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RESEARCH ARTICLE

Design, Synthesis, Characterization and Antimicrobial Screening of Novel 2-[Imino(Pyridin-2-yl)Methyl]-*N*-Phenylhydrazinecarbothioamide

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ABSTRACT

Microbial metabolite or synthetic analogs which inhibit the growth and survival of microorganism without serious toxicity to the host are called antibacterial agents. An "antifungal drug" is a medication used to treat fungal infections such as athlete's foot, ringworm, candidiasis (thrush), and others. Thiocarbamate derivatives exhibit a wide range of biological effects such as antibacterial, antifungal and etc. Pyridyl-2-amidrazone derivatives exhibit antitubercular and antifungal activities. Drug-likeness is a qualitative concept used in drug design for how "drug-like" a substance is with respect to factors like bioavailability. It is estimated from the molecular structure before the substance is even synthesized and tested. Molinspiration supports also internet chemistry community by offering free on-line cheminformatics services for calculation of important molecular properties (for example log P, polar surface area, number of hydrogen bond donors and acceptors). Synthesize thiocarbamate incorporated pyridyl-2-amidrazone and then evaluating them for antibacterial & antifungal activities. Drug-likeliness was calculated based on "Lipinski's rule of five". The score is computed using many commercial and academic softwares like Molinspiration etc. To evaluate druglikeness better, the rules have spawned many extensions, for example one from a 1999 paper by Ghose et al. Molinspiration tools are written in Java, therefore are available practically on any computer platform.

KEYWORDS

Carbothioamides, Druglikeness Score, Molinspiration Server, Lipinski's Rule of Five, Anti-Fungal

INTRODUCTION

A chemical substance of known biological activity is chosen to synthesize a new medicinal compound and different steps have been attempted in order to design a highly active compound with less steric effects, side effects and toxic effects, thereby enhancing the biological activities.

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Thiocarbamate[1-2] derivatives exhibit a wide range of biological effects such as antibacterial, antifungal, anti-inflammatory, antitubercular activities etc. Xiaoning Si et al.[3] Pyrrolidine dithiocarbamate (PDTC) reported to inhibit ubiquitin-proteasome-mediated proteolysis, reduction of CVB3 viral RNA synthesis. Nadia.M et al.[4] reported *N, N* disubstituteddithio carbamates, against *Geotrichumcandidum* and *Mucorcircinelloides.* Pyridyl-2-amidrazone derivatives exhibit antitubercular and antifungal activities. E.Banfi et al.[5] synthesized a series of 2-pyridinecarboxamidrazone derivatives and

evaluated for their inhibitory activity against *mycobacterium avium* isolates. They compounds exhibited good activity. Therefore we aimed to synthesize thiocarbamate incorporated pyridyl-2-amidrazone and then evaluating them for antibacterial & antifungal activities. Bozena et al.[6] N^1-2,4-dihydroxybenzenecarbothio-N^3-phenyl-2-picoline-amidrazone for the estimation of potential activity *in vitro*. The compound showed strongest fungistatic activity. M. D. Aytemir et al.[7] several derivatives of 6-phenylhexahydropyrimidine-2,4-dione having piperidine dithiocarbamate, active against Gram-negative *E. coli*, yeast-like fungi *Candidaalbicans.* Michael D. Coleman et al.[8] reported the preliminary in vitro toxicological evaluation of a series of 2-pyridylcarboxamidrazone candidate anti-tuberculosis compounds. The investigation that was carried out during the course of the work was divided into 4 steps. Step-I. Lead optimization of the drug molecule Step II .Synthesis of novel 2-[Imino (Pyridin-2-yl)Methyl]-*N*-Phenylhydrazinecarbothioamide. Step III. Characterization of the synthesized drug molecule. Step IV. Antifungal & Antibacterial screening for the lead molecule.

MATERIALS AND METHOD

Melting points were determined in open capillaries and were uncorrected. Reactions were monitored by Thin Layer Chromatography (TLC) on a pre-coated silica gel G plates using Iodine vapour as visualizing agent. Purity of the compounds was recorded on JASCO V-530 UV/VIS Spectrophotometer in the Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore, and Tamil Nadu. IR spectra were recorded on Spectrum one: FT-IR Spectrometer, The Sophisticated Analytical Instruments Facility (SAIF), Department of Science and Technology (DST), IIT, Chennai, Tamil Nadu. Mass spectra were recorded on The JEOL GCMATE II GC-MS, The Sophisticated Analytical Instruments Facility (SAIF), Department of Science and Technology (DST), IIT, Chennai, Tamil Nadu. softwares and databases used, Molinspiration server, ChemSketch, ChemDraw Ultra 7.0 All the *in-silico* works are carried out in the Department of Pharmaceutical Chemistry, Sasikanth Reddy College of Pharmacy, Nellore, Andhra Pradesh India.

Experiment/Methodology

Drug-likeness score[9-10] For the better oral absorption of the ligand the drug-likeness scores are constructed for getting information about solubility, diffusion, log P, molecular weight etc. One of the ideal methods for this is using Lipinski's rule of five with the Molinspiration server. The drug likeliness score computation is done for the ligand [4a].

Calculation of Lipinski's Rule of Five

1. Open the Molinspiration home page
2. Click Calculation of Molecular Properties and Drug-likeness
3. Draw the structure of 4a in JME window
4. Click calculate properties
5. Save the properties

For calculating using Molinspiration it requires JAVA in the computer.

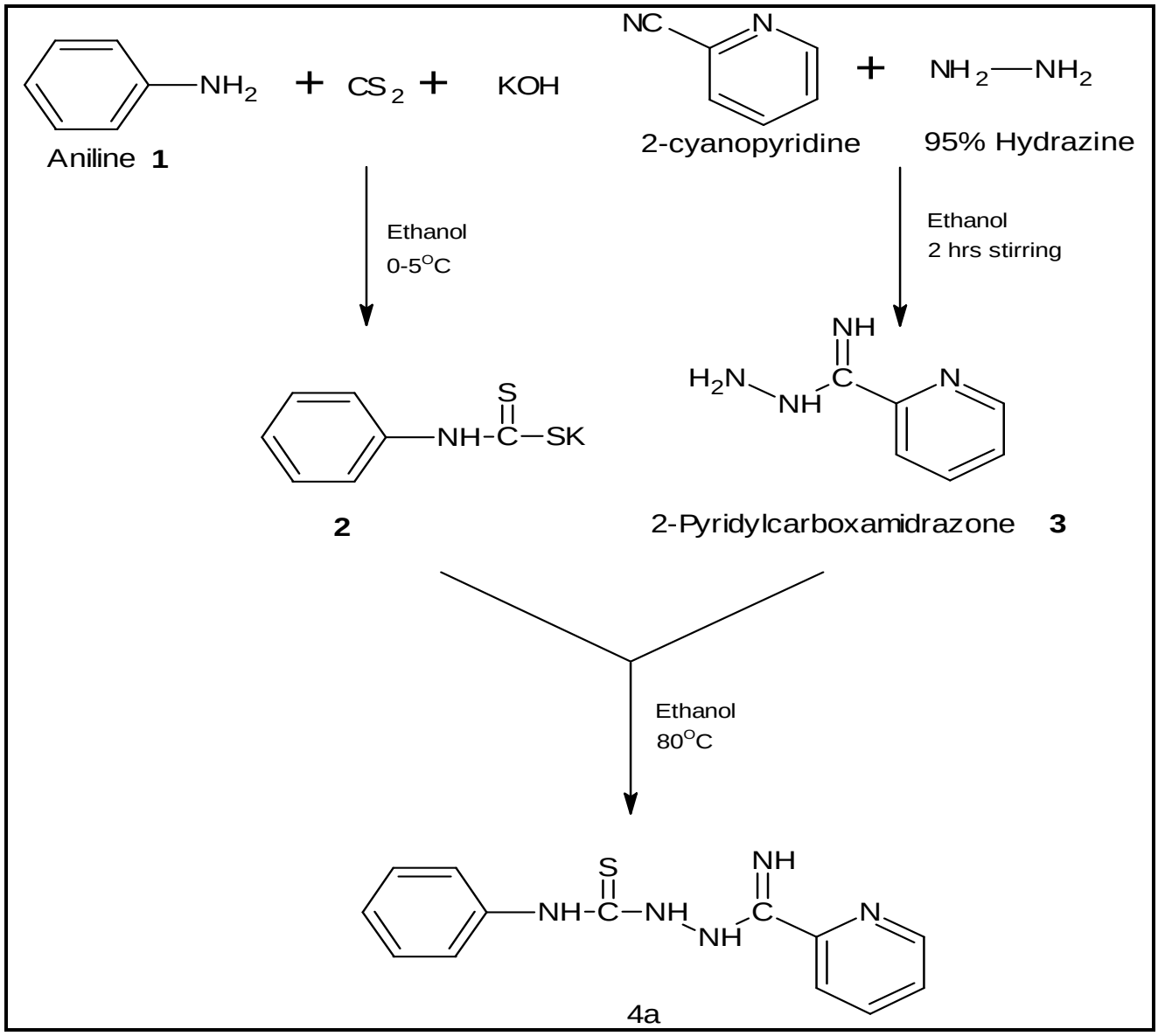
Synthesis

Synthesis of pyridyl-2-amidrazone[11]

Equimolar amounts of 2-cyano-pyridine (Aldrich) and hydrazine hydrate were mixed together, after which a small amount of ethanol was added until a clear solution was visible. Upon standing overnight at room temperature (15° C), the white crystals of 2-pyridyl-amidrazone were filtered off and washed with a small amount of ether and air dried. The crystals were further purified by recrystallation from benzene.

General Method for the Preparation of Compound 4a[12]

To a solution of aniline (0.01 mol) in absolute ethanol (20 ml) was added potassium hydroxide (0.01 mol) and carbon disulphide (0.75 ml), and the mixture was stirred at 0-5^{o}C for 1 h to form a potassium salt of substituted phenyl



dithiocarbamate. To the stirred mixture of phenyl thiocarbamate salt was added pyridyl-2-amidrazone (0.01 mol) and the stirring was continued at 80°C for 1 h and on adding crushed ice to obtain 2-[Imino(Pyridin-2-yl)Methyl]-*N*-Phenylhydrazinecarbothioamide (4a).

Anti-fungal Activity

Anti-fungal activity of synthesized compound 4a were assessed against *Candida albicans*, were collected from The National Chemical Laboratory, Pune and stored in the Pharma lab, sanniyasikumpam, Pondicherry. Drug 4a (500 µg/ml) against standard drug Fluconazole (10 µg/ml), DMF (Dimethyl Formamide) used as control. 30g of Sabouraud dextrose agar was suspended in 1000ml of distilled water and boiled to dissolve the medium completely. The selected strains were preserved by sub-culturing them periodically on agar slants and storing them under frozen conditions.

Antibacterial Activity

The anti-bacterial activity of synthesized drug 4a *Bacillus subtilis, Escherichia coli,* were collected from The National Chemical Laboratory, Pune and stored in the Pharma lab, sanniyasikumpam, Pondicherry. Drug 4a (500 µg /ml) against *kanamycin &* Amikacin (10 µg /ml) as standard using Dimethyl sulfoxide (Control). 15-20 ml of Mueller Hinton agar was transferred to test tubes and sealed with non-

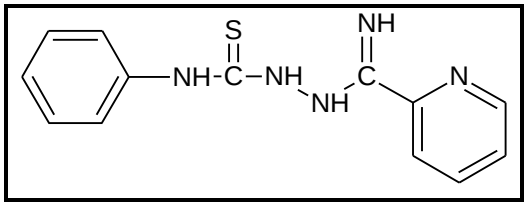
absorbent cotton. They were then autoclaved at a pressure of 15 psi (121^0C) for not less than 15 minutes. The strains were confirmed for their purity and identity by Gram's staining method and their characteristic biochemical reactions.

RESULTS AND DISCUSSION

The structure of the synthesized compounds were established on the basis of IR, UV and Mass spectral data.

Spectral Studies of Compound 4a

Structure :



Chemical Name : 2-[Imino(Pyridin-2-yl)Methyl]-*N*- Phenylhydrazinecarbothioamide

Molecular Formula : $C_{13}H_{13}N_5S$

Percentage yield : 88%

Molecular weight : 271.34

UV Spectra Data

Solvent : Methanol

λ_{max} : 473

IR Spectra

The IR spectrum of the compound (**4a**) showed characteristic absorption bands in the following regions. C-H out-of-plane bending (2-substituted pyridine) 755cm^{-1}, In-plane ring bending (2-substituted pyridine) 644 cm^{-1}, Imines (N-H Stretching) 3201 cm^{-1}, N-H in-plane bending 1589 cm^{-1}, Aromatic C-H Stretching 3035 cm^{-1}, C=S Stretching, 1449 cm^{1}.

The novel 2-[Imino(Pyridin-2-yl)Methyl]-*N*-Phenylhydrazinecarbothioamide is synthesized by the scheme given above, and the structures of the synthesized compounds were established on the basis of their physical (Melting Point and TLC (Thin Layer Chromatography) and spectral (UV, IR and MASS) data. The compound 4a exhibited a better solubility, diffusion, Log P, molecular weight etc. with no violations making the ligand pharmacodyanamically active and better oral absorptive series.

Table 1: Characterization Data

Characterization Data of the Synthesized Compound[4a]				
Compound Code	**Molecular Formula**	**Molecular weight**	**Melting Point °C**	**% yield**
4a	$C_{13}H_{13}N_5S$	271.34	167	88

Recrystallisation solvent : water

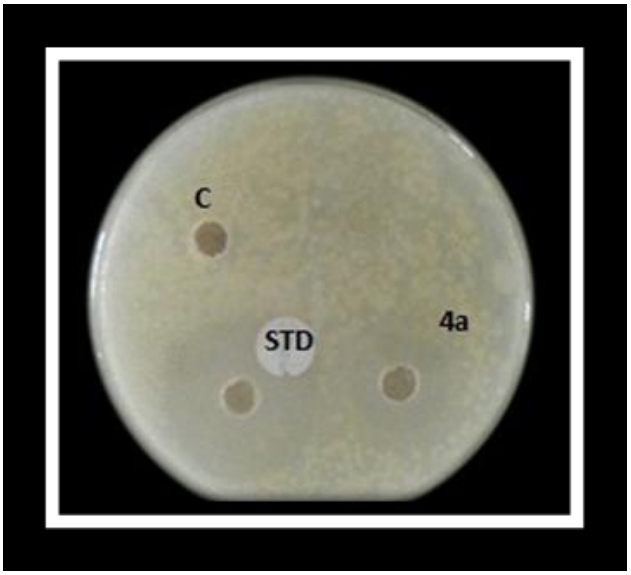
TLC solvent* : ethanol

Visualizing agent* : Iodine vapour

Biological Activity

Antifungal Activity

The synthesized compounds were screened for their antifungal activity against *Candida albicans by* cup plate method at a concentration of 500µg/well. *Candida albicans* was found to be moderately sensitive to the test compound 4a. The zone of inhibition of ligand is sited in Fig.1.



*C: Control , *STD: Standard.

Figure 1: Anti-Fungal Activity: Candida Albicans

Table 2: Zone of Inhibition for the Test Compounds

S.No	Compound code	Zone of inhibition (mm)* *Candida albicans*
1	**4a**	15
2	FLUCONAZOLE (STD)	24

*Zone of inhibition

< 12mm - categorized as resistant

12-18mm - categorized as moderately sensitive

>18mm - categorized as sensitive

Antibacterial Activity

The newly synthesized compound 4a were screened for their antibacterial activity against *Bacilus subtilis* and *Escherichia coli by* cup plate method at a concentration of 500µg/well. The compound was found to be inactive.

CONCLUSION

Drug likeness score is an *In-silico* is an important criteria for a new designed molecule which conserve the time for trails of to test pharmacodynamically active or inactive. Reaction process confirmed by TLC. Synthesized compound 4a was confirmed by the Infrared spectra and mass spectra, biological activity also confirmed which exhibits anti-fungal activity and not anti-bacterial activity. Activity confirms and can be opted for the further research on the compound 4a. Drug likeness score is an easy method to estimate a compound does exhibits pharmacodynamically active or inactive, which pave to save the economy and time.

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