Synthesis, anticancer activity and structure-activity relationship of some anticancer agents based on Cyclopenta (b) thiophene scaffold

Mohamed Said and Hosam Elshihawy*
Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Suez Canal University, Egypt

Abstract: Methods for the synthesis of new heterocyclic systems of thieno (3,2-d)-(1,2,3)-triazine derivatives and N-(3-cyano-5,6-dihydro-4H-cyclopenta (b) thiophene derivatives have been developed. The newly synthesized compounds were tested in vitro against human breast carcinoma cell line (MCF-7). Compounds 7 and 9 have shown the highest activity among the two synthesized series. The results of this study have led to the identification of two lead compounds with good inhibitory activities that can confirm the design of the next generation inhibitors of tyrosine kinase with fewer side effects such as hepatotoxicity and resistance.

Keywords: synthesis, tyrosine kinase, carcinoma, inhibitory activity, MCF-7.

INTRODUCTION

Intra- or inter-cellular communication disorders are a major cause of pathogenic mechanisms. For this reason, modern drug research has become increasingly focused on signal transduction therapy and many of the recently validated targets are transduction-related macromolecules, especially kinases. Kinase activity is known to be over-expressed in a large percentage of clinical cancer of various types (Pamula et al., 2007; Jardines et al., 1993) (e.g., breast, ovarian, colon, prostate) and to be closely related to a poor prognosis in patients (Hickey et al. 1994; Herbst et al., 2003). Accordingly, kinases had become an important target for drug design (Dobrusin and Fry, 1992). Protein kinases (PTKs) catalyze the phosphorylation of tyrosine and serine/threonine residues in various proteins involved in the regulation of all functions (Jordan et al., 2000).

Protein phosphorylation mechanism is one of the most significant signal transduction mechanisms by which inter-cellular signals regulate crucial intra-cellular processes such as ion transport, cellular proliferation and differentiation, and hormone responses. All PTKs have a region in their active site that recognizes ATP, which is the phosphorylating agent in all cases, as well as another for their substrates. They can be broadly classified as receptor such as EGFR, or non-receptor kinases. In spite of having a common substrate, the ATP binding sites are relatively different for different kinases, some selectivity in the inhibition is possible (Wullschleger et al., 2006). Most clinically used inhibitors act in the ATP recognition sites, such as dasatinib (I), lpatinib (II) and gefitinib (ZD1839, Iressa®) (III) (Vansteenkiste, 2004; Bonomi 2003).

Intensive research in the area of tyrosine kinase inhibitors led to development of enormous number of active compounds which must be devoid of side effects reported for tyrosine kinase inhibitors like hepatotoxicity, acquired resistance, renal failure and cardiotoxicity (Rowinsky et al., 2004; Kluthko et al., 2006; Yue-Mei et al., 2004; Hennequin et al., 2006; Peter et al. 2006; Alessandra et al., 2006; Madhavi et al., 2007; Yi et al. 2005; Abouzid et al. 2008; Richard et al., 2007; Rahul et al., 2010; Yi et al., 2013, Yi-fan et al., 2011; Gafter-Gvili et al., 2010; Abouzid et al., 2008).

In the same direction, and in continuing effort to find more potent selective lead compound, herein, we describe the design and synthesis of two series of 5,6-dihydro-4H-cyclopenta (b) thiophene derivatives (IV, V) using thiophene-3-carbonitrile to mimic the effect of dasatinib and other active thiophene-3-carbonitrile containing compounds in addition of using triazine moiety instead of pyrimidine containing compound such as gefitinib as possible anticancer agents that may act in the ATP recognition sites as TK inhibitors.

Chemistry

Thiophene intermediate 3-amino-5,6-dihydro-4H-cyclopenta (b) thiophene-2-carbonitrile (1) was synthesized by the condensation of cyclopentanone, elemental sulphur and malononitrile (El-Ayaan et al., 2006; Taylor et al., 1997; Bossemeyer, 1995; Gewald, 1965; Sauter et al., 1995). Compound (2) was prepared by treating the thiophene 2-amino-5,6-dihydro-4H-cyclopenta[b] thiophene-carbonitrile (1) with sodium nitrite in the presence of hydrochloric and acetic acids at 0-5°C (Paronikyan et al., 2006).

N-chloroacetylated derivative (2) was in turn allowed to react with sulfonamides in dry DMF or with aminophenol in dioxane and few drops of triethylamine to give the
corresponding N-phenylaminoacetamide derivatives (4-8) (El-Subbagh and Al-Obaid, 1996).

On the other hand, compounds (9-13) were obtained by refluxing 4-chlorothieno[3,2-d]-1,2,3-triazines (3) with aromatic amines in dry pyridine, pyridinium salts formed, were removed by washing the separated solids with ice water. However, in the case of sulfa drugs (3) was added portionwise with stirring to the hot solution of sulfonamides in dry pyridine (Al-Obaid et al., 2009; Monge et al., 1981). The structures of the newly synthesized compounds were confirmed by microanalysis and other spectral methods.

MATERIALS AND METHODS

Experimental

2-Amino-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carboxylic acid (1)

Sulfur (127 mg, 3.96 mmol) was added to a solution of cyclopentanone (3.96 mmol) in (25mL) and malononitrile (3.96 mmol). The mixture was stirred at 45°C for 15 minutes. The mixture was further stirred at 60°C for 18 hrs. The mixture was filtered while hot. The resulting crystals were washed with 30% EtOH, dried and recrystallized from the appropriate solvent. m.p. 135°C, (yield 80%) (Gewald, 1965).

2-Chloro-N-(3-cyano-5,6-dihydro-4H-cyclopenta[b]thiophen-2-yl) acetamide (2)

Chloroacetyl chloride (10 mmol) was added dropwise to a solution of (1) (10 mmol) in dioxane (30 mL) and triethylamine (1 mL). The solution continued stirring and cooled for few minutes. The reaction mixture was subsequently refluxed for 10 hrs, then cooled and poured onto crushed ice. The crude product was filtered and recrystallized from DMF/ethanol mixture in a ratio of (1:10) respectively. m.p. 148°C, (yield 62%) (El-Shafei et al., 1992).

N-(3-cyano-5,6-dihydro-4H-cyclopenta[b]thiophen-2-yl)-2-(hydroxyphenylamino) acetamide derivatives (4, 5)

An equimolar amount (2mmol) of N-chloroacetylated derivatives (2) and the appropriate aminophenol were dissolved in dioxane (10mL). The reaction mixture was treated with (1mL) triethylamine, heated under reflux for 15 and 14 hrs respectively. The precipitated was collected by filtration, washed with water, dried, and recrystallized from the appropriate solvent (El-Subbagh and Al-Obaid 1996).

N-(3-Cyano-5,6-dihydro-4H-cyclopenta[b]thiophen-2-yl)-2-(4-hydroxyphenylamino) acetamide (4)

IR (KBr, cm−1): 1500 (C=C), 3780 (OH). 1H-NMR (DMSO, 300 MHz): 1.76-3.10 (m, 6H, CH2 cyclopentane), 3.42 (s, 3H, CH3 oxazole), 3.92 (s,2H,COCH2), 5.84 (s, 1H, OH), 7.17-7.98 (m,4H, ArH), 9.21 (s,1H, OH), 11.41 (s, 1H, CONH). Anal.Calcd. for C16H15N3O2S: C, 61.35; H, 5.00; N, 13.66. Found: C, 61.70; H, 4.97; N, 13.66.

N-(3-Cyano-5,6-dihydro-4H-cyclopenta[b]thiophen-2-yl)-2-(3-hydroxyphenylamino) acetamide (5)

IR (KBr, cm−1): 1500 (C=C), 1170 (SO2), 1580 (C=O), 1800 (C=O), 2100 (CN), 3730 (NH). 1H-NMR (DMSO, 300 MHz): 1.66-3.12 (m,6H, CH2 cyclopentane), 3.42 (s, 2H, CH2CO), 6.56 (s, 2H, ArH+NHAr), 6.86-8.57 (m, 3H, ArH), 9.78 (s,1H, OH), 11.19 (s, 1H, CONH). Anal.Calcd. for C16H15N3O2S: C, 61.32; H, 4.82; N, 13.41. Found: C, 61.70; H, 4.97; N, 13.66.

N-(3-Cyano-5,6-dihydro-4H-cyclopenta[b]thiophen-2-yl)-2-(4-(N-substituted-sulfamoyl) phenylamino)acetamide derivatives (6-8)

A mixture of N-chloroacetylated derivatives (2) (1 mmol), N, N-dimethylformamide (5mL), the appropriate sulfa drug (1 mmol), and (0.1ml) triethylamine was heated under reflux as mentioned in table 1. The reaction mixture was cooled and poured into crushed ice. The crude product was filtered and recrystallized from the appropriate solvent (El-Subbagh and Al-Obaid 1996).

N-(3-Cyano-5,6-dihydro-4H-cyclopenta[b]thiophen-2-yl)-2-(4-(N-methylisoxazol-3-yl)sulfonyl) phenylamino) acetamide (6)

IR (KBr, cm−1): 1100 (C-O-C, oxazole), 1710 (SO2), 1580 (C=O), 1580 (C=O), 1800 (C=O), 2100 (CN), 3730 (NH). 1H-NMR (DMSO, 300 MHz): 1.75-1.82 (m,2H,CH2 cyclopentanone), 2.72-2.83 (m, 4H, CH2CH2 cyclopentanone), 3.10 (s,3H,CH3 oxazole), 3.92 (s,2H,COCH2), 5.84 (s, 1H, CH oxazole), 6.53 (s,1H,NHAr), 7.17-7.98 (m,4H, ArH), 12.31 (br s,2H, CONH+S SO2NH). Anal.Calcd. for
3-(5,6-Dihydro-7H-cyclopenta[4:5]thieno[2,3-d]-1,2,3-triazin-4-ylamino)phenol (10)
IR (KBrs cm⁻¹): 1450 (C=C), 1600-1700 (C=N), 3630 (NH), 3720 (OH). Anal.Calcd. for C_{14}H_{12}N_{6}O_{3}S_{2}: C, 59.14; H, 4.25; N, 19.70. Found: C, 58.92; H, 3.90; N, 19.37.

4-(5,6-Dihydro-7H-cyclopenta[4:5]thieno[2,3-d]-1,2,3-triazin-4-ylamino)-N-substituted benzenesulfonamides (11-13)
4-Chlorothienotriazine (3) (3 mmol) was added portionwise with stirring to the appropriate sulfa drug (3 mmol) in dry pyridine (10mL). The reaction mixture was heated under reflux as mentioned in table 1. The mixture was filtered while hot, washed with ice water, dried and recrystallized from the suitable solvent (Monge et al., 1981).

4-(5,6-Dihydro-7H-cyclopenta[4:5]thieno[3,2-d]-1,2,3-triazin-4-ylamino)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (11)
IR (KBrs cm⁻¹): 1100 (C=O, oxazole), 1170 (SO₂), 1570 (C=N), 3520 (NH). Mass spectrum: m/z: 428 (M⁺, 4.74%), 156 (100%), 108 (52.15%). Anal.Calcd. for C_{18}H_{15}N_{7}O_{2}S_{2}: C, 50.45; H, 3.76; N, 19.61. Found: C, 50.85; H, 4.05; N, 19.75.

4-(5,6-Dihydro-7H-cyclopenta[4:5]thieno[2,3-d]-1,2,3-triazin-4-ylamino)-N-(quinazolin-2-yl)benzenesulfonamide, sodium salt (12)
IR (KBrs cm⁻¹): 1150 (SO₂), 1500 (C=C), 1580 (C=N), 3700(NH). Anal.Calcd. for C_{18}H_{16}N_{7}O_{2}S_{2}: C, 48.21; H, 3.37; N, 21.86. Found: C, 48.52; H, 3.70; N, 22.10.

4-(5,6-Dihydro-7H-cyclopenta[4:5]thieno[2,3-d]-1,2,3-triazin-4-ylamino)-N-(quinazolin-2-yl)benzenesulfonamide, sodium salt (13)
IR (KBrs cm⁻¹): 1100 (SO₂), 1500 (C=C), 1620 (C=N), 3710 (NH). Anal.Calcd. for C_{22}H_{17}N_{7}O_{2}S_{2}: C, 53.00; H, 3.44; N, 19.67. Found: C, 52.64; H, 3.25; N, 19.56.

Anticancer activity
Skehan's method
The biological testing was done on the human tumor cell line (MCF-7) obtained as a gift from NCI, Meriyland, USA. The cytotoxic activity was measured in vitro for the newly synthesized compounds using the Sulforhodamine-B stain SRB assay using the method of Skehan Cells was plated in 96-multiwell microtiter plate (10⁴cells/well) for 24 hrs before treatment with the compound (s) to allow attachment of cell to the wall of the plate. Tested compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the compound under investigation (0.1, 2.5, 5 and 10 µg/mL) were added to the cell monolayer.
Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37°C and in an atmosphere of 5% CO₂. After 48 hrs, Cells were fixed, washed and stained for 30 minutes with 0.4% (wt/vol) with SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration was plotted to get the survival curve for breast tumor cell line after the specified time (Skehan et al., 1990).

The inhibitory activities (IC₅₀) of tested compounds are given in table 2. IC₅₀ values are the average of at least three independent experiments.

RESULTS

Chemistry

o-Amino carbonitriles of thiophenes (1) were prepared according to the reported procedures, utilizing the Gewald’s thiophene synthesis. It involves a multi-component condensation between elemental sulfur, α-methylene carbonyl compound and activated nitrile such as α-cyanonitriles (malononitrile) in the presence of morpholine to afford the corresponding 2-aminothiophene (1). The N-chloroacetylated intermediate (2) were obtained in a good yield upon treating the starting material (1) with chloroacetyl chloride and triethylamine in a nucleophilic substitution reaction to afford the corresponding N-chloroacetylated derivatives (El-Shafei et al., 1992).

The final step in the synthesis of this series involved nucleophilic displacements of the chlorine atom of compound 2 with a variety of substituted sulphonamides and amino phenols (El-Subbagh and Al-Obaid 1996).
On the other hand, the dinitrity of 2-amino-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carbonitrile resulted in the formation of 4-chlorothieno(3,2-d)-1,2,3-triazine derivative (3) which in turn was allowed to react with various primary and secondary aromatic amines to give the target 4-anilino derivatives (9-13) (Al-Obaid et al., 2002). Intensive investigations were done on numerous and different types of cell lines by our scientific group and we have revealed that EGFR-TK is highly over-expressed in cell line (MCF-7) rather than other different cell lines therefore our research investigations were concentrated on this type of cell line (Abouzid and Shouman 2008). The ATP binding site in kinase family is considered as a suitable target of an expanding class of anticancer drugs that are specific kinase inhibitors.

**DISCUSSION**

In the present study, we developed a new series of cyano-substituted compounds based on cyclopenta[b]thiophene scaffold that are promising inhibitors against the growth of cultured human breast carcinoma cell line (MCF-7). Intensive investigations were done on numerous and different types of cell lines by our scientific group and we have revealed that EGFR-TK is highly over-expressed in cell line (MCF-7) rather than other different cell lines therefore our research investigations were concentrated on this type of cell line (Abouzid and Shouman 2008). The ATP binding site in kinase family is considered as a suitable target of an expanding class of anticancer drugs that are specific kinase inhibitors.
Table 1: It shows Yields and Physicochemical Characteristics of Compounds 4-13

<table>
<thead>
<tr>
<th>Cpd. No.</th>
<th>Recrystallization Solvent</th>
<th>Yield (%)</th>
<th>Reaction time, hours</th>
<th>M.P. (°C)</th>
<th>Molecular formulae (M. Wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Ethanol</td>
<td>50</td>
<td>15</td>
<td>250</td>
<td>C_{16}H_{15}N_{3}O_{2}S (313)</td>
</tr>
<tr>
<td>5</td>
<td>Ethanol</td>
<td>48</td>
<td>14</td>
<td>250</td>
<td>C_{16}H_{15}N_{3}O_{2}S (313)</td>
</tr>
<tr>
<td>6</td>
<td>Acetone</td>
<td>46</td>
<td>15</td>
<td>&gt;300</td>
<td>C_{20}H_{10}N_{6}O_{2}S (457)</td>
</tr>
<tr>
<td>7</td>
<td>Acetone</td>
<td>40</td>
<td>15</td>
<td>265</td>
<td>C_{20}H_{18}N_{2}NaO_{2}S (477)</td>
</tr>
<tr>
<td>8</td>
<td>Acetone</td>
<td>45</td>
<td>15</td>
<td>270</td>
<td>C_{24}H_{20}N_{6}NaO_{2}S (527)</td>
</tr>
<tr>
<td>9</td>
<td>Methanol</td>
<td>45</td>
<td>16</td>
<td>280</td>
<td>C_{14}H_{12}N_{2}OS (284)</td>
</tr>
<tr>
<td>10</td>
<td>Methanol</td>
<td>40</td>
<td>14</td>
<td>&gt;300</td>
<td>C_{14}H_{12}N_{2}OS (284)</td>
</tr>
<tr>
<td>11</td>
<td>Ethanol</td>
<td>65</td>
<td>16</td>
<td>&gt;300</td>
<td>C_{18}H_{16}N_{2}NaO_{2}S (428)</td>
</tr>
<tr>
<td>12</td>
<td>Ethanol</td>
<td>70</td>
<td>16</td>
<td>&gt;300</td>
<td>C_{18}H_{16}N_{2}NaO_{2}S (448)</td>
</tr>
<tr>
<td>13</td>
<td>Ethanol</td>
<td>50</td>
<td>16</td>
<td>&gt;300</td>
<td>C_{22}H_{17}N_{2}NaO_{2}S (498)</td>
</tr>
</tbody>
</table>

IC50, drug concentration resulting in a 50% inhibition of the EGFR tyrosine kinase

Table 2: It shows anticancer Activity of Compounds (4, 7, 9 and 10) Compounds 7 and 9 have shown the highest activity among the two synthesized series

<table>
<thead>
<tr>
<th>Cpd No</th>
<th>Surviving fraction (%)</th>
<th>IC50 (nmol/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (µg/mL)</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>82</td>
<td>57</td>
</tr>
<tr>
<td>7</td>
<td>80</td>
<td>55</td>
</tr>
<tr>
<td>9</td>
<td>75</td>
<td>44</td>
</tr>
<tr>
<td>10</td>
<td>76</td>
<td>58</td>
</tr>
</tbody>
</table>

The continuous decrease in the percentage of surviving fraction clearly defined the inhibition activity of the series of compounds, 4 (at 5 µg/ml/82% survival), (at 12 µg/ml/57% survival), (at 25 µg/ml/28% survival), (at 50 µg/ml/20% survival); and 7 (at 5 µg/ml/80% survival), (at 12 µg/ml/55% survival), (at 25 µg/ml/26% survival), (at 50 µg/ml/21% survival). Likewise, the replacement of pyrimidine ring by triazine moiety in gefitinib like compounds (scheme 3) was also effective. This was ensured by the cytotoxic effect of compounds 9 and 10 (table 2); compound 9 (at 5 µg/ml/75% survival), (at 12 µg/ml/44% survival), (at 25 µg/ml/13% survival); and compound 10 (at 5 µg/ml/76% survival), (at 12 µg/ml/58% survival) (at 25 µg/ml/12% survival). So, Compounds 7 and 9 were the highest active compounds among the two synthesized series.

CONCLUSION

The overall outcome expected from this study revealed that: The aromatic ring (hydrophobic region) attached to NH fragment (H-bonding donor region) and a hydrophobic region represented by cyclopenta (b) thiophene core are three essential points that play an important role in cytotoxicity. The presence of one hydrogen atom attached to the nitrogen of anilino moiety as a hydrogen bonding donor is essential for activity. The m-substituted hydroxyl derivative 10 is less active than the p-isomer 9.

The presence of aryl moiety at the 4-amino position is necessary for the activity as hydrophobic region. (fig. 3)

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REFERENCES


