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

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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-4*H*-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 (Pannala *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), lapatinib (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 me be devoid of side effects reported for tyrosine kinase inhibitors like hepatotoxicity, acquired resistance, renal failure and cardiotoxicity (Rowinsky *et al*, 2004; Klutchko *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-4*H*-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-4*H*-cyclopenta (*b)* thiophene-2-carbonitrile (1) was synthesized by the condensation of cyclopentanone, elemental sulphur and malononitrile (El-Ayaan *et al*., 2007; Taylor *et al*., 1997; Bossemeyer, 1995; Gewald, 1965; Sauter *et al*., 1995). Compound (2) was prepared by treating the thiophene *o*-amino nitrile (1) with chloroacetyl chloride and triethylamine to afford the corresponding *N*-chloroacetylated derivative (2) (El-Shafei *et al*., 1992). The triazine ring was obtained by dinitriting 2-amino-5,6-dihydro-4*H*-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

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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

Melting points were measured in open capillary tubes using Stuart melting point apparatus SMP10 and are uncorrected. Infrared (IR) spectra were recorded using KBr discs on a Shimadzu Spectrophotometer (υ_{max} in cm^{-1}). Proton Magnetic Resonance (^{1}H-NMR) spectra were recorded on Mercury-300 BB (NMR300) spectrometer (300MHz). Chemical shifts are reported in δ values (parts per million, ppm) relative to tetramethylsilane (TMS) as internal standard. Abbreviation used in NMR analysis are as follows: d=doublet, dd=doublet of doublets, m=multiplet, q=quartet, s=singlet, t=triplet. Electron impact mass spectra (EI-MS) were recorded on DI Analysis Shimadzu QP-2010 Plus mass spectrometer. IR, ^{1}H-NMR, EI-MS and elemental analyses were performed in the micro analytical center, Cairo University, Egypt. The TLC analyses were conducted on silica gel 60F254 plates and visualized with UV light eluted in acetone-hexane-pyridine 1:4:1 (compounds 1-3), chloroform-ether 1:3 (compounds 4-8) or chloroform-ether 1:4 (compounds 9-13) solvent mixtures.

Experimental

2-Amino-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carbonitrile (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. Morpholine (5.54 mmol) was added dropwise over 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), 1600 (C=N), 1680 (C=O), 2100 (CN), 3610 (NH), 3780 (OH). ^{1}H-NMR (DMSO, 300 MHz): 1.76-3.10 (m, 6H, CH_2 cyclopentane), 4.01 (s, 2H, $COCH_2$), 6.52 (s, 1H, NHAr), 6.73-8.51 (m, 4H, ArH), 9.21 (s,1H, OH), 11.41 (s, 1H, CONH). Anal.Calcd. for $C_{16}H_{15}N_3O_2S$: 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-(3-hydroxyphenylamino) acetamide (5)

IR (KBr, cm^{-1}): 1500 (C=C), 1600 (C=N), 1680 (C=O), 2100 (CN), 3700 (NH), 3800 (OH). ^{1}H-NMR (DMSO, 300 MHz): 1.66-3.12 (m,6H, CH_2 cyclopentane), 3.42 (s, 2H, $COCH_2$), 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 $C_{16}H_{15}N_3O_2S$: C, 61.32; H, 4.82; N, 13.41. Found: C, 61.35; H, 5.00; N, 13.50.

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

IR (KBr, cm^{-1}): 1100 (C-O-C, oxazole), 1170 (SO_2), 1500 (C=N), 1580 (C=O), 1800 (C=O), 2100 (CN), 3730 (NH). ^{1}H NMR (DMSO, 300 MHz): 1.75-1.82 (m,2H,CH_2 cyclopentane), 2.72-2.83 (m, 4H, $(CH_2)_2$ cyclopentane), 3.10 (s,3H,CH_3 oxazole), 3.92 (s,2H,$COCH_2$), 5.84 (s, 1H,CH oxazole), 6.53 (s,1H,NHAr), 7.17-7.98 (m,4H, ArH), 12.31 (br s,2H, CONH+SO_2NH). Anal.Calcd. for

$C_{20}H_{19}N_5O_4S_2$: C, 52.50; H, 4.19; N, 15.31. Found: C, 52.12; H, 4.20; N, 14.98.

N-(3-Cyano-5,6-dihydro-4H-cyclopenta[b]thiophen-2-yl)-2-(4-(N-(pyrimidin-2yl) sulfamoyl,sodiumsalt) phenylamino) acetamide (7)

IR (KBr, cm^{-1}): 1170 (SO_2), 1500 (C=C), 1580 (C=N), 1680 (C=O), 2100 (CN), 3750 (NH). ^{1}H-NMR (DMSO, 300 MHz): 1.34-1.41 (m, 4H, $(CH_2)_2$ cyclopentane), 3.85 (s, 2H, $COCH_2$), 4.36-4.470 (m, 2H, CH_2 cyclopentane), 6.21 (s, 1H, NHAr), 7.24-9.16 (m, 7H, ArH), 12.03 (s, 1H, SO_2NH), 12.33 (s, 1H, CONH). Anal.Calcd. for $C_{20}H_{18}N_6NaO_3S_2$: C, 50.31; H, 3.80; N, 17.60. Found: C, 50.00; H, 3.48; N, 17.35.

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

IR (KBr, cm^{-1}): 1100 (SO_2), 1500 (C=C), 1580 (C=N), 1720 (C=O), 2100 (CN), 3800 (NH). ^{1}H-NMR (DMSO, 300 MHz): 1.85-2.32 (m, 4H, $(CH_2)_2$ cyclopentane), 2.81-2.92 (m,2H, CH_2 cyclopentane), 3.99 (s,2H, $COCH_2$), 5.95 (s,1H, NHAr), 6.86-7.07 (m, 4H, ArH), 7.49-7.66 (m, 4H, ArH), 8.08 (s, 1H, ArH), 10.95 (s,1H, SO_2NH), 11.92 (s,1H, CONH). Anal.Calcd. for $C_{24}H_{20}N_6NaO_3S_2$: C, 54.64; H, 3.82; N, 15.93. Found: C, 54.42; H,3.45; N,15.63.

4-Chlorocyclopenta[4:5]thieno[2,3-d]-1,2,3-triazine (3)

A solution of sodium nitrite (1 gm, 16 mmol) was added to a stirred mixture of compound 1 (10 mmol), HCl (10 mL, 5%) and glacial acetic acid (10mL). The mixture was stirred at 0-5°C for 1 hr then continued stirred for 2 hrs at 20°C. The mixture was poured into 100 ml of water, and the precipitate was separated by filtration, washed with water, and dried to obtain compound (3). m.p. 200˚C (31) (yield 81.8%) (Paronikyan *et al*., 2006).

(5,6-Dihydro-7H-cyclopenta[4:5]thieno[2,3-d]-1,2,3-triazin-4-ylamino)phenols (9, 10)

A mixture of equimolar amounts (0.01 mol) of 4-chlorothienotriazine (3) and the appropriate aminophenol in dry pyridine (10 mL) was refluxed for 16 and 14 hrs respectively. The mixture was filtered while hot and washed with ice-water to remove the pyridinium salts formed. The precipitate was then recrystallized from the suitable solvent (Al-Obaid *et al.,* 2009).

4-(5,6-Dihydro-7H-cyclopenta (4:5) thieno (2,3-d)-1,2,3-triazin-4-ylamino)phenol (9)

IR (KBr, cm^{-1}): 1500 (C=C), 1600-1700 (C=N), 3500 (NH), 3800 (OH). ^{1}H NMR (DMSO, 300 MHz): 1.75-2.33 (m, 2H, CH_2 cyclopentane), 2.72-3.39 (m, 4H, $(CH_2)_2$ cyclopentan), 6.52-8.51 (m, 4H, ArH), 9.63 (s, 2H, NHAr + OH). Anal.Calcd. for $C_{14}H_{12}N_4OS$: C, 59.14; H, 4.25; N, 19.70. Found: C, 58.74; H, 3.89; N, 19.31.

3-(5,6-Dihydro-7H-cyclopenta[4:5]thieno[2,3-d]-1,2,3-triazin-4-ylamino)phenol (10)

IR (KBr, cm^{-1}): 1450 (C=C), 1600-1700 (C=N), 3630 (NH), 3720 (OH). Anal.Calcd. for $C_{14}H_{12}N_4OS$: 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 (KBr, cm^{-1}): 1100 (C-O-C, oxazole), 1170 (SO_2), 1500 (C=C), 1570 (C=N), 3520 (NH). Mass spectrum: m/z: 428 (M^+, 4.74%), 156 (100%), 108 (52.15%). Anal.Calcd. $C_{18}H_{16}N_6O_3S_2$: 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-(pyrimidin-2-yl)-benzenesulfonamide, sodium salt (12)

IR (KBr, cm^{-1}): 1150 (SO_2), 1500(C=C), 1580 (C=N), 3700(NH). Anal.Calcd. for $C_{18}H_{15}N_7NaO_2S_2$: 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-(quinoxalin-2-yl)-benzenesulfonamide, sodium salt (13)

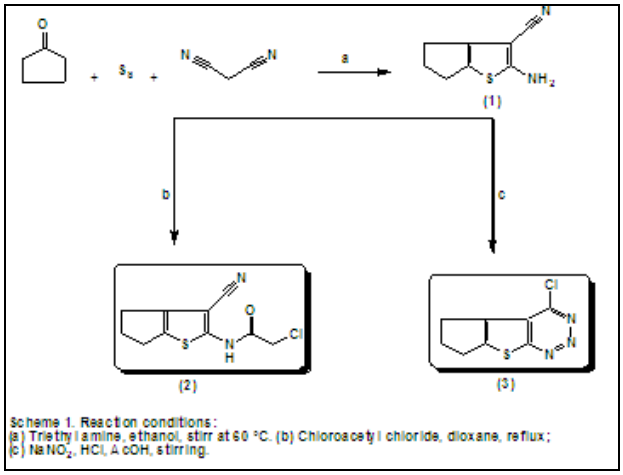
IR (KBr, cm^{-1}): 1100 (SO_2), 1500 (C=C), 1620 (C=N), 3710 (NH). Anal.Calcd. for $C_{22}H_{17}N_7NaO_2S_2^+$: C, 53.00; H, 3.44; N, 19.67. Found: C, 52.64; H, 3.25; N, 19.56.

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 Sulfo-Rhodamine-B stain SRB assay using the method of *Skehan* Cells was plated in 96-multiwell microtiter plate (10^4cells/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% CO_2. 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).



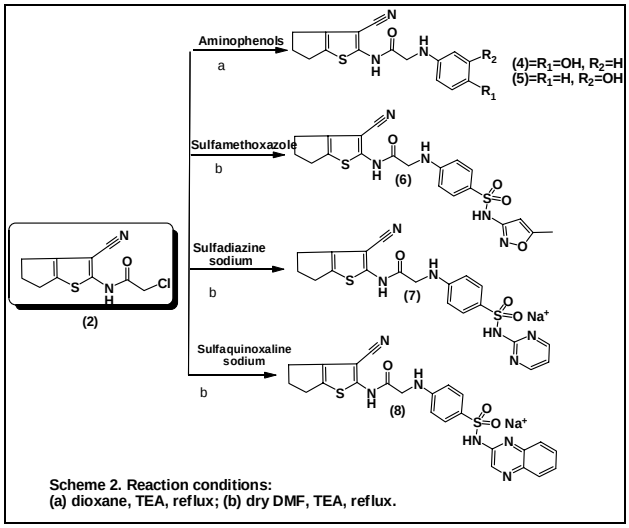
Scheme 1: it shows preparation of Thiophene intermediates (2 and 3). 3-amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-2-carbonitrile (1) was synthesized by the condensation of cyclopentanone, elemental sulphur and malononitrile following Gewald's method. Compound (2) was prepared by treating the thiophene *o*-amino nitrile (1) with chloroacetyl chloride and triethylamine to afford the corresponding *N*-chloroacetylated derivative (2). The triazine ring was obtained by dinitriting 2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-carbonitrile (1) with sodium nitrite in the presence of hydrochloric and acetic acids at 0-5°C .

The inhibitory activities (IC_{50}) of tested compounds are given in table 2. IC_{50} values are the average of at least three independent experiments.

RESULTS

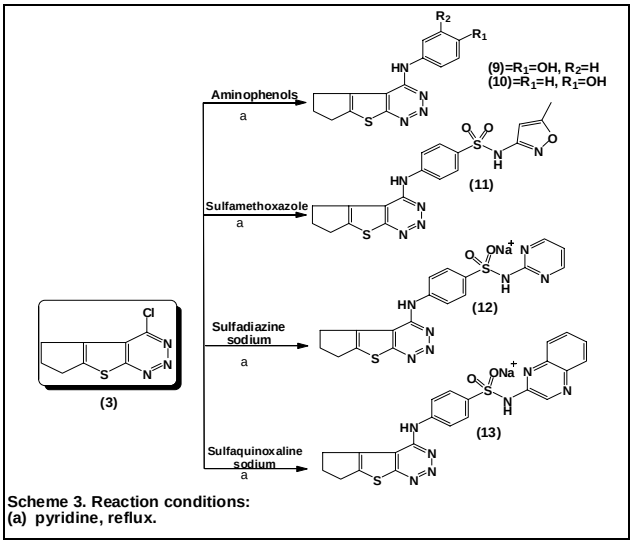
Chemistry

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).



Scheme 2: *N*-chloroacetylated derivative (**2**) was allowed to react with sulfonamides in dry DMF or with amino-phenol in dioxane and few drops of triethylamine to give the corresponding *N*-phenylaminoacetamide derivatives (4-8).

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).



Scheme 3: 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.

On the other hand, the dinitriting of 2-amino-5,6-dihydro-4*H*-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*., 2009; Monge *et al*., 1981).

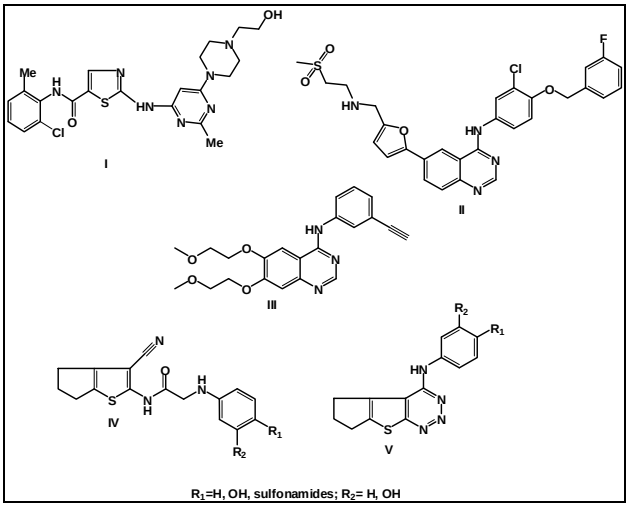


Fig. 1: It shows most clinically used tyrosine kinase inhibitors act in the ATP recognition sites, such as dasatinib (I), lapatinib (II) and gefitinib (ZD1839, *Iressa*®) (III) and two series of 5,6-dihydro-4*H*-cyclopenta (*b*) thiophene derivatives (IV, V) using thiophene-3-carbonitrile to mimic the effect of dasatinib and other active thiophene-3-carbonitrile containing compounds in addition of using triazine moiety instead of pyrimidine in compounds containing it like gefitinib as possible anticancer agents that may act in the ATP recognition sites as TK inhibitors

The NMR spectra of compounds (4-8) showed the NHAr peak at around 12 ppm which confirmed the formation of the product, a characteristic singlet peak at around 3-5 ppm representing the methylene group of acetamide moiety while those of compounds (9-13) were characterized by two important regions: a highly shielded region consisting of aliphatic multiplet signals corresponding to cyclopentane, and a highly deshielded region consisting of aromatic and/or heteroaromatic multiplets corresponding to the 4-anilino moiety, the benzene ring and the heterocycles of the sulfonamide functions. Furthermore, the NMR spectra showed the highly deshielded NH peak at around 11.2 ppm as broad singlet which confirmed the formation of the product. The elemental analyses showed that all the newly synthesized compounds were having purity within ±0.4% of theoretical values.

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.

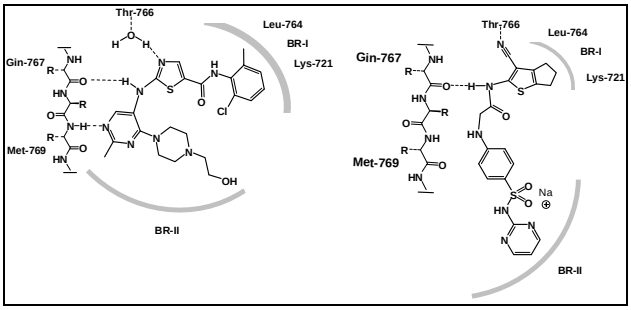


Fig. 2: This figure compares binding modes of dasatinib and **7**, the most active compound in the cyano-substituted series, at the ATP-site of protein tyrosine kinases.

From fig. 2 we concluded that the replacement of thiazole nitrogen in dasatinib structure with a C-CN function could result in a novel series of TK inhibitors because there is no need for water bridging as dasatinib does

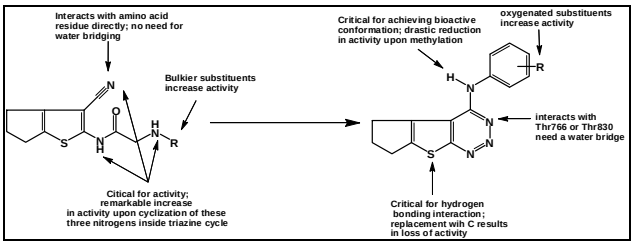


Fig. 3: It shows structure-activity relationship of cyclopenta (*b*) thiophenes for the both prepared series.

These inhibitors are able to form hydrogen bonds with the protein backbone while peripheral groups are oriented towards two hydrophobic pockets called BR-I (binding region-I) and BR-II (Capdeville *et al*., 2002).

The Binding mode of dasatinib was compared and illustrated with compound (**7)** which is the most active compound in the cyano-substituted series, at the ATP-site of protein tyrosine kinases in figs. 2 and 3.

We concluded that the replacement of thiazole nitrogen in dasatinib structure with a C-CN function could result in a novel series of EGFR-TK inhibitors because there is no need for water bridging as dasatinib does. This was ensured by the cytotoxic effect of compounds **4** and 7 (fig. 2) (table 2).

Table 1: It shows Yields and Physicochemical Characteristics of Compounds 4-13

Cpd. No.	Recrystallization Solvent	Yield (%)	Reaction time, hours	M.P. (˚C)	Molecular formulae (M. Wt.)
4	Ethanol	50	15	250	$C_{16}H_{15}N_3O_2S$ (313)
5	*Ethanol*	48	14	250	$C_{16}H_{15}N_3O_2S$ (313)
6	Acetone	46	15	>300	$C_{20}H_{19}N_5O_4S_2$ (457)
7	Acetone	40	15	265	$C_{20}H_{18}N_6NaO_3S_2$ (477)
8	Acetone	45	15	270	$C_{24}H_{20}N_6NaO_3S_2$ (527)
9	Methanol	45	16	280	$C_{14}H_{12}N_4OS$ (284)
10	Methanol	40	14	280	$C_{14}H_{12}N_4OS$ (284)
11	Ethanol	65	16	>300	$C_{18}H_{16}N_6O_3S_2$ (428)
12	Ethanol	70	16	>300	$C_{18}H_{15}N_7NaO_2S_2$ (448)
13	Ethanol	50	16	>300	$C_{22}H_{17}N_7NaO_2S_2$ (498)

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

Cpd No.	Surviving fraction (%) Concentration (µg/mL)				IC_{50} (nmol/mL)
	5	12	25	50	
4	82	57	28	20	49.52
7	80	55	26	21	30.8
9	75	44	13	20	38.73
10	76	58	12	21	52.11

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

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)

ACKNOWLEDGMENT

The authors would like to thank Dr. Samia Shouman, national cancer institute, Cairo Egypt, for carrying out the anticancer activity for the synthesized compounds.

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