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SYNTHESIS AND BIOLOGICAL EVALUATION OF 2-ISONICOTINYL 3, 5-DIARYL -2H-(3-TETRAZOLIUM) CHLORIDE DERIVATIVES

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ABSTRACT

A synthesis of 2-Isonicotinyl 3, 5-diaryl -2H-(3-tetrazolium) chloride (5) was performed starting from isonicotinic hydrazide (1). The treatment of 1 with various substituted aromatic aldehydes afforded the corresponding Schiff's bases 3. The Schiff's bases are converted into a series of aryl formazan via diazocoupling which underwent cyclization in the presence of H_2O_2 and $KMnO_4$ to give substituted tetrazolium derivatives. The titled compounds were characterized physically and analysed structurally by spectroscopic techniques and Elemental analysis. The compounds S_1-S_{12} were screened for antibacterial activity against *Escherichia coli(ATCC 14580), Bacillus subtilus(ATCC 700294) and Staphylococcus aureus(ATCC14580)*, anti fungal activity against *Aspergillus niger(NCIM 627)* and anti oxidant activity by DPPH method. All the compounds exhibited significant antimicrobial activity and the compound 2-isonicotinoyl-5-(4-methoxyphenyl)-3-(4-sulfamoylphenyl)-2H-(3-tetrazolium) chloride (S_3) revealed good inhibitory activity against bacterial and fungal strains. The presences of electron withdrawing substituents in the phenyl ring at 3 and 5 position of tetrazole resulted in more potent compounds for the above evaluated activities.

KEYWORDS: INH, Tetrazole, DPPH, Schiff's base

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INTRODUCTION

Tetrazole are class of synthetic organic heterocyclic compounds consisting of five membered ring of four nitrogen and one carbon atom. The development of tetrazole chemistry has been largely associated with wide scale of applications in these compounds: medicine, biochemistry, agriculture, photography, as well as corrosion inhibitors, components of gas generating compositions, and explosives[1-3]. Although tetrazoles were discovered more than one hundred years ago a systematic examination of these compounds was initiated in the latter half of the 20th century. A considerable advance in the tetrazole chemistry has been made by Russian scientists. A series of analogues and derivatives of tetrazole have been reported to establish various biological activities such as analgesic[4], antiinflammatory[5], antinociceptive[6], anticonvulsant[7] and anticancer[8] activities. Tetrazole was first prepared by the reaction of anhydrous hydrazoic acid and hydrogen cyanide under pressure.[9-10] Keeping these in mind efforts were taken to synthesise a series of new synthons bearing tetrazole nucleus.

EXPERIMENTAL PROTOCOLS

Melting points were determined by open end capillary tube method and were uncorrected. The IR spectra (by using KBr pellets) were recorded using Perkin Elmer FTIR spectrometer and ^{1}HNMR spectra were recorded on a Brucker 400MHz spectrometer using TMS as internal standard. Mass spectra were recorded on a LC-MSD-TRAMP-SL 2010 A SHIMADZU. Microwave irradiation was carried out with Catalyst Commercial Microwave.TLC was performed on precoated silica gel GF 254 [E.Merck]. The elemental analysis was carried out on PERKIN ELMER Series II 2400 CHN Elemental Analyzer.

RESULTS AND DISCUSSION

The synthetic pathway for the preparation of tetrazolium derivatives are shown in the Figure 1.

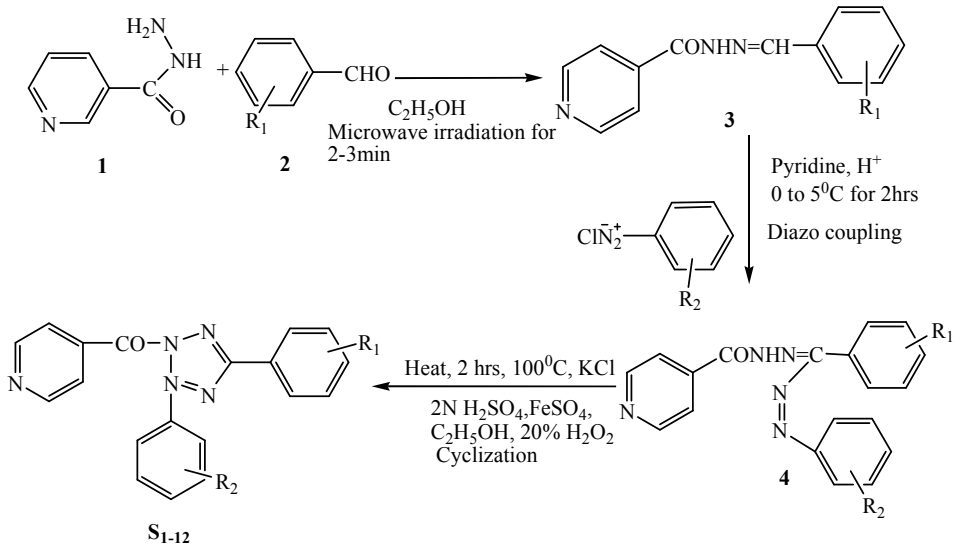

Figure 1
Scheme of Synthesis of Compounds S_{1-12}

Spectroscopic analysis

The structural evaluation of novel tetrazolium derivatives were performed by various spectroscopic techniques. The IR spectra of the synthesized compounds revealed the presence of NH_2OH stretching vibration between 3300-3500 cm^{-1}, 1500-1600cm^{-1} indicates the presence of C=C and 1600-1700

cm^{-1} indicates C=O in the target compounds S_{1-12}. The ^1H NMR spectra of all the compounds showed respective downfields that supported the structure of various synthesized tetrazolium chloride derivatives. The compounds S_1, S_5, S_7, and S_{10} showed downfield between 4.8-5.1ppm which confirms the presence of -OH group in those structures. The compounds S_3 and S_{10} showed downfield between 2-2.2ppm which confirms the presence of NH_2 group in the structures. Compound S_5, S_6, and S_{10} showed downfield at 10.9 to 11.2 ppm, which reveals the presence of acidic -OH group. Aromatic protons showed characteristic downfield 6.8 to 8.8 ppm in the target nucleus. The synthesized compounds exhibited molecular ion peak which confirms the structure of the synthesized compounds. Data obtained by elemental analysis are consistent with the structures of the synthesized compounds which ascertain the structure and purity of synthesized compounds.

Antimicrobial activity

Anti microbial activity of the synthesized compounds were evaluated by cup plate method. The results of antimicrobial activity were given in Table 1. The zone of inhibition of the synthesized compounds revealed that S_3, S_4, S_5, S_8 and S_{11} showed better inhibition when compared to the standard Ciprofloxacin at 100 µg/ml against all the three biological strains *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus*. Antifungal screening revealed that the test compounds S_3, S_7, and S_9 showed good inhibition compared to the standard Ketoconazole against *Aspergilus niger.*

Table 1
Biological activities of Synthesised Compounds S_{1-12}

Compound	R_1	R_2	Antimicrobial activity (Zone of Inhibition mm)				Antioxidant activity IC_{50} values (µg/ml)
			B.subtilis	*S.aurens*	*E.coli*	*A.niger*	
S_1	-OH	-H	16±0.07	21±0.12	20±0.16	21±0.52	78±0.26
S_2	$-OCH_3$	-H	14±0.06	14±0.02	14±0.36	14±0.24	66±0.02
S_3	$-OCH_3$	$-SO_2NH_2$	21±0.08	22±0.28	22±0.38	24±0.46	40±0.04
S_4	Cl	-COOH	21±0.12	21±0.14	21±0.34	18±0.28	72±0.06
S_5	-OH	-COOH	23±0.34	21±0.26	21±0.04	21±0.18	47±0.26
S_6	-H	-COOH	20±0.22	20±0.18	20±0.06	17±0.14	39±0.08
S_7	-OH	Cl	20±0.64	22±0.06	19±0.24	23±0.28	70±0.42
S_8	Cl	-H	23±0.13	24±0.14	21±0.08	19±0.02	66±0.36
S_9	-H	Cl	18±0.08	21±0.18	20±0.12	22±0.14	78±0.48
S_{10}	-OH	$-SO_2NH_2$	18±0.15	19±0.26	20±0.06	18±0.34	70±0.04
S_{11}	$-OCH_3$	-COOH	23±0.28	20±0.16	23±0.08	20±0.12	56±0.24
S_{12}	-H	-H	15±0.16	15±0.18	15±0.42	15±0.32	103±0.35
Standard	-	-	25±0.24	29±0.12	21±0.04	24±0.18	38±0.26

Antioxidant activity

All the compounds were subjected to *in vitro* anti oxidant activity by DPPH free radical scavenging method, using Ascorbic acid as standard. All The compounds showed significant activity with IC_{50} value in the range of 103µg/ml to39µg/ml. The compound S_3 and S_6 showed greater antioxidant activity when comparing with other compounds and equipotent activity with that of the standard.

EXPERIMENTAL PROCEDURE

Synthesis of Isonicotinyl aryl hydrazones (3):
About 0.01moles of Isoniazid (1) and substituted aromatic aldehyde (2) in ethanol (30ml) was taken in a round bottom flask and irradiated in microwave oven for 2-3min. Then the solvent was removed and recrystallized using ethanol to get compound 3.

Synthesis of 3-Aryl-1-isonicotinyl-5-aryl formazan (4)

Aromatic amine (0.02mol) in a mixture of glacial acetic acid and hydrochloric acid was diazotized with a solution of sodium nitrite at 0-5ºC. Then this reactant was coupled with compound 3(0.01mol) in pyridine in an ice cold condition. The reaction mixture was kept in ice bath for 2 hrs and poured an ice water. The

dark coloured mass obtained were recrystallized from ethanol.

Synthesis of 2-Isonicotinoyl-3, 5-diaryl-2H (3-tetrazolium) chloride (S_{1-12})

A mixture of formazan (0.01mol) and H_2SO_4 containing a trace of $FeSO_4$ was suspended in ethanol then the mixture was treated for 2hrs at 100ºC. After the completion of oxidation, the excess of ethanol was distilled off and KCl was added to the residue for the precipitation of tetrazolium chloride. The product formed was recrystallised from petroleum ether.

S1:*5-(4-hydroxyphenyl)-2-isonicotinoyl-3-phenyl-2H-(3-tetrazolium) chloride*

Yield:75%; m.p:243ºC; FTIR (cm^{-1}): 3441(OH), 1700(C=O), 1598 (C=C), 1412(C=N), 1316(C-N),1040(N-N),1482(N=N); ^1H NMR(ppm): δ 6.83-8.6 (13H, m, Ar-H), 5.0 (1H, S, OH); Mass(m/z): 379.40 Anal.cal for $C_{19}H_{14}ClN_5O_2$ C-60.08, H-3.71, N-18.43 Found: C-60.07, H-3.72, N-18.40.

S_2:*2-isonicotinoyl-5-(4-methoxy phenyl)-3-phenyl-2H-(3-tetrazolium) chloride*

Yield:70.5%; m.p:179ºC; FTIR (cm^{-1}): 1674(C=O), 1601(C=C), 1413(C=N), 1218(C-O-C) 1046(N-N),1488(N=N); ^1H NMR(ppm): δ 6.83-7.96(13H, m, Ar-H), 3.73(3H, S, OCH_3); Mass(m/z): 339.30; Anal.cal for $C_{20}H_{16}ClN_5O_2$ (393.83): C-60.99, H-4.09, N-17.78: Found: C-60.89, H-4.14, N-17.70.

S_3:*2-isonicotinoyl-5-(4-methoxy phenyl)-3-(4-sulfamoylphenyl)-2H-(3-tetrazolium) chloride*

Yield:73%; m.p:138ºC; FTIR (cm^{-1}): 3057(C-H), 1656(C=O), 1600(C=C), 1477(C=N), 1316(C-N) 1050(N-N),1492(N=N); ^1H NMR(ppm): δ 6.8-8.8(12H, m, Ar-H), 3.9(3H, S, OCH_3), 2.0(2H,S,NH_2); Mass(m/z): 472.10 Anal.cal for $C_{20}H_{17}ClN_6O_4S$ (472.9): C-50.8, H-3.62, N-17.77: Found: C-50.79, H-3.62, N-17.74.

S_4:*3-(4-carboxyphenyl)-5-(2-chlorophenyl)-2-isonicotinoyl-2H-(3-tetrazolium)chloride*

Yield:79%; m.p:109ºC; FTIR (cm^{-1}): 3438(O-H), 1659(C=O), 1584(C=C), 1415(C=N), 1375(C-N), 748(C-Cl) 1055(N-N),1510(N=N); ^1H NMR(ppm): δ 7.3-8.1(12H, m, Ar-H), 11.0(1H, S, COOH); Mass(m/z): 407.20 Anal.cal for $C_{20}H_{13}Cl_2N_5O_3$ (442.25): C-54.28, H-2.92, N-15.83: Found: C-54.28, H-2.92, N-15.86.

S_5:*3-(4-carboxyphenyl)-5-(4-hydroxyphenyl)-2-isonicotinoyl-2H-(3-tetrazolium)chloride*

Yield:92%; m.p:175ºC; FTIR (cm^{-1}): 3441(O-H), 1656(C=O), 1597(C=C), 1411(C=N), 1316(C-N) 1059(N-N),1494(N=N); ^1H NMR(ppm): δ 6.8-8.9(12H, m, Ar-H), 4.8(1H, S, OH), 11.0(1H,S,COOH); Mass(m/z): 423.40 Anal.cal for $C_{20}H_{14}ClN_5O_4$ (407.81): C-56.68, H-3.32, N-16.52: Found: C-56.47, H-3.36, N-16.56.

S_6: *3-(4-carboxyphenyl)-2-isonicotinoyl-5-phenyl-2H-(3-tetrazolium) chloride*

Yield:80%; m.p:105ºC; FTIR (cm^{-1}): 3433(O-H), 1676(C=O), 1602(C=C), 1459(C=N), 1302(C-N) 1062(N-N),1520(N=N); ^1H NMR(ppm): δ 7.2-8.45(13H, m, Ar-H), 10.9(1H, S, OH); mass (m/z): 406.74; Anal.cal for : C-58.90, H-3.45, N-17.17: Found: C-58.88, H-3.42, N-17.14.

S_7: *3-(3-chlorophenyl)-5-(4-hydroxyphenyl)-2-isonicotinoyl-2H-(3-tetrazolium) chloride*

Yield:85%; m.p:120ºC; FTIR (cm^{-1}): 3420(O-H), 1683(C=O), 1615(C=C), 1464(C=N), 1315(C-N) 1046(N-N),1486(N=N); ^1H NMR(ppm): δ 6.79-8.6(12H, m, Ar-H), 4.7(1H, S, OH); mass (m/z):413.01 Anal.cal for: C-55.08, H-3.16, N-16.90: Found: C-55.10, H-3.16, N-16.94.

S_8: *5-(2-chlorophenyl)-2-isonicotinoyl-3-phenyl-2H-(3-tetrazolium) chloride*

Yield:62%; m.p:127ºC; FTIR (cm^{-1}): 1692(C=O), 1602(C=C), 1462(C=N), 1365(C-N); 1054(N-N),1488(N=N) ^1H NMR(ppm): δ 7.16-8.45(13H, m, Ar-H); mass (m/z)-398.25: Anal.cal for: C-57.47, H-3.04, N-17.63: Found: C-57.44, H-3.02, N-17.58.

S_9: *3-(3-chlorophenyl)-2-isonicotinoyl-5-phenyl-2H-(3-tetrazolium) chloride*

Yield:76%; m.p:96ºC; FTIR (cm^{-1}): 1654(C=O), 1598(C=C), 1448(C=N), 1354(C-N) 1048(N-N),1478(N=N); ^1H NMR(ppm): δ 7.1-8.65(13H, m, Ar-H); mass (m/z):397.05 Anal.cal for (398.25): C-57.47, H-3.29, N-17.63: Found: C-57.42, H-3.26, N-17.58.

S_{10}: *5-(4-hydroxyphenyl)-2-isonicotinoyl-3-(4-sulfamoylphenyl)-2H-(3-tetrazolium) chloride*

Yield:80%; m.p:95ºC; FTIR (cm^{-1}): 3438(OH), 1659(C=O), 1609(C=C), 1415(C=N), 1308(C-N) 1074(N-N),1482(N=N); ^{1}H NMR(ppm): δ 6.8-8.7(12H, m, Ar-H); 2.2(2H,s, NH_2), 5.1(1H, s, OH); Mass (m/z): 458.04 Anal.cal for : C-49.81, H-2.85, N-18.42: Found: C-49.86, H-2.88, N-18.38.

***S_{11}**: 3-(4-carboxyphenyl)-2-isonicotinoyl-5-(4-methoxylphenyl)-2H-(3-tetrazolium)chloride*
Yield:95%; m.p:130ºC; FTIR (cm^{-1}): 3432(OH), 1677(C=O), 1602(C=C), 1462(C=N), 1302(C-N) 1042(N-N),1512(N=N); ^{1}H NMR(ppm): δ 6.83-8.55(12H, m, Ar-H); 3.7(2H, s, CH_3), 11.25(1H, s, OH); Mass (m/z): 437.84 Anal.cal for : C-57.60, H-3.68, N-15.99: Found: C-57.64, H-3.72, N-15.96.

***S_{12}**: 2-isonicotinoyl-3,5-diphenyl-2H-(3-tetrazolium)chloride*
Yield: 59%; m.p:172ºC; FTIR (cm^{-1}): 1598(C=C), 1448(C=N), 1314(C-N) 1056(N-N),1520(N=N); ^{1}H NMR(ppm): δ 7.3-8.63(14H, m, Ar-H); Mass (m/z): 363.09 Anal.cal for : C-62.72, H-3.87, N-19.24: Found: C-62.68, H-3.89, N-19.24.

Biological activity

Anti microbial activity assessment

All test micro organisms, Gram negative bacteria**:** *E. coli* (ATCC 700722), Gram positive bacteria: *S aureus* (ATCC 700294), *B.subtilis* (ATCC 14580) and yeast like fungi: *A.niger* is obtained from NCRI, Pune. The newly synthesized compounds **S_{1-12}** were weighed and dissolved in dimethyl suphoxide to prepare stock solution of 10,000µg/ml. The anti microbial effects of the substances were tested quantitatively [11] in respective broth media by using cup plate method and zone of inhibition values were determined. Anti bacterial and anti fungal assays were performed in Muller-Hinton broth (MH) at pH 7.2 using Phosphate buffer. Ciprofloxacin and Ketoconazole were used as standards for anti bacterial and anti fungal activities respectively.

Anti oxidant activity by DPPH method

The free radical scavenging ability of sample under study was carried out by DPPH method [12-13] Different concentration of the sample was treated with 0.1M solution of DPPH in ethanol and absorbance was measured at 570 nm. Ascorbic acid was used as reference standard and 0.1M solution of DPPH in ethanol was used as control. Tests were done in triplicate for each concentration of sample and percentage inhibition was calculated.

CONCLUSION

Twelve new analogues of tetrazole heterocycles were synthesized, characterized and confirmed by spectral analysis. All the compounds exihibited significant antimicrobial activity and *in vitro* anti oxidant activity by DPPH free radical scavenging method. From the above study it can be concluded, the presences of electron withdrawing substituents in the phenyl ring at 3 and 5 position of tetrazole resulted in more potent compounds for the evaluated activities. Thus from these observations, *in vivo* methods of analysing the biological activities are planned in the near future.

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