These conjugates bear a nucleobase for DNA recognition or self-assembly through base-pair complementarity, a biocompatible linker for interfacing with biological system, and a *p*-aminobenzamide moiety for pharmacological applications. The use of hydrophilic or lipophilic linkers may tune the dispersibility of these conjugates in different solvents, as well as impart different properties. In the preliminary experiments the versatility and application of these linkers has been tested for functionalization of SWCNTs.

Keywords. Nucleobase; substituted benzamide; adenine; triethyleneglycol; aminocaproic acid.

1. Introduction

Nucleobases (i.e., adenine, guanine, cytosine, thymine and uracil) are key constituents of nucleic acids (i.e., DNA and RNA). Nucleic acids can exist in different structural forms and this diversity leads to different biological functions.[1–5] In addition to Watson–Crick base-pairing interactions, other interactions such as reverse Watson–Crick, Hoogesteen interactions, and both π-stacking and hydrophobic interactions alternatively help to stabilize different structures based on nucleic acid self-assembly.[6–9] Among the different nucleobases, adenine ring is the most ubiquitous nitrogen-containing heterocycle in nature. It contains an exocyclic amino group and four imino nitrogens and thus offers five potential sites for interaction: the N^6 amino group and N^1, N^3, N^7, and N^9 imino nitrogens.[10] By the judicious choice of the nature of the substituents that can be attached on the N^1, N^3, N^6, N^7 and N^9 centres, the biological function displayed by the adenine could be tuned.[11,12] This heterocyclic adenine also has an ability to coordinate a variety of metal ions, that could be used for the stabilization of superstructures as well as to support metal-aided catalytic transformations.[13–17] Moreover, the N^7 imino nitrogen is most preferred site for metal ion binding because it remains exposed in the major groove of the DNA duplex. Based on the capacity of nucleobases to generate well-defined 3D-structures, the concept of base pairing has shifted in the last decade from the biological realm to the field of supramolecular chemistry.[13,15]

Benzamides are used clinically as analgesics, gastrointestinal motility stimulants and to treat psychiatric conditions. It was also noticed that substituted benzamide, exhibits excellent fungicidal activities. The benzamide derivatives may also be useful for inhibiting hepatic gluconeogenesis, and may also be effective to relieve the effects of endogenous glucocorticoids in diabetes mellitus, obesity, neuronal loss and/or the cognitive impairment of old age. The benzamide derivatives are also useful for modulation of numerous metabolic functions.[18–21]

We have used a range of hydrophobic and hydrophilic spacers,[22] like alkyl chains of various lengths (ethyl chain and aminocaproic acid) and triethylene glycol (TEG) to connect the adenine and benzamide groups. Aminocaproic acid is a derivative and analogue of the amino acid lysine, works as an antifibrinolytic or anti-proteolytic. Aminocaproic acid is used to treat excessive postoperative bleeding. A meta-analysis found that aminocaproic acid significantly reduced blood loss in patients undergoing coronary

artery bypass grafting.[23–25] Triethylene glycol (TEG) is a colourless, water soluble liquid. Triethylene glycol may be used directly as a plasticizer, for dehydration of natural gas and could be used for further transformation.[26,27]

Here, we report the synthesis of novel adenine-benzamide conjugates linked by means of a range of hydrophobic and hydrophilic spacers, such as triethylene glycol (TEG) and alkyl chains of various lengths (ethyl chain and aminocaproic acid). First, we synthesized adenine derivatives which are modified with reactive functional group at N^9 position and then we synthesized substituted benzamide derivatives which are functionalized with versatile spacer. Finally, the two components were successfully coupled via standard peptide coupling procedure (figure 1). The nucleobase-benzamide conjugates were characterized by different spectroscopic techniques. These derivatives comprising both nucleobase and benzamide would be extremely interesting in view of the remarkable properties of benzamide and recognition properties of nucleobases and could find potential applications as sensors, in medicinal chemistry, azide/amide chemistry and in dying industry. Preliminary results show the pristine SWCNTs on functionalization with TEG linker leads to exfoliation of the nanotubes with high degree of functionalization (1 functional group for every 111 C atoms).

2. Experimental

Reagents and solvents were purchased from Fluka, Acros, Aldrich, and used without further purification unless otherwise stated. Moisture-sensitive reactions were performed under Ar atmosphere. CH_2Cl_2 was freshly distilled from CaH_2, THF from Na/benzophenone, and DMF dried over 4 Å molecular sieves. Chromatographic purification was done with silica gel (from Merck, Kiesegel 60, 40–60 μm, 230–400 mesh ASTM) in standard column. TLC was performed on aluminium sheets coated with silica gel 60 F254 (Merck, Darmstadt). ^1H and ^{13}C NMR spectra were recorded on Bruker DPX 300 (operating at 300 and 75 MHz, respectively). The peak values were obtained as ppm (δ), and referenced to the solvent. Abbreviations used for splitting patterns are s = singlet, bs = broad singlet, t = triplet, q = quartet, m = multiplet, dd = double doublet. The thermogravimetric analyses were performed using a TGA Q500 TA instrument with a ramp of 10°C/min under N_2 from 100 to 900°C. Transmission electron microscopy was performed on a Hitachi H600 microscope and a Philips 208 working at different accelerating voltage and at different magnification. The samples were dispersed (5 μg/ 5 ml) in methanol:water (1:1) by ultrasonication for 5 min and kept for 10–12 h. The solution was again ultrasonicated for 5 min before depositing and 10 μL was deposited onto a holey-carbon TEM grid and dried. The images are typical and representative of the samples under observation. Adenine-benzamide derivatives consisting linker, reactive functionality and adenine nucleobase **1**, **2** and **3** were synthesized as described below. Adenine derivatives **7** and **12** were synthesized according to slightly modified literature procedures and characterized spectroscopically.[28,29] Boc-NH$(CH_2CH_2O)_2$-$CH_2CH_2NH_2$ **4** was synthesized according to the literature procedure.[30]

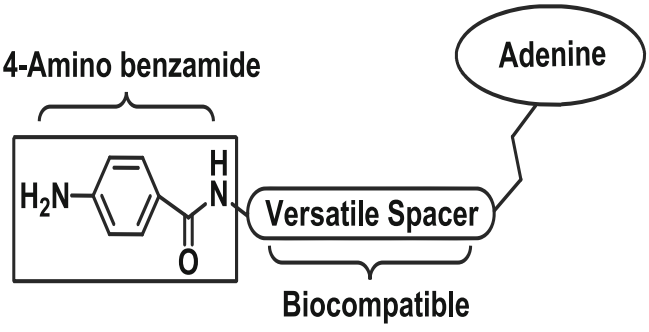


Figure 1. Proposed design for nucleobase-benzamide conjugates linked via versatile spacer.

2.1 *General procedure for synthesis of nucleobase-benzamide conjugate **1***

2.1a *Synthesis of tert-butyl 2-(2-(2-(4-nitrobenzamido) ethoxy)ethoxy)ethyl-carbamate (**5**)*: 4-Nitro benzoic acid (1.0 g, 6.0 mmol) was dissolved in thionyl chloride ($SOCl_2$) (0.85 g, 0.52 mL, 7.0 mmol) and refluxed for 5 h. Few drops of dry dimethylformamide (DMF) were also added to catalyse the reaction. The excess of $SOCl_2$ was evaporated under vacuum to obtain quantitative yield of 4-nitrobenzoyl chloride. The 4-nitrobenzoyl chloride was co-evaporated with diethyl ether to remove the traces of $SOCl_2$. Then, 4-nitrobenzoyl chloride (1.0 g, 5.4 mmol) was dissolved in dry toluene (50 mL) and amine **4** (1.47 g, 5.9 mmol), dissolved in dry toluene (10 mL), was immediately added to this solution under Ar atmosphere followed by addition of triethylamine (1.81 mL, 13.0 mmol). The reaction mixture was stirred for 12 h at 65°C under Ar. After completion of the reaction (via TLC), the mixture was cooled down to room temperature and diethyl ether was added. The solid precipitate was filtered off, washed with diethyl ether and dried under vacuum.

The residue was purified by column chromatography using 5–15% methanol in dichloromethane to isolate pure **5** as a white solid (1.69 g, 78.9% yield). R_f = 0.6 (dichloromethane:methanol, 90:10). ^{1}H NMR (300 MHz) ($CDCl_3$): δ 1.42 (s, 9H, 3xCH_3 of Boc), 3.30–3.31 (m, 2H, CH_2); 3.56–3.70 (m, 10 H, 5 x CH_2); 4.92 (bs, 1H, NHCO carbamate); 6.95 (bs, 1H, NHCO), 8.09 (dd, $J_1 = 9.0$ Hz, $J_2 = 2.5$ Hz, 2H, ArH); 8.30 (dd, $J_1 = 8.8$ Hz, $J_2 = 2.4$ Hz, 2H, ArH) ppm. ^{13}C NMR (75 MHz) ($CDCl_3$): δ 28.26, 39.96, 40.16, 69.39, 70.04, 70.20, 70.62, 79.31, 123.56, 128.20, 140.025, 149.35, 155.88, 165.39 ppm. LC-MS *m/z* 420 $[M+Na]^+$.

2.1b *Synthesis of N-(2-(2-(2-aminoethoxy)ethoxy) ethyl)-4-nitrobenzamide.TFA (**6**)*: The compound **5** (0.5 g, 1.8 mmol) was dissolved in 1:1 solution (4 mL) of trifluoroacetic acid (TFA) and dichloromethane and stirred for 2 h at room temperature under Ar atmosphere. After the completion of the reaction (TLC), the solvent was evaporated under vacuum and the resulting solid was triturated with diethyl ether and washed successively with more diethyl ether and re-precipitated by dissolving in methanol and diethyl ether and resulting solid was further dried under vacuum to get **6** as white solid, quantitative yield. ^{1}H NMR (300 MHz) ($CDCl_3$): δ 2.95 (q, $J = 5.7$ Hz, 2H, CH_2); 3.46 (q, $J = 5.7$ Hz, 2H, CH_2); 3.55–3.60 (m, 8H, 4 x CH_2); 7.89 (bs, 2H, NH_2), 8.07 (dd, $J_1 = 9.0$ Hz, $J_2 = 2.4$ Hz, 2H, ArH); 8.31 (dd, $J_1 = 9.0$ Hz, $J_2 = 2.4$ Hz, 2H, ArH); 8.90 (t, $J = 5.4$ Hz, 1H, NHCO) ppm. ^{13}C NMR (75 MHz) ($CDCl_3$): δ 39.97, 40.68, 70.17, 114.26, 123.47, 129.49, 150.67, 167.12. LC-MS *m/z* 298 $[M^+]$.

2.1c *Synthesis of N-(2-(2-(2-(3-(6-amino-9H-purin-9-yl) propanamido)ethoxy)ethoxy)ethyl)-4-nitrobenzamide (**8**)*: A solution of compound **7** (1.0 g, 4.8 mmol) in dry dichloromethane:dimethylformamide (1:1) (30 mL) was cooled down to 0°C and HOBt (*N*-hydroxybenzotriazole) (0.783 g, 5.8 mmol) was added, followed by the addition of EDC·HCl (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) (1.11 g, 5.8 mmol). After 1 h, the mixture was allowed to return to room temperature and then amine **6** (1.49 g, 5.3 mmol) dissolved in dichloromethane: dimethylformamide (1:1) (5 mL) was added, followed by the addition of diisopropylethylamine (1.85 mL, 10.6 mmol). The reaction mixture was stirred for 24 h. The solvent was evaporated under high vacuum and ethyl acetate (100 mL) was added. The ethyl acetate layer was washed with 10% $NaHCO_3$ solution (2 × 40 mL), and finally with saturated brine solution (2 × 20 mL). The collected organic layer was dried over anhydrous sodium sulphate and evaporated under vacuum to get the crude compound (2.89 g). The crude product obtained was further purified by column chromatography on silica using gradient of 10–20% methanol in dichloromethane to give pure **8** as a yellow solid (1.74 g, 74.3% yield). R_f = 0.58 (dichloromethane:methanol, 80:20). ^{1}H NMR (300 MHz) (DMSO-d_6) : δ 2.66 (t, $J = 6.9$ Hz, 2H, CH_2); 3.15 (q, $J = 5.7$ Hz, 2H, CH_2); 3.30–3.35 (m, 2H, CH_2); 3.41–3.56 (m, 8H, 4xCH_2); 4.33 (t, $J = 6.9$ Hz, 2H, CH_2); 7.17 (s, 2H, NH_2 of adenine); 7.98 (s, 1H, ArH-C2 of adenine); 8.00–8.02 (m, 1H, NHCO); 8.06 (dd, $J_1 = 9.0$ Hz, $J_2 = 2.4$ Hz, 2H, ArH); 8.13 (s, 1H, ArH-C8 of adenine); 8.31 (dd, $J_1 = 9.0$ Hz, $J_2 = 2.4$ Hz, 2H, ArH); 8.87 (t, $J = 5.4$ Hz, 1H, NHCO) ppm. ^{13}C NMR (75 MHz) (DMSO-d_6): δ 35.03, 38.45, 39.30, 39.40, 68.57, 68.89, 69.46, 118.58, 123.37, 128.71, 139.91, 140.87, 148.87, 149.29, 152.27, 155.81, 164.57, 169.44 ppm. LC-MS *m/z* 487.2 $[M+H]^+$.

2.1d *Synthesis of 4-amino-N-(2-(2-(2-(3-(6-amino-9H-purin-9-yl)propanamido)ethoxy)ethoxy)ethyl) benzamide (**1**)*: To a methanol solution of compound **8** (1.0 g, 2.0 mmol), Pd/C (100 mg) was added slowly. The flask was degassed and saturated with hydrogen and reaction was stirred for 15 h under H_2 atmosphere at room temperature. After completion of the reaction (TLC), the Pd/C was filtered off on celite pad, washed with methanol and combined solvent was evaporated under vacuum to isolate the compound **1** as off-white solid (0.88 g, 75% yield). $R_f = 0.3$ (dichloromethane:methanol, 80:20). ^{1}H NMR (300 MHz) (DMSO-d_6): δ 2.67 (t, $J = 6.6$ Hz, 2H, CH_2); 3.15 (q, $J = 6.0$ Hz, 2H, CH_2); 3.31–3.37 (m, 4H, 2 x CH_2); 3.45–3.48 (m, 6H, 3 x CH_2); 4.33 (t, $J = 6.6$ Hz, 2H, CH_2); 5.60 (s, 2H, NH_2 of aniline); 6.52 (d, $J_1 = 9.0$ Hz, 2H, ArH); 7.17 (s, 2H, NH_2 of adenine); 7.55 (d, $J_1 = 9.0$ Hz, 2H, ArH); 7.99 (s, 1H, ArH-C2 of adenine); 8.01–8.06 (m, 2H, NHCO); 8.13 (s, 1H, ArH-C8 of adenine) ppm. ^{13}C NMR (75 MHz) (DMSO-d_6): δ 34.99, 38.44, 38.80, 39.45, 68.90, 69.07, 69.42, 112.41, 118.58, 120.95, 128.60, 140.83, 149.28, 151.49, 152.25, 155.81, 166.21, 169.39 ppm. LC-MS *m/z* 457.2 $[M+H]^+$.

2.2 *General procedure for synthesis of nucleobase-benzamide conjugate **2***

2.2a *Synthesis of methyl 6-aminohexanoate hydrochloride salt (**9**)*: 6-Aminocaproic acid (5.0 g,

38.0 mmol) was dissolved in dry methanol (100 mL). To this solution $SOCl_2$ (6.92 mL, 95.0 mmol) was added drop-wise at time interval of 2 h, maintaining the temperature at 0°C. The reaction was further stirred for 5 h at room temperature. The methanol was evaporated under vacuum and resulting solid was co-evaporated with diethyl ether several times to isolate the compound **9** as white solid, (6.59 g, 95.6% yield). ^{1}H NMR (300 MHz) ($CDCl_3$): δ 1.44 (quintuplet, J_1 = 6.6 Hz, J_2 = 14.7 Hz, 2H, CH_2), 1.66 (quintuplet, J_1 = 7.2 Hz, J_2 = 14.7 Hz, 2H, CH_2); 1.80 (broad quintuplet, J = 6.3 Hz, 2H, CH_2); 2.33 (t, J = 8.7 Hz, 2H, CH_2); 3.01, (bt, 2H, CH_2); 3.66 (s, 3H, OCH_3); 8.26 (bs, 3H, NH_3^+) ppm. ^{13}C NMR (75 MHz) ($CDCl_3$): δ 24.04, 25.78, 27.01, 33.48, 39.66, 51.43, 173.74 ppm. LC-MS *m/z* 259.2 $[M + TFA]^+$.

2.2b *Synthesis of methyl 6-(4-nitrobenzamido) hexanoate (**10**)*: 4-Nitrobenzoyl chloride (1.0 g, 5.4 mmol) was dissolved in dry toluene (50 mL) and to this solution amine **9** (1.07 g, 5.9 mmol) also dissolved in dry toluene (10 mL) was added immediately under Ar atmosphere followed by addition of triethylamine (0.97 mL, 7.0 mmol). The reaction mixture was stirred for 12 h at 65°C under Ar atmosphere. After completion of the reaction (TLC), the mixture was cooled to room temperature and diethyl ether was added. The solid precipitate was filtered off, washed with diethyl ether and dried under vacuum. The residue was column chromatographed on silica using gradient of 0.5–5% methanol in dichloromethane to isolate pure **10** as white solid (1.18 g, 67%). R_f = 0.74 (dichloromethane:methanol, 98:2). ^{1}H NMR (300 MHz) ($CDCl_3$): δ 1.43 (quintuplet, J_1 = 7.2 Hz, J_2 = 15.9 Hz, 2H, CH_2), 1.61–1.73 (m, 4H, CH_2), 2.34 (t, J = 7.2 Hz, 2H, CH_2); 3.49 (q, J = 6.9 Hz, 2H, CH_2); 3.66 (s, 3H, OCH_3); 6.46 (bs, 1H, NHCO); 7.94 (dd, J_1 = 9.0 Hz, J_2 = 2.1 Hz, 2H, ArH); 8.27 (dd, J_1 = 9.0 Hz, J_2 = 2.1 Hz, 2H, ArH) ppm. ^{13}C NMR (75 MHz) ($CDCl_3$): δ 24.10, 26.18, 28.85, 33.62, 39.89, 51.48, 123.61, 128.10, 140.26, 149.36, 165.45, 174.06 ppm. LC-MS *m/z* 295.1 $[M+H]^+$.

2.2c *Synthesis of 6-(4-nitrobenzamido)hexanoic acid (**11**)*: The compound **10** (2.0 g, 7.0 mmol) was dissolved in minimum volume of methanol and NaOH (0.033 g, 8.0 mmol) as its 1N solution was added and stirred for 24 h. Then, methanol was evaporated under vacuum and 20 mL of water was added in ice cold condition. The solution was acidified to pH 2–3 and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated to get pure product **11** as light green solid (1.70 g, 89.5%yield). R_f = 0.2 (dichloromethane:methanol, 90:10), ^{1}H NMR (300 MHz) (DMSO-d_6): δ 1.33 (quintuplet, J_1 = 7.2 Hz, J_2 = 14.7 Hz, 2H, CH_2), 1.54 (quintuplet, J_1 = 7.2 Hz, J_2 = 14.7 Hz, 4H, CH_2); 2.20 (t, J = 7.2 Hz, 2H, CH_2); 3.27 (q, J = 6.9 Hz, 2H, CH_2); 8.06 (dd, J_1 = 9.0 Hz, J_2 = 2.1 Hz, 2H, ArH); 8.28 (dd, J_1 = 9.0 Hz, J_2 = 2.1 Hz, 2H, ArH); 8.73 (t, J = 5.7 Hz, 1H, NHCO) ppm. ^{13}C NMR (75 MHz) (DMSO-d_6): δ 24.16, 25.94, 28.58, 33.52, 39.22, 123.40, 128.55, 140.23, 148.81, 164.35, 174.36 ppm. LC-MS *m/z* 281.1 $[M+H]^+$.

2.2d *Synthesis of N-(6-(2-(6-amino-9H-purin-9-yl)ethylamino)-6-oxohexyl)-4-nitrobenzamide (**13**)*: A solution of compound **11** (2.0 g, 7.0 mmol) in dry dichloromethane:dimethylformamide (1:1) (20 mL) was cooled to 0°C and HOBt (*N*-hydroxybenzotriazole) (1.158 g, 8.5 mmol) was added, followed by addition of EDC·HCl (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) (1.643 g, 8.5 mmol). After 1 h the mixture was allowed to come at room temperature and then adenine amine as TFA salt **12** (3.174 g, 7.8 mmol) dissolved in dichloromethane:dimethylformamide (1:1) (20 mL) was added, followed by addition of diisopropylethylamine (4.35 mL, 24.9 mmol). The reaction mixture was stirred for 24 h. The solvent was evaporated under high vacuum and ethyl acetate (100 mL) was added. The ethyl acetate layer was washed with 10% $NaHCO_3$ solution (2 × 40 mL), and finally with saturated brine solution (2 × 20 mL). The collected organic layer was dried over anhydrous sodium sulphate and organic layer was evaporated under vacuum to get the crude compound (2.89 g). The crude product obtained was further column chromatographed on silica using 15–25% gradient of methanol in dichloromethane to give pure **13** as yellow solid (2.02 g, 64.4%yield). R_f = 0.5 (dichloromethane:methanol, 80:20). ^{1}H NMR (300 MHz) (DMSO-d_6): δ 1.21 (quintuplet, J_1 = 6.6 Hz, J_2 = 15.0 Hz, 2H, CH_2); 1.40–1.54 (m, 4H, 2 x CH_2); 2.00 (t, J = 7.5 Hz, 2H, CH_2); 3.25 (q, J = 6.9 Hz, 2H, CH_2); 3.45 (q, J = 6.6 Hz, 2H, CH_2); 4.17 (t, J = 6.0 Hz, 2H, CH_2); 7.16 (s, 2H, NH_2 of adenine); 7.91 (t, J = 6.0 Hz, 1H, NHCO); 8.01 (s, 1H, ArH-C2 of adenine); 8.06 (dd, J_1 = 9.0 Hz, J_2 = 2.4 Hz, 2H, ArH); 8.13 (s, 1H, ArH-C8 of adenine); 8.30 (dd, J_1 = 9.0 Hz, J_2 = 2.4 Hz, 2H, ArH); 8.77 (t, J = 5.4 Hz, 1H, NHCO) ppm. ^{13}C NMR (75 MHz) (DMSO-d_6): δ 24.83, 26.03, 28.65, 35.21, 42.64, 45.48, 39.28, 118.71, 123.38, 128.58, 140.24, 140.9, 148.80, 149.60, 152.23, 155.85, 164.39, 172.39 ppm. LC-MS *m/z* 441.2 $[M+H]^+$.

2.2e *Synthesis of 4-amino-N-(6-(2-(6-amino-9H-purin-9-yl)ethylamino)-6-oxo-hexyl) benzamide (**2**)*: To a methanol solution of **13** (1.0 g, 2.2 mmol), Pd/C (100 mg) was added slowly. The flask was then degassed and saturated with hydrogen and reaction was stirred for 15 h under H_2 atmosphere. After completion of the reaction (TLC), the Pd/C was filtered off on celite pad, washed with methanol and combined solvent was evaporated under vacuum to isolate the compound **2** as transparent liquid (0.877 g, 94.2% yield). R_f = 0.22 (dichloromethane:methanol, 80:20); ^{1}H NMR (300 MHz) (DMSO-d_6): δ 1.18 (quintuplet, J_1 = 5.7 Hz, J_2 = 12.9 Hz, 2H, CH_2); 1.43 (quintuplet, J_1 = 7.2 Hz, J_2 = 14.4 Hz, 4H, 2xCH_2), 1.99 (t, J = 7.5 Hz, 2H, CH_2); 3.15 (q, J = 6.9 Hz, 2H, CH_2); 3.45 (q, J = 5.7 Hz, 2H, CH_2); 4.18 (t, J = 5.7 Hz, 2H, CH_2); 5.55 (s, 2H, NH_2 of aniline); 6.51 (d, J = 8.7 Hz, 2H, ArH); 7.16 (s, 2H, NH_2 of adenine); 7.54 (d, J = 8.4 Hz, 2H, ArH); 7.88–7.95 (m, 2H, two triplets merge with each other, NHCO); 8.01 (s, 1H, ArH-C2 of adenine); 8.13 (s, 1H, ArH-C8 of adenine); ^{13}C NMR (75 MHz) (DMSO-d_6): δ 24.88, 26.10, 29.12, 35.25, 38.21, 38.77, 42.63, 112.41, 118.70, 121.38, 128.52, 140.86, 149.57, 151.32, 152.20, 155.83, 166.00, 172.36 ppm. LC-MS *m/z* 411.2 $[M+H]^+$.

2.3 *General procedure for synthesis of nucleobase-benzamide conjugate **3***

2.3a *Synthesis of N-(2-(6-amino-9H-purin-9-yl) ethyl)-4-nitrobenzamide (**14**)*: 4-Nitrobenzoyl chloride (1.0 g, 5.4 mmol) was dissolved in dry toluene (50 mL) and to this solution amine **12** (1.05 g, 5.9 mmol) also dissolved in dry toluene (10 mL) was added immediately under Ar atmosphere followed by addition of triethylamine (2.61 mL, 19.0 mmol). The reaction mixture was stirred for 12 h at 65°C under Ar atmosphere. After completion of the reaction (TLC), the mixture was cooled to room temperature and diethyl ether was added. The solid precipitate was filtered off, washed with diethyl ether and dried under vacuum. The residue was column chromatographed on silica using gradient of 5–15% methanol in dichloromethane to isolate pure **14** (1.056 g, 54.2% yield). ^{1}H NMR (300 MHz) (DMSO-d_6): δ 3.70 (q, J = 5.4 Hz, 2H, CH_2); 4.37 (t, J = 5.4 Hz, 2H, CH_2); 7.18 (s, 2H, NH_2 of adenine); 7.98 (d, J = 9.0 Hz, 2H, ArH); 8.10 (s, 1H, ArH-C2 of adenine); 8.11 (s, 1H, ArH-C8 of adenine); 8.28 (d, J = 9.0 Hz, 2H, ArH); 9.02 (t, J = 5.4 Hz, 1H, NHCO) ppm. ^{13}C NMR (75 MHz) (DMSO-d_6): δ 39.38, 42.45, 118.70, 123.38, 128.57, 139.82, 140.92, 148.89, 149.67, 152.24, 155.83, 164.84 ppm. LC-MS *m/z* 328.11 $[M+H]^+$.

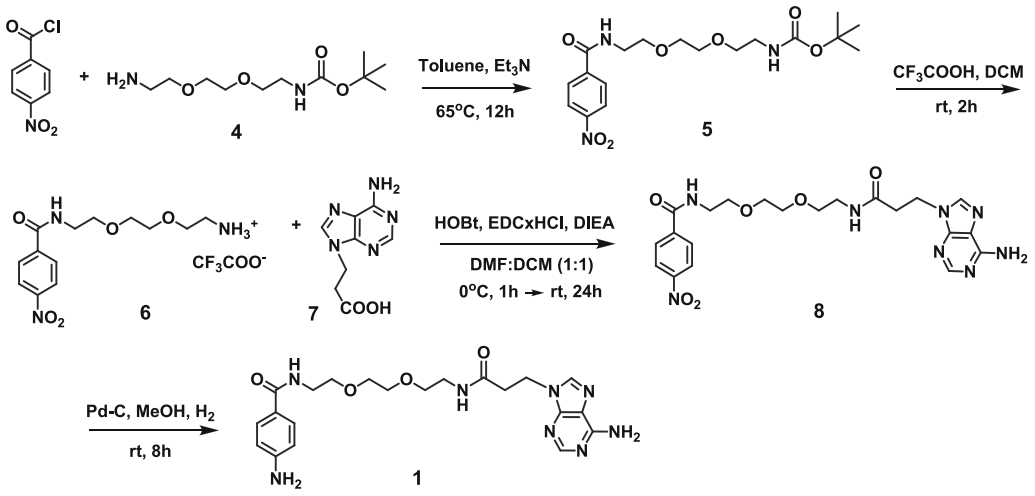
2.3b *Synthesis of 4-amino-N-(2-(6-amino-9H-purin-9-yl)ethyl)benzamide (**3**)*: To a methanol solution of compound **20** (0.5 g, 1.5 mmol), Pd/C (50 mg) was added slowly. The flask was then degassed and saturated with hydrogen and reaction was stirred for 8 h under H_2 atmosphere. After completion of the reaction (TLC), the Pd/C was filtered off on celite pad, washed with methanol and combined solvent was evaporated under vacuum to isolate the compound **9** as transparent liquid (0.382 g, 84.3% yield). ^{1}H NMR (300 MHz) (DMSO-d_6): δ 3.61 (q, J = 5.7 Hz, 2H, CH_2); 4.29 (t, J = 6.0 Hz, 2H, CH_2); 5.61 (s, 2H, NH_2 of aniline); 6.51 (d, J = 8.4 Hz, 2H, ArH); 7.17 (s, 2H, NH_2 of adenine); 7.49 (d, J = 8.4 Hz, 2H, ArH); 8.00 (s, 1H, ArH-C2 of adenine); 8.14 (s, 1H, ArH-C8 of adenine); 8.17 (t, J = 5.4 Hz, 1H, NHCO) ppm.^{13}C NMR (75 MHz) (DMSO-d_6): 42.78, 45.44, 112.38, 118.72, 120.79, 128.60, 140.90, 149.56, 151.583, 152.17, 155.82, 166.46 ppm. LC-MS *m/z* 298.2 $[M+H]^+$.

3. Results and discussion

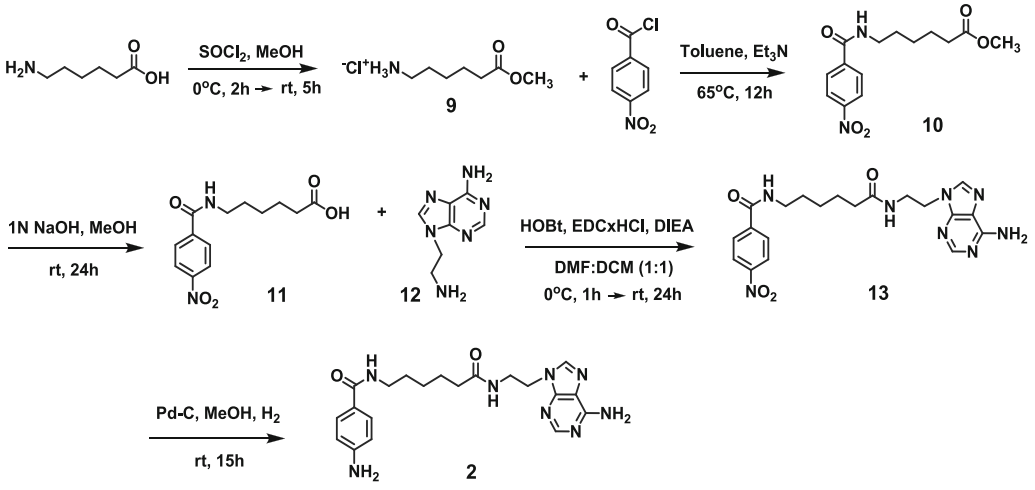
For the synthesis of the new adenine-benzamide conjugates **1–3**, possessing versatile spacers, two structural units can be envisaged as building blocks: adenine equipped with reactive functional group at N⁹-position and substituted benzamide equipped with versatile spacer (figure 1). The reaction pathway leading to the formation of adenine-benzamide conjugates such as N⁹-adenine-TEG-benzamide (**1**), N⁹-adenine-caproic acid-benzamide (**2**) and N⁹-adenine-ethyl-banzamide (**3**) are depicted in schemes 1, 2 and 3.

The adenine containing reactive functional group at N⁹-position [i.e., 3-(9-adeninyl)propionic acid **7** and 9-(2-aminoethyl)adenine **12**] were prepared by following the reported literature procedure by slight modification. Briefly, the 3-(9-adeninyl)propionic acid **7** was prepared via Michael addition reaction between adenine and ethyl acrylate to give the corresponding ethyl ester derivative, which was later hydrolysed under acidic conditions to afford the desired N⁹-adenine derivative **7** terminated with acid group. On the other hand, for the synthesis of 9-(2-aminoethyl)adenine **12**, the base catalysed nucleophilic substitution reaction of adenine was performed using Boc protected 2-bromoethylamine as electrophile, followed by removal of the Boc protective group by the addition of the trifluoroacetic acid in dichloromethane to give desired N⁹-adenine derivative **12** terminated with amine group.

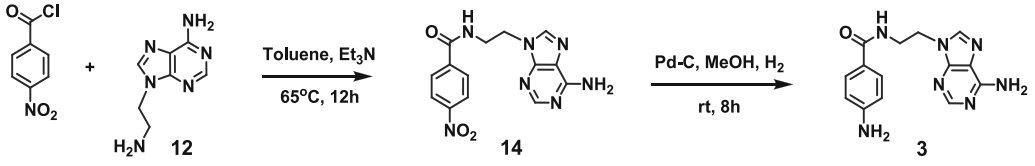
For the synthesis of substituted benzamide derivatives with different spacer, we started with substituted benzoic acid. For this purpose, 4-nitrobenzoic acid was chosen and it was refluxed for 5 h in the presence of



Scheme 1. Synthesis of nucleobase-benzamide conjugate **1** linked by means of TEG chain.



Scheme 2. Synthesis of nucleobase-benzamide conjugate **2** linked by means of aminocaproic acid chain.



Scheme 3. Synthesis of nucleobase-benzamide conjugate **3** linked by means of ethyl chain.

thionyl chloride to afford the 4-nitrobenzoyl chloride derivative which was later isolated and stored under desiccator at −20°C. In the next step, 4-nitrobenzoyl chloride moiety was then coupled with mono-Boc protected triethyleneglycol amine in anhydrous toluene at 65°C to obtain the compound **5** in good yield. Triethylamine was also added to neutralize the HCl liberated during the reaction. The Boc protective group in compound **5** was then removed by the addition of the trifluoroacetic acid to give compound **6** in good yield. Adenine nucleobase was then introduced by amidation reaction between the amino functions of **6** and 3-(9-adeninyl)propionic acid **7** in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and 1-hydroxy-benzotriazole (HOBT) to afford **8** in 74% yield after column chromatography. Finally, the adenine derivative **8** was subjected to reduction in the presence of Pd–C to yield adenine-benzamide conjugate **1** in 75% yield and structure was confirmed spectroscopically. We observed a very

clean conversion of the NO_2 group into NH_2 group in a very short time if the flask was degassed and saturated with hydrogen at regular interval of time.

We extended the synthetic strategy to prepare other adenine-benzamide conjugate **2** starting from aminocaproic acid. While doing the preparation of building units containing aminocaproic acid group, one should keep in mind the nucleophilic nature of the amine group. For this purpose, the aminocaproic acid was dissolved in methanol and protected by means of activation of the COOH groups using thionyl chloride under ice cool temperature to yield **9** as hydrochloride salt in quantitative yield. By using a similar approach as described above, we prepared substituted benzamide having caproic linker (**10**) by amidation reaction between nitrobenzoyl chloride and **9** in 67% yield after column chromatography. The ester protective group was then cleaved via basic hydrolysis with NaOH in methanol to obtain compound **11** in good yield. The carboxylic acid groups of **11** were activated using EDC·HCl and HOBt, and then treated with the primary amine function of N-9-(2-aminoethyl)adenine **12** to yield **13** in 64% after chromatography using gradient of methanol in dichloromethane. Compound **13** was then subjected to reduction with Pd–C to afford the final adenine-benzamide conjugate **2** in 94% yield.

There are few interesting features in this present strategy for adenine-benzamide conjugate synthesis that merit comments. First, the presence of exocyclic amine at N^6 position of adenine plays an important role on the regioselectivity as well as the yield of the reaction, due to the reactivity of N^6 amine towards acid chloride. Due to this reason, the alternate synthetic strategy, which involves amidation of the more reactive 4-nitrobenzoyl chloride with adenine derivatives containing either TEG or caproic acid, has not been adopted. Second, the ^{1}H NMR spectra of the final conjugates **1**–**3** are displayed in figure 2. We can clearly see the signals of N^6 exocyclic amine, amide linkages and methylene groups for the spacer, thus confirming the presence of nucleobase, benzamide and spacer, respectively. Third, the present procedure employs toluene as a solvent, for synthesis of substituted benzamide-spacer analogue, because the purification procedure becomes easy and straightforward due to precipitation of the product in the reaction mixture. Fourth, in peptide coupling step, the present optimised procedure utilises EDC·HCl as dehydrating agent instead of DCC, due to water solubility of either EDC·HCl and side product, hereby helping in the purification process. Finally, in all cases, $>$ 99% high purities was evidenced by LC-MS data.

Our planning and execution of the synthetic strategy was straightforward as supported by the synthesis of adenine-benzamide conjugate **3** (as a control molecule). For this purpose, 4-nitrobenzoyl chloride was directly condensed with 9-(2-aminoethyl)adenine (**12**) in dry toluene to obtained **14** in moderate yield which was later purified by column chromatography using gradient of methanol in dichloromethane. TLC shows the formation of two types of product, probably due to reaction of acid chloride with aliphatic amine and N^6 exocyclic amine. However, we optimized the procedure and found that addition of excess of 9-(2-aminoethyl)adenine (**12**) (2-3 equivalents), the product formation due to N-6 amine is reduced to $<$ 10%. The NO_2 group in **14** was then reduced to NH_2 using Pd–C method to afford adenine-benzamide conjugate **3** in 84% yield.

Adenine shows poor solubility in all solvents, which is an obstacle for many applications such as for developing sensors, as a solubilizing agent for carbon nanotubes (CNTs) via exploitation of base pairing, azide and amide chemistry. However, the present conjugates show very good solubility in common organic solvents. We have outlined few applications in figure 3, where our adenine-benzamide conjugates could find potential uses.

Encouraged by these observations, we explored the possibility for the synthesis of adenine-CNT hybrids where these derivatives have been directly attached onto the endwalls of CNTs via amidation chemistry and preliminary results are presented here. For this purpose we covalently functionalized the oxidized-SWCNTs with conjugate **1** via amidation reaction. Oxidized SWCNTs lost 13.0% of mass up to 500°C, while thermogravimetric analysis (TGA) curve of the SWCNT-**1** conjugate display gradual weight loss of 8.93% at 500°C. On the basis of the weight loss, we estimated that the amount of functional groups per gram (f_w, mmol/g) is 0.184 corresponding to a degree of functionalization of one functional group each 411 carbon atoms of CNTs.

Moreover, the versatility of the triethylene glycol (TEG) linker used here was confirmed by their capacity to modulate the dispersibility properties of the SWCNTs. Pristine SWCNTs were reacted with 4-aminobenzoyl derivatives (which was obtained by reduction of the nitro group of **5**) via C-C addition reaction to produce sidewall functionalized TEG-SWCNTs. Compare to pristine SWCNTs which are completely insoluble in water, TEG-SWCNT hybrids are easily dispersed in water:methanol (1:2) solution. We observed high degree of functionalization of approximately one functional group each 111 carbon atoms of CNTs with more exfoliated nanotubes seen in the transmission electron microscope.

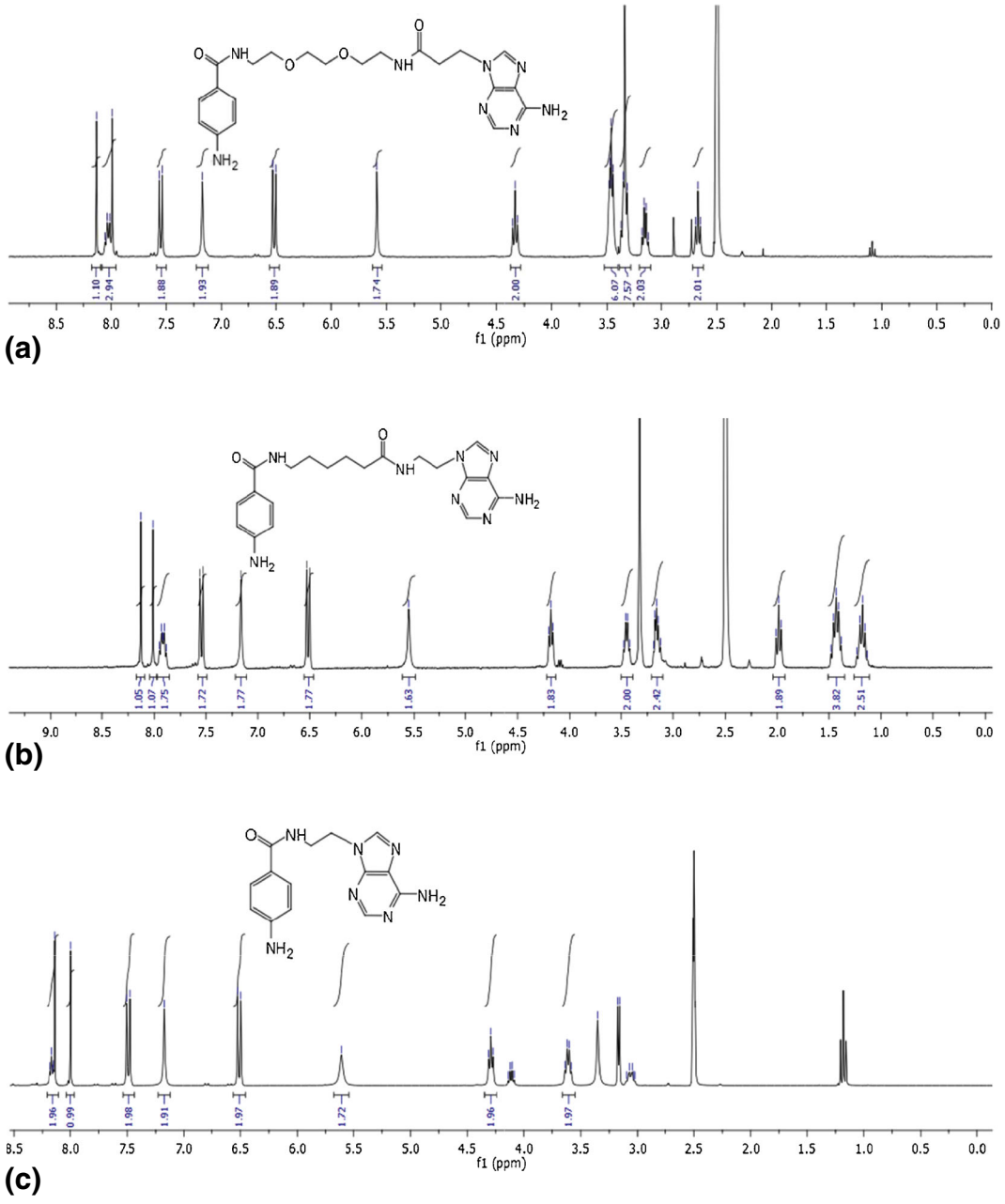


Figure 2. The ^{1}H NMR spectrum of the adenine-benzamide conjugates **1**–**3**.

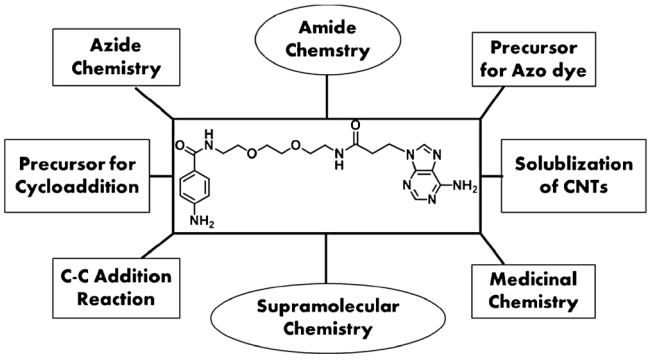


Figure 3. Overview of the proposed applications of benzamide-nucleobase conjugates in various research fields.

4. Conclusions

In summary, we propose the synthesis of novel adenine-benzamide conjugates, where modified nucleobases equipped with reactive functional groups such as carboxylic acid and amine have been attached onto the substituted benzamide by means of amidation reaction using versatile spacer such as triethylene glycol (TEG), aminocaproic acid and ethyl chains. In view of the remarkable properties of benzamide and recognition properties of nucleobases these conjugates could find potential applications as sensors, in medicinal chemistry, azide/amide chemistry and for making dyes.

The possibility of developing adenine-benzamide-CNT hybrids would bear a nucleobase for DNA recognition or self-assembly through base-pair complementarity and a biocompatible linker for interfacing with biological system.

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