



## Original article

# Synthesis, characterization and derivatization of some novel types of mono- and bis-imidazolidineiminothiones and imidazolidineiminodithiones with antitumor, antiviral, antibacterial and antifungal activities – part I

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## ABSTRACT

Halogenated and alkylated *N*-arylcyanothioformanilides were reacted with the nucleophilic reagents triethylamine, hydrazine and diphenyldiazomethane to produce *N*-arylcyanothioformanilide ammonium salts, a thiosemicarbazide and a 2-(arylamino)-3,3-diphenylacrylonitrile, respectively. They also underwent several types of electrophilic reactions with aryl-, arylbisocyanates and arylisothiocyanates to yield mono- and bis-imidazolidineiminothiones and imidazolidineiminodithiones. Treatment of imidazolidineiminothiones with hydrogen sulfide, substituted *ortho*-phenylenediamines and thiocarbonyldrazide afforded the corresponding thiohydantoin, quinoxaline and imidazotriazine derivatives. Several of the synthesized products were subjected to in vitro biological evaluation against antitumor, antiviral, antimicrobial and antifungal strains. Most tested compounds showed significant activities.

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## 1. Introduction

Cyanothioformanilides are versatile reagents and have been extensively utilized for the synthesis of various fused heterocycles which display interesting medicinal and pharmacological properties. A variety of “one-pot” ring closure reactions of cyanothioformanilides [1–3] give rise to a plethora of heterocyclic cores which include pyrroles [4,5], pyrrolothiazoles [6], imidazoles [7–9], benzimidazoles [10], oxazoles [11,12], benzoxazoles [10,12], thiazoles [13,14], and quinazolinonethione [10,12].

Our keen interest in the chemistry of cyanothioformanilide **I** [15,16] and derivatives thereof containing aliphatic, aromatic [17,18], and various heterocyclic ring systems [6], as well as the related cyanothiosulfonamide congeners [19] led us to synthesize many bioactive heterocyclic compounds with a wide variety of pharmacological effects [20–27]. In these transformations, various derivatives of cyanothioformanilide **I** reacted with a range of electrophiles, nucleophiles, and *ortho*-substituted aniline reagents

[12,28]. In addition, bis-cyanothioformanilides derived from 1,4-bis-isothiocyanatobenzene and 2,4-bis-isothiocyanatotoluene reacted with different reagents to produce novel bis-heterocycles [5,29]. In the present study, the reactions of a series of previously reported substituted cyanothioformanilides **1a–g** [30] (Fig. 1) were investigated with various electrophiles and nucleophiles, leading to several new mono- and bis-imidazolidineiminothiones and imidazolidineiminodithiones. Structures of the prepared compounds were elucidated by the use of 1D/2D <sup>1</sup>H NMR, <sup>13</sup>C NMR, IR and GC mass spectral techniques. Finally, some selected compounds were evaluated for cytotoxic activity against various tumor cell lines (brain tumor cell line U251, human hepatocellular carcinoma cell line HEPG2, human breast adenocarcinoma cell line MCF-7, human epithelial carcinoma cell line HELA and colon carcinoma cell line HCT116), antiviral (HAV, HSV1 and CoxB4), antibacterial (*Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) and antifungal strains (*Candida albicans* and *Aspergillus niger*).

## 2. Chemistry

Prior to delving into the synthesis and biological evaluation of heterocycles (*vide post*), we decided to investigate some of the cyanothioformanilides shown in Fig. 1 by various 1D and 2D NMR

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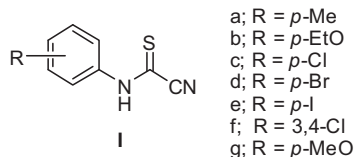


Fig. 1. Structure of cyanothioformanilide I.

techniques. Although these were reported in the literature by us and others, they were not fully characterized and no NMR spectral data has yet been reported. Analysis of cyanothioformanilides **1a–g** by NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) indicated the presence of tautomeric mixtures of thione and thiol forms, in which the former predominates. For instance, the  $^1\text{H}$  NMR spectrum of **1f** (tautomeric mixture; major:minor; 84:16) revealed at  $\delta = 11.01$  ppm an exchangeable proton (broad s, 1H, NH) which disappeared upon deuteration with  $\text{D}_2\text{O}$ . The aromatic ring displayed a set of three signals for the major tautomer as follows: Ar-H(2) as a doublet (1H,  $J = 2.5$  Hz), Ar-H(5) as a doublet ( $J = 8.8$  Hz), and Ar-H(6) as doublet of doublets ( $J = 8.8, 2.5$  Hz) (Fig. 2). Protons for the minor tautomer exhibited well separated chemical shifts and also appeared as a set of three signals with similar multiplicity patterns and  $J$ -values. The  $^{13}\text{C}$  spectrum of cyanothioformanilide **1f** showed eight signals for the major form which were assigned to the structure with the aid of DEPT 90/135 as well as COSY and HSQC 2D techniques. Also, it showed a farthestmost downfield signal for the thione group ( $\text{C}=\text{S}$ ) at  $\delta$  164.2 ppm, three signals corresponding to the CH groups, another three signals stemming from the quaternary aromatic carbons, and an upfield signal resulting from the nitrile group ( $\delta$  114.1 ppm). The mass spectrum exhibited a molecular ion peak at  $m/z$  203 (100%). In addition, IR measurements displayed absorptions at:  $\nu$  ( $\text{cm}^{-1}$ ) NH (3250),  $\nu$  ( $\text{cm}^{-1}$ ) SH (2600–2550),  $\nu$  ( $\text{cm}^{-1}$ ) CN (2119) and  $\nu$  ( $\text{cm}^{-1}$ ) CS–N (1450, 1170). Interestingly, the extended conjugation of the thiol form does not seem to impart enough thermodynamic stability to drive the equilibrium forward, rendering it the major tautomer. However, it is noted that cyanothioformanilides **1a**, **1b** and **1g** comprise a much higher ratio of the thiol form (thione:thiol; 58:42; 56:44; and 57:43 respectively) compared to compounds **1c–f** (thione:thiol; 70:30; 68:32; 63:36; and 84:16 respectively). This is understandable as the electron-donating substituents in the former series place a charge in the para position of the aromatic ring which can only be delocalized in the thiol form to give a stabilized anion positioned  $\alpha$  to the nitrile group. In any case, it appears that the superior basicity and electronegativity of the thioamide nitrogen atom renders the thione form more favorable. Accordingly, such reagents are ambident nucleophiles and may react via the sulfur or nitrogen atom leading to different heterocyclic cores in ring closure reactions. Indeed, Ketcham et al. observed that *N*-substituted cyanothioformanilides react with alkyl or aryl isothiocyanates to give 5-imino-1,3-disubstituted-2,4-imidazolidinedithiones or 5-(phenylimino)-4-imino-2-thiazolidinedithiones depending upon the reaction conditions and the reactants [13].

The chemical behavior of cyanothioformanilides **1a–g** toward a variety of basic and nucleophilic reagents such as triethylamine, hydrazine hydrate and diphenyldiazomethane was studied. Thus, room temperature reactions of equimolar amounts of *p*-bromocyanothioformanilide **1d** or its iodo- and 3,4-dichloro-derivatives with triethylamine in diethyl ether as a solvent immediately gave the corresponding triethylammonium salts **1la–c** which crystallized out of solution upon standing (Fig. 3). Interestingly, the  $^1\text{H}$  and  $^{13}\text{C}$  spectra of these salts indicated that each comprised of a mixture of two tautomeric products whose ratio slightly differed from that of the starting cyanothioformanilide. For instance, whereas *p*-bromocyanothioformanilide **1a** consisted of a 75:25 mixture of tautomers, its iodo analogue **1b** exhibited a much higher tautomeric mixture of 91:9. The dichloro derivative **1c** displayed an intermediate tautomeric ratio of 80:20. The  $^1\text{H}$  NMR spectrum of **1a** and the iodo analogue **1b** each exhibited a set of two aromatic doublets for each tautomer (4H), a set of two aliphatic quartets (6H,  $J = 7.3$  Hz,  $3\text{XCH}_2$ ), and a set of two aliphatic triplets (9H,  $J = 7.3$  Hz,  $3\text{XCH}_3$ ). As expected, the  $^1\text{H}$  NMR spectrum of **1c** showed five sets of signals for each tautomer. The proton of the triethylammonium salt appeared as a broad signal at 6.97–6.65 ppm. The  $^{13}\text{C}$  spectrum of the thione tautomer displayed ten signals which was assigned by DEPT 90 and DEPT 135 to six aromatic carbons, two quaternary thione and nitrile carbons, and two aliphatic methylene and methyl carbons.

Having 3,4-dichlorocyanothioformanilide (**1f**) in hand, we decided to explore its reactivity at room temperature with hydrazine hydrate using ethanol as a solvent. As one might anticipate, the reaction led, via the intermediacy of **A**, to the formation of the thiosemicarbazide derivative **III**, following the elimination of one molecule of HCN (Scheme 1). The thiosemicarbazide product (**III**) was analyzed by  $^1\text{H}$  and  $^{13}\text{C}$  NMR techniques, where the former showed the presence of three sets of aromatic proton signals and two sets of exchangeable signals integrating for four protons, whereas the latter revealed the presence of seven signals which were assigned to the  $\text{C}=\text{S}$  group, three quaternary carbons and three methine aromatic protons. As further evidence to support the assigned structure, the infrared spectrum showed a broad band at  $\nu$  ( $\text{cm}^{-1}$ ) 3400–3150 arising from overlapping absorptions of the NH and  $\text{NH}_2$  groups.

Unexpectedly, room temperature reaction of an excess of ethereal diphenyldiazomethane with *p*-iodocyanothioformanilide (**1e**) produced the acrylonitrile derivative **IV** (Scheme 2). Interestingly, a related reaction involving treatment of thiobenzophenone with excess diazomethane at  $20^\circ\text{C}$  gave 4,4,5,5-tetraphenyl-1,3-dithiolane exclusively [39]. The reaction mode consisted of two 1,3-dipolar cycloadditions separated by a 1,3-dipolar cycloreversion. It was reported that reverse addition produced 22% yield of 1,1-diphenylethane as a mixture with the corresponding thiirane precursor. A plausible mechanistic rationale for the formation of product **IV** involves a twofold extrusion reaction in which initial formation of an intermediate 1,3,4-thiadiazoline via a 1,3-dipolar cycloaddition of

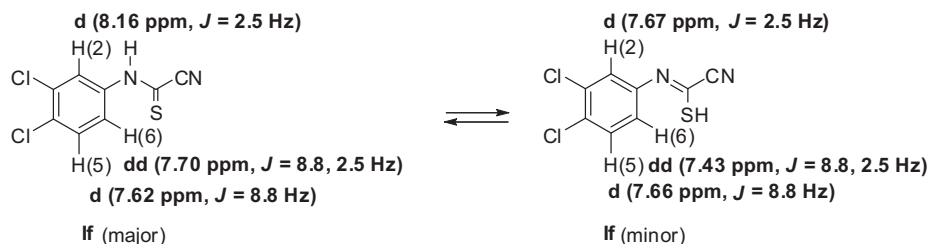


Fig. 2.  $^1\text{H}$  NMR splitting pattern and coupling of the aromatic protons of cyanothioformanilide **1f**.

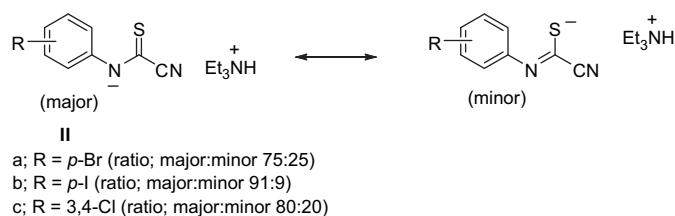
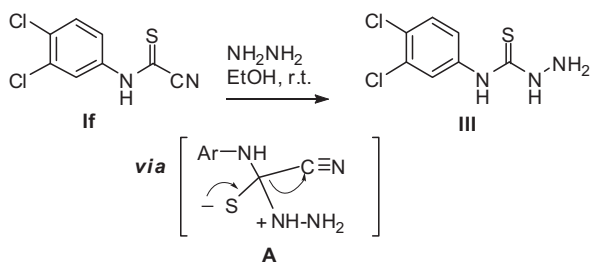


Fig. 3. Reaction products of cyanothioformanilides **la–c** with triethylamine in ether at room temperature.

diphenyldiazomethane to the thione function, is ensued by the extrusion of molecular nitrogen and formation of a transient thiocarbonyl ylide. Subsequent rapid electrocyclic of the ylide gives rise to a thiirane. Related thiirane intermediates have been previously isolated by our group in the reaction of diphenyldiazomethane with 5-bromo-2-*p*-tolylisindoline-1,3-dithione and 2-phenylisindoline-1,3-dithione [40]. However, the resulting aromatic spiro[isindoline-1,2'-thiirane]-3-thiones were stable to sulfur extrusion. In the current case, subsequent extrusion of sulfur affords the corresponding tetra-substituted acrylonitrile derivative **IV**. Clearly, this mode of cyclization leading to a thiirane is more competitive as the alternative one would lead to a highly hindered dithiolane product. The preceding transformation is particularly noteworthy as both intermediates proved unstable to isolation and directly extruded nitrogen and sulfur under ambient conditions. This is in sharp contrast to twofold extrusion reactions where a substrate must be induced thermally or photochemically to extrude stable species, particularly when the chalcogen atom is sulfur or selenium. Indeed as reported by Huisgen et al., a  $t_{1/2} \sim 16$  h at 20 °C was reported for the sulfur extrusion from a thiirane ring [39]. As such, this result marks the first example of the conversion of a cyanothioformanilide into an alkene at room temperature and warrants a separate investigative study into its scope and potential synthetic utility. It is possible that the driving force toward extrusion is provided by the exocyclic nitrogen atom of the thiadiazoline and thiirane intermediates which facilitates the extrusion process by initial formation of an iminium ion intermediate in each step.

The  $^1\text{H}$  NMR spectrum of **IV** showed an AB system typical of a *para*-substituted aromatic system integrating for four protons. Further, the aniline exchangeable proton appeared as a broad singlet which disappeared upon treatment with  $\text{D}_2\text{O}$ . Finally, three sets of aromatic signals integrating for ten protons were observed for the two phenyl groups. Further analysis of the product by  $^{13}\text{C}$  and DEPT experiments indicated the presence of fifteen signals (6 quaternary aromatic carbons, 8  $\text{CH}$ 's, and CN). The mass spectrum of **IV** showed a molecular ion peak at  $m/z$  422 (100%, base peak), suggesting a high stability of the product against fragmentation. The IR spectrum clearly showed the nitrile and NH stretchings, and more informatively, did not display any  $\text{C}=\text{S}$  stretch.

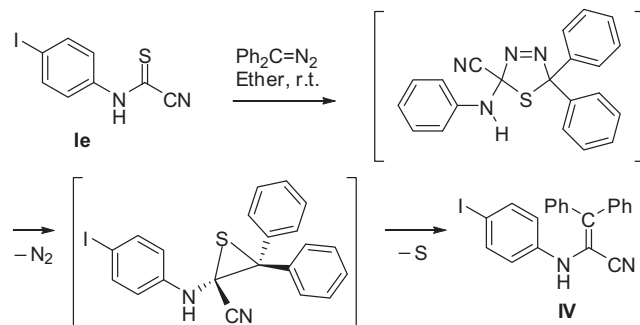


Scheme 1. Reaction of cyanothioformanilide **If** with hydrazine hydrate in ethanol at room temperature.

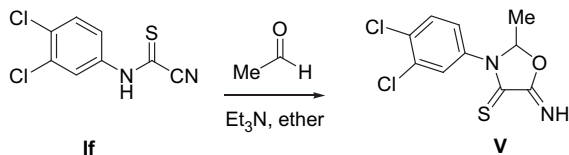
At the other end of their reactivity spectrum, the cyanothioformanilides were reacted with several electrophilic reagents such as acetaldehyde. Thus, according to the general protocol of Ketcham and Schaumann [11], reaction of 3,4-dichlorocyanothioformanilide (**If**) with acetaldehyde in the presence of triethylamine produced the corresponding oxazolidineiminothione **V** (Scheme 3). The  $^1\text{H}$  NMR spectrum of **V** exhibited six sets of signals, where the imine proton displayed the usual broad exchangeable signal, and the aromatic protons showed their anticipated pattern of two doublets and one doublet of doublets. More informative were those signals stemming for the methine and methyl groups which showed up as a quartet at  $\delta$  6.09 ppm and as a doublet at  $\delta$  1.59 ppm, respectively. Further analysis of the product by  $^{13}\text{C}$  and DEPT experiments indicated the presence of ten signals (3 quaternary aromatic carbons, 3  $\text{CH}$ 's,  $\text{O}-\text{CH}$ , and  $\text{CH}_3$ ). The IR spectrum clearly showed an NH stretching at  $\nu$  ( $\text{cm}^{-1}$ ) 3270 and a  $\text{CS}-\text{N}$  stretchings at  $\nu$  ( $\text{cm}^{-1}$ ) 1490, 1170, respectively.

Imidazoles and their fused derivatives are key components of great many bioactive compounds (such as histidine, purine, biotin and hydantoin) of both natural and synthetic origin [31]. Hydantoin derivatives are used in therapy as anticonvulsants and chemotherapeutic agents [32]. Antimicrobial activity was reported for derivatives possessing aromatic substituents at the imidazole nitrogen. Such active compounds include *N*-acyl and 5-arylidene derivatives of hydantoin and 4-thiohydantoin [33–35]. Moreover, previous reports demonstrated that synthetic imidazoles act either as inhibitors of  $\alpha$ -adrenoreceptor mediated events in platelets [36] or inducers of platelets activation [37].

In continuation with our previous work in this area [22–27], we decided to prepare and evaluate the biological activity of poly-halogenated imidazolidineiminothiones containing trichlorophenyl, chloro-trifluoromethylphenyl and *p*-chloro, *p*-bromo or *p*-iodo phenyl groups. As a synthetic entry point to access these derivatives, heterocyclic ring closing reactions of various aromatic cyanothioformanilides (**la–g**) containing 3,4-dichloro-, *p*-chloro, *p*-bromo, *p*-iodo, *p*-methyl, *p*-methoxy, and *p*-ethoxyphenyl groups were carried out with aromatic phenyl-, *p*-chlorophenyl-, *p*-chloro-3-



Scheme 2. Reaction of cyanothioformanilide **Ie** with diphenyldiazomethane in ether at room temperature.



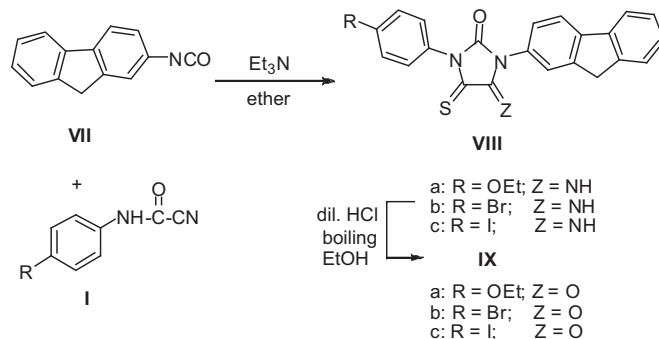
**Scheme 3.** Reaction of cyanothioformanilide **If** with acetaldehyde in ether at room temperature.

trifluoromethyl- and 2,4,5-trichlorophenylisocyanates in ether with triethylamine as a base to furnish the corresponding 5-imino-4-thioxo-2-imidazolidinones (**VI**) (Scheme 4). These products were purified by crystallization and obtained in 65–70% yields. Compounds **Vla,h** [8], **Vlb** [21] and **Vlc** [5] were previously reported by us but were not evaluated in any biological assays.

The  $^1\text{H}$  NMR spectrum of **Vle** exhibited five sets of signals, where the imine proton displayed the usual broad exchangeable signal, and the aromatic protons for the *p*-iodophenyl and 2,4,5-trichlorophenyl groups showed their anticipated pattern of two doublets and two singlets, respectively. The  $^{13}\text{C}$  NMR, however, displayed only twelve of the expected thirteen signals. Further analysis by DEPT experiments revealed the presence of four CH groups (6 carbons), one of  $\text{C}=\text{S}$ ,  $\text{C}=\text{O}$ ,  $\text{C}=\text{N}$ , and  $\text{C}-\text{I}$ , and four quaternary aromatic carbons. When an inverse gated coupling experiment was carried out on **Vle**, the aromatic carbons integrated for five, suggesting that the missing aromatic quaternary carbon is overlapping with one of the other four aromatic carbons. Infrared measurements clearly showed the presence of  $\text{NH}$ ,  $\text{C}=\text{O}$ , and  $\text{CS}-\text{N}$  stretchings at their expected frequencies.

The  $^1\text{H}$  NMR spectrum of **Vlf** exhibited six sets of signals; broad singlet for the imine proton, two doublets and one doublet of doublets for the aromatic protons of 3,4-dichlorophenyl group, and a multiplet for the *p*-iodophenyl group. The  $^{13}\text{C}$  NMR, displayed the expected thirteen signals for  $\text{C}=\text{S}$ ,  $\text{C}=\text{N}$ ,  $\text{C}=\text{O}$ , five quaternary aromatic carbons and seven aromatic CH protons. The mass spectrum exhibited a molecular ion peak at  $m/z$  385 (70%) and a base peak for a 3,4-dichloroisothiocyanate fragment at  $m/z$  203. The isotopic distribution of the molecular ion peak clearly corroborates a molecular structure containing three atoms of chlorine.

In addition to the above compounds, we prepared other new types of imidazolidineiminothiones (**VIII**) containing 9H fluoren moiety (Scheme 5). Thus, reactions of *p*-ethoxy-, *p*-bromo-, and *p*-iodocyanothioformanilides **Ib**, **Id** and **Ie** with 9H fluoren-2-ylisocyanate (**VII**) in the presence of triethylamine afforded imidazolidineiminothiones **VIIIa–c**. These were all crystalline solids and were therefore purified by recrystallization from ethanol. The  $^1\text{H}$  NMR spectrum of compound **VIIIa** exhibited ten sets of signals;

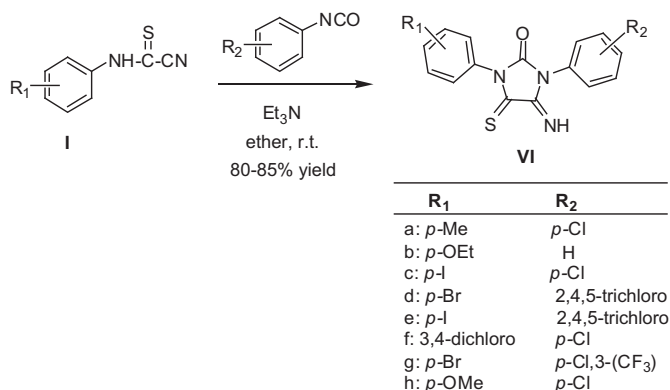


**Scheme 5.** Preparation of imidazolidineiminothiones containing 9H fluoren moiety.

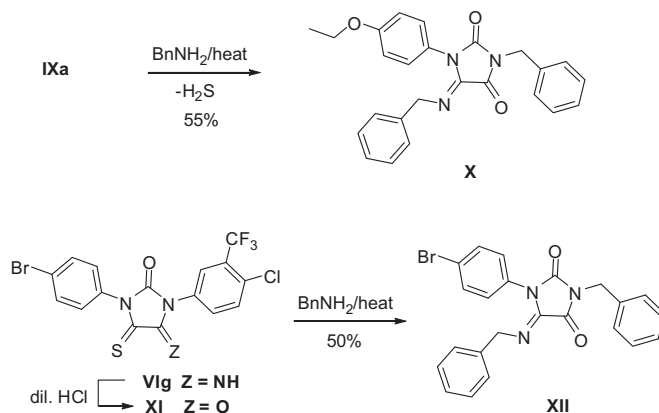
a broad exchangeable singlet for the imine proton, three aromatic doublets and one broad signal, as well as two multiplets integrating for eleven protons. More informative were those signals stemming from the allylic methylene group which appeared as a singlet and the ethoxy group which exhibited its usual multiplicity pattern of a quartet and a triplet. The  $^{13}\text{C}$  NMR, displayed the expected twenty two signals for  $\text{C}=\text{S}$ ,  $\text{C}=\text{N}$ ,  $\text{C}=\text{O}$ ,  $\text{C}-\text{O}$ , six quaternary aromatic carbons and nine aromatic CH protons, as well as two methylene and one methyl carbons. Infrared measurements of **VIIIa** showed the presence of an  $\text{NH}$  ( $3251\text{ cm}^{-1}$ ),  $\text{C}=\text{O}$  ( $1767\text{ cm}^{-1}$ ) and  $\text{C}=\text{N}$  ( $1669\text{ cm}^{-1}$ ) stretches at their expected frequencies. The  $^{13}\text{C}$  spectrum corroborated the proton and IR data, clearly displaying the expected 22 signals ( $\text{C}=\text{S}$ ,  $\text{C}-\text{O}$ ,  $\text{C}=\text{O}$ ,  $\text{C}=\text{N}$ , 6C, 9CH,  $\text{CH}_2\text{O}$ ,  $\text{CH}_2$ , and  $\text{CH}_3$ ). Further structural evidence emerged from the hydrolysis of **VIII** with dilute HCl in boiling ethanol which afforded diones **IXa–c**.

The  $^1\text{H}$  NMR spectrum of **IXc** exhibited nine signals integrating for thirteen protons as expected. This data, however, could not stand on its own in providing unequivocal proof of structure for the parent heterocycles **VIIIa–c**. More convincing evidence stemmed from the  $^{13}\text{C}$  NMR and DEPT spectra which showed the presence of 20 signals representing 22 carbon atoms;  $\text{C}=\text{S}$ ,  $2\text{XC}=\text{O}$ , 6C, 9CH,  $\text{C}-\text{I}$ , and  $\text{CH}_3$ . More significantly, a chemical shift at 180 ppm was indicative of a  $\text{C}=\text{S}$  moiety which is the only group that could possibly possess such a distinctive downfield shift. The infrared spectrum proved as useful in tracing the disappearance of the  $\text{NH}$  stretch in the parent compound and the appearance of a  $\text{C}=\text{O}$  stretch. It is noted that all the mass spectra of **VIII** and **IX** share a common base peak at  $m/z$  207 stemming from a 2-isocyanato-9H-fluorene fragment.

For the purpose preparing analogues for further structural evidence of compounds **VIIIa–c** and their dione analogues, 4-thioxoimidazolidine-2,5-dione (**IXa**) reacted successfully with excess



**Scheme 4.** Preparation of imidazolidineiminothiones.



**Scheme 6.** Preparation of imidazolidinebenzyliminodiones heterocycles.



benzylamine at the thione moiety to produce the corresponding benzylimine derivative as a single geometric isomer, following the elimination of  $\text{H}_2\text{S}$  (Scheme 6). Surprisingly, the  $^1\text{H}$  and  $^{13}\text{C}$  data were not consistent with the presence of a fluorenyl moiety. For instance, the  $^1\text{H}$  NMR spectrum indicated the presence of 7 signals, corresponding to 23 protons. The benzylimine methylene appears as one singlet, at  $\delta$  5.35 ppm indicating one geometric isomer. The  $^{13}\text{C}$  NMR and DEPT spectra showed the presence of 27 signals representing 31 carbon atoms;  $\text{C}=\text{N}$ ,  $2\text{XC}=\text{O}$ , 7C, 12 types of protonated carbons corresponding to 8 CH types, 3  $\text{CH}_2$  groups, and a  $\text{CH}_3$ . Extensive 1D/2D NMR analysis led us to suggest structure **X** as the product. Further structural evidence stemmed from the mass spectrum which corroborates the spectral data and the proposed structure, giving a molecular ion peak with  $m/z$  413. In our further attempts to prepare benzylimine derivatives, compound **Vig** was subjected to iminothiolysis to afford **XI**, which was subsequently treated with excess benzylamine in boiling ethanol. Similarly, **XII** was isolated as the major product of the reaction and was fully characterized by spectroscopic and mass spectral techniques. Proton NMR indicated the presence of two benzylic methylene singlets ( $\delta$  5.35 and 4.93 ppm) which were correlated to their respective carbons appearing at  $\delta$  53.1 and 43.0.  $^{19}\text{F}$  and  $^{13}\text{C}$  NMR data confirmed the absence of the trifluoromethyl moiety which often has a characteristic fluorine chemical shift of  $-63$  ppm and a quartet multiplicity in the carbon that usually appears at 122.2 ppm ( $J = 273.5$  Hz).

Formation of **X** and **XII** can be rationalized according to the mechanism shown in Scheme 7 for **XII**. Reaction of the thione moiety of **XI** with benzylamine leads to the formation of an imidazolidinebenzyliminodione intermediate, which upon further reaction with a second molecule of benzylamine affords **XII**. Presumably, ring opening is facilitated by the longer reaction time and excess benzylamine used for the reaction. Interestingly, the increased steric bulkiness provided by the 9H-fluorenyl moiety compared to the *p*-chloro-3-trifluoromethylphenyl group does not seem to maintain the intactness of the group under the same reaction conditions.

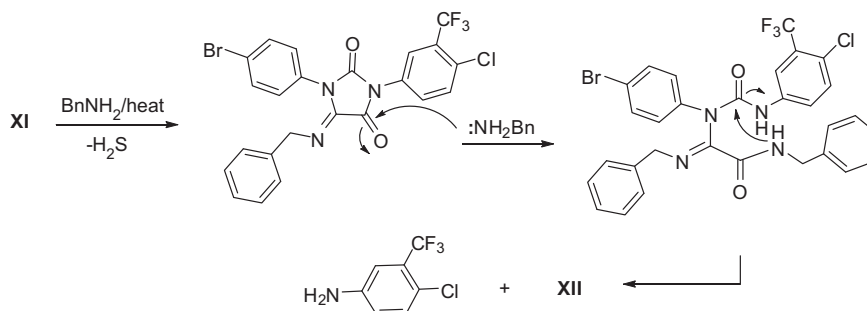
Imidazolineiminothiones containing a naphthyl group were also synthesized as single isomers and in good yields via reaction of cyanothioformanilides **Ic**, **Id** and **Ig** with 1-isocyanatonaphthalene to produce **XIIIa** and **XIIIb**, respectively (Fig. 4). The mass spectrum of **XIIIa** exhibited a base peak at  $m/z$  365 which clearly corresponded to the molecular ion peak. In addition, proton and carbon NMR data were consistent with the proposed structure.

Since a considerable number of bis-heterocyclic compounds (e.g. bis-quinazoline derivatives) have been reported to exhibit superior antibacterial and antifungal activities [38] than their mono-heterocyclic counterparts, we opted to prepare some bis-imidazolidineiminothione derivatives. Thus, reaction of two

equivalents of cyanothioformanilides **Ib** with one equivalent of bis(3,5-diethyl-4-isocyanatophenyl)methane resulted in the formation of the desired bis-imidazolidineiminothione **XIV** (Fig. 5). Similarly, reaction of **If** and with 4,4'-oxybis(isocyanatobenzene) afforded the novel bis-imidazolidineiminothione **XV** as a single regioisomer. The  $^1\text{H}$  NMR spectrum of **XIV** indicated the presence of 9 signals, corresponding to 46 protons. The benzyl methylene appears as one singlet, at  $\delta$  4.04 ppm in the proton NMR and at 41.7 ppm in the  $^{13}\text{C}$  carbon, suggesting one regioisomeric product. The  $^{13}\text{C}$  NMR and DEPT spectra showed the presence of 15 signals representing 33 carbon atoms;  $2\text{XC}=\text{N}$ ,  $2\text{XC}=\text{O}$ , 7C, 8 types of protonated carbons corresponding to 3 CH types, 3  $\text{CH}_2$  and 2  $\text{CH}_3$  groups. Mass spectral data strongly supports the proposed structure, giving a molecular ion peak with  $m/z$  774. Similarly, compound **XV** was fully characterized and gave satisfactory NMR and mass spectral data.

We also had a long standing interest in preparing derivatives of thiohydantoin which have been shown to exhibit a spectrum of biological and pharmacological activities. Earlier, we reported the preparation of 4-thiohydantoin derivative **XVIa** [5] via reduction of the imine function of the parent iminothione with hydrogen sulfide [7]. We now report the preparation of its 3,4-dichloro derivative **XVIb** following the same experimental protocol that had been used earlier (Fig. 6). Its mass spectrum exhibited a molecular ion base peak at  $m/z$  370 and the IR clearly showed the absence of the NH absorbance band present in the parent compound. The product proved air sensitive as slow and progressive air oxidation to the corresponding disulfide **XVII** was observed during preparation and storage. Presumably, tautomerization of the thiohydantoin to the enethiol, followed by air oxidation leads to the dihydantoin disulfide as suggested by the mechanism shown in Scheme 8. Indeed, when pure thione **XVIb** was left standing at room temperature, it underwent partial conversion to give a 2.1:1 mixture of the enethiol and disulfide as determined by the aid of  $^1\text{H}$  NMR,  $^{13}\text{C}$ , DEPT 90/135 as well as COSY and HSQC 2D techniques. As such, we were able to assign all the observed chemical shifts in the proton and carbon NMR of the mixture to the enethiol hydantoin tautomer and its dihydantoin disulfide oxidative product.

The imidazolidineiminothiones **Vla**, **Vib** and **Vif** underwent cyclization with substituted *ortho*-phenylenediamines to give sulfur-free products, following the elimination of  $\text{H}_2\text{S}$  and  $\text{NH}_3$  molecules (Fig. 7). As anticipated, the products of **Vla** and **Vif** (**XVIIIa** and **XVIIIc**, respectively) each consisted of two regioisomers which were formed in almost equal amounts according to  $^1\text{H}$  NMR integration of the methyl and methoxy signals of the respective mixtures. These were inseparable by column chromatography and were therefore characterized as mixtures. The molecular ion peak in the mass spectra of **XVIIIa–c** was the base peak as well, pointing to the stability of such compounds.



Scheme 7. Mechanism of formation of imine **XII**.

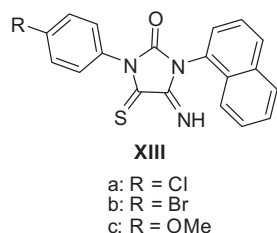


Fig. 4. Structures of naphthyl-containing imidazolineiminothiones.

We were also interested in preparing imidazotriazines of type **XIX** due to an anticipated potential biological activity. Previously we had anticipated that reactions of the iminothiones **VI** with thiosemicarbazide would afford the desired triazine products **XIX** ( $R=NH_2$ ) through elimination of two moles of  $H_2S$  as shown in Scheme 9 [22]. To our dismay, several attempts to prepare **XIX** failed and we observed the evolution of only one mole of  $H_2S$ . Close examination of the reaction mixture revealed that the non-cyclized intermediate product **XX** had formed almost quantitatively and apparently resisted cyclization. Attempted ring closure of **XX** by refluxing in ethanol also failed.

As an alternate route to the preparation of imidazotriazines, imidazolidineiminothione **VIc** [5] and its *p*-chloro- and *p*-bromoisiminothione derivatives [8] were reacted with benzophenonehydrazine and triethylamine in refluxing ethanol. Unfortunately, cyclization did not proceed under the reaction conditions and we were only able to isolate the corresponding 4-((diphenylmethylene)hydrazono)-5-iminoimidazolidin-2-one **XXI** products (Scheme 10).

Previously, we reported preparation attempts toward imidazotriazine of type **XXIII** from the reactions of imidazolidineiminothiones **VI** with phenyl or diphenyldiazomethane [16] (Fig. 8). This was based on the premise that addition of the diazomethane and subsequent loss of sulfur would form the desired triazine. In the present investigation, reactions of **VIa,c,f** and their *p*-ethoxy [21] analogue with diphenyldiazomethane did not produce the imidazotriazines. Instead, 1-(4-chlorophenyl)-3-(3,4-dichlorophenyl or 4-(substituted)-4-(diphenylmethylene)-5-iminoimidazolidin-2-ones (**XXIV**) was isolated and their structures were confirmed by spectroscopic and mass spectral techniques. Presumably, a twofold extrusion reaction involving a [2 + 3] dipolar cycloaddition of diphenyldiazomethane to the thione function, followed by the extrusion of molecular nitrogen and sulfur has occurred, affording the tetra-substituted alkene **XXIV**. Alternatively and more justifying to our approach, an intermediate triazine structurally similar to **XXIII** is a plausible precursor to **XXIV**.

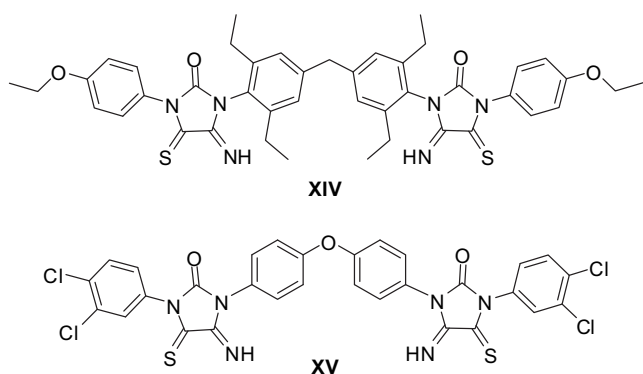


Fig. 5. Structures of bis-imidazolidineiminothione derivatives.

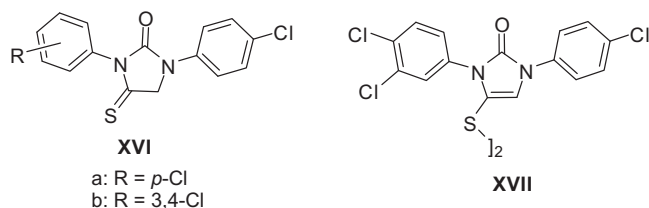


Fig. 6. Novel thiohydantoin derivative and its dihydantoin disulfide side product.

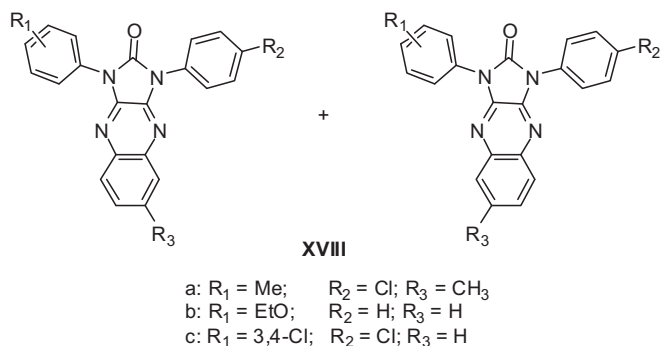
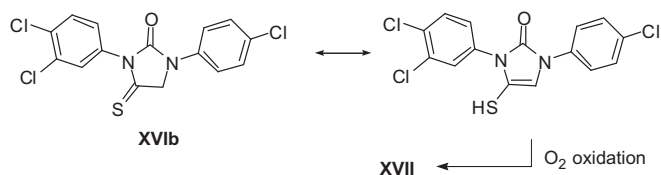


Fig. 7. Cyclization reactions of imidazolidineiminothiones with *ortho*-phenylenediamines.

Because of stability issues, the former readily extrudes molecular nitrogen at room temperature affording **XXIV**.

Finally after extensive experimentations, the desired imidazotriazine core could be constructed via the reactions of imidazolidineiminothiones **VI** and related compounds with thiocarbonylhydrazide, eliminating two molecules of hydrogen sulfide to produce triazines **XXV** as shown in Scheme 11. While triazines **XXVa–c** are all novel, **XXVd** has been reported earlier by our group [8], although it was never evaluated in any biological assay. In fact, preliminary positive results obtained by the evaluation of **XXVd** against certain types of cancer cell lines (*vide post*) provided the impetus to search for synthetic routes (*vide supra*) to construct these types of systems and eventually led to the preparation of its triazine analogues **XXVa–c**.

As further addition to our pool of new heterocycles, we decided to prepare a series of imidazolidineiminothiones (Fig. 9). These types of compounds are known to exhibit enhanced bioactivity over their imidazolidineiminothione counterparts. Thus, several dithiones were synthesized via the respective reactions of *p*-ethoxy (**Ib**), *p*-bromo (**Id**) and *p*-iodocyanthioformanilide (**Ie**) with phenyl, *p*-bromo and *p*-iodophenylisothiocyanate to furnish the desired 5-imino-2,4-dithioxoimidazolidines (**XXVla–c**). Whereas **XXVla,c** were produced and isolated as mixtures of 5-imino-2,4-imidazolidinedithione and 4,5-diiminothiazolidine, **XXVlb** was obtained as a single dithione isomer following recrystallization. Thus, we elected to utilize the latter for biological testing and we were also selective in the products we subjected to testing.



Scheme 8. Air oxidation of a thiohydantoin derivative.

### 3. Results and discussion

#### 3.1. Antitumor properties

The following set of compounds **XIV**, **XVIa**, **XVIIIa,c**, **XXVd** and **XXVIb** were initially subjected to screening tests against Ehrlich Ascites Carcinoma cells (EAC). They were then evaluated for cytotoxic activity against various tumor cell lines (brain tumor cell line U251, human hepatocellular carcinoma cell line HEPG2, human breast adenocarcinoma cell line MCF-7, human epithelial carcinoma cell line HELA and colon carcinoma cell line HCT116).

##### 3.1.1. Screening of compounds **XIV**, **XVIa**, **XVIIIa,c**, **XXVd** and **XXVIb** against EAC cells

Compounds **XIV**, **XVIa**, **XVIIIa,c**, **XXVd** and **XXVIb** were screened against EAC cells at concentrations of 100, 50 and 25  $\mu\text{g/mL}$  as shown in Table 1. All compounds produced over 90% inhibition of cell viability at the 100  $\mu\text{g/mL}$  concentration. Compounds **XVIa** and **XXVd** were highly effective at all concentration range and only marginal drop in % inhibition of cell viability was observed with decreasing concentration. On the other hand, % inhibition of cell viability was more sensitive to concentration changes with the remaining compounds as more pronounced drop was observed at lower dose.

##### 3.1.2. Cytotoxic activity of compounds **XIV**, **XVIa**, **XVIIIa,c**, **XXVd** and **XXVIb** against various tumor cell lines

The cytotoxicity of compounds **XIV**, **XVIa**, **XVIIIa,c**, **XXVd** and **XXVIb** was evaluated against brain tumor cell line U251, human hepatocellular carcinoma cell line HEPG2, human breast adenocarcinoma cell line MCF-7, human epithelial carcinoma cell line HELA and colon carcinoma cell line HCT116. The median inhibition concentration (IC<sub>50</sub>) values are shown in Table 2 and the results are summarized in Table 3 and represented graphically in Fig. 10.

The IC<sub>50</sub> data shown in Table 2 and the dose response curves of Fig. 10 clearly underscore the superiority of bis-imidazolidineiminothione **XIV** against U251, MCF7, HELA and HCT116 and point to the effectiveness of triazine **XXVd** against HEPG2 cell lines. Notably, 5-imino-2,4-imidazolidinedithione **XXVIb** was markedly active against U251, MCF7 and HCT116 cell line. Generally, all cell lines were responsive to dose increase as indicated by the ensuing reduction in surviving fraction, although dose response was variable as seen in Table 3.

#### 3.2. Antiviral properties

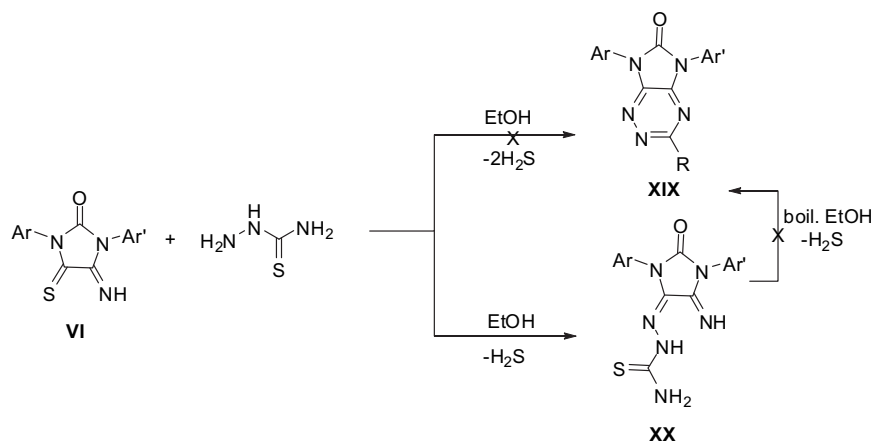
Initially, the maximum non-toxic concentration (MNTC) of compounds **Vle-g**, **VIII-c**, **IXa,b** and **XXVIb** on Vero cells was determined using five 10-fold serial dilutions of each compound starting from  $10^{-1}$  until  $10^{-5}$  dilution of the initial sample concentration of 5 mg/mL (DMSO). Cells were monitored for any physical signs of toxicity for three days. The MNTC data is shown in Table 4 and was selected for further anti-infectivity studies against hepatitis A virus (HAV), herpes simplex virus 1 (HSV1) and Coxsackie B4 (COxB4) viral strain. The results are summarized in Table 4. Imidazolidineiminothiones **VIIIc** containing a 9H fluorene moiety was the most effective against all three viral strains, reducing virus plaque count of each one from 50 to 70%. Interestingly, its bromo derivative **VIIIa** showed no effect, while its ethoxy derivative **VIIIa** was active against HSV1. Notably, the hydrolysis product of the latter thioxoimidazolidinedione **IXa** proved highly active against all tested strains, while the hydrolysis derivative of the former **IXb** affected HSV1 only. Imidazolidineiminothiones **Vle** and **Vlg** also showed activity against HAV and HSV1, respectively, while the related analogue **Vlf** was inactive. Finally, imidazolidineiminothione **XXVIb** was effective against HSV1.

#### 3.3. Antimicrobial properties

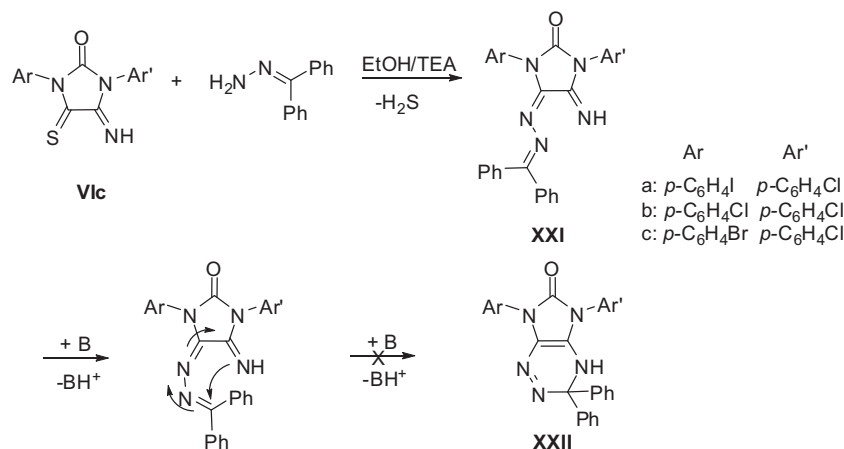
Two sets of heterocycles were selected and compounds within each series were tested under the same conditions for antimicrobial activity against Gram negative and Gram positive bacteria as follows: series 1: **VId-g**, **VIIIa-c** and **IXa-b**; series 2: **If**, **Ilc**, **V**, **VIf**, **XIIIa**, **XIV**, **XV**, **XVIb** and **XXVIb,c**.

##### 3.3.1. Antimicrobial activities of compounds **VId-g**, **VIIIa-c** and **IXa-b** against Gram negative and Gram positive bacteria

The following first series of selected compounds **VId-g**, **VIIIa-c** and **IXa-b** were tested in vitro for antibacterial activity against the following bacterial strains: Gram-negative bacteria, *Escherichia coli* NCTC 10416, *Salmonella typhi* NCIMB 9331, and Gram positive bacteria, *B. subtilis* NCTC 1040, *S. aureus* NCTC 7447. The results are summarized in Table 5 and represented graphically in Fig. 11. The compounds were tested for antimicrobial activity by the agar disk diffusion technique using a 1 cm microplate well diameter and a 100  $\mu\text{L}$  of each concentration. Chloramphenicol was used as a standard antibacterial agent. All compounds were dissolved in



Scheme 9. Attempted preparation of imidazotriazines **XIX**.



**Scheme 10.** Attempted preparation of imidazotriazines **XXII**

*N,N*-dimethylformamide (5 mg/mL) except compounds **VIg** and **IXb** which were concentrated at 4 and 1 mg/mL respectively.

The data displayed in Table 5 and Fig. 11 show that all heterocycles tested in the antimicrobial assays possess significant antibacterial activities against the growth of *E. coli* and *S. typhi* on solid media, where compounds **VI** and **IXa** were found to possess the highest antibacterial activities in the series against Gram-negative bacteria. Substituting the *para* bromine substituent in **VId** with an iodine atom as in **VIe** had negligible effect on activity. Further refinement of substituents on both aryl groups as in **VI**, **g** produced slight improvement in activity against *E. coli*. While compounds **VIe**, **VIIIa** and **IXa** were equally as effective against *B. subtilis*, **VIIIa** showed the highest antibacterial activity against both strains of Gram-positive bacteria in the series. Interestingly, the hydrolysis product (**IXa**) of compound **VIIIa** showed a relatively enhanced activity against the Gram negative bacteria *E. coli* and reduced activity against the Gram positive bacteria *S. aureus*. It is noted that exchanging the ethoxy group of **IXa** with a bromine atom as in **IXb** resulted in a significant drop in activity against both classes of bacteria.

### 3.3.2. Antimicrobial activities of compounds **If**, **IIC**, **V**, **VI**, **g**, **XIIIa**, **XIV**, **XV**, **XVI** and **XXVI**, **c** against Gram negative and Gram positive bacteria

The following second series of selected compounds **If**, **IIC**, **V**, **VI**, **g**, **XIIIa**, **XIV**, **XV**, **XVI** and **XXVI**, **c** were tested in vitro for antibacterial activity against the following bacterial strains: Gram-negative bacteria, *Pseudomonas aeruginosa* NCIB 9016, and Gram-positive bacteria, *B. subtilis* NCIB 3610. The results are summarized in Table 6 and represented graphically in Fig. 12. The compounds were tested for antimicrobial activity by the agar disk diffusion technique under the same conditions of the first series of compounds with the exception that antimicrobial activities were measured using three different sample concentrations. All compounds were dissolved in *N,N*-dimethylformamide at 1, 2.5 and 5 mg/mL concentrations.

The data displayed in Table 6 and Fig. 12 show that all of the compounds surveyed in the antimicrobial assays possess significant antibacterial activities against the growth of *P. aeruginosa* and *B. subtilis* on solid media. Cyanothioformanilide **If** was remarkably active at all concentrations and possessed the highest antibacterial activity in the series against both, Gram negative and Gram positive bacteria except at the 5 mg/mL concentration for *P. aeruginosa*. Interestingly, oxazolidineiminothione **V** which is derived from cyanothioformanilide **If** exhibited the highest activity against *P. aeruginosa* at the 5 mg/mL concentration and ranked second in its activity against *B. subtilis*. Further, antimicrobial activity was most

responsive and increased significantly with concentration increases of compound **V**. It is noted that compounds **XXVI** and **XXVII** show distinct antimicrobial activities and rank third in activity against *P. aeruginosa* and *B. subtilis*, respectively.

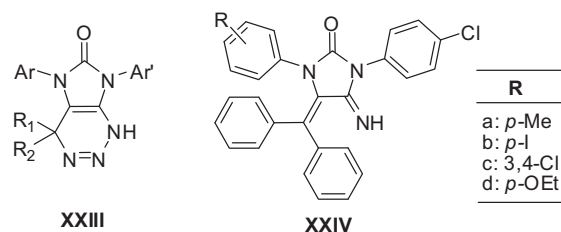
### 3.4. Antifungal properties

The same two sets of heterocycles that were tested for antimicrobial activity were selected (except **IIC**) and compounds within each series were tested under the same conditions for antifungal activity using *C. albicans* IMRU 3669 and *A. niger* IMI 31276 as unicellular and filamentous fungi. The two sets are as follows: series 1: **VId**–**g**, **VIIIa**–**c** and **IXa**, **b**; series 2: **If**, **V**, **VI**, **XIIIa**, **XIV**, **XV**, **XVI** and **XXVI**, **c**.

#### 3.4.1. Antifungal activities of compounds **VId**–**g**, **VIIIa**–**c** and **IXa**–**b** against *C. albicans* and *A. niger*

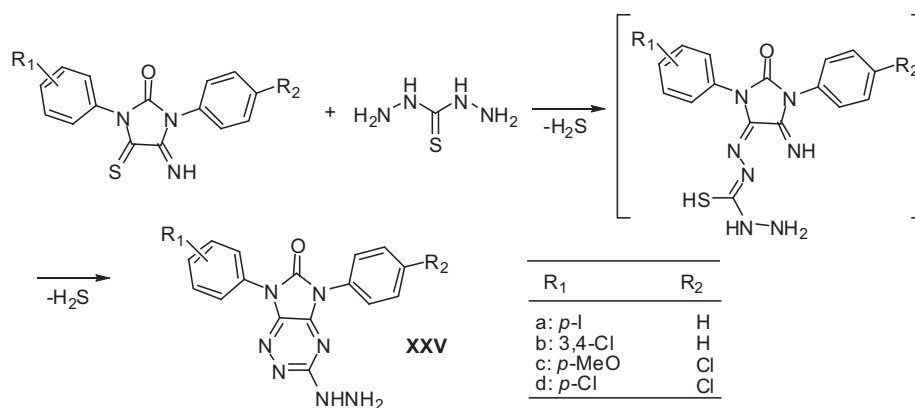
The following first series of selected compounds **VId**–**g**, **VIIIa**–**c** and **IXa**, **b** were tested in vitro for antifungal activity against the following fungal strains: *C. albicans* IMRU 3669 and *A. niger* IMI 31276. The results are summarized in Table 7 and represented graphically in Fig. 13. The compounds were tested for antifungal activity by the agar disk diffusion technique using a 1 cm microplate well diameter and a 100  $\mu$ L of each concentration. Griseofluvin was used as a standard antifungal agent. All compounds were dissolved in *N,N*-dimethylformamide (5 mg/mL) except compounds **VI** and **IXb** which were concentrated at 4 and 1 mg/mL respectively.

All of the tested compounds were found to possess antifungal activity against the two microorganisms. In particular, imidazolidineiminothiones **VI** and **VI** exhibited the highest antifungal activities and were almost equally as effective against *C. albicans* and *A. niger*. It is noted that compound **VIIIa** was also highly effective against *A. niger* but was not as potent against *C. albicans*.



**Fig. 8.** Reaction products of a selection of imidazolidineiminothiones **VI** with diphenyldiazomethane.





Scheme 11. Preparation of a series of imidazotriazines.

### 3.4.2. Antifungal activities of compounds **If**, **V**, **VIf**, **XIIIa**, **XIV**, **XV**, **XVib** and **XXVib,c** against *C. albicans* and *A. niger*

The following second series of selected compounds **If**, **V**, **VIf**, **XIIIa**, **XIV**, **XV**, **XVib** and **XXVib,c** were tested in vitro for antifungal activity against the same fungal organisms used earlier (*C. albicans* and *A. niger* Ferm-BAM C-21). The results are summarized in Table 8 and represented graphically in Fig. 14. The compounds were tested for antifungal activity by the agar disk diffusion technique under the same conditions of the first series of compounds with the exception that antimicrobial activities were measured using three different sample concentrations. All compounds were dissolved in *N,N*-dimethylformamide at 1, 2.5 and 5 mg/mL concentrations.

All tested samples were found to possess antifungal activity against both microorganisms. As observed earlier in antimicrobial testing, cyanothioformanilide **If** was also remarkably active at all concentrations and possessed the highest antifungal activity in the series against both *C. albicans* and *A. niger*. Also as before, the derivative of cyanothioformanilide **If**, oxazolidineiminothione **V** showed high antifungal activity at all concentrations and ranked second in activity and was relatively more potent against *C. albicans*. Both **If** and **V** produced dramatic enhancement in antifungal activity with concentration increase, whereas the remaining species showed only marginal improvement with the exception of **XVib**. Although the latter was not tested against *A. niger*, it showed distinct antimicrobial activity against *C. albicans* and ranked third in activity.

In addition to the preliminary evaluation of the potential biological activities of a large selection of the preceding heterocycles by the agar disk diffusion technique, we tested the antimicrobial and antifungal activity of a narrow selection of potential leads by evaluating their minimum inhibitory concentrations (MIC). Thus, the antibacterial and antifungal properties of compounds **XXVib,c**, **XVib** and **If** were evaluated by the microtube dilution procedure (Table 9) following the guidelines of the Clinical and Laboratory Standard Institute (CLSI) [41,42] (Table 9), formerly National

Committee for Clinical and Laboratory Standards (NCCLS), against a series of 4 bacterial strains and 2 fungal species. To carry out the antimicrobial and antifungal evaluation, concentrations of compounds up to 667 µg/mL were incorporated to growth media according to published procedures [39,42]. As shown in Table 9, all compounds tested showed strong inhibitory effect, low enough to render them as targets for further studies. Interestingly, both 5-imino-2,4-dithioxoimidazolidine **XXVib** and its diiodo analogue **XXVic** showed similar MIC values, pointing to a negligible effect achieved by exchanging the bromine by another halogen atom. They both showed strong MIC values for the Gram negative bacteria *S. typho* (MIC 1.95 µg/mL) and the fungi *C. albicans* (MIC 1.95 µg/mL) and *A. niger* (MIC 0.49 µg/mL). Slightly higher MIC values were observed for the rest of the bacterial strains, but low enough to warrant further investigation. The thiohydantoin derivative **XVib** showed high activity against the Gram negative bacteria *S. typho* (MIC 0.49 µg/mL) and the fungus *C. albicans* (MIC 0.97 µg/mL) and slightly higher MIC values for *B. subtilis* and *K. pneumonia*. On the other hand, moderate MIC values were observed for the remaining strains. Finally, the cyanothioformanilide **If** showed the most consistency in yielding very strong MIC values (ranging from as low as 0.24–3.9 µg/mL) for the first five strains shown in Table 9 and a moderate value for *A. niger* (15.6 µg/mL).

## 4. Conclusion

In summary, the synthesis and characterization of a new series of mono- and bis-imidazolidineiminothiones and imidazolidineiminodithiones have been described. Several heterocycles were also derivatized for structural elucidation and biological evaluation purposes. Most compounds displayed antitumor, antiviral, antibacterial and antifungal activities and could therefore serve as lead chemical entities for further modification to render them clinically useful drug agents.

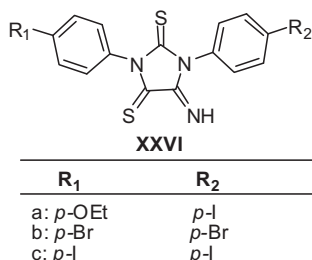


Fig. 9. A series of novel imidazolidineiminodithiones.

Table 1  
Inhibition of EAC cell viability.

Sample no.	% Inhibition of cell viability		
	µg/mL		
	100	50	25
XIV	90	80	60
XVIa	100	100	90
XVIIIa	90	60	35
XVIIIc	95	70	30
XXVd	100	100	95
XXVib	90	70	50

**Table 2**

IC50 values of compounds **XIV**, **XVIa**, **XVIIIa,c**, **XXVd** and **XXVib** obtained against cell lines U251, HEPG2, MCF7, HELA and HCT116.

Sample no.	IC50 (μg/mL)				
	Cell line				
	U251	HEPG2	MCF7	HELA	HCT116
<b>XIV</b>	1.01	5.44	2.15	2.42	4.36
<b>XVIa</b>	ND <sup>a</sup>	NA	9.36	NA	ND
<b>XVIIIa</b>	NA <sup>b</sup>	4.43	NA	8.32	NA
<b>XVIIIc</b>	NA	7.11	NA	7.38	NA
<b>XXVd</b>	ND	1.54	NA	NA	NA
<b>XXVib</b>	1.28	NA	3.09	8.93	4.56

<sup>a</sup> ND: Not determined.

<sup>b</sup> NA: Could not determine from data as surviving fraction was over 0.50 at the maximum sample dose of 10 μg/mL.

## 5. Experimental

### 5.1. Chemistry

IR spectra were recorded (KBr) on a Perkin Elmer 1650 spectrophotometer. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded on Avance II Bruker FT NMR spectrometer 400 (400 MHz) using CDCl<sub>3</sub> or DMSO-*d*<sub>6</sub> as solvents and TMS as an internal standard. 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 & S for all compounds were within ±0.3 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 10.

**Table 3**

Evaluation of compounds **XIV**, **XVIa**, **XVIIIa,c**, **XXVd** and **XXVib** against brain tumor cell line U251, human hepatocellular carcinoma cell line HEPG2, human breast adenocarcinoma cell line MCF-7, human epithelial carcinoma cell line HELA and colon carcinoma cell line HCT116.

Cell line	Sample conc. (μg/mL)	Surviving fraction					
		<b>XIV</b>	<b>XVIa</b>	<b>XVIIIa</b>	<b>XVIIIc</b>	<b>XXVd</b>	<b>XXVib</b>
<b>U251</b>	0	1.00	ND <sup>a</sup>	1.00	1.00	ND	1.00
	1	0.51	ND	0.98	0.98	ND	0.53
	2.5	0.31	ND	0.91	0.94	ND	0.46
	5.0	0.28	ND	0.86	0.80	ND	0.33
	10.0	0.26	ND	0.76	0.72	ND	0.31
<b>HEPG2</b>	0	1.00	1.00	1.00	1.00	1.00	1.00
	1	0.76	0.61	0.94	0.87	0.56	0.92
	2.5	0.67	0.65	0.56	0.54	0.46	0.85
	5.0	0.65	0.59	0.49	0.52	0.50	0.84
	10.0	0.39	0.52	0.41	0.51	0.49	0.71
<b>MCF7</b>	0	1.00	1.00	1.00	1.00	1.00	1.00
	1	0.77	0.71	0.81	0.87	1.00	0.83
	2.5	0.48	0.59	0.75	0.73	1.01	0.54
	5.0	0.48	0.58	0.71	0.70	0.66	0.36
	10.0	0.44	0.49	0.67	0.67	0.67	0.28
<b>HELA</b>	0	1.00	ND	1.00	1.00	1.00	1.00
	1	0.67	ND	0.97	0.94	0.97	0.67
	2.5	0.49	ND	0.92	0.85	0.92	0.65
	5.0	0.44	ND	0.64	0.66	0.64	0.64
	10.0	0.39	ND	0.42	0.40	0.42	0.46
<b>HCT116</b>	0	1.00	ND	1.00	1.00	1.00	1.00
	1	0.78	ND	0.95	0.92	0.95	0.88
	2.5	0.64	ND	0.84	0.80	0.84	0.64
	5.0	0.46	ND	0.56	0.67	0.56	0.44
	10.0	0.40	ND	0.56	0.62	0.56	0.42

<sup>a</sup> ND: Not determined.

5.1.1. For the preparation of the known cyanothioformanilides **1a–g**, see Ref. [30]

5.1.1.1. *4-Tolylcarbamothioyl cyanide (1a)*. Tautomeric ratio 58:42; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 9.61 (br s, 1 H, NH, major and minor), 7.66 (d, *J* = 8.5 Hz, 2H, Ar–H, major), 7.28–7.26 (m, 4H, Ar–H, minor), 7.24 (d, *J* = 8.5 Hz, 2H, Ar–H, major), 2.38 (s, 3H, minor), 2.37 (s, 3H, major); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 165.6 (C=S, major), 161.4 (C=N, minor), 139.3 (Me–C, minor), 138.5 (Me–C, major), 134.4 (C–N, major), 134.3 (C–N, minor), 130.5 (o-CH, minor), 129.8 (o-CH, major), 122.8 (*m*-CH, minor), 122.4 (*m*-CH, major), 113.6 (CN, major), 112.0 (CN, minor), 21.2 (CH<sub>3</sub>, major), 21.1 (CH<sub>3</sub>, minor).

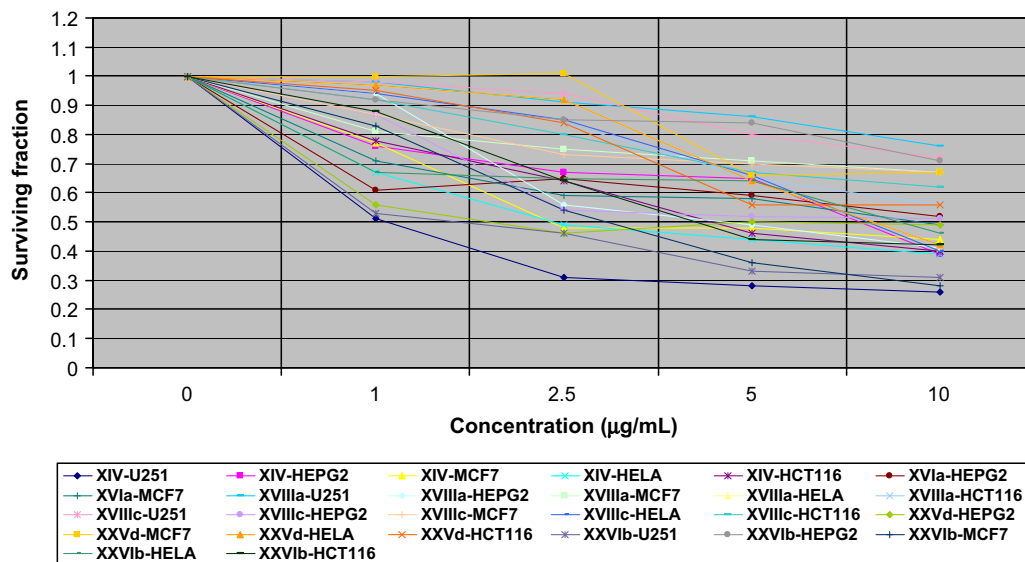
5.1.1.2. *(4-Ethoxyphenyl)carbamothioyl cyanide (1b)*. Tautomeric ratio 56:44; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 9.78 (br s, 1 H, NH, major), 9.63 (br s, 1 H, NH, minor), 7.72 (d, *J* = 9.0 Hz, 2H, Ar–H, major), 7.31–7.25 (m, 2H, Ar–H, minor), 6.97–6.89 (m, 2H, Ar–H, major and minor), 4.05 (q, *J* = 7.0 Hz, 2H, major and minor), 1.43 (t, *J* = 7.0 Hz, 3H, minor), 1.42 (t, *J* = 7.0 Hz, 3H, major); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 165.7 (C=S, major), 160.6 (C=N, minor), 159.3 (O–C, minor), 158.3 (O–C, major), 129.9 (C–N, major), 129.7 (C–N, minor), 124.8 (*m*-CH, minor), 124.0 (*m*-CH, major), 115.5 (o-CH, minor), 114.9 (o-CH, major), 113.6 (CN, major), 112.0 (CN, minor), 64.6 (OCH<sub>2</sub>, minor), 63.9 (OCH<sub>2</sub>, major), 14.7 (CH<sub>3</sub>, major), 14.6 (CH<sub>3</sub>, minor).

5.1.1.3. *(4-Chlorophenyl)carbamothioyl cyanide (1c)*. Tautomeric ratio 70:30; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 9.81 (br s, 1 H, NH, major), 9.54 (br s, 1 H, NH, minor), 7.77 (d, *J* = 9.0 Hz, 2H, Ar–H, major), 7.45 (d, *J* = 9.0 Hz, 2H, Ar–H, minor), 7.41 (d, *J* = 9.0 Hz, 2H, Ar–H, minor), 7.34 (d, *J* = 9.0 Hz, 2H, Ar–H, major); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 165.6 (C=S, major), 161.6 (C=N, minor), 135.4 (C–Cl, major), 135.3 (C–N, major), 134.8 (C–N, major), 133.5 (C–Cl, minor), 130.2 (o-CH, minor), 129.5 (o-CH, major), 124.2 (*m*-CH, minor), 123.6 (*m*-CH, major), 113.4 (CN, major), 111.8 (CN, minor).

5.1.1.4. *(4-Bromophenyl)carbamothioyl cyanide (1d)*. Tautomeric ratio 68:32; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 9.57 (br s, 1 H, NH, major and minor), 7.70 (d, *J* = 9.0 Hz, 2H, Ar–H, major), 7.61 (d, *J* = 9.0 Hz, 2H, Ar–H, minor), 7.57 (d, *J* = 9.0 Hz, 2H, Ar–H, major), 7.29 (d, *J* = 9.0 Hz, 2H, Ar–H, minor); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 165.5 (C=S, major), 161.8 (C=N, minor), 135.8 (C–Br, major), 135.7 (C–Br, minor), 133.2 (o-CH, minor), 132.5 (o-CH, major), 124.3 (*m*-CH, minor), 123.8 (*m*-CH, major), 122.6 (C–N, minor), 121.4 (C–N, major), 113.4 (CN, major), 111.8 (CN, minor).

5.1.1.5. *(4-Iodophenyl)carbamothioyl cyanide (1e)*. Tautomeric ratio 64:36; IR (KBr) 3250 (NH), 3080 (CH arom.), 2225 (CN) cm<sup>−1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 9.44 (br s, 1H, NH, both isomers), 8.11 (d, *J* = 8.8 Hz, 2H, Ar–H, minor), 7.78 (d, *J* = 8.8 Hz, 2H, Ar–H, major), 7.56 (d, *J* = 8.8 Hz, 2H, Ar–H, major), 7.16 (d, *J* = 8.8 Hz, 2H, Ar–H, minor); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 160.4 (C=S), 152.1 (C=N), 152.0, 139.1 (o-CH, minor), 138.5 (o-CH, major), 124.3 (*m*-CH, minor), 123.9 (*m*-CH, major), 125.7 (CH), 119.7 (CN), 93.8 (C–I), 92.7 (C–I); MS (*m/z*, %) 261 (33, M + −HCN), 219 (30), 134 (14).

5.1.1.6. *(3,4-Dichlorophenyl)carbamothioyl cyanide (1f)*. Tautomeric ratio 84:16; IR (KBr) 3275 (NH), 3090 (CH arom.), 2240 (CN) cm<sup>−1</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>CN, 400 MHz) δ 11.01 (br s, 1H, NH), 8.16 (d, *J* = 2.5 Hz, 1H, Ar–H, major), 7.70 (dd, *J* = 8.8, 2.5 Hz, 1H, Ar–H, major), 7.67 (d, *J* = 2.5 Hz, 1H, Ar–H, minor), 7.66 (d, *J* = 8.8 Hz, 1H, Ar–H, minor), 7.62 (d, *J* = 8.8 Hz, 1H, Ar–H, major), 7.43 (dd, *J* = 8.8, 2.5 Hz, 1H, Ar–H, minor); <sup>13</sup>C NMR (CD<sub>3</sub>CN, 100 MHz) δ 164.2 (C=S, major), 138.0 (C, minor), 137.7 (C–N), 133.0 (C), 132.7 (C, minor), 132.3 (CH, minor), 131.9 (CH, major), 131.5 (C), 126.1 (CH, minor), 125.1 (CH, major), 124.2 (CH, minor), 123.5 (CH), 114.1 (CN, major), 114.1



**Fig. 10.** Cytotoxicity of XIV, XVIa, XVIIIa,c, XXVd and XXVib against brain tumor cell line U251, human hepatocellular carcinoma cell line HEPG2, human breast adenocarcinoma cell line MCF-7, human epithelial carcinoma cell line HELA and colon carcinoma cell line HCT116.

(CN, minor). MS ( $m/z$ , %) 203 (100, M + -HCN), 204 (19), 205 (73), 206 (12), 207 (28), 161 (16).

**5.1.1.7. (4-Methoxyphenyl)carbamothioyl cyanide (Ig).** Tautomeric ratio 57:43;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  9.62 (br s, 1 H, NH, major), 9.51 (br s, 1 H, NH, minor), 7.72 (d,  $J = 9.0$  Hz, 2H, Ar-H, major), 7.31 (d,  $J = 9.0$  Hz, 2H, Ar-H, minor), 9.67 (d,  $J = 9.0$  Hz, 2H, Ar-H, minor), 6.95 (d,  $J = 9.0$  Hz, 2H, Ar-H, major), 3.85 (s, 3H, minor), 3.84 (s, 3H, major);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  165.9 (C=S, minor), 161.0 (C=N), 159.9 (O-C, minor), 159.0 (O-C, major), 129.9 (C-N, major), 129.8 (C-N, minor), 124.9 ( $m$ -CH, minor), 124.2 ( $m$ -CH, major), 115.0 ( $o$ -CH, minor), 114.4 ( $o$ -CH, major), 113.7 (CN, major), 112.0 (CN, minor), 55.7 ( $\text{OCH}_3$ , minor), 55.6 ( $\text{OCH}_3$ , major).

**5.1.2. Preparation of compounds IIa–c; the triethylammonium salts were prepared according to the following general procedure**

Equimolar amounts (5.0 mmol) of the corresponding cyanothioformanilide and triethylamine in dry diethyl ether (20 mL) were stirred at room temperature for 25 min. The resulting salt was filtered off, washed with a minimum amount of ether, air-dried and recrystallized to give IIa–c (Table 10).

**Table 4**

Anti-infectivity effect of compounds VIe–g, VIII–c, IXa,b and XXVib against HAV, HSV1 and COxB4 viral strains.

Sample no.	Selected dilution <sup>a</sup>	Anti-infectivity effect		
		Viral strain		
		HAV	HSV1	CoxB4
VIe	$10^{-4}$	40%	–ve <sup>b</sup>	–ve
VIf	$10^{-4}$	–ve	–ve	–ve
VIg	$10^{-4}$	–ve	50%	–ve
VIIIa	$10^{-3}$	–ve	50%	–ve
VIIIb	$10^{-4}$	–ve	–ve	–ve
VIIIc	$10^{-3}$	50%	70%	50%
IXa	$10^{-2}$	50%	50%	60%
IXb	$10^{-2}$	–ve	40%	–ve
XXVib	$10^{-4}$	–ve	50%	–ve

<sup>a</sup> Sample dilution at which no cytotoxicity on Vero cells was detected for 3 days.

<sup>b</sup> No anti-infectivity effect was observed.

**5.1.2.1. Triethylammonium (4-bromophenyl)carbamothioyl cyanide (IIa).** Tautomeric ratio 75:25;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.47 (d,  $J = 8.8$  Hz, 2H, Ar-H, major and minor), 7.19 (d,  $J = 8.8$  Hz, 2H, Ar-H, major), 6.95 (d,  $J = 8.8$  Hz, 2H, Ar-H, minor), 6.37–5.89 (br s, 1H, NH), 3.07 (q,  $J = 7.3$  Hz, 6H), 1.31 (t,  $J = 7.3$  Hz, 9H).

**5.1.2.2. Triethylammonium (4-iodophenyl)carbamothioyl cyanide (IIb).** Tautomeric ratio 91:9;  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 400 MHz)  $\delta$  7.67 (d,  $J = 8.5$  Hz, 2H, Ar-H, minor), 7.60 (d,  $J = 8.8$  Hz, 2H, Ar-H, major), 7.02 (d,  $J = 8.8$  Hz, 2H, Ar-H, major), 6.79 (d,  $J = 8.8$  Hz, 2H, Ar-H, minor), 3.83–2.76 (br s, 1H, NH), 3.09 (q,  $J = 7.3$  Hz, 6H), 1.22 (t,  $J = 7.3$  Hz, 9H);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{CN}$ , 100 MHz)  $\delta$  160.4 (C=S), 152.1, 152.0, 138.9 (CH, minor), 138.1 (CH), 125.7 (CH), 119.7 (CN), 86.4 (C-I), 47.8 ( $\text{CH}_2$ ), 9.3 ( $\text{CH}_3$ ).

**5.1.2.3. Triethylammonium (3,4-dichlorophenyl)carbamothioyl cyanide (IIc).** Tautomeric ratio 80:20;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.39 (d,  $J = 2.3$  Hz, 1 H, Ar-H), 7.38 (d,  $J = 8.6$  Hz, 1H, Ar-H), 7.07 (dd,  $J = 8.6$ , 2.3 Hz, 1H, Ar-H), 7.03 (d,  $J = 2.5$  Hz, 1H, Ar-H, minor), 6.97–6.65 (br s, NH), 6.81 (dd,  $J = 8.6$ , 2.5 Hz, 1H, Ar-H, minor), 3.83–2.26 (br s, 1H, NH), 3.15 (q,  $J = 7.4$  Hz, 2H), 1.36 (t,  $J = 7.4$  Hz, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  159.8 (C=S, major), 157.0 (C=S, minor), 149.0 (C, major), 149.8 (C, minor), 132.7 (C, minor), 132.2 (C,

**Table 5**

Antimicrobial effect of compounds VIId–g, VIIIa–c and IXa–b against Gram negative and Gram positive bacteria.

Compound no.	Mean values of inhibition zone diameter (mm)			
	<i>E. coli</i>	<i>S. typhi</i>	<i>B. subtilis</i>	<i>S. aureus</i>
VIId	16.0	18.0	16.0	16.5
VIe	16.5	17.0	17.0	18.0
VIf	19.0	17.0	16.0	16.0
VIg	18.0	16.5	16.0	17.5
VIIIa	17.0	18.0	17.0	19.0
VIIIb	14.0	16.0	13.0	ND <sup>a</sup>
VIIIc	15.0	17.0	13.5	13.5
IXa	19.0	17.5	17.0	15.5
IXb	11.0	12.0	14.0	13.0

<sup>a</sup> ND: Not determined.

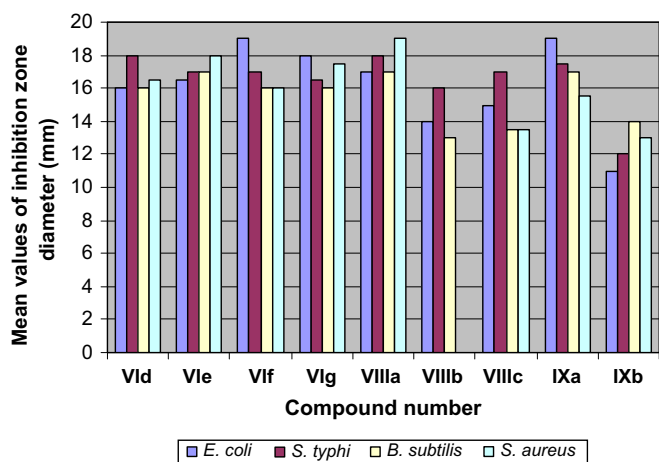


Fig. 11. Antibacterial activities of compounds **VId–g**, **VIIa–c** and **IXa–b** against Gram negative and Gram positive bacteria.

major), 130.8 (CH, minor), 130.3 (CH, major), 128.2 (C, minor), 127.3 (C, major), 123.7 (CH, major), 123.3 (CH, minor), 121.9 (CH, major), 121.0 (CH, minor), 118.6 (CN, major), 113.4 (CN, minor), 46.3 (CH<sub>2</sub>), 8.89 (CH<sub>3</sub>).

**5.1.2.4. N-(3,4-Dichlorophenyl)hydrazinecarbothioamide (III).** A solution of **If** (5 mmol) in ethanol (10 mL) was treated with excess hydrazine hydrate (15 mmol) and the reaction mixture was stirred at room temperature for 15 min, then diluted with cold water. The obtained product was filtered off, washed with cold water, air-dried and recrystallized to give **III** (Table 10): IR (KBr) 3400–3150 (NH & NH<sub>2</sub>), 1490 & 1150 (CSN) cm<sup>-1</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>CN, 400 MHz)  $\delta$  9.15 (br s, 1 H, NH), 7.17 (d,  $J$  = 8.6 Hz, 1H, Ar–H), 6.77 (d,  $J$  = 2.8 Hz, 1H, Ar–H), 6.56 (dd,  $J$  = 8.6, 2.8 Hz, 1 H, Ar–H), 4.41 (br s, 2H, NH<sub>2</sub>); <sup>13</sup>C NMR (CD<sub>3</sub>CN, 100 MHz)  $\delta$  189.6 (C=S), 149.3 (C), 132.7 (C), 128.6 (C), 131.6 (CH), 116.3 (CH), 115.4 (CH).

**5.1.2.5. 2-(4-Iodophenylamino)-3,3-diphenylacrylonitrile (IV).** To a well stirred ethereal solution of diphenyldiazomethane (excess), a solution of **Ie** (5 mmol) in dry ether (20 mL) was added dropwise and the mixture was stirred for 25 min. The obtained product was filtered off, washed with a minimum amount of ether, air-dried and recrystallized to give **IV** (Table 10): IR (KBr) 3285 (NH), 2225 (CN) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.59 (d,  $J$  = 8.8 Hz, 2H), 7.42–7.39 (m, 5H), 7.37–7.34

(m, 3H), 7.23–7.19 (m, 2H), 6.72 (d,  $J$  = 8.8 Hz, 2H), 5.43 (br s, 1H, NH); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  143.7 (C), 141.5 (C), 138.2 (CH), 137.6 (C), 137.4 (C), 130.1 (CH), 129.3 (2CH), 129.2 (CH), 129.0 (CH), 128.4 (CH), 118.4 (CH), 115.8 (C), 109.7 (CN), 84.1 (C–I); MS ( $m/z$ , %) 422 (100, M<sup>+</sup>), 295 (11), 77 (17)

**5.1.2.6. 3-(3,4-Dichlorophenyl)-5-imino-2-methyloxazolidine-4-thione (V).** To a solution of **If** (5 mmol) in ether (25 mL), acetaldehyde (5 mmol) was added, followed by three 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 to give **V** (Table 10): IR (KBr) 3270 (NH), 3090 (CH arom.), 1490 & 1170 (CSN) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  8.83 (br s, 1 H, NH), 7.68 (d,  $J$  = 2.5 Hz, 1 H, Ar–H), 7.64 (d,  $J$  = 8.8 Hz, 1 H, Ar–H), 7.38 (dd,  $J$  = 8.8, 2.5 Hz, 1 H, Ar–H), 6.09 (q,  $J$  = 5.6 Hz, 1 H, CH), 1.59 (d,  $J$  = 5.6 Hz, 3 H, CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  176.8 (C=S), 164.7 (C=N), 134.7 (C), 134.2 (C), 133.8 (C), 131.7 (CH), 127.1 (CH), 124.4 (CH), 91.2 (OCH), 20.3 (CH<sub>3</sub>); MS ( $m/z$ , %) 274 (<1, M<sup>+</sup>), 127 (100).

**5.1.3. Preparation of compounds VIa–g, VIIa–c and XIIIa–c; the preceding compounds were prepared according to the following general procedure**

A solution of the corresponding cyanothioformanilide (5 mmol) in ether (15 mL) was treated with a solution of the respective isocyanate (0.005 mol) in ether (10 mL), followed by three drops of triethylamine. The reaction mixture was magnetically stirred for 25 min. The obtained product was filtered off, washed with a minimum amount of ether, air-dried and recrystallized to give **VIa–g**, **VIIa–c** and **XIIIa–c** (Table 10).

**5.1.3.1. chlorophenyl)imidazolidin-2-one (VId).** IR (KBr) 3250 (NH), 1765 (C=O), 1669 (C=N), 1500 & 1210 (CSN) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  9.53 (br s, 1H, NH), 7.72 (s, 1H), 7.69 (d,  $J$  = 8.8 Hz, 2H), 7.62 (s, 1H), 7.36 (d,  $J$  = 8.8 Hz, 2H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  180.5 (C, C=S), 152.9 (C, C=O), 152.3 (C, C=N), 135.5 (C), 132.8 (C–H), 132.3 (C), 132.2 (C), 131.9 (CH), 131.8 (CH), 131.3 (C), 128.7 (C), 128.6 (CH); MS ( $m/z$ , %) 461 (7, M<sup>+</sup>), 229 (100), 428 (59), 213 (62), 75 (34).

**5.1.3.2. 4-Imino-1-(4-iodophenyl)-5-thioxo-3-(2,4,5-trichlorophenyl)imidazolidin-2-one (VIe).** <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  9.53 (br s, 1H, NH), 7.89 (d,  $J$  = 8.8 Hz, 2H), 7.71 (s, 1H), 7.62 (s, 1H), 7.23 (d,  $J$  = 8.8 Hz, 2H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  180.5 (C, C=S), 152.9 (C, C=O), 152.3 (C, C=N), 138.8 (C–H), 135.5 (C), 132.3 (C), 132.1 (C), 132.0 (C), 131.9 (CH), 131.8 (CH), 128.7 (CH), 95.7 (C–I).

**5.1.3.3. 1-(4-Chlorophenyl)-3-(3,4-dichlorophenyl)-5-imino-4-thioxoimidazolidin-2-one (VIg).** IR (KBr) 3257 (NH), 1761 (C=O), 1665 (C=N), 1495 & 1200 (CSN) cm<sup>-1</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>CN, 400 MHz)  $\delta$  9.62 (br s, 1 H, NH), 7.76 (d,  $J$  = 8.4 Hz, 1 H, Ar–H), 7.69 (d,  $J$  = 2.4 Hz, 1 H, Ar–H), 7.58–7.56 (m, 4H), 7.48 (dd,  $J$  = 8.4, 2.4 Hz, 1 H, Ar–H); <sup>13</sup>C NMR (DMSO, 100 MHz)  $\delta$  182.5 (C=S), 153.7 (C=O), 153.2 (C=O), 133.1 (C), 132.7 (C), 132.5 (C), 131.6 (C), 131.5 (CH), 131.3 (C), 129.9 (CH), 129.1 (2XCH), 128.8 (2XCH), 128.5 (CH); Note: the aromatic signals which are stemming from the *p*-substituted chlorobenzene and having chemical shifts ranging from 7.58 to 7.52 appear as two doublet with DMSO as a solvent  $\delta$  9.87 (br s, 1 H, NH), 7.92 (d,  $J$  = 8.8 Hz, 1 H, Ar–H), 7.89 (d,  $J$  = 2.3 Hz, 1 H, Ar–H), 7.65 (d,  $J$  = 8.8 Hz, 2 H, Ar–H), 7.59 (d,  $J$  = 8.0 Hz, 2 H, Ar–H); Note: the aromatic dd overlaps with one of the *p*-substituted chlorobenzene signals. MS ( $m/z$ , %) 385 (70, M<sup>+</sup>), 203 (100), 153 (91), 126 (14).

**5.1.3.4. 1-(4-Bromophenyl)-3-(4-chloro-3-(trifluoromethyl)phenyl)-4-imino-5-thioxoimidazolidin-2-one (VIg).** IR (KBr) 3263 (NH), 1770

**Table 6**  
Antimicrobial effect of compounds **If**, **Iic**, **V**, **VIg**, **XIIIa**, **XIV**, **XV**, **XXVib** and **XXVibc** against Gram negative and Gram positive bacteria.

Mean values of inhibition zone diameter (mm)						
Sample conc.	Gram negative			Gram positive		
	Test organism					
	<i>P. aerogenosa</i>			<i>B. subtilis</i>		
	1 mg/mL	2.5 mg/mL	5 mg/mL	1 mg/mL	2.5 mg/mL	5 mg/mL
<b>If</b>	35	37	38	40	41	43
<b>Iic</b>	13	16	19	15	16	17
<b>V</b>	15	29	41	20	27	33
<b>VIg</b>	15	16	18	13	13	14
<b>XIIIa</b>	15	16	18	17	18	19
<b>XIV</b>	–	13	15	13	14	15
<b>XV</b>	15	18	20	15	19	20
<b>XXVb</b>	29	32	37	19	20	20
<b>XXVib</b>	18	19	21	21	25	32
<b>XXVlc</b>	15	17	20	19	21	24



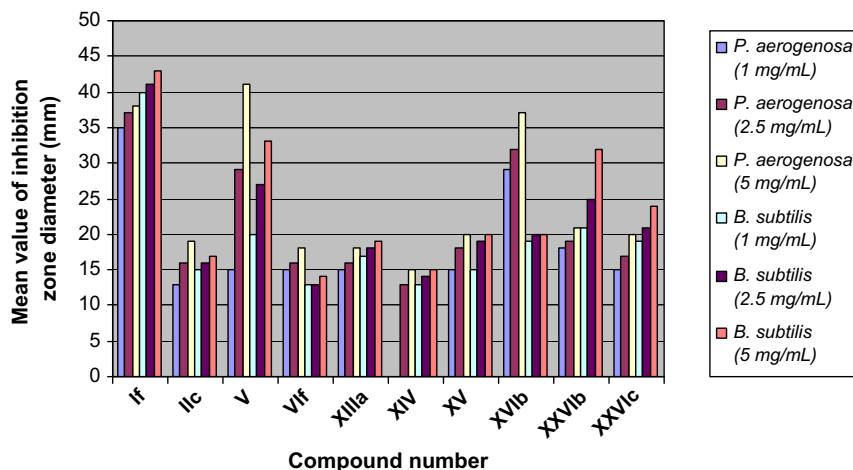


Fig. 12. Antibacterial activities of compounds If, Ilc, V, VIf, XIIIa, XIV, XV, XVib and XXVib,c against Gram-negative and Gram-positive bacteria.

(C=O), 1674 (C=N), 1512 & 1238 (CSN)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  9.70 (br s, 1H, NH), 8.02 (d,  $J$  = 2.4 Hz, 1H), 7.79 (dd,  $J$  = 8.8, 2.4 Hz, 1H), 7.70 (d,  $J$  = 8.8 Hz, 2H, Ar-H), 7.67 (d,  $J$  = 8.8 Hz, 1H), 7.37 (d,  $J$  = 8.8 Hz, 2H, Ar-H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  180.4 (C, C=S), 153.2 (C, C=O), 153.0 (C, C=N), 132.9 (CH), 132.4 (CH), 132.3 (C), 131.3 (C-Cl), 130.7 (C-N), 130.3 (CH), 129.5 (q,  $J$  = 32.3 Hz, C), 128.6 (CH), 125.5 (q,  $J$  = 5.3 Hz, CH), 124.0 (C-Br), 122.2 (q,  $J$  = 273.5 Hz,  $\text{CF}_3$ ).  $^{19}\text{F}$  NMR ( $\text{CDCl}_3$ , 376 MHz)  $\delta$  -63.0; MS ( $m/z$ , %) 461 (48,  $\text{M}^+$ ), 213 (100).

5.1.3.5. 1-(4-Ethoxyphenyl)-3-(9H-fluoren-2-yl)-4-imino-5-thioxoimidazolidin-2-one (VIIIa). IR (KBr) 3251 (NH), 1767 (C=O), 1669 (C=N), 1490 & 1200 (CSN)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  9.51 (br s, 1H, NH), 7.93 (d,  $J$  = 8.2 Hz, 1H), 7.83 (d,  $J$  = 7.5 Hz, 1H), 7.74 (br s, 1H, Ar-H), 7.76–7.55 (m, 2H, Ar-H), 7.44–7.32 (m, 4H, Ar-H), 7.05 (d,  $J$  = 8.8 Hz, 2H, Ar-H), 4.10 (q,  $J$  = 7.0 Hz, 2H), 3.99 (s, 2 H,  $\text{CH}_2$ ), 1.44 (t,  $J$  = 7.0 Hz, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  182.4 (C, C=S), 159.7 (C, C=O), 154.8 (C, C=O), 154.4 (C, C=N), 144.3 (C), 143.7 (C), 142.2 (C), 140.7 (C), 130.6 (C), 128.5 (CH), 127.5 (CH), 127.1 (CH), 125.7 (CH), 125.4 (C), 125.3 (CH), 123.7 (CH), 120.5 (CH), 120.4 (CH), 115.2 (CH), 64.0 ( $\text{CH}_2$ ), 37.1 ( $\text{CH}_2$ ), 14.8 ( $\text{CH}_3$ ); MS ( $m/z$ , %) 413 (96,  $\text{M}^+$ ), 207 (100), 179 (34).

5.1.3.6. 1-(4-Bromophenyl)-3-(9H-fluoren-2-yl)-4-imino-5-thioxoimidazolidin-2-one (VIIIb). IR (KBr) 3255 (NH), 1770 (C=O), 1670 (C=N), 1500 & 1210 (CSN)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  9.61 (br s, 1H, NH), 7.92 (d,  $J$  = 8.4 Hz, 1H), 7.82 (d,  $J$  = 7.2 Hz, 1H), 7.74–7.67 (m, 3H, Ar-H), 7.59–7.53 (m, 2H, Ar-H), 7.44–7.38 (m, 3H, Ar-

H), 7.35 (td,  $J$  = 7.3, 1.3 Hz, 1H, Ar-H), 3.98 (s, 2 H,  $\text{CH}_2$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  181.2 (C, C=S), 154.3 (C, C=O), 153.6 (C, C=N), 144.3 (C), 143.6 (C), 142.4 (C), 140.6 (C), 132.8 (CH), 131.7 (C), 130.0 (C), 128.7 (CH), 127.4 (CH), 127.0 (CH), 125.3 (CH), 125.1 (CH), 123.7 (C, C-Br), 123.3 (CH), 120.5 (CH), 120.3 (CH), 37.0 ( $\text{CH}_2$ ); MS ( $m/z$ , %) 447 (55,  $\text{M}^+$ ), 213 (13), 207 (100), 165 (59).

5.1.3.7. 1-(9H-Fluoren-2-yl)-5-imino-3-(4-iodophenyl)-4-thioxoimidazolidin-2-one (VIIIc). IR (KBr) 3247 (NH), 1761 (C=O), 1670 (C=N), 1491 & 1195 (CSN)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  9.61 (br s, 1H, NH), 7.93–7.87 (m, 3H), 7.81 (d,  $J$  = 7.7 Hz, 1H), 7.71 (d,  $J$  = 1.3 Hz, 1H), 7.58–7.53 (m, 2H, Ar-H), 7.40 (t,  $J$  = 7.4 Hz, 1H, Ar-H), 7.35 (td,  $J$  = 7.3, 1.3 Hz, 1H, Ar-H), 7.29–7.24 (m, 2H, Ar-H), 3.98 (s, 2 H,  $\text{CH}_2$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  181.1 (C, C=S), 154.3 (C, C=O), 153.6 (C, C=N), 144.3 (C), 143.6 (C), 142.4 (C), 140.6 (C), 138.7 (CH), 132.4 (C), 130.0 (C), 128.8 (CH), 127.4 (CH), 127.0 (CH), 125.3 (CH), 125.1 (CH), 123.3 (CH), 120.5 (CH), 120.3 (CH), 95.5 (C, C-I), 37.0 ( $\text{CH}_2$ ); MS ( $m/z$ , %) 495 (99,  $\text{M}^+$ ), 261 (22), 207 (100), 165 (49).

5.1.4. Preparation of compounds IXa–c; the preceding compounds were prepared according to the following general procedure

The corresponding diones VIIIa–c (5 mmol) were dissolved in boiling ethanol (20 mL) and treated with dil. HCl (1:1 molar ratio). The obtained product was filtered off, washed with cold water, air-dried and recrystallized to give IXa–c (Table 10).

Table 7

Antifungal effect of compounds VId–g, VIIIa–c and IXa–b against *C. albicans* and *A. niger*.

Compound no.	Mean values of inhibition zone diameter (mm)	
	<i>C. albicans</i>	<i>A. niger</i>
VId	14.0	ND <sup>a</sup>
VIe	17.0	20.0
VIf	17.0	21.0
VIg	16.0	16.0
VIIIa	15.0	20.0
VIIIb	13.0	ND
VIIIc	13.0	ND
IXa	14.5	ND
IXb	13.0	13.0

<sup>a</sup> ND: Not determined.

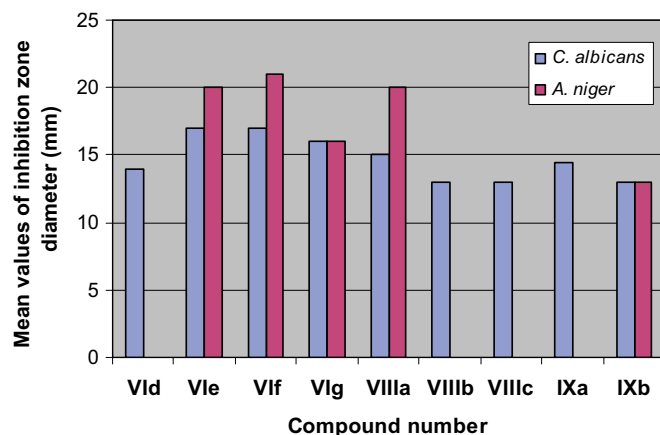


Fig. 13. Antifungal activities of compounds VId–g, VIIIa–c and IXa,b against *C. albicans* and *A. niger*.

**Table 8**Antifungal effect of compounds **If**, **V**, **VIf**, **XIIIa**, **XIV**, **XV**, **XVIb** and **XXVIb,c** against *C. albicans* and *A. niger*.

Mean values of inhibition zone diameter (mm)						
Sample conc.	Test organism					
	<i>C. albicans</i>			<i>A. niger</i>		
	1 mg/mL	2.5 mg/mL	5 mg/mL	1 mg/mL	2.5 mg/mL	5 mg/mL
<b>If</b>	37	40	44	29	45	56
<b>V</b>	21	33	39	ND <sup>a</sup>	11	20
<b>VIf</b>	ND	13	14	ND	15	17
<b>XIIIa</b>	ND	12	14	ND	ND	12
<b>XIV</b>	12	14	15	ND	ND	13
<b>XV</b>	15	17	18	ND	ND	ND
<b>XVIb</b>	25	29	32	ND	ND	ND
<b>XXVIb</b>	18	20	23	15	18	20
<b>XXVIc</b>	19	20	21	12	15	17

<sup>a</sup> ND: Not determined.

**5.1.4.1. 1-(4-Ethoxyphenyl)-3-(9H-fluoren-2-yl)-5-thioxoimidazolidine-2,4-dione (IXa).** IR (KBr) 1770 (2XC=O), 1509 & 1205 (CSN)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  8.05 (d,  $J = 8.1$  Hz, 1H), 7.96 (d,  $J = 7.3$  Hz, 1H), 7.82 (d,  $J = 1.3$  Hz, 1H, Ar-H), 7.72 (d,  $J = 7.3$  Hz, 2H, Ar-H), 7.65 (dd,  $J = 8.1$ , 2.0 Hz, 2H, Ar-H), 7.59–7.47 (m, 4H, Ar-H), 7.21–7.16 (m, 2H, Ar-H), 4.23 (q,  $J = 7.0$  Hz, 2H), 4.12 (s, 2 H,  $\text{CH}_2$ ), 1.60 (t,  $J = 7.0$  Hz, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  182.7 (C, C=S), 159.8 (C, C=O), 153.6 (C, C=O), 153.1 (C, C=O), 144.3 (C), 143.6 (C), 142.7 (C), 140.4 (C), 128.5 (C), 128.4 (CH), 127.5 (CH), 127.0 (CH), 125.2 (CH), 124.6 (CH), 124.4 (C), 122.7 (CH), 120.5 (CH), 120.4 (CH), 115.3 (CH), 63.9 ( $\text{CH}_2$ ), 37.0 ( $\text{CH}_2$ ), 14.8 ( $\text{CH}_3$ ); MS ( $m/z$ , %) 414 (84,  $\text{M}^+$ ), 207 (100), 179 (69), 165 (29), 151 (95).

**5.1.4.2. 1-(4-Bromophenyl)-3-(9H-fluoren-2-yl)-4-imino-5-thioxoimidazolidin-2-one (IXb).** IR (KBr) 1770 (2XC=O)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.92 (d,  $J = 8.3$  Hz, 1H), 7.82 (d,  $J = 7.2$  Hz, 1H), 7.73–7.68 (m, 2H, Ar-H), 7.68–7.65 (m, 1H, Ar-H), 7.58 (d,  $J = 7.6$  Hz, 1H, Ar-H), 7.50 (dd,  $J = 8.4$ , 2.0 Hz, 1H, Ar-H), 7.44–7.34 (m, 4H), 3.98 (s, 2 H,  $\text{CH}_2$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  182.0 (C, C=S), 153.1 (C, C=O), 152.8 (C, C=O), 144.4 (C), 143.6 (C), 142.8 (C), 140.4 (C), 132.9 (CH), 131.0 (C), 128.8 (CH), 128.3 (C), 127.6 (CH), 127.1 (CH), 125.2 (CH), 124.6 (CH), 124.0 (C), 122.6 (CH), 120.5 (CH), 120.4 (CH), 37.0 ( $\text{CH}_2$ ); MS ( $m/z$ , %) 448 (37,  $\text{M}^+$ ), 213 (11), 207 (100), 165 (52).

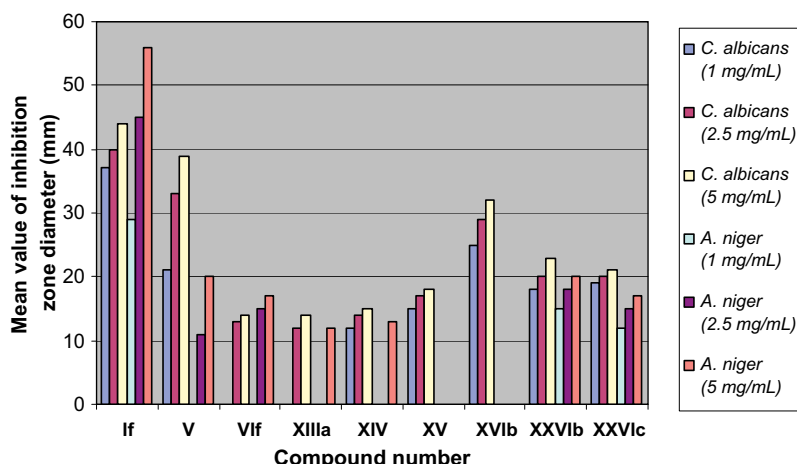
**5.1.4.3. 3-(9H-fluoren-2-yl)-1-(4-iodophenyl)-5-thioxoimidazolidine-2,4-dione (IXc).**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.94–7.89 (m, 3H), 7.83 (d,  $J = 7.3$  Hz, 1H), 7.67 (d,  $J = 1.3$  Hz, 1H), 7.58 (d,  $J = 7.4$  Hz,

1H), 7.50 (dd,  $J = 8.3$ , 2.0 Hz, 1H), 7.42 (t,  $J = 7.4$  Hz, 1H, Ar-H), 7.37 (td,  $J = 7.5$ , 1.3 Hz, 1H, Ar-H), 7.27–7.23 (m, 2H, Ar-H), 4.00 (s, 2 H,  $\text{CH}_2$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  181.9 (C, C=S), 153.0 (C, C=O), 152.8 (C, C=O), 144.4 (C), 143.6 (C), 142.8 (C), 140.4 (C), 138.9 (CH), 131.7 (C), 128.9 (CH), 128.3 (C), 127.6 (CH), 127.1 (CH), 125.2 (CH), 124.6 (CH), 122.6 (CH), 120.5 (CH), 120.4 (CH), 95.7 (C, C-I), 37.0 ( $\text{CH}_2$ ).

**5.1.4.4. 3-Benzyl-5-(benzylimino)-1-(4-ethoxyphenyl)imidazolidine-2,4-dione (X).** A solution of **IXa** (5 mmol) in ethanol (25 mL) was treated with excess benzylamine (15 mmol). The reaction mixture was heated under reflux for 1 h, cooled down and poured into cold dil. HCl (1:1 molar ratio). The obtained product was filtered off, washed with cold water, air-dried and recrystallized to give **X** (Table 10):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.49 (dd,  $J = 8.1$ , 2.0 Hz, 2H), 7.42–7.26 (m, 12H, Ar-H), 5.35 (s, 2 H,  $\text{CH}_2$ ), 4.92 (s, 2 H,  $\text{CH}_2$ ), 4.05 (q,  $J = 7.0$  Hz, 2H), 1.43 (t,  $J = 7.0$  Hz, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  158.9 (C, C=N), 155.0 (C, C=N), 153.2 (C, C=O), 140.6 (C), 139.9 (C), 136.0 (C), 122.7 (C), 129.2 (CH), 128.6 (CH), 128.0 (CH), 124.4 (C), 127.6 (CH), 127.4 (CH), 126.9 (CH), 115.0 (CH), 63.8 ( $\text{CH}_2$ ), 53.0 ( $\text{CH}_2$ ), 42.9 ( $\text{CH}_2$ ), 14.7 ( $\text{CH}_3$ ); MS ( $m/z$ , %) 413 (33,  $\text{M}^+$ ), 322 (42), 148 (79), 91 (100).

**5.1.4.5. 1-(4-Bromophenyl)-3-(4-chloro-3-(trifluoromethyl)phenyl)-5-thioxoimidazolidine-2,4-dione (XI).** Imidazolidineiminothione **Vlg** (5 mmol) was dissolved in boiling ethanol (20 mL) and treated with dil. HCl (1:1 molar ratio). The obtained product was filtered off, washed with cold water, air-dried and recrystallized to give **XI** (Table 10):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.94 (d,  $J = 2.1$  Hz, 1H), 7.73–7.69 (m, 3H), 7.55–7.48 (m, 1H), 7.34 (d,  $J = 8.8$  Hz, 2H, Ar-H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  180.8 (C=S), 152.3 (C=O), 151.7 (C=O), 133.0 (CH), 132.7 (CH), 130.6 (C), 129.9 (C), 129.6 (CH), 129.4 (q,  $J = 32.0$  Hz, C), 129.0 (C), 128.7 (CH), 124.8 (q,  $J = 5.2$  Hz, CH), 124.3 (C-Br), 122.0 (q,  $J = 272.7$  Hz,  $\text{CF}_3$ ).

**5.1.4.6. 3-Benzyl-5-(benzylimino)-1-(4-bromophenyl)imidazolidine-2,4-dione (XII).** The benzylimine **XII** (Table 10) was prepared following the same procedure used to prepare its analogue benzylimine **X**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.61 (d,  $J = 8.84$  Hz, 2H), 7.51–7.42 (m, 2H), 7.41–7.28 (m, 10H), 5.35 (s, 2H,  $\text{NCH}_2$ ), 4.93 (s, 2H,  $\text{NCH}_2$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  154.3 (C), 152.5 (C), 140.2 (C), 139.7 (C), 135.7 (C), 132.4 (CH), 129.5 (C), 129.3 (CH), 128.7 (CH), 128.5 (CH), 128.1 (CH), 127.6 (CH), 127.0 (CH), 127.3 (CH), 127.0 (CH), 122.4 (C), 53.1 ( $\text{CH}_2$ ), 43.0 ( $\text{CH}_2$ ).

**Fig. 14.** Antifungal activities of compounds **If**, **V**, **VIf**, **XIIIa**, **XIV**, **XV**, **XVIb** and **XXVIb,c** against *C. albicans* and *A. niger*.

**Table 9**

Minimum inhibitory concentrations of compounds **XXVib**, **XXVlc**, **XVib** and **If** against various strains of Gram positive and Gram negative bacteria and fungi.

Minimum inhibitory concentration MIC ( $\mu\text{g/mL}$ )						
Compound no.	Test organism					
	Gram positive bacteria		Gram negative bacteria		Fungus	
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>K. pneumonia</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>A. niger</i>
<b>XXVib</b>	3.9	15.6	7.8	1.95	1.95	0.49
<b>XXVlc</b>	3.9	15.6	7.8	1.95	1.95	0.49
<b>XVib</b>	1.95	15.6	3.9	0.49	0.97	31.3
<b>If</b>	0.97	3.9	1.95	0.24	0.97	15.6

### 5.1.5. Preparation of **XIIIa–c**: see the general procedure described earlier for the preparation of compounds **Vla–g**, **VIIIa–c** and **XIIIa–c**

**5.1.5.1. 1-(4-Chlorophenyl)-4-imino-3-(naphthalen-1-yl)-5-thioxoimidazolidin-2-one (XIIIa)**. IR (KBr) 3261 (NH), 1760 (C=O), 1670 (C=N), 1510 & 1190 (CSN)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  9.52 (br s, 1H, NH), 8.01 (dd,  $J$  = 6.8, 2.4 Hz, 1H), 7.98–7.93 (m, 1H), 7.73–7.66 (m, 1H), 7.63–7.48 (m, 8H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  181.3 (C=S), 154.5 (C=O), 153.7 (C=N), 135.6 (C), 134.6 (C), 131.1 (C), 130.7 (CH), 129.8 (CH), 129.7 (C), 128.9 (CH), 128.5 (CH), 128.1 (C), 127.5 (CH), 127.0 (CH), 126.9 (CH), 125.6 (CH), 122.1 (CH); MS ( $m/z$ , %) 365 (100,  $\text{M}^+$ ), 212 (85), 169 (63), 153 (11).

**5.1.5.2. 1-(4-Bromophenyl)-4-imino-3-(naphthalen-1-yl)-5-thioxoimidazolidin-2-one (XIIIb)**. IR (KBr) 3261 (NH), 1760 (C=O), 1670 (C=N), 1510 & 1190 (CSN)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  9.51 (br s, 1H, NH), 8.03–7.91 (m, 2H), 7.72–7.64 (m, 1H), 7.67 (d,  $J$  = 8.8 Hz, 2H), 7.62–7.52 (m, 4H), 7.43 (d,  $J$  = 8.8 Hz, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  181.1 (C=S), 154.5 (C=O), 153.6 (C=N), 134.6 (C), 132.7 (CH), 131.6 (C), 130.6 (CH), 129.6 (C), 128.8 (CH), 128.6 (CH), 128.0 (C), 127.4 (CH), 126.9 (CH), 126.8 (CH), 125.5 (CH), 123.7 (C), 122.0 (CH); MS ( $m/z$ , %) 409 (84,  $\text{M}^+$ ), 212 (100), 168 (80), 153 (11).

**5.1.5.3. 1-(4-Methoxy)-4-imino-3-(naphthalen-1-yl)-5-thioxoimidazolidin-2-one (XIIIc)**.  $^1\text{H}$  NMR (DMSO, 400 MHz)  $\delta$  9.58 (s, 1H, NH), 8.20–8.15 (m, 1H), 8.12 (d,  $J$  = 8.4 Hz, 1H), 8.10–8.05 (m, 1H), 7.78 (dd,  $J$  = 7.2, 1.2 Hz, 1H), 7.72–7.67 (m, 1H), 7.65–7.60 (m, 2H), 7.58 (d,  $J$  = 9.2 Hz, 2H), 7.15 (d,  $J$  = 9.2 Hz, 2H), 3.84 (s, 3H,  $\text{OCH}_3$ );  $^{13}\text{C}$  NMR (DMSO, 100 MHz)  $\delta$  183.5 (C=S), 159.6 (C), 154.9 (C), 154.3 (C), 133.8 (C), 130.1 (C), 129.6 (CH), 129.3 (C), 129.1 (CH), 128.1 (CH), 127.4 (CH), 126.9 (CH), 126.6 (CH), 126.0 (C), 125.7 (CH), 123.6 (CH), 114.3 (CH), 55.4 ( $\text{OCH}_3$ ).

### 5.1.6. Preparation of bis-imidazolidineiminothiones **XIV** and **XV**

To a solution of **Ib** or **If** (10 mmol, 2.0 equiv.) in ether (20 mL), a solution of the corresponding bisocyanate (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 re-crystallized to give **XIV** and **XV** (Table 10), respectively.

**5.1.6.1. 3,3'-(4,4'-Methylenebis(2,6-diethyl-4,1-phenylene))bis(1-(4-ethoxyphenyl)-4-imino-5-thioxoimidazolidin-2-one) (XIV)**. IR (KBr) 3228 (NH), 1779 (C=O), 1662 (C=N), 1531 & 1185 (CSN)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  9.42 (br s, 2H, NH), 7.39 (d,  $J$  = 8.8 Hz, 4H), 7.10 (s, 4H), 7.03 (d,  $J$  = 8.8 Hz, 4H), 4.08 (q,  $J$  = 7.0 Hz, 4H), 4.04 (s, 2H), 2.53 (q,  $J$  = 7.5 Hz, 8H), 1.45 (t,  $J$  = 7.0 Hz, 6H), 1.20 (t,  $J$  = 7.5 Hz, 12H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  181.9 (C=S), 159.6 (C), 154.4 (C), 154.2 (C), 142.4 (C), 128.3 (CH), 127.7 (CH), 126.8 (C), 125.1 (C), 115.1

(CH), 63.8 ( $\text{OCH}_2$ ), 41.7 ( $\text{CH}_2$ ), 24.6 ( $\text{CH}_2$ ), 14.7 ( $\text{CH}_3$ ), 14.4 ( $\text{CH}_3$ ); MS ( $m/z$ , %) 774 (51,  $\text{M}^+$ ), 151 (100), 362 (19), 361 (26).

**5.1.6.2. 3,3'-(4,4'-Oxybis(4,1-phenylene))bis(1-(3,4-dichlorophenyl)-4-imino-5-thioxoimidazolidin-2-one) (XV)**.  $^1\text{H}$  NMR (DMSO, 400 MHz)  $\delta$  9.80 (br s, 1H, NH), 7.92 (d,  $J$  = 8.5 Hz, 2H), 7.91 (d,  $J$  = 2.3 Hz, 2H), 7.62 (dd,  $J$  = 8.5, 2.3 Hz, 2H), 7.61 (d,  $J$  = 8.8 Hz, 4H), 7.28 (d,  $J$  = 8.8 Hz, 4H);  $^{13}\text{C}$  NMR (DMSO, 100 MHz)  $\delta$  182.6 (C=S), 155.9 (C), 154.0 (C), 153.3 (C), 133.1 (C), 132.4 (C), 131.5 (C), 131.4 (CH), 129.9 (CH), 129.2 (CH), 128.4 (CH), 127.9 (C), 119.2 (CH).

**5.1.6.3. 1-(4-Chlorophenyl)-3-(3,4-dichlorophenyl)-4-thioxoimidazolidin-2-one (XVib)**. To a solution of **Vif** (5 mmol) in benzene (25 mL), triethylamine (3 drops) was added, then a stream of  $\text{H}_2\text{S}$  gas was bubbled in for 15 min. The obtained product was filtered off, washed with a minimum amount of dry benzene and recrystallized to give **XVib** (Table 10). The benzene mother liquor upon concentration yielded a product which crystallized to give **XVII** (Table 10): IR (KBr) 1750 (C=O), 1490 & 1150 (CSN)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.82–7.79 (m, 1 H, Ar-H), 7.55–7.48 (m, 4 H, Ar-H), 7.39–7.34 (m, 2 H, Ar-H), 4.83 (s, 2 H,  $\text{CH}_2$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  194.9 (C=S), 152.9 (C=O), 136.2 (C), 135.6 (C), 133.6 (C), 131.4 (C), 131.1 (C), 131.1 (CH), 129.7 (CH), 128.9 (CH), 128.6 (C), 119.8 (CH), 117.2 (CH), 59.4 ( $\text{NCH}_2$ ); MS ( $m/z$ , %) 370 (62,  $\text{M}^+$ ), 187 (24), 153 (16).

**5.1.6.4. 1-(4-Chlorophenyl)-3-(3,4-dichlorophenyl)-4-mercapto-1H-imidazol-2(3H)-one and 4,4'-disulfanediybis(1-(4-chlorophenyl)-3-(3,4-dichlorophenyl)-1H-imidazol-2(3H))-one (XVII)**. (mix. of thiol and disulfide: 68:32). IR (KBr) 1740 (C=O)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.64 (d,  $J$  = 8.5 Hz, 1H, major), 7.61 (m, 1H, minor), 7.57–7.52 (m, 2H, both isomers), 7.44–7.35 (m, 3H, both), 7.33–7.29 (m, 1H), 6.89 (d,  $J$  = 2.5 Hz, 1H, major), 6.80 (d,  $J$  = 2.5 Hz, 1H, minor), 6.75–6.64 (m, 2H, both);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  150.2 (C=O, minor), 150.1 (C=O, major), 135.3 (C), 135.2 (C), 134.8 (C), 133.8 (C), 133.6 (C), 133.1 (C, major), 133.0 (C, minor), 132.6 (C), 131.8 (C), 131.7 (2XC), 131.2 (CH, major), 131.1 (CH, minor), 130.8 (CH, major), 130.7 (CH, minor), 130.2 (CH, major), 129.9 (CH, major), 129.8 (CH, minor), 129.6 (CH), 129.5 (CH), 128.0 (CH), 127.5 (CH, minor), 127.4 (CH, major), 126.5 (CH, minor), 126.3 (CH, minor), 124.0 (CH, minor), 123.9 (CH, major), 123.4 (C, minor), 123.2 (C, major), 117.0 (C), 116.8 (C), 116.0 (C); MS ( $m/z$ , %) 740 (6,  $\text{M}^+$ ), 706 (17), 187 (5), 171 (19), 153 (6), 64 (100).

### 5.1.7. Preparation of compounds **XVIIIa–c**; the preceding compounds were prepared according to the following general procedure

A mixture of the corresponding imidazolidineiminothione **VI** (5 mmol) and the requisite *o*-phenylenediamine or its derivatives (5 mmol) in ethanol (25 mL) was heated under reflux until the evolution of ammonia and  $\text{H}_2\text{S}$  gases were ceased as detected by their odor (approx. 7 h). The obtained product was filtered off, washed with a minimum amount of ethanol and crystallized to give **XVIIIa–c** (Table 10).

**5.1.7.1. 3-(4-Chlorophenyl)-6-methyl-1-p-tolyl-1H-imidazo[4,5-b]quinoxalin-2(3H)-one (XVIIIa)**. (two regioisomers: 1:1). IR (KBr) 1730 (C=O), 1630 (C=N)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.89–7.83 (m, 3H), 7.76 (s, 1H), 7.71–7.65 (m, 2H), 7.56 (d,  $J$  = 8.8 Hz, 2H), 7.46–7.38 (m, 3H), 2.54 (s, 3H, major), 2.53 (s, 3H, minor), 2.46 (s, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  152.1 (C=O), 139.2 (C), 138.9 (C), 138.8 (C), 138.7 (C), 138.3 (C), 138.0 (C), 137.9 (C), 137.3 (C), 137.1 (C), 133.7 (C), 130.1 (C), 130.0 (CH), 129.7 (CH), 129.6 (CH), 129.5 (CH), 21.5 (CH), 21.3 ( $\text{CH}_3$ ); MS ( $m/z$ , %) 400 (100,  $\text{M}^+$ ), 289 (11), 246 (27), 91 (19).

**Table 10**

Physical data of the synthesized compounds.

Compd. no.	Cryst. solvent M.P [°C]	Yield %	Mol. formula M.Wt.	Elemental analysis			
				Calcd./Found [%]			
				C	H	N	S
<b>IIa</b>	<sup>a</sup> C/H	73	C <sub>14</sub> H <sub>20</sub> BrN <sub>3</sub> S (341)	49.12	5.89	12.28	9.37
	80			49.00	5.80	12.20	9.20
<b>IIb</b>	C/H	75	C <sub>14</sub> H <sub>20</sub> IN <sub>3</sub> S (389)	43.19	5.18	10.79	8.24
	100			43.10	5.20	10.65	8.20
<b>IIc</b>	C/H	50	C <sub>14</sub> H <sub>19</sub> Cl <sub>2</sub> N <sub>3</sub> S (331)	50.60	5.76	12.65	9.65
	120			50.50	5.60	12.60	9.50
<b>III</b>	<sup>b</sup> E/W (1:1)	30	C <sub>7</sub> H <sub>7</sub> Cl <sub>2</sub> N <sub>3</sub> S (235)	35.61	2.99	17.80	13.58
	220			35.50	2.90	17.65	13.50
<b>IV</b>	C/H	40	C <sub>21</sub> H <sub>15</sub> IN <sub>2</sub> (422)	59.73	3.58	6.63	–
	190			59.60	3.50	6.55	–
<b>V</b>	C/H	55	C <sub>10</sub> H <sub>8</sub> Cl <sub>2</sub> N <sub>2</sub> OS (274)	43.65	2.93	10.18	11.65
	87			43.50	2.90	10.10	11.60
<b>VIa</b>	E <sup>c</sup>	65	C <sub>15</sub> H <sub>7</sub> Cl <sub>3</sub> BrN <sub>3</sub> OS (461.5)	38.86	1.52	9.06	6.92
	189			38.70	1.40	8.95	6.90
<b>VIe</b>	E	63	C <sub>15</sub> H <sub>7</sub> Cl <sub>3</sub> IN <sub>3</sub> OS (509.5)	35.29	1.38	8.23	6.28
	149			35.20	1.20	8.20	6.20
<b>VIg</b>	E	67	C <sub>15</sub> H <sub>8</sub> Cl <sub>3</sub> N <sub>3</sub> OS (383.5)	46.76	2.29	10.92	8.32
	176			46.70	2.20	10.80	8.20
<b>VIIg</b>	E	70	C <sub>16</sub> H <sub>8</sub> F <sub>3</sub> ClBrN <sub>3</sub> OS (461.5)	41.54	1.74	9.08	6.93
	142			41.50	1.60	8.95	6.80
<b>VIIIa</b>	E	65	C <sub>24</sub> H <sub>19</sub> N <sub>3</sub> O <sub>2</sub> S (413)	69.71	4.63	10.16	7.75
	196			69.60	4.50	10.00	7.70
<b>VIIIb</b>	E	60	C <sub>22</sub> H <sub>14</sub> BrN <sub>3</sub> OS (447)	58.94	3.15	9.37	7.15
	186			58.80	3.10	9.20	7.10
<b>VIIIc</b>	E	70	C <sub>22</sub> H <sub>14</sub> IN <sub>3</sub> OS (495)	53.34	2.85	8.48	6.47
	202			53.20	2.80	8.40	6.40
<b>IXa</b>	E/W (1:1)	45	C <sub>24</sub> H <sub>18</sub> N <sub>2</sub> O <sub>3</sub> S (414)	69.55	4.35	6.76	7.73
	195			69.40	4.30	6.70	7.60
<b>IXb</b>	E/W (1:1)	50	C <sub>22</sub> H <sub>13</sub> BrN <sub>2</sub> O <sub>2</sub> S (448)	58.76	2.89	6.23	7.12
	205			58.60	2.80	6.20	7.00
<b>IXc</b>	E/W (1:1)	55	C <sub>22</sub> H <sub>13</sub> IN <sub>2</sub> O <sub>2</sub> S (496)	53.24	2.64	5.64	6.46
	240			53.10	2.60	5.60	6.30
<b>X</b>	e/H <sup>d</sup>	50	C <sub>25</sub> H <sub>23</sub> N <sub>3</sub> O <sub>3</sub> (413)	72.64	5.57	10.17	–
	120			72.60	5.50	10.00	–
<b>XI</b>	e/H	53	C <sub>16</sub> H <sub>7</sub> F <sub>3</sub> ClBrN <sub>2</sub> O <sub>2</sub> S (462.5)	41.51	1.51	6.05	6.92
	125			41.40	1.40	5.90	6.82
<b>XII</b>	e/H	51	C <sub>23</sub> H <sub>8</sub> BrN <sub>3</sub> O <sub>2</sub> (447)	63.69	3.82	8.92	–
	175			63.60	3.70	8.80	–
<b>XIIIa</b>	C/H	69	C <sub>19</sub> H <sub>12</sub> ClN <sub>3</sub> OS (365.5)	62.38	3.28	11.49	8.76
	205			62.20	3.20	11.40	8.70
<b>XIIIb</b>	C/H	72	C <sub>19</sub> H <sub>12</sub> BrN <sub>3</sub> OS (409)	55.62	2.93	10.27	7.82
	190			55.50	2.80	10.20	7.70
<b>XIIIc</b>	C/H	75	C <sub>20</sub> H <sub>15</sub> N <sub>3</sub> O <sub>2</sub> S (361)	66.48	4.15	11.63	8.86
	210			66.40	4.10	11.50	8.80
<b>XIV</b>	C/H	65	C <sub>43</sub> H <sub>46</sub> N <sub>6</sub> O <sub>4</sub> S <sub>2</sub> (774)	66.67	5.94	10.85	8.27
	200			66.50	5.90	10.80	8.20
<b>XV</b>	C/H	60	C <sub>30</sub> H <sub>16</sub> Cl <sub>4</sub> N <sub>6</sub> O <sub>3</sub> S <sub>2</sub> (714)	50.43	2.26	11.76	8.98
	230			50.30	2.20	11.70	8.80
<b>XVIb</b>	E	55	C <sub>15</sub> H <sub>9</sub> Cl <sub>3</sub> N <sub>2</sub> OS (371.5)	48.47	2.44	7.54	8.63
	239			48.40	2.30	7.50	8.50
<b>XVII</b>	E	50	C <sub>30</sub> H <sub>16</sub> Cl <sub>6</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> (740)	48.65	2.16	7.57	8.65
	195			48.60	2.10	7.50	8.50
<b>XVIIIa</b>	E	63	C <sub>23</sub> H <sub>17</sub> ClN <sub>4</sub> O (400)	69.00	4.25	14.00	–
	253			68.80	4.10	13.90	–
<b>XVIIIb</b>	E	60	C <sub>23</sub> H <sub>18</sub> N <sub>4</sub> O <sub>2</sub> (382)	72.25	4.71	14.66	–
	220			72.10	4.60	14.50	–
<b>XVIIIc</b>	Dioxane	65	C <sub>21</sub> H <sub>11</sub> Cl <sub>3</sub> N <sub>4</sub> O (440)	57.27	2.50	12.73	–
	317			57.10	2.40	12.60	–
<b>XXIa</b>	E	75	C <sub>28</sub> H <sub>19</sub> ClIN <sub>5</sub> O (603)	55.72	3.15	11.61	–
	200			55.60	3.10	11.50	–
<b>XXIb</b>	E	77	C <sub>28</sub> H <sub>19</sub> Cl <sub>2</sub> N <sub>5</sub> O (511)	65.75	3.72	13.70	–
	190			65.70	3.60	13.60	–
<b>XXIc</b>	E	73	C <sub>28</sub> H <sub>19</sub> ClBrN <sub>5</sub> O (555)	60.54	3.42	12.61	–
	200			60.50	3.30	12.50	–
<b>XXIVa</b>	E	55	C <sub>28</sub> H <sub>19</sub> ClBrN <sub>3</sub> O (527)	63.76	3.60	7.97	–
	170			63.70	3.50	7.70	–
<b>XXIVb</b>	E	51	C <sub>28</sub> H <sub>19</sub> ClIN <sub>3</sub> O (575)	58.43	3.30	7.30	–
	157			58.40	3.20	7.20	–
<b>XXIVc</b>	E	59	C <sub>28</sub> H <sub>18</sub> Cl <sub>3</sub> N <sub>3</sub> O (517)	64.99	3.48	8.10	–
	191			64.90	3.40	8.00	–



Table 10 (continued)

Compd. no.	Cryst. solvent M.P [°C]	Yield %	Mol. formula M.Wt.	Elemental analysis			
				Calcd./Found [%]			
				C	H	N	S
<b>XXIVd</b>	E	50	C <sub>30</sub> H <sub>24</sub> ClN <sub>3</sub> O <sub>2</sub> (497)	73.02	4.87	8.59	–
	185			72.90	4.80	8.50	–
<b>XXVa</b>	E	45	C <sub>16</sub> H <sub>12</sub> IN <sub>7</sub> O (444)	43.24	2.70	22.07	–
	205			43.10	2.60	21.90	–
<b>XXVb</b>	E	52	C <sub>16</sub> H <sub>11</sub> Cl <sub>2</sub> N <sub>7</sub> O (387)	49.61	2.84	25.32	–
	185			69.70	2.80	25.20	–
<b>XXVc</b>	E	50	C <sub>17</sub> H <sub>14</sub> ClN <sub>7</sub> O (367)	55.59	3.82	26.70	–
	190			55.60	2.70	26.60	–
<b>XXVIa</b>	C/H	55	C <sub>17</sub> H <sub>14</sub> IN <sub>4</sub> OS <sub>2</sub> (467)	43.68	3.00	8.99	13.71
	130			43.60	2.90	8.90	13.60
<b>XXVIb</b>	C/H	60	C <sub>15</sub> H <sub>9</sub> Br <sub>2</sub> N <sub>3</sub> S <sub>2</sub> (453)	39.74	1.99	9.27	14.13
	220			39.60	1.90	9.20	14.10
<b>XXVIc</b>	C/H	57	C <sub>15</sub> H <sub>9</sub> I <sub>2</sub> N <sub>3</sub> S <sub>2</sub> (549)	32.78	1.64	7.65	11.66
	170			32.80	1.50	7.50	11.50

<sup>a</sup> C/H: Chloroform/*n*-hexane.<sup>b</sup> E/W: Ethanol/water.<sup>c</sup> E: Ethanol.<sup>d</sup> e/H: Diethylether/*n*-hexane.

5.1.7.2. 1-(4-Ethoxyphenyl)-3-phenyl-1*H*-imidazo[4,5-*b*]quinoxalin-2(3*H*)-one (**XXVIIIb**). IR (KBr) 1735 (C=O), 1635 (C=N) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.96 (dd, *J* = 6.2, 3.5 Hz, 2H), 7.88–7.84 (m, 2H), 7.70 (d, *J* = 8.9 Hz, 2H), 7.63–7.56 (m, 4H), 7.50–7.45 (m, 1H), 7.10 (d, *J* = 8.9 Hz, 2H), 4.12 (q, *J* = 7.0 Hz, 2H), 1.47 (t, *J* = 7.0 Hz, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 158.8 (C=O), 152.5 (C), 139.5 (C), 139.2 (2XC), 139.1 (C), 132.3 (C), 129.3 (CH), 128.2 (CH), 128.0 (2XCH), 127.5 (CH), 127.4 (CH), 125.8 (CH), 124.5 (C), 115.2 (CH), 63.8 (CH<sub>2</sub>), 14.8 (CH<sub>3</sub>); MS (*m/z*, %) 382 (100, M<sup>+</sup>), 354 (66), 353 (39).

5.1.7.3. 1-(4-Chlorophenyl)-3-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-*b*]quinoxalin-2(3*H*)-one (**XXVIIIc**). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 8.11 (d, *J* = 2.4 Hz, 1H), 8.03–7.95 (m, 2H), 7.86–7.81 (m, 1H), 7.83 (d, *J* = 8.8 Hz, 2H), 7.70–7.63 (m, 3H), 7.59 (d, *J* = 8.8 Hz, 2H); MS (*m/z*, %) 440 (97, M<sup>+</sup>), 352 (29), 329 (13), 187 (10), 111 (10).

5.1.8. Preparation of compounds **XXIa–c**; the preceding compounds were prepared according to the following general procedure

A solution of the corresponding imidazolidineiminothione **VI** (5 mmol) in boiling ethanol was treated with benzophenone hydrazone (5 mmol), followed by triethylamine (3 drops). The reaction mixture was heated under reflux for 3 h. The obtained product was filtered off, washed with a minimum amount of ethanol and crystallized to give **XXIa–c** (Table 10).

5.1.8.1. (*E*)-1-(4-chlorophenyl)-4-((diphenylmethylene)hydrazono)-5-imino-3-(4-iodophenyl)imidazolidin-2-one (**XXIa**). IR (KBr) 3271 (NH), 1760 (C=O), 1627 (C=N) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 10.5 (br s, 1H, NH), 7.77–7.68 (m, 2H, Ar–H), 7.56 (d, *J* = 8.8 Hz, 2H), 7.54–7.35 (m, 10H), 7.29–7.24 (m, 2H), 7.04 (d, *J* = 8.8 Hz, 2H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 169.9 (C), 151.9 (C), 149.1 (C), 140.0 (C), 136.7 (C), 134.9 (C), 134.0 (C), 131.5 (C), 137.6 (CH), 131.3 (CH), 130.2 (C), 130.0 (C), 129.6 (CH), 129.4 (CH), 129.3 (2XCH), 128.5 (CH), 128.2 (CH), 127.9 (2XCH), 92.8 (C–I); MS (*m/z*, %) 575 (48, M<sup>+</sup> – N<sub>2</sub>), 574 (100), 526 (34), 357 (27), 77 (56).

5.1.8.2. (*E*)-1,3-bis(4-chlorophenyl)-4-((diphenylmethylene)hydrazono)-5-iminoimidazolidin-2-one (**XXIb**). IR (KBr) 3271 (NH), 1760 (C=O), 1627 (C=N) cm<sup>-1</sup>; MS (*m/z*, %) 511 (100, M<sup>+</sup>), 434 (80), 317 (15), 193 (27), 168 (18).

5.1.8.3. (*E*)-1-(4-bromophenyl)-3-(4-chlorophenyl)-5-((diphenylmethylene)hydrazono)-4-iminoimidazolidin-2-one (**XXIc**). IR (KBr)

3271 (NH), 1760 (C=O), 1627 (C=N) cm<sup>-1</sup>; MS (*m/z*, %) 557 (13, M<sup>+</sup> + 2), 402 (14), 361 (14), 165 (100), 77 (44).

5.1.9. Preparation of compounds **XXIVa–d**; the preceding compounds were prepared according to the following general procedure

To a solution of the respective imidazolidineiminothione **VI** (5 mmol) in dry ether (20 mL), a solution of diphenyldiazo-methane (15 mmol; excess) in dry ether (20 mL) was added. The reaction mixture was stirred at room temperature for 30 min. The obtained product was filtered off, washed with a minimum amount of dry ether, air-dried and crystallized to give **XXIVa–d** (Table 10).

5.1.9.1. 1-(4-Chlorophenyl)-4-(diphenylmethylene)-5-imino-3-*p*-tolylimidazolidin-2-one (**XXIVa**). IR (KBr) 3229 (NH), 1757 (C=O), 1657 (C=N), 1600 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.57–7.29 (m, 11H, NH & Ar–H), 6.97–6.85 (m, 4H, Ar–H), 6.84–6.78 (m, 4H, Ar–H), 2.16 (s, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 156.2 (C), 153.9 (C), 138.9 (C), 138.1 (C), 136.5 (C), 133.4 (C), 132.2 (C), 131.4 (C), 130.1 (CH), 129.7 (CH), 129.6 (CH), 129.1 (CH), 129.0 (CH), 128.7 (CH), 127.7 (C), 127.5 (CH), 127.3 (CH), 126.3 (CH), 125.1 (C), 20.9 (CH<sub>3</sub>); MS (*m/z*, %) 463 (35, M<sup>+</sup>), 462 (100), 165 (23), 77 (44).

5.1.9.2. 1-(4-Chlorophenyl)-4-(diphenylmethylene)-5-imino-3-(4-iodophenyl)imidazolidin-2-one (**XXIVb**). IR (KBr) 3229 (NH), 1757 (C=O), 1657 (C=N), 1600 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.50–7.41 (m, 7H, 1 NH & 6Ar–H), 7.40–7.33 (m, 4H, Ar–H), 7.08–7.02 (m, 2H, Ar–H), 6.99–6.93 (m, 2H, Ar–H), 6.85–6.79 (m, 4H, Ar–H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 155.8 (C), 153.5 (C), 138.4 (C), 137.9 (C), 137.5 (C), 134.6 (C), 133.6 (C), 131.2 (C), 137.1 (CH), 130.0 (CH), 129.8 (CH), 129.6 (CH), 129.3 (CH), 129.1 (CH), 128.7 (CH), 128.1 (CH), 128.0 (CH), 127.7 (CH), 124.4 (C), 91.4 (C–I); MS (*m/z*, %) 575 (35, M<sup>+</sup>), 574 (100, M<sup>+</sup> – H), 447 (13), 165 (41).

5.1.9.3. 1-(4-Chlorophenyl)-3-(3,4-dichlorophenyl)-4-(diphenylmethylene)-5-iminoimidazolidin-2-one (**XXIVc**). IR (KBr) 3229 (NH), 1757 (C=O), 1657 (C=N), 1600 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.50 (br s, 1 H, NH), 7.48–7.39 (m, 7H, Ar–H), 7.38–7.33 (m, 2H, Ar–H), 7.10 (d, *J* = 8.8 Hz, 1H), 7.07 (d, *J* = 2.3 Hz, 1H), 7.04–6.96 (m, 4H, Ar–H), 6.87–6.80 (m, 2H, Ar–H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 155.5 (C), 153.4 (C), 138.2 (C), 138.0 (C), 134.0 (C), 133.8

(C), 131.9 (C), 131.1 (C), 130.6 (C), 130.0 (CH), 129.9 (CH), 129.6 (CH), 129.5 (CH), 129.3 (CH), 129.1 (CH), 128.7 (CH), 128.6 (C), 128.4 (CH), 128.2 (CH), 127.8 (CH), 125.9 (CH), 124.3 (C); MS ( $m/z$ , %) 517 (32,  $M^+$ ), 516 (100,  $M^+ - H$ ), 165 (30).

**5.1.9.4. 1-(4-Chlorophenyl)-4-(diphenylmethylene)-3-(4-ethoxyphenyl)-5-iminoimidazolidin-2-one (XXIVd).** IR (KBr) 3220 (NH), 1750 (C=O), 1655 (C=N), 1605 (C=C)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.51–7.11 (m, 10H, NH & Ar-H), 7.00–6.87 (m, 5H, Ar-H), 6.84–6.77 (m, 2H, Ar-H), 6.57–6.49 (m, 2H, Ar-H), 3.88 (q,  $J = 7.0$  Hz, 2H), 1.32 (t,  $J = 7.0$  Hz, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  157.3 (C), 156.1 (C), 154.0 (C), 138.9 (C), 138.0 (C), 133.3 (C), 131.5 (C), 127.5 (C), 127.4 (C), 130.0 (CH), 129.7 (CH), 129.6 (CH), 129.0 (CH), 128.9 (CH), 128.6 (CH), 127.7 (CH), 127.6 (CH), 127.3 (CH), 125.3 (C), 114.1 (CH), 63.6 ( $\text{CH}_2$ ), 14.6 ( $\text{CH}_3$ ); MS ( $m/z$ , %) 493 (72,  $M^+$ ), 492 (100,  $M^+ - H$ ), 165 (26).

**5.1.10. Preparation of compounds XXVa–c; the preceding compounds were prepared according to the following general procedure**

A mixture of the requisite imidazolidineiminothione **VI** (5 mmol) and thiocarbonylhydrazide (5 mmol) in ethanol (25 mL) was heated under reflux for 7 h. Liberation of  $\text{H}_2\text{S}$  could be easily detected through reaction time. The obtained product was filtered off, washed with a minimum amount of ethanol, air-dried and crystallized to give **XXVa–c** (Table 10).

**5.1.10.1. 3-Hydrazinyl-7-(4-iodophenyl)-5-phenyl-5H-imidazo[4,5-e][1,2,4]triazin-6(7H)-one (XXVa).** IR (KBr) 3431 ( $\text{NH}_2$ ), 3297 (NH), 1760 (C=O)  $\text{cm}^{-1}$ ; MS ( $m/z$ , %) 445 (1<,  $M^+$ ), 245 (71), 230 (16), 203 (15), 119 (44), 103 (27), 90 (100), 77 (91).

**5.1.10.2. 5,7-Bis(4-chlorophenyl)-3-hydrazinyl-5H-imidazo[4,5-e][1,2,4]triazin-6(7H)-one (XXVb).** IR (KBr) 3431 ( $\text{NH}_2$ ), 3297 (NH), 1760 (C=O)  $\text{cm}^{-1}$ ; MS ( $m/z$ , %) 388 (13,  $M^+ + 1$ ), 387 (1<,  $M^+$ ), 353 (18), 268 (12), 200 (6), 187 (100), 119 (58).

**5.1.10.3. 5-(4-Chlorophenyl)-3-hydrazinyl-7-(4-methoxyphenyl)-5H-imidazo[4,5-e][1,2,4]triazin-6(7H)-one (XXVc).** IR (KBr) 3430 ( $\text{NH}_2$ ), 3290 (NH), 1765 (C=O)  $\text{cm}^{-1}$ ; MS ( $m/z$ , %) 384 (19,  $M^+ + 1$ ), 383 (1<,  $M^+$ ), 353 (20), 235 (5), 200 (6), 153 (48), 149 (100).

**5.1.11. Preparation of compounds XXVla–c; the preceding compounds were prepared according to the following general procedure**

An equimolar mixture of the requisite cyanothioformamide **I** and isothiocyanate (5 mmol) and triethylamine (3 drops) in dry ether (25 mL) was stirred at room temperature for 30 min. The reaction mixture was triturated several times with light petroleum ether until solidification. The resulting solid was collected and recrystallized to give **XXVla–c** (Table 10).

**5.1.11.1. 3-(4-Ethoxyphenyl)-5-imino-1-(4-iodophenyl)imidazolidine-2,4-dithione & 5-(4-ethoxyphenylimino)-4-imino-3-(4-iodophenyl)thiazolidine-2-thione (XXVla).** Ratio dithione:dimiminothiazole 34:66;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  9.50 (br s, 1 H, N-H, major), 9.48 (br s, 1 H, N-H, minor), 7.93–7.84 (m, 3H, 2H major and 1H minor overlapping), 7.27–7.19 (m, 4H, 2H major and 2H minor overlapping), 7.09 (d,  $J = 8.7$  Hz, 2H, major), 7.02 (d,  $J = 9.0$  Hz, 1H, minor), 6.97 (d,  $J = 8.8$  Hz, 2H, major), 4.07 (q,  $J = 7.0$ , 2 H,  $\text{CH}_2$ ), 1.44 (t,  $J = 7.0$  Hz, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  192.1 (C, C=S, major), 180.2 (C=S, minor), 180.0 (C=S, minor), 161.2 (C, major), 159.8 (C, major), 158.9 (C, major), 156.1 (C, minor), 145.1 (C, major), 139.7 (C, major), 138.9 (CH, major), 138.6 (CH, minor), 135.2 (C, major), 133.6 (C, minor), 130.3 (CH, major), 130.0 (CH, minor), 129.2 (CH, minor), 127.3 (C, minor), 127.2 (C, minor), 123.9 (CH, major),

115.3 (CH, major), 115.1 (CH, minor), 95.7 (C, major), 95.3 (C, minor), 63.8 ( $\text{CH}_2$ , major), 63.7 ( $\text{CH}_2$ , minor), 14.7 ( $\text{CH}_3$ );

**5.1.11.2. 1,3-Bis(4-bromophenyl)-5-iminoimidazolidine-2,4-dithione (XXVib).** IR (KBr) 3250 ( $\text{NH}_2$ ), 1495 & 1150 (CS–N)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  9.53 (br s, 1H, NH), 7.70 (d,  $J = 8.8$  Hz, 2H, Ar-H), 7.69 (d,  $J = 8.8$  Hz, 2H, Ar-H), 7.37 (d,  $J = 8.8$  Hz, 2H, Ar-H), 7.26 (d,  $J = 8.8$  Hz, 2H, Ar-H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  179.6 (2XC, C=S), 156.0 (C, C=N), 134.0 (2XC–N), 132.9 (CH), 132.8 (CH), 130.0 (CH), 129.9 (CH), 124.4 (C–Br), 123.4 (C–Br); MS ( $m/z$ , %) 455 (14,  $M^+$ ), 453 (17), 374 (12), 240 (16).

**5.1.11.3. 5-Imino-1,3-bis(4-iodophenyl)imidazolidine-2,4-dithione & 4-imino-3-(4-iodophenyl)-5-(4-iodophenylimino)thiazolidine-2-thione (XXVic).** Ratio prior to crystallization was dithione:dimiminothiazole 35:65; ratio after re-crystallization was dithione:dimiminothiazole 68:32; IR (KBr) 3250 ( $\text{NH}_2$ ), 1495 & 1150 (CS–N)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  9.57 (br s, 1H, NH, minor), 9.52 (br s, 1H, NH, major), 7.93–7.88 (app ddd consisting of three overlapping doublets, 6H, minor 2H and major 4H), 7.80 (d,  $J = 8.8$  Hz, 2H, Ar-H, minor), 7.24 (d,  $J = 8.8$  Hz, 2H, Ar-H, major), 7.13 (d,  $J = 8.8$  Hz, 2H, Ar-H, major), 7.10 (d,  $J = 8.8$  Hz, 2H, Ar-H, minor), 6.93 (d,  $J = 8.8$  Hz, 2H, Ar-H, minor);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  191.0 (C=S, minor), 179.6 (C=S major), 179.5 (C=S major), 160.7 (C=N, minor), 156.0 (C=N, major), 150.5 (C), 147.4 (C), 139.1 (CH), 138.9 (CH), 138.8 (CH), 138.8 (CH), 135.1 (C), 134.7 (C), 133.5 (C), 130.3 (CH), 130.1 (CH), 130.0 (CH), 122.7 (CH), 96.2 (C–I), 96.0 (C–I), 95.5 (C–I), 92.7 (C–I); MS ( $m/z$ , %) 549 (19,  $M^+$ ), 288 (5), 261 (57).

## 5.2. Biological evaluation

### 5.2.1. Screening studies of compounds XIV, XVIa, XVIIIa,c, XXVd and XXVib against EAC cells

Anti tumor activity (in vitro) studies were done at Cairo University, National Cancer Institute, Cancer Biology Department, Pharmacology Unit.

#### 5.2.1.1. Reagents.

1. RPMI 1640 medium (Sigma)
2. Ehrlich Ascites Carcinoma cells (EAC) suspension ( $2.5 \times 10^5$  /mL)
3. Trypan blue dye: A stock solution was prepared by dissolving 1 g of the dye in (100 mL) distilled water. The working solution was then prepared by diluting (1 mL) of the stock solution with (9 mL) of distilled water. The stain was used then for staining the dead EAC cells.
4. Compounds tested: **XIV**, **XVIa**, **XVIIIa,c**, **XXVd** and **XXVib**.

#### 5.2.1.2. Procedure.

1. EAC cells were obtained by needle aspiration of ascetic fluid from the preinoculated mice under aseptic conditions.
2. 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.
3. The ascetic fluid was diluted to 1:10 with saline to contain  $2.5 \times 10^6$  cells on a hemocytometer.
4. In a set of sterile 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  $\mu\text{g}$ ) were mixed. The test tubes were incubated at  $37^\circ\text{C}$  for 2 h. Trypan blue exclusion test was carried out to calculate the percentage of non viable cells. Compounds producing more than 70% non-viable cells are considered active [43].

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

### 5.2.2. Cytotoxic activity studies of **XIV**, **XVIa**, **XVIIIa,c**, **XXVd** and **XXVib** against various cell lines

Compounds **XIV**, **XVIa**, **XVIIIa,c**, **XXVd** and **XXVib** were tested at concentrations between 1 and 10 µg/mL using SRB assay for cytotoxic activity against the following tumor cell lines:

1. Brain tumor cell line (U251)
2. Liver carcinoma cell line (HEPG2)
3. Breast carcinoma cell line (MCF7)
4. Cervix carcinoma cell line (HELA)
5. Colon carcinoma cell line (HCT116)

### 5.2.3. Measurement of potential cytotoxicity by SRB assay

Potential cytotoxicity of compounds **XIV**, **XVIa**, **XVIIIa,c**, **XXVd** and **XXVib** was tested using the method of Skehan et al. [44], as follows:

- Cells were plated in 96 multiwell plate (10<sup>4</sup> cells/well) for 24 h before treatment with the compound(s) to allow attachment to the wall of the plate.
- Different concentrations of the compounds (0, 1, 2.5, 5 and 10 µg/mL) were added to the cell monolayer triplicate wells were prepared for each individual dose.
- Monolayer cells were incubated with the compound(s) for 48 h at 37 °C in atmosphere of 5% CO<sub>2</sub>.
- After 48 h, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain.
- Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer.
- Color intensity was measured in an ELISA reader.
- The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line after the specified compound.

### 5.2.4. Virucidal activity studies

Compounds **Vle-g**, **VIII-c**, **IXa,b** and **XXVib** were tested against HAV, HSV1 and COxB4 viral strains and viral activity was assayed by the plaque formation method.

#### 5.2.4.1. Determination of the anti-infectivity effect of compounds **Vle-g**, **VIII-c**, **IXa,b** and **XXVib** against HAV, HSV1 and COxB4 viral strains.

1. 0.1 mL of (10<sup>3</sup>) virus suspension were mixed with 0.1 mL of sample at the selected dilution shown in Table 4 (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<sup>3</sup>) 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 incubated 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 the plaque formation method [45].

### 5.2.5. Antimicrobial and antifungal studies of compounds **VId-g**, **VIIIa-c** and **IXa-b**

Antimicrobial and antifungal studies were carried out by the diffusion agar technique [46] using a 1 cm microplate well diameter and a 100 µL of a sample concentration of 5 mg/mL, unless otherwise indicated. The tests were done by Dr. Samy El-Henawy, Tarik M.A. Al-Raheem and Deya A. Ftooh at the Fermentation Biotechnology and Applied Microbiology (Ferm-BAM) Center, Al-Azhar University, Cairo, Egypt. The tested organisms were Gram-negative bacteria (*E. coli*, NCTC-10416 & *S. typhi*, NCIMB-9331), Gram-positive bacteria (*S. aureus*, NCTC-7447 & *B. subtilis*, NCTC-1040) and fungi (*C. albicans*, IMRU-3669 & *A. niger*, IMI-31276). 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.

### 5.2.6. Antimicrobial and antifungal studies of compounds **If**, **V**, **VIf**, **XIIIa**, **XIV**, **XV**, **XVib** and **XXVib,c**

The above compounds were tested in vitro for antimicrobial activity against *B. subtilis*, MCIB-3610 and *P. aeruginosa*, NCIB9016 and for antifungal activity against *C. albicans* and *A. niger* Ferm-BAM C-21 using a 1 cm microplate well diameter and a 100 µL of each of the following three concentrations: 1, 2.5 and 5 mg/mL. The compounds were tested for antifungal activity by the agar disk diffusion technique under the same conditions of the first series of compounds with the exception that antimicrobial activities were measured using three different sample concentrations. All compounds were dissolved in DMF.

### 5.2.7. Antimicrobial and antifungal susceptibility testing of compounds **XXVib,c**, **XVib**, and **If**

The Minimum Inhibitory Concentration (MIC) of the four tested compounds was determined by the microtube dilution technique following the guidelines of the National Committee for Clinical Laboratory Standards for bacteria [41] and for filamentous fungi [42]. The four Compounds were tested for their Minimum Inhibitory Concentration (MIC) against the Gram positive bacteria *B. subtilis*, NCTC-1040 & *S. aureus*, NCTC-7447, the Gram negative bacteria *Klebsiella pneumonia*, NCIMB-9111 & *S. typhi*, NCIMB-9331 and against filamentous fungi (*A. niger*, Ferm-BAM C-21) and unicellular fungi (*C. albicans*, IMRU-3669). The results are summarized in Table 9. The bacteria and fungi were maintained on nutrient broth medium and Czapek's Dox medium, respectively.

#### 5.2.7.1. Procedure

- 1) Prepare 15 sterile capped test tubes for each test microorganism.
- 2) All of the following steps are carried out using aseptic technique.
- 3) Add 2.0 mL of dimethylformamide and 2 mg of the tested compound to the first tube and mix the contents well.
- 4) Add 1.0 mL of sterile broth medium to all other tubes.
- 5) Transfer 1.0 mL from the first tube to the second tube.
- 6) Using a separate pipette, mix the contents of this tube and transfer 1.0 mL to the third tube.
- 7) Continue dilutions in this manner to tube number 14, being certain to change pipettes between tubes to prevent carry-over of tested compound on the external surface of the pipette.
- 8) Remove 1.0 mL from tube 14 and discard it. The fifteenth tube, which serves as a control, receives no tested compound.
- 9) Suspend to an appropriate turbidity several colonies of the culture to be tested in 5.0 mL of broth medium to give a slightly turbid suspension.

- 10) Dilute this suspension by aseptically pipetting 0.2 mL of the suspension into 40 mL.
- 11) Add 1.0 mL of the diluted culture suspension to each of the tubes. The final concentration of tested compound is now one-half of the original concentration in each tube.
- 12) Incubate all tubes at 37 °C overnight for bacteria and 30 °C and 72 h for fungi.
- 13) Examine the tubes for visible signs of microbial and fungal growth. The highest dilution without growth is the minimal inhibitory concentration (MIC).

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