SYNTHESIS AND IN VITRO ANTIMICROBIAL ACTIVITY OF SUGAR HYDRAZINO BENZOTHIOZOLYL THIOCARBAMIDE

K.M. Heda

*Department of Chemistry,Shri R. L. T. College of Science,Akola – 444001(M.S.) India
kavitaheda25@gmail.com

ABSTRACT

Benzothiazoles are bicyclic ring system with multiple applications. The synthesis of novel glycosides derivatives and investigation of their chemical and biological behaviour have gained more importance in recent decades. Serial of 1-tetra-O-benzoyl-β-D-glucosyl-3-(2)-hydrazino-1, 3-substituted benzothiozolyl thiocarbamide has been synthesized by the interaction of two pharmocophores, tetra-O-benzoyl-β-D-glucosyl isothiocyanates and substituted 2-hydrazino-1,3-benzothiazoles in acetone medium. The reaction mixture was kept at room temp for 24 hrs. Acetone is evaporated then product is recrystallised by petroleum ether (60-80%). Benz-fused compounds have been employed in the synthesis of various compounds which show very potential pharmacological activities. Carbohydrate is the key element in variety of biological phenomena and its N-linked sugar derivatives also exhibit wide range of medicinal activities. Keeping in this view, when one biological active molecule is linked to another, the resultant molecule generally has increased potency. The identities of these newly synthesised 1-tetra-O-benzoyl-β-D-glucosyl-3-(2)-hydrazino-1, 3-substituted benzothiozolyl thiocarbamide have been established on the basis of usual chemical transformations and IR , ^{1}H NMR and Mass spectral studies. The antibacterial and antifungal activities of also reported. Some of these derivatives exhibit significant antimicrobial activity. These compounds show appreciable activity towards these microorganisms like Escherichia coli, Proteus vulgaris, Staphylococcus aureus, Salmonella typhimurium, Psudomonas aeruginosa, Aspergillus Niger and Candida albicance.

Keyword: *2-hydrazino-1,3 benzothiozole , substituted benzothiozolyl thiocarbamide, tetra-O-benzoyl-β-D-glucosyl isothiocyanates, Biological studies.*

Introduction

In spite of tremendous advance made in modern medicine, there are still a large number of aliments for which suitable drugs are yet to be found. Today, there is an need to develop safer drug for the treatment of pain. Hydrazino benzothiazole and isatin derivatives are an important class of organic heterocycles because of their potential activities are reported to be effective in CNS disorders such as convulsion[1] and depressions[2]. Indole and benzothiazoles its analogs constitute the active class of the compounds possessing wide spectrum of antimicrobial[3], anthelmintic[4], analgesic[5],anti-inflammatory[6] and anti-tuberculosis[7]activities.

Benzothiozoles constitute an importance's class of compounds. In recent year heterocyclic compound analogues and derivatives have attracted strong interest due to their useful biological and pharmacological properties. Benzothiazole, a multifaceted nucleus, has been under research for the last two decades. Being a heterocyclic compound, benzothiazole finds use in research as a starting material for the synthesis of larger, usually bioactive structures. Its aromaticity makes it relatively stable, although as a heterocycle, it has reactive sites which allow for functionalization. From the literature survey, it has been found that extensive work has been reported on 2-substituted benzothiazole derivatives in past and evaluated for different activities like antibacterial[8], anticancer[9], antiviral[10], antitumor[11], anticonvulsant[12], neuroprotective[13], a topical carbonic anhydrase inhibitor and an antihypoxic. Taking this into view, and in continuation of our search for biologically potential benzothiazole derivatives, a certain new derivatives were synthesized taking benzothiazole as the basic moiety. Different benzothiazoles react with hydrazine and this hydrazino benzothiazoles then focused to fuse with *N*-lactosylated compound[14]. Hence, in present work, different benzothiazoles react with hydrazine and this hydrazino benzothiazoles then focused to fuse with *N*-glucosylatd compound.

Results and discussion

Herein, we report the synthesis of various 1-tetra-O-benzoyl-β-D-glucosyl-3-(2)-hydrazino-1, 3-substituted benzothiozolyl thiocarbamide **III(a-d)** by interaction of tetra-O-benzoyl-β-D-

glucosyl isothiocyanates **(I)** and substituted 2-hydrazino-1,3-benzothiazole **II(a-d)** in acetone medium. All products were crystallized from ethanol before recording the physical data (Table-1). The purity of compounds was checked by TLC. The spectral analysis[15-17] IR, 1H NMR and Mass spectra of the product were observed.

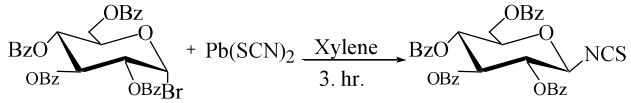
Experimental
Material and Methods

All chemicals were research grade. Melting points determined are uncorrected. IR spectra were recorded in KBr on a FT-IR Perkin-Elmer RXI(4000-450cm^{-1}) spectrophotometer. ^{1}H NMR measurements were performed on a Bruker DRX-300 (300 MHz FT NMR) NMR spectrometer in $CDCl_3$ solution with TMS as internal reference. The Mass spectra were recorded on a THERMO Finnigan LCQ Advantage max ion trap Mass spectrometer. Thin layer chromatography (TLC) was performed on silica Gel G and spots were visualized by iodine vapour. The compounds were screened for their antibacterial and antifungal activities by the disc diffusion assay method [18]. The compounds describe in this paper were first time synthesized by the multistep reaction protocol.

1] Preparation of tetra-O-benzoyl-β-D-glucosyl isothiocyanates: [19]

To a suspension of tetra-O-benzoyl-β-D-glucosyl bromide (21 g) in sodium dried xylene (80 ml) was added lead thiocyanate (5 g). The reaction mixture was refluxed gently for 3 hr.with frequent shaking .This solution was then cooled and liberated lead bromide was removed by filtration .The xylene filtrate was then treated with petrolium ether.(60-80) with stirring ,the pale yellow solid obtained (12 g).this solid was expected tetra–O-benzoyl-β-D-glucosylisothiocyanate .It was purified by dissolving it in a minimum quantity of chloroform and reprecipitating with petroleum ether.m.p 115-120^{0}C.

Scheme I



Tetra-O-Benzoyl-α-D-Glucosyl Bromide — Tetra-O-Benzoyl-β-D-Glucosyl Isithiocyanate

2] Preparation of 2- hydrazino-1,3-benzothiozole

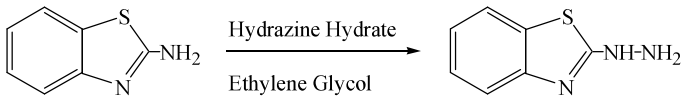
a) Preparation of benzothiozole

The required substituted benzothiazoles has been prepared by the oxidative cyclization of aryl thiocarbamide with the help of molecular bromine. To the chloroformic paste of phenyl thiocarbamide (5gm in 10ml) molecular bromine (20%) was added gradually with constant stirring until a slight excess of bromine was added as evident from an orange red colour. It was then allow to stand for 5 to 6 hrs. to resultant acidic solid was treated with cold ethanol, the solid dissolved in ethanol. On basification with dilute ice cold NH_4OH 2-aminobenzothiozole was separated out as a white solid (3gm). M.p. 130^{o}C.

b) Preparation of 2- hydrazino-1,3-benzothiozole

Concentrated HCl (1mL) was added drop wise to hydrazine hydrate (0.2 M, 1mL 80%) at 5-10^{o}C followed by ethylene glycol (20mL). To the above solution 2-aminobenzothiazole (0.01 M, 1.85g) was added in portions. It was then refluxed for 3 h, cooled and poured onto crushed ice. The separated solid was filtered, dried and recrystalized from ethanol. **II(a-g). (Scheme-II)**

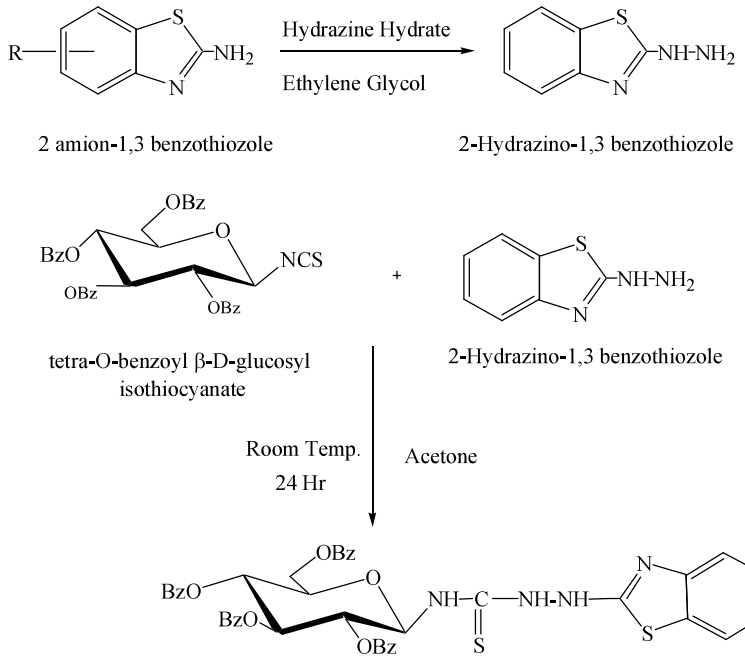
Sceme 2:



2 amion-1,3 benzothiozole — 2-Hydrazino-1,3 benzothiozole

3] Preparation of 1-tetra-O-benzoyl-β-D-glucosyl-3-(2)-hydrazino-1, 3-substituted benzothiozolyl thiocarbamide

A acetone solution of tetra-O-benzoyl-β-D-glucosyl isothiocyanates (0.025M, 2.5g in 20mL) was mixed with acetone solution of 2-hydrazino-1,3-benzothiazole (0.025M, 0.37g in 10mL), and mixture after shaking for sometime was kept at room temperature for 24 hr. Acetone was distilled off to obtained sticky residue. This residue was triturated several times with petroleum ether to afford a light coloured solid. **III(a-d). (Scheme-III).**



1-tetra-O-benzoyl β-D-Glucosyl-3-(2)-hydrazino-1,3-benzothiazolyl Thiocarbamide

Where, R= (a) Phenyl, (b) *o*-tolyl, (c) *m*-tolyl , (d) *p*-tolyl, And Bz = Benzoyl

3a: IR (KBr):υ 3400.29 (N-H), 3066.82 (Ar-H), 1730.15 (C=O), 1598.99 (C=N), 1263.37 (C-N), 1140.07 (C=S), 920.05 (Characteristics of glucose), 709.80 (C-S), H NMR (δ in ppm, $CDCl_3$): δ 5.7 -5.21 (3H, s, N-H), δ 7.5 (24H, m, aromatic protons), δ 4.5 (7H, m, glucosyl proton). Mass (m/z): 801 (M^+), 723, 579, 475, 374, 305; Anal. Calcd for $C_{42}H_{33}O_9N_4S_2$.: C, 62.92; H, 4.11; N, 6.99; S, 7.99; Found: C, 62.90; H, 4.17; N, 7.01; S, 7.95.

On the basis of all above facts the product with m. p. 200°C was assigned the structure 1-tetra-O-benzoyl-β-D-glucosyl-3-(2)-hydrazino-1, 3-substituted Phenyl-benzothiozolyl thiocarbamide When the reaction of tetra-O-benzoyl-β-D-glucosyl isothiocyanates was extended to several other 2- hydrazino-1,3-benzothiozole corresponding 1-tetra-O-benzoyl-β-D-glucosyl-3-(2)-hydrazino-1, 3-substituted -benzothiozolyl thiocarbamide has been synthesized.

3b: : IR (KBr):υ 3433.29 (N-H), 3064.89 (Ar-H), 1730 (C=O), 1600.92 (C=N), 1140.07 (C=S), 939.33 (Characteristics of glucose), 1265.30 (C-N), 783.10 (C-S), H NMR (δ in ppm, $CDCl_3$): δ 5.7 -5.21 (3H, s, N-H), δ 7.5 (24H, m, aromatic protons), δ 4.5 (7H, m, glucosyl proton), δ 0.9 (3H, s, Methyl proton,). Mass (m/z): 815 (M^+), 723, 579, 475, 374, 305; Anal. Calcd for $C_{43}H_{35}O_9N_4S_2$.: C, 62.92; H, 4.11; N, 6.99; S, 7.99; Found: C, 62.90; H, 4.17; N, 7.01; S, 7.95.

On the basis of all above facts the product with m. p. 190°C was assigned the structure 1-tetra-O-benzoyl-β-D-glucosyl-3-(2)-hydrazino-1, 3-substituted o-tolyl-benzothiozolyl thiocarbamide

Table -1: Physical data for characterization of compounds (3a-d)

Compd	Yield %	R_f	M.P. ^{0}C	Analysis (%): Found (calcd)	
				N	S
3a	60.00	0.48	200	7.01(6.99)	7.95(7.99)
3b	75.00	0.69	190	6.60 (6.64)	7.80(7.87)
3c	80.00	0.52	185	6.70 (6.64)	7.85(7.87)
3d	64.00	0.60	173	6.69(6.64)	7.90(7.87)

C and H analysis was found satisfactory in all cases.

Antimicrobial Studies

All the compounds have been screen for both antimicrobial and antifungal activity using cup plate agar diffusion method18-20 by measuring the inhibition zone in mm. the compounds were taken at a concentration of 1 mg/mL using Dimethyl Sulphoxide (DMSO) as solvent. Amikacin (100 µg/mL) was used as standard for antibacterial activity and Fluconazole (100 µg/mL) as standard for antifungal activity. The compounds were screen for antibacterial activity against *Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa* and *Klebsiyella species* by using Nutrient Agar medium and antifungal activity against *Thrichoderma harzianum* and *Verticillium species* was determined by using Potato Dextrose Agar medium. These sterilized agar media were poured into Petri dishes and allowed to solidify. On the surface of the media microbial suspensions were spread with the help of sterilized cotton swab. After inoculation the well was punched by using sterile stainless steel cork borer of 6mm diameter. In to these wells were added 0.1 mL portion of the test compounds in solvent. The drug solution was allowed to diffuse for an hour into the medium. The plate was incubated at 37^{o}C for 24 h and 30^{o}C for 48 h for antibacterial and for antifungal activities respectively. The zone of inhibition observed around the cups after respective incubation was measured. The results are presented in Table 2. Antibacterial studies of these compounds indicated that compounds exhibited most significant activity against All the other compounds exihibited low to moderate activity.

(Table2)

Sr. no	*E. c.*	S. *a.*	*P.v*	*P.a*	*S.t*	*K.p*	*A.n*	*C.a*
1(3a)	17	20	20	19	18	21	19	20
2(3b)	10	19	15	12	20	19	20	21
3(3c)	18	14	19	17	15	18	17	19
4(3d)	14	20	18	18	19	20	20	19
Amikacin	18	21	23	19	20	21		
Fluconazole							24	24

Sample	**Disc content**	**Resistant**	**Intermediate**	**Sensitive**
Amikacin	100ug/ml	≤ 15 mm	16-20 mm	≥ 21 mm
Fluconazole	100ug/ml	≤ 15 mm	16-20 mm	≥ 21 mm

Conclusion

Derivatives were synthesized and characterized for their structure elucidation. As outline in synthesis process, important novel -1,3-substituted benzothiozolyl thiocarbamide has been synthesized. All the structure of the above compounds was in good agreement with Spectral and Analytical data. Various chemical and spectral data supported the structures. Some of the compounds synthesized showed promising antimicrobial activities. The newly synthesized thiocarbamides exhibits comparable antibacterial and antifungal activities against the organisms tested. The method adopted in this investigation is simple,

efficient and inexpensive and is useful in synthesizing pharmacologically important molecules.

Acknowledgement

Authors are thankful to SAIF, CDRI Chandigarh for providing the spectral data. Authors are also thankful to Dr. Rupali Mantri (M. D. Microbiology), Assistant Professor, G. M. C., Akola for her help in doing antimicrobial activity and also Dr. V. D. Nanoty for encouragement and necessary facilities.

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