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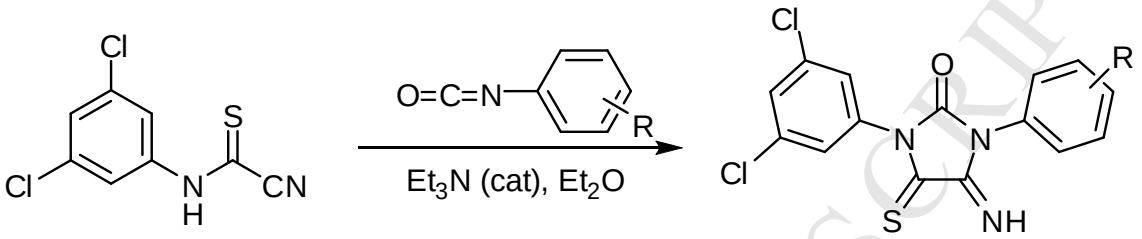

Cytotoxicity IC_{50} (µg):-

R= 4-CH_3: 0.014 for HEPG2 and 0.009 for MCF7

R = 2,4,5-Cl_3: 0.015-0.017 for HEPG2, MCF7, HCT116, PC3

Antiviral effect (%):-

R = 4-Cl: HAV (100), CoxB4 (41.6) and HSV1 (36.4)

R = 2,4,5-Cl_3: HAV (80.3), CoxB4 (20.6) and HSV1(96.7)

Antibacterial and antifungal activities (MIC values µg/mL):-

0.78-6.25 for R = 3-OCH_3, 4-SCH_3 and 2-CH_3,5-F

A series of new imidazolidineiminothiones with various substituents were synthesized. *In vitro* biological evaluation of such compounds showed significant antitumor, antiviral, antibacterial and antifungal activities

New imidazolidineiminothione derivatives: Synthesis, spectral characterization and evaluation of antitumor, antiviral, antibacterial and antifungal activities

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Abstract: A series of new imidazolidineiminothione derivatives with various halogenated and alkylated aromatic substituents at N-(1) and at N-(3) was synthesized through the reaction of *N*-arylcyanothioformamides with arylisocyanate derivatives. Structure of imidazolidineiminothione derivatives were established based on spectroscopic IR, ^{1}H NMR, ^{13}C NMR, ^{1}H,^{1}H-COSY, HSQC, ^{19}F NMR, MS and elemental analyses data. Evaluation of antitumor, antiviral, antibacterial and antifungal activities for the synthesized compounds were carried out to probe their activities. Most of the synthesized compounds displayed antitumor activity. The presence of 3,5-dichlorophenyl moiety at N-(1) and trichlorophenyl moiety on N-(3) (**2f)** resulted the highest cytotoxic activity. The presence of 9*H*-fluorenyl moiety on N-(3) resulted in the lowest cytotoxic activity. The antiviral screening displayed that **2d** and **2f** were markedly active against one or two viral strains. Compound **2d** (3,5-dichlorophenyl moiety at N-(1) and 4-chlorophenyl moiety on N-(3)) showed 100 % antiviral effect toward HAV. Compound **2f** showed 96.7 % antiviral effect toward HSV1 and 80.3 % antiviral effect toward HAV. The antimicrobial activity suggested that all of the imidazolidineiminothione derivatives possess significant antimicrobial activity against most of the test organisms. Some imidazolidineiminothione derivatives showed MIC values of antibacterial and antifungal activities ranged from 0.78 to 6.25 µg/mL.

Keywords: *N*-Arylcyanothioformamides; Imidazolidineiminothiones; NMR Spectra; Antitumor; Antiviral; Antibacterial; Antifungal activities

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1. Introduction

Cancer is one of the major health problems in all countries. Numerous advances chemotherapeutic management have been made for some patients, but the discovering of new anticancer agents remain critically important. Conventional chemotherapy does not discriminate between tumour cells and rapidly dividing normal cells. Targeted therapy is promising approach to cancer therapy which leading to beneficial clinical effects [1-3].

Imidazole derivatives are key components of great many bioactive compounds of both natural and synthetic origin [4, 5]. Also, imidazoles are intermediates in the biosynthesis of nucleotides, and some of their metal complexes are found to be active as cytotoxic metallopharmaceuticals [6].

Also, viral infection diseases are one of the major health problems. Hepatitis A virus (HAV) infections remain a major cause of acute hepatitis [7], acute kidney injury [8] and hemophagocytic syndrome [9]. HAV is usually transmitted by the fecal-oral route [10]. Despite the successful results of hepatitis A vaccination programs, the death of HAV infected children were increased [10]. Although there are safe and effective vaccines against HAV, it is also important to discover new and potential drugs for the treatment of HAV. Infection with Cox B can cause fever, headache, chest pain and other problems. Currently, there is no specific treatment or vaccine available for Coxsackie virus infections [11]. Herpes simplex viruses (HSV-1) infection may result several diseases such as genital herpes, labial herpes and chronic mucocutaneous ulceration [12,13]. HSV infections are usually treated with nucleoside analogues such as acyclovir [14]. However, the efficacy of these drugs is limited by the recent increase in the resistance of virus and recurrence of latent virus [15]. Thus, the development of new anti-HSV agents is highly desirable. As general, the medical community faces a serious problem against virus infections and needs search for effective antiviral agents [16].

The search for newer antibacterial and antifungal agents continues due to the rapid development the resistance among bacteria and fungi to the existing antimicrobial agents [17-20]. Even though novel broad spectrum antimicrobial agents were reported in recent years, the emergence of resistance has become an obstacle. Imidazolidineiminothione derivatives and the various heterocycles derived from them were shown to exhibit an interesting and a wide range of pharmacological effects [21-27]. In view of these facts, it was of interest to synthesize a novel series of imidazolidineiminothiones, and preliminary evaluation of the antitumor, antiviral, antimicrobial and antifungal properties of the synthesized compounds.

2. Results and discussion

2.1. Chemistry

N-Arylcyanothioformamide derivatives **1a-e** were prepared by treating arylisothiocyanate derivatives with potassium cyanide according to literatures procedure [20, 21, 28, 29]. It is noteworthy to mention that analysis of the *N*-arylcyanothioformamides by NMR (^{1}H and ^{13}C) indicated the presence of tautomeric mixtures of thiol and thione forms, in which the latter predominates. The tautomeric nature of the *N*-arylcyanothioformamides reflects their nucleophilic character in which they may react *via* the sulfur or nitrogen atom leading to different heterocylic cores in ring closing reactions. Comprehensive study on the tautomeric nature of the *N*-arylcyanothioformamide was carried out by El-Sharief *et al* [21]. Treating an ethereal solution of various *N*-arylcyanothioformamides **1a**-**e** with an equimolar amount of

various isocyanate derivatives, followed by addition of a few drops of triethylamine as catalyst afforded imidazolidineiminothione derivatives **2-6**. 5-Imino-4-thioxo-2-imidazolidinone derivatives **2-6** were furnished as the sole products, indicating that the ring closing reaction proceeds *via* a single path which involves attack *via* the nitrogen atom. These products were purified by crystallization and obtained in 75-90 % yields. The structure of compounds **2-6** was inferred from their correct elemental analyses and spectral data.

Scheme 1: Synthesis of imidazolidineiminothione derivatives

2.1.1. IR spectral analysis

Infrared measurements of 5-imino-4-thioxo-2-imidazolidinone derivatives **2-6** showed diagnostic absorbance bands in region 3244-3221 cm^{-1} for the imino NH functional group, 1773-1766 cm^{-1} for the C=O functional group, and 1667-1658 cm^{-1} for the C=N functional group. The thione stretch appeared at its expected frequency from 1126 to 1065 cm^{-1}. Aliphatic C-H stretching vibration bands are observed in the region of 2832-3063 cm^{-1}. Aromatic C-H bands are obtained in the region of 3018-3063 cm^{-1}. Moreover, IR spectra showed disappearance of bands for cyano group. IR spectrum of **2a**, as representative example, exhibited characteristic bands at 3231, 1772, 1665 and 1110 for NH, C=O, C=N and C=S functional group, respectively.

2.1.2. ^{1}H NMR spectral analysis

^{1}H NMR spectrum of compound **2f** as representative example (Fig. 1) exhibited four signals for aromatic protons in the region of 7.42 - 7.72 ppm. Douplet signal appeared at 7.42 ppm with two proton integral value is assigned to proton at C-2 and C-6 carbons of 3,5-dichlorophenyl moiety. The latter two protons resonated as douplet signal not two singlet signals due to the *meta*-coupling of each proton at C-2 and C-6 carbons of 3,5-dichlorophenyl moiety with the proton at C-4 carbon. Triplet signal appeared at 7.51 ppm with one proton integral value is assigned to proton at C-4 carbon of 3,5-dichlorophenyl moiety. By similar manner, The latter proton resonated as a triplet signal not singlet signal due to the *meta*-coupling of this proton with the two protons at C-2 and C-6 carbons of 3,5-dichlorophenyl moiety. The remaining two singlet signals at 7.61 and 7.72 ppm are assigned to two protons at C-3 and C-6 carbons of 2,4,5-trichlorophenyl moiety. Beside the latter four aromatic signals, a broad exchangeable single at 9.56 ppm are assigned for the imine proton. All ^{1}H NMR spectra of 5-imino-4-thioxo-2-imidazolidinone derivatives **2-6** exhibited broad exchangeable signal for the imine proton about 9.60 ppm. ^{1}H, ^{1}H-COSY spectra of 5-imino-4-thioxo-2-imidazolidinone derivatives supported these assignments.

Fig. 1: ^{1}H NMR spectrum of compound **2f**.

2.1.3. ^{1}H, ^{1}H-COSY spectra spectral analysis

^{1}H,^{1}H-COSY supported the interpretations of structure elucidation of 5-imino-4-thioxo-2-imidazolidinone derivatives **2-6**. ^{1}H,^{1}H-COSY of compound **2f** as representative example (Fig. 2) revealed the following information. The two protons at C-2 and C-6 carbons of 3,5-dichlorophenyl moiety appeared at 7.42 ppm are *meta*-coupled with the proton at C-4 carbon. The proton at C-4 carbon appeared at 7.51 ppm is *meta*-coupled with the two protons at C-2 and C-6 carbons of 3,5-dichlorophenyl moiety. Moreover ^{1}H,^{1}H-COSY showed that the two protons at C-3 and C-6 carbons of 2,4,5-trichlorophenyl moiety do not coupled with any other protons.

Fig. 2: ^{1}H, ^{1}H-COSY spectrum of compound **2f**

2.1.4. ^{13}C NMR spectral analysis

^{13}C NMR spectra (on-resonance & DEPT) spectral assignment has been made based on characteristic signal positions of the functional groups. ^{13}C NMR spectra were most informative and displayed the expected three signals for the 4-imino-5-thioxoimidazolidine-2-one core 153-161 ppm for C=N, 153-163 ppm for C=O and 180-182 ppm for C=S. In general, the aromatic carbons could be readily distinguished from the other carbons due to their characteristic absorption in the region of 110-150 ppm. ^{13}C NMR spectrum of compound **3c** as representative example is shown in Fig. 3. ^{13}C NMR spectrum of compound **3c** showed three signals in the aliphatic region. The signals at 15.7, 55.9 and 56.4 ppm are due to SCH_3 and $2OCH_3$ carbons, respectively. The signals appeared in the range 113.5-148.9 ppm are due to aromatic carbons. The signals resonated in the deshielded region of 153.4, 153.6, 154.4 and 181.9 ppm are assigned to C=N, C=O, C-O and C=S carbons, respectively. ^{1}H, ^{13}C Heteronuclear Single Quantum Coherence (HSQC) spectra of 5-imino-4-thioxo-2-imidazolidinone derivatives **2-6** supported these assignments and added a strong evidence for these interpretations.

Fig. 3: ^{13}C NMR spectrum of compound **3c**

2.1.5. ^{1}H, ^{13}C Heteronuclear Single Quantum Coherence (HSQC) spectral analysis

From the ^{1}H-^{13}C HSQC spectrum of **3c**, as representative example, (Fig. 4), the following important assignments can be made: Singlet signal at 2.51 ppm showed cross peak with the signal at 15.7 ppm. This suggests that the carbon signal is due to SCH_3 carbon. Two singlet signal at 3.78 and 3.79 ppm show cross peak with the signal at 55.9 and 56.4 ppm. This suggests that the carbon signals are due to two OCH_3 carbons. Doublet signal at 6.88 ppm showed cross peak with the signal at 113.5 ppm. This suggested that this carbon signal is due

to C-6 carbon of 2,5-dimethoxyphenyl moiety. Multiplet signal at 7.04-7.01 ppm with two proton integral value showed cross peak with the carbon signals at 114.7 and 116.9 ppm; it suggested that these carbon signals is due to C-3,4 carbons of 2,5-dimethoxyphenyl moiety. Two doublet signals at 7.37 and 7.51 ppm showed cross peak with the signals at 126.7 and 126.9 ppm. This confirmed that these carbons signals are due to C-2,6 and C-3,5 of 4-(methylthio)phenyl moiety. The assignment of NH proton is confirmed as there are no correlations for NH by HSQC spectral analysis. In the higher frequency region of the ^{13}C spectrum, apart from the C=S signal at 181.9 ppm there are seven signals without correlations respectively at 121.6 (C-N), 129.0 (C-N), 139.4 (C-S), 148.9 (C-O), 153.4 (C=N), 153.6 (C=O) and 154.4 (C-O) ppm.

Fig. 4: HSQC spectrum of compound **3c**

2.1.6. ^{19}F NMR spectral analysis

^{19}F NMR spectral measurement of 5-imino-4-thioxo-2-imidazolidinone derivative **2g** showed diagnostic signal at -62.9 for fluorine atom in trifluoromethyl moiety (Fig 5).^{19}F NMR spectral measurements of 5-imino-4-thioxo-2-imidazolidinone derivative **2h** and **6** showed signals at -114.6 and -114.6, respectively. These signals are attributed to fluorine atom at 5-fluoro-2-methylphenyl moiety.

Fig. 5: ^{19}F NMR spectra of compounds **2g** and **2h**

2.1.7. Mass spectral analysis

Additional structural information can be deduced from mass spectroscopy. The electron impact mass spectra of the 4-imino-5-thioxoimidazolidine-2-one derivatives **2-6** confirmed the proposed formula by showing a peak at: *m/z* value corresponding to the 4-imino-5-thioxoimidazolidine-2-one moiety. Also, their mass spectra showed two characterized peaks attributable to the raw starting materials fragments of the 4-imino-5-thioxoimidazolidine-2-one isothiocyanate and isocyanate derivatives as shown in the fragmentation pattern in Fig. 6. Beside the two beaks, there are other beaks attributed to fragments of aryl or phenyl moieties.

Fig 6: Fragmentation pattern of 4-imino-5-thioxoimidazolidine-2-one

2.2. Biological activity

The aim of the present investigation is to synthesize series of imidazolidineiminothiones which bearing various substituent at N-(1) and others at N-(3). The antitumor, antiviral, antibacterial and antifungal activities of these derivatives were measured. The effect of each

substituent at N-(1) and those at N-(3) on these activities was studied and make a comparative study between them to deduce a structure activity relationship. A series of imidazolidineiminothione **2a-h** which contain 3,5-dichlorophenyl moiety at N-(1) and various moieties at N-(3) was synthesized. The effect of each substituent on N-(3) of the imidazolidine ring on the activities of the imidazoslidines **2a-h** was studied. Thus, changing the substituent on N-(3) from 4-tolyl to 4-anizyl to 4-metylthiophenyl (**2a → 2b → 2c**) was carried out to show the difference between CH_3, OCH_3 and SCH_3 on the effect of the biological activities. Changing the substituent on N-(3) from momo- to di- to trichlorophenyl (**2d → 2e → 2f**) was carried out for the same reason. Also, changing the substituent on N-(3) from 3-(CF_3)-4-Cl-phenyl to 2-(CH_3)-5-F-phenyl (**2g → 2h**) was carried out for the same study. Another imidazolidineiminothiones **3a-d** which contain 2,5-dimethoxyphenyl moiety at N-(1) and various moiety at N-(3) was synthesized. Changing the substituent on N-(3) from *p*-tolyl to *p*-anizyl to *p*-metylthiophenyl to 9*H*-fluorenyl (**3a → 3b → 3c → 3d**) was carried out to show the difference between each substituent on the effect of the biological activities. Changing the substituent on N-(1) from dichlorophenyl to dimethoxyphenyl and let the substituent on N-(3) not changed was carried out such as **2a → 3a, 2b → 3b** and **2c → 3c**. Moreover, substituent on N-(1) was changed from 3,5-dichlorophenyl to 2,4-dimethoxyphenyl while the substituent at N-(3) is not changed (3-chlorophenyl) this in order to study the difference between **2d** and **4**. Finally, the same study was carried out between **2e** and **5,** and **2h** and **6** where substituent at N-(3) not changed but that at N-(1) was changed

2.2.1. Antitumor properties

The cytotoxic activity was evaluated against human hepatocellular carcinoma cell line (HEPG2), human breast cancer cell line (MCF7), colon carcinoma cell line (HCT116) and prostate carcinoma cell line (PC3). Doxorubicin (CAS-23214-92-8) was used as the reference drug, which considers one of the most effective anticancer agents. Potential cytotoxicity of the compounds was tested using the method of Skehan *et al*. [30]. IC_{50} value was summarized in Table **1**. The obtained IC_{50} values for these compounds suggest that all of the evaluated imidazolidineiminothione derivatives possess significant cytotoxic activity against most of the tested cell lines used in these assays. A moderate difference in cytotoxic activity is noted between the tested compounds, this indicate that the main effect related to the presence of the imidazolidineiminothione moiety which contain adjacent imino and thione functions groups. Certain aspects of the structure activity relationships for these compounds can be more clearly highlighted.

Regarding the effect of each substituent on N-(3) (4-tolyl (**2a**), 4-anizyl (**2b**) and 4-metylthiophenyl (**2c**)); the presence of 4-tolyl (**2a**) resulted in the highest cytotoxic activity followed by 4-metylthiophenyl. Compound **2a** showed half potency of doxorubicin in inhibiting the growth of HEPG2 and MCF7 cell lines. Regarding the effect of changing the substituent on N-(3) from momo- to di- to trichlorophenyl (**2d → 2e → 2f**); the presence of trichlorophenyl resulted in the highest cytotoxic activity among all of the tested compounds. Compound **2f** showed IC_{50} about 0.015 µg toward all the tested cell lines.

Imidazolidineiminothiones **3a-d** which contain 2,5-dimethoxyphenyl moiety at N-(1) and different moieties on N-(3), like structure **2a-c**, the presence of 4-tolyl (**3a**) resulted in the highest cytotoxic activity. Increasing the size of the substituent, as in compound **3d** had a detrimental effect on cytotoxic activity. The presence of 9*H*-fluorenyl moiety resulted in the lowest cytotoxic activity among all of the tested compounds. Changing the substitution on N-(1) from 3,5-dichlorophenyl to 3,5-dimethoxyphenyl on N-(1) while the substituent at N-(3) is not changed (**2d** to **4)** had a good effect on cytotoxic activity.

Table 1

2.2.2. Antiviral activities.

The synthesized compounds were subjected to *in vitro* testing of antiviral activity against the following three viral strains: hepatitis A virus (HAV), human herpes simplex virus 1 (HSV1) and Coxsackie B4 (CoxB4) viral strain. Viral infectivity assay was carried out using the plaque formation method [31]. A plaque is a localized focus of virus-infected cells which under optimal conditions originates from a single infectious virus particle. Counting of these foci for serial dilution of virus suspension is a highly quantitative method for assay of viral infectivity. Under these conditions, reduction in virus plaque counts provides a very sensitive mean for measuring antiviral activity of potential antiviral. The results of the plaque reduction assay are summarized in Table **2**. The percentage of antiviral effect obtained for the synthesized compounds suggested that most of the imidazolidineiminothione derivatives evaluated possess significant antiviral activity against most of the test virus used in these assays. Certain aspects of the structure activity relationships for these compounds can be more clearly highlighted.

Regarding the effect of changing the substituent on N-(3) from *p*-tolyl to *p*-anizyl to *p*-metylthiophenyl (**2a** → **2b** → **2c**); the presence of *p*-tolyl (**2a**) resulted in the highest antiviral activity. Regarding the effect of changing the substituent on N-(3) from momo- to di- to trichlorophenyl (**2d** → **2e** → **2f**); the presence of momochlorophenyl and trichlorophenyl moieties resulted in the highest antiviral activity among all of the tested compounds. Compound **2d** showed 100 % antiviral effect toward HAV. Compound **2f** showed 80.3 % antiviral effect toward HAV and 96.7 % antiviral effect toward HSV1.

Imidazolidineiminothiones **3a-d** which contain 2,5-dimethoxyphenyl moiety at N-(1) and different moieties on N-(3). The presence of 4-metylthiophenyl (**3c**) resulted in the highest antiviral activity followed by 4-anizyl moiety. Compound **3c** showed 85.7 % antiviral effect toward HSV1. Increasing the size of the substituent, as in compound **3d** had a detrimental effect on antiviral activity. Changing substitution on N-(1) from 3,5-dichlorophenyl to 3,5-dimethoxyphenyl on N-(1) while the substituent at N-(3) is not changed (**2d** → **4)** had a detrimental effect on antiviral activity. Also, Changing the substitution on N-(1) from 3,5-dichlorophenyl to phenyl on N-(1) while the substituent at N-(3) is not changed (**2e** → **5)** had

detrimental effect on antiviral activity. Finally, Changing the substitution on N-(1) from 3,5-dichlorophenyl to bromophenyl on N-(1) while the substituent at N-(3) is not changed (**2h** and **6**) had slight effect on antiviral activity.

Table 2

2.2.3. Antibacterial activities

The synthesized compounds were tested *in vitro* for antibacterial activity against the following bacterial strains: three Gram-positive bacteria, *Micrococcus luteus* (ATCC10240), *B. subtilis* (NCTC-10400) and *B. Pumilus* and three Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC25619), *E. coli* (ATCC10536) and *S. lutea*, and the results are summarized in Table **3**. Antimicrobial tests were carried out by the agar well diffusion method [32] using 100 μL of tested compound solution prepared by dissolving 5 mg of the chemical compound in 1 ml of dimethyl sulfoxide (DMSO). The inoculated plates were then incubated for 24 h at 37 °C. The antibacterial agent Erythromycin (5 mg/ml) was used as a standard. After incubation time, antimicrobial activity was evaluated by measuring the inhibition zone diameters against the test organisms and compared with standard zone size ranges that determine susceptibility, intermediate susceptibility, or resistance to the screened compounds. Visual bacterial growth is observed only in areas in which the drug concentrations are below those required for growth inhibition. The experiment was carried out in triplicate and the average zone of inhibition was calculated. The mean values of the inhibition zone diameter obtained for these compounds suggest that all of the imidazolidineiminothione derivatives evaluated possess significant antibacterial activity against most of the test organisms used in these assays. A moderate difference in antibacterial activity is noted between the tested compounds, this indicate that the main effect related to the presence of the imidazolidineiminothione moiety which contain adjacent imino and thione functions groups. The results of the antibacterial screening demonstrated the following assumptions about the structural activity relationship (SAR).

Regarding the effect of changing the substituent on N-(3) from *p*-tolyl to *p*-anizyl to *p*-metylthiophenyl (**2a** → **2b** → **2c**); the presence of *p*-anizyl (**2b**) resulted in the highest antibacterial activity followed by *p*-metylthiophenyl. The presence of *p*-tolyl moiety resulted in the lowest antibacterial activity. Regarding the effect of changing the substituent on N-(3) from momo- to di- to trichlorophenyl (**2d** → **2e** → **2f**); the presence of momochlorophenyl resulted in the highest antibacterial activity followed by trichlorophenyl. The lowest antibacterial activity was exhibited by dichlorophenyl moiety. Compounds **2g,h** had the same activity. Imidazolidineiminothiones **3a-d** which contain 2,5-dimethoxyphenyl group at N-(1) and different substituent on N-(3). Also, like structure **2a-c**, the presence of *p*-anizyl (**3b**) resulted in the highest antibacterial activity followed by *p*-metylthiophenyl, the presence of *p*-anizyl and *p*-metylthiophenyl moieties resulted in the highest antimicrobial activity among all the compounds investigated in this study. Increasing the size of the substituent, as

in compound **3d** had a detrimental effect on antibacterial activity. The presence of to 9*H*-fluorenyl moiety resulted in the lowest antibacterial activity among all of the tested compounds. Changing the substitution on N-(1) from 3,5-dichlorophenyl to 3,5-dimethoxyphenyl while the substituent at N-(3) is not changed (**2d → 4)** had a detrimental effect on antibacterial activity. Also, changing the substitution on N-(1) from 3,5-dichlorophenyl to phenyl while substituent at N-(3) is not changed **(2e → 5)** had no effect on antibacterial activity. Finally, changing the substitution on N-(1) from 3,5-dichlorophenyl to bromophenyl while the substituent at N-(3) is not changed (**2h → 6)** had slight effect on antibacterial activity.

Table 3

2.2.4. Antifungal activity

The synthesized compounds were tested *in vitro* for antifungal activity against the following three fungal strains: *Candida albicans* (IMRU3669), *A. niger* and *P. chrysogenum* in the antifungal susceptibility tests. Antifungal agents are evaluated against clinical isolates of standard strains of fungi by the agar well diffusion method [32] using 100 μL of tested compound solution prepared by dissolving 5 mg of the chemical compound in 1 ml of dimethyl sulfoxide. The antifungal agent Metronidazole was used as a standard at concentration 5 mg/mL and was tested under the same conditions. The inoculated plates were then incubated. After incubation time, antifungal activity was evaluated by measuring the inhibition zone against the test organisms and compared with that of the standard. Antifungal activities were expressed as inhibition zone diameter in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated. The results are tabulated in Table **3**. In general, some compounds were very effective antifungal agents and in several cases achieved similar level of activity as the standard antifungal agent Metronidazole. A moderate difference in antifungal activity is noted between the tested compounds, this indicate that the main effect related to the presence of the imidazolidineiminothione moiety which contain adjacent imino and thione functions groups. Regarding the effect of changing the substituents on N-(1) and N-(3), the results of screening demonstrated the presence of good correlation between the antifungal and antibacterial activities.

2.2.5. Minimum inhibitory concentrations against Gram positive bacteria, Gram negative bacteria and fungi

Minimum inhibitory concentrations of the more active synthesized compounds **2b, 2c, 2h, 3b** and **3c** was then evaluated *in vitro* using the twofold serial dilution technique [33] against various strains of Gram positive and Gram negative bacteria and fungi. These techniques provide a quantitative assessment of *in vitro* antimicrobial activity. Hence, further to the

preceding tests of the potential antibacreial and antifungal activities. Minimum inhibitory concentration (MIC) The lowest concentration showing no growth was taken as the minimum inhibitory concentration (MIC). The results of minimum inhibitory concentration were reported in Table **4**.

Table 4

As shown in Table 4, most of the tested compounds revealed MIC 0.78 µg/ml against *Pseudomonas aeroginosa*. Most of the tested compounds showed MIC 6.25 µg/ml against most of the tested organisms.

3. Conclusion

The synthesis, characterization and biological activity of a new series of imidazolidineiminothiones with variable aromatic substituents at N-(1) and N-(3) atoms have been described. Most compounds displayed antitumor, antiviral, antibacterial and antifungal activities. A moderate difference in cytotoxic activity is noted between the tested compounds, this indicate that the main effect related to the presence of the imidazolidineiminothione moiety which contain adjacent imino and thione functions groups. Regarding the effect of changes the substituents on N-(3); the presence of trichlorophenyl resulted in the highest cytotoxic activity followed among all of the tested compounds. The presence of to 9*H*-fluorenyl moiety resulted in the lowest cytotoxic activity among all of the tested compounds. The antiviral effect of all compounds were investigated, where **2a,d**,**f** were markedly active against one or two viral strains. Structure activity relationship studies revealed several matching pairs of aromatic substituents on N-(1) and N-(3) which could serve to optimize structural features for optimal activity to eventually render such compounds clinically useful drug agents. The antimicrobial activity obtained for these compounds suggest that all of the imidazolidineiminothione derivatives evaluated possess significant antimicrobial activity against most of the test organisms used in these assays. A moderate difference in antimicrobial activity is noted between the tested compounds, this indicate that the main effect related to the presence of the imidazolidineiminothione moiety which contain adjacent imino and thione functions groups.

4. Experimental

IR spectra were recorded (KBr) on a Perkin Elmer 1650 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Avance II Bruker FT NMR spectrometer 400 (400 MHz) using $CDCl_3$ as solvents and TMS as an internal standard. $CFCl_3$ was used as an internal standard for all ^{19}F NMR measurements. Chemical shifts are expressed as δ ppm units. NMR spectra were recorded at Department of Chemistry, Faculty of Science, Taibah University, Almadinah lmunawarrah, Saudi Arabia Mass spectra were recorded on Shimadzu GC-MS QP

100 EX (70 eV) at the Micro Analytical Center at Cairo University. Melting points were obtained on a Fisher–Johns melting points apparatus and are uncorrected.

4.1. General procedure for the synthesis of imidazolidineiminothione derivatives 2a-j, 3a-d, 4, 5 and 6

A solution of the cyanothioformanilide derivative (0.01 mol) and the corresponding isocyanate derivative (0.01 mol) in dry ether (30 mL) was treated with three drops of triethylamine. The reaction mixture was stirred for 15 min. The obtained product was filtered off, washed with ether, air-dried and recrystallized from chloroform/ n-hexane to give imidazolidineiminothione derivatives **2a-j**, **3a-d**, **4**, **5** and **6**.

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4.1.1. 1-(3,5-Dichlorophenyl)-4-imino-5-thioxo-3-p-tolylimidazolidin-2-one (2a)

Yield 73 %; mp 146-147 °C; IR: ν/cm^{-1}: 3231 (NH), 1772 (C=O), 1665 (C=N), 1110 (C=S); ^{1}H NMR: δ/ppm = 2.41 (s, 3H, CH_3), 7.33 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.40 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.43 (d, 2H, *J* = 1.8 Hz, Ar-H), 7.49 (t, 1H, *J* = 1.8 Hz, Ar-H), 9.57 (br, 1H, NH); ^{13}C NMR: 21.3 (CH_3); 126.0 (2CH), 126.3 (2CH), 128.9 (C-N), 129.9 (CH), 130.1 (2CH), 134.2 (C-N), 135.7 (2C-Cl), 139.1 (C-CH_3), 153.1 (C=N), 154.0 (C=O), 180.8 (C=S); MS (*m/z*, %): 363 (M^+, 40), 364 (M+1, 16), 365 (M+2, 29), 366 (M+3, 10), 362 (M-1, 16), 203 ($Cl_2C_6H_3NCS$, 51), 133 ($CH_3C_6H_4NCO$, 100); Anal. Calcd for $C_{16}H_{11}Cl_2N_3OS$ (364.25): C, 52.76; H, 3.04; N, 11.54; Found: C, 52.84; H, 3.08; N, 11.37

4.1.2. 1-(3,5-Dichlorophenyl)-4-imino-3-(4-methoxyphenyl)-5-thioxoimidazolidin-2-one (2b)

Yield 71 %; mp 140-141°C; IR: ν/cm^{-1}: 3240 (NH), 1767 (C=O), 1664 (C=N), 1125 (C=S); ^{1}H NMR: δ/ppm = 3.85 (s, 3H, OCH_3), 7.04 (d, 2H, *J* = 8.9 Hz, Ar-H), 7.41-7.45 (m, 4H, Ar-H), 7.50 (t, 1H, *J* = 1.9 Hz, Ar-H), 9.55 (br, 1H, NH); ^{13}C NMR: 55.6 (OCH_3), 114.8 (CH), 124.1 (2C), 126.0 (CH), 127.9 (2C), 129.9 (2C), 134.2 (C-N), 135.7 (2C-Cl), 153.2 (C=N), 154.1 (C=O), 159.7 (C-O), 180.8 (C=S); MS, m/z (%): 379 (M^+, 10.4), 380 (M+1, 4.1), 381 (M+2, 9.1), 378 (M-1, 5.8), 203 ($Cl_2C_6H_3NCS$, 27.0), 149 ($OCH_3C_6H_4NCO$, 100); Anal. Calcd for $C_{16}H_{11}Cl_2N_3O_2S$ (380.25): C, 50.54; H, 2.92; N, 11.05; Found: C, 50.49; H, 2.88; N, 11.13

4.1.3. 1-(3,5-Dichlorophenyl)-4-imino-3-(4-(methylthio)phenyl)-5-thioxoimidazolidin-2-one (2c)

Yield 71 %; mp 191-192 °C; IR: ν/cm^{-1}: 3241 (NH), 1772 (C=O), 1666 (C=N), 1118 (C=S); ^{1}H NMR: δ/ppm = 2.54 (s, 3H, SCH_3), 7.34 (d, 2H, *J* = 8.9 Hz, Ar-H), 7.41 - 7.44 (m, 4H, Ar-H), 7.63 (t, 1H, *J* = 1.9 Hz, Ar-H), 11.55 (br, 1H, NH); ^{13}C NMR: 14.8 (CH_3), 123.5 (C-N), 126.1 (CH), 127.1 (CH), 127.9 (CH), 132.2 (CH), 132.8 (C-N), 137.5 (C-Cl), 147.2 (C-

S), 151.0 (C=N), 152.2 (C=O), 176.4 (C=S); MS, m/z (%): 395 (M^+, 8.5), 396 (M+1, 12.2), 203 ($Cl_2C_6H_3NCS$, 47.6), 165 ($SCH_3C_6H_4NCO$, 100); Anal. Calcd for $C_{16}H_{11}Cl_2N_3OS_2$ (396.31): C, 48.49; H, 2.80; N, 10.60; Found: C, 48.42; H, 2.76; N, 10.73

4.1.4. 1-(3-Chlorophenyl)-3-(3,5-dichlorophenyl)-5-imino-4-thioxoimidazolidin-2-one (2d)

Yield 74 %; mp 162-163 °C; IR: ν/cm^{-1}: 3219 (NH), 1778 (C=O), 1663 (C=N), 1128 (C=S); ^{1}H NMR: δ/ppm = 7.39-7.49 (m, 5H, Ar-H), 7.51 (s, 1H, Ar-H), 7.60 (t, 1H, *J* = 1.9 Hz, Ar-H), 9.66 (br, 1H, NH); ^{13}C NMR: 124.5 (CH), 125.9 (CH), 126.6 (CH), 130.0 (2CH), 130.1 (CH), 130.3 (CH), 132.7 (C-N), 134.0 (C-N), 134.9 (C-Cl), 135.8 (2C-Cl), 152.8 (C=N), 153.3 (C=O), 180.2 (C=S); MS, m/z (%): 385 (M^+, 33.8), 386 (M+1, 11.7), 387 (M+2, 11.5), 384 (M-1, 23.9), 203 ($Cl_2C_6H_3NCS$, 100), 153 (ClC_6H_4NCO, 43.6); Anal. Calcd for $C_{15}H_8Cl_3N_3OS$ (384.67): C, 46.84; H, 2.10; N, 10.92; Found: C, 46.72; H, 2.04; N, 10.82

4.1.5. 3-(2,6-Dichlorophenyl)-1-(3,5-dichlorophenyl)-4-imino-5-thioxoimidazolidin-2-one (2e)

Yield 74 %; mp 220-222 °C; IR: ν/cm^{-1}: 3232 (NH), 1769 (C=O), 1658 (C=N), 1118 (C=S); ^{1}H NMR: δ/ppm = 7.44-7.45 (m, 3H, Ar-H), 7.50 (d, 2H, *J* = 1.9 Hz, Ar-H), 7.53 (t, 1H, *J* = 1.9 Hz, Ar-H), 9.54 (br, 1H, NH); ^{13}C NMR: 126.0 (2CH), 127.6 (C-N), 129.0 (2CH), 130.1 (CH), 131.9 (CH), 134.0 (C-N), 135.4 (2C-Cl), 135.7 (2C-Cl), 151.6 (C=N), 151.9 (C=O), 180.4 (C=S); MS, m/z (%): 419 (M^+, 29.9), 420 (M+1, 15.9), 418 (M-1, 8.2), 203 ($Cl_2C_6H_3NCS$, 100), 187 ($Cl_2C_6H_3NCO$, 30.0); Anal. Calcd for $C_{15}H_7Cl_4N_3OS$ (419.11): C, 42.99; H, 1.68; N, 10.03; Found: C, 42.84; H, 1.73; N, 10.10

4.1.6. 1-(3,5-Dichlorophenyl)-4-imino-5-thioxo-3-(2,4,5-trichlorophenyl)imidazolidin-2-one (2f)

Yield 75 %; mp 173-175 °C; IR: ν/cm^{-1}: 3217 (NH), 1777 (C=O), 1661 (C=N), 1126 (C=S); ^{1}H NMR: δ/ppm = 7.42 (d, 2H, *J* = 1.6 Hz, Ar-H), 7.51 (t, 1H, *J* = 1.6 Hz, Ar-H), 7.61 (s, 1H, Ar-H), 7.72 (s, 1H, Ar-H), 9.56 (br, 1H, NH); ^{13}C NMR: 125.9 (2CH), 128.5 (C-N), 130.2 (CH), 131.7 (CH), 131.8 (CH), 132.0 (C-N), 132.4 (C-Cl), 133.8 (C-Cl), 135.6 (C-Cl), 135.8 (2C-Cl), 151.9 (C=N), 152.6 (C=O), 180.1 (C=S); MS, m/z (%): 453 (M^+, 5.7), 454 (M+1, 3.1), 452 (M-1, 2.1), 203 ($Cl_2C_6H_3NCS$, 70), 195 ($Cl_3C_6H_2NCO$, 100); Anal. Calcd for $C_{15}H_6Cl_5N_3OS$ (453.56): C, 39.72; H, 1.33; N, 9.26; Found: C, 39.64; H, 1.37; N, 9.28

4.1.7. 1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(3,5-dichlorophenyl)-5-imino-4-thioxoimidazolidin-2-one (2g)

Yield 69 %; mp 152-153 °C; IR: ν/cm^{-1}: 3255 (NH), 1773 (C=O), 1663 (C=N), 1126 (C=S); ^{1}H NMR: δ/ppm = 7.42 (d, 2H, *J* = 1.7 Hz, Ar-H), 7.52 (t, 1H, *J* = 1.7 Hz, Ar-H), 7.68 (d, 1H, *J* = 8.7 Hz, Ar-H), 7.78 (dd, 1H, *J* = 8.7, 2.4 Hz, Ar-H), 8.01 (d, 1H, *J* = 2.4 Hz, Ar-H), 9.72 (br, 1H, NH); ^{13}C NMR: 122.2 (q, *J* = 273.4 Hz, CF_3), 125.5 (q, *J* = 5.9 Hz, CH), 125.9

(2CH), 126.3 (C-N), 129.5 (q, *J* = 32.2 Hz, C-CF_3), 130.2 (CH), 130.3 (CH), 130.5 (C-N), 132.4 (CH), 133.8 (C-Cl), 135.9 (2C-Cl), 152.6 (C=N), 152.9 (C=O), 180.0 (C=S); ^{19}F NMR: -62.98; MS, m/z (%): 453 (M^+, 34.6), 454 (M+1, 9.2), 452 (M-1, 14.6), 203 ($Cl_2C_6H_3NCS$, 100), 221 ($Cl(CF_3)C_6H_3NCO$, 12.2); Anal. Calcd for $C_{16}H_7Cl_3F_3N_3OS$ (452.67): C, 42.45; H, 1.56; N, 9.28; Found: C, 42.41; H, 1.52; N, 9.34

4.1.8. 1-(3,5-Dichlorophenyl)-3-(5-fluoro-2-methylphenyl)-4-imino-5-thioxoimidazolidin-2-one (2h)

Yield 69 %; mp 125-126 °C; IR: ν/cm^{-1}: 3445 (NH), 1766 (C=O), 1661 (C=N), 1120 (C=S); ^{1}H NMR: δ/ppm = 2.25 (s, 3H, CH_3), 7.08 (dd, 1H, *J* = 8.5, 2.6 Hz, Ar-H), 7.14 (td, 1H, *J* = 8.3, 2.6 Hz, Ar-H), 7.38-7.33 (m, 1H, Ar-H), 7.44 (d, 2H, *J* = 1.8 Hz, Ar-H), 7.51 (t, 1H, *J* = 1.8 Hz, Ar-H), 9.53 (br, 1H, NH); ^{13}C NMR: 17.4 (CH_3), 115.8 (d, *J* = 23.4 Hz, CH), 117.3 (d, *J* = 20.5 Hz, CH), 125.9 (2CH), 130.0 (CH), 131.0 (d, *J* = 9.6 Hz, C-N), 132.2 (d, *J* = 3.6 Hz, C), 132.5 (d, *J* = 8.1 Hz, C-H), 134.0 (C-N), 135.8 (2C-Cl), 153.3 (C=O), 152.6 (C=N), 161.2 (d, *J* = 246 Hz, C-F), 181.6 (C=S); ^{19}F NMR: -114.6; MS, m/z (%): 381 (M^+, 76.1), 382 (M+1, 25.2), 383 (M+2, 52.8), 203 ($Cl_2C_6H_3NCS$, 20), 150 ($F(CH_3)C_6H_3NCO$, 100); Anal. Calcd for $C_{16}H_{10}Cl_2FN_3OS$ (382.24): C, 50.28; H, 2.64; N, 10.99; Found: C, 50.32; H, 2.61; N, 10.86

4.1.9. 1-(2,5-Dimethoxyphenyl)-4-imino-5-thioxo-3-p-tolylimidazolidin-2-one (3a)

Yield 64 %; mp 135-136 °C; Yield 77 %; mp 131-132 °C; IR: ν/cm^{-1}: 3439 (NH), 1769 (C=O), 1665(C=N), 1117 (C=S) cm^{-1}; ^{1}H NMR: δ/ppm = 2.40 (s, 3H, CH_3), 3.77 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 6.88 (dd, 1H, *J* = 2.4, 0.8 Hz, Ar-H), 7.03-7.01 (m, 2H, Ar-H), 7.31 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.45 (d, 2H, *J* = 8.4 Hz, Ar-H), 9.47 (br, 1H, NH); ^{13}C NMR: 21.2 (CH_3), 55.9 (OCH_3), 56.4 (OCH_3), 113.5 (CH), 114.7 (CH), 116.8 (CH), 121.7 (C-N), 126.3 (2CH), 129.4 (C-N), 129.9 (2CH), 138.6 (C), 148.9 (C-O), 153.5 (C=N), 153.6 (C=O), 154.6 (C-O), 182.1 (C=S); Anal. Calcd for $C_{18}H_{17}N_3O_3S$ (355.41): C, 60.83; H, 4.82; N, 11.82; Found: C, 60.87; H, 4.79; N, 11.90

4.1.10. 1-(2,5-Dimethoxyphenyl)-4-imino-3-(4-methoxyphenyl)-5-thioxoimidazolidin-2-one (3b)

Yield 67 %; mp 205-206 °C; IR: ν/cm^{-1}: 3439 (NH), 1769 (C=O), 1665(C=N), 1117 (C=S) cm^{-1}; ^{1}H NMR: δ/ppm = 3.79 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.90-6.87 (m, 1H, Ar-H), 7.05-7.00 (m, 4H, Ar-H), 7.48 (d, 2H, *J* = 9.3 Hz, Ar-H), 9.44 (br, 1H, NH); ^{13}C NMR: 55.4 (OCH_3), 55.9 (OCH_3), 56.4 (OCH_3), 113.4 (CH), 114.6 (2CH), 114.7 (CH), 116.8 (CH), 121.7 (C-N), 124.6 (C-N), 127.9 (2CH), 148.9 (C-O), 153.6 (C=O and C=N), 154.7 (C-O), 159.4 (C-O), 182.1 (C=S); Anal. Calcd for $C_{18}H_{17}N_3O_4S$ (371.41): C, 58.21; H, 4.61; N, 11.31; Found: C, 58.17; H, 4.58; N, 11.23

4.1.11. 1-(2,5-Dimethoxyphenyl)-4-imino-3-(4-(methylthio)phenyl)-5-thioxoimidazolidin-2-one (3c)

Yield 60 %; mp 170-171 °C; IR: ν/cm^{-1}: 3439 (NH), 1769 (C=O), 1665 (C=N), 1117 (C=S) cm^{-1}; ^{1}H NMR: δ/ppm = 2.51 (s, 3H, SCH_3), 3.78 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 6.88 (d, 1H, *J* = 2.5 Hz, Ar-H), 7.04-7.01 (m, 2H, Ar-H), 7.37 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.51 (d, 2H, *J* = 8.8 Hz, Ar-H), 9.50 (br, 1H, NH); ^{13}C NMR: 15.7 (SCH_3), 55.9 (OCH_3), 56.4 (OCH_3), 113.5 (CH), 114.7 (CH), 116.9 (CH), 121.6 (C-N), 126.7 (2CH), 126.9 (2CH), 129.0 (C-N), 139.4 (C-S), 148.9 (C-O), 153.4 (C=N), 153.6 (C=O), 154.4 (C-O), 181.9 (C=S); Anal. Calcd for $C_{18}H_{17}N_3O_3S_2$ (387.48): C, 55.80; H, 4.42; N, 10.84; Found: C, 55.93; H, 4.37; N, 10.74

4.1.12. 1-(2,5-Dimethoxyphenyl)-3-(9H-fluoren-2-yl)-4-imino-5-thioxoimidazolidin-2-one (3d)

Yield 66 %; mp 205-206 °C; IR: ν/cm^{-1}: 3422 (NH), 1779 (C=O), 1666 (C=N), 1132 (C=S); ^{1}H NMR: δ/ppm = 3.77 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 6.88 - 7.45 (m, 12H, Ar-H), 9.47 (br, 1H, NH); Anal. Calcd for $C_{24}H_{19}N_3O_3S$ (429.49): C, 67.12; H, 4.46; N, 9.78; Found: C, 67.34; H, 4.49; N, 9.41

4.1.13. 1-(3-Chlorophenyl)-3-(2,4-dimethoxyphenyl)-5-imino-4-thioxoimidazolidin-2-one (4)

Yield 62 %; mp 162-163 °C; IR: ν/cm^{-1}: 3439 (NH), 1769 (C=O), 1665 (C=N), 1117 (C=S) cm^{-1}; ^{1}H NMR: δ/ppm = 3.79 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.63-7.58 (m, 2H, Ar-H), 7.20 (d, 1H, *J* = 9.3 Hz, Ar-H), 7.39-7.35 (m, 1H, Ar-H), 7.43 (t, 1H, *J* = 7.9 Hz, Ar-H), 7.55-7.51 (m, 1H, Ar-H), 7.67-7.65 (m, 1H, Ar-H), 9.55 (br, 1H, NH); ^{13}C NMR: 55.6 (OCH_3), 55.9 (OCH_3), 99.8 (CH), 105.0 (CH), 114.1 (C-N), 124.5 (CH), 126.6 (CH), 128.5 (CH), 129.7 (CH), 130.1 (CH), 133.3 (C-N), 134.7 (C-Cl), 153.4 (C=N), 154.0 (C=O), 155.7 (C-O), 162.3 (C-O), 182.1 (C=S); Anal. Calcd for $C_{17}H_{14}ClN_3O_3S$ (375.83): C, 54.33; H, 3.75; N, 11.18; Found: C, 54.27; H, 3.64; N, 11.23

4.1.14. 1-(2,6-Dichlorophenyl)-5-imino-3-phenyl-4-thioxoimidazolidin-2-one (5)

Yield 65 %; mp 125-126 °C; IR: ν/cm^{-1}: 3439 (NH), 1769 (C=O), 1665 (C=N), 1117 (C=S) cm^{-1}; ^{1}H NMR: δ/ppm = 7.45-7.40 (m, 1H, Ar-H), 7.61-7.47 (m, 7H, Ar-H), 9.49 (br, 1H, NH); ^{13}C NMR: 127.2 (2CH), 127.9 (C-N), 129.0 (2CH), 129.5 (2CH), 129.8 (CH), 131.7 (CH), 132.6 (C-N), 135.5 (2C-Cl), 152.3 (C=O and C=N), 181.3 (C=S); MS, m/z (%): 349.9 (M^+, 8.9), 350.9 (M+1; 13.4), 351.9 (M+2; 5.4), 348.9 (M-1; 18.9), 135 (C_6H_5NCS, 100), 77 (C_6H_5; 81.0); Anal. Calcd for $C_{15}H_9Cl_2N_3OS$ (350.22): C, 51.44; H, 2.59; N, 12.00; Found: C, 51.53; H, 2.62; N, 12.11

4.1.15. 1-(4-Bromophenyl)-3-(5-fluoro-2-methylphenyl)-4-imino-5-thioxoimidazolidin-2-one (6)

Yield 68 %; mp 122-123 °C; IR: ν/cm^{-1}: 3439 (NH), 1769 (C=O), 1665 (C=N), 1117 (C=S) cm^{-1}; ^{1}H NMR: δ/ppm = 2.26 (s, 3H, CH_3); 7.17-7.07 (m, 2H, Ar-H), 7.36-7.33 (m, 1H, Ar-H), 7.39 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.70 (d, 2H, *J* = 8.8 Hz, Ar-H), 9.51 (br, 1H, NH); ^{13}C NMR: 17.5 (CH_3), 115.8 (d, *J* = 23.5 Hz, CH), 117.3 (d, *J* = 20.4 Hz, CH), 123.8 (C-Br), 128.6 (2CH), 131.2 (d, *J* = 9.7 Hz, C-N), 131.5 (C-N), 132.2 (d, *J* = 3.7 Hz, C), 132.5 (d, *J* = 8.7 Hz, CH), 132.8 (2CH), 153.0 (C=N), 153.5 (C=O), 161.1 (d, *J* = 247 Hz, C-F), 181.0 (C=S); ^{19}F NMR: -114.7; Anal. Calcd for $C_{16}H_{11}BrFN_3OS$ (392.25): C, 48.99; H, 2.83; N, 10.71; Found: C, 48.84; H, 2.86; N, 10.61

4.2. Cytotoxic activity studies of synthesized compounds against various tumor cell lines

Cytotoxicity of synthesized compounds was tested using the method of Skehan *et al*. [28]. Cells were plated in a 96 multiwell plate (104 cells/well) for 24 hrs before treatment with the compounds to allow attachment to the wall of the plate. Different concentrations of the compounds (0, 5, 12.5, 25, and 50 μg/mL) were added to the cell monolayer, where triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 hrs at 37 °C in an atmosphere of 5% CO_2. After 48 hrs, cells were fixed, washed and stained with SRB stain, where excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Finally, color intensity was measured in an ELISA reader and the relationship between surviving fraction and drug concentration was plotted to get the survival curve of each tumor cell line for each compound. The median inhibition concentration (IC_{50}) which is the required concentration to reduce the survival to 50% is then determined from the survival curve.

4.3. Virucidal activity studies

The synthesized compounds were tested against HAV, HSV1 and CoxB4 viral strains where viral activity was assayed by the plaque formation method. Initially, the cytotoxicity of synthesized compounds on Vero cells was determined. The method used was that described by Vanden Berghe *et al* [31]. Viral infectivity was assayed by titration of viruses by the plaque formation method [34].

4.4. Antimicrobial activity

Chemical compounds were individually tested against a panel of gram positive and gram negative bacterial pathogens and fungi. Antimicrobial tests were carried out by the agar well diffusion method [32] using 100 μL of suspension containing 1 x10^8 CFU/mL of pathological tested bacteria and 1 x10^6 CFU/ml of yeast spread on nutrient agar (NA) and Sabouraud dextrose agar (SDA) media, respectively. After the media had cooled and solidified, wells (10 mm in diameter) were made in the solidified agar and loaded with 100 μL of tested compound solution prepared by dissolving 5 mg of the chemical compound in one ml of dimethyl sulfoxide (DMSO). The inoculated plates were then incubated for 24 h at 37 °C. Negative controls were prepared using DMSO employed for dissolving the tested compound.

Erythromycin and Metronidazole (5 mg/ml) were used as standard for antibacterial and antifungal activity, respectively. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard. The observed zone of inhibition is presented in Table 3. Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated.

4.5. Minimal inhibitory concentration (MIC) measurement

Screening was performed following the procedure outlined in the Manual of Clinical Microbiology [33]. All the bacteria were incubated and activated at 30 °C for 24 h inoculation into nutrient broth and the fungi were incubated in malt extract broth for 48 h. The compounds were dissolved in DMSO and then diluted using cautiously adjusted Mueller-Hinton broth. Two-fold serial concentrations of the compounds were employed to determine the (MIC). The final concentrations of the solutions were 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 μg/ml. In each case triplicate tests were performed and the average was taken as the final reading. The tubes were then inoculated with the test organisms, grown in their suitable broth at 37 °C for 24 hours for tested microorganisms (1×10^8 CFU/ml for bacteria and 1 $x10^6$ CFU/ml of fungi), each 5 ml received 0.1 ml of the above inoculum and incubated at 37 °C for 24 hours. The lowest concentration showing no growth was taken as the minimum inhibitory concentration (MIC).

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Figure captions:

Scheme 1: Synthesis of imidazolidineiminothione derivatives

Figure 1: ^{1}H NMR spectrum of compound **2f**

Figure 2: ^{1}H, ^{1}H-COSY spectrum of compound **2f**

Figure 3: ^{13}C NMR spectrum of compound **3c**

Figure 4: HSQC spectrum of compound **3c**

Figure 5: ^{19}F NMR spectra of compounds **2g** and **2h**

Figure 6: Fragmentation pattern of 4-imino-5-thioxoimidazolidine-2-one

Table 1: IC_{50} values of synthesized compounds obtained against tumour cell lines

Table 2: The percentage of anti-infectivity effect of the synthesized against viral strains.

Table 3: Antimicrobial activity of the synthesized compounds against the pathological organisms expressed as inhibition diameter zones in millimeters (mm) based on well diffusion assay

Table 4: Minimum inhibitory concentration MIC (µg/mL) of the most potent synthesized compounds against the pathological organisms

Table 1: IC_{50} values of synthesized compounds obtained against tumour cell lines

Compd. No.	IC_{50} µg of Cytotoxicity			
	Cell lines			
	HEPG2	MCF7	HCT116	PC3
2a	0.014	0.009	0.053	0.035
2b	0.037	0.027	0.056	0.056
2c	0.029	0.018	0.031	0.049
2d	0.025	0.011	0.041	0.031
2e	0.039	0.020	0.039	0.064
2f	0.017	0.015	0.015	0.017
2g	0.028	0.024	0.044	0.039
2h	0.050	0.011	0.034	0.064
3a	0.037	0.015	0.014	0.048
3b	0.032	0.031	0.051	0.051
3c	0.029	0.028	0.050	0.047
3d	0.048	0.052	0.047	0.056
4	0.012	0.020	0.023	0.031
Doxorubicin	0.007	0.005	0.007	0.008

Table 2: The percentage of anti-infectivity effect of the synthesized against viral strains.

Compd. No.	Anti viral effect (%)		
	Viral strain		
	HAV	CoxB4	HSV1
2a	66.0	43.6	71.6
2b	0	0	13.3
2c	0	0	3.4
2d	100	41.6	36.4
2e	70.2	0	33.6
2f	80.3	20.6	96.7
2g	0	0	9.6
2h	20.6	31.1	45.3
3a	30.7	0	50.2
3b	60.1	0	42.0
3c	0	18.5	85.7
3d	29.9	0	32.4
4	36.2	2.3	47.4
5	0	0	33.3
6	36.2	0	43.1

Table 3: Antimicrobial activity of the synthesized compounds against the pathological organisms expressed as inhibition diameter zones in millimeters (mm) based on well diffusion assay

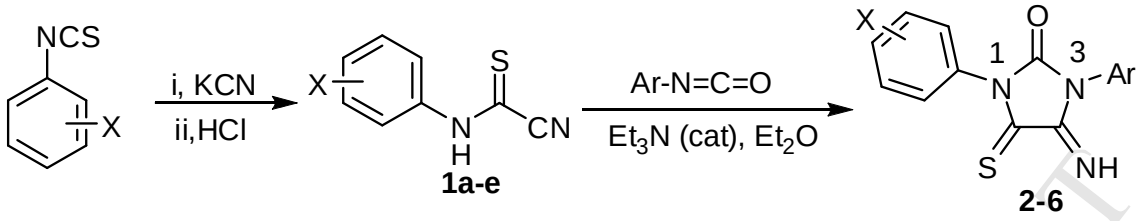
Compd. No.	Mean values of inhibition zone diameter in millimeters (mm)								
	Gram +ve			Gram -ve			Fungi		
	M l	*B s*	*B p*	*P a*	*E c*	*S l*	*C a*	*A n*	*P c*
2a	17	16	20	16	16	28	21	18	19
2b	20	25	26	20	25	30	27	21	23
2c	18	24	25	21	23	26	25	19	20
2d	16	30	15	0	30	24	24	16	21
2e	16	19	17	17	19	27	23	15	18
2f	16	27	21	18	27	21	21	15	20
2g	21	20	21	21	18	23	22	12	20
2h	20	20	21	22	18	24	18	22	21
3a	17	16	20	14	16	26	17	16	17
3b	17	28	23	26	28	27	29	19	20
3c	17	29	20	25	29	31	27	20.0	22
3d	16	12	17	0	18	24	14	14.0	18
4	14	20	17	16	20	28	27	18.0	20
5	14	18	18	15	18	26	23	17.0	16
6	22	22	21	21	20	23	22	20.0	21
Erythromycin	32	40	32	30	37	44	----	----	----
Metronidazole	----	----	----	----	----	----	27	33	25

where, *m l = m. luteus, b s = b. subtilis , b p = b. pumilus, p a = p aeruginosa, e c = e. coli, s l = s. lutea, c a = c. albicans, a n = a. niger* and *p c = p. chrysogenum*

Table 4: Minimum inhibitory concentration MIC (µg/mL) of the most potent synthesized compounds against the pathological organisms

Compd. No.	*P. aeruginosa*	*S. lutea*	*B. pumilus*	*M. luteus*	*C. albicans*
2b	0.78	3.12	3.12	6.25	1.56
2c	0.78	6.25	6.25	6.25	6.25
2h	3.12	3.12	6.25	6.25	6.25
3b	0.78	6.25	3.12	6.25	6.25
3c	0.78	6.25	6.25	6.25	6.25

Scheme 1: Synthesis of imidazolidineiminothione derivatives



1a X = 3,5-Cl_2
1b X = 2,5-$(OCH_3)_2$
1c X = 2,4-$(OCH_3)_2$
1d X = H
1e X = 4-Br

2a X = 3,5-Cl_2 Ar = C_6H_4-CH_3-4
2b X = 3,5-Cl_2 Ar = $C_6H_4OCH_3$-3
2c X = 3,5-Cl_2 Ar = $C_6H_4SCH_3$-4
2d X = 3,5-Cl_2 Ar = C_6H_4Cl-3
2e X = 3,5-Cl_2 Ar = $C_6H_3Cl_2$-2,6
2f X = 3,5-Cl_2 Ar = $C_6H_2Cl_3$-2,4,5
2g X = 3,5-Cl_2 Ar = C_6H_3Cl-4, CF_3-3
2h X = 3,5-Cl_2 Ar = $C_6H_3CH_3$-2, F-5

3a X = 2,5-$(OCH_3)_2$ Ar = $C_6H_4CH_3$-4
3b X = 2,5-$(OCH_3)_2$ Ar = $C_6H_4OCH_3$-4
3c X = 2,5-$(OCH_3)_2$ Ar = $C_6H_4SCH_3$-4
3d X = 2,5-$(OCH_3)_2$ Ar = 9*H*-fluoren-2-yl

4 X = 2,4-$(OCH_3)_2$ Ar = C_6H_4Cl-3
5 X = H Ar = $C_6H_3Cl_2$-2,6
6 X = 4-Br Ar = $C_6H_3CH_3$-2,F-5

Fig. 1: ^{1}H NMR spectrum of compound **2f**.

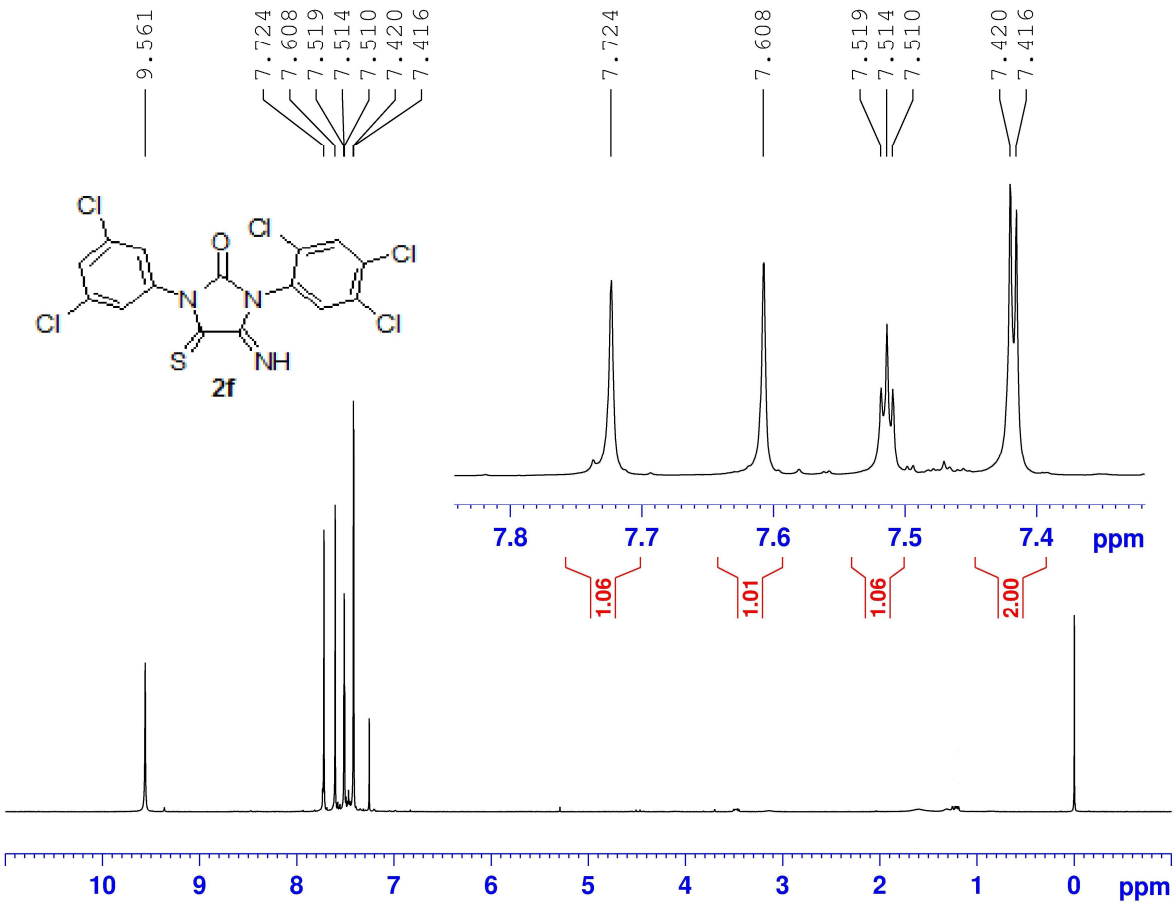


Fig. 2: ^{1}H, ^{1}H-COSY spectrum of compound **2f**

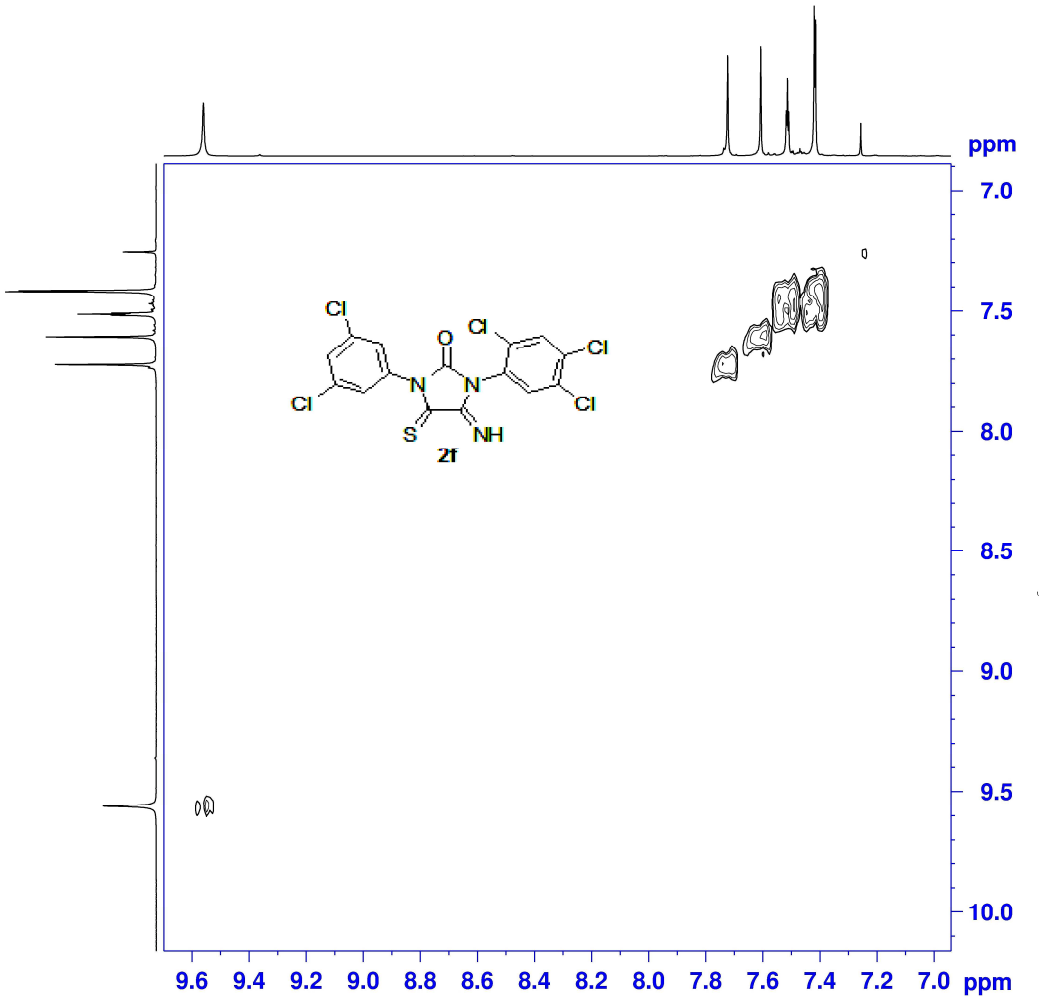


Fig. 3: ^{13}C NMR spectrum of compound **3c**

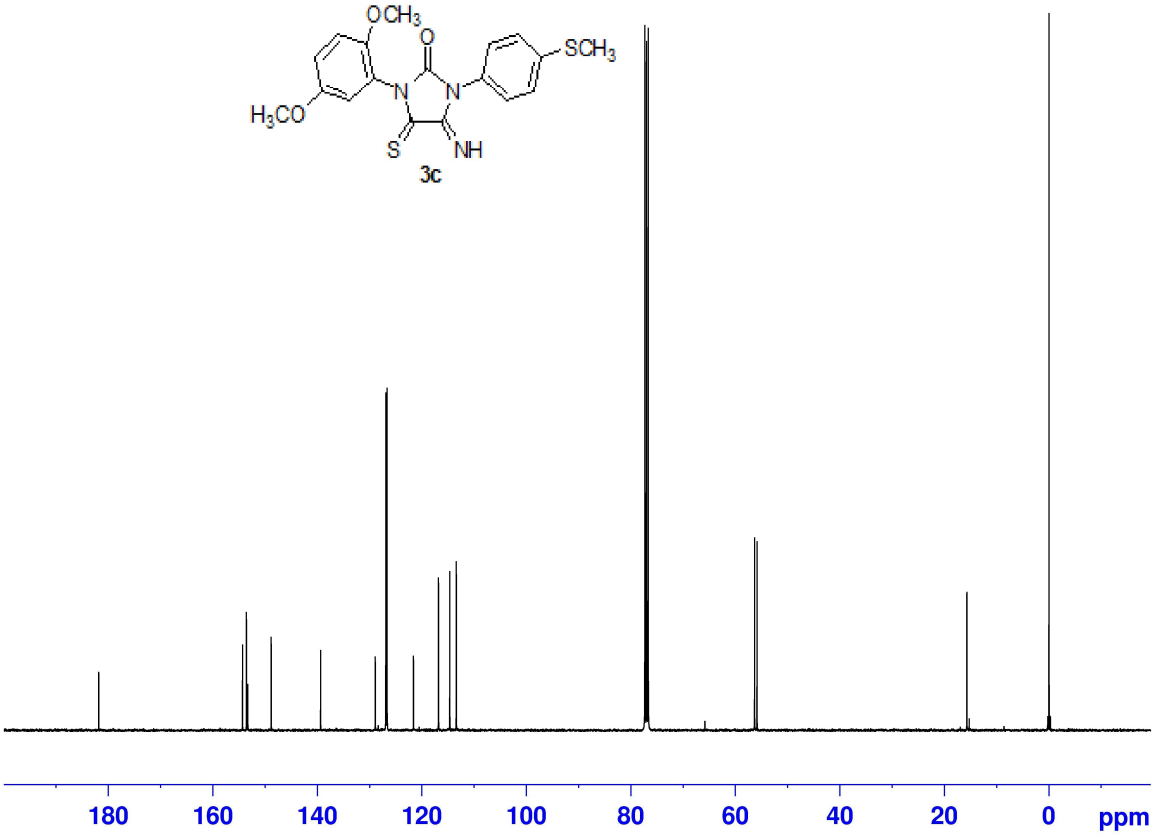


Fig. 4: HSQC spectrum of compound **3c**

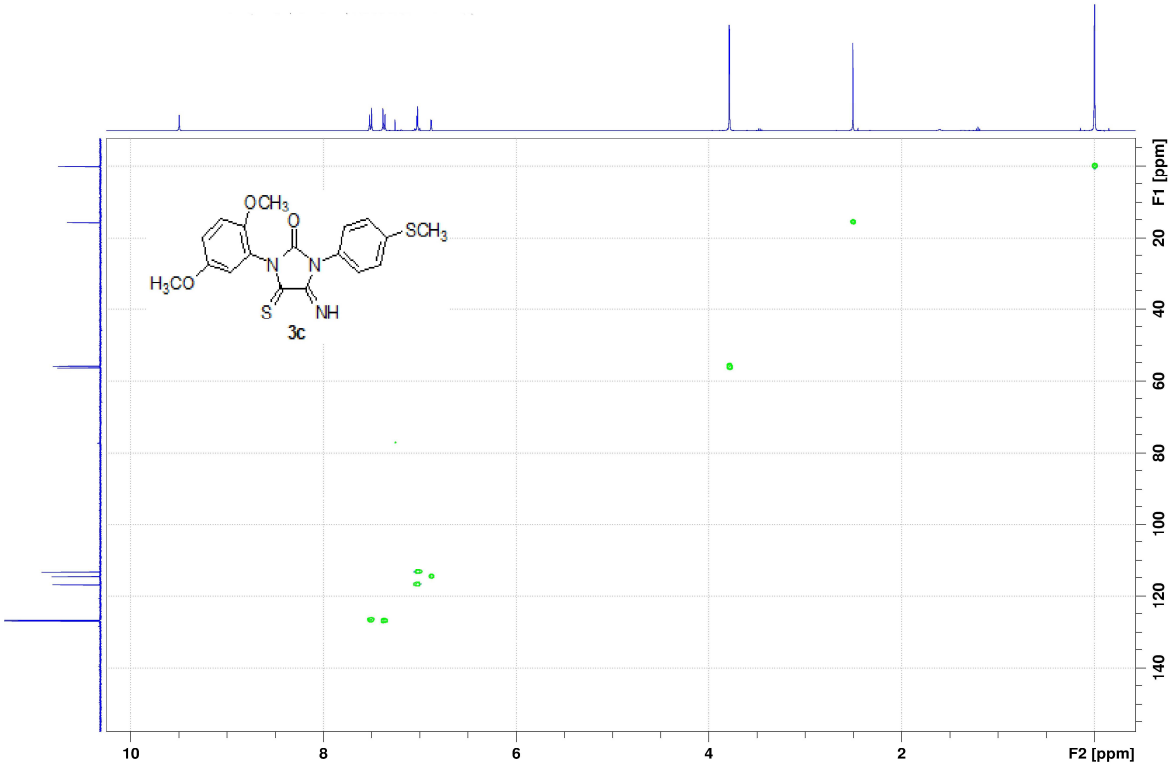


Fig. 5: ^{19}F NMR spectra of compounds **2g** and **2h**

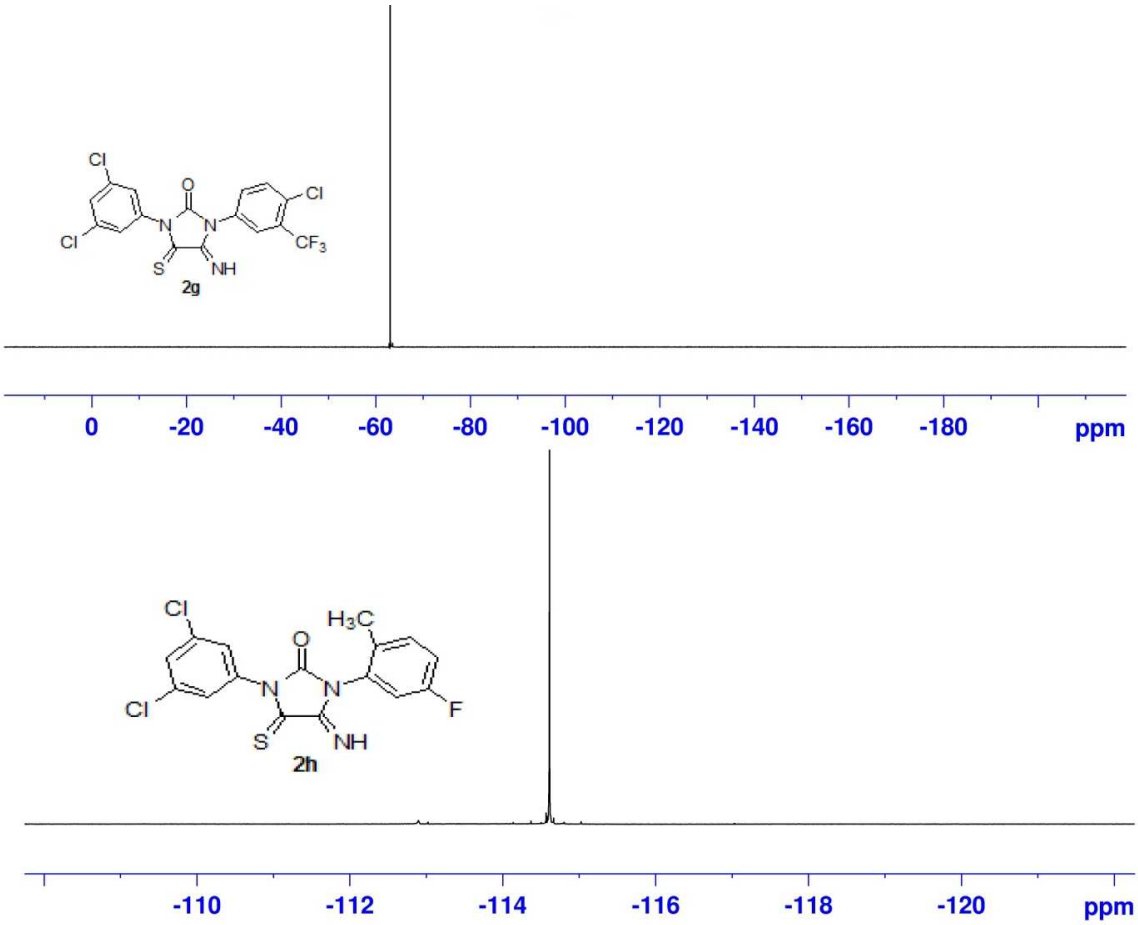
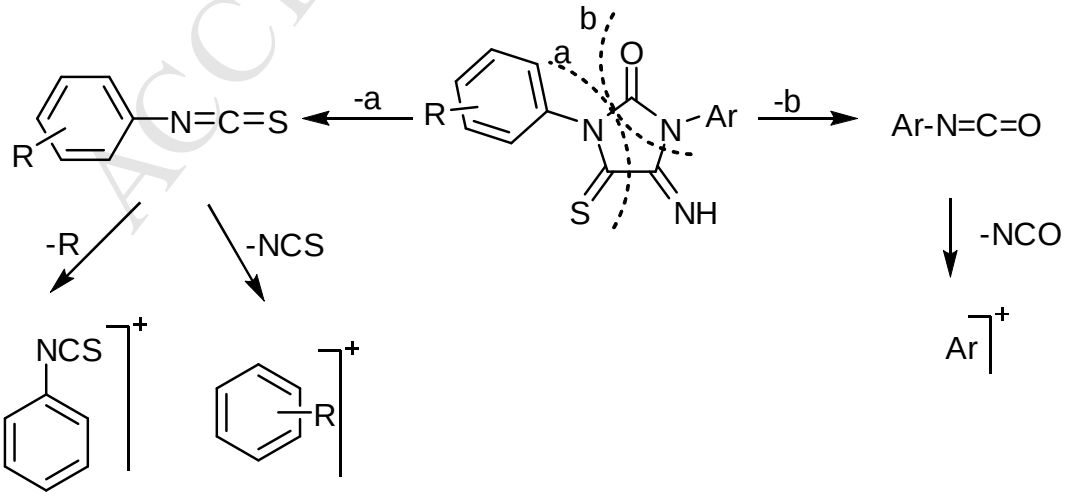


Fig. 6: Fragmentation pattern of 4-imino-5-thioxoimidazolidine-2-one



- Synthesis of *N*-arylcyanothioformamides derivatives
- Using the cyanothioformamides for synthesizing imidazolidineiminothiones.
- Antitumor activity was determined.
- Antiviral activity was determined.
- Antibacterial and antifungal activities were determined.