Utilizing of 4-(benzothiazol-2-yI)phenylamine as a precursor of bioactive agents

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ABSTRACT

Several heterocyclic compounds show either anticancer or antimicrobial activity, the initial goal of this study was directed towards combining some of those heterocyclic moieties - that have either activity - together to test whether the newly formed compounds will demonstrate both activity or one activity will predominate over the other or both activity will diminish. This was accomplished via Scheme 1 and Scheme 2 where diazotization of 4-(benzothiazol-2-yI)phenylamine 1 followed by reacting this functionalized hydrazone with a variety of binucleophiles to give the key intermediates that reacted with amino reagents viz. hydrazine, urea or thiourea to yield some of the goal compounds (Scheme 1). Reacting compound 1 with carbonyl or isothiocyanate reagents followed by cyclization of the given key intermediates afforded a set of novel target compounds (Scheme 2). These final compounds were tested for in vitro antimicrobial and anticancer activity. Two compounds showed both antimicrobial and anticancer activity while the other compounds were highly active either as antimicrobial only or as anticancer only.

KEYWORDS

4-(benzothiazol-2-yI)phenylamine; Pharmacophores; Anticancer; Antimicrobial; Improving chemotherapy regimen design

INTRODUCTION

It is worthy to mention that 4-(benzothiazol-2-yl)phenylamine (1) shows anticancer activity via induction of cytochrome enzyme (CYP1A1), catalyzed biotransformation of aminophenylbenzothiazole to generate electrophilic species, which covalently bind to DNA, exerting lethal damage to sensitive tumor cells in vitro and in vivo⁴⁻⁵. It is also important to mention that polymerase (topoisomerase or DNA gyrase enzyme) main target for antimicrobial agents plays a significant role in modulating cellular sensitivity to DNA-targeting anticancer agents. When compared with normal human cells, polymerase deficient cells derived were 3-10 fold more sensitive to targeting anticancer agents. Cellular and biochemical evaluation strongly suggested that the higher sensitivity of polymerase deficient cells to these agents was due to the inability of polymerase deficient cells to help resume the DNA replication process stopped by the anticancer agents which introduced DNA lesions. These results indicated that polymerase could play an important role in determining the cellular sensitivity to
therapeutic agents. The findings not only illuminate polymerase as a potential pharmacological target for developing new anticancer agents but also provide new directions for improving future chemotherapy regimen design\cite{6-9}. The strategy of this work is of great interest, where a hybrid of the anticancer agent compound 1 and other nuclei having antimicrobial activity are synthesized to disclose the activity of the resulting compounds whether compounds having both anticancer and/or antimicrobial, or both activity will be diminish. The following pharmacophores are used; pyrazolyl\cite{10,12}, pyrimidinonyl and thiopyrimidinonyl\cite{13,14}, thiazolidinonyl\cite{15-19}, cyclic imides\cite{20}, urea\cite{21} and finally triazolyl moieties\cite{12,16,22,23} due to their potential antimicrobial activity.

**RESULTS AND DISCUSSION**

**Chemistry**

The synthetic approaches utilized for the preparation of the target compounds are summarized in Scheme 1 and 2.

**Scheme 1**

The initial goal of this scheme is directed towards the synthesis of pyrazolo- and pyrimidinonylphenyl hydrazonobenzothiazoles. This was achieved via condensing the diazonium salt of compound 1 with some active methylene-containing reagents e.g. malononitrile, diethyl malonate and ethyl acetoacetate to give compounds 2, 3 and 4 respectively. Examining the $^1$H NMR spectrum of compound 4 revealed the presence of the two geometrical isomers \([Z \text{ and } E]\) nearly in equal percentages since the apex of the signals of the methyl groups and methylene group are forked i.e. there are two CO\(_2\text{H}\) and two COOC\(_2\text{H}_5\) so there is no significant steric effect between the two isomers.

The main synthetic route to compounds 5, 6 and 7 is the hydrazinolysis of compounds 2, 3, and 4 sequentially using absolute hydrazine in refluxing ethanol for 30 min and their structures were confirmed as was attested by elemental analyses and spectral data. Moreover, condensation of compound 4 with either urea or thiourea in the presence of sodium ethoxide was found to be a conventional path for the formation of pyrimidinonylphenylhydrazonobenzothiazole and its thio derivative 8a,b. All the new compounds were characterized by mp, elemental analyses and spectral date ($^1$H NMR, IR and MS).

**Scheme 2**

This scheme is concerned with the preparation of the goal compounds 14, 15a-c and 16. The key intermediate 9 was prepared in a good yield by condensing ethyl isothiocyanate with compound 1 in boiling methylene chloride in the presence of few drops of TEA.
investigate the structure-activity relationship with respect to the required dual anticancer and antimicrobial activity, cyclization of this thiourea derivative 9 using chloroacetic acid/ anhydrous sodium acetate in absolute ethanol to afford one of the desired compounds 13. The structure of compound 13 was identified on the basis of its spectral data as well as its chemical transformation, where it is condensed with 4-chlorobenzaldehyde in glacial acetic acid and in the presence of anhydrous sodium acetate to yield the aryldene derivative 14. Furthermore, the intermediates 10a-c represent a versatile building block for the synthesis of new heterocycles incorporating 2-arylthiazolidinone nucleus, was synthesized by reacting compound 1 with different aromatic aldehydes in refluxing ethanol for three hours and may be this slightly long refluxing period is the main reason for the formation of the more thermodynamically stable geometrical isomer of compound 10a-c and this was ascertain from the $^1$H NMR of these Schiff’s basis$^{25,26}$ since it shows only one kind of N=CH proton. Subsequent synthesis of compounds 15a-c were performed via reacting equimolar amounts of these Schiff’s basis with 2-mercaptoacetic acid in dry benzene for 24 hours, $^1$H NMR spectra of these target compounds showed characteristic dd signal at 3.98-4.02 ppm for the CH$_2$ of the thiazolidinone ring due to gem coupling of the two protons of C-2 $^1$H at 6.65-6.84 ppm and the disappearance of the methylene proton of the Schiff’s basis. Meanwhile treatment of compound 1 with ethyl chloroacetate/ potassium carbonate in absolute ethanol and few drops of glacial acetic acid for three hours provided the parent ester 11 which upon hydrazinolysis using hydrazine in absolute ethanol gave the newly formed hydrazide 12 that was cyclized using ethyl isothiocyanate adopting the same conditions as that used in synthesizing compound 9 to provide compound 16.

Scheme 2

Antimicrobial

Several target compounds were explored to evaluate antimicrobial activity by assessment of the minimum inhibitory concentrations (MICs) of these compounds against different microbial isolates by agar-dilution method (Heuritt and Vincent, 1999). MIC was defined as the lowest concentration of the test compound that yielded no visible growth on the plate. The test organisms included gram-positive bacteria (Staph. aureus and B. subtilis), gram-negative bacteria (E. coli and
Ps. aeruginosa) and fungi (C. albicans). The organisms were grown overnight in brain-heart infusion (MHA) broth (Oxoid, England) at 37°C. Serial dilutions were done to the stock solution (2µg/mL) for each compound in MHA agar to obtain different concentrations ranging from 25 to 400 µg/mL. The plates were incubated with approximately 10^4 organisms spot, and then incubated at 37°C for 18 hours.

Regarding Ps. aeruginosa, compound 14 was shown to be highly effective while 15a was moderate in action. Concerning Staph. Aureus, compound 14 was the most effective. Referring to E. coli, compound 14 was the only compound with a high activity against E. coli. B. subtilis was found to be highly sensitive 7, 14 and 16. Concerning C. albicans, compounds 8b and 16 revealed a good activity. (TABLE 1)

**TABLE 1: The minimum inhibitory concentrations (MIC) of some of the target compounds**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ps. aeruginosa</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>B. subtilis</th>
<th>C. albicans</th>
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<td>&lt;12.50</td>
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<td>&gt;400</td>
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<tr>
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<td>&gt;400</td>
<td>&gt;400</td>
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<td>&lt;200</td>
<td>&lt;100</td>
<td>&lt;200</td>
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<td>&gt;400</td>
<td>&gt;400</td>
<td>&lt;100</td>
<td>&lt;200</td>
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</tbody>
</table>

**Anticancer**

Compounds 5, 6, 7, 8b, 13, 14, 15a and 16 were tested for their cytotoxic activity using Dox (doxorubicin dihydrochloride) as a reference drug and using sulphorhodamine B (SRB) assay provided a rapid and sensitive method for measuring the drug induced cytotoxicity in attached breast cancer cell line (MCF-7) cultures in 96-well (10^4 cells/ well) micro titer plates. Different concentrations of the tested compounds (0, 1, 2.5, 5 and 10 µg/mL DMSO) were assessed in triplate wells for each individual dose and the IC_{50} for each compound was recorded where IC_{50} is the dose which reduces survival to 50 %. SRB binds to protein basic aminoacid residues in TCA fixed cells to provide a sensitive index of cellular protein content. At the end of the staining period, SRB was removed and cultures were quickly rinsed with 1 % acetic acid to remove unbounded dye. Bound dye was solubilised with 10 mM unbuffered Tris base pH 10.5. The optical density of the solubilised dye measured in an ELISA reader, is directly proportional to the surviving fraction of the cell. The present study demonstrated that compounds 13, 14, 15a and 16 exerted anticancer activity with IC_{50} 4.9 µg/mL, 8.72 µg/mL, 7.38 µg/mL and 7.52 µg/mL respectively. The rest of the tested compounds showed no activity at all.

**EXPERIMENTAL**

**Chemistry**

Melting points were determined in capillary tubes using Griffin apparatus and are uncorrected. Chemical analyses were carried out at the Microanalytical Center, Cairo University, Giza, Egypt. Infrared spectra were measured on a Schimadzu IR 435 spectrometer. Proton magnetic resonances (1H NMR) were measured at 300 MHz on Varian Gemini spectrophotometer using tetramethylsilane as internal standard (chemical shifts are reported in 6 ppm). Mass spectra were obtained on Hewlett Packard 5988 spectrometer.

Compound 1, 2 and 3 were prepared according to reported procedure^[27,24] (ZE) Ethyl-2-[4-(Benzothiazol-2-yl)phenyl hydrazono]-3-oxo-butyrate (4)

To an ice-cold solution of 1 (2.26g, 0.01 mol) in hydrochloric acid (2.5 mL) and distilled water (5 mL), a solution of sodium nitrite (0.90g, 0.013 mol) in distilled water (5mL) was added portionwise, Then this solution was added portionwise to a well-stirred ice-cold solution of ethyl acetoacetate (1.30g, 0.01 mol) in aqueous ethanol (10 mL, 50%) containing sodium acetate (0.82g, 0.011 mol). After completion of addition, the reaction mixture was kept in ice for 2 h then filtered. The product was dried and crystallized from ethanol to give 2.97 g of compound 4 (75%); m.p. 137-141 °C; IR (KBr): 3250 (NH), 2987-2927 (CHaliphatic), 1693, 1656 (C=O) cm^{-1}; 1H NMR (300 MHz, DMSO-d_6): δ1.19 (t, 3H, CH_3CH_3), 4.00 (s, 3H,
CH$_3$), 4.13 (q, 2H, CH$_2$CH$_3$) 4.32 (s, 1H, NH, D$_2$O exchangeable) and 6.86-8.07 (m, 8H, ArH) ppm; EIMS: m/z 367 (M$^+$) (68%) and (224) (100%). Anal. Calcd for C$_{19}$H$_{17}$N$_3$O$_3$S: C, 62.11; H, 4.66; N, 11.44. Found; C, 62.50; H, 4.44; N, 11.35%.

4-[4-(Benzothiazol-2-yl)phenylhydrazono]-4H-pyrazole-3,5-diamine (5), 4-[4-(Benzothiazol-2-yl)phenylhydrazono]pyrazolidine-3,5-dione (6) and 4-[4-(Benzothiazol-2-yl)phenylhydrazono]-5-methyl-2,4-dihydropyrazol-3-one (7)

**General method**

A mixture of either 2, 3 or 4 (0.01mol) and hydrazine hydrate (99%) (0.011mol) in ethanol (20 mL) was refluxed for 0.5 h. The reaction mixture was evaporated, the residue was washed with water, dried and crystallized from the suitable solvent.

4-[4-(Benzothiazol-2-yl)phenylhydrazono]-4H-pyrazole-3,5-diamine (5)

Yield 82% (DMF); m.p. > 300 ºC; IR (KBr): 3520-3000 (NH$_2$, NH) and 1600-1580 (C=N) cm$^{-1}$; $^1$H NMR (DMSO-d$_6$): $\delta$ 6.03 (s, 2H, NH$_2$, D$_2$O exchangeable), 6.54 (s, 2H, NH$_2$, D$_2$O exchangeable), 7.45-8.10 (m, 8H, Ar-H) and 10.90 (s, 1H, HN-N=C, D$_2$O exchangeable) ppm; EIMS: m/z 335 (M$^+$) (100%). Anal. Calcd for C$_{16}$H$_{13}$N$_7$S: C, 57.30; H, 3.91; N, 29.23. Found; C, 57.59; H, 4.12; N, 29.12%.

4-[4-(Benzothiazol-2-yl)phenylhydrazono]pyrazolidine-3,5-dione (6)

Yield 60% (DMF/MeOH), m.p. >300 ºC; IR (KBr): 3441 (NH), 3060 (CH aromatic) and 1720 (C=O) cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-d$_6$): $\delta$ 7.43-8.13 (m, 8H, ArH), 10.00-10.10 (brs, 1H, NH, D$_2$O exchangeable), 12.00-12.10 (brs, 1H, NH, D$_2$O exchangeable) ppm; EIMS: m/z 337 (M$^+$) (7%) and (226) (100%). Anal. Calcd for C$_{16}$H$_{11}$N$_5$O$_2$S: C, 56.96; H, 3.29; N, 20.76. Found C, 57.59; H, 4.12; N, 29.12 %.

4-[4-(Benzothiazol-2-yl)phenylhydrazono]-5-methyl-2,4-dihydropyrazol-3-one (7)

Yield 70% (EtOH); m.p. 278-280 ºC; IR (KBr): 3398 (NH), 2887-2815 (CH aliphatic) and 1666 (C=O) cm$^{-1}$; $^1$HNMR (300 MHz, DMSO-d$_6$): $\delta$ 1.73(s, 3H, CH$_3$) 6.65(s, 1H, NH of pyrazolone, D$_2$O exchangeable), 7.27-8.07 (m, 8H, Ar-H) and 11.59(s, 1H, HN-N=C, D$_2$O exchangeable) ppm; EIMS: m/z 335 (M$^+$) (100%). Anal. Calcd for C$_{17}$H$_{13}$N$_5$O$_3$S: C, 60.88; H, 3.91; N, 20.88. Found; C, 61.11; H, 4.21; N, 20.73 %.

5-[4-(Benzothiazol-2-yl)phenylhydrazono]pyrimidine-2,4,6-trione (8a) and 5-[4-(Benzothiazol-2-yl)phenylhydrazono]-2-thioxo-dihydropyrimidine-4,6-dione (8b)

**General method**

To an ethanolic solution of sodium ethoxide (0.01 mol) [sodium (0.23g) and absolute ethanol (5mL)], compound 3 (3.97 g, 0.01 mol) was added followed by hot solution of either dry urea or dry thiourea (0.01 mol) in ethanol (30mL). The reaction mixture was heated under reflux for 7 h in an oil-bath at 110 ºC, cooled then treated with hot water (10mL) and hydrochloric acid till acidic to litmus paper. The resulting solid was filtered, washed with water, dried and crystallized from dioxan / EtOH.

5-[4-(Benzothiazol-2-yl)phenylhydrazono]pyrimidine-2,4,6-trione (8a)

Yield 55%; m.p. > 300 ºC; IR (KBr): 3423 (NH), 3082 (CH aromatic) and 1703, 1695 (C=O) cm$^{-1}$; $^1$H NMR (300MHz, DMSO-d$_6$): $\delta$ 7.44-8.16 (m, 8H, ArH), 11.90-12.00 (brs, 3H, NH and/or OH, D$_2$O exchangeable) ppm; EIMS: m/z 365 (M$^+$) (74%) and (55) (100%). Anal. Calcd for C$_{17}$H$_{11}$N$_5$O$_3$S: C, 55.88; H, 3.03; N, 19.17. Found; C, 55.79; H, 3.05; N, 19.27%.

5-[4-(Benzothiazol-2-yl)phenylhydrazono]-2-thioxo-dihydropyrimidine-4,6-dione (8b)

Yield 63%; m.p. > 300 ºC; IR (KBr): 3423 (NH), 3082 (CH aromatic) and 1710, 1690 (C=O) cm$^{-1}$; $^1$H NMR (300MHz, DMSO-d$_6$): $\delta$ 7.44-8.16 (m, 8H, ArH), 11.90-12.00 (brs, 3H, NH and/or OH, D$_2$O exchangeable) ppm; EIMS: m/z 381 (M$^+$) (12.7%) and (55) (100%). Anal. Calcd for C$_{17}$H$_{11}$N$_5$O$_2$S$_2$: C, 53.53; H, 2.91; N, 18.36. Found; C, 53.70; H, 2.81; N, 18.26%.

1-[4-(Benzothiazol-2-yl)phenyl]-3-ethylthiourea (9) and 5-[4-(Benzothiazol-2-yl)phenylamino-methyl]-4-ethyl-4H-[1,2,4]triazole-3-thiol (16)

**General method**

A mixture of 1 or 2 (0.01 mol), ethyl isothiocyanate (0.87g, 0.01mol) and few drops of
triethylamine in methylene chloride (30 mL) was re-fluxed for 24h for compound 9 and 48h for compound 16, evaporated under reduced pressure and the resi-due crystallized from ethanol.

1-[4-(Benzothiazol-2-yl)phenyl]-3-ethylthiourea (9)

Yield 70%; m.p. 191-193 °C; IR (KBr): 3361 (NH) cm\(^{-1}\); \(^1\)H NMR (300MHz, DMSO-d\(_6\)): \(\delta\) 1.14 (t, 3H, CH\(_2\)CH\(_3\)), 3.54 (q, 2H, CH\(_2\)CH\(_3\)), 7.41-8.10 (m, 8H, ArH), 8.13 (s, 1H, NH, D\(_2\)O exchangeable) and 9.75 (s, 1H, NH, D\(_2\)O exchangeable) ppm; EIMS: m/z 313 (M\(^+\)) (22.9%) and (279) (100%). Anal. Calcd for C\(_{16}\)H\(_{15}\)N\(_3\)S\(_2\): C, 61.31; H, 4.82; N, 13.41. Found; C, 61.22; H, 4.48; N, 13.33%.

5-[4-(Benzothiazol-2-yl)phenylamino-methyl]-4-ethyl-4H-[1,2,4]triazole-3-thiol (16)

Yield 60 %; m.p. 237-239 °C; IR (KBr): 3421-3334 (NH), and 2896 (CH aliphatic) cm\(^{-1}\); \(^1\)H NMR (300MHz, DMSO-d\(_6\)):\(\delta\) 1.22 (t, 3H, CH\(_2\)CH\(_3\)), 4.03 (q, 2H, CH\(_2\)CH\(_3\)), 4.50 (d, 2H, CH\(_2\)), 7.35 (s, 1H, NH, D\(_2\)O exchangeable), 6.79-8.04 (m, 8H, ArH) and 13.60 (s, 1H, SH, D\(_2\)O exchangeable) ppm; EIMS:m/z 367 (M\(^+\)) (100%). Anal. Calcd for C\(_{18}\)H\(_{17}\)N\(_5\)S\(_2\): C, 58.83; H, 4.66; N, 19.06. Found; C, 58.70; H, 4.80; N, 18.66%.

2-[4-(Arylidenameino)phenyl]benzothiazole (10a-c)

**General method**

To a solution of 1 (2.26 g, 0.01 mol) in absolute ethanol (30 mL) and glacial acetic acid (0.5 mL), the appropriate aromatic compound was added and the mixture was refluxed for 3h. The reaction mixture was evaporated under reduced pressure, and the residue was crystallized.

(10a-c): IR (KBr): 3400-3200 (N=CH) and 1520-1500 (C=N of azomethine) cm\(^{-1}\); \(^1\)H NMR (300MHz, DMSO-d\(_6\)): \(\delta\) 7.42-8.16 (m, 13H, ArH) and 8.71 (s, 1H, N=CH) ppm; EIMS: m/z 314 (M\(^+\)) (100%); Anal. Calcd for C\(_{20}\)H\(_{14}\)N\(_2\)S: C, 76.40; H, 4.49; N, 8.91. Found; C, 76.19; H, 4.54; N, 8.61 %.

(4-Benzothiazol-2-yl-phenyl)benzylideneamine (10a)

Yield 65% (EtOH); m.p. 110-112 °C; \(^1\)H NMR (300MHz, DMSO-d\(_6\)): \(\delta\) 7.44-8.16 (m, 12H, ArH) and 8.73 (s, 1H, N=CH) ppm; EIMS: m/z 348 (M\(^+\)) (100%). Anal. Calcd for C\(_{20}\)H\(_{13}\)ClN\(_2\)S: C, 68.86; H, 3.76; N, 8.03. Found; C, 68.90; H, 4.30; N, 8.13 %.

(4-Benzothiazol-2-yl-phenyl)-4-chlorobenzylideneamine (10b)

Yield 69% (EtOH); m.p. 125-127 °C; \(^1\)H NMR (300MHz, DMSO-d\(_6\)): \(\delta\) 7.44-8.16 (m, 12H, ArH) and 8.73 (s, 1H, N=CH) ppm; EIMS: m/z 348 (M\(^+\)) (100%). Anal. Calcd for C\(_{20}\)H\(_{13}\)ClN\(_2\)S: C, 68.86; H, 3.76; N, 8.03. Found; C, 68.90; H, 4.30; N, 8.13 %.

(4-Benzothiazol-2-yl-phenyl)-4-nitro-benzylideneamine (10c)

Yield 82% (DMF/ Toluene); m.p. 240-242 °C; IR (KBr): 3400-3200 (N=CH) and 1520-1500 (C=N of azomethine) cm\(^{-1}\); \(^1\)H NMR (300MHz, DMSO-d\(_6\)):\(\delta\) 7.47-8.41 (m, 12H, ArH) and 8.91 (s, 1H, N=CH) ppm; EIMS: m/z 359 (M\(^+\)) (100%). Anal. Calcd for C\(_{20}\)H\(_{13}\)N\(_3\)O\(_2\)S: C, 66.84; H, 3.65; N, 11.69. Found; C, 66.91; H, 4.10; N, 11.59 %.

Ethyl 2-[4-(Benzothiazol-2-yl)phenylamino]acetate (11)

A well-stirred mixture of 1 (2.26g, 0.01 mol), anhydrous potassium carbonate (1.38g, 0.01 mol) and ethyl chloroacetate (1.22g, 0.01 mol) in dry acetone (100 mL) was refluxed for 24 h, filtered while hot and evaporated under reduced pressure. The residue was washed with water, filtered dried and crystallized from acetone to give 2.5 g of 11 (80%); m.p. 78-80 °C; IR (KBr): 3378 (NH), 2981-2903 (CH aliphatic) and 1728 (C=O) cm\(^{-1}\); \(^1\)H NMR (300MHz, DMSO-d\(_6\)):\(\delta\) 1.27 (t, 3H, CH\(_2\)CH\(_3\)), 3.50 (s, 2H, COCH\(_2\)), 4.29 (q, 2H, CH\(_2\)CH\(_3\)), 7.36-8.07 (m, 8H, ArH) and 11.66 (s, 1H, NH, D\(_2\)O exchangeable) ppm; EIMS: m/z 312 (M\(^+\)) (33.4%) and (239)(100%). Anal. Calcd for C\(_{17}\)H\(_{16}\)N\(_2\)O\(_2\)S: C, 65.36; H, 4.66; N, 19.06. Found; C, 65.70; H, 4.80; N, 18.66%.

2-[4-(Benzothiazol-2-yl)phenylamino]acetic acid hydrazide (12)

A well-stirred mixture of 11 (3.13g, 0.01mol) and hydrazine hydrate (99%) (0.011 mol) in absolute ethanol (20 mL) was heated under reflux for 6h then cooled and filtered. The solid was crystallized from ethanol to give 2.73 g of 12 (85%); m.p. 114-116 °C; IR (KBr): 3350 (NH\(_2\)), 3291(NH), and 1661 (C=O) cm\(^{-1}\); \(^1\)H NMR (300MHz,DMSO-d\(_6\)): \(\delta\) 3.64 (s, 2H, COCH\(_2\)), 3.79 (s, 2H, NH\(_2\), D\(_2\)O exchangeable) and 6.95-8.03 (m, 8H, ArH) and 11.00 (s, 1H, NH, D\(_2\)O exchangeable) ppm; EIMS: m/z 298 (M\(^+\)) (19.56%) and (226)(100%). Anal.Calcd for C\(_{15}\)H\(_{14}\)N\(_4\)O\(_2\)S: C, 60.38; H, 4.73; N, 18.78. Found; C, 60.45; H, 4.59; N,18.80%.
2-[(ZE)-4-(Benzothiazol-2-yl)phenylimino]-3-ethylthiazolidin-4-one (13)

A mixture of 9 (3.13 g, 0.01 mol), chloroacetic acid (0.95 g, 0.01 mol) and anhydrous sodium acetate (0.82 g, 0.01 mol) in absolute ethanol (30 mL) was refluxed for 20 h. filtered while hot and the filtrate was concentrated under reduced pressure. The residue washed with ethanol, dried and crystallized from aqueous acetic acid to give 2.45 g of 13 (70%); m.p. 291-293 °C; IR (KBr): 2989-2876 (CH aliphatic), and 1727 (C=O) cm^{-1}; 'H NMR (300 MHz, DMSO-d_6): δ 1.03 (t, 3H, CH_2CH_3), 3.76 (q, 2H, CH_2CH_3), 3.91-4.07 (dd, 2H, CH_2, C_5H), and 7.12-8.14 (m, 8H, ArH) ppm; EIMS: m/z 353 (M^+ 24.3%) and (57) (100%). Anal. Calcd for C_{18}H_{15}N_{3}O_{2}: C, 61.16; H, 4.28; N, 11.89. Found; C, 61.30; H, 4.20; N, 11.51%.

3-(4-Benzothiazol-2-yl-phenyl)-2-phenylthiazolidin-4-one (15a)

Yield 65% (acetic acid); m.p. 185-187 3; IR (KBr): 3000-2900 (CH aliphatic) and 1700-1680 (C=O) cm^{-1}; 'H NMR (300 MHz, DMSO-d_6): δ 3.95-4.09 (dd, 2H, CH_2, C_5H), 6.65 (s, 1H, C*2 H) and 7.22-8.12 (m, 13H, ArH) ppm; EIMS: m/z 388 (M^+ 24.3%) and (91) (100%); Anal. Calcd for C_{22}H_{16}ClN_{2}O_{3}: C, 68.01; H, 4.15; N, 7.21. Found; C, 68.40; H, 4.43; N, 7.27%.

3-(4-Benzothiazol-2-yl-phenyl)-2-(4-chlorophenyl)thiazolidin-4-one (15b)

Yield 65% (dioxan/EtOH); m.p. 220-222 3; IR (KBr): 390-4.13 (dd, 2H, CH_2, C_5 H), 6.69 (s, 1H, C*=2 H) and 7.34-8.15 (m, 12H, ArH) ppm; EIMS: m/z 422 (M^+) (52.57%) and (256) (100%); Anal. Calcd for C_{22}H_{16}ClN_{2}O_{3}: C, 62.47; H, 3.57; N, 6.62. Found; C, 62.69; H, 3.75; N, 6.52%.

3-(4-Benzothiazol-2-yl-phenyl)-2-(4-nitrophenyl)thiazolidin-4-one (15c)

Yield 55% (dioxan/EtOH); m.p. 219-221 3; IR (KBr): 2972-2916 (CH aliphatic), and 1727 (C=O) cm^{-1}; 'H NMR (300 MHz, DMSO-d_6): δ 3.94-4.13 (dd, 2H, CH_2, C_5 H), 6.84 (s, 1H, C*=2 H) and 7.59-8.16 (m, 12H, ArH) ppm; EIMS: m/z 433 (M^+ 84.56%) and (256) (100%); Anal. Calcd for C_{22}H_{16}ClN_{3}O_{3}: C, 60.95; H, 3.49; N, 9.69. Found; C, 60.93; H, 3.19; N, 9.55%.

CONCLUSION

Concerning the antimicrobial activity; compounds 7, 8b, 14 and 16 showed antimicrobial activity ranging from moderate to high activity. It is worth to mention that the presence of two atoms- spacer in between the benzothiazole moiety and the other pharmacophore improves the antifungal activity over the antibacterial e.g. compounds 8b and 16 while the presence of only one atom spacer with a hydrophobic part e.g. compound 14 increases greatly the antibacterial activity over the antifungal. Moreover when there is no spacer there is no antimicrobial activity e.g. compound 15a.

Regarding anticancer activity, joining 2-(4-aminophenyl)benzothiazole (1) with pyrazolyl, pyrimidinyl or thiopyrimidinyl through a two nitrogen atoms spacer diminishes the cytotoxic activity e.g. compounds 5, 6, and 8b. Replacing one of the two
nitrogen atoms spacer with a methylene group (CH₂) gave compound 16 with IC₅₀ 7.52 µg/mL (moderate anticancer activity). It is worth mentioning that using only one nitrogen atom spacer increases the anticancer activity e.g. compounds 13 and 14. Compound 13 showed a high anticancer activity as compared to the reference drug IC₅₀ values 4.90 and 2.97 µg/mL, respectively. On the other hand, compound 14 revealed the lowest reactivity due to the presence of the phenyl group (in the same plane with the rest of the compound) which increases both the bulkiness and the hydrophobicity of the compound. For compound 15 which have no spacer in between the benzothiazole moiety and the antimicrobial pharmaphore as well as having a phenyl group, it showed a better activity than compound 14 since the phenyl group is not in the same plane of the compound as it is carried by chiral carbon atom so it may be above or below and not coplaner with the rest of the compound, so it only increases the hydrophobicity but not the bulkiness. So this is potentially explaining the observation that 15 has better cytotoxicity compared to compound 14. Finally compounds 14 and 16 accomplished the main work objective where they show both antimicrobial and anticancer activity.

REFERENCES