



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



## Design, synthesis, and biological evaluation of *N*-arylpiperazine derivatives as interferon inducers

Yeonjeong Chu<sup>a,b,e</sup>, B. Raja Sekhara Reddy<sup>c,e</sup>, V. Pratap Reddy Gajulapalli<sup>b,e</sup>, K. Sudhakar Babu<sup>c,\*</sup>, Eunha Kim<sup>b,\*</sup>, Sanghee Lee<sup>a,d,\*</sup>

<sup>a</sup> Brain Science Institute, Korea Institute of Science and Technology, Seoul, South Korea

<sup>b</sup> Department of Molecular Science and Technology, Ajou University, Suwon, South Korea

<sup>c</sup> Department of Chemistry, Sri Krishnadevaraya University, Anantapur, India

<sup>d</sup> Department of HY-KIST Bio-convergence, Hanyang University, Seoul, South Korea

### ARTICLE INFO

#### Keywords:

Interferon inducer  
type I Interferon  
Arylpiperazine  
Anti-viral agent  
Innate immunity

### ABSTRACT

Type I Interferon (IFN) signaling plays an important role in the immune defense system against virus infection and in the innate immune response, thus IFNs are widely used as anti-viral agents and treatment for immune disorder or cancer. However, there is a growing demand for novel small-molecule IFN inducer due to tolerance, toxicity, or short duration of action following direct administration of IFNs. In this study, we assessed arylpiperazine (ARP) as a new core skeleton of IFN inducer. To investigate structure–activity relationship, we designed and synthesized a series of ARP analogues and evaluated the ability to stimulate IFN response in THP-1 human monocyte cells. Compound **5i** was identified as a potent type I IFN inducer as it significantly increased cytokine secretion and increased expression of various IFN-stimulating genes which are representative biomarkers of type I IFN pathway. Our results suggested a beneficial therapeutic potential of **5i** as an anti-viral agent.

Interferons (IFNs) are essential signaling peptides which act as the first line of surveillance in defense system and modulate innate and adaptive immune activation.<sup>1,2</sup> In response to virus infection, IFNs are released by host cells, after which they bind to their specific receptors, thereby stimulating JAK/STAT signaling pathway and initiating transcription of various interferon-stimulated genes (ISGs) such as CXCL10, IRF7, IFIT3, and OAS etc.<sup>3–7</sup> Activation of IFN signaling pathway regulates not only anti-viral response but also induces cellular immunity by stimulating macrophage or monocyte.<sup>8</sup>

Based on the class of binding receptor and signaling cascade, IFNs are categorized into three major group: type I, type II and type III. Type I IFNs including IFN $\alpha$  and IFN $\beta$  are considered as therapeutic targets for treatment of hepatitis B and C infections and multiple sclerosis.<sup>9,10</sup> In addition, IFNs are used in cancer treatments of hematological malignancy such as leukemia and lymphomas.<sup>11,12</sup> Despite their therapeutic potential, there are several limitations of IFNs treatments including toleration, dose-related toxicity and side-effects such as dizziness, headache, muscle pain, and depression.<sup>13</sup> Moreover, IFNs are typically administrated by intramuscular injection for therapeutic use. PEGylated IFNs with enhancing stability are generally used in clinical treatment to

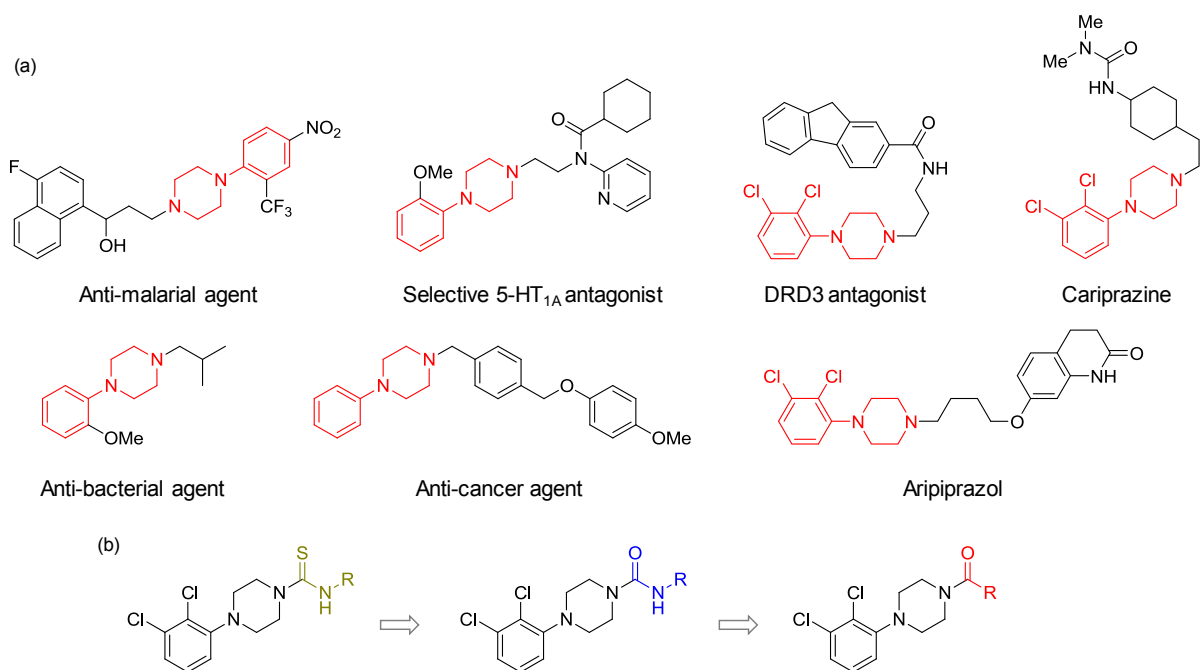
overcome the short duration of effect. However, PEGylated IFNs are not able to be used on patients with hyperbilirubinemia.<sup>14,15</sup> For these reasons, continuous efforts have been made to develop small-molecule modulators so as to stimulate IFNs. For example, tilorone is the first synthetic small-molecule drugs used as orally active IFN inducer which showed efficient anti-viral activity against broad spectrum of viruses including ebola virus and middle east respiratory syndrome-related coronavirus (MERS-Cov), especially.<sup>16</sup>

Therefore, we aimed to develop new small-molecule regulators to stimulate type I IFN effect and identified that particular arylpiperazine (ARP) derivatives exhibited the desired biological activity using phenotypic screening. Remarkably, the ARP core structure was reported to exert anti-malarial, anti-microbial, anti-cancer activity or selective 5-HT<sub>1A</sub> antagonistic effect (Fig. 1a).<sup>17–20</sup> In addition, 2,3-dichloro substituent in ARP pharmacophore plays important role in particular drugs such as cariprazine, aripiprazol or bioactive DRD3 antagonist (Fig. 1a).<sup>21–23</sup> Based on these important characteristics of the ARP skeleton, we designed and synthesized a series of 2,3-dichloro ARP derivatives by further *N*-modification on piperazine part with different linker moiety such as thiourea, urea, and carbonyl functional group in

\* Corresponding authors at: Brain Science Institute, Korea Institute of Science and Technology, Seoul, South Korea (S. Lee).

E-mail addresses: [drksbabu9@gmail.com](mailto:drksbabu9@gmail.com) (K. Sudhakar Babu), [ehkim01@ajou.ac.kr](mailto:ehkim01@ajou.ac.kr) (E. Kim), [slee19@kist.re.kr](mailto:slee19@kist.re.kr) (S. Lee).

<sup>e</sup> These authors equally contributed to this work.

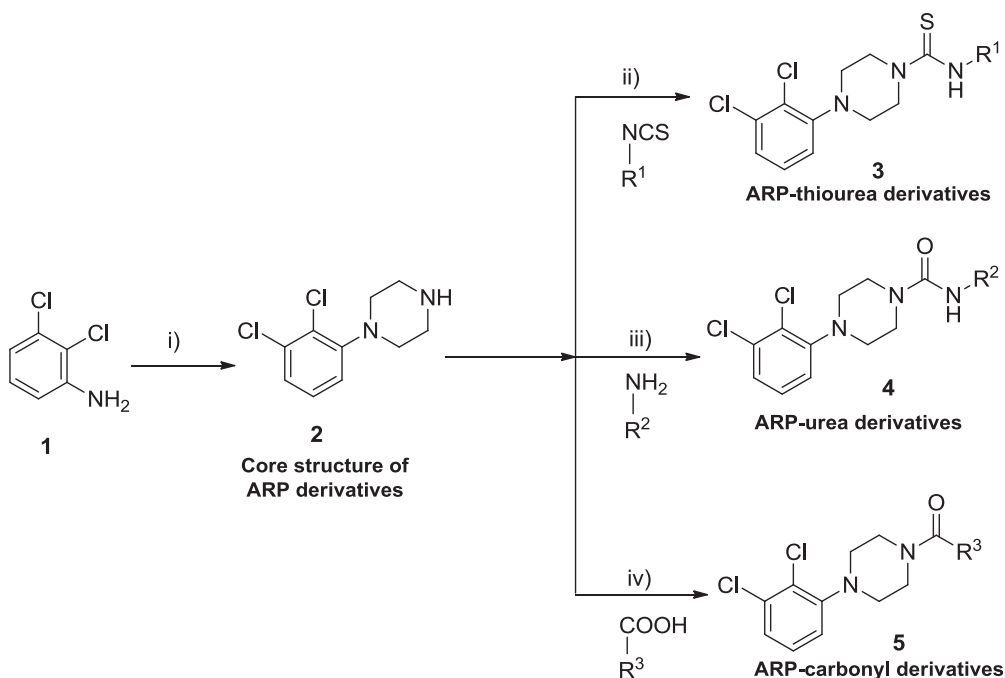


**Fig. 1.** (a) Various bioactive small molecules embedded in an arylpiperazine (ARP) and a di-chloro ARP core structure. (b) Design strategy of ARP derivatives for type I IFN inducers with a different linker moiety.

order to assess structure–activity relationship (SAR; Fig. 1b), then we evaluated their biological activities associated with the immune response.

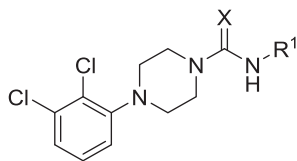
The series of ARP derivatives (3, 4, and 5) was completed in a two-step approach, as shown in Scheme 1. In the first step, 2,3-dichloroaniline (1) reacted with bis(2-chloroethyl)ethylamine to obtain 1-(2,3-dichlorophenyl) piperazine hydrochloride. Subsequently, 1-(2,3-dichlorophenyl)piperazine hydrochloride in the presence of NH<sub>4</sub>OH was converted to a free amine (–NH<sub>2</sub>) compound, 1-(2,3-

dichlorophenyl)piperazine (2). The crude product 2 was modified with diverse substituents based on thiourea (3), urea (4), and a carbonyl linker (5). For instance, derivatives 3a to 3f were produced from the reaction between 2 and various thiocyanates with triethylamine (TEA) in dry DCM. The intermediate 2 reacted with aromatic amines in the presence of triphosgene (1.5 equiv.) to produce compounds 4a to 4h (see Supplementary material). The amide coupling reaction between 2 and acid derivatives (1 equiv.) in presence of HATU and DIPEA (3 equiv.) in DMF produced compounds 5a to 5k (see Supplementary



**Scheme 1.** Synthesis of Arylpiperazine derivatives.<sup>a</sup> <sup>a</sup>Reaction conditions and reagents: (i) bis(2-chloroethyl)ethylamine, PTSA, tetrabutylammonium bromide, xylene, 135 °C, 48 h, reflux, NH<sub>4</sub>OH, (yield 88%); (ii) triethylamine, DCM, 15 min, room temperature, (yield 85–95%); (iii) Triphosgene, triethylamine, DCM, room temperature, 1 h, (yield 85–95%); (iv) HATU, DIPEA, dry DMF, room temperature, 12 h, (yield 85–90%).

**Table 1**  
ISRE reporter assay of ARP derivatives with thiourea or urea linker.



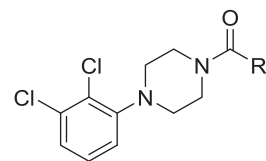
Compound	X	R <sup>1</sup>	RU <sup>a</sup>	Cell viability (%) <sup>b</sup>
3a	S		1.2 ± 0.4	84 ± 15
3b	S		1.1 ± 0.3	97 ± 2
3c	S		1.3 ± 0.3	81 ± 7
3d	S		1.2 ± 0.2	99 ± 4
3e	S		0.4 ± 0.2	46 ± 54
3f	S		1.5 ± 0.2	85 ± 9
4a	O		1.0 ± 0.3	90 ± 10
4b	O		2.1 ± 1.7	95 ± 11
4c	O		3.9 ± 1.4	61 ± 16
4d	O		4.2 ± 1.3	96 ± 11
4e	O		3.3 ± 2.0	98 ± 16
4f	O		3.3 ± 1.1	76 ± 17
4g	O		3.0 ± 1.3	70 ± 26
4h	O		2.7 ± 1.0	87 ± 15

<sup>a</sup> Relative unit of ISRE reporter signal. Normalized response using DMSO as a control. <sup>b</sup> THP-1 cell viability. RU and cell viability were measured by treatment with 40 μM of each compound and are shown as mean ± standard deviation.

material). Most of the compounds occurred at moderate yield (85–90%) and were characterized by low-resolution mass spectroscopy, <sup>1</sup>H NMR, and <sup>13</sup>C NMR. Purity of the compounds was analyzed using high-performance liquid chromatography (HPLC) before performing biological assays.

All the synthesized compounds were evaluated using ISRE reporter assay on THP-1 human monocyte cells for monitoring immune response. In this system, ISG54 minimal promoter in conjunction with five ISRE elicits transcription of secreted luciferase reporter gene upon

**Table 2**  
ISRE reporter assay of ARP derivatives with carbonyl linkers.



Compound	R <sup>1</sup>	RU <sup>a</sup>	Cell viability (%)
5a		1.3 ± 0.7	89 ± 16
5b		2.5 ± 0.8	89 ± 8
5c		2.8 ± 0.3	105 ± 20
5d		1.8 ± 0.1	90 ± 15
5e		3.7 ± 0.9	81 ± 16
5f		1.9 ± 1.1	83 ± 14
5g		1.4 ± 0.6	84 ± 16
5h		2.3 ± 1.2	86 ± 18
5i		5.2 ± 1.5	98 ± 22
5j		2.8 ± 1.7	101 ± 15
5k		2.5 ± 1.4	96 ± 12

<sup>a</sup> Relative unit for ISRE reporter signal. Normalized response by DMSO as a control. <sup>b</sup> THP-1 cell viability. RU and cell viability were measured by treatment with 40 μM of each compounds and are shown as mean ± standard deviation.

stimulation of the type I IFN pathway, and IFN-related immune activation was measured based on luminescence.<sup>24</sup>

As a result of the SAR study (Table 1.), all thiourea derivatives revealed negligible or very weak potency, regardless of alkyl and aryl moiety at R<sup>1</sup> (3a to 3f). No significant effects of *p*-nitro, fluoro, chloro substituents were observed on the aryl ring, however, the *p*-bromo substitution revealed undesired cellular toxicity (3c to 3e). In the aryl part, *meta* substitution lead to slightly increased activity compared to *para* substitution (3e and 3f).

To enhance activity, we introduced a urea moiety as a linker with diverse substitution on the aryl ring, which increased potency of ARP analogues for immune stimulation. Compared with the thiourea linker,

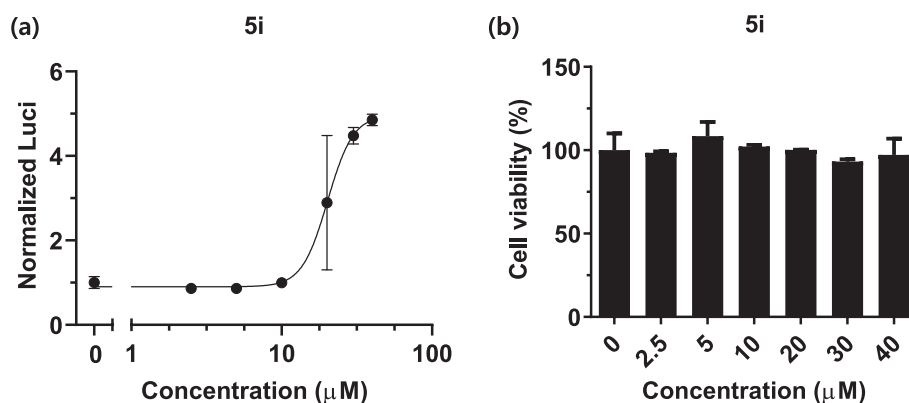


Fig. 2. (a) Dose-response curve of the ISRE reporter assay. (b) THP-1 cell viability in 5i treatments.

*para* substitution on the phenyl ring exhibited stronger potency than *meta* substitution in most urea linker cases (4a to 4h). In *para*-substituted derivatives, no significant activity was observed in the nitro moiety (4a) which corresponded to thiourea linker, whereas the fluoro and trifluoromethyl moieties showed increased activity (4b and 4c). However, compound 4c showed undesired cellular toxicity, thereby a trifluoromethyl substituent was replaced by a trifluoromethoxy moiety which substantially enhanced potency (4.2-fold change) and generated no toxicity (4d).

Considering the interesting results of thiourea and urea linker, we next incorporated carbonyl moiety as a linker for ARP derivatives (Table 2). Somewhat weaker or stronger potency was observed in case of trifluoromethyl and methoxy substituents, compared to individual functional group in urea linker (5c to 5e). For the further investigation, aliphatic chain or heteroaryl group were introduced at R<sup>1</sup> position which produced no significant difference and only marginally increased activity (5a and 5b). Furthermore, disubstituted ARP analogues were considered to increase activity (5f to 5h) that resulted in weak activity which was below that of *p*-methoxy analogue (5e).

To increase potency of ARP derivatives, we examined the effects on carbon element by incorporating a benzyl group with a trisubstituted functional group. For this purpose, trimethoxy benzyl (5i) or tri-fluoro benzyl (5j) were introduced at the phenyl ring. As a result, immune response was considerably improved due to tri-methoxy substitution on the aryl ring (5.2-fold change), whereas tri-fluoro benzyl substitution showed no drastic change on ISRE result with a bit increase of activity (2.8-fold change). In addition, we confirmed no effect on cell viability by benzyl group. For the further modification, we inserted a phenethyl moiety at R<sup>1</sup> position (5k) to assess effects of carbon chain which showed activity similar to that of dichlorophenyl (5h) or trifluorobenzyl (5j) moiety (2.5, 2.3 and 2.8, respectively) Based on these

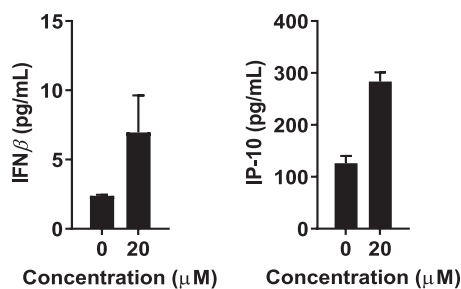


Fig. 3. Evaluation of the effect on IFN $\beta$  and IP-10 secretion by compound 5i. Cytokine levels were measured using ELISA.

SAR results, we identified the compound 5i as an effective type I IFN inducer and conducted further biological evaluation.

To estimate the potency of compound, we tested dose-dependency of immune responses elicited by 5i, which clearly induced stimulation of ISRE reporter signal. EC<sub>50</sub> was calculated as 13.1  $\mu$ M and E<sub>max</sub> was interpolated as 4.9-fold change (Fig. 2a). No cellular toxicity to THP-1 cells was observed at treatment dosages of up to 40  $\mu$ M (Fig. 2b).

We further evaluated whether 5i-mediated activation of ISRE reporter signal would be correlated with activation of innate immunity. Using ELISA assay, we measured extracellular release of IFN $\beta$  and IP-10 which are associated with activation of the type I IFN pathway. The results confirmed that 5i certainly induced secretion of IFN $\beta$  and IP-10 (Fig. 3).

In response to virus infection, host cells released various cytokines such as IFNs and the cytokine-mediated anti-viral status is established and maintained by production of various ISGs. To validate effects of 5i on anti-viral state, we explored the impact of 5i on type I IFN-induced ISG expression. Corresponding to the cytokine level, mRNA expression of IFN $\beta$  and CXCL10 was clearly increased by 5i treatment (Fig. 4). In addition to IFN $\beta$  and CXCL10, 5i significantly elicited transcription of various ISGs such as IRF7, IFIT3, and OAS1 which are considered biomarkers of type I IFN signaling pathway (Fig. 4). In conclusion, all these results indicated the potential ability of 5i as an efficient type I IFN inducer without undesired toxicity. Although, it is hard to conclude that 5i showed superior potency compared to tilorone, an efficient small molecule anti-viral agent inducing type I IFN, due to the different evaluating system. Considering 50 mg/kg required to treat Ebola virus by tilorone,<sup>25</sup> the fact that 5i induced significant increased IFN $\beta$  secretion at 20  $\mu$ M suggested structure insight for developing new anti-viral therapy.

In conclusion, we confirmed ARP motif as a new core skeleton of type I IFN regulation. Moreover, we synthesized various ARP analogues and investigated their biological activity for anti-viral state and innate immunity. Based on the SAR analysis, we verified the importance of the carbonyl linker and the benzyl moiety with a trimethoxy attachment on adjacent aryl ring in the ARP pharmacophore. As a result, compound 5i was identified as a potent type I IFN inducer. Further investigation about cytokine secretion and IFN-mediated ISG expression by 5i indicated effective stimulation of type I IFN pathway. All these results suggest a beneficial therapeutic potential of 5i for anti-viral agent.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

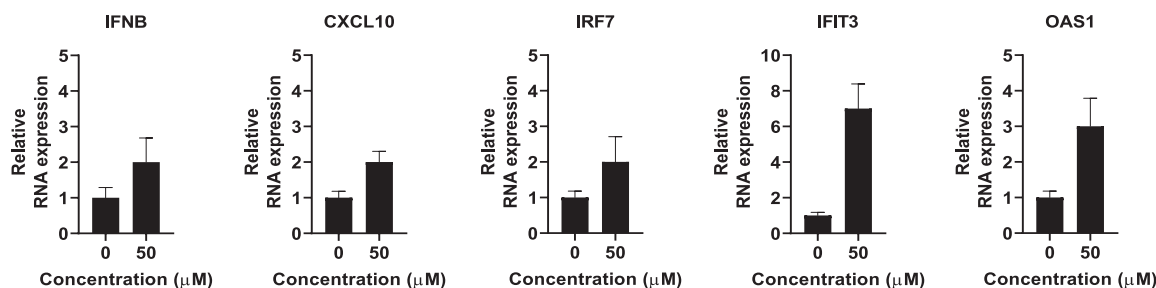


Fig. 4. Gene expression results of various interferon-stimulated genes. mRNA expression level of IFNB, CXCL10, IRF7, IFIT3, OAS1 was examined by qPCR and was normalized using GAPDH expression. Graph are shown as the average value and RQmax.

## Acknowledgments

This study was financially supported by Korea Institute of Science and Technology (KIST) Institutional Program (2E30180) and Bio & Medical Technology Development Program (NRF-2019M3E5D4066905) of the National Research Foundation funded by Korean government (MSIT), a grant from Priority Research Centers Program (2019R1A6A1A11051471), 2020R1C1C1010044 funded by the National Research Foundation of Korea (NRF) and Convergence brain research through cross-institute collaboration (KBRI 20-BR-03-01/ IBS-R001-D1-2020-b02).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bmcl.2020.127613>.

## References

- Hall JC, Rosen A. Type I interferons: crucial participants in disease amplification in autoimmunity. *Nat Rev Rheumatol*. 2010;6:40–49.
- Le Bon A, Etchart N, Rossmann C, et al. Cross-priming of CD8+ T cells stimulated by virus-induced type I interferon. *Nat Immunol*. 2003;4:1009–1015.
- Liu M, Guo S, Hibbert JM, et al. CXCL10/IP-10 in infectious diseases pathogenesis and potential therapeutic implications. *Cytokine Growth Factor Rev*. 2011;22:121–130.
- Honda K, Takaoka A, Taniguchi T. Type I interferon gene induction by the interferon regulatory factor family of transcription factors. *Immunity*. 2006;25:349–360.
- Liu X-Y, Chen W, Wei B, Shan Y-F, Wang C. IFN-induced TPR protein IFIT3 potentiates antiviral signaling by bridging MAVS and TBK1. *J Immunol*. 2011;187:2559–2568.
- Sadler AJ, Williams BRG. Interferon-inducible antiviral effectors. *Nat Rev Immunol*. 2008;8:559–568.
- Schoggins JW, Rice CM. Interferon-stimulated genes and their antiviral effector functions. *Curr Opin Virol*. 2011;1:519–525.
- Munder BM, Mallo M, Eichmann K, Modolell M. A novel pathway of autocrine macrophage activation. *J Exp Med*. 1998;187:2103–2108.
- Reder AT, Feng X. How type I interferons work in multiple sclerosis and other diseases: Some unexpected mechanisms. *J Interf Cytokine Res*. 2014;34:589–599.
- Villamil A, Mullen E, Casciato P, Gadano A. Interferon beta 1a-induced severe autoimmune hepatitis in patients with multiple sclerosis: Report of two cases and review of the literature. *Ann Hepatol*. 2015;14:273–280.
- Tamura K, Makino S, Araki Y, Imamura T, Seita M. Recombinant interferon beta and gamma in the treatment of adult T-cell leukemia. *Cancer*. 1987;59:1059–1062.
- Gill PS, Harrington W, Kaplan MH, et al. Treatment of adult t-cell leukemia-lymphoma with a combination of interferon alfa and zidovudine. *N Engl J Med*. 1995;332:1744–1748.
- Parker BS, Rautela J, Hertzog PJ. Antitumour actions of interferons: implications for cancer therapy. *Nat Rev Cancer*. 2016;16:131–144.
- Ishigami M, Hayashi K, Katano Y, Itoh A, Hirooka Y, Goto H. Impact of early elevation of serum bilirubin during treatment with pegylated interferon and ribavirin in patients with chronic hepatitis C. *Hepatol Res*. 2010;40:963–970.
- Zwirter R, Narasimhan P, Wind-Rotolo MM, et al. Mechanisms of hyperbilirubinemia during peginterferon lambda-1a therapy for chronic hepatitis c infection: a retrospective investigation. *J Interf Cytokine Res*. 2016;36:644–651.
- Ekins S, Madrid PB. Tilorone, a broad-spectrum antiviral for emerging viruses. *Antimicrob Agents Chemother*. 2020;64.
- Darmani NA. The silent and selective 5-HT(1A) antagonist, WAY 100635, produces via an indirect mechanism, a 5-HT(2A) receptor-mediated behaviour in mice during the day but not at night. *J Neural Transm*. 1998;105:635–643.
- Chen H, Yu YZ, Tian XM, et al. Synthesis and biological evaluation of arylpiperazine derivatives as potential anti-prostate cancer agents. *Bioorganic Med Chem*. 2019;27:133–143.
- Chaudhary P, Kumar R, Verma AK, et al. Synthesis and antimicrobial activity of N-alkyl and N-aryl piperazine derivatives. *Bioorganic Med Chem*. 2006;14:1819–1826.
- Quiliano M, Pabón A, Moles E, et al. Structure-activity relationship of new anti-malarial 1-aryl-3-substituted propanol derivatives: Synthesis, preliminary toxicity profiling, parasite life cycle stage studies, target exploration, and targeted delivery. *Eur J Med Chem*. 2018;152:489–514.
- Maramai S, Gemma S, Brogi S, et al. Dopamine D3 receptor antagonists as potential therapeutics for the treatment of neurological diseases. *Front Neurosci*. 2016;10:1–16.
- Grady MA, Gasperoni TL, Kirkpatrick P. Aripiprazole. *Nat Rev Drug Discov*. 2003;2:427–428.
- Ágai-Csöngör É, Domány G, Nógrádi K, et al. Discovery of cariprazine (RGH-188): A novel antipsychotic acting on dopamine D3/D2 receptors. *Bioorganic Med Chem Lett*. 2012;22:3437–3440.
- Levy DE, Kessler DS, Pine R, Reich N, Darnell JE. Interferon-induced nuclear factors that bind a shared promoter element correlate with positive and negative transcriptional control. *Genes Dev*. 1988;2:383–393.
- Ekins S, Lingerfelt MA, Comer JE, et al. Efficacy of Tilorone Dihydrochloride against Ebola Virus Infection. *Antimicrob Agents Chemother*. 2018;62:e01711–e1717.