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Synthesis, characterization, and derivatization of some novel types of fluorinated mono- and bis-imidazolidineiminothiones with antitumor, antiviral, antibacterial, and antifungal activities

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ABSTRACT

A series of thirty eight novel imidazolidineiminothiones (**6a–g**, **10a–h**, **13a,b**, **15a–d**, and **16a**), 5-thioxoimidazolidine-2,4-diones (**7a–d**, **11a–e**, **14a,b**, and **16b**), and bis-imidazolidineiminothiones (**17–20**) with various fluorinated aromatic substituents at *N*-(1) and *N*-(3) were prepared in 75–85% yields. The imidazolidineiminothiones were synthesized from fluorinated *N*-arylcyanothioformanilides and substituted aromatic isocyanates, and by the reactions of fluorinated aromatic isocyanates with fluorinated and non-fluorinated aromatic *N*-arylcyanothioformanilides. Subsequent hydrolysis of selected products produced the corresponding 5-thioxoimidazolidine-2,4-diones. Preliminary screening of several compounds against Ehrlich ascites carcinoma (EAC) cells indicated that **6f** and **16a** were the most active (90% and 80% inhibition, respectively). Further evaluation for cytotoxicity against other tumor cell lines gave IC_{50} values ranging from 0.67 to 3.83 μg/mL, where compounds **15a** and **16a** were markedly active against all cell lines. This highlights the synergistic effect of the suitably positioned fluorinated substituents on *N*-(1) and *N*-(3) of the imidazolidineiminothiones. Compounds **6a**,**e–g**, **10a–c**, **13b**, **15a–d**, and **17–20** were tested against microbial organisms (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Sarcina lutea*), and fungal strains (*Candida albicans*, *Aspergillus niger*, and *Aspergillus flavus*). Whereas compound **6a** exhibited the highest antibacterial activity against Gram positive and Gram negative bacteria, **13b** displayed the strongest antifungal activity against all fungal strains, reaching as high as 30 mm. Finally, **15a**,**b**,**d** were subjected to in vitro testing of antiviral activity against hepatitis A virus (HAV), human herpes simplex virus 1 (HSV1), and Coxsackie B4 (COxB4) viral strain, where **15b** was the most effective, reducing virus plaque count of HSV1 and COxB4 by 50% and 60%, respectively.

1. Introduction

Fluorinated compounds have proved invaluable as antibacterial and antifungal agents, and have been used for the treatment of obesity and various diseases associated with the cardiovascular and central nervous systems [1–5]. Intrinsic properties of the fluorine atom, such as high electronegativity, small atomic radius [6], and low polarisability of the C–F bond, impart significant improvement on the biological activity of fluorinated molecules. Thus, fluorine substitution remains an attractive means in the design and development of more active and selective pharmaceutical drug agents.

As a part of our ongoing synthetic effort in the preparation of biologically active molecules, we have been exploring and developing annulation reactions of aromatic cyanothioformanilides (**1**), which are themselves bioactive [7–9]. These have been earlier prepared by treating *N*-aryl isothiocyanates with cyanide [10–19] and by other classical methods [20]. Recent approaches have employed environmentally-friendly *N*-arylimino-1,2,3-dithiazoles which transform readily to the corresponding cyanothioformanilides under various conditions [21–36]. *N*-Arylcyanothioformanilides have found wide utility in organic synthesis [11,15,37–43] and we have used them to prepare various heterocycles with promising pharmacological properties. Such reagents are ambident nucleophiles as reflected by their existence as tautomeric mixtures [44,45], and thus may react at the sulfur or nitrogen atom. The nitrile function serves as an electrophilic center and may participate in subsequent reactions. These properties have been exploited in the development of a variety of "one-pot"

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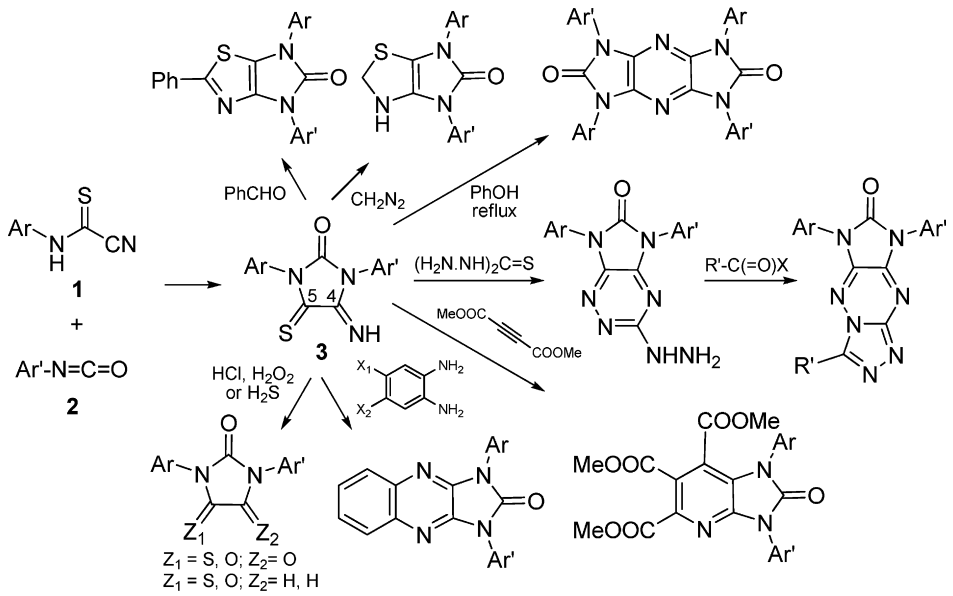


Scheme 1. Previous preparation and some selected reactions of imidazolidineiminothiones.

ring-closure reactions of *N*-arylcyanothioformanilides [13,18,42,46–48] to yield various heterocyclic cores which include pyrroles [14,49–51], pyrrolothiazoles [52], imidazoles [10,13,53–61], benzimidazoles [62], oxazoles [11,43,47,48], benzoxazoles [43,47,48,62], thiazoles [52,63], quinazolinonethiones [42,60], quinoxaline [42], imidazoquinoxalines [63,64], among others [65–71].

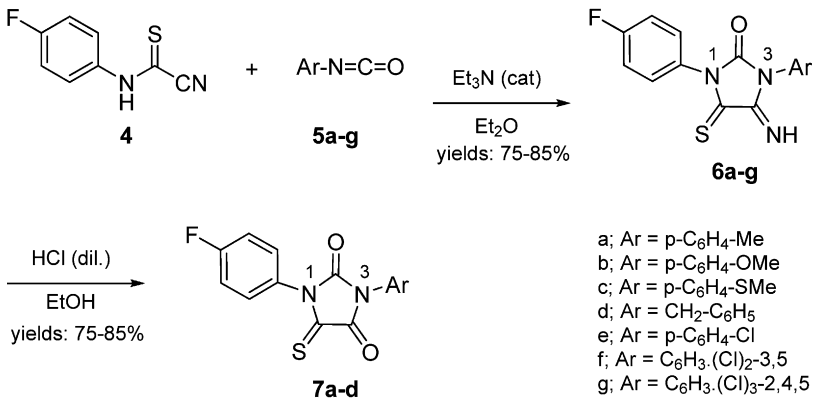
We have recently reported the preparation, derivatization, and in vitro biological evaluation of imidazolidineiminothiones (**3**) [14,44,45,52,53,68]. These were synthesized from *N*-arylcyanothioformanilides via a heterocyclic ring closing reaction with various isocyanates (**2**) to afford heterocyles containing adjacent imino and thione functions in the 4 and 5 positions, respectively (Scheme 1). These groups are believed to be the pharmacophore responsible for the broad spectrum of biological activities exhibited by such compounds. Conveniently, such arrangement has served as a reactive template in subsequent ring closing reactions in which one or both of the exocyclic functional groups have incorporated into additional fused heterocyclic rings. Imidazolidineiminothiones and their derivatives were shown to exhibit a wide range of activity against tumor cell lines, and various viral, microbial, and fungal strains (Scheme 1).

2. Results and discussion

2.1. Chemistry

Fluorinated imidazolidineiminothiones and derivatives thereof are virtually unknown and remain unexplored in many respects. It is noted that literature search returned, in addition to two fluorinated imidazolidineiminothione compounds reported by our group [44,64], two references detailing the preparation and transition metal ion binary complexes of 1-(4-fluorophenyl)-4-imino-3-phenyl-5-thioxoimidazolidine-2-one [72], and fungicidal action of 1-(4-chloro-3-(trifluoromethyl)phenyl)-4-imino-3-methyl-5-thioxoimidazolidine-2-one [57]. Preliminary bioactivity data obtained from investigating the antiviral properties (anti-infectivity effect) of one of the fluorinated imidazolidineiminothiones [44] against hepatitis A virus, human herpes simplex virus 1, and Coxsackie B4 (COxB4) viral strain demonstrated its superiority relative to the non-fluorinated counterparts. Interestingly, among the eight imidazolidineiminothiones belonging to the same series, six non-fluorinated analogs were completely inactive, whereas only one non-fluorinated imidazolidineiminothione along with the fluorinated analog displayed strong anti-infectivity effects (reduced virus plaque count by 50%). In addition, the fluorinated imidazolidineiminothione possessed significantly higher antibacterial activities in most cases against the growth of Gram positive and Gram negative bacteria (*Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, and *Staphylococcus aureus*) [44]. Thus, encouraged by the preliminary results obtained with the fluorinated imidazolidineiminothione and the reported bioactivity of the related imidazolidineiminothiones and their derivatives, versatile reactivity which permits facile chemoselective structural modifications, and scarcity of fluorinated imidazolidineiminothiones and related compounds, the present investigation reports on the preparation and biological activities of a series of thirty eight novel fluorinated mono- and bis-imidazolidineiminothiones and a selection of the hydrolysed 5-thioxo-imidazolidine-2,4-dione analogs. They were synthesized by various combinations of fluorinated *N*-arylcyanothioformanilides with substituted aromatic isocyanates, and by the reactions of fluorinated aromatic isocyanates with substituted *N*-arylcyanothioformanilides. Whereas reactions of fluorinated *N*-arylcyanothioformanilides with substituted aromatic isocyanates positioned the fluorinated substituents at *N*-(1) and in proximity to the thione group, various combinations of fluorinated aromatic isocyanates with substituted *N*-arylcyanothioformanilides placed the fluorinated substituents at *N*-(3) near the imino group. Further, a new class of related analogs where fluorinated substituents have been incorporated into both, the *N*-(1) and *N*-(3) positions, was prepared from fluorinated *N*-arylcyanothioformanilides and fluorinated aryl isocyanates. Hydrolysis of the selected products afforded several novel 5-thioxo-imidazolidine-2,4-diones. Finally, a series of symmetrical and non-symmetrical fluorinated bis-imidazolidineiminothiones was prepared from p-fluorocyanothioformanilide and various bis-isocyanates.

The preparation of imidazolidineiminothiones **6a–g** with a fluorinated *N*-(1) substituent is illustrated in Scheme 2. Thus, commercial isocyanates **5a–g** with various aromatic substituents (p-tolyl, p-methoxyphenyl, p-thiomethylphenyl, benzyl, p-chlorophenyl, 3,5-dichlorophenyl, and 2,4,5-trichlorophenyl) were

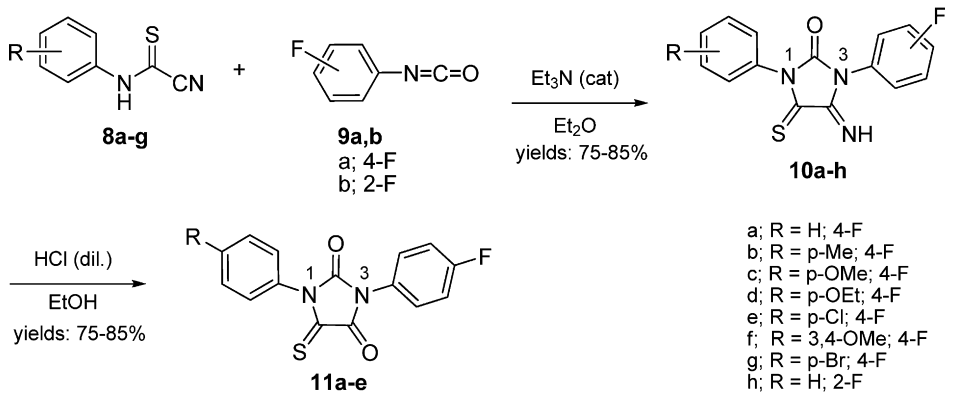


Scheme 2. Preparation of *N*-(1) fluorinated imidazolidineiminothiones and 5-thioxoimidazolidine-2,4-diones with differing aromatic substituents at *N*-(3).

reacted with an equimolar amount of p-fluorocyanothioformanilide (**4**) in ether in the presence of a few drops of triethylamine as a catalyst to furnish the corresponding 5-imino-4-thioxo-2-imidazolidinones **6a**–**g** as shown in Scheme 2. p-Fluorocyanothioformanilide (**4**) was prepared by treating an ethanolic solution of commercial 4-fluorophenyl isothiocyanate with an aqueous solution of KCN according to literature protocols [10–19]. The ring closing reaction proceeded by attack of the nitrogen atom of the cyanothioformanilide onto the isocyanate carbon, where the ensuing annulation proceeds by attack of the resulting nitrogen anion onto the electrophilic nitrile group. The products were obtained as crystalline solids in 75–85% yields and were purified by recrystallization from chloroform/n-hexane. These compounds could be stored at room temperature for prolonged periods of time without showing any signs of decomposition or developing odors which indicates instability. Hydrolysis of **6a**–**d** with dilute HCl in boiling ethanol afforded diones **7a**–**d** as crystalline solids in 75–85% yields which were purified by recrystallization from chloroform/n-hexane. Due to the significant and broad spectrum bioactivity of imidazolidineiminothiones which presumably stems from the iminothione group, herein we investigate iminothiones **6a**–**g** as potential antitumor, antimicrobial, antifungal, and antiviral agents that contain the pharmacophore motif, a fluorinated *N*-(1) substituent, and variants at *N*-(3). Therefore, a comparative study of activities should uncover the effect exerted by the *N*-(3) substituent and reveal any potential synergistic or opposing effect between the fluorinated *N*-(1) group and *N*-(3) substituents which will ultimately enable structural optimization and the design of highly active heterocycles. On the other hand, evaluating the activities of diones **7a**–**d** in which the imino group has been hydrolysed will shed light on the role played by the iminothione function in this particular series.

Structures of the prepared compounds were further elucidated by the use of 1D/2D ^{1}H NMR, ^{13}C NMR (see Supplementary material section), and IR techniques. Hence, infrared measurements showed diagnostic absorbances in the range of 3429–3455 cm^{-1} for the imino NH, 1769–1778 cm^{-1} for the C=O group, and 1661–1676 cm^{-1} for the C=N function. The thione stretch appeared at its expected frequency from 1065 to 1126 cm^{-1}. The ^{1}H NMR spectra of all compounds except **6f** exhibited the usual broad exchangeable singlet for the imine proton which ranged from δ 10.02 to 9.37 ppm. It is noted that the ^{1}H NMR of **6f** was recorded in d1-TFA/$CDCl_3$ which catalyzed rapid exchange of the imine proton. Integration values and multiplicities were in agreement with the assigned structures. The aromatic signals were observed in all cases as multiplets for the p-fluorophenyl protons due to F–H and H–H couplings. On the other hand, the aromatic signals for the *N*-(3) substituents appeared in most cases as singlets, resolved doublets or triplets. More diagnostic signals were those stemming from the benzyl methylene, methoxy, methylthio, and aromatic methyl groups which appeared as singlets at δ 4.99, 3.85, 2.51 and 2.41 ppm, respectively. The ^{13}C NMR spectra were most informative and displayed the expected three signals for the 4-imino-5-thioxoimidazolidine-2-one core (δ 182.9–176.4 ppm for C=S, δ 154.6–152.0 ppm for C=O, and δ 153.8-150.3 ppm for C=N). The aromatic signals ranged from δ 164.3 to 114.7 ppm and corroborated the number of quaternary and methine groups present, whereas the distinctive benzyl methylene, methoxy, methylthio, and aromatic methyl signals appeared at their anticipated locations at δ 44.4, 55.3, 15.6, and 21.2 ppm, respectively. The ^{13}C aromatic signals of the fluorophenyl group appeared as four doublets ranging from δ 164.3 to 162.1 ppm for the C–F (d, J = 246.9–253.4 Hz), δ 129.5–126.8 ppm for the C–N (d, J = 2.9–3.7 Hz), δ 117.7–116.4 ppm for the α C–H (d, J = 22.8–23.5 Hz), and δ 130.1–129.1 ppm for the β C–H (d, J = 8.8–9.6 Hz). The multiplicity patterns and coupling constants proved useful during the assignment of the aromatic protons to the appropriate carbons in the HSQC spectra. Finally, the ^{19}F chemical shifts ranged from δ −108.5 to −110.2 ppm. The hydrolysis products **7a**–**d** were characterized by similar means where all spectroscopic and microanalytical data corroborated the proposed structures. Most notable, the disappearance of the NH resonance from the proton NMR was observed.

The preparation of the second series of fluorinated imidazolidineiminothiones **10a**–**h** with a fluorinated *N*-(3) substituent is shown in Scheme 3. Thus, commercial fluorophenyl isocyanates **9a**,**b** were reacted with ethereal solutions containing equimolar amounts of cyanothioformanilides **8a**–**g** with various aromatic substituents (phenyl, p-tolyl, p-methoxyphenyl, p-ethoxyphenyl, p-chlorophenyl, 3,4-dimethoxyphenyl, and p-bromophenyl). As before, the reactions were catalyzed by the addition of a few drops of triethylamine to furnish the corresponding 4-imino-5-thioxo-2-imidazolidinones **10a**–**h** as shown in Scheme 3. The products were obtained as pure crystalline solids in 75–85% yields and were fully characterized by standard spectroscopic and analytical methods (see Experimental and Supplementary material sections). The arylcyanothioformanilides **8a**–**g** were prepared from either commercial arylisothiocyanates and KCN according to literature protocols [10–19] or through the preparation of dithiocarbamates from aromatic amines, followed by conversion to the corresponding isothiocyanates and the subsequent cyanation to afford the desired aryl cyanothioformanilides. Hydrolysis of **10a**–**e** with dilute HCl in boiling ethanol afforded diones **11a**–**e** as crystalline solids. It is noted that this series contains fluorinated *N*-(3) substituents and variants at *N*-(1) which is anticipated to illustrate the effect exerted by the *N*-(3) fluorinated substituents and reveal matching and mismatching enhancement effect with the *N*-(1) substituents on biological activity. Further, the hydrolysed



Scheme 3. Synthesis of *N*-(3) fluorinated imidazolidineiminothiones and 5-thioxoimidazolidine-2,4-diones with differing aromatic substituents at *N*-(1).

products will reveal enhancing or opposing effect of the imino function in this series on biological activity.

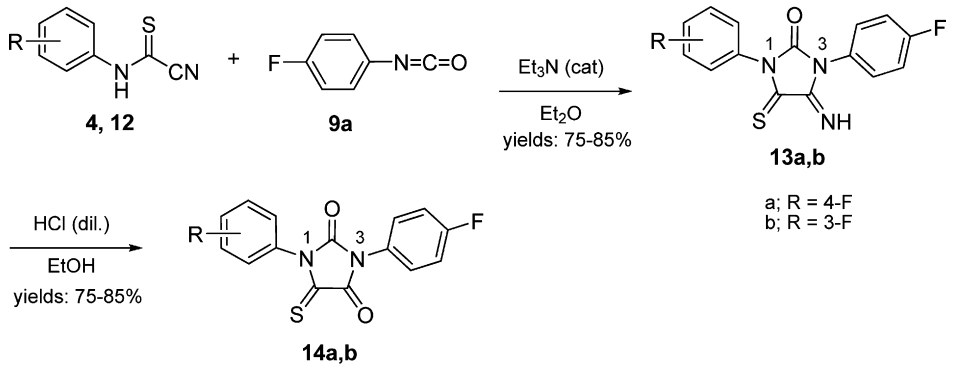
The synthesis of the next series of imidazolidineiminothiones in which both *N*-(1) and *N*-(3) contain fluorinated substituents is outlined in Scheme 4. Thus, bis-fluorinated imidazolidineiminothiones **13a**,**b** were prepared from 4-fluorophenyl isocyanate (**9a**) and fluorocyanothioformanilides **4** and **12** which contain fluorine in the 4 and 3 positions of the phenyl ring, respectively. Hydrolysis of **13a**,**b** furnished the corresponding 5-thioxo-imidazolidine-2,4-diones **14a**,**b** as crystalline solids in 75–85%. It is noted that this series contains fluorinated *N*-(1) and *N*-(3) substituents in which the fluorine atom is directly bonded to the aromatic phenyls. While **13a**,**b** and their hydrolysed analogs **14a**,**b** will serve to illustrate the effect of bis-fluorination in the presence and absence of the imino group, we decided to prepare one last series of bis-fluorinated imidazolidineiminothiones (**15a**–**d** and **16a**,**b**) in which either the *N*-(1) or *N*-(3) aromatic substituent is fluorinated with a trifluoromethyl group and the remaining phenyl is substituted with a p-methoxy, p-ethoxy, p-fluoro, and p-iodo groups (Scheme 5). In this case, the impact of indirectly bonded fluorine to the aromatic group and any potential synergy with the remaining functions will be uncovered.

There has been a recent surge in the preparation of symmetrical and non-symmetrical bis-heterocycles (e.g. bis-quinazoline derivatives) which demonstrated superior antibacterial and antifungal activities than their mono-heterocyclic counterparts [73]. In this regard, we and others have reported the preparation and biological activities of several variously-substituted bis-imidazolidineiminothiones. Indeed, such heterocycles were markedly active in the 1–5 μg/mL scale against a broad range of carcinoma cell lines, as well as microbial and fungal strains [44]. So far, fluorinated bis-imidazolidineiminothiones are unknown and yet to be investigated. Consequently, we opted to prepare some symmetrical and non-symmetrical fluorinated bis-imidazolidineiminothione derivatives as shown in Fig. 1. Thus, reaction of two molar equivalents of p-fluorocyanothioformanilide (**4**) under the usual standard conditions with one molar equivalent of each of 2,2′-dimethoxy-4,4′-biphenylene diisocyanate, 4,4′-oxybis(isocyanatobenzene), isophorone diisocyanate, and hexamethylene diisocyanate gave the corresponding bis-imidazolidineiminothiones **17**–**20**, respectively. The diisocyanates are all commercially available and were used as received. Whereas **17** and **18** are characterized by structural rigidity, **19** and **20** contain longer and flexible tethers connecting the two heterocyclic units.

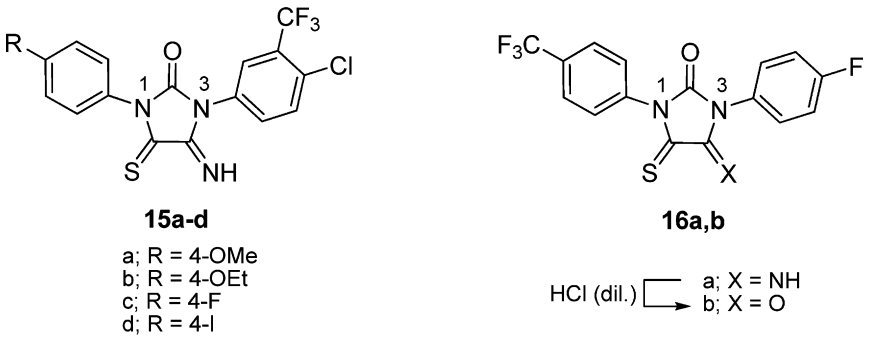
2.2. Biological activities

2.2.1. Antitumor properties

Imidazolidineiminothiones exhibit broad spectrum pharmacological properties attributable to the adjacent imino and thione groups in the 4 and 5 positions. We have previously prepared many heterocycles containing these moieties [44] and investigated them against viral, microbial, and fungal strains, as well as various tumor cell lines. Several compounds underwent derivatization by functionalizing either of the imino and thione groups, or both. Many compounds showed antitumor, antiviral, antibacterial, and antifungal properties, and emerged as lead chemical entities. In an attempt to render them clinically useful drug agents, the most promising leads were subjected to further structural optimization by varying the substituents on *N*-(1) and *N*-(3) [45]. Subsequent assessment of the bioactivity of such derivatives revealed the effects of the imino, thione, *N*-(1), and *N*-(3) groups on activity, and



Scheme 4. Preparation of bis-fluorinated imidazolidineiminothiones and 5-thioxoimidazolidine-2,4-diones with differing fluorinated aromatic substituents at *N*-(1) and *N*-(3).



Scheme 5. Preparation of *N*-(1) and *N*-(3) fluoromethylated imidazolidineiminothiones and 5-thioxoimidazolidine-2,4-diones.

showed the most effective and synergetic combination of functional groups needed to maximize biological activity. In continuation with our efforts in this area, and due to the absence of biological activity data on fluorinated imidazolidineiminothiones, a large selection of the prepared compounds (**6a**–**g**, **10d**,**f**,**g**, **13a**, **15a**,**c**, **16a**, **17**, and **19**) underwent a preliminary screening test against Ehrlich ascites carcinoma cells (EAC). Measurement of antitumor activity was carried out by inhibition studies of EAC cell viability using the trypan blue dye exclusion assay. In this method, an EAC suspension (2.5×10^5/mL) is treated with 0.8 mL RPMI 1640 media and 0.1 mL of each tested compound (corresponding to either 100, 50, or 25 μg) with subsequent incubation at 37 °C for a duration of 2 h. The percentage of non-viable cells is then calculated as follows: % of non-viable cells = (no. of non-viable cells/no. of cells) × 100. Compounds producing over 70% non-viable cells are considered highly active.

2.2.2. *Screening of compounds 6a–g, 10d,f,g, 13a, 15a,c, 16a, 17, and 19 against EAC cells*

Compounds **6a**–**g**, **10d**,**f**,**g**, **13a**, **15a**,**c**, **16a**, **17**, and **19** were screened against EAC cells at concentrations of 25, 50, and 100 μg/mL as shown in Table 1 and represented graphically in Fig. 2. Compounds **6f** (*N*-(1): 4-fluorophenyl; *N*-(3): 3,5-dichlorophenyl), and **16a** (*N*-(1): 4-trifluoromethylphenyl; *N*-(3): 4-fluorophenyl) were the most active as they displayed the highest % inhibition of EAC cell viability (90% and 80%, respectively). Modest, albeit reasonable % inhibition values of cell viability were observed for compounds **6a**,**d** and **19** (30%, 60%, and 30%, respectively; **6a**: *N*-(1): 4-fluorophenyl; *N*-(3): p-tolyl; **6d**: *N*-(1): 4-fluorophenyl; *N*-(3): benzyl; **19**: *N*-(1): 4-fluorophenyl; *N*-(1): 4-fluorophenyl). Interestingly, the non-fluorinated analog of **6a** [45] only produced a 10% inhibition of EAC cell viability, pointing to the enhancement of fluorine substitution on biological activity. Whereas compounds **6b**,**c**,**e**,**g**, **10f**, and **13a** were completely inactive, the remaining fluorinated imidazolidineiminothiones **10d**,**g**, **15a**,**c**, and **17** displayed % inhibition values of 20% for **15c** and **17** (**15c**: *N*-(1): 4-fluorophenyl; *N*-(3): 4-chloro-3-trifluoromethylphenyl; **17**: *N*-(1): 4-fluorophenyl; *N*-(1): 4-fluorophenyl), and values ranging from 10% to 5% for **10d**,**g**, and **15a** (**10d**: *N*-(1): 4-ethoxyphenyl; *N*-(3): 4-fluorophenyl; **10g**: *N*-(1): 4-bromophenyl; *N*-(3): 4-fluorophenyl; **15a**: *N*-(1): 4-methoxyphenyl; *N*-(3): 4-chloro-3-trifluoromethylphenyl). In order to evaluate the effects bestowed by the *N*-(1) and *N*-(3) substituents on bioactivity, a comparative study of activity was carried out within group **6a**–**g** where the 4-fluorophenyl group is invariant. Fig. 2 shows that while the p-tolyl and benzyl groups resulted in high % inhibition values in the **6a**–**g** series (30% and 60% inhibition, respectively), the methoxy, thiomethyl, p-chloro, and 2,4,5-trichlorophenyl groups formed complete mismatched pairs with the 4-fluorophenyl (0.0% inhibition). Optimal synergy was achieved between the 4-fluorophenyl and 3,5-dichlorophenyl groups as demonstrated by the highest value of % inhibition obtained for **6f** (90% inhibition). It is noted that **6f** is the most active among the remaining compounds as well. One of the most interesting observations made pertaining to the enhancing effect of fluorine substitution is visible in compound **15c** (20% inhibition) and **6e** (0.0% inhibition). The deleterious effect of the p-chloro group in **6e** has been clearly offset by the trifluormethyl substituent as shown by the enhancement in the activity of **15c**. Indeed, the dominant role played by fluorine substitution is further evident in **16a** which is the second most active among all compounds (80% inhibition). In this case, *N*-(3) is substituted with a 4-fluorophenyl function and *N*-(1) bears a trifluormethylphenyl group.

2.2.3. *Cytotoxic activity of compounds 15a and 16a against various tumor cell lines*

The encouraging results obtained during the screening against EAC cells prompted further evaluation of a selection of heterocycles for cytotoxic activity against the following tumor cancer cell lines: human hepatocellular carcinoma cell line (HEPG2), human

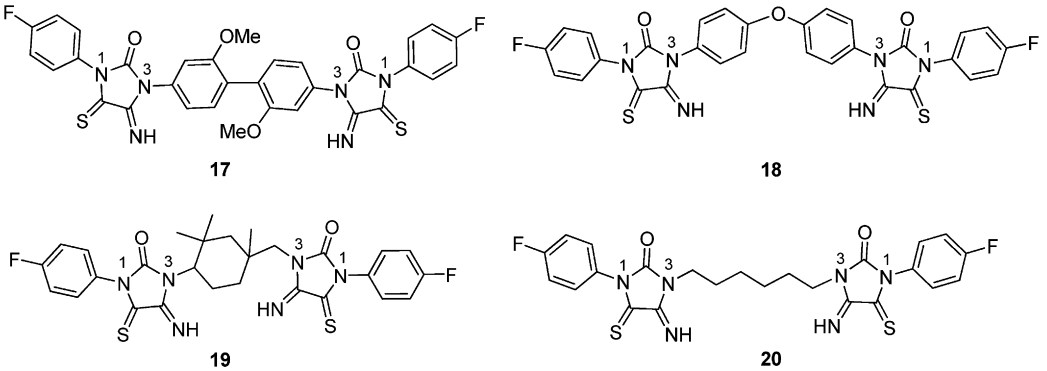


Fig. 1. Preparation of symmetrical and non-symmetrical fluorinated bis-imidazolidineiminothiones.

Table 1
Inhibition of Ehrlich ascites carcinoma cells (EAC) viability.

Compound no. and Concentration	% Inhibition of cell viability		
	100 μg/mL	50 μg/mL	25 μg/mL
6a	30	ND[a]	ND
6b	0	0	0
6c	0	0	0
6d	60	ND	ND
6e	0	0	0
6f	90	45	23
6g	0	0	0
10d	10	0	0
10f	0	0	0
10g	10	0	0
13a	0	0	0
15a	5	0	0
15c	20	ND	ND
16a	80	ND	ND
17	20	ND	ND
19	30	15	5

[a] ND: not determined.

laryngeal carcinoma cell line (HEP2), human breast adenocarcinoma cell line (MCF7), human epithelial carcinoma cell line (HELA), and colon carcinoma cell line (HCT116). Since **15a** and **16a** ranked among the least and most active compounds against EAC, respectively, we were interested in exploring their full potential and general applicability to other tumor cell lines. Thus, the cytotoxicity was further evaluated against HEPG2, HEP2, MCF7 HELA, and HCT116 carcinoma cell lines. The compounds were tested at concentrations between 5 and 50 μg/mL using the sulforhodamine B (SRB) assay (Table 2) and the relationship between surviving fraction and drug concentration is plotted in Fig. 3 to get the survival curve of each tumor cell line for each compound. The sulforhodamine B (SRB) assay was developed by Skehan et al. [74] to measure drug-induced cytotoxicity for large-scale drug-screening applications. Under mild acidic conditions, the protein dye sulforhodamine B binds electrostatically on protein basic amino acid residues of trichloroacetic acid-fixed cells. However, under mild basic conditions it can be extracted from cells and dissolved for measurement. The SRB assay possesses a colorimetric end point, is indefinitely stable, and is nondestructive. These practical advantages render the assay an appropriate and sensitive assay to measure drug-induced cytotoxicity.

Generally, all cell lines were responsive to dose increase as indicated by the ensuing reduction in surviving fraction, although

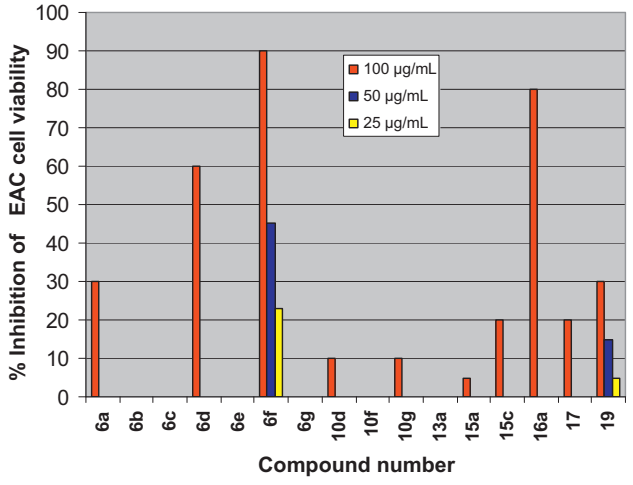


Fig. 2. Cytotoxicity of compounds **6a–g**, **10d**,**f**,**g**, **13a**, **15a**,**c**, **16a**, **17**, and **19** against Ehrlich ascites carcinoma cells (EAC).

Table 2
Evaluation of compounds **15a** and **16a** against human hepatocellular carcinoma cell line (HEPG2), human laryngeal carcinoma cell line (HEP2), human breast adenocarcinoma cell line (MCF7), human epithelial carcinoma cell line (HELA), and colon carcinoma cell line (HCT116).

Cell line	Sample conc. (μg/mL)	Surviving fraction		
		15a	Sample conc. (μg/mL)	**16a**
HEPG2	0	1.00	0	1.00
	1.00	0.64	5.00	0.26
	2.50	0.19	12.5	0.24
	5.00	0.12	25.0	0.24
	10.0	0.12	50.0	0.31
HEP2	0	1.00	0	1.00
	1.00	0.39	5.00	0.24
	2.50	0.34	12.5	0.20
	5.00	0.33	25.0	0.20
	10.0	0.31	50.0	0.28
MCF7	0	1.00	0	1.00
	1.00	0.83	5.00	0.29
	2.50	0.60	12.5	0.21
	5.00	0.40	25.0	0.28
	10.0	0.28	50.0	0.29
HELA	0	1.00	0	1.00
	1.00	0.48	5.00	0.18
	2.50	0.33	12.5	0.19
	5.00	0.29	25.0	0.25
	10.0	0.33	50.0	0.29
HCT116	0	1.00	0	1.00
	1.00	0.81	5.00	0.24
	2.50	0.37	12.5	0.29
	5.00	0.34	25.0	0.27
	10.0	0.34	50.0	0.36

dose response was less pronounced between 5 and 10 μg/mL and 25–50 μg/mL for **15a** and **16a**, respectively (Table 2 and Fig. 3). Due to the encumbered and overlapping nature of the dose response curve profiles, Fig. 3 was impractical for the purpose of a comparative study. Thus, we opted to use the median inhibition concentration (IC_{50}) values determined from Fig. 3 as they comprise a convenient means for comparing activity of **15a** and **16a** as antitumor agents. Thus, the IC_{50} values measured in this manner are tabulated in Table 3 and plotted in Fig. 4.

The IC_{50} values shown in Table 3 and represented graphically in Fig. 4 clearly demonstrate the effectiveness of fluorinated imidazolidineiminothione **15a** (*N*-(1): 4-methoxyphenyl; *N*-(3): 4-chloro-3-trifluoromethylphenyl) and **16a** (*N*-(1): 4-trifluoromethylphenyl; *N*-(3): 4-fluorophenyl) against HEPG2, HEP2, MCF7, HELA, and HCT116 carcinoma cell lines. Surprisingly, while **15a** was ineffective against EAC cells, it proved extremely active against all carcinoma cell lines used, producing similar and consistent IC_{50} values for all cell lines in exception to MCF7 (Fig. 4). The IC_{50} values ranged from 0.67 to 3.83 μg/mL and were consistently higher than those obtained with **16a**. Notably, **16a** was markedly active against all cell lines and consistently produced low IC_{50} values in all cases (ranging from 2.99 to 3.58 μg/mL). This clearly underlines the synergistic effect of the suitably positioned 4-trifluoromethylphenyl and 4-fluorophenyl groups on the aromatic rings of *N*-(1) and *N*-(3) of the imidazolidineiminothione. The dominant role of the *N*-(1) and *N*-(3) substituents is seen within **15a** and **16a** which show uniform bioactivity almost against all cell lines with very negligible disparity in median inhibition concentration values.

2.2.4. Antibacterial and antifungal properties

The following selected 16 compounds **6a**,**e–g**, **10a–c**, **13b**, **15a–d**, and **17–20** were tested under the same conditions for

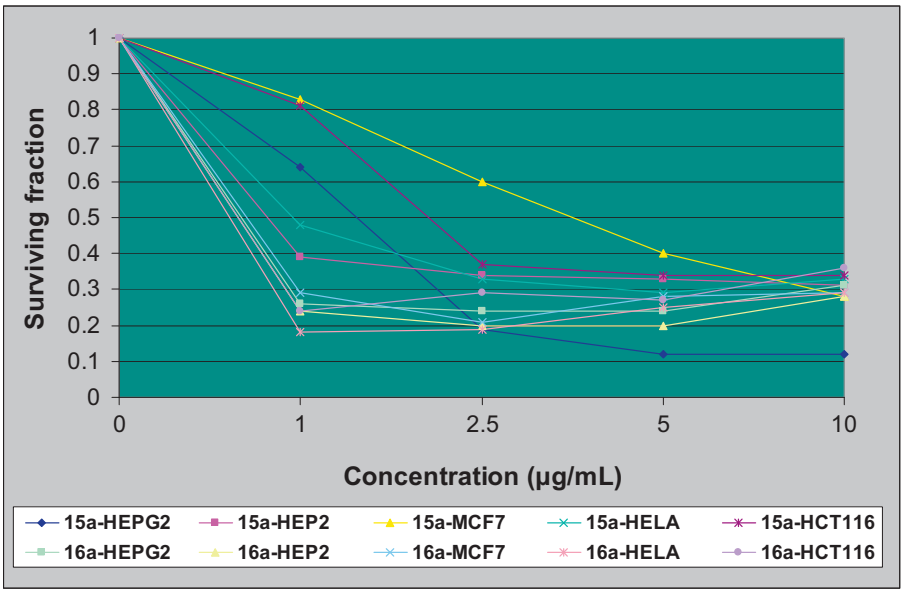


Fig. 3. Cytotoxicity of compounds **15a** and **16a** against human hepatocellular carcinoma cell line HEPG2, human laryngeal carcinoma cell line HEP2, human breast adenocarcinoma cell line MCF7, human epithelial carcinoma cell line HELA, and colon carcinoma cell line HCT116.

Table 3
IC_{50} values of compounds **15a** and **16a** obtained against human hepatocellular carcinoma cell line (HEPG2), human laryngeal carcinoma cell line (HEP2), human breast adenocarcinoma cell line (MCF7), human epithelial carcinoma cell line (HELA), and colon carcinoma cell line (HCT116).

Sample no.	IC_{50} (μg/mL) Cell line				
	HEPG2	HEP2	MCF7	HELA	HCT116
15a	1.28	0.67	3.83	0.82	1.61
16a	3.25	3.28	3.58	2.99	3.12

antimicrobial activity against Gram positive and Gram negative bacteria, and fungi.

2.2.4.1. Antibacterial activities of compounds 6a,e–g, 10a–c, 13b, 15a–d, and 17–20 against Gram positive and Gram negative bacteria. The following selected heterocycles **6a**,**e**–**g**, **10a**–**c**, **13b**, **15a**–**d**, and **17**–**20** were tested in vitro for antibacterial activity against the following bacterial strains: Gram-positive bacteria, *B. subtilis* NCTC-1040, *S. aureus* NCTC-7447, and Gram-negative bacteria, *E. coli* NCTC-10416, *S. typhi NCIMB*-9331, and *Sarcina lutea* ATCC-9341, and the results are summarized in Table 4 and represented graphically in Fig. 5. All compounds were dissolved in *N*,*N*-dimethylformamide at a concentration of 5 mg/mL and tested for antimicrobial activity by the agar disk diffusion method [75] using a 1 cm microplate well diameter and a 100 μL of each concentration. The antibacterial agent Chloramphenicol was used as a positive standard at a concentration of 50 μg/mL in *N*,*N*-dimethylformamide and was tested under the same conditions. In the disk diffusion technique for susceptibility testing, compound-impregnated disks are placed on an agar plate containing a standard suspension of the desired microorganism. The heterocycle being tested then diffuses from the disk in a concentration gradient out into the agar. The plate is incubated for 24 h at 35 °C and visual bacterial growth is observed only in areas in which the drug concentrations are below those required for growth inhibition. The diameters of the zones of inhibition are measured with calipers or automated scanners and are compared with standard zone size ranges that determine susceptibility, intermediate susceptibility, or resistance to the screened compounds.

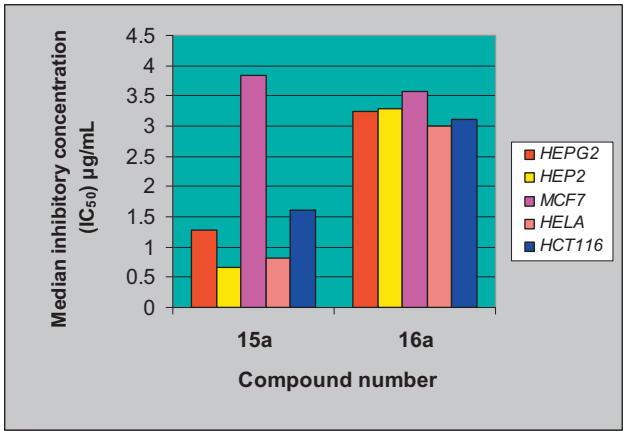


Fig. 4. Median inhibitory concentration (IC_{50}) values obtained for compounds **15a** and **16a** against human hepatocellular carcinoma cell line HEPG2, human laryngeal carcinoma cell line HEP2, human breast adenocarcinoma cell line MCF7, human epithelial carcinoma cell line HELA, and colon carcinoma cell line HCT116.

The mean values of inhibition zone diameter summarized in Table 4 and plotted in Fig. 5 show that all fluorinated imidazolidineiminothiones tested in the antimicrobial assays possess significant antibacterial activities against the growth of *B. subtilis*, *S. aureus*, *E. coli*, *S. typhi*, and *S. lutea* on solid media, with the exception of **19**. Whereas compound **6a** (*N*-(1): p-fluorophenyl; *N*-(3): p-tolyl) exhibited the highest antibacterial activity against Gram positive bacteria in the series (23.5 and 23.0 mm against *B. subtilis*, and *S. aureus*, respectively), bis-fluoroimidazolidineiminothione **19** (*N*-(1): p-fluorophenyl) was the least active (11.0 and 0.00 mm against *B. subtilis* and *S. aureus*, respectively). The dominant effect of the p-fluorophenyl and the key role of the *N*-(1) and *N*-(3) substituents is clearly seen in series **6a**,**e**–**g** and **10a**–**c**. It is noteworthy that a much higher antibacterial activity was realized in the former series with p-fluorophenyl at the *N*-(1) position (23.5–18.0 mm of zone inhibition diameter values) than that in which the p-fluorophenyl is located in the *N*-(3) position as in the latter series (14.0–18.0 mm of zone inhibition diameter values). Consistent with these results are those obtained with series **15a**–**d** in which the *N*-(3) substituent is fluorinated. In this

Table 4
Antibacterial and antifungal activity of compounds **6a**,**e**–**g**, **10a**–**c**, **13b**, **15a**–**d**, and **17**–**20** against Gram positive and Gram negative bacteria, and fungi.

Compound no.	Mean values of inhibition zone diameter (mm) Test organism							
	Gram positive bacteria		Gram negative bacteria			Fungus		
	B. subtilis	*S. aureus*	*E. coli*	*S. typhi*	*S. lutea*	*C. albicans*	*A. niger*	*A. flavus*
6a	23.5	23.0	26.0	–	25.0	19.5	–	16.0
6e	20.5	20.0	16.5	–	16.0	23.0	_	15.0
6f	22.0	21.0	23.0	–	22.0	17.0	–	14.0
6g	19.0	18.0	24.0	–	22.0	23.0	–	16.0
10a	16.0	14.0	21.0	19.0	–	13.0	12.0	–
10b	16.0	16.0	16.5	19.0	–	13.5	13.0	–
10c	18.0	18.0	16.0	14.0	–	19.0	14.0	–
13b	20.0	18.5	16.5	17.0	–	19.0	30.0	–
15a	16.0	15.0	19.5	–	17.0	16.5	–	14.0
15b	20.0	18.0	15.5	15.0	–	17.0	17.0	–
15c	17.0	17.5	16.0	15.0	–	17.0	16.5	–
15d	16.0	17.5	17.0	16.5	–	14.5	0.00	–
17	22.0	22.5	20.5	–	19.0	21.0	–	16.0
18	18.5	17.5	15.0	–	14.0	17.0	–	15.0
19	11.0	0.00	15.0	15.0	–	0.00	0.00	–
20	17.0	16.0	16.0	–	15.0	16.5	–	14.0
ST	24.0	20.0	24.0	20.0	21.0	23.0	21.0	19.0

–: Test was not performed; **ST**: Chloramphenicol and Griseofulvin were used as standard antibacterial and antifungal agents, respectively.

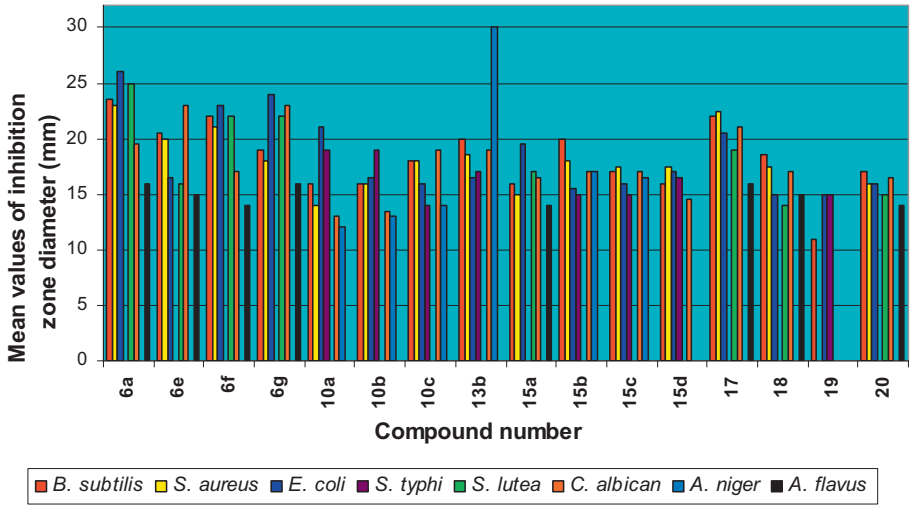


Fig. 5. Antibacterial and antifungal activity of compounds **6a**,**e**–**g**, **10a**–**c**, **13b**, **15a**–**d**, and **17**–**20**.

case, the zone inhibition diameter values ranged from 17.5 to 20.0 mm and are clearly inferior to those obtained from series **6a**,**e**–**g**. While **6a** proved again the most active antibacterial agent against Gram negative bacteria (26.0 and 25.0 mm against *E. coli* and *S. lutea*, respectively), several heterocycles achieved the same lower level of activity which averaged between 14.0 and 15.0 mm of inhibition zone diameter values. As with Gram positive bacteria, it appears in the case of Gram negative bacteria and where comparison can be made, generally higher antibacterial activity is seen with the p-fluorophenyl being located at the *N*-(1) position (as deduced from comparing series **6a**,**e**–**g** with series **10a**–**c**, and **15a**–**d**). Finally, the rigid fluorinated bis-imidazolidineiminothione **17** manifested higher activity than its increasingly flexible analogs **18**–**20**. Generally, the screened compounds were effective against Gram-positive and Gram-negative bacteria, displaying negligible disparity in antibacterial activity for individual heterocycles across all strains of tested microorganisms. However, a clear difference in antibacterial activity can be observed between compounds which is a direct reflection of the reinforcing or opposing effects induced by *N*-(1) and *N*-(3) substituents. The antibacterial positive standard Chloramphenicol achieved similar levels of inhibition at a much lower concentration, thus underscoring its superiority as an antibacterial drug.

2.2.4.2. Antifungal properties. There is a constant search for newer antifungal agents as a result of the emergence of resistance among fungi against common known antifungal agents. Usually, new antifungal agents are evaluated against clinical isolates of standard strains of fungi by the broth dilution and disc diffusion methods, whereby the former follows the standard method of NCCLS M38A [76,77]. The emergence of resistance among *Aspergillus* has been noted as a major problem and is of great concern. For instance, invasive aspergillosis, a mycotic infection caused mainly by *Aspergillus flavus*, has emerged worldwide as a cause of infection among immunocompromised patients. Consequently, we chose *A. flavus* and *Aspergillus niger* as filamentous fungi, and *Candida albicans* as a unicellular fungus in the antifungal susceptibility tests. Even though novel broad spectrum antifungal agents such as triazoles and related analogs thereof were reported in recent years, the simultaneous emergence of resistance has become an issue. Thus, new classes of such agents are needed. As such, fluorinated imidazolidineiminothiones may

comprise a new class of antifungal agents. Thus, compounds **6a**,**e**–**g**, **10a**–**c**, **13b**, **15a**–**d**, and **17**–**20** were all tested under the same conditions for antifungal activity using the preceding filamentous and unicellular fungi.

2.2.4.2.1. Antifungal activities of compounds 6a,e–g, 10a–c, 13b, 15a–d, and 17–20 against C. albicans, A. niger, and A. flavus. Heterocycles **6a**,**e**–**g**, **10a**–**c**, **13b**, **15a**–**d**, and **17**–**20** were subjected to in vitro testing of antifungal activity against the following three fungal strains: *C. albicans*, *A. niger*, and *A. flavus*. The results are displayed in Table 4 and represented graphically in Fig. 5. The compounds were tested for antifungal activity by the agar disk diffusion method using a 1 cm microplate well diameter and a 100 μL of each concentration [75]. The antifungal agent Griseofulvin was used as a positive standard at a concentration of 50 μg/mL in *N*,*N*-dimethylformamide and was tested under the same conditions. All compounds were dissolved in *N*,*N*-dimethylformamide (5 mg/mL) which itself was shown to exhibit no activity in the above assay. Most of the tested compounds were found to possess strong antifungal activity against the three fungi where the mean value of the inhibition zone diameter fluctuated from as high as 30 mm (**13b**: *N*-(1): 3-fluorophenyl; *N*-(3): 4-fluorophenyl) to as low as 0.00 mm (**15d**: *N*-(1): 4-iodophenyl; *N*-(3): 4-chloro-3-trifluoromethylphenyl, and **19**: *N*-(3): 4-fluorophenyl). In general, most compounds were very effective antifungal agents, although displaying significant variation in antifungal activity for individual heterocycles across the three strains of tested fungi. As expected, a clear difference in antifungal activity is noted between compounds which points to the effect of the *N*-(1) and *N*-(3) groups, and to the dominant effect of the 3-fluorophenyl and 4-fluorophenyl functions in the case of **13b**. In general, almost all tested compounds proved effective antifungal agents, producing high inhibition zone diameter values. Thus, these compounds comprise a new class of promising and potential broad-spectrum antifungal agents. The antifungal positive standard Griseofulvin achieved high levels of inhibition that ranged from 19 to 23 mm at a much lower concentration, thus underscoring its effectiveness as an antifungal drug.

2.2.5. Antiviral properties

Antiviral agents are a class of medication that specifically target viral infections. Unlike antibiotics for bacteria which may exhibit broad spectrum activity, very often specific antiviral drugs are used for specific viruses. Unlike most antibiotics which destroy their target pathogen, antivirals inhibit their replication and development. Most commercial antivirals are designed to treat influenza A and B viruses, HIV, herpes viruses, and the hepatitis B and C viruses. Almost all antivirals are subject to drug resistance as microorganisms mutate over time, becoming less susceptible or even completely immune. Developing safe and effective antiviral drugs is challenging as viruses utilize the host's cells to replicate, thus introducing a risk of harming the host cells. At the moment, there is a need to develop new agents and extend the range of antivirals to other families of pathogens.

2.2.5.1. Antiviral activities of compounds 15a,b,d against HAV, HSV1, and COxB4. Heterocycles **15a**,**b**,**d** 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. Initially, the maximum non-toxic concentration (MNTC) of compounds **15a**,**b**,**d** on the uninfected African green monkey kidney (Vero) host cells was determined using four 10-fold serial dilutions of each compound starting from 10^{-1} until 10^{-4} dilution of the initial sample concentration of 5 mg/mL (DMSO) [78]. Cytotoxicity was determined by microscopic examination of cell morphology, where cells were monitored for any physical signs of toxicity including partial or complete loss of the monolayer, rounding, shrinkage, cell granulation, or lysis for up to three days. The MNTC data is shown in Table 5 and the appropriate non-toxic concentration dilution for each compound (dilution number: **15a**: 10^{-4}; **15b**: 10^{-2}; **15c**: 10^{-4}) was selected for further anti-infectivity studies against HAV, HSV1 and COxB4 viral strain. Viral infectivity assay was carried out using the plaque formation method [79,80] which has proved superior to the quantal assay. In the latter, the concentration of a viral suspension is titrated by determining the highest dilution of the suspension which produces degeneration in 50% of the inoculated cell culture. This dilution is the 50% tissue culture infective dose ($TCID_{50}$). On the other hand, in the plaque formation method, a plaque is a localized focus of virus-infected cells which under optimal conditions originates from a single infectious virus particle. Enumeration of these foci for serial dilution of virus suspension is a highly quantitative method for assay of viral infectivity. Consequently, plaque assay is more precise than the $TCID_{50}$, which justified our choice of such a technique. Under these conditions, reduction in virus plaque counts provides a very sensitive mean for measuring antiviral activity of potential antivirals. The results of the plaque reduction assay are summarized in Table 6. While fluorinated imidazolidineiminothione **15a** (*N*-(1): 4-methoxyphenyl; *N*-(3): 4-chloro-3-trifluoromethylphenyl) was completely inactive against all three viral strains, its ethoxy analog **15b** (*N*-(1): 4-ethoxyphenyl; *N*-(3): 4-chloro-3-trifluoromethylphenyl) was effective, reducing virus plaque count of HSV1 and COxB4 by 50% and 60%, respectively. This sharp difference in activity is a testament to the effect exerted by manipulating the *N*-(1) and *N*-(3) substituents and how subtle differences in substitution pattern may have a marked effect on activity. Finally, the iodo analog **15d** (*N*-(1): 4-iodophenyl; *N*-(3): 4-chloro-3-trifluoromethylphenyl) showed significant activity against HSV1, reducing the virus plaque count by 50%. It is noted

Table 5
Cytotoxicity of compounds **15a**,**b**,**d** on Vero cells.

Sample no.	Days	**15a**	**15b**	**15d**
Dilution		Cytotoxicity on Vero cell		
−1	1st	+4[b]	+4	+4
−1	2nd	+4	+4	+4
−1	3rd	+4	+4	+4
−2	1st	+4	–[a]	+4
−2	2nd	+4	–	+4
−2	3rd	+4	–	+4
−3	1st	+4	–	+4
−3	2nd	+4	–	+4
−3	3rd	+4	–	+4
−4	1st	–	–	–
−4	2nd	–	–	–
−4	3rd	–	–	–

[a] Sample dilution at which no cytotoxicity on Vero cells was detected.
[b] Sample dilution at which cytotoxicity on Vero cells was significant; +1 partial or complete loss of cell monolayer; +2 rounding; +3 shrinkage; +4 cell granulation or lysis.

Table 6
Anti-infectivity effect of compounds **15a**,**b**,**d** against HAV, HSV1, and COxB4 viral strains.

Sample no.	Selected dilution[a]	Anti-infectivity effect		
		Viral strain		
		HAV	HSV1	CoxB4
15a	10^{-4}	−ve[b]	−ve	−ve
15b	10^{-2}	−ve	50%	60%
15d	10^{-4}	−ve	50%	−ve

[a] Sample dilution at which no cytotoxicity on Vero cells was detected for 3 days.
[b] No anti-infectivity effect was observed.

that **15b** showed negative anti-infectivity effect toward HAV, and **15d** was inactive toward HAV and COxB4, pointing to the precedent specificity of antivirals.

3. Conclusions

In summary, the synthesis and characterization of a new series of novel imidazolidineiminothiones, 5-thioxoimidazolidine-2,4-diones, and bis-imidazolidineiminothiones with variable fluorinated aromatic substituents at *N*-(1) and *N*-(3) has been described. The products were obtained in 75–85% yields. Most compounds displayed antitumor, antibacterial, antifungal, and antiviral activities. Screening of several compounds against EAC cells indicated that imidazolidineiminothiones **6f** (*N*-(1): 4-fluorophenyl; *N*-(3): 3,5-dichlorophenyl) and **16a** (*N*-(1): trifluoromethylphenyl; *N*-(3): 4-fluorophenyl) were the most active, displaying the highest % inhibition of cell viability (90% and 80%, respectively). Subsequent evaluation of selected compounds for cytotoxic activity against HEPG2, HEP2, MCF7, HELA, and HCT116 tumor cell lines was conducted. The IC_{50} values ranged from 0.67 to 3.83 μg/mL where compound **15a** (*N*-(1): 4-methoxyphenyl; *N*-(3): 4-chloro-3-trifluoromethylphenyl) and **16a** (*N*-(1): trifluoromethylphenyl; *N*-(3): 4-fluorophenyl) were highly active against all cell lines, consistently producing low IC_{50} values in all cases. This underlines the matching effect of the suitably positioned fluorinated substituents on *N*-(1) and *N*-(3) of the imidazolidineiminothiones. Heterocyles **6a**,**e**–**g**, **10a**–**c**, **13b**, **15a**–**d**, and **17**–**20** were tested against microbial organisms (*B. subtilis*, *S. aureus*, *E. coli*, *S. typhi*, and *S. lutea*), and fungal strains (*C. albicans, A. niger,* and *A. flavus*). Whereas **6a** (*N*-(1): 4-fluorophenyl; *N*-(3): p-tolyl) was the most active against Gram positive and Gram negative bacteria, **13b** (*N*-(1): 3-fluorophenyl; *N*-(3): 4-fluorophenyl) showed the strongest antifungal activity against all fungal strains reaching as high as 30 mm. Finally, the antiviral effect of **15a**,**b**,**d** was investigated against hepatitis A virus (HAV), human herpes simplex virus 1 (HSV1) and Coxsackie B4 (COxB4) viral strain, where **15b** (*N*-(1): 4-ethoxyphenyl; *N*-(3): 4-chloro-3-trifluoromethylphenyl) proved most active, reducing virus plaque count of HSV1 and COxB4 by 50% and 60%, respectively. 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.

4. Experimental

4.1. Chemistry

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 d1-TFA/$CDCl_3$ (1/1), $CDCl_3$, or DMSO-d6 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. Mass spectra were recorded on Shimadzu GC–MS QP 100 EX (70 eV) at the Micro Analytical Center at Cairo University. Found: C, H, N and S for all compounds were within ±0.4 from the theoretical value. Melting points were obtained on a Fisher-Johns melting points apparatus and are uncorrected. The physical data of the synthesized compounds are shown in Table 7 (also see Supplementary material section).

4.1.1. For the preparation of the known cyanothioformanilides 8a–g, see Ref. [64–69]. Also see Ref. [44] and references therein

The physical and spectral data of **8b**–**e**,**g** were in accord to those reported [44]. **8a**,**e** are commercially available.

4.1.2. Typical procedure for the preparation of imidazolidineiminothiones 6a–g, 10a–h, 13a,b, 15a–d, and 16a

A solution of the cyanothioformanilide (5 mmol) and the corresponding isocyanate (5 mmol) in dry ether (30 mL) was treated with three drops of triethylamine. The reaction mixture was magnetically stirred for 15 min. The obtained product was filtered off, washed with ether, air-dried, and recrystallized from chloroform/n-hexane. The corresponding imidazolidineiminothiones **6a**–**g**, **10a**–**h**, **13a**,**b**, **15a**–**d**, and **16a** (Table 7, also see Supplementary material section) were obtained as yellow or orange solids in yields ranging from 75% to 85%.

4.1.3. Typical procedure for the preparation of diones 7a–d, 11a–e, 14a,b, and 16b

The requisite imidazolidineiminothiones (5 mmol) were dissolved in boiling ethanol (20 mL) and treated with dil. HCl (1:1 molar ratio). The obtained products were filtered off, washed with cold water, air-dried, and recrystallized from chloroform/n-hexane to give the corresponding diones **7a**–**d**, **11a**–**e**, **14a**,**b**, and **16b** as yellow or orange solids (Table 7, also see Supplementary material section) in yields ranging from 75% to 85%.

4.1.4. Typical procedure for the preparation of bis-imidazolidineiminothiones 17–20

To a solution of p-fluorocyanothioformanilide (**4**) (10 mmol, 2.0 equiv.) in ether (20 mL), a solution of the corresponding bis-isocyanate (5 mmol, 1.0 equiv.) in ether (10 mL) was added, followed by 3 drops of triethylamine. The reaction mixture was stirred for 25 min. The obtained product was filtered off, washed with a minimum amount of ether, air-dried, and recrystallized from chloroform/n-hexane. The corresponding bis-imidazolidineiminothiones **17**–**20** (Table 7, also see Supplementary material section) were obtained as yellow or orange solids in yields ranging from 75% to 85%.

4.1.5. 1-(4-Fluorophenyl)-4-imino-5-thioxo-3-p-tolylimidazolidin-2-one (6a)

Mp 178–180 °C; IR (KBr): ν 3433 (NH), 1778 (C=O), 1667 (C=N), 1119 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.55 (s, 1H, NH), 7.50–7.44 (m, 2H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.27–7.20 (m, 2H), 2.41 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.6 (C=S), 162.7 (d, *J* = 251.0 Hz, C–F), 154.2 (C=O), 153.7 (C=N), 138.9 (C), 130.0 (CH), 129.2 (C–N), 129.1 (d, *J* = 8.9 Hz, CH), 128.6 (d, *J* = 3.0 Hz, C–N), 126.3 (CH), 116.6 (d, *J* = 22.8 Hz, CH), 21.2 (CH_3); ^{19}F ($CDCl_3$, 376.5 MHz) δ −110.2; MS (*m*/*z*, %) 313 (50, M^+), 153 (100, p-F·C_6H_4·NCS), 133 (63).

4.1.6. 1-(4-Fluorophenyl)-4-imino-3-(4-methoxyphenyl)-5-thioxoimidazolidin-2-one (6b)

Mp 153–155 °C; IR (KBr): ν 3439 (NH), 1769 (C=O), 1665 (C=N), 1256 (C–O), 1179 (C–O), 1117 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.53 (s, 1H, NH), 7.49–7.42 (m, 4H), 7.26–7.20 (m, 2H), 7.03 (d, *J* = 9.0 Hz, 2H), 3.85 (s, 3H, OMe); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.6 (C=S), 162.7 (d, *J* = 250.7 Hz, C–F), 159.6 (C–O), 154.4 (C=O), 153.8 (C=N), 129.1 (d, *J* = 8.9 Hz, CH), 128.6 (d, *J* = 3.7 Hz, C–N), 127.8 (CH), 124.3 (C–N), 116.9 (d, *J* = 22.8 Hz, CH), 114.7 (CH), 55.3 (CH_3); ^{19}F ($CDCl_3$, 376.5 MHz) δ −110.2; MS (*m*/*z*, %) 329 (29, M^+), 153 (66, p-F·C_6H_4·NCS), 149 (100, p-MeO·C_6H_4·NCO).

4.1.7. 1-(4-Fluorophenyl)-4-imino-3-(4-(methylthio)phenyl)-5-thioxoimidazolidin-2-one (6c)

Mp 174–178 °C; IR (KBr): ν 3455 (NH), 1778 (C=O), 1661 (C=N), 1090 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.57 (s, 1H, NH), 7.51–7.42 (m, 4H), 7.37 (d, *J* = 8.7 Hz, 2H), 7.27–7.20 (m, 2H), 2.51 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.4 (C=S), 162.7 (d,

Table 7
Physical data of compounds **6a–g**, **7a–d**, **10a–h**, **11a–e**, **13a,b**, **14a,b**, **15a–d**, **16a,b**, and **17–20**.

Compound no.[a,b]	Mol. formula M.Wt.	Elemental analysis Calcd./found [%]			
		C	H	N	S
6a	$C_{16}H_{12}FN_3OS$	61.33	3.86	13.41	10.23
	(313.35)	61.50	3.80	13.30	10.10
6b	$C_{16}H_{12}FN_3O_2S$	58.35	3.67	12.76	9.74
	(329.35)	58.50	3.60	12.60	9.50
6c	$C_{16}H_{12}FN_3OS_2$	55.63	3.50	12.17	18.57
	(345.41)	55.70	3.50	12.00	18.30
6d	$C_{16}H_{12}FN_3OS$	61.33	3.86	13.41	10.23
	(313.35)	61.50	3.80	13.30	10.00
6e	$C_{15}H_9ClFN_3OS$	53.98	2.72	12.59	9.61
	(333.01)	54.00	2.70	12.70	9.50
6f	$C_{15}H_8Cl_2FN_3OS$	48.93	2.19	11.41	8.71
	(368.21)	49.00	2.20	11.30	8.60
6g	$C_{15}H_7\ Cl_3FN_3OS$	44.74	1.75	10.44	7.96
	(402.66)	44.90	1.70	10.30	7.80
7a	$C_{16}H_{11}FN_2O_2S$	61.14	3.53	8.91	10.20
	(314.33)	61.00	3.50	8.70	10.00
7b	$C_{16}H_{11}FN_2O_3S$	58.17	3.36	8.48	9.71
	(330.33)	58.00	3.30	8.30	9.60
7c	$C_{16}H_{11}FN_2O_2S_2$	55.48	3.20	8.09	18.51
	(346.40)	55.30	3.30	8.00	18.30
7d	$C_{16}H_{11}FN_2O_2S$	61.14	3.53	8.91	10.20
	(314.33)	61.00	3.50	8.70	10.10
10a	$C_{15}H_{10}FN_3OS$	60.19	3.37	14.04	10.71
	(299.32)	60.00	3.30	14.00	10.50
10b	$C_{16}H_{12}FN_3OS$	61.33	3.86	13.41	10.23
	(313.35)	61.50	3.80	13.30	10.10
10c	$C_{16}H_{12}FN_3O_2S$	58.35	3.67	12.76	9.74
	(329.35)	58.50	3.60	12.50	9.50
10d	$C_{17}H_{14}FN_3O_2S$	59.46	4.11	12.24	9.34
	(343.38)	49.50	4.10	12.10	9.10
10e	$C_{15}H_9\ ClFN_3OS$	53.98	2.72	12.59	9.61
	(333.77)	54.00	2.70	12.40	9.50
10f	$C_{17}H_{14}FN_3O_3S$	56.82	3.93	11.69	8.92
	(359.374)	57.00	3.90	11.50	8.90
10g	$C_{15}H_9\ BrFN_3OS$	47.63	2.40	11.11	8.48
	(378.22)	47.70	2.40	11.10	8.40
10h	$C_{15}H_{10}FN_3OS$	60.19	3.37	14.04	10.71
	(299.05)	60.00	3.30	14.10	10.50
11a	$C_{15}H_9FN_2O_2S$	59.99	3.02	9.33	10.68
	(300.31)	60.00	3.00	9.10	10.50
11b	$C_{16}H_{11}FN_2O_2S$	61.14	3.53	8.91	12.20
	(314.33)	61.30	3.50	8.80	10.00
11c	$C_{16}H_{11}FN_2O_3S$	58.17	3.36	8.48	9.71
	(330.33)	58.30	3.30	8.40	9.50
11d	$C_{17}H_{13}FN_2O_3S$	59.29	3.81	8.13	9.31
	(344.36)	59.40	3.80	8.00	9.20
11e	$C_{15}H_8ClFN_2O_2S$	53.82	2.41	8.37	9.58
	(334.75)	53.90	2.40	8.10	9.30
13a	$C_{15}H_9F_2N_3OS$	56.78	2.86	13.24	10.11
	(317.31)	56.90	2.80	13.00	10.00
13b	$C_{15}H_9F_2N_3OS$	56.78	2.86	13.24	10.11
	(317.31)	56.80	2.80	13.10	10.10
14a	$C_{15}H_8F_2N_2O_2S$	56.60	2.53	8.80	10.07
	(318.30)	56.70	2.50	8.70	10.10
14b	$C_{15}H_8F_2N_2O_2S$	56.60	2.53	8.80	10.07
	(318.30)	56.80	2.60	8.60	10.10
15a	$C_{17}H_{11}ClFN_3O_2S$	49.34	2.68	10.15	7.75
	(413.80)	49.50	2.70	10.00	7.50
15b	$C_{18}H_{13}ClF_3N_3O_2S$	50.53	3.06	9.82	7.49
	(427.83)	50.50	3.00	9.70	7.40
15c	$C_{16}H_8ClF_4N_3OS$	47.83	2.01	10.46	7.98
	(401.77)	48.00	2.00	10.30	7.90
15d	$C_{16}H_8ClF_3IN_3OS$	37.70	1.58	8.24	6.29
	(508.67)	37.90	1.50	8.10	6.30
16a	$C_{16}H_9F_4N_3OS$	52.32	2.47	11.44	8.73
	(367.32)	52.50	2.40	11.30	8.50
16b	$C_{16}H_8F_4N_2O_2S$	52.18	2.19	7.61	8.71
	(368.31)	52.30	2.20	7.50	8.80
17	$C_{32}H_{22}F_2N_6O_4S_2$	58.53	3.38	12.80	9.77
	(656.68)	58.70	3.30	12.70	9.80
18	$C_{30}H_{18}F_2N_6O_3S_2$	58.82	2.96	13.72	10.47
	(612.63)	59.00	3.00	13.50	10.30
19	$C_{28}H_{28}F_2N_6O_2S_2$	57.72	4.84	14.42	11.01
	(582.69)	57.90	4.80	14.30	11.10
20	$C_{24}H_{22}F_2N_6O_2S_2$	54.53	4.20	15.90	12.13
	(528.60)	54.70	4.20	15.80	12.00

[a] All products were crystallized from chloroform/n-hexane.
[b] The yields of all these products ranged between 75% and 85%.

J = 250.1 Hz, C–F), 154.0 (C=O), 153.6 (C=N), 139.8 (C–S), 129.1 (d, J = 8.8 Hz, CH), 128.6 (C–N), 128.5 (d, J = 3.0 Hz, C–N), 126.9 (CH), 126.7 (CH), 116.6 (d, J = 23.5 Hz, CH), 15.6 (CH_3); ^{19}F ($CDCl_3$, 376.5 MHz) δ −110.1; MS (m/z, %) 345 (66, M^+), 153 (50, p-F·C_6H_4·NCS).

*4.1.8. 1-Benzyl-3-(4-fluorophenyl)-5-imino-4-thioxoimidazolidin-2-one (**6d**)*

Mp 123–125 °C; IR (KBr): ν 3430 (NH), 1775 (C=O), 1674 (C=N), 1067 (C=S) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.39 (s, 1H, NH), 7.54–7.48 (m, 2H), 7.41–7.27 (m, 5H), 7.23–7.15 (m, 2H), 4.99 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.9 (C=S), 162.6 (d, J = 250.6 Hz, C–F), 154.6 (C=O), 153.5 (C=N), 135.2 (C), 129.1 (CH), 129.0 (d, J = 9.6 Hz, CH), 128.8 (CH), 128.5 (d, J = 3.5 Hz, C–N), 128.3 (CH), 116.5 (d, J = 23.0 Hz, CH), 44.4 (CH_2); ^{19}F ($CDCl_3$, 376.5 MHz) δ −110.4; MS (m/z, %) 313 (23, M^+), 153 (16, p-F·C_6H_4·NCS), 91 (100, $C_6H_5{\cdot}CH_2^+$).

*4.1.9. 1-(4-Chlorophenyl)-3-(4-fluorophenyl)-5-imino-4-thioxoimidazolidin-2-one (**6e**)*

Mp 189 °C; IR (KBr): ν 3432 (NH), 1773 (C=O), 1676 (C=N), 1065 (C=S) cm^{-1}; ^1H NMR (DMSO, 400 MHz) δ 9.77 (s, 1H, NH), 7.66–7.58 (m, 6H), 7.44 (t, J = 8.8 Hz, 2H); ^{13}C NMR (DMSO, 100 MHz) δ 182.9 (C=S), 162.1 (d, J = 246.9 Hz, C–F), 153.8 (C=O), 153.5 (C=N), 132.5 (C), 131.4 (C), 130.1 (d, J = 9.0 Hz, CH), 129.5 (d, J = 2.9 Hz, C), 129.0 (CH), 128.8 (CH), 116.4 (d, J = 23.4 Hz, CH); MS (m/z, %) 333 (20, M^+), 153 (100, p-F·C_6H_4·NCS).

*4.1.10. 1-(3,5-Dichlorophenyl)-3-(4-fluorophenyl)-5-imino-4-thioxoimidazolidin-2-one (**6f**)*

Mp 198–200 °C; IR (KBr): ν 3431 (NH), 1776 (C=O), 1675 (C=N), 1069 (C=S) cm^{-1}; ^1H NMR (d1-TFA/$CDCl_3$; 1/1, 400 MHz) δ 7.71 (t, J = 1.8 Hz, 1H), 7.49 (d, J = 1.8 Hz, 2H), 7.48–7.43 (m, 2H), 7.36–7.25 (m, 2H); ^{13}C NMR (d1-TFA/$CDCl_3$; 1/1, 100 MHz) δ 176.4 (C=S), 164.3 (d, J = 253.4 Hz, C–F), 152.0 (C=O), 150.3 (C=N), 138.0 (C–Cl), 133.2 (CH), 129.2 (C–N), 129.1 (d, J = 9.6 Hz, CH), 126.8 (d, J = 3.2 Hz, C–N), 125.7 (CH), 117.7 (d, J = 23.5 Hz, CH); ^{19}F (DMSO, 376.5 MHz) δ −108.5; MS (m/z, %) 367 (24, M^+), 231 (11), 195 (26), 153 (100, p-F·C_6H_4·NCS), 95 (56).

*4.1.11. 1-(4-Fluorophenyl)-4-imino-5-thioxo-3-(2,4,5-trichlorophenyl)imidazolidin-2-one (**6g**)*

Mp 205–207 °C; IR (KBr): ν 3429 (NH), 1777 (C=O), 1674 (C=N), 1069 (C=S) cm^{-1}; ^1H NMR (DMSO, 400 MHz) δ 10.02 (s, 1H, NH), 8.22 (s, 1H), 8.07 (s, 1H), 7.62 (dd, J = 9.2, 4.8 Hz, 2H), 7.46 (t, J = 8.8 Hz, 2H); ^{13}C NMR (DMSO, 100 MHz) δ 182.4 (C=S), 162.2 (d, J = 247.1 Hz, C–F), 152.7 (C=O), 152.4 (C=N), 133.6 (C), 132.3 (C), 132.2 (CH), 131.5 (CH), 130.5 (C), 130.1 (d, J = 9.5 Hz, CH), 129.1 (d, J = 2.9 Hz, C–N), 116.5 (d, J = 23.4 Hz, CH).

*4.1.12. 1-(4-Fluorophenyl)-5-thioxo-3-p-tolylimidazolidine-2,4-dione (**7a**)*

Mp 134–135 °C; IR (KBr): ν 1748 (C=O), 1096 (C=S) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.47–7.41 (m, 2H), 7.39–7.30 (m, 4H),

7.27–7.20 (m, 2H), 2.42 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.4 (C=S), 162.8 (d, J = 250.6 Hz, C–F), 153.2 (C=O), 152.8 (C=O), 139.4 (C), 130.1 (CH), 129.2 (d, J = 8.8 Hz, CH), 127.9 (d, J = 3.1 Hz, C–N), 127.6 (C–N), 125.6 (CH), 116.7 (d, J = 23.4 Hz, CH), 21.2 (CH_3); ^{19}F ($CDCl_3$, 376.5 MHz) δ −109.9.

4.1.13. 1-(4-Fluorophenyl)-3-(4-methoxyphenyl)-5-thioxoimidazolidine-2,4-dione (7b)

Mp 194–196 °C; IR (KBr): ν 1746 (C=O), 1254 (C–O), 1163 (C–O), 1115 (C=S) cm^{-1}; ^{1}H NMR (DMSO, 400 MHz) δ 7.61–7.54 (m, 2H), 7.47–7.39 (m, 4H), 7.10 (d, J = 9.0 Hz, 2H), 3.81 (s, 3H, OMe); ^{13}C NMR (DMSO, 100 MHz) δ 185.2 (C=S), 162.3 (d, J = 247.2 Hz, C–F), 159.6 (C–O), 154.2 (C=O), 153.8 (C=O), 130.3 (d, J = 8.8 Hz, CH), 129.3 (d, J = 3.0 Hz, C–N), 128.4 (CH), 123.5 (C–N), 116.6 (d, J = 23.2 Hz, CH), 114.7 (CH), 55.7 (CH_3); ^{19}F (DMSO, 376.5 MHz) δ −112.2.

4.1.14. 1-(4-Fluorophenyl)-3-(4-(methylthio)phenyl)-5-thioxoimidazolidine-2,4-dione (7c)

Mp 166–168 °C; IR (KBr): ν 1749 (C=O), 1096 (C=S) cm^{-1}; ^{1}H NMR (DMSO, 400 MHz) δ 7.54–7.48 (m, 2H), 7.46–7.36 (m, 6H), 2.51 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.4 (C=S), 162.7 (d, J = 250.1 Hz, C–F), 154.0 (C=O), 153.6 (C=O), 139.8 (C–S), 129.1 (d, J = 8.8 Hz, CH), 128.6 (C–N), 128.5 (d, J = 3.0 Hz, C–N), 126.9 (CH), 126.7 (CH), 116.6 (d, J = 23.5 Hz, CH), 15.6 (CH_3); ^{19}F ($CDCl_3$, 376.5 MHz) δ −110.1; MS (m/z, %) 346 (19, M$^+$), 165 (100, p-$CH_3S{\cdot}C_6H_4{\cdot}NCO$), 153 (46, p-$F{\cdot}C_6H_4{\cdot}NCS$).

4.1.15. 3-Benzyl-1-(4-fluorophenyl)-5-thioxoimidazolidine-2,4-dione (7d)

Mp 138–140 °C; IR (KBr): ν 1736 (C=O), 1088 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.52–7.46 (m, 2H), 7.42–7.32 (m, 5H), 7.24–7.17 (m, 2H), 4.89 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.9 (C=S), 162.7 (d, J = 251.0 Hz, C–F), 154.0 (C=O), 153.4 (C=O), 134.4 (C), 129.2 (CH), 129.1 (d, J = 8.8 Hz, CH), 129.0 (CH), 128.7 (CH), 127.8 (d, J = 3.6 Hz, C–N), 116.6 (d, J = 23.5 Hz, CH), 43.9 (CH_2); ^{19}F ($CDCl_3$, 376.5 MHz) δ −110.1.

4.1.16. 1-(4-Fluorophenyl)-5-imino-3-phenyl-4-thioxoimidazolidin-2-one (10a)

Mp 185–187 °C; IR (KBr): ν 3447 (NH), 1775 (C=O), 1667 (C=N), 1126 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.57 (s, 1H, NH), 7.61–7.43 (m, 7H), 7.28–7.18 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.3 (C=S), 162.1 (d, J = 249.9 Hz, C–F), 154.1 (C=O), 153.7 (C=N), 132.7 (C–N), 129.8 (CH), 129.5 (CH), 128.4 (d, J = 8.8 Hz, CH), 127.9 (d, J = 3.0 Hz, C–N), 127.1 (CH), 116.4 (d, J = 23.5 Hz, CH); ^{19}F ($CDCl_3$, 376.5 MHz) δ −112.0; MS (m/z, %) 301 (7, M$^+$+2), 300 (22, M$^+$+1), 299 (99, M$^+$), 298 (20, M$^+$−1), 162 (37, $C_6H_5{\cdot}CNH{\cdot}NCS$), 137 (24, p-$F{\cdot}C_6H_4{\cdot}NCO$), 135 (100, $C_6H_5{\cdot}NCS$).

4.1.17. 1-(4-Fluorophenyl)-5-imino-4-thioxo-3-p-tolylimidazolidin-2-one (10b)

Mp 176–178 °C; IR (KBr): ν 3462 (NH), 1771 (C=O), 1668 (C=N), 1113 (C=S) cm^{-1}; ^{1}H NMR (DMSO, 400 MHz) δ 9.63 (s, 1H, NH), 7.64–7.57 (m, 2H), 7.41–7.33 (m, 6H), 2.37 (CH_3); ^{13}C NMR (DMSO, 100 MHz) δ 183.0 (C=S), 161.5 (d, J = 246.2 Hz, C–F), 154.4 (C=O), 154.0 (C=N), 139.4 (C), 130.9 (C–N), 129.9 (CH), 129.7 (d, J = 8.8 Hz, CH), 129.0 (d, J = 3.0 Hz, C–N), 127.6 (CH), 116.0 (d, J = 22.8 Hz, CH), 21.0 (CH_3); ^{19}F (DMSO, 376.5 MHz) δ −113.1; MS (m/z, %) 314 (16, M$^+$+1), 313 (74, M$^+$), 312 (15, M$^+$−1), 149 (94, p-$CH_3{\cdot}C_6H_4{\cdot}NCS$), 137 (24, p-$F{\cdot}C_6H_4{\cdot}NCO$), 135 (100, $C_6H_5{\cdot}NCS$), 91 (100, $C_6H_4{\cdot}CH_3^+$).

4.1.18. 1-(4-Fluorophenyl)-5-imino-3-(4-methoxyphenyl)-4-thioxoimidazolidin-2-one (10c)

Mp 168–170 °C; IR (KBr): ν 3455 (NH), 1778 (C=O), 1667 (C=N), 1254 (C–O), 1177 (C–O), 1113 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.55 (s, 1H, NH), 7.60–7.53 (m, 2H), 7.37 (d, J = 9.0 Hz, 2H), 7.24–7.17 (m, 2H), 7.05 (d, J = 9.0 Hz, 2H), 3.81 (s, 3H, OMe); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.6 (C=S), 162.1 (d, J = 248.8 Hz, C–F), 160.3 (C–O), 154.1 (C=O), 153.9 (C=N), 128.3 (d, J = 8.8 Hz, CH), 128.2 (CH), 127.9 (d, J = 3.2 Hz, C–N), 125.2 (C–N), 116.3 (d, J = 23.2 Hz, CH), 114.8 (CH), 55.5 (CH_3); ^{19}F ($CDCl_3$, 376.5 MHz) δ −112.1.

4.1.19. 1-(4-Ethoxyphenyl)-3-(4-fluorophenyl)-4-imino-5-thioxoimidazolidin-2-one (10d)

Mp 175–176 °C; IR (KBr): ν 3462 (NH), 1775 (C=O), 1665 (C=N), 1256 (C–O), 1177 (C–O), 1115 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.55 (s, 1H, NH), 7.60–7.53 (m, 2H), 7.36 (d, J = 9.1 Hz, 2H), 7.25–7.17 (m, 2H), 7.03 (d, J = 9.1 Hz, 2H), 4.09 (q, J = 7.0 Hz, 2H), 1.45 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.6 (C=S), 162.1 (d, J = 249.5 Hz, C–F), 159.7 (C–O), 154.2 (C=O), 153.9 (C=N), 128.3 (d, J = 8.8 Hz, CH), 128.2 (CH), 128.0 (d, J = 3.3 Hz, C–N), 125.0 (C–N), 116.3 (d, J = 22.7 Hz, CH), 115.2 (CH), 63.8 (CH_2), 14.7 (CH_3); ^{19}F ($CDCl_3$, 376.5 MHz) δ −112.1; MS (m/z, %) 343 (100, M$^+$), 179 (42, p-EtO-$C_6H_4{\cdot}NCS$), 151 (87), 111 (98).

4.1.20. 1-(3,4-Dimethoxyphenyl)-3-(4-fluorophenyl)-4-imino-5-thioxoimidazolidin-2-one (10e)

Mp 192–194 °C; IR (KBr): ν 3455 (NH), 1769 (C=O), 1667 (C=N), 1215 (C–O), 1165 (C–O), 1130 (C=S) cm^{-1}; ^{1}H NMR (DMSO, 400 MHz) δ 9.73 (s, 1H, NH), 7.64–7.57 (m, 2H), 7.41–7.33 (m, 3H), 6.77 (d, J = 2.5 Hz, 1H), 6.69 (dd, J = 8.8, 2.5 Hz, 1H), 3.83 (s, 3H, OMe), 3.77 (s, 3H, OMe); ^{13}C NMR (DMSO, 100 MHz) δ 182.9 (C=S), 161.3 (d, J = 245.6 Hz, C–F), 161.7 (C–O), 155.6 (C–O), 153.9 (C=O), 153.5 (C=N), 130.0 (CH), 129.4 (d, J = 8.8 Hz, CH), 128.6 (d, J = 2.9 Hz, C–N), 115.9 (d, J = 23.0 Hz, CH), 114.1 (C–N), 105.6 (CH), 99.4 (CH), 56.0 (CH_3), 55.6 (CH_3); ^{19}F (DMSO, 376.5 MHz) δ −112.9; MS (m/z, %) 359 (44, M$^+$), 191 (40), 111 (100).

4.1.21. 1-(4-Chlorophenyl)-3-(4-fluorophenyl)-4-imino-5-thioxoimidazolidin-2-one (10f)

Mp 146–148 °C; IR (KBr): ν 3430 (NH), 1771 (C=O), 1668 (C=N), 1117 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.59 (s, 1H, NH), 7.59–7.50 (m, 4H), 7.43 (d, J = 8.8 Hz, 2H), 7.26–7.18 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.0 (C=S), 162.2 (d, J = 250.2 Hz, C–F), 153.9 (C=O), 153.5 (C=N), 135.7 (C–Cl), 131.0 (C–N), 129.8 (CH), 128.4 (CH), 128.4 (d, J = 8.8 Hz, CH), 127.7 (d, J = 2.9 Hz, C–N), 116.4 (d, J = 22.7 Hz, CH); ^{19}F ($CDCl_3$, 376.5 MHz) δ −111.8; MS (m/z, %) 333 (29, M$^+$), 169 (100, p-Cl-$C_6H_4{\cdot}NCS$).

4.1.22. 1-(4-Bromophenyl)-3-(4-fluorophenyl)-4-imino-5-thioxoimidazolidin-2-one (10g)

Mp 200–202 °C; IR (KBr): ν 3460 (NH), 1773 (C=O), 1667 (C=N), 1119 (C=S) cm^{-1}; ^{1}H NMR (DMSO, 400 MHz) δ 9.71 (s, 1H, NH), 7.80 (d, J = 8.8 Hz, 2H), 7.64–7.57 (m, 2H), 7.51 (d, J = 8.8 Hz, 2H), 7.42–7.35 (m, 2H); ^{13}C NMR (DMSO, 100 MHz) δ 182.8 (C=S), 161.6 (d, J = 245.5 Hz, C–F), 154.3 (C=O), 153.7 (C=N), 132.8 (C–N), 132.6 (CH), 130.1 (CH), 129.7 (d, J = 8.8 Hz, CH), 128.9 (d, J = 2.9 Hz, C–N), 122.9 (C–Br), 116.1 (d, J = 22.7 Hz, CH); ^{19}F (DMSO, 376.5 MHz) δ −113.0; MS (m/z, %) 377 (43, M$^+$), 212 (86), 136 (99, p-$F{\cdot}C_6H_4{\cdot}NCNH$), 90 (100).

4.1.23. 1-(2-Fluorophenyl)-4-imino-3-phenyl-5-thioxoimidazolidin-2-one (10h)

Mp 130–134 °C; IR (KBr): ν 3435 (NH), 1782 (C=O), 1672 (C=N), 1096 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.51 (s, 1H, NH), 7.56–7.40 (m, 7H), 7.31–7.21 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.4 (C=S), 157.7 (d, J = 253.1 Hz, C–F), 153.5 (C=O), 153.0 (C=N), 132.6 (C), 131.3 (d, J = 8.0 Hz, CH), 129.7 (d, J = 7.4 Hz, CH), 129.4 (CH), 127.0 (CH), 124.8 (d, J = 3.6 Hz, CH), 119.4

(d, J = 13.1 Hz, C), 116.9 (d, J = 19.8 Hz, CH); ^{19}F ($CDCl_3$, 376.5 MHz) δ −118.4; MS (m/z, %) 299 (25, M^+), 135 (91), 77 (100, $C_6H_5^+$).

*4.1.24. 3-(4-Fluorophenyl)-1-phenyl-5-thioxoimidazolidine-2,4-dione (**11a**)*

Mp 174–176 °C; IR (KBr): ν 1746 (C=O), 1115 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.61–7.47 (m, 5H), 7.46–7.41 (m, 2H), 7.27–7.18 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.0 (C=S), 162.4 (d, J = 249.9 Hz, C–F), 153.1 (C=O), 152.6 (C=O), 132.0 (C–N), 129.9 (CH), 129.6 (CH), 127.7 (d, J = 8.8 Hz, CH), 127.2 (CH), 126.3 (d, J = 3.6 Hz, C–N), 116.6 (d, J = 23.4 Hz, CH); ^{19}F ($CDCl_3$, 376.5 MHz) δ −111.1.

*4.1.25. 3-(4-Fluorophenyl)-5-thioxo-1-p-tolylimidazolidine-2,4-dione (**11b**)*

Mp 165–168 °C; IR (KBr): ν 1750 (C=O), 1115 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.55–7.48 (m, 2H), 7.36 (d, J = 8.6 Hz, 2H), 7.31 (d, J = 8.6 Hz, 2H), 7.25–7.19 (m, 2H), 2.44 (CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.2 (C=S), 162.4 (d, J = 250.5 Hz, C–F), 153.2 (C=O), 152.7 (C=O), 140.3 (C), 130.3 (CH), 129.4 (C–N), 127.7 (d, J = 8.8 Hz, CH), 126.9 (CH), 126.3 (d, J = 3.7 Hz, C–N), 116.5 (d, J = 23.5 Hz, CH), 21.4 (CH_3); ^{19}F ($CDCl_3$, 376.5 MHz) δ −111.2.

*4.1.26. 3-(4-Fluorophenyl)-1-(4-methoxyphenyl)-5-thioxoimidazolidine-2,4-dione (**11c**)*

Mp 186–188 °C; IR (KBr): ν 1750 (C=O), 1254 (C–O), 1160 (C–O), 1113 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.54–7.49 (m, 2H), 7.35 (d, J = 9.0 Hz, 2H), 7.25–7.19 (m, 2H), 7.06 (d, J = 9.0 Hz, 2H), 3.87 (s, 3H, OMe); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.3 (C=S), 162.4 (d, J = 249.4 Hz, C–F), 160.4 (C–O), 153.3 (C=O), 152.7 (C=O), 128.4 (CH), 127.7 (d, J = 8.8 Hz, CH), 126.3 (d, J = 3.4 Hz, C–N), 124.5 (C–N), 116.6 (d, J = 22.8 Hz, CH), 114.9 (CH), 55.6 (CH_3); ^{19}F ($CDCl_3$, 376.5 MHz) δ −111.2.

*4.1.27. 1-(4-Ethoxyphenyl)-3-(4-fluorophenyl)-5-thioxoimidazolidine-2,4-dione (**11d**)*

Mp 182–184 °C; IR (KBr): ν 1750 (C=O), 1227 (C–O), 1159 (C–O), 1115 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.55–7.47 (m, 2H), 7.33 (d, J = 9.0 Hz, 2H), 7.25–7.18 (m, 2H), 7.03 (d, J = 9.0 Hz, 2H), 4.09 (q, J = 7.1 Hz, 2H), 1.45 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.3 (C=S), 162.4 (d, J = 250.2 Hz, C–F), 159.8 (C–O), 153.3 (C=O), 152.7 (C=O), 128.3 (CH), 127.7 (d, J = 8.8 Hz, CH), 126.3 (d, J = 2.9 Hz, C–N), 124.2 (C–N), 116.5 (d, J = 23.4 Hz, CH), 115.3 (CH), 63.9 (CH_2), 14.7 (CH_3); ^{19}F ($CDCl_3$, 376.5 MHz) δ −111.2; MS (m/z, %) 344 (19, M^+), 179 (62, p-EtO-C_6H_4·NCS), 151 (100), 111 (47).

*4.1.28. 1-(4-Chlorophenyl)-3-(4-fluorophenyl)-5-thioxoimidazolidine-2,4-dione (**11e**)*

Mp 168–171 °C; IR (KBr): ν 1744 (C=O), 1094 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.58–7.45 (m, 4H), 7.44–7.37 (m, 2H), 7.27–7.17 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.7 (C=S), 162.5 (d, J = 250.3 Hz, C–F), 152.9 (C=O), 152.4 (C=O), 136.0 (C–Cl), 130.3 (C–N), 129.9 (CH), 128.5 (CH), 127.7 (d, J = 8.8 Hz, CH), 126.1 (d, J = 3.7 Hz, C–N), 116.6 (d, J = 22.8 Hz, CH); ^{19}F ($CDCl_3$, 376.5 MHz) δ −110.8; MS (m/z, %) 334 (34, M^+), 169 (100, p-Cl-C_6H_4·NCS), 111 (52, p-Cl-C_6H_4).

*4.1.29. 1,3-Bis(4-fluorophenyl)-4-imino-5-thioxoimidazolidin-2-one (**13a**)*

Mp 200–202 °C; IR (KBr): ν 3462 (NH), 1771 (C=O), 1667(C=N), 1123 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.58 (s, 1H, NH), 7.60–7.52 (m, 2H), 7.49–7.43 (m, 2H), 7.28–7.18 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.3 (C=S), 162.8 (d, J = 250.8 Hz, C–F), 162.1 (d, J = 250.0 Hz, C–F), 154.0 (C=O), 153.6 (C=N), 129.1 (d, J = 8.9 Hz, CH), 128.5 (d, J = 3.8 Hz, C–N), 128.4 (d, J = 8.8 Hz, CH), 127.8 (d, J = 3.7 Hz, C–N), 116.7 (d, J = 23.4 Hz, CH), 116.4 (d, J = 22.8 Hz, CH); ^{19}F ($CDCl_3$, 376.5 MHz) δ −110.0, −111.8; MS (m/z, %) 317 (33, M^+), 153 (100, p-F-C_6H_4·NCS).

*4.1.30. 1-(3-Fluorophenyl)-3-(4-fluorophenyl)-4-imino-5-thioxoimidazolidin-2-one (**13b**)*

Mp 160–162 °C; IR (KBr): ν 3462 (NH), 1775 (C=O), 1667 (C=N), 1121 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.59 (s, 1H, NH), 7.62–7.48 (m, 3H), 7.33–7.16 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.9 (C=S), 162.7 (d, J = 249.2 Hz, C–F), 162.2 (d, J = 249.2 Hz, C–F), 153.9 (C=O), 153.4 (C=N), 133.7 (d, J = 9.5 Hz, CH), 130.7 (d, J = 8.8 Hz, CH), 128.4 (d, J = 8.8 Hz, CH), 127.7 (d, J = 3.0 Hz, C–N), 122.9 (d, J = 3.0 Hz, C–N), 117.0 (d, J = 20.4 Hz, CH), 116.4 (d, J = 22.8 Hz, CH), 114.9 (d, J = 24.9 Hz, CH); ^{19}F ($CDCl_3$, 376.5 MHz) δ −109.9, −111.8; MS (m/z, %) 317 (40, M^+), 153 (100, p-F-C_6H_4·NCS).

*4.1.31. 1,3-Bis(4-fluorophenyl)-5-thioxoimidazolidine-2,4-dione (**14a**)*

Mp 190–192 °C; IR (KBr): ν 1744 (C=O), 1115 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.54–7.38 (m, 4H), 7.30–7.18 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.0 (C=S), 162.9 (d, J = 251.5 Hz, C–F), 162.4 (d, J = 250.3 Hz, C–F), 153.0 (C=O), 152.5 (C=O), 129.2 (d, J = 8.8 Hz, CH), 127.8 (d, J = 3.7 Hz, C–N), 127.7 (d, J = 8.8 Hz, CH), 126.1 (d, J = 3.7 Hz, C–N), 116.8 (d, J = 23.4 Hz, CH), 116.6 (d, J = 23.5 Hz, CH); ^{19}F ($CDCl_3$, 376.5 MHz) δ −109.6, −110.9.

*4.1.32. 1-(3-Fluorophenyl)-3-(4-fluorophenyl)-5-thioxoimidazolidine-2,4-dione (**14b**)*

Mp 140–144 °C; IR (KBr): ν 1746 (C=O), 1115 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.58–7.44 (m, 3H), 7.30–7.14 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.6 (C=S), 162.7 (d, J = 249.0 Hz, C–F), 162.5 (d, J = 250.2 Hz, C–F), 152.8 (C=O), 152.4 (C=O), 133.0 (d, J = 10.2 Hz, CH), 130.9 (d, J = 8.8 Hz, CH), 127.7 (d, J = 8.8 Hz, CH), 126.1 (d, J = 2.9 Hz, C–N), 123.0 (d, J = 3.7 Hz, C–N), 117.1 (d, J = 20.6 Hz, CH), 116.6 (d, J = 23.4 Hz, CH), 115.0 (d, J = 24.3 Hz, CH); ^{19}F ($CDCl_3$, 376.5 MHz) δ −109.6, −110.8.

*4.1.33. 1-(4-Chloro-3-(trifluoromethyl)phenyl)-5-imino-3-(4-methoxyphenyl)-4-thioxoimidazolidin-2-one (**15a**)*

Mp 170 °C; IR (KBr): ν 3459 (NH), 1762 (C=O), 1662 (C=N), 1125 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.67 (br s, 1H, NH), 8.04 (d, J = 2.6 Hz, 1H), 7.81 (dd, J = 8.6, 2.5 Hz, 1H), 7.66 (d, J = 8.6 Hz, 1H), 7.37 (d, J = 9.0 Hz, 2H, Ar–H), 7.06 (d, J = 9.0 Hz, 2H, Ar–H), 3.87 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.0 (C=S), 160.4 (C–O), 153.5 (C=N), 153.4 (C=O), 132.3 (CH), 132.0 (C–N), 130.9 (C), 130.3 (CH), 129.4 (q, J = 32.0 Hz, C–N), 128.3 (CH), 125.5 (q, J = 5.3 Hz, CH), 124.9 (C–N), 122.3 (q, J = 273.7 Hz, CF_3), 114.9 (CH), 55.6 (CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −63.0; MS (m/z, %) 413 (37, M^+), 165 (100, p-MeO-C_6H_4·NCS), 150 (48).

*4.1.34. 1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(4-ethoxyphenyl)-5-imino-4 thioxoimidazolidin-2-one (**15b**)*

Mp 117–118 °C; IR (KBr): ν 3462 (NH), 1763 (C=O), 1663 (C=N), 1123 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.66 (br s, 1H, NH), 8.04 (d, J = 2.5 Hz, 1H), 7.81 (dd, J = 8.5, 2.5 Hz, 1H), 7.66 (d, J = 8.5 Hz, 1H), 7.36 (d, J = 9.0 Hz, 2H, Ar–H), 7.04 (d, J = 9.0 Hz, 2H, Ar–H), 4.09 (q, J = 7.0 Hz, 2H), 1.45 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.0 (C=S), 159.8 (C–O), 153.5 (C=N), 153.4 (C=O), 132.3 (CH), 132.0 (C), 130.9 (C), 130.3 (CH), 129.4 (q, J = 32.0 Hz, C–N), 128.2 (CH), 125.5 (q, J = 5.3 Hz, CH), 124.6 (C–N), 122.3 (q, J = 272.0 Hz, CF_3), 115.3 (CH), 63.9 (CH_2), 14.7 (CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −63.0; MS (m/z, %) 427 (60, M^+), 179 (47, p-EtO-C_6H_4·NCS), 151 (100).

*4.1.35. 1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(4-fluorophenyl)-5-imino-4-thioxoimidazolidin-2-one (**15c**)*

Mp 169 °C; IR (KBr): ν 3462 (NH), 1764 (C=O), 1660 (C=N), 1119 (C=S) cm^{-1}; ^1H NMR (DMSO, 400 MHz) δ 9.93 (s, 1H, NH), 8.14 (d, *J* = 2.4 Hz, 1H), 7.98 (d, *J* = 8.4 Hz, 1H), 7.94 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.59 (dd, *J* = 9.2, 5.2 Hz, 2H), 7.46 (t, *J* = 8.8 Hz, 2H); ^{13}C NMR (DMSO, 100 MHz) δ 182.6 (C=S), 162.1 (d, *J* = 246.0 Hz, C–F), 153.4 (C=O), 153.3 (C=N), 132.6 (CH), 132.5 (CH), 131.9 (C), 130.0 (d, *J* = 9.0 Hz, CH), 129.3 (d, *J* = 3.0 Hz, C–N), 127.0 (q, *J* = 31 Hz, C), 126.1 (q, *J* = 5.5 Hz, CH), 122.4 (q, *J* = 271 Hz, CF_3), 116.4 (d, *J* = 23.0 Hz, CH); MS (*m*/*z*, %) 401 (25, M^+), 153 (100, p-F-C_6H_4·NCS), 95 (17, p-F-$C_6H_4^+$).

*4.1.36. 1-(4-Chloro-3-(trifluoromethyl)phenyl)-5-imino-3-(4-iodophenyl)-4-thioxoimidazolidin-2-one (**15d**)*

Mp 134–135 °C; IR (KBr): ν 3460 (NH), 1761 (C=O), 1662 (C=N), 1121 (C=S) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.69 (br s, 1H, NH), 8.02 (d, *J* = 2.5 Hz, 1H), 7.90 (d, *J* = 8.8 Hz, 2H, Ar–H), 7.78 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.66 (d, *J* = 8.5 Hz, 1H), 7.22 (d, *J* = 8.8 Hz, 2H, Ar–H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.3 (C=S), 153.2 (C=O), 153.0 (C=N), 138.8 (CH), 132.4 (CH), 132.2 (C), 132.0 (C), 130.7 (C), 130.3 (CH), 129.5 (q, *J* = 32 Hz, C), 128.7 (CH), 125.5 (q, *J* = 5.3 Hz, CH), 122.2 (q, *J* = 272.0 Hz, CF_3), 95.8 (C–I); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −62.9; MS (*m*/*z*, %) 509 (67, M^+), 261 (100, p-I-C_6H_4·NCS), 134 (48).

*4.1.37. 1-(4-Fluorophenyl)-5-imino-4-thioxo-3-(4(trifluoromethyl)phenyl)imidazolidin-2-one (**16a**)*

Mp 110–112 °C; IR (KBr): ν 3462 (NH), 1763 (C=O), 1663 (C=N), 1123 (C=S) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.60 (s, 1H, NH), 7.61–7.51 (m, 4H), 7.44–7.34 (m, 2H), 7.28–7.17 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.0 (C=S), 162.2 (d, *J* = 249.5 Hz, C–F), 153.9 (C=O), 153.5 (C=N), 149.6 (C), 130.8 (C), 128.8 (CH), 128.4 (d, *J* = 8.8 Hz, CH), 127.7 (d, *J* = 3.4 Hz, C–N), 121.7 (CH), 116.4 (d, *J* = 23.4 Hz, CH), 120.3 (q, *J* = 258.8 Hz, CF_3); ^{19}F ($CDCl_3$, 376.5 MHz) δ −57.8, −111.8; MS (*m*/*z*, %) 368 (1, M^++1), 109 (100, p-F-C_6H_4·N), 69 (94).

*4.1.38. 3-(4-Fluorophenyl)-5-thioxo-1-(4-(trifluoromethyl)phenyl)imidazolidine-2,4-dione (**16b**)*

Mp 155–158 °C; IR (KBr): ν 1744 (C=O), 1115 (C=S) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.54–7.47 (m, 4H), 7.44–7.38 (m, 2H), 7.25–7.19 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.7 (C=S), 162.5 (d, *J* = 250.2 Hz, C–F), 152.9 (C=O), 152.4 (C=O), 149.7 (C), 130.1 (C), 128.9 (CH), 127.7 (d, *J* = 8.8 Hz, CH), 126.1 (d, *J* = 3.7 Hz, C–N), 121.8 (CH), 116.6 (d, *J* = 22.8 Hz, CH), 120.3 (q, *J* = 260.2 Hz, CF_3); ^{19}F ($CDCl_3$, 376.5 MHz) δ −57.8, −110.8; MS (*m*/*z*, %) 368 (1, M^+), 219 (100), 203 (8, p-CF_3-C_6H_4·NCS), 137 (62, p-F-C_6H_4·NCO).

*4.1.39. 3,3′-(2,2′-Dimethoxybiphenyl-4,4′-diyl)bis(1-(4-fluorophenyl)-4-imino-5-thioxoimidazolidin-2-one) (**17**)*

Mp 215–218 °C; IR (KBr): ν 3441 (NH), 1777 (C=O), 1668 (C=N), 1234 (C–O), 1113 (C=S) cm^{-1}; ^1H NMR (DMSO, 400 MHz) δ 9.72 (s, 1H, NH), 7.69–7.59 (m, 3H), 7.57–7.41 (m, 4H), 3.95 (s, 3H); ^{13}C NMR (DMSO, 100 MHz) δ 183.1 (C=S), 162.3 (d, *J* = 247.5 Hz, C–F), 155.7 (C–O), 154.0 (C=O), 153.5 (C=N), 142.8 (C), 131.7 (CH), 130.3 (d, *J* = 9.6 Hz, CH), 129.6 (d, *J* = 2.6 Hz, C–N), 120.5 (C), 119.8 (CH), 116.6 (d, *J* = 23.5 Hz, CH), 111.8 (CH), 56.4 (CH_3); ^{19}F (DMSO, 376.5 MHz) δ −111.2; MS (*m*/*z*, %) 657 (40, M^++1), 656 (33, M^+), 624 (28, M^+-OMe), 477 (70), 267 (100).

*4.1.40. 3,3′-(4,4′-Oxybis(4,1-phenylene))bis(1-(4-fluorophenyl)-4-imino-5-thioxoimidazolidin-2-one) (**18**)*

Mp 195–200 °C; IR (KBr): ν 3440 (NH), 1775 (C=O), 1665 (C=N), 1115 (C=S) cm^{-1}; ^1H NMR (DMSO, 400 MHz) δ 9.71 (br s, 2H, NH), 7.66–7.59 (m, 8H), 7.45 (t, *J* = 8.8 Hz, 4H), 7.27 (d, *J* = 8.8 Hz, 4H); ^{13}C NMR (DMSO, 100 MHz) δ 183.0 (C=S), 162.0 (d, *J* = 246.9 Hz, C–F), 155.9 (C–O), 154.2 (C=O), 153.7 (C=N), 130.1 (d, *J* = 9.0 Hz, CH), 129.6 (d, *J* = 2.9 Hz, C–N), 129.1 (CH), 128.0 (C–N), 119.1 (CH), 116.2 (d, *J* = 23.4 Hz, CH); ^{19}F (DMSO, 376.5 MHz) δ −111.3; MS (*m*/*z*, %) 612 (5, M^+), 252 (100), 153 (96), 95 (74).

*4.1.41. 1-(4-Fluorophenyl)-3-((5-(3-(4-fluorophenyl)-5-imino-2-oxo-4-thioxoimidazolidin-1-yl)-1,3,3-trimethylcyclohexyl)methyl)-4-imino-5-thioxoimidazolidin-2-one (**19**)*

Mp 128–130 °C; IR (KBr): ν 3441 (NH), 1777 (C=O), 1665 (C=N), 1092 (C=S) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.43 (s, 1H, NH), 9.39 (s, 1H, NH), 7.46–7.35 (m, 4H), 7.24–7.15 (m, 4H), 4.72–4.62 (m, 1H), 3.67 (s, 2H), 2.43–2.34 (m, 1H), 2.27–2.19 (m, 1H), 1.70–1.63 (m, 1H), 1.62–1.55 (m, 1H), 1.42–1.37 (m, 2H), 1.27 (s, 3H), 1.16 (s, 3H), 1.03 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.9 (C=S), 181.8 (C=S), 162.6 (d, *J* = 251.2 Hz, C–F), 162.5 (d, *J* = 251.2 Hz, C–F), 155.6 (C=O), 155.3 (C=O), 154.2 (C=N), 153.7 (C=N), 129.0 (d, *J* = 8.8 Hz, CH), 128.7 (d, *J* = 2.5 Hz, C–N), 128.6 (d, *J* = 2.5 Hz, C–N), 116.6 (d, *J* = 23.5 Hz, CH), 116.5 (d, *J* = 23.5 Hz, CH), 54.4 (CH_2), 48.2 (CH), 47.2 (CH_2), 41.0 (CH_2), 38.3 (C), 37.9 (CH_2), 35.1 (CH_3), 32.2 (C), 27.5 (CH_3), 23.5 (CH_3); ^{19}F ($CDCl_3$, 376.5 MHz) δ −110.3, −110.5; MS (*m*/*z*, %) 582 (51, M^+), 359 (55), 149 (98), 123 (100).

*4.1.42. 3,3′-(Hexane-1,6-diyl)bis(1-(4-fluorophenyl)-4-imino-5-thioxoimidazolidin-2-one) (**20**)*

Mp 172–175 °C; IR (KBr): ν 3443 (NH), 1775 (C=O), 1663 (C=N), 1090 (C=S) cm^{-1}; ^1H NMR (d1-TFA/$CDCl_3$; 1/1, 400 MHz) δ 11.71 (s, 1H, NH), 7.45–7.36 (m, 2H), 7.33–7.24 (m, 2H), 4.20–4.02 (m, 2H), 2.00–1.77 (m, 2H), 1.63–1.42 (m, 2H); ^{13}C NMR (d1-TFA/$CDCl_3$; 1/1, 100 MHz) δ 177.2 (C=S), 164.3 (d, *J* = 254.6 Hz, C–F), 153.6 (C=O), 150.5 (C=N), 129.1 (d, *J* = 8.8 Hz, CH), 126.9 (d, *J* = 2.9 Hz, C–N), 117.7 (d, *J* = 23.4 Hz, CH), 43.7 (CH_2), 27.0 (CH_2), 25.9 (CH_2); ^{19}F (d1-TFA/$CDCl_3$; 1/1, 376.5 MHz) δ −109.1; MS (*m*/*z*, %) 528 (44, M^+), 304 (39), 224 (31), 154 (41), 153 (100, p-F-C_6H_4·NCS).

4.2. Biological evaluation

*4.2.1. Screening studies of compounds **6a–g**, **10d,f,g**, **13a**, **15a,c**, **16a**, **17**, and **19** against EAC cells*

Antitumor activities (in vitro) studies were carried out at Cairo University, National Cancer Institute, Cancer Biology Department, Pharmacology Unit, using RPMI 1640 medium (Sigma), Ehrlich ascites carcinoma cells (EAC) suspension (2.5×10^5/mL), and trypan blue dye. A stock solution was prepared by dissolving 1.0 g of the dye in 100 mL of H_2O. Subsequent dilution of 1 mL of the stock solution with 9 mL of distilled water was done. The stain used for staining dead EAC cells was obtained by needle aspiration of ascetic fluid from preinoculated mice under aseptic conditions. The cells were tested for viability and contamination by staining certain cell volume of this fluid by an equal volume of the working solution of trypan blue dye. The ascetic fluid was diluted to 1:10 with saline to contain 2.5×10^6 cells on a hemocytometer. In a set of test tubes, 0.1 mL of tumor cells suspension, 0.8 mL RPMI 1640 media, and 0.1 mL of each tested compound (corresponding to 25, 50, and 100 μg) were mixed and incubated at 37 °C for 2 h. Trypan blue exclusion test was carried out to calculate the percentage of non viable cells [81].

$$\%\ \text{of non-viable cells} = \frac{\text{no. of non-viable cells}}{\text{no. of cells}} \times 100$$

*4.2.2. Cytotoxic activity studies of compounds **15a** and **16a** against various tumor cell lines*

Compounds **15a** and **16a** were tested at concentrations between 1 and 50 μg/mL using SRB assay for cytotoxic activity against HEPG2, HEP2, MCF7, HELA, and HCT116 tumor cell lines.

4.2.3. Measurement of potential cytotoxicity by SRB assay

Potential cytotoxicity of heterocycles **15a** and **16a** was tested using the method of Skehan et al. [74]. Cells were plated in a 96 multiwell plate (10^4 cells/well) for 24 h before treatment with the compounds to allow attachment to the wall of the plate. Different concentrations of the compounds (**15a**: 0, 1, 2.5, 5, and 10 μg/mL; **16a**: 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 h at 37 °C in an atmosphere of 5% CO_2. After 48 h, 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.

4.2.4. Antibacterial and antifungal susceptibility testing of compounds 6a,e–g, 10a–c, 13b, 15a–d, and 17–20

Antimicrobial and antifungal studies were carried out by the diffusion agar technique [74] using a 1 cm microplate well diameter and a 100 μL of a sample concentration of 5 mg/mL, unless otherwise indicated. The tested organisms were Gram-negative bacteria (*E. coli*, NCTC-10416, *S. typhi*, *NCIMB*-9331, and *S. lutea*, ATCC-934), Gram-positive bacteria (*B. subtilis*, NCTC-1040 and *S. aureus*, NCTC-7447), and fungi (*C. albicans*, *IMRU-3669*, *A. niger*, and *A. flavus*). The bacteria and fungi were maintained on nutrient agar and Czapek's Dox agar medium, respectively. All chemical compounds were dissolved in *N*,*N*-dimethylformamide (DMF) (5 mg/mL), except if noted otherwise. DMF showed no inhibition activity. The results are tabulated in Table 4.

4.2.5. Virucidal activity studies

Heterocycles **15a**,**b**,**d** were tested against HAV, HSV1 and COxB4 viral strains where viral activity was assayed by the plaque formation method.

4.2.5.1. Determination of cytotoxicity of **15a,b,d** *on Vero cells.* Initially, the cytotoxicity of compounds **15a**,**b**,**d** on Vero cells was determined. The method used was that described by Vanden Berghe et al. [78]. Propagation of healthy Vero cell line by enzyme treatment and assessment of cytotoxicity effect of **15a**,**b**,**d** was carried out as follows:

1. The media overlaying cell monolayer was decanted and cells were released from tissue culture flask by treatment with 5 mL of pre-warmed trypsin–EDTA solution.
2. The trypsin was aspirated with a pipette, then 2 mL of trypsin was dispensed, the bottle rocked, and was incubated at 37 °C for more than 10 min.
3. Cells were suspended in about 8 mL of growth media.
4. Cells were counted using a hemocytometer and using trypan blue vital stain.
5. About 10 mL of 2×10^5 VERO cell suspension was transferred to a 50 cm^3 TC bottle (Falcon) tightly closed then was incubated at 37 °C. Cells were sub-cultured once weekly.
6. For seeding 96 well plate, 0.1 mL (2×10^5 cells) was transferred to each flat-bottom well and incubated at 37 °C for 24–48 h to develop a complete monolayer sheet.
7. The 6 or 12 well plates used for plaque assay and for cytotoxicity study were seeded with 2–3 mL cell suspension per well. Confluent monolayer was obtained after 24 h incubation at 37 °C.
8. Compounds **15a**,**b**,**d** were each dissolved in 1 mL of DMSO.
9. Growth medium was decanted from 96 well micro titer plates after confluent sheet of VERO cell was formed, cell monolayer was washed twice with wash media, then about 1 mL of wash media was added and the plates were incubated at room temperature for 5–10 min.
10. Ten-fold serial dilutions of different compounds were made in Eagle's minimum essential medium (MEM), starting from 10^{-1} till 10^{-4} dilution.
11. 0.2 mL of each dilution was tested in different wells leaving 6 wells as control, receiving only maintenance medium.
12. The plate was incubated at 37 °C and examined frequently for up to 3 days. Cells were checked for any physical signs of toxicity, e.g. partial or complete loss of the monolayer, rounding, shrinkage/retraction, cell granulation, and lysis.
13. The maximum non-toxic concentration (MNTC) of each compound on the cultured cells was determined and was used in antiviral tests.

4.2.5.2. Determination of the anti-infectivity effect of compounds **15a,b,d** *against HAV, HSV1, and COxB4 viral strains.*

1. 0.1 mL of (10^3) virus suspension was mixed with 0.1 mL of sample at the selected dilution shown in Table 6 (initial sample concentration 5 mg/mL DMSO). The mixtures were incubated at room temperature for 1 h in sterile screw-capped vials.
2. Non-treated virus infected control set was done by mixing 0.1 mL of (10^3) of virus suspension in MEM, with 0.1 mL of MEM medium (virus control).
3. 12-well plates seeded with VERO cells were washed with wash solution (total washes 2) then added 1 mL wash solution and incubate at room-temperature for 5–10 min.
4. 0.2 mL of either test or a control vial was inoculated. One well/each plate was left neither inoculated with virus nor treated with any compound as cell control.
5. Plates were incubated at 37 °C to allow virus adsorption.
6. Cell monolayers were washed twice with PBS, then overlaid with 2× MEM/agarose mixture. Viral infectivity was assayed by titration of viruses by the plaque formation method [79].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jfluchem.2011.06.015.

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