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Article

# Discovery of *N*-arylsulfonyl-3-acylindole benzoyl hydrazone derivatives as anti-HIV-1 agents

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The discovery and development of novel inhibitors with activity against variants of human immunodeficiency virus type 1 (HIV-1) is pivotal for overcoming treatment failure. As our ongoing work on research of anti-HIV-1 inhibitors, 32 *N*-arylsulfonyl-3-acylindole benzoyl hydrazone derivatives were prepared by introduction of the hydrazone fragments on the *N*-arylsulfonyl-3-acylindolyl skeleton and preliminarily screened *in vitro* as HIV-1 inhibitors for the first time. Among of all the reported analogues, eight compounds exhibited significant anti-HIV-1 activity, especially *N*-(3-nitro)phenylsulfonyl-3-acetylindole benzoyl hydrazone (**18**) and *N*-(3-nitro)phenylsulfonyl-3-acetyl-6-methylindole benzoyl hydrazone (**23**) displayed the most potent anti-HIV-1 activity with $EC_{50}$ values of 0.26 and 0.31 μg/mL, and TI values of >769.23 and >645.16, respectively. It is noteworthy that introduction of $R^3$ as the methyl group and $R^2$ as the hydrogen group could result in more potent compounds. This suggested that introduction of $R^3$ as the methyl group could be taken into account for further preparation of these kinds of compounds as anti-HIV-1 agents.

**Keywords:** Benzoyl hydrazone. Human immunodeficiency virus type-1. Inhibitor of virus replication. Anti-HIV-1 agent.

## INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is mainly caused by human immunodeficiency virus type 1 (HIV-1) infection and remains one of the biggest medical barriers for human health, since it was first reported in 1981 (Gottlieb *et al*., 1981). The reverse transcriptase (RT) of the HIV-1 plays a significant role in the viral replication process, which makes it a pivotal target for anti-HIV-1 inhibitor discovery (Jonckheere, Anné, De Clercq, 2000; Yisma *et al*., 2014). Although numerous RT inhibitors, including primarily the nucleoside/nucleotide RT inhibitors (NRTIs), *e.g.*, zidovudine, and non-nucleoside RT inhibitors (NNRTIs), *e.g.*, nevirapine, delavirdine and efavirenz, have been developed, like other anti-HIV inhibitors, effectiveness of now approved NRTIs and NNRTIs have been hampered because of the fast development of resistance (Boone, 2006; De Clercq, 2002; Sluis-Cremer, Wainberg, Schinazi, 2015; Yu *et al*., 2011). It is estimated that 36.9 million people (including 2.6 million children) were living with HIV infection in the year 2014 according to *UNAIDS*-2015 report, and 1.2 million people died due to HIV as well as related diseases (Chander *et al*., 2016). To circumvent this challenge, there is an urgent need to discover and develop safe, green, efficient, selective and novel anti-HIV inhibitors having significant potency against drug-resistant RT viral strains as well as less toxicity (Chander *et al*., 2016; Huang *et al*., 2007; Polanski *et al*., 2006; Safakish *et al*., 2017).

Hydrazones are excellent candidates for the research of antiprotozoal agents (Carvalho *et al*., 2014; de Sá Alves, Barreiro, Fraga, 2009), multidentate ligands (Bessy Raj, Prathapachandra Kurup, Suresh, 2008), pesticidal agents (Che *et al*., 2013b; Guo *et al*., 2012) and gelatinase inhibitors (Yang *et al*., 2016). Meanwhile, *N*-arylsulfonylindoles or *N*-arylsulfonyl-3-acylindoles are excellent candidates for the study of anti-HIV-1 inhibitors (Che *et al*., 2016; Fan *et al*., 2009; Ran *et al*., 2010), and especially some *N*-arylsulfonyl-3-acetylindoles showed potent anti-HIV-1 activity. Nevertheless, the anti-HIV-1 activity of the *N*-arylsulfonyl-3-acylindole benzoyl

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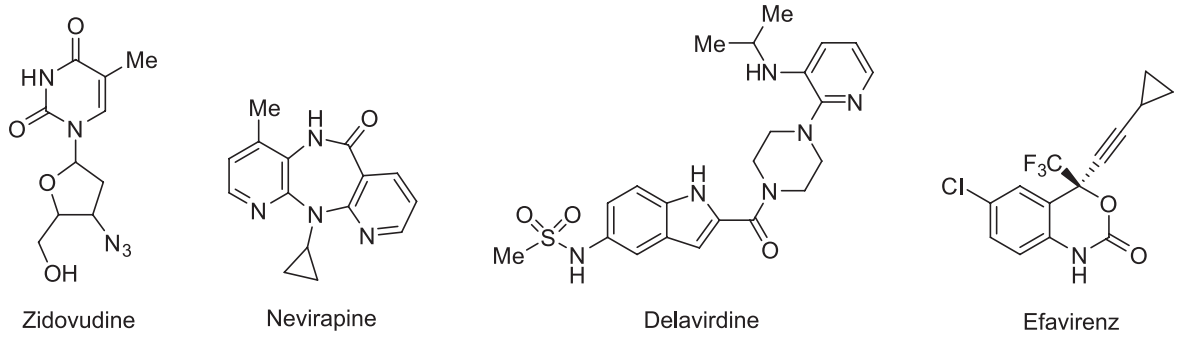



**FIGURE 1** - Structures of currently approved NRTIs and NNRTIs by the U.S. FDA.

hydrazones has not been previously published. Inspired by these previous observations, and the goal in this program is to discover new compounds with potent biological activity (Che *et al*., 2013a; Che *et al*., 2015; Che *et al*., 2016; Xu, Che, Wang, 2011), we report here the results of the anti-HIV-1 activity of 32 *N*-arylsulfonyl-3-acylindole benzoyl hydrazone derivatives for the first time.

## RESULTS AND DISCUSSION

### Chemistry

32 *N*-Arylsulfonyl-3-acylindole benzoyl hydrazone analogs (**1-32,** Figure 2) were smoothly synthesized as shown in Scheme 1. *N*-Arylsulfonyl-3-acetylindoles (**A**) reacted with the corresponding benzoyl hydrazides (**B**) in the presence of AcOH at reflux, and compounds **1-32** were prepared, as well as characterized by satisfactory proton nuclear magnetic resonance ($^1$H NMR), carbon nuclear magnetic resonance ($^{13}$C NMR), electrospray ion trap mass spectrometry (ESI-TRAP-MS), high-resolution mass spectra (HR-MS), as well as melting point (Che *et al*., 2013b). Owing to the steric hindrance, the substituents on the C−N bond of all compounds adopted a *trans* configuration (*E* configuration) (Carvalho *et al*., 2014; Che *et al*., 2013b; Lourenço *et al*., 2008).

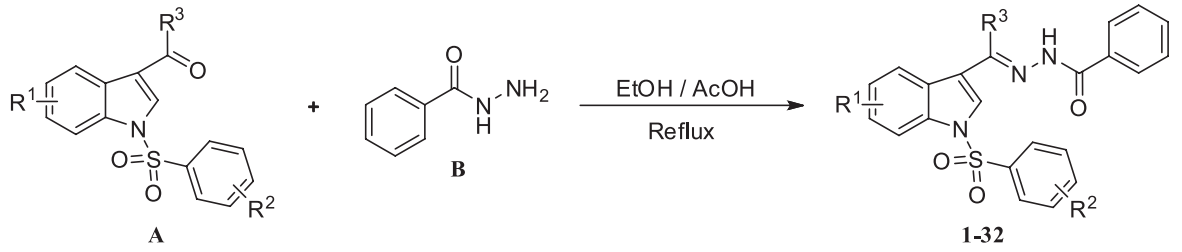
Interestingly, all reactants dissolved well in anhydrous ethanol in the beginning of the reaction. As the reaction progressed, some insoluble species were gradually precipitated out, as well as at the end of the reaction, a large amount of solid accumulated in the bottom of the flask. As a result of this behavior, all the desired *N*-arylsulfonyl-3-acylindole benzoyl hydrazone analogs (**1-32**) could be obtained by filtration. This procedure imbues the synthetic methodology with green credentials.

Moreover, target compounds **1-32** were analysed by reverse phase high-performance liquid chromatography (RP-HPLC), and all compounds purity were >95%.

### Biological activities

As our ongoing work on research of anti-HIV-1 inhibitors, purified and characterized *N*-arylsulfonyl-3-acylindole benzoyl hydrazones **1-32** were screened *in vitro* for their inhibitory activity against HIV-1 replication in acutely infected C8166 cells, and 3′-azido-3′-deoxythymidine (AZT) was used as a positive control presented in Table I.

The results of anti-HIV-1 viral replication assay revealed that, out of thirty-two evaluated compounds, two (**18** and **23**) showed significant anti-HIV-1 activity ($EC_{50}$ values of 0.26 and 0.31 $\mu$g/mL, TI values of >769.23 and >645.16, respectively), six (**10**, **14**, **19**, **20**, **24** and **27**) showed moderate anti-HIV-1 activity ($EC_{50}$ values of 1.02, 0.52, 0.49, 1.65, 0.67 and 0.50 μg/mL, TI values of 116.33, >384.61, >408.16, >121.21, >298.50 and >249.86, respectively), while the rest of tested compounds displayed weak anti-HIV-1 activity ($EC_{50}$ values of 1.71 to




**SCHEME 1** - Synthetic route for the preparation of *N*-arylsulfonyl-3-acylindole benzoyl hydrazone derivatives **1-32**.

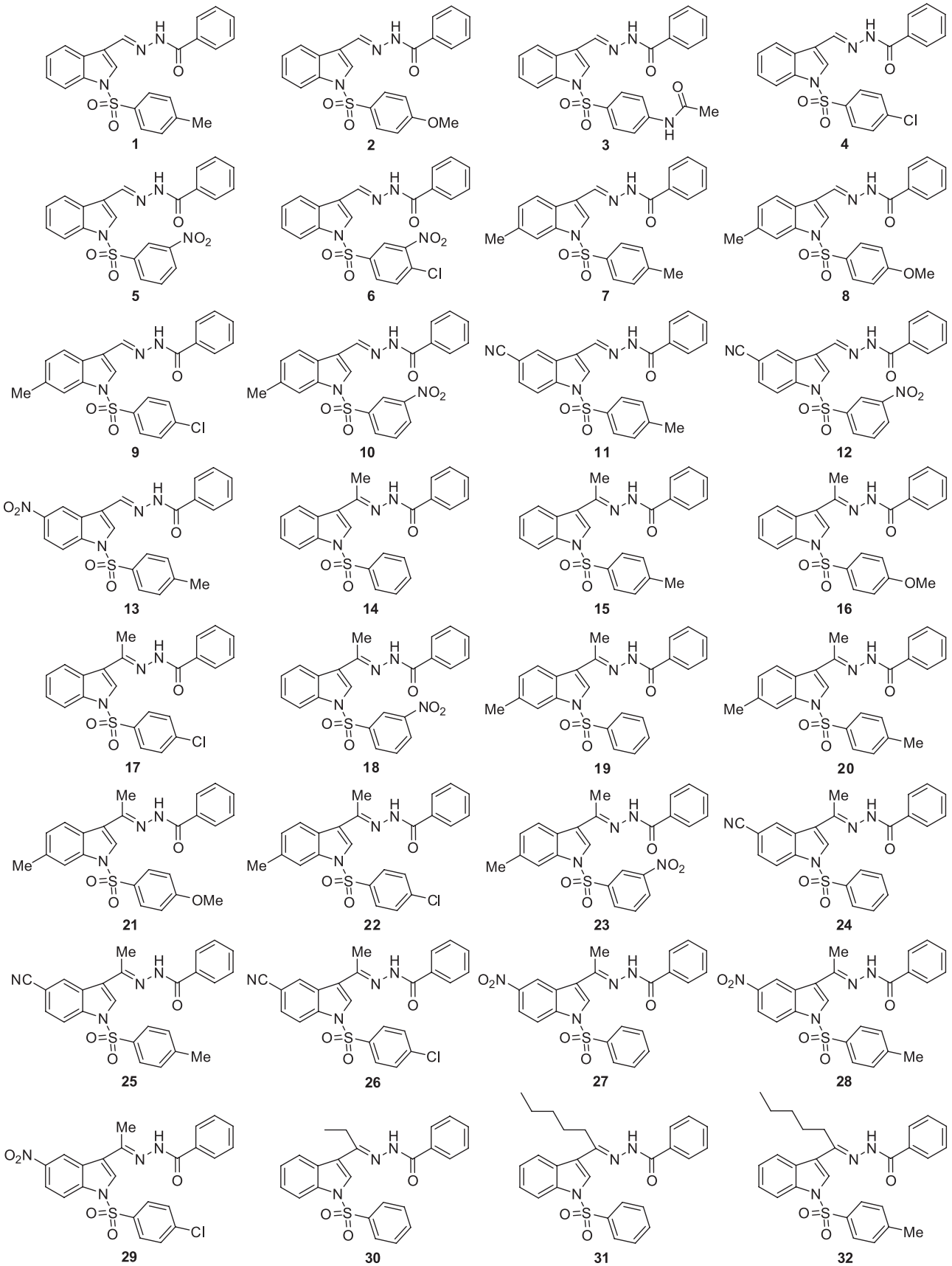
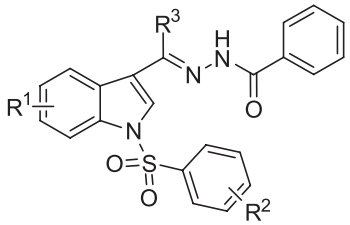



**FIGURE 2 -** Chemical structures of *N*-arylsulfonyl-3-acylindole benzoyl hydrazone derivatives **1-32**.

**TABLE I -** Anti-HIV-1 activity of *n*-arylsulfonyl-3-acylindole benzoyl hydrazone derivatives 1-32 *in vitro*[a].



**1-32**

| Compd | $R^1$ | $R^2$ | $R^3$ | $CC_{50}$[b](μg/mL) | $EC_{50}$[c](μg/mL) | TI[d] |
|---|---|---|---|---|---|---|
| **1** | H | 4-Me | H | 89.93 | 11.02 | 8.16 |
| **2** | H | 4-OMe | H | 192.02 | 3.02 | 63.58 |
| **3** | H | 4-NHAc | H | 18.31 | 1.71 | 10.70 |
| **4** | H | 4-Cl | H | 53.15 | 9.92 | 5.36 |
| **5** | H | 3-$NO_2$ | H | 27.09 | 4.06 | 6.67 |
| **6** | H | 3-$NO_2$, 4-Cl | H | 1.77 | 59.99 | 0.03 |
| **7** | 6-Me | 4-Me | H | 41.74 | 8.25 | 5.06 |
| **8** | 6-Me | 4-OMe | H | 74.86 | 3.66 | 20.45 |
| **9** | 6-Me | 4-Cl | H | 0.68 | 3.64 | 0.19 |
| **10** | 6-Me | 3-$NO_2$ | H | 118.66 | 1.02 | 116.33 |
| **11** | 5-CN | 4-Me | H | 123.68 | 3.13 | 39.51 |
| **12** | 5-CN | 3-$NO_2$ | H | 21.47 | 3.13 | 6.85 |
| **13** | 5-$NO_2$ | 4-Me | H | 73.09 | 20.82 | 3.51 |
| **14** | H | H | Me | >200 | 0.52 | >384.61 |
| **15** | H | 4-Me | Me | 8.79 | 0.93 | 9.45 |
| **16** | H | 4-OMe | Me | 1.30 | 0.16 | 8.13 |
| **17** | H | 4-Cl | Me | >200 | 125.16 | >1.60 |
| **18** | H | 3-$NO_2$ | Me | >200 | 0.26 | >769.23 |
| **19** | 6-Me | H | Me | >200 | 0.49 | >408.16 |
| **20** | 6-Me | 4-Me | Me | >200 | 1.65 | >121.21 |
| **21** | 6-Me | 4-OMe | Me | >200 | 12.37 | >16.16 |
| **22** | 6-Me | 4-Cl | Me | 96.71 | 2.51 | 38.53 |
| **23** | 6-Me | 3-$NO_2$ | Me | >200 | 0.31 | >645.16 |
| **24** | 5-CN | H | Me | >200 | 0.67 | >298.50 |
| **25** | 5-CN | 4-Me | Me | >200 | 59.99 | >3.33 |
| **26** | 5-CN | 4-Cl | Me | 19.76 | 11.72 | 1.68 |
| **27** | 5-$NO_2$ | H | Me | 124.93 | 0.50 | 249.86 |
| **28** | 5-$NO_2$ | 4-Me | Me | >200 | 2.55 | >78.43 |
| **29** | 5-$NO_2$ | 4-Cl | Me | >200 | 7.27 | >27.51 |
| **30** | H | H | Et | >200 | 14.53 | >13.76 |
| **31** | H | H | *n*-pentyl | >200 | 38.23 | >5.23 |
| **32** | H | 4-Me | *n*-pentyl | >200 | 52.23 | >3.83 |
| **AZT[e]** | \ | \ | \ | 1139.47 | 0.00324 | 351688.3 |

[a]Values are means of two separate experiments (the values exhibited standard deviation (SD) less than ±5% from mean). [b]$CC_{50}$ (50% cytotoxic concentration), concentration of drug that causes 50% reduction in total C8166 cell number. [c]$EC_{50}$ (50% effective concentration), concentration of drug that reduces syncytia formation by 50%. [d]In vitro therapeutic index ($CC_{50}$ value/$EC_{50}$ value). [e]AZT was used as a positive control.

>125.16 μg/mL, except **16**, the $EC_{50}$ value of 0.16 μg/mL, and TI values of 0.03 to 78.43).

In order to elucidate the anti-HIV-1 activity of compounds **1-32** at a molecular basis and to reveal structural features critical for their anti-HIV-1 activity, a brief investigation of structure activity relationship (SAR) was determined, which revealed how the substituents on **1-32** were related to the anti-HIV-1 activity. In general, (1) $R^3$ = Me was more vital for the anti-HIV-1 activities than $R^3$ = H, Et, or *n*-pentyl. For example, when $R^1$ and $R^2$ were H, introduction of $R^3$ as the methyl group could lead to the pronounced compound (**14** *vs* **30** and **31**, $EC_{50}$ values of 0.52, 14.53 and 38.23 μg/mL, TI values of >384.61, >13.76 and >5.23, respectively; that is, the TI value of **14** was close to 28 times of that of **30** and was more than 73 times of that of **31**). (2) When $R^1$ = H or 6-Me and $R^2$ = 3-$NO_2$, compounds with $R^3$ = Me exhibited significant inhibitory potential against the HIV viral replication (**18** *vs* **5**, $EC_{50}$ values of 0.26 and 4.06 μg/mL, TI values of >769.23 and 6.67, respectively, especially the TI value of **18** was more than 115 times of that of **5**; **23** *vs* **10**, $EC_{50}$ values of 0.31 and 1.02 μg/mL, TI values of >645.16 and 116.33, respectively). (3) $R^2$ as the 3-nitro group also plays an important role in the activities against the HIV viral replication. For example, when $R^3$ = H and $R^1$ = 6-Me, the corresponding compound **10** usually displayed more potent anti-HIV-1 activity (TI = 116.23); When $R^3$ = Me and $R^1$ = H or 6-Me, the corresponding compounds **18** and **23** also exhibited the most remarkable anti-HIV-1 activity (TI >769.23 and >645.16, respectively). (4) It is noteworthy that introduction of $R^3$ as the methyl group and $R^2$ as the hydrogen group could result in more potent compounds. For example, the $EC_{50}$ and TI values of compounds **14**, **19**, **24** and **27** were 0.52, 0.49, 0.67, 0.50 μg/mL, and >384.61, >408.16, >298.50, 249.86, respectively. This suggested that introduction of $R^3$ as the methyl group could be taken into account for further preparation of these kinds of compounds as anti-HIV-1 agents.

## EXPERIMENTAL SECTION

### Compound purity assessment

The purity of *N*-arylsulfonyl-3-acylindole benzoyl hydrazone derivatives **1-32** were recorded on a Shimadzu LC-15C liquid chromatograph [SPD-15C UV-vis spectrophotometric detector (190-700 nm)] using a flow rate of 1.0 mL/min ($MeOH/H_2O$ = 5/1) and a Hypersil ODS $C_{18}$ column (5 $\mu$m, 4.6 × 150 mm) as the stationary phase, and all compounds purity were >95%.

### Anti-HIV-1 activity assay

#### *Cells and virus*

The cell line (C8166) and the laboratory-derived virus (HIV-$1_{IIIB}$) were obtained from the Medical Research Council, AIDS Reagent Project, London, UK. C8166 was maintainedin RPMI-1640 medium supplemented with 10% heat-inactivated newborn calf serum (Gibco, Grand Island, NY, USA). The cells used in all experiments were in log-phasegrowth. The 50% HIV-$1_{IIIB}$ tissue culture infectious dose ($TCID_{50}$) in C8166 cells was determined and calculated by the Reed and Muench method. Virusstocks were stored in small aliquots at -70°C.

#### *MTT-based cytotoxicity assay*

Cellular toxicity of compounds **1-32** on C8166 cells was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method as described previously. Briefly, cells were seeded in a 96-well microtiter plate in the absence or presence of various concentrations of compounds in triplicate and incubated at 37 °C in a humid atmosphere of 5% $CO_2$ for 3 days. The supernatants were discarded and MTT reagent (5 mg/mL in PBS) was added to each well, then incubated for 4 h, after which 100 $\mu$L of 50% DMF–20% SDS was added. After the formazan was dissolved completely, the plates were read on a BioTekElx800 ELISA reader (BioTek, Winooski, VT, USA) at 595/630 nm. Thecytotoxic concentration that caused the reduction of viable C8166cells by 50% ($CC_{50}$) was determined from the dose-response curve.

#### *Syncytia assay*

In the presence of 100 $\mu$L of various concentrations of compounds **1-32**, C8166 cells ($4\times10^5$/mL) were infected with HIV-$1_{IIIB}$ at a multiplicity of infection (M.O.I) of 0.06. The final volume per well was 200 μL. Control assays were performed without the test compounds in HIV-$1_{IIIB}$ infected and uninfected cultures. After 3 days of culture, the cytopathic effect (CPE) was measured by counting the number of syncytia. Percentage inhibition of syncytia formationwas calculated, and the 50% effective concentration ($EC_{50}$) was calculated. 3′-Azido-3′-deoxythymidine (AZT; Sigma-Aldrich, St. Louis, MO, USA) was used as a positive control. The therapeutic index (TI) was calculated as $CC_{50}/EC_{50}$.

## CONCLUSIONS

In the present study, 32 *N*-arylsulfonyl-3-acylindole benzoyl hydrazone derivatives were prepared and screened *in vitro* as HIV-1 inhibitors for the first time.

Among the reported analogues, compounds **10**, **14**, **18-20**, **23**, **24** and **27** exhibited significant anti-HIV-1 activity with $EC_{50}$ values of 1.02, 0.52, 0.26, 0.49, 1.65, 0.31, 0.67 and 0.50 μg/mL, and TI values of 116.33, >384.61, >769.23, >408.16, >121.21, >645.16, >298.50 and >249.86, respectively. Especially *N*-(3-nitro) phenylsulfonyl-3-acetylindole benzoyl hydrazone (**18**) and *N*-(3-nitro)phenylsulfonyl-3-acetyl-6-methylindole benzoyl hydrazone (**23**) displayed the highest anti-HIV-1 activity.

## ACKNOWLEDGEMENTS

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## CONFLICT OF INTEREST

The authors have reported no conflict of interest.

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