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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Syntheses and in vitro biological screening of 1-aryl-10*H*-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-*b*]indoles

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Abstract Structurally diverse 1-aryl-10*H*-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-*b*]indoles **4a–v** were synthesized by regiospecific heterocyclizations. The designed molecular diversity was evaluated in vitro in parallel cell-based assays for cytotoxicity of viruses multiplication supporting cell lines and antiviral activity against viruses representative of two of three genera of the *Flaviviridae* family. The compound library was also tested against Retrovirus (HIV-1), two Picornaviruses (CVB-2 and Sb-1), and *Paramyxoviridae* (VSV) representative. Among double-stranded RNA (dsRNA) viruses, *Reoviridae* representative (Reo-1) was tested. Two representatives of DNA virus families were also included—HSV-1 (*Herpesviridae*) and VV (*Poxviridae*). The compounds **4m** and **4o** were found cytotoxic, having CC_{50} values ranging from 4 to 30 μM. Moreover, compound **4v** has exhibited significant activity ($EC_{50} = 3$ μM) against BVDV.

Keywords 1,2,4-Triazines · Indoles · Triazoles · In vitro · Cytotoxicity · Antiviral

Introduction

The inherent toxicity and appearance of drug-resistant viruses restricted the use of most antiviral agents, hence the identification of novel antivirals and "lead" compounds are of great interest from which new antivirals could be synthesized (Buckwold *et al.*, 2004, Kossakowski *et al.*, 2009, Cesarini *et al.*, 2010). A livestock infection by the category of *Pestivirus* genus (*Flaviviridae* family) creates significant economic losses, and hence the development of safer, efficacious, and relatively cheap anti-pestiviruses vaccine is an unmet medical need (Paeshuyse *et al.*, 2006). These viruses cause a variety of clinical indications, such as teratogenesis, abortion, respiratory problems, chronic wasting disease, immune system dysfunction, and predisposition to secondary viral and bacterial infections (Tonelli *et al.*, 2010). The *Flaviviridae* family contains viruses with single-stranded positive sense RNA genomes ($ssRNA^+$), and encompasses three genera and several viruses those are presently not classified to specific genera (Tonelli *et al.*,

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2010). The *Hepacivirus* genus includes the hepatitis C virus (HCV) (Tonelli *et al.*, 2010). Viruses such as GB virus-A and GB virus-A-like agents, GB virus-D and GBV-C or hepatitis G virus, which are currently not classified within the *Hepacivirus* genus, are closely related to HCV and represents unassigned members of *Flaviviridae* (Tonelli *et al.*, 2010; Carta *et al.*, 2007). Moreover, this family is also consisting the *Flavivirus* genus, with viruses, for instance, Dengue Fever, Yellow Fever (YFV), West Nile, Japanese encephalitis, and tick-borne encephalitis (TBEV), and the *Pestivirus* genus, which consists of Bovine Viral Diarrhea (BVDV), Border Disease, and Classical Swine Fever viruses (Carta *et al.*, 2011). Other important ssRNA$^+$ viruses are those belonging to the *Picornaviridae* family. These viruses cause a variety of diseases, such as cold, heart infection, conjunctivitis, meningitis, and hepatitis (Carta *et al.*, 2007; Carta *et al.*, 2011). This family includes nine genera, some of which comprises major human pathogens, such as *Enterovirus* (including *Poliovirus*, *Echovirus*, *Coxsackievirus*), *Rhinovirus*, and *Hepatovirus* (Giampieri *et al.*, 2009). Currently, no particular vaccine is available for the treatment of *Picornaviridae* infections as well as for *Flaviviridae* members, except YFV.

Consequently, the development of novel, efficacious, and inexpensive antiviral "lead" molecules to combat this human pathogen is a pressing need (Stachulski *et al.*, 2011). BVDV is the prototype of the genus because it is also used as a surrogate for HCV to develop new anti-HCV agents (Tonelli *et al.*, 2011). We have recently evaluated various 9-aminoacridines (Tonelli *et al.*, 2011) for in vitro antiviral activity against a panel of RNA and DNA viruses. Moreover, we have also probed in vitro a series of new angular and linear [4,7]phenanthroline *N*-tricyclic systems against the ssRNA$^+$ viruses (Carta *et al.*, 2007). The compounds were also evaluated against viruses representative of an additional genus of ssRNA$^+$ genomes [Human immunodeficiency virus (HIV-1)], of double-stranded RNA genomes (dsRNA) [Reovirus (Reo-1)], and of single-stranded, negative-sense RNA genomes (ssRNA$^-$) [respiratory syncytial virus (RSV) and vesicular stomatitis virus (VSV)]. Notably, phenantrolines were found promising against members of the *Flaviviridae* and *Picornaviridae* families.

Azoles and azines are ubiquitous heterocycles, which are well-known for their versatile biological activities (Li *et al.*, 2009). Importantly, the fusion of the heterocyclic nuclei enhances the pharmacological activities than its parent nucleus (Abdel-Rahman *et al.*, 2010). In a search of novel therapeutic agents, versatile heterocyclic moieties were appended in the 1,2,4-triazine nucleus via the interaction between functionalized 1,2,4-triazines with various nucleophilic and electrophilic reagents (Abdel-Rahman *et al.*, 2010). In particular, 1,2,4-triazine motif is exploited for pharmacological activities, as herbicides and pesticides, as well as in dyes. In drug discovery, for examples, pyrrolo-triazines were found as potent PI3K inhibitors (Wang *et al.*, 2012) and various kinase inhibitors (Abraham *et al.*, 2011; Dyckman *et al.*, 2011; Mesaros *et al.*, 2012). Moreover, they were also found as potent adenosine A_{2A} antagonists (Congreve *et al.*, 2012), antitumors (Sztanke *et al.*, 2011), anticonvulsants (Sun *et al.*, 2009), antimalarials (Ban *et al.*, 2010), etc. Significant biological activities were also associated with indole-fused 1,2,4-triazines (Sivendran *et al.*, 2010; Shelke and Bhosale, 2010; Gupta *et al.*, 2010; Ashour *et al.*, 2012; Maarouf *et al.*, 2012). Importantly, the indolo-triazine (Buckwold *et al.*, 2004) (VP32947) was discovered as viral RNA-dependent RNA-polymerase (EC_{50} = 0.03 μM, SI >111). Though remarkable therapeutic worth of 1,2,4-triazine motif, the screening in antiviral therapeutic area is still in its infancy (Modzelewska-Banachiewicza and Kaminska, 2001; Elghandour *et al.*, 2006; Sztanke *et al.*, 2007) (Fig. 1). Inspired by literature reports, we have envisioned for the syntheses and screening of a molecular diversity based on 1-aryl-10*H*-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-*b*]indoles **4a**–**v**.

Results and discussion

Chemistry

At the outset, compound **2** was synthesized according to literature described procedure (Joshi and Chand, 1980). The compounds **3a**–**v** was synthesized by acid-catalyzed condensation, utilizing aromatic aldehydes bearing versatile electron donor and acceptors. Finally, they were

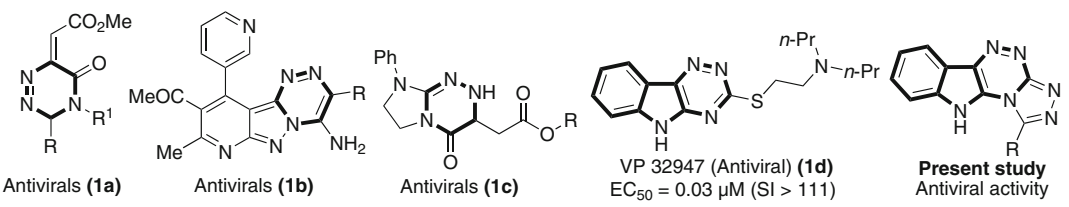
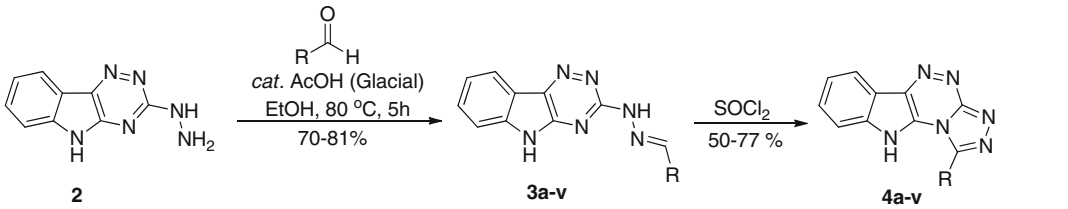


Fig. 1 Antiviral activity of 1,2,4-triazines condensed with other heterocycles (**1**)

cyclized using thionyl chloride as a cyclizing agent, afforded the target molecules **4a–v** in moderate to good yields with a wide range of functional group tolerance (Scheme 1). The title compounds thus obtained generated contradictions regarding their structural assignment. In literatures, regioselectivity of electrocyclization of functionalized 1,2,4-triazines, such as *N*-[1,2,4-triazine-3-yl] nitrilimines (Shawali and Gomha, 2002), 3-azido-1,2,4-triazines (Mojzych *et al.*, 2005; Goodman and Paudler, 1977), and 3-hydrazino-1,2,4-triazino[5,6-*b*]indole (Joshi and Chand, 1980), revealed that the comparative nucleophilicities of N^2 or N^4 in the 1,2,4-triazine ring governs the course of orientation. In our case, the cyclization of compounds **3a–v** with one carbon cyclizing precursors were only be electronically controlled and led to angularly annulated 1,2,4-triazolo[3′,4′:3,4]-1,2,4-triazino[5,6-*b*]indoles (compounds **4a–v**), and not linearly cyclized, 1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-*b*]indoles. The mechanism for the preparation of compounds **4a–v** is proposed in Fig. 2. In the absence of steric hindrance in the compounds **3a–v**, the basicity of N^4 became predominant over N^2 of the 1,2,4-triazine ring, and hence forced the orientation to afford angularly cyclized products; however, regio specificity was unaffected by the nature of the functional groups presents in the compounds **3a–v**. The mass spectra of the synthesized compounds were proved to be most important tool to elucidate the structures of the molecules. The *m*/*z* values of the relevant fragments suggested about the significant structural features like the mode of cyclization and aromatic nature of the molecules. The appearance of an intense molecular ion peak revealed the aromatic nature of the triazolotriazinoindole fused system, the molecular ion, in this case, underwent fragmentation with loss of corresponding benzonitrile, which was due to the benzonitrile di-radical obtained by initial fragmentation of the triazole ring and nitrogen molecule. An ion at *m*/*z* 103 was obtained by the loss of nitrogen molecule and HCN (Fig. 3).



Scheme 1 Synthesis of compounds **4a–v**

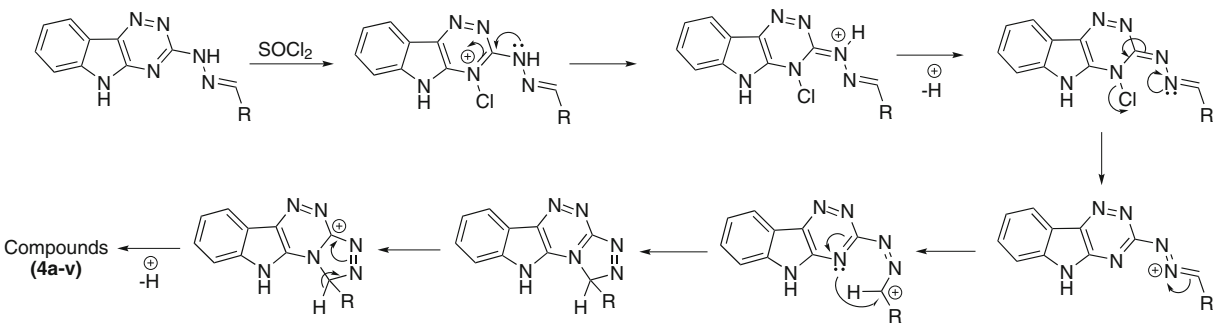


Fig. 2 Proposed mechanism for the synthesis of compounds **4a–v**

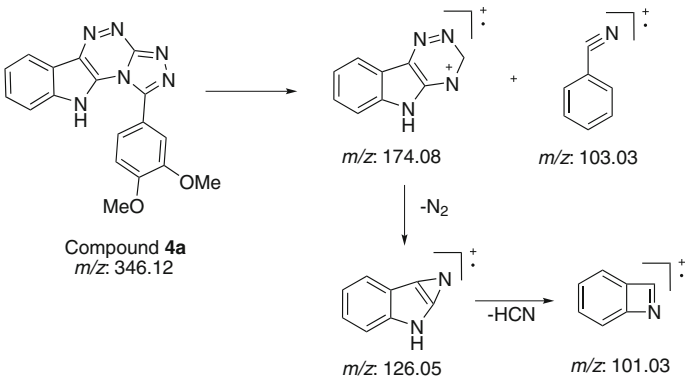


Fig. 3 Mass fragmentation of compound **4a**

Biology

The cytotoxicity and antiviral activities of the compounds **4a–v** are summarized in Table 1 against viruses representative of two of three genera of the *Flaviviridae* family, *i.e.*, *Flaviviruses* (YFV) and *Pestiviruses* (BVDV). The title compounds were also screened against representatives of other virus families. Among $ssRNA^+$ was a *Retrovirus* (HIV-1), two *Picornaviruses* [Coxsackie Virus type B2 (CVB-2), and Poliovirus type-1 Sabin strain (Sb-1)]; among $ssRNA^-$ were *Paramyxoviridae* [*Rhabdoviridae*, VSV, RSV] representative. Among dsRNA viruses, was a *Reoviridae* representative [Respiratory Enteric Orphan Virus type-1(Reo-1)]. Two representatives of DNA virus families were also included: Herpes Simplex type 1 (HSV-1, *Herpesviridae*) and Vaccinia Virus (VV, *Poxviridae*). The indolo-triazine derivatives were presented antiviral activities varied from moderate (**4a**, **4c**, and **4p**) to significant (**4t** and **4v**). The data may serve as a good subject for discussion of the influence of molecular structure of a compound on its biological activity. It seems that antiviral activity depends on the factors, such as type of substituents, regioisomerism, and steric interactions associated with the parent scaffold. The most active compound **4v** (R = 9-anthryl) bearing sterically hindered group exerted EC_{50} value 3 μM against BVDV strain without cytotoxic to parent MDBK cell-line at CC_{50} value >100 μM. Incorporation of electron-donating group at *para* position (**4c**, R = 4-SMe Ph) and moderately electronegative group (**4p**, R = 3-Cl Ph) exerted moderate antiviral activity with EC_{50} values 45 and 69 μM against Reo-1 and BVDV strains without causing cytotoxicity to their parent BHK-21 and MDBK cell lines, respectively. The regioisomerism of the aryl substituents sincerely affected cytotoxicity and antiviral activities. As such, 3,4-disubstituted compound **4a** (R = 3,4-diOMe Ph) exerted EC_{50} value of 55 μM against Reo-1 strain without being cytotoxic to its parent BHK-21 cell-line at CC_{50} value >100 μM, while **4t** (R = 4-OH, 3-OMe Ph) exerted antiviral activity at EC_{50} value 90 μM against BVDV and 25 μM against Reo-1 strains, but cytotoxic against BHK-21 cell-line with CC_{50} value 45 μM, whereas 2,5-disubstituted compound **4m** was inactive to all viruses representatives; however, it exerted cytotoxicity to MT-4, MDBK, BHK-21, and Vero-76 cell lines with CC_{50} values 4, 12, 30, and 15 μM, respectively. In addition, notable cytotoxicities were exerted by the compounds **4n** (R = 3-OMe Ph) with CC_{50} values 47, 64, and 65 μM on MT-4, MDBK, and Vero-76, **4o** (R = 2-OMe Ph) to all cell lines with CC_{50} values 5, 17, 23, and 20 μM, **4p** (R = 3-Cl Ph) with CC_{50} values 29, 55, and 60 μM on MT-4, BHK-21 and Vero-76 cell lines, respectively. Whereas, rest of the compounds were neither active against any of the virus representative nor cytotoxic to any cell lines employed.

Table 1 Cytotoxicity and antiviral activities of compounds **4a–v**

Compds	MT-4	HIV-1	MDBK	BVDV	SI[1]	BHK-21	YFV	Reo-1	Vero-76	HSV-1	VV	VSV	CVB-2	Sb-1	RSV
	CC_{50}^{a} μM	EC_{50}^{b} μM	CC_{50}^{c} μM	EC_{50}^{d} μM		CC_{50}^{e} μM	EC_{50}^{f} μM	EC_{50}^{f} μM	CC_{50}^{g} μM	EC_{50}^{h} μM	EC_{50}^{h} μM	EC_{50}^{h} μM	EC_{50}^{h} μM	EC_{50}^{h} μM	EC_{50}^{h} μM
4a	>100	>100	>100	>100	–	>100	>100	55	>100	>100	>100	>100	>100	>100	>100
4b	>100	>100	>100	>100	–	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
4c	>100	>100	>100	>100	–	>100	>100	45	90	>90	>90	>90	>90	>90	>90
4d	>100	>100	>100	>100	–	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
4e	>100	>100	>100	>100	–	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
4f	>100	>100	>100	>100	–	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

Table 1 continued

Compds	MT-4 CC_{50} μM	HIV-1 EC_{50} μM	MDBK CC_{50} μM	BVDV EC_{50} μM	SI[1]	BHK-21 CC_{50} μM	YFV EC_{50} μM	Reo-1 EC_{50} μM	Vero-76 CC_{50} μM	HSV-1 EC_{50} μM	VV EC_{50} μM	VSV EC_{50} μM	CVB-2 EC_{50} μM	Sb-1 EC_{50} μM	RSV EC_{50} μM
4g	>100	>100	>100	>100	–	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
4h	>100	>100	>100	>100	–	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
4i	>100	>100	>100	>100	–	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
4j	>100	>100	>100	>100	–	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
4k	>100	>100	>100	>100	–	>100	>100	>100	>100	>100	>100	>100	100	>100	>100
4 l	>100	>100	>100	>100	–	100	>100	>100	80	>80	>80	>80	>80	>80	>80
4m	4	>4	12	>12	–	30	>30	>30	15	>15	>15	>15	>15	>15	>15
4n	47	>47	64	>64	–	>100	>100	>100	65	>65	>65	>65	>65	>65	>65
4o	5	>5	17	>17	–	23	>23	>23	20	>20	>20	>20	>20	>20	>20
4p	29	>29	>100	69	>1.45	55	>55	>55	60	>60	>60	>60	>60	>60	>60
4q	>100	>100	>100	>100	–	>100	>100	>100	70	>70	>70	>70	>70	>70	>70
4r	>100	>100	>100	>100	–	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
4s	>100	>100	>100	>100	–	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
4t	58	>58	>100	90	>1.11	45	>45	25	80	>80	>80	>80	>80	>80	>80
4u	>100	>100	>100	>100	–	>100	>100	>100	≥100	>100	>100	>100	>100	>100	>100
4v	56	>56	>100	3	>33.33	>100	>100	>100	≥100	>100	>100	>100	>100	>100	>100
AZT[i]	50	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–
NM 108[j]	–	–	–	1.8	–	–	2.5	–	–	–	–	–	–	–	–
NM 176[k]	–	–	–	–	–	–	–	–	–	–	–	–	23	18	–
M 5255[l]	–	–	–	–	–	–	–	–	–	–	1.8	–	–	–	–
ACG[m]	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–

Data represent mean value for three independent determinations. Variation among duplicate samples was less than 15 %. Antiviral activity is given as EC_{50} (median effective concentration-the concentration of a drug (μM) required to induce a 50 % effect), and cytotoxicity is given as CC_{50} (cytotoxic concentration-the amount of a drug (μM) at which 50 % cell become dead). SI (selectivity index) was determined as the ratio between CC_{50} and EC_{50} for MDBK and BVDV (SI[1])

[a] Compd. concentration (μM) required to reduce the viability of mock-infected MT-4 ($CD4^+$ human T-cells containing an integrated HTLV-1 genome) cells by 50 %, as determined by the colorimetric MTT method

[b] Compd. concentration (μM) required to reduce the viability of mock-infected MDBK (bovine normal kidney) cells by 50 %, as determined by the MTT method

[c] Compd. concentration (μM) required to reduce the viability of mock-infected BHK (Hamster normal kidney fibroblast) monolayers by 50 %, as determined by the MTT method

[d] Compd. concentration (μM) required to reduce the viability of mock-infected VERO-76 (Monkey normal kidney) monolayers by 50 %, as determined by the MTT

[e] Compd. concentration (μM) required to achieve 50 % protection of MT-4 cells from the HIV-1-induced cytopathogenicity, as determined by the MTT method

[f] Compd. concentration (μM) required to achieve 50 % protection of MDBK cells from the BVDV (Bovine Viral Diarrhea Virus)-induced cytopathogenicity, as determined by the MTT method

[g] Compd. concentration (μM) required to achieve 50 % protection of BHK (Kidney fibroblast) cells from the YFV (Yellow Fever Virus) and Reo (Reovirus-1)-induced cytopathogenicity, as determined by the MTT method

[h] Compd. concentration (μM) required to reduce the plaque number of HSV-1 (Herpesvirus-1), VV (Vaccinia Virus), VSV (Vesicular Stomatitis Virus), CVB-2 (Coxsackievirus B2), Sb-1 (Poliovirus 1) and RSV (Respiratory Syncytial Virus) by 50 % in VERO-76 monolayers

[i] AZT (3′-azido-thymidine)

[j] NM108 (2′-β-methyl-guanosine)

[k] NM176 (2′-ethynyl-D-citidine)

[l] M5255 (mycophenolic acid)

[m] ACG (acyclo-guanosine)

Conclusions and future directions

In summary, a versatile 1-aryl-10*H*-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-*b*]indoles were designed, synthesized, and evaluated in vitro in parallel cell-based assays for cytotoxicity and antiviral activity against representative strains of emergent and re-emergent human and cattle viral infectious diseases. The screening results revealed that the compound **4v** exerted the most promising activity with 3 μM EC_{50} value against BVDV strain devoid of causing cytotoxicity to its parent MDBK cell-line. While four distinct candidates **4a**, **4c**, **4p**, and **4t** have presented antiviral activities from moderate to significant range with diminutive cytotoxicity. In addition to these two derivatives, **4m** and **4o** exerted significant cytotoxicity against all RNA virus multiplication supporting cell lines and will be evaluated for antiproliferative activity against hematological and solid tumor cell lines.

Methods and materials

Chemistry

Chemicals and solvents were purchased from commercial sources and were used without further purification. Melting points were determined in open capillary tubes using Electrothermal-9200 melting point apparatus and are uncorrected. Yields refer to isolated compounds, estimated to be >95 % pure as determined by ^{1}H-NMR. TLC: Macherey–Nagel, TLC plates Alugram® Sil G/UV254. Detection under UV light at 254 nm. ^{1}H-NMR spectra were recorded on Bruker Avance II 300 MHz spectrometer in DMSO-d_6. Chemical shifts (δ) are given in ppm relative to TMS, coupling constants (J) are in Hz. All IR spectra were recorded on Shimadzu FTIR 8400 Spectrophotometer by KBr pellet method. All ESI–MS spectra were recorded on Shimadzu GCMS-QP2010. The microanalysis was performed on EuroVector EA3000.

*Synthesis of 3-hydrazino-5H-[1,2,4]triazino[5,6-b]indole (**2**)*

It was prepared according to literature described procedure. Yields: 60 %, m.p. 275–278 °C (lit. m.p. 276–277 °C) (Joshi and Chand, 1980).

*General procedure for the syntheses of compounds (**3a–v**) (Ram et al., 1987; Monge et al., 1987)*

The above compounds were synthesized according to literature described method. To a three-neck flask, substituted aromatic aldehydes (0.01 mol), compound **2** (0.01 mol) and glacial acetic acid (10.0 mol%) were added in ethanol and heated at 80 °C for 5 h. On cooling the reaction mass at ambient temperature, the crude products were precipitate out. Ethanol was removed by filtration, and the products were washed with diethyl ether and *n*-pentane and dried under vacuum. No further purification was attempted.

*General procedure for the syntheses of compounds (**4a–v**)*

To an appropriate hydrazones **3a–v** (5.0 mmol), thionyl chloride (10 ml) was added under nitrogen atmosphere. The resulting reaction mixture was heated at 75 °C in oil bath for 4h. The progress of reaction was checked by TLC (dichloromethane/methanol, 95/5). The excess of thionyl chloride was removed by distillation under reduced pressure. The residue was quenched in ice to leave the precipitates. The crude products were filtered and washed with water and isopropanol and dried under vacuum to afford pure title compounds.

*1-(3,4-Dimethoxy)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4a**)*

Yields: 77 % as a white solid, m.p. (°C): 289–291 (dec.). IR (KBr, cm^{-1}): 3352, 3128, 1517, 1487, 1463, 1298, 744. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 12.28 (brs, 1H), 8.23 (d, J = 7.5 Hz, 1H, ArH), 8.08 (d, J = 6.0 Hz, 1H, ArH), 7.99 (s, 1H, ArH), 7.74 (*t*, J = 7.7, 7.2 Hz, 1H, ArH), 7.70 (d, J = 8.1 Hz, 1H, ArH), 7.46 (*t*, J = 7.8, 7.2 Hz, 1H, ArH), 7.20 (d, J = 8.4 Hz, 1H, ArH), 3.90 (s, 6H, 2 × OMe). ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 158.2, 155.5, 150.3, 149.3, 148.8, 137.9, 125.4, 123.7, 122.5, 119.2, 118.2, 116.9, 114.8, 112.9, 111.9, 109.6, 56.9, 55.2. ESI–MS: *m/z* 346, 331, 304, 174, 126, 103, 101. Anal. calcd for $C_{19}H_{15}N_6O_2$: C, 62.42; H, 4.07; N, 24.27. Found: C, 62.46; H, 4.11; N, 24.32.

*1-(4-Methoxy)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4b**)*

Yields: 72 % as a white solid, m.p. (°C): 282–284. IR (KBr, cm^{-1}): 3412, 3128, 1612, 1581, 1558, 1504, 1257, 840. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 13.04 (brs, 1H), 8.38 (d, J = 9.0 Hz, 2H, ArH), 8.33 (d, J = 7.8 Hz, 1H, ArH), 7.82 (*t*, J = 7.5, 4.1 Hz, 1H, ArH), 7.58 (d, J = 8.1 Hz, 1H, ArH), 7.46 (*t*, J = 7.5, 3.5 Hz, 1H, ArH), 7.24 (d, J = 9.0 Hz, 2H, ArH), 3.90 (s, 3H, OMe). ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 158.6, 158.3, 154.5, 151.1, 139.0, 127.1, 125.1, 123.2, 122.0, 119.1, 117.3, 115.7, 114.2, 112.8, 55.9. ESI–MS: *m/z* 316, 301, 273, 174, 158, 126, 103, 101. Anal. calcd for $C_{18}H_{13}N_6O$: C, 64.55; H, 3.82; N, 26.57. Found: C, 64.61; H, 3.79, N, 26.62.

*1-(4-Methylsulfanyl)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4c**)*

Yields: 69 % as a yellow solid, m.p. (°C): 241–243. IR (KBr, cm^{-1}): 3396, 3124, 1618, 1589, 1548, 1367, 891. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.67$ (brs, 1H), 8.23 (d, $J = 7.5$ Hz, 1H, ArH), 7.80 (d, $J = 8.8$ Hz, 2H, ArH), 7.71 (*t*, $J = 7.7$, 4.0 Hz, 1H, ArH), 7.69 (d, $J = 8.1$ Hz, 1H, ArH), 7.49 (*t*, $J = 7.8$, 4.2 Hz, 1H, ArH), 7.19 (d, $J = 8.4$hz, 1H, ArH), 2.45 (s, 3H, SMe). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 158.3$, 153.1, 151.0, 139.6, 135.3, 133.1, 131.4, 128.2, 122.3, 121.9, 119.0, 118.1, 115.2, 113.2, 15.3. ESI–MS: *m/z* 332, 317, 174, 126, 103, 101. Anal. calcd for $C_{18}H_{13}N_6S$: C, 61.43; H, 3.64; N, 25.58. Found: C, 61.49; H, 3.59; N, 25.65.

*1-(3-Bromophenyl)-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4d**)*

Yields: 75 % as a yellow solid, m.p. (°C): >300. IR (KBr, cm^{-1}): 3409, 3101, 1616, 1535, 1465, 1299, 898. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.28$ (brs, 1H), 8.34 (*t*, $J = 3.7$, 4.1 Hz, 1H, ArH), 7.72 (d, $J = 8.1$ Hz, 1H, ArH), 7.54 (*t*, $J = 7.7$, 7.2 Hz 1H, ArH), 7.46 (d, $J = 4.2$ Hz, 1H, ArH), 7.15 (*t*, $J = 7.7$, 3.5 Hz, 1H, ArH), 7.23–6.60 (m, 3H, ArH). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 158.2$, 154.1, 152.0, 139.2, 133.2, 131.1, 130.1, 129.9, 125.9, 123.7, 123.0, 122.0, 118.1, 117.3, 115.1, 113.4. ESI–MS: *m/z* 366, 367, 181, 174, 126, 103. Anal. calcd for $C_{16}H_9BrN_6$: C, 52.62; H, 2.48; N, 23.01. Found: C, 52.69; H, 2.54; N, 23.05.

*1-(4-Fluoro)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4e**)*

Yield: 67 % as a white solid, m.p. (°C): 164–165. IR (KBr, cm^{-1}): 3410, 3132, 1612, 1588, 1461, 1238, 756. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.22$ (brs, 1H), 8.08 (d, $J = 6.8$ Hz, 2H, ArH), 7.89 (d, $J = 6.9$ Hz, 1H, ArH), 7.66 (*t*, $J = 7.3$, 3.6 Hz, 1H, ArH), 7.55 (d, $J = 7.9$ Hz, 1H, ArH), 7.39 (*t*, $J = 6.7$, 3.6 Hz, 1H, ArH), 7.33 (d, $J = 8.7$ Hz, 2H, ArH). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 165.2$, 161.2, 159.1, 154.3, 152.4, 139.5, 129.3, 127.9, 124.2, 123.1, 119.2, 118.1, 116.2, 115.1, 113.7. ESI–MS: *m/z* 305, 174, 126, 121, 103. Anal. calcd for $C_{16}H_9FN_6$: C, 63.16; H, 2.98; N, 27.62. Found: C, 63.22; H, 2.92; N, 27.64.

*1-(2-Chloro)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4f**)*

Yield: 70 % as a yellow solid, m.p. (°C): 247–249. IR (KBr, cm^{-1}): 3410, 3132, 1612, 1588, 1461, 1238, 756. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.59$ (brs, 1H), 8.45 (d, $J = 7.9$, 1H, ArH), 7.75–7.63 (m, 3H, ArH), 7.55–7.35 (m, 4H, ArH). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 157.9$, 152.8, 150.2, 139.5, 132.6, 131.5, 129.9, 128.9, 128.8, 127.5, 124.5, 123.0, 119.5, 118.3, 115.7, 113.4. ESI–MS: *m/z* 321, 322, 174, 137, 126, 103. Anal. calcd for $C_{16}H_9ClN_6$: C, 59.92; H, 2.83; N, 26.20. Found: C, 59.98; H, 2.87; N, 26.21.

*1-(4-Chloro)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4g**)*

Yield: 73 % as a white solid, m.p. (°C): >300. IR (KBr, cm^{-1}): 3410, 3118, 1618, 1537, 1470, 1238, 829. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.12$ (brs, 1H), 8.23 (d, $J = 7.9$ Hz, 2H, ArH), 7.65–7.57 (m, 2H, ArH), 7.55 (d, $J = 7.6$ Hz, 2H, ArH), 7.51–7.29 (m, 2H, ArH). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 158.3$, 153.1, 152.0, 139.0, 135.2, 133.8, 130.5, 128.1, 123.2, 122.3, 119.2, 118.0, 115.3, 113.2. ESI–MS: *m/z* 322, 321, 174, 137, 126, 103. Anal. calcd for $C_{16}H_9ClN_6$: C, 59.92; H, 2.83; N, 26.20. Found: C, 59.94; H, 2.85; N, 26.24.

*1-(3-Nitro)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4h**)*

Yield: 63 % as a yellow solid, m.p. (°C): >300. IR (KBr, cm^{-1}): 3408, 3116, 1620, 1523, 1455, 1299, 875. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.23$ (brs, 1H), 8.67 (*t*, $J = 3.2$ Hz, 1H, ArH), 8.62–8.22 (m, 2H, ArH), 8.19–8.17 (m, 1H, ArH), 7.77–7.63 (m, 2H, ArH), 7.52–7.32 (m, 2H, ArH). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 158.3$, 153.5, 152.0, 148.2, 139.4, 133.6, 132.0, 131.1, 124.1, 123.9, 122.8, 120.8, 119.3, 118.2, 113.9, 112.9. ESI–MS: *m/z* 332, 174, 126, 103. Anal. calcd for $C_{16}H_9N_7O_2$: C, 58.01; H, 2.74; N, 29.60. Found: C, 58.09; H, 2.81; N, 29.65.

*1-(4-Nitro)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4i**)*

Yield: 64 % as a yellow solid, m.p. (°C): 253–255. IR (KBr, cm^{-1}): 3494, 3116, 1620, 1523, 1461, 1299, 856. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.19$ (brs, 1H), 8.35 (d, $J = 7.2$ Hz, 2H, ArH), 8.12–8.09 (m, 1H, ArH), 8.02 (d, $J = 6.9$ Hz, 2H, ArH), 7.59–7.57 (m, 1H, ArH), 7.53-7.35 (m, 2H, ArH). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 158.3$, 154.1, 152.1, 147.4, 139.3, 133.1, 125.9, 125.2, 123.1, 122.9, 117.9, 117.2, 115.3, 113.4. ESI–MS: *m/z* 332, 174, 126, 103. Anal. calcd for $C_{16}H_9N_7O_2$: C, 58.01; H, 2.74; N, 29.60. Found: C, 58.05; H, 2.80; N, 29.59.

*1-Phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino [5,6-b]indole (**4j**)*

Yield: 62 % as a white solid, m.p. (°C): 221–223. IR (KBr, cm^{-1}): 3425, 3114, 1614, 1587, 1485, 1230, 750. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 13.03$ (1H, brs), 8.43 (d, $J = 6.6$ Hz, 2H, ArH), 8.34 (d, $J = 7.8$ Hz, 1H), 7.82 (*t*, $J = 7.5$, 7.8 Hz, 1H, ArH), 7.67 (m, 3H, ArH), 7.58 (d, $J = 7.8$ Hz, 1H, ArH), 7.45 (*t*, $J = 7.5$ Hz, 1H, ArH). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 158.6$, 154.2, 152.4, 139.4, 131.4, 129.9, 129.0, 126.4, 123.8, 123.1, 119.2, 118.1, 115.2, 113.3. ESI–MS: *m/z* 285, 176, 126, 103, 101. Anal. calcd for $C_{16}H_{10}N_6$: C, 67.12; H, 3.52; N, 29.35. Found: C, 67.15; H, 3.57; N, 29.41.

*1-(2-Nitro)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4k**)*

Yield: 57 % as a yellow solid, m.p. (°C): 279–281. IR (KBr, cm^{-1}): 3404, 3124, 1610, 1583, 1465, 1220, 846. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.21$ (brs, 1H, NH exchangeable), 8.15–7.99 (m, 3H, ArH), 7.91–7.67 (m, 3H, ArH), 7.51–7.31 (m, 2H, ArH). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 156.3$, 152.6, 151.0, 149.9, 139.2, 134.5, 130.4, 127.0, 126.4, 123.8, 123.0, 122.4, 119.5, 118.1, 115.2, 111.9. ESI–MS: *m/z* 332, 174, 126, 103. Anal. calcd for $C_{16}H_9N_7O_2$: C, 58.01; H, 2.74; N, 29.60. Found: C, 58.07; H, 2.73; N, 29.63.

*1-(3-Phenoxy)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4l**)*

Yield: 59 % as a white solid, m.p. (°C): 283–285. IR (KBr, cm^{-1}): 3434, 3126, 1610, 1563, 1400, 1225, 750. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.17$ (brs, 1H, NH exchangeable), 8.14–8.07 (m, 2H, ArH), 7.63–7.51 (m, 3H, ArH), 7.47–7.29 (m, 4H, ArH), 7.25–7.12 (m, 3H, ArH). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 158.5$, 157.1, 156.0, 154.3, 152.7, 139.8, 133.4, 131.1, 130.5, 123.9, 123.3, 122.6, 121.9, 121.2, 119.7, 118.0, 117.8, 115.6, 115.0, 111.7. ESI–MS: *m/z* 379, 195, 126, 103. Anal. calcd for $C_{22}H_{14}N_6O$: C, 69.83; H, 3.73; N, 22.21. Found: C, 69.87; H, 3.79; N, 22.24.

*1-(2,5-Dimethoxy)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4m**)*

Yield: 72 % as a white solid, m.p. (°C): 249–250. IR (KBr, cm^{-1}): 3434, 3128, 1620, 1552, 1400, 1271, 894. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.17$ (brs, 1H, NH exchangeable), 8.14–8.11 (m, 2H, ArH), 7.67–7.56 (m, 2H, ArH), 7.34–7.31 (m, 1H, ArH), 6.98 (d, $J = 6.9$ Hz, 1H, ArH), 6.81 (d, $J = 6.2$ Hz, 1H, ArH), 3.95 (s, 3H, OMe), 3.89 (s, 3H, OMe). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 160.1$, 155.4, 152.4, 150.0, 139.3, 130.1, 123.1, 122.9, 118.3, 116.2, 115.2, 112.6, 110.1, 107.7, 56.6, 55.9. ESI–MS: *m/z* 346, 331, 304, 174, 126, 103, 101. Anal. calcd for $C_{19}H_{15}N_6O_2$: C, 62.42; H, 4.07; N, 24.27. Found: C, 62.45; H, 4.11; N, 24.32.

*1-(3-Methoxy)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4n**)*

Yield: 69 % as a yellow solid, m.p. (°C): 205–207. IR (KBr, cm^{-1}): 3404, 3143, 1608, 1554, 1456, 1315, 885. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.20$ (brs, 1H, NH exchangeable), 8.14–8.10 (m, 2H, ArH), 7.85–7.77 (m, 3H, ArH), 7.41–7.25 (m, 3H, ArH), 3.91 (s, 3H, OMe). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 162.2$, 158.3, 154.1, 152.3, 139.3, 131.2, 128.4, 123.2, 122.8, 118.9, 118.7, 117.8, 115.9, 113.9, 112.7, 112.0, 55.6. ESI–MS: *m/z* 316, 301, 273, 174, 158, 126, 103, 101. Anal. calcd for $C_{18}H_{13}N_6O$: C, 64.55; H, 3.82; N, 26.57. Found: C, 64.59; H, 3.84; N, 26.62.

*1-(2-Methoxy)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4o**)*

Yield: 73 % as a yellow solid, m.p. (°C): 225–227. IR (KBr, cm^{-1}): 3423, 3124, 1616, 1587, 1483, 1253, 735. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.21$ (brs, 1H, NH exchangeable), 8.17–8.13 (m, 2H, ArH), 7.68–7.63 (m, 2H, ArH), 7.50–7.22 (m, 2H, ArH), 7.35–7.05 (m, 2H, ArH), 3.94 (s, 3H, OMe). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 158.1$, 153.3, 152.1, 151.9, 139.3, 128.8, 128.5, 124.4, 123.8, 123.3, 119.2, 118.8, 118.5, 114.4, 114.2, 113.1, 55.7. ESI–MS: *m/z* 316, 301, 273, 174, 158, 126, 103, 101. Anal. calcd for $C_{18}H_{13}N_6O$: C, 64.55; H, 3.82; N, 26.57. Found: C, 64.60; H, 3.80; N, 26.61.

*1-(3-Chloro)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4p**)*

Yield: 68 % as a white solid, m.p. (°C): 234–236. IR (KBr, cm^{-1}): 3419, 3132, 1608, 1585, 1477, 1232, 896. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.23$ (brs, 1H, NH exchangeable), 8.15–8.11 (m, 2H, ArH), 8.01–7.97 (m, 2H, ArH), 7.76–7.62 (m, 1H, ArH), 7.45–7.37 (m, 1H, ArH), 7.34–7.21 (m, 2H, ArH). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 158.3$, 155.1, 152.1, 139.4, 134.2, 131.4, 130.1, 128.3, 127.2, 125.2, 124.3, 123.7, 119.3, 118.1, 115.4, 111.9. ESI–MS: *m/z* 322, 321, 174, 137, 126, 103. Anal. calcd for $C_{16}H_9ClN_6$: C, 59.92; H, 2.83; N, 26.20. Found: C, 59.97; H, 2.87; N, 26.26.

*1-(2-Hydroxy)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4q**)*

Yield: 56 % as a yellow solid, m.p. (°C): >300 (dec.). IR (KBr, cm^{-1}): 3404, 3120, 1614, 1589, 1539, 1253, 740. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.18$ (brs, 1H, NH exchangeable), 8.13–8.10 (m, 2H, ArH), 7.67–7.61 (m, 1H, ArH), 7.57–7.55 (m, 1H, ArH), 7.47–7.31 (m, 2H, ArH), 7.24–7.07 (m, 2H, ArH), 4.11 (brs, 1H, OH exchangeable). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 157.4, 154.3, 153.1, 152.1, 139.2, 130.5, 129.7, 124.1, 122.7, 121.5, 119.7, 119.1, 118.1, 115.2, 113.1, 111.3$. ESI–MS: *m/z* 303, 174, 126, 103. Anal. calcd for $C_{16}H_{10}N_6O$: C, 63.57; H, 3.33; N, 27.80. Found: C, 63.63; H, 3.37; N, 27.78.

*1-(4-Hydroxy)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4r**)*

Yield: 53 % as a yellow solid, m.p. (°C): >300 (dec.). IR (KBr, cm^{-1}): 3406, 3136, 1608, 1554, 1458, 1272, 844. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.20$ (brs, 1H, NH exchangeable), 8.09–8.05 (m, 2H, ArH), 7.95 (d, $J = 6.6$ Hz, 2H, ArH), 7.67–7.62 (m, 1H, ArH), 7.54–7.49 (m, 1H, ArH), 7.27–7.21 (m, 2H, ArH), 6.83 (d, $J = 6.5$ Hz, 2H, ArH), 5.21 (brs, 1H, OH exchangeable). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 158.8, 157.3, 154.2, 152.1, 139.0, 128.3, 123.1, 122.3, 122.9, 119.4, 118.2, 115.3, 114.4, 113.2$. ESI–MS: *m/z* 303, 174, 126, 103. Anal. calcd for $C_{16}H_{10}N_6O$: C, 63.57; H, 3.33; N, 27.80. Found: C, 63.62; H, 3.35; N, 27.82.

*1-(3-Hydroxy)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4s**)*

Yield: 50 % as a yellow solid, m.p. (°C): 254–256. IR (KBr, cm^{-1}): 3304, 3136, 1610, 1554, 1490, 1319, 877. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.18$ (brs, 1H, NH exchangeable), 8.14–8.11 (d, $J = 7.2$ Hz, 2H, ArH), 7.84–7.67 (m, 2H, ArH), 7.50–7.38 (m, 2H, ArH), 7.29–6.93 (m, 2H, ArH), 4.48 (brs, 1H, OH exchangeable). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 158.5, 157.1, 154.1, 151.9, 139.1, 131.3, 130.9, 122.8, 122.4, 119.9, 118.4, 117.5, 117.2, 115.2, 114.1, 112.2$. ESI–MS: *m/z* 303, 174, 126, 103. Anal. calcd for $C_{16}H_{10}N_6O$: C, 63.57; H, 3.33; N, 27.80. Found: C, 63.60; H, 3.36; N, 27.87.

*1-(3-Methoxy-4-Hydroxy)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b] indole (**4t**)*

Yield: 70 % as a yellow solid, m.p. (°C): 258–260. IR (KBr, cm^{-1}): 3404, 3128, 1609, 1560, 1460, 1288, 788. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.17$ (brs, 1H, NH exchangeable), 8.15–8.07 (m, 1H, ArH), 7.63–7.61 (m, 1H, ArH), 7.50–7.45 (m, 2H, ArH), 7.35–7.17 (m, 2H, ArH), 6.95–6.89 (m, 1H, ArH), 4.87 (brs, 1H, OH exchangeable), 3.96 (s, 3H, OMe). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 158.4, 154.5, 152.1, 149.1, 148.9, 139.0, 123.1, 122.9, 122.1, 120.1, 118.9, 118.2, 115.3, 113.1, 112.7, 110.1, 57.2$. ESI–MS: *m/z* 332, 317, 174, 126, 103. Anal. calcd for $C_{17}H_{12}N_6O_2$: C, 61.44; H, 3.64; N, 25.29. Found: C, 63.49; H, 3.65; N, 25.32.

*1-(4-Methyl)phenyl-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4u**)*

Yield: 71 % as a white solid, m.p. (°C): 287–289. IR (KBr, cm^{-1}): 3419, 3126, 1610, 1554, 1498, 1220, 844. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.20$ (brs, 1H, NH exchangeable), 8.59 (d, $J = 7.1$ Hz, 2H, ArH), 8.08–8.05 (m, 1H, ArH), 7.65–7.54 (m, 2H, ArH), 7.37–7.33 (m, 1H, ArH), 7.25 (d, $J = 6.8$ Hz, 2H, ArH), 2.21 (s, 3H, Me). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 158.7, 153.1, 151.2, 139.2, 137.2, 131.7, 129.8, 125.7, 123.9, 122.1, 119.2, 117.8, 115.3, 113.2, 20.9$. ESI–MS: *m/z* 301, 174, 126, 117, 103. Anal. calcd for $C_{17}H_{12}N_6$: C, 67.99; H, 4.03; N, 27.98. Found: C, 68.03; H, 4.09; N, 27.95.

*1-(9-Anthryl)-10H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[5,6-b]indole (**4v**)*

Yield: 76 % as a yellow solid, m.p. (°C): >300 (dec.). IR (KBr, cm^{-1}): 3404, 3128, 1614, 1554, 1480, 1271, 759. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta = 12.37$ (brs, 1H, NH exchangeable), 7.72 (d, $J = 7.1$ Hz, 1H, ArH), 7.68 (*t*, $J = 6.8$, 3.4hz, 1H, ArH), 7.62 (d, $J = 7.8$ Hz, 1H, ArH), 7.51 (*t*, $J = 7.2$, 3.3 Hz, 1H, ArH), 7.40–6.56 (m, 11H, ArH). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta = 156.2, 152.2, 141.3, 139.3, 135.3, 133.4, 131.3, 130.1, 127.5, 126.9, 126.2, 125.2, 123.1, 122.2, 119.2, 118.3, 115.2, 113.1$. ESI–MS: *m/z* 387, 279, 174, 126, 103. Anal. calcd for $C_{24}H_{14}N_6$: C, 74.60; H, 3.65; N, 21.75. Found: C, 74.67; H, 3.70; N, 21.81.

Biology

Cell-based assays

Compounds were dissolved in DMSO at 100 mM and then diluted in culture medium. Cell lines were purchased from American Type Culture Collection (ATCC). The absence of mycoplasma contamination was checked periodically by the Hoechst staining method. Cell lines supporting the multiplication of RNA viruses were the following: $CD4^+$ human T-cells containing an integrated HTLV-1 genome (MT-4); Madin Darby Bovine Kidney (MDBK); Baby Hamster Kidney (BHK-21); and Monkey kidney (Vero 76) cells.

Cytotoxicity assays

The compounds were probed for their in vitro cytotoxicity and antiviral activities against representatives of different virus families by cell-based assays. Compounds were dissolved in DMSO at 100 mM and then diluted in culture medium. The cytotoxicity tests were run in parallel with antiviral assays. MDBK, BHK, and Vero 76 cells were resuspended in 96-multi-well plates at an initial density of 6×10^5, 1×10^6, and 5×10^5 cells/ml, respectively, in maintenance medium, with or without serial dilutions of test compounds. Cell viability was determined after 48–120 h at 37 °C in a humidified CO_2 (5 %) atmosphere by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method. The cell numbers of Vero 76 monolayers were determined by staining with the crystal violet dye. For cytotoxicity evaluations, exponentially growing cells derived from human hematological tumors [$CD4^+$ human T-cells containing an integrated HTLV-1 genome (MT-4)] were seeded at an initial density of 1×10^5 cells/ml in 96-well plates in RPMI-1640 medium, supplemented with 10 % fetal calf serum (FCS), 100 U/ml penicillin G, and 100 μg/ml streptomycin. Cell cultures were then incubated at 37 °C in a humidified, 5 % CO_2 atmosphere in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 96 h at 37 °C by MTT method (Pauwels *et al.*, 1998).

Antiviral assays

The activity of compounds against HIV-1 was based on the inhibition of virus-induced cytopathogenicity in MT-4 cells acutely infected with a multiplicity of infection (m.o.i.) of 0.01. Briefly, RPMI (50 μl) containing 1×10^4 MT-4 was added to each well of flat-bottom microtitre trays containing RPMI (50 μl), with or without serial dilutions of test compounds. Then, HIV-1 suspension (20 μl) containing 100 $CCID_{50}$ was added. After 4-days incubation, cell viability was determined by MTT method. Activity of compounds against YFV and Reo-1 was based on the inhibition of virus-induced cytopathogenicity in acutely infected BHK-21 cells. Activities against BVDV, in infected MDBK cells, were also based on inhibition of virus induced cytopathogenicity. BHK and MDBK cells were seeded in 96-well plates at a density of 5×10^4 and 3×10^4 cells/well, respectively, and were allowed to form confluent monolayers by incubating overnight in growth medium at 37 °C in a humidified CO_2 (5 %) atmosphere. Cell monolayers were then infected with 50 μL of a proper virus dilution (in serum-free medium) to give a m.o.i = 0.01. One hour later, MEM Earle's medium (50 μL), supplemented with inactivated FCS, 1 % final concentration, with or without serial dilutions of test compounds, were added. After 3–4 days incubation at 37 °C, cell viability was determined by the MTT method (Pauwels *et al.*, 1998). Activity of compounds against CVB-2 strain, Sb-1, RSV, VSV, VV, and HSV-1, in infected Vero 76 cells, was determined by plaque reduction assays in Vero 76-cell monolayers. To this end, Vero 76 cells were seeded in 24-well plates at a density of 2×10^5 cells/well and were allowed to form confluent monolayers by incubating overnight in growth medium at 37 °C in a humidified CO_2 (5 %) atmosphere. Then, monolayers were infected with appropriate virus dilutions (250 μl) to give 50–100 PFU/well. Following the removal of unadsorbed virus, Dulbecco's modified Eagle's medium (500 μl), supplemented with 1 % inactivated FCS and 0.75 % methyl cellulose, with or without serial dilutions of test compounds, were added. Cultures were incubated at 37 °C for 2 (Sb-1 and VSV) or 3 (CVB-2, VV and HSV-1) or 5 days (RSV) and then fixed with PBS containing 50 % ethanol and 0.8 % crystal violet, washed, and air-dried. Plaques were then counted. 50 % effective concentrations (EC_{50}) were calculated by linear regression technique. The cytotoxicity was evaluated in parallel with the antiviral activity. AZT (3′-Azido-thymidine), NM 108 (2′-β-Methyl-guanosine), NM 176 (2′-Ethynyl-D-citidine), M 5255 (Mycophenolic acid), and ACG (Acyclo-guanosine) were used as reference inhibitors of $ssRNA^+$, $ssRNA^-$, and DNA viruses, respectively.

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Conflict of interest The authors declare that they have no conflict of interest.

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