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Synthesis, Characterization and Antimicrobial Activity of Novel Substituted Purine Derivatives

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Abstract

In the present work, three new compounds were synthesized (49a, 49b, 51). Compounds were synthesized as the reaction sequences outlined in scheme1. Nitration of the theophylline was carried out as per the literature method. In this theophylline was reacted with the 65% HNO_3 in presence of the acetic anhydride at the 0-5°C. Acetic anhydride was used as a dehydrating agent in the nitration reactions. Reaction was carried out at a controlled temperature because; the high temperature may cause polynitration and oxidative breakdown of aromatic ring. Product was characterized by the FTIR, ^1H NMR and mass spectra. The N-alkylation of 8-nitro theophylline can be done by using 1-chloro3-iodopropane as alkylating agent and K_2CO_3 as base, at controlled temperature condition to prevent polymerization and get better yield. Then the reaction of 7(2-chloro ethyl) 8-nitro theophylline, substituted aryl amines and KI was carried out in microwave oven at 140W for 15 minutes to obtain 1,3-dimethyl-7-{2-[(4-aryl amino] ethyl}-8-nitro-3, 7-dihydro-1*H*-purine-2, 6-dione.

Keywords: Purine, Theophylline, 8-nitrotheophylline, Anti-microbial activity.

INTRODUCTION:

Broadly, all microbes that can grow in the absence of oxygen are called anaerobic bacteria. They include clostridia, a spore-bearing anaerobe, and Gram-negative bacteria like bacteroides and fusobacteria. Microbes that require oxygen to grow are called aerobic bacteria. Those that grow in the presence of some oxygen (but not a lot) are called microaerophilic; they include *E. coli, neisseria, haemophilus* and others. Gram (1853-1938) classified all bacteria by the colour they take with the Gram's stain. Those that take a blue colour (Gentian violet) are called Gram-positive, and those that take the red stain (eosin) are called Gram-negative.[1]

Diseases caused by bacteria, viruses, fungi and other parasites are major causes of death, disability, social and economic disruption for millions of people.[2] Infectious diseases raise awareness of our global vulnerability, the need for strong health care systems and the potentially broad and borderless impact of disease.[3] According to World health statistics 2008 report published by WHO, the infection will be one of the most serious problem in 2030.[4]

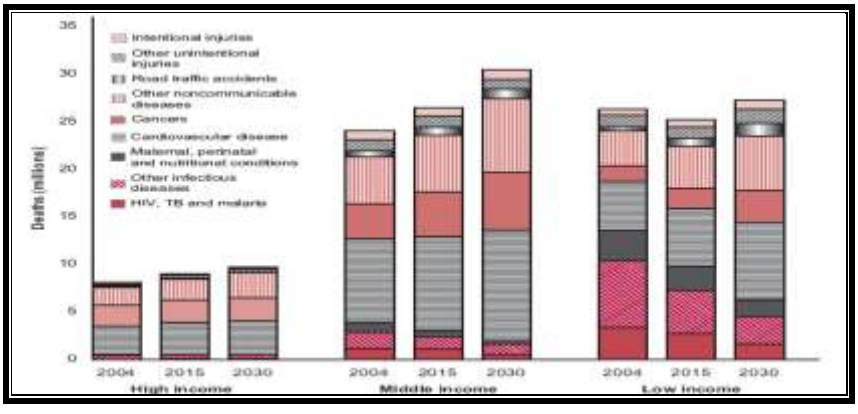


Figure 1: Epidemiology of infection world wide

The human body exists in a state of dynamic equilibrium with microorganism. In a healthy individual this balance is maintained as peaceful co-existence and lack of disease. But sometimes, micro-organisms cause an infection or a disease.

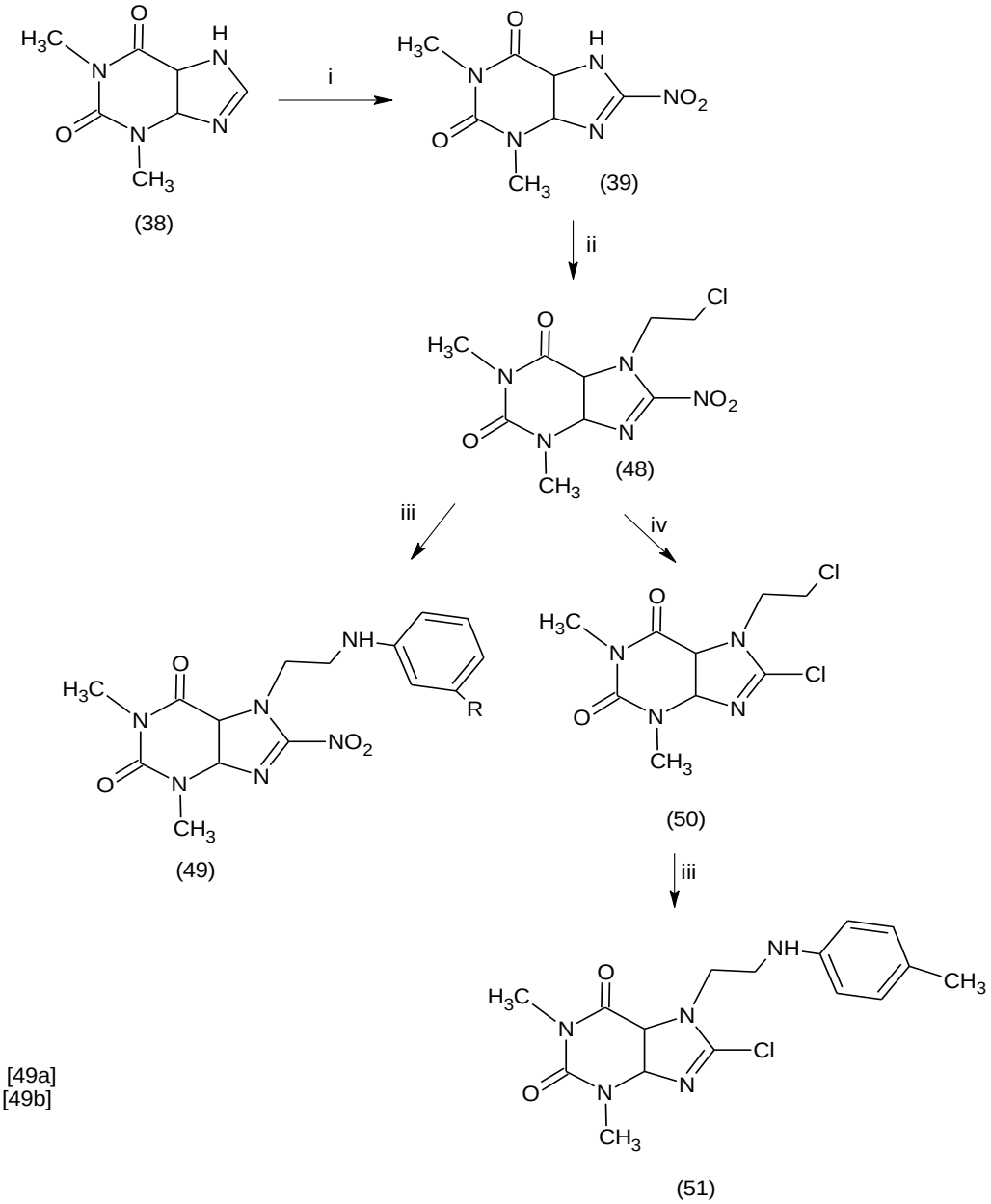
MATERIALS AND METHODS:

Materials:

All chemicals were procured from, Rankem, E-Merck, Qualigens, Hi-Media, and S.D. Fine chemicals. All solvents were redistilled and dried before use. Reactions were routinely monitored by thin layer chromatography and spots were visualized by exposure to iodine vapour or UV light. All the synthesized compounds were purified by column chromatography followed by recrystallization. Melting points were determined by using open capillary method and are uncorrected. Fourier Transform Infra Red spectra (FTIR) were recorded on Shimadzu FTIR-8400S spectrophotometer using potassium bromide pellets and sodium chloride cell. Nuclear Magnetic Resonance spectra (^{1}H-NMR) were recorded on JEOL-300 MHz spectrophotometer in $CDCl_3$ using TMS as an internal standard. Chemical shifts (δ) are expressed in parts per million (ppm). Mass spectra were recorded on HEWLETT 180017, PACKARD GCD System mass spectrophotometer using electron ionization detector.

Methods:

Synthetic Scheme:



i- Acetic anhydride, 65% HNO_3

ii- K_2CO_3, 1-chloro-2-iodo ethane, DMF, 80^{0}C

iii- ACN, KI, Substituted aryl amines, MW 140W for 15 minutes

iv- Concentrated HCl

SYNTHETIC PROCEDURE

Synthesis of 8-nitro theophylline [39][29]

In acetic anhydride (50 ml) theophylline (38) (5.4g, 30 mmole) was suspended and after cooling to 0-5^{0}C, 65% nitric acid (4.5ml, 10 mmole) was added drop wise with stirring and continued stirring for 2 hours. The mixture was heated to 40^{0}C for 10 minutes and then cooled to room temperature. The reaction mixture was then diluted with 100 ml of Ethanol: water (1:1) and precipitate obtained was collected at pump. A recrystallized sample was obtained from 0.5 N HCl in the form of yellowish crystals.

Synthesis of 7(2-chloro ethyl) 8-nitro theophylline [48][29]

In anhydrous dimethylformamide (DMF) (30ml), 8-nitrotheophylline (39) (1.5g, 6.7 mmoles), potassium carbonate (1.0g) and 1-chloro-2-iodo ethane (1ml, 16 mmoles), were heated to 80^{0}C for 30 minutes and subsequently stirred at room temperature for 36 hours. The solvent was evaporated under reduced pressure using rotary evaporator. The residue was collected, subjected to column chromatography using silica gel (60-120#) as a stationary phase and Ethyl acetate: n-hexane (30:70) as a mobile phase, pure compound **48** was obtained. R_f value = 0.5

Synthesis of 1, 3-dimethyl-7-{2-[(4-methylphenyl) amino] ethyl}-8-nitro-3, 7-dihydro-1*H*-purine-2, 6-dion [49a][30]

To the solution of 7(2-chloro ethyl) 8-nitro theophylline (48) (0.100g, 0.44mmoles), in acetonitrile (ACN) (3ml) in microwave vial *p*-toluidine (0.107g, 0.9mmoles) and potassium iodide (0.005g, 0.1 mmoles) were added. The vial was sealed and irradiated in microwave oven at 140W for 15 minutes. Solvent was evaporated under reduced pressure using rotary evaporator. The residue was collected, subjected to column chromatography using silica gel (60-120#) as a stationary phase and Toluene: Ethyl acetate (80:20) as a mobile phase, pure compound **49a** was obtained. R_f value = 0.6

Synthesis of 1,3-dimethyl-8-nitro-7-[2-(phenylamino)ethyl]-3,7-dihydro-1*H*-purine-2,6-dione [49b][30]

Procedure same as 49a, only instead of *p*-toluidine the amine used was aniline. Pure compound **49b** was obtained. R_f value = 0.67

Synthesis of 7(2-chloro ethyl) 8-chloro theophylline [50][29]

In RBF concentrated hydrochloric acid (15ml) and 7(2-chloro ethyl) 8-nitro theophylline (48) (0.3g, 13.3 mmoles) was refluxed for 30 minutes. The reaction mixture was then treated with water (10ml) and the pH adjusted to 5 by ammonia. The precipitate was collected, recrystallized from hot water (20ml) and colorless crystals was obtained.

Synthesis of 8-chloro-1,3-dimethyl-7-{2-[(4-methylphenyl)amino] ethyl}-3,7-dihydro-1*H*-purine-2,6-dione [51][30]

Procedure same as 49a, only 7(2-chloro ethyl) 8-chloro theophylline (50) was used instead of 7(2-chloro ethyl) 8-nitro theophylline (48). Pure compound **51** was obtained. R_f value = 0.5

ANTI-BACTERIAL ACTIVITY:

All the compounds synthesize in the present investigation willscreen for their anti-bacterial activity by Cup plate Method. Antibacterial activities will test on nutrient medium against, Staphylococcus aureus, and Escherchia coli which are representative types of gram positive and gram negative organisms respectively. The antibacterial activities of the compounds will assess by disc-diffusion method.

Preparation of test solutions:

10 mg of the compound will dissolve in 10 ml of DMF. From this 1 ml of solution willtake and dilute up to 10 ml with DMF. Now the concentration of the test solution will 100 µg/ml. From the stock solution 1ml of solution will take and dilute with 1ml of DMF now the concentration is 50µg/ml[31].

Preparation of Standard Antibiotic Solution:

Amoxicillin will use as standard antibiotics for comparison and solutions will prepare by using sterile water, as they were water-soluble. The solutions are dilute by using sterile water so that the concentrations of the solutions will 100 µg/ml and 50 µg/ml.

Method of Testing:

The sterilize media will cool to 45^{0}C with gentle shaking to bring about uniform cooling and then inoculate with 18-24 hrs old culture under aseptic conditions, mixwell by gentle shaking. This will pour in to sterile Petri dishes (properly labeled) and allow the medium to set. After solidification all the Petri dishes will transfer to laminar flow unit. Then the discs which were previously prepared will carefully kept on the solidified media by using sterilized forceps. These Petri dishes will kept as it is for one-hour diffusion at room temperature and then for incubation at 37^{0}C for 24 hours in an incubator. The extent diameter of inhibition after 24 hours will measure as the zone of inhibition in millimeters.

RESULT AND DISCUSSION:

Table 1: Data for Analysis of synthesize compound

Particulars	Compound Number					
	39	48	49a	49b	50	51
Molecular formula	$C_7H_7N_5O_4$	$C_9H_{12}N_5O_4Cl$	$C_{16}H_{18}N_6O_4$	$C_{15}H_{15}N_6O_4$	$C_9H_{10}O_2N_4Cl_2$	$C_{16}H_{18}N_5O_2Cl$
Molecular weight	225.00 g/mol	289.00 g/mol	358.00 g/mol	343.00 g/mol	201.00 g/mol	347.00 g/mol
Theoretical yield	6.75 g	2.06 g	0.123 g	0.118 g	0.31 g	0.124 g
Practical yield	4.50 g	1.22 g	0.080 g	0.060 g	0.2 g	0.074 g
Percentage yield	66.00 % w/w	59.00% w/w	65.00% w/w	50.00% w/w	67.00% w/w	59.00% w/w
Melting point	282-284^{0}C	126-128^{0}C	144-146^{0}C	242-244^{0}C	128-130^{0}C	118-120^{0}C

The FTIR spectra shows characteristic peaks at 3500.92 cm^{-1} (secondary N-H Stretching), 2956.01 and 2855.71 cm^{-1} (Aliphatic -C-H stretching), 1553.71cm^{-1} (-N-O Stretching), 1325.14 cm^{-1} (-N-C Stretching) and 985.66 cm^{-1} (-C-N stretching for nitro compound). The ^1H NMR spectra shows chemical shifts at 3.205 (*s*, 3H), 3.387 (*s*, 3H), 3.999 (*s*, -NH). The mass spectra shows peaks at *m/z* = 223 (M-2), 169.

When alkyl bromides or alkyl chlorides are used as alkylating agents, the reactions proceed slowly and several polyalkylation/ halogenated by-products are observed.[30] Initially we have carried out alkylation of the 8-nitro theophylline with 1-bromo-2-chloro ethane in DMF at its boiling point. Due to high temperature of reaction it may causing polymerization and desired product was obtained in poor yield. To avoid polymerization and to increase yield reaction was carried out at controlled temperature 80^0C and 1-iodo 2-chloro ethane was used instead of 1-bromo 2-chloro ethane. The product obtained was characterized by FTIR, ^1H NMR and mass spectra. The FTIR spectra shows characteristic peaks at 2956.01, 2923.22 and 2853.78 cm^{-1} (Aliphatic -C-H stretching), 1663.66 and 1709.95 cm^{-1} (-C=O Stretching), 1553.71 cm^{-1} (-N-O Stretching), 1339.61 cm^{-1} (-C-N Stretching), and 747.44 cm^{-1} (-C-Cl Stretching). ^1H NMR shows chemical shifts at 4.979 [*t*, -CH_2 (c)], 3.616 [*s*, -CH_3 (a)], 3.451[*s*, -CH_3 (b)], 3.253 [*t*, -CH_2 (e)] and 2.506 [*m*, -CH_2 (d)]. The mass spectra shows peaks at *m/z* = 169, 88, 59, 43.

Synthesis of 1, 3-dimethyl-7-{2-[(4-methyl phenyl)amino] ethyl}-8-nitro-3, 7-dihydro-1*H*-purine-2, 6-dione was carried out by microwave method in which 7(3-chloro propyl) 8-nitro theophylline, KI and *p*-toluidine irradiated at 140W for 15 minute in ACN as solvent. Compound was characterized by FTIR, ^1H NMR, mass spectra. The FTIR spectra shows characteristic peaks at 3379.40 cm^{-1} (–N-H stretching), 2951.19, 2922.25 and 2854.74 cm^{-1} (Aliphatic –C-H stretching), 1705.13 and 1662.69 cm^{-1} (-C=O Stretching), 1552.67 cm^{-1} (-N-O Stretching) and 1340.57 cm^{-1} (-C-N Stretching). ^1H NMR shows chemical shifts at 7.2627 [*s*, -NH], 7.01 [*d*, -CH_2 aromatic (g_1, g_2)], 6.5045 [*d*, -CH aromatic (f_1, f_2)], 5.005 [*t*, -CH_2 (e)], 3.5953 [*s*, -CH_3 (a)], 3.4377 [*s*, -CH_3 (b)], 3.2406 [*t*, -CH_2 (c)] and 2.2322 [*s*, -CH_3 aromatic (h)]. The mass spectra shows peaks at *m/z* = 169, 149, 92, 88, 59, 43.

Synthesis of 7(2-chloro ethyl) 8-chloro theophylline was carried out by refluxing 7(2-chloro ethyl) 8-nitro theophylline with concentrated HCl for 30 minutes. Product obtained characterized by FTIR spectra which shows characteristic peaks at 2974.33 cm^{-1} (Aliphatic –C-H stretching), 1678.13 and 1629.90 cm^{-1} (-C=O stretching) and 752.26 cm^{-1} (-C-Cl stretching).

Similarly as synthesis of 1, 3-dimethyl-7-{2-[(4-aryl amino]ethyl}-8-nitro-3, 7-dihydro-1*H*-purine-2, 6-dione, synthesis of 8-chloro-1, 3-dimethyl-7-{2-[(4-aryl amino] ethyl}-3, 7-dihydro-1*H*-purine-2,6-dione was carried out. Compound was characterized by FTIR and mass spectra. FTIR spectra shows characteristic peaks at 3344.68 cm^{-1} (-N-H Stretching), 2968.55 and 2852.81cm^{-1} (Aliphatic –C-H stretching), 1697.41 and 1656.91 cm^{-1} (-C=O Stretching) and 744.55 cm^{-1} (-C-Cl Stretching). The mass spectra shows peaks at *m/z* = 169, 149, 92, 88, 59, 43.

Antimicrobial activity:

Zone of inhibition (ZOI):

All the synthesized compounds were evaluated for antimicrobial activity against four bacterial and two fungal strains against chloramphenicol and nystatin as standard. Antimicrobial susceptibility testing was performed by the standardized well method at the concentration of 100µg/well, measuring the zone of inhibition after 24 hours of incubation. The results of antimicrobial screening are given in table below.

Table 2: Zone of Inhibition of compounds (49a, 49b, 51)

Comp. Name	Zone of inhibition (mm)					
	Antibacterial activity (100 µg/well)				Antifungal activity (100 µg/well)	
	E. coli	*P. aeruginosa*	*B. subtilis*	*S. aureus*	*A. niger*	*C. albicans*
49a	22.65	16.8	7.8	10.52	30.38	25.6
49b	20.52	13.14	15.87	17.6	24.08	22.78
51	12.4	12.6	9.23	12.97	-	-
Chloramphenicol	30.1	25.2	30.1	33.1	NA	NA
Nystatin	NA	NA	NA	NA	20.5	22.1

NA – Not applicable

All of the compounds tested have moderate antibacterial and good antifungal activity when compared with the reference drug. Among which the 49a, 49b good antifungal activity against *A. niger and C. albicans.*

CONCULSION:

The N-alkylation of 8-nitro theophylline can be done by using 1-chloro2-iodoethane as alkylating agent and K_2CO_3 as base, at controlled temperature condition to prevent polymerization and get better yield. Then the reaction of 7(2-chloro ethyl) 8-nitro theophylline, substituted aryl amines and KI was carried out in microwave oven at 140W for 15 minutes to obtain 1,3-dimethyl-7-{2-[(4-aryl amino] ethyl}-8-nitro-3, 7-dihydro-1*H*-purine-2, 6-dione. All the compounds were evaluated for antimicrobial activity against gram positive bacteria *Bacillus subtilis, Staphylococcus aureus*, gram negative bacteria. The presence of electron withdrawing group at position 8 is important for the antimicrobial activity. All the three compounds showed moderate antibacterial and good antifungal activity.

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