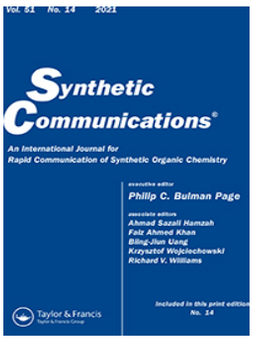

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Synthesis and antiviral evaluation of 5-(arylazo)salicylaldehyde thiosemicarbazone derivatives as potent anti-bovine viral diarrhea virus agents

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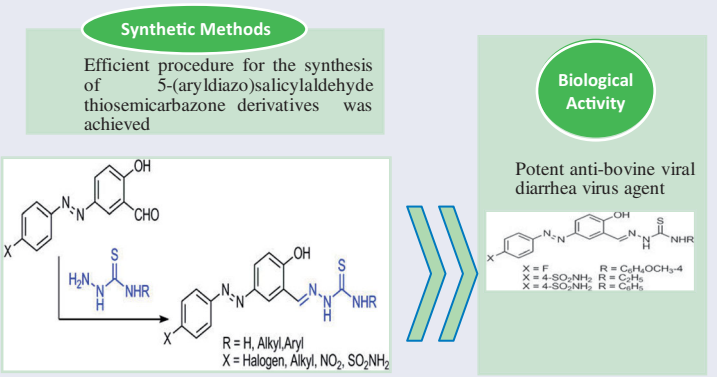
ABSTRACT

Thiosemicarbazones which previously characterized as a new class that inhibit anti-bovine viral diarrhea virus (BVDV) prompted us for synthesizing a series of new thiosemicarbazone derivatives. Thus, in this study, we reported the synthesis and antiviral evaluation of a series of 5-(arylazo)salicylaldehyde thiosemicarbazone derivatives for their expected antiviral activity. The desired products were synthesized from the condensation of 5-(arylazo)salicylaldehyde derivatives with *N*-(4)-substituted thiosemicarbazide derivatives. Antiviral screening was performed to test the anti-bovine viral diarrhea virus properties. From the obtained results, compounds **5**, **23** and **24** showed highly selective activity against BVDV by blocking the viral RNA synthesis in cell culture. The BVDV in antiviral drug studies is valuable surrogate for the hepatitis C virus (HCV); so, the above results provided a novel candidate for the development of anti-HCV agents.

GRAPHICAL ABSTRACT



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Supplemental data for this article can be accessed on the publisher's website.

Introduction

Thiosemicarbazones are versatile building blocks in the synthesis of densely substituted heterocycles.[1–3] Thiosemicarbazones have been reported as first antiviral compounds recognized to have a broad spectral antiviral activities against range of DNA and RNA viruses.[4] Modified aromatic and substituted thiosemicarbazones were investigated as broad spectra of antiviral activities including Hepatitis C Virus (HCV) subgenomic RNA replicon inhibitors and human immunodeficiency.[5–8] The *N*-methylisatin-*β*-thiosemicarbazone (methisazone & marboran) (Figure 1) was demonstrated as an effective antiviral drug in the chemoprophylaxis of small pox in South India.[9] Also, thiosemicarbazones and their metal complexes represent an essential structural unit with a wide range of biological and pharmacological properties such as anticancer and antimicrobial activities. Owing to their versatile chemistry, research has been progressed to design and synthesize broad spectra of thiosemicarbazone derivatives to verify their antibacterial, antifungal, antitumor and antiviral properties.[10–15]

Therefore, the present study was aimed to synthesize several newer thiosemicarbazone derivatives with screening for their antiviral properties hoping to obtain potent antiviral thiosemicarbazone derivatives with fewer side effects, rapid clearance rate or less incidence of relapse.

Results and discussion

The 5-(arylazo)salicylaldehyde precursors **I-V** were prepared as shown in Scheme 1 from reacting salicylaldehyde with the appropriate 4-substituted-aryldiazonium chloride according to the procedure described previously.[16–18] Hydroxyl group has positive resonance effect (+R) which is the main factor responsible for the high nucleophilicity of position-5 at salicylaldehyde. So, according to the effect of hydroxyl group, the coupling reaction will orient to position-5 which is *para* to hydroxyl group. Formyl group at salicylaldehyde has -R effect. So, the formyl group will orient the coupling reaction to *meta* position of formyl group (position-5 at salicylaldehyde). Moreover, Hammett σ constants supported these results. The Hammett values revealed that the position-5 at salicylaldehyde is more electron rich site. According to the both effects of hydroxyl and formyl groups at salicylaldehyde, the coupling reaction will orient to position-5 which is *para* to hydroxyl group and *meta* to formyl group to produce 5-(arylazo)salicylaldehyde precursors **I-V** as clean cut product.

The thiosemicarbazone derivatives **1-25** were synthesized in high yields through two different synthetic methodologies (Scheme 2). The first approach (Method A) was a multicomponent coupling reaction (one-pot synthesis, catalyst-free) of

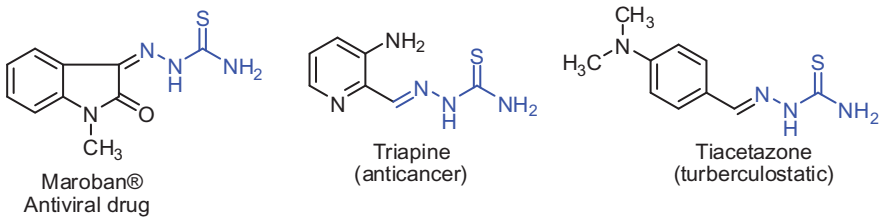
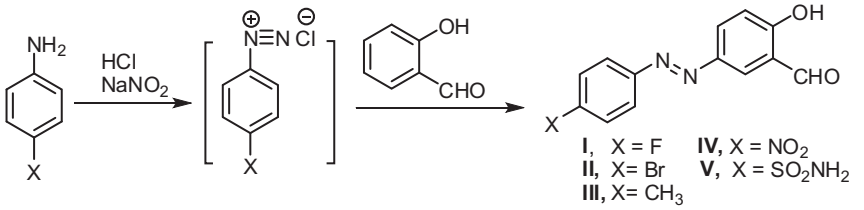
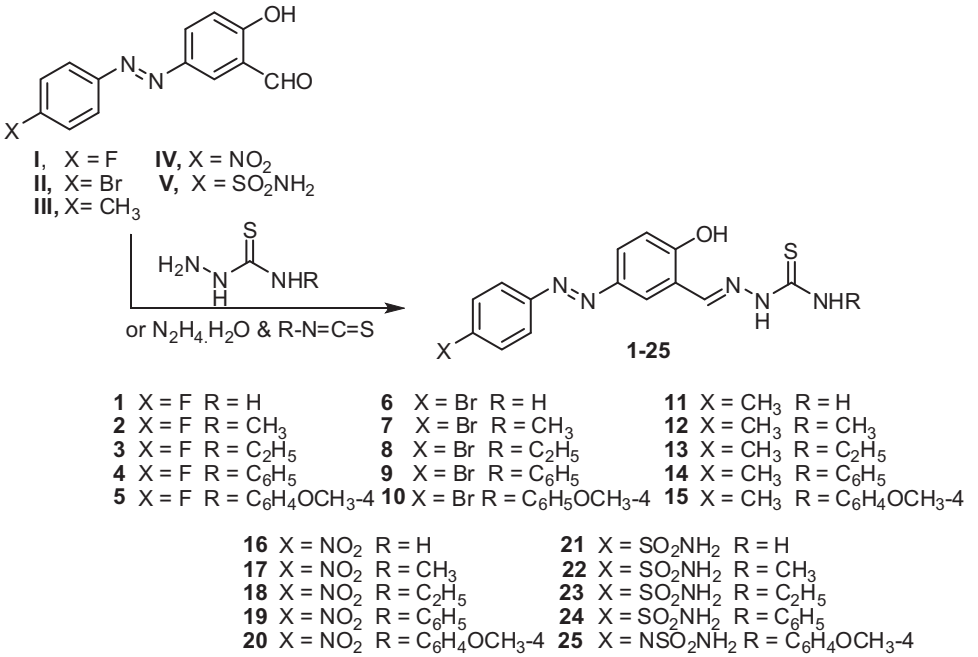


Figure 1. Representative bioactive thiosemicarbazones.



Scheme 1. Synthesis of 5-(4-substitutedphenylazo)salicylaldehyde derivatives **I–V.**



Scheme 2. Synthesis of 5-(4-substituted-phenylazo)salicylaldehyde thiosemicarbazones **1–25**.

5-(arylazo)- salicylaldehydes **I-V**, isothiocyanates and hydrazine hydrate, in short reaction times and high yields. The second one (Method B, Scheme 2) involved a procedure comprising two steps: the first one, is the synthesis of thiosemicarbazide derivatives from the reaction of the corresponding isothiocyanates with hydrazine hydrate, followed by reacting them with the 5-(arylazo)salicylaldehydes **I-V** to afford the desired thiosemicarbazone derivatives **1-25** in good yields. The synthesized thiosemicarbazone derivatives were characterized by bearing various substituents at arylazo and *N*-(4)-substituted thiosemicarbazone moieties. Structure of the synthesized *N*-(4)-substituted thiosemicarbazones **1-25** was inferred from their correct elemental analyses and careful studying of their spectral data. The infrared spectra the obtained products were characterized by the presence of intense bands due phenolic group ν(OH) in the region 3468–3409 cm^{-1}. The spectra showed also other bands in the regions 3300–3100 cm^{-1}, 1618–1614 cm^{-1}, and 1281–1223 cm^{-1} attributed to NH, CH=N and C=S diagnostic groups for thiosemicarbazones with disappearance of carbonyl group bands. ^{1}H NMR spectra of products **1–25** were characterized, generally, by the presence of broad signals integrated as one

Table 1. Screening of anti BVDV activity of the synthesized thiosemicarbazone derivatives.

Compound No.	10 μg/ml	Notes
4	Toxic effect	–
5	$2 \times 10^{-4.6}$ PFU	Active
10	Toxic effect	–
14	Toxic effect	–
15	Toxic effect	–
19	Toxic effect	–
23	$2 \times 10^{-4.6}$ PFU	Active
24	$2 \times 10^{-4.6}$ PFU	Active
25	Toxic effect	–
Positive control	$85 \times 10^{-4.6}$ PFU	–

hydrogen at $\delta = 10.32$–11.21 ppm region (D_2O-exchangeable) assigned to the phenolic OH protons. The spectra of the 4-amino derivatives **1**, **6**, **11**, **16,** and **21** revealed NH_2 proton signals as two signals at $\delta = 8.09$–8.12 ppm and 8.17–8.19 ppm regions (D_2O-exchangeable). The spectra of N^4-substituted derivatives were characterized by the presence of NH-alkyl and NH-aryl proton signals at $\delta = 8.56$–8.65 ppm and 10.14–10.24 ppm regions (D_2O-exchangeable), respectively. The spectra revealed also signals due to NH–N = protons at $\delta = 11.46$–11.87 ppm region (D_2O-exchangeable) beside the other characteristic signals. ^{13}C NMR spectral assignments were based on characteristic signal positions of the functional groups. In ^{13}C NMR spectra the signals resonated in the deshielded region of δ about 177 ppm assigned to C = S.

Anti-viral screening of the new synthesized compounds

Anti-viral screening of selected examples from the synthesized products was carried out on the Bovine Viral Diarrhea Virus (BVDV), which is a single stranded positive RNA virus classified as a member of the same family as Hepatitis C Virus (HCV) i.e. *Flaviviridae*. The synthesized compounds were tested *in vitro* for their antiviral activity. Viral infectivity assay was carried out using the plaque formation method.[19] A plaque is a localized focus of virus (*BVDV*)-infected (*Madin-Darby Bovine Kidney, MDBK*) cells which under optimal conditions, the autophagy induced cell death originates from a single infectious virus particle. Counting of these foci for a serial dilution of virus suspension is a precise method for quantification of viral infectivity. Under these conditions, reduction in virus plaque counts provides a sensitive mean for measuring antiviral activity. The results of the plaque reduction assay are summarized in Table 1. The antiviral effect obtained for the synthesized compounds suggested that the tested thiosemicarbazone derivatives (**4**, **10**, **14**, **15**, **19** and **25**) have no antiviral activity or have a toxic effect on the cells. On the other hand, cell cultures containing thiosemicarbazone derivatives (**5**, **23,** and **24**) displayed >40 fold decrease in viral infectivity ($2 \times 10^{-4.6}$ versus $85 \times 10^{-4.6}$ PFU) when compared with mock BVDV infected MDBK cells in absence of tested thiosemicarbazone derivatives.

Structure activity relationship (SAR)

From the results obtained from Table 1**:** It was noticed that**:** Compounds **5, 23,** and **24** have a strong antiviral activity. Compounds 4, **10, 14, 15, 19** and **25** were of toxic effect on the cells. Using the general structures provided in Figure 2, certain aspects of the structure

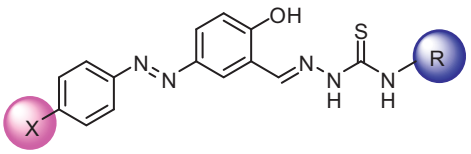


Figure 2. General formula of the synthesized compounds **1–25**.

activity relationships for these compounds can be more clearly highlighted. As anticipated, a clear difference in antiviral activity is noted between compounds **1–25** within and between each series, pointing to the reinforcing and opposing effects of X and R groups, where X were alkyl, halides, nitro and sulfonamide moieties; R were H, alkyl and aryl moieties.

From the obtained antiviral results it was noticed that, activity of thiosemicarbazone derivatives **5, 23,** and **24** can be attributed to the presence of the 5-(arylazo) salicylaldehyde moiety and dependence on the other substituents. Thus, the results indicated that the presence of 4-fluoro- and 4-methoxyphenyl- substituents at arylazo and *N*-(4)-thiosemicarbazone moieties respectively (compound **5)** showed a strong antiviral activity. Moreover, the presence of sulphonamido- at arylazo and ethyl-**23**, phenyl-**24** at N-(4)-thiosemicarbazone moieties revealed a strong antiviral activity. Therefore, this study showed a discovery of a new lead molecule to design more potent anti-viral agents. Further studies for the active compounds **5, 23,** and **24** as an anti-HCV candidate is currently in progress.

Conclusion

We have synthesized a series of new 5-(arylazo) salicylaldehyde-thiosemicarbazone derivatives bearing various substituents at arylazo and *N*-(4)-substituted thiosemicarbazone moieties. The expected anti-BVDV property of the synthesized derivatives was tested. The results indicated that introduction of fluoro- and 4-methoxyphenyl- substituents at arylazo and N-(4)-thiosemicarbazone moieties respectively (i.e. compound **5)** showed a strong anti-BVDV activity. Also, the presence of sulphonamido- at arylazo and ethyl- (**23**), phenyl- (**24**) at N-(4)-thiosemicarbazone moieties exhibited a potent anti- BVDV activity. We, therefore have report on a lead molecule to design more potent anti-viral agents.

Experimental part

All melting points are recorded on digital Gallen Kamp MFB-595 instrument and may be uncorrected. The IR spectra (KBr) (cm^{-1}) were measured on a JASCO spectrophotometer. ^{1}H NMR spectra were recorded on Joel spectrometers (at 500 MHz) and are reported relative to deuterated solvent signals in deuterated dimethylsulfoxide (DMSO-d_6). ^{13}C NMR spectra were recorded on Joel Spectrometers (at 125 MHz) in deuterated dimethylsulfoxide (DMSO-d_6).

Synthesis of the 5-(arylazo) salicylaldehydethiosemicarbazone derivatives 1-25

Method A

A mixture of salicylaldehyde derivatives **I–V** (0.01 mol), hydrazine hydrate (0.012 mol) and isothiocyanate (0.01 mol) in ethanol (25 mL) was heated under reflux for 20 min.,

then left to cool. The solid product obtained was filtered off and crystallized from the proper solvent to give the thiosemicarbazones **1–25**.

Method B

A mixture of salicylaldehyde derivatives **I–V** (0.01 mol) and the desired thiosemicarbazide derivatives (namely thiosemicarbazide, *N*-(methyl) thiosemicarbazide, *N*-(ethyl) thiosemicarbazide, *N*-(phenyl) thiosemicarbazide) (0.01 mol) in ethanol (25 mL) was heated under reflux for 30 min., then left to cool. The solid product obtained was filtered off and crystallized from the proper solvent to give the thiosemicarbazones **1–25**.

2-(5-((4-Fluorophenyl)diazenyl)-2-hydroxybenzylidene)hydrazinecarbothioamide (1): Yield 82%; m.p. 268–270 °C; IR: $\nu/cm^{-1} = 3425, 3244, 3153$ (NH_2 NH), 1602 (C=N); ^1H NMR (500 MHz, DMSO): δ/ppm: 7.01 (d, 1H, $J = 8.7$ Hz, Ar-H), 7.37 (m, 2H, Ar-H), 7.72 (d, 1H, $J = 6.5$ Hz, Ar-H), 7.83 − 7.92 (m, 2H, Ar-H), 8.09, 8.18 (2 s, 2H, NH_2), 8.41 (s, 1H, Ar-H), 8.54 (s, 1H, CH=N), 10.82 (br, 1H, OH), 11.48 (s, 1H, NH); ^{13}C NMR (126 MHz, DMSO): 116.7, 116.9, 117.4, 123.7, 124.6, 124.9, 125.0, 139.1, 145.8, 149.3, 160.0, 162.9, 164.8, 178.3 (C=S); MS, *m/z* (%): 317 (M^+; 49.4); Anal. Calcd. for $C_{14}H_{12}FN_5OS$ (317.3): C, 52.99; H, 3.81; N, 22.07; Found: C, 53.14; H, 3.78; N, 21.97%.

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