DESIGN AND SYNTHESIS OF NOVEL THIOPHENES BEARING BIOLOGICALLY ACTIVE ANILINE, AMINOPYRIDINE, BENZYLAMINE, NICOTINAMIDE, PYRIMIDINE AND TRIAZOLOPYRIMIDINE MOIETIES
SEARCHING FOR CYTOTOXIC AGENTS

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Abstract: To discover new bioactive lead compounds for medicinal purposes, herein, (E)-3-(substituted amino)-1-thiophen-2-yl-prop-2-en-1-ones 3–8, aminopyridines 9–11, benzylamine 12, nicotinamide 13, pyrimidines 14, 15, hexanoic acid 16 and triazolopyrimidine 19 were prepared and tested for cytotoxic activity. Results showed that the tested compounds exhibited a remarkable activity, especially compounds 3 and 19 with IC50 values (55.2 and 50.49 µM, respectively) compared to doxorubicin (IC50 = 71.8 µM) as a reference drug.

Keywords: thiophene, aniline, aminopyridine, nicotinamide, pyrimidine, triazolo-pyrimidine, cytotoxic activity

Thiophenes have been reported to possess interesting biological and pharmacological activities and several derivatives with this ring are used as antibacterial (1–4), anti-inflammatory (5), anticancer (6–12), and antiviral (13) agents. Moreover, from the literature survey it was found that aniline, pyridine, nicotinamide, pyrimidine, triazolopyrimidine derivatives showed wide spectrum of biological activities, especially anticancer activities (14–19). As a part of our ongoing research program directed towards developing new approaches to a variety of heterocyclic ring systems for anticancer activity, especially those containing sulfur compounds (20–27), we report herein the utility of (E)-3-(dimethylamino)-1-(thiophen-2-yl)prop-2-en-1-one 2 (28) for the synthesis of target compounds.

EXPERIMENTAL

Chemistry

Melting points are uncorrected and were determined on BUCHI melting point apparatus B-545 (BUCHI Laborteknik AG CH-9230 Flawil, Switzerland). Elemental analyses (C, H, N) were performed on Carlo Erba 1108 Elemental Analyzer. All these data were within ±0.4% of the theoretical values. The IR spectra (KBr) were measured on Shimadzu IR 110 spectrophotometer (Shimadzu, Kyoto, Japan), 1HNMR and 13CNMR spectra were obtained by a Bruker proton NMR-Avance 500 instrument (500 MHz) (Bruker, Germany), in dimethyl sulfoxide-d6 as a solvent, using tetramethylsilane (TMS) as an internal standard. All reactions were monitored by thin layer chromatograph using precoated aluminium sheets (Silica gel Merck 60 F254) and were visualized by UV lamp (Merck, Germany). All chemicals were commercially supplied from Sigma-Aldrich, USA.

(E)-3-(dimethylamino)-1-thiophen-2-yl-prop-2-en-1-one (2)

Compound 2 was prepared according to previously described method (28).

(E)-3-(3-substituted phenylamino)-1-thiophen-2-yl-prop-2-en-1-ones (3–8)

General procedure

A mixture of 2 (28) (1.81 g, 0.01 mol) and the appropriate amine, namely: 3-ethylaniline, 4-
ethoxyaniline, 3,4,5-trimethoxyaniline, 2-chloro-5-nitroaniline, 2-methyl-4-nitroaniline and 2,4-dibromoaniline (0.01 mol) in ethanol (20 mL) containing acetic acid (5 mL) was refluxed for 2 h. The precipitated product was collected by filtration, washed with ethanol and crystallized from dioxane to give 3–8, respectively.

\((E)-3-(3\text{-ethylphenylamino})-1\text{-thiophen-2-yl-prop-2-en-1-one}\ (3)\)

Yield 88%; m.p. 110.5°C; IR (KBr, cm\(^{-1}\)): 3464 (NH), 2960, 2836 (CH aliph.), 2960, 2836 (CH aliph.), 1624 (C=O), 1H-NMR (DMSO-d\(_6\), \(\delta\) ppm): 1.19 (t, 3H, CH\(_3\)), 2.6 (q, 2H, CH\(_2\)), 6.0, 6.3 (2d, 2H, CH=CH, \(J = 7.3\) Hz), 7.0–8.1 (m, 7H, Ar-H), 11.7 (d, 1H, NH, \(J = 7.1\) Hz). Analysis: calcd. for C\(_{15}\)H\(_{15}\)NOS (257.35): C 70.01, H 5.87, N 5.44%; found: C 70.32, H 6.20, N 5.60%.

\((E)-3-(4\text{-ethoxyphenylamino})-1\text{-thiophen-2-yl-prop-2-en-1-one}\ (4)\)

Yield 78%; m.p. 135.6°C; IR (KBr, cm\(^{-1}\)): 3430 (NH), 3070 (CH arom.), 2970, 2866 (CH aliph.), 1632 (C=O). 1H-NMR (DMSO-d\(_6\), \(\delta\) ppm): 1.3 (t, 3H, CH\(_3\)), 3.9 (q, 2H, CH\(_2\)), 5.9, 6.9 (2d, 2H, CH=CH, \(J = 7.3\), 7.4 Hz), 7.4–7.9 (m, 7H, Ar-H), 11.7 (d, 1H, NH, \(J = 8.1\) Hz). Analysis: calcd. for C\(_{15}\)H\(_{15}\)NO\(_2\)S (273.35): C 65.91, H 5.53, N 5.12%; found: C 65.68, H 5.22, N 5.40%.

\((E)-3-(3,4,5\text{-trimethoxyphenylamino})-1\text{-thiophen-2-yl-prop-2-en-1-one}\ (5)\)

Yield 82%; m.p. 99.5°C; IR (KBr, cm\(^{-1}\)): 3406 (NH), 3089 (CH arom.), 2939, 2870 (CH aliph.), 1636 (C=O), 115.3, 115.4, 116.9, 117.6, 129.2, 133.2, 134.0, 144.4, 154.9, 159.4, 180.4. Analysis: calcd. for C\(_{16}\)H\(_{17}\)NO\(_4\)S (319.38): C 60.17, H 5.37, N 4.39%; found: C 59.88, H 5.09, N 4.72%.

\((E)-3-(2\text{-chloro-5-nitrophenylamino})-1\text{-thiophen-2-yl-prop-2-en-1-one}\ (6)\)

Yield 69%; m.p. 177.7°C; IR (KBr, cm\(^{-1}\)): 3429 (NH), 3089, 2860 (CH aliph.), 1562, 1346 (NO\(_2\)), 732 (C-Cl). 1H-NMR (DMSO-d\(_6\), \(\delta\) ppm): 6.2, 6.8 (2d, 2H, CH=CH, \(J = 7.0\), 7.1 Hz), 7.3–8.0 (m, 6H, Ar-H), 10.6 (d, 1H, NH, \(J = 7.7\) Hz). Analysis: calcd. for C\(_{13}\)H\(_9\)ClN\(_2\)O\(_3\)S (308.74): C 50.57, H 2.94, N 9.07%; found: C 50.20, H 3.30, N 9.38%.

\((E)-3-(2\text{-methyl-4-nitrophenylamino})-1\text{-thiophen-2-yl-prop-2-en-1-one}\ (7)\)

Yield 69%; m.p. 217.2°C; IR (KBr, cm\(^{-1}\)): 3468 (NH), 3100 (CH arom.), 2970, 2840 (CH aliph.), 1581, 1373 (NO\(_2\)), 1H-NMR (DMSO-d\(_6\), \(\delta\) ppm): 2.1 (s, 3H, CH\(_3\)), 6.4, 6.9 (2d, 2H, CH=CH, \(J = 7.0\), 7.1 Hz), 7.0–7.9 (m, 6H, Ar-H), 10.8 (d, 1H, NH, \(J = 7.4\) Hz). Analysis: calcd. for C\(_{14}\)H\(_{12}\)N\(_2\)O\(_3\)S (288.32): C 58.08, H 4.20, N 9.72%; found: C 58.08, H 4.12, N 9.41%.

\((E)-3-(2,4\text{-dibromophenylamino})-1\text{-thiophen-2-yl-prop-2-en-1-one}\ (8)\)

Yield 82%; m.p. 143.5°C; IR (KBr, cm\(^{-1}\)): 3448 (NH), 3048 (CH arom.), 2970, 2870 (CH aliph.), 1628 (C=O), 732 (C-Br). 1H-NMR (DMSO-d\(_6\), \(\delta\) ppm): 6.1, 6.8 (2d, 2H, CH=CH, \(J = 7.4\), 7.5 Hz), 7.1–7.9 (m, 6H, Ar-H), 12.0 (d, 1H, NH, \(J = 8.1\) Hz). Analysis: calcd. for C\(_{13}\)H\(_9\)Br\(_2\)NOS (387.09): C 40.10, H 2.62, N 3.33%.

\((E)-3-(\text{substituted amino})-1\text{-thiophen-2-yl-prop-2-en-1-one}\ (9–11)\)

A mixture of 2 (1.81 g, 0.01 mol) and 4-aminopyridine or 3-amino-2-chloropyridine and/or 2-amino-4-chloropyridine (0.01 mol) in ethanol (20 mL) containing acetic acid (5 mL) was refluxed for 8 h. The reaction mixture was filtered while hot and crystallized from dimethylformamide/ethanol to give 9–11, respectively.

\((E)-3-(\text{pyridin-4-ylamino})-1\text{-thiophen-2-yl-prop-2-en-1-one}\ (9)\)

Yield 90%; m.p. 213.4°C; IR (KBr, cm\(^{-1}\)): 3433 (NH), 3093 (CH arom.), 2970, 2860 (CH aliph.), 1639 (C=O), 1620 (C=N). 1H-NMR (DMSO-d\(_6\), \(\delta\) ppm): 6.4, 6.8 (2d, 2H, CH=CH, \(J = 7.6\) Hz), 7.1–8.3 (m, 7H, Ar-H), 10.8 (d, 1H, NH, \(J = 7.2\) Hz). Analysis: calcd. for C\(_{13}\)H\(_9\)ClN\(_2\)O\(_3\)S (319.38): C 59.88, H 5.09, N 4.72%.

\((E)-3-(\text{pyridin-4-ylamino})-1\text{-thiophen-2-yl-prop-2-en-1-one}\ (9)\)

Yield 90%; m.p. 213.4°C; IR (KBr, cm\(^{-1}\)): 3433 (NH), 3093 (CH arom.), 2970, 2860 (CH aliph.), 1639 (C=O), 1620 (C=N). 1H-NMR (DMSO-d\(_6\), \(\delta\) ppm): 6.4, 6.8 (2d, 2H, CH=CH, \(J = 7.6\) Hz), 7.1–8.3 (m, 7H, Ar-H), 10.8 (d, 1H, NH, \(J = 7.2\) Hz). Analysis: calcd. for C\(_{13}\)H\(_9\)ClN\(_2\)O\(_3\)S (319.38): C 59.88, H 5.09, N 4.72%.
The obtained solid was crystallized from dioxane-acetic acid (20 mL) was heated under reflux for 12 h. The obtained solid was crystallized from dioxane-ethanol mixture to give (E)-3-(4-chloropyridin-2-ylamino)-1-thiophen-2-yl-prop-2-en-1-one (11)

Yield 76%; m.p. 200.8°C; IR (KBr, cm⁻¹): 3448 (NH), 3060 (CH arom.), 2980, 2872 (CH aliph.), 1632 (C=O), 1592 (C=N), 775 (C-Cl). H-NMR (DMSO-d₆, δ, ppm): 6.4, 6.9 (2d, 2H, CH=CH, J = 7.6, 7.7 Hz), 7.2–8.0 (m, 5H, Ar-H, 8.3 (s, 1H, N=CH), 10.6 (d, 1H, NH, J = 8.2 Hz). ¹³C-NMR (DMSO-d₆, δ, ppm): 97.3, 115.6, 123.4, 131.3, 133.1, 134.2, 148.8, 151.3, 152.8, 153.8, 163.4, 190.2. Analysis: calcd. for C₁₂H₁₂ClN₂OS (264.01): C 54.63, H 3.81, N 10.21%.

(E)-3-(4-chlorobenzylamino)-1-thiophen-2-yl-prop-2-en-1-one (12), (E)-N-(3-oxo-3-thiophen-2-yl-prop-1-enyl-amino)-1-thiophen-2-yl-prop-2-en-1-one (13) and (E)-3-(pyrimidin-2-yl-amino)-1-thiophen-2-yl-prop-2-en-1-one (14)

A mixture of 2 (1.81 g, 0.01 mol) and 4-chlorobenzylamine or nicotinamide and/or 2-aminopyrimidine (0.01 mol) in ethanol (20 mL) and acetic acid (20 mL) was added appropriate amine, namely: 5-aminouracil or 6-aminocaproic acid and/or 2H-1,2,4-triazol-3-amine (16) and 7-thiophen-2-yl[1,2,4]triazolo[1,5-a]pyrimidine (19)

To a solution of 2 (1.81 g, 0.01 mol) in acetic acid (20 mL) was heated under reflux for 12 h. The obtained solid was crystallized from dioxane to give 12–14, respectively.

Yield 76%; m.p. 200.8°C; IR (KBr, cm⁻¹): 3448 (NH), 3060 (CH arom.), 2980, 2872 (CH aliph.), 1632 (C=O), 1592 (C=N), 775 (C-Cl). H-NMR (DMSO-d₆, δ, ppm): 6.4, 6.9 (2d, 2H, CH=CH, J = 7.6, 7.7 Hz), 7.2–8.0 (m, 5H, Ar-H, 8.3 (s, 1H, N=CH), 10.6 (d, 1H, NH, J = 8.2 Hz). ¹³C-NMR (DMSO-d₆, δ, ppm): 97.3, 115.6, 123.4, 131.3, 133.1, 134.2, 148.8, 151.3, 152.8, 153.8, 163.4, 190.2. Analysis: calcd. for C₁₂H₁₂ClN₂OS (264.01): C 54.63, H 3.81, N 10.21%.

Yield 76%; m.p. 200.8°C; IR (KBr, cm⁻¹): 3448 (NH), 3060 (CH arom.), 2980, 2872 (CH aliph.), 1632 (C=O), 1592 (C=N), 775 (C-Cl). H-NMR (DMSO-d₆, δ, ppm): 6.4, 6.9 (2d, 2H, CH=CH, J = 7.6, 7.7 Hz), 7.2–8.0 (m, 5H, Ar-H, 8.3 (s, 1H, N=CH), 10.6 (d, 1H, NH, J = 8.2 Hz). ¹³C-NMR (DMSO-d₆, δ, ppm): 97.3, 115.6, 123.4, 131.3, 133.1, 134.2, 148.8, 151.3, 152.8, 153.8, 163.4, 190.2. Analysis: calcd. for C₁₂H₁₂ClN₂OS (264.01): C 54.63, H 3.81, N 10.21%.
with saline to the appropriate volume. Different concentrations of the compounds under test (5, 12.5, 25 and 40 \( \mu \text{mol/L} \)) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37\(^\circ\)C and in atmosphere of air and 5% CO\(_2\). After 48 h, cells were fixed, washed and stained for 30 min. with 0.4\% (w/v) 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 (Sunostick Medical Technology, SPR-960B, U.K.). Negative control was added by using the cell lines with the

![Scheme 1. Synthetic pathways for compounds 2–16](image)

### Table 1. \textit{In vitro} cytotoxic activity of some newly synthesized compounds against human breast cancer cell line (MCF7).

<table>
<thead>
<tr>
<th>Compd. no.</th>
<th>Control</th>
<th>Compound concentration (( \mu \text{mol/L} ))</th>
<th>IC(_{50}) ( \mu \text{M} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>12.5</td>
</tr>
<tr>
<td>DOX</td>
<td>1.00</td>
<td>0.721 ± 0.020</td>
<td>0.546 ± 0.020</td>
</tr>
<tr>
<td>3</td>
<td>1.00</td>
<td>0.614 ± 0.002</td>
<td>0.519 ± 0.009</td>
</tr>
<tr>
<td>6</td>
<td>1.00</td>
<td>0.810 ± 0.058</td>
<td>0.647 ± 0.026</td>
</tr>
<tr>
<td>9</td>
<td>1.00</td>
<td>0.887 ± 0.032</td>
<td>0.672 ± 0.024</td>
</tr>
<tr>
<td>12</td>
<td>1.00</td>
<td>0.683 ± 0.085</td>
<td>0.680 ± 0.016</td>
</tr>
<tr>
<td>13</td>
<td>1.00</td>
<td>0.806 ± 0.008</td>
<td>0.631 ± 0.033</td>
</tr>
<tr>
<td>15</td>
<td>1.00</td>
<td>0.829 ± 0.017</td>
<td>0.636 ± 0.015</td>
</tr>
<tr>
<td>19</td>
<td>1.00</td>
<td>0.577 ± 0.044</td>
<td>0.421 ± 0.012</td>
</tr>
</tbody>
</table>

\( \mu \text{M} = 3. \text{DOX} = \text{doxorubicin.} \)
solvent without drug. The relation between surviving fraction and drug concentration was plotted to get the survival curve of each tumor cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC$_{50}$) was calculated and compared with the reference drug doxorubicin and the results are given in Table 1.

RESULTS AND DISCUSSION

Chemistry

Schemes 1 and 2 outline the synthetic pathway used to obtain compounds 3-16, 19. The starting material (E)-3-(dimethylamino)-1-thiophen-2-yl-prop-2-en-1-one 2 was prepared via reaction of acetylthiophene 1 with dimethylformamide-dimethylacetal (DMF-DMA) (28). Treatment of compound 2 with aniline derivatives gave the corresponding secondary amine derivatives 3ñ8, respectively (Scheme 1). The structure of the later products were assigned on the basis of their analytical and spectral data. IR spectra of compounds 3ñ8 exhibited, in each case, NH absorption bands in the region 3408ñ3468 cm$^{-1}$. Also, the enaminone 2 reacted with 4-aminopyridine or 3-amino-2-chloropyridine and/or 2-amino-4-chloropyridine in ethanol/acetic acid to give the corresponding pyridine derivatives 9ñ11, respectively (Scheme 1). When compound 2 was reacted with 4-chlorobenzylamine in refluxing ethanol/acetic acid, it furnished the corresponding (E)-3-(4-chlorobenzylamine)-1-thiophene-2-yl-prop-2-en-1-one 12. On the other hand, nicotinamide 13, pyrimidine 14 and 15 derivatives were obtained in good yield via reaction of compound 2 with nicotinamide or 2-aminopyrimidine or aminouracil. (E)-4-(3-oxo-3-thiophen-2-yl-prop-1-enylamino)butanoic acid 16 was obtained via reaction of 2 with 6-aminocaproyc acid (Scheme 1). The reactivity of enaminone 2 towards some heterocyclic amines was also examined. Thus, reaction of 2 with 5-amino-1,2,4-triazole in ethanol/acetic acid yielded the respective 1,2,4-triazolo[1,5-a]pyrimidine derivative 19 through the formation of intermediate 18. (Scheme 2). To account for the formation of the product 19 it is suggested that the studied reaction started with Michael-type addition of the exocyclic amino group of the amine used to the activated double bond of 2 followed by in-situ tandem elimination of dimethylamine to give the intermediate 18. (Scheme 2). The 'H-NMR spectrum of 19 revealed two doublets signals at 7.5, 7.9 ppm with $J = 7.8$ Hz assignable to two vicinal protons of the pyrimidine ring residue.

In vitro cytotoxic activity

Some newly synthesized compounds were evaluated for their in vitro cytotoxic activity against human breast cancer cell line (MCF7). Doxorubicin, which is one of the most effective anticancer agents, was used as the reference drug in this study. The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of breast cancer cell line (MCF7). The response parameter calculated was the IC$_{50}$ value, which corresponds to the concentration required for 50% inhibition of cell viability. Table 1 shows the in vitro cytotoxic activity of the tested compounds compared to the reference drug. It was found that, in the negative control, solvent has no effect on the
cells as the surviving fraction is 1.00, the most potent compounds were the aniline derivative 3 having ethyl group at 3-position (IC\textsubscript{50} = 55.2 µM) and the triazolopyrimidine derivative 19 (IC\textsubscript{50} = 50.49 µM), which were found to be more potent than the reference drug doxorubicin (IC\textsubscript{50} = 71.8 µM). Also, the aniline derivative 6 (IC\textsubscript{50} = 72.7 µM) was found to be nearly as potent as the reference drug. Isosteric replacement of the benzene ring with pyridine led to a drop in the activity as in compound 9 (IC\textsubscript{50} = 100 µM) and also a drop in the activity was shown by replacing the aniline ring with benzyl amine moiety as in compound 12 (IC\textsubscript{50} = 112.1 µM), an improvement in the activity was observed upon incorporation of nicotinamide moiety in compound 13 (IC\textsubscript{50} = 79.06 µM), while the pyrimidine derivative 15 (IC\textsubscript{50} = 83.3 µM) showed less activity.

CONCLUSION

From the above results, we can conclude that administration of the tested compounds on human breast (MCF7) cell lines showed promising cytotoxic activity. The most potent compounds are (E)-3-(3-ethylphenylamino)-1-thiophen-2-yl-prop-2-en-one 3 (IC\textsubscript{50} = 55.20 µM) and 7-thiophen-2-yl-[1,2,4]triazolo[1,5-a]pyrimidine 19 (IC\textsubscript{50} = 50.49 µM), which were found to be more potent than doxorubicin (IC\textsubscript{50} = 71.8 µM), and aniline 6 carrying chloro moiety at 2-position, nitro group at 5-position (IC\textsubscript{50} = 72.7 µM), which is as potent as the reference drug.

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