Introduction

Pro-inflammatory cytokines are involved in the pathogenesis of a large number of disease processes. Interleukin 6 (IL-6) and tumour necrosis factor-α (TNF-α) are two multi-functional pro-inflammatory cytokines that are involved in the pathogenesis of inflammation, cardiovascular diseases, cancer and neurodegenerative disease through a series of cytokine signalling pathways [1, 2]. Hence, inhibition of such cytokines has currently become a major target of drug development. It is, however, important that such potential therapeutic agents demonstrate inhibitory bioactivity with respect to these cytokines [2, 3].

Curcumin (diferuloylmethane) is an orange–yellow compound from turmeric (Curcuma longa), a spice found in curry powder. Traditionally known for its anti-inflammatory effects, curcumin has established itself in the last two decades to be a potent immunomodulatory agent that can regulate the activation of a variety of immunocytes and the expression of inflammatory factors. Considering that the β-diketone moiety of curcumin may result in its instability and poor metabolic property, we previously designed a series of mono-carbonyl analogues of curcumin with enhanced stability by deleting this moiety. These compounds demonstrate improved pharmacokinetic profiles both in vitro and in vivo. In this study, we reported a total of 44 mono-carbonyl analogues, which have been evaluated for the inhibitory activities against LPS-induced TNF-α and IL-6 release in the macrophages. Based on the screening results of these analogues, five active compounds A01, A03, A13, B18 and C22 were investigated to inhibit TNF-α and IL-6 release in a dose-dependent manner, three of which further demonstrated inhibitory effects on LPS-induced TNF-α, IL-1β, IL-6, MCP-1, COX-2, PGES, INOS and p65 NF-κB mRNA production. The results indicated that these mono-carbonyl analogues may possess anti-inflammatory activities similar to curcumin despite the absence of the β-diketone. These mono-carbonyl analogues may be a favourable alternative for the development of curcumin-based anti-inflammatory drugs both pharmacokinetically and pharmacologically. We further examined the biological properties of A13, the only hydrosoluble analogue when combined with hydrochloric acid. The results showed a dose-dependent inhibition of LPS-induced cytokine production. These data further indicated that compound A13 may be explored as a promising anti-inflammatory molecule.

Abstract

Curcumin (diferuloylmethane) is an orange–yellow compound from turmeric (Curcuma longa), a spice found in curry powder. Traditionally known for its anti-inflammatory effects, curcumin has established itself in the last two decades to be a potent immunomodulatory agent that can regulate the activation of a variety of immunocytes and the expression of inflammatory factors. Considering that the β-diketone moiety of curcumin may result in its instability and poor metabolic property, we previously designed a series of mono-carbonyl analogues of curcumin with enhanced stability by deleting this moiety. These compounds demonstrate improved pharmacokinetic profiles both in vitro and in vivo. In this study, we reported a total of 44 mono-carbonyl analogues, which have been evaluated for the inhibitory activities against LPS-induced TNF-α and IL-6 release in the macrophages. Based on the screening results of these analogues, five active compounds A01, A03, A13, B18 and C22 were investigated to inhibit TNF-α and IL-6 release in a dose-dependent manner, three of which further demonstrated inhibitory effects on LPS-induced TNF-α, IL-1β, IL-6, MCP-1, COX-2, PGES, INOS and p65 NF-κB mRNA production. The results indicated that these mono-carbonyl analogues may possess anti-inflammatory activities similar to curcumin despite the absence of the β-diketone. These mono-carbonyl analogues may be a favourable alternative for the development of curcumin-based anti-inflammatory drugs both pharmacokinetically and pharmacologically. We further examined the biological properties of A13, the only hydrosoluble analogue when combined with hydrochloric acid. The results showed a dose-dependent inhibition of LPS-induced cytokine production. These data further indicated that compound A13 may be explored as a promising anti-inflammatory molecule.

Keywords: curcumin analogues • anti-inflammatory activity • SAR • inflammatory factor • COX-2
arthritis, inflammatory eye diseases, inflammatory bowel disease, chronic pancreatitis and cancers in several independent phase I and phase II clinical trials [10].

Although curcumin can be safely used with an oral dose as high as 12 g/day, its clinical applications have been significantly limited by its instability and poor metabolic properties [11–13]. As demonstrated in clinical studies, curcumin possesses poor pharmacokinetic profiles, such as low bioavailability and rapid metabolism. In a recent phase II trial, the curcumin level in plasma reached only up to 22–41 ng/ml even after administering a daily oral dose of 8.0 g/day for 4 weeks [14].

It has been suggested that β-diketone moiety may be responsible for such rapid degradation and poor bioavailability of curcumin. Recent reports have demonstrated the β-diketone moiety in curcumin is a specific substrate for liver aldo-keto reductases. Presence of β-diketone may be one of major factors contributing to the rapid metabolism of curcumin in vivo [15, 16]. In our previous publication, we have demonstrated design and synthesis of a series of curcumin mono-carbonyl analogues without the β-diketone moiety (shown in Fig. 1) [17, 18]. We have also showed that these analogues, without β-diketone, exhibited enhanced stability in vitro and improved pharmacokinetic profiles in vivo [19]. We evaluated a total of 87 analogues for anti-inflammatory properties using LPS-stimulated mouse J774.1A macrophages, 43 of which were reported previously [18]. The purpose of this communication is to examine whether deletion of reactive β-diketone moiety have any effects on the anti-inflammatory activity compared with the lead compound. In this study, we focus on the rest 44 mono-carbonyl analogues of curcumin and report their anti-inflammatory activities. Following initial screening, we further studied three bioactive compounds A01, A13 and B18 with regards to their abilities to prevent LPS-induced inflammatory mRNA expression. We further expanded our studies and characterized bioactivity of only water-soluble compound A13.

Materials and methods

Chemical synthesis

Curcumin (Sigma-Aldrich, St. Louis, MO) and its synthetic analogues were dissolved in DMSO at 20 mmol/l as stock solution. The general procedure of synthesis of the present analogues of curcumin is outlined below. Following the addition of 7.5 mmol ketone to a solution of 15 mmol arylaldehyde in MeOH (10 ml), the solution was stirred at room temperature for 20 min. and was followed by dropwise addition of 20% NaOH (1.5 ml, 7.5 mmol). The mixture was stirred at room temperature and monitored with TLC. Following completion of the reaction, the residue was poured into saturated NH4Cl solution and filtered. The precipitate was washed sequentially with water, cold ethanol, cold acetone and vacuum dried. The solids were purified by chromatography over silica gel using CH2Cl2/CH3OH as the eluent for compounds 03–21. The synthesis of compounds 01 and 02 has previously been reported by us [17]. A stock solution of A13-HCl at 40 mM was obtained by dissolving 40 μmol A13 in 1 ml of 80 mM HCl solution that was used in appropriate dilution with water for experiments.

Melting points were determined on a Fisher–Johns melting apparatus and are uncorrected. 1HNMR spectra were recorded on a Varian INOVA-400 spectrometer (Varian Medical Systems Inc., Palo Alto, CA, USA). The chemical shifts are presented in terms of parts per million with TMS as the internal reference. Electron-spray ionization mass spectra in positive mode (ESIMS) data were recorded on a Bruker Esquire 3000+ spectrometer (Bruker BioSpin Ltd., Milton, Ontario, Canada). Column chromatography purifications were carried out on Silica Gel 60 (E. Merck, 70–230 mesh). Chemical reagents, structural characterization in 1H NMR and MS, physical properties and molecular formulas of the new derivative compounds are being submitted as supporting information (Supplementary file).

Cell line and reagents

Mouse J774A.1 macrophages were obtained from the Molecular Pharmacology Lab (Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA). Cell culture reagents and NuPAGE Novex Bis–Tris and Tris–acetate Gels were obtained from Invitrogen (Carlsbad, CA). Foetal bovine serum was from Atlanta Biologicals (Norcross, GA) and was heat-inactivated for 30 min. at 65°C. Antibodies against COX-2 and horseradish peroxidase-conjugated donkey anti-goat IgG were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Bio-Rad protein assay reagent, horseradish peroxidase-conjugated goat anti-rabbit IgG and Precision Plus Protein Kaleidoscope Standards were obtained from Bio-Rad (Hercules, CA). BioMax MS film was obtained from Eastman Kodak (Rochester, NY). RNAqueous total RNA isolation kit was purchased from Ambion (Austin, TX). High-Capacity cDNA archive kit and gene expression kits for mouse ATP-binding cassette were bought from Applied Biosystems (Foster City, CA). All other chemical reagents were obtained from Sigma Chemicals (St. Louis, MO).

Cell treatment

Mouse J774A.1 macrophages were maintained in DMEM media supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C with 5% CO2. Human lung cancer H460 cells
were incubated for 1–24 hrs. and primers for mouse TNF-
medium, respectively. The synthetic process of
cyclohexanone, cyclopentanone or acetone in an alkaline
obtained by coupling the appropriate aromatic aldehyde with
routes we previously reported [17], compounds
single carbonyl group (shown in Fig. 2). Using the synthetic
in supplementary file.

Enzyme-linked immunosorbent assay (ELISA)

After treatment, the culture media and cells were collected separately. The levels of TNF-α and IL-6 in the media were determined by ELISA using specific mouse antibody and ELISA Max™ Set Deluxe Kits (Biolegend, San Diego, CA, USA). The tests were performed according to the manufac-
turer's instruction. The cells collected were lysed with the total lysis
buffer (Tris–HCl, 20 mM; NP40, 1%; NaCl, 150 mM; EDTA, 2 mM; SDS,
0.1%; NaF, 20 mM; Na3VO4, 20mM; H2O). The total protein concentrations
of the viable cell pellets were determined using Bio-Rad protein assay
reagents. Total amount of the inflammatory factor in the media were nor-
malized to the total protein amount of the viable cell pellets.

RNA isolation and real-time quantitative PCR

Total cellular RNA was isolated from mouse J774A.1 macrophages after
treatment with LPS and compounds or vehicle control for 24 hrs, using
an Ambion RNAqueous kit. Total RNA (10 μg) was used for first-strand
cDNA synthesis using High-Capacity cDNA archive kit (Applied Biosystems). The mRNA levels of TNF-α, IL-1β, IL-6, MCP-1, COX-2
and NF-κB were quantified using the specific gene expression assay kits
and primers for mouse TNF-α, IL-1β, IL-6, MCP-1, COX-2 and NF-κB on
an iQ5 multi-color real-time PCR detection system. The mRNA values
for each gene were normalized to internal control β-actin mRNA. The ratio of normalized mean value for each treatment groups to vehicle
control was calculated.

Results

Synthesis of curcumin analogues

Three series of dienones, 1,5-diyanyl-1,4-pentadiene-3-ones(B),
together with cyclopentanone(A) and cyclohexanone(C) ana-
logues, were designed by displacing β-diketone moiety with a
single carbonyl group (shown in Fig. 2). Using the synthetic
routes we previously reported [17], compounds 1–19 were
obtained by coupling the appropriate aromatic aldehyde with
cyclohexanone, cyclopentanone or acetone in an alkaline
medium, respectively. The synthetic process of 01 and 02
through hydroxyl protection and deprotection were reported pre-
viously [17]. The synthetic yields, melting points, 1H NMR and
ESI-MS analysis of unpublished compounds are being described in
supplementary file.

Inhibition of the LPS-induced TNF-α and IL-6 release by curcumin analogues

The stimulation by microbial endotoxins may induce an inflam-
matory process in the macrophages through cytokine signalling
pathways. Lipopolysaccharide (LPS), an endotoxin from the
walls of Gram-negative bacteria, is a potent stimulator of
inflammatory cytokines in macrophages. Hence, curcumin and
its 44 synthetic analogues in the 5-carbon linker series were
evaluated for their inhibitory abilities against LPS-induced
TNF-α and IL-6 release in the mouse macrophages. Cells were
pre-treated with compounds for 2 hrs and then incubated with
LPS for 22 hrs. The amount TNF-α and IL-6 in media were
detected by ELISA and normalized by protein concentration of
cells harvested in the homologous cultural plates. Their
inhibitory activities are shown in Fig. 3. The initial screening of
these analogs at 10 μM concentration showed that the majority
of them inhibited the expression of TNF-α and IL-6 induced by
LPS and their inhibitory abilities were comparable to or some-
times more pronounced than that of the leading curcumin at the
same concentration. The results of the anti-inflammation assay of three classes of
analogues are shown in Fig. 3A and B, respectively. Among
these compounds, 24 compounds were found to be more potent
than curcumin in inhibiting LPS-induced TNF-α expression, and
17 compounds showed better inhibitory effects than curcumin
did on LPS-induced IL-6 expression. Compounds A01, A08,
A13, A19, B01, B03, B06, B09, B11, B17, B18, C01, C02, C11
and C12 exhibited stronger inhibition against both TNF-α
and IL-6 than curcumin did. B03, a 2′,5′-dimethyl-substituted com-
 pound without the phenolic groups, showed the strongest
inhibitory effect on LPS-induced TNF-α and IL-6 release among
tested analogues and its inhibitory effects reached 34.3 and
15.9%, respectively.

Active compounds inhibit the TNF-α and IL-6 release in dose-dependent manner

Of the active analogues above, four compounds A01, A03, A13,
B18 and one previously reported compound C22 (2,6-bis(4-(ally-
loxy)benzylidene)cyclohexanone) [18], which demonstrated low
cytotoxicities (data not shown) in macrophages, were selected for
further assessment of their dose-dependent inhibitory effects
against LPS-induced TNF-α and IL-6 release. J774A.1 macrophages
were pre-treated with curcumin or its analogues in a series of
concentration (2.5, 5.0, 10 and 20 μM) for 2 hrs and were subse-
quently incubated with LPS (0.5 μg/ml) for 22 hrs. The results
(shown in Fig. 4) indicated a dose-dependent inhibition of LPS-
induced TNF-α and IL-6 release by curcumin and all five ana-
logues. Compounds A01, A13 and B18 demonstrated comparable
inhibitory effects to curcumin. Interestingly, curcumin was found
to be more effectively with respect to the inhibition of LPS-induced
IL-6 release, whereas A01, A13 and B18 showed better inhibitory activities against TNF-α release. B18, the only heterocyclic ring-containing analogues, was observed to possess, in the concentration of 20 μM, most potent effect in reducing TNF-α release and a comparable IL-6-inhibitory ability reaching a level similar to curcumin.

A01, A13 and B18 inhibited mRNA expression of inflammatory cytokines induced by LPS

LPS has been reported to augment mRNA expression of inflammatory factors, including TNF-α, IL-6, IL-1β and monocyte chemoattractant protein 1 (MCP-1), which are of importance in...
the genesis and development of inflammation [20, 21]. Hence, the effects of compounds A01, A13 and B18 were further evaluated with respect to such mRNA expression. The cells were treated with compounds and LPS and total RNA was extracted. Specific mRNAs were detected by real-time RT-PCR (shown in Fig. 5). Compared to the vehicle control, LPS increased the level of the mRNAs of TNF-α/H9251, IL-6, IL-1β/H9252 and MCP-1. When cells were co-incubated with LPS and curcumin or these three analogues at 10 μM, increases in the levels of inflammatory mRNAs were prevented, indicating that A01, A13, B18 and curcumin have inhibitory effects on these mRNAs expression.

Cyclooxygenase 2 (COX-2), prostaglandin E synthase (PGES) and inducible NO synthase (iNOS) have been demonstrated as inflammatory enzymes that are altered in inflammation and in various disease states in humans. They have also been reported to be responsive to the LPS stimulation [9, 20–22]. To determine whether curcumin and its analogs also have any inhibitory effects on the expression of inflammatory enzymes in macrophages, total mRNA was extracted and mRNA of COX-2, PGES and iNOS was, respectively, analyzed by real-time RT-PCR after cells were treated with compounds and LPS for 24 hrs. As shown in Fig. 5B, LPS-induced mRNA expressions of three inflammatory enzymes were significantly reduced by A01, A13, B18 and curcumin at 10 μM concentration. Although the inhibitory effects of analogues were not as pronounced as that of curcumin in COX-2 and iNOS groups, the majority of them reduced LPS-induced expressions of mRNAs below the level of the control group. In PGES group, A13 and B18 exhibited slightly stronger inhibitory effects than curcumin.

Compounds A01, A13 and B18 inhibit the NF-κB p65 mRNA expression

NF-κB is an LPS-inducible transcription factor that plays a central role in the mammalian innate immune response and inflammation. The activation of NF-κB is exerted through the regulation of downstream target genes that encode pro-inflammatory cytokines and inducible enzymes, such as TNF-α, IL-6, IL-1β and COX-2 [23, 24]. A number of studies reported showed that curcumin abrogates NF-κB activation and reduces NF-κB over-expression induced by LPS, TNF-α or H2O2 [25, 26]. In this section, we investigated the effects of NF-κB p65 mRNA expression by curcumin and active analogues in order to know about their abilities in the level of transcription factor. As shown in Fig. 6, LPS-induced increase of NF-κB p65 mRNA level were prevented by curcumin and its synthetic analogues at 10 μM concentration. Hence, similar to
curcumin, these mono-carbonyl analogues can also inhibit LPS-induced NF-κB expression.

**Water-soluble compound A13-HCl inhibit the expression of inflammatory cytokines in a dose-dependent manner**

Among active analogues above, A13 possesses two N,N-dimethyl groups in its structure, which provides water solubility of A13 as a quaternary ammonium salt when conjugated with two hydrochloric acid molecules. The advantage of hydrosolubility of a drug is of significant importance. Thus, water-soluble A13-HCl was prepared for the study of its dose-dependent effects. As shown in Fig. 7, the quaternary ammonium salt form of A13 inhibited LPS-induced TNF-α, IL-6 and IL-1β release in a dose-dependent manner, indicating that the combination of A13 and HCl dose not decrease the anti-inflammatory ability of A13.

**Discussion**

The main findings of this study are that we have developed several mono-carbonyl analogues of curcumin with potent anti-inflammatory...
activities. We have also demonstrated that the water-soluble compound (A13) has dose-dependent anti-inflammatory activity and has the potential to be developed as a therapeutic agent.

As an excellent leading compound, curcumin has been investigated in depth in the field of medicinal chemistry. A number of analogues of curcumin have also been designed and synthesized for the development of new anti-inflammatory and anti-cancer drugs [27–31]. Considering that the presence of β-diketone moiety may result in the instability and poor metabolic properties of curcumin, we have previously designed a series of mono-carbonyl analogues of curcumin with enhanced stability in vitro and improved pharmacokinetic profiles in vivo [17–19]. In the present study, 44 mono-carbonyl analogues were evaluated for the inhibitory activities against LPS-induced TNF-α and IL-6 release in macrophages in an attempt to identify compounds with potent biological activities, which can be targeted for development as pharmaceutical agents.

Curcumin can influence functions of different cells in a variety of ways [32]. Several structural moieties of curcumin, such as the β-diketone and phenolic hydroxyl group, have been reported to be responsible for the bioactivities of this compound [29–31, 33]. As previously reported, the reactive β-diketone was characterized as an important bioactivity-inducing moiety and may contribute to the anti-oxidant properties of curcumin [33, 34]. However, there

Fig. 5 Curcumin, A01, A13 and B18 inhibited LPS-induced inflammatory mRNA expression in J774A.1 macrophages. Cells were pre-treated with compounds at 10 μM or vehicle control for 2 hrs and treated with LPS (0.5 μg/ml) for 22 hrs. The mRNA levels of inflammatory cytokines TNF-α, IL-6, IL-1β and MCP-1 (A), and inflammatory enzymes COX-2, PGES, and iNOS (B) were quantified by real-time quantitative PCR. The mRNA values for each gene were normalized to internal control β-actin mRNA and were expressed as a ratio to DMSO.
are no studies that have directly addressed the relationship of \(\beta\)-diketone and anti-inflammatory property of curcumin until now. Compounds \(\text{A13}\), which are just derived from the replacement of \(\beta\)-diketone in curcumin by a mono-carbonyl group, exhibited comparable bioactivities to curcumin, suggesting that the \(\beta\)-diketone moiety may not have a role on curcumin’s anti-inflammatory property. According to the screening results, we discussed the possible structure-activity relationship of this kind of analogues.

The present acetone-derived \(\text{B}\)-class compounds are slightly more effective than cyclopanetanone-derived \(\text{A}\)-class and cyclohexanone-derived \(\text{C}\)-class compounds, whereas our previous publication has suggested that the \(\text{C}\)-class compounds showed relatively higher activities than \(\text{A}\)- and \(\text{B}\)-class compounds according to the previous 43 compounds \[18\]. Taken all 87 analogues together, it is indicated that the structure of 5-carbonyl linker may have a role on such activities and acetone (\(\text{B}\)) and cyclohexanone (\(\text{C}\)) linkers are more favourable for the anti-inflammatory property than cyclopanetanone linker (\(\text{A}\)). It is interesting to note that the analogues that retained a phenyl substituent showed pronounced biological activities than curcumin. Among the analogues containing heterocyclic ring, only \(\text{A19}\), \(\text{B17}\) and \(\text{B18}\) exhibited higher activities than curcumin. These data indicate that the phenyl structure of curcumin may be necessary to retain its activity. Among the 2-’halogen–containing analogues \(\text{A7}\) (2-’-F), \(\text{A8}\) (2-’-Cl) and \(\text{A9}\) (2-’-CF3), compounds \(\text{A9}\) exhibited stronger inhibitory effects against LPS-induced inflammation than \(\text{A7}\), \(\text{A8}\) and even compared with curcumin. Thus, these results indicate that the bioactivity of analogues against inflammation induced by LPS is associated with electronegativity of the 2-’-substituent.

Among analogues with 4-’-phenolic group, the 3-’-methoxy-containing compounds \(\text{A1}\) showed the excellent inhibitory activities, whereas compounds \(\text{A2}\) demonstrated minimal inhibitory activities or even stimulatory effects, suggesting that the presence of a 3-’-methoxy group is critical to curcumin’s activity. Previous
reports [33, 35] have demonstrated the formation of hydrogen bonding between 3′-OCH₃ and 4′-OH of curcumin decreases the electron-donating ability of 4′-OH. Thus, our data further confirm that reduction of the electron-donating ability of the 4′-substituent may increase the anti-inflammatory abilities of the mono-carbonyl analogues. Alkylation is a common approach to reduce the electron-donating ability of OH. As demonstrated in Fig. 3, N,N-dimethyl-propyl-alkylized A13 and 3′,4′-(O-CH₂-O)-containing B11 showed marked inhibitory activities against LPS-induced TNF-α and IL-6. Compounds 03, 06 and 12, lacking electron-effective moiety in the 4′-position, also exhibited excellent anti-inflammatory properties. We further analyzed the SAR of 4′-position, and showed that, a weak electron-donating substituent at the 4′-position augments the anti-inflammatory activity of the mono-carbonyl analogue, whereas strong electron-donating moiety may reduce or remove such bioactivity.

TNF-α and IL-6 are two versatile pleiotropic cytokines that induce growth stimulation and play a crucial role as an immunomodulator and mediator of host resistance to many infectious agents [1, 2]. The inhibition of TNF-α and IL-6 release by A01, A03, A13, B18 and C22 in dose-dependent manner (Fig. 4) demonstrate their potential to be developed as anti-inflammatory agents. Besides TNF-α and IL-6, curcumin has been reported to exert its therapeutic effects via inhibiting various cytokines such as IL-1β and MCP-1, and key enzymes involved in the inflammatory response such as COX-2, PEGS and iNOS [9, 22–24, 36, 37]. As shown in Fig. 5, three active analogues remarkably reduced LPS-induced mRNAs expressions of TNF-α, IL-1β, IL-6 MCP-1, COX-2, PEGS and iNOS, further establishing their anti-inflammatory properties. They also inhibited mRNA expressions of both inflammatory factors and transcription factor NF-κB p65. Therefore, despite the removal of β-diketone, the mono-carbonyl analogues retained the anti-inflammatory properties at the molecular level. Combined with our previous studies with regards to their stability and pharmacokinetics [19], these data indicate that these mono-carbonyl analogues without β-diketone may lend themselves favourably for the development of curcumin-based anti-inflammatory drug development from both pharmacokinetic and pharmacological standpoints.

The biological properties of A13, the only hydrosoluble analogue when combined with HCl, were further investigated in macrophages. Our results from RT-PCR studies in macrophages confirmed the anti-inflammatory ability of A13-HCl in water solution. Hence, the new analogue A13, in the form of its quaternary ammonium salt with the advantages of both hydrophilicity and stability, may be considered as a promising anti-inflammatory candidate to treat various inflammatory diseases. However, further studies are necessary to establish such notion. Such studies should include testing of these new anti-inflammatory analogues of curcumin in animal models and examination of the underlying molecular mechanisms at the transcriptional or post-transcriptional level.

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