Med Chem Res (2008) 17:412–424
DOI 10.1007/s00044-007-9076-x

MEDICINAL
CHEMISTRY
RESEARCH

ORIGINAL RESEARCH

# Synthesis of some novel 2-substituted benzoxazoles as anticancer, antifungal, and antimicrobial agents

**P. K. Jauhari · A. Bhavani · Subhash Varalwar ·
Kiran Singhal · Prem Raj**

Received: 14 November 2007 / Accepted: 27 November 2007 / Published online: 10 January 2008


**Abstract** Benzoxazole derivatives show various types of biological properties such as antiviral, antineoplastic, anti-HIV-1, antitubercular, anthelmintic, antimicrobial, and antifungal activities. In the last few years 2-substituted benzoxazole derivatives have been studied extensively for their antitumor, antiviral, and antimicrobial activities. In an effort to identify new candidates that may be of value in designing new, potent, selective, and less toxic anticancer, antiviral, and/or antimicrobial agents, we synthesized 2-[(arylhydrazono) cyanomethyl]-5-chloro benzoxazoles (**II**), 2-[(arylidene)cyanomethyl]-5-halo benzoxazoles (**III**), and 2-[(cycloalkylidine)cyanomethyl]-5-chlorobenzoxazoles (**IV**), and tested them for anticancer, antifungal, and antibacterial activities. Some of these (compounds **11**, **14**) were found to possess anticancer activity and remarkable antifungal as well as antibacterial activities.

**Keywords** 5-halo-2-cyano methyl benzoxazole · 2-[(arylhydrazono) cyanomethyl]-5-halobenzoxazoles · 2-[(arylidene)cyanomethyl]-5-halo benzoxazoles · 2-[(cycloalkylidine) cyanomethyl]-5-halo benzoxazoles · Anticancer · Antifungal · Antimicrobial agents

## Introduction

Malignant tumors represent one of the most common human diseases, and their clinical prognosis remains relatively poor. The discovery and development of new

P. K. Jauhari (✉) · A. Bhavani · S. Varalwar
Vivimed Labs Research Centre, Nacharam, Hyderabad 500007, India
e-mail: jauhari.pk@gmail.com

K. Singhal · P. Raj
Chemistry Department, Lucknow University, Lucknow 226001, India

treatments for these diseases is urgently needed due to problems with currently available drugs, such as toxicities and drug resistance (Haskell, 2001), leaving ample space for the development of new therapeutic strategies. During last few years 2-substituted benzoxazole analogues have been screened for their antitumor (Ueki *et al.*, 1993; Cheng *et al.*, 1993; Shi *et al.*, 1996; Hall *et al.*, 1999; Kumar *et al.*, 2002; Easmon *et al.*, 2001; Michel *et al.*, 1984), antiviral (Balani *et al.*, 1992; Hoffman *et al.*, 1993; Saari *et al.*, 1992; Perrin *et al.*, 1996; Staszewski *et al.*, 1995; Olsen *et al.*, 1994; Prudhomme *et al.*, 1986), and antimicrobial activities (Arpaci *et al.*, 2002; Ersan *et al.*, 1997; Oren *et al.*, 1997; Temiz *et al.*, 1998; Sener *et al.*, 1997; Yalcin *et al.*, 1992; Reiner, 1982).

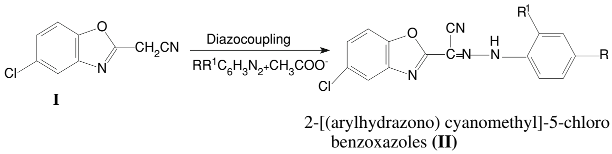
2-Substituted benzoxazoles have also been shown to exert analgesic (Bartsch and Erker, 1991), fungicidal, insecticidal, nematocidal (Yalcin *et al.*, 1992), potent protease inhibitory, and anticancer activities and serve as a topoisomerase I poison (Dumez *et al.*, 2002; Kim *et al.*, 1996).

Bearing these results in mind we have taken up the synthesis of 2-substituted benzoxazoles due to their structural similarity with some benzoxazoles and likelihood that they will exhibit important biological effects such as antibacterial, antitumor, anticancer, and antifungal activities.

## Chemistry

We have adopted a concise synthetic route for the preparation of 2-[(arylhydrazono)cyanomethyl]-5-chloro benzoxazoles (**II**), 2-[(arylidene)cyanomethyl]-5-halo benzoxazoles (**III**), and 2-[(cycloalkylidene)cyanomethyl]-5-chlorobenzoxazoles (**IV**). The starting synthon for these was 5-halo-2-cyano methylbenzoxazole (**I**). Diazocoupling of 5-chloro-2-cyanomethyl benzoxazole with the appropriate diazonium acetate gave the corresponding 2-[(arylhydrazono) cyanomethyl]-5-chlorobenzoxazoles as shown in Scheme 1, compound **1**–**7** (see also Table 1). Condensation of **I** (X = Cl, F) with the appropriate aromatic aldehyde in the presence of a catalytic amount of benzyl dimethylamine gave the corresponding 2-[(arylidene)cyanomethyl]**-**5-halo benzoxazoles as shown in Scheme 2, compounds **8–11** and **14** (Table 2). 2-[(cycloalkylidine) cyanomethyl]-5-chlorobenzoxazoles were prepared by condensing compound **I** (X = Cl**)** with cyclic ketone, as shown in Scheme 3, compounds **12** and **13** (Table 3, Table 4, Table 5, Table 6**)** .

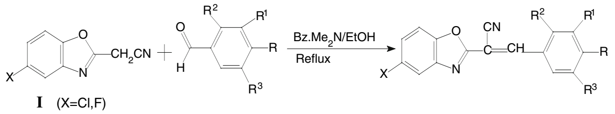
The structure of the compounds in Schemes 1, 2, and 3 were confirmed by infrared spectroscopy (IR), proton magnetic resonance (PMR), carbon-13 magnetic resonance spectroscopy (CMR), and microanalysis.




**Scheme 1**

**Table 1** Synthesis of 2-[(arylhydrazono) cyanomethyl]-5-chlorobenzoxazoles

| Compound | R | $R^1$ | Time (h) | Yield (%) | m.p. (°C) | Colour | Solv. cryst. |
|---|---|---|---|---|---|---|---|
| **1** | $OCH_3$ | H | 1.5 | 80 | 202–206 | Light orange | MeOH |
| **6** | Br | H | 2.5 | 85 | 204–206 | Orange | EtOH |
| **4** | Cl | H | 3 | 82 | 236–238 | Yellow | MeOH |
| **7** | H | H | 2.5 | 90 | 202–205 | Yellow | MeOH |

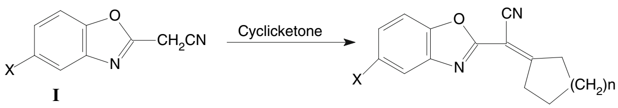



2-[(arylidene) cyanomethyl]
-5-halobenzoxazoles **(III)**

**Scheme 2**

**Table 2** Synthesis of 2-[(arylidene )cyanomethyl] -5-halobenzoxazoles

| Compound | X | R | $R^1$ | $R^2$ | $R^3$ | Time (h) | Yield (%) | m.p. (°C) | Color | Solv. |
|---|---|---|---|---|---|---|---|---|---|---|
| **8** | Cl | $OCH_3$ | H | H | H | 4 | 90 | 184–185 | Light green | MeOH |
| **9** | Cl | OH | H | H | H | 4.5 | 82 | 274–276 | Yellow | EtOH |
| **10** | Cl | H | H | H | H | 6 | 85 | 194–197 | Beige | EtOH |
| **11** | Cl | $OCH_3$ | $OCH_3$ | H | $OCH_3$ | 5.5 | 86 | 163–165 | Bright yellow | MeOH |
| **14** | F | $OCH_3$ | $OCH_3$ | H | $OCH_3$ | 6 | 93 | 220–222 | Lemon yellow | EtOH |




X= Cl, F

2-[(cycloalkylidine) cyanomethyl]
-5-halo benzoxazoles **(IV)**

**Scheme 3**

**Table 3** Synthesis of 2-[(cycloalkylidine) cyanomethyl]-5-chlorobenzoxazoles

| Compound | X | n | Time (h) | Yield (%) | m.p. (°C) | Color | Solv. cryst. |
|---|---|---|---|---|---|---|---|
| **12** | Cl | 1 | 4 | 90 | 135–137 | Beige | MeOH |
| **13** | Cl | 2 | 4.5 | 95 | 138–140 | Half white | MeOH |

**Table 4** Antiproliferation activity against various cancer cell lines

| Compound | HeLa | Activity* | WiDr | Activity* | HepG-2 | Activity* | MCF-7 | Activity* |
|---|---|---|---|---|---|---|---|---|
| 1 | 13.28 ± 0.91 | (+) | 14.28 ± 0.98 | (+) | 18.82 ± 1.12 | (+) | 9.35 ± 0.62 | (+) |
| 4 | 19.57 ± 5.20 | (−) | 16.78 ± 5.24 | (−) | 23.8 ± 2.75 | (−) | 10.12 ± 0.97 | (+) |
| 6 | 13.32 ± 08.5 | (+) | 17.46 ± 1.92 | (−) | 16.66 ± 0.62 | (+) | 9.82 ± 0.82 | (+) |
| 7 | 13.89 ± 0.95 | (+) | 19.23 ± 2.23 | (−) | 17.36 ± 1.12 | (+) | 11.89 ± 1.05 | (−) |
| 8 | 13.72 ± 0.86 | (+) | 14.12 ± 0.92 | (+) | 17.22 ± 1.10 | (+) | 9.52 ± 0.86 | (+) |
| 9 | 13.29 ± 0.93 | (+) | 15.28 ± 1.20 | (−) | 20.06 ± 1.12 | (+) | 11.89 ± 1.05 | (−) |
| 10 | 17.52 ± 3.20 | (−) | 18.76 ± 5.20 | (−) | 21.20 ± 2.15 | (−) | 10.12 ± 0.97 | (+) |
| 11 | 11.72 ± 0.86 | (+)(+) | 12.22 ± 0.82 | (+) | 11.22 ± 1.10 | (+) | 7.22 ± 0.76 | (+) |
| 12 | 12.18 ± 0.91 | (+) | 13.28 ± 0.98 | (+) | 18.32 ± 1.12 | (+) | 9.35 ± 0.62 | (+) |
| 13 | 13.24 ± 0.85 | (+) | 14.22 ± 0.94 | (+) | 16.26 ± 0.68 | (+) | 11.74 ± 1.15 | (−) |
| 14 | 11.40 ± 0.72 | (+)(+) | 11.70 ± 0.75 | (+)(+) | 11.24 ± 0.7 | (+)(+) | 11.42 ± 0.81 | (+)(+) |
| Control[a] | 15.26 ± 1.15 | (−) | 14.72 ± 1.06 | (−) | 20.12 ± 1.15 | (−) | 10.22 ± 1.12 | (−) |

HeLa = human cervical carcinoma cell line

WiDr = human colon carcinoma cell line

Hep G-2 = human hepatoma cell line

MCF-7 = human breast adenocarcinoma cell line

*Activity is expressed as no. of cell count divided by $10^4$, and so are dimensionless

[a] Control: culture medium only

Concentration: 50 μg/mL; incubation period: 48 h; colony diameter in mm

**Table 5** Antifungal activity

| S. no. | Sample | *Aspergillus flavus* (colony diameter in mm) | Inhibition | *Aspergillus niger* (colony diameter in mm) | Inhibition |
|---|---|---|---|---|---|
| 1 | **1** | 1.0 | 50% | 0.7 | 65% |
| 2 | **4** | 0.7 | 65% | 0.6 | 70% |
| 3 | **8** | 0.7 | 65% | 0.7 | 65% |
| 4 | **9** | 0.8 | 60% | 1.0 | 50% |
| 5 | **10** | 0.8 | 60% | 0.7 | 65% |
| 6 | **11** | 0.3 | 85% | 0.4 | 80% |
| 7 | **12** | 0.8 | 60% | 0.5 | 75% |
| 8 | **13** | 0.5 | 75% | 0.8 | 60% |
| 9 | **14** | 0.3 | 90% | 0.4% | 95% |

**Table 6** Antibacterial activity

| S. no. | Sample | Pseudomonas aeruginosa | Staphylococcus aureus | Klebsiella pneumoniae |
|---|---|---|---|---|
| 1 | **1** | + | + + | + |
| 2 | **6** | + + | + + | + + |
| 3 | **4** | + + | + | + + |
| 4 | **7** | + + | + + | + + |
| 5 | **8** | + + | + | + + |
| 6 | **9** | + + | + + | + + |
| 7 | **10** | + + | + + | + + |
| 8 | **11** | + + + | + + + | + + |
| 9 | **12** | + + | + + + | + + |
| 10 | **13** | + + | + + | + |
| 11 | **14** | + + + + | + + + + | + + + |

Method: disc diffusion method; concentration 10 μg/ml; control: culture media only; incubation period: 24 h

+ = diameter 5–10 mm

+ + = diameter 10–15 mm

+ + + = diameter 15–20 mm

+ + + + = diameter 20–25 mm

## Biological results and discussion

### Anticancer activity

All the compounds were tested for the antiproliferation activity against four tumor cell lines. Compounds **6**, **12**, and **13** were found to be active against human cervical carcinoma cell line (HeLa). However, compound **11** showed much higher activity against HeLa. Compounds **1** and **8** were found to be active against all four cell lines. Compound **9** showed activity against the human cervical carcinoma and human hepatoma cell line, but did not show any activity against colon carcinoma and human breast adenocarcinoma cell lines. Compound **10** showed activity against the human breast adenocarcinoma cell line but was inactive against the other three cell lines. Compounds **4**, **6**, and **7** did not show any activity against the human colon carcinoma cell line (WiDr) but compounds **11**, **12**, and **13** were found to be active against WiDr. Compounds **6**, **7**, **11**, **12**, and **13** were active against the human hepatoma cell line (Hep G2) but compound **4** was found to be inactive against Hep G2. Similarly, compounds **4**, **11** and **12** showed activity against the human breast adenocarcinoma cell line (MCF-7). However, compound **13** did not show any activity. Compound **14**, which contains a fluoro substituent, was found to be significantly highly active against all four cell lines tested (HeLa, WiDr, Hepa 2, and MCF-7). Thus it may be concluded that compounds with fluoro and chloro substitutents at position 5 of the benzoxazole ring coupled with three methoxy groups in the phenyl ring exhibited much higher activity.

## Antifungal activity

Three series of compounds, namely aryl hydrazono benzoxazoles (**1**, **4**, **8**, **9**, and **10**), arylidene benzoxazoles (**11** and **14**), and cycloalkylidene benzoxazoles (**12** and **13**)**,** were assayed for antifungal activity against *Aspergillus flavus* and *Aspergillus niger*. Antifungal testing was carried out using the potato dextrose agar plate diffusion method. Compounds **4**, **8**, and **9** exhibited inhibition in the range of 60–70%. Compound **4**, i.e., 2-[(4-aryl hydrazono) cyanomethyl]-5-chloro benzoxazole showed 70% inhibition against *Aspergillus niger*. Compounds **12** and **13** showed much higher activity (percentage inhibition). 2-[(cyclohexylidene)cyanomethyl]-5-chloro-benzoxazole (**12**) showed 75% inhibition against *Aspergillus niger* but was less active against *Aspergillus flavus* (60%). In contrast, compound **13** i.e., (2-[(cyclopentylidene)cyanomethyl]-5-chloro-benzoxazole showed higher activity against *A. flavus* but less inhibition against *A. niger* (60%). Compound **11**, i.e., 2-[(3,4,5-trimethoxy arylidene]-5-chloro benzoxazole, showed remarkable activity against both *A. flavus* (85%) as well as *A. niger* (80%). Compound **14** with a fluoro substituent exhibited exceptional activity against both *A. flavus* and *A. niger* (>90%).

As a generalized observation, chloro and methoxy groups at the 4-position in the aromatic ring enhanced antifungal activity. Therefore, it is not surprising that the introduction of 3-methoxy groups (compound **11**) further enhanced antifungal activity. The introduction of the fluoro group in place of the chloro at position 5 in the benzoxazole ring greatly enhanced antifungal activity against both *A. niger* and *A. flavus*, primarily due to the enhanced solubility of the fluoro substituent as well as due to the –I effect compared to the chloro group, which is electron withdrawing.

Antibacterial activity

All the compounds were tested for antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. Compound **1** was active against *Staphylococcus aureus*. Compounds **6** and **7** were active against all three bacteria. Compounds **4** and **8** were not very active against *Staphylococcus aureus*. Compounds **9** and **10** were active against all three bacteria. Compound **11** was highly active against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, but not against *Klebsiella pneumoniae*. Compound **12** exhibited a much higher activity than **13** against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Compound **14** was significantly active against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*.

## Experimental chemistry

Melting points were taken in open glass capillaries and are uncorrected. IR spectra was determined by using a Perkin-Elmer FT-IR-Spectrometer in KBr phase ($\gamma_{max}$ in $cm^{-1}$). PMR spectra were recorded on a Varian 200 MHz spectrometer using TMS as an internal standard. CMR spectra were recorded on a $^{13}C$ Advance Brucker 300 MHz spectrometer. Microanalyses were done at IICT, Hyderabad. Mass spectra were recorded on an Autospec fast atom bombardment ($FAB^+$) magnet with a 7 kV accelerator voltage and 25 kV gun voltage.

General procedure for the synthesis of compounds **1**, **4**, **6**, and 7

5-Chloro-2-cyanomethyl benzoxazole (**I**, 0.01 M) was taken in acetic acid (10 mL), the solution was cooled to 0°C and to this solution was added dropwise during half an hour, aryl diazonium acetate, prepared from aryl amine (0.01 M) in acetic acid (15 mL) and $NaNO_2$ (0.015 M). The reaction was continued for a specific period as mentioned in Table 1. To the reaction mass was added water (40 mL) and the solid product was isolated after filtration and washing with water. It was then recrystallized with the appropriate solvent as mentioned in Table 1 to achieve the percentage yield also listed therein.

2-[(4′-Methoxy aryl hydrazono)cyanomethyl]-5-chlorobenzoxazole (**1**)

IR(KBr): 3421(NH), 2222(C≡N),1608,1553,1461(C = N,NH,C = C), 1252, 1137, 1080(C-O-C); $^1H$-NMR($CDCl_3$): $\delta$ 3.90(s,3H;$OC\underline{H}_3$), 7.32(s,1H,benzoxazole $C_4$-$\underline{H}$), 7.46-7.83(m,6H,4Ar-$\underline{H}$ and benzoxazole $C_{6,7}$ –$\underline{H}$, 13.57(s,1H,N$\underline{H}$ , $D_2O$ exchangable); MS m/z: 326($M^+$); $^{13}C$-NMR ($CDCl_3$) $\delta$: 112.05, 117.28, 127.1, 119.5, 146.6, 157.2, 157.88, 131.1, 134.9, 115.09, 114.9, 112.05, 55.61;

Calculated for $C_{16}H_{11}ClN_4O_2$: C,58.80;H,3.36;N,17.15: found C, 58.65;H,3.30;N,17.02.

2-[(4′-Chloro aryl hydrazono)cyanomethyl]-5-chlorobenzoxazole (**4**)

IR(KBr): 3416(NH),2228(C≡N), 1597,1541,1445(C = N,NH,C = C),1262,1197, 1134,1101(C-O-C)

$^1$H-NMR($CDCl_3$):7.42(s,1H,benzoxazole$C_4$-H),7.48-7.8(m,6H,4-ArH and benzoxazole $C_{6,7}$-H), 13.46(s,1H,NH,$D_2O$ exchangeable. MS: m/z 331($M^+$)

Calculated for $C_{15}H_8$ Cl $_2$ N4O: C,54.38;H,2.41;N,16.91: found C,54.20; H,2.35; N,16.78.

2-[(4′-Bromo aryl hydrazono)cyanomethyl)-5-chlorobenzoxazole (**6**)

IR(KBr): 3418(NH),2230(C≡N),1615,1545,1463(C = N,NH,bendingC = C); 1262,1096

(C-O-C). $^1$H-NMR($CDCl_3$): δ7.38(s,1H,benzoxazole$C_4$-H), 7.43–7.73( $C_{6,7}$-H)

13.65(s,1H,NH,$D_2O$ exchangeable,4.03(s,3H;$OCH_3$). MS: m/z 376(M + 1)

Calculated for $C_{15}H_8$ $BrClN_4O$: C,47.93;H,2.13;N,14.91: Found C,47.85,H, 2.03,N,14.78.

2-[(Aryl hydrazono) cyanomethyl]-5-chlorobenzoxazole (**7**)

IR(KBr): 3415(NH),2224(C≡N),1610,1545,1476(C = N,NH bending,C = C), 1262,1136,1100(C-O-C) $^1$H-NMR($CDCl_3$): 7.37(s,1H,benzoxazole$C_4$-H),7.46–7.74(m,6H,4-ArH and benzoxazole $C_{6,7}$-H), 13.40(s,1H,NH,$D_2O$ exchangeable. MS: m/z 297($M^+$)

Calculated for $C_{15}H_9$ $ClN_4$ O: C,60.70;H,3.03;N,18.88: found C, 60.57;H,2.94; N,18.70.

2-[4′-Methoxy arylidene)cyanomethyl]-5-chlorobenzoxazole (**8**)

IR(KBr): 2230(C≡N), 1590,1534(C = N,C = C), 1175,1046(C-O-C) $^1$H-NMR($CDCl_3$): 3.91 (s,3H,$OCH_3$), 7.47(s,1H,benzoxazole $C_4$-H), 7.5–7.74 (m,6H,4Ar-H and benzoxazole $C_{6,7}$-H), 8.23(s,1H, = CH). MS: m/z 311(M + 1)

Calculated for $C_{17}H_{11}$ $ClN_2O_2$: C,65.70;H,3.54;N,9.01: found C, 65.51;H,3.37;N,8.90.

2-[(4′-Hydroxy arylidene)cyanomethyl]-5-chlorobenzoxazole (**9**)

IR (KBr): 2222(C≡N), 1594,1565(C = N,C = C),1288,1175,1047(C-O-C) 6.01(brs,1H,Ar-4′OH,exchangeable on $D_2O$ shake $^1$H-NMR($CDCl_3$): 7.38 (s,1H,benzoxazole $C_4$-H),7.47–7.67(m,6H,4 Ar-H and benzoxazole $C_{6,7}$-H), 8.21(s,1H, = CH). MS:m/z:297(M + 1)

Calculated for $C_{16}H_9$ $ClN_2O_2$: C,64.75; H,3.03; N,9.44: Found c,64.60;H, 2.95; N,9.26.

2-[(Arylidene)cyanomethyl]-5-chlorobenzoxazole (**10**)

IR (KBr): 2226(C≡N), 1592,1513(C = N,C = C),1260,1180,1047(C-O-C) $^1$H-NMR($CDCl_3$): 7.5 (s,1H,benzoxazole $C_4$-H), 7.58–8.06(m,7H,5 Ar-H and benzoxazole $C_{6,7}$-H), 8.32(s,1H, = CH)

MS:m/z 280($M^+$)

Calculated for $C_{16}H_9$ $ClN_2O$: C, 68.44;H,3.20;N,9.98: found C,68.24; H,3.09; N,9.79.

2-[(3′,4′,5′-Trimethoxy arylidene)cyanomethyl]-5-chlorobenzoxazole (**11**)

IR (KBr): 2229 (C≡N), 1576,1501 (C = N,C = C),1246,1128,1036(C-O-C) $^1$H-NMR($CDCl_3$): 3.95(s,9H,-$OCH_3$),7.48(s,1H,benzoxazole $C_4$-H), 7.50–7.73 (m,4H,2Ar-H and benzoxazole

$C_{6,7}$-H),8.21(s,1H, = CH) MS:m/z 371 (M + 1) $^{13}$C-NMR($CDCl_3$) $\delta$: 160.42,153.36,149.58,14923, 142.69,130.77,127.07,126.31, 120.31,115.18,114.51, 111.37,108.87, 108.14,56.31.

Calculated for $C_{19}H_{15}ClN_2O_4$; C, 61.53; H, 4.04; N, 7.55; Found C, 61.40; H, 4.19; N, 7.38.

2-[3′,4′,5′-Trimethoxy arylidene) cyanomethyl] -5-fluorobenzoxazole (**14**)

IR (KBr): 2232(C≡N), 1580,1492(C = N,C = C), 1230(Ar-F), 1246,1128,1036(C-O-C).

$^1$H-NMR($CDCl_3$): 4.0(s,9H,-$OCH_3$),7.36(s,1H,benzoxazole $C_4$-H), 7.5–7.82 (m,4H,2Ar-H and benzoxazole $C_{6,7}$-H), 8.25(s,1H, = CH) $^{13}$C-NMR($CDCl_3$) $\delta$: 161.32, 160.78,159.40,153.36, 149.33,147.00,142.49,127.07,115.18, 113.61,111.02, 110.94,108.17,106.67, 56.30 MS: m/z 355(M + 1)

Calculated for $C_{19}H_{15}FN_2O_4$: C, 64.4; H,4.23;N,7.90: found C,64.21; H,4.37; N, 7.63.

2-[(Cyclopentylidene)cyanomethyl]-5-chlorobenzoxazole (**12**)

IR (KBr): 2226 (C≡N), 1605,1552,1446,(C = N,C = C),1256, 1060(C-O-C) $^1$H-NMR($CDCl_3$): 1.60–1.95(m,4H,cyclopentyl-$C_{3,4}$-$H_2$); 2.93–3.16(two t, each 2H,J = 6.4,7.2 Hz, cyclopentyl-$C_{2,5}$-$H_2$); 7.26(s,1H,benzoxazole-$C_4$-H); 7.31–7.47 (m,2H,benzoxazole-$C_{6,7}$-H) MS: m/z 259(M + 1)

Calculated for $C_{14}H_{11}ClN_2O$: C,64.99;H,4.25; N,10.83: found C,64.79;H,4.42; N,10.64.

2-[Cyclohexylidene)cyanomethyl]-5-chlorobenzoxazole (13)

IR (KBr) 2222(C≡N), 1620,1549,1449(C = N,C = C), 1263,1060(C-O-C) $^{1}$H-NMR($CDCl_3$): 1.63–1.88(m,6H,Cyclohexyl-$C_{3,4,5}$-$\underline{H}_2$); 2.78–3.19 (two t,each 2H, J = 6.5,6.2 Hz,cyclohexyl-$C_{2,6}$-$\underline{H}_2$); 7.28(s,1H,benzoxazole $C_4$-$\underline{H}$) 7.33–7.5 (m,2H,benzoxazole $C_{6,7}$-$\underline{H}$); MS: m/z 272,(M + 1)

Calculated for $C_{15}$ $H_{13}ClN_2O$: C, 66.0; H,4.77;N,10.27: found C, 66.12; H, 4.61; N,10.32.

## Experimental biology

### Antitumor activity

The in vitro antitumor activity of the compounds was carried out by the [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] (MTT) method to estimate the effect of each compound on cell growth. The principle behind this assay depends upon the reduction of the tetrazolium salt. The yellow-colored tetrazolium MTT is reduced by metabolically active cells in part by the action of dehydrogenase enzymes to generate reducing equivalents such as nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH). The resulting intracellular purple zones were solubilized and quantified by using a spectrophotometer. The [3-(4,5-215 dimethylthiazolyl-2)-2,5 diphenyltetrazolium bromide (MTT) was dissolved in phosphate buffer solution (PBS) at a concentration of 5 mg/mL. Then, 50 μL of the MTT solution was added to each well of 96-well culture plates, containing the 100 μL medium and incubated at 37°C for 4 h. The medium was then removed carefully without disturbing the purple-colored formazon crystals. Then, 50 mL of dimethyl sulfoxide (DMSO) was added to each well and mixed thoroughly to dissolve the formazon crystals . The plates were then read on a microplate reader at a wavelength of 570 nm. The readings were presented as an optical density.

Four cell lines were used: the human hepatoma cell line HEPG-2, the human cervical carcinoma cell line HeLa, the human colon carcinoma cell line WiDr, and the human breast adenocarcinoma cell line MCF-7.

### Antibacterial activity

The antibacterial activity of the compounds was determined by the disc diffusion method. In this technique, sterile discs of 5 mm diameter of filter paper (Whatmann no. 1), impregnated with the test compounds (10 μg/mL of ethanol), were placed on nutrient agar plates at 37°C for 24 h. The inhibition zones around the dried impregnated discs were measured after 24 h. The activity was classified as “highly active” (diameter = 20–25 mm), “active” (diameter = 15–20 mm) or “slightly active” (diameter = 5–10 mm). A diameter of less than 5 mm was regarded as “inactive”.

### Antifungal activity

The antifungal activity of these compounds was tested by the agar plate diffusion method against the two human pathogenic fungal strains *Aspergillus flavus* and *Aspergillus niger*. One milliliter of each compound was poured into a Petri dish containing about 20 mL of molten potato dextrose agar. As the medium solidified the Petri dishes were inoculated separately with the fungal isolates and kept at 27°C for 48 h. All the values (percentage inhibition) were recorded.

## Summary

Benzoxazoles and their analogues are known to possess various biological effects such as antitumor, antiviral, and antimicrobial activities. 2-Substituted benzoxazoles have also been shown to exert analgesic, fungicidal, insecticidal, nematocidal, potent protease inhibitory, and anticancer activities and to act as a topoisomerase I poison. The cyano function present in antibiotics like cephactrile, cefmetazole, and toyomycin are needed to *enhance biological activity.*

Based on these structural findings and in an effort to synthesize new biologically active molecules that may be less toxic and possess anticancer, antiviral, and antimicrobial activity, we have taken up the synthesis of three series of compounds, namely 2-[(arylhydrazono) cyanomethyl]-5-chloro benzoxazoles, 2-[(arylidene)cyanomethyl]-5-halo benzoxazoles, and 2-[(cycloalkylidene) cyanomethyl]-5-chlorobenzoxazoles. These were then tested for their anticancer, antifungal, and antimicrobial activities.

Fluro and chloro substitution at position 5 of the benzoxazole ring coupled with 3-methoxy groups in the phenyl ring exhibited much higher activity. Compound **14**, containing a fluoro substituent, was found to be significantly highly active against all four cell lines tested (HeLa, WiDr, Hepa 2, and MCF-7). As a generalized observation, chloro and methoxy groups at the 4-position in the aromatic ring enhanced antifungal activity; therefore, it is not surprising that the introduction of 3-methoxy groups (compound **11**) further enhanced antifungal activity. The introduction of a fluoro group in place of the chloro substituent at position 5 in the benzoxazole ring greatly enhanced antifungal activity against both *Aspergillus niger* and *Aspergillus flavus*.

Therefore, it was observed that compound **11** and **14** were found to possess both anticancer activity and remarkable antifungal and antibacterial activities.

## References

Arpaci T, Sener A, Yalcin I, Altanlar N (2002) Synthesis and antimicrobial activity of some 2-[p-substituted-phenyl]benzoxazol-5-yl-arylcarboxyamides. Arch Pharm (WeinHeim) 335:283–288

Arpaci T, Oeren I, Altanlar N (2002) Synthesis and antimicrobial activity of some novel 2-(p-substituted-phenyl)-5-substituted-carbonylaminobenzoxazoles. IL Farmaco 57:175–181

Arpaci T, Sener A, Yalcin I, Altanlar N (2002) Synthesis and microbiological activity of some novel N-[2-(p-substitutedphenyl)-5-benzoxazolyl]-cyclohexyl carboxamide, -cyclohexyl acetamide and -cyclohexyl propionamide derivatives. IL Farmaco 57:771–775

Balani SK, Pitzenberger SM, Kauffman LR, Arison BH, Ramjit HG, Goldman ME, O'Brien JA, King JD, Hoffman JM, Rooney CS, Theoharides AD (1992) Metabolism of a new HIV-1 reverse transcriptase inhibitor, 3-[2- (benzoxazol-2-yl)ethyl]-5-ethyl-6-methylpyridin-2(1H)-one (L-696,229), in rat and liver slices. Drug Metab Dispos 20:869–876

Bartsch H, Erker T (1991) Chemistry of O,N- and S,N-heterocycles, X: Synthesis and biological activity of 2-substituted 2-ethylbenzoxazoles. Arch Pharm 324:79–82

Cheng CC, Liu DE, Chou TC (1993) Design of antineoplastic agents on the basis of the "2-phenyl naphthalene-type" structural patterns. Heterocycles 35:775–789

Dumez E, Snaith JS, Jackson REW, Mc Elroy AB, Overington J, Wythes MJ (2002) Synthesis of macrocyclic, potential protease inhibitors using a generic scaffold. J Org Chem 67:4882–4892

Easmon J, Puerstinger G, Roth T, Fiebg HH, Jenny M, Heinisch G, Hofmann J (2001) 2-benzoxazolyl and 2-benzimidazolyl hydrazones derived from 2-acetylpyridine: Anovel class of antitumour agents. Int J Cancer 94:89–96

Ersan S, Nacak S, Berkem R, Ozden T (1997) Synthesis and antimicrobial activities of 2-[(α-methylbenzylidene)-hydrazeno]benzoxazoles. Arzneim Forsch 47:963–965

Hall IH, Peaty NJ, Henry JR, Easmon J, Heinisch G, Purshinger G (1999) Investigation on the mechanism of action of the novel anti tumor agents 2-benzothiazolyl, 2benzoxazolyl, and 2-benzimidazolyl hydrazones derived from 2-acetylpyridine. Arch Pharm (Weinheim) 332:115–123

Haskell CM (2001) Introduction, In Cancer treatment, 5th ed., Chapter 1, Saunders,W.B., Philadelphia. Chapter 1

Hoffman JM, Smith AM, Rooney CS, Fisher TE, Wai JS, Thomas CM, Bamberger DL, Barnes JL, Williams TM, Jones JH, Olson BD, O'Brien JA, Goldman ME, Nunberg JH, Quintero JC, Schleif WA, Emini EA, Anderson PS (1993) Synthesis and evaluation of 2-pyridinone derivatives as HIV-1-specific reverse transcriptase inhibitors. 4. 3-[2-(Benzoxazol-2-yl)ethyl]-5-ethyl-6-methylpyridin-2(1H)-one and analogs. J Med Chem 36:953–966

Kim JS, Sun Q, Gatto B, Yu C, Liu LE, LoVoie EJ (1996) Structure-activity relationships of benzimidazoles and related heterocycles as topoisomerase I poisons. Bioorg Med Chem 4:621–630

Kumar D, Jacob MR, Reynolds MB, Kerwin SM, (2002) synthesis and evaluation of anticancer benzoxazoles and benzimidazoles related to UK-1. Bioorg Med Chem 10:3997–4004

Michel KH, Boeck LD, Hoehn MM, Jones ND, Chaney MO (1984) The discovery, fermentation, isolation and structure of antibiotics A33853 and its tetraacetyl derivatives. J Antibiot 37:441–445

Olsen DB, Carroll SS, Culberson JC, Shafer JA, Kuo LC (1994) Effect of template secondary structure on the inhibition of HIV-1 reverse transcriptase by a pyridinone non-nucleoside inhibitor. Nucleic Acids Res 22:1437–1443

Oren I, Temiz O, Yalcin I, Sener E, Akin A, Ucarturk N (1997) Synthesis and antimicrobial activity of 5 (or 6) –methyl-2-substituted benzoxazoles and benzimidazole derivatives. Arzneim Forsch 47:1393–1400

Perrin L, Rakik A, Yearly S, Baumberger C, Kinloch-de- Loies S, Pechiere M, Hirschel B (1996) Combined therapy with Zidovudine and L-697,661 in primary HIV infection. AIDS 10:1233–1237

Prudhomme M, Guyat J, Jeminet G (1986) Semisynthesis of A23187(calcimycin) analogues. II Introduction of a methyl group on the benzoxazole ring. J Antibiotics 39:934–937

Reiner R (1982) An Introduction to Antibiotics. Georg Thiem Verlag, Stuttgart–New York., pp. 151

Saari WS, Wai JS, Fisher TE, Thomas CM, Hoffman JM, Rooney CS, Smith AM, Jones JH, Bamberger DL, Goldman ME, O'Brien JA, Nunberg JH, Quintero JC, Schleif WA, Emini EA, Anderson PS (1992) Synthesis and evaluation of 2-pyridinone derivatives as HIV-1-specific reverse transcriptase inhibitors. 2. Analogs of 3-aminopyridin-2(1H)-one. J Med Chem 35:3792–3802

Sener E, Yalcin I, Temiz O, Oren I, Akin A, Ucarturk N (1997) synthesis & structural activity relationships of some 2,5-disubstituted benzoxazoles & benzimidazoles as antimicrobial agents. IL Farmaco 52:99–103

Shi D, Bradshaw TD, Wrigley S, McCall CJ, Leieveld P, Fichtner I, Stevens MFG (1996) Antitumor benzothiazoles. 3.1 Synthesis of 2-(4aAminophenyl)benzothiazoles and evaluation of their activities against breast cancer cell lines in vitro and in vivo. J Med Chem 39:3375–3384

Staszewski S, Massari FE, Kaber A, Gohler R, Durrr S, Anderson KW, Schneider CL, Waterbury JA, Bakshi KK, Taylor VI, Hildebrand CS, Kriesl C, Haffstedt B, Schleif WA, Byrnes VW (1995) Combination therapy with zidovudine prevents selection of human immunodeficiency virus type 1

variants expressing high-level resistance to L-697,661, a nonnucleoside reverse transcriptase inhibitor. J Infect Dis 171:1159–1165
Temiz O, Oren I, Sener E, Ucarturk N (1998) Synthesis and microbiological activity of some novel 5- or 6-methyl-2-(2,4-disubstituted phenyl) benzoxazole derivatives. IL Farmaco 53:337–341
Ueki M, Ueno K, Miyadoh S, Shibata K, Taniguchi M, Oi SJ (1993) UK-1, a novel cytotoxic metabolite from streptomyces sp. 517–02.I. Taxonomy, fermentation, isolation, physicochemical and biological properties. Antibiotics 46:1089–1094
Yalcin I, Oren I, Akin A, Ucarturk N (1992) The synthesis and the structure-activity relationships of some substituted benzoxazoles, oxazolo(4,5-b)pyridines, benzothiazoles and benzimidazoles as antimicrobial agents. Eur J Med Chem 27:401–406