Synthesis and anti-bacterial screening of ethyl 6-oxo-3-phenyl-1,6-dihydropyrano[3,2-*e*]indole-2-carboxylate and 7-phenyl-5*H*-pyrano [3',2':4,5]indolo[1,2-*a*]quinoxaline-6,10-dione

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Ethyl 6-oxo-3-phenyl-1,6-dihydropyrano[3,2-*e*]indole-2-carboxylate **3a–c** were synthesised from ethyl 2-[(2-oxo-2*H*-1-benzopyran-6-yl)-hydrazono]-3-phenylpropanoate **2a–c.** Compounds **2a–c** was in turn prepared by reacting diasotised solution of 6-aminocoumarin and ethyl-2-benzylacetoacetate. *N*-nitroarylation of ethyl 6-oxo-3-phenyl-1,6-dihydropyrano[3,2-*e*]indole-2-carboxylate **3a–c** was carried out with 2-chloronitrobenzene to give ethyl 1-(2-nitrophenyl)-6-oxo-3-phenyl-1,6-dihydropyrano[3,2-*e*]indole-2-carboxylate **4a–c**, which on catalytic reductive cyclisation with H_2/Ni affords 7-phenyl-5*H*-pyrano[3',2':4,5]indolo[1,2-*a*]quinoxaline-6,10-dione **5a–c**. The structures of all these compounds have been established on the basis of analytical and spectral data. All the compounds show significant antibacterial activity.

Keywords: 6-amino-coumarin, indole-2-carboxylate, N-nitroarylation, quinoxaline, antibacterial activity

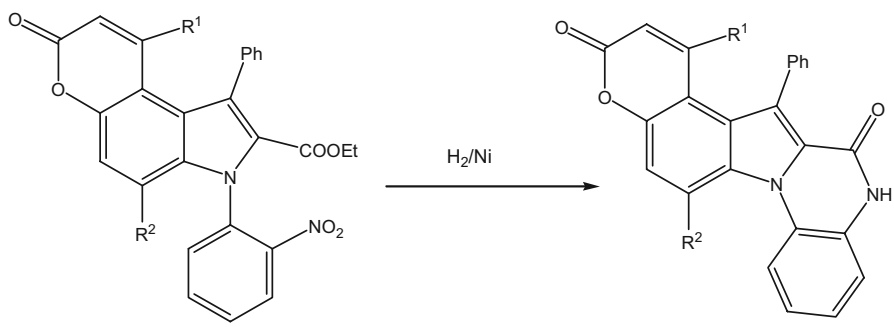
Coumarins constitute an important class of naturally occurring compounds with useful pharmacological activity[1-6] as antibacterial[7-12] and antifungal agents.[13-17] The result of the variety of the physiological activity of pyrroloindole derivatives[18-19] and also the possibility of their use in the synthesis of alkaloids and alkaloid related substances. We report here the synthesis of quinoxalin-6,10-dione **5a–c** from ethyl 1-(2-nitrophenyl)-6-oxo-3-phenyl-1,6-dihydropyrano[3,2-*e*] indole-2-carboxylate **4a–c**. Compound **4a–c** was obtained from successive steps using 6-aminocoumarin as starting material. Diasotised solution of 6-aminocoumarin was treated with ethyl-2-benzylacetoacetate to yield ethyl 2-[(2-oxo-2*H*-1-benzopyran-6-yl)-hydrazono]-3-phenylpropanoate **2a–c.** In its ^{1}H NMR it showed singlet at δ 4.11 for two protons of CH_2–Ph along with the other signals. Compounds **2a–c** on refluxing with thionyl chloride underwent Fischer-indole cyclisation to yield ethyl 6-oxo-3-phenyl-1,6-dihydropyrano[3,2-*e*]indole-2-carboxylate **3a–c**. In the ^{1}H NMR of compound **3c,** it shows signals as triplet for CH_3 protons at δ 1.08 and as a quartet at δ 4.16 for methylene protons and a signal at δ 9.45 for –NH group which is D_2O exchangeable. It also shows absence of CH_2–Ph protons which were observed in compound **2c** as a singlet at δ 4.11. The mass spectra of **3c** exhibit peaks at *m/z* 361(50%) corresponding to molecular ion peak of the compound, other significant peaks were observed at *m/z* 347 (40%), 315 (100%)**.**

N-Nitroarylation of carboxylates **3a–c** was achieved via Ullamann reaction[20-21] by refluxing pyranoindole-2-carboxylate **3a–c** with 2-choronitrobenzene using anhydrous K_2CO_3 and cupric oxide in dry pyridine to afford ethyl 1-(2-nitrophenyl)-6-oxo-3-phenyl-1,6-dihydropyrano[3,2-*e*] indole-2-carboxylate **4a–c**. Its IR spectra did not show any peak beyond 3050 cm^{-1}, indicating the absence of –NH stretching. Compounds **4a–c** on catalytic reductive cyclisation with H_2/Ni gave 7-phenyl-5*H*-pyrano[3',2':4,5]indolo[1,2-*a*] quinoxaline-6**,**10 **5a–c**. IR spectra of **5c** showed a peak at 3432 cm^{-1} for –NH stretching and a peak at 1655 cm^{-1} for –NH–C=O in addition to carbonyl stretch of coumarin. The ^{1}H NMR of **5c** exhibited a signal at δ 8.85 for –NH proton which is D_2O exchangeable.

Antibacterial activity

All the synthesised compounds **3a–c, 4a–c** and **5a–c,** were screened for their antibacterial activity against *Staphylococcus aureus*, *S. typhi* and *Escherichia coli* (Table 1), by disc diffusion method.[22] The zone of inhibition was measured in mm and was compared with standard drug. DMSO was used as a blank and Streptomycin was used as antibacterial standard. All the compounds were tested at 50 and 100 μgm mL^{-1} concentration.

From the antibacterial screening of the compounds amongst **3a–c** to **5a–c,** it could be concluded that **3b**, **4b**, and **5b** were less active as compared to **3a**, **3c**, **4a**, **4c** and **5a**, **5c**. It was observed that presence of methyl group in benzopyran moiety possess moderate activity, indicating the importance of methyl substitution.[23]



4a-c **5a-c**

4a : R^1 = H, R^2 = CH_3
4b : R^1 = H, R^2 = H
4c : R^1 = CH_3, R^2 = CH_3

5a : R^1 = H, R^2 = CH_3
5b : R^1 = H, R^2 = H
5c : R^1 = CH_3, R^2 = CH_3

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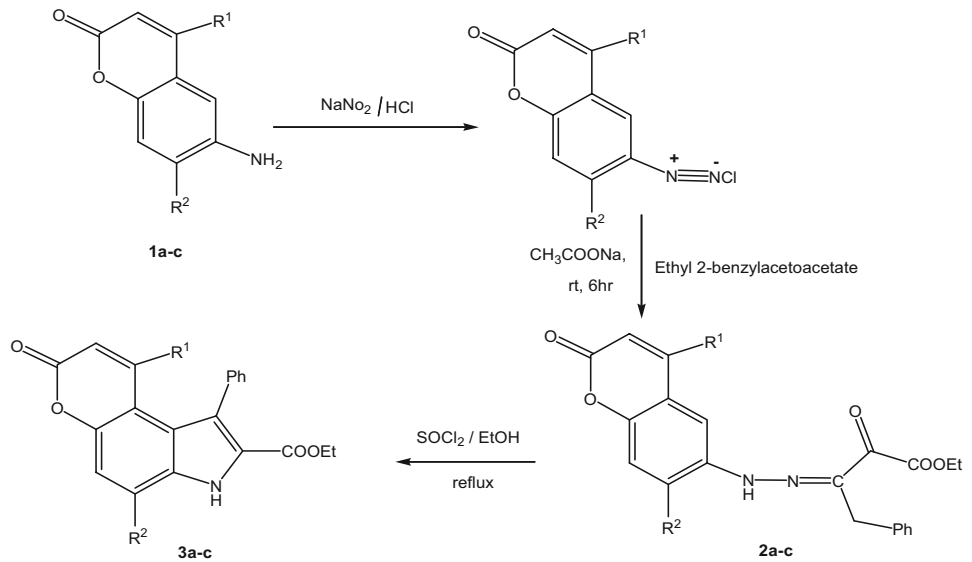
Table 1 Antibacterial activity of compounds (**3a–c**), (**4a–c**) and (**5a–c**)

Compound	Antibacterial activity/mg mL^{-1}					
	S. aureus		*S. typhi*		E. coli	
	50	100	50	100	50	100
3a	12	14	10	14	13	14
3b	10	12	11	13	12	12
3c	13	15	13	16	17	18
4a	12	13	12	14	13	13
4b	11	14	10	13	11	14
4c	13	15	14	16	15	17
5a	13	14	12	13	13	13
5b	12	13	11	14	13	15
5c	14	17	13	17	16	20
Sm	16	23	15	24	17	25

Disc size: 6.25 mm
Duration: 24 h.

Sm: Streptomycin
resistant (11 mm/less)
sensitive(15 mm/more)

Control: DMSO
intermediate (12–14 mm)



Scheme 1

Experimental

Melting points were taken in open capillaries and are uncorrected. IR spectra (ν_{max} in cm^{-1}) were recorded on a Perkin Elmer FTIR, ^{1}H NMR on 300 MHz JEOL NMR AL300 using TMS as standard and $CDCl_3$ as a solvent. Mass spectra (GC–MS) were recorded on Shimadzu GC–MS QP-2010. Elemental analyses were carried out at IIT, Mumbai. All products are purified by recrystallisation. The reaction are followed up and purity of the products is carried out on pre-coated TLC plates (Silica gel 60 F254, Merck), visualising the spots in UV light.

Synthesis of ethyl 2-[(2-oxo-2H-1-benzopyran-6-yl)hydrazono]-3-phenylpropanoate (**2a–c**): A solution of 6-aminocoumarin **1a–c** (9.45 g, 0.05 mol) in a mixture of 25 mL of hydrochloric acid and 50 mL of glacial acetic acid was diasotised at 0 °C with sodium nitrite solution (4 g. (0.055 mol) dissolved in 10 mL of water). The mixture is kept at this temperature for 1 h. The resulting solution of diazonium salt was filtered and added into mixture of 60 mL of glacial acetic acid, 0.051 mol of ethyl 2-benzylacetoacetate (97%) and 50 g. (0.37 mol) of sodium acetate (AcONa $3H_2O$) at 0 to 5 °C at pH = 5.5.The mixture was left for 6 h. After that an equal volume of water was added into the mixture. The crude product was collected, washed with ethanol, then water and recrystallised from ethanol to give compound as red product **2a–c.**

2a: M.p. 130 °C, Yield 65%; IR: (KBr) 3438 (NH), 3054, 2950 (–CH), 1730, 1710 (>C=O), 805 cm^{-1}, *etc.* ^{1}H NMR ($CDCl_3$) 1.43(t, 3H, J = 6.0 Hz, CH_2-CH_3), 2.20(s, 3H, CH_3), 4.15(s, 2H, CH_2–Ph), 4.42(q, 2H, J = 6.0 Hz, CH_2–CH_3), 6.24(d, 1H, J = 9.3 Hz, C_3–H), 6.98(s, 1H, C_8–H), 7.27–7.36(m, 5H, Arom-H), 7.60(s, 1H, C_5–H), 7.65(d, 1H, J = 9.3 Hz, C_4–H), 8.01(s, 1H, NH, D_2O-exchangable). Anal. Calcd for $C_{21}H_{20}N_2O_4$: C, 69.22; H, 5.53; N, 7.69. Found: C, 69.40; H, 5.50; N, 7.74%.

2b: M.p. 124 °C, Yield 60%; IR: (KBr) 3430 (NH), 3050, 2952 (–CH), 1735, 1715 (>C=O), 810 cm^{-1}, *etc.* ^{1}H NMR ($CDCl_3$) 1.39(t, 3H, J = 6.0 Hz, CH_2–CH_3), 4.12(s, 2H, CH_2–Ph), 4.40(q, 2H, J = 6.0 Hz, CH_2–CH_3), 6.21(d, 1H, J = 9.3 Hz, C_3–H), 6.98(d, 1H, J = 9.0 Hz, C_8–H), 7.27–7.36(m, 5H, Arom-H), 7.38(d, 1H, J = 9 Hz, C_7–H), 7.60(s, 1H, C_5–H), 7.65(d, 1H, J = 9.3 Hz, C_4–H), 8.01(s, 1H, NH, D_2O-exchangable). Anal. Calcd for $C_{20}H_{18}N_2O_4$: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.72; H, 5.29; N, 8.06%.

2c: M.p. 134 °C, Yield 62%; IR: (KBr) 3420 (NH), 3050, 2950 (–CH), 1732, 1712 (>C=O), 805 cm^{-1}, *etc.* ^{1}H NMR ($CDCl_3$) 1.42(t, 3H, J = 6.0 Hz, CH_2–CH_3), 2.18(s, 3H, CH_3), 2.38 (s, 3H, CH_3), 4.11(s, 2H, CH_2–Ph), 4.40(q, 2H, J = 6.0 Hz, CH_2–CH_3), 6.23 (s, 1H, C_3–H), 6.98(s, 1H, C_8–H), 7.27–7.35(m, 5H, Arom-H), 7.60 (s, 1H, C_5–H), 8.01(s, 1H, NH, D_2O-exchangable). MS, *m/z* (%): (M +) 378(75), 188(100), 160(40). Anal. Calcd for $C_{22}H_{22}N_2O_4$: C, 69.83; H, 5.86; N, 7.40. Found: C, 70.02; H, 5.91; N, 7.45%.

Synthesis of ethyl 6-oxo-3-phenyl-1,6-dihydropyrano[3,2-e]indole-2-carboxylate (**3a–c**)

Compound **2a–c** (0.028 mol) was added to a mixture of 35 mL ethanol and 12 mL $SOCl_2$. The reaction mixture was refluxed for 8 h, cooled and precipitate was filtered off, washed with ethanol, then water. The compound **3a–c** was recrystallised from ethanol.

3a: M.p. 200 °C, Yield 62%; IR: (KBr) 3368 (NH), 3058, 2981 (–CH), 1736, 1712 (>C=O), 811 cm^{-1}, *etc.* ^{1}H NMR ($CDCl_3$) 1.12 (t, 3H, J = 6.8 Hz, CH_2–CH_3), 2.50(s, 3H, CH_3), 4.18(q, 2H, J = 6.8 Hz CH_2–CH_3), 6.10(d, 1H, J = 9.3 Hz, C_3–H), 6.90(s, 1H, C_8–H), 7.19–7.37(m, 5H, Arom-H), 7.68(d, 1H, J = 9.0 Hz, C_4–H), 9.40(s, 1H, NH, D_2O-exchangable). Anal. Calcd for $C_{21}H_{17}N_1O_4$: C, 72.61; H, 4.93; N, 4.03. Found: C, 72.73; H, 4.96; N, 4.09%.

3b: M.p.192 °C, Yield 60%; IR: (KBr) 3376 (NH), 3052, 2981 (–CH), 1735, 1715 (>C=O), 812 cm^{-1}, *etc.* ^{1}H NMR ($CDCl_3$) 1.15(t, 3H, J = 6.8 Hz, CH_2–CH_3), 4.25(q, 2H, J = 6.8 Hz CH_2–CH_3), 6.18(d, 1H, J = 9.3 Hz, C_3–H), 6.95(d, 1H, J = 9 Hz, C_8–H), 7.19–7.37(m, 5H, Arom-H), 7.50(d, 1H, J = 9 Hz, C_7–H), 7.68(d, 1H, J = 9.0 Hz, C_4–H), 9.35(s, 1H, NH, D_2O-exchangable). Anal. Calcd for $C_{20}H_{15}N_1O_4$: C, 72.06; H, 4.54; N, 4.20. Found: C, 72.20; H, 4.58; N, 4.14%.

3c: M.p. 205 °C, Yield 65%; IR: (KBr) 3370 (NH), 3050, 2950 (–CH), 1730, 1718 (>C=O), 806 cm^{-1}, *etc.* ^{1}H NMR ($CDCl_3$) 1.08(t, 3H, J = 6.8 Hz, CH_2–CH_3), 2.25(s, 3H, CH_3), 2.65(s, 3H, CH_3), 4.16(q, 2H, J = 6.8 Hz, CH_2–CH_3), 6.07(s, 1H, C_3–H), 7.19(s, 1H, C_8–H), 7.27–7.35(m, 5H, Arom-H), 9.45(s, 1H, NH, D_2O-exchangable). MS, *m/z* (%): (M +) 361(50), 347(40), 315(100), 287(60). ^{13}C NMR ($CDCl_3$): 14.8(CH_3–COOEt), 17.70(CH_3), 18.08(CH_3), 60.20(CH_2–COOEt), 113.10(–CH=C), 111.50–138.23(13-Arom-C), 152.50, 153.54, 159.51(C=O), 160.2(C=O). Anal. Calcd for $C_{22}H_{19}N_1O_4$: C, 73.11; H, 5.30; N, 3.88. Found: C, 73.25; H, 5.32; N, 3.93%.

Synthesis of ethyl 1-(2-nitrophenyl)-6-oxo-3-phenyl-1,6-dihydropyrano[3,2-e]indole-2-carboxylate (**4a–c**)

A mixture of pyranoindole-2-carboxylate **3a–c** (50 mmol), appropriate 2-chloronitrobenzene (50 mmol), anhydrous potassium carbonate (5 g) and cupric oxide (0.20 g) were taken in dry pyridine and the mixture was refluxed for 26 h. It was then cooled and filtered, the residue was washed with hot pyridine and the combined filtrate was poured in ice/cold dilute HCl to get the solid product which was washed with water. The product was crystallised from ethanol to give yellow crystals.

4a: M.p. 238 °C, Yield 52%; IR: (KBr) 3045, 2930 (–CH-arom), 1732, 1718 (>C=O), 1550, 1330 (NO_2), 701 cm^{-1}, *etc.* ^{1}H NMR ($CDCl_3$) 1.33(t, 3H, J = 6.8 Hz, CH_2–CH_3), 2.45(s, 3H, CH_3), 4.20(q, 2H, J = 6.8 Hz, CH_2–CH_3), 6.21(d, 1H, J = 9.0 Hz, C_3–H), 6.98(s, 1H, C_8–H), 7.17–7.85(m, 9.0H, Arom-H), 7.68(d, 1H, J = 9.0 Hz, C_4–H). Anal. Calcd for $C_{27}H_{20}N_2O_6$: C, 62.22; H, 4.30; N; 5.98. Found: C, 62.44; H, 4.35; N, 6.03%.

4b: M.p. 220 °C, Yield 50%; IR: (KBr) 3040, 2935 (–CH), 1738, 1721(>C=O), 1548, 1335 (NO_2), 982 cm^{-1}, *etc.* ^{1}H NMR ($CDCl_3$) 1.38(t, 3H, J = 6.8 Hz, CH_2–CH_3), 4.15(q, 2H, J = 6.8 Hz, CH_2–CH_3), 6.23(d, 1H, J = 9.0 Hz, C_3–H), 7.10(d, 1H, J = 9.3 Hz, C_8–H), 7.15(d, 1H, J = 9.0 Hz, C_7–H), 7.20–7.58(m, 9H, Arom-H), 7.73(d, 1H, J = 9.0 Hz, C_4–H). Anal. Calcd for $C_{26}H_{18}N_2O_6$: C, 68.71; H, 3.99; N, 6.16. Found: C, 68.92; H, 3.89; N, 6.18%.

4c: M.p. 232 °C, Yield 48%; IR: (KBr) 3042, 2930 (–CH), 1730, 1723(>C=O), 1552, 1330, (NO_2), 980 cm^{-1}, *etc.* ^{1}H NMR ($CDCl_3$) 1.33(t, 3H, J = 6.8 Hz, CH_2–CH_3), 2.30(s, 3H, CH_3), 2.50(s, 3H, CH_3), 4.20(q, 2H, J = 6.8 Hz, CH_2–CH_3), 6.25(s, 1H, C_3–H), 6.98(s, 1H, C_8–H), 7.17–7.85(m, 9H, Arom-H). Anal. Calcd for $C_{28}H_{22}N_2O_6$: C, 69.70; H, 4.59; N, 5.80. Found: C, 69.43; H, 4.65; N, 5.86%.

Synthesis of 7-phenyl-5H-pyrano[3',2':4,5]indolo[1,2-a]quinoxaline-6,10-dione (**5a–c**)

Compound **4a–c** was subjected to reductive cyclisation in DMF (50 mL) with freshly prepared Ranay nickel (2 g) and hydrogen in paar low hydrogenator. The catalyst was removed by filtration and repeatedly washed with dimethylformamide. The solvent was removed under reduced pressure and product was obtained as light yellow solid.

5a: M.p. 260 °C, Yield 55%; IR: (KBr) 3389(–NH), 2978(–CH-arom), 1723, 1655(–CONH), 978 cm^{-1}, *etc.* ^{1}H NMR ($CDCl_3$) 2.32(s, 3H, CH_3), 6.20(d, 1H, J = 9.0 Hz, C_3–H), 6.85(s, 1H, C_8–H), 7.20–7.80(m, 9H, Arom-H), 7.73(d, 1H, J = 9.0 Hz, C_4–H), 8.85 (s, 1H, NH, D_2O-exchangable). Anal. Calcd for $C_{25}H_{16}N_2O_3$: C, 76.53; H, 4.08; N, 7.14. Found: C, 76.73; H, 4.15; N, 7.17%.

5b: M.p. 258 °C, Yield 50%; IR: (KBr) 3382(–NH), 2970 (–CH-arom), 1723, 1660(–CONH), 1050 cm^{-1}, *etc.* ^{1}H NMR ($CDCl_3$) 6.23(d, 1H, J = 9.0 Hz, C_3–H), 6.85(d, 1H, J = 9.0 Hz, C_8–H), 7.20–7.80(m, 9H, Arom-H), 7.85(d, 1H, J = 9.0 Hz, C_7–H), 7.70(d, 1H, J = 9.3 Hz, C_4–H), 8.80 (s, 1H, NH, D_2O-exchangable). Anal. Calcd for $C_{24}H_{14}N_2O_3$: C, 76.18; H, 3.73; N, 7.40. Found: C, 76.34; H, 3.75; N, 7.18%.

5c: M.p. 262 °C, Yield 58%; IR: (KBr) 3432(–NH), 2955(–CH-arom), 1719, 1655(–CONH), 1060 cm^{-1}, *etc.* ^{1}H NMR ($CDCl_3$) 2.15(s, 3H, CH_3), 2.56(s, 3H, CH_3), 6.20(s, 1H, C_3–H), 6.95(s, 1H, C_8–H), 7.20–7.80(m, 9H, Arom-H), 8.85 (s, 1H, NH, D_2O-exchangable). ^{13}C NMR ($CDCl_3$): 16.80(CH_3), 17.90(CH_3), 111.50–136.80(18-aromatic-C), 150.21, 151.80, 156.30(C=O), 160.0(C=O). Anal. Calcd for $C_{26}H_{18}N_2O_3$: C, 76.83; H, 4.44; N, 6.89. Found: C, 76.71; H, 4.50; N, 6.92%.

Antibacterial activity

The newly prepared compounds were screened for their antibacterial activity against *Staphylococcus aureus*, *S. typhi* and *Escherichia coli* bacterial strains by disc diffusion method.[22] A standard inoculum was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6.25 mm in diameter were prepared from Whatman no. 1 filter paper and sterilised by dry heat at 140 °C for 1 h. The sterile discs previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were also kept. The plates were inverted and incubated for 24 h at 37 °C. Streptomycin was used as a standard drug. Inhibition zones were measured and compared with the standard. The bacterial zones of inhibition values are given in Table 1.

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References

1 C.M. Chiliro, S. Katsuno, M. Omura, H. Tokuda, H. Nishino and H. Furukawa, *J. Nat. Prod*., 2000, **63**, 1218.
2 A.Z. Abyshev, G.I. D'Yachuk, E.V. Semenov and M.P. Pukhov, *Pharm. Chem J.,* 1993, **27**, 66.
3 C.A. Kontogiorgis and D.J. Hadjipavlou, *Bioorg. Med. Chem. Lett*., 2004, **14**, 611.
4 A.Z. Abyshev, A.T. Alekseev, V.G. Platonov and I.A. Byrkin, *Pharm. Chem. J.,* 1996, **30**, 441.
5 C. Teran, L. Santana, E. Uriarte, Y. Fall, L. Lena and B.R. Tolf, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 3570.
6 T.C. Wang, Y.L. Chen, C.C. Cherng and C.M. Teng, *Helv. Chim. Acta*, 1996, **79**, 1620.
7 A.M. El-Syed, A.G. Ghattas, M.T. El-Wassimy and O.A. Abd Allah, *Farmaco*, 1999, **54**, 56.
8 S.A. Mayekar and V.V. Mulwad, *Ind. J. Chem*., 2008, **47B**, 1254.
9 V.V. Mulwad and A.C. Chaskar, *Ind. J. Chem*., 2006, **45B**, 1710.
10 V.V. Mulwad and R.B. Pawar, *Ind. J. Chem*., 2003, **42B**, 2091.
11 V.V. Mulwad and M.V. Lohar, *Ind. J. Chem*., 2003, **42B**, 1937.
12 V.V. Mulwad and J.M. Shirodkar, *J. Het. Chem*., 2003, **40**, 377.
13 V.V. Mulwad, R.B. Pawar and A.C. Chaskar, *J. Kor. Chem. Soc*., 2008, **52**, 3, 249.
14 V.V. Mulwad and D.S. Satwe, *Ind. J. Chem*., 2006, **45B**, 1210.
15 B.P. Choudhari and V.V. Mulwad, *Ind. J. Chem*., 2006, **45B**, 309.
16 V.V. Mulwad, A.C. Chaskar and J.M. Shirodkar, *Ind. J. Chem*., 2005, **44B**, 1465.
17 B.P. Choudhari and V.V. Mulwad, *Ind. J. Chem*., 2006, **45B**, 314.
18 S. Chikvaidze, S.A. Samsoniya and Z.E. Saliya, *Chem. Het. Compounds*., 2000, **36**, 12.
19 A. Guiotto, A. Chilin, P. Manzini and P. Rodighiero, *Farmaco.,* 1995, **50**, 479.
20 M.A. Khan and J.B. Polya, *J. Chem. Soc*. (C)., 1970, 85.
21 P.E. Fanta, *Synthesis,* 1974, **1**, 9.
22 R. Cruickshank, J.P. Duguid, B.P. Marmion and R.H.A. Swain, *Medicinal microbiology*, 12 edn, Vol. II, Churchill Livingstone, London, 1975, pp. 196–202.
23 V.V. Mulwad and D.S. Satwe, *Ind. J. Chem*., 2004, **43B**, 2727.