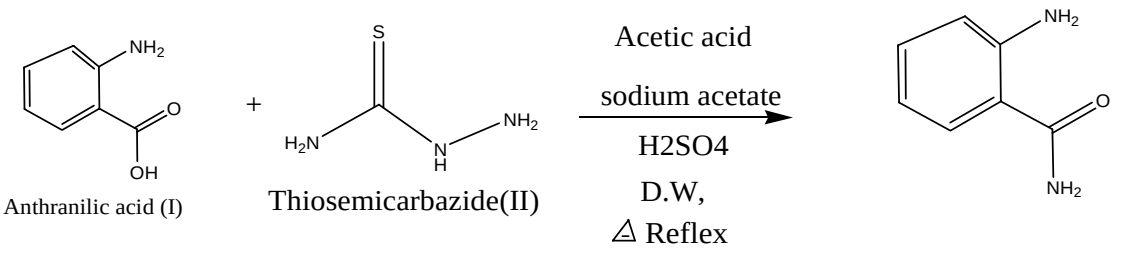
Synthesis and Antibacterial Activity of 2-aminobenzamide in Presence of Thiosemicarbazide, Acetic Acid, H_2SO_4

Pokharkar Raghunath D, Funde Prasad E, Pingale Shirish S

Department of Chemistry, Sangamner College, Ahmednagar, Maharashtra., P.D.F. Applied research and Development Institute, Ahmadnagar

Summary

Anthranilic acid *(I)* reflux with Thiosemicarbazide(II) in prisons of Acetic acid, H_2SO_4 - to From 2-aminobenzamide (III) the stretchers was confirmed with IR-Spectral data the homogeneity and purity of the compound were checked through T.L.C. this stretcher are confirmed by 1HNMR,MASS , Spectral data and their antibacterial activity was evaluated.



Anthranilic acid (I) Thiosemicarbazide(II) 2-aminobenzamide(III)

. .

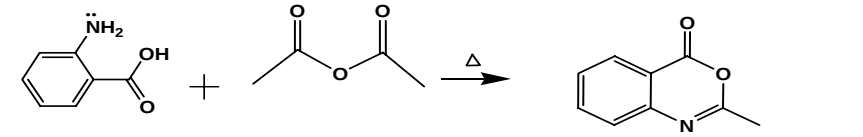
Keywords Anthranilic acid (I), Thiosemicarbazide(II), 2-aminobenzamide (III),H_2SO

Correspondence to: Dr.Pokharkar Raghunath D., Funde Prasad E Department of Chemistry, Sangamner College, Ahmednagar, Maharashtra.India., P.D.F. Applied research and Development Institute, Ahmednagar, Maharashtra At/Po: Funde takali ,Tal: Pathardi, Di:A-Nagar, 414102 E-mail:- pef@rediffmail.com Phone-no. 91-9881447393

Introduction

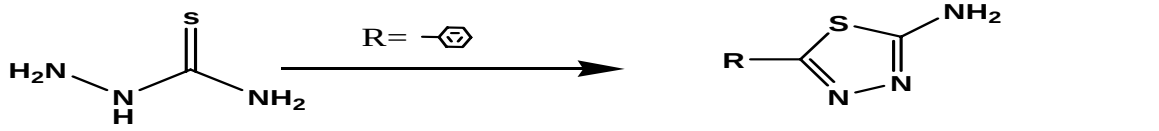
The area of the reaction of Anthranilic acid (I) with the different reagents for e.g. HCl/Br_2 (p764-Finar)acetic anhydrides are very well-known where the nucleophilic interaction takes place e.g. in particular reaction of Anthranilic acid with Acetic Anhydride by heating heating process results in the formation of (V)which is a form of six members ring where O & N both are present Here the cycligtion can be performed with Refluxing process.

In the seam way one another Reaction of Anthranilic acid (I) is observed where the five me bored heterocyclic Ring can be formed with the help of Thiosemicarbazide(II), reagent e.g.



anthranilic acid [IVa] **acetic anhydride** **2-methyl-4H benzo[d][1,3] oxazin-4-one [V]**

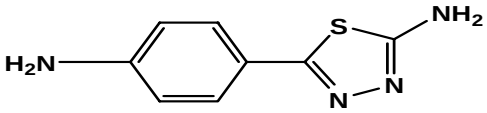
In the seam way one another Reaction of Anthranilic acid (I) is observed where the five me bored heterocyclic Ring can be formed with the help of Thiosemicarbazide(II), reagent e.g.



Thiosemicarbazide **5-phenyl-2H-1,2,4-thiazole-2-amine [IIIb]**

The preparation of 5-(4-aminophenyl)-1,3,4-thiadiazol-2-amine(IIIb),5-(4-aminophenyl)-2H-1,2,4-triazol-3(4H)-thione reactively was described in many reaction as the initial substance, 1-benzoylthiosemicarbazide,respectively ,is used

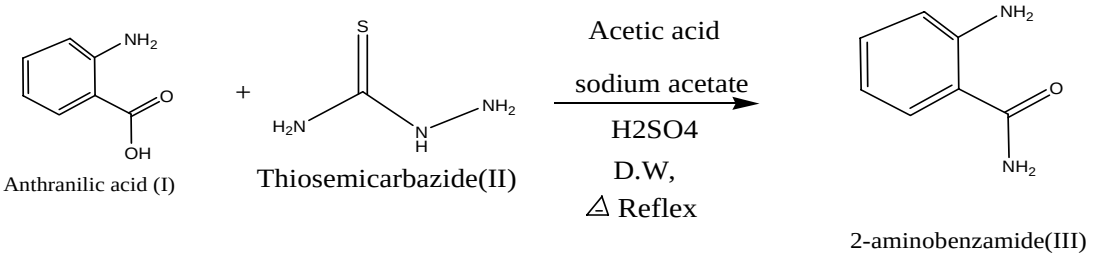
Their cyclization can be performed in the presence of various substances e.g. concentrated sulphuric acid , phosphoric acid, acylchloride and hydroxides, respectively .In other cases Thiosemicarbazide. It Helf was used as the initial substance, which then reacted with benzoylchloride.



5-(4-aminophenyl)-1,3,4-thiadiazol-2-amine [IIIb]

The reaction of p-amino benzoic acid with thiosemicarbazide respectively. Which would originate in hetrocycles , are not yet described in the literature. The originated hetrocycles would correspond to the following stretchers.Based on the knowledge mentioned above.

It was very catchy idea to find the conditions, under which the 1,2,4- triazoles & 1,3,4-thiadiazol respectively could originate.
This techniques is used to developed synthesis of 2-aminobenzamide (III) from Anthranilic acid in presence of Thiosemicarbazide(II) , Acetic acid, H_2SO_4 sodium acetate then reaction is as follow.



Anthranilic acid (I) Thiosemicarbazide(II) 2-aminobenzamide(III)

Here structure Were supported by IR-Spectral data the homogeneity and purity of the compound were checked through T.L.C. this structure are confirmed by 1HNMR,MASS Spectral data then acetic acid by separated distillation methods and is use as another reaction e.g. Anthranilic acid (I) and Thiosemicarbazide(II) by preparation of 2-aminobenzamide (III) in the presence of Acetic acid, H_2SO_4 this phenomenon is obese green chemistry approach. and their antibacterial activity was evaluated.

Materials and Methods

Chemicals

Anthranilic acid (I), Thiosemicarbazide(II), 2-aminobenzamide (III),H_2SO

IR-Spectral data were recorded in KBr on a DR-Pro410M Jasco FTIR Spectroscopic methods the homogeneity and purity of the compound wear checked through T.L.C.1HNMR spectra were recorded in DMSO on JEOL-FX-100 Spectroscopic methods and MASS Spectral data.

Synthesis of 2-aminobenzamide (III)

(2.7 g) Anthranilic acid(I) Dissolved in 20 ml Acetic acid (1.4 gm)of Thiosemicarbazide (II) Dissolved in 20 ml Acetic acid add 0.5 g sodium acetate dissolved complete Thiosemicarbazide(II)the added 20 ml 18% H_2SO_4 Anthranilic acid(I) and Thiosemicarbazide (II) mixture added drop by drop star continuously . This mixture is reflux for one(1) hors at 90 ^{0} C temperature with stirring. At the time of reaction the fumes of acetic acid removed After 1 hors stop heating and cool at room temp then white needle shaped crystals are separated wash the crystals with distilled water M.P.= 199 ^{0} C yield = 87 % Then filtrates mix are separated out acetic acid by distillation methods and then acetic acid is use as another reaction e.g. Anthranilic acid (I) and Thiosemicarbazide(II) by preparation of 2-aminobenzamide (III) in prisons of Acetic acid, H_2SO_4 this phenomenon is obese green chemistry approach

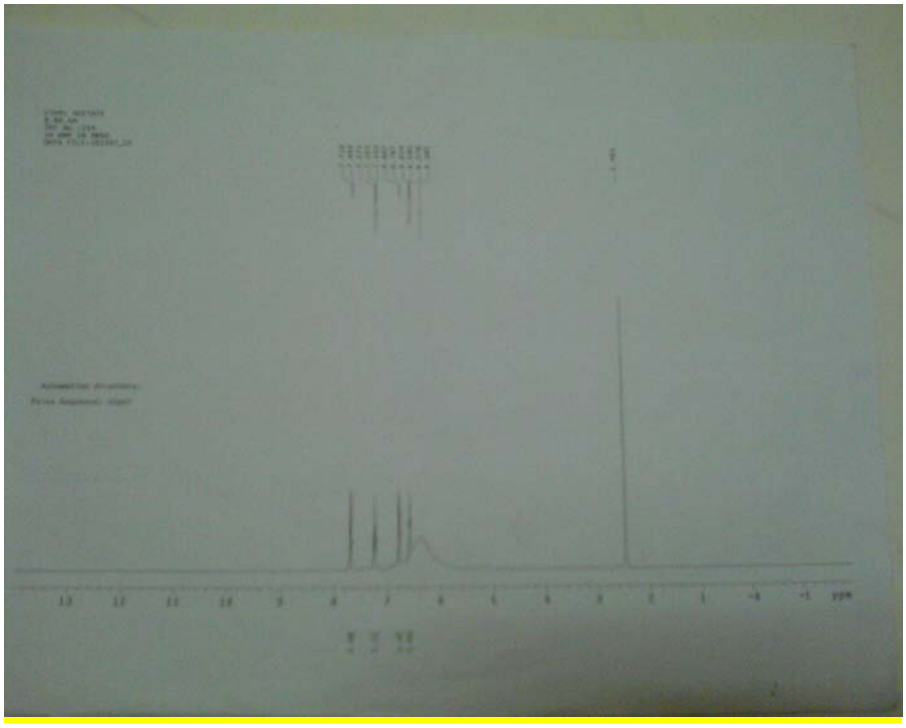
Disc diffusion bioassay

Antibacterial Activity of the 2-aminobenzamide (III)from synthesis of Anthranilic acid (I), Thiosemicarbazide(II)was studied against ten bacterial strains by the agar well diffusion method (15). Mueller Hinton agar no. 2 (Hi Media, India) was used as the bacteriological medium. The 2-aminobenzamide (III)from synthesis of Anthranilic acid (I), Thiosemicarbazide(II) were diluted in 100% dimethylsulphoxide (DMSO) at the concentrations of 5 mg/mL and 2.5 mg/mL. The antibacterial activity was evaluated at two different concentrations viz. 500 µg/ well and 250 µg/ well. The Mueller Hinton agar was melted and cooled to 48 - 50ºC and a standardized inoculum (1.5×108 CFU/mL, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile Petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (100 µl) was introduced in the well (8.5 mm). The plates were incubated overnight at 37ºC. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well. The experiment was performed three times to minimize the error and the mean values are presented.

Results

IR (KBr, cm^{-1}): 1389, 3628, 3684, 1635, 1521,1436.

1HNMR



:

2.418(S,1H,SH thiocarboxylic acid),

6.38(NH2 ,aromatic C-NH),

6.59(1H ,t,CH,1benzene –N,-C(=O)S),

6.80(1H,d,CH,1benzene –N,-C(=O)S),

7.25(1H,t,CH, 1benzene –N,-C(=O)S),

7.71(1H,d,CH, 1benzene –N,-C(=O)S).

MASS

: 135.9, 136.9, 137.9.

Table 1. Antibacterial activity of 2-aminobenzamide (III)from synthesis of Anthranilic acid (I), Thiosemicarbazide(II)by agar well diffusion method.

Microorganisms	Inhibition Zone (mm)* 2-aminobenzamide (III)
Staphylococcus aureus (NCIM No. 5021, ATCC No. 25923)	42 mm
Escherichia coli (NCIM No: 2931, ATCC No. 25922)	28 mm

*: values include cup borer diameter (8.5 mm) and are mean of three replicates.

Discussion

Synthesis of 2-aminobenzamide (III) is the very easily synthesized from Anthranilic acid react Thiosemicarbazide in prisons of Acetic acid, H_2SO_4sodium acetate and distilled water and total spectral data are confirm struck-char IR, 1HNMR and MASS spectroscopic data and their antibacterial activity was evaluated.

The Synthesis new ruts 2-aminobenzamide (III) may be potential antibacterials. The results of the antibacterial study (Table 2) shows that all tested compounds inhibit the growth of *Staphylococcus aureus* (NCIM No. 5021, ATCC No. 25923),*Escherichia coli* (NCIM No: 2931, ATCC No. 25922) bacteria inhibit the growth of both Gram positive and Gram negative bacteria at lower concentration. The highest activity was obtained.

Acknowledgments

The are thankful to guide Dr R. D. Pokharkar Head of the P.G. & Research center Department, Department of chemistry, Sangamner College Sangamner affiliated by Poona University , Pune, for research facilities and motivation.

References

1.ahidi Bonjar, G. H. *Asian. J. Plant Sci*. **2004**, *3*, 56.

2.(a) Stephen Babu, M. F.; Anasuyamma, U.; Venugopal, M.; Naga Raju, C.; Suresh Reddy,C. *Indian J. Chem*. **2005**, *44B*, 1248. (b) Naga Raju, C.; Naidu, M. S. R.; John, E. O.;Reddy, M. C. *Magn. Reson. Chem*. **1990**, *28*, 908.

3.Thomas, L. C.; Chittenden, R. A. *J. Chem. Soc*. **1961,** 1913.

4 Andersson, D. I. Persistence of antibiotic resistant bacteria. *Curr. Opin. Microbiol*. **2003**, *6*, 452–456.

5 DaSilva, E.; M. Iaccarino, M. Emerging diseases: a global threat. *Biotech. Adv*. **1999**, *17*, 363–384.

6 Projan, S. J. Why is big Pharma getting out of antibacterial drug discovery? *Curr. Opin. Microbiol*. **2003**, *6*, 427–430.

7 Silver, L. L. Novel inhibitors of bacterial cell wall synthesis. *Curr. Opin. Microbiol*. **2003**, *6*, 431-438.

8 Tripathi R. P.; Tewari, N.; Dwivedi, N.; Tiwari, V. K. Fighting tuberculosis: an old disease with new challenges. *Med. Res. Rev.* **2005**, *25*, 93–131.

9 Lawn S. D., Bekker, L.G.; Miller, R. F. Immune reconstitution disease associated with mycobacterial infections in HIV-infected individuals receiving antiretrovirals. *Lancet Inf. Dis*. **2005**, *5*, 361-373.

10 Ballell, L.; Field, R. A.; Duncan, K.; Young, R. J. New small-molecule synthetic antimycobacterials. *Antimic. Agent. Chemoth*. **2005**, *49*, 2153–2163.

11 Zhang, Y. The magic bullets and tuberculosis drug targets. *Ann. Rev. Pharm. Tox*. **2005**, *45*, 529–64.

12 Deivy Clementino de Lima, Paula Alvarez Abreu, Cı´ cero Carlos de Freitas, Dilvani Oliveira Santos, Rodrigo Oliveira Borges, Tereza Cristina dos Santos, et al. Snake venom: any clue for antibiotics and CAM? Evid Based Complement Alternat Med 2005;2:39–47.

13. Anjaria, J.; Parabia, M.; Dwivedi, S.; (2002). Ethnovet heritage Indian ethnoveterinary medicine – An Overview,; Pathik Enterprise, Ahmedabad (Gujarat), India.
14. Barbour, E.K.; Al Sharif, M.; Sagherian, V.K.; Habre, A.N.; Talhouk, R.S.; Talhouk, S.N.; (2004). Screening of selected indigenous plants of Lebanon for antimicrobial acitivity. *J. Ethnopharmacol*., 93, 1-7.
15. Motsei, M.L.; Lindsey, K.L.; van Staden, J.; Jaeger, A.K.; (2003). Screening of traditionally used South African plants for antifungal activity against *Candida albicans*. *J. Ethnopharmacol*., 86, 235-241.
16. Shahidi, G.H.; Karimi Nik, A. (2004). Antibacterial activity of some medicinal plants of Iran against *Pseudomonas aeruginosa* and *P. fluorescens. Asian J. Plant Sci*., 3, 61-64.
17. Machado, T.B.; Pinto, A.V.; Pinto, M.C.F.R.; Leal, I.C.R.; Silva, M.G.; Amaral, A.C.F.; Kuster, R.M.; Netto-dosSantos, K.R.; (2003). *In vitro* activity of Brazilian medicinal plants, naturally occurring naphthoquinones and their analogues, against methicillin-resistant *Staphylococcus aureus*. *Int. J. Antimicrob*., 21, 279-284.
18. Perez, C.; Paul, M.; Bazerque, P.; (1990). An antibiotic assay by the agar-well diffusion method. *Acta Biol. Med. Exp*., 15, 113-115.