Cytotoxic effects of new synthesis heterocyclic derivatives of Amoxicillin on some cancer cell lines

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Abstract. A new Schiff base [I] was prepared by refluxing Amoxicillin trihydrate and 4-Hydroxy-3,5-dimethoxybenzaldehyde in aqueous methanol solution using glacial acetic acid as a catalyst. The new 1,3-oxazepine derivative [II] was obtained by Diels- Alder reaction of Schiff base [I] with phthalic anhydride in dry benzene. The reaction of Schiff base [I] with thioglycolic acid in dry benzene led to the formation of thiazolidin-4-one derivative [III]. While the imidazolidin-4-one [IV] derivative was produced by reacting the mentioned Schiff base [I] with glycine and triethylamine in ethanol for 9 hrs. Tetrazole derivative [V] was synthesized by refluxing Schiff base [I] with sodium azide in dimethylformamid DMF. The structure of synthesized compounds[I-V] was characterized by their melting points, elemental analysis CHN-S and by their spectral data; FTIR and ¹H NMR spectroscopy. Two cancer cell lines include: (RD) human pelvic rhabdomyosarcoma and (L20B) the mice intestines carcinoma cell line (which expresses the genes for human cellular receptor for Polio viruses) were used in this study. The cytotoxic effect of different concentrations of all the synthesized compounds for 48 hrs was examined. All compounds except [IV] and [V] showed less than 50% inhibition for (L20B), while these compounds exhibit inhibition more than 50% inhibition for (RD).

Key Words: Oxazepine, Thiazolidin-4-one, Imidazolidin-4-one, Tetrazole, β-lactam, Amoxicillin, Anticancer, Cell Line, Cytotoxic effects.

1.Introduction
Amoxicillin is a bacteriolytic containing the β-lactam antibiotic drug of class penicillin [1-2], the first antibiotic developed for the treatment of bacterial infectious diseases have also found new applications as anti-cancer prodrugs. Recently studies have reported on the anticancer properties of the β-lactams: N-methylthio β-lactam and N-ethylthio β-lactams, figure (1), a new group of drugs was found to induce apoptotic behavior in a number of cancer cell lines, like human breast, leukemia, prostate, etc. [3-4].
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Figure (1). N-methylthio β-lactam and N-ethylthio β-lactams

Heterocyclic compounds containing varies ring are associated with diverse pharmacological activities such as antimicrobial, anti-inflammatory, analgesic, anticancer against some the cancer cell lines. The rapid development of resistance to existing antimicrobial drugs generates a serious challenge to the scientific community [5].

1,3-oxazepine is unsaturated non homologous seven membered heterocycle, consist of oxygen in position 1 and nitrogen in position 3 in addition to the 5 carbon atoms. 1,3-oxazepine derivative as an antitumor agent and colorectal adenocarcinoma [6-7].

Thiazolidin-4-one is used as antioxidant, anticancer, anti-inflammatory, antimicrobial, antifungi may be associated with their affinity to anticancer biotargets, such as non-membrane protein tyrosine phosphatase, JNK-stimulating phosphatase-1, and tumor necrosis factor [8-11].

Imidazoles such as all azoles are five membered ring systems, occurs in the nucleus of purine and in histidine, several imidazolidin derivatives have been examines for antitumor activity against various human tumors, which have shown higher cytotoxic activity and good inhibitory effect at the ovarian cancer cell line [12-13].

Tetrazole cycle is a promising pharmacophore fragment frequently used in the development of anticancer drugs. Over recent 10-15 years, various isomeric forms of tetrazole have been successfully used in the design of promising anticancer drugs [14].

In this study we have used amoxicillin to changes in parent chemical structures and modern classes of antibiotics to evaluate its anti-cancer activity against two cancer cell lines human pelvic rhabdomyosarcoma (RD) and the mouse cell line (L20B), an attention for being promise anticancer products.

2. EXPERIMENTAL

2.1. Materials and Instrumentation
All the chemical used in the synthesis were supplied from BDH and Sigma-Aldrich. Standard antibiotic drugs: Amoxicillin trihydrate was supplied by the State Company of Drug Industries and Medical Appliances in Iraq-Samara.

Melting points were registered using electro thermal melting point apparatus and are uncorrected. Infrared spectra was recorded as KBr discs on SHIMADZU 8400s spectrophotometer. 1H NMR spectra was recorded on a Bruker - 500 MHz instrument using DMSO as a solvent and TMS as internal reference (ppm), measurement were made at Central lab, Tahran University (Iran). Elemental analysis (C.H.N.S) were carried out using an EuroEA Elemental Analyzer. All reactions was monitored by thin layer chromatography (TLC) and spots were visualized using iodine chamber.
2.2. Synthesis Procedures
All derivatives [I-V] were synthesized according to scheme (1), and all compounds [I-V] gave acceptable elemental analysis, FTIR and \(^1\)H NMR spectra that matched data reported in the quote references.

Scheme (1)

2.2.1. Preparation of Schiff Base: 6-{2-[(4-Hydroxy-3,5-dimethoxybenzilidene) amino]-2-(4-hydroxyphenyl) acetyl] amino}-3,3-dimethyl-7-oxo-1-aza-bicyclo[3.2.0] heptane-2-carboxylic acid [I]:
To the stirred solution of amoxicillin trihydrate (0.419gm, 1 mmol) dissolved in 10 ml aqueous methanol (1:1) was added a stirred solution of 4-hydroxy-3,5-dimethoxybenzaldehyde (syringaldehyde) (0.182gm, 1mmol) dissolved in 10 ml methanol and 2 drops of glacial acetic acid. The mixture was stirred and refluxed for 10 hrs at 30 °C. The solvent was evaporated under vacuum. The pale orange solid obtained was filtered, washed and recrystallized from ethanol. Yield: 71%; m.p. = 205-207 °C; IR(KBr) \(\nu\) cm\(^{-1}\): 3275-3420 (s & br, Ph-OH; & -COOH; O-H); 3010 (s, C-H arom.); 2966 & 2918 (s, C-H aliph.); 1647 (s, C=N), 1665 (s, C=O amide); \(^1\)H NMR (TMS) \(\delta\) ppm: 1.58 (s, 6H, CH\(_3\)); 2.49 (1H, CH-S); 3.87 (s, 6H, 2OCH\(_3\)); 5.62 (1H,Ph-OH); 6.72-7.12 (4H, Ar-OH); 8.23 (1H,CH= N); 9.76 (1H, H of COOH). Anal. Calc. for C\(_{25}\)H\(_{27}\)N\(_3\)O\(_8\)S: C, 56.71; H, 5.10; N,7.93; S,6.04. Found: C, 56.83; H, 4.99; N,7.15; S,5.88.
2.2.2. Preparation of 6-{2-[(4-Hydroxy-3,5-dimethoxyphenyl)-2,3-dihydro benz[1,2e][1,3]-oxazepine-4,7-diones]-2- (4-hydroxyphenyl) acetyl] amino}-3,3-dimethyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0] heptane -2-carboxylic acid [II]:
A mixture of equimolar amounts (0.53gm, 1 mmol) of Schiff base [I] and phthalic anhydride (0.148gm, 1 mmol) in dry benzene as a solvent was refluxed for 4 hrs[7], the solvent was removed and the resulting colored solid was recrystallized from petroleum ether to obtained 1,3-oxazipenes [II]. Yield: 78%; m.p. = 220-222 °C; IR(KBr) cm⁻¹: 3290-3400 (s & br, Ph-OH ; & -COOH; O-H); 3026 (s, C-H arom.); 2972 & 2920 (s, C-H aliph.); 1750 (s, C=O lactone ring), 1665 (s, C=O amide) ; 1280 and 1103 cm⁻¹ (br, asymmetric and symmetric C-O-C) band. ¹H NMR (TMS) δ ppm: 1.28 (s, 6H, CH₃); 2.49 (1H, CH-S); 3.88 (s,6H,2OCH₃); 5.40 (s,1H, Ph-OH); 6.67-7.26 (4H, Ar-OH); 8.203-8.64 (m, 6H, Ar-); 9.40 (1H, H of COOH) . Anal. Calc. for C₃₃H₃₁N₃O₁₁S: C, 58.49; H, 4.57; N,6.20; S,4.72. Found: C, 58.98; H, 4.10; N,6.08; S,4.18.

2.2.3. Preparation of 6-{2-[(4-Hydroxy-3,5-dimethoxyphenyl)-1,3-thiazolidin-4-one]-2- (4-hydroxyphenyl) acetyl] amino}-3,3-dimethyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0] heptane -2-carboxylic acid [III]:
Compound of Schiff base [I] (0.53gm, 1 mmol) and thioglycolic acid (1 mmol) was refluxed in dry benzene (10 mL) for 8hrs [10] . The solvent was evaporated and the reaction mixture was neutralized with sodium bicarbonate solution, filtered off, the product was off white, and recrystallized from petroleum ether (50-60)°C. Yield : 66% m.p. = 180-182 °C, IR(KBr) cm⁻¹: 3286-3415(s & br, Ph-OH ; & -COOH; O-H); 3026 (s, C-H arom.); 2976 & 2929 (s, C-H aliph.); 1680 (C=O of thiazolidine ring) ;1662 (s, C=O amide), 1614 (C=C); 896 (C-S); ¹H NMR (TMS) δ ppm: 1.17 (s, 6H, CH₃); 2.48 (1H, CH-S); 3.34 (s,2H, CH₂-S); 3.83 (s, 6H, 2OCH₃); 6.69 (s, 1H,Ph-OH); 7.78-7.20 (6H, Ar-H); 9.76 (s,1H, H of COOH); Anal. Calcd. for C₂₇H₂₉N₃O₉S₂: C, 53.73; H, 4.80; N, 6.96; S,10.61. Found: C, 52.99; H, 4.64; N, 6.09; S,10.82.

2.2.4. Preparation of 6-{2-[(4-Hydroxy-3,5-dimethoxyphenyl) imidazolidin-4-one]-2- (4-hydroxyphenyl) acetyl] amino}-3,3-dimethyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0] heptane -2-carboxylic acid [IV]:
A mixture of Schiff base [I] (0.53gm, 1 mmol), glycine (0.075gm, 1mmol) and triethylamine (1mL) in ethanol (20mL) was refluxed for 9hrs[13] . The reaction mixture was neutralized with diluted HCl and then poured into ice-cold water. The precipitate was filtered off, washed with water and recrystallized from ethanol to give [IV]. Yield : 71% m.p = 198-200 °C; IR(KBr) cm⁻¹: 3380(br, NH),3176-3404(s & br, Ph-OH ; & -COOH; O-H); 3006 (s, C-H arom.); 2979 & 2929 (s, C-H aliph.); 1680 (C=O of imidazolidine ring) ;1670 (s, C=O amide); ¹H NMR (TMS) δ ppm: 9.76 (s,1H, H of COOH); 8.57 (s, 2H, NH-C ); 7.02-7.19 (6H, Ar-); 6.69 (s, 1H,Ph-OH); 7.78-7.20 (6H, Ar-H); 6.69 (s, 1H,Ph-OH); 3.82 (s,6H,2OCH₃); 3.77 (s,2H, CH₂-N); 2.49 (s ,1H, CH-N) ; 1.19 (s, 6H, CH₃); Anal. Calcd. for C₂₇H₃₀N₄O₉S: C, 55.29; H,5.11; N, 9.55.; S, 5.46 Found: C, 55.89; H, 4.96; N, 9.50; S, 5.01.

2.2.5. Preparation of 6-{5-[(4-Hydroxy-3,5-dimethoxyphenyl) tetrazole]-2- (4-hydroxy phenyl) acetyl] amino}-3,3-dimethyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0] heptane -2-carboxylic acid [V]:
Sodium azide (0.065gm, 1 mmol) was added to a stirring solution of Schiff base [I] (0.53gm, 1 mmol), in dimethylformamid (10 mL), and the mixture was refluxed for 4 hrs with stirring [15], then cooled at room temperature and the precipitate was filtered , washed with cold water. Recrystallized from ethanol to give Yield : 58% m.p = 233-235°C; IR(KBr) cm⁻¹: 3246-3400 (s & br, Ph-OH ; & -COOH; O-H); 3383(br, NH), 3041 (s, C-H arom.); 2964-2806 (s, C-H aliph.); 1658 (s, C=O amide); 1581 (C=C); 1639 (C=N for tetrazole ring); 1496 (N=N); ¹H NMR (TMS) δ ppm: 1.70 (s, 6H, CH₃); 3.82 (s, 6H, 2OCH₃); 6.67 (1H,Ph-OH); 7.17-7.14 (6H, Ar-OH); 9.34(s,1H, H of COOH) . Anal. Calcd. for C₂₅H₂₇N₆O₈S: C, 52.53; H, 4.72; N,14.71; S, 5.60. Found: C, 52.93; H, 4.60; N,14.00; S,5.43.
3. Results and Discussion

A major number of pharmacologically active molecules like amoxicillin that have been found for clinical use and synthesized through the derivatization of heterocyclic antibiotics as anticancer drugs clearly form an important part of chemotherapeutics with medicinal properties, with its use as antibacterials and also anticancer drugs.

Aim of the present work is describes the synthesis of new heterocyclic compounds [I-V] derived from amoxicillin to produce bio-active compounds. The new Schiff base [I] was synthesized by refluxing equimolar of amoxicillin trihydrate with syringaldehyde in dry benzene with some drops of glacial acetic in good yield. These Schiff base [I] was identified by their melting points, elemental analysis C,H,N,S, FTIR, and 1HNMR spectroscopy. FTIR absorption spectra showed the demise of absorption bands due to NH2 and C=O groups of the starting materials together with presence of new band at 1620 cm\(^{-1}\) which is due to C=N stretching vibration. The 1,3-oxazepine derivative [II] was obtained by Diels-Alder reaction of Schiff base [I] with phthalic anhydride in dry benzene. Thiazolidin-4-one derivative [III] was synthesized by refluxing Schiff base [I] with molybdenum thioglycolic acid was refluxed in dry benzene for 8 hrs. The novel limidazolidin-4-one derivative [IV] was synthesized by refluxing Schiff base [I] with glycine and triethylamine in ethanol for 9hrs. Tetrazole derivative [V] was obtained by addition reaction of Schiff base [I] with sodium azide in dimethylformamid for 7hrs. and recorded in DMSO and show all expected protons. The suggested mechanism to obtain the target products are outlined [III] and [IV], scheme (2).

![Scheme (2): Mechanism for the derivative compounds [III] and [IV].](image-url)
3.1 Anticancer screening

All compounds of these study were selected for testing for their anticancer activity, at Bio-technology research center in Al-Nahrain University, Baghdad, Iraq. Two cell lines were used for the evaluation: human pelvic rhabdomyosarcoma (RD) and the mouse cell line (L20B) according to the method described by Freshney [17]. Cytotoxicity assay of this demonstrated that synthesized heterocyclic compounds [I –V] caused inhibitory effect on the growth of (RD) and (L20B) cell lines except compound [IV] table (1). All compounds except [IV] and [V] showed less than 50% inhibition for mice intestines carcinoma cell line(L20B), while these compounds exhibit inhibition more than 50% inhibition for human pelvic rhabdomyosarcoma (RD). As the table (1) compound [II] showed there was a potent toxic effect on both cell lines RD & L20B 67.7% and 49.8%, respectively. The heterocyclic derivatives of amoxicillin [I-V] exhibit cytotoxic effects on both cancer cell lines (except [IV] ) need to further investigation to know mechanism by which the heterocyclic compounds act in comparison to traditional anticancer drug that might get the amoxicillin derivatives an attention for being promise anticancer product.

Table (1): The cytotoxic effect as percent inhibition rate (%IR) of different concentration (µl/well) for all synthesized compounds of after 48 hours exposure on RD and L20B cell lines.

| Comp. | Inhibition of cells growth% | Inhibition of cells growth% |
|-------|---------------------------|---------------------------|
| [I]   | 50.8                      | 40.7                      |
| [II]  | 67.7                      | 49.8                      |
| [III] | 58.0                      | 50.0                      |
| [IV]  | 64.2                      | -                         |
| [V]   | 51.0                      | 30.4                      |

4. Conclusions

Heterocyclic compounds derived from amoxicillin were synthesized and structurally characterized using spectroscopic techniques. The synthetic way started from reaction between amoxicillin and adequate syringaldehyde in aqueous methanol.

The heterocyclic compounds containing amoxicillin moiety have been estimated for their anticancer activity on both cancer cell lines RD & L20B.

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