International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.1, No.4, pp 1548-1553, Oct-Dec 2009

In vitro Antitubercular activity of novel 3-(4-Methoxyphenyl)-1-isonicotinoyl-5-(substituted phenyl)-formazans

Jignesh P. Raval*[1], Purvesh Patel[1] and Pradip S. Patel[1]

[1]Department of Pharmaceutical Chemistry, Ashok & Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences (ARIBAS), New Vallabh vidyanagar - 388121(Gujarat-India)

**Corresponding author E-mail: drjpraval@yahoo.co.in*

Abstract: In the present investigation, a series of 3-(4-methoxyphenyl)-1-isonicotinoyl-5-(substituted phenyl)-formazans **(5a-r)** were synthesized by condensation of schiff base **(3)** and diazonium salt of various substituted aromatic amines, **(3a-r)**. The intermediate schiff base **(3)** was itself synthesized by condensation of isonicotinic acid hydrazide with 4-methoxybenzaldehyde **(2)**. The structures of the compound have been confirmed by elemental analysis and spectral analysis. Newly synthesized compounds were tested for their in vitro anti-tubercular activity against Mycobacterium tuberculosis H37Rv using the BACTEC 460 radiometric system. Among the synthesized compounds some of them have shown significant antitubercular activity.

Keywords: Schiff base, Formazans, Isonicotic acid hydrazide, Antitubercular activity, Antimycobacterial activity, *Mycobacterium tuberculosis H37Rv*.

Introduction

The high number of multidrug-resistant Mycobacterium tuberculosis strains circulating worldwide has increased concern that tuberculosis (TB) may once again become an incurable disease and has emphasized the need for new drugs to treat this infection. Over the last few years, extensively drug-resistant TB, defined as TB caused by M. tuberculosis strains resistant to isoniazid (INH), rifampin, any fluoroquinolone, and one of the injectable drugs amikacin, kanamycin, and capreomycin, has become a major concern for TB treatment and control in the global setting[1-9]. In addition, strains with resistance to all of the major clinically used antitubercular drugs are known[10], thus answering the question posed by Dye and Espinal, "Will tuberculosis become resistant to all antibiotics?" [11].

In the 1960s and 1970s, several monosubstituted isonicotinohydrazides were reported to possess appreciable *in vivo* antimycobacterial activity linked to the release of parental INH[12-15]. Also, our research group has previously published several papers, in which the synthesis and primary biological valuation of a large number of heterocyclic derivatives were described[16-21]. To our knowledge, formazans with such anti-infective properties have not been reported before. Thus, to extend our antitibuercular research[22,23], different formazans derivatives using isoniazid were synthesized and evaluated as antibacterial agents[24]. Some of them showed good *in vitro* parameters. In this report, we detail the *in vitro* antitubercular activities of various formazan derivatives**(Scheme – 1)**.

Experimental

All the melting points were taken in open capillaries tube and are uncorrected. The purity of compounds was checked routinely by TLC (0.5 mm thickness) using silica gel – G coated Al – plates (Merck) and spots were visualized by exposing the dry plates in iodine vapours. IR spectra (ν_{max} in cm^{-1}) were recorded on Shimadzu FTIR spectrophotometer using KBr or Nujol technique. ^1H & ^{13}C NMR spectra on a Bruker's WM 400 FT MHz NMR instrument using $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal reference (chemical shifts in δ ppm). The elemental analysis (C, H, N) of compounds was performed on Carlo Erba – 1108 elemental analyzer.

N'-(4-methoxybenzylidene)isonicotinohydrazide 3.

Title compound was synthesized using earlier method.[24, 25].

3-(4-methoxyphenyl)-1-isonicotinoyl-5-phenylformazan, 5a.

Aniline **(4a)** (0.01 mole) in aqueous HCl(10ml) was reacted with N'–(4–methoxybenzylidene)isonicotinohydrazide (0.01 mole) following the procedure described in reported literature[24,25]. The progress of reaction was monitored by TLC using acetone:ethanol:chloroform (1:3:6) as eluent. The dark coloured product obtained was crystallized from ethanol. Yield 83%, mp 144~45°C. IR(ν_{max} in cm^{-1}): 3310(NH), 1330 (CN), 1680(CO) 1610(N=C of schiff base), 1590(N=N), 1256(OCH_3). ^{1}H NMR($CDCl_3$) δ ppm: 3.81(s, 3H, OCH_3), 12.15 (s, 1H, CONH, D_2O exchangeable), 7.79-8.10(m,13H, ArH). ^{13}C NMR($CDCl_3$) δ ppm: 55.7(OCH_3), 151.5(N=C), 163.2(CO), 114.9, 120.7, 122.8, 127.1, 129.0, 130.1, 141.9, 148.1, 161.0, (Ar-C).

Other compounds, **5b-r** were prepared in similar manner and characterization data of **5b-m** are reported in literature[24], while **5n-r** are given in Table – 1.

Selected spectral data of the products, 5n-r.

3-(4-methoxyphenyl)-5-(2,4-dichlorophenyl)-1-isonicotinoyl-formazan, 5n.

IR (ν_{max} in cm^{-1}): 3100 (Aromatic CH str), 3340(NH), 1345(CN), 2800 (OCH_3), 1670(CO), 1612(N=C of Schiff base), 1572(N=N of formazans), 812(C-Cl). ^{1}H NMR(DMSO-d_6) δ ppm: 3.91(s, 3H, OCH_3), 10.12(s, 1H, CONH, D_2O exchangeable), 7.15-7.85(m, 11H, Ar-H). ^{13}C NMR(DMSO-d_6) δ ppm: 61.2(OCH_3), 155.0(N=C), 163.7(CO), 112.6, 119.7, 124.9, 125.0, 127.9, 128.4, 129.6, 133.7, 138.8, 147.9, 160.1(Ar-C).

3-(4-methoxyphenyl)-5-(2,5-dichlorophenyl)-1-isonicotinoyl-formazan, 5o.

IR (ν_{max} in cm^{-1}): 3111 (Aromatic CH str), 3335(NH), 1340(CN), 2811 (OCH_3), 1675(CO), 1615(N=C of Schiff base), 1577(N=N of formazans), 814(C-Cl). ^{1}H NMR(DMSO-d_6) δ ppm: 3.94(s, 3H, OCH_3), 10.14(s, 1H, CONH, D_2O exchangeable), 7.25-7.90(m, 11H, Ar-H). ^{13}C NMR(DMSO-d_6) δ ppm: 60.9(OCH_3), 155.0(N=C), 164.1(CO), 111.8, 120.1, 125.1, 126.2, 128.1, 129.5, 130.7, 134.8, 139.9, 148.1, 161.3 (Ar-C).

3-(4-methoxyphenyl)-5-(2,6-dichlorophenyl)-1-isonicotinoyl-formazan, 5p.

IR (ν_{max} in cm^{-1}): 3121 (Aromatic CH str), 3340(NH), 1345(CN), 2821 (OCH_3), 1670(CO), 1612(N=C of Schiff base), 1579(N=N of formazans), 818(C-Cl). ^{1}H NMR(DMSO-d6) δ ppm: 10.18(s, 1H, CONH, D_2O exchangeable), 7.25-7.92(m, 11H, Ar-H). ^{13}C NMR(DMSO-d_6) δ ppm: 60.1(OCH_3), 155.7(N=C), 164.1(CO), 113.9, 121.2, 127.3, 129.2, 129.9, 135.9, 139.1, 149.9, 160.9 (Ar-C).

3-(4-methoxyphenyl)-5-(2-hydroxyphenyl)-1-isonicotinoyl-formazan, 5q.

IR (ν_{max} in cm^{-1}): 3129 (Aromatic CH str), 3560 (OH), 3325(NH), 1325(CN), 2829(OCH_3), 1684(CO), 1621(N=C of Schiff base). ^{1}H NMR(DMSO-d_6) δ ppm : 3.99(s, 3H, OCH_3), 10.22(s, 1H, CONH, D_2O exchangeable), 5.29(s, 1H, OH), 7.80-8.87 (m, 13H, Ar-H). ^{13}C NMR(DMSO-d_6) δ ppm: 61.7(OCH_3), 162.7 (N=C), 169.1(CO), 114.9, 119.1, 121.7, 127.2, 127.9, 129.2, 130.7, 131.9, 134.7, 137.9, 148.1, 160.1(Ar-C).

3-(4-methoxyphenyl)-5-(4-hydroxyphenyl)-1-isonicotinoyl-formazan, 5r.

IR (ν_{max} in cm^{-1}): 3127 (Aromatic CH str), 3569 (OH), 3329(NH), 1327(CN), 2829(OCH_3), 1683(CO), 1627(N=C of Schiff base). ^{1}H NMR(DMSO-d_6) δ ppm : 4.01(s, 3H, OCH_3), 10.21(s, 1H, CONH, D_2O exchangeable), 5.18(s, 1H, OH), 7.82-8.89 (m, 13H, Ar-H). ^{13}C NMR(DMSO-d_6) δ ppm: 62.6(OCH_3), 162.1 (N=C), 169.2(CO), 114.1, 115.7, 121.3, 127.4, 129.2, 130.3, 131.2, 134.6, 138.2, 160.1 (Ar-C).

Pharmacology

Anti-mycobacterial activity[26,27]

The primary screening was conducted at concentration of 12.5 or 6.25 mg/ml (or molar equivalent of highest molecular weight compound in a series of congeners) against M. tuberculosis H37Rv (ATCC27294) in BACTEC 12B medium using the BACTEC 460 radiometric system[26, 27]. Compounds demonstrating at least 90% inhibition in the primary screen were re-examined at lower concentration (MIC) in broth micro-dilution assay with Almar Blue. The MIC was defined as the lowest concentration inhibiting 99% of the inoculum. Concurrent with the determination of MICs, compounds were tested for cytotoxicity (IC50) in VERO at concentration equal to and greater than the MIC for M. tuberculosis H37Rv after 72 h of exposure, viability is assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 Non-radioactive Cell proliferation assay. The data are presented in **Table 2.**

Results and discussion

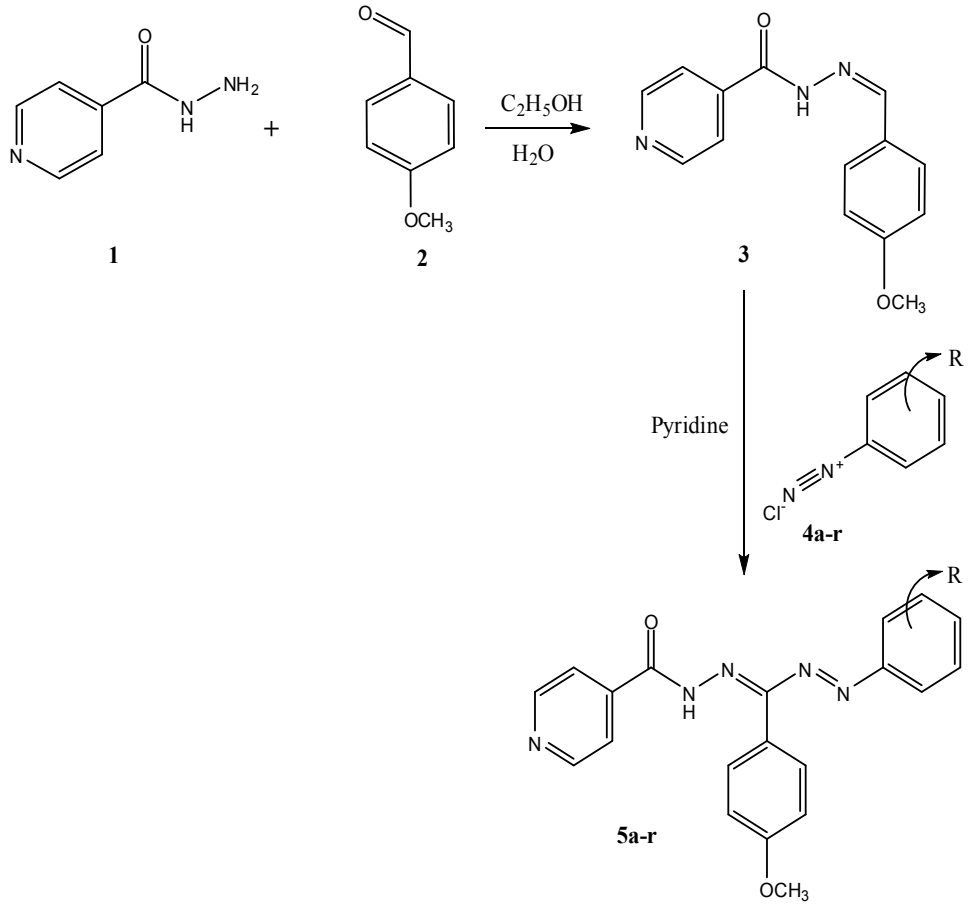
Chemistry

Target compound **(5a-r)** were prepared according to **Scheme 1.** Condensation of Isonicotinic acid hydrazide **(1)** with 4-methoxybenzaldehyde in presence of ethanol as a reaction medium gave schiff base **(3).** Formation of **(3)** was confirmed by appearance of IR band in the region 1625 cm^{-1} due to -N=CH- group, 1665 cm^{-1} due to >C=O group of amide, 3340 cm^{-1} due to -NH- group (secondary amine) of **(3)** and disappearance of IR band in the region 3378 cm^{-1} & 1710 cm^{-1} corresponding to NH_2 group and CHO group of isonicotinic acid hydrazide **(1)** and 4-methoxybenzaldehyde **(2)** respectively. ^{1}H NMR spectra showed a singlet at δ 8.86 ppm due to >N=CH- (1H) of schiff base **(3)** and disappearance of signal at δ 2.60 ppm due to $–NH_2$ (2H) of isonicotinic acid hydrazide **(1)** and δ 9.5 ppm due to –CHO (1H) of 4-methoxybenzaldehyde **(2)**. Similarly, ^{13}C NMR spectra showed a signal at δ 145.6 ppm due to N=C of schiff base **(3).** Further reaction of schiff base **(3)** and diazonium salt of substituted aromatic amines **(4a-r)** in pyridine at 0–5 ºC afforded

substituted formazans **(5a-r).** These compound show IR absorption band at 1600-1615 cm^{-1} due to –C=N- group, 1660-1675 cm^{-1} due to >C=O group of amide, 3335-3355 cm^{-1} due to -NH- group (secondary amine) and 1570-1585 cm^{-1} due to -N=N- group and the disappearance of bands at 1625 cm^{-1} (N=C) also confirmed the formation of **(5a-r).** ^1H NMR spectra of formazans **(5a-r)** shows disappearance of singlet δ 8.96 ppm due to >C=NH– of compound **(3).** The synthetic route of above mentioned compounds is shown in **Scheme 1**.

Pharmacology

All the newly synthesized substituted formazan derivatives **(5a-r)** were tested for their anti-mycobacterial activity in vitro against M. tuberculosis H37Rv using the BACTEC 460 radiometric system. The results are summarized in Tables 1 and 2 with INH, a standard used for comparison. Among them, compound 3-(4-methoxyphenyl)-5-(2,4-dichlorophenyl)-1-isonicotinoyl-formazan **(5n)** and 3-(4-methoxyphenyl)-5-(2,6-dichlorophenyl)-1-isonicotinoyl-formazan **(5p)** produced highest efficacy and exhibited >90% inhibition at a concentration of 0.0179 µM and 0.0149 µM followed by 3-(4-methoxyphenyl)-5-(2-methoxyphenyl)-1-isonicotinoyl-formazan **(5b),** 3-(4-methoxyphenyl)-5-(4-methoxyphenyl)-1-isonicotinoyl-formazan **(5d)** and 3-(4-methoxyphenyl)-5-(2,5-dichlorophenyl)-1-isonicotinoyl-formazan **(5o)** which showed moderate inhibitory activity with 0.0277 µM, 0.0203 µM, 0.0292 µM respectively. Thus, the 2,4- dichloro and 2,6- dichloro groups substitution derivatives displayed relatively higher inhibitory activity in general. However, the electron rich groups such as, 2,4-dichloro, 2,5-dichloro, 2,6-dichloro, 2-methoxy, 3- methoxy and 4-methoxy substituted analogues produced significant increase in inhibitory activity against *M. tuberculosis H37Rv*. On the other hand analogues with methyl group substitution **(5k-m)** and phenyl substitution **(5a)** showed relatively low inhibitory activity against *M. tuberculosis H37Rv*. Instead (OH) group, (CH_3) group and (NO_2) group substitution at phenyl ring in formazan analogues worsens the anti-mycobacterial activity in comparison to (Cl) group analogues.



Scheme-1: Synthetic route to 3-(4-methoxyphenyl)-1-isonicotinoyl-5-(substituted phenyl)-formazans 5a-r.

Table – 1: Characterization data of 3 and 5a-r.

Compd.	R	Melting point(^{O}C)	Yield (%)	λmax in nm (DMF)	Anal. Calcd. (found)/ %		
					C	H	N
3	-	160 ~ 62	70 ~ 71	310	64.19 (64.29)	4.14 (4.18)	17.28 (17.38)
5a	H	144 ~ 45	83 ~ 84	282	66.84 (66.92)	4.77 (4.82)	19.49 (19.34)
5b	2-OCH_3	142 ~ 44	72 ~ 74	371	64.77 (64.82)	4.92 (4.87)	17.98 (17.77)
5c	3-OCH_3	146 ~ 47	81 ~ 82	372	64.77 (64.72)	4.92 (4.90)	17.98 (17.79)
5d	4-OCH_3	149 ~ 50	77 ~ 79	379	64.77 (64.76)	4.92 (4.87)	17.98 (17.89)
5e	2-NO_2	129 ~ 31	69 ~ 71	275	59.40 (59.37)	3.99 (3.89)	20.78 (20.89)
5f	3-NO_2	134 ~ 36	62 ~ 67	272	59.40 (59.67)	3.99 (3.79)	20.78 (20.81)
5g	4-NO_2	127 ~ 29	69 ~ 71	277	59.40 (59.43)	3.99 (3.77)	20.78 (20.84)
5h	2-Cl	136 ~ 38	70 ~ 73	302	60.99 (60.79)	4.09 (4.09)	17.78 (17.79)
5i	3-Cl	139 ~ 40	76 ~ 77	303	60.99 (60.79)	4.09 (4.11)	17.78 (17.91)
5j	4-Cl	148 ~ 49	79 ~ 81	307	60.99 (60.82)	4.09 (4.18)	17.78 (17.92)
5k	2-CH_3	130 ~ 33	80 ~ 82	281	67.55 (67.67)	5.13 (5.18)	18.76 (18.82)
5l	3-CH_3	135 ~ 36	84 ~ 86	286	67.55 (67.72)	5.13 (5.21)	18.76 (18.78)
5m	4-CH_3	139 ~ 40	87 ~ 89	287	67.55 (67.77)	5.13 (5.18)	18.76 (18.96)
5n	2,4-$(Cl)_2$	183 ~ 84	81 ~ 82	261	56.09 (56.28)	3.53 (3.69)	16.35 (16.21)
5o	2,5-$(Cl)_2$	181 ~ 83	74 ~ 76	272	56.09 (56.17)	3.53 (3.77)	16.35 (16.27)
5p	2,6-$(Cl)_2$	187 ~ 89	71 ~ 72	266	56.09 (56.22)	3.53 (3.66)	16.35 (16.38)
5q	2-OH	166 ~ 69	61 ~ 62	311	63.99 (64.11)	4.56 (4.73)	18.66 (18.72)
5r	4-OH	172 ~ 74	64 ~ 66	318	63.99 (64.18)	4.56 (4.79)	18.66 (18.79)

All the newly synthesized compounds **(5a-r)** were tested for cytotoxicity (IC50) in VERO cells at concentrations of 62.5 mg/ml or 10 times. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 Non-radioactive Cell proliferation method. Most of the active compounds were found to be non-toxic till 62.5 mg/ml. Among the newer derivatives, it is conceivable that derivatives showing anti-mycobacterial activity can be further modified to exhibit better potency than the standard drugs. Thus, newly synthesized formazan derivatives discovered in this study may provide valuable therapeutic intervention for the treatment of anti-tubercular diseases.

Conclusion

Formazans derivatives were synthesized and characterized for their structure elucidation. Various chemical and spectral data supported the structures thought of. Antimycobacterial data of compounds **5n** and **5p** are showing highest efficacy and exhibited >90% inhibition at lower concentration.

Table – 2: Anti-mycobacterial activity substituted formazans, 5a-r

Comp.	Primary Screen (6.25 µg/mL)	% Inhibition	Concentration (µM)	Actual MIC (µg/mL)
5a	>12. 5	48	0.0949	-
5b	>6.25	87	0.0277	-
5c	>6.25	82	0.0308	-
5d	<6.25	89	0.0203	6.25
5e	>12. 5	22	0.1902	-
5f	>12. 5	27	0.1101	-
5g	>12. 5	36	0.1477	-
5h	>12. 5	66	0.0650	-
5i	>12. 5	67	0.0640	-
5j	>12. 5	69	0.0643	-
5k	>12. 5	26	0.2809	-
5l	>12. 5	24	0.2000	-
5m	>12. 5	28	0.2109	-
5n	<6.25	92	0.0179	6.00
5o	>6.25	85	0.0292	-
5p	<6.25	94	0.0149	6.25
5q	>12. 5	32	0.1022	-
5r	>12. 5	46	0.0979	-

Isoniazid (0.025 – 0.05 mg/mL).

Acknowledgments

The authors gratefully acknowledge the encouragement and support of **Charutar Vidya Mandal** (Vallabh Vidyanagar, Gujarat-India). Thanks to **SICART** & **Department of Chemistry**(Navinbhai), Sardar Patel University, Vallabh Vidyanagar for providing analytical and NMR spectral facilities.

References

1. Benatar S. R., Extensively drug resistant tuberculosis: problem will get worse in South Africa unless poverty is alleviated., Br. Med. J., 2006, 333, 705.
2. Dahle U. R., Extensively drug resistant tuberculosis: beware patients lost to follow-up. Br. Med. J., 2006, 333, 705.
3. Espinal M. A., The global situation of MDR-TB., Tuberculosis., 2003, 83, 44–51.
4. Gandhi N. R., Moll A., Sturm A. W., Pawinski R., Govender T., Lalloo U., Zeller K., Andrews J., and Friedland G., Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet 2006, 368, 1575–1580.
5. Goldman R. C., Plumley K. V. and Laughon B. E., The evolution of extensively drug resistant tuberculosis (XDR-TB): history, status and issues for global control. Infect. Disord. Drug Targets., 2007, 7, 73–91.
6. Lawn S. D. and Wilkinson R., Extensively drug resistant tuberculosis: a serious wake-up call for global health., Br. Med. J., 2006, 333, 559–560.
7. Manissero D. and K. Fernandez de la Hoz., Extensive drug-resistant TB: a threat for Europe? Euro Surveill., 2006, 11:E060928.
8. Shah N. S., Pratt R., Althomsons S. and Cegielski J. P., Extensively drug-resistant tuberculosis, United States, 1993–2006., JAMA 2007, 297: 1871–1873. [Reprinted from MMWR Morb. Mortal. Wkly. Rep. 56:250– 253, 2007.]
9. World Health Organization: Strategic and technical meeting on intensified control of neglected tropical diseases. A renewed effort to combat entrenched communicable diseases of the poor. World Health Organization, Geneva, Switzerland. http://www.popline.org/ docs/312474. Posting date, March 2006.
10. Migliori G. B., De Iaco G., Besozzi G., Centis R. and Cirillo D. M., First tuberculosis cases in Italy resistant to all tested drugs. Euro Surveill., 2007, 12:E070517.1.
11. Dye C. and Espinal M. A., Will tuberculosis become resistant to all antibiotics? Proc. Biol. Sci., 2001, 268, 45–52.
12. Fox H. H. and Gibas J. T., Synthetic tuberculostats. IX. Dialkyl derivatives of isonicotinylhydrazine., J. Org. Chem., 1955, 20, 60–69.
13. Lewis A. and Shepherd R. G., Antimycobacterial agents, p. 409–491 In A. Burger (ed.), Medicinal chemistry, 3rd ed. Wiley-Interscience, New York, N.Y., 1970.

14. McMillan F. H., Leonard F., Meltzer R. I. and King J. A., Antitubercular substances. II. Substitution products of isonicotinic hydrazide. J. Am. Pharm. Assoc., 1953, 42, 457–464.
15. Sensi P. and Gialdroni Grassi G., Antimycobacterial agents, p. 575– 635. In M. E. Wolff (ed.), Burger's medicinal chemistry and drug discovery, 5th ed. John Wiley & Sons, Inc., New York, N.Y., 1996.
16. Raval J.P. and Desai K.R., Synthesis and antimicrobial activity of new triazolopyridinyl phenothiazines., ARKIVOC., 2005, 21-28.
17. Raval J.P., Desai J.T., Desai C.K. and Desai K.R., A comparative study of microwave assisted and conventional synthesis of 2,3-dihydro-2-aryl-4-[4-(2–oxo–2H–chromen–3–yl)–1,3-thiazol–2–ylamino]-1,5–benzothiazepines and its antimicrobial activity, ARKIVOC., 2008, 233-244.
18. Raval J.P., Desai K.G. and Desai K.R., Neat Reaction Technology for the synthesis of 4-oxothiazolidines derived from 2-SH-Benzothiazole and antimicrobial screening of some synthesized 4-thiazolidinones. J. Iran Chem. Soc., 2006, 3, 233-241.
19. Raval J.P. and Desai K.R., A Comparative study of Microwave assisted and Conventional Synthesis of novel 2-(4-diethylamino-2-hydroxyphenyl)-3-substituted-thiazolidin-4-one derivatives., Chemija., 2009, 101-108.
20. Raval J.P. and Desai K.R., Synthesis of 3-Phenyl-4[4-(m-nitrophenyl)-N-2-(2'-arylureido / arylthioureido-4'-N-morpholino-s-triazin)-benzo-[6,7]-coumarins and their Applicatiions., E-J. Chem., 2004; 1, 211 – 215.
21. Raval J.P., Rai A.R., Patel N.H., Patel H.V. and Patel P.S., Synthesis and *in vitro* antimicrobial activity of N'-(4-(arylamino)-6-(pyridin-2-ylamino)-1,3,5-triazin-2-yl)benzohydrazide. Int. J ChemTech., 2009, 1(3), 616-620.
22. Raval J.P., Malaviya A.J., Patel N.H., Patel H.V. and Patel P.S., Synthesis and In Vitro Antimycobacterial Activity of Trisubstituted s-Triazines., Sci.Pharm., 200X; 7X: XXX–XXX, doi:10.3797/scipharm.0905-15.
23. Raval J.P., Akhaja T.N., Patel N.H., Patel H.V. and Patel P.S., Synthesis, characeterization and *in vitro* pharmacological activity of novel 3-(4-Fluorophenyl)-1-isonicotinoyl-5-(substituted phenyl)-formazans., Int. J. Pharm. Res., 2009, 1(2),62-71.
24. Raval J.P., Patel P.R., Patel N.H., Patel P.S., Bhatt V.D. and Patel K.N., Synthesis, Characeterization and in vitro antibacterial activity of novel 3-(4-methoxyphenyl)-1-isonicotinoyl-5-(substituted phenyl)-formazans. Int. J ChemTech. , 2009, 1(3), 610-615.
25. Desai K.G. and Desai KR, Microbial screening of novel synthesized formazans having amide linkages., J. Heterocycl. Chem., 2006, 43, 1083-1089.
26. Collins L. and Franzblau S.G., Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against Mycobacterium tuberculosis and Mycobacterium avium., Antimicrob. Agents Chemother., 1997, 41, 1004–1009.
27. Interleid B., Antibiotic in Laboratory Medicine., In: V. Lorian (Ed.), third ed. Williams and Wilkins, Baltimore, 1991; 134.

*****