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RECENT DEVELOPMENT ON THE SYNTHESIS OF THIOUREA DERIVATIVES AND EFFECT OF SUBSTITUENTS ON THE ANTICANCER ACTIVITY: A SHORT REVIEW

(Perkembangan Terkini Sintesis Sebatian Terbitan Tiourea dan Kesan Kumpulan Penukargantian Terhadap Aktiviti Antikanser: Ulasan Ringkas)

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Abstract

Thiourea is a carbon, hydrogen, sulphur, and nitrogen-based organic molecule. The thiourea group has been improved by replacing various substituents for its hydrogen atoms at the nitrogen terminal, resulting in a wide range of biological activities, especially on anticancer properties. Despite the fact that thiourea derivatives have remarkable anticancer potential, finding a novel anticancer agent that is both safe and selective remains a challenge. This paper summarizes recent research on thiourea compounds that can be used to treat malignant cell lines. Several synthesis methods and the effects of substituents as well as their anticancer therapeutic potential in a variety of healthy and malignant cell lines are discussed. As a result, it is envisaged that this review will guide the design and synthesis of new thiourea derivatives in the future for the development of highly effective and selective anticancer

drugs.

Keywords: thiourea derivatives, synthesis method, anticancer activity, substituents

Abstrak

Tiourea merupakan sebatian organik yang mempunyai atom karbon, hidrogen, sulfur dan nitrogen. Modifikasi sebatian organik tiourea ini dengan menggantikan atom hidrogen pada terminal nitrogen dengan pelbagai kumpulan penukargantian yang terpilih telah menghasilkan sebatian yang mempunyai pelbagai aktiviti biologi. Salah satu daripada aktiviti biologinya adalah sebagai agen antikanser. Meskipun sebatian terbitan tiourea ini dilaporkan mempunyai aktiviti antikanser yang signifikan, namun masih terdapat cabaran untuk mencari sebatian terbitan tiourea baru yang selamat dan bersifat selektif. Kertas ini meringkaskan kajian terkini mengenai sebatian terbitan tiourea yang boleh diaplikasi untuk kajian merawat sel kanser. Pelbagai kaedah sintesis, kesan kumpulan penukargantian serta potensi sebatian ke atas pelbagai jenis sel termasuk sel sihat dan malignan telah dibincangkan. Ulasan ringkas ini dapat memberi panduan untuk meningkatkan kemajuan dalam mencipta dan mensintesis sebatian antikanser baru yang berkesan dan selektif berasaskan tiourea.

Kata kunci: terbitan tiourea, kaedah sintesis, aktiviti antikanser, kesan kumpulan penukargantian

Introduction

The incidence and mortality of cancer-related diseases are quickly growing year after year, with 19.3 million new cases and 10 million deaths reported in 2020. Despite the availability of advanced treatments including immunotherapy, radiotherapy and chemotherapy, the number of cancer cases has not decreased in the last 30 years [1]. This pattern was linked to acquired resistance, rapid mutability as well as bad effects that occurred for a year after taking current anticancer drugs [2, 3]. Therefore, finding a new anticancer drug that is efficient and safe is crucial to solving the alarming issue. The anticancer properties of thiourea derivatives are undeniable. Besides that, they also play an important role in a wide range of biological activities such as anti-thyroid [4], anti-bacterial [5], anti-inflammatory [6], anti-malaria [7], antioxidant [5], antihypertensive [8], anti-viral [9] and anticancer [10].

The discovery of 5-fluorouracil (5-FU), sorafenib, and tenovin medicines spurred substantial research into thiourea derivatives as potential anticancer therapies (Figure 1). A compound of 5-FU, cyclic fluorinated urea has been used to treat colorectal cancer [11] and other solid tumours (gastrointestinal tract, pancreas, ovary, liver, brain, and breast) for over 50 years, whereas Sorafenib is a diaryl urea-containing compound that has been clinically tested for lung, breast, colon, ovarian, pancreatic, and gastric cancer. Meanwhile, tenovin with thiourea-based compounds exerted inhibitation towards the colon, prostate, gastric and lung cancer [12, 13].

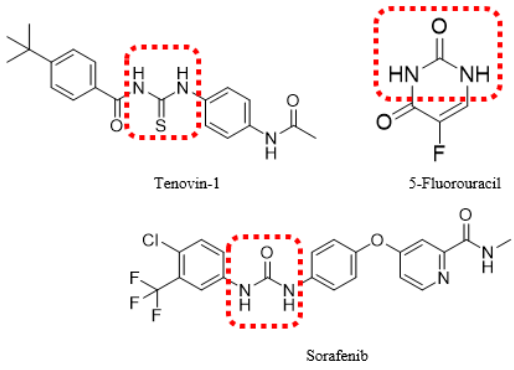


Figure 1. Thio(urea) scaffold-containing anticancer chemotherapeutics [12, 13]

Over the years, many reports have been published on the synthesis of thiourea derivatives and their evaluation as anticancer agents along with the structure-activity relationships (SAR). The nature and different types of substitutions on nitrogen atoms of thiourea moiety considerably boosted the anticancer activity and determined whether the thiourea was mono-substituted, di-substituted or multi-substituted [14]. However, the substitution of one hydrogen atom at each thiourea nitrogen atom with desirable moieties dominates the structural pattern of thiourea derivatives as an active chemical [14, 15]. It is concluded that coupling the thiourea scaffold with other substituents results in

pharmacophore molecules which are extremely good anticancer agents [15, 5, 6]. The derivatives were reported to demonstrate potent cytotoxic activities against a series of cancer cells including breast [16], lung [17], renal [18], colon [5], prostate [19], liver [20] and ovarian [18] cancers. These have sparked an interest in expanding the research by synthesising novel thiourea compounds as possible anticancer drugs. Thus, the present review aims to summarize the recent development in thiourea derivatives in terms of the synthesis pathway, anticancer activities as well as the effect of substituents on the anticancer activities.

Chemistry of thiourea

Thiourea or thiocarbamide moiety is the first urea analogue which was synthesized for the first time by Marceli Nencki, a Polish scientist [21]. It is defined by the substitution of a sulfur atom for the oxygen atom. A lot of research articles are focused on thiourea scaffold as anticancer chemotherapeutics than urea moiety because of their remarkable polarity, reactivity, and efficient process of preparation [22]. A sulfur atom has a large size and is less electronegative resulting in the electrons being more polarizable as well as a stronger nucleophile for better reactivity than an oxygen atom [23]. Thiourea is considered an important group in drug design due to its extensive biological properties [22]. The thiourea scaffold serves as an excellent building block in constructing anticancer molecules with the presence of C=S regarded as an excellent acceptor, while thiourea N-H moiety is a favourable hydrogen bond donor. This dual nature of thiourea functionality is crucial in the drug's aqueous solubility and permeability [24]. The unique feature of thiourea moiety with desirable electron-rich characteristics is beneficial for thiourea derivatives which allows them to easily bind with a variety of enzymes and receptors in biological systems. Moreover, these interactions play a key role in the stability of ligand-receptor interactions subsequently, inhibiting the progression of cancer [25].

Thiourea (Figure 2a) compound is a needle-like glossy crystal with a colourless to pale yellow, bitter-tasting and ammonia-like odour. Thiourea is slightly soluble in cold water and soluble in hot water with a boiling point of 150-160 ºC [17]. Figure 2b shows the resonance forms of thiourea in which the amphoteric character of thiourea is demonstrated by its resonance, which indicates electrophilic attack at the C-1 position; nucleophilic attack at either N-1 or N-2. Meanwhile, the tautomerization at H-atom that is attached to either N-1 or N-2 position would form two pairs of isomers namely thione and thiol (Figure 2c).

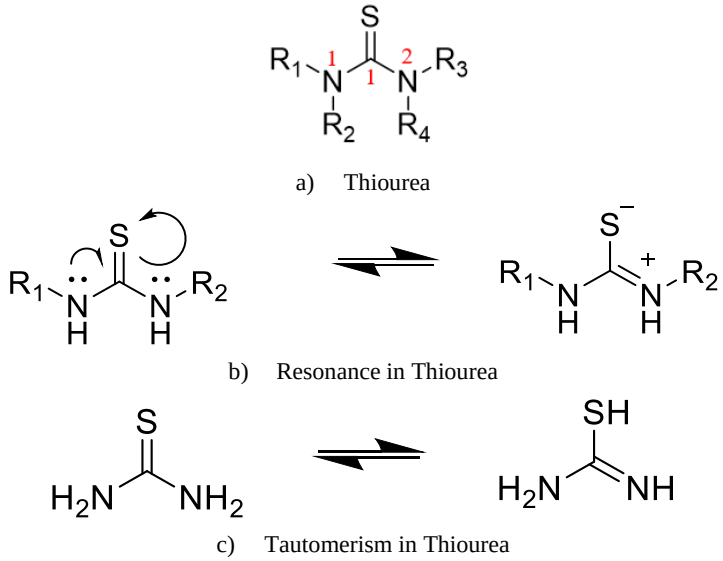


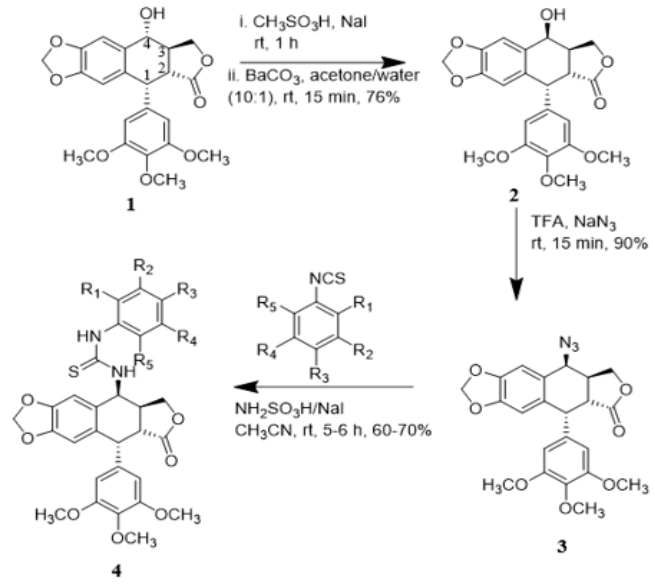
Figure 2. Thiourea scaffold showing numbering (a), resonance (b) and tautomerism (c)

Thiourea is a chemically interesting compound over the years due to its planar and flexible structure which exhibits greater π-electron delocalisation which also would enhance the binding ability to a variety of proteins in the biological system. The thiourea moiety is easily protonated under acidic conditions and interact with important amino acid residues on cancer cells such as carboxyl and phosphate groups, thus enhancing the anticancer activities [26].

Synthesis pathway of thiourea derivatives and their anticancer activities

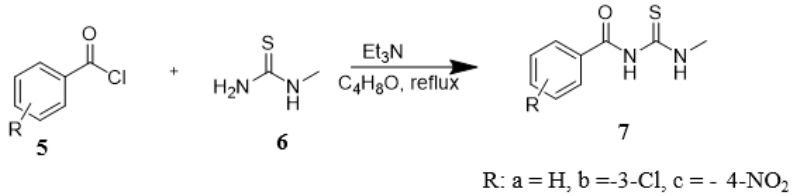
A lot of research on the thiourea moiety is currently ongoing to identify novel lead compounds to combat various cancers. The thiourea pharmacophore (-HN-C(=S)-NH-) is important in designing novel anticancer chemotherapeutics which leads to further development through structural modification. This pharmacophore acquires specific binding sites such as the hydrogen binding area thus increasing the binding affinity of the ligand-protein complex. The presence of C=S and N-H functional groups on thiourea which represents weak hydrogen bond acceptor as well as hydrogen bond donor respectively would significantly enhance the blocking of the enzymatic activity and simultaneously be a potent anticancer agent.

A novel of podophyllotoxin–thiourea derivatives (Scheme 1) were synthesized and evaluated for their in vitro cytotoxicity against A549 (human lung cancer), MDA MB-231 (human breast carcinoma), DU-145 (human prostate cancer), LNCaP (androgen-sensitive human prostate adenocarcinoma) and HGC- 27 (human gastric carcinoma) cell lines by MTT assays [27]. The synthesis strategy of podophyllotoxin–thiourea congeners **4a–u** was successfully performed by reaction with various isothiocyanates in the presence of NH_2SO_3H and NaI from 4ß-azido intermediate **(3)** and 4ß-deoxy-epipodophyllotoxin **(2)**. Among the compounds synthesized, compound **4a** was found to be effective in the DU-145 cell line compared to etoposide (standard drug) with half-maximal inhibitory concentration (IC_{50}) values less than 10 µM which is the range of 0.50-7.89µM. These compounds were further assayed for cytotoxicity on normal human prostate cell line (RWPE-1) and were shown as safe to be used. The introduction of bulky groups at the C-4 position at the podophyllotoxin thiourea derivative (**4**) significantly aids the antitumor activity [28]. The *N*-substituted benzene thiourea derivatives at the C-4 position showed that R_3 substituent (**4**) was significant for anticancer activity. For example, the presence of a small group such as methoxy **(4a)** increases the electron density and activates the ring. However, a bulky substituent at the R_3 position of the phenyl ring resulted in no cytotoxic activity.



Scheme 1. Synthesis of novel podophyllotoxin-thiourea congeners

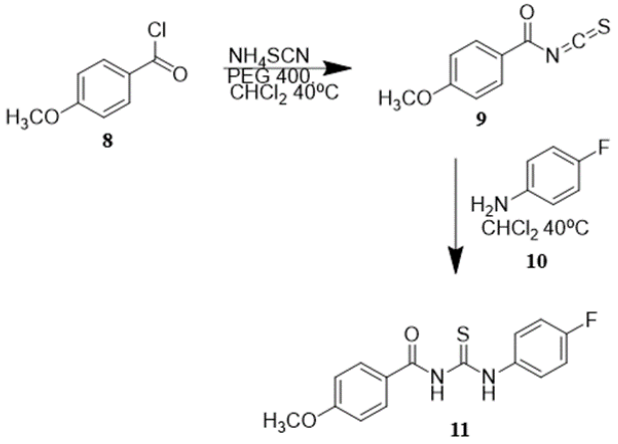
The 1-benzoyl-3-methyl thiourea derivatives were designed with different substitutions at *meta* and *para* positions on the phenyl ring of 1-benzoyl-3-methyl thiourea [29]. The compounds **7a-c** were synthesized from the reaction of starting material *N*-methylthiourea **(6)** with several ring-substituted benzoyl chlorides **(5)** (Scheme 2). The standard MTT assay was used to assess in vitro cytotoxicity against cervical cancer cell lines (HeLa). All of the compounds had higher cytotoxicity, with IC_{50} values ranging from 160 to 383µg/mL, than hydroxyurea (HU) as a reference drug, which had an IC_{50} of 428 µg/mL. It is revealed that the presence of benzoyl and methyl group on thiourea would increase the resonance within the compounds thus improving the compound stability and enhancing cytotoxic activity. The nitro substituents on the *para*-position **(7c)** had the highest activity, making it more active than HU. The electron-withdrawing group (EWG) substitutions such as chloro at the *meta*-position and nitro at the *para*-position of the aromatic ring improve the lipophilicity of molecules and are responsible for increased cytotoxicity [29]. The strong EWG would withdraw electrons through a π-bond due to the overlap of a *p* orbital on the substituent with a *p* orbital on the aromatic ring and stabilized by the electron-donating group (EDG) from methyl by resonance. Meanwhile, the absence of a sidechain on benzoyl showed the lowest activity among the synthesized compounds **(7a)**. These findings suggest that 1-benzoyl-3-methylthiourea derivatives could be used as anticancer agents in HeLa cells.



Scheme 2. Synthesis reaction of the 1-benzoyl-3-methylthioureas

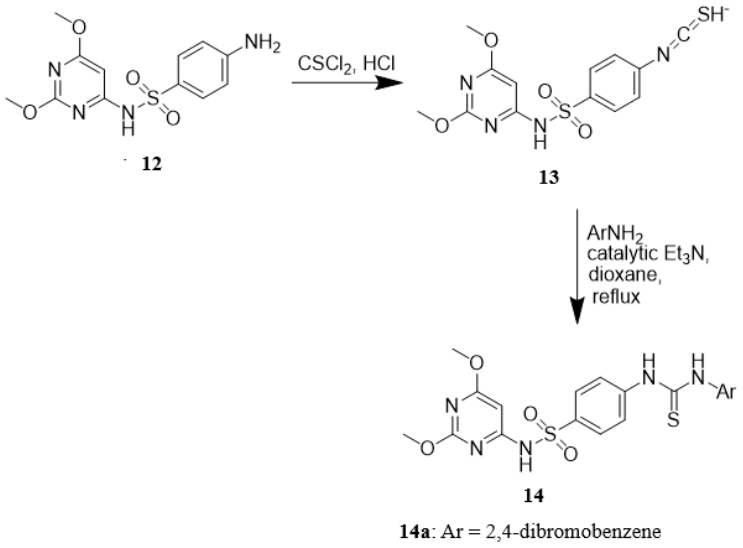
Encouraged by the outstanding anticancer activity from the presence of EWG as substituents at phenyl rings, the effect of EDG on the phenyl ring was also studied. The EDG group has been introduced to the

phenyl ring of benzoyl moiety linked thiourea in a compound of *N*-4-methoxybenzoyl-*N'*-(4-fluorophenyl)thiourea **(11)** and tested against HeLa cell line and compared with HU as a standard drug [30]. Scheme 3 shows that the compound was synthesized by the reaction of 4-methoxybenzoyl chloride **(8)** with ammonium thiocyanate to give an intermediate compound namely 4-methoxybenzoylisothiocyanate (**9**). Further reaction of **(9)** with 4-fluoroaniline **(10)** yielded the desired compound. The MTT results displayed that the compound has a lower inhibitory concentration IC_{50} value (0.720 ± 0.07 mM) which is more potent than HU (16.535 ± 2.092 mM). The presence of *p*-methoxy on benzoyl moiety and *p*-fluoro on phenyl ring enhanced the cytotoxic activity due to the lipophilic, electronic and steric properties. The methoxy (EDG) is a strongly activating group with the electron donor which imparts the resonance effect whereas fluorine (EWG) deactivates the ring by withdrawing the electron of the ring and stabilizing the compound. Thus, the combination of EWG and EDG in a thiourea compound potentially enhanced anticancer properties.



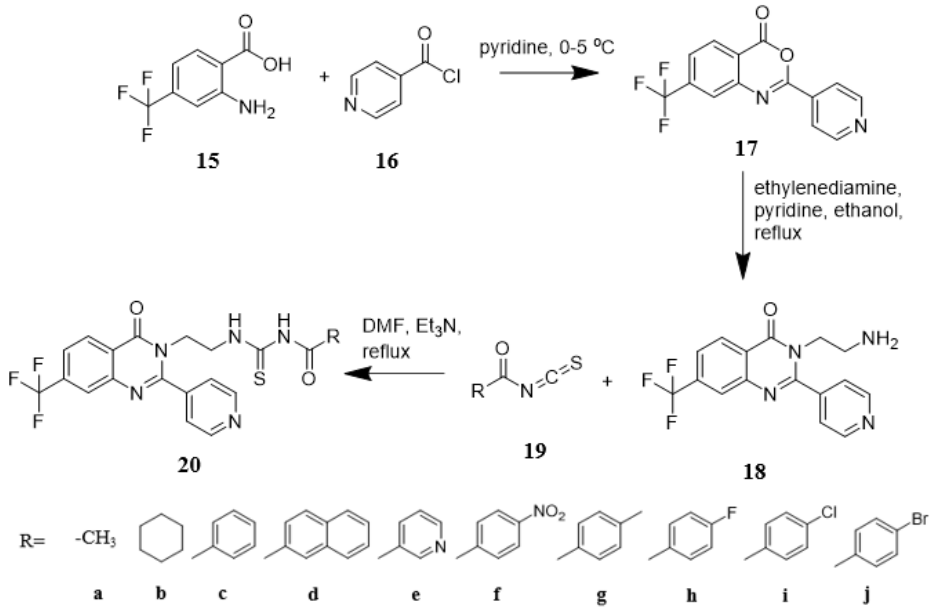
Scheme 3. Synthesis of *N*-methoxybenzoyl-*N'*-(4-fluorophenyl)thiourea derivatives

Subsequently, several novel thiourea derivatives bearing sulfonamide were synthesized and screened against HepG2, MCF-7, Caco-2, HCT-116, PC-3 cancer cell lines and Vero-B normal cell line [6]. The compounds were synthesized from the starting compound *N*-(2,6-dimethoxypyrimidin-4-yl)-4-isothiocyanatobenzenesulfonamide **(13)** as represented in Scheme 4. Among the compounds synthesized, compound **14a** which contains 2,4-dibromobenzene as a substituent exhibited broad selective cytotoxicity against cancer cells HepG2, MCF-7, Caco-2, and PC-3 with IC_{50} values 19.3, 20.5, 15.08 15.6 µg/ml respectively. This compound is a potent anticancer agent which might be due to the substitution of EWG (bromine atom) at *ortho* and *para* positions which activates the benzene ring through inductive effect, thus enhancing the anticancer properties.



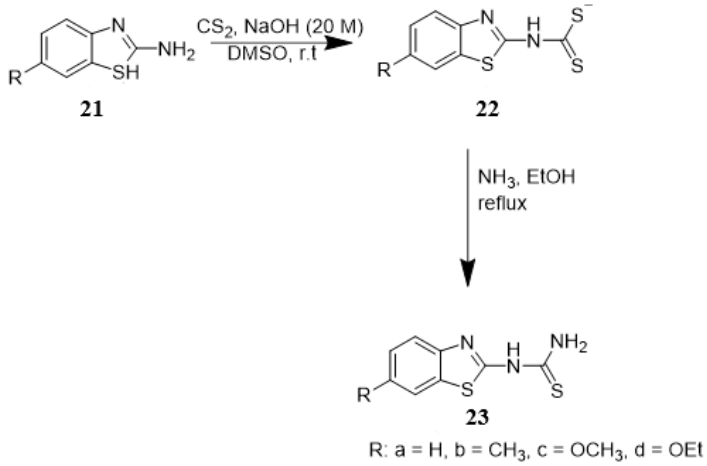
Scheme 4. Synthesis thioureas derivatives bearing a sulfonamide moiety

Another series is the novel 7-trifluoromethyl-2-pyridylquinazolin-4(3H)-one-based acyl thiourea derivatives **(20a-j)** as potential antiproliferative agents. The compounds were synthesized and tested for cytotoxicity in both cancerous human ovarian cancer (SK-OV-3), cervical cancer (HeLa), renal cancer (Caki-2) as well as noncancerous human umbilical vein endothelial cell (HUVEC) cell lines [18]. From the MTT bioassay results, the fluorine substitution on the benzoyl group was the most advantageous compared to other EWG due to the high electronegativity, highest carbon-fluorine bond strength, and smallest atomic radius (50 pm) as well as increased hydrophobic properties [31]. Compounds containing halogen atoms have higher potency overall. The presence of nicotinoyl and acetyl as the substituents contributed more to electron delocalization in the compound resulting in outstanding efficacy, whereas the absence of any functional or donor group on the benzoyl ring resulted in inert compounds. In addition, the conjugation bonds contributed by the quinazoline group in substituted thiourea derivatives correlated to the increase of lipophilic properties which is a salient feature for inhibition of DNA replication thus leading to the increased antiproliferative activity. However, all compounds showed desirable cytotoxicity towards the noncancerous cell line. It was discovered that modifying the substituent at the thiourea linkage gave different cytotoxic potentials to specific cell lines as summarized in Scheme 5. Specifically, thiourea-bearing quinazoline compounds with 4-fluorophenyl functionality **(20h)** and pyridyl moieties **(20e)** showed encouraging anticancer effects and could be used as a platform to design further chemotherapeutic agents.



Scheme 5. Synthesis of acyl thiourea derivatives of 7-trifluoromethyl-2-pyridylquinazolin-4(3H)-one

A new series of benzothiazole thiourea derivatives (**23a-d**) were tested for anticancer activity on human breast cancer cells (MCF-7), human cervix epithelial carcinoma (HeLa), human colon cancer cell line (HT-29), human leukaemia cell line (K-562), and mouse neuroblastoma cell line (Neuro-2a) [5]. The derivatives were prepared by the treatment of substituted 2-amino benzothiazole **(21)** with carbon disulfide and the ammonolysis of intermediate **(22)** in ethanol under reflux conditions as shown in Scheme 6. The compounds showed weakly to moderately cytotoxic activity with IC_{50} values ranging from 0.39 µM and up to 200 µM. Compound **23d** exhibited the most potent cytotoxic activity which was roughly four times more effective than cisplatin against the two cancer cell lines MCF-7 and HT-29, as depicted in Table 1. The synthesized compounds showed no cytotoxic effects on normal cells (L-929). The highest cytotoxicity of compound **23d** reflects the higher EDG nature of the ethoxy group and its steric effect. Therefore, combining benzothiazole and thiourea moieties might contribute to the improvement of the antiproliferation action.



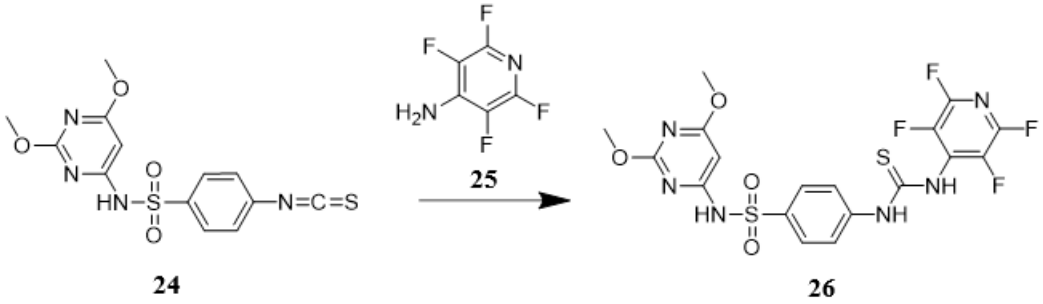
Scheme 6. General synthesis of the benzothiazole thiourea derivatives

Table 1. Cytotoxic activities of compounds (**23a–d**) against MCF-7, HeLa, HT-29, K-562, and Neuro-2a cancer cell lines

	Cell Lines (μM)					
Compound	**MCF-7**	**HeLa**	**HT-29**	**K-562**	**Neuro-2a**	**L-929**
23a	8.55	21.7	8.1	69.7	57.4	134.1
23b	11.9	47.3	14.8	85.3	235.3	135.0
23c	12.8	45.2	15.0	98.1	200.5	230.7
23d	6.72	4.97	3.90	40.5	22.7	107.6
Cisplatin	6.25	0.39	16.2	25.8	211.0	0.7

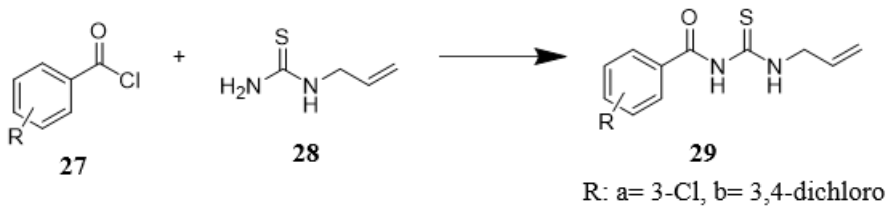
* The agent with IC_{50} > 100 μM is considered to be inactive

Inspired by the activity profile of a previous report [6] on thiourea-based sulfonamide derivatives, a new compound was developed namely *N*-(2,6-dimethoxypyrimidin-4-yl)-4-(3-(arylthioureido)benzenesulfonamides derivatives [20]. The desired compound was synthesized and tested for antiproliferative activity. The reaction scheme is depicted in Scheme 7. The antiproliferative assay showed that compound **(26)** with more EWG (fluorine atom) substituted to phenyl ring showed broad selective cytotoxicity against HepG2, MCF-7, Caco-2, and PC-3 cancer cells with IC_{50} values ranging from 15.08 μg/mL to 20.5 μg/mL. This compound also exhibited no cytotoxicity toward healthy cells (Vero-B). This work is in line with the previous study [6,20,29,18,15] which stated that EWG attached to phenyl ring bearing thiourea moiety would enhance the anticancer activity.



Scheme 7. Synthesis of thiourea derivatives bearing sulfonamides 26

Besides, two novel compounds namely *N*-(allylcarbamothioyl)-3-chlorobenzamide (**29a**) and *N*-(allylcarbamothioyl)-3,4-dichlorobenzamide **(29b)** were synthesized by reacting ally thiourea **(28)** and *N*-benzoyl chloride **(27)** as presented in Scheme 8 [15]. The compounds that have a different number of chlorine atoms on the phenyl ring were screened for cytotoxicity study on human breast cancer cell lines (T47D) using the MTT assay. This study concluded that an extra chlorine atom in compound **29b** contributed to higher cytotoxicity activity than compound **29a** and standard drug 5-FU with IC_{50} values of 86 μg/mL, 128 μg/mL and 213 μg/mL, respectively. The increased number of chlorine (EWG) would reduce the electron density and deactivate the ring but was stabilized by the presence of ally thiourea. Thus, the effect of the allyl group, as well as more EWG on thiourea structure, showed a substantial increase in antiproliferative activity as the conjugation makes the compounds more stable.



Scheme 8. Synthesis thiourea derivatives **29a-b**

Next, potent anticancer drugs were synthesized featuring the novel 4-*tert*-butylbenzoyl-3-allylthiourea framework and screened against MCF-7 cells overexpressed by HER-2 (MCF-7/HER-2) [32]. From the results of the MTT assay, compound **30** showed the best antiproliferative activity in comparison with positive control HU **(31)** with IC_{50} values of 0.15±6.48 mM and 2.01±1.05 mM, respectively (Figure 3). The *tert*-butyl functional group is EDG which will activate the *para* position of the phenyl ring due to more electron donation from C-H subsequently increasing compound conjugation. Thus, this study confirmed that a compound with allyl group and EDG is significantly cytotoxic and has the potential to be developed as a breast anticancer agent with HER-2 overexpression.

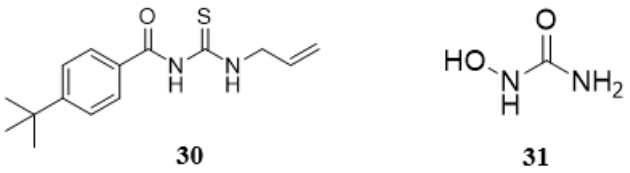
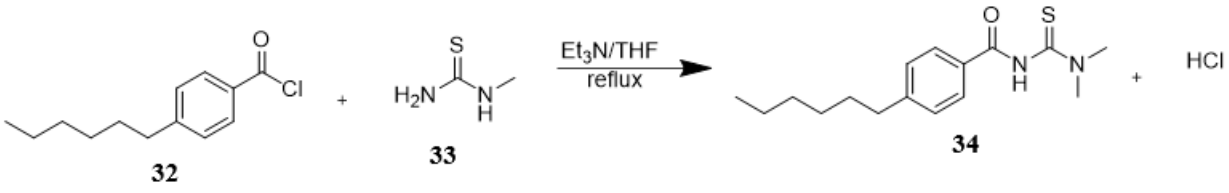


Figure 3. Structure of 4-t-butylbenzoyl-3-allylthiourea (30) and hydroxyurea (31)

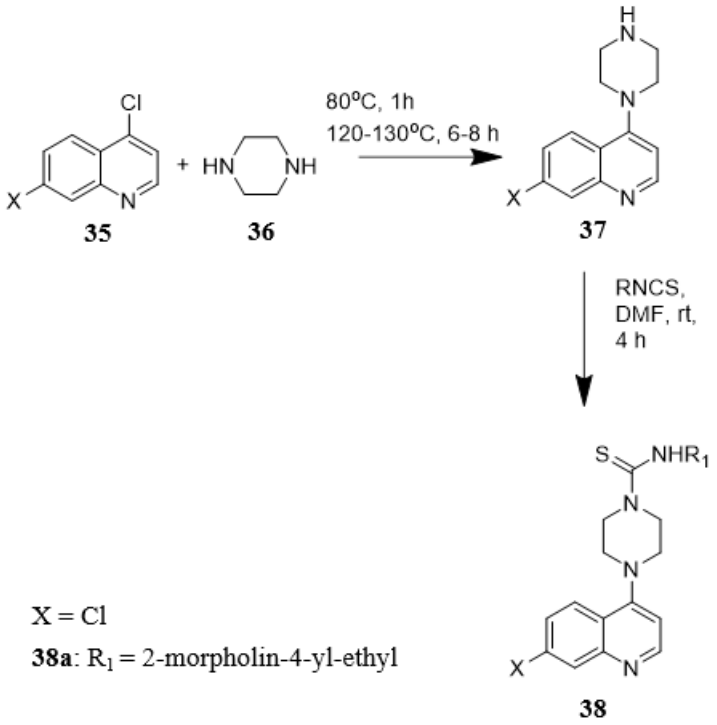
A new potent compound was discovered by reacting 4-hexylbenzoyl chloride **(32)** and *N*-methylthiourea **(33)** as shown in Scheme 9 [33]. The compound 1-(4-hexylbenzoyl)-3-methylthiourea **(34)** displayed significant anticancer activity against four cancer cell lines as compared to the control (hydroxyurea). This compound revealed low IC_{50} values; HeLa = 412 µM (5632 µM), MCF-7 = 390 µM (2829 µM), WiDr = 433 µM (1803 µM), and T47D = 179 µM (1803 µM). The incorporation of *n*-heptane aliphatic substituent would enhance the activity due to the increased lipophilic as well as pharmacokinetic properties. However, replacing the aliphatic chain with a tricyclic aliphatic reduces anticancer activity.



Scheme 9. The reaction between 4-hexyl benzoyl chloride with *N*-methylthiourea

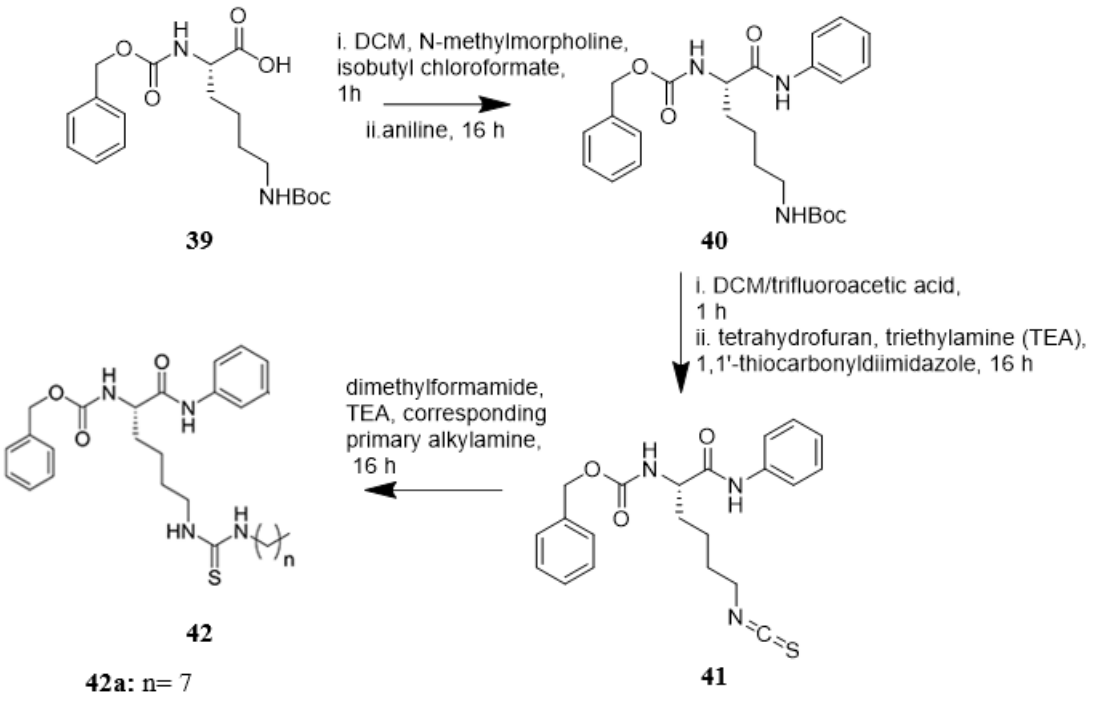
A new compound 4-piperazinylquinoline derived thioureas (Scheme 10) was developed and examined for their antiproliferative activity on three human breast tumour cell lines (MDA-MB231, MDA-MB468, and MCF7) as well as two non-cancer breast epithelial cell lines (184B5 and MCF10A) [16]. Among the novel compounds synthesized, compound **38a** namely (4-(7-chloro-quinoline-4-yl)-piperazine-1-carbothioic acid

(2-morpholine-4-yl-ethyl)-amide) showed significantly improved antigrowth/antiproliferative activity against breast cancer cells; MDA-MB231, MDA-MB468 and MCF7 with IC_{50} values ranging from 3.0 to 4.6 µM which is 7 to 11-fold selective to cancer cells and non-toxic to non-cancer cells. Attachment of quinoline, piperazine and morpholine on thiourea significantly enhanced antiproliferative activity with the cytotoxicity value 7 to11-fold higher in cancer than in noncancerous cells. This might be due to the heterocyclic compounds possessing hydrogen bond donor-acceptor capability which subsequently would enhance the lipophilic properties [34,35,36].



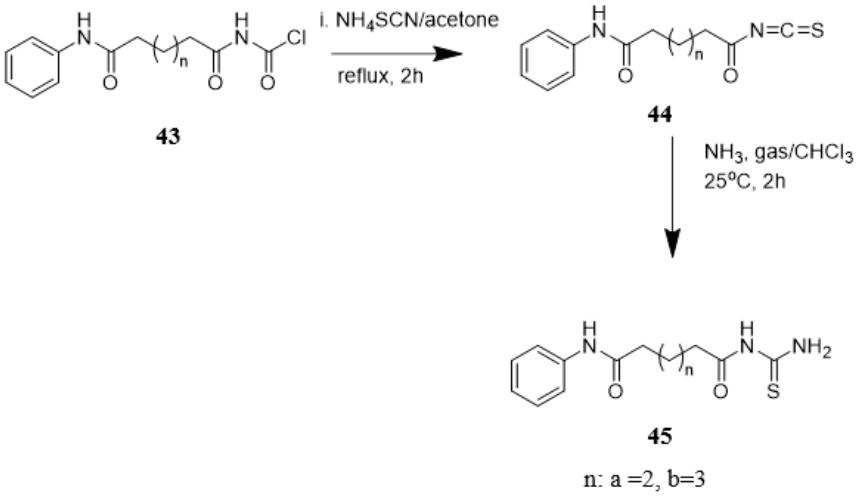
Scheme 10. Synthesis of 4-piperazinyl quinoline derived thiourea

Sirtuin 2 (SIRT 2) is an enzyme that is responsible for tumour suppressors in general, although knocking it down or inhibiting it has a broad anticancer effect in human breast cancer cell lines through boosting c-Myc degradation [37]. A novel lysine-derived thioureas as SIRT2 inhibitors were synthesized against MCF7 (breast cancer), MDA-MB-468 (breast cancer), MDA-MB-231(breast cancer), BxPC-3 (pancreatic cancer), NCI-H23 (lung cancer), A549 (lung cancer), SW948 (colorectal cancer), HCT116 (colorectal cancer), and CCD841 CoN (colon cell) [38]. The chemical reaction to synthesize the compounds is shown in Scheme 11. In comparison to thioamide-type inhibitor (reference drug), compound **42a** was highly selective and demonstrated broad cytotoxicity amongst cancer cell lines, specifically, human colorectal cancer cell line HCT116 with half-maximal growth inhibitory (GI_{50}) values of ~7 µM. None of the inhibitors was toxic to noncancerous HME1 epithelial cells.



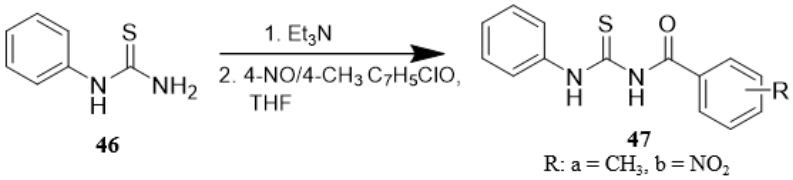
Scheme 11. Synthesis of Thiourea derivatives with varying chain lengths

Other novel compounds that were discovered are namely *N*-adipoyl monoanilide thiourea **(45a)** and *N*-pimeloyl monoanilide thiourea **(45b)** as histone deacetylase (HDAC) inhibitors [39]. Generally, HDAC is important to regulate epigenetic or non-epigenetic, as well as in cancer cell death, apoptosis, and cell cycle arrest [40]. The compounds were synthesized as shown in Scheme 12. Both compounds showed better inhibition against human colon adenocarcinoma (HRT-18) and mouse hepatoblastoma (HC-04) cells with a low cytotoxic effect on normal human breast cells (HBL-100) through MTT assay. It was also found that compound **45b** had higher antiproliferative activity than compound **45a** against the tested cancer cells with IC_{50} values of 21.44 µM and 24.12 µM and 27.37 µM and 30.42 µM, respectively against HC-04 and HRT-18 cell lines. The increased chain of carbon would increase the lipophilicity of the compound and subsequently enhance the cytotoxicity [41].



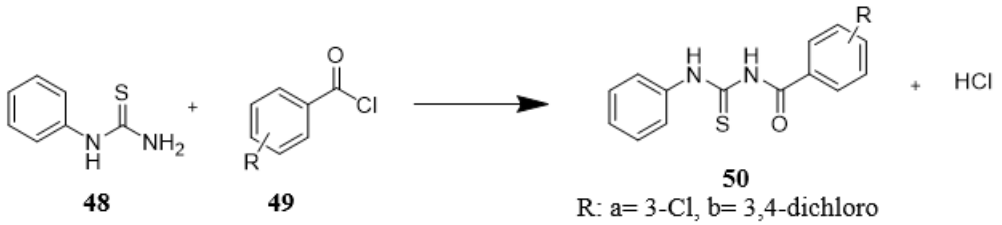
Scheme 12. Synthesis of the target compounds (45a-b)

Two compounds of *N*-(phenylcarbamothyoil) benzamide derivatives **(47)** were synthesized by reacting *N*-phenylthiourea, triethylamine **(46)**, and 4-nitro/4-methylbenzoyl chloride as presented in Scheme 13 [42]. This study investigated the effect of electronic and lipophilic substitution on anticancer activity. Compound **47b** with a strong electronic effect of 4-NO_2 (σ = 0.78) is more potent with lower IC_{50} values compared to compound **47a** with a strong lipophilic effect of 4-CH_3 (π =0.56) with IC_{50} values of 0.12±0.014 and 1.08±0.013 mM, respectively. The effect of the EWG is stronger than the EDG. Overall, the tested compounds have better activity than HU (4.58 ±0.019 mM) and are non-toxic to healthy Vero cells. The study suggested that *N*-(phenylcarbamothyoil) benzamide derivatives compound has a stronger electronic effect than lipophilic effect.



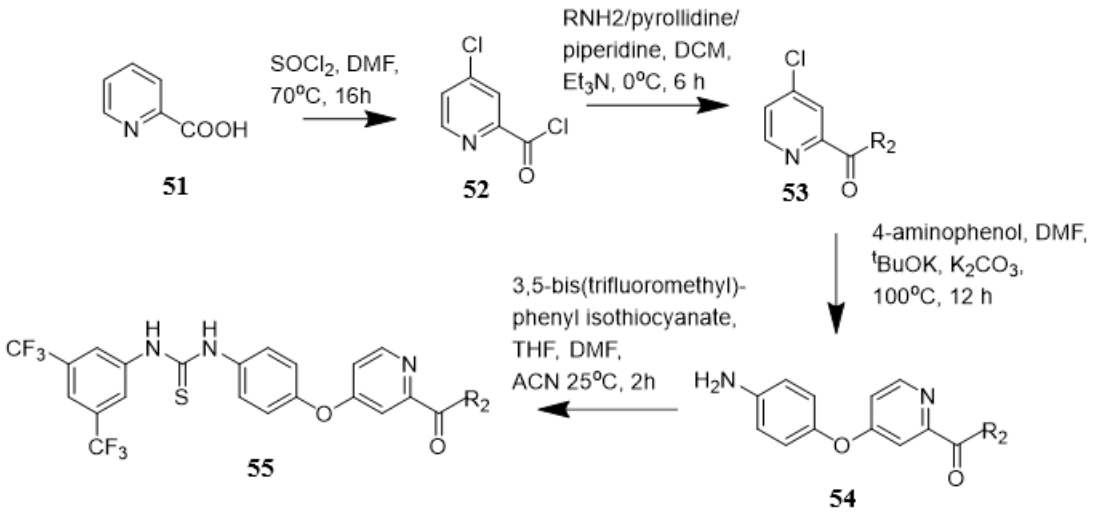
Scheme 13. Synthesis of *N*-(phenylcarbamothyoil) benzamide derivatives

In response to the strong EWG effect in cytotoxicity, another two compounds of *N*-benzoyl-*N'*-phenylthiourea derivatives were designed, namely *N*-(3-chloro)benzoyl-*N'*-phenylthiourea **(50a)** and N-(3,4-dichloro) benzoyl-*N'*-phenylthiourea **(50b)** [43]. The test compounds were synthesized from *N*-phenylthiourea (**48**) with benzoyl chloride derivatives (**49)** via acyl nucleophilic substitution reactions as represented in Scheme 14. A cytotoxicity study obtained from the MTT assay showed that **50a** has a higher cytotoxic effect than **50b** with IC_{50} values of 0.43 mM and 0.85 mM respectively. Overall, all compounds showed better anticancer activity compared with HU (IC_{50} = 4.58 mM). The activities of these two novel compounds were projected to be cell-targeted because they were harmful to cancer cells but not to Vero normal cells.



Scheme 14. Synthesis of *N*-benzoyl-*N'*-phenylthiourea or *N*-(phenylcarbamothioyl)-benzamide derivatives

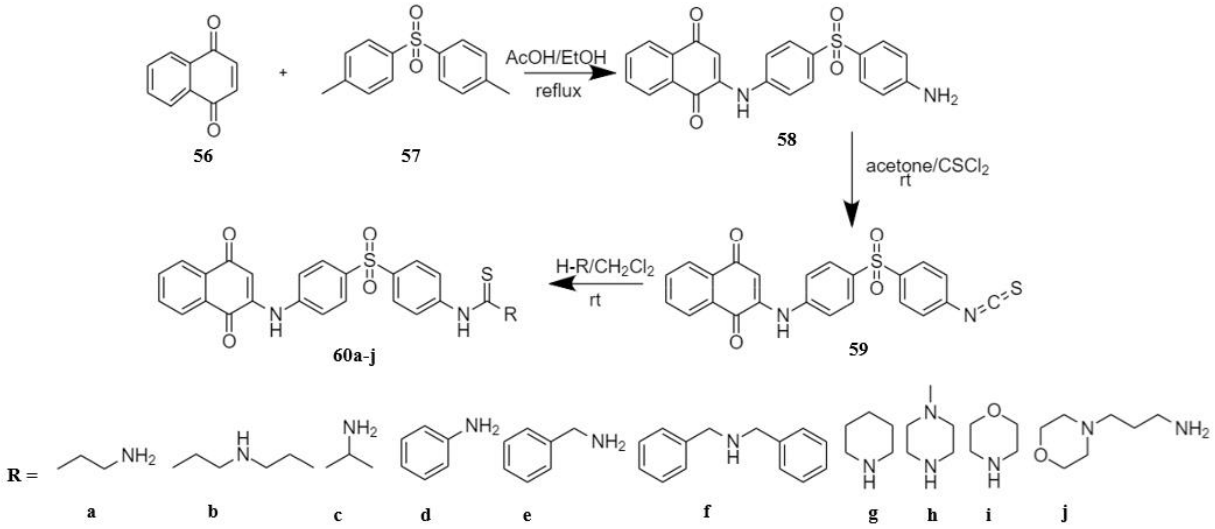
The synthesis of a series of novel thiourea derivatives containing 3,5-bis(trifluoromethyl)phenyl moiety (R_1) at terminal thiourea and phenylamino (R_2) at the terminal acyl position is shown in Scheme 15 [44]. Compound (**55a)** showed the best cytotoxicity against seven cancer cell lines: NCI-H460, Colo-205, HCT116, MDA-MB-231, MCF-7, HEpG2 and PLC/PRF/5 with IC_{50} values of 1.86, 9.92, 6.42, 8.21, 9.19, 6.21 and 7.82µM, respectively. Compound **55a** was found to be most potent with higher antiproliferative activities than Sorafenib and has excellent activity against normal cell HUVEC (IC_{50}: 2.81µM). When 3,5-bis(trifluorophenyl) was replaced with a nitro group (**55b**), good antiproliferative activity was observed. Meanwhile, none and weak activities were reported for unsubstituted (**55c**) and EDG substituent ring **(55d**) on phenyl ring respectively.



Scheme 15. Synthesis novel thiourea derivatives **55a-d**

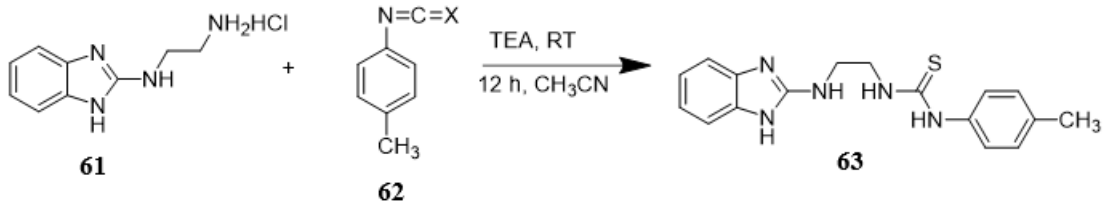
Another novel thiourea compounds that are potent anticancer chemotherapeutic are thiourea bearing dapsone-naphthoquinone derivatives [45]. The synthesis pathways of the desired compounds **(60a-j)** are illustrated in Scheme 16. The reaction between 1,4-naphthoquinone **(56)** and dapsone **(57)** produces 2-[4-(4-aminobenzenesulfonyl)-phenylamino]-[1,4]naphthoquinone **(58)**. The 2-[4-(4-isothiocyanato-benzenesulfonyl)-phenylamino]-[1,4]naphthoquinone **(59)** was obtained by adding thiophosgene to the **(58)** suspension in acetone. Finally, the synthesis of thiourea derivatives **(60a-j)** was completed by adding primary or secondary amines to the isothiocyanato intermediate **(59)**. The most effective compound was found to be a thiourea derivative containing *N*-methyl piperazine (**60h**) with an IC_{50} of 5.8±0.55 µM against chronic

myelogenous leukaemia (CML) (K562 cell lines). The anticancer action of thiourea derivatives was diminished by increasing the inhibitory effect of nucleophile steric hindrance. The anticancer activity of compounds **(60d–f)** containing phenyl, benzyl, and dibenzyl amines, respectively is significantly reduced. The anticancer activity of compounds **(60g-j)** revealed that adding N-CH_3 group to the thiourea skeleton considerably increased the anticancer activity of **60h**, with an IC_{50} of 5.8±0.55 µM. Furthermore, the anticancer activity of **60h** was 5.2 times more active than the carbon-substituted compound 60g (IC_{50}=30.37±2.21 µM) and 5 times more active than the oxygen-substituted compound **60i** (IC_{50}=29.06±2 µM).



Scheme 16. Preparation of the aimed compound (**60a–j**)

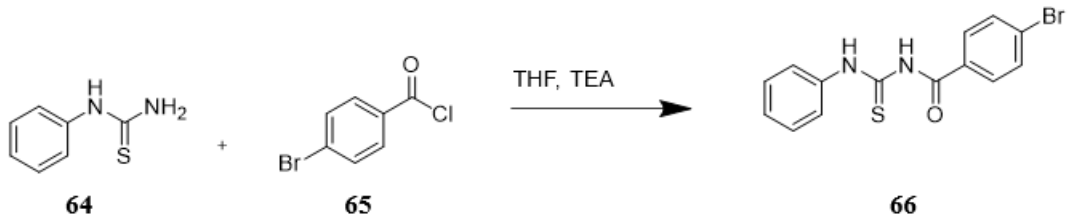
A new thiourea benzimidazole derivatives were synthesized, as outlined in Scheme 17 [47]. The cytotoxic activity of the compounds was assessed in human breast cancer cell lines viz MDA-MB-231 and MCF-7. The compound 1-(2-(1*H*-benzo[d]imidazol-2-yl-amino)ethyl)-3-*p*-tolylthiourea **(63)** was found with higher activity in MCF-7 with IC_{50} values of 25.8 µM, as compared with MDA- MB-231 cells (54.3µM). It was revealed that benzimidazole is connected to thiourea moiety through ethylenediamine linker contributed to the anticancer activity due to its resonance structure. These findings could lead to the development of novel thiourea benzimidazole compounds with significant anticancer properties.



Scheme 17. Synthesis thiourea benzimidazole derivatives **63**

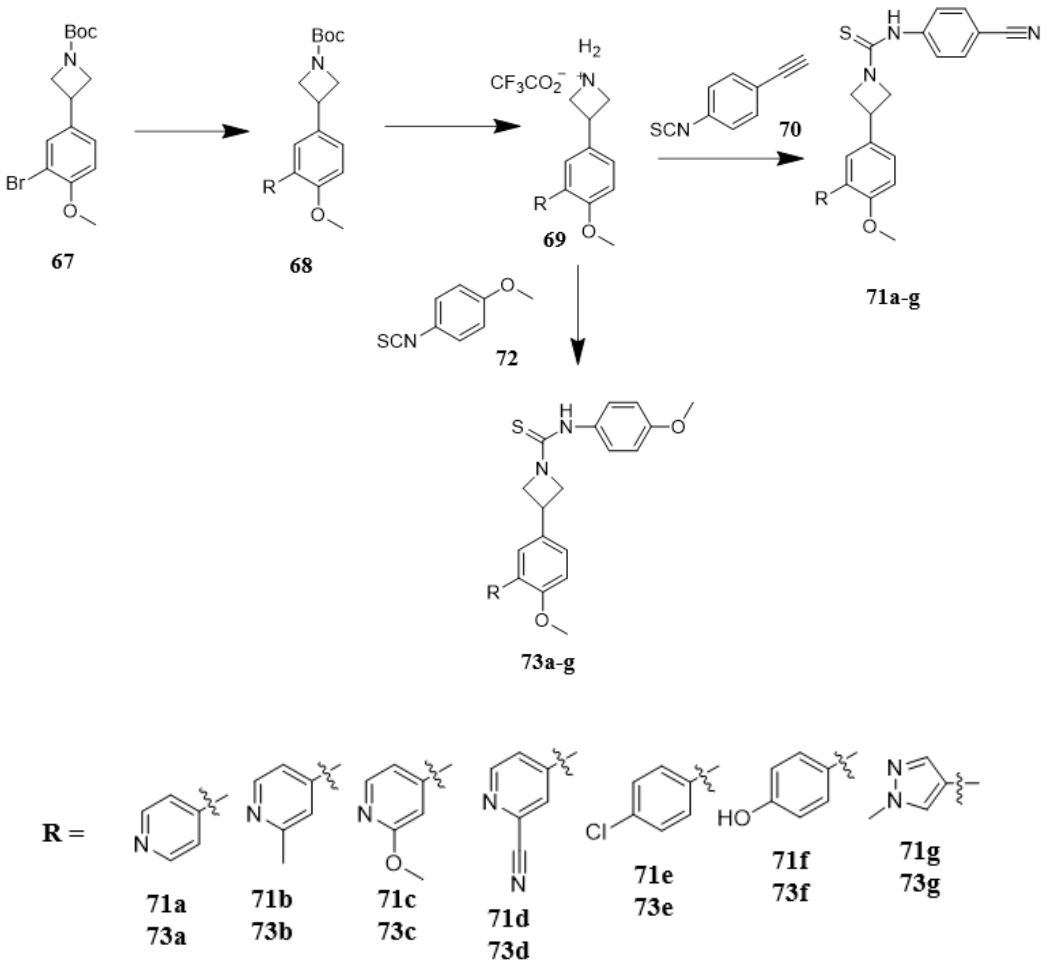
Next, a potent compound was revealed namely *N*-(4-bromo)-benzoyl-*N'*-phenylthiourea **(66)** [47]. The compound was synthesized by reaction of N-phenylthiourea **(64)** and 4-bromobenzoyl chloride **(65)** through acyl nucleophilic substitution as depicted in Scheme 18. In vitro cytotoxicity assay was carried out on HER2-positive primary breast cancer cells and produced IC_{50} values of 0.54 mM, lower than standard

drug hydroxyurea with an IC_{50} value of 11.61mM. This finding is correlated to the previous study [42] which confirmed that the inductive effect of EWG showed greater cytotoxicity than the lipophilic effect of EDG. Thus, the substituent of EWG for benzoyl ring of thiourea is important and could be further developed as an anticancer candidate for HER2-positive breast cancer.



Scheme 18. Synthesis of *N*-(4- bromo)-benzoyl-*N*'-phenylthiourea **66**

Other than that, a novel series of compounds were developed with thiourea-azetidine hybrid **(71a-g)** and **(73a-g)** [48]. The synthesis pathway of these compounds is illustrated in Scheme 19. All compounds were evaluated for their in vitro anticancer activity against various human cancer cell lines viz; lung (A549), prostate (PC3), breast (MCF7), liver (HepG2), colon (HCT116), ovarian (SKOV3), skin (A431), brain (U251), and kidney (786-O). With medium effective dose (EC_{50}) values of 0.25, 0.6, 0.03, and 0.03 µM, the compound of 3-(4-methoxy-3-(2-methoxypyridin-4-yl)phenyl)-*N*-(4-methoxyphenyl)azetidine-1-carbothioamide **(73c)** was shown to be the most potent member against PC3, U251, A431, and 786-O cancer cell lines, respectively, and demonstrated more potency than doxorubicin. In the A431 cell line, the median effective concentration (EC_{50}) values for compounds **73a-g** ranged from 0.03 to 12.55 µM. For A431 and 786-O cell lines, compound 3-(4-methoxy-3-(pyridin4-yl)phenyl)-N-(4-methoxyphenyl)azetidine-1-carbothioamide **(73a)** was shown to be highly effective, with EC_{50} values of 0.77 and 0.73 µM, respectively. All of the substances have a good to moderate level of toxicity. The study revealed that thiourea derivatives containing EDG (–OCH_3) on the phenyl ring and pyridine **(73a)**, substituted pyridine derivatives **(73b-d)** and substituted pyrazole derivative **(73g)**, respectively were found to be the most potent than compounds with substituted pyridine **(71a-d)** and pyrazole heterocycle derivatives **(71g)**, which contained EWG (–CN, in place of –OCH_3). In their series, the compound with *p*–chloro substitution and EWG (–CN) on the phenyl ring **(71e)** was found to have significantly higher activity against HepG2 and HCT-116 cell lines than phenyl derivatives with EDG (*p*-OH) **(71f)**. Out of nine cancer cell lines studied, the derivatives with EDG on both sides (–OH and –OCH_3) **(73f)** showed good to moderate activity on cell lines of A431, A549, HepG2, and HCT-116. Thus, this study confirmed that EDG substituent would enhance the cytotoxic activity.



Scheme 19. The synthesis of thiourea-azetidine derivatives (71a-g and 73a-g)

Another study is the synthesis and evaluation of several thioureas and benzylidine moieties as carbonic anhydrase-II (CA-II) inhibitors [49]. The CA-II enzyme is important to catalyse the reversible hydration of carbon dioxide, which results in the creation of bicarbonate and proton. The inhibition of this enzyme is used as a biomarker and therapeutic target in the treatment of leukaemia, cystic fibrosis, glaucoma, and epilepsy [50]. Within this study, several thiourea derivatives and compound **84** of the benzylidene series showed outstanding inhibitory activity against CA. The cytotoxic evaluation against 3T3 cell lines (Mouse Fibro-blast) using MTT assay exhibited prominent inhibition activity with IC_{50} values ranging from 1.90 ± 1.30 to 25.90 ± 2.05 µM compared to standard inhibitor acetazolamide (IC50 = 0.13 ± 0.05). Meanwhile, benzylidene derivatives showed weak inhibition potential with IC_{50} values in the range of 52.68 ± 0.47 to 348.57 ± 3.32 µM. Also, the study reported that *N*-(trifluoromethyl) benzoyl moiety in thiourea is important for CA II inhibition. The structures of thiourea derivatives are shown in Figure 4. It was revealed that compound **(82)**, with ethoxy substituents (EDG) on the N-phenyl ring, significantly enhance the potency of the compound with an IC_{50} value of 1.90 µM. The presence of EDG increased the electron density and activate the ring. Unsubstituted *N*-phenyl ring **(80)** showed a slight decrease in anticancer activity. However, the presence of EWG such as F, CN, NO_2 and Br group on the *N*-

phenyl ring for compounds (**74-83)** suppresses the activity with IC_{50} values ranging from 4.40 ± 1.80 µM to 25.90 ± 2.05 µM. On the other hand, the presence of furylmethylene ring increased the potency of compound **84**. Overall, it is indicated that EDG on the benzene ring of thiourea derivatives and benzylidene have significantly enhanced the anticancer activity.

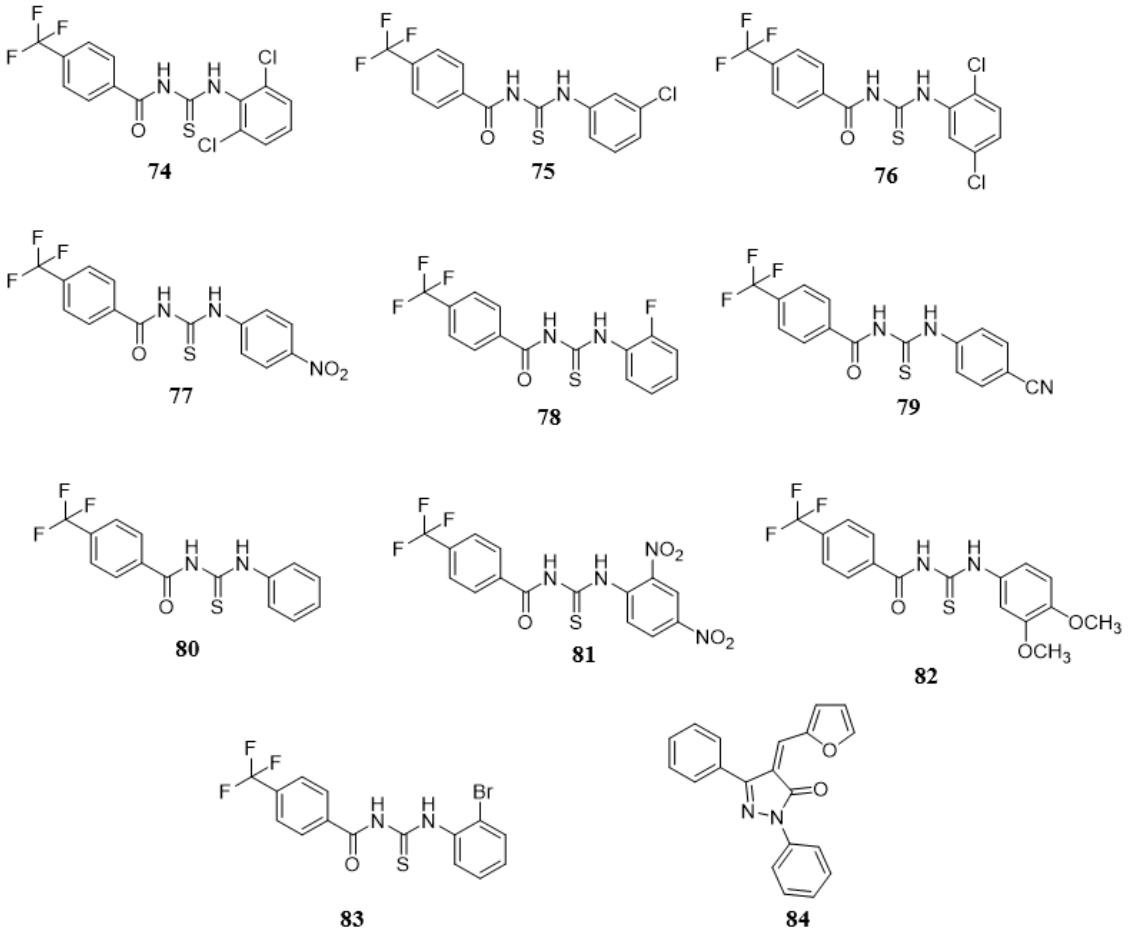
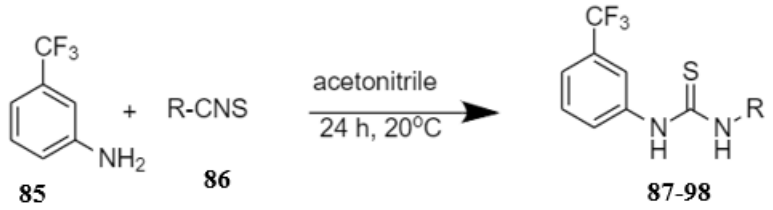


Figure 4. Structure of thiourea derivatives **74-84**

The potent *N*-(trifluoromethyl) benzoyl moiety linked to thiourea potential for anticancer activity led to the synthesis of 1,3-disubstituted thioureas **(87-98)** [12]. The synthesis pathway of these compounds involves a single-step reaction of 3-(trifluoromethyl)aniline **(85)** with various isothiocyanates **(86)**, belonging to a group of dihalogenophenyl **(87-90)**, halogenomethylphenyl **(91-92)**, alkylphenyl **(96)**, or monophenyl-substituted **(91–95)** derivatives (Scheme 20). Then, the cytotoxicity of the compounds was investigated against four human carcinoma cell lines, such as SW480 (primary colon cancer), SW620 (metastatic colon cancer), PC3 (metastatic prostate cancer), and K-562 (chronic myelogenous leukaemia), as well as against the normal cell line HaCaT (immortalized human keratinocytes), the IC_{50} values are summarized in Table 2. This study summarized the increasing impact of cytotoxicity according to the terminal benzene moiety functionalities as follows: 4-bromo- **(97)** < 2-(trifluoromethyl)- **(93)** < 2-methyl-5-chloro- **(92)** < 4-cyano- **(96)** < 2-methyl-3-chloro- **(91)** < 2,3-dichloro- **(90)** << 2,4-dichloro- **(89)** < 4-chloro- **(95)** < 3-chloro-4- fluoro- **(87)** < 4-(trifluoromethyl)- **(94)** < 3,4-dichloro- **(88)**. Dihalogenophenyl

derivatives **(87-90)**, compounds **94- 95** were the most active among the series towards all tumour cell lines with $IC_{50} \leq 10$ µM. The most promising drug candidates **(87-90, 94, 95)** were weakly cytotoxic towards normal HaCaT cell lines. Meanwhile, the introduction of the unsubstituted alkylphenyl group **(98)** to the thiourea branch dramatically decreased the compound's bioactivity. The structural modifications of the thiourea terminal moieties indicated the dihalogenophenyl derivative **(88)**, followed by its isomer **(89)** and para-substituted analogue **(94)**, as the most effective in cancer treatment. Therefore, this study highlighted the significance of EWG substituent in enhancing anticancer activity.



85 **86** **87-98**

87, R= 3-Cl, 4-F-Ph **90**, R= 2-Cl, 3-Cl-Ph **93**, R= 2-CF_3-Ph **96**, R= 4-CN-Ph
88, R =3-Cl, 4-Cl-Ph **91**, R =2-CH_3, 3-Cl-Ph **94**, R =4-CF_3-Ph **97**, R =4-Br-Ph
89, R = 2-Cl, 4-Cl-Ph **92**, R = 2-CH_3, 5-Cl-Ph **95**, R = 4-Cl-Ph **98**, R = -CH_2CH_2-Ph

Scheme 20. Synthesis procedure for 3-(trifluoromethyl)phenylthiourea derivatives **87-98**.

Table 2. Cytotoxic activity (IC_{50}, µM) of the studied compounds estimated by the MTT assay

No.	R	Cancer Cells				Normal Cells
		SW480	SW620	PC-3	K-562	HaCaT
87	3-Cl,4-F-Ph	12.7	9.4	53.6	6.8	43.6
88	3-Cl,4-Cl-Ph	9.0	1.5	31.7	6.3	24.7
89	2-Cl,4-Cl-Ph	30.1	5.8	13.7	54.3	52.1
90	2-Cl,3-Cl-Ph	22.5	14.0	10.5	35.9	13.9
91	2-CH_3, 3-Cl-Ph	7.3	23.2	51.8	52.5	55.6
92	2-CH_3, 5-Cl-Ph	15.6	22.1	65.9	40.3	14.5
93	2-CF_3-Ph	>100	30.1	17.1	76.5	65.7
94	4-CF_3-Ph	8.9	7.6	6.9	54.8	41.3
95	4-Cl-Ph	38.1	6.7	22.6	10.2	71.5
96	4-CN-Ph	20.6	18.7	66.2	12.9	50.1
97	4-Br-Ph	41.5	16.2	76.6	74.2	17.2
98	-CH_3CH_3-Ph	25.3	38.2	26.7	23.8	55.4
Cisplatin	-	10.4	6.7	13.2	8.2	6.3

Conclusion

This review summarizes the recently synthesized thiourea compounds and their anticancer activities. It shows that thiourea moiety is important in discovering potent, effective and safer drugs to treat cancer. As described above, there were numerous synthesis pathways to prepare the thiourea derivatives. Based on the evaluation of thiourea derivatives as an anticancer agent, modification of thiourea moiety with suitable substituents at N1 and N2 improved the cytotoxicity properties of the compounds. The study also indicates that the substituent containing electron donating and electron withdrawing groups play an excellent role in improving anticancer activities. Even though most of these reported compounds are in clinical trials, the urgent need for further derivation and cytotoxicity studies will provide an opportunity for managing therapeutic values more efficiently and with greater efficacy. In short, the thiourea derivatives scaffold is a valuable candidate in the design and development of novel compounds that warrants further investigation with a clinical trial.

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