Int J Pharm Bio Sci 2013 July; 4(3): (P) 767 - 777

Research Article Medicinal Chemistry

International Journal of Pharma and Bio Sciences ISSN 0975-6299

SYNTHESIS OF 8-SUSBTITUTED-3,4-DIHYDRO-6-METHYL-4-PHENYLIMIDAZO[1,5-*b*][1,2,4]TRIAZIN-2(8*H*)-ONE DERIVATIVES AS NOVEL ANTIMICROBIAL AGENTS.

A A BARVKAR*[1] AND S D SAWANT[2]

[1] Department of Pharmaceutical Chemistry, Pharmacy Faculty, University of pune, SVPM's College of Pharmacy, Malegaon (Bk), Pune, MS. 413115. India.
[1] Department of Pharmaceutical Chemistry, Pharmacy Faculty, Karpagam University, Coimbatore, TN. 641021. India.
[2] Department of Pharmaceutical Chemistry, Pharmacy Faculty, University of Pune, STES's SKN College of Pharmacy, Kondhwa, Pune, MS. 411048. India.

ABSTRACT

A series of new 6-methyl-3,4-dihydroimidazo[1,5-*b*][1,2,4]triazin-2(8*H*)-one derivatives have been synthesized with the initial reaction of various aromatic aldehydes and N-acetylglycine in acetic anhydride and anhydrous sodium acetate by Erlenmeyer-Azlactone synthesis to give oxazol-5(4*H*)-one derivatives which on reaction with substituted phenylhydrazine in dry benzene gave 1*H*-imidazol-5(4*H*)-one and lastly condensation with chloroacetamide in N,N-dimethylformamide gave final compounds. All synthesized compounds were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, LC-MS and HPTLC etc and are well supported by spectroscopic data. These novel compounds are investigated for their in vitro antimicrobial activity using zone of inhibition using cup plate method. The antibacterial activities were compared with standard drug such as ciprofloxacin and antifungal drug such as cotrimazole at concentration of 250, 500 and 750 µg/L. Synthesized analogues showed favorable antimicrobial effect.

KEYWORDS:Erlenmeyer-Azlactone synthesis, antimicrobial, ciprofloxacin, cotrimazole.

A A BARVKAR
Department of Pharmaceutical Chemistry, , University of pune, SVPM's College of Pharmacy, Malegaon (Bk), Pune, MS. 413115. India.

1. INTRODUCTION

Heterocyclic compounds have taken a justified place in the market because they have wide variety of medicinal applications as compared to carbocyclic compounds. Therefore it is important factor to develop and synthesize novel heterocyclic compounds for better health of social community [1]. Though many effective antimicrobial drugs exist in the market, we could not cease the research for development of novel antimicrobial agents in order to find and develop more effective medicines. In many times medication for treating microbial infections are waiting for outcome of challenging drug e.g. tuberculosis or AIDS. This is mainly because of important factor so called "multi-drug resistance" by pathogenic microorganisms. Hence there is thirsty need for development of new categories of antimicrobial agents [2,3,4,5].Triazine and fused triazines have diverse medicinal, agricultural and industrial applicability. One of fused ring system, imidazotriazines is an important class of condensed bicyclic heterocyclic compounds that shows a various medicinal apllications such as anticancer [6], antimicrobial [7], anti-inflammatory [8], neuroprotection [9] etc. Wide coverage of the medicinal activities indicates that 1,2,4-triazine ring has wide scope in synthetic chemistry as well as natural chemistry [10]. Even also 1,2,4-triazine class has major applications in agrochemicals [11,12]. Some newer fused 1,2,4-triazine-4(6*H*)-ones 1A has selective cytotoxic effect in micro quantity against a wide variety of cancerous cells [6,13], while other derivatives of 1A showed analgesic [13], antibacterial, and antiviral effects [7]. Drugs with the 1,2,3,4-tetrahydroimidazo[1,2-*b*][1,2,4]triazine ackbone 2A has potential application as interleukin-1(IL-1) and tumor necrosis factor (TNF) inhibitors [8]. Now a days, specific substituted imidazo[1,5-*f*][1,2,4]triazines 3A is reported as kinase inhibitors with tremendous anticancer effects [13]. The 1,2,4-triazines 3A also showed against glycogen synthase kinase 3 (GSK3β), and inhibition of phosphodiesterase 10 (PDE10) inhibition, which is mainly important for the treatment of neurodegenerative disorders, like Parkinson's disease [12,13]. Motivated by this above-mentioned facts herein is reported the synthesis and antimicrobial activity of novel 6-methyl-3,4-dihydroimidazo[1,5-*b*][1,2,4]triazin-2(8*H*)-one series. Also the literature survey reveals that there are not many and not same the examples of triazines fused with imidazole.

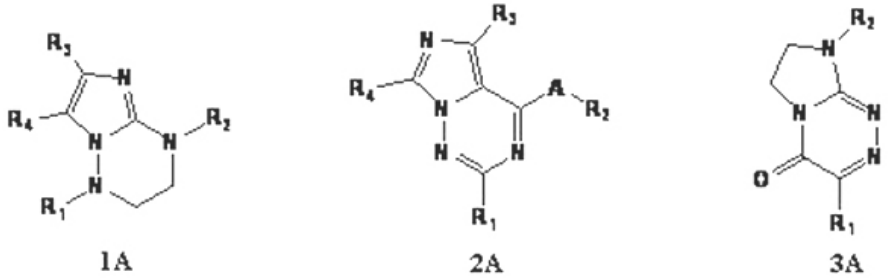

Figure 1
Biologically important imidazo[1,2,4]triazine derivatives 1A-3A.

2A=1,2,3,4-tetrahydroimidazo[2,1-b][1,2,4]triazine, 2A= Imidazo [1,5-f][1,2,4]triazine, 3A=7,8-dihydroimidazo[2,1-c][1,2,4]triazine-4(6H)-one
R_1=R_2=Alkyl, acyl, CONHNH$_2$, acetate or Ar/Het-Ar, R_3=R_4=(cyclo)alkyl or Ar/Het-Ar, A=O-, S-, NH-alkyl or N-cycloalkyl

2. MATERIALS AND METHODS

All reagents were used as purchased from E. Merck and used without further purification. Melting points were determined by using a digital melting point determination apparatus (Remi) and are uncorrected. Purity of compounds were checked by High Performance Thin-layer chromatography (HPTLC) and was performed on CAMAG twin with applicator Linomat-IV and plate specifications are Merck precoated silica gel 60 F_{254} with 0.2 mm thickness. Spectroscopic data were recorded by using FT-IR (Shimadzu spectrophotometer 8400 using KBr), ^{1}H NMR (Varian Mercury 400, Model- Unity AS400, serial- S0121719, frequency 400 MHz using DMSO as a solvent and tetramethylsilane (TMS) as an internal standard and chemical shifts were expressed as δ values in ppm), ^{13}C NMR (INOVA-300 with 75 MHz frequency DMSO as a solvent and tetramethylsilane (TMS) as an internal standard), LC-MS (Benchtop Agilent 1100 series LC–MSD (Agilent Technologies, Waldbronn, Germany), Column: C18, preparation on ODS (octadecylsilica) Hypersil column (Agilent Technologies), Flow-rate was 0.25 mL/min to 0.50 mL/min). Antimicrobial activity was done using cup plate agar diffusion method.

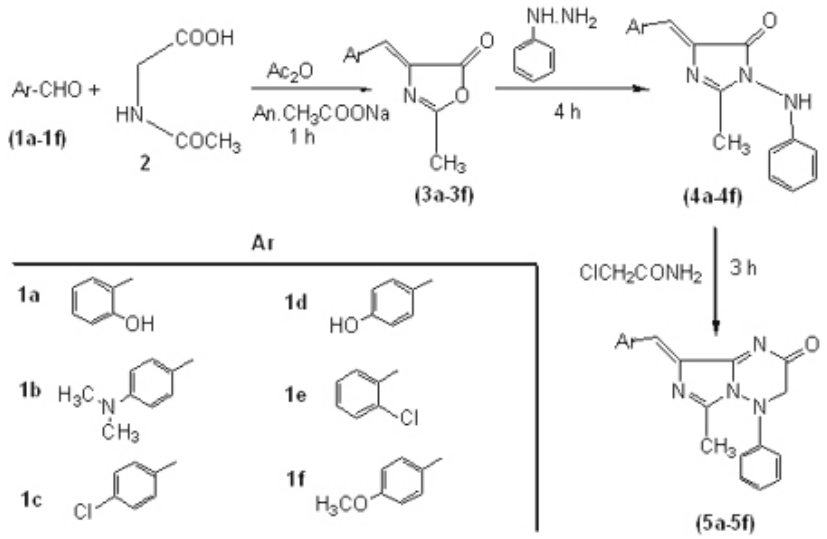

Figure 2
Route of synthesis.

2.1. General procedure for synthesis of compounds (3a-3f) by Erlenmeyer-Azlactone synthesis [15]

Warm mixture of 29 g (0.25 mol) of *N*-acetylglycine, 37.5 ml (0.37 mol) of aromatic aldehydes (1a-1f), 15 gm (0.183 mol) of anhydrous sodium acetate and 59 mL (0.62 mol) of acetic anhydride in 500 mL flask equipped with a reflux condenser; on water bath with occasional shaking until solution is complete (10-20 min). Boil the resulting solution for 1 h, cool and leave in a refrigerator overnight. Stir the solid mass of yellow crystals with 60 mL of cold water, transfer to a Buchner funnel and wash well with cold water. Washed with a little ether. Crystallized from carbon tetrachloride and used for next step of synthesis.

4-(2-hydroxybenzylidene)-2-methyloxazol-5(4H)-one [3a]

Mol. Form. $C_{11}H_9NO_3$ (203.19); Ar = (-C_6H_5-*o*-OH); mp 305-307 ºC; yield: 89%; IR (KBr, V_{max}, cm $^{-1}$): 855 (C-H bend), 1294 (C-O str), 1355 (C-N str), 1520 (C=C str), 1605 (C=N str), 1671 (C=O str), 2970 (CH_3 str), 3041 (C-H str), 3324

(O-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz): 1.42 (s, 1H, CH_3), 6.68 (s, 1H, CH), 6.71 (d, 1H, ArH), 6.77 (t, 1H, ArH), 6.97 (t, 1H, ArH), 7.13 (d, 1H, ArH), 11.32 (s, 1H, OH).

4-[4-(dimethylamino)benzylidene]-2-methyloxazol-5(4H)-one [3b]

Mol. Form. $C_{13}H_{14}N_2O_2$ (230.26); Ar = [-C_6H_5-*p*-*N*-$(CH_3)_2$]; m.p 82-85 ºC; yield: 82%;IR (KBr, V_{max}, cm $^{-1}$): 802 (C-H bend), 1263 (C=C str), 1544 (C=N str), 1637 (C=N str), 1759 (C=O str), 2967 (CH_3 str), 3077 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz): 2.25 (s, 1H, CH_3), 2.85 (s, 6H, 2 × CH_3), 6.58 (s, 1H, CH), 7.14 (d, 2H, ArH), 7.65 (d, 2H, ArH).

4-(4-chlorobenzylidene)-2-methyloxazol-5(4H)-one [3c]

Mol. Form. $C_{11}H_8ClNO_2$ (221.63); Ar = (-C_6H_5-*p*-Cl); mp175-176 ºC; yield: 76%; IR (KBr, V_{max}, cm $^{-1}$): 763 (C-Cl str), 824 (C-H bend), 1313 (C-N str), 1585 (C=C str), 1650 (C=N str), 1790 (C=O str), 2979 (CH_3 str), 3086 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz): 2.34 (s, 1H, CH_3), 7.22 (d, 2H, ArH), 7.23 (d, 2H, ArH), 7.63 (s, 1H, CH).

4-(4-hydroxybenzylidene)-2-methyloxazol-5(4H)-one [3d]

Mol. Form. $C_{11}H_9NO_3$ (203.19); Ar = (-C_6H_5-*p*-OH); mp 140-142 ºC; yield: 92%; IR (KBr, V_{max}, cm $^{-1}$): 863 (C-H bend), 1197 (C-O str), 1296 (C-N str), 1635 (C=C str), 1685 (C=N str), 1757 (C=O str), 2974 (CH_3 str), 3036 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz): 2.35 (s, 1H, CH_3), 5.01 (s, 1H, CH), 6.69 (d, 2H, ArH), 7.14 (d, 2H, ArH), 7.64 (s, 1H, OH).

4-(2-chlorobenzylidene)-2-methyloxazol-5(4H)-one [3e]

Mol. Form. $C_{11}H_8ClNO_2$ (221.63); Ar = (-C_6H_5-*o*-Cl); mp 85-88 ºC; yield: 95%; IR (KBr, V_{max}, cm $^{-1}$): 736 (C-Cl str), 888 (C-H bend), 1296 (C-N str), 1519 (C=C str), 1632 (C=N str), 1706 (C=O str), 2951 (CH_3 str), 3057 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz): 2.34 (s, 1H, CH_3), 7.08-7.25 (m, 4H, ArH), 7.91 (s, 1H, CH).

4-(3-methoxybenzylidene)-2-methyloxazol-5(4H)-one [3f]

Mol. Form. $C_{12}H_{11}NO_3$ (221.63); Ar = (-C_6H_5-*m*-OCH_3); mp 195-197 ºC; yield: 88%; IR (KBr, V_{max}, cm $^{-1}$): 881 (C-H bend), 1170 (C-O str), 1300 (C-N str), 1509 (C=C str), 1680 (C=N str), 1772 (C=O str), 2902 (CH_3 str), 3068 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz): 2.34 (s, 1H, CH_3), 3.73 (s, 1H, OCH_3), 6.75 (d, 1H, ArH), 6.81 (s, 1H, ArH), 6.86 (d, 1H, ArH), 7.10 (t, 1H, ArH), 7.64 (s, 1H, CH).

2.2. Typical procedure for synthesis of compounds [4a-4f]

A solution of **3a-3f** (6 mmole) in dry benzene (30 mL) and phenylhydrazine (5 mmole) was heated under reflux for 4 h. Then the mixture was poured upon water. The precipitated solid was filtered off, dried and crystallized from ethanol to get the desired compounds.

5-(2-hydroxybenzylidene)-2-methyl-3-(phenylamino)-3,5-dihydro-4H-imidazol-4-one [4a]

Mol. Form. $C_{17}H_{15}N_3O_2$ (293.31); Ar = (-C_6H_5-*o*-OH); mp 90-92 ºC, yield: 72%; HPTLC: R_f 0.62, Chloroform: methanol: water (6:2:2); IR (KBr, V_{max}, cm $^{-1}$): 884 (C-H bend), 1133 (C-O str), 1231 (C-N str), 1617 (C=C str), 1637 (C=N str), 1747 (C=O str), 2971 (CH_3 str), 3128 (C-H str), 3431 (N-H str), 3674 (O-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.13 (s, 3H, CH_3), 6.21 (s, 1H, NH), 6.32 (s, 1H, CH), 6.64 (d, 3H, ArH), 6.74 (t, 2H, ArH), 6.96 (t, 1H, ArH), 7.17 (d, 1H, ArH), 7.19 (t, 2H, ArH), 11.71 (s, 1H, OH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 21.31, 108.55, 113.31, 115.86, 116.12, 119.21, 121.23, 127.81, 129.65, 130.61, 144.72, 151.81, 158.91, 166.13; LC-MS (m/z): 294.37 [M+1].

5-[4-(dimethylamino)benzylidene]-2-methyl-3-(phenylamino)-3,5-dihydro-4H-imidazol-4-one [4b]

$C_{19}H_{20}N_4O$ (320.38); Ar = [-C_6H_5-*p*-*N*-$(CH_3)_2$]; mp 109-111 ºC; yield: 69%; HPTLC: R_f 0.51, Chloroform: methanol: water (6:3:1); IR (KBr, V_{max}, cm $^{-1}$): 889 (C-H bend), 1341 (C-N str), 1519 (C=C str), 1631 (C=N str), 1752 (C=O str),

2969 (CH_3 str), 3069 (C-H str), 3431 (N-H str); ^{1}H- NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.24 (s, 3H, CH_3), 2.85 (s, 6H, 2 × CH_3), 6.29 (s, 1H, NH), 6.55 (d, 2H, ArH), 6.63 (d, 2H, ArH), 6.69 (s, 1H, CH), 6.71 (d, 2H, ArH), 7.15 (t, 1H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 20.93, 40.87, 108.98, 113.93, 114.65, 119.45, 124.65, 127.35, 129.46, 130.76, 144.24, 149.67, 151.87, 166.13; LC-MS (m/z): 321.77 [M+1].

5-(4-chlorobenzylidene)-2-methyl-3-(phenylamino)-3,5-dihydro-4H-imidazol-4-one [4c]

Mol. Form. $C_{17}H_{14}ClN_3O$ (311.76); Ar = (-C_6H_5-*p*-Cl); mp 275-276 ºC; yield: 81%; HPTLC: R_f 0.58, Chloroform: methanol: water (6:3:1); IR (KBr, V_{max}, cm $^{-1}$): 766 (=C-Cl), 873 (C-H bend), 1334 (C-N str), 1543 (C=C str), 1618 (C=N str), 1743 (C=O str), 3036 (CH_3 str), 3127 (C-H str), 3429 (N-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : .34 (s, 3H, CH_3), 6.66 (d, 2H, ArH), 6.71 (t, 1H, ArH), 7.19 (t, 2H, ArH), 7.22 (d, 2H, ArH), 7.24 (d, 2H, ArH), 7.58 (s, 1H, CH), 8.92 (s, 1H, NH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 20.76, 108.87, 113.24, 119.35, 127.89, 128.78, 129.65, 130.76, 133.23, 134.21, 144.87, 151.09, 166.12; LC-MS (m/z): 312.09 [M+1].

5-(4-hydroxybenzylidene)-2-methyl-3-(phenylamino)-3,5-dihydro-4H-imidazol-4-one [4d]:

Mol. Form. $C_{17}H_{15}N_3O_2$ (293.31); Ar = (-C_6H_5-*p*-OH); mp 69-71 ºC, yield; 78%;HPTLC: R_f 0.51, Chloroform: methanol: water (7:2:1); IR (KBr, V_{max}, cm $^{-1}$): 836 (C-H bend), 1334 (C-N str), 1542 (C=C str), 1632 (C=N str), 1752 (C=O str), 3051 (CH_3 str), 3163 (C-H str), 3451 (N-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.34 (s, 3H, CH_3), 5.21 (s, 1H, OH), 6.67 (d, 2H, ArH), 6.68 (d, 2H, ArH), 6.73 (t, 1H, ArH), 7.13 (d, 2H, ArH), 7.19 (t, 2H, ArH), 7.54 (s, 1H, CH), 7.98 (s,1H, NH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 20.11, 108.27, 113.24, 115.54, 119.87, 127.83, 129.32, 130.27, 144.97, 152.09, 157.98, 166.85; LC-MS (m/z): 294.36 [M+1].

5-(2-chlorobenzylidene)-2-methyl-3-(phenylamino)-3,5-dihydro-4H-imidazol-4-one [4e]:

Mol. Form. $C_{17}H_{14}ClN_3O$ (311.76); Ar = (-C_6H_5-*o*-Cl); mp 209-210 ºC; yield: 65%; HPTLC: R_f 0.63, Chloroform: methanol: water (6:3:1); IR (KBr, V_{max}, cm $^{-1}$): 746 (=C-Cl), 864 (C-H bend), 1346 (C-N str), 1551 (C=C str), 1645 (C=N str), 1745 (C=O str), 3034 (CH_3 str), 3176 (C-H str), 3417 (N-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.35 (s, 3H, CH_3), 6.64 (d, 2H, ArH), 6.69 (t, 1H, ArH), 7.08 (dd, 1H, ArH), 7.09 (dd, 1H, ArH), 7.18 (t, 2H, ArH), 7.21 (d, 1H, ArH), 7.25 (d, 1H, ArH),7.81 (s, 1H, ArH), 7.92 (s, 1H, CH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz): 21.38, 108.76, 113.54, 119.54, 126.87, 127.34, 128.65, 129.65, 130.54, 131.24, 133.76, 144.87, 151.14, 164.20; LC-MS (m/z): 312.22 [M+1].

5-(3-methoxybenzylidene)-2-methyl-3-(phenylamino)-3,5-dihydro-4H-imidazol-4-one [4f]:

Mol. Form. $C_{18}H_{17}N_3O_2$ (307.34); Ar = (-C_6H_5-*p*-OCH_3); mp 176-178 ºC, yield: 83%; HPTLC: R_f 0.61, Chloroform: methanol: water (8:1:1); IR (KBr, V_{max}, cm $^{-1}$): 851 (C-H bend), 1334 (C-N str), 1519 (C=C str), 1634 (C=N str), 1723 (C=O str), 3032 (CH_3 str), 3134 (C-H str), 3485 (N-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.34 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 6.65 (d, 1H, ArH), 6.66 (t, 1H, ArH), 6.71 (d, 2H, ArH), 6.81 (s, 1H, ArH), 6.86 (d, 1H, ArH), 7.10 (t, 1H, ArH), 7.19 (t, 2H, ArH), 7.56 (s, 1H, CH), 7.94 (s, 1H, NH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz): 20.87, 55.09, 108.04, 112.67, 113.02, 113.85, 118.45, 119.54, 129.63, 129.73, 130.40, 137.54, 144.76, 161.89, 166.43; LC-MS (m/z): 308.92 [M+1].

2.3. Typical procedure for synthesis of compounds [5a-5f]

A solution of 4a-4f (8 mmole) and chloroacetamide (8 mmole) was refluxed with boiling *N, N*-dimethylformamide (30 mL) for 3 h. Then the mixture was poured into water. The precipitated solid was filtered off, dried and crystallized from ethanol to get the desired compounds.

8-(2-hydroxybenzylidene)-3,4-dihydro-6-methyl-4-phenylimidazo[1,5-b][1,2,4]triazin-2(8H)-one [5a]

Mol. Form. $C_{19}H_{16}N_4O_2$ (332.35); Ar = (-C_6H_5-*o*-OH); m.p: 82-83 ºC; yield: 79%; HPTLC: R_f 0.63, Chloroform: methanol: water (7:1:2); IR (KBr, V_{max}, cm $^{-1}$): 885 (C-H bend), 1110 (C-O str), 1223 (C-N str), 1617 (C=C str), 1631 (C=N str), 1764 (C=O str), 2928 (=CH_2 str, sym), 2851 (=CH_2 str, asym), 3074 (CH_3 str), 3164 (C-H str), 3613 (O-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz): 2.82 (s, 3H, CH_3), 4.23(s, 2H, CH_2), 6.64 (d, 3H, ArH), 6.78 (t, 1H, ArH), 6.89 (t, 1H, CH & 2H, ArH), 6.93 (t, 1H, ArH), 7.18 (d,1H, ArH), 7.22 (d, 1H, ArH), 11.72 (s, 1H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz): 21.38, 62.55, 102.66, 113.21, 116.12, 115.81, 119.23, 121.33, 127.11, 127.98, 129.21, 129.99, 144.72, 151.44, 158.31, 164.56, 200.21; LC-MS (m/z): 333.16 [M+1].

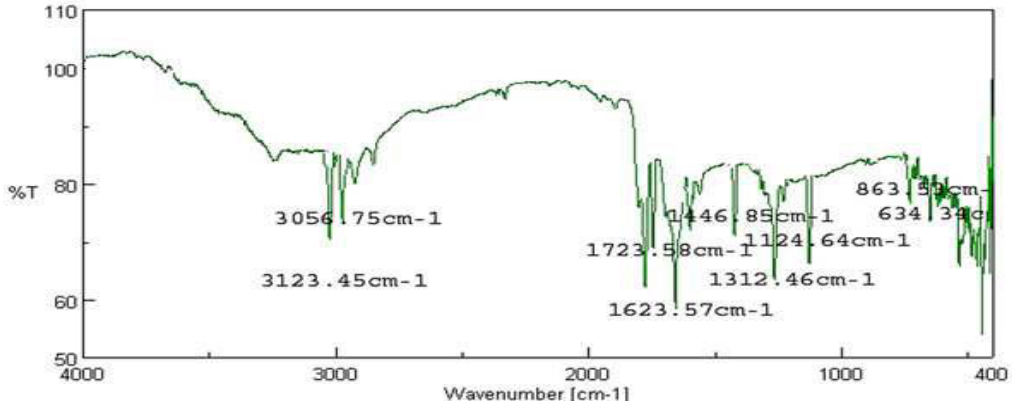

Figure 3
IR spectrum of 5a.

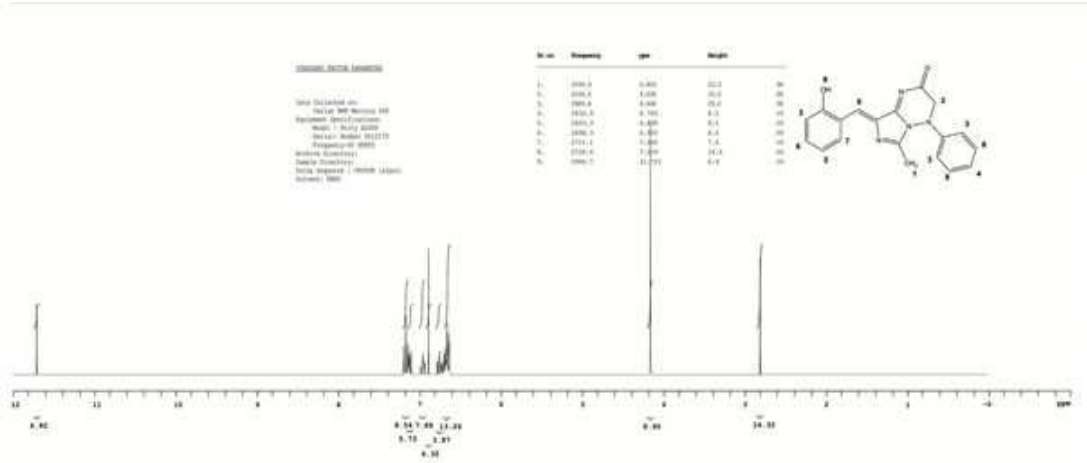

Figure 4
^{1}H NMR of 5a.

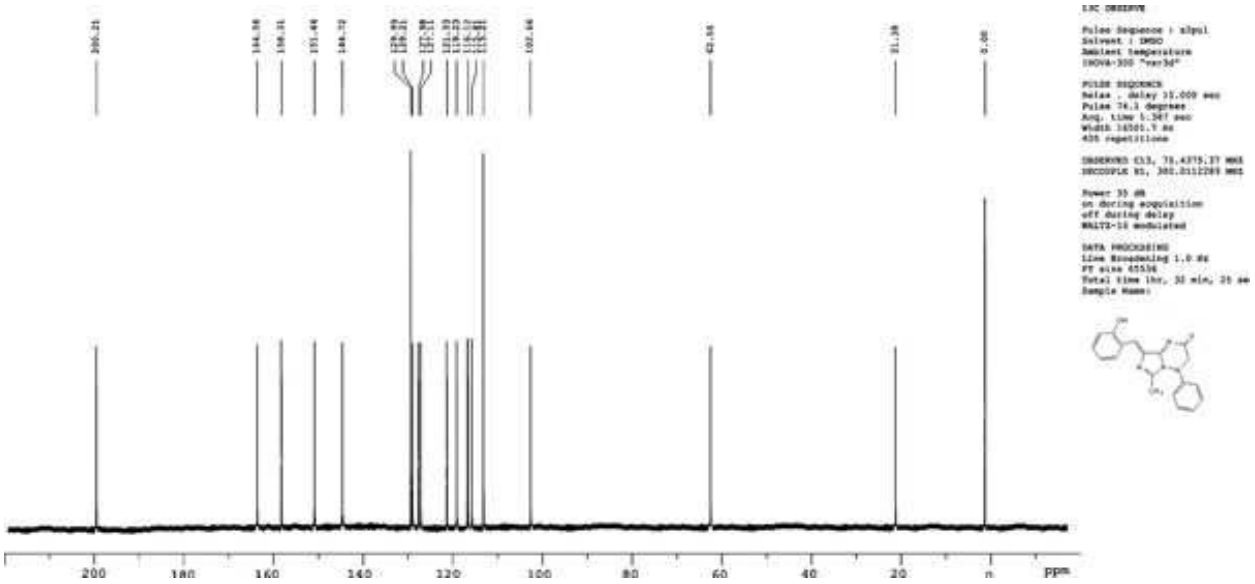

Figure 5
^{13}C NMR of 5a.

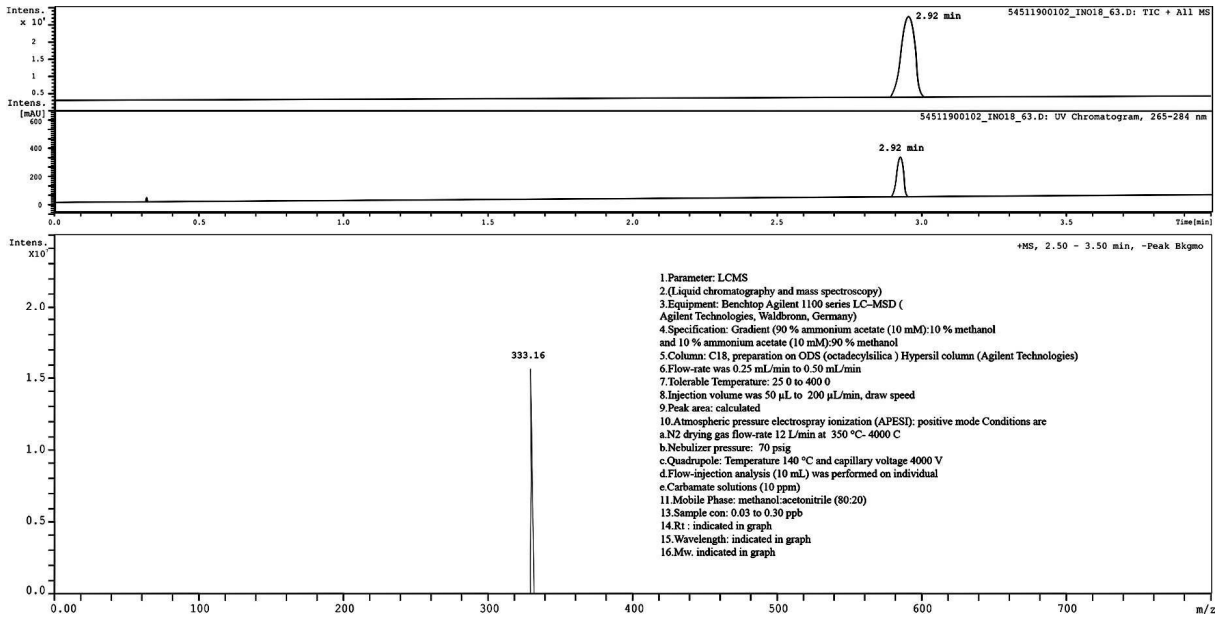

Figure 6
LC-MS of 5a.

8-[4-(dimethylamino)benzylidene]-3,4-dihydro-6-methyl-4-phenylimidazo[1,5-b][1,2,4]triazin-2(8H)-one [5b]

Mol. Form. $C_{21}H_{21}N_5O$ 359.42; Ar = [-C_6H_5-*p*-*N*-$(CH_3)_2$]; mp 75-77 ºC; yield: 81%; HPTLC: R_f 0.70, Chloroform: methanol: water (8:1:1); IR (KBr, V_{max}, cm $^{-1}$): 878 (C-H bend), 1358 (C-N str), 1528 (C=C str), 1628 (C=N str), 1767 (C=O str), 2847 (=CH_2 str, sym), 2931 (=CH_2 str, asym), 3036 (CH_3 str), 3127 (C-H str), 3431 (N-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.67 (s, 3H, CH_3), 2.82 (s, 6H, 2 × CH_3), 4.14 (s, 3H, CH_2), 6.57 (d, 2H, ArH), 6.67 (s, 1H, CH), 6.68 (d, 2H, ArH), 6.72 (t, 1H, ArH), 7.13 (d, 2H, ArH), 7.19 (t, 2H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz): 21.76, 4078, 62.87, 102.76, 113.98, 114.45, 119.23, 124.73, 127.00, 127.96, 129.67, 144.72, 148.93,

151.27, 164.29, 200.37' LC-MS (m/z): 360.28 [M+1].

8-(4-chlorobenzylidene)-3,4-dihydro-6-methyl-4-phenylimidazo[1,5-b][1,2,4]triazin-2(8H)-one [5c]:

Mol. Form. $C_{21}H_{21}N_5O$ (350.80); Ar = (-C_6H_5-*p*-Cl); mp 54-56 ºC; yield: 72%; HPTLC: R_f 0.68, Chloroform: methanol: water (8:1:1); IR (KBr, V_{max}, cm $^{-1}$): 764 (=C-Cl), 871 (C-H bend), 1343 (C-N str), 1537 (C=C str), 1625 (C=N str), 1725 (C=O str), 2847 (=CH_2 str, sym), 2927 (=CH_2 str, asym), 3031 (CH_3 str), 3126 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.34 (s, 3H, CH_3), 4.17 (s, 2H, CH_2), 6.06 (s, 1H, CH), 6.67 (d, 2H, ArH), 6.72 (t, 1H, ArH), 7.18 (t, 2H, ArH), 7.23 (d, 2H, ArH), 7.24 (d, 2H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz): 21.98, 62.45, 102.66, 113.87, 119.23, 127.01, 127.98, 129.18, 130.12, 133.12, 134.03, 144.23, 151.65, 167.34, 200.13; LC-MS (m/z): 351.04 [M+1].

8-(4-hydroxybenzylidene)-3,4-dihydro-6-methyl-4-phenylimidazo[1,5-b][1,2,4]triazin-2(8H)-one [5d]:

Mol. Form. $C_{19}H_{16}N_4O_2$ (332.35); Ar = (-C_6H_5-*p*-OH); mp 160-161 ºC; yield: 77%; HPTLC: R_f 0.62, Chloroform: methanol: water (7:1:2); IR (KBr, V_{max}, cm $^{-1}$): 854 (C-H bend), 1365 (C-N str), 1537 (C=C str), 1635 (C=N str), 1754 (C=O str), 2833 (=CH_2 str, sym), 2942 (=CH_2 str, asym), 3052 (CH_3 str), 3165 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.34 (s, 3H, CH_3), 4.17 (s, 2H, CH_2), 5.02 (s, 1H, OH), 6.61-6.67 (t, 1H, CH & d, 2H, ArH), 6.68 (d, 2H, ArH), 6.72 (t, 1H, ArH), 7.13 (d, 2H, ArH), 7.21 (t, 2H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz): 21.39, 62.45, 102.98, 113.56, 115.86, 119.32, 127.96, 128.46, 129.30, 144.67, 151.45, 157.98, 164.56, 200.17; LC-MS (m/z): 333.29 [M+1].

8-(2-chlorobenzylidene)-3,4-dihydro-6-methyl-4-phenylimidazo[1,5-b][1,2,4]triazin-2(8H)-one [5e]:

Mol. Form. $C_{19}H_{15}ClN_4O$ (350.80); Ar = (-C_6H_5-*o*-Cl); mp 105-107 ºC; yield: 89%; HPTLC: R_f 0.70, Chloroform: methanol: water (7:2:1); IR (KBr, V_{max}, cm $^{-1}$): 734 (=C-Cl), 863 (C-H bend), 1312 (C-N str), 1522 (C=C str), 1623 (C=N str), 1723 (C=O str), 2871 (=CH_2 str, sym), 2912 (=CH_2 str, asym), 3056 (CH_3 str), 3123 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.35 (s, 3H, CH_3), 4.17 (s, 2H, CH_2), 6.66 (d, 2H, ArH), 6.71 (t, 1H, ArH), 6.91 (s, 1H, CH), 7.08 (dd, 1H, ArH), 7.09 (d, 1H, ArH), 7.18 (d, 2H, ArH), 7.22 (d, 1H, ArH), 7.24 (d, 1H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz): 20.87, 62.87, 102.76, 113.54, 119.21, 126.16, 127.17, 127.87, 128.54, 129.45, 130.45, 131.24, 133.76, 144.54, 151.87, 164.98, 200.56; LC-MS (m/z): 351.20 [M+1].

8-(3-methoxybenzylidene)-3,4-dihydro-6-methyl-4-phenylimidazo[1,5-b][1,2,4]triazin-2(8H)-one [5f]:

Mol. Form. $C_{20}H_{18}N_4O_2$ (346.38); Ar = (-C_6H_5-*p*-OCH_3); mp 120-122 ºC; yield: 90%; HPTLC: R_f 0.61, Chloroform: methanol: water (8:1:1); IR (KBr, V_{max}, cm $^{-1}$): 876 (C-H bend), 1365 (C-N str), 1523 (C=C str), 1633 (C=N str), 1745 (C=O str), 2872 (=CH_2 str, sym), 2902 (=CH_2 str, asym), 3054 (CH_3 str), 3121 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.34 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 4.17 (s, 2H, CH_2), 6.62 (s, 1H, CH), 6.66 (d, 1H, ArH), 6.67 (d, 2H, ArH), 6.72 (t, 1H, ArH), 6.80 (s, 1H, ArH), 6.85 (d,1H, ArH), 7.11 (t, 1H, ArH), 7.19 (t, 1H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz): 21.30, 56.65, 62.40, 102.62, 113.65, 119.65, 123.26, 126.86, 127.70, 128.26, 128.62, 129.43, 136.20, 138.26, 144.70, 151.42, 164.86, 200.56; LC-MS (m/z): 347.64 [M+1].

3. ANTIMICROBIAL ACTIVITY

All compounds (5a-5f) were screened for in vitro antibacterial activity and antifungal activity using cup plate agar diffusion method by measuring the zone of inhibition in mm [16,17]. Antibacterial activity was checked using gram positive bacteria *Bacillus subtilis* and pathogenic gram negative bacteria *Escherichia coli* and antifungal activity was checked against fungus *Asparegillus niger*. Standard drug used for antibacterial activity was ciprofloxacin and cotrimazole was for antifungal activity. Synthesized compounds and standard drugs are used after making dilutions in DMSO in

such a way that each mL should contain 250, 500 and 750 µg/mL of compound and drug. The solvent control used was dimethylsulfoxide DMSO). . The zones of inhibition are reported in Table.

4. RESULT AND DISCUSSION

Table 1
Antimicrobial activity of synthesized compounds (5a-5f).

Compound code	Concentration (µg/mL)	Zone of inhibition (mm)		
		Gram positive	Gram negative	Fungi
		B. subtilis	*E. coli*	*A. niger*
5a	750	09	11	20
	500	07	09	19
	250	05	07	18
5b	750	22	21	18
	500	21	20	13
	250	20	19	10
5c	750	15	14	09
	500	12	12	07
	250	10	09	05
5d	750	16	15	15
	500	12	10	12
	250	10	08	10
5e	750	10	10	14
	500	09	08	13
	250	07	05	10
5f	750	16	16	15
	500	14	14	13
	250	12	09	12
Ciprofloxacin	750	28	29	NA
	500	26	27	NA
	250	25	26	NA
Cotrimazole	750	NA	NA	23
	500	NA	NA	21
	250	NA	NA	20
DMSO	---	---	---	---

NA=Not applicable. Diameter of cup is 8 mm, DMSO-Dimethylsulfoxide.

All synthesized compounds were evaluated using physical data such ad melting point and R_f values as well as spectroscopic methods IR, ^1H NMR, ^{13}C NMR, LC-MS etc and showed best match with the same. These compounds were also evaluated for their antibacterial and antifungal activity using cup plate agar diffusion method (Table 1). It has been observed that all the compounds tested showed mild to moderate activity against bacteria and fungi. Compound 5b showed highest antibacterial activity against *Bacillus subtilis* as well as *E. coli* but compounds 5a is highest active against *A. niger*. Compound 5a and 5e showed mild antibacterial activity and compound 5c showed weak antifungal activity.

Antimicrobial activity.

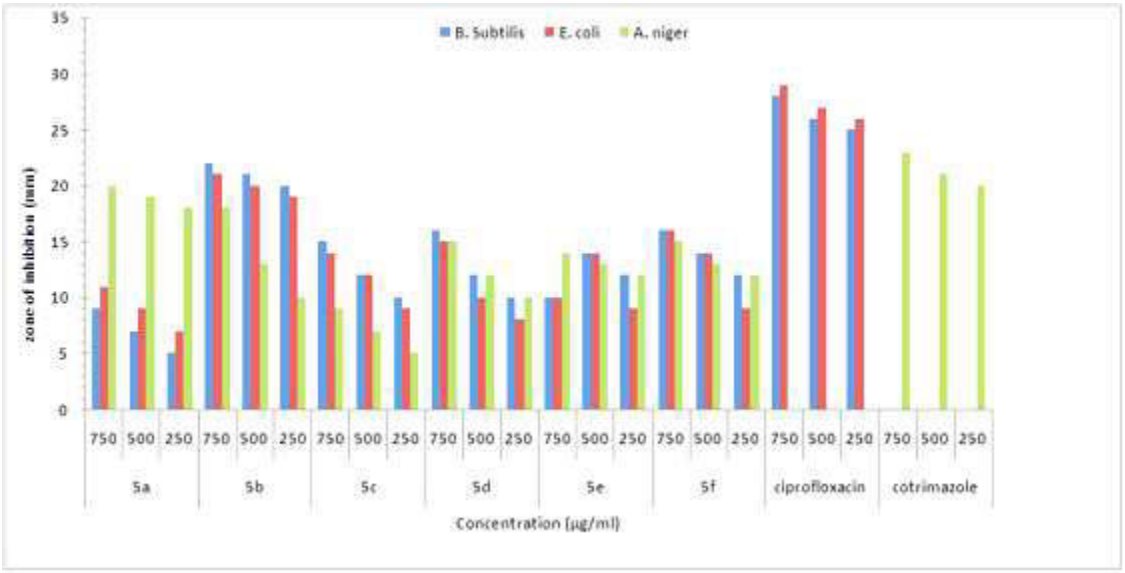

Figure 8
Antimicrobial activity.

5. CONCLUSION

The method reported is simple, efficient and inexpensive. The synthesized compounds showed mild to moderate antimicrobial activity as compared to standard drugs. From this result, it can be concluded that, compound containing strong electropositive group showed the highest antibacterial activity, but this is not applicable to antifungal activity of the compounds. In future, expanded set of this series will be investigated and synthesized in order to get more potent pharmacologically active compounds.

6. ACKNOWLEDGEMENT

Authors are thankful to R. J. Marathe, Y. B. Phatake, C. D. Dhumal, SPM Science College, Sharadanagar, for providing laboratory facilities for doing antimicrobial activity and to Dr. S. J. Sathe, HOD, Microbiology Department, T. C. College, Baramati, for proofreading this article.

7. REFERENCES

1. Goker H, Kus C, Abbasoglu U, Arch Pharm, 328: 425-430, (1995).
2. Kraemer W, Buechel KH, Plempel M, Haller I, Chem Abstr, 90: 115460, (1979).
3. Hassan E, Al-Ashmawi MI, Abdel-Fattah B, Pharmazie, 38: 833-835, (1983).
4. Goswami BN, Kataky JCS, Baruah JNJ, Heterocycl Chem, 23: 1439-1442, (1986).
5. Eweiss NF, Bahajaj AA, Elsherbini EAJ, Heterocycl Chem, 23: 1451-1458, (1986).
6. Sztanke K, Pasternak K, Rzymowska J, Sztanke M, Kandefer-Szerszn M, Eur J Med Chem, 43: 1085–1094, (2008).
7. Sztanke K, Pasternak K, Rajtar B, Sztanke M, Majek M, Polz-Dacewicz M, Bioorg Med Chem, 15: 5480–5486, (2007).
8. T. Oku, Y. Kawai, H. Marusawa, H. Tanaka. Imidazotriazine derivatives. WO001992012154A1, July 23, 1992.

9. S. Siegel, A. Wilmen, S. Rohrig, N. Svenstrup, M.J. Gnoth, S. Heitmeier, U. Rester. Imidazo-Pyrazolopyrazine and Imidazotriazine und ihre Verwendung. DE102007032349A1, Jan 15, 2009.
10. Maechling S, Good J, Lindell SDJ, Comb Chem, 12: 818–821, (2010).
11. Kirkman JK, Lindell SD, Maechling S, Slawin AMZ, Moody CJ, Org Biomol Chem, 6: 4452–4459, (2008).
12. Tzvetkov NT, Eurel H, Muller CE, Bielstein J Org Chem, 8: 1584-1593, (2012).
13. Sztanke K, Tuzimski T, Sztanke M, Rzymowska J, Pasternak K, Bioorg Med Chem, 19: 5103–5116, (2011).
14. M. Cheung, N.P. King, K.W. Kuntz, R.A. Mook, M.A. Pobanz, J.M. Salovich, B.J. Wilson, Imidazotriazine compounds. US000007462614B2, Dec 9, 2008.
15. Furniss BS, Hannaford AJ, Smith PWG, Tatchell AR, Vogel's Textbook of Practical Organic Chemistry, 5th Edn . Pearson Education, London,2006,pp.1155.
16. Kavanagh F, Analytical Microbiology, Academic Press, New York,1963,pp.133 and 398.
17. British Pharmacopoeia-II, Biological Assay and Tests, The stationary Office Ltd., London, 1998,A-205.