Indian Journal of Chemistry
Vol. 60B, November 2021, pp. 1496-1501

# Synthesis, characterization and biological evaluation of some 2-arylbenzoxazole acetic acid derivatives as potential anticancer agents

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*Received 26 March 2020; accepted (revised) 17 September 2021*

A series of 2-arylbenzoxazole compounds possessing a cytotoxic activity as potential anticancer agents has been synthesised. Oxidative coupling of benzaldehyde with *o*-aminophenol utilizing lead tetraacetate approach has been used to realize the synthesis of compounds **1-11**. The cytotoxicity of **1-11** have been screened against breast cancer cell line MCF-7 and human colon cancer cell line HCT-116 utilizing doxorubicin as reference drug. Among these compounds, 2-(3-benzyloxyphenyl)benzoxazole-5-acetic acid **5** and 2-(4-methoxyphenyl)benzoxazol-5-acetic acid **10**, are found to be promising cytotoxic compounds against MCF-7 cell line. In addition, this study shows that the presence of acetic acid group at position 5 of benzoxazole nucleus enhances the activity. Moreover, it is noticed that the presence of oxygen atom directly linked to the phenyl substituent improves activity. The results offer a new benzoxazole based template to design and develop novel antineoplastic agents.

**Keywords**: Cytotoxicity, arylbenzoxazoles, MCF-7, structure-activity relationships, lead tetraacetate

The discovery of novel, effective and safe anticancer compounds represents a great challenge for researchers despite the long time and efforts that have been spent to achieve this objective. The existing anticancer agents are still far from optimum. This fact is reflected with the number of deaths caused by cancer as well as by the toxic effects of the existing anticancer drugs[1]. Literature showed that 2-arylbenzoxazoles possess a very wide range of biological activities including anticancer activities[2-4]. Some of the reported benzoxazoles with antitumor activities are presented in Figure 1. Ueki *et al*. isolated a benzoxazole, UK-1, as a novel cytotoxic antibiotic produced from *streptomyces* sp. 517-2, which exhibits inhibitory activity against various cancer cells[5]. Based on UK-1, Kumar[6] and co-workers reported the synthesis of a structural analogue of UK-1, compound IA, which showed to maintain selectivity and activity against cancer cells lines MCF-7 and HT-29, with $IC_{50}$ values in the 1.5 & 9.1 µM, respectively. In another work, the 5- flourobenzoxazole 2A exhibits potent activity *in vitro*, submicromolar, in tow cell lines ($GI_{50}$ = 0.36 µM and 0.27 µM on MCF-7 and MDA 468)[7]. In addition, The 5-aminolbenzoxazole compound 3A demonstrated to be active against some cancer cell lines[8]. More recently, Al-Harthy *et al*.[9] investigated the potential anticancer activity of some 2-methyl benzoxazoles upon testing them against human A-549 lung carcinoma cells. Also Khajondetchairit *et al*.[10] reported a very potent arylbenzoxazoles with $IC_{50}$ of 3.3 µM in the KB cell line.

On the other hand, some arylbenzoxazole derivatives have been tested as topoisomerase II inhibitors which in known to be as potential drug target, and its inhibition have become important strategy in the treatment of neoplastic diseases. In this context, Pinar and co-workers tested the efficiency of a series of benzoxazoles as eukaryotic topoisomerase II inhibitors. Their study showed that several 2-arylbenzoxazole derivatives had significant inhibition activities on topoisomerase II enzyme, and among these compounds, **4A** was found to be more potent than the reference drug etoposide ($IC_{50}$ value = 18.8 µM, Figure 1)[11]. While Oksuzoglu *et al*.[12] showed that 5-chlorotolylbenzoxazole **5A** was to be significant DNA topoisomerase II inhibitor ($IC_{50}$ value = 22.3 µM). As reported in literature review mentioned above, the 2- arylbenzoxazole scaffold can tolerate a wide range of substituents without compromising the anticancer activities. The tolerability of benzoxazole nucleus could be attributed to a different biomolecular targets that it's possibly interacts with.

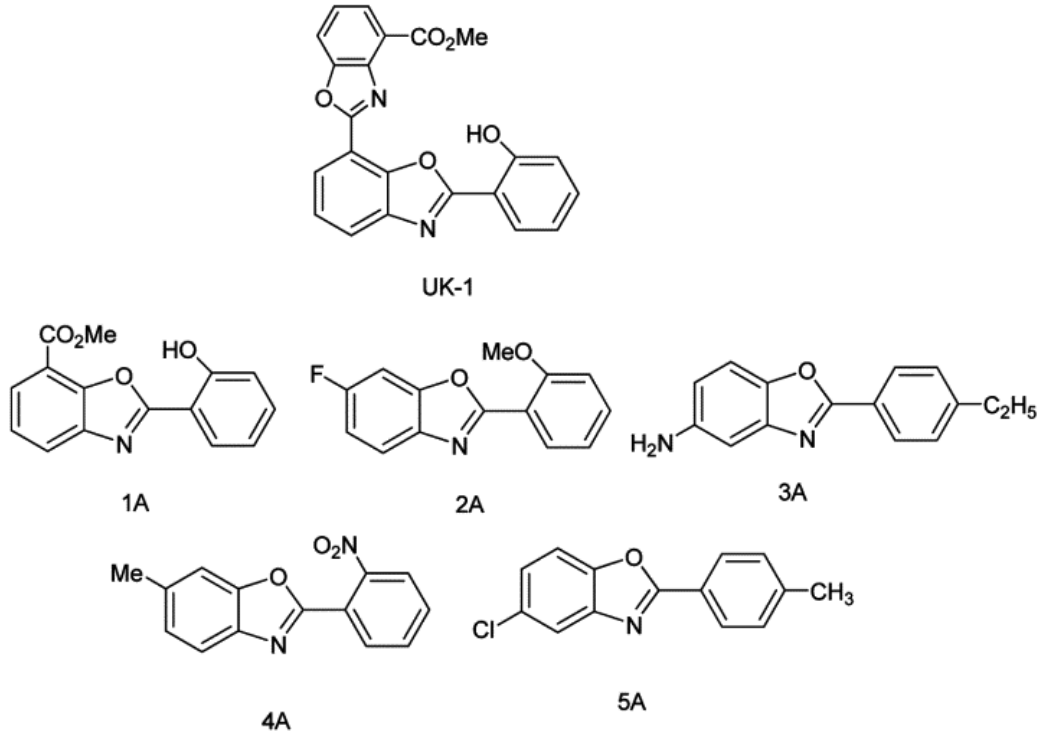



Figure 1 — Some benzoxazoles with anticancer activities

In this study, a number of 2-arylbenzoxazole-5-acetic acid derivatives were synthesized, characterized and their cytotoxic activities were biologically evaluated *in vitro* against MCF-7 and HCT-116 cell lines.

## Results and Discussion

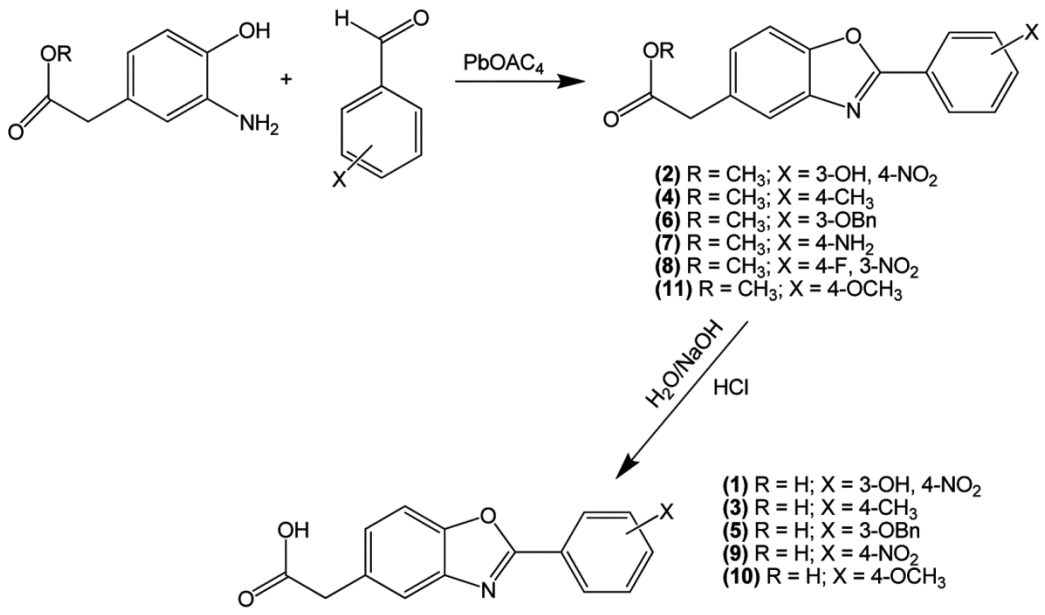
### Synthetic chemistry

A novel series of 2-arylbenzoxazole derivatives were designed and synthezied as cytotoxic agents. According to literature, several synthetic methods are available to prepare 2-arylbenzoxazole target compounds[13]. Oxidative coupling of benzaldehyde with *o*-aminophenol utilizing lead tetraacetate represents one of these synthetic methods. Although it is not among the popular methods, possibly due to the oxidative power of lead tetraacetate[14,15]. It is reported that lead tetraacaetae can cause oxidative decarboxylation of organic carboxylic compounds[16].

The unwanted reactivity of lead tetraacetate could be minimized by either avoiding compounds with susceptible groups and by using protective strategy. In our synthetic attempts, we found that the presence of hydroxyl groups as well as free carboxylic acid groups decreases the yield dramatically. In such cases, using protective groups would be helpful; therefore, in order to prepare carboxylic acid compounds, we block the carboxylic acid groups by converting them to the corresponding methyl ester derivatives, and then the oxidative coupling was achieved. The deprotection of carboxylic acids was achieved by hydrolysis of ester with NaOH solution in ethanol followed by acidification with concentrated HCl (Scheme I).

### Cytotoxicity

The synthesized benzoxazole derivatives were biologically evaluated *in vitro* for their anticancer activity. In order to examine the cytotoxic activity of compounds **1**-**11** (Table I), we performed cell viability analysis on two cancers cell lines, namely breast (MCF-7) and colorectal (HCT-116). The anticancer agent doxorubicin was used as the standard reference to compare their cytotoxic activity. Cells were incubated with the compounds at different concentrations that range between 0-200 μg/mL for 72 hours and then cell growth was evaluated using MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay**.** If no cytotoxicity was observed at 200 μg/mL, the compound was assumed to have no cytotoxic activity against the two types of cancerous cell lines and it is represented as resistant (R) in Table I. Table I summarizes the results of




Scheme I — Synthesis of 2-arylbenzoxazole derivatives 1-11

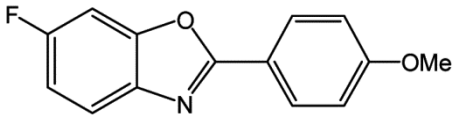
Table I — Growth inhibitory activity of compounds **1-11** and the reference drug doxorubicin, against human cancer MCF-7 and HCT-116 cell lines. $IC_{50}$ (μM) values after 72 h incubation period.

| Compd | MCF-7 $IC_{50}$ (μM) | HCT-116 $IC_{50}$ (μM) |
|---|---|---|
| Doxorubicin | 0.13 | 0.12 |
| **1** | 14.30 | 41.95 |
| **2** | 57.31 | 58.35 |
| **3** | 121.31 | 15.02 |
| **4** | 84.70 | 20.00 |
| **5** | 1.49 | 84.54 |
| **6** | 5.24 | 40.03 |
| **7** | R | R |
| **8** | R | R |
| **9** | R | R |
| **10** | 2.02 | 4.26 |
| **11** | 3.41 | 5.98 |

R, resistant.

cytotoxicity tests against MCF-7 and HCT-116 cell lines.

From the obtained results, as can be seen from Table I, compounds **5**, **6**, **10** and **11** demonstrates potent anti-proliferative activities against MCF-7 cell line, while, compounds **10** and **11** exhibits potent cytotoxic activities against HCT-116 cell line, and these results shows that previously mentioned compounds can be considered as promising antineoplastic agents. Conclusions were supported by comparing the $IC_{50}$ s of the tested compounds with the $IC_{50}$ of doxorubicin obtained under the same




**6A**

Figure 2 — Structure of compound **6A**

conditions (Table I). For example, compound **5** exhibits one tenth the activity of doxorubicin on MCF-7 cell line which means that optimization of **5** could yield a compound with comparable activity with doxorubicin. Unlike doxorubicin, **5**, **6**, **10** and **11** shows higher selectivity against MCF-7 compared to their activity against HCT-116.

Regarding a hypothesized structure-activity relationships (SAR) that could be concluded from our work, it seems that the presence of acetic acid moiety or its methyl ester at position 5 enhances the cytotoxic activity of arylbenzaoxazole scaffold. This can be noticed by comparing 4-methoxyphenyl-5-flurobenzoxazole (compound **6A** shown in Figure 2) with $IC_{50}$ value of 22.7 μM on MCF7 with compound **10**, which possess acetic acid group instead of fluoro atom at position 5, with $IC_{50}$ value of 2.02 μM on the same cell line.[7]

On the other hand, it seems that the presence of oxygen atom at positions 3 or 4 (on the phenyl group) potentiate the activity unlike the presence of nitro, fluoro and amino substituents. Additionally, this study show that compounds with free carboxylic group (**1**, **5** and **10**)

are more active than their corresponding methyl esters on MCF-7 cells, while the HCT-116 cells seem to be insensitive to this variation. Compounds **5** and **6**, which have oxygen atom at position 3 exhibit higher selectivity against MCF-7 compared to HCT-116. Furthermore, the ester derivative **2** shows no selectivity, while its acid derivative **1** exhibits high selectivity against MCF-7. The ester **11** doesn't differentiate much between the two cell lines. Among the tested compounds, **3** and its methyl ester **4** exhibits significant higher activity against HCT-116 compared to MCF-7. Concerning the possibility that the mechanism of action of tested compounds to be by the inhibition of topoisomerase II, previous studies showed that the presence of nitro and amino group at position 4 (on the phenyl group) decreases topoisomerase II inhibition activity.

At the best of our knowledge, all the previously reported arylbenzoxazoles that showed good cytotoxic activities, they did not contain alkanoic moiety on the benzoxazole ring. Therefore, this is the first study to demonstrate that the presence of acetic/acetate moiety attached to the benzoxazole ring potentiates the cytotoxic activity. Concluding, synthesized compounds in this work as **5**, **6**, **10** and **11** were found to possess strong anti-proliferative activity and may provide promising opportunities to design and develop novel antineoplastic agents.

## Materials and methods

### *Reagents and chemicals*

All chemical reagents were purchased from commercially available sources and utilized without purification. $^1$H NMR spectra were obtained using a Varian Utility 300 spectrometer (Varian Medical systems Inc, Palo Alto, CA, USA), and chemical shifts were reported as parts per million (ppm) relative to internal standard TMS. FT-IR spectra were obtained with a Nicolet Impact 410 (Nicolet Instrument Corp, Fitchburg, WI, USA). Mass spectrometry (MS) data were obtained by VG 7070 mass spectrometer (M-scan Inc, West Chester PA, USA).

### General method for the synthesis of the tested compounds 1-11

2-arylbenzoxazole-5-acetic acids and their methyl esters (compounds **1-11** shown in Scheme I) were prepared as following: methyl ester derivatives were firstly prepared by addition of equimolar amount of methyl 3-amino-4-hydroxyphenylacetate to a solution of the suitably substituted benzaldehyde in absolute ethanol. The formed solution was heated under reflux for about 4 h. The ethanol was evaporated to give thick product which was dissolved in hot glacial acetic acid. Suitable amount of lead tetraacetate was added, and the mixture was allowed to cool to RT yielding solid precipitate which was collected by filtration. Purification of the final product, if required, can be achieved by recrystallization from hot ethanol.

To get the corresponding acids, hydrolysis of the methyl esters were performed by stirring a solution of the methyl ester compounds in suitable volume of NaOH solution in 90% ethanol at RT for 3 h. During this time a solid was formed and it was collected on a Buchner funnel, washed with acetone, and dissolved in suitable amount of $H_2O$. The aqueous solution was acidified with concentrated HCl giving a solid precipitate which was collected and purified by recrystallization from aqueous ethanol.

### Cytotoxicity

Human cell cultures: human breast cancer cells (MCF-7) and human colorectal carcinoma cells (HCT-116) were obtained from the American Type Culture Collection (ATCC). They were grown as monolayer's in T75 flasks at 37°C in a humidified atmosphere with 5% $CO_2$ in Dulbecco's modified Eagle's medium (DMEM) (Capricorn Scientific, Germany) supplemented with 10% (v/v) fetal bovine serum and 1% penicillin-streptomycin (EuroClone Diagnostica, Italy). Cell lines were split twice weekly with 0.25% trypsin (EuroClone Diagnostica, Italy).

### MTT Cell Proliferation Assay

Cells were plated at 10,000 cells per well in a 96-well plate and incubated overnight in DMEM and subsequently treated with the compounds at various concentrations starting with 100 µg/mL followed by two-fold dilutions. The cell viability was measured after 72 h post-treatment to determine the $IC_{50}$ of each compound. By the end of the incubation period, 20 µL of MTT solution (5 mg/mL, Calbiochem, Darmstadt, Germany) was added to each well and incubated for three hours until purple precipitate became visible. The solution was removed and 200 µl of dimethyl sulfoxide (DMSO) was then added to each well to dissolve the crystals. The plates were then read using a microtiter plate reader at the wavelength of 570 nm (Bio-tek Instruments). Triplicates were carried out for each cell line. Equation 1 formula was used to determine the percentage of viable cells:

$$\text{Per centage of Cell Viability} = \frac{\text{OD Sample}}{\text{OD control}} \times 100\% \quad \ldots (1)$$

## Experimental Section

### NMR spectroscopy

**2-( 3-Hydroxy-4-nitrophenyl) benzoxazole-5-acetic acid, 1**

Yield 9%. m.p.249-250°C. $^1$H NMR (DMSO-$d_6$): δ 3.74 (s, 2H, benzylic $CH_2$), 7.38 (d, 1H, ArH), 7.73 (m, 3H, ArH), 7.91( s, 1H, ArH), 8.08 (d, 1H, ArH), 11.58 (br, 1H), 12.39 (br, 1H); MS: *m/z* 315.

**Methyl 2-(3-hydroxy-4-nitrophenyl) benzoxazole-5-acetate, 2**

Yield 30%. m.p.176-179°C. $^1$H NMR ($CDCl_3$): δ 3.6 (s, 3H, $OCH_3$), 3.86 (s, 2H, benzylic $CH_2$), 7.4 (d, 1H, ArH), 7.76 (m, 3H, ArH), 7.91(s, 1H, ArH), 8.07 (d, 1H, ArH); MS: *m/z* 329.

**2-(4-Methylyphenyl) benzoxazole-5-acetic acid, 3**

Yield 61%. m.p.164-166°C. $^1$H NMR (DMSO-$d_6$): δ 2.43 (s, 3H, $CH_3$), 3.80 (s, 2H, benzylic $CH_2$), 7.09 (d, 2H, ArH, J = 10 Hz), 7.30 (dd, 1H, ArH), 7.58 (d, 2H, ArH), 7.74 (d, 1H, ArH), 7.78 (d, 2H, ArH), 8.19 (d, 2H, ArH); MS: *m/z* 268.

**Methyl 2-(4-methylyphenyl) benzoxazole-5-acetate, 4**

Yield 53%. m.p.146-148°C. $^1$H NMR (DMSO-$d_6$): δ 2.45 (s, 3H, $ArCH_3$), 3.65 (s, 3H, $OCH_3$), 3.85 (s, 2H, benzylic $CH_2$), 4.65 (s, 3H, $ArCH_3$), 7.09 (d, 2H, ArH, J = 10 Hz), 7.30 (dd, 1H, ArH), 7.58 (d, 2H, ArH), 7.74 (d, 1H, ArH), 7.78 (d, 2H, ArH), 8.19 (d, 2H, ArH); MS: *m/z* 282.

**2-(3-Benzyloxyphenyl) benzoxazole-5-acetic acid, 5**

Yield 57%. m.p.194-195°C. $^1$H NMR: (DMSO-$d_6$): δ 3.68 (s, 2H, benzylic $CH_2$), 5.19 (s, 2H, $ArOCH_2Ph$), 7.42 (m, 12H, ArH), 12.27 (br, 1H, COOH); MS: *m/z* 361.

**Methyl 2-(3-benzyloxyphenyl)benzoxazoe-5-lacetate, 6**

Yield 69%. m.p.130-131°C. $^1$H NMR: (DMF-d7): δ 3.7 (s, 3H, $COOCH_3$), 3.9 (s, 2H, benzylic $CH_2$), 5.32 (s, 2H, $PhCH_2OR$), 7.62 (m, 12H, ArH), MS: *m/z* 3757.

**Methyl 2-(4-aminophenyl) benzoxazole-5-acetate, 7**

Yield 70%. m.p.185-186°C ; $^1$H NMR: ($CDCl_3$): δ 3.65 (s, 3H, $OCH_3$), 3.75 (s, 2H, $CH_2$), 4.0 (s, 2H, $NH_2$), 6.75 (d, 2H, ArH), 7.15 (dd, 1H, ArH), 7.45 (d, 1H, ArH), 7.6 (d, ArH), 8.05 (d, 2H, ArH); MS: *m/z* 283.

**Methyl- 2-(3-fluoro-4-nitrophenyl) benzoxazole-5-acetate, 8**

Yield 66%. m.p.136-141°C. $^1$H NMR: ($CDCl_3$): δ 3.6 (s, 3H, $OCH_3$), 3.86 (s, 2H, benzylic $CH_2$), 7.4 (d, 1H, ArH), 7.76 (m, 3H, ArH), 7.91(s, 1H, ArH), 8.07 (d, 1H, ArH); MS: *m/z* 331.

**Methyl 2-(4-nitrophenyl) benzoxazole-5-acetate, 9**

Yield: 82%. m.p.195-196°C. $^1$H NMR: ($CDCl_3$): δ 3.5 (s, 3H, $OCH_3$), 3.7 (s, 2H, $CH_2$), 7.3 (d, 1H, ArH, J= 8.43 Hz), 7.6 (d, 1H, ArH, J = 8.4 Hz), 7.75 (s, 1H, ArH), 8.4 (m, 4H, ArH); MS: *m/z* 313.

**2-(4-Methoxyphenyl) benzoxazole-5-acetic acid, 10**

Yield: 80%. m.p.112-115°C. $^1$H NMR: ($CDCl_3$): δ 3.74 (s, 2H, $CH_2$), 3.80 (s, 3H, $OCH_3$), 7.10 (d, 2H, ArH, J = 10 Hz), 7.27 (dd, 1H, ArH), 7.50 (d, 1H, ArH, J = 10 Hz), 7.70 (s, 1H, ArH), 8.21 (d, 2H, ArH, J = 10 Hz); MS: *m/z* 283.

**Methyl 2-(4-methoxyphenyl) benzoxazole-5-acetate, 11**

Yield 50%. m.p.103-105°C. $^1$H NMR: ($CDCl_3$): δ 3.60 (s, 3H, $COOCH_3$), 3.72 (s, 2H, $CH_2$), 3.85 (s, 3H, $OCH_3$), 6.99 (d, 2H, ArH, J = 10 Hz), 7.2 (dd, 1H, ArH), 7.46 (d, 1H, ArH, J = 10 Hz), 7.61 (s, 1H, ArH), 8.15 (d, 2H, ArH, J = 10 Hz); MS: *m/z* 297.

## Conclusion

A series of 2-arylbenzoxazole derivatives were synthesized using oxidative coupling of the suitably substituted benzaldehyde with methyl ester of 3-amino-4-hydroxyphenylacetates. The cytotoxicity of synthesized compounds was evaluated using MTT assay and the $IC_{50}$ for each compound was reported. The findings of this study showed that some compounds (**10** & **11**) exhibit promising activity in both cancer cell lines (MCF-7 & HCT-116), while **5** and **6** demonstrate significant activity against MCF-7 cancer cell line. These results offer a new benzoxazole based template to design and develop novel antineoplastic agents, therefore, additional *in vitro* and *in vivo* studies are required to reveal their possible mechanism of action.

## Aknowledgment

The authors would like to thank the deanship of research in the Jordan University of Science and Technology and Isra University for supporting this work.

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