Synthesis, Cytotoxic and Anti-proliferative Activity of Novel Thiophene, Thieno[2,3-b]pyridine and Pyran Derivatives Derived from 4,5,6,7-tetrahydrobenzo[b]thiophene Derivative

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Abstract

Novel tetrahydrobenzo[b]thienopyrrole derivatives are synthesized from 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophene (1) through its reaction with α-chloroacetone to give the corresponding N-alkyl derivative 3. Compound 3 undergoes ready cyclization in sodium ethoxide solution to give the tetrahydrobenzo[b]thienopyrrole 4. The latter compound 4 is used as the key starting material for the synthesis of thiophene, thieno[2,3-b]pyridine and pyran derivatives. The cytotoxicity of the synthesized products towards the human cancer cell lines namely gastric cancer (NUGC), colon cancer (DLD-1), liver cancer (HA22T and HEPG-2), breast cancer (MCF-7), nasopharyngeal carcinoma (HONE-1) and normal fibroblast (WI-38) cell lines are measured. Compounds 4, 7a, 7b, 8a, 8b, 10c, 10d, 10f, 12a, 12b, 14b and 15b exhibit the optimal cytotoxic effect against cancer cell lines. Compounds 7b and 14b show the maximum inhibitory effect and these are much higher than the reference CHS-828 (pyridyl cyanoguanidine). On the other hand, the anti-proliferative evaluations of these compounds with high potency against the cancer cell lines L1210, Molt4/C8, CEM, K562, K562/4 and HCT116 show that compounds 7b and 8b give IC₅₀’s against Molt4/C8 and CEM cell lines higher than that of the reference, doxorubicin.

Keywords: Tetrahydrobenzo[b]thiophene, pyran, thiophene, cytotoxicity, anti-proliferative activity

1. Introduction

Sulfur containing heterocycles paved way for the active research in the pharmaceutical Chemistry. Nowadays benzothiophene derivatives in combination with other ring systems have been used extensively in pharmaceutical applications.¹⁻³ A large number of compounds containing thiophene system have been investigated because of their broad spectrum of biological activities which include analgesic,⁴ antibacterial,⁵ antifungal,⁶ antiparasitic,⁷ antiviral,⁸ anti-inflammatory,⁹ anti-convulsant,¹⁰ anti-nociceptive,¹¹ DNA cleavage,¹² herbicidal,¹³ antitubercular,¹⁴ protein kinase inhibition,¹⁵ respiratory syndrome protease inactivation,¹⁶ an active ester in the peptide synthesis and agonists of peroxisome proliferator activated receptors.¹⁷ In addition to these considerable biological applications, tetrahydrobenzo[b]thiophenes are important intermediates, protecting groups and final products in organic synthesis. Recently, our research group was involved through comprehensive program aiming for the synthesis of 4,5,6,7-tetrahydrobenzo[b]thiophene derivatives followed by their antitumor evaluations.¹⁸,¹⁹ Moreover, we reported the multi-component reactions with 3-(α-bromoacetyl)coumarin to give pyran and pyrididine derivatives.²⁰ In continuation of this program we are demonstrating the use of 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophene for the synthesis of tetrahydrobenzo[b]thienopyrrole derivatives followed by their cytotoxic and the anti-proliferative evaluations.²¹,²²
2. Results and Discussion

The reaction of the 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophene (1) with α-chloroacetone in the presence potassium carbonate afforded the 2-((2-oxo-propyl)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (3). Compound 3 was characterized by 1H-NMR and 13C-NMR. Thus, the 1H-NMR spectrum display the presence of beside the expected tetrahydro-benzene moiety, a singlet at δ 5.20 ppm indicating the presence of the N-CH2 group, a singlet at δ 2.88 ppm assigned to the CH3 group and a broad singlet at δ 8.30 ppm due to the NH group. Moreover, the 13C-NMR spectrum showed δ: 19.6 (CH3), 20.3, 22.0, 25.7 and 34.6 (4 CH2), 55.6 (CH2), 116.8 (CN), 124.1, 124.9, 128.7 and 139.5 (thiophene C), 164.8 (C=O). Compound 3 under-
went ready cyclization when heated in sodium ethoxide solution in a boiling water bath to yield the 1-(3-amino-4,5,6,7-tetrahydro-1H-benzo[4,5]thieno[2,3-b]pyrrol-2-yl)ethanone (4) (Scheme 1).

Compound 4 showed interesting reactivity towards different reagents, thus, it reacted with either malononitrile (5a) or ethyl cyanoacetate (5b) in the presence of ammonium acetate in an oil bath at 120 °C afforded the Knoevenagel condensed products 6a and 6b, respectively. The latter products underwent ready cyclization in sodium ethoxide solution to give the annulated products 7a and 7b, respectively (Scheme 2). The structures of the latter products were established on the basis of the analytical and spectral data. Thus, the 1H-NMR spectrum of 7a showed the presence of δ 2.89 ppm assigned to the CH3 group, a singlet at δ 4.89 ppm indicating the NH2 group and a singlet at δ 8.33 ppm confirming the presence of the NH group. Moreover, the 13C-NMR spectrum showed δ 19.8 (CH3), 20.1, 22.7, 25.2 and 34.6 (4 CH2), 116.8 (CN), 120.1, 122.6, 123.8, 124.2, 125.3, 127.2, 135.6, 142.3 (thiophene, pyrrole, pyridine C) and 168.2 (C=N).

Compound 4 was studied to produce thiophene derivatives through the Gewald’s reaction23–26 as many thiophenes were used as anticancer drugs. Thus, the reaction of compound 4 with either of malononitrile or ethyl cyanoacetate and elemental sulphur gave the thiophene derivatives 8a and 8b, respectively. On the other hand, the one pot reaction of compound 4 with either malononitrile or ethyl cyanoacetate and any of benzaldehyde, 4-chlorobenzaldehyde or 4-methoxybenzaldehyde gave the pyran derivatives 10a-f, respectively. The 1H-NMR and 13C-NMR spectra 10a-f were consistent with their respective structures. Further confirmations for the structure of compounds 10a-f were obtained through their synthesis via another synthetic root.

Thus, the reaction of compound 4 with the cinnamoylitrile derivatives 11a-f in the presence of a catalytic amount of

\[ 4 + S_8 + 5a,b \xrightarrow{1,4-dioxane/Et_3N} 8a, X = CN \]
\[ b, X = COOEt \]

\[ 4 + H_2C\text{-}\text{CN} + 9a, X = CN \]
\[ b, X = COOEt \]
\[ b, Ar = C_6H_5 \]
\[ b, Ar = 4-Cl-C_6H_4 \]
\[ c, Ar = 4-OCH_3 \]
\[ 10a, Ar = C_6H_5, R = NH_2 \]
\[ b, Ar = C_6H_5, R = OH \]
\[ c, Ar = 4-Cl-C_6H_4, R = NH_2 \]
\[ d, Ar = 4-Cl-C_6H_4, R = OH \]
\[ e, Ar = 4-OCH_3-C_6H_4, R = NH_2 \]
\[ f, Ar = 4-OCH_3-C_6H_4, R = OH \]

\[ 4 + 11a, X = CN \]
\[ b, Ar = C_6H_5, X = COOEt \]
\[ c, Ar = 4-Cl-C_6H_4, X = CN \]
\[ d, Ar = 4-Cl-C_6H_4, X = COOEt \]
\[ e, Ar = 4-OCH_3, X = CN \]
\[ f, Ar = 4-OCH_3, R = COOEt \]

Shema 3. Synthesis of compounds 8a,b and 10a-f.
triethylamine gave the same products 10a-f, respectively (m.p., mixed m.p. and fingerprint IR) (Scheme 3).

Moreover, the reaction of either of compound 8a or 8b with ethyl cyanoacetate in refluxing dimethylformamide afforded the 2-amido derivatives 12a and 12b, respectively. Formation of the latter products was explained on the condensation of ethyl cyanoacetate with the 2-aminothiophene moiety not to the 3-aminopyrrol moiety on the basis of the 1H-NMR spectra of such products. Thus, the 1H-NMR spectrum of either 12a or 12b displayed the missing of the NH₂ group that attached to thiophene ring which is expected to appear within the range δ 5.10-5.24 ppm while that of the 3-aminopyrrole moiety existing at δ 4.81-4.83 ppm. Similar acylation of the 2-aminothiophene was reported before in literature. The high yield of compound 12a, encouraged us to make further work. Thus, the reaction of 12a with either of the aryl diazonium salts 13a-d gave the aryl hydrazo derivatives 14a-d, respectively. Moreover, compounds 12a,b underwent ready cyclization in sodium ethoxide to produce the thieno[2,3-b]pyridine derivatives 15a and 15b, respectively (Scheme 4).

2.2. Anti-tumor Cell Activity

2.2.1. Chemicals and Cell cultures

Fetal bovine serum (FBS) and L-glutamine, were purchased from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was purchased from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, CHS-828, penicillin, streptomycin and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (Saint

![Scheme 4. Synthesis of compounds 12a,b-15a,b.](image-url)
Louis, USA). The cell cultures was obtained from the European Collection of cell Cultures (ECACC, Salisbury, UK) and human gastric cancer (NUGC), human colon cancer (DLD-1), human liver cancer (HA22T and HEPG-2), human breast cancer (MCF-7), nasopharyngeal carcinoma (HONE-1) and normal fibroblast cells (WI-38) were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 lg/mL), at 37 °C in a humidified atmosphere containing 5% CO2. Exponentially growing cells were obtained by plating 1.5 × 10^5 cells/mL for the six human cancer cell lines including cells derived from 0.75 × 10^4 cells/mL followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

2.2.2. In vitro Cytotoxicity Assay

The heterocyclic compounds, prepared in this study, were evaluated according to standard protocols28,29 for their in vitro cytotoxicity against the six human cancer cell lines including cells derived from human gastric cancer (NUGC), human colon cancer (DLD-1), human liver cancer (HA22T and HEPG-2), human breast cancer (MCF-7), nasopharyngeal carcinoma (HONE-1) and a normal fibroblasts cell (WI-38). All of IC50 values were listed in Table 1. Some heterocyclic compounds were observed with significant cytotoxicity against most of the cancer cell lines tested (IC50=10–1000 nM). Normal fibroblasts cells (WI-38) were affected to a much lesser extent (IC50>10,000 nM). The reference compound used was the CHS-828 which is the pyridyl cyanoguanidine anti-tumor agent.30 It is a new chemotherapeutic drug in addition it has low toxicity and lacks known patterns of multidrug resistance.31

2.2.3. Structure-activity Relationship

From Table 1 it is clear that the thiophene moiety was found to be crucial for the cytotoxic effect of the cyclic compounds 3-15a,b. Compounds 4, 7a, 7b, 8a, 8b, 10c, 10d, 10f, 12a, 12b, 14b and 15b exhibited optimal cytotoxic effect against cancer cell lines, with IC50’s in the nM range. Comparing the cytotoxicity of the tetrahydrobenzothiophene 3 and the cyclized product 4, it is obvious that the cytotoxicity of compound 4 is higher than that of compound 3. The presence of the pyrrol ring through the tetrahydrobenzo[b]thiophene in compound 4 is responsible for its high potency. The condensation reac-

<table>
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<th>HA22T b</th>
<th>HEPG-2 b</th>
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<td>740</td>
<td>253</td>
<td>2210</td>
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Table 1. Cytotoxicity of the newly synthesized products against a variety of cancer cell lines [IC50 (nM)]

* Drug concentration required to inhibit tumor cell proliferation by 50% after continuous exposure of 48 h.

b NUGC, gastric cancer; DLD-1, colon cancer; HA22T, liver cancer; HEPG-2, liver cancer; HONE-1, nasopharyngeal carcinoma; MCF-7, breast cancer; WI-38, normal fibroblast cells. NA: Not Active.
tion of compound 4 with either malononitrile or ethyl cyanocetate to produce compounds 5a and 5b, respectively showed a decrease of cytotoxicity. On the other hand, the cyclization of compounds 6a and 6b to the benzo[4',5']thieno[3',2':4,5]pyrrolo[3,2-b]pyridine derivatives 7a and 7b showed remarkable increase of the cytotoxicity. Moreover, it is clear that compound 7b showed more cytotoxicity than 7a, this is attributed to the presence of the oxygen rich COOE-t group. The introduction of the second thiophene moiety to compound 4 that gives both of compounds 8a and 8b showed high potency especially in case of compounds 8b which was attributed due to the presence of the COOEt. Considering the pyran derivatives 10a-f, the cytotoxicity of compounds 10c and 10d showed the highest values among the six compounds. However, compound 10c showed high cytotoxicity against the four cancer cell lines HUGC, DLD-1, HA22T and HEPG-2, but it is of great value to notice that compound 10d showed high cytotoxicity against five cancer cell lines and such cytotoxicity is higher than that of compound 10c. The high cytotoxicity of compound 10d is attributed to the presence of the OH and the Cl group as well.

The thiophene derivatives 12a and 12b showed high cytotoxicity similar to that of compounds 8a, b. Moreover, compound 12b with the COOE-t showed high potency than that of compound 12a. The coupling of the diazonium salts 13a-d with compound 12a afforded the arylhydrazine derivatives 14a-d. Compound 14b with the Cl group showed the maximum cytotoxicity among the arylhydrazine derivatives 14a-d. Finally, considering the thieno[2,3-b]pyridine derivatives 15a, b where the presence of the OH in compound 15b conserved an interesting cytotoxicity against the cancer cell lines HA22T, HEPG-2 and HONE-1 with the IC$_{50}$'s 377, 740, 253 nM, respectively. It is of great value to notice that compounds 7b, 8b and 12b showed the maximum cytotoxicity among the tested compounds.

2. 2. 4. Anti-proliferative Cell Activity Against Cancer Cell Lines

We used a panel of tumor cell lines to test the cytotoxicity of the new compounds, especially those showed high potency against the six cancer cell lines through Table 2. Importantly, this panel included the cell lines and their isoegenic sub-lines with the determinants of drug resistance: murine leukemia L1210, T-lymphocyte cell lines Molt4/C8 and CEM, human leukemia K562 and its MDR subline K562/4 that over expressed P-glycoprotein, and the colon carcinoma HCT116. The above determinants alter the response of cells to many anticancer drugs including doxorubicin. Data on cytotoxic (anti-proliferative) activity are presented in Table 2 in which IC$_{50}$ values represent the concentrations that inhibit cell proliferation by 50%. It is clear from Table 2 that tested compounds 4, 7a, 7b, 8a, 8b, 10c, 10d, 10f, 12a, 12b, 14b and 15b showed high potency against the cell lines. The benzo[4',5']thieno[3',2':4,5]pyrrolo[3,2-b]pyridine derivative 7b and the benzo[4,5]thieno-[2,3-b]pyrrol-2-yl)-thiophene derivative 8b showed high cytotoxicity against Molt4/C8 and CEM cell lines and their IC$_{50}$'s are higher than that of the reference doxorubicin. It is clear from Table 2 that the twelve tested compounds showed high IC$_{50}$ against K562/4 cell line than doxorubicin.

3. Experimental

3. 1. General

All melting points were determined on an electro-thermal apparatus (Büchi 535, Switzerland) in an open capillary tube and are uncorrected. $^{13}$C-NMR and $^1$H-NMR spectra were recorded on Bruker DPX200 instrument in DMSO with TMS as internal standard for protons and solvent signals as internal standard for carbon spectra. Chemical shift values are mentioned in ä (ppm). Mass spectra

<table>
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<tr>
<th>Compound</th>
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<th>Molt4/C8</th>
<th>Cytotoxicity (IC$_{50}$ in nM)</th>
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<td></td>
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</tr>
<tr>
<td>4</td>
<td>1.5 ± 0.5</td>
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<td>0.3 ± 0.01</td>
</tr>
<tr>
<td>7a</td>
<td>0.4 ± 0.1</td>
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</tr>
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<td>7b</td>
<td>0.3 ± 0.08</td>
<td>0.4 ± 0.04</td>
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<td>8b</td>
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<td>0.02 ± 0.002</td>
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<tr>
<td>12b</td>
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<td>Dox.</td>
<td>0.37 ± 0.07</td>
<td>0.20 ± 0.02</td>
<td>0.06 ± 0.02</td>
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</table>

Doxorubicin (Dox.) was used as the reference drug.
were recorded on EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. Elemental analyses were carried out by the Microanalytical Data Unit Ludwig-Maximilians-Universitat-Munchen, Germany. The progress of all reactions was monitored by TLC on 2 × 5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck).

3. 1. 1. Synthesis of 2-((2-Oxopropyl)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (3)

To a solution of compound 1 (1.78 g, 0.01 mol) in 1,4-dioxiane (40 mL) containing sodium carbonate (1.00 g) α-chloroacetic acid (0.94 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then poured onto ice/water and the formed solid product was collected by filtration and crystallized from ethanol.

White crystals; yield: 2.01 g (86%); mp: 182–183 °C; IR (KBr, cm−1): 3488–3334 (NH, NH 2), 3054 (CH aromatic), 2227, 2222 (2CN), 1620 (C=C); 1H-NMR (DMSO-d6) δ : 1.80–1.85 (m, 4H, 2CH2), 2.22–2.26 (m, 4H, 2CH2), 2.66 (s, 3H, CH3), 4.88 (s, 2H, NH2, D2O exchangeable); 13C-NMR (DMSO-d6) δ : 19.6, 20.3, 22.0, 25.7, 34.6, 55.6, 116.8, 124.1, 124.9, 128.7, 139.5, 164.8; MS electron impact (EI): m/z 234 (M+).

Anal. Calcd for C12H14N4S: C, 63.80; H, 4.93; S, 29.86. Found: C, 63.72; H, 4.93; S, 29.80.

3. 1. 2. General Procedure for the Synthesis of Thieno[2,3-b]pyrrolo Derivatives 6a and 6b

To the dry solid of compound 3 (2.34 g, 0.01 mol) in sodium ethoxide (0.02 mol) prepared by dissolving metallic sodium (0.46 g, 0.02 g) in absolute ethanol (20 mL) was added followed by ammmonium acetate (0.50 g, 0.01 mol). The whole reaction mixture was heated in an oil bath at 120 °C for 1 h then left to cool. The solidified product was boiled with ethanol then left to cool. The formed solid product was collected by filtration and crystallized from acetic acid.

2-(1-(3-Amino-4,5,6,7-tetrahydro-1H-benzo[4,5]thieno[2,3-b]pyrrolo-2-yl)ethylidene)-malononitrile (6a)

Yellow crystals; yield: 1.92 g (68%); mp: 167–168 °C; IR (KBr, cm−1): 3488–3334 (NH, NH2), 3054 (CH aromatic), 2227, 2222 (2CN), 1620 (C=C); 1H-NMR (DMSO-d6) δ : 1.79–1.86 (m, 4H, 2CH2), (m, 4H, 2CH2), 2.69 (s, 3H, CH3), 4.86 (s, 2H, NH2, D2O exchangeable), 8.29 (s, 1H, NH, D2O exchangeable); 13C-NMR (DMSO-d6) δ : 19.4, 20.3, 22.2, 25.6, 34.5, 116.3, 116.9, 122.3, 123.8, 124.0, 124.9, 135.2; MS (EI): m/z (%) 282 (M+). Anal. Calcd for C17H17N4O5S: C, 61.70; H, 5.00; N, 19.84; S, 11.36. Found: C, 61.72; H, 4.93; N, 20.05; S, 11.59.

Ethyl 3-(3-Amino-4,5,6,7-tetrahydro-1H-benzo[4,5]thieno[2,3-b]pyrrol-2-yl)-2-cyanobut-2-enoate (6b)

Yellow crystals; yield: 2.46 g (75%); mp: 121–122°C; IR (KBr, cm−1): 3473–3330 (NH, NH2), 3054 (CH aromatic), 2222 (CN), 1640 (C=C); 1H-NMR (DMSO-d6) δ : 1.13 (t, 3H, J = 7.26 Hz, CH3), 1.80–1.86 (m, 4H, 2CH2), 2.22–2.27 (m, 4H, 2CH2), 2.66 (s, 3H, CH3), 4.22 (q, 2H, J = 7.26 Hz, CH2), 4.88 (s, 2H, NH, D2O exchangeable), 8.27 (s, 1H, NH, D2O exchangeable); 13C-NMR (DMSO-d6) δ : 16.3, 19.6, 20.2, 22.5, 25.6, 34.8, 116.6, 122.0, 123.5, 124.6, 124.7, 127.2, 134.8, 166.1; MS (EI): m/z (%) 329 (M+). Anal. Calcd for C17H16N4O6S: C, 61.98; H, 5.81; N, 12.76; S, 9.73. Found: C, 62.08; H, 6.07; N, 12.59; S, 9.88.
2-Amino-4-methyl-7,8,9,10-tetrahydro-1H-benzo[4',5']thieno[3',2-b:4,5]pyrrolo[3,2-b]pyridine-3-carbonitrile (7a)

Yellow crystals; yield: 2.27 g (80%); mp: 232–233 ºC; IR (KBr, cm–1): 3474–3314 (NH, NH 2), 3056 (CH aromatic), 2220 (CN), 1626 (C=C); 1H-NMR (DMSO-d 6) δ: 1.76–1.84 (m, 4H, 2CH 2), 2.21–2.26 (m, 4H, 2CH 2), 2.89 (s, 3H, CH 3), 4.89 (s, 2H, NH 2, D 2O exchangeable), 8.33 (s, 1H, NH, D 2O exchangeable); 13C-NMR (DMSO-d 6) δ: 19.8, 20.1, 22.7, 25.2, 34.6, 116.8, 120.1, 122.6, 123.8, 124.2, 125.3, 127.2, 132.7, 135.6, 142.3, 168.2; MS (EI): m/z (%) 361 (M+).

Ethyl 2-amino-4-methyl-7,8,9,10-tetrahydro-5H-benzo[4',5']thieno[3',2-b:4,5]pyrrolo[3,2-b]pyridine-3-carboxylate (7b)

Yellow crystals; yield: 2.24 g (68%); mp: 195–196 ºC; IR (KBr, cm–1): 3466–3327 (NH, NH 2), 3056 (CH aromatic), 1640 (C=C); 1H-NMR (DMSO-d 6) δ: 1.82–1.86 (m, 4H, 2CH 2), 2.20–2.27 (m, 4H, 2CH 2), 2.88 (s, 3H, CH 3), 4.24 (q, 2H, J = 7.07 Hz, CH 2), 4.84 (s, 2H, NH 2, D 2O exchangeable), 8.32 (s, 1H, NH, D 2O exchangeable); 13C-NMR (DMSO-d 6) δ: 16.2, 19.8, 20.1, 22.7, 25.2, 34.6, 55.6, 120.3, 122.4, 123.8, 124.6, 124.7, 127.6, 133.9, 143.2, 164.4, 168.9; MS (EI): m/z (%) 325 (M+). Anal. Calcd for C 17H 19N 3O 2S: C, 57.80; H, 4.87; N, 18.99; S, 20.44. Found: C, 57.72; H, 4.90; N, 18.97; S, 20.48.


To a solution of compound 4 (2.34 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.5 mL) and elemental sulfur (0.32 g, 0.01 mol) either of malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h and the formed solid product produced from the hot solution was collected by filtration and crystallized from ethanol. Thin layer chromatography revealed just a single spot which proved the presence of a single product.

Method (B): To a solution of compound 4 (2.34 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.5 mL), either of the cinnamonitrile derivatives 11a-f (0.01 mol) were added. The reaction mixture was heated under reflux for 2 h and the formed solid product produced from the hot solution was collected by filtration and crystallized from ethanol. Thin layer chromatography revealed just a single spot which proved the presence of a single product.

2-Amino-6-(3-amino-4,5,6,7-tetrahydro-1H-benzo[4,5]thieno[2,3-b]pyrrol-2-yl)-thiophene-3-carbonitrile (10a)

Pale yellow crystals; yield: 3.10 g (80%); mp: 167–168°C; IR (KBr, cm–1): 3489–3321 (NH, NH 2), 3056 (CH aromatic), 2220 (CN), 1630 (C=C); 1H-NMR (DMSO-d 6) δ: 1.76–1.85 (m, 4H, 2CH 2), 2.21–2.27 (m, 4H, 2CH 2), 4.83, 5.41 (2s, 4H, NH 2, D 2O exchangeable), 8.32 (s, 1H, thiophene H-5), 8.26 (s, 1H, NH, D 2O exchangeable); 13C-NMR (DMSO-d 6) δ: 20.4, 22.9, 25.0, 34.6, 116.6, 120.3, 123.1, 123.8, 124.2, 125.3, 127.2, 139.3, 140.6, 142.3; MS (EI): m/z (%) 314 (M+). Anal. Calcd for C 15H 14N 3S 2: C, 61.98; H, 5.81; N, 12.76; S, 19.44. Found: C, 61.68; H, 5.63; N, 12.42; S, 19.74.

3. 1. 5. General Procedure for the Synthesis of Pyran Derivatives 10a-f

Method (A): General Procedure: To a solution of compound 4 (2.34 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.5 mL), either of malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) and either of benzaldehyde (1.06 g, 0.01 mol), 4-chlorobenzaldehyde (1.40 g, 0.01 mol) or 4-methoxybenzaldehyde (1.36 g, 0.01 mol) were added. The reaction mixture was heated under reflux for 1 h and the formed solid product produced from the hot solution was collected by filtration and crystallized from ethanol. Thin layer chromatography revealed just a single spot which proved the presence of a single product.

Method (B): To a solution of compound 4 (2.34 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.5 mL), either of the cinnamonitrile derivatives 11a-f (0.01 mol) were added. The reaction mixture was heated under reflux for 2 h and the formed solid product produced from the hot solution was collected by filtration and crystallized from ethanol. Thin layer chromatography revealed just a single spot which proved the presence of a single product.

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139.3, 140.6, 141.8, 142.3; MS (EI): m/z (%) 388 (M+). Anal. Caled for C_{22}H_{20}N_{4}O_{2}S: C, 67.84; H, 4.92; N, 10.79; S, 8.23. Found: C, 67.60; H, 4.73; N, 10.99; S, 8.40.

6-(3-Amino-4,5,6,7-tetrahydro-1H-benzo[4,5]thieno[2,3-b]pyrrol-2-yl)-2-hydroxy-4-phenyl-4H-pyran-3-carbonitrile (10b)

Pale yellow crystals; yield: 2.57 g (66%); mp: 264–265 °C; IR (KBr, cm–1): 3520–3341 (NH, NH2, OH), 2222 (CN), 1622 (C=C); 1H-NMR (DMSO-d$_6$) δ: 1.78–1.85 (m, 4H, 2CH$_2$), 2.18–2.25 (m, 4H, 2CH$_2$), 4.86, 5.40 (2s, 4H, 2NH$_2$, D$_2$O exchangeable), 10.28 (s, 1H, OH, D$_2$O exchangeable); 13C-NMR (DMSO-d$_6$): 20.3, 22.9, 25.4, 34.7, 52.7, 116.9, 117.2, 120.4, 122.6, 123.9, 124.3, 125.3, 125.6, 125.7, 126.9, 128.8, 130.6, 130.9, 140.9, 144.3; MS (EI): m/z (%) 423 (M+). Anal. Caled for C$_{22}$H$_{22}$ClN$_3$O$_2$S: C, 62.33; H, 4.28; N, 14.42; Cl, 7.66; S, 7.39.

2-Amino-6-(3-amino-4,5,6,7-tetrahydro-1H-benzo[4,5]thieno[2,3-b]pyrrol-2-yl)-4-(4-chlorophenyl)-4H-pyran-3-carbonitrite (10c)

Pale yellow crystals; yield: 2.87 g (68%); mp: 274–275 °C; IR (KBr, cm–1): 3474–3330 (NH, NH$_2$), 3055 (CH aromatic), 2222 (CN), 1622 (C=C); 1H-NMR (DMSO-d$_6$) δ: 1.77–1.86 (m, 4H, 2CH$_2$), 2.20–2.27 (m, 4H, 2CH$_2$), 4.86 (s, 2H, NH$_2$, D$_2$O exchangeable), 3.68–5.87 (2d, 2H, pyran H-4, H-5), 5.67–5.74 (2d, 2H, pyran H-4, H-5), 7.32–7.38 (m, 4H, C$_6$H$_4$), 8.25 (s, 1H, NH, D$_2$O exchangeable); 13C-NMR (DMSO-d$_6$): 20.2, 22.6, 25.8, 34.3, 39.8, 116.5, 120.2, 122.6, 123.7, 123.9, 125.7, 126.9, 127.4, 130.2, 130.4, 130.6, 140.9, 141.3, 142.0, 142.8; MS (EI): m/z (%) 424 (M+). Anal. Caled for C$_{22}$H$_{20}$N$_2$O$_2$: C, 66.61; H, 5.30; N, 13.39; S, 7.66. Found: C, 66.24; H, 5.48; N, 13.19; S, 7.80.

Orange crystals; yield: 3.01 g (72%); mp: 167–168 °C; IR (KBr, cm–1): 3531–3312 (NH, NH$_2$), 3058 (CH aromatic), 2223 (CN), 1628 (C=C); 1H-NMR (DMSO-d$_6$) δ: 1.74–1.86 (m, 4H, 2CH$_2$), 2.20–2.28 (m, 4H, 2CH$_2$), 3.01 (s, 3H, OCH$_3$), 4.86, 5.22 (2s, 4H, 2NH$_2$, D$_2$O exchangeable), 5.67–5.74 (2d, 2H, pyran H-4, H-5), 7.32–7.38 (m, 4H, C$_6$H$_4$), 8.25 (s, 1H, NH, D$_2$O exchangeable); 13C-NMR (DMSO-d$_6$): 20.0, 22.8, 25.8, 34.8, 30.8, 39.6, 116.9, 120.6, 122.6, 123.4, 123.9, 125.7, 126.9, 127.6, 130.4, 134.9, 141.7, 142.3, 143.6; MS (EI): m/z (%) 418 (M+). Anal. Caled for C$_{22}$H$_{20}$ClN$_3$O$_3$: C, 66.61; H, 5.30; N, 13.39; S, 7.66. Found: C, 66.24; H, 5.48; N, 13.19; S, 7.80.


To a solution of either compound 8a (3.14 g, 0.01 mol) or 8b (3.61 g, 0.01 mol) in dimethylformamide (40 mL) ethyl cyanoacetate was added. The reaction mixture was heated under reflux for 2 h then poured onto ice/water. The formed solid product was collected by filtration and crystallized from ethanol.

N-(4-(3-Amino-4,5,6,7-tetrahydro-1H-benzo[4,5]thieno[2,3-b]pyrrol-2-yl)-3-cyano-thiophen-2-yl)-1-cyanoacetamide (12a)

Yellow crystals; yield: 3.43 g (90%); mp: 184–185 °C; IR (KBr, cm–1): 3482–3323 (NH, NH$_2$), 3055 (CH aromatic), 2225, 2220 (2CN), 1705 (C=O), 1630 (C=C); 1H-NMR (DMSO-d$_6$) δ: 1.79–1.83 (m, 4H, 2CH$_2$), 2.25–2.26 (m, 4H, 2CH$_2$), 4.83 (s, 2H, NH$_2$, D$_2$O exchangeable), 5.20 (s, 2H, CH$_2$), 6.20 (s, 1H, thiophene H-5), 7.30–7.44 (m, 4H, C$_6$H$_4$), 8.25 (s, 1H, NH, D$_2$O exchangeable); 13C-NMR (DMSO-d$_6$): 20.3, 22.9, 25.4, 34.7, 52.7, 116.9, 117.2, 120.3, 123.1, 124.1, 124.6, 125.3, 127.2, 138.8, 141.2.

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142.6, 168.2; MS (EI): m/z (%) 381 (M⁺). Anal. Calcd for C₁₇H₁₄N₅O₂S: C, 56.67; H, 3.96; N, 18.36; S, 16.81. Found: C, 56.88; H, 3.58; N, 18.56; S, 16.93.

Ethyl 4-(3-amino-4,5,6,7-tetrahydro-1H-benzo[4,5]thieno[2,3-b]pyrrrol-2-yl)-2-(2-cyano-acetamido) thiophene-3-carboxylate (12b)

Orange crystals; yield: 2.99 g (70%); mp: 194–195 °C; IR (KBr, cm–1): 3453–3320 (NH, NH₂), 3056 (CH aromatic), 2223, 1702, 1688 (2CN), 1702 (C=O), 1630 (C=C); 1H-NMR (DMSO-d₆): δ 1.13 (t, 3H, J = 6.83 Hz, CH₃), 1.81–1.87 (m, 4H, 2CH₂), 2.22–2.25 (m, 4H, 2CH₂), 4.23 (q, 2H, J = 6.83 Hz, CH₂), 4.81 (s, 2H, NH₂, D₂O exchangeable); 13C-NMR (DMSO-d₆): δ 20.6, 22.4, 25.8, 34.9, 116.8, 117.3, 120.0, 121.4, 123.1, 124.0, 124.1, 124.8, 125.3, 127.2, 138.8, 140.4, 141.2, 143.4, 164.8, 168.6; MS (EI): m/z (%) 520 (M⁺). Anal. Calcd for C₂₃H₂₈ClN₇O₂S: C, 55.70; H, 3.62; N, 18.59; S, 12.48.

3.1.8. General Procedure for the Synthesis of Hydrazoacetamide Derivatives 14a-d

To a cold solution (0–5 °C) of compound 12a (3.81 g, 0.01 mol) in ethanol (50 mL) containing sodium acetate (3.50 g, 0.05 mol) either benzenediazonium chloride (0.01 mol), 4-chlorobenzenediazonium chloride (0.01 mol), 4-methoxybenzenediazonium chloride (0.01 mol) or 4-methylaniline (0.01 mol) [prepared by adding a cold solution of sodium nitrite (0.70 g, in water (10 mL)) to a cold solution (0–5 °C) of either aniline oil (0.93 g, 0.01 mol), 4-chloroaniline (1.27 g, 0.01 mol) or 4-methoxybenzenediazonium chloride (1.24 g, 0.01 mol) or 4-methylaniline (1.07 g, 0.01 mol) in concentrated hydrochloric acid (12 mL) with continuous stirring] was added with continuous stirring. The whole reaction mixture was left at room temperature for 1 h then the formed solid product was collected by filtration and crystallized from acetic acid.

2-((4-(3-Amino-4,5,6,7-tetrahydro-1H-benzo[4,5]thieno[2,3-b]pyrrrol-2-yl)-3-cyanothiophen-2-yl)amino)-2-oxo-N’-(p-tolyl)acetohydrazonoyl cyanide (14a)

Red crystals; yield: 3.78 g (78%), mp: 129–130 °C; IR (KBr, cm⁻¹): 3428–3318 (NH, NH₂), 3057 (CH aromatic), 2223, 2220 (2CN), 1712 (C=O), 1638 (C=C); 1H-NMR (DMSO-d₆): δ 1.74–1.82 (m, 4H, 2CH₂), 2.13–2.20 (m, 4H, 2CH₂), 3.38 (s, 3H, OCH₃), 4.88 (s, 2H, NH₂, D₂O exchangeable), 6.13 (s, 1H, thiophene H-5), 7.30–7.38 (m, 4H, C₆H₄), 8.21, 8.32, 8.45 (3s, 3H, 3NH, D₂O exchangeable); 13C-NMR (DMSO-d₆): δ 20.8, 22.7, 25.8, 34.3, 55.3, 116.3, 117.0, 120.3, 121.4, 123.8, 124.0, 124.0, 124.8, 125.9, 127.0, 133.2, 138.2, 140.8, 141.9, 164.9, 168.6; MS (EI): m/z (%) 516 (M⁺). Anal. Calcd for C₂₃H₂₈ClN₇O₂S: C, 58.24; H, 4.11; N, 19.02; S, 12.44. Found: C, 58.40; H, 4.26; N, 19.11; S, 12.29.

2-((4-(3-Amino-4,5,6,7-tetrahydro-1H-benzo[4,5]thieno[2,3-b]pyrrrol-2-yl)-3-cyanothiophen-2-yl)amino)-2-oxo-N’-(4-methoxyphenyl)-2-oxoacetohydrazonoyl cyanide (14b)

Reddish brown crystals; yield: 4.63 g (90%); mp: 168–169 °C; IR (KBr, cm⁻¹): 3462–3335 (NH, NH₂), 3053 (CH aromatic), 2227, 2221 (2CN), 1720 (C=O), 1638 (C=C); 1H-NMR (DMSO-d₆): δ 1.74–1.82 (m, 4H, 2CH₂), 2.13–2.20 (m, 4H, 2CH₂), 3.38 (s, 3H, OCH₃), 4.88 (s, 2H, NH₂, D₂O exchangeable), 6.13 (s, 1H, thiophene H-5), 7.30–7.38 (m, 4H, C₆H₄), 8.21, 8.32, 8.45 (3s, 3H, 3NH, D₂O exchangeable); 13C-NMR (DMSO-d₆): δ 20.8, 22.7, 25.8, 34.3, 55.3, 116.3, 117.0, 120.3, 121.4, 123.8, 124.0, 124.0, 124.8, 125.9, 127.0, 133.2, 138.2, 140.8, 141.9, 164.9, 168.6; MS (EI): m/z (%) 516 (M⁺). Anal. Calcd for C₂₃H₂₈ClN₇O₂S: C, 58.24; H, 4.11; N, 19.02; S, 12.44. Found: C, 58.40; H, 4.26; N, 19.11; S, 12.29.

3.1.9. General Procedure for the Synthesis of Thieno[2,3-b]pyridine Derivatives 15a and 15b

A suspension of either compound 12a (3.81 g, 0.01 mol) or 12b (4.28 g, 0.01 mol) in sodium ethoxide (0.02 mol) was added with continuous stirring. The whole reaction mixture was left at room temperature for 1 h then the formed solid product was collected by filtration and crystallized from acetic acid.
mol) prepared by dissolving metallic sodium (0.46 g, 0.02 mol) in absolute ethanol (20 mL) was heated in a boiling water bath for 12 h then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration and crystallized from 1,4-dioxane.

4-Amino-3-(3-amino-4,5,6,7-tetrahydro-1H-benzo[4,5]thieno[2,3-b]pyrrol-2-yl)-6-hydroxy-thieno[2,3-b]pyridine-5-carbonitrile (15a)

Yellow crystals; yield: 2.29 g (60%); mp: > 300 °C; IR (KBr, cm–1): 3578–3345 (NH, NH2, OH), 3056 (CH aromatic), 2224 (CN), 1628 (C=C); 1H-NMR (DMSO-d6): δ: 1.75–1.85 (m, 4H, 2CH2), 2.23–2.27 (m, 4H, 2CH2), 4.68, 5.09 (2s, 4H, 2NH2, D2O exchangeable), 6.16 (s, 1H, thiophene H-5), 8.28 (s, 1H, NH, D2O exchangeable), 9.90 (s, 1H, OH, D2O exchangeable); 13C-NMR (DMSO-d6): δ: 20.8, 22.9, 25.8, 34.7, 116.7, 120.2, 121.7, 123.1, 124.6, 125.3, 126.5, 127.0, 129.6, 138.8, 142.8, 144.5, 162.8; MS (EI): m/z (%): 381 (M+). Anal. Calcd for C18H15N5OS2: C, 56.67; H, 3.96; N, 18.36; S, 16.81. Found: C, 56.98; H, 3.85; N, 18.36; S, 17.00.

3-(3-Amino-4,5,6,7-tetrahydro-1H-benzo[4,5]thieno[2,3-b]pyrrol-2-yl)-4,6-dihydroxy-thieno[2,3-b]pyridine-5-carbonitrile (15b)

Yellow crystals; yield: 2.79 g (73%); mp: 289–290 °C (IR (KBr, cm–1): 3578–3345 (NH, NH2, OH), 3056 (CH aromatic), 2222 (CN), 1628 (C=C); 1H-NMR (DMSO-d6): δ: 1.79–1.85 (m, 4H, 2CH2), 2.23–2.27 (m, 4H, 2CH2), 4.86 (s, 2H, NH2, D2O exchangeable), 6.17 (s, 1H, thiophene H-5), 8.26 (s, 1H, NH, D2O exchangeable), 10.29, 10.34 (2s, 2H, D2O exchangeable, 2OH); 13C-NMR (DMSO-d6): δ: 20.3, 22.8, 25.8, 34.7, 116.6, 120.2, 121.6, 123.1, 124.4, 124.8, 125.3, 126.8, 127.5, 133.2, 140.8, 143.8, 144.2, 162.9; MS (EI): m/z (%): 382 (M+). Anal. Calcd for C18H15N5O5S2: C, 56.53; H, 3.69; N, 14.65; S, 16.77. Found: C, 56.72; H, 3.46; N, 14.80; S, 16.37.

4. Conclusions

Novel 4,5,6,7-tetrahydro-1H-benzo[4,5]thieno[2,3-b]pyrrol-derivatives were synthesized in good yields. Some compounds were used to produce annulated products. The cytotoxicity of the newly synthesized compounds indicates that compounds 4, 7a, 7b, 8a, 8b, 10c, 10d, 10f, 12a, 12b, 14b and 15b showed the highest potency among the tested compounds. In addition, the anti-proliferative evaluations of these twelve compounds indicated that the benzo[4,5]thieno[3′,2′:4,5]pyrrol[3,2-b]pyridine derivative 7b and the benzo[4,5]thieno[2,3-b]pyrrol-2-yl-thiophene derivative 8b showed high potency against Molt4/C8 and CEM cell lines and their IC50’s are higher than the reference drug “doxorubicin”.

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6. References


Povzetek

Iz 2-amino-3-ciano-4,5,6,7-tetrahidrobenzo[b]tiofena (1) smo z reakcijo z α-kloroacetonom sintetizirali N-alkil derivat (3), tetrahidrobenzo[b]tienopirol. Spojino 3 smo v raztopini natrijevega etoksida s ciklizacijo pretvorili v tetrahidrobenzo[b]tienopirol (4), ki smo ga uporabili naprej za sinteze derivatov tiofena, tieno[2,3-b]piridina in pirana. Cito- toksičnost sintetiziranih spojij smo preverili na rakavih celicah želodčnega (NUGC), črevesnega (DLD-1), jetrnega (HA22T in HEPG-2) ter nazofaringealnega karcinoma (HONE-1), raka dojk (MCF-7) in na normalnih fibroblastnih celicah (WI-38). Izkazalo se je, da imajo spojine 4, 7a, 7b, 8a, 8b, 10c, 10d, 10f, 12a, 12b, 14b in 15b optimalni cito- toksični učinek na rakeve celice. Spojini 7b in 14b kažeta maksimalni inhibicijski efekt, ki je precej večji od efekta referenčne spojine CHS-828 (piridil cianogvanidina).