Original Article

Synthesis And Characterisation Of Novel Derivative Of ^{2}fluoro Adenosine

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Abstract

The main approach of this research paper to introduce new derivatives of adenosine. Novel alkyl derivative of 2'-Fluoro-2'-deoxyadenosine were synthesized and characterised by ^{1}H ^{13}C, IR and mass spectroscopy. This paper also describes simple synthetic pathway of n-benzyl fluoro adenosine. The synthesis pathway are suitable for industrial scale with high yield and purity.

Keywords: ^{2}Fluoro Adenosine, Alkyl derivative, Synthesis, Characterisation, HPLC.

INTRODUCTION

All of the body's cells contain adenosine, a naturally occurring nucleoside, in one form or another. It is a crucial part of the body's mechanisms for generating and using energy.2'-Fluoro-2'-deoxyadenosine is a unique analogue of adenosine. [1]

Since 1929, a number of researchers have studied the adenosine nucleoside; primarily focusing on its distribution, characterization, and biological significance as well as the synthetic chemistry this type of molecule has undergone to produce a number of its derivatives. [2]

According to several studies, including those by Cristalli et al., a number of derivatives of 2-alkynyladenosine and 2-alkynyladenosine-5'-N-ethyluronamide have been formed. A cross-coupling reaction involving palladium used to complete the first series, and a novel nucleoside called N-ethyl-1'-deoxy-1'-(6-amino-2-iodo -9H-purine-9-yl)—D-ribofuranuron-amide used to complete the second series [3].

Similar to this, a 2-substitution of 6-benzyladenosine-5'-uronamide analogues as agonists of the A3 receptor was proposed. Using methyl—Dribofuranoside (2) as a precursor, which subjected to this nucleophilic substitution reaction, this procedure proposed in 10 stages [4-5]. A series of 8-alkylamino of N6-cyclopentyladenosine compounds, which were demonstrated to be partial agonists with the A1 receptor type cardiovascular by in vivo studies, were proposed by Van Schaick et al. [6]. Three different approaches were used to synthesise such series.

According to several investigations, other methods like acylation, reduction, and deprotection processes can be used to synthesise adenosine derivatives. It happens in the Lescrinier et al. synthesis, which is based on a coupling process that involves reducing N6-acylated 2',3'-O-isopropylidene adenosine [8] with LiAlH4 and then removing the isopropylidene group protection [9].

In Previous, work numerous ways to make 2'-deoxy-2'-fluoronucleosides, one of which is through nucleophilic displacement of 2'-0 trifluoromethanesulfonylarabinosides.

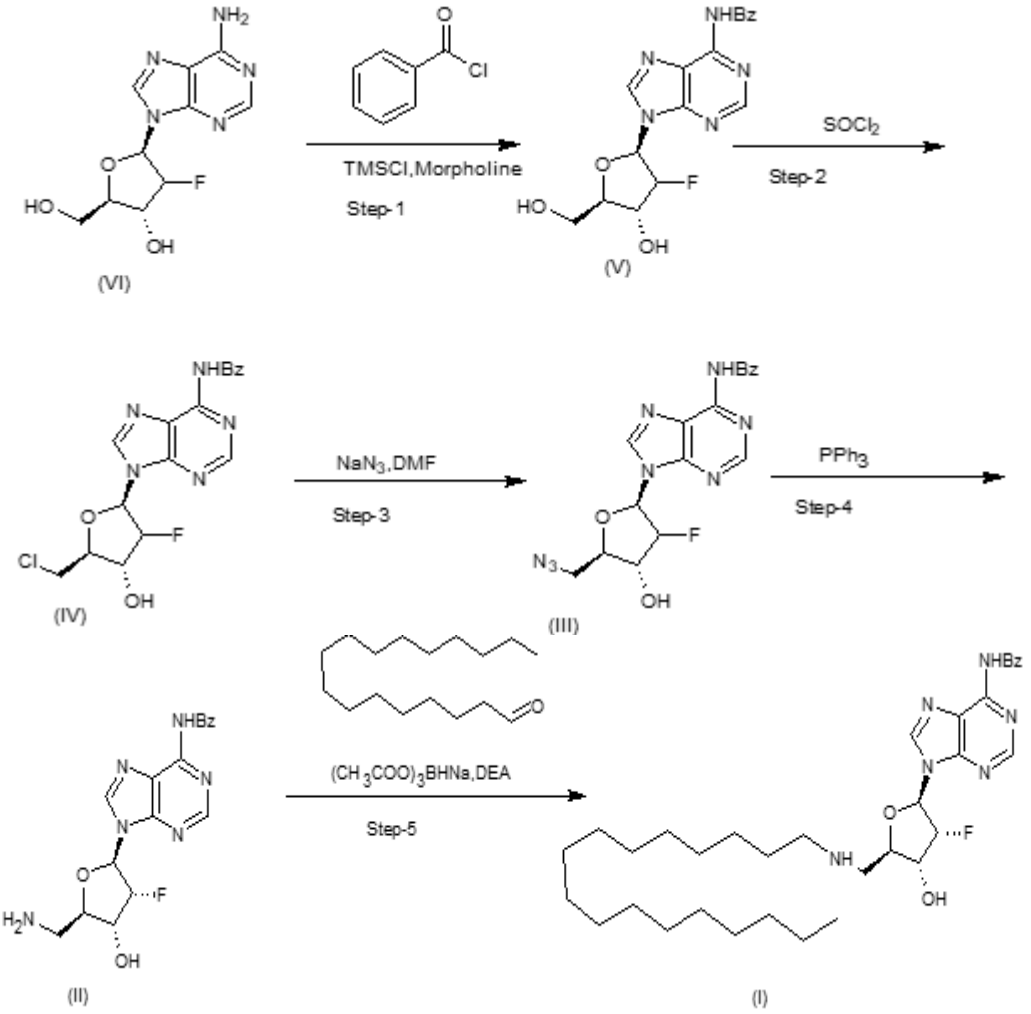
The worker described the preparation of 4'-methoxyuridine', 4'-fluoro derivatives of purine3 and pyrimidine nucleosides, and 4'-hydroxymethyl derivatives of purine and pyrimidine nucleosides In addition, some 4'-azidonucleosides have also been prepared and the general subject of 4'-substituted nucleosides has been reviewed'. Certain fluoro adenosine containing functionalised carbon substituents at C-10-17 have also been the subject of investigation in other laboratories [10].

SUBJECTS AND METHODS

We report herein industrially scalable, efficient synthesis of N-amniobenzyl fluoro adenosine by using safe reagents and experimental conditions. Further chlorination, azido adenosine and finally we have synthesized alkylated C-18 2-fluoro deoxyadenosine compound. To confirm the product and to study the structural conformation details analytical methods of purity by HPLC also reported.

All the key raw materials used for synthesis were obtained from, Sigma Aldrich and was used without further purification. All other solvents and reagents were used analytical or HPLC grade. Melting points were calculated by Spectra Lab device. Using a Shimadzu 8300 IR Spirit Fourier transform infrared spectrometer, infrared (KBr) spectra were examined. In DMSO and CDCl3 solutions, 1 H and 13 C NMR were captured on Bruker spectra at 400 and 100 MHz, respectively. On a Waters TQD device with an ionisation potential of 110 V, ESI mass spectra were captured. Using a Thermofisher-chromeon 7, 2, HPLC analysis was carried out. On 0.25 mm silica gel plates, thin layer chromatography (TLC) experiments were carried out (60F254, Merck). UV light at a wavelength of 254 nm was used for visualisation.

Route of synthesis



Synthesis procedure

Synthesis of N-{9-[(2R,4R,5R)-3-fluoro-4-hydroxy-5-(hydroxymethyl) tetrahydrofuran-2-yl]-9H-purin-6-yl} benzamide (V)

In a clean and dried RBF charged Morpholine (0.012 mole) and (2R, 3R,5R)-5-(6-amino-9H-purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol (0.01 Mole) followed by this added Trimethylsilyl chloride (0.03 mole) slowly drop wise into the reaction mass. Stirred the reaction mass for 5-10 hrs for reaction completion and reaction monitor by TLC. Reaction mass spotted against starting material. After reaction completion, reaction mass cooled to 0°C. Then Benzoyl chloride slowly drop wise (preferably in 2-3 hrs) was added. Increased reaction mass the temperature to at 25-35°C. Stirred the reaction mass for 3 to 4 hrs at 25-35°C .Charged 4v water to the reaction mass. Then slowly added Methanolic Ammonia in drop wise manner and stirred for 15 min. Distilled out above reaction mass under reduced atmosphere. Cooled to 0 to 5°C.filter the mass and collected solid wet cake. Dried the wet material at 40-50°C.
White colour solid compound (V) Yield 85%.

HPLC Purity 98.7%, MS (ES+) m/z 374.2 [M+1] melting range 180-182°C;
IR (KBr, vmax, cm-1): 3310, 3064, 2900, 2850, 1690, 1330, 1240, 1140, 720;
H NMR (400 MHz, DMSO-d6) : 3.61-3.62 (s, 1H), 3.67-3.68 (s, 1H), 3.69-3.71 (s, 1H), 4.00- 4.02 (m, 1H), 4.37-4.44 (m, 1H), 5.17-5.20 (s, 1H), 5.34-5.35 (s, 1H), 6.16-6.17 (s, 1H), 7.53-7.57 (m, 2H), 7.63-7.66 (m, 1H, 8.03-8.05 (d,2H) 8.75-8.77 (s 2H) 11.24(s, 1H). **^{13}C NMR (100 MHz, DMSO-*d*6):** 164.8, 152.3, 150.8, 150.5, 143.1, 133.5, 133.0, 128.9, 128.2, 124.6 ,89.5 88.3, 82.7, 70.4, 63.1, 44.3.

Synthesis of N-{9-[(2R,4R,5S)-5-(chloromethyl)-3-fluoro-4-hydroxytetrahydrofuran-2-yl]-9H-purin-6-yl} benzamide (IV)
Charged dichloromethane in clean glass reactor. Charged N-{9-[(2R,4R,5R)-3-fluoro-4-hydroxy-5-(hydroxymethyl) tetrahydrofuran-2-yl]-9H-purin-6-yl} benzamide (V) (0.022 mol) and stirred for 10 min. Added slowly thionyl chloride (2 mol) in dropwise manner at 5-10°C.Slowly raised the reaction mass temp.to 25-30°C and stirred for 2 hrs. at this temperature. Further raised the temp.to 20-25°C and maintain reaction same temperature for 2-3 hrs. Reaction monitored by TLC (5 Hexane: Ethyl acetate).After reaction completion evaporated the excess solvent under reduce pressure up-to 40° C till solid material observed .Charged water 5v slowly and stir for 2 hrs at 10-20°C.Filtered the reaction mass and washed with water till pH of mother liquor comes above2.0.Purified the crude wet cake in water. Dried the solid wet material under reduced pressure below 40°C.
White to light yellow compound (IV) Yield 76%.

HPLC Purity: 98.2%, MS (ES+) m/z 392.2 [M+1] melting range 197-199°C.
IR (KBr, vmax, cm-1) 3398, 3292, 2108, 1620, 1294, 1076, 732;
H NMR (400 MHz, DMSO-*d*6) : 3.86-3.94 (s, 1H), 3.90-4.0 (s, 1H), 4.16-4.18 (s, 1H), 4.67- 4.73 (m,1H), 5.60-5.61 (s, 1H), 5.98-6.0(m, 1H), 6.38-6.43 (m, 1H), 7.52-7.56 (m, 2H), 7.62-7.65 (m, 1H), 8.02-8.04 (m,2H), 8.61 (s, 1H), 8.76 (s, 1H), 11.23 (s, 1H).), **^{13}C NMR (100 MHz, DMSO-*d*6):** 165.6, 152.2, 151.2, 149.8, 142.0, 133.7, 132.9, 128.7, 128.2, 123.5, 87.1 83.3, 83.2, 69.9, 44.2.

Synthesis of N-{9-[(2R,4R,5R)-5-(azidomethyl)-3-fluoro-4-hydroxytetrahydrofuran-2-yl]-9H-purin-6-yl} benzamide (III)
Charged Dimethyl formamide 10v in a clean and dried RBF and charge N-{9-[(2R,4R,5S)-5-(chloromethyl)-3-fluoro-4-hydroxytetrahydrofuran-2-yl]-9H-purin-6-yl}benzamide (IV) (0.010 mole). Charge sodium azide (0.04 mole) under inert atmosphere at 20-30°C. Raised the reaction mass temperature to 80-90°C and stirred the reaction mass 4 hrs at same temperature. TLC in H2SO4 stain monitored the reaction. After reaction completion, added ice-cold water20 v and stired for 30 min at 5-10°C. Filtered, and washed wet solid with chilled water. Solid wet cake was dried 60-65°C. The off-white solid compound formula (III) Yield 81.0%.

IR (KBr, vmax, cm-1) 3321, 3116, 2858, 1697, 1261, 1095, 707;
HPLC Purity: 99.06%, MS (ES+) m/z 399.2 [M+1]; Melting range 176-178°C.
H NMR (400 MHz, DMSO-*d*6) : 3.58-3.73 (m, 1H), 3.74-3.77 (m, 1H), 4.11-4.12 (s, 1H), 4.72- 4.82 (m, 1H), 5.59-5.73 (s, 1H), 5.96-5.97 (s, 1H), 6.40-6.45(m, 1H), 7.53-7.57 (m, 2H), 7.62-7.64 (m, 1H), 8.03-8.05 (m,2H), 8.64-8.66 (s, 1H), 8.78 (s, 1H), 11.27 (s, 1H). **^{13}C NMR (100 MHz, DMSO-*d*6):** 166.2, 162.9, 152.5, 152.1, 151.1, 143.9, 133.6, 133.0, 129.0, 128.9, 126.1, 86.2, 84.2, 82.0, 69.8, 40.4.

Synthesis of N-{9-[(2R,3R,4R,5R)-5-(aminomethyl)-3-fluoro-4-hydroxytetrahydrofuran-2-yl]-9 H-purin-6-yl} benzamide (II)
Charged Tetrahydrofuran 8v in a clean and dried glass assembly. Charged N-{9-[(2R, 4R,5R)-5-(azidomethyl)-3-fluoro-4-hydroxytetrahydrofuran-2-yl]-9H-purin-6-yl}benzamide (III) (0.05 mole) at 5-10°C.Charge triphenyl phosphine (0.055 mole). Charged water 0.5v and Raised the reaction mass temperature to 15-20°C and stir for 25-30 hrs. Reaction monitored by TLC (mobile phase Ethyl aceate: Hexane) .After reaction completion water was added slowly into the reaction mass in dropwise manner at 20-30°C. Stirred the reaction mass 10-12 hrs at 20-30°C, and white precipitate was observed. Filtered the reaction mass and washed with mixture of methanol and water. The wet material dried under reduced pressure at 35-40°C. White crystalline solid compound (II) yield 78.0%.

HPLC purity : 98.5%, MS (ES+) m/z 373.2 [M+1], Melting range 158-150°C.
IR (KBr, vmax, cm-1) :3304, 2918, 2848, 1681, 1456, 1082, 740;
H NMR (400 MHz, DMSO-*d*6) : 2.49-2.90 (m, 1H), 2.91-3.01 (m, 1H), 3.04 -3.07 (s, 1H), 3.15 (s, 1H), 4..04- 4.59 (m, 1H), 4.60- 4.67 (m, 1H), 5.53- 5.55 (s, 1H), 5.67-5.68 (s, 1H), 6.21-6.27 (d, 1H), 6.36-6.41(d, 1H), 7.37 (d, 1H), 7.53-7.57 (m, 2H), 7.62-7.66 (m, 1H) 8.05-8.15 (m, 2H), 8.76-8.77 (s, 2H), 11.27 (s, 1H). **^{13}C NMR (100 MHz, DMSO-*d*6):** 166.3, 152.5, 152.1, 151.0, 143.9, 134.0, 132.9, 129.1, 128.9, 126.5, 87.5, 86.0, 82.5, 69.3, 40.5.

The HPLC purity of compound (V), IV), (III) and (II) was performed by separating a simple method by high performance liquid chromatography (HPLC) with the following condition.

Chromatographic parameters:
Instrument: HPLC equipped with UV/PDA detector software

Mode: Gradient
Column : YMC-Pack ODS-AQ (250 mm X 4.6 mm), 5 ⌈m
Wavelength: 260 nm

Synthesis of formula N-(9-{(2R,3R,4R,5R)-5-[-(heptadecylamino)]-3-fluoro-4-hydroxytetrahydrofuran-2-yl}-9H-purin-6-yl) benzamide (I)

Charged dichloromethane 5v and methanol 5v followed by activated molecular sieve and stirred for 4 hrs at 25-30°C under nitrogen atmosphere. Then checked the moisture content it should be less than 0.05%. Charged N-{9-[(2R,3R,4R,5R)-5-(aminomethyl)-3-fluoro-4-hydroxytetrahydrofuran-2-yl]-9H-purin-6-yl}benzamide (0.05 mole). Then Diethyl amine (0.06 mole) added slowly into the reaction mass. Stirred the reaction mass for 2.0 hrs at the same temperature. Charged heptadecanal (0.06 mole) and raised the reaction mass temperature to 40°C. Stirred the reaction mass for 6-12 hrs at 35-40°C.Check reaction progress by TLC (Mobile phase (Ethyl acetate 5: Hexane5). After reaction completion. Distilled out the reaction mass under reduced pressure and charged dichloromethane and stir for 30 min at 25-30°C, the clear solution of reaction mass observed. Charged aqueous solution of sodium bicarbonate solution and stirred for 30 minutes and lower organic layer separated. Washed the aqueous layer twice with dichloromethane. Combine both organic layer and evaporated under reduced pressure to get Crude product. The crude compound purified by column chromatography with using 60-120 silica ethyl acetate in Hexane to elute to get a pure product. The off-white solid compound (I) Yield: 70%.

HPLC Purity: 99.5%, MS (ES+) m/z 611.2 [M+1], Melting range 98-100°C.
H NMR (400 MHz, DMSO-*d*6) □: 0.81-0.92 (d, 3H), 1.12-1.35(d, 31H), 1.60-1.69(s, 2H), 2.51-2.65(s, 2H) 3.26-3.32(m, 1H), 3.30-3.38(m, 1H), 4.10-4.13 (s, 1H), 4.61- 4.71 (m, 1H), 5.58-5.82(d, 1H), 6.30-6.42 (t, 1H), 7.74-7.86 (m, 3H), 8.15-8.25(d, 2H), 8.72-8.76 (d, 2H). **^{13}C NMR (100 MHz, DMSO-*d*6):** 166.1, 152.1, 152.0, 151.0, 143.9, 133.7, 132.9, 129.0, 128.9, 126.3, 92.6, 86.9, 86.6, 82.0, 70.5, 70.3, 50.4, 49.5, 40.5, 40.3, 39.7, 39.5, 31.8, 29.5, 29.4, 29.3, 28.9, 27.0, 22.5,14.4.

The purity of formula (I) was determined by separating a simple method by high performance liquid chromatography (HPLC) under the following condition.

Chromatographic parameters:

Instrument	:	HPLC equipped with UV/PDA detector and suitable software
Mode	:	Gradient
Column	:	ACE 5 C8 (150 mm X 4.6 mm), 5⌈m
Wavelength	:	230 nm

RESULTS & CONCLUSION:

In summary, our discovery is to introduce new derivative of adenosine N-(9-{(2R,3R,4R,5R)-5-[-(heptadecylamino)]-3-fluoro-4-hydroxytetrahydrofuran-2-yl}-9H-purin-6-yl)benzamide (I).We demonstrated a facile route to synthesize formula -I starting from and (2R,3R,5R)-5-(6-amino-9H-purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol (2'-Fluoro-2'-deoxya-denosine). Knowledge of the different possible metabolites and their synthetic routes. This new molecule may have potential as an aspirant, to be designed as a novel therapeutic against challenging diseases like antiarrhythmic, cancer, and diabetes, anti-inflammatory.

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REFERENCES:

1. George S. Mahler, in Analytical Profiles of Drug Substances and Excipients, 1998.
2. Gutierrez, M., Valdes, F., Luna, V., Arevalo, B., & Brown, N. (2018).
3. Cristalli, G.; Eleuteri, A.; Vittori, S.; Volpini, R.; Lohse,
4. M.J.;Klotz, K.N. 2-Alkynyl derivatives of adenosine and adenosine-5'-
5. J. Med. Chem., 1992, 35(13), 2363-2368.
6. Van Schaick, E.A.; Mathôt, R.A.A.; Gubbens-Stibbe, J.M.;
7. Langemeijer, M.W.E.; Roelen, H.; IJzerman,
8. Johnston-Cox, H.A.; Ravid, K. Adenosine and blood platelets. Purinergic Signal., 2011, 7(3), 357-365.
9. Ciancetta, A.; Jacobson, K.A. Structural probing and molecular modeling of the A3 adenosine receptor: A Focus on agonist binding. Molecules, 2017, 22(3), 449.
10. Maruyama, T., Utzumi, K., Sato, Y., & Richman, D. D. (1994). Synthesis and Anti-HIV Activity of 6-Substituted Purine 2′-Deoxy-2′-fluororibosides. Nucleosides and Nucleotides, 13(1-3), 527–537. doi:10.1080/15257779408013260.