International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

GREEN SYNTHESIS OF NICOTINIC ACID HYDRAZIDE SCHIFF BASES AND ITS BIOLOGICAL EVALUATION

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ABSTRACT

A series of biologically active nicotinic acid hydrazide schiff bases have been synthesized from nicotinic acid hydrazide and variety of aldehydes using lemon juice as natural catalyst, in moderate to good yields. The schiff bases synthesized, exhibited excellent anti-tubercular activity in comparison to standard drugs used.

Keywords: Schiff bases, nicotinic acid hydrazides, lemon juice, anti-tubercular

INTRODUCTION

Schiff bases form an important class of organic compounds, as they are the backbone for the development of various heterocyclic units such as oxadiazole, azetidinone, benzoxazole etc. C=N bond or azomenthine functionality are the condensation products of a primary amine and a carbonyl compound. Schiff bases of various heterocyclic derivatives have been screened biologically for their pharmacological activities. That include isatin derivatives,[1] pyrazole compounds,[2] macrocycles[3] etc. for anti-microbial. Benzocoumarins[4] and quinoline[5] schiff bases were screened for antioxidant activities. Quinazolinone based compounds exhibited anthelmintic activity.[6] Oxadiazole[7] and triazoles[8] for analgesic and anti-inflammatory activity, D-mannitol derived[9] and isonicotinic hydrazide based[10] for their anti-tubercular activity. Isoniazid based hydrazones for their anti-cancer activities.[11] Thus, schiff bases form an important pharmacophore unit that can help in the design and development of new drug entities.[12]

Nicotinic acid hydrazide schiff bases have been converted to their azetidin-2-one derivatives and screened for their biological activities.[13],[14] Such schiff bases have also been evaluated for their anti-oxidant activities[15], anti-fungal activities[16] and anti-tubercular activities.[17] QSAR studies have also been carried out on nicotinic acid benzylidene hydrazide derivatives.[18] Besides, Schiff bases are well known to be used for analytical purposes in the determination of metal ions, as they have an affinity for transition metals such as Cu, Mn, Co, Ni and Fe. So, there has also been continued interest in transition metal complexes of Schiff bases, having increased biological activities.[19] They are also used as having numerous applications as catalysts, pigments, dyes, intermediates in organic synthesis, polymer stabilizers and corrosion inhibitors.

The best known conventional method for nicotinic acid hydrazide schiffs base preparation has been using glacial acetic acid as catalyst under refluxing conditions.[20] There is a method reported using aqueous methanolic medium at room temperature.[17] The only green synthesis known is for schiff bases of isonicotinic acid hydrazide under microwave conditions.[21] In the need to use environmentally benign reaction conditions, waste-free reaction systems, the role of green catalysts plays an important role. The best known green catalyst are the natural, biocatalyst obtained from fruit juices. The advantages of using such natural catalysts include biocatalytic effect, eco-friendly nature, cost-effective,

non-hazardous, easily available. In view of this lemon juice, which has variety of biological properties, acidic pH can be a good natural catalyst.[22] Being an extract of citrus limonium species, it contains about 5-7% citric acid. It is also soluble in water. Its use as catalysts in organic synthesis has been enormous such as in Biginelli reaction, Knoevanagel condensation, Schiff base preparation, bis-indolyl methane synthesis etc. There are few reports, wherein natural catalysts have been effectively used for schiff base preparation,[23],[24],[25]. Although there is a report for green synthesis of isonicotinic acid hydrazide schiff bases,[21] no such reports are available for nicotinic acid hydrazide schiffbases.

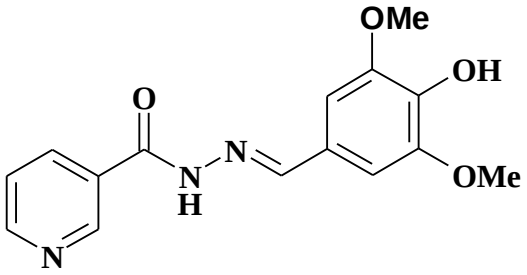
MATERIALS AND METHODS

All chemicals are purchased from S. D. fine Chemicals Pvt Ltd and Avra Synthesis Pvt Ltd and are used after purification. Solvents used have been purified by distillation via standard procedure. Nicotinic acid hydrazide was synthesized using literature known procedure via nicotinyl chloride.[26] The lemon juice extract was obtained by pressing lemon and then filtering through cotton plug, to remove any traces or particles. The filtered light yellow coloured lemon juice was used as such. Thin layer chromatography was performed on Merck-precoated silicagel 60-F_{254} plates. The IR spectra of the synthesized compounds were recorded on a Shimadzu FTIR spectrophotometer using DRS method. The ^{1}H and $^{13}CNMR$ were recorded on Bruker Avance II 400 NMR Spectrometer. Chemical shifts (δ) are reported in ppm.

Typical experimental procedure for the green synthesis of nicotinic acid hydrazide Schiff base (2): Nicotinic acid hydrazide (0.01mol) was dissolved in ethanol (5.0 ml) and lemon juice (2.0 ml) was added to it with swirling. Aldehyde (0.01mol) was added and the reaction was stirred at room temperature for 15 minutes. The thin layer chromatography showed that the reaction reached to completion. It was then poured into crushed ice and the solid obtained was filtered through Buchner funnel. The solid was recrystallised using alcohol.

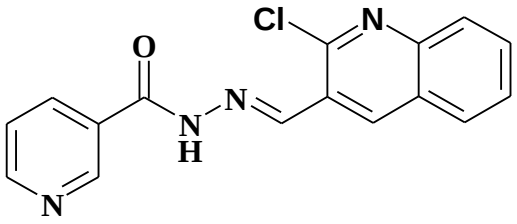
Spectroscopic data of Schiff bases (2)

Nicotinic acid (4-hydroxy-3,5-dimethoxy-benzylidene)-hydrazide (2a)



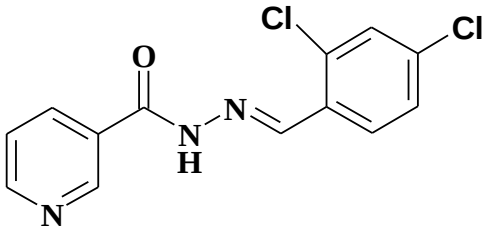
Off-white solid, m.p.128-130^{0}C. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 12.25(s, 1H, NH), 11.88 (s, 1H, OH), 9.10 (s,1H), 8.60 (d, 1H), 8.20 (s, 1H, CH=N), 8.10-8.13 (d, 1H), 7.88 (dd, 1H), 6.90 (s, 2H), 3.82 (s, 6H); IR (DRS method-KBr): cm^{-1} 3575, 3500, 1670, 1510,1425, 1125.

Nicotinic acid (2-chloro-quinolidene)-hydrazide (2b)



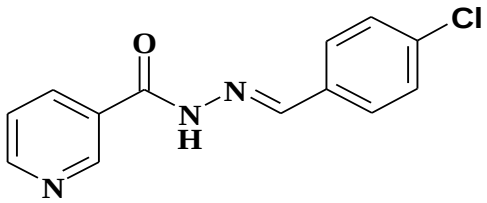
Off-white solid, m.p.203-205^{0}C. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm:
12.10 (s, 1H, NH), 9.15 (s,1H), 8.55 (d, 1H), 8.36 (s, 1H, CH=N), 8.05-8.10 (d, 1H), 8.28 (s, 1H), 7.8-8.0 (m, 4H), 7.55 (dd, 1H).; IR (DRS method-KBr): cm^{-1} 3350, 1675, 1490, 1425, 1125, 720, 575.

Nicotinic acid (2,4-dichloro-benzylidene)-hydrazide (2c)[27]



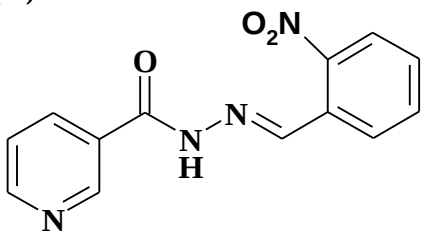
Off-white solid, m.p.138-140^{0}C. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm:
12.25 (s, 1H, NH), 9.10 (s,1H), 8.60 (d, 1H), 8.1 (s, 1H, CH=N), 8.0 (d, 1H), 7.5 (dd, 1H), 7.28-7.4 (m, 2H), 7.22 (s, 1H) ; IR (DRS method-KBr): cm^{-1} 3100,1645,1575,1475,710, 550.

Nicotinic acid (4-chloro-benzylidene)-hydrazide (2d)[17]



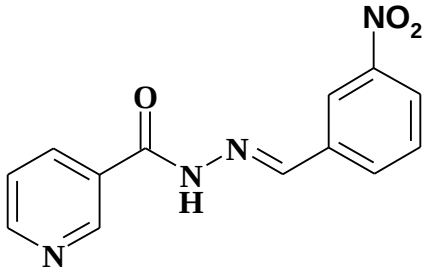
Yellow solid, m.p.185-187^{0}C. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm:
12.20 (s, 1H, NH), 9.0 (s,1H), 8.5 (d, 1H), 8.11 (s, 1H, CH=N), 8.0 (d, 1H), 7.6 (dd, 1H), 7.55 (d, 2H), 7.11 (d, 2H) ; IR (DRS method-KBr): cm^{-1} 3450, 1660, 1600, 1250, 700.

Nicotinic acid (2-nitro-benzylidene)-hydrazide (2e)[17]



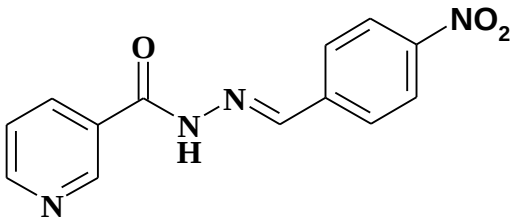
Yellow solid, m.p.190-192^{0}C. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm:
12.27 (s, 1H, NH), 9.0 (s,1H), 8.5 (d, 1H), 8.4 (s, 1H, CH=N), 8.0 (d, 1H), 7.55-8.0 (m,4H), 7.5 (dd, 1H) ; IR (DRS method-KBr): cm^{-1} 3450, 1650, 1600, 1530, 1350, 700, 580.

Nicotinic acid (3-nitro-benzylidene)-hydrazide (2f)[17]



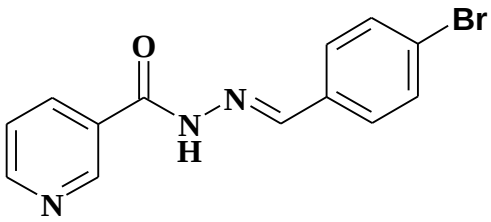
Pale Yellow solid, m.p.185-187^{0}C. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm:
12.27 (s, 1H, NH), 9.0 (s,1H), 8.6 (s, 1H), 8.5 (d, 1H), 8.2 (s, 1H, CH=N), 8.0 (d, 1H), 7.55-8.2 (m,3H), 7.5 (dd, 1H).; IR (DRS method-KBr): cm^{-1} 3450, 1670, 1600, 1520, 1330, 700.

Nicotinic acid (4-nitro-benzylidene)-hydrazide (2g)[17]



Light yellow solid, m.p.258-260^{0}C. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm:
12.25 (s, 1H, NH), 9.0 (s,1H), 8.55 (d, 1H), 8.4 (s, 1H, CH=N), 8.25 (d, 2H), 8.0 (d, 1H), 7.7 (d,2H), 7.5 (dd, 1H) ; IR (DRS method-KBr): cm^{-1}3550, 1675, 1600, 1540, 1320, 700.

Nicotinic acid (4-bromo-benzylidene)-hydrazide (2h)[16]



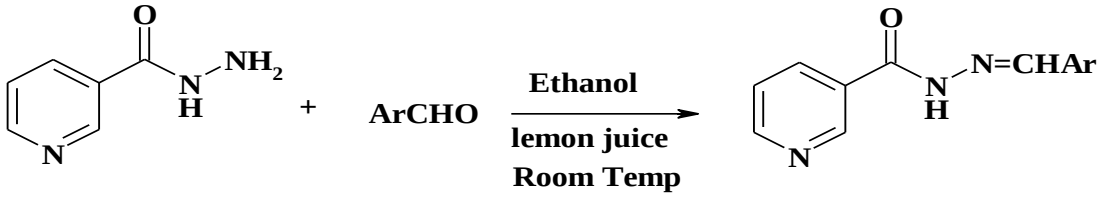
Yellow solid, m.p.190-192^{0}C. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm:
12.10 (s, 1H, NH), 9.0 (s,1H), 8.4 (s, 1H, CH=N), 8.35 (d, 1H), 8.0 (d, 1H), 7.7 (d,2H), 7.52 (d, 2H), 7.1 (d, 2H) ; IR (DRS method-KBr): cm^{-1}3350, 1670, 1600, 700.

RESULTS AND DISCUSSION

As reported, nicotinic acid hydrazide schiff bases have been synthesized by reacting with aromatic aldehydes in presence of glacial acetic acid under refluxing ethanolic conditions. But, we carried out, conventional synthesis of schiff bases of nicotine hydrazide with appropriate aldehydes in presence of glacial acetic acid in ethanol **was carried out** at room temperature. In all eight schiff bases were prepared (Table 1). In view of simple condensation of aromatic amine with carbonyl compounds being carried out using natural catalyst such as lemon juice and the need for working within this framework of green chemistry, it was visualized to get important schiff bases from nicotino hydrazide by this method. (Scheme 1)

Initially, we tried the reaction of equimolar quantities of nicotinic acid hydrazide and p-chlorobenzaldehyde with few ml of lemon juice extract in ethanol as solvent at room temperature. The tlc showed completion of the reaction and the reaction and so it was poured into crushed ice to give the schiff base in 60% yield. In all, we prepared eight schiff bases in good yields. The product 2 was obtained in quick time at room temperature. (Table 1)

Scheme 1:



(1) **(2)**

Table 1: Synthesis of schiff bases (2a-h) using both conventional and green method

Compound	Ar	% Yield (Conventional Method)	% Yield (Green Method)	M.P ^{0}C
2a	4-OH-3,5-$(OMe)_2$	60	70	128-130
2b	2-Cl-3-Quinolinyl	52	72	203-205
2c	2,4$(Cl)_2$	55	70	138-140
2d	4-Cl	65	60	185-187
2e	2-NO_2	65	55	190-192
2f	3-NO_2	55	60	185-187
2g	4-NO_2	55	65	258- 260
2h	4-Br	60	65	190-192

Biological Evaluation: The Schiff base compounds (2) synthesized were evaluated for their antibacterial and anti-tubercular activities.

Anti-bacterial activity: The synthesized compounds were screened for their antibacterial activity against two microorganisms and the minimum inhibitory concentrations were determined. Bacterial strains used were *Escherichia. Coli* (Gram-negative) and *Staphylococcus. Aureus* (Gram-positive). These bacterial strains were maintained on brain heart infusion medium for 24h at 37^{0}C. The agar plates were inoculated to a McFarland 0.5 turbidity standard. The inoculated plates were allowed to stand for atleast 3 minutes, before making wells. Different concentrations of the test compounds were added into each of the wells. Once applied, the plates are kept for incubation for 18-24 hrs at 37^{0}C. The inhibition zones were measured after 24 h. The minimum inhibitory concentration (MIC) was defined as the intercept of the grave of logarithm concentrations versus diameter of the inhibition zones. (Table 2)

Table 2: Antibacterial activity of compounds (2a-h)

Compound	Zone of Inhibition in (mm) E.Coli	Zone of Inhibition in (mm) S. Aureus
2a	10	14
2b	12	12
2c	12	12
2d	14	10
2e	12	-
2f	12	-
2g	12	-
2h	12	-
1	18	16
Ciprofloxacin	32	26

Anti-tubercular activity: The synthesized compounds (2) were also evaluated for their anti-tubercular activity against standard strain $H_{37}RV$. The method used was microplate Alamar Blue assay (MABA). Being a non-toxic method, it has several advantages such as thermal stability of the reagent, and good correlation with BACTEC radiometric method. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After addition of Alamar Blue reagent and incubating for 24 hrs, the results were observed. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. (Table 3)

Table 3: Anti-Tubercular activity of compounds (2)

Compounds	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
2a	S	S	S	S	S	S	S	R
2b	S	S	S	S	S	S	R	R
2c	S	S	S	S	S	S	R	R
2d	S	S	S	S	S	S	S	R
2e	S	S	R	R	R	R	R	R
2f	S	S	S	S	R	R	R	R
2g	S	S	S	S	S	S	S	R
2h	S	S	S	S	S	S	S	S
1	S	S	S	R	R	R	R	R
Pyrazinamide	S	S	S	S	S	S	R	R
Streptomycin	S	S	S	S	S	R	R	R
Ciprofloxacin	S	S	S	S	S	S	R	R

S - Sensitive R - Resistant , Strain used: ***M.tuberculosis*** (H37 RV strain)

CONCLUSION

In conclusion, we have developed a new, simple and convenient environmentally benign synthesis of nicotinic acid hydrazide schiff bases in good yields. The role of natural catalyst like lemon juice in the synthesis of biologically active molecules has been well demonstrated. Variety of schiff bases have been synthesized proving the versatility of the green methodology. The anti-microbial testing indicated that the schiff base compounds do exhibit better activity against *E-Coli* than *S. Aureus*, showing its specificity. Most of the synthesized compounds showed excellent anti-tubercular action, superior to that of standard drugs pyrazinamide, streptomycin and ciprofloxacin. Such schiff bases can be good lead compounds for the development of new drug entities in future.

ACKNOWLEDGEMENTS

The authors are thankful to the Management of Dnyanprassarak Mandals College, Goa for providing the facilities. The authors are also thankful to Department of Science and Technology-Goa, for financial assistance and Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belgaum, for providing bioactivity data.

REFERENCES

1. Prakash CR, Raja S. J Saudi Chem Soc, 2011.
2. Malladi S, Isloor AM, Isloor S, Akhila DS. Arabian J Chem, 2013; 6 (3): 335–340.
3. Essa HH, Kandil F, Falah A. Iraqi J Sci, 2012; 53 (2): 230–240.
4. Sashidhara KV, Kumar A, Bhatia G, Khan MM, Khanna AK, Saxena JK. Eur J Med Chem, 2009; 44 (4):1813–1818.
5. Sashidhara KV, Rosaiah JN, Bhatia G, Saxena JK. Eur J Med Chem, 2008; 43 (11): 2592–2596.
6. Revanasiddappa D, Prasad KS, Kumar LS, Jayalakshmi B. Int J Chem Tech Res, 2010; 2 (2): 1344–1349.
7. Bhandari SV, Bothara KG, Raut MK, Patil AA, Sarkate AP, Mokale VJ. Bio Med Chem, 2008; 16 (4), 1822–1831.
8. Nithinchandra, Kalluraya B, Aamir S, Shabaraya AR. Eur J Med Chem, 2012; 54: 597–604.
9. Ferreira MDL, Vasconcelos TRA, de Carvalho EM, Lourenco MCS, Wardell SMSV, Wardell JL, Ferreira VF, Souza MVN. Carbohydr Res, 2009; 344 (15) 2042–2047.
10. Hearn MJ, Cynamon MH, Chen MF, Coppins R, Davis J, Kang HJ, Noble A, Tu-Sekine B, Terrot MS, Trombino D, Thai M, Webster ER, Wilson R. Eur J Med Chem, 2009; 44 (10): 4169–4178.
11. Rodrigues Felipe AR, Oliveira Augusto CA, Cavalcanti BC, Pessoa C, Pinheiro AC, DeSouza Markus VN. Sci Pharm. 2014; 82: 21-28.
12. Kajal A, Bala S, Kamboj S, Sharma N, Saini V. J Catal, 2013; 1-14.
13. Sharma MC, Sahu NK, Kohli DV, Chaturvedi SC, Sharma S. Digest Journal of Nanomaterials and Biostructures, 2009; 4(2): 361-367.
14. Revanasiddappa BC, Subrahmanyam EVS, Satyanarayan D. Int J Chem Tech Res, 2010; 2(1): 129-132.
15. Praveen Kumar P, Rani BL. Int J Chem Tech Res, 2011; 3(1): 155-160.
16. Madhavi N, Rani BL. Int J Pharm Res. Dev, 2013; 5(7): 7-12.
17. Adhi H, Zaker S, Manakaresi H. Jour Rep Pharm Sci, 2012; 1(1): 60-66.
18. Narang R, Narasimhan B, Sharma S, Sriram D, Yogeeswari P, De Cleraq E, Pannecouque C, Batzarini J. Med Chem Res, 2012; 21:1557-1576.
19. Chohan ZH, Praveen M, Ghaffar A. Met-Based Drugs, 1997; 4(5): 267–272.
20. Dhar DN, Taploo CL. J Sci Ind Res, 1982; 41(8): 501–506.
21. Thomas AB, Tupe PN, Badha RV, Nanda RK, Kothapalli IP, Paradkar OP, Sharma PA, Deshpande AD. Green Chem Lett Rev, 2009; 2(1): 23-27.
22. Pal R. Open J Org Chem, 2013; 1(4): 47-56.
23. Patil SS, Jadhav SD, Shinde SK. Org Chem Inter, 2012; 2012: 1-5.
24. Patil SS, Jadhav SD, Patil UP. Arch Appl Sci Res, 2012; 4(2): 1074-1078.
25. Yadav G, Mani JV. Int J Sci Res, 2015; 4(2): 121-127.
26. Ramalakshmi N, Deepa S, Srinivas KS, Puratchikody A, Arunkumar S. Rasayan J Chem, 2009; 2(2): 393-396.
27. Revanasiddappa BC. Jour Pharm Res, 2009; 8(3): 122-124