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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of some benzoxazinyl pyrazolone arylidenes as potent antimicrobials and antioxidants

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Abstract 2-[2-(3-Methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethyl]-2*H*-1,4-benzoxazin-3(4*H*)-one (**3**) was obtained starting from methyl-(3,4-dihydro-3-oxo-2*H*-1,4-benzoxazin-2-yl) acetate (**1**) through the corresponding hydrazide (**2**). Condensation of (**3**) with different aromatic aldehydes under Knoevengel condensation afforded 2-{2-[3-methyl-4-(2-methylbenzylidine)-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl]-2-oxoethyl}-2*H*-1,4-benzoxazin-3(4*H*)-ones (**4a–k**). The structures of the compounds were determined by FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectral data, and elemental analysis. All the synthesized compounds were screened for their in vitro antimicrobial and antioxidant studies.

Keywords Benzoxazine · Pyrazole · Knoevengel condensation · Antimicrobial activity · Antioxidant activity

Introduction

The pharmaceutical applications of nitrogen and oxygen containing heterocycles have owed the researchers to focus their research on these classes of compounds. Benzoxazines are an important class of nitrogen and oxygen containing heterocycles which have been reported to possess antimicrobial (Dabholkar and Gavande, 2012), antioxidant (Largeron *et al*., 1999), anticancer (Korolyov *et al*., 2010), and antitubercular activities (Xiaokai *et al*., 2010). Various alkyl benzoxazine derivatives are reported to possess free radical scavenging property owing to its capacity to inhibit oxidative stress (Blattes *et al*., 2005). Reactive oxygen species like superoxide, hydrogen peroxide, etc., can be neurotoxic thus damaging critical cellular components necessary for cell viability. This is considered as one underlying cause for cerebral palsy. Benzoxazine derivatives were identified as potent neuroprotective antioxidants possessing the antioxidant effect comparable to the standard α-tocopherol (Largeron *et al.,* 2001). Pyrazole derivatives have also gained the attraction of researchers as it is reported to possess excellent potential as antimicrobial (Urmila *et al.*, 2005; Mohammad *et al.*, 2010), antioxidant (Manojkumar *et al.*, 2009), and anticancer activities (Puthiyapurayil *et al.*, 2012). Pyrazoles and pyrazolones are reported for their effect as antioxidants in inhibiting the oxidation of LDL, which otherwise responsible for causing atherosclerosis (Jeong *et al*., 2004). The development of new antimicrobial agents is the urgent need in drug discovery perspective, as the development of resistance of pathogenic bacteria to the available antibiotics is rapidly becoming a major world-wide problem. Keeping in mind about the potential of new drug entities obtained by the modification of structures of the reported compounds or the incorporation of one or more hetero moieties possessing potential activity and also about the reports on benzoxazines and pyrazoles, an attempt was made here to incorporate benzoxazines and pyrazoles and evaluate their antimicrobial and antioxidant potency.

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Results and discussion

Chemistry

The scheme of the present work shows that methyl-(3,4-dihydro-3-oxo-2*H*-1,4-benzoxazin -2-yl) acetate **1** was formed by the reaction of 2-amino phenol with maleic anhydride and this with hydrazine hydrate has given 3,4-dihydro-3-oxo-2*H*-1,4-benzoxazin-2-yl acetic acid hydrazide **2**. 2-[2-(3-Methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethyl]-2*H*-1,4-benzoxazin-3-(4*H*)-one **3** was obtained by treating **2** with ethyl acetoacetate in the presence of methanol. The formation of pyrazole ring was confirmed by the IR absorption band obtained at 1721.22, 1610.80, 1501.02 corresponding to C=O, C=N, N–N, respectively. The signal recorded at δ 2.61 in the ^{1}H NMR spectra and at δ 38.88 in the ^{13}C NMR spectra corresponded to the CH_3 of the pyrazolone. The CH_2 of the pyrazole was obtained as signals at δ 4.90 and δ 66.24 in the ^{1}H NMR and ^{13}C NMR spectra, respectively. The pyrazole derivative **3** on Knoevengel condensation with different aromatic aldehydes gave 2-{2-[4-(substituted) benzylidene-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl]-2-oxoethyl}-2*H*-1,4-benzoxazin-3(4*H*)-one **(4a–k)**. The formation of **(4a–k)** was confirmed by the IR absorption band obtained for **4e** at 1654.31 corresponding to the C=CH linkage. The absorption bands at 1627.55 and 1535.78 corresponded for the pyrazole ring. The formation of the compound was further confirmed by the ^{1}H NMR signal obtained as singlet at around δ 6.23 and ^{13}C NMR at around δ 112.33 corresponding to C=CH group. The aromatic protons were shown as multiplets at δ 7.24–7.76 and aromatic carbons in the range of δ 118.70–132.15. The formation of the compound was further confirmed by the mass spectral data where molecular ion peak was obtained corresponding to the molecular weight. The successful formation of the compound was further confirmed by the elemental analysis data. All the derivatives were obtained in quite good yields.

Antimicrobial screening

All the newly synthesized compounds **(4a–k)** were screened for their in vitro antibacterial activity against *Staphylococcus aureus* NCIM 2079, *Bacillus subtilis* NCIM 2063, *Escherichia coli* NCIM 2118, and *Pseudomonas aeruginosa* NCIM 2036 and antifungal screening against *Aspergillus niger* NCIM 545 and *Candida albicans* NCIM 3100. The antibacterial studies revealed that compounds **4c**, **4f**, and **4j** containing the methoxy, dimethyl amino, and trimethoxy moieties, respectively, showed the minimum inhibitory concentration as low as 15.6 μg/ml against all the bacterial strains. Also compound **4b** with fluro substitution was found to be active against *Pseudomonas aeruginosa* at MIC 15.6 μg/ml. Other compounds in the series also showed moderate antibacterial activity. In the antifungal studies, the compounds substituted by fluro, methoxy, and bromo group, i.e., **4c**, **4b**, and **4k** showed good activity with MIC at 15.6 and 31.25 μg/ml against the fungal strains *Aspergillus niger* and *Candida albicans*. The excellent antimicrobial potential shown by the fluro derivative may be due to its optimum lipophilic nature and the electron-rich methoxy and trimethoxy derivatives has attributed their antibacterial effect due to its electron donating nature and the solubilizing effect.

Antioxidant activity

The antioxidant potential of all the newly synthesized compounds was done by evaluating their scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The antioxidant studies by DPPH assay revealed that compounds **4b**, **4e**, and **4k** with fluro, chloro, and bromo moieties exhibited good scavenging activity. The free radical scavenging effect of the halogen derivatives may be due to its electron attracting and lipophilic nature.

Conclusion

In the present work, we have synthesized eleven benzoxazinyl pyrazolone arylidenes aiming as antimicrobial and antioxidant agents. The antimicrobial studies revealed that all the titled compounds possessed significant antimicrobial activity. In the antibacterial study performed, the derivatives with fluro, methoxy, dimethylamino, and trimethoxy substituent were found to be potential. Also, compounds possessing fluro and methoxy substituent showed excellent antifungal activity. In the antioxidant studies, the excellent free radical scavenging activity is attributed to the presence of alkyl group attached to the benzoxazine and the derivatives with fluro, chloro, and bromo substitution showed good free radical scavenging activity. To conclude with, the novel series of benzoxazinyl pyrazolo arylidenes synthesized by the incorporation of pyrazole moiety in the benzoxazinyl structure through an alkyl link may be considered as a promising lead for future design of effective antimicrobial and antioxidant agents.

Experimental

Chemistry

General informations for chemicals and instruments

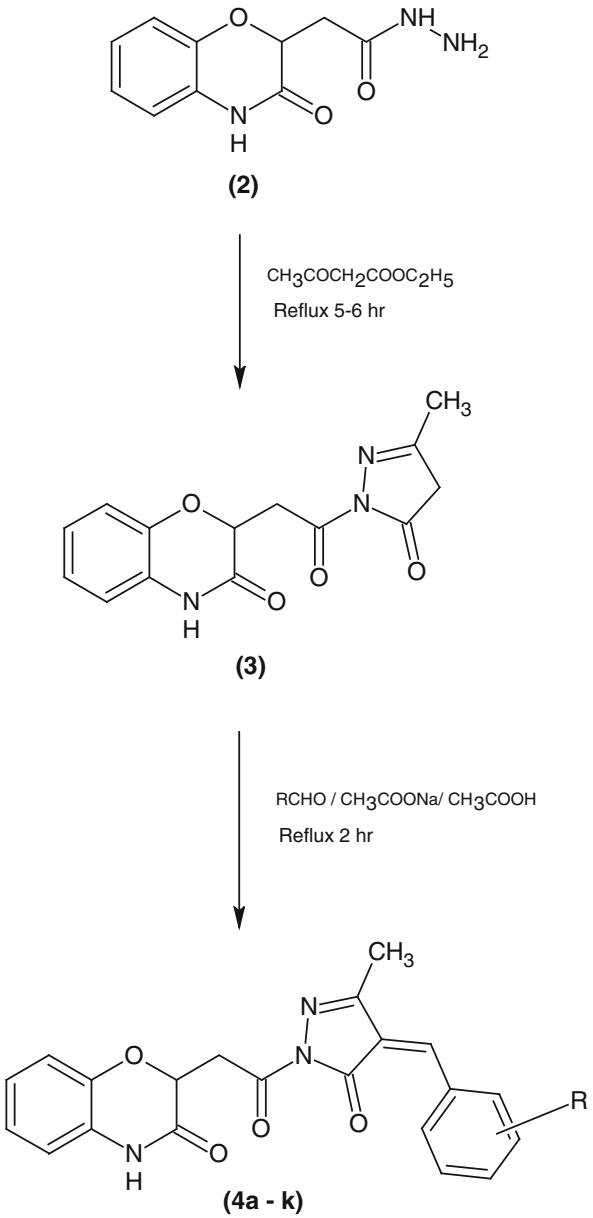
Melting points were determined by using melting point apparatus MR-VIS (MR08190508). IR spectra were

recorded on JASCO FT/IR-410 spectrometer on KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 300 MHz NMR spectrometer. Mass spectra were recorded on Shimadzu, LCMS 2010 EV. The elemental analysis was done on Vario EL III CHNS and the analysis data (C, H, and N) were within the range of ±0.4 % of the calculated values. Purity of all the compounds were checked by thin layer chromatography using silica gel—G as adsorbent and iodine vapor as detecting agent.

The synthetic pathway is enumerated in Scheme 1. Methyl-(3,4-dihydro-3-oxo-2*H*-1,4-benzoxazin-2-yl) acetate **1** was formed by the reaction of 2-amino phenol with maleic anhydride and the reaction of **1** with hydrazine hydrate gave 3,4-dihydro-3-oxo-2*H*-1,4-benzoxazin-2-yl acetic acid hydrazide **2** (Jayamma *et al*., 1989).

*Synthesis of 2-[2-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl]-2H-1,4 benzoxazin-3(4H)-one (**3**)*

The aryl hydrazide **2** and ethyl acetoacetate both in equimolar amounts (0.002 mol) were refluxed in methanol (20 ml) for 5–6 h. After cooling the reaction mixture, a



Scheme 1 Formation of benzoxazinyl pyrazolo methylidenes **4a–k**

R = H, F, OCH_3, OH (OCH_3), Cl, $N(CH_3)_2$, CH_3, OH, NO_2, $(OCH_3)_3$, Br

solid which was separated out was filtered and dried after washing with pet ether (60–80 °C). Recrystallization was done from methanol. The solvent system used was methanol:benzene (3:7).

Yield: 63 %; m.p. 160 °C. Spectroscopic analysis: IR(KBr) ν_{max}/cm^{-1}: 3218.06, 3103.27, 1721.22, 1610.80, 1501.02,1219.63, 753.52; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.61 (s, 3H, CH_3), 3.55 (s, 2H,CH_2 of pyrazole), 4.90 (s, 2H, CH_2), 5.13 (s, 1H, CH), 7.26–7.48 (m, 4H, Ar–H), 10.92 (bs, 1H, NH of benzoxazine); ^{13}C NMR (DMSOd_6, 300 MHz, δ ppm): 38.88 (CH_3 of pyrazolone), 66.24 ($\underline{C}H_2$–CO), 113.90 (C_4 of pyrazolone), 118.40–132.61 (aromatic carbons), 166.14 (>NCO), 171.80 (C=O of pyrazolone), 172.14 (C=O of benzoxazinone); $(M + 1)^+$ ion peak at 288; Anal. Calcd. (%) for $C_{14}H_{13}N_3O_4$: C, 58.53; H, 4.53; N, 14.63. Found: C, 58.50; H, 4.57; N, 14.68.

*Synthesis of 2-{2-[4-(substituted) benzylidene-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl]-2-oxoethyl}-2H-1,4-benzoxazin-3(4H)-one (**4a–k**)*

To compound **3** (0.01 mol) dissolved in glacial acetic acid (10 ml) was added fused sodium acetate (0.015 mol) and various aldehydes (0.01 mol) and the mixture was refluxed for 2 h. It was then cooled and poured into crushed ice. The resulting solid was washed and recrystallized from aqueous ethanol. The solvent system used was methanol:benzene (4:6).

*2-{2-[3-Methyl-5-oxo-4-(phenylmethylidene)-4,5-dihydro-1H-pyrazol-1-yl]-2-oxoethyl}-2H-1,4-benzoxazin-3(4H)-one (**4a**)* Yield: 58 %; m.p. 184 °C. Spectroscopic analysis: IR(KBr) ν_{max}/cm^{-1}: 3027.09, 2965.22, 1675.14, 1629.21, 1560.01,1220.65,1035.65, 752.73; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.58 (s, 3H, CH_3), 4.85 (s, 2H, CH_2), 5.12 (s, 1H, CH), 6.12 (s, 1H, C=CH), 7.18–7.73 (m, 9H, Ar–H), 9.83 (bs, 1H, NH of benzoxazine); ^{13}C NMR (DMSOd_6, 300 MHz, δ ppm): 38.68 (CH_3 of pyrazolone), 65.56 (CH_2–CO), 112.43 (C=CH), 119.43–132.05 (aromatic carbons), 166.29 (>NCO), 170.54 (C=O of pyrazolone), 172.34 (C=O of benzoxazinone); $(M)^+$ ion peak at 375; Anal. Calcd. (%) for $C_{21}H_{17}N_3O_4$: C, 67.20; H, 4.53; N, 11.12. Found: C, 67.24; H, 4.49; N, 11.09.

*2-(2-{4-[(4-Fluorophenyl) methylidene]-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl}-2-oxoethyl)-2H-1,4-benzoxazin-3(4H)-one (**4b**)* Yield: 65 %; m.p. 222 °C. Spectroscopic analysis: IR(KBr) ν_{max}/cm^{-1}: 3003.31, 2950.32, 1680.41, 1628.42, 1556.12,1227.20,1056.35, 762.52; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.56 (s, 3H, CH_3), 4.96 (s, 2H, CH_2), 5.22 (s, 1H, CH), 6.22 (s, 1H, C=CH), 7.23–7.65 (m, 8H, Ar–H), 10.23 (bs, 1H, NH of benzoxazine); ^{13}C NMR (DMSOd_6, 300 MHz, δ ppm): 38.45 (CH_3 of pyrazolone), 65.68 (CH_2–CO), 112.33 (C=CH), 120.19–131.15 (aromatic carbons), 165.62 (>NCO), 170.39 (C=O of pyrazolone), 172.78 (C=O of benzoxazinone); $(M)^+$ ion peak at 393; Anal. Calcd. (%) for $C_{21}H_{16}N_3O_4F$: C, 64.12; H, 4.07; N, 10.61. Found: C, 64.22; H, 4.12; N, 10.58.

*2-(2-{4-[(4-Methoxyphenyl)methylidene]-3-methyl-5-oxo-4, 5-dihydro-1H-pyrazol-1-yl} -2-oxoethyl)-2H-1,4-benzoxazin-3(4H)-one (**4c**)* Yield: 57 %; m.p. 226 °C. Spectroscopic analysis: IR(KBr) ν_{max}/cm^{-1}: 3107.35, 2981.23, 1667.75, 1628.63, 1565.71, 1221.19, 1064.33, 771.53; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.53 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 4.66 (s, 2H, CH_2), 5.16 (s, 1H, CH), 6.18 (s, 1H, C=CH), 7.24–7.83 (m, 8H, Ar–H), 10.42 (bs, 1H, NH of benzoxazine); ^{13}C NMR (DMSOd_6, 300 MHz, δ ppm): 38.36 (CH_3 of pyrazolone), 65.72 (CH_2–CO), 112.23 (C=CH), 118.20–130.15 (aromatic carbons), 165.16 (>NCO), 170.89 (C=O of pyrazolone), 172.67 (C=O of benzoxazinone); $(M + 1)^+$ ion peak at 406; Anal. Calcd. (%) for $C_{22}H_{19}N_3O_5$: C, 65.18; H, 4.69; N, 10.37. Found: C, 65.23; H, 4.71; N, 10.43.

*2-(2-{4-[(3-Hydroxy-4-methoxyphenyl) methylidene]-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl}-2-oxoethyl)-2H-1,4-benzoxazin-3(4H)-one (**4d**)* Yield: 62 %; m.p. 203 °C. Spectroscopic analysis: IR(KBr) ν_{max}/cm^{-1}: 3110.42, 3098.45, 2978.32, 1670.67, 1629.25, 1560.43, 1219.23, 1053.64, 754.64; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.54 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 4.56 (s, 2H, CH_2), 5.23 (s, 1H, CH), 6.20 (s, 1H, C=CH), 7.16–7.63 (m, 7H, Ar–H), 9.87 (bs, 1H, NH of benzoxazine), 11.02 (s, 1H, OH); ^{13}C NMR (DMSOd_6, 300 MHz, δ ppm): 38.38 (CH_3 of pyrazolone), 65.76 (CH_2–CO), 112.23 (C=CH), 118.19–129.20 (aromatic carbons), 165.66 (>NCO), 171.03 (C=O of pyrazolone), 172.78 (C=O of benzoxazinone); $(M + 1)^+$ ion peak at 422; Anal. Calcd. (%) for $C_{22}H_{19}N_3O_6$: C, 62.70; H, 4.51; N, 9.97. Found: C, 62.66; H, 4.46; N, 9.92.

*2-(2-{4-[(4-Chlorophenyl)methylidene]-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl}-2-oxoethyl)-2H-1,4-benzoxazin-3(4H)-one (**4e**)* Yield: 59 %; m.p. 240 °C. Spectroscopic analysis: IR(KBr) ν_{max}/cm^{-1}: 3106.70, 2934.45, 1654.31,1627.55, 1535.78, 1229.19, 1064.76, 763.21; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.46 (s, 3H, CH_3), 4.44 (s, 2H, CH_2), 5.36 (s, 1H, CH), 6.13 (s, 1H, C=CH), 7.24–7.76 (m, 8H, Ar–H), 10.34 (bs, 1H, NH of benzoxazine); ^{13}C NMR (DMSOd_6, 300 MHz, δ ppm): 38.24 (CH_3 of pyrazolone), 65.56 (CH_2–CO), 112.56 (C=CH), 118. 20–132.15 (aromatic carbons), 165.56 (>NCO), 170.43 (C=O of pyrazolone), 172.67 (C=O of benzoxazinone); $(M)^+$ ion peak at 409; Anal. Calcd. (%) for $C_{21}H_{16}N_3O_4Cl$: C, 61.61; H, 3.91; N, 10.26. Found: C, 61.67; H, 3.96; N, 10.30.

*2-(2-{4-[(4-Dimethylaminophenyl)methylidene]-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl}-2-oxoethyl)-2H-1,4-benzoxazin-3(4H)-one (**4f**)* Yield: 67 %; m.p. 190 °C. Spectroscopic analysis: IR(KBr) ν_{max}/cm^{-1}: 3006.65, 2945.34, 1652.43, 1626.14, 1532.34, 1230.34, 1072.32, 756.90; ^1H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.39 (s, 3H, CH_3), 3.07 (s, 6H, $N(CH_3)_2$), 4.52 (s, 2H, CH_2), 5.37 (s, 1H, CH), 6.22 (s, 1H, C=CH), 7.28–7.82 (m, 8H, Ar–H), 10.44 (bs, 1H, NH of benzoxazine); ^{13}C NMR ($DMSOd_6$, 300 MHz, δ ppm): 38.16 (CH_3 of pyrazolone), 65.28 (CH_2–CO), 112.09 (C=CH), 118.43–130.11 (aromatic carbons), 165.60 (>NCO), 170.56 (C=O of pyrazolone), 172.56 (C=O of benzoxazinone); $(M + 1)^+$ ion peak at 419; Anal. Calcd. (%) for $C_{23}H_{22}N_4O_4$: C, 66.02; H, 5.26; N, 13.39. Found: C, 66.09; H, 5.22; N, 13.43.

*2-(2-{3-Methyl-4-[(4-methylphenyl)methylidene]-5-oxo-4,5-dihydro-1H-pyrazol-1-yl}-2-oxoethyl)-2H-1,4-benzoxazin-3(4H)-one (**4g**)* Yield: 62 %; m.p. 214 °C. Spectroscopic analysis: IR(KBr) ν_{max}/cm^{-1}: 3023.78, 2938.45, 1645.43, 1627.41, 1536.32, 1228.34, 1068.65, 754.56; ^1H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.38 (s, 3H, CH_3), 4.43 (s, 2H, CH_2), 5.36 (s, 1H, CH), 6.18 (s, 1H, C=CH), 7.26–7.83 (m, 8H, Ar–H), 9.35 (bs, 1H, NH of benzoxazine); ^{13}C NMR ($DMSOd_6$, 300 MHz, δ ppm): 38.55 (CH_3 of pyrazolone), 39.68 (CH_3 of phenyl) 65.72 (CH_2–CO), 112.44 (C=CH), 121.13–132.17 (aromatic carbons), 165.26 (>NCO), 170.45 (C=O of pyrazolone), 172.54 (C=O of benzoxazinone); $(M)^+$ ion peak at 389; Anal. Calcd. (%) for $C_{22}H_{19}N_3O_4$: C, 67.86; H, 4.88; N, 10.79. Found: C, 67.82; H, 4.93; N, 10.74.

*2-(2-{4-[(4-Hydroxyphenyl)methylidene]-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl}-2-oxoethyl)-2H-1,4-benzoxazin-3(4H)-one (**4h**)* Yield: 58 %; m.p. 174 °C. Spectroscopic analysis: IR(KBr) ν_{max}/cm^{-1}:3112.34, 3044.84, 2956.45, 1645.43, 1629.85, 1540.87, 1230.97, 1072.33, 765.33; ^1H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.43 (s, 3H, CH_3), 4.44 (s, 2H, CH_2), 5.40 (s, 1H, CH), 6.23 (s, 1H, C=CH), 7.22–7.65 (m, 8H, Ar–H), 9.44 (bs, 1H, NH of benzoxazine), 11.03 (s, 1H, OH); ^{13}C NMR ($DMSOd_6$, 300 MHz, δ ppm): 38.64 (CH_3 of pyrazolone), 65.56 (CH_2–CO), 112.33 (C=CH), 119.32–130.19 (aromatic carbons), 165.76 (>NCO), 170.56 (C=O of pyrazolone), 172.96 (C=O of benzoxazinone); $(M + 1)^+$ ion peak at 392; Anal. Calcd. (%) for $C_{21}H_{17}N_3O_5$: C, 64.45; H, 4.34; N, 10.74. Found: C, 64.49; H, 4.38; N, 10.79.

*2-(2-{4-[(4-Nitrophenyl)methylidene]-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl}-2-oxoethyl)-2H-1,4-benzoxazin-3(4H)-one (**4i**)* Yield: 62 %; m.p. 121 °C. Spectroscopic analysis: IR(KBr) ν_{max}/cm^{-1}: 3056.73, 2943.34, 1644.23, 1628.34, 1535.34, 1224.72, 1087.45, 766.32; ^1H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.40 (s, 3H, CH_3), 4.40 (s, 2H, CH_2), 5.38 (s, 1H, CH), 6.25 (s, 1H, C=CH), 7.18–7.67 (m, 8H, Ar–H), 9.42 (bs, 1H, NH of benzoxazine); ^{13}C NMR ($DMSOd_6$, 300 MHz, δ ppm): 38.59 (CH_3 of pyrazolone), 65.50 (CH_2–CO), 112.28 (C=CH), 119.30–130.23 (aromatic carbons), 165.68 (>NCO), 170.51 (C=O of pyrazolone), 172.76 (C=O of benzoxazinone); $(M + 1)^+$ ion peak at 421; Anal. Calcd. (%) for $C_{21}H_{16}N_4O_6$: C, 60.32; H, 3.81; N, 13.33. Found: C, 60.49; H, 3.88; N, 13.36.

*2-(2-{4-[(3,4,5-Trimethoxyphenyl)methylidene]-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl}-2-oxoethyl)-2H-1,4-benzoxazin-3(4H)-one (**4j**)* Yield: 60 %; m.p. 156 °C. Spectroscopic analysis: IR(KBr) ν_{max}/cm^{-1}: 3036.82, 2963.56, 1656.32, 1628.72, 1542.21, 1231.09, 10782.33, 761.52; ^1H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.56 (s, 3H, CH_3), 3.68 (s, 9H, $(OCH_3)_3$), 4.50 (s, 2H, CH_2), 5.46 (s, 1H, CH), 6.32 (s, 1H, C=CH), 7.10–7.82 (m, 6H, Ar–H), 9.50(bs, 1H, NH of benzoxazine); ^{13}C NMR ($DMSOd_6$, 300 MHz, δ ppm): 38.23 (CH_3 of pyrazolone), 64.65 (CH_2–CO), 114.23(C=CH), 118.34–132.01(aromatic carbons), 163.57 (>NCO), 171.06 (C=O of pyrazolone), 171.29 (C=O of benzoxazinone); $(M)^+$ ion peak at 465; Anal. Calcd. (%) for $C_{24}H_{23}N_3O_7$: C, 61.93; H, 4.94; N, 9.03. Found: C, 61.78; H, 4.88; N, 9.10.

*2-(2-{4-[(4-Bromophenyl)methylidene]-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl}-2-oxoethyl)-2H-1,4-benzoxazin-3(4H)-one (**4k**)* Yield: 54 %; m.p. 203 °C. Spectroscopic analysis: IR(KBr) ν_{max}/cm^{-1}: 3063.87, 2966.56, 1643.25, 1628.53, 1542.65, 1228.14, 1070.22, 756.65; ^1H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.47 (s, 3H, CH_3), 4.48 (s, 2H, CH_2), 5.36 (s, 1H, CH), 6.27 (s, 1H, C=CH), 7.20–7.80 (m, 8H, Ar–H), 9.38 (bs, 1H, NH of benzoxazine); ^{13}C NMR ($DMSOd_6$, 300 MHz, δ ppm): 37.23(CH_3 of pyrazolone), 65.56 (CH_2–CO), 111.56(C=CH), 121.03–131.87 (aromatic carbons), 164.54 (>NCO), 168.71 (C=O of pyrazolone), 170.95(C=O of benzoxazinone); $(M + 1)^+$ ion peak at 455; Anal. Calcd. (%) for $C_{21}H_{16}BrN_3O_4$: C, 55.51; H, 3.52; N, 9.25. Found: C, 55.64; H, 3.48; N, 9.32.

Biological activity

Antimicrobial activity

The synthesized compounds were screened for their in vitro antibacterial activity against *Staphylococcus aureus* NCIM 2079, *Bacillus subtilis* NCIM 2063, *Escherichia coli* NCIM 2118, and *Pseudomonas aeruginosa* NCIM 2036 and antifungal activity against *Aspergillus niger* NCIM 545 and *Candida albicans* NCIM 3100 by twofold serial dilution method (Linnette *et al.*, 1985).

The in vitro antimicrobial activities of the compounds were tested in Muller Hinton broth for bacteria and in Sabourauds dextrose broth for fungal strains by twofold serial dilution method. The test compounds were dissolved in dimethylsulfoxide (DMSO) to obtain 1 mg/ml stock solutions.

The final inoculum size was 10^5 cfu/ml for antibacterial assay and 10^2 cfu/ml for antifungal assay and testing was performed at pH 7.4 ± 0.2. The tubes were incubated at 37 ± 1 °C for bacteria and 28 ± 1 °C for fungi. The minimum inhibitory concentrations (MICs) were recorded by visual observations after 24 h for bacteria and 48 h of incubation for fungi. Ciprofloxacin and fluconazole were used as standards, respectively, for bacterial and fungal studies. The antimicrobial results are given in Table 1.

Antioxidant activity

The antioxidant potential of all the newly synthesized compounds were done by evaluating their scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by using ascorbic acid as the reference compound (Demetrios *et al.*, 1998).

1.5 ml methanolic solution of the synthesized compounds (0.2 mM) was added to 1.5 ml (0.2 mM) solution of DPPH radical in methanol to make the final concentration of DPPH and synthesized compounds to 0.1 mM. The mixture was shaken vigorously and allowed to stand for 30 min. After this, the absorbance at 517 nm was determined and the percentage of scavenging activity was calculated using the formula shown below. Ascorbic acid was used as the reference compound. All tests and analyses were undertaken on three replicates and the results were averaged. The results are given in Table 2.

$$\text{Scavenging activity } (\%) = \{[(\text{Ab} + \text{As}) - \text{Am}]/\text{Ab}\} \times 100\,\%$$

Ab: absorbance of 0.1 mM methanolic solution of DPPH at 517 nm, As: absorbance of 0.1 mM methanolic solution of test compound at 517 nm, Am: absorbance of methanolic mixture of the drug and DPPH at 517 nm.

Table 2 Antioxidant activity data of synthesized compounds (**4a–k**)

Compound	Scavenging activity (%)
4a	53
4b	82
4c	65
4d	69
4e	86
4f	35
4g	76
4h	78
4i	64
4j	65
4k	86
Ascorbic acid	98

Table 1 Antimicrobial activity data of synthesized compounds (**4a–k**)

Compound	MIC (μg/ml)					
	Antibacterial activity				Antifungal activity	
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. niger*	*C. albicans*
4a	62.5	31.25	125	15.625	125	62.5
4b	15.625	31.25	31.25	15.625	31.25	31.25
4c	15.625	31.25	15.625	31.25	15.625	15.625
4d	31.25	125	62.5	250	31.25	62.5
4e	125	31.25	31.25	250	250	31.25
4f	31.25	15.625	15.625	31.25	125	62.5
4g	62.5	250	125	62.5	62.5	31.25
4h	125	62.5	31.25	125	31.25	62.5
4i	62.5	31.25	125	125	62.5	62.5
4j	31.25	15.625	31.25	15.625	15.625	31.25
4k	7.812	62.5	62.5	125	31.25	31.25
Ciprofloxacin	31.25	3.91	3.91	1.953		–
Fluconazole	–	–	–	–	3.91	1.9

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