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Synthetic Studies and Antibacterial Activity of Nucleobases and their N- and S-Glucosides from 2-Amino Benzoic Acid and its Benzamido Derivatives

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ABSTRACT

A series of S-glucosides 11a-14a and their benzamido derivatives 11b-14b have been synthesized by reacting D-glucose with thiol groups of 5-(2'-aminophenylene)-1,3,4-oxadiazole-2-thioles 7(a,b), 5-(2'-aminophenylene)-1,3,4-thiadiazole-2-thiols 8(a,b), 5-(2'-aminophenylene)-1,2,4-triazole-3-thiols 9(a,b) and 5-(2'-aminophenylene)-4-N-amino-1,2,4-triazole-3-thiols 10(a,b). The thiols 7(a,b)-10(a,b) have been synthesized from hydrazides 3(a,b) which already been synthesized from 2-aminobenzoic acid and its benzamido derivative. All synthesized compounds were characterized by IR, UV,^{1}H- and^{13}C- NMR. Nucleobases and a representative of S-glycoside were tested *in vitro* against the following microorganisms: two Gram-positive bacteria *Staphylococcus aureus* and *Bacillus cereus*and two Gram-negative bacteria *Escherichia coli, Pseudomonas aeruginosa* and they exhibited significant effects. Amykacine was used as positive standard.

Keywords: Nucleobases, glycosides, benzamide derivatives, synthesis, antibacterial activity.

INTRODUCTION

Natural nucleosides and glycosides are glycosylamines consisting of nucleobases (often referred to as pyrimidine and purine derivatives). They are bound to a ribose or deoxyribose sugar via a beta-glycosidic linkage.[1] In molecular biology, several analogues of the sugar backbone exist e.g. locked nucleic acid (LNA) and peptide nucleic acid (PNA) or having different nucleobases like morpholine.[2]In medicine several nucleosides analogues are used as antiviral,[3] anticancer,[4] antibacterial[5] and antifungal[6] agents. Synthetic modified nucleosides designed to pair in unusual ways with natural or unnatural nucleobases have many potential applications in biology; biotechnology and medicine have been reported.[7]

Since the discovery of nucleoside ribavirin[8] which made of triazole as its nucleobase, proved that it is not only possesses inhibitory activity against a range of DNA and RNA viruses[9] but also displays antitumor activity in mice.[10]

The modern synthesis of nucleosides and glycosides covered almost any kind of sugar-beta-glycoside linkage and heterocyclic rings. Recently many C-, O-, N- and S-glycosides[11-14] have received considerable attention, because they are widely employed as biological inhibitors, inducers, and ligands for affinity chromatography for carbohydrate processing-enzymes and proteins[15].

Much attention has been focused on thiol derivatives of1,3,4- oxa, thia-diazoles, 1,2,4-triazole and amino- 1,2,4-triazolefor their broad-spectrum activities, such as antitumor, anticonvulsant, antifungal[16-19], herbicidal, and plant growth regulatory activities[20].

Till now, many of these diazole derivatives have been synthesized, and some of them have been patented for commercial uses [21]. The synthesis and biological evaluation of N-glucosides[16,22] and C-glucosides containing 1,2,4-triazole have been greatly emphasized, but only a few S-glucosides containing nucleobases of five membered heterocycles with three hetero atoms have been reported.

Recently, it has been reported that 4-benzamidobenzoic acid hydrazide derivatives exhibited considerable *in-vitro* soluble epoxide hydrolase inhibitory activity[23,24]. We also observed that the benzamidobenzoic derivatives of all synthesized intermediates had better solubility and showed some different biological effects.

EXPERIMENTAL

General

All reactions monitored by TLC, silica gel F254, made by Merck, Germany. The melting points measured with a BÜCHI 540 melting point apparatus and are uncorrected. The IR spectra exhibited as (wave number: v cm-^{1}) were recorded using KBr discs in a JASCO V-530 spectrophotometer at University of Oran-1, Algeria. The UV spectra were recorded on a ZUZI Split-Beam UV-Vis 4418PC (4418SPC) spectrophotometer exhibited as (λmax in nm).The ^{1}H NMR and ^{13}C NMR (250 MHz) spectra in DMSO-*d6* exhibited as (δ/ppm) were recorded at University of Oran-1, Algeria.

Microorganisms in this study supplied by the university hospital of Oran and identified by the laboratory of applied microbiology, University of Oran-1. The Mueller Hinton medium supplied by (Difco).

Synthesis

General procedures for esterification of 2-amino benzoic acid. (2-Amino-methyl and 2-amino-ethyl benzoates 2A and 2B)

Methyl esterification, catalysed by Argil (Montmorillonite) to give 2-amino methylbenzoate2A

An acid 1 (7g., 0.051 mole) dissolved in methanol (200 ml) added to it dried argil – preheated to 100°C for 1 hr (7.0g) and the mixture was refluxed on water bath with an aid of magnetic stirring for 4 hr. After cooling, a saturated aqueous solution of NaOH (20ml) added and extracted twice with dichloromethane (20 ml). The combined extracts dried over anhydrous Na_2SO_4, filtered and evaporated to dryness to give 2-aminomethylbenzoate 2A as colourless syrup (5.1 g., 65%). Lit m.p. 24°C. IR, ν_{max}/ cm^{-1} 3427 , 1729 (CO).

Ethyl esterification catalysed by $H_2SO_4$to give 2-amino ethylbenzoate 2B

An acid 1 (5g. 0.02 mole) was dissolved in ethanol (200 ml) added to it, drops of H_2SO_4 (5 ml.). The mixture heated under reflux at 80°C for 8 hours. The reaction was monitored by TLC eluted with chloroform/cyclohexane (6/4). The reaction mixture was cooled down to room temperature and neutralized by solid $NaHCO_3$, filtered off and washed with alcohol (2 ml.). The filtrate was dried over $MgSO_4$ anhydrous, filtered and washed with alcohol (2ml), the combined filtrates were evaporated to dryness to give 2-amino ethylbenzoate 2B as colourless syrup (1.8 g., 30%).Lit m.p. 13°-15°C. IR,ν_{max}/ cm^{-1} 3340 (NH_2), 1670 (CO) , UV , λmax 192 nm. Logε 0.117; 194 nm. logε 0.119; 222nm. logε2.067; 248 nm. logε0.763 ; 336 nm. logε0.542

Benzoamidation of 2- amino benzoic acid (2-Benzoamido benzoic acid (2C)

2-Amino benzoic acid 1(5.0 g, 0.033 mole), dissolved in chloroform (100 ml), benzoyl chloride (50 ml) was added gradually, and the mixture was cooled into an ice bath. Pyridine (5.0 ml) was added dropwise with an aid of stirring. The reaction mixture was refluxed at 110°C for 10 hr. Reaction was monitored by TLC ($CHCl_3$/cyclohexane, 6:4). Solvents were distilled under vacuum to give crystalline solid which on recrystallization from ethanol/hexane gave a colourless crystalline 2-benzamido benzoic acid (2C) (07.92 g, 90 %):M.p. 182-183°C. IR,ν_{max}/ cm^{-1} 3332 (NH), 1697(O-C=O), 1672 (N-C=O).

Esterification of 2-benzoamido benzoic acid (2C), (2-Benzamidomethylbenzoate (2D)

2-Benzoamido benzoic acid (2C) was esterified with methanol and H_2SO_4–as above- to give white crystalline 2- bezamidomethylbenzoate (*2D)*, recrystallized from chloroform to give (4.5 g, 85.6%). M.p.92-94°C. IR,ν_{max}/ cm^{-1} 3340 (NH), 1721 (O-C=O), 1672 (N-C=O).

2-Benzamidoethylbenzoate (2E)

2-Benzoamido benzoic acid (2C) was esterified with ethanol and H_2SO_4 to give white crystalline 2- bezamidoethylbenzoate (*2E)* as white crystalline, recrystallized from chloroform to give (3.7 g.,66 %): M.p.94-98°C. IR,ν_{max}/ cm^{-1} 3340 (NH), 1720 (O-C=O), 1672 (N-C=O).

General procedure for preparation of hydrazides 3a and 3b

The esters 2(A-D) (3 g.) dissolved in ethanol (80 ml), added to it dropwise a hydrazine hydrate 64% (5ml.) and the mixtures were heated under reflux on a water bath 90°C (20 hr. for 2(A-C) and 12 hr for 2D. The reaction monitored by TLC. The solvents were evaporated under reduced pressure to give white solids which were washed with a small quantity of ether (few mls) to give corresponding crystalline products

2-aminobenzoichydrazide (3a): White crystalline (2.4 g. from 2A and 2.7 g. from 2B, 90%): M.p. 119-122°C*;* IR,λ_{max}/ cm^{-1} 3333 (NH), 2626 (SH), 1631 (N-CO), 1610 (N-N-C=O), 1382 (C=S). UV ,λ_{max} 192, 195, 208, 214, 216, 248, 326, loge 0.539, 0.617, 2.814, 3.077, 3.039, 1.451, 0.68 respectively. ^{1}H-NMR.$_{\delta H}$ 11.2 (s, 1H,NH),7.9-6.2 (m.4H,H_{ar}), 5.42 (s, H, NH), 4.1 (d, 2H, NH_2), 3.3 (SH). ^{13}C_NMR, 205 (C=S), 170.27 (N=C-SH), 169 (N-C-O-), [132.76, 126.89, 117.30, 116.80, 77.44, 76.60 (C_{ar})].

2-Benzamido benzoic hydrazide (3b)*:* White crystalline (2.5 g., from 2D and 2.3 g., from 2E, 84%). M.p. 195°C.IR,ν_{max}/ cm^{-1} 3342 (NH),2626 (SH),1630 (N-C=O).1610 (N-N-C=O), 1399 (C=S),UV, ^{1}H and ^{13}C-NMR are similar to above.

General procedure for preparation of 5-(2'-benzamidophenylene)-1,3,4-oxadiazole-2-thiones 4(a,b) or thioles7(a,b)

The hydrazides 3a, (0.26 g.0.002 mole) or 3b,(1.0g., 0.004 mole) in an ethanolic solution of KOH 85% (from KOH ,0.5 g. in ethanol 80 ml), added to them dropwise CS_2 (2.5 ml for 3a and 10ml for 3b) and the mixture was refluxed at 80°C for 7 to 22 hr. The bulk of the solvents removed under vacuum at 50°C, the remaining solid washed with iced diluted HCl in filter paper. The solid dissolved in a small amount of ethanol then left into the refrigerator to give fine crystalline products:

5-(2'-aminophenylene)-1,3,4-oxadiazole-2-thiones(4a or 7a)

White crystalline (0.233 g, 70%), m.p. 210°C .IR,ν_{max}/ cm^{-1} 3410.2 strong and broad (free and bonded NH), 2626 (SH), 1632 (C=N), 1403 (C=S).UV λ_{max} 240, 350 nm. loge 6.0, 6.0 respectively. NMR.$_{\delta H}$ 9.7 (s, 1H, NH $_{oxadiazole\ ring}$), 7.1-6.65 (m, 4H, H_{ar}) ,6.2 (s,2H,NH_2), 3.4 (s, 1H,SH).^{13}C-NMR (δ/ppm): 205.54(C=S), 162.11(C=N), 164.56, 155.4, 132.16, 129.8,126.5, 116.94, (C_{ar}).

5-(2'-benzoamidophenylene)-1,3,4-oxadiazole-2-thiones(4b or 7b)

White crystalline (0.90 g, 78%), m.p. 278°C. IR,ν_{max}/ cm^{-1},3333 strong and broad (free and bonded NH), 2626 (SH), 1502 (C=O), 1382 (C=S).UV λ_{max} 242, 348 nm. loge 6.0, 6.0 respectively. ^{1}H-NMR (δ/ppm) 10.2 (s, 1H, NH $_{oxadiazole\ ring}$), 7.8-6.30 (m, 9H, H_{ar}) , 6.2 (s,H,NH), 3.4 (s, 1H,SH).^{13}C-NMR (δ/ppm): 198.56(C=S), 164.34(C=N), 166.36-,115.23(C_{ar})

General procedure for preparation of 2-aminophenylenethiosemicarbazides 5a and 5b

The hydrazides 3a (2.0 g., 0.013 mole) and

3b (1.0 g., 0.003 mole) dissolved in ethanol (200 ml for 3a and 80 ml for 3b) added to it NH_4SCN (1.7 g., for 3a and 0.85 g., for 3b). HCl (15 ml for 3a and 10 ml for 3b were added dropwise, refluxed at 80°C for 14 hr. Bulk of ethanol was removed by vacuum, the thiosemicarbazides 5a and 5b were precipitated, filtered off and recrystallized from methanol to give:

2-Aminophenylenethiosemicarbazide(5a). 2.4 g., 86%. M.p. 180°C.IR,ν_{max}/ cm^{-1} 3290 (NH), 1631 (C=O), 1404 (C=S).

2-Benzamidophenylenethiosemicarbazide(5b). 0.98 g., 80%. M.p. 222°C.IR,ν_{max}/ cm^{-1} 3233 (NH), 1620 (C=O), 1404 (C=S).

General procedure for preparation of thiadiazoles 8a and 8b

The thiosemicarbazides 5a and 5b(1.0g.) were dissolved in absolute ethanol (100 ml), KOH (0.5 g) was added with an aid of strring, followed by addition of CS_2 (1.0ml). The mixture was refluxed at a temperature 75-80°C for 5 hr., cooled and acidified with HCl to pH=1to give :

5-(2'-Aminophenylene)-1,3,4-thiadiazole-2-thiols(8a): white crystalline, (0.8 g., 80%),m.p. 167°C. IR,n_{max}/ cm^{-1} 3453 (NH), 1632 (C=N), 1403 (C=S). NMR.$_{\delta H}$ 9.7 (s, 1H, NH $_{oxadiazole\ ring}$), 7.1-6.65 (m, 4H, H_{phenyl}) , 6.2 (s,2H,NH_2), 3.4 (s, 1H,SH).^{13}C-NMR (δ/ppm): 205.54 (C=S), 162.11 (C=N), 164.56, 155.4, 132.16, 129.8,126.5, 116.94, (C_{ar}).

5-(2'-Benzamidophenylene)-1,3,4-thiadiazole-2-thiols(8b): white crystalline,(O.78 g.,78%),m.p. 267°C. IR,ν_{max}/ cm^{-1} 3290 (NH), 1642(N-C=O), 1402 (C=S). NMR.$_{\delta H}$10.2 (s, 1H, NH $_{oxadiazole\ ring}$), 7.3-6.85 (m, 4H, H_{ar}) , 6.5 (s,2H,NH_2), 3.2 (s, 1H,SH).^{13}C-NMR (δ/ppm): 198.88(C=S), 165.88 (C=O), 162.45 (C=N),[160.65- 110.24, (C_{ar})].

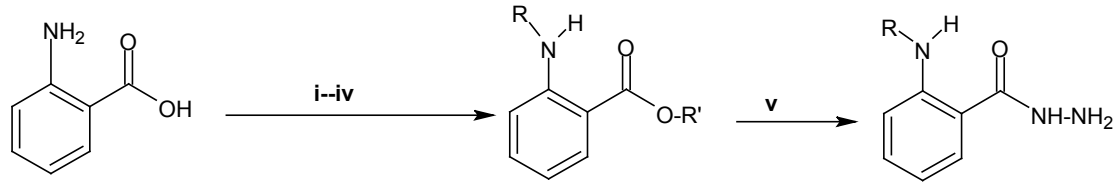
General procedure for preparation of 1,2,4-triazoles 9a and 9b

The thiosemicarbazides 6a (1.0g., 0.005 mole) dissolved in water (100 ml). An aqueous solution of NaOH (0.42 g.NaOH, in 30 ml of H_2O) was added and the mixture wax refluxed for 10 hr. Water was evaporated under vacuum, solid formed, recrystallized from methanol to give brownish crystalline:

5-(2'-Aminophenylene)-1H-1,2,4-triazole-3-thiol(9a): (0.86 g., 89%). M.p. 179°C. IR,ν_{max}/ cm^{-1} , 3426 (NH, free), 3223 (NH, bonded), 2600 (SH), 1631 (C=N), 1403(C=S). ^{1}H-NMR.$_{\delta H}$ 9.5 (s, 1H, NH $_{triazole}$), 7.9-7.3 (m, 4H, H_{ar}) , 3.4 (s, 1H,SH).^{13}C-NMR (δ/ppm): 204.60(C=S), 147.9(C=$N_{triazole}$),[130.56-116.94, (C_{ar})].

The thiosemicarbazides 6b (1.0g., 0.003 mole) dissolved in water (100 ml). An aqueous solution of KOH (0.53g.KOH, in 20 ml of H_2O) was added and the mixture wax refluxed for 8 hr. Water was evaporated under vacuum, solid formed, recrystallized from ethanol to give brownish crystalline:

5-(2'-Benzamidophenylene)-1H-1,2,4-triazole-3-thiol(9b): (0.86 g., 91%). M.p. 252°C. IR, ν_{max}/ cm^{-1} . 3350 (NH, free and bonded), 2604 (SH), 1664 (N-C=O),1631 (C=N),1402 (C=S).



1

2A,R=H;R'=CH_3
2B,R=H;R'=C_2H_5
2C,R=COph;R'=H
2D,R=COph;R'=CH_3
2E,R=COph;R'=C_2H_5

3a,R=H
3b,R=COph

i-CH_3OH,H_2SO_4 ; ii-C_2H_5OH,H_2SO_4;iii-C_2H_5OH;Argile; iv-,PhCOCl;iv-$NH_2NH_2H_2O$

Scheme1: Synthesis of hydrazides3a and 3b

NMR.$_{\delta H}$ 10.2 (s, 1H, NH $_{triazole}$), 7.6-6.68 (m, 9H, H_{ar}), 3.5 (s, 1H,SH).^{13}C-NMR (δ/ppm): 199.60(C=S), 165(C=O),136.8(C=$N_{triazole}$), [132.44-112.94, (C_{ar})].

General procedure for preparation of 4-amino-1, 2, 4-triazoles 10a and 10b

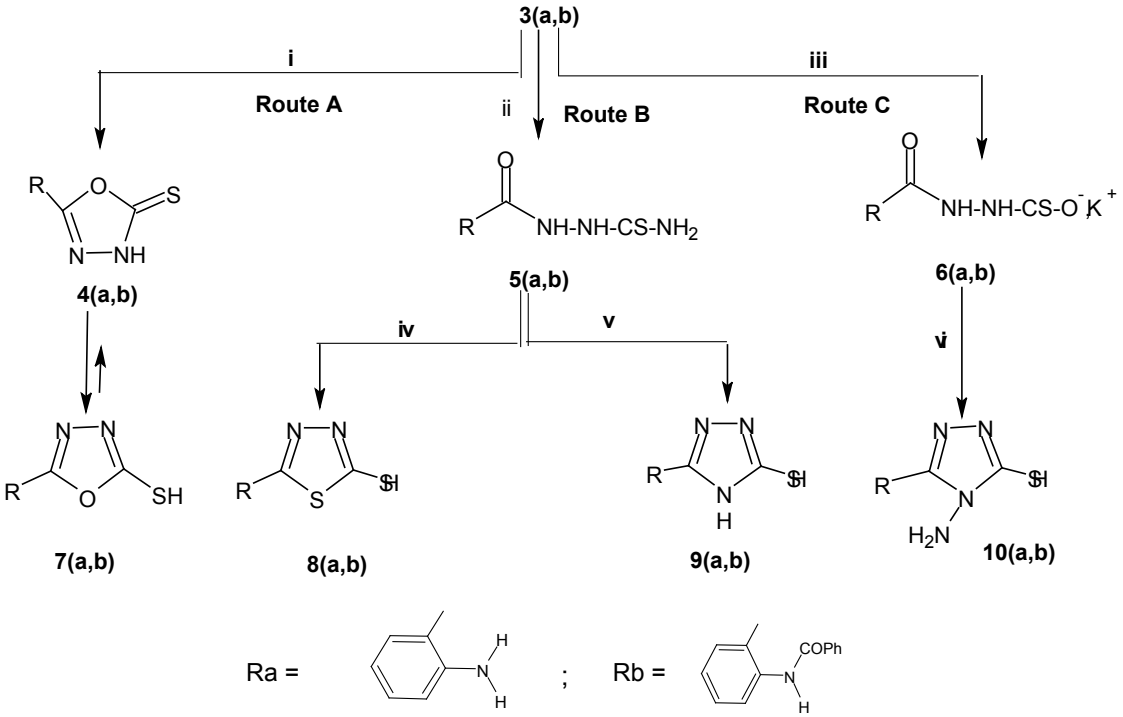
The hydrazides 3a and 3b (1.0 g., 0.007 mole and 0.003 mole respectively) dissolved in methanol (50 ml), KOH (1.86 g) was added followed by dropwise addition of CS_2 (5 ml). The mixture was stirred at room temperature for 15 hr. Diethyl ether (30 ml) added and stirring continued for further 1.0 hr to give a white ppt of salt 6a and 6b. Salts 6(a,b)–individually-, without further identification, were added to it hydrazine hydrate 64% (2 ml) and the reaction mixture was refluxed at 80°C for 8 hr. Cooled to 5°C and acidified with HCl to pH=1 to give colourless crystalline, recrystallized from ethanol :

5-(2'-Aminophenylene)-4-N-amino-1,2,4-triazole-3-thiol(10a)(1.27g., 93%),m.p.160°C;IR, ν_{max}/ cm^{-1} 3423 (NH), 3063 (CH_{ar}), 2473 (SH), 1622 (C=N).NMR.$_{\delta H}$ 8.9 (s, 1H, $NH_{triazole}$), 7.6-6.8 (m, 4H, H_{ar}) , 6.52 (s,2H,NH_2), 3.4 (s, 1H,SH).^{13}C-NMR (δ/ppm): 147.9(C=$N_{triazole}$),[130.56-116.94, (C_{ar})].

5-(2'-Benzoamidophenylene)-4-N-amino-1,2,4-triazole-3-thiol(10b) (1.06 g., 80%), m.p. 225°C.IR,ν_{max}/ cm^{-1} 3290 (NH), 2630 (SH), 1643 (N-C=O), 1624(C=N), 1398 (C=S).^{1}H-NMR.$_{\delta H}$ 9.6 (s, 1H, NH $_{triazole}$), 7.8-6.35 (m, 9H, H_{ar}), 3.2 (s, 1H,SH).^{13}C-NMR (δ/ppm): 202.40 (C=S), 163 (C=O),135.0(C=$N_{triazole}$),[133.44-110.5, (C_{ar})].

General procedure for preparation of S-nucleosides 11(a,b)- 14(a,b)

Thiols 7(a,b)-10(a,b) (0.01 mole) and D-glucose (0.01 mole) were dissolved in ethanol (100 ml) added to it HCl (8 ml) and the mixture was refluxed at 80°C for 5-8 hr. Proceeding of the reaction was monitored by tlc. Volatile solvent removed by vacuum, the syrup or the solids remain were washed with ether to give:



i: CS_2, KOH, Reflux; ii: NH_4SCN; iii: KOH,CS_2,Room temp; iv: KOH, CS_2, EtOH, HCl; : NaOH, H_2O; vi: NH_2NH_2, KOH

Scheme2: Synthesis of nucleobases 7(a, b)-10(a, b) from hydrazides 3(a,b)

5-(2'-Aminophenylene)-1,3,4-oxadiazole-2-S-glucoside (11a), yellow syrup. IR, ν_{max}/ cm^{-1} 3423 (OH), 3290 (NH), 3067 (CH), 2998 (CH_{ar}),1630 (C=N). RMN, 8.3 (s,1H,NH), 8-7.5 (m, 4H, H_{ar}),4.5(m, 4H,O-H); 3.7(m,8H, C-H),3.2(d,2H, H2-C-O).^{13}C-NMR (δ/ppm):167(C=N), 140-120(C_{ar}), 78-72 (C_{sugar})

*5-(2'-Benzamidophenylene)-1,3,4-oxadiazole-2-S-glucoside (11b):*Yellow syrup.IR,ν_{max}/ cm^{-1} 342O (OH), 3330 (NH), 3067 (CH), 2990 (CH_{ar}), 1632 (C=N). ^{1}H-RMN, 12.3 (s,1H,NH), 8-7.2 (m, 9H, H_{ar}),4.0(m, 4H,O-H); 3.7(m,8H, C-H), 3.2(d,2H, H2-C-O).13C-NMR ,171 (C=O), 167(C=N), 136-118(C_{ar}), 76-60 (C_{sugar})

5-(2'-Aminophenylene)-1,3,4-thiadiazole-2-S-glucoside (12a): Yellow syrup, . IR, ν_{max}/ cm^{-1} 3360 (broad,OH and NH), 3070 (CH), 2996 (CH_{ar}), 1632 (C=N).^{1}H-RMN, 8.6 (s,1H,NH), 7.5-6.5 (m, 4H, H_{ar}),4.2 (m, 4H,O-H); 3.57(m,8H, O-C-H), 3.5 (d,2H, H_2C-O).^{13}C-NMR: 175 and 160 (C=N), 150 (C_{ar}), 60 (C_{sugar}).

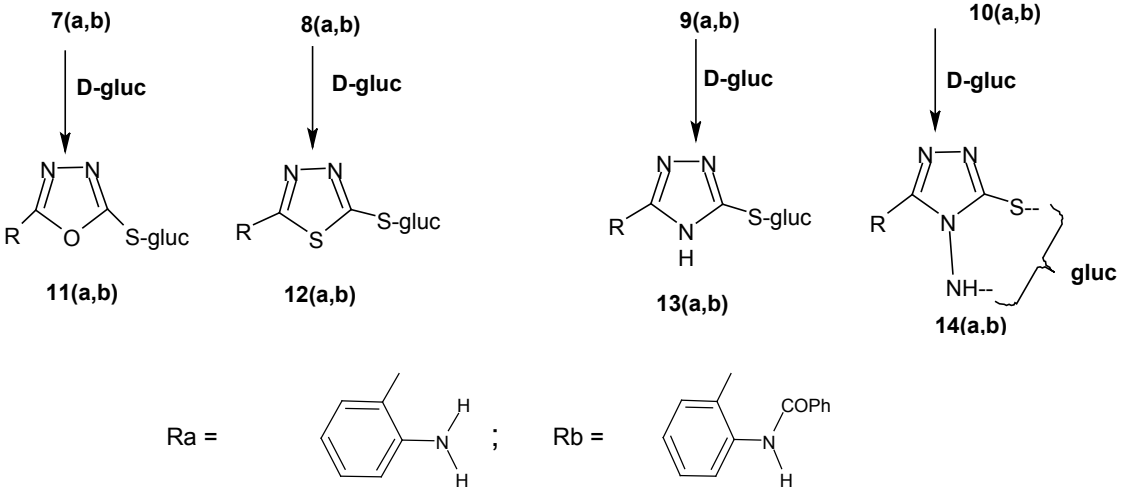
5-(2'-Benzamidophenylene)-1,3,4-thiadiazole-2-S-glucoside(12b): Yellow syrup,IR, ν_{max}/ cm^{-11} 342O (OH), 3330 (NH), 3067 (CH), 2990 (CH_{ar}), 1632 (C=N).^{1}H-NMR, 12.3 (s,1H,NH), 8-7.2 (m, 9H, H_{ar}),4.0(m, 4H,O-H); 3.7(m,8H, C-H), 3.2(d,2H, H_2-C-O).^{13}C-NMR (δ/ppm): 169 (C=O), 164 (C=N), 136-118(C_{ar}), 78-63 (C_{sugar})

5-(2'-Aminophenylene)-1,3,4-triazole-2-S-glucoside (13a) : Recrystallized from ethanol: M.p. 77°C. IR, ν_{max}/ cm^{-}3423 (OH), 3290 (NH), 3067 (CH), 2998 (CH_{ar}), 1630 (C=N).1H-NMR, 8.4 (s,1H,NH), 7.5-7(m, 4H, H_{ar}),4.5(m, 4H,O-H); 3.6(m,8H,H-C-O), 3.3(d,2H, H_2C-O).^{13}C-NMR (δ/ppm):168 and 166 (C=N), 137-133 (C_{ar}), 70 (C_{sugar}).

5-(2'-Benzamidophenylene)-1,3,4-triazole-2-S-glucoside(13b): IR, ν_{max}/ cm^{-1}342O (OH), 3330 (NH), 3067 (CH), 2990 (CH_{ar}), 1632 (C=N). ^{1}H-NMR, 9.5 (s,1H,NH), 7.8-6.7(m, 9H, H_{ar}),5.0(m, 4H,O-H); 3.5 (m,8H, H-C-O), 3.2(d,2H, H_2-C-O).^{13}C-NMR (δ/ppm):169 (C=O), 164 (C=N), 134-120 (C_{ar}), 75-70 (C_{sugar})

5-(2'-Aminophenylene)-4-amino-1,3,4-triazole-2-S-glucoside(14a): recrystallized from ethanol, m.p. 78°C.IR, ν_{max}/ cm^{-1}3423 (OH), 3290 (NH), 3067 (CH), 2998 (CH_{ar}), 1630 (C=N). ^{1}H-NMR, 9.6 (s, 2H, NH_2), 8.2 (s,1H,NH), 8.3-7.6(m, 4H, H_{ar}),4.3(m, 4H,O-H); 3.7(m,8H, H-C-O), 3.2(d,2H, H_2-C-O).^{13}C-NMR (δ/ppm): 169 (C=O), 164 (C=N), 134-120 (C_{ar}), 75-70 (C_{sugar})

*5-(2'-Benzamidophenylene)-4-amino-1,3,4-triazole-2-S-glucoside (14b):*IR, ν_{max}/ cm^{-1} 342O (OH), 3330 (NH), 3067 (CH), 2990 (CH_{ar}), 1632 (C=N).^{1}H-NMR, 9.8 (s,1H,NH2),8.9(s,1H,NH), 8-7.2 (m, 9H, H_{ar}),4.2(m, 4H,O-H); 3.5 (m,8H, H-C-O), 3.5(d,2H, H_2-C-O).^{13}C-NMR (δ/ppm):171 (C=O), 163 (C=N), 134-120 (C_{ar}), 72-70 (C_{sugar})



Scheme 3: Synthesis of N- and S-glycosides 11(a,b)-14(a,b)

The antibacterial Tests

The filter paper disc method was performed in duplicate using fresh Mueller Hinton agar medium. This agar medium was inoculated with 0.5mL of cultures containing about 106 CFU/mL. Filter paper discs (5 mm diameter) saturated with solutions of each compound (concentrations 10µg mL^{-1} DMSO) was placed on the indicated agar mediums. The incubation time was 24 h at 37 °C. The blank test disc with DMSO and positive reference amykacine were used. Inhibitory activity was evaluated by measuring the diameter of clear zone observed around the disc (in mm).

Minimum inhibition concentration (MIC) Tests

Each 1 mL of the original concentration [c] (10 µg mL^{-1}) in DMSO of the compounds7(a,b)-10(a,b) and 12(a,b)were diluted with DMSO for four times to1/2 [c], 1/4[c] , 1/8 [c] ,1/16[c] , and

Table 1: Inhibition of microorganisms by compounds 7(a,b)-10(a,b)and 12(a,b)in mm

Compounds*	Gram positive		Gram negative	
	S.aureus	*B. cereus*	*E.coli*	*P.aeroginosa*
7a	0	20	31	11
7b	10	11	12	0
8a	19	20	0	33
8b	21	0	16	19
9a	0	16	13	12
9b	18	0	17	20
10a	11	19	13	21
10b	19	25	0	11
12a	14		10	
12b	0		0	
Amykacine	20mm	19mm	22mm	0

*concentration 10 µg mL^{-1}

Table 2: minimum inhibition concentrations (MIC)*

Com.	*S. aureus* µg mL^{-1}				*B. cereus* µg mL^{-1}				*E.coli* µg mL^{-1}				*P. aeroginosa* µg mL^{-1}			
	i	ii	iii	iv	i	ii	iii	iv	i	ii	iii	iv	i	ii	iii	iv
7a	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
7b	+	+	-	-	+	+	+	-	+	+	-	-	-	-	-	-
8a	+	+	+	-	+	+	+	+	-	-	-	-	+	+	+	+
8b	+	+	+	+	-	-	-	-	+	+	+	-	+	+	+	-
9a	-	-	-	-	+	+	+	-	+	+	+	-	+	+	+	-
9b	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+
10a	+	+	-	-	+	+	+	-	+	+	-	-	+	+	+	+
10b	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-
12a	+	+	+	+					+	+	-	-				
12b	-	-	-	-					-	-	-	-				

i =1/2.C; ii=1/4.C; iii=1/8.C; iv=1/16.C

*(C= initial concentration = 10 µg mL^{-1})

optical density was measured at 0 Hr, 18 Hr, 24 Hr and 48 Hr.

RESULTS AND DISCUSSION

Synthesis of hydrazides 3(a,b)

Esters and benzamido acid 2(A-E) and the corresponding hydrazides 3(a,b) are the common intermediates for the synthesis described in this work. They should be available in relatively large quantities to utilize for further synthesis. In best opportunity, 2-aminomethylbenzoate 2A was obtained by classical esterification of 2-aminobanzoic acid 1 with methanol catalysed by $H_2SO_4$to give a modest yield (12%). Since 1 showed a better solubility in ethanol, classical esterification of 1 using H_2SO_4 or HCl as catalysts gave a best yield of 2-aminoethylbenzoate 2B in 30% yield. Generally, the yield of both methyl and ethyl 2-aminobenzoates 2(A,B) respectively were considered very poor and unsuitable for stepwise synthesis of nucleobases 7-10(a,b) (see Scheme 1). The yield percentages of esterification been raised firstly by using montmorillonite argil as catalyst and secondly by protecting the amino group *via* N-benzoylation of 1 to give 2-benzamido benzoic acid 2C prior to esterification. Methyl and ethyl 2-aminobenzoates 2(A,B) resulted from argil as catalyst gave higher yield (65%) . Similirlay 2-benzamido methyl and ethylbenzoates 2(D, E) were also produced in (65%) yield.

Hydrazides 3(a,b) were obtained in excellent yields 3a(90%) and 3b (84%) by refluxing the corresponding esters 2(A-E) with hydrazine hydrate 51% in methanol solution. Hydrazides 3a and 3b characterized by IR, UV, ^{1}H-NMR and ^{13}C-NMR (see experimental).

Synthesis of nucleobases 7(a, b)-10(a, b)

The synthesis of heterocyclic thiols (nucleobases) 7(a,b)-10(a,b) have been obtained from the hydrazides3a and 3b into three synthetic routes A, B and C.

Route A: gave rise to oxadiazoles 4a and 4b, which obtained by treating –indvidually- the hydrazides 3a and 3b with ethanolic KOH followed by addition of CS_2 under reflux for 14 hours. IR spectra suggested the presence of keto-enol tautomers 4(a,b) $\rightleftarrows$ 7(a,b) detected by the presence of NH and SH bands in the region 3333 cm^{-1}and 2626 cm^{-1} respectively and for C=S group in 1404cm^{-1}. While the NMR spectra exhibited signals at 11.2 ppm and 3.3 ppm for the NH and the SH respectively while ^{13}C-NMR showed both thione at 205ppmandat170.27 ppm for thiol form (N=C-SH) .

Route B: gave rise to the synthesis of thiadiazole thiols 8(a, b) and triazoles 9(a, b). Reaction of the hydrazides 3(a, b) with ammoniumthiocyanide resulted into formation of thiosemicarbazides 5(a, b) in excellent yields (86% for 5a and 80% for 5b). IR_{5a} spectra showed a broad band cantered at 3290 cm-1 for NH, 1631 for (C=O) and 1404 for(C=S). IR_{5b}, 3233 (NH), 1620 (C=O), 1404 (C=S).

Treatment of 5(a,b) with alcoholic KOH followed by acidification with HCl to pH=1 resulted into very good yields of thiadiazoles 8a in 80% yield and 8b in 78% yield. While treatment of thiosemicarbazides 5(a, b) with aqueous NaOH or KOH furnished 1,2,4-triazole thiols 9(a,b) in excellent yields (89% for 9a, and 91% for 9b).

Route C: dealt with synthesis of 2'-amino-1, 2, 4-triazole thiols 10(a,b) which involving treatment of the hydrazides 3(a,b) with CS_2 in presence of KOH to give potassium salts of hydrazinic acids 6(a,b). The latters without further identification when treated with hydrazine hydrate 64% and KOH resulted the 2'-amino phenelyn-1,2,4-triazole thiols10(a,b) in very good yields (10a, 93% and 10b, 80%).

Synthesis of glycosides: 11(a,b)-14(a,b)

The second target of this work was the synthesis of *S*-glycosides; those been achieved by Fischer glycosidation reaction of unprotected monosaccharides with thiol groups in the presence of an acid catalyst. Thus, refluxing of nucleobases 7(a,b)-10(a,b) with D- glucose they yielded *S*-glucosides 11(a,b)-14(a,b) (see Scheme 3). Thin layer chromatography have shown formation of one spot after each reaction with disappearance of nucleobases spots except for 14(a,b).The latter always exhibited two very close spots were difficult to separate by column chromatography. They related to mixture of S- and N-glucosides. No further work was done to separate these mixtures. IR and NMR measurements and melting points supported the tlc for formation of S-glycosides 11(a,b)-14(a,b).

Biological Tests

Nucleobases 7(a,b)-10(a,b) and one type of S-glucosides namely 5-(2'-amino and 2'-benzamidophenylene)-1,3,4-thiadiazole-2-S-glucosides (12a,b) were tested against representative microorganisms. Two Gram-positive bacteria *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* and two Gram-negative bacteria *Escherichia coli* ATCC 25924*, Pseudomonas aeruginosa* ATCC 27835.The filter paper disk method (NCCLS) [25]was employed in duplicate for *in vitro* study of antibacterial effects and amykacine was used as a positive reference. The inhibitory effects summarized in Table 1.

From Table 1, it is shown that almost all synthetic nucleobases 7(a,b)-10(a,b) and glycoside 12a exhibited various effect on the representative bacteria. It seemed that it is difficult to generalize whether there are differences in activity between the aminophenylene nucleobases 7a-10a and their benzamido derivatives 7b-10b. There are only some cases where the effect exceeded the positive reference amykacine as in oxadiazole 7a on G(-) *E.coli* , activity of thiadiazole 8a upon G(+) *B. cereus* and activity of all tested nucleobases 7(a,b)-10(a,b) on G(-)*P. aeraginosa*. 5-(2'-Aminophenylene)-1,3,4-thiadiazole-2-S-glucoside (12a) exhibited a moderate effect upon G(+) *S. aureus* and G(-) *E. coli* ,while its benzamido derivative 12b showed no effect toward the two organisms.

Minimum inhibition concentration (MIC) test were performed only on compounds showed an effect in initial concentration 10 ug.mL^{-1}. Tests were done in duplicate and the average results shown in Table 2.

CONCLUSIONS

Esterification and benzamidation of 2-amino benzoic acid proved to be synthetically valuable. 2-Benzamido benzoic acid exhibited better solubility in alcohols which lead to higher yield of esters and ultimately give higher yield of hydrazides 3(a,b). Since the hydrazides 3(a,b) were considered the key intermediates for the synthesis of nucleobases 7(a,b)-10(a,b) and glycosides 11(a,b)-14(a,b). Also some differences observed in biological activity caused by benzamido derivatives of 2-amino benzoic acid particularly in synthesizing nucleobases and S-glucosides 12(a, b).

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