Synthesis, design of triazinopyrimidines containing benzothiazole and benzoxazole derivatives as anticancer agents

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ABSTRACT
New series of cytotoxic agents triazolino[4,3-a]pyrimidines containing benzoxazole or benzothiazole moieties 4 and 5 were prepared from the reaction of each of 1-(2-(benzo[d]oxazol-2-yl)hydrazono)-1-chloropropan-2-one and 1-(2-(benzo[d]thiazol-2-yl)hydrazono)-1-chloropropan-2-one with certain pyrimidine thiones. Some of the newly synthesized compounds were screened against certain cancer tumors.

INTRODUCTION
Cytotoxic drugs and chemotherapy still remain the most important area of research. Enhancement and approaches to anticancer drugs seemed to be a major area of investigation, despite the continuous progress of anticancer agents, overall control of cancer is still a dream[1]. Great effort was exerted to develop new anticancer agents with high toxicity toward cancer cells and with a minimal toxicity against normal cells[2]. Some cytotoxic drugs which are kinase inhibitors share common properties, low molecular weight (small molecules), hydrophobic heterocycles and act by competing with ATP for binding in kinase ATP binding site. From these inhibitors, a number of small molecule epidermal growth factor receptor (EGFR) kinase inhibitors have been evaluated in cancer clinical trials. For example, anilinoquinazoline-containing compounds erlotinib (Tarceva™)[3] and gefitinib (Iressa™)[4] have been approved for the chemotherapeutic treatment of patients with advanced non-small lung cancer. Also, lapatinib (Tykerb™)[5] was approved for treatment of HER2-positive advanced or metastatic breast cancer. Taking these structure in guid and in addition, 2-(4-aminophenyl)-benzothiazole 1b and their analogues are a novel class of potent and selective antitumor agents[6-10]. In view of these report and continuation of the previous work[11-16], and in our way to investigate their anticancer activity, here in we designed some new anticancer agent. Such design was formed of a benzothiazole-triazolopyridimidine hybrid and evaluated for their co-adherent cytotoxic properties against breast cancer (MCF-7). Herein, we described the design and the synthesis of a new series of 4-substituted aminopyrazolo[3,4-d]pyrimidines as antitumor agents. The rational for the design of target compounds was based upon some...
Structural modifications on the general features of anilinoquinazoline-containing compounds Figure 1. These modifications comprise a replacement of benzene moiety in quinazoline skeleton by a triazinopyrimidine moiety hence it is an isostear with the naturally occurring in body purine bases and this expected to be more intensive for cytotoxic activity.

RESULTS AND DISCUSSION

Chemistry

Treatment of each diazotized [4-(1,3-benzoazol-2-yl)phenyl]amine (1a) and diazotized [4-(1,3-benzothiazol-2-yl)phenyl]amine (1b) with 2-chloro-2,4-pentandione in ethanolic sodium acetate gave 1-(2-(benzo[d]oxazol-2-yl)hydrazono)-1-chloropropan-2-one (2a) and 1-(2-(benzo[d]thiazol-2-yl)hydrazono)-1-chloropropan-2-one (2b). Structure 2 was elucidated by elemental analysis and spectral data. 1H NMR spectrum of 2a showed signals at δ = 2.10 (s, 3H, CH₃CO), 7.40 (d, 2H, J = 8Hz, ArH’s), 7.50 (d, 2H, J = 8Hz, ArH’s), 8.00 (d, 2H, J = 8Hz, ArH’s), 8.07 (d, 2H, J = 8Hz, ArH’s) and 10.89 (s, br., 1H, NH)(Scheme 1). Thus, compound 2a reacted with ethyl 4-methyl-6-phenyl-2-thioxo-1,3,6-trihydropyrimidine-5-carboxylate (3a) in chloroform and triethylamine gave the 1,2,4-triazolo[4,3-a]pyrimidine-5-carboxylates 4a. The structure of 5a was elucidated on the base of elemental analysis, spectra, and alternative synthesis. 1H NMR spectrum of 4a showed signals at δ = 1.18 (d, 3H, CH₂CH₂), 2.10 (s, 3H, CH₃CO), 2.21 (s, 3H, CH₃), 4.21 (q, 2H, CH₂CH₂), 6.62 (s, 1H, CH), and 7.17–8.35 (m, 13H, ArH). Its IR spectrum re-
revealed bands at $\nu = 1735$ cm$^{-1}$ (CO). Similar, treatment of the appropriate 2a,b with the appropriate 3a-e gave triazolo[4,3-a]pyrimidines 4b-e and 5a-e, respectively Scheme 1. The reaction was supposed to occur via illustrated mechanism in scheme 2 and also the reaction was proved by alternative route of synthesis as seen in scheme 3.

Another synthetic pathway to the same compound 4a is operated in order to confirm their structure and this involve reaction of Ethyl 6-methyl-4-[4-phenyl-2-methylthio-3,4-dihydropyrimidine-5-carboxylate (9) reacted with 2a in boiling ethanolic sodium ethoxide solution gave products identical in all aspects (mp, mixed mp, and spectra) with the corresponding 7a. (Scheme 3). The formation of 7 can be explained via 1,3-dipolar cycloaddition or 1,3-addition of nitrile imides 4 (prepared in situ from hydrazonoyl halides 2 with triethylamine or sodium ethoxide) to C=S of 6 (or NH of 10) to give intermediate 6 (or 10), with ring opening and ring closure to afford the final products 4 and 5 by elimination of hydrogen sulfide from 6 (or methyl mercapta from 10). (scheme3)

**Antitumor activity**

Some of the newly synthesized compounds were evaluated for their in vitro cytotoxic activity against human breast cell line (MCF7) using doxorubicin as the reference drug according to the method described as reported$^{[19]}$. The cytotoxicity was assessed
TABLE 1: Results of in vitro cytotoxic activity of the synthesized compounds on human breast cancer cell line (MCF7)

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>IC_{50} in µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>0.87</td>
</tr>
<tr>
<td>7a</td>
<td>2.55</td>
</tr>
<tr>
<td>7b</td>
<td>1.79</td>
</tr>
<tr>
<td>7c</td>
<td>1.75</td>
</tr>
<tr>
<td>7d</td>
<td>-ve</td>
</tr>
<tr>
<td>8a</td>
<td>0.98</td>
</tr>
<tr>
<td>8b</td>
<td>-ve</td>
</tr>
<tr>
<td>8c</td>
<td>0.98</td>
</tr>
</tbody>
</table>

The relation between surviving fraction and drug concentration was plotted to obtain the survival curve of MCF7 tumor cell line after addition of the specified compound. The parameter used here is IC_{50}, which corresponds to the concentration required for 50% inhibition of cell viability. The IC_{50} of the synthesized compounds compared to the reference drug are shown in TABLE 1.

The obtained data revealed that most of the newly synthesized compounds showed potent antitumor activity. Among the tested compounds, the most potent cytotoxic effect against MCF-7 cell line was...
CONCLUSION

Some of synthesized compounds show reasonable activity when compared with reference doxorubicin as shown in compounds 8a and 8c and 7c. Some compounds were not active as 7d and 8b that showed no inhibitory activity towards tumor cells.

EXPERIMENTAL

All melting points were determined on an electrothermal apparatus and are uncorrected. IR spectra were recorded (KBr discs) on a Shimadzu FT-IR 8201 PC spectrophotometer. 1H NMR spectra were recorded in CDCl3 and (CD3)2SO solutions on a Varian Gemini 300 MHz spectrometer and chemical shifts were expressed in δ units using TMS as internal reference. Elemental analyses were carried out at the Microanalytical Center of the Cairo University. Compounds 2a,b and pyrimidine thione 3a-d and 9a-d were prepared as previously reported[17,18]. Cytotoxic activity was preceded in National Cancer of research, Cairo University, Egypt.

Synthesis of 1-(2-(benzo[d]oxazol-2-yl)hydrazono)-1-chloropropan-2-one (2a) and 1-(2-(benzo[d]thiazol-2-yl)hydrazono)-1-chloropropan-2-one (2b)

The appropriate amount of diazotized [4-(1,3-benzoazol-2-yl)phenyl]amine (1a) and diazotized [4-(1,3-benzothiazol-2-yl)phenyl]amine (1b) (0.005 mol) were added dropwise to cold solution (0 ºC) of 3-chloro-2,4-pentanone (1.65 g, 0.005 mol) and sodium acetate trihydrate (0.65 g, 0.005 mol) while stirring. The yellow precipitate was collected and recrystallized from ethanol to give 2a and 2b

2a

yellow (yield 85%), m.p. 199-202 ºC (EtOH), νmax/cm⁻¹(KBr) 3258 (NH), 3055 (CH, aromatic), 2981 (CH, aliphatic), 1655 (CO), 1605 (C=C), 1365 (CH3); 1H NMR: δ = 2.10 (s, 3H, CH3CO), 7.40 (d, 2H, J = 8Hz, ArH’s), 7.50 (d, 2H, J = 8Hz, ArH’s), 8.00 (d, 2H, J = 8Hz, ArH’s), 8.07 (d, 2H, J = 8Hz, ArH’s) and 10.89 (s, br., 1H, NH). (calcd for C16H12ClN3O2: C, 61.25; H, 3.86; Cl, 11.30; N, 13.39 %). Found C, 61.00; H, 3.75; Cl, 11.40; N, 13.42%.

2b

yellow (yield 80%), m.p. 228-229 ºC (EtOH), νmax/cm⁻¹(KBr) 3132 (NH), 3055 (CH, aromatic), 2926 (CH, aliphatic), 1650 (CO), 1605 (C=C), 1335 (CH3); 1H NMR: δ = 2.11 (s, 3H, CH3), 7.40 (d, 2H, J = 8Hz, ArH’s), 7.50 (d, 2H, J = 8Hz, ArH’s), 8.20 (d, 2H, J = 8Hz, ArH’s), 8.37 (d, 2H, J = 8Hz, ArH’s) and 10.89 (s, br., 1H, NH). (calcd for C16H12ClN3OS: C, 58.27; H, 3.67; Cl, 10.75; N, 12.74; S, 9.72 %). Found C, 58.15; H, 3.75; Cl, 10.91; N, 12.65; S, 9.85%.

Synthesis of 1,2,4-triazolo[4,3-a]pyrimidines (7 and 8)a–d

Method A

A mixture of the appropriate hydrazonoyl halides 2a,b (0.005 mol) and the appropriate 3a-d (1.9 g, 0.005 mol) in chloroform containing triethylamine (0.75 ml, 0.005 mol) was refluxed for 10 h. Chloroform was evaporated under reduced pressure and the residue solid was crystallized from DMF to give (7, 8)a-d, respectively.

Method B

Equimolar amounts of the hydrazonoyl halides 2a,b, 9a-d, and sodium ethoxide (0.005 mol each) in ethanol (20 ml) were refluxed for 3 h. The reaction mixture was cooled and the resulting solid was collected and crystallized from ethanol to give products identical in all respects (mp, mixed mp, and spectra) with corresponding products obtained by method A.

7a

yellow (yield 80%), m.p. 298-300 ºC, νmax/cm⁻¹(KBr) 3055 (CH, aromatic), 2983 (CH, aliphatic), 1735 (CO, ester), 16560 (CO), 1620 (C=N), 1600 (C=C), 1470 (CH2), 1361 (CH3); 1H NMR: δ = 1.18 (d, 3H, CH2CH2), 2.10 (s, 3H, CH3CO), 2.21 (s, 3H, CH3), 4.21 (q, 2H, CH2CH2), 6.62 (s, 1H, CH), and 7.17-8.35 (m, 13H, ArH); (calcd for C30H25N5O4: C, 69.35; H, 4.85; N, 13.48 %). Found C, 69.15; H, 4.75; N, 13.40 %.

7b
Orange (yield 67%), m.p. 234-36°C, \( v_{\text{max}} \) cm\(^{-1}\): \( ^1{(\text{KBr})} 3055 \) (CH, aromatic), 2983 (CH, aliphatic), 1730 (CO, ester), 16560 (CO), 1620 (C=N), 1600 (C=C), 1470 (CH\(_3\)), 1361 (CH\(_3\)); \(^1{\text{H NMR}}: \delta = 1.15 \) (t, 3H, CH\(_2\)), 2.20 (s, 3H, CH\(_3\)), 2.68 (s, 3H, CH\(_3\)), 3.82 (s, 3H, OCH\(_3\)), 3.87 (s, 3H, OCH\(_3\)), 4.24 (q, 2H, CH\(_2\)), 5.65 (s, 1H, pyrimidine H-4), 7.42-8.33 (m, 13H, ArH’s); (cited for C\(_{33}\)H\(_{31}\)N\(_5\)O\(_2\): C, 66.31; H, 5.04; N, 12.13 %). Found C, 66.22; H, 5.14; N, 12.13 %.

Yellow (yield 85%), m.p. 274-76°C, \( v_{\text{max}} \) cm\(^{-1}\): \( ^1{(\text{KBr})} 3055 \) (CH, aromatic), 2983 (CH, aliphatic), 1725 (CO, ester), 16560 (CO), 1620 (C=N), 1600 (C=C), 1470 (CH\(_3\)), 1361 (CH\(_3\)); \(^1{\text{H NMR}}: \delta = 1.15 \) (t, 3H, CH\(_2\)), 1.18 (d, 6H, (CH\(_2\))\(_2\)), 2.20 (s, 3H, CH\(_3\)), 2.68 (s, 3H, CH\(_3\)), 2.88 (sex, 1H, (CH\(_2\))\(_2\)), 4.24 (q, 2H, CH\(_2\)), 5.65 (s, 1H, pyrimidine H-4), 7.42-8.33 (m, 13H, ArH’s); (cited for C\(_{33}\)H\(_{31}\)N\(_5\)O\(_2\): C, 68.61; H, 5.41; N, 12.12; S, 5.55%. Found C, 68.54; H, 5.41; N, 12.12; S, 5.55%.

8a

Yellow (yield 85%), m.p. >300°C, \( v_{\text{max}} \) cm\(^{-1}\): \( ^1{(\text{KBr})} 3055 \) (CH, aromatic), 2983 (CH, aliphatic), 1725 (CO, ester), 16560 (CO), 1620 (C=N), 1600 (C=C), 1470 (CH\(_3\)), 1361 (CH\(_3\)); \(^1{\text{H NMR}}: \delta = 1.18 \) (t, 3H, CH\(_2\)), 2.20 (s, 3H, CH\(_3\)), 2.65 (s, 3H, CH\(_3\)), 4.24 (q, 2H, CH\(_2\)), 5.65 (s, 1H, pyrimidine H-4), 7.42-8.33 (m, 13H, ArH’s); (cited for C\(_{30}\)H\(_{29}\)N\(_5\)O\(_2\): C, 69.35; H, 4.85; N, 13.48 %). Found C, 69.35; H, 4.85; N, 13.48 %.

In vitro antitumor activity measurement

The cytotoxicity was carried out using sulphorhodamine-B (SRB) assay. Cells will be seeded in 96 well microtiter plates at a concentration of 1000-2000 cells/well, 100 µl/well. After 24 hrs, cells will be incubated for 72 h with various concentrations of drugs 1, 2.5, 5, 10 µg/ml). For each derivative concentration and doxorubicin, 3 wells were used. The plates were incubated for 72 hours. The medium is discarded. The cells were fixed with 150 µl cold trichloroacetic acid 10% final concentration for 1 hour at 4°C. The plates were washed.
with distilled water using (automatic washer Tecan, Germany) and stained with 50 µl 0.4 % SRB dissolved in 1 % acetic acid for 30 minutes at room temperature in dark. The plates were washed with 1 % acetic acid to remove unbound dye and air-dried [24 hrs.]. The dye was solubilized with 150 µl/well of 10 m Mtris base (PH 7.4) for 5 min on a shaker at 1600 rpm. The optical density (OD) of each well will be measured spectro photometrically at 490 nm with an ELISA microplate reader. The mean background absorbance was automatically subtracted and mean values of each derivative and doxorubicin concentration was calculated. The experiment was repeated 3 times. The percentage of cell survival was calculated as follows: Surviving fraction = O.D. (treated cells)/ O.D. (control cells).

REFERENCES