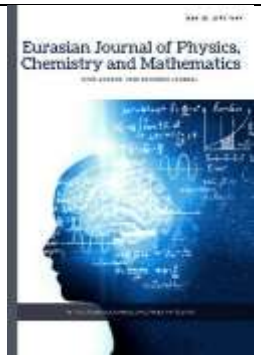

Spectrum Antimicrobial Agent Displayed Potent Growth Inhibitory Effect Toward Non-Small Cell Lung Cancer

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ABSTRACT

Following the synthesis of a variety of novel 6-aryl-5-cyano thiouracil derivatives, research was conducted to explore the antibacterial, antifungal, and anticancer effects of these compounds. When tested against the Gram-positive bacteria S. aureus and B. subtilis, the findings of the antimicrobial test indicated that compounds 7b and 7c had a greater degree of antibacterial activity than the reference drug amoxicillin. Compound 4i was shown to be an antibacterial agent with a broad spectrum, and it also displayed the highest antifungal activity against C. albicans. In addition to this discovery, compound 4i was found to be an anticancer agent. In point of fact, the antifungal activity of this medication was significantly higher than that of the standard treatment amphotericin B (MIC = 2.34, compared to 3.00 g/mL for amphotericin B). In line with the methodology devised by the National Cancer Institute (NCI) in the United States of America, certain compounds were examined for their in vitro cytotoxicity at a single concentration of 105 M. When evaluated for their potential to kill kidney cancer cell lines UO-31 and/or A498, the great majority of the compounds were found to have only a modest level of cytotoxic activity, as indicated by the findings of the first screening. Despite this, compounds 6d and 6i displayed a significant growth inhibiting influence on the leukaemia MOLT-4 cell line as well as the non-small cell lung cancer HOP-92 cell line.

Keywords: 6-aryl-5-cyano thiouracils; antibacterial; antifungal; anticance

Introduction

Important component of nucleic acids

Pyrimidines are an essential part of nucleic acids and have been put to use in the pharmaceutical industry as building blocks in the manufacturing of antiviral, anticancer, antibacterial, and antifungal medicines. Pyrimidines are also a significant component of ribonucleic acids. In a similar vein, the related thiouracil derivatives have the potential to be used as therapeutic agents in the treatment of viral infections, cancer, and infectious diseases. For instance, it was just recently discovered that products of S-alkylation and N-alkylation work as new antibacterial agents, cytotoxic agents, and one-of-a-kind HIV reverse transcriptase inhibitors. In addition, a search of the relevant literature showed that the thiouracilcarbonitrile ring system has a prominent place in the design and synthesis of new chemotherapeutic drugs that exhibit outstanding anticancer and antibacterial effects (Figure 1). Particularly, 2-[(1H-benzoimidazol-2-yl) methylthio]-4-hydroxy-6-phenylpyrimidine-5-carbonitrile (I) showed considerable antiproliferative efficacy throughout a broad range when tested in vitro. In addition to this, thiouracil quinoxaline hybrids II showed substantial inhibitory effects on the EBV-EA activation while also having a chemopreventive impact against carcinogenesis in Raji cells. The nitrofuran analogue III, on the other hand, exhibited a unique inhibitory action against a variety of Gram-positive bacteria. In the meanwhile, a variety of 4-anilino- and 4-hydrazinothiopyrimidine-5-carbonitriles, as well as their condensed heterocycles, shown

promise chemotherapeutic action as antibacterial and anticancer agents. Additionally, it was of great interest that specifically functionalized S-aralkylated 6-aryl-5-cyano-2-thiouracils may possess specific biological properties. These properties include the inhibition of bacterial protein translocase SecA (compound IV), hepatitis C viral NS5B RNA dependent RNA polymerase (compound V), and potent antagonist of Epac protein—a therapeutic target of cancer—(compound VI).

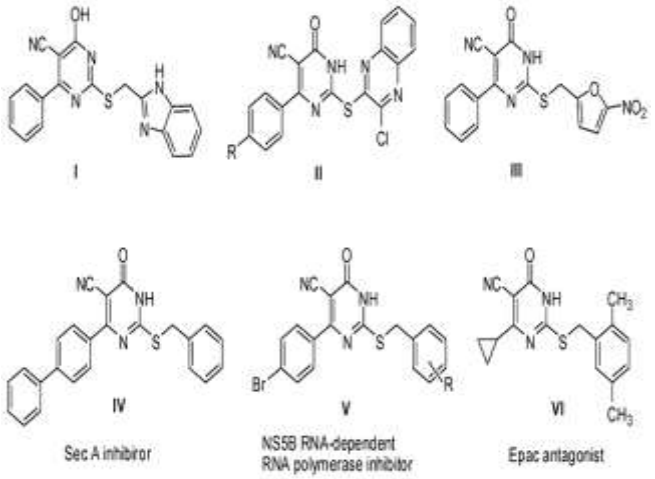
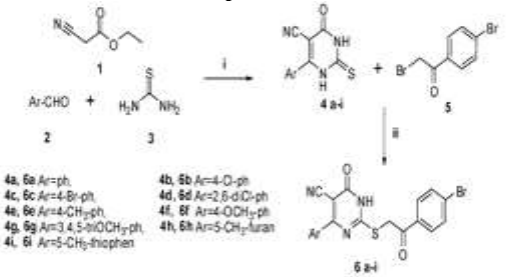


Figure 1. Structures of several very effective anticancer and antibacterial agents Lead compounds with a 6-aryl-5-cyano-2-thiouracil structure

We propose the synthesis and biological evaluation of novel 6-aryl-5-cyano-2-thiouracil derivatives 6a–i and 7a–c as potential antibacterial and cytotoxic drugs. These derivatives were shown to inhibit the growth of bacterial cells. The decision to do this was made in consideration of the biological significance of the thiouracils that were discussed before. In a previous report, it was stated that for a series of 5-substituted-2-anilinopyrimidinones, a systematic increase in antimicrobial potency was observed upon elongation of the alkyl spacer between the phenyl ring and the pyrimidinone pharmacophore from one to three carbons. This information was used as the basis for the design of the target compounds 6a–i. This insight served as the foundation for the development of the compounds 6a–i that were intended as targets. A novel series of thiouracil-5-carbonitile derivatives 6a–i have been produced in a method that is comparable to the one that is being used in this strategy. The modification of the structure of these compounds has centred on changing the aryl methyl moiety in the lead compounds I, II, and IV to a bromobenzoylmethyl moiety while simultaneously introducing a variety of substituted aryl groups at the position 6 of the thiouracil ring. This has allowed the compounds to have a structure that is more favourable for the production of the desired compound. In order to bestow onto the molecules different lipophilic and electrical environments, a substituent was selected to put on the aryl group, and this was done in order to fulfil the aforementioned goal.

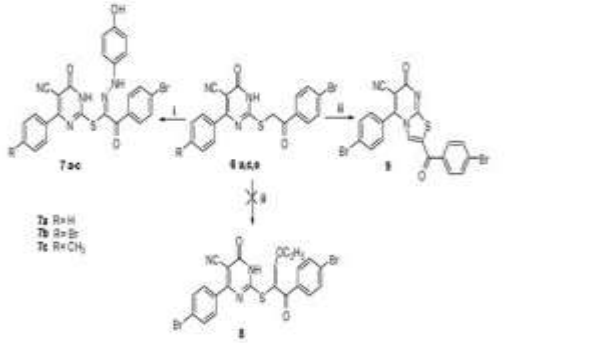
In addition, the 4-hydroxyphenylhydrazono derivatives 7a–c were generated by exploiting the active methylene site present in 6 in order to include an additional pharmacophoric group. This was done in order to make the compound. Hydrazones are an important class of compounds that display specific antifungal and antibacterial activities in addition to their broad-spectrum anticancer activity. Hydrazones have been shown to inhibit the growth of cancerous cells.

Schemes 1 and 2 respectively depict the synthetic processes that were utilised in order to produce the target compounds 6–9. The structural makeups of the newly synthesised compounds have been identified based on the elemental analyses and spectrum data collected from the compounds..



Reagents and conditions: (i) anhydrous K_2CO_3, absolute ethanol, reflux 12 h; (ii) anhydrous K_2CO_3, dry benzen, reflux, 24 h.

Figure 2 Synthetic pathway for target compounds 4a–i and 6a–i.



Reagents and conditions: (i) *p*-aminophenol, glacial acetic acid, $NaNO_2$, stir at −5 °C, 30 min. (ii) $(C_2H_5O)_3CH$, acetic anhydride, reflux, 8 h.

Figure 3. Various routes of synthesis for compounds 7a–c and 9.

The 6-aryl-4-oxo-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carbonitrile (thiouracils) 4a–i were created through the tertiary condensation of ethyl cyanoacetate 1 with the appropriate aldehyde 2 and thiourea 3 in the presence of anhydrous potassium carbonate. This reaction was carried out in the presence of anhydrous potassium carbonate. The infrared spectra of these compounds may be differentiated from one another based on the presence of NH stretching bands in the range of 3410–3124 cm1, CN bands in the range of 2214–2152 cm1, C=O bands in the range of 1652–1625 cm1, and C=S bands in the range of 1253–1222 cm1.

In order to produce compounds 6a–i, a selective S-alkylation of 4a–i was carried out in refluxing dry benzene with bromophenacyl bromide 5 utilising potassium carbonate as the base catalyst. This reaction was carried out in order to produce compounds 6a–i. The chemicals were created as a result of this method. The infrared spectral data for compounds 6a–i indicated that there were no absorption bands for C=S. However, the data also showed that there was an additional benzoyl C=O band at 1735–1693 cm1. Their 1H-NMR spectra showed the existence of a singlet signal that resonated at 5.98–4.51 ppm and might be attributed to the SCH2 structure. The tautomeric forms A and B are both possibilities for the presence of the compounds 6a–i in nature. (Figure 2). In order to distinguish between all of these distinct forms, 13C-NMR spectra of compounds 6b–f were collected and analysed. The spectra showed that there were two carbonyl signals, one corresponding to the benzoyl C=O at 194.50–194.09 ppm and the other corresponding to the pyrimidinone C=O at 166.61–160.36 ppm. The nature of the nitrogen atom that is near to the pyrimidinone carbonyl has been discovered to have a considerable influence on the chemical shift of the pyrimidinone carbonyl, according to the findings that were published in the academic literature. According to the values of the pyrimidinone C=O in compounds 6b–f, the N-(3) that is next to the C=O may be sp3-hybridized (pyrrole type). Because of this, it is comparable to that which is found in the methyl derivative 10, and it is separate from the C=O that is close to sp2-hybridized nitrogen (pyridine type), which emerges at 175–170 ppm. This is because it is akin to that which is found in the methyl derivative 10. (compound 11). (Figure 2). As a direct consequence of this, compounds 6a–i are only ever found in a single tautomeric form, which is indicated by the letter A as opposed to the letter B.

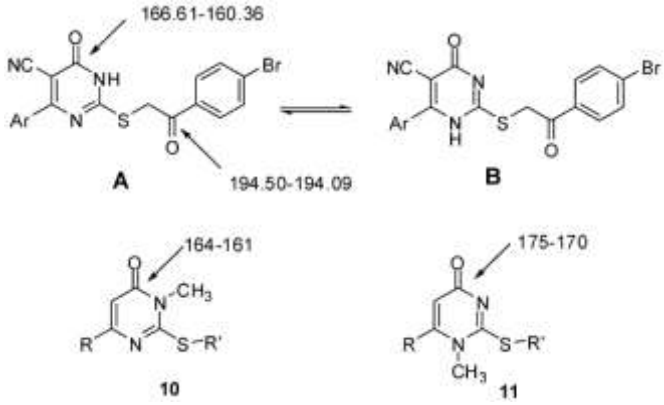


Figure 2. Possible tautomeric structures of compounds 6a–i, as well as the 13C-NMR chemical shifts of previously described thiouracils.

Compound 6a, Compound 6c, or Compound 6e underwent a reaction with diazotized p-aminophenol in an aqueous solution of sodium hydroxide at a temperature of 5 degrees Celsius, which resulted in the formation of the corresponding arylhydrazono derivatives 7a–c (Scheme 2). Compounds 7a–c are capable of occurring in any one or more than four distinct tautomeric forms, which are denoted by the letters C–F. In comparison to the enolazo tautomeric forms, the data from their infrared spectrum seems to be more consistent with the hydrazone structures (C or D) (E or F). For example, each of the compounds contained two

carbonyl bands in the ranges 1654–1632 and 1670–1660 cm1, which matched to the stretching vibrations of the pyrimidinone and the benzoyl carbonyl groups, respectively. These carbonyl bands were present in all of the compounds. Within the middle portion of the carbonyl spectrum, several locations were discovered. The low value of the wave number that was assigned for the subsequent C=O stretching band appears to be the result of chelation with NH and conjugation with the C=N double bond, both of which are required by the hydrazone form C or D. The hydrazone form C or D can only exist in one of these two configurations. In the region of 9.41–13.20 ppm, the phenolic OH, the hydrazone NH, and the pyrimidinone NH were all contributors to the three exchangeable singlet signals that were seen by the 1H-NMR. It was discovered that the signals that were created by SCH2 were absent from the system. The 13C-NMR spectrum of compound 7c was recorded and then compared to the spectra of compounds 6b–f in order to differentiate between the C and D tautomers. This was the final step in the process. In a manner that is analogous to the findings presented in 6b–f and 10, it was determined that pyrimidinone C=O was detected at a concentration of 161.04 ppm. From these observations, one may draw the conclusion that the form C of the hydrazone derivatives 7a–c is the one that occurs with the greatest frequency.

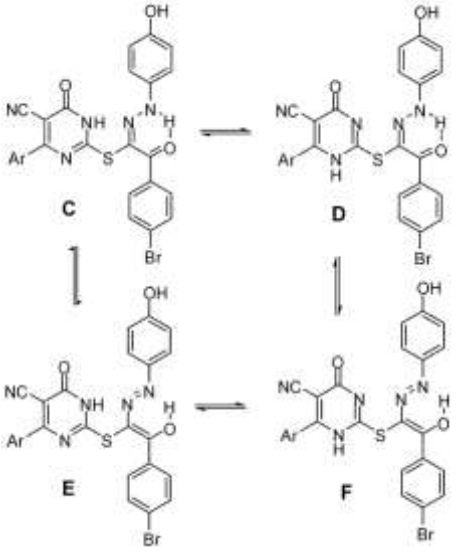


Figure 3. Possible tautomeric structures of compounds 7a–c

When the chemical 6c was refluxed with triethyl orthoformate in acetic anhydride, the thiazolo[3,2-a]pyrimidine derivative 9 was formed as a consequence of the intramolecular cyclization of the latter. This occurred as a result of the thiazolo[3,2-a]pyrimidine derivative 9 being created. It was hypothesised that this would result in the formation of derivative 8 of ethoxy methene. It was discovered that the SCH2 signal had vanished from the 1H-NMR spectrum of the product, which also demonstrated the appearance of a singlet signal at 7.65 ppm, which was determined to be linked to the thiazole CH. In the 1H-NMR spectrum, the important characteristic was the absence of the triplet-quartet pattern of the ethoxy group as well as the D2O exchangeable (NH) signal. This absence highlighted the production of the cyclic thiazolo[3,2-a]pyrimidine derivative 9, which was a beneficial outcome. Moreover, this derivative was successful.

Objectives Of the Study

1. To study on different cancer types: leukemia, melanoma, lung, colon, central nervous system (CNS), ovarian, renal, prostate and breast cancers
2. To study on synthesized compounds expressed as minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and concentration

Methodology

Experimental

Chemistry

The Gallenkamp melting point apparatus MFB-595-010M is used to make the determinations in one-end open capillary tubes, and the melting points are determined without any corrections (Gallenkamp, London, England). The microanalysis was performed at Al-Azhar University's Micro-analytical Unit, which is part of the Regional Centre for Microbiology and Biotechnology. Using potassium bromide discs, infrared spectra were recorded using a Shimadzu FT-IR 8400S spectrophotometer (Shimadzu, Kyoto, Japan), and the findings are given in wave numbers (cm1). The NMR

spectra were obtained by using a Varian Mercury VX-300 NMR spectrometer to get the readings. In dimethylsulphoxide, 1H- spectra were acquired at a frequency of 300 MHz, while 13C- spectra were acquired at a frequency of 75.46 MHz (DMSO-d6). The chemical shifts are denoted by the symbol and are connected to the solvents' properties. Both a Hewlett Packard Varian (Varian, Palo Alto, California, United States) and a Shimadzu Gas Chromatograph Mass Spectrometer-QP 1000 EX were utilised in order to record mass spectra (Shimadzu, Kyoto, Japan). TLC was carried out with the use of Art.DC-Plastikfolien, Kieselgel 60 F254 sheets (Merck, Darmstadt, Germany), the developing solvents were chloroform/methanol (9:1), and the spots were seen at 366 and 254 nm by UV Vilber Lourmat 77,202. (Vilber, Marne La Vallee, France). Compounds 4a–c and 4e–g were produced by following the processes that were detailed, whereas compound 6a is accessible for purchase in commercial settings.

Anticancer Activity

The Developmental Therapeutic Program at the National Cancer Institute (NCI) chose thirteen different compounds (4d, 4g–i, and 6a–i) to test for their anticancer efficacy in vitro. These compounds were divided into three groups. The anticancer tests were carried out in line with the protocol that was established by the Drug Evaluation Branch of the National Cancer Institute in Bethesda. In the main anticancer assay, the compounds were tested with a single dosage against a panel of about 60 cancer lines at a concentration of 105 M. The results of this test were then analysed. The human tumour cell lines were obtained from nine distinct forms of cancer, including leukaemia, melanoma, lung, colon, central nervous system (CNS), ovarian, kidney, prostate, and breast cancers respectively. In order to determine the cell survival and growth rate, a methodology for drug exposure lasting for 48 hours was utilised, and a sulforhodamine B (SRB) protein test was carried out. The results for each tested agent were given as the percentage growth of the treated cells in comparison to the untreated control cells. Additionally, the results for each tested agent were shown as a mean graph of the growth that was seen.

Secondary sources : we use journal , magazines , news paper , article , websites etc.

Result

Biological Evaluation

Antimicrobial Activity

Using the microbroth dilution method, the newly synthesised compounds were tested for their antibacterial activity in vitro against Staphylococcus aureus ATCC 6538P, Bacillus subtilis ATCC CC33, Escherichia coli ATCC 5087, and Pseudomonas aeruginosa ATCC 9027. Additionally, the compounds were tested for their antifungal activity against Candida albicans ATCC 60193 and Asper The minimum bactericidal concentration (MBC), which reflects the bactericidal activity of the tested compounds, was calculated at g/mL. The minimum inhibitory concentration (MIC) and concentration that inhibit 50% of microorganisms (IC50) serve as measures of the microbial inhibitory activity, while the minimum bactericidal concentration (MIC) serves as a measure of the bactericidal activity (Table1). Compounds 6a and 6c were shown to have good to fair antibacterial activity throughout a broad spectrum, according to the findings that were provided in Table 1. On the other hand, compounds 4d, 4h, 6f, 6g, and 7a–c were only active against Gram positive strains. Compound 4g had moderate antifungal activity against C. albicans and A. niger, but compound 9 evoked only modest antifungal activity against A. niger together with Gram-positive antibacterial activity. Compound 4i alone showed significant antibacterial and antifungal activity throughout a broad spectrum when compared to the other compounds that were examined. On the other hand, at concentrations of up to 50 g/mL, the other compounds 6b, 6d, 6e, and 6i showed no significant action against any of the strains that were tested (Figure 4).

Table 1. Antimicrobial activity of the synthesised compounds against pathogenic bacteria, represented as minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and concentration that

inhibits 50% of microorganisms (IC50) in g /mL.

Table 1 shows the antimicrobial activity of the compounds that were synthesised, expressed as minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and concentration that inhibits 50% of microorganisms (IC50) in g / mL against the pathological strains based on the two-fold serial dilution technique..

Compound		Gram positive bacteria		Gram negative bacteria		Fungi	
		S. aureus	B. subtlis	E. coli	P. aeruginosa	C. albicans	A. niger
4d	MIC	9.38	9.38	>50	>50	>50	>50
	MBC	9.30	9.30	>50	>50	>50	>50
	IC50	4.20	6.25	>50	>50	>50	>50
4g	MIC	>50	>50	>50	>50	9.38	18.75
	MBC	>50	>50	>50	>50	9.30	18.75
	IC50	>50	>50	>50	>50	6.25	12.50
4h	MIC	1.17	1.17	>50	>50	>50	>50
	MBC	1.56	1.56	>50	>50	>50	>50
	IC50	0.40	0.78	>50	>50	>50	>50
4i	MIC	2.34	9.38	9.38	9.38	2.34	4.69
	MBC	2.30	9.30	9.30	9.30	2.30	6.25
	IC50	1.17	6.25	3.13	3.13	1.17	3.13
6a	MIC	4.69	9.38	18.75	37.50	>50	>50
	MBC	3.80	9.00	18.75	37.50	>50	>50
	IC50	2.30	6.25	12.50	25.00	>50	>50
6b	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC50	>50	>50	>50	>50	>50	>50
6c	MIC	37.50	37.50	37.50	37.50	> 50	> 50
	MBC	37.50	37.50	37.50	37.50	> 50	> 50
	IC50	12.50	25.00	25.00	25.00	> 50	> 50
6d	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC50	>50	>50	>50	>50	>50	>50
6e	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC50	>50	>50	>50	>50	>50	>50
6f	MIC	2.34	4.69	>50	>50	>50	>50
	MBC	3.13	6.25	>50	>50	>50	>50
	IC50	1.56	3.13	>50	>50	>50	>50
6g	MIC	4.69	4.69	>50	>50	>50	>50
	MBC	4.70	4.70	>50	>50	>50	>50
	IC50	1.56	1.56	>50	>50	>50	>50
6h	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC50	>50	>50	>50	>50	>50	>50
6i	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC50	>50	>50	>50	>50	>50	>50
7a	MIC	18.75	18.75	>50	>50	>50	>50
	MBC	18.75	18.75	>50	>50	>50	>50

	IC50	12.50	12.50	>50	>50	>50	>50
7b	MIC	1.17	2.34	>50	>50	>50	>50
	MBC	1.17	2.30	>50	>50	>50	>50
	IC50	0.78	1.17	>50	>50	>50	>50
7c	MIC	0.19	1.17	>50	>50	>50	>50
	MBC	0.20	1.17	>50	>50	>50	>50
	IC50	0.15	0.40	>50	>50	>50	>50
9	MIC	37.50	37.50	>50	>50	>50	37.50
	MBC	37.50	50.00	>50	>50	>50	50.00
	IC50	12.50	37.50	>50	>50	>50	37.50
Amoxicillin	MIC	1.25	150.00	NA	NA	NA	NA
Gentamicin	MIC	NA	NA	1.00	8.00	NA	NA
Amphotericin B	MIC	NA	NA	NA	NA	3.00	1.25

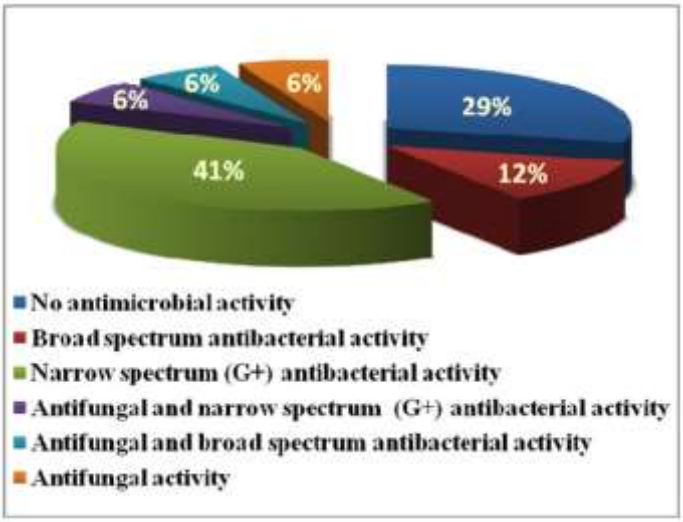


Figure 4. The antimicrobial spectrum of tested compounds

It was determined, based on the structure-activity connection of the compounds that were investigated, that the beginning 6-aryl-5-cyano-2-thiouracil derivatives 4d, 4g–i possessed moderate to powerful antibacterial activity. This conclusion was reached after the structure-activity connection of the compounds that were investigated was examined. It would appear that the aryl substituent that is located in the thiouracil ring at position 6 is essential to both the antibacterial spectrum as well as the efficacy of these compounds. Compound 4d, which included a 2,6-dichlorophenyl, had no effect on Gram negative bacteria but did have an effect on Gram positive bacteria. The methylfuran analogue 4h showed an increase in inhibitory activity against the same bacterial isolates that was eight times higher than that of the control group. This was determined by comparing the results to a standard. As can be seen in Figure 4i, the addition of methylthiophene to the thiouracil ring widened the antibacterial spectrum, which had previously been restricted. The kind of bacteria that responded to 4i with the highest sensitivity was S. aureus, followed by B. subtilis, E. coli, and P. aeruginosa in that order. In addition, it demonstrated the most potent antifungal activity against C. albicans, even more potent than the medication that was used as a reference, amphotericin B (MIC = 2.34, 3.00 g/mL respectively), and it demonstrated activity that was reasonably effective against A. niger. Both of these findings were based on the fact that it inhibited the growth of the fungus. On the other hand, our research revealed that the trimethoxyphenyl congener 4g demonstrated just a minute level of antifungal activity.

After being put through a series of tests to determine whether or not the S-bromobenzoylmethyl thiouracil derivatives 6a–i had any antifungal activities, the findings indicated that they do not. Because of this, researchers came to the conclusion that S-alkylation had a negative impact on the antifungal activity of the thiouracils that were manufactured in a lab (4g versus 6g and 4i versus 6i). On the other hand, it would appear that the type of aryl substituent that is present on the thiouracil scaffold has an influence on the antibacterial activity of the compounds in issue. [Citation needed] Compound 6a, which contained a phenyl group that was not substituted, demonstrated a high level of activity against Gram-positive bacteria such as S. aureus and B. subtilis, but only a moderate to low level of activity against Gram-negative bacteria such as E. coli and P. aeruginosa. This difference in activity levels was due to the presence of the phenyl group. Compounds 6f and 6g were created as a result of the incorporation of a phenyl-bearing mesomeric electron-donating methoxy group into the mesomeric structure. Only Gram-positive bacteria are susceptible to the enhanced action of these chemicals. On the other hand, analogues with heteroaryl substituents 6h and 6i or phenyl linked to inductive electron-donating or electron-drawing atoms such as in 6b–e were either ineffective or mildly active against the bacteria that were the subject of the investigation. This is supported by the presumption that the electronic properties of the substituents are of the highest significance.

Compounds 7a–c, which demonstrated a powerful inhibitory effect against Gram-positive S. aureus and B. subtilis, were produced by attaching a 4-hydroxyphenylhydrazono moiety to the active methylene found in compounds 6a, 6c, and 6e. This addition led to the development of compounds 7a–c. The amount of activity displayed by compound 7a, which included a phenyl substituent in the 6-position of the thiouracil ring, was about in the centre of the spectrum. When contrasted with the conventional antibiotic amoxicillin, the more lipophilic p-bromophenyl 7b and p-tolyl 7c counterparts displayed much greater levels of antibacterial activity. In particular, 7c was found to be the most effective antibacterial agent in this study, with a minimum inhibitory concentration (MIC) of 0.19 g/mL against S. aureus and 1.17 g/mL against B. subtilis, respectively. This finding was made possible by the fact that 7c was tested against both strains of bacteria. Based on this discovery, it appears that the presence of an electron-donating methyl group is more advantageous to the antibacterial action of this class of compounds than the presence of an electron-withdrawing bromine atom is in this regard. In addition, the fact that compounds 7a–c contain a 4-hydroxyphenylhydrazono moiety has the potential to explain for the exceptional antibacterial activity that these compounds exhibit. It has been demonstrated that phenolic compounds possess the ability to sensitise the phospholipid bilayer of the cytoplasmic membrane of microorganisms. This can lead to increased permeability, the inaccessibility of essential intracellular constituents, and/or the impairment of bacterial enzyme systems that are involved in the production of energy.

The cyclization of compound 6c into the corresponding thiazolo pyrimidine derivative 9 had an influence on the microbiological spectrum, but it did not have an effect on the efficacy of the medicine. In contrast to compound 6c, which demonstrated moderate antibacterial activity across a wide range but no antifungal activity, compound 9 demonstrated only little antimicrobial activity against Gram-positive bacterial strains as well as the fungus A. niger. Because of the little difference in concentration between the minimum inhibitory concentration (MIC) and the maximum inhibitory concentration (MBC) of active compounds, we may conclude that these compounds do not have bacteriostatic effects; rather, they have antibacterial effects

Anticancer Activity

The RPMI 1640 medium with 5% foetal bovine serum and 2 mM L-glutamine is used to cultivate the human tumour cell lines that are used in the cancer screening panel. In a typical screening experiment, cells are seeded into 96

well microtiter plates in a volume of 100 L at plating densities that range anywhere from 5,000 to 40,000 cells per well. These plating densities are determined by the doubling time of each specific cell line. After cells have been seeded onto the microtiter plates, the plates are given a 24-hour incubation at 37 degrees Celsius, 5% carbon dioxide, 95% air, and 100% relative humidity before any investigational medications are added. After waiting for 24 hours, two plates of each cell line are then fixed in situ with trichloroacetic acid (TCA), which represents a measurement of the cell population for each cell line throughout the time of drug addiction (Tz). Before being put to use, unproven medicines are first dissolved in dimethyl sulfoxide at a concentration that is 400 times higher than the highest final test concentration that is intended, and then they are frozen. When it comes time to administer the medicine, an aliquot of the frozen concentrate will be thawed and then diluted to a concentration that is twice as high as the highest final concentration that is wanted using complete medium that contains 50 g/mL of gentamicin. The needed final compound concentrations are achieved by adding aliquots of the compound dilution that are each 100 microliters in volume and are introduced to the appropriate microtiter wells that already contain 100 microliters of medium. After the injection of the chemical, the plates are put back into the incubator for an additional forty-eight hours at a temperature of 37 degrees Celsius, 5% carbon dioxide, 95% air, and 100% relative humidity. The addition of cold TCA brings an end to the experiment when it is performed on adherent cells. After the cells have been cemented in place by the careful injection of 50 L of cold TCA at a concentration of 50% (w/v), the cells are placed in an incubator at 4 degrees Celsius for an hour.

The supernatant is thrown away, and the plates are rinsed with running water five times before being dried off in the air. A solution of sulforhodamine B (SRB) containing 0.4% weight-per-volume sulforhodamine B in 1% acetic acid is added to each well, and the plates are then incubated at room temperature for ten minutes. After the plates have been stained, any unbound dye is removed by washing them five times in acetic acid 1% solution, and then they are air dried. After that, the bound dye is dissolved in trizma base at a concentration of 10 mM, and the absorbance is measured using an automatic plate reader at a wavelength of 515 nm. The protocol is the same for suspension cells, with the exception that the test is finished by fixing settling cells at the bottom of the wells by carefully adding 50 L of 80% TCA (with a final concentration of 16% TCA). This brings the total concentration of TCA to 16%. The percentage of growth is computed at each of the drug concentrations levels by using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)]. The formula for calculating the percentage of growth inhibition is as follows: [(Ti-Tz)/(C-Tz)] 100 for concentrations in which Ti is more than or equal to Tz, or [(Ti-Tz)/Tz] 100 for concentrations in which Ti is less than Tz.

Conclusions

This research describes the synthesis of compounds 4a–i, 6a–i, 7a–c, and 9 that are based on 6-aryl-5-cyanothiouracil and have the potential to act as antibacterial and anticancer agents. When compared to the reference medications amoxicillin, gentamicin, and amphotericin B, the antibacterial activity of a few newly synthesised compounds showed significant promise. The bulk of the active compounds exhibited action against the gram-positive bacteria S. aureus and, to a lesser extent, B. subtilis. This activity was observed across the board. In comparison to the other analogues, the activity of the final compounds 6f and 6g, which contain mesomeric electron-donating methoxy substituents on the phenyl at the 6 position of the thiouracil scaffold, was found to be significantly higher than that of the other analogues when tested against both Gram-positive strains. In addition to this, the incorporation of the 4-hydroxyphenylhdrazono moiety in compounds 7b and 7c contributed to an exceptional level of efficacy against the same bacterial strains. In vitro cytotoxicity testing of chosen compounds 4d, 4g–i, and 6a–i at a

single dose of 105 M demonstrated that the majority of the compounds exhibited only a moderate amount of cytotoxic activity against kidney cancer cell lines UO-31 and A498. Compounds 6d and 6i, on the other hand, displayed a substantial growth inhibitory impact against the non-small cell lung cancer HOP-92 cell line and the leukaemia MOLT-4 cell line, respectively.

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