Design, synthesis and biological potential of heterocyclic benzoxazole scaffolds as promising antimicrobial and anticancer agents

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Abstract

**Background:** Benzoxazole is the most important class of heterocyclic compound in medicinal chemistry. It has been incorporated in many medicinal compounds making it a versatile heterocyclic compound that possess a wide spectrum of biological activities.

**Results:** The molecular structures of synthesized benzoxazole derivatives were confirmed by physicochemical and spectral means. The synthesized compounds were further evaluated for their in vitro biological potentials i.e. antimicrobial activity against selected microbial species using tube dilution method and antiproliferative activity against human colorectal carcinoma (HCT 116) cancer cell line by Sulforhodamine B assay.

**Conclusion:** In vitro antimicrobial results demonstrated that compounds 4, 5, 7 and 16 showed promising antimicrobial potential. The in vitro anticancer activity indicated that compounds 4 and 16 showed promising anticancer activity against human colorectal cancer cell line (HCT 116) when compared to standard drug and these compounds may serve as lead compound for further development of novel antimicrobial and anticancer agents.

**Keywords:** Benzoxazole molecules, Synthesis, Antimicrobial activity, Anticancer activity

Background

Colorectal cancer is one of the most dangerous forms of cancer, causing the deaths of many patients every year [1]. As such, a significant progress is being made continuously towards developing novel chemotherapeutic agents [2, 3]. One of the standard drugs for treatment of colorectal cancer is 5-fluorouracil (5-FU). However it is associated with a lot of side effects as it not only affects the cancer cells but also the normal cells [3–7]. In order to overcome the undesirable side effects of available anticancer agents there is a need to develop novel chemotherapeutic agents for more effective cancer treatment [2].

The number of cases of multidrug resistant bacterial infections is increasing at an alarming rate and clinicians have become reliant on vancomycin as the antibiotic for serious infections resistant to traditional agents which indicated that there is a need for the development of new classes of antimicrobial agents [8]. Hence there is a need to develop those agents whose chemical characteristics clearly differ from those existing agents and can overcome the problem of resistance [9].

Benzoxazole belongs to one of the most important class of heterocyclic compounds which are very significant for medicinal field. It has been incorporated in many medicinal compounds that made it versatile heterocyclic compound possessing wide spectrum of biological activities viz: antimicrobial [10, 11], analgesic/anti-inflammatory
[12], antitumor [13], antidiabetic activity [14] etc. Keeping in view of the pharmacological importance of benzoxazole derivatives, the present study had synthesized some new benzoxazole derivatives and evaluate their antimicrobial and antiproliferative activities. The design of benzoxazole molecules with antimicrobial and anticancer potential was based on literature as shown in Fig. 1.

**Results and discussion**

**Chemistry**

A series of benzoxazole derivatives (1–20) was synthesized using synthetic procedures as outlined in Scheme 1. Initially, 2-chloro-N-(substituted phenyl)acetamide (I) was prepared by reacting substituted aniline with chloroacetyl chloride in the presence of acetone and powdered potassium carbonate. To prepare 2-azido-N-(substituted phenyl)acetamide (II) reaction was carried out between I in dry DMF and sodium azide at room temperature. Benzo[d]oxazole-2-thiol (III) was prepared from 2-aminophenol in methanol, potassium hydroxide followed by the addition of carbon-di-sulphide. Further, to a solution of III in acetone was added anhydrous potassium carbonate powder followed by slow addition of 3-bromo-prop-1-yne at 0 °C and the obtain 2-(prop-2-yn-1-ylthio) benzo[d]oxazole (IV). Finally, II and IV were dissolved in a mixture of t-BuOH:H2O:DMF followed by the addition of sodium ascorbate and copper (II) sulfate so as to obtain target benzoxazole derivatives (1–20). The synthesized compounds were confirmed by physicochemical properties (Table 1) i.e. melting point, molecular formula, Rf value, % yield and spectral interpretation details (Table 2) i.e. FT-IR, NMR and Mass, which are in agreement with

![Fig. 1 Design of benzoxazole molecules for antimicrobial and anticancer potential based on literature](image-url)
the proposed molecular structures. The three obvious peaks in the IR spectra of the title compounds at 1689–1662 cm$^{-1}$, 3315–2986 cm$^{-1}$ and 1499–1408 cm$^{-1}$ are attributed to C=N group of oxazole ring, C–H and C=C groups of aromatic ring, respectively. The absorption peak of C–F group in aromatic fluoro compounds (11 and 18) appeared at 1235–1207 cm$^{-1}$ whereas bands at 738–622 cm$^{-1}$ correspond to C–Br stretching of aromatic bromo derivatives (7, 12, 14 and 16). The presence of aryl alkyl ether group (C–O–C, Ar–OCH$_3$) in compound 3 showed a band at 1194 cm$^{-1}$. Further the presence of chloro group (Ar–Cl) in compounds 5, 6, 10, 13, 19 and 20 showed IR stretches at 744–739 cm$^{-1}$. The IR band at 1653–1578 cm$^{-1}$ indicated the presence of CONH group of synthesized compounds. The compounds 1, 2 and 4 displayed IR stretching around 1394–1341 cm$^{-1}$ that
corresponds to C-N symmetric stretching of aromatic NO\textsubscript{2} group.

In \textsuperscript{1}H-NMR spectra the multiplet signals between 6.70 and 8.57 ppm are assigned to the presence of aromatic protons of synthesized compounds (1–20). The compound 3 showed a singlet at 3.71 ppm due to the existence of –OCH\textsubscript{3} of Ar–OCH\textsubscript{3} in its structure. All the synthesized compounds showed a singlet at 7.34–7.14 ppm which corresponds to the presence of N–CH of triazole. Compounds, 6, 7, 9, 15 and 17 showed singlet around 2.50 ppm due to the existence of –CH\textsubscript{3} group at ortho and para position. The appearance of singlet at 4.72–4.77 ppm and 8.27–7.82 ppm are due to –CH\textsubscript{2} and –NH group, respectively. \textsuperscript{13}C-NMR spectral data showed the confirmation of carbon atom in the assigned molecular structures of the synthesized compounds. The mass spectra of title compounds shows consistency between [M]+ ion absorption signal and the calculated molecular weight. The synthesized benzoxazole derivatives (1–20) were screened for their pharmacological activity i.e. antimicrobial and antiproliferative activities against selected microbial (bacterial and fungal) organisms and cancer cell line (HCT 116), respectively (using standard protocol shown in experimental section). The

\begin{table}[h]
\centering
\begin{tabular}{llllll}
\hline
\textbf{Comp.} & \textbf{Molecular mass} & \textbf{M. formula} & \textbf{m.p. °C} & \textbf{R\textsubscript{f} value} & \textbf{% yield} \\
\hline
1: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(3-nitrophenyl) acetamide & 410.41 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 152–154 & 0.17 & 76 \\
2: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-nitrophenyl) acetamide & 410.41 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 165–167 & 0.18 & 81 \\
3: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-methoxyphenyl) acetamide & 395.43 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 102–104 & 0.23 & 75 \\
4: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(2-chloro-4-nitrophenyl) acetamide & 444.85 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 144–146 & 0.20 & 86 \\
5: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(2,5-trichlorophenyl) acetamide & 468.74 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 189–191 & 0.21 & 79 \\
6: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(3-chloro-2-methylphenyl) acetamide & 413.88 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 138–140 & 0.22 & 82 \\
7: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-bromo-2-methyl-phenyl) acetamide & 458.33 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 127–129 & 0.22 & 85 \\
8: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-ethylphenyl) acetamide & 393.46 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 118–120 & 0.23 & 85 \\
9: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(2,4-dimethylphenyl) acetamide & 393.49 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 108–110 & 0.23 & 81 \\
10: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(2-chlorophenyl) acetamide & 399.85 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 144–146 & 0.19 & 86 \\
11: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-fluorophenyl) acetamide & 383.40 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 119–121 & 0.19 & 90 \\
12: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-bromophenyl) acetamide & 444.31 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 172–174 & 0.20 & 77 \\
13: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(3,4-dichlorophenyl) acetamide & 434.30 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 169–171 & 0.19 & 80 \\
14: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(2-bromophenyl) acetamide & 444.31 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 131–133 & 0.19 & 81 \\
15: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(3,5-dimethylphenyl) acetamide & 393.46 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 125–127 & 0.23 & 79 \\
16: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(3-bromophenyl) acetamide & 444.31 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 151–153 & 0.18 & 78 \\
17: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(2,3-dimethylphenyl) acetamide & 393.46 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 133–135 & 0.24 & 82 \\
18: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(2-fluorophenyl) acetamide & 383.40 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 119–120 & 0.20 & 89 \\
19: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(3-chlorophenyl) acetamide & 399.85 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 161–163 & 0.19 & 86 \\
20: 2-(5-(Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-chlorophenyl) acetamide & 399.85 & C\textsubscript{20}H\textsubscript{19}N\textsubscript{5}O\textsubscript{2}S & 166–168 & 0.19 & 82 \\
\hline
\end{tabular}
\caption{Physicochemical properties of synthesized benzoxazole derivatives}
\end{table}
Table 2: Spectral data of synthesized compounds (1–20)

| Comp. | FT-IR (KBr cm\(^{-1}\)) | C–H str. (Ar) | C=O str. (Ar) | N=CH str. | C–N str. | C–O–C str. | C–H str. | CONH str. | C–S str. | Other str. | \(^1\)H NMR (δ, DMSO) | \(^{13}\)C NMR (δ, DMSO) | MS: m/z |
|-------|--------------------------|---------------|---------------|-----------|----------|------------|----------|-----------|---------|------------|-------------------|-------------------|--------|
| 1     | 3088                     | 1477          | 1688          | 1262      | 1134     | 2834       | 1647     | 672       | 1350 NO\(_2\) str. | 7.63–8.57 (m, 8H, Ar–H), 7.33 (s, 1H, –CH of triazole), 4.76 (s, 2H, –N–CH\(_3\)), 7.95 (s, 1H, –NH) | 1649, 151.2, 147.9, 141.1, 139.4, 130.3, 125.7, 125.1, 124.4, 118.2, 113.3, 110.2, 52.3, 26.3 | 411    |
| 2     | 3133                     | 1460          | 1685          | 1259      | 1194     | 2973       | 1649     | 687       | 1394 NO\(_2\) str. | 7.66–8.35 (m, 8H, Ar–H), 7.34 (s, 1H, –CH of triazole), 4.75 (s, 2H, –N–CH\(_3\)), 7.82 (s, 1H, –NH) | 1652, 151.2, 144.4, 141.1, 124.6, 124.4, 118.3, 110.2, 52.3, 26.3 | 411    |
| 3     | 3138                     | 1452          | 1662          | 1241      | 1178     | 2831       | 1618     | 674       | –        | 6.88–7.59 (m, 8H, Ar–H), 7.32 (s, 1H, –CH of triazole), 4.77 (s, 2H, –N–CH\(_3\)), 8.25 (s, 1H, –NH) | 1641, 155.4, 151.1, 140.8, 131.4, 124.7, 124.5, 120.7, 118.2, 113.9, 110.2, 52.1, 52.2, 26.3 | 396    |
| 4     | 3072                     | 1451          | 1697          | 1275      | 1177     | 2887       | 1647     | 671       | 1341 NO\(_2\) str. | 7.34–8.23 (m, 7H, Ar–H), 7.33 (s, 1H, –CH of triazole), 4.74 (s, 2H, –N–CH\(_3\)), 8.21 (s, 1H, –NH) | 1657, 151.3, 143.5, 141.1, 124.6, 124.3, 123.7, 118.3, 110.2, 52.3, 26.3 | 445    |
| 5     | 3077                     | 1499          | 1689          | 1277      | 1181     | –          | 1653     | 676       | 743 C–Cl str. | 7.35–7.66 (m, 6H, Ar–H), 7.34 (s, 1H, –CH of triazole), 4.73 (s, 2H, –N–CH\(_3\)), 8.09 (s, 1H, –NH) | 1653, 151.3, 141.1, 134.3, 130.6, 129.8, 124.9, 124.6, 124.3, 118.3, 110.2, 52.1, 263 | 469    |
| 6     | 3121                     | 1499          | 1672          | 1272      | 1132     | 3067       | 1578     | 669       | 739 C–Cl str. | 7.20–7.66 (m, 7H, Ar–H), 7.29 (s, 1H, –CH of triazole), 4.74 (s, 2H, –N–CH\(_3\)), 8.22 (s, 1H, –NH) | 1645, 151.2, 141.1, 136.9, 133.8, 130.3, 126.8, 124.6, 124.3, 118.3, 110.2, 51.9, 263 | 414    |
| 7     | 2986                     | 1494          | 1674          | 1291      | 1185     | 2877       | 1601     | 742       | 622 C–Br str. | 7.338–7.45 (m, 7H, Ar–H), 4.19 (s, 2H, –CH\(_2\)S), 7.33 (s, 1H, –CH of triazole), 4.76 (s, 2H, –N–CH\(_3\)), 8.24 (s, 1H, –NH), 2.51 (s, 3H, –CH\(_3\)) | 1643, 151.1, 141.1, 134.1, 128.8, 128.8, 124.6, 118.2, 110.2, 52.1, 17.4 | 459    |
| 8     | 3080                     | 1449          | 1662          | 1237      | 1130     | 2965       | 1606     | 709       | –        | 7.14–7.66 (m, 8H, Ar–H), 7.31 (s, 1H, –CH of triazole), 4.74 (s, 2H, –N–CH\(_3\)), 8.20 (s, 1H, –NH), 1.16 (s, 3H, –CH\(_3\)), 258 (s, 2H, –CH\(_3\)) | 16381, 151.3, 141.2, 136.1, 128.1, 124.6, 124.3, 118.3, 110.2, 52.2, 27.5, 15.5 | 394    |
| 9     | 3037                     | 1486          | 1676          | 1293      | 1141     | 2993       | 1592     | 668       | –        | 6.94–7.58 (m, 7H, Ar–H), 4.18 (s, 2H, –CH\(_2\)S), 7.31 (s, 1H, –CH of triazole), 4.77 (s, 2H, –N–CH\(_3\)), 8.27 (s, 1H, –NH), 2.50 (s, 6H, –CH\(_3\), –CH\(_2\)) | 1641, 151.1, 140.9, 134.6, 132.8, 131.4, 130.8, 126.5, 124.7, 124.4, 118.2, 110.2, 52.1, 26.3, 17.6 | 394    |
| 10    | 3121                     | 1408          | 1675          | 1220      | 1137     | 2985       | 1641     | 675       | 743 C–Cl str. | 7.15–7.58 (m, 8H, Ar–H), 7.34 (s, 1H, –CH of triazole), 4.74 (s, 2H, –N–CH\(_3\)), 8.21 (s, 1H, –NH) | 1641, 151.3, 141.2, 125.6, 124.6, 124.3, 118.3, 110.2, 52.1, 26.4 | 400    |
| 11    | 3048                     | 1491          | 1677          | 1289      | 1132     | 2879       | 1589     | 686       | 1235 C–F str. | 7.19–7.65 (m, 8H, Ar–H), 7.31 (s, 1H, –CH of triazole), 4.75 (s, 2H, –N–CH\(_3\)), 8.24 (s, 1H, –NH) | 1647, 151.3, 141.1, 134.1, 129.5, 124.6, 124.3, 118.3, 110.2, 52.1, 26.4 | 384    |
| Comp. | FT-IR (KBr cm\(^{-1}\)) | \(^1\)H NMR (δ, DMSO) | \(^13\)C NMR (δ, DMSO) | MS: m/z |
|-------|-------------------------|------------------------|------------------------|--------|
|       | C–H str. (Ar) | C≡C str. (Ar) | N=CH str. | C–O–C str. | C–H str. | CONH str. | C–S str. | Other str. |
| 12    | 3018 | 1488 | 1682 | 1288 | 1178 | 2949 | 1597 | 753 | 738 | C–Br str. | 7.33–7.52 (m, 8H, Ar–H), 7.332 (s, 1H, –CH of triazole), 4.73 (s, 2H, –N–CH\(_2\)), 8.21 (s, 1H, –NH) |
| 13    | 3315 | 1470 | 1677 | 1291 | 1130 | 2992 | 1588 | 689 | 744 | C–Cl str. | 7.33–7.58 (m, 7H, Ar–H), 7.34 (s, 1H, –CH of triazole), 4.74 (s, 2H, –N–CH\(_2\)), 8.21 (s, 1H, –NH) |
| 14    | 3054 | 1495 | 1674 | 1286 | 1134 | 2880 | 1584 | 740 | 684 | C–Br str. | 7.14–7.60 (m, 8H, Ar–H), 7.31 (s, 1H, –CH of triazole), 4.73 (s, 2H, –N–CH\(_2\)), 8.21 (s, 1H, –NH) |
| 15    | 3144 | 1496 | 1676 | 1261 | 1179 | 3001 | 1607 | 687 | – | C–Br str. | 6.7–7.33 (m, 7H, Ar–H), 7.34 (s, 1H, –CH of triazole), 4.73 (s, 2H, –N–CH\(_2\)), 8.18 (s, 1H, –NH), 2.50 (s, 6H, (–CH\(_3\))\(_2\)) |
| 16    | 3308 | 1475 | 1681 | 1226 | 1132 | – | 1619 | 743 | 676 | C–Br str. | 7.29–7.91 (m, 8H, Ar–H), 7.30 (s, 1H, –CH of triazole), 4.75 (s, 2H, –N–CH\(_2\)), 8.24 (s, 1H, –NH) |
| 17    | 3016 | 1460 | 1671 | 1211 | 1133 | 2947 | 1585 | 737 | – | C–Br str. | 7.02–7.34 (m, 7H, Ar–H), 7.14 (s, 1H, –CH of triazole), 4.72 (s, 2H, –N–CH\(_2\)), 8.18 (s, 1H, –NH), 2.50 (s, 6H, (–CH\(_3\))\(_2\)) |
| 18    | 3124 | 1454 | 1679 | 1293 | 1149 | 3057 | 1616 | 711 | 1207 | C–F str. | 7.16–7.67 (m, 8H, Ar–H), 7.29 (s, 1H, –CH of triazole), 4.73 (s, 2H, –N–CH\(_2\)), 8.20 (s, 1H, –NH) |
| 19    | 3310 | 1467 | 1678 | 1271 | 1130 | 3136 | 1595 | 680 | 742 | C–Cl str. | 7.14–7.66 (m, 8H, Ar–H), 7.33 (s, 1H, –CH of triazole), 4.73 (s, 2H, –N–CH\(_2\)), 8.20 (s, 1H, –NH) |
| 20    | 3126 | 1488 | 1670 | 1275 | 1148 | 3041 | 1589 | 671 | 739 | C–Cl str. | 7.31–7.59 (m, 8H, Ar–H), 7.33 (s, 1H, –CH of triazole), 4.73 (s, 2H, –N–CH\(_2\)), 8.22 (s, 1H, –NH) |

Str.: stretching, Ar: aromatic
structure–activity relationship study of the synthesized compounds indicated that the compounds bearing electron withdrawing group at different position of the substituted portion showed the promising antimicrobial and anticancer potentials.

**In vitro antimicrobial activity**

The synthesized benzoxazole compounds (1–20) were investigated for their antimicrobial potential against selected Gram-positive (S. aureus, B. subtilis), Gram-negative (E. coli, K. pneumoniae, S. typhi) bacterial and fungal (C. albicans, A. niger) organisms by tube dilution method (Table 3, Figs. 2 and 3). In case of Gram-positive bacteria, compound 5 (MIC<sub>SI</sub> = 13.3 µM and MIC<sub>se</sub> = 26.7 µM) showed the significant activity against S. typhi and S. aureus, respectively. Other side, compound 4 (MIC<sub>SI</sub> = 28.1 µM and MIC<sub>se</sub> = 14 µM) showed promising activity against S. aureus, A. niger and E. coli, respectively. Compound 7 (MIC<sub>SI</sub> = 27.3 µM) exhibited good activity against K. pneumoniae and C. albicans. Whereas, compound 16 was found to be most active one against A. niger with MIC value of 28.1 µM. In this series compound 4 having high antimicrobial potential among the synthesized compounds may be taken as lead compound for the development of novel antimicrobial agent.

**In vitro anticancer activity**

The antiproliferative activity of the benzoxazole derivatives was assessed against the human colorectal cancer cell line (HCT 116 (ATCC CCL-247)). Antiproliferative screening results (Table 4) revealed that compounds 4 (IC<sub>50</sub> = 22.5 µM) and 16 (IC<sub>50</sub> = 38.3 µM) displayed most promising antiproliferative activity in reference to the standard drug 5-flourouracil (IC<sub>50</sub> = 12.2 µM).

**Structure activity relationship (SAR)**

The structure activity relationship for antimicrobial and anticancer activities of synthesized benzoxazole derivatives (SAR, Fig. 4) can be deduced as follows:

- Presence of two heterocyclic moieties i.e. benzoxazole and triazole in the synthesized compounds, showed the promising in vitro antimicrobial and anticancer activities against the selected microbial organisms and cancer cell line, respectively.
- Presence of electron withdrawing groups (Cl and NO<sub>2</sub>) at ortho and para-positions, respectively of the substituted portion (Compound 4), enhanced the antimicrobial activity against S. aureus, E. coli, A. niger and antiproliferative activity against HCT 116 cancer cell line.
- Presence of electron releasing group (CH<sub>3</sub>) at ortho and electron withdrawing group (Br) at para-position of the substituted portion (Compound 7) enhanced the antimicrobial activity against K. pneumoniae and C. albicans.
- Electron withdrawing group (Br) at meta-position of the substituted portion (Compound 16), enhanced the antifungal and antiproliferative activities against A. niger and HCT 116 cancer cell line, respectively, as well as compound 5 have electron withdrawing group (Cl) at ortho and para-position of the substituted portion played an effective role in improving the antibacterial activity against B. subtilis and S. typhi.

The structure–activity relationship of the synthesized benzoxazole derivatives indicated that the compounds bearing electron withdrawing and electron releasing groups at different position of the substituted portion plays an excellent role in improving the antimicrobial and antiproliferative activities. The aforementioned facts are supported by the earlier research findings [21–23].

**Experimental section**

**Material and reagents**

The materials required to carry out this research work were obtained from commercial sources and were used with no further purification. Reaction monitoring was carried by thin-layer chromatography using 0.25 mm silica gel plates, using chloroform and methanol (9:1) as mobile phase and iodine vapours helped in observing the spots which were visualized in UV light. Melting point of compounds was determined by open capillary tube technique. An infrared spectrum was recorded (ATR, cm<sup>-1</sup>) in Bruker 12060280, software: OPUS 7.2.139.1294 spectrometer. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR were recorded at 600 and 150 MHz, respectively on Bruker Avance III 600 NMR spectrometer by appropriate deuterated solvents. The results are conveyed in parts per million (δ, ppm) downfield from tetramethylsilane (internal standard). <sup>1</sup>H-NMR spectral details of the synthesized derivatives are represented with multiplicity like singlet (s); doublet (d); triplet (t); multiplet (m) and the number hydrogen ion. Waters Micromass Q-ToF Micro instrument was utilized for obtaining the Mass spectra.

**General procedure for synthesis of benzoxazole derivatives (1–20)**

**Step A: Synthesis of 2-chloro-N-(substituted phenyl) acetamide derivatives (I)**

To a stirred solution of substituted aniline (10 mmol) in acetonitrile (35 ml) at 0 ºC was added powdered potassium carbonate (50 mmol). After stirring the mixture for 30 min at 0 ºC, chloroacetyl chloride (20 mmol) was added dropwise with vigorous stirring. The mixture was
then continuously stirred at room temperature for 3 h. The mixture was then poured into water (400 ml) with stirring. The separated solid was filtered and washed with hexane (50 ml) to give the desired intermediate I in good yield.

**Step B: Synthesis of 2-azido-N-(substituted phenyl)acetamide derivatives (II)**

To a stirred solution of I (3.0 mmol) in dry DMF (15 ml) was slowly added sodium azide (6.0 mmol). The resulting reaction mixture was then stirred for 12 h at room temperature.
temperature. The mixture was then poured into ice cold water (100 ml) with stirring. The separated solid was filtered and washed with water (50 ml) to give the desired compound II in good yield.

Step C: Synthesis of benzo[d]oxazole-2-thiol (III)

To a solution of 2-aminophenol (100 mmol) in methanol (150 ml) was added aqueous potassium hydroxide (130 mmol) in water (30 ml), followed by addition of carbon-di-sulfide (150 mmol). Resulting mixture was refluxed at 65 °C for 5 h. After the completion of reaction, reaction mixture was poured in water (500 ml), which was neutralized with conc. hydrochloric acid and the solid separated was filtered and washed with hexane to afford the pure compound III (Yield: 90%). MP: 168–170 °C.

| Compound no. | Cancer cell line (HCT 116) | Compound no. | Cancer cell line (HCT 116) |
|--------------|-----------------------------|--------------|-----------------------------|
| 1            | 97.5                        | 11           | 130.4                       |
| 2            | 73.1                        | 12           | >225.1                      |
| 3            | 108.7                       | 13           | >230.3                      |
| 4            | 22.5                        | 14           | 90.0                        |
| 5            | 85.3                        | 15           | 40.7                        |
| 6            | 84.6                        | 16           | 38.3                        |
| 7            | 72.0                        | 17           | 177.9                       |
| 8            | >254.2                      | 18           | 148.7                       |
| 9            | 66.1                        | 19           | 50.0                        |
| 10           | 175.1                       | 20           | 200.1                       |
| 5-Fluorouracil | 12.2                        | 5-Fluorouracil | 12.2                       |

Table 4 Anticancer activity results of synthesized compounds

Anticancer screening results (IC<sub>50</sub> = µM)

Fig. 3 Antifungal screening results of the synthesized benzoxazole derivatives

Fig. 4 Structure activity relationship of benzoxazole derivatives
**Step D: Synthesis of 2-(prop-2-ynylthio)benzo[d]oxazole (IV)**
To a solution of III (50 mmol) in acetone (150 ml) was added anhydrous potassium carbonate powder (100 mmol) with stirring. After 5 min, propargyl bromide (55 mmol) was added slowly at 0 °C and allowed to stir for 30 min at room temperature. After completion of the reaction, followed by TLC, the mixture was quenched with ice cold water (500 ml) with vigorous stirring. The solid product separated was filtered followed by washing with water (50 ml) which afforded the desired intermediate IV (Yield: 8.7 g, 92%). MP: 188–190 °C.

**Step E: Synthesis of target compounds (1–20)**
The intermediates IV (1.5 mmol) and II (1.5 mmol) were dissolved in a mixture of t-BuOH:H2O:DMF mixture (6 ml, 1:1:1). Sodium ascorbate (0.75 mmol) was added, followed by copper (II) sulfate (0.3 mmol). The mixture was stirred vigorously at room temperature until TLC indicated the disappearance of the starting materials (30 min). After completion of the reaction as monitored by TLC (CHCl3:MeOH/9:1, Rf: 0.17), solid separated in the reaction mass was then filtered and washed with water (10 ml) followed by methanol (10 ml) to give pure benzoxazole derivatives.

**In vitro antimicrobial assay**
The antimicrobial testing of the benzoxazole derivatives (1–20) was done by tube dilution method [24] against ofloxacin (antibacterial) and fluconazole (antifungal) as standard drugs using Gram-positive (B. Subtilis MTCC-441; S. aureus, MTCC-3160) and Gram-negative bacteria (E. coli, MTCC-443; S. typhi, MTCC-98; K. pneumoniae, MTCC-530). The antifungal activity was assayed against (C. albicans, MTCC-227) and mould (A. niger, MTCC-281). Serial dilutions of the test compounds and reference drugs were prepared in double strength nutrient broth I.P. (bacteria) or sabouraud dextrose broth I.P. (fungi) [25]. The stock solution of the test and reference compounds was prepared in dimethyl sulfoxide. The samples were incubated at 37 ± 1 °C for 24 h (bacteria), at 25 ± 1 °C for 7 days (A. niger) and at 37 ± 1 °C for 48 h (C. albicans), respectively and the results were recorded in terms of MIC. The MIC was the lowest concentration of the tested compound that yields no visible growth of microorganisms in the test tube.

**In vitro anticancer assay**
The antiproliferative effect of benzoxazole derivatives was determined against the human colorectal carcinoma [HCT 116] cancer cell line using the Sulfonhodamine-B (SRB) assay. HCT 116 was seeded at 2500 cells/well (96 well plate). The cells were allowed to attach overnight before being exposed to the respective compounds (0.001–100 µg/mL) for 72 h. The highest concentration of each compound tested (100 µg/mL) contained only 0.1% DMSO (non-cytotoxic). SRB assay [26] was then performed. Trichloroacetic acid was used to fix the cell. Staining with 0.4% (w/v) Sulfonhodamine B mixed with 1% acetic acid was performed for 30 min. After five washes of 1% acetic acid solution, protein-bound dye was extracted with 10 mM tris base solution. Optical density was read at 570 nm and IC50 (i.e. concentration required to inhibit 50% of the cells) of each compound was determined. Data was presented as mean IC50 of at least triplicates.

**Conclusion**
In this study, new benzoxazole derivatives were designed and synthesized. These benzoxazole derivatives were evaluated for their biological potentials (antimicrobial and anticancer). In vitro antimicrobial results demonstrated that compounds 5, 4, 7 and 16 showed most promising antimicrobial activity against selected microbial species in reference to the standard drugs and in vitro antiproliferative screening results indicated that compounds 4 and 16 showed promising anticancer potential against human colorectal cancer cell line in reference to the standard drugs. These compounds may serve as lead compounds for further development into novel antimicrobial and anticancer agents.

**Authors’ contributions**
Authors BN, SK and SK have designed, synthesized and carried out the antimicrobial and anticancer assay. In reference to the standard drugs. These compounds may serve as lead compounds for further development into novel antimicrobial and anticancer agents.

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