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Functional Structure/Activity Relationships

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# Synthesis of Dithiolethiones and Identification of Potential Neuroprotective Agents via Activation of Nrf2-Driven Antioxidant Enzymes

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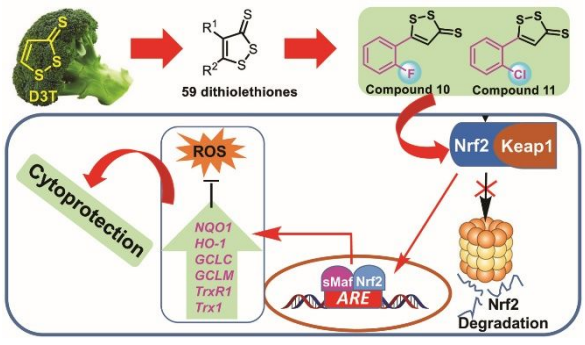
## Abstract

Oxidative stress is implicated in the pathogenesis of a wide variety of neurodegenerative disorders, and accordingly, dietary supplement of exogenous antioxidants or/and upregulation of endogenous antioxidant defense system is promising for therapeutic intervention or chemoprevention of neurodegenerative diseases. Nrf2, a master regulator of the cellular antioxidant machinery, cardinally participates in the transcription of cytoprotective genes against oxidative/electrophilic stresses. Herein, we report the synthesis of 59 structurally diverse dithiolethiones and evaluation of their neuroprotection against 6-hydroxydopamine- or $H_2O_2$-induced oxidative damages in PC12 cells, a neuron-like rat pheochromocytoma cell line. Initial screening identified compounds **10** and **11** having low cytotoxicity but conferring remarkable protection on PC12 cells from oxidative-mediated damages. Further studies demonstrated that both compounds upregulated a battery of antioxidant genes as well as corresponding genes' products. Significantly, silence of Nrf2 expression abolishes cytoprotection of **10** and **11**, indicating targeting Nrf2 activation is pivotal for their cellular functions. Taken together, the two lead compounds discovered here with potent neuroprotective functions against oxidative stress *via* Nrf2 activation merit further development as therapeutic or chemopreventive candidates for neurodegenerative disorders.


## TOC

# 1. Introduction

Reactive oxygen species (ROS) are a wide variety of reactive molecules that are produced, transformed, and consumed as a consequence of aerobic metabolism in living organisms. Under physiological conditions, ROS are maintained at a low level and play critical roles in cellular signaling; however, when the ROS homeostasis is disturbed with a favor of ROS accumulation, generally arising from excessive ROS generation or/and impairment of antioxidant defense, oxidative stress occurs.[1-3] Oxidative stress causes deleterious effects on biomolecules, which ultimately contributes to many pathophysiological conditions,[4] such as cancer, cardiovascular diseases and neurodegenerative disorders.

The central nervous system (CNS) is particularly susceptible to oxidative stress due to its high oxygen consumption, remarkable levels of transition metals, and enrichment of polyunsaturated fatty acids, rendering it more vulnerable to ROS-mediated damage.[4-7] Furthermore, dopaminergic neurons may suffer additional oxidative burden owing to auto-oxidation and metabolism of dopamine, resulting in electrophilic dopamine quinones (DAQs). The subsequent metabolites of DAQs as well as DAQs themselves are able to consume reducing equivalents NADPH, react with vital nucleic acid, and deplete thiol antioxidant glutathione (GSH), all of which processes are accompanied by concomitant production of large amounts of ROS.[8] As a consequence, neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS), are all considered to be associated with oxidative stress.[5-10] Given the fact that elevated oxidative stress and the progressive loss of neuronal cell populations are characteristics of the development of the neurodegenerative diseases, alleviating oxidative stress is a reasonable strategy to ameliorate the symptoms or arrest/reverse the progression of these diseases.[6, 8] As a result, potential therapeutic pharmacological interventions based on charging exogenous or/and endogenous antioxidants have been developed during the past years. Compared to supplement of exogenous antioxidants, activation of endogenous antioxidant defense pathway has emerged as a preferred strategy

in mitigating the detrimental effects of ROS as such regulation is usually at the transcriptional level and sustainable.[11-14]

Nuclear factor erythroid 2-related factor 2 (Nrf2), a member of cap 'n' collar family of basic region leucine zipper transcription factors, is a master regulator of antioxidant response signaling. Many antioxidant and detoxifying enzymes, such as NAD(P)H quinone oxidoreductase 1 (NQO1), heme oxygenase-1 (HO-1), glutathione (GSH) biosynthetic enzymes glutamate cysteine ligase (GCL), thioredoxin 1 (Trx1) and thioredoxin reductase 1 (TrxR1), are regulated by Nrf2 at the transcription level.[15-16] Under basal conditions, Nrf2 is anchored in the cytoplasm to its repressor protein Keap1 (Kelch-like ECH-associated protein 1), a substrate specific adaptor component of cullin3-based E3 ubiquitin ligase complexes targeting Nrf2 for ubiquitination and subsequent proteasomal degradation.[17-18] As such, this maintains Nrf2 at a low concentration in nucleus and keeps subsequent transcription of target genes at a basal level. Upon exposure to stressed conditions, Keap1 acts as a stress sensor on account of its several hypersensitive cysteine residues that can be covalently modified by ROS and electrophiles,[19] giving rise to dissociation of Nrf2 from Keap1-cullin3-Nrf2 complexes and abrogation of Keap1-dependent depression of Nrf2. As a result thereafter, Nrf2 translocates to the nucleus and constitutes a heterodimer with other transcription activators, *e.g.*, small musculoaponeurotic fibrosarcoma (sMAF) proteins, and binds to the enhancer sequence of antioxidant response elements (AREs) in the regulatory promoter regions of a wide range of cytoprotective genes, which ultimately initiates the expression of abundant protective genes. On this ground, activation of Keap1-Nrf2-ARE pathway is promising in chemoprevention of oxidative stress-related diseases or is of therapeutic potential in treatment of such disorders.[11-14, 20-23]

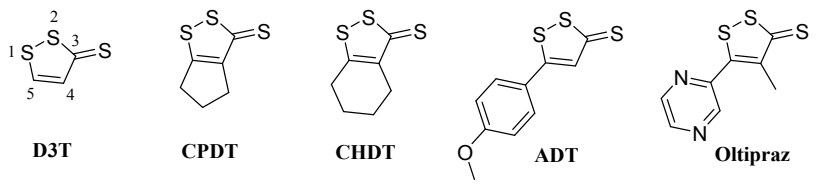
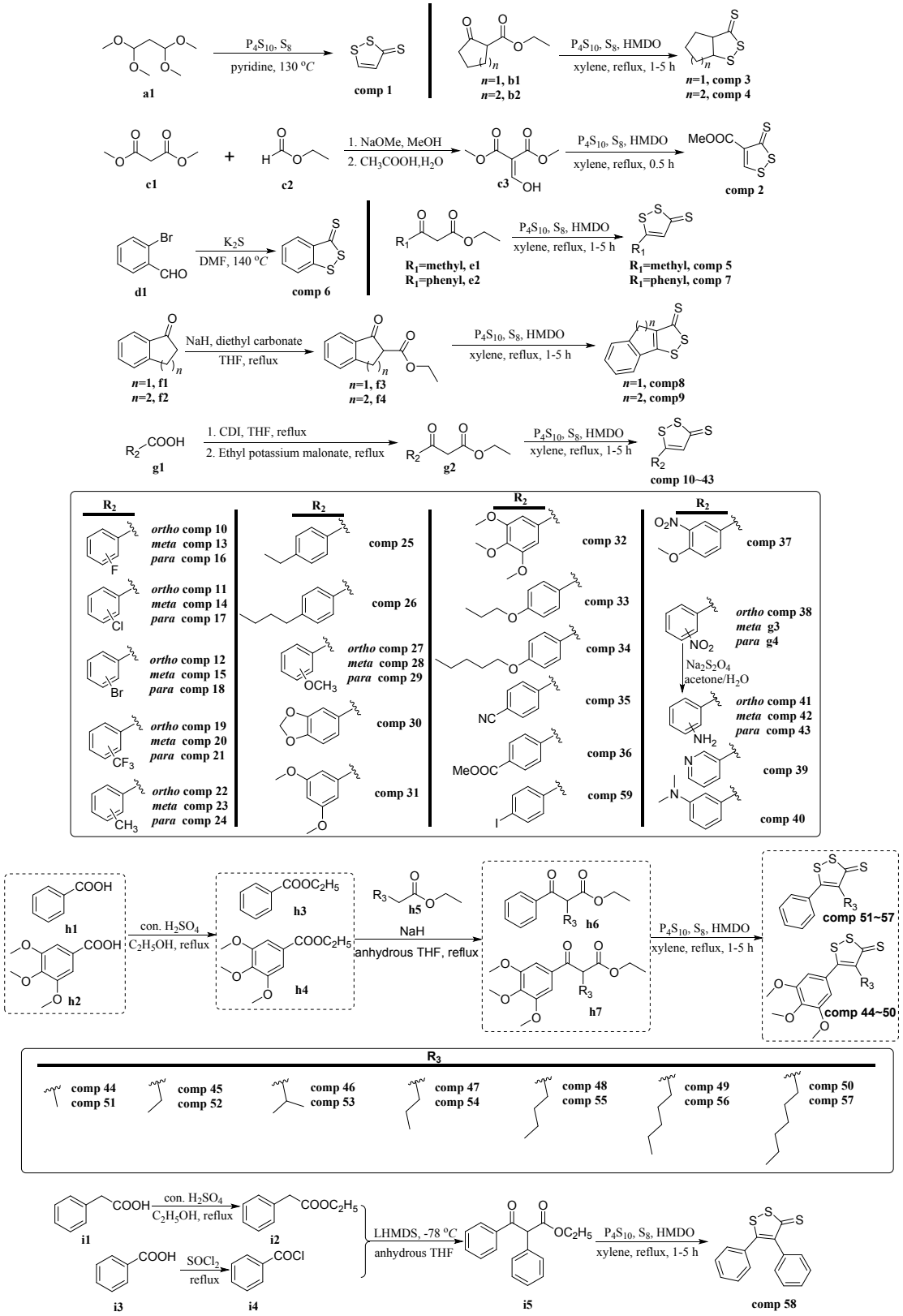



**Figure 1.** Structures of typical dithiolethiones.

Small molecules have been continually attracting extensive attention in the discovery and development of antioxidant agents targeting Keap1-Nrf2-ARE pathway.[24-30] Encouragingly, past decades have witnessed tremendous progress in successful identification of Nrf2 activators, and works by our group also unveiled several naturally occurring and synthetic molecules that target Keap1-Nrf2-ARE pathway and sequentially protected cells from oxidative insults.[28, 31-37] Recently, numerous lines of evidences demonstrated that 3H-1,2-dithiole-3-thione (D3T) and its several synthetic derivatives (*e.g.*, ADT, CPDT, CHDT, and Oltipraz) (**Figure 1**) are of medicinal application potential. All of these molecules are analogues of naturally occurring dithiolethiones isolated from cruciferous vegetables and abundant especially in broccoli. These molecules possess protective effects against carcinogenesis[38-43] and play neuroprotective roles in various cell lines and animal models.[44-48] Although several of this class of sulfur-containing heterocyclic molecules represent promising therapeutic or preventive potential in combating with neurodegenerative disorders, the orchestrating mechanism of action underlying their neuroprotective effects and their structure-activity relationships (SAR) have not been thoroughly elucidated.[47-48] With the intention of advancing our development of novel small molecules with redox-regulating properties, we reported herein the synthesis of 59 structurally diverse dithiolethiones and evaluation of them as potential neuroprotective agents against 6-hydroxydopamine (6-OHDA)- or $H_2O_2$-induced oxidative damages in a neuron-like rat pheochromocytoma cell line (PC12 cells). After examination of their cytotoxicity and neuroprotective effects, preliminary SAR was delineated and summarized, which will facilitate further optimization of dithiolethiones as potential chemotherapeutic or chemopreventive candidates. Two compounds (**10** and **11**) were demonstrated to exhibit low cytotoxicity and high cytoprotective activity, and thereby were selected for further studies. Treatment of PC12 cells with compounds **10** and **11** promoted the upregulation of a variety of Nrf2-driven antioxidant species (*e.g.*, GSH, HO-1, NQO1, TrxR and Trx). Silence of Nrf2 expression by RNA interference drastically abolished the cytoprotective effects of compounds **10** and **11**, supporting the essential participation of

Nrf2 for the cellular performance of the agents.




**Scheme 1.** General synthetic procedures for various intermediates and target compounds.

# 2. Results and Discussion

## 2.1. Chemistry

As is shown in **scheme 1**, target compounds **1** and **3~7** were synthesized from commercially available chemicals as starting materials, and compound **2** was synthesized through intermediate **c3**, which was obtained *via* the reaction of **c1** with **c2** by the method described by Coates and Hobbs.[49] Intermediates **f3** and **f4** were obtained by the methods described by Che *et al.*[50] and Unsworth *et al.*[51] from commercially available **f1** and **f2**, respectively. Various commercially available starting materials **g1** were converted into intermediates **g2** in high yields without further purification after a concise operation by the method described by Jae *et al*.[52] Key intermediates **h6** and **h7** were easily obtained by reactions of various **h5** with **h3** and **h4**[53]. Both **h3** and **h4** were achieved by ethyl esterification of **h1** and **h2** in ethyl alcohol with catalytic amount of concentrated $H_2SO_4$ in high yields. Easily synthetic intermediates **i2** and **i4** reacted in a condition described by Taylor *et al.*,[54] providing the key intermediate **i5**.

Three methods were utilized in the synthesis of 59 target dithiolethiones in this paper, namely, compound **1** by method of Behringer *et al.*,[55] compound **6** by method of Jin *et al.*,[56] and the other compounds by method of Curphey.[57] All target compounds were characterized through $^{1}H$ NMR, $^{13}C$ NMR and MS. HPLC was employed to determine the purity of all compounds used for bio-evaluation.

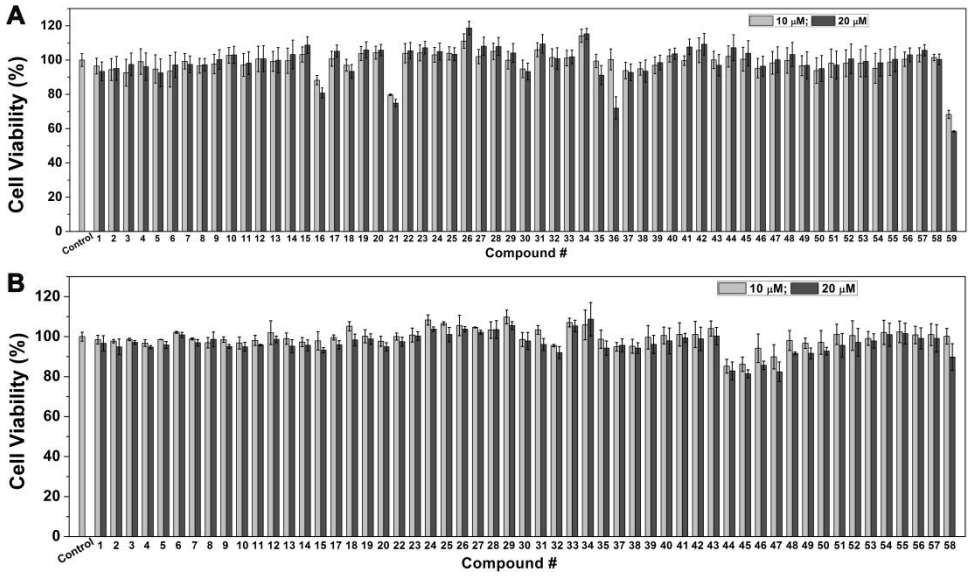



**Figure 2.** Cytotoxicity screening of dithiolethiones against (A) PC12 cells and (B) BEAS-2B cells. The cells were seeded in 96-well plates for 24 h, and then treated with indicated concentrations of compounds for another 24 h. The cell viability was measured by the MTT assay. Representative graphs are from three independent experiments, with errors bars representing SD values.

## 2.2. Initial Screening and SAR Studies

As displayed in **Figure 2**, all target compounds were firstly screened for cytotoxicity against PC12 cells by using the MTT assay, and compounds **16**, **21**, **36** and **59** were found to exhibit apparent toxicity toward PC12 cells. Next, except for compounds **16**, **21**, **36** and **59**, the other 55 target compounds were evaluated for toxicity against BEAS-2B cells (human bronchial epithelial cell line) by using the same method as used for PC12 cells. Obviously, the overwhelming majority of the target compounds show little toxicity toward the two cell lines in different concentrations. Compounds **16**, **21**, **36** and **59** exhibit apparent toxicity against PC12 cells, while compounds **44**~**47** possess slight inhibition activity to the growth of BEAS-2B cells. In addition to compounds with distinct cytotoxicity (**comp 16**, **21 36**, **44**~**47,** and **59**), the other compounds were chosen for evaluating their neuroprotective activity in two well established cellular models of neurodegenerative diseases[28, 31-37, 58], and the levodopa (L-DOPA) is used as the positive compound. Impressively, all compounds are capable of rescuing PC12 cells from 6-OHDA- and $H_2O_2$-induced injury and a large number of compounds are better than L-DOPA in

preventive effects, with compounds **10** and **11** being the top two most efficacious agents (**Figure 3**).

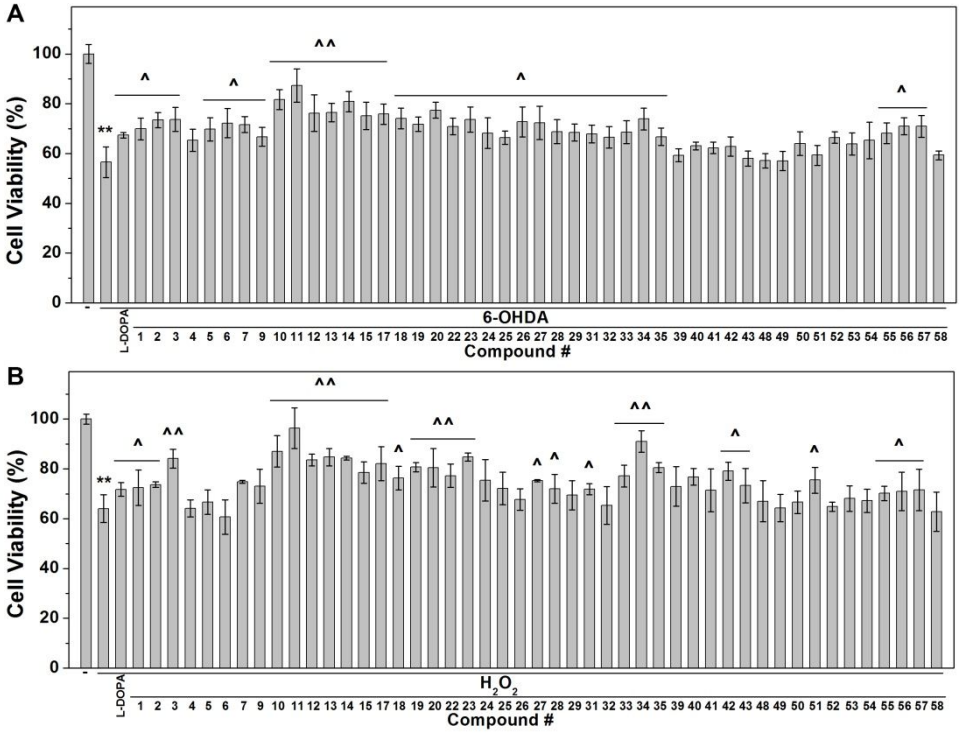



**Figure 3.** Preventive activity screening of dithiolethiones against (A) 6-OHDA- or (B) $H_2O_2$-induced PC12 cell damage. The cells were cultured for 24 h and subsequently subjected to 20 μM indicated compounds or the positive compound L-DOPA for another 24 h. Then the old medium was discarded followed by addition of new medium containing (A) 6-OHDA (200 μM) or (B) $H_2O_2$ (500 μM) for another 12 h. The cell viability was evaluated by the MTT assay. Each bar represents the means ±SD of three independent experiments. $^{**}P < 0.01$ vs the control group; $^{\wedge}P < 0.05$, and $^{\wedge\wedge}P < 0.01$ vs the 6-OHDA or $H_2O_2$ treatment.

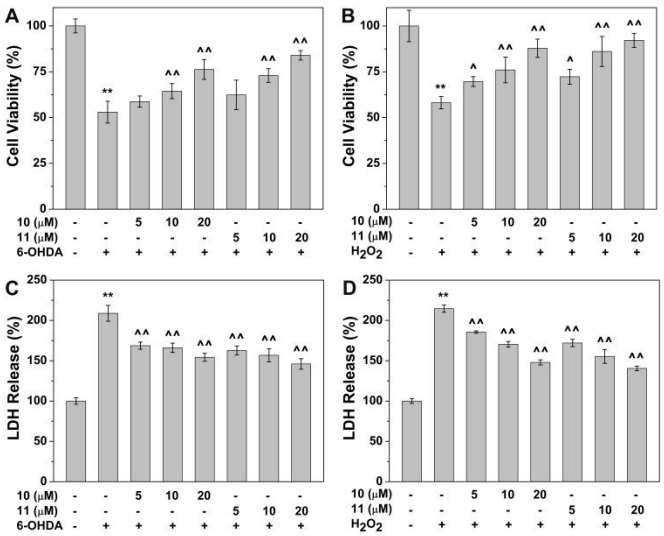
The preliminary structure-activity relationship (SAR) analysis showed that the structurally simplest compound **1** (D3T) without any substitution have moderate preventive activity. Compound **2** with electron withdrawing group on the 4th position of D3T showed slightly increase in its activity. Five membered ring (compound **3**, CPDT) is more favored than six membered ring (compound **4**, CHDT). Phenyl substitution (compound **7**) at the 5th position of D3T showed good preventive activity, while compounds **16**, **21**, **35**, **36** and **59** with electron withdrawing groups on the *para* position of phenyl exhibit apparent cytotoxicity to PC12 cells, and both **44**~**50** bearing 3,4,5-trimethoxyphenyl and **58** bearing two phenyl substitutions are somewhat toxic[59] to BEAS-2B cells. With

regard to compounds **10**~**18**, appropriate atom size and electron withdrawing ability play important roles in preventive activity of these compounds, as chlorine substituted compounds (**11**, **14** and **17**) are the most active ones. In addition, *ortho*-halogen substituted compounds showed better activity than *meta*-halogen substituted ones, and the *para*-halogen substituted ones are the least. As for compounds **27**~**34**, the amounts of electron donating groups make little difference to preventive activity, and the *ortho*-methoxy group (compound **27**) is more favored. What is more, it is also worth mentioning that *n*-pentyloxy (compound **34**) causes significantly beneficial effect on its preventive activity. When exploring the effect of chain length on activity of compounds (**48**~**57**), we noticed that chain length makes little effect on the activity. Taken together, aromatic substitutions at the 5$^{th}$ position of D3T are tolerated and favored, and especially the *ortho*-substituted halogen with suitable electron withdrawing ability plays pivotal roles in improving the preventive activity of compounds. Further studies on optimization of the 4$^{th}$ position of D3T are underway in our lab.

## 2.3. Rescue of PC12 Cells from Oxidative Damages by Compounds 10 and 11

Because of the better preventive activity and lower cytotoxicity of compounds **10** and **11**, they were picked up for the follow-up studies. 6-OHDA, a neurotoxin, whose toxicity is mainly ascribed to its ability to produce excessive amounts of ROS, was applied to generate a cellular model of PD[60]. As depicted in **Figure 4A**, PC12 cells challenged with 6-OHDA solely were about half alive in comparison with control cells. When cells were pretreated with **10** or **11**, the 6-OHDA-induced cell death was alleviated remarkably in a dose-dependent manner. Prominently, compounds **11** rescued PC12 cells up to more than 80% at the concentration of 20 μM. $H_2O_2$, a well-known intrinsic signal molecule as well as a kind of ROS species, was used to produce cell neurotoxicity mimicking the endogenous process of pathogenesis of PD. As shown in **Figure 4B**, $H_2O_2$ treatment leads to roughly 40% death of PC12 cells in comparison with the control group. Likewise, pretreatment of PC12 cells with **10** or **11** also markedly relieved cells from the $H_2O_2$-mediated damage with elevation of cells viability up to impressive 90% at the

179 concentration of 20 μM. With the intention of confirming the results displayed in **Figure 4A** and **Figure 4B**, the
180 lactate dehydrogenase (LDH) leakage assay was further conducted.[28, 31] As displayed in **Figure 4C** and **Figure 4D**,
181 the contents of released LDH in two models were 2-fold more than the control, while this process could be
182 diminished evidently by pretreatment of the cells with **10** or **11**, which was in accordance with the results of MTT
183 assay. In order to consolidate the results obtained above, we further determined the preventive effect of **10** and **11**
184 in N2a cells (mouse neuroblastoma N2a cells), and the results showed that both **10** and **11** exhibited robust
185 preventive effect against $H_2O_2$-induced oxidative stress (**Figures** S1-S3, Supporting Information).
186 Given the fact that overproduction of ROS is considered to be persistently implicated in the pathogenesis of a broad
187 range of both acute and chronic neuropathologies,[5, 9-10] we then determined whether compounds **10** and **11** are able
188 to mitigate ROS generation in PC12 cells caused by 6-OHDA or $H_2O_2$. Dichlorodihydrofluorescein diacetate
189 (DCFH-DA), a non-fluorescent probe that can be converted into a highly fluorescent molecule by reacting with
190 ROS in cells, was utilized as a detector probe of ROS. As shown in **Figure 5**, after treatment of 6-OHDA or $H_2O_2$
191 solely, PC12 cells showed strong fluorescent emission compared with the control groups. However, preincubation
192 of PC12 cells with either **10** or **11** could reduce the fluorescence signal to a great extent, which demonstrates their
193 ability to alleviate the accumulation of ROS.




194

195 **Figure 4.** Preventive activity of **10** and **11** against 6-OHDA- or $H_2O_2$-induced injury in PC12 cells. (A, B) Protection of PC12 cells from 6-OHDA- or

$H_2O_2$-mediated damage by pretreatment with indicated concentrations of **10** and **11**. PC12 cells were cultured for 24 h and subsequently exposed to indicated concentrations of **10** and **11** for another 24 h, and afterwards the old medium was discarded followed by addition of new medium containing (A) 6-OHDA (200 μM) or (B) $H_2O_2$ (500 μM) for another 12h. The cell viability was evaluated by the MTT assay. (C, D) Preventive activity of **10** and **11** verified by LDH release assay. PC12 cells were cultured for 24 h, and indicated concentrations of **10** and **11** were added for another 24 h followed by addition of (C) 6-OHDA (200 μM) or (D) $H_2O_2$ (500 μM) for further 12 h. Afterwards determination of the activity of released LDH was conducted. Representative graphs are from three independent experiments, with errors bars representing SD values. $^{**}P < 0.01$ vs the control group; $^{\wedge}P < 0.05$, and $^{\wedge\wedge}P < 0.01$ vs the 6-OHDA or $H_2O_2$ treatment.

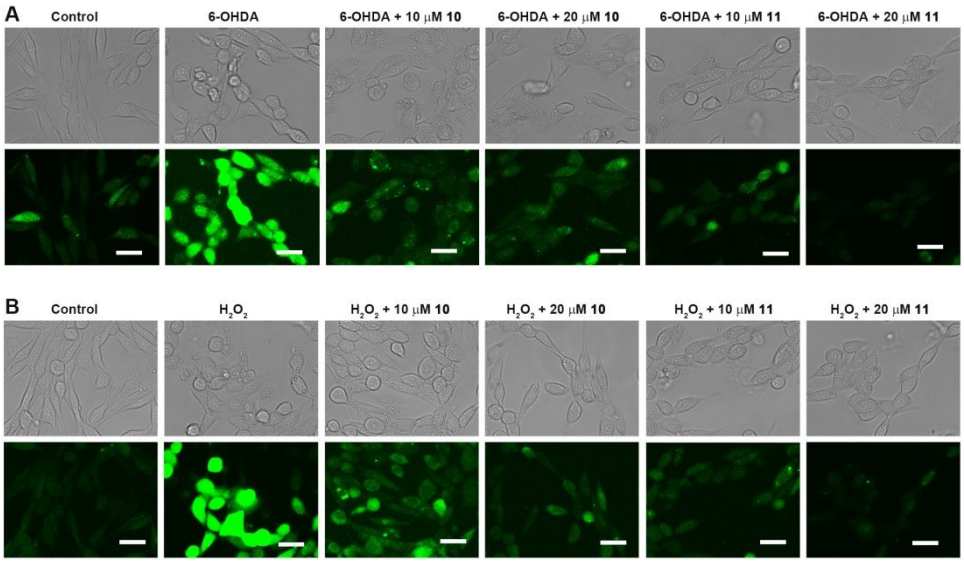



**Figure 5.** Alleviation of ROS production by **10** and **11** in PC12 cells. The cells were cultured for 24 h, subsequently treated with indicated concentrations of **10** and **11** for another 24 h, and then exposed to (A) 6-OHDA or (B) $H_2O_2$ for 12 h. ROS levels were determined by DCFH-DA staining.

## 2.4. Relief of Apoptosis by Compounds 10 and 11

Since the cytotoxicity of 6-OHDA and $H_2O_2$ to PC12 cells is mainly mediated by induction of apoptotic cell death,[28, 31] we then asked if compounds **10** and **11** could attenuate 6-OHDA- or $H_2O_2$-induced apoptosis of PC12 cells. Apoptotic cells are generally characterized by condensed nuclear chromatins, which could be revealed by fluorescence imaging accomplished with the aid of a DNA staining dye Hoechst 33342. As depicted in **Figure 6A**

and **Figure 6B**, both 6-OHDA and $H_2O_2$ lead to apoptosis of PC12 cells, manifested by the occurrence of highly fluorescent pycnotic nuclei, while weak fluorescence emission was observed in control groups. Apparently, pretreatment of PC12 cells with **10** and **11** significantly assuaged cell apoptosis in a dose-dependent manner. To confirm the results evidenced by cellular morphological changes above, we next determined the activity of caspase-3, a biochemical indicator of apoptosis. Both 6-OHDA and $H_2O_2$ elevated activity of cellular caspase-3 to approximately 2.5-fold higher than the control cells, whereas, with addition of compounds **10** or **11**, activity of cellular caspase-3 was ablated conspicuously (**Figure 6C** and **Figure 6D**). Finally, we employed flow cytometry to quantify the cell populations with Annexin V-FITC/PI double staining assay in order to further consolidate the results obtained above. As shown in scattergrams **Figure 7A** and **7B** and quantification results from **Figure 7C** and **7D**, both 6-OHDA and $H_2O_2$ result in death of PC12 cells to a fairly high level. Nevertheless, pretreatment of PC12 cells with **10** and **11** notably mitigate the oxidative stress-induced insult in a dose-dependent manner. Taken together, the results above imply that **10** and **11** could relieve PC12 cells of oxidative stress-induced apoptosis.

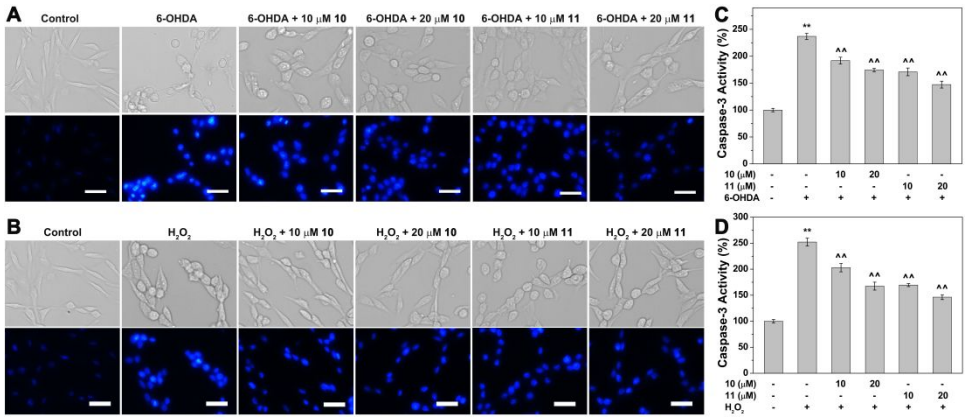



**Figure 6.** Attenuation of 6-OHDA- or $H_2O_2$-induced apoptosis of PC12 cells by **10** and **11**. PC12 cells were incubated with indicated concentrations of **10** and **11** for 24 h, and then were exposed to (A) 6-OHDA or (B) $H_2O_2$ for further 12 h. Hoechst 33342 staining assay was employed to visualize and image apoptotic PC12 cells. Inhibition of activity of caspase-3 by preincubation of PC12 cells with indicated concentrations of **10** and **11** followed by exposure to (C) 6-OHDA or (D) $H_2O_2$. The cellular caspase-3 activity was measured by a colorimetric assay. Representative graphs are from three independent experiments, with errors bars representing SD values. $^{**}P < 0.01$ vs the control group. $^{\wedge\wedge}P < 0.01$ vs the 6-OHDA or $H_2O_2$ treatment.

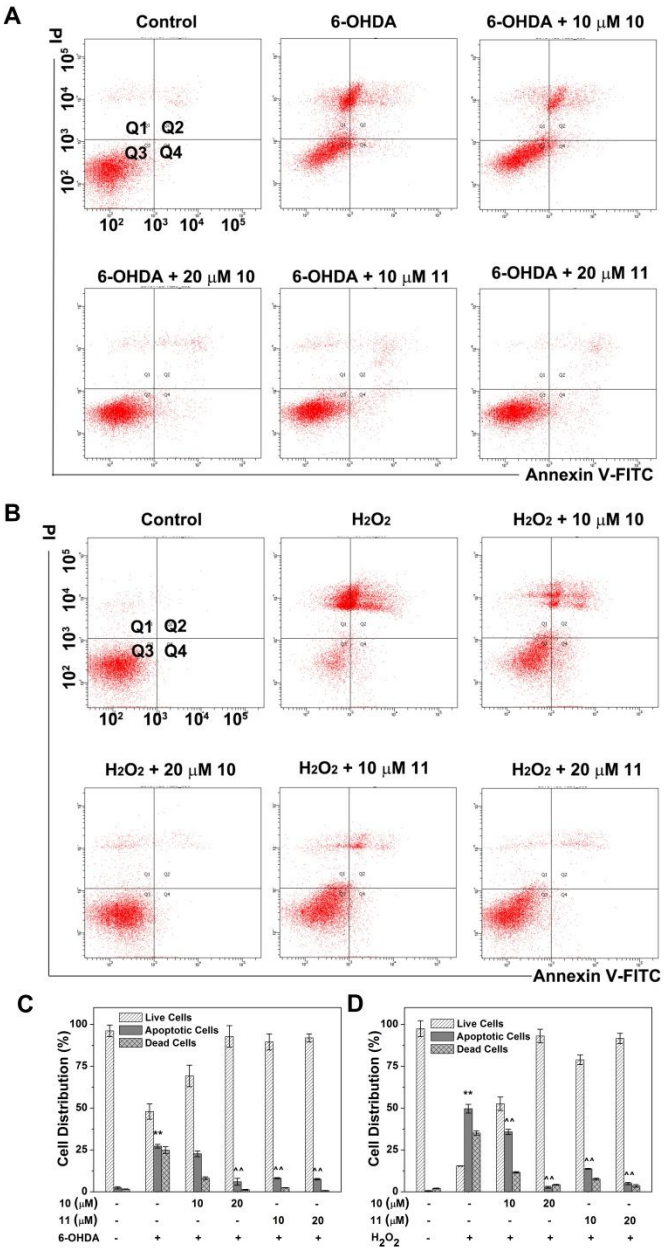



**Figure 7.** Preventive effect of compounds **10** and **11** in PC12 cells analyzed by flow cytometry. PC12 cells were incubated with indicated concentrations of **10** or **11** for 24 h followed by (A) 6-OHDA or (B) $H_2O_2$ treatment for 12 h, and then the population of live cells, apoptotic cells and dead cells was analyzed by Annexin V/PI-double-staining assays. The cells are divided into four different populations: Q3 (lower left) is the live cell population with double-negative staining, Q1 (upper left) in the dead cell population with PI-positive-and Annexin V-negative staining, Q4 (lower right) is the early apoptotic cell population with Annexin V-positive- and PI-negative staining, Q2 (upper right) is the late apoptotic cell population with Annexin V/PI-double staining. The quantification results of preventive effect are shown in (C) and (D), and dead cells (Q1), apoptotic cells (Q2 and Q4), and normal cells (Q4) are illustrated. Representative graphs are from three independent experiments, with errors bars representing SD values. ***P*

238 < 0.01 vs the control group. ^^$P$ < 0.01 vs the 6-OHDA or $H_2O_2$ treatment.

## 239 2.5. Upregulation of the Antioxidant Defence System by Compounds 10 and 11

240 Several studies have demonstrated that certain dithiolethiones exerted their anticarcinogenic or protective effects
241 *via* arousing the antioxidant defence mechanism of Keap1-Nrf2-ARE through covalent modification[61], oxidation[62]
242 or persulfidation[63] of cysteine sulfhydryls in Keap1. Then we measured whether compounds **10** and **11** could
243 upregulate the Nrf2-directed antioxidant genes in PC12 cells, such as *HO-1*, *NQO1*, *TrxR*, *Trx*, *GCLC* (GCL
244 catalytic subunit), and *GCLM* (GCL regulatory subunit). Also, the effects of **10** and **11** on *Nrf2* mRNA transcription
245 level were evaluated. As shown in **Figure 8A** and **Figure 8B**, compounds **10** and **11** could boost these detoxifying
246 genes to a dramatically high level except for *Nrf2*, with *HO-1* being increased to about 10- and 22-fold higher than
247 the control group, and the mRNA levels reached the peak at 6 h after treatment of PC12 cells with **10** or **11**. It is
248 worth noting that neither **10** nor **11** had any impact on *Nrf2* mRNA level, suggesting that *Nrf2* gene transcription or
249 its message stability was not affected by the compounds. Furthermore, the content and activity of the antioxidant
250 molecules encoded by these genes was determined. As shown in **Figure 9**, the expression content of the proteins
251 HO-1, NQO1, TrxR, and Trx in PC12 cells were elevated remarkably by compounds **10** and **11** treatment in a dose
252 dependent manner, with HO-1 being promoted most notably, which is consistent with the genes expression level
253 result above. Accordingly, activities of NQO1 (**Figure 10B**), HO-1 (**Figure 10C**), TrxR (**Figure 10D**) and Trx
254 (**Figure 10E**) were all promoted to varying degrees in a dose-dependent manner. GCLC and GCLM, which are the
255 catalytic and regulatory subunits of the GSH biosynthesis enzyme GCL, form a heterodimer who catalyzes the
256 rate-limiting step in biosynthesis of GSH. GSH, a cardinal component of the glutathione system and a ubiquitous
257 cysteine-containing tripeptide antioxidant, can detoxify ROS and thereby play an important role in maintaining
258 intracellular redox homeostasis. As displayed in **Figure 10A**, total GSH level was elevated strikingly by treatment
259 of PC12 cells with **10** or **11**. Collectively, compounds **10** or **11** showed great ability to upregulate the endogenous

antioxidant species.

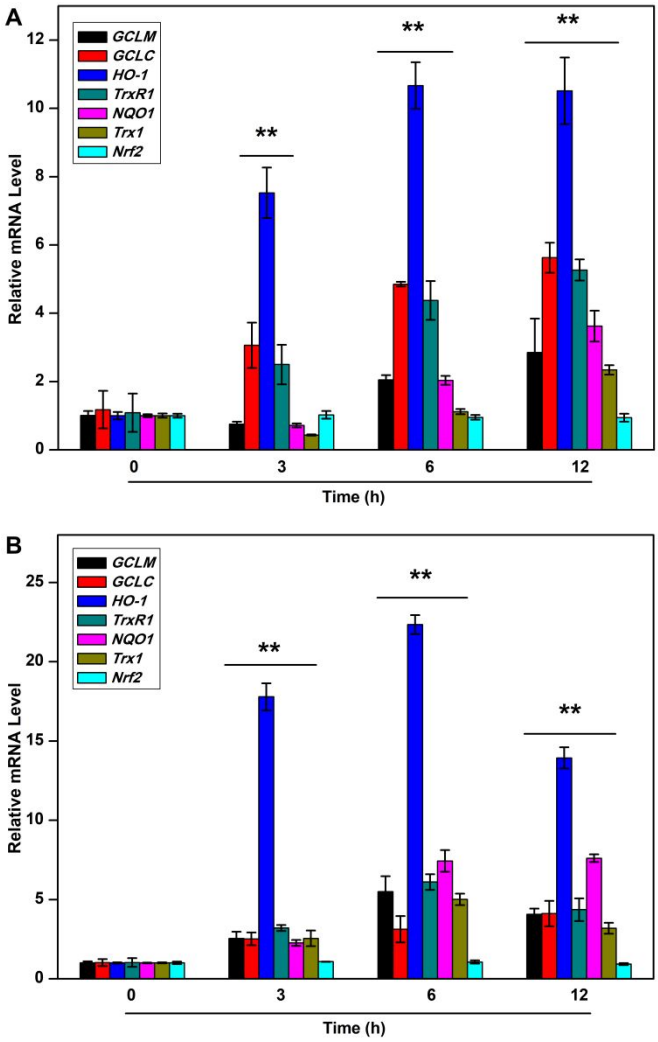



**Figure 8.** Effect of compounds **10** and **11** on transcriptional expression of antioxidant genes and *Nrf2*. PC12 cells treated with (A) **10** and (B) **11** by 20 μM were collected at indicated time and then the total RNA were extracted. *Nrf2* and Phase II genes levels were normalized using GAPDH as an internal control, and analyzed by RT-PCR. Representative graphs are from three independent experiments, with errors bars representing SD values. $^{**}P < 0.01$ vs the control group.

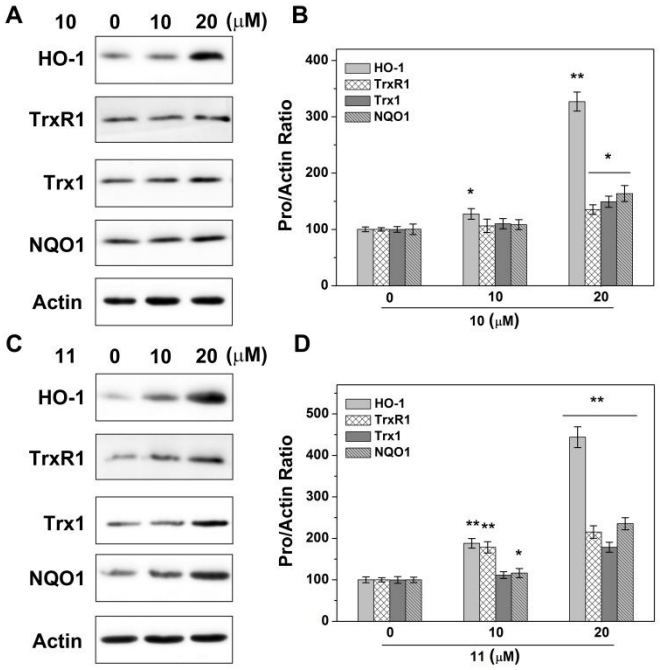



**Figure 9.** Elevation of the content of corresponding antioxidant enzymes by compounds **10** and **11** in PC12 cells. (A and C) Promotion of the expression of antioxidant enzymes determined by Western blots. After treatment with indicated concentrations of compounds **10** and **11** for 24 h, the expression levels of HO-1, TrxR1, Trx1, and NQO1 in PC12 cells were determined. Quantification results of the Western blots are shown in B and D. Representative graphs are from three independent experiments, with errors bars representing SD values. *$P < 0.05$ and **$P < 0.01$ vs the control group.

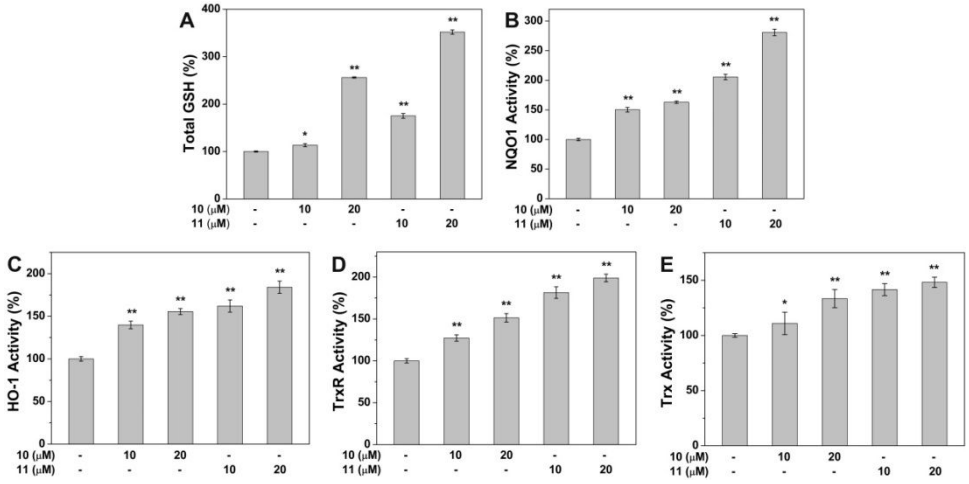



**Figure 10.** Upregulation of activity or content of antioxidant species by **10** and **11** in PC12 cells. Elevation of (A) total GSH, (B) NQO1activity, (C) HO-1 activity, (D) TrxR activity and (E) Trx activity by treatment of PC12 cells with indicated concentrations of **10** and **11**. PC12 cells were treated with indicated concentration of **10** and **11** for 24 h. Then antioxidant enzymes and GSH levels were determined. Representative graphs are from three

independent experiments, with errors bars representing SD values. *$P < 0.05$ and **$P < 0.01$ vs the control group.

## 2.6. Promotion of the Expression Level and Nuclear Translocation of Nrf2 by Compounds 10 and 11.

The translocation of Nrf2 from cytoplasm to nucleus is prerequisite for the Nrf2-dependent cytoprotective pathway, which has attained broad consensus among scientists in this field. We then determined whether Nrf2 could accumulate in nucleus by stimulation from compounds **10** and **11**. After preparation of different fractions of PC12 cells treated by **10** and **11**, western blots assays were employed to quantify the contents of Nrf2 in different parts of the cells. As is displayed in **Figure 11**, the total Nrf2 was elevated slightly followed by **10** (**Figure 11 A-B**) or **11** (**Figure 11 E-F**) treatment, although the *Nrf2* mRNA level was not influenced by these two compounds, indicating that elevation of Nrf2 expression is conferred by post-transcription processes. Obviously, after **10** or **11** treatment, cytoplasmic Nrf2 content declined gradually (**Figure 11 A**, **D**, **E**, and **H**), whereas the nuclear Nrf2 content increased coincidently (**Figure 11 A**, **C**, **E**, and **G**), which indicates Nrf2 translocates to nucleus from cytoplasm. Taken together, the results above demonstrate that **10** and **11** could promote both the nuclear translocation and expression level of Nrf2, which would facilitate the binding of Nrf2 to ARE sequence so as to initiate the transcription of antioxidant genes.

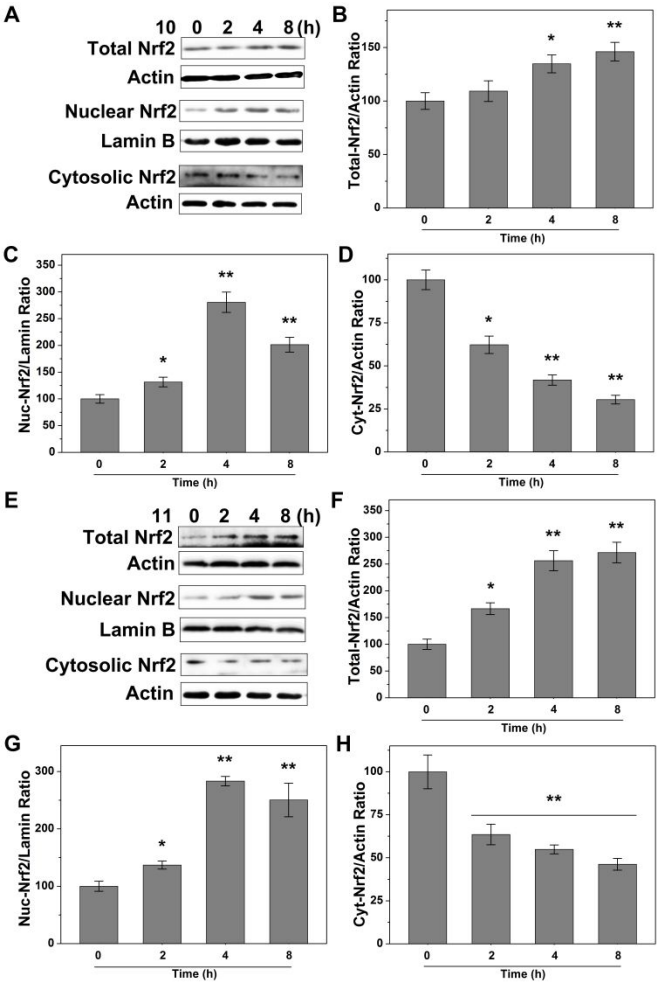



**Figure 11.** Promotion of the level of Nrf2 expression and nuclear translocation of Nrf2 by compounds **10** or **11** treatment in PC12 cells. PC12 cells were treated with 20 μM compounds **10** or **11** for the indicated time, and then cells were collected and their fractions were prepared. (A and E) Western blots analysis of total Nrf2, nuclear Nrf2, and cytosolic Nrf2. Quantification results of the Western blots are shown in B−D and F-H. Representative graphs are from three independent experiments, with errors bars representing SD values. *$P < 0.05$ and **$P < 0.01$ vs the control group.

## 2.7. Essential Involvement of Nrf2 for Actions of Compounds 10 and 11

We next assessed the contribution of Nrf2 for the preventive activity of **10** and **11**. On this account, Nrf2 knockdown assay was conducted by transfecting PC12 cells with a sh*Nrf2* plasmid specifically targeting *Nrf2* to generate stably transformed PC12 cells (PC12-sh*Nrf2* cells). The control cells (PC12-sh*NT*) were transfected with a non-targeting sh*RNA* plasmid. The transfection efficiency was examined by Western blot as shown in **Figure 12A**, with the Nrf2 protein in PC12-sh*Nrf2* cells decreased to 30% of the control PC12-sh*NT* cells (**Figure 12B**). Then,

we determined the preventive effect of **10** and **11** on oxidative stress-induced damages of both PC12-sh*Nrf2* cells and PC12-sh*NT* cells. As shown in **Figure 12C** and **Figure 12D**, compounds **10** and **11** exerted their preventive effects on PC12-sh*NT* cells, while such preventive effects were almost entirely abrogated in PC12-sh*Nrf2* cells, indicating that Nrf2 is critically involved in the neuroprotection of PC12 cells by **10** and **11**.

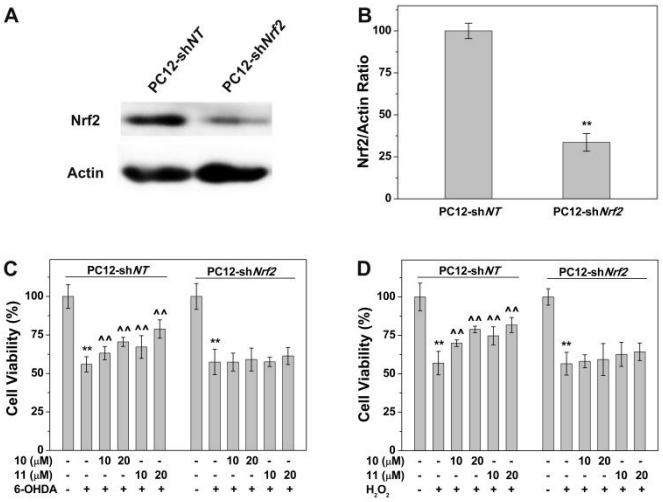



**Figure 12.** Necessity of Nrf2 for cytoprotective activity of **10** and **11**. (A, B) Determination of Nrf2 expression level in PC12-sh*NT* and PC12-sh*Nrf2* cells. PC12-sh*NT* and PC12-sh*Nrf2* cells were treated with indicated concentrations of **10** and **11** for 24 h, and then exposed to (C) 6-OHDA (200 μM) or (D) $H_2O_2$ (500 μM) for 12 h. The cell viability was evaluated by the MTT assay. Representative graphs are from three independent experiments, with errors bars representing SD values. $^{**}P < 0.01$ vs the control group; $^{\wedge\wedge}P < 0.01$ vs the 6-OHDA or $H_2O_2$ treatment.

Continuous generation of ROS is a physiological outcome due to the aerobic metabolism of organisms, and indeed ROS are essential to numerous physiological processes. However, once the ROS generation is beyond the control of antioxidant defense mechnism, oxidative stress occurs, which results in lipid peroxidation, protein oxidation, DNA damage, and ultimately cell death. PD is characterized by a progressive loss of dopaminergic neurons in the substantia nigra pars compacta and the presence of intracellular protein deposition designated as Lewy bodies. Although the molecular etiopathogenesis of PD is not fully understood, increasing evidences suggest that oxidative stress acts as a critical role in the propagation stage of cellular damage during the development of PD. As a consequence, a considerable amount of strategies to suppress oxidative stress are devoted to retarding or reversing

the neurodegenerative progression.

Dithiolethiones have long been known to possess cancer chemopreventive activity that is ascribed to their ability to induce phase 2 enzymes and antioxidant molecules by activation of Nrf2.[40-42, 64] Additionally, several studies demonstrated that certain dithiolethiones bearing different chemical structures had beneficial effects on neurodegenerative diseases.[44-48, 65] However, these sporadic studies limited the elucidation of the SAR of dithiolethiones. In addition, the subtle mechanism of action is not well clarified in these studies. According to classifications of Nrf2 activators by Hu *et al*.[24], dithiolethiones are one of the important categories and are able to arouse endogenous antioxidant defence mechanism of Nrf2-ARE. Under the frame of our ongoing researches regarding the discovery and development of redox active small molecules as therapeutic or preventive agents,[28, 31-37] we described here the synthesis and evaluation of a series of structurally diverse dithiolethiones, and discovered compounds **10** and **11** as the most potent neuroprotective agents to protect PC12 cells from the 6-OHDA- and $H_2O_2$-mediated neurotoxicity. The preliminary SAR of these dithiolethiones in preventing oxidative stress-induced damages of PC12 cells was summarized, which would guide further development and optimization of such type of compounds. Although the exact mechanism by which dithiolethiones activate Keap1-Nrf2-ARE pathway still remains to be elusive, the available research data demonstrate that at least three possibilities should be considered. The hypersensitive sulfydryls of Keap1 could be covalently modified or oxidized or S-sulfhydrated by dithiolethiones themselves and their metabolites. On the whole, these three mechanisms could be integrated as covalent modification because of the alteration of the sulfydryls of the Keap1 protein. As firmly and elegantly demonstrated by Kobayashi *et al*.[61] and Smirnova *et al.*[66], D3T is able to react with Cys151 of Keap1 followed by stabilization of Nrf2 and subsequent upregulation of antioxidant species. In this context, compounds **10** and **11** are expected to exert their preventive effect in the similar way as D3T. Specifically, compounds **10** and **11** are presumed to have an impact on cysteine-rich Keap1 protein by direct covalent modification of sulfydryl in Keap1

and thereby disrupt the interaction between Keap1 and Nrf2, giving rise to the release of Nrf2 from cytoplasm to migrating to nucleus where it stimulates the transcriptional activation of a panel of ARE-dependent cytoprotective genes. These genes' products then eliminate and detoxify the ROS and therefore confer the neuroprotection on PC12 cells in combating with oxidative stress-induced damage. Genetic knockdown of Nrf2 expression almost entirely abolishes the preventive effects of compounds **10** and **11**, highlighting targeting Nrf2 is physiologically important in exerting their neuroprotective effects in PC12 cells.

Regularly dietary intake of cruciferous vegetables, especially broccoli, could prevent our body from being damaged by oxidative/electrophilic stress. However, it is worth mentioning that routine supplement of such vegetables is a chemopreventive strategy against neurodegenerative diseases rather than a chemotherapeutic intervention tactics in treatment of such disorders. Similarly, compounds **10** and **11** are thus postulated to exert their potent chemopreventive effect in preventing the pathogenesis of neurodegenerative diseases or retarding the progression of such disorders.

In summary, 59 structurally diverse dithiolethiones were synthesized and evaluated for neuroprotective activity in PC12 cells, and compounds **10** and **11** were identified as the most active compounds, showing markedly better preventive effects than famous D3T, CPDT and compound **2**. Mechanistic studies demonstrated that activation of the transcription factor Nrf2 is cardinally responsible for the neuroprotective effects of **10** and **11**. Furthermore, information obtained from SAR analysis would facilitate our further optimization of **10** and **11** to develop potential neuroprotective candidates.

# 4. Experimental Section

## 4.1. Chemistry

### 4.1.1. General Information

A Bruker AMX spectrometer was employed to obtain the $^{1}H$ NMR and $^{13}C$ NMR spectra using TMS as reference.

The Shimadzu LCMS-2020 system or the Hewlett-Packard 5988A spectrometer is employed to analyze mass spectra. All reagents and solvents were of reagent grade or purified according to standard methods before use. Silica-gel plates (GF254) (Qingdao Haiyang Chemical Co., Ltd.) were used for monitoring the reaction under UV light (254 nm). Silica gel (200~300 mesh) (Qingdao Haiyang Chemical Co., Ltd.) were used for column chromatography.

### 4.1.2. Compounds Purity Analysis

The purity of all 59 final target compounds was determined by HPLC analysis, which was performed on Shimadzu LC-20A system with a Wondasil C-18 Superb reversed-phase column (5 mm, 4.6 mm × 150 mm). Methanol and water were used as mobile phase, and the flow rate is 0.6 mL/min. The tested compounds were dissolved in chromatographic grade methanol, and the injection volume is 5 μL. The detection wavelength in the range of 210~400 nm was employed to search for the maximal absorbance of the compounds, the purity of which (compounds **1**−**59**) is > 95% (RT stands for retention time).

### 4.1.3. Synthesis of Intermediates

Intermediates **a1**, **b1**, **b2**, **c1**, **c2**, **d1**, **e1**, **e2**, **f1**, **f2**, **g1**, **h1**, **h2**, **h5**, **i1** and **i3** were all obtained from commercially available sources. Commercially available **h1**, **h2** and **i1** (70 mmol) were dissolved in anhydrous ethyl alcohol (180 mL) with injection of 2 mL of concentrated $H_2SO_4$. Then it was refluxed 12 hours and was directly evaporated to dryness. Then the residues were extracted dichloromethane and washed with large amount of water. Concentration of combined organic phases to afford the crude intermediates **h3**, **h4** and **i2**. These crude intermediates were used for the next step without further purification. Intermediate acyl chloride **i4** was obtained from the reaction of benzoic acid **i3** (8 mmol) in thionyl chloride (30 mL) as solvent under reflux for 3 h. Then the reaction was evaporated under vacuum to obtain a colorless oil, which was reacted in the next step reaction directly. Key intermediates **c3**[49], **f3**[50], **f4**[51], **h6**[53], **h7**[53] and **i5**[54] were synthesized according to the procedures described without

any further modification.

Intermediates **g2** were synthesized according to the method developed by Jae *et al.*[52] with a little optimization. In brief, the mixture of ethyl malonate potassium salt (2 eq), $MgCl_2$ (1 eq) and TEA (2 eq) in anhydrous THF, which was stirred for 6 h at 25 $^oC$, was as **A** solution. The mixture of corresponding substituted benzoic acid (0.6 eq) and 1, 1'-carbonyldiimidazole (CDI) (0.8 eq) in anhydrous THF, which was refluxed to obtain a clear solution, was as **B** solution. Then **B** was transferred to **A**, the mixture of which was refluxed overnight. The reaction was placed under ice, and appropriate volume of 5 M HCl was added until pH 5~6. After extraction with EtOAc, workup as described to provide the crude intermediates for further reaction without purification.

***Ethyl 2-methyl-3-oxo-3-phenylpropanoate*** (**h6-1**) ($R_3$=$CH_3$). Briefly, to an anhydrous THF solution of ethyl propionate (2 eq) under ice bath was added NaH (3 eq, 60% in mineral oil) portionwise. After 10 min, addition of **h3** (1 eq) was followed by refluxing overnight. The cooled reaction was directly poured into saturated aqueous $NH_4Cl$, which was extracted using EtOAc. After concentration of the combined organic layer, it was purified with a short plug of silica gel through column chromatography. Yield: 92.3%; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.003-7.974 (m, 2H), 7.615-7.572 (m, 1H), 7.505-7.467 (m, 2H), 4.382 (q, 1H, *J*=7.2 Hz, -CH($CH_3$)-), 4.302 (q, 2H, *J*=7.2 Hz, -$OCH_2$-), 1.501 (d, 3H, *J*=6.8 Hz, -$CH_3$), 1.171 (t, 3H, *J*=7.2 Hz, -$CH_3$); MS-ESI m/z: 229.0 $[M+Na]^+$.

***Ethyl 2-benzoylbutanoate*** (**h6-2**) ($R_3$=$C_2H_5$). A similar method for **h6-1** was employed to get compound **h6-2** exploiting ethyl butyrate and **h3** as starting materials. Yield: 94.6%; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.006-7.985 (m, 2H), 7.613-7.571 (m, 1H), 7.504-7.466 (m, 2H), 4.239-4.124 (m, 3H, -CH($C_2H_5$)-, -$OCH_2$-), 2.084-2.008 (m, 2H, -$CH_2$-), 1.176 (t, 3H, *J*=7.2 Hz, -$CH_3$), 1.001 (t, 3H, *J*=7.2 Hz, -$CH_3$); MS-ESI m/z: 243.0 $[M+Na]^+$.

***Ethyl 2-benzoyl-3-methylbutanoate*** (**h6-3**) ($R_3$=*iso*-propyl). A similar method for **h6-1** was employed to get compound **h6-3** exploiting ethyl isovalerate and **h3** as starting materials. Yield: 66.4%; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.029 (d, 2H, *J*=7.2 Hz), 7.590 (t, 1H, *J*=7.6 Hz), 7.483 (t, 2H, *J*=7.6 Hz), 4.167-4.084 (m, 3H, -CH($C_3H_7$)-,

-$OCH_2$-), 2.705-2.615 (m, 1H, -$CH(CH_3)_2$), 1.182 (t, 3H, *J*=7.2 Hz, -$CH_3$), 1.052 (d, 3H, *J*=6.4 Hz, -$CH_3$), 0.943 (d, 3H, *J*=6.4 Hz, -$CH_3$); MS-ESI m/z: 257.0 $[M+Na]^+$.

***Ethyl 2-benzoylpentanoate*** (**h6-4**) ($R_3$=*n*-propyl). A similar method for **h6-1** was employed to get compound **h6-4** exploiting ethyl valerate and **h3** as starting materials. Yield: 75.6%; $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 7.997 (d, 2H, *J*=7.6 Hz), 7.590 (t, 1H, *J*=7.2 Hz), 7.485 (t, 2H, *J*=8.0 Hz), 4.310 (t, 1H, *J*=6.8 Hz, -$CH(C_3H_7)$-), 4.149 (q, 2H, *J*=7.2 Hz, -$OCH_2$-), 2.057-1.915 (m, 2H, -$CH_2$-), 1.435-1.343 (m, 2H, -$CH_3$), 1.175 (t, 3H, *J*=7.2 Hz, -$CH_3$), 0.953 (t, 3H, *J*=7.6 Hz, -$CH_3$); MS-ESI m/z: 257.0 $[M+Na]^+$.

***Ethyl 2-benzoylhexanoate*** (**h6-5**) ($R_3$=*n*-butyl). A similar method for **h6-1** was employed to get compound **h6-5** exploiting ethyl hexanoate and **h3** as starting materials. Yield: 80.3%; $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 8.009-7.984 (m, 2H), 7.616-7.573 (m, 1H), 7.506-7.464 (m 2H), 4.290 (t, 1H, *J*=7.2 Hz, -$CH(C_4H_9)$-), 4.178-4.121 (m, 2H, -$OCH_2$-), 2.053-1.952 (m, 2H, -$CH_2$-), 1.397-1.305 (m, 4H, -$CH_2$-), 1.176 (t, 3H, *J*=6.8 Hz, -$CH_3$), 0.897 (t, 3H, *J*=6.8 Hz, -$CH_3$); MS-ESI m/z: 271.0 $[M+Na]^+$.

***Ethyl 2-benzoylheptanoate*** (**h6-6**) ($R_3$=*n*-pentyl). A similar method for **h6-1** was employed to get compound **h6-6** exploiting ethyl heptanoate and **h3** as starting materials. Yield: 77.8%; $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 8.003-7.981 (m, 2H), 7.612-7.569 (m, 1H), 7.503-7.465 (m 2H), 4.288 (t, 1H, *J*=6.8 Hz, -$CH(C_5H_{11})$-), 4.176-4.120 (m, 2H, -$OCH_2$-), 2.044-1.926 (m, 2H, -$CH_2$-), 1.359-1.290 (m, 6H, -$CH_2$-), 1.174 (t, 3H, *J*=7.2 Hz, -$CH_3$), 0.871 (t, 3H, *J*=6.8 Hz, -$CH_3$); MS-ESI m/z: 285.0 $[M+Na]^+$.

***Ethyl 2-benzoyloctanoate*** (**h6-7**) ($R_3$=*n*-hexyl). A similar method for **h6-1** was employed to get compound **h6-7** exploiting ethyl caprylate and **h3** as starting materials. Yield: 74.5%; $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 8.003-7.981 (m, 2H), 7.614-7.571 (m, 1H), 7.509-7.466 (m 2H), 4.288 (t, 1H, *J*=6.8 Hz, -$CH(C_6H_{13})$-), 4.176-4.119 (m, 2H, -$OCH_2$-), 2.029-1.962 (m, 2H, -$CH_2$-), 1.351-1.252 (m, 8H, -$CH_2$-), 1.174 (t, 3H, *J*=7.2 Hz, -$CH_3$), 0.865 (t, 3H, *J*=7.2 Hz, -$CH_3$); MS-ESI m/z: 277.1 $[M+H]^+$.

***Ethyl 2-methyl-3-oxo-3-(3,4,5-trimethoxyphenyl)propanoate*** (**h7-1**) ($R_3$=$CH_3$). A similar method for **h6-1** was employed to get compound **h7-1** exploiting ethyl propionate and **h4** as starting materials. Yield: 88.9%; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.268 (s, 2H), 4.382 (q, 1H, $J$=7.2 Hz, -CH($CH_3$)-), 4.302 (q, 2H, $J$=7.2 Hz, -$OCH_2$-), 3.931-3.906 (m, 9H, -$OCH_3$), 1.502 (d, 3H, $J$=6.8 Hz, -$CH_3$), 1.203 (t, 3H, $J$=7.2 Hz, -$CH_3$); MS-ESI m/z: 297.0 $[M+H]^+$.

***Ethyl 2-(3,4,5-trimethoxybenzoyl)butanoate*** (**h7-2**) ($R_3$=$C_2H_5$). A similar method for **h6-1** was employed to get compound **h7-2** exploiting ethyl butyrate and **h4** as starting materials. Yield: 91.4%; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.286 (s, 2H), 4.190-4.137 (m, 3H, -CH($C_2H_5$)-, -$OCH_2$-), 3.929 (s, 3H, -$OCH_3$), 3.924 (s, 6H, -$OCH_3$), 2.086-2.013 (m, 2H, -$CH_2$-), 1.205 (d, 3H, $J$=7.2 Hz, -$CH_3$), 1.002 (t, 3H, $J$=7.2 Hz, -$CH_3$); MS-ESI m/z: 311.1 $[M+H]^+$.

***Ethyl 3-methyl-2-(3,4,5-trimethoxybenzoyl)butanoate*** (**h7-3**) ($R_3$=*iso*-propyl). A similar method for **h6-1** was employed to get compound **h7-3** exploiting ethyl isovalerate and **h4** as starting materials. Yield: 55.7%; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.319 (s, 2H), 4.193-4.095 (m, 2H, -$OCH_2$-), 4.012 (d, 1H, $J$=9.6 Hz, -CH($C_3H_7$)-), 3.931 (s, 9H, -$OCH_3$), 2.733-2.609 (m, 1H, -CH($CH_3$)$_2$), 1.212 (t, 3H, $J$=7.2 Hz, -$CH_3$), 1.052 (d, 3H, $J$=6.8 Hz, -$CH_3$), 0.951 (d, 3H, $J$=6.8 Hz, -$CH_3$); MS-ESI m/z: 325.0 $[M+H]^+$.

***Ethyl 2-(3,4,5-trimethoxybenzoyl)pentanoate*** (**h7-4**) ($R_3$=*n*-propyl). A similar method for **h6-1** was employed to get compound **h7-4** exploiting ethyl valerate and **h4** as starting materials. Yield: 80.6%; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.281 (s, 2H), 4.255 (t, 1H, $J$=7.2 Hz, -CH($C_3H_7$)-), 4.162 (q, 2H, $J$=7.2 Hz, -$OCH_2$-), 3.930 (s, 3H, -$OCH_3$), 3.923 (s, 6H, -$OCH_3$), 2.061-1.923 (m, 2H, -$CH_2$-), 1.434-1.340 (m, 2H, -$CH_3$), 1.205 (t, 3H, $J$=7.2 Hz, -$CH_3$), 0.969 (t, 3H, $J$=7.2 Hz, -$CH_3$); MS-ESI m/z: 325.0 $[M+H]^+$.

***Ethyl 2-(3,4,5-trimethoxybenzoyl)hexanoate*** (**h7-5**) ($R_3$=*n*-butyl). A similar method for **h6-1** was employed to get compound **h7-5** exploiting ethyl hexanoate and **h4** as starting materials. Yield: 82.3%; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.282 (s, 2H), 4.233 (t, 1H, $J$=7.2 Hz, -CH($C_4H_9$)-), 4.162 (q, 2H, $J$=7.2 Hz, -$OCH_2$-), 3.930 (s, 3H, -$OCH_3$),

3.923 (s, 6H, -$OCH_3$), 2.035-1.980 (m, 2H, -$CH_2$-), 1.410-1.311 (m, 4H, -$CH_2$-), 1.204 (t, 3H, *J*=7.2 Hz, -$CH_3$), 0.909 (t, 3H, *J*=6.8 Hz, -$CH_3$); MS-ESI m/z: 339.0 $[M+H]^+$.

***Ethyl 2-(3,4,5-trimethoxybenzoyl)heptanoate*** (**h7-6**) ($R_3$=*n*-pentyl). A similar method for **h6-1** was employed to get compound **h7-6** exploiting ethyl heptanoate and **h4** as starting materials. Yield: 74.2%; $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 7.281 (s, 2H), 4.234 (t, 1H, *J*=7.2 Hz, -$CH(C_5H_{11})$-), 4.162 (q, 2H, *J*=7.2 Hz, -$OCH_2$-), 3.929 (s, 3H, -$OCH_3$), 3.922 (s, 6H, -$OCH_3$), 2.030-1.970 (m, 2H, -$CH_2$-), 1.377-1.325 (m, 6H, -$CH_2$-), 1.204 (t, 3H, *J*=7.2 Hz, -$CH_3$), 0.897-0.864 (m, 3H, -$CH_3$); MS-ESI m/z: 353.0 $[M+H]^+$.

***Ethyl 2-(3,4,5-trimethoxybenzoyl)octanoate*** (**h7-7**) ($R_3$=*n*-hexyl). A similar method for **h6-1** was employed to get compound **h7-7** exploiting ethyl caprylate and **h4** as starting materials. Yield: 58.6%; $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 7.280 (s, 2H), 4.234 (t, 1H, *J*=7.2 Hz, -$CH(C_6H_{13})$-), 4.161 (q, 2H, *J*=7.2 Hz, -$OCH_2$-), 3.930 (s, 3H, -$OCH_3$), 3.923 (s, 6H, -$OCH_3$), 2.031-1.974 (m, 2H, -$CH_2$-), 1.361-1.187 (m, 11H, -$CH_2$-, -$CH_3$), 0.891-0.857 (m, 3H, -$CH_3$); MS-ESI m/z: 389.1 $[M+Na]^+$.

### 4.1.4. Target Compounds Synthesis and Characterization

Compound **1** and compound **6** were synthesized by the methods invented by Behringer *et al.*[55] and Jin *et al.*[56] without any modification, respectively. Except for compounds **1** and **6**, the other target compounds were synthesized in the light of the procedure developed by Curphey *et al.*[57] with a little optimization. In brief, ~20 mL anhydrous xylene containing various intermediates **g2** (5 mmol) was injected dropwise into the suspension of the $P_4S_{10}$ (3 mmol), HMDO (15 mmol), and sulfur (5 mmol) in 30 mL of anhydrous xylene under reflux. The refluxed reaction was terminated until no original materials **g2** were observed monitored by TLC. Addition of $K_2CO_3$ solution (6.68 mmol $K_2CO_3$/mmol of $P_4S_{10}$) was conducted at 0 °*C*. Then addition of ~25 mL acetone (equal to half of the reaction solvent) was followed by stirring for thirty min at 0 °*C*. Extraction of the obtained maroon material with toluene was followed by purification through a short column of activated carbon (~4 g) to obtain a clear

maroon solution, which was concentrated and purified through column chromatography.

Because of inaccessibility of pure **g3** and **g4** as target compounds, the crude **g3** and **g4** were directly reduced according to Reddy *et al.*[67] in order to provide target compounds **42** and **43**. In the same way, **41** was obtained by reduction of **38**.

***3H-1,2-dithiole-3-thione*** (**Compound 1**). Compound **1** was synthesized according to a published procedure[55] to give a khaki solid. Yield: 69.6%; $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 8.980 (d, 1H, $J$=5.6 Hz), 7.296 (d, 1H, $J$=5.6 Hz); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 216.703, 160.307, 139.686; MS-ESI m/z: 135.00 $[M+H]^+$; Purity determination: RT=4.28 min, purity 99.90%, 80:20 $CH_3OH/H_2O$.

***Methyl 3-thioxo-3H-1,2-dithiole-4-carboxylate*** (**Compound 2**). A published method[39] was employed to afford Compound **2** as a red solid. Yield: 47.2%; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$: 9.206 (s, 1H), 3.897 (s, 3H, -COOMe); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 211.164, 165.548, 161.210, 137.959, 52.853; MS-ESI m/z: 193.00 $[M+H]^+$; Purity determination: RT=3.96 min, purity 98.53%, 80:20 $CH_3OH/H_2O$.

***5,6-dihydrocyclopenta[c][1,2]dithiole-3(4H)-thione*** (**Compound 3**). Compound **3** was obtained from **b1** as starting material following the general procedure described by Curphey *et al.*[57] to provide a khaki solid. Yield: 66.7%; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$: 2.970-2.934 (m, 2H), 2.698-2.571 (m, 4H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 208.088, 175.347, 155.680, 34.089, 30.435, 29.011; MS-ESI m/z 175.05 $[M+H]^+$; Purity determination: RT=5.30 min, purity 99.75%, 85:15 $CH_3OH/H_2O$.

***4,5,6,7-tetrahydro-3H-benzo[c][1,2]dithiole-3-thione*** (**Compound 4**). A similar method for compound **3** was employed to get compound **4** exploiting **b2** as starting material to afford a light khaki solid. Yield: 49.2%; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$: 2.817 (t, 2H, $J$=5.2 Hz), 2.581 (t, 2H, $J$=6.0 Hz), 1.860-1.767 (m, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 215.311, 169.343, 143.390, 29.653, 27.522, 21.381, 20.806; MS-ESI m/z: 189.00 $[M+H]^+$; Purity determination: RT=6.43 min, purity 99.92%, 85:15 $CH_3OH/H_2O$.

***5-methyl-3H-1,2-dithiole-3-thione*** (**Compound 5**). A similar method for compound **3** was employed to get compound **5** exploiting **e1** as starting material to afford a maroon oil. Yield: 81.3%; $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.214 (s, 1H), 2.523 (d, 3H, $J$=0.8 Hz, -$CH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 215.665, 175.420, 138.955, 17.826; MS-ESI m/z: 149.00 $[M+H]^+$; Purity determination: RT=4.40 min, purity 99.93%, 85:15 $CH_3OH/H_2O$.

***3H-benzo[c][1,2]dithiole-3-thione*** (**Compound 6**). Compound **6** was prepared from **d1** as starting material following method developed by Jin *et al.*[56] to provide an orange solid. Yield: 74.5%; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.225 (d, 1H, $J$=8.0 Hz), 7.750-7.667 (m, 2H), 7.485-7.445 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 215.563, 173.073, 135.973, 132.281, 131.622, 129.689, 126.959; MS-ESI m/z: 185.00 $[M+H]^+$; Purity determination: RT=9.75 min, purity 99.92%, 80:20 $CH_3OH/H_2O$.

***5-phenyl-3H-1,2-dithiole-3-thione*** (**Compound 7**). A similar method for compound **3** was employed to get compound **7** exploiting **e2** as starting material to afford a metallic shiny yellow solid. Yield: 80.9%; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.684-7.655 (m, 2H), 7.590-7.547 (m, 1H), 7.519-7.474 (m, 2H), 7.452 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 216.812, 152.856, 140.785, 132.804, 128.571, 126.089, 124.398; MS-ESI m/z: 211.00 $[M+H]^+$; Purity determination: RT=8.57 min, purity 99.56%, 80:20 $CH_3OH/H_2O$.

***Indeno[1,2-c][1,2]dithiole-3(4H)-thione*** (**Compound 8**). A similar method for compound **3** was employed to get compound **8** exploiting **f3** as intermediate to afford a khaki solid. Yield: 37.5%; $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 8.033 (d, 1H, $J$=7.6 Hz), 7.670 (d, 1H, $J$=7.6 Hz), 7.576 (t, 1H, $J$=7.6 Hz), 7.493 (t, 1H, $J$=7.2 Hz), 3.746 (s, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 205.638, 171.415, 152.826, 148.981, 137.381, 130.581, 127.858, 126.311, 122.529, 34.949; MS-ESI m/z: 223.00 $[M+H]^+$; Purity determination: RT=9.86 min, purity 99.02%, 80:20 $CH_3OH/H_2O$.

***4,5-dihydro-3H-naphtho[1,2-c][1,2]dithiole-3-thione*** (**Compound 9**). A similar method for compound **3** was employed to get compound **9** exploiting **f4** as intermediate to afford a red solid. Yield: 86.8%; $^{1}$H NMR (400 MHz,

517 DMSO-$d_6$) $\delta$: 7.799 (d, 1H, $J$=8.0 Hz), 7.548 (td, 1H, $J$=7.2 Hz, 1.2 Hz), 7.459 (d, 1H, $J$=7.6 Hz), 7.401 (t, 1H,
518 J=7.6 Hz); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 211.575, 165.473, 142.636, 137.149, 132.655, 129.853, 128.602,
519 127.727, 125.423, 27.329, 24.645; MS-ESI m/z: 237.00 $[M+H]^+$; Purity determination: RT=14.03 min, purity
520 99.96%, 80:20 $CH_3OH/H_2O$.

521 ***5-(2-fluorophenyl)-3H-1,2-dithiole-3-thione*** (**Compound 10**). A similar method for compound **3** was employed to
522 get compound **10** exploiting corresponding **g2** as intermediate to afford a maroon solid. Yield: 84.2%; $^1$H NMR
523 (400 MHz, DMSO-$d_6$) $\delta$: 7.951 (td, 1H, $J$=8.0 Hz, 2Hz), 7.702-7.644 (m, 2H), 7.481 (ddd, 1H, $J$=9.6 Hz, 8.4 Hz,
524 1.2 Hz), 7.392 (td, 1H, $J$=7.6 Hz, 1.2 Hz); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 214.741, 166.546, 166.512, 159.984,
525 157.471, 138.249, 138.202, 134.084, 133.994, 129.917, 129.899, 125.575, 125.542, 118.929, 118.811, 116.961,
526 116.744; MS-ESI m/z: 229.00 $[M+H]^+$; Purity determination: RT=9.60 min, purity 98.94%, 75:25 $CH_3OH/H_2O$.

527 ***5-(2-chlorophenyl)-3H-1,2-dithiole-3-thione*** (**Compound 11**). A similar method for compound **3** was employed to
528 get compound **11** exploiting corresponding **g2** as intermediate to afford an orange solid. Yield: 92.3%; $^1$H NMR
529 (400 MHz, DMSO-$d_6$) $\delta$: 7.760 (dd, 1H, $J$=7.6 Hz, 1.6 Hz), 7.698 (dd, 1H, $J$=8.0 Hz, 1.6 Hz), 7.603 (td, 1H, $J$=7.6
530 Hz, 2 Hz), 7.518 (td, 1H, $J$=7.6 Hz, 1.2 Hz), 7.490 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 215.295, 170.267,
531 140.020, 132.646, 131.250, 131.227, 130.652, 129.582, 127.961; MS-ESI m/z: 244.95 $[M+H]^+$; Purity
532 determination: RT=10.04 min, purity 99.81%, 80:20 $CH_3OH/H_2O$.

533 ***5-(2-bromophenyl)-3H-1,2-dithiole-3-thione*** (**Compound 12)**. A similar method for compound **3** was employed to
534 get compound **12** exploiting corresponding **g2** as intermediate to afford a light orange solid. Yield: 89.3%; $^1$H NMR
535 (400 MHz, DMSO-$d_6$) $\delta$: 7.847 (dd, 1H, $J$=8.0 Hz, 1.6Hz), 7.711 (dd, 1H, $J$=7.6 Hz, 2 Hz), 7.557 (td, 1H, $J$=7.6 Hz,
536 1.6 Hz), 7.054 (td, 1H, $J$=7.6Hz, 1.6 Hz), 7.428 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 215.431, 172.111,
537 140.044, 133.712, 132.632, 131.538, 131.245, 128.320, 121.172; MS-ESI m/z: 288.90 $[M+H]^+$; Purity
538 determination: RT=10.05 min, purity 99.91%, 80:20 $CH_3OH/H_2O$.

***5-(3-fluorophenyl)-3H-1,2-dithiole-3-thione*** (**Compound 13**). A similar method for compound **3** was employed to get compound **13** exploiting corresponding **g2** as intermediate to afford a metallic shiny maroon solid. Yield: 85.7%; $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.866 (s, 1H), 7.824 (dt, 1H, $J$=10.0 Hz, 2 Hz), 7.738 (d, 1H, $J$=7.6 Hz), 7.620-7.565 (m, 1H), 7.475 (td, 1H, $J$=8.4 Hz, 2 Hz); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 215.418, 171.529, 171.502, 163.475, 161.028, 136.322, 133.147, 133.064, 131.649, 131.565, 123.206, 123.177, 119.022, 118.811, 114.079, 113.842; MS-ESI m/z: 229.00 $[M+H]^+$; Purity determination: RT=6.54 min, purity 96.95%, 85:15 $CH_3OH/H_2O$.

***5-(3-chlorophenyl)-3H-1,2-dithiole-3-thione*** (**Compound 14**). A similar method for compound **3** was employed to get compound **14** exploiting corresponding **g2** as intermediate to afford a metallic shiny maroon solid. Yield: 87.2%; $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 8.011 (t, 1H, $J$=2.0 Hz), 7.884 (s, 1H), 7.860 (dq, 1H, $J$=8.0 Hz, 0.8 Hz), 7.692 (dq, 1H, $J$=8.4 Hz, 1.2 Hz), 7.572 (t, 1H, $J$=8.0 Hz); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 215.591, 171.505, 136.503, 134.314, 133.019, 131.862, 131.358, 126.695, 125.781; MS-ESI m/z: 244.95 $[M+H]^+$; Purity determination: RT=11.25 min, purity 99.60%, 80:20 $CH_3OH/H_2O$.

***5-(3-bromophenyl)-3H-1,2-dithiole-3-thione*** (**Compound 15)**. A similar method for compound **3** was employed to get compound **15** exploiting corresponding **g2** as intermediate to afford a khaki solid. Yield: 82.1%; $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 8.130 (t, 1H, 2 Hz), 7.913-7.887 (m, 2H), 7.824 (dq, 1H, $J$=8.0 Hz, 0.8 Hz), 7.501 (t, 1H, $J$=8.0 Hz); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 215.519, 171.416, 136.495, 134.786, 133.229, 131.541, 129.433, 126.139, 122.805; MS-ESI m/z: 288.85 $[M+H]^+$; Purity determination: RT=12.20 min, purity 99.82%, 80:20 $CH_3OH/H_2O$.

***5-(4-fluorophenyl)-3H-1,2-dithiole-3-thione*** (**Compound 16**). A similar method for compound **3** was employed to get compound **16** exploiting corresponding **g2** as intermediate to afford a metallic shiny orange solid. Yield: 91.6%; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.702-7.652 (m, 2H), 7.398 (s, 1H), 7.229-7.171 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 215.498, 171.628, 166.284, 163.753, 135.990, 129.202, 129.113, 127.975, 127.941, 117.135, 116.914;

MS-ESI m/z: 228.95 $[M+H]^+$; Purity determination: RT=7.51 min, purity 99.84%, 80:20 $CH_3OH/H_2O$.

***5-(4-chlorophenyl)-3H-1,2-dithiole-3-thione*** (**Compound 17**). A similar method for compound **3** was employed to get compound **17** exploiting corresponding **g2** as intermediate to afford an orange solid. Yield: 85.4%; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.961-7.926 (m, 2H), 7.857 (s, 1H), 7.637-7.602 (m, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 215.520, 172.063, 137.124, 136.066, 130.038, 129.643, 128.907; MS-ESI m/z: 244.95 $[M+H]^+$; Purity determination: RT=9.73 min, purity 99.70%, 80:20 $CH_3OH/H_2O$.

***5-(4-bromophenyl)-3H-1,2-dithiole-3-thione*** (**Compound 18)**. A similar method for compound **3** was employed to get compound **18** exploiting corresponding **g2** as intermediate to afford a red solid. Yield: 89.4%; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.654-7.621 (m, 2H), 7.546-7.513 (m, 2H), 7.405 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 215.523, 171.287, 136.171, 132.987, 130.620, 128.310, 126.953; MS-ESI m/z: 288.90 $[M+H]^+$; Purity determination: RT=10.62 min, purity 99.82%, 80:20 $CH_3OH/H_2O$.

***5-(2-(trifluoromethyl)phenyl)-3H-1,2-dithiole-3-thione*** (**Compound 19**). A similar method for compound **3** was employed to get compound **19** exploiting corresponding **g2** as intermediate to afford a metallic shiny orange solid. Yield: 88.5%; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.975-7.952 (m, 1H), 7.866-7.781 (m, 3H), 7.331 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 215.445, 170.326, 140.296, 140.274, 132.938, 131.755, 131.396, 128.726, 128.705, 127.128, 127.015, 126.964, 126.913, 126.862, 126.826, 124.832, 122.109; MS-ESI m/z: 278.95 $[M+H]^+$; Purity determination: RT=8.28 min, purity 99.95%, 80:20 $CH_3OH/H_2O$.

***5-(3-(trifluoromethyl)phenyl)-3H-1,2-dithiole-3-thione*** (**Compound 20**). A similar method for compound **3** was employed to get compound **20** exploiting corresponding **g2** as intermediate to afford a metallic shiny maroon solid. Yield: 85.3%; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 8.252-8.190 (m, 2H), 7.996-7.980 (m, 2H), 7.789 (t, 1H, $J$=7.6 Hz); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 215.765, 171.379, 136.956, 132.182, 131.129, 130.793, 128.507, 128.471, 128.432, 123.843, 123.806, 123.767; MS-ESI m/z: 278.95 $[M+H]^+$; Purity determination: RT=10.22 min, purity

99.93%, 80:20 $CH_3OH/H_2O$.

***5-(4-(trifluoromethyl)phenyl)-3H-1,2-dithiole-3-thione*** (**Compound 21**). A similar method for compound **3** was employed to get compound **21** exploiting corresponding **g2** as intermediate to afford a metallic shiny red solid. Yield: 84.9%; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 8.133-8.113 (m, 2H), 7.920-7.895 (m, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 215.637, 171.043, 136.946, 134.854, 134.839, 131.754, 131.434, 127.909, 126.320, 126.283, 126.246, 126.208, 124.942, 122.233; MS-ESI m/z: 278.95 $[M+H]^+$; Purity determination: RT=9.14 min, purity 99.68%, 80:20 $CH_3OH/H_2O$.

***5-(o-tolyl)-3H-1,2-dithiole-3-thione*** (**Compound 22**). A similar method for compound **3** was employed to get compound **22** exploiting corresponding **g2** as intermediate to afford an orange solid. Yield: 93.4%; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.446-7.404 (m, 2H), 7.345-7.268 (m, 2H), 7.156 (s, 1H), 2.438 (s, 3H, -$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 215.920, 173.277, 139.420, 136.158, 131.531, 131.131, 130.675, 129.356, 126.574, 20.634; MS-ESI m/z: 225.05 $[M+H]^+$; Purity determination: RT=9.63 min, purity 99.92%, 80:20 $CH_3OH/H_2O$.

***5-(m-tolyl)-3H-1,2-dithiole-3-thione*** (**Compound 23**). A similar method for compound **3** was employed to get compound **23** exploiting corresponding **g2** as intermediate to afford a maroon solid. Yield: 92.8%; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.474-7.446 (m, 3H), 7.401-7.366 (m, 2H), 2.432 (s, 3H, -$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 215.557, 173.306, 139.685, 135.883, 133.092, 131.629, 129.560, 127.551, 124.153, 21.465; MS-ESI m/z: 225.00 $[M+H]^+$; Purity determination: RT=6.34 min, purity 99.50%, 90:10 $CH_3OH/H_2O$.

***5-(p-tolyl)-3H-1,2-dithiole-3-thione*** (**Compound 24**). A similar method for compound **3** was employed to get compound **24** exploiting corresponding **g2** as intermediate to afford a khaki solid. Yield: 96.4%; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.547 (d, 2H, $J$=8.4 Hz), 7.424 (s, 1H), 7.284 (d, 2H, $J$=8.0 Hz), 2.419 (s, 3H, -$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 215.389, 173.421, 143.238, 135.379, 130.366, 128.851, 126.849, 21.727; MS-ESI m/z: 225.00 $[M+H]^+$; Purity determination: RT=10.93 min, purity 99.87%, 80:20 $CH_3OH/H_2O$.

***5-(4-ethylphenyl)-3H-1,2-dithiole-3-thione*** (**Compound 25**). A similar method for compound **3** was employed to get compound **25** exploiting corresponding **g2** as intermediate to afford a maroon solid. Yield: 90.2%; $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.815 (t, 3H, $J$=7.6 Hz), 7.386 (d, 2H, $J$=8.0 Hz), 2.675 (q, 2H, $J$=7.6 Hz, -$CH_2$-), 1.200 (t, 3H, $J$=7.6 Hz, -$CH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 215.171, 173.804, 148.884, 134.995, 128.984, 128.695, 127.077, 28.078, 15.058; MS-ESI m/z: 239.00 $[M+H]^+$; Purity determination: RT=6.82 min, purity 99.33%, 90:10 $CH_3OH/H_2O$.

***5-(4-butylphenyl)-3H-1,2-dithiole-3-thione*** (**Compound 26**). A similar method for compound **3** was employed to get compound **26** exploiting corresponding **g2** as intermediate to afford a maroon solid. Yield: 93.8%; $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.830-7.798 (m, 3H), 7.370 (d, 2H, $J$=8.0 Hz), 2.649 (t, 2H, $J$=7.6 Hz, -$CH_2$-), 1.613-1.537 (m, 2H, -$CH_2$-), 1.355-1.262 (m, 2H, -$CH_2$-), 0.899 (t, 3H, $J$=7.6 Hz, -$CH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 215.141, 173.750, 147.519, 134.957, 129.460, 128.666, 126.975, 34.663, 32.612, 21.718, 13.690; MS-ESI m/z: 267.05 $[M+H]^+$; Purity determination: RT=8.68 min, purity 99.69%, 90:10 $CH_3OH/H_2O$.

***5-(2-methoxyphenyl)-3H-1,2-dithiole-3-thione*** (**Compound 27**). A similar method for compound **3** was employed to get compound **27** exploiting corresponding **g2** as intermediate to afford a red wine-colored solid. Yield: 86.9%; $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.987 (dd, 1H, $J$=8.0 Hz, 1.6 Hz), 7.881 (s, 1H), 7.617-7.574 (m, 1H), 7.299 (d, 1H, $J$=8.0 Hz), 7.133-7.093 (m, 1H), 3.982 (s, 3H, -$OCH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 212.960, 169.410, 157.036, 136.730, 133.747, 129.375, 121.544, 119.531,112.963, 56.384; MS-ESI m/z: 241.00 $[M+H]^+$; Purity determination: RT=8.90 min, purity 99.24%, 80:20 $CH_3OH/H_2O$.

***5-(3-methoxyphenyl)-3H-1,2-dithiole-3-thione*** (**Compound 28**). A similar method for compound **3** was employed to get compound **28** exploiting corresponding **g2** as intermediate to afford a metallic shiny kakhi solid. Yield: 84.8%; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.433 (s, 1H), 7.397 (t, 1H, $J$=8.0 Hz), 7.244 (dq, 1H, $J$=8.0 Hz, 0.8 Hz), 7.144 (t, 1H, $J$=2.4 Hz), 7.103-7.074 (m, 1H), 3.867 (s, 3H, -$OCH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 215.585,

627 172.869, 160.337, 136.154, 132.879, 130.812, 119.383, 117.865, 112.379, 55.660; MS-ESI m/z: 240.95 $[M+H]^+$;
628 Purity determination: RT=10.14 min, purity 99.84%, 80:20 $CH_3OH/H_2O$.
629 ***5-(4-methoxyphenyl)-3H-1,2-dithiole-3-thione*** (**Compound 29**). A similar method for compound **3** was employed
630 to get compound **29** exploiting corresponding **g2** as intermediate to afford a metallic shiny kakhi solid. Yield:
631 86.2%; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 7.639-7.602 (m, 2H), 7.395 (s, 1H), 7.000-6.963 (m, 2H), 3.882 (s, 3H,
632 $-OCH_3$); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 215.151, 173.155, 163.036, 134.686, 128.679, 124.235, 115.093, 55.738;
633 MS-ESI m/z: 240.95 $[M+H]^+$; Purity determination: RT=9.18 min, purity 99.94%, 80:20 $CH_3OH/H_2O$.
634 ***5-(benzo[d][1,3]dioxol-5-yl)-3H-1,2-dithiole-3-thione*** (**Compound 30**). A similar method for compound **3** was
635 employed to get compound **30** exploiting corresponding **g2** as intermediate to afford a yellow solid. Yield: 46.9%;
636 $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 7.348 (s, 1H), 7.233 (dd, 1H, $J$=8.0 Hz, 1.6 Hz), 7.094 (d, 1H, $J$=2.0 Hz), 6.082 (s,
637 2H, $-OCH_2O-$); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta$: 214.871, 173.572, 151.029, 148.486, 134.734, 125.204,
638 122.506, 109.194, 107.152, 102.354; MS-ESI m/z: 254.95 $[M+H]^+$; Purity determination: RT=5.52 min, purity
639 99.40%, 90:10 $CH_3OH/H_2O$.
640 ***5-(3,5-dimethoxyphenyl)-3H-1,2-dithiole-3-thione*** (**Compound 31**). A similar method for compound **3** was
641 employed to get compound **31** exploiting corresponding **g2** as intermediate to afford a metallic shiny red solid.
642 Yield: 85.9%; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.872 (s, 1H), 6.987 (d, 2H, $J$=2.4 Hz), 6.744 (t, 1H, $J$=2.4 Hz),
643 3.825 (s, 6H, $-OCH_3$); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta$: 215.316, 173.399, 161.030, 136.023, 132.918, 104.978,
644 104.044, 55.671; MS-ESI m/z: 271.00 $[M+H]^+$; Purity determination: RT=6.60 min, purity 99.37%, 90:10
645 $CH_3OH/H_2O$.
646 ***5-(3,4,5-trimethoxyphenyl)-3H-1,2-dithiole-3-thione*** (**Compound 32**). A similar method for compound **3** was
647 employed to get compound **32** exploiting corresponding **g2** as intermediate to afford an orange solid. Yield: 86.3%;
648 $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 7.412 (s, 1H), 6.847 (s, 2H), 3.928 (s, 9H, $-OCH_3$); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$:

215.151, 173.174, 153.854, 141.420, 135.721, 126.964, 104.226, 61.164, 56.445; MS-ESI m/z: 300.95 $[M+H]^+$; Purity determination: RT=4.90 min, purity 99.54%, 90:10 $CH_3OH/H_2O$.

***5-(4-propoxyphenyl)-3H-1,2-dithiole-3-thione*** (**Compound 33**). A similar method for compound **3** was employed to get compound **33** exploiting corresponding **g2** as intermediate to afford a kakhi solid. Yield: 89.2%; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.880-7.842 (m, 2H), 7.747 (s, 1H), 7.087-7.049 (m, 2H), 4.030 (t, 2H, $J$=6.4 Hz, -$OCH_2$-), 1.795-1.707 (m, 2H, -$CH_2$-), 0.983 (t, 3H, $J$=7.6 Hz, -$CH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 214.567, 173.653, 162.083, 133.952, 128.866, 123.411, 115.328, 69.410, 21.848, 10.217; MS-ESI m/z: 269.00 $[M+H]^+$; Purity determination: RT=9.58 min, purity 99.90%, 85:15 $CH_3OH/H_2O$.

***5-(4-(pentyloxy)phenyl)-3H-1,2-dithiole-3-thione*** (**Compound 34**). A similar method for compound **3** was employed to get compound **34** exploiting corresponding **g2** as intermediate to afford a metallic shiny maroon solid. Yield: 87.5%; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.883-7.845 (m, 2H), 7.758 (s, 1H), 7.086-7.048 (m, 2H), 4.061 (t, 2H, $J$=6.8 Hz, -$OCH_2$-), 1.768-1.699 (m, 2H, -$CH_2$-), 1.436-1.297 (m, 4H, -$CH_2CH_2$-), 0.896 (t, 3H, $J$=6.8 Hz, -$CH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 214.645, 173.759, 162.140, 134.023, 128.946, 123.462, 115.373, 67.993, 28.230, 27.631, 21.912, 13.921; MS-ESI m/z: 297.00 $[M+H]^+$; Purity determination: RT=8.83 min, purity 99.76%, 90:10 $CH_3OH/H_2O$.

***4-(3-thioxo-3H-1,2-dithiol-5-yl)benzonitrile*** (**Compound 35**). A similar method for compound **3** was employed to get compound **35** exploiting corresponding **g2** as intermediate to afford a dark red solid. Yield: 31.9%; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 8.109-8.079 (m, 2H), 8.026-7.996 (m, 2H), 7.918 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 215.766, 170.933, 137.278, 135.262, 133.376, 127.942, 118.056, 114.207; MS-EI m/z: 235 ($M^+$, 56.81), 170 (100), 127 (21.24), 171 (14.82); Purity determination: RT=4.59 min, purity 97.92%, 85:15 $CH_3OH/H_2O$.

***Methyl 4-(3-thioxo-3H-1,2-dithiol-5-yl)benzoate*** (**Compound 36**). A similar method for compound **3** was employed to get compound **36** exploiting corresponding **g2** as intermediate to afford a maroon solid. Yield: 72.8%;

671 $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 8.081-7.900 (m, 4H), 7.900 (s, 1H), 3.892 (s, 3H, -$COOCH_3$); $^{13}$C NMR (100
672 MHz, DMSO-$d_6$) $\delta$: 215.731, 171.738, 165.364, 136.839, 135.240, 132.374, 130.155, 127.556, 52.571; MS-EI m/z:
673 268 ($M^+$, 100), 203 (96.59), 86 (17.35), 129 (16.61), 269 (15.90), 270 (14.57), 204 (13.84), 101 (13.83), 144
674 (13.76); Purity determination: RT=6.42 min, purity 98.25%, 85:15 $CH_3OH/H_2O$.

675 ***5-(4-methoxy-3-nitrophenyl)-3H-1,2-dithiole-3-thione*** (**Compound 37**). A similar method for compound **3** was
676 employed to get compound **37** exploiting corresponding **g2** as intermediate to afford a kakhi solid. Yield: 53.4%;
677 $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.174 (d, 1H, *J*=2.4 Hz), 7.838 (dd, 1H, *J*=8.8 Hz, 2.4 Hz), 7.387 (s, 1H), 7.217 (d,
678 1H, *J*=8.8 Hz), 4.058 (s, 3H, -$OCH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 215.275, 169.434, 155.526, 139.797,
679 136.007, 132.416, 124.385, 124.032, 114.714, 57.196; MS-ESI m/z: 285.95 $[M+H]^+$; Purity determination:
680 RT=4.60 min, purity 96.97%, 90:10 $CH_3OH/H_2O$.

681 ***5-(2-nitrophenyl)-3H-1,2-dithiole-3-thione*** (**Compound 38**). A similar method for compound **3** was employed to
682 get compound **38** exploiting corresponding **g2** as intermediate to afford a red solid. Yield: 61.2%; $^{1}$H NMR (400
683 MHz, DMSO-$d_6$) $\delta$: 8.239 (d, 1H, *J*=8.4 Hz), 7.920-7.818 (m, 3H), 7.425 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$)
684 $\delta$: 215.521, 170.217, 147.226, 139.247, 134.122, 132.325, 131.745, 125.370, 125.344; MS-ESI m/z: 278.05
685 $[M+Na]^+$; Purity determination: RT=4.76 min, purity 98.11%, 85:15 $CH_3OH/H_2O$.

686 ***5-(pyridin-3-yl)-3H-1,2-dithiole-3-thione*** (**Compound 39**). A similar method for compound **3** was employed to get
687 compound **39** exploiting corresponding **g2** as intermediate to afford a red solid. Yield: 48.5%; $^{1}$H NMR (400 MHz,
688 DMSO-$d_6$) $\delta$: 9.091 (d, 1H, *J*=1.6 Hz), 8.778 (dd, 1H, *J*=4.4 Hz, 1.2 Hz), 8.329-8.299 (m, 1H), 7.919 (s, 1H),
689 7.599-7.567 (m, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 215.801, 170.608, 152.840, 147.445, 136.815, 134.955,
690 127.742, 124.640; MS-ESI m/z: 212.00 $[M+H]^+$; Purity determination: RT=4.11 min, purity 99.48%, 90:10
691 $CH_3OH/H_2O$.

692 ***5-(3-(dimethylamino)phenyl)-3H-1,2-dithiole-3-thione*** (**Compound 40**). A similar method for compound **3** was

693 employed to get compound **40** exploiting corresponding **g2** as intermediate to afford a maroon solid. Yield: 46.2%;
694 $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.830 (s, 1H), 7.327 (t, 1H, $J$=7.6 Hz), 7.096 (dd, 1H, $J$=7.6 Hz, 1.2 Hz), 7.037
695 (t, 1H, $J$=2.0 Hz), 6.959 (dd, 1H, $J$=7.6 Hz, 1.6 Hz), 2.973 (s, 6H, -N($CH_3$)$_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$:
696 215.234, 175.081, 150.749, 135.289, 131.823, 130.153, 115.951, 114.318, 109.711, 39.927; MS-ESI m/z: 254.00
697 $[M+H]^+$; Purity determination: RT=9.78 min, purity 99.46%, 85:15 $CH_3OH/H_2O$.

698 ***5-(2-aminophenyl)-3H-1,2-dithiole-3-thione*** (**Compound 41**). A similar method for compound **3** was employed to
699 get compound **41** exploiting compound **38** as intermediate to afford a maroon solid. Yield: 55.8%; $^{1}$H NMR (400
700 MHz, $CDCl_3$) $\delta$: 7.428 (s, 1H), 7.365 (dd, 1H, $J$=7.6 Hz, 1.6 Hz), 7.316-7.273 (m, 1H), 6.852-6.789 (m, 2H), 4.157
701 (bs, 2H, -$NH_2$); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 216.176, 171.771, 143.999, 137.801, 132.794, 129.955, 119.174,
702 117.134, 116.465; MS-ESI m/z: 226.00 $[M+H]^+$; Purity determination: RT=4.23 min, purity 99.42%, 90:10
703 $CH_3OH/H_2O$.

704 ***5-(3-aminophenyl)-3H-1,2-dithiole-3-thione*** (**Compound 42**). A similar method for compound **3** was employed to
705 get compound **42** exploiting corresponding **g3** as intermediate to afford a yellow solid. Yield: 38.9%; $^{1}$H NMR (400
706 MHz, DMSO-$d_6$) $\delta$: 7.625 (s, 1H), 7.188-7.148 (m, 1H), 7.001-6.985 (m, 2H), 6.799-6.771 (m, 1H), 5.513 (bs, 2H,
707 -$NH_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 215.062, 174.993, 149.739, 134.867, 131.727, 130.251, 117.778,
708 114.290, 111.256; MS-ESI m/z: 226.00 $[M+H]^+$; Purity determination: RT=4.50 min, purity 99.64%, 85:15
709 $CH_3OH/H_2O$.

710 ***5-(4-aminophenyl)-3H-1,2-dithiole-3-thione*** (**Compound 43**). A similar method for compound **3** was employed to
711 get compound **43** exploiting corresponding **g4** as intermediate to afford a maroon solid. Yield: 35.7%; $^{1}$H NMR
712 (400 MHz, DMSO-$d_6$) $\delta$: 7.632-7.597 (m, 2H), 7.577 (s, 1H), 6.635-6.599 (m, 2H), 6.308 (bs, 2H, -$NH_2$); $^{13}$C NMR
713 (100 MHz, DMSO-$d_6$) $\delta$: 212.531, 175.376, 153.716, 130.981, 129.060, 117.839, 113.702; MS-ESI m/z: 226.00
714 $[M+H]^+$; Purity determination: RT=3.94 min, purity 99.79%, 90:10 $CH_3OH/H_2O$.

***4-methyl-5-(3,4,5-trimethoxyphenyl)-3H-1,2-dithiole-3-thione*** (**Compound 44**). A similar method for compound **3** was employed to get compound **44** exploiting corresponding **h7** as intermediate to afford an orange solid. Yield: 86.5%; $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 6.925 (s, 2H), 3.838 (s, 6H, -$OCH_3$), 3.743 (s, 3H, -$OCH_3$), 2.142 (s, 3H, -$CH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 214.961, 169.258, 153.391, 141.325, 139.396, 128.143, 106.283, 60.181, 56.298, 16.512; MS-ESI m/z: 314.95 $[M+H]^+$; Purity determination: RT=6.29 min, purity 95.00%, 85:15 $CH_3OH/H_2O$.

***4-ethyl-5-(3,4,5-trimethoxyphenyl)-3H-1,2-dithiole-3-thione*** (**Compound 45**). A similar method for compound **3** was employed to get compound **45** exploiting corresponding **h7** as intermediate to afford an orange solid. Yield: 84.6%; $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 6.884 (s, 2H), 3.837 (s, 6H, -$OCH_3$), 3.748 (s, 3H, -$OCH_3$), 2.605 (q, 2H, $J$=3.2 Hz, -$CH_2$-), 1.037 (t, 3H, $J$=7.2 Hz, -$CH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 214.717, 169.869, 153.316, 146.489, 139.302, 127.792, 105.960, 60.081, 56.166, 22.780, 12.437; MS-ESI m/z: 328.95 $[M+H]^+$; Purity determination: RT=7.10 min, purity 95.14%, 85:15 $CH_3OH/H_2O$.

***4-isopropyl-5-(3,4,5-trimethoxyphenyl)-3H-1,2-dithiole-3-thione*** (**Compound 46**). A similar method for compound **3** was employed to get compound **46** exploiting corresponding **h7** as intermediate to afford an orange solid. Yield: 86.2%; $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 6.856 (s, 2H), 3.822 (s, 6H, -$OCH_3$), 3.739 (s, 3H, -$OCH_3$), 3.259-3.187 (m, 1H, -$CH(CH_3)_2$), 1.244 (s, 3H, -$CH_3$), 1.226 (s, 3H, -$CH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 215.494, 171.600, 152.984, 148.328, 139.069, 127.504, 106.459, 60.123, 56.200, 30.412, 19.169; MS-ESI m/z: 343.00 $[M+H]^+$; Purity determination: RT=7.63 min, purity 98.93%, 85:15 $CH_3OH/H_2O$.

***4-propyl-5-(3,4,5-trimethoxyphenyl)-3H-1,2-dithiole-3-thione*** (**Compound 47**). A similar method for compound **3** was employed to get compound **47** exploiting corresponding **h7** as intermediate to afford a yellow solid. Yield: 84.2%; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$: 6.678 (s, 2H), 3.937 (s, 3H, -$OCH_3$), 3.908 (s, 6H, -$OCH_3$), 2.665-2.625 (m, 2H, -$CH_2$-), 1.628-1.533 (m, 2H, -$CH_2$-), 0.908 (t, 3H, $J$=7.2 Hz, -$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 215.932,

737 168.916, 153.748, 146.043, 139.845, 128.536, 105.765, 61.146, 56.419, 32.116, 21.699, 14.412; MS-ESI m/z:
738 343.00 $[M+H]^+$; Purity determination: RT=6.06 min, purity 97.48%, 90:10 $CH_3OH/H_2O$.

739 ***4-butyl-5-(3,4,5-trimethoxyphenyl)-3H-1,2-dithiole-3-thione*** (**Compound 48**). A similar method for compound **3**
740 was employed to get compound **48** exploiting corresponding **h7** as intermediate to afford a maroon solid. Yield:
741 86.4%; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$: 6.887 (s, 2H), 3.832 (s, 6H, $-OCH_3$), 3.743 (s, 3H, $-OCH_3$), 2.620-2.580
742 (m, 2H, $-CH_2-$), 1.463-1.387 (m, 2H, $-CH_2-$), 1.256-1.164 (m, 2H, $-CH_2-$), 0.764 (t, 3H, $J$=7.2 Hz, $-CH_3$); $^{13}C$ NMR
743 (100 MHz, DMSO-$d_6$) $\delta$: 214.949, 169.947, 153.308, 145.368, 139.294, 127.857, 106.021, 60.114, 56.180, 29.431,
744 28.902, 22.096, 13.449; MS-ESI m/z: 357.00 $[M+H]^+$; Purity determination: RT=6.94 min, purity 95.98%, 90:10
745 $CH_3OH/H_2O$.

746 ***4-pentyl-5-(3,4,5-trimethoxyphenyl)-3H-1,2-dithiole-3-thione*** (**Compound 49**). A similar method for compound **3**
747 was employed to get compound **49** exploiting corresponding **h7** as intermediate to afford an orange solid. Yield:
748 81.1%; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$: 6.886 (s, 2H), 3.832 (s, 6H, $-OCH_3$), 3.743 (s, 3H, $-OCH_3$), 2.611-2.572
749 (m, 2H, $-CH_2-$), 1.478-1.404 (m, 2H, $-CH_2-$), 1.179-1.152 (m, 4H, $-CH_2-$), 0.762 (t, 3H, $J$=6.8 Hz, $-CH_3$); $^{13}C$ NMR
750 (100 MHz, DMSO-$d_6$) $\delta$: 214.928, 169.901, 153.306, 145.404, 139.299, 127.867, 106.013, 60.088, 56.154, 31.043,
751 29.081, 26.814, 21.541, 13.592; MS-ESI m/z: 371.05 $[M+H]^+$; Purity determination: RT=7.85 min, purity 99.87%,
752 80:20 $CH_3OH/H_2O$.

753 ***4-hexyl-5-(3,4,5-trimethoxyphenyl)-3H-1,2-dithiole-3-thione*** (**Compound 50**). A similar method for compound **3**
754 was employed to get compound **50** exploiting corresponding **h7** as intermediate to afford a red solid. Yield: 85.4%;
755 $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$: 6.883 (s, 2H), 3.831 (s, 6H, $-OCH_3$), 3.743 (s, 3H, $-OCH_3$), 2.618-2.579 (m, 2H,
756 $-CH_2-$), 1.466-1.392 (m, 2H, $-CH_2-$), 1.211-1.086 (m, 6H, $-CH_2-$), 0.778 (t, 3H, $J$=6.4 Hz, $-CH_3$); $^{13}C$ NMR (100
757 MHz, DMSO-$d_6$) $\delta$: 214.924, 169.885, 153.304, 145.387, 139.298, 127.863, 106.000, 60.065, 56.138, 30.658,
758 29.059, 28.465, 27.058, 21.804, 13.730; MS-ESI m/z: 385.05 $[M+H]^+$; Purity determination: RT=9.14 min, purity

95.46%, 90:10 $CH_3OH/H_2O$.

***4-methyl-5-phenyl-3H-1,2-dithiole-3-thione*** (**Compound 51**). A similar method for compound **3** was employed to get compound **51** exploiting corresponding **h6** as intermediate to afford a metallic shiny orange solid. Yield: 83.5%; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.554-7.513 (m, 3H), 7.505-7.488 (m, 2H), 2.206 (s, 3H, -$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 215.811, 168.153, 141.911, 133.418, 130.883, 129.354, 128.777, 16.781; MS-ESI m/z: 225.00 $[M+H]^+$; Purity determination: RT=6.12 min, purity 99.73%, 90:10 $CH_3OH/H_2O$.

***4-ethyl-5-phenyl-3H-1,2-dithiole-3-thione*** (**Compound 52**). A similar method for compound **3** was employed to get compound **52** exploiting corresponding **h6** as intermediate to afford a maroon solid. Yield: 81.5%; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.623-7.566 (m, 5H), 2.547 (q, 2H, $J$=7.6 Hz, -$CH_2$-), 0.975 (t, 3H, $J$=7.2 Hz, -$CH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 214.861, 169.766, 146.619, 132.553, 130.785, 129.330, 128.360, 22.451, 12.361; MS-ESI m/z: 239.00 $[M+H]^+$; Purity determination: RT=8.98 min, purity 95.00%, 85:15 $CH_3OH/H_2O$.

***4-isopropyl-5-phenyl-3H-1,2-dithiole-3-thione*** (**Compound 53**). A similar method for compound **3** was employed to get compound **53** exploiting corresponding **h6** as intermediate to afford an orange solid. Yield: 86.4%; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.536-7.475 (m, 3H), 7.455-7.425 (m, 2H), 3.387-3.280 (m, 1H, -$CH(CH_3)_2$), 1.232 (s, 3H, -$CH_3$), 1.214 (s, 3H, -$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 216.395, 170.405, 149.387, 132.913, 130.475, 128.907, 30.981, 19.823; MS-ESI m/z: 253.00 $[M+H]^+$; Purity determination: RT=9.80 min, purity 96.56%, 85:15 $CH_3OH/H_2O$.

***5-phenyl-4-propyl-3H-1,2-dithiole-3-thione*** (**Compound 54**). A similar method for compound **3** was employed to get compound **54** exploiting corresponding **h6** as intermediate to afford a maroon solid. Yield: 82.6%; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.559-7.506 (m, 3H), 7.485-7.445 (m, 2H), 2.634-2.594 (m, 2H, -$CH_2$-), 1.554-1.486 (m, 2H, -$CH_2$-), 0.839 (t, 3H, $J$=7.6 Hz, -$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 215.952, 168.978, 146.308, 133.329, 130.706, 129.293, 128.622, 31.703, 21.440, 14.248; MS-ESI m/z: 253.00 $[M+H]^+$; Purity determination: RT=10.39

781 min, purity 99.84%, 80:20 $CH_3OH/H_2O$.

782 ***4-butyl-5-phenyl-3H-1,2-dithiole-3-thione*** (**Compound 55**). A similar method for compound **3** was employed to
783 get compound **55** exploiting corresponding **h6** as intermediate to afford a maroon solid. Yield: 84.8%; $^1$H NMR
784 (400 MHz, $CDCl_3$) $\delta$: 7.570-7.497 (m, 3H), 7.483-7.429 (m, 2H), 2.659-2.620 (m, 2H, -$CH_2$-), 1.500-1.423 (m, 2H,
785 -$CH_2$-), 1.287-1.195 (m, 2H, -$CH_2$-), 0.802 (t, 3H, $J$=7.2 Hz, -$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 215.714,
786 168.736, 146.318, 133.239, 130.614, 129.192, 128.537, 30.001, 29.335, 22.641, 13.687; MS-ESI m/z: 267.00
787 $[M+H]^+$; Purity determination: RT=12.81 min, purity 98.26%, 85:15 $CH_3OH/H_2O$.

788 ***4-pentyl-5-phenyl-3H-1,2-dithiole-3-thione*** (**Compound 56**). A similar method for compound **3** was employed to
789 get compound **56** exploiting corresponding **h6** as intermediate to afford a red solid. Yield: 82.6%; $^1$H NMR (400
790 MHz, $CDCl_3$) $\delta$: 7.575-7.506 (m, 3H), 7.486-7.446 (m, 2H), 2.646-2.605 (m, 2H, -$CH_2$-), 1.519-1.445 (m, 2H,
791 -$CH_2$-), 1.257-1.165 (m, 4H, -$CH_2$-), 0.806 (t, 3H, $J$=6.4 Hz, -$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 215.774,
792 168.841, 146.452, 133.245, 130.678, 129.244, 128.564, 31.713, 29.583, 27.576, 22.188, 14.016; MS-ESI m/z:
793 281.00 $[M+H]^+$; Purity determination: RT=9.92 min, purity 99.42%, 90:10 $CH_3OH/H_2O$.

794 ***4-hexyl-5-phenyl-3H-1,2-dithiole-3-thione*** (**Compound 57**). A similar method for compound **3** was employed to
795 get compound **57** exploiting corresponding **h6** as intermediate to afford a red solid. Yield: 84.1%; $^1$H NMR (400
796 MHz, $CDCl_3$) $\delta$: 7.579-7.505 (m, 3H), 7.485-7.445 (m, 2H), 2.648-2.608 (m, 2H, -$CH_2$-), 1.510-1.423 (m, 2H,
797 -$CH_2$-), 1.250-1.132 (m, 6H, -$CH_2$-), 0.819 (t, 3H, $J$=6.8 Hz, -$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 215.949,
798 168.824, 146.565, 133.387, 130.715, 129.302, 128.652, 31.356, 29.697, 29.293, 27.899, 22.610, 14.149; MS-ESI
799 m/z: 295.05 $[M+H]^+$; Purity determination: RT=11.85 min, purity 97.73%, 80:20 $CH_3OH/H_2O$.

800 ***4,5-diphenyl-3H-1,2-dithiole-3-thione*** (**Compound 58**). A similar method for compound **3** was employed to get
801 compound **58** exploiting corresponding **i5** as intermediate to afford a red solid. Yield: 76.9%; $^1$H NMR (400 MHz,
802 $CDCl_3$) $\delta$: 7.407-7.280 (m, 6H), 7.251-7.229 (m, 2H), 7.150-7.118 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$:

215.063, 170.600, 145.740, 134.152, 133.205, 130.988, 130.812, 129.027, 128.967, 128.609, 128.546; MS-ESI m/z: 287.00 $[M+H]^+$; Purity determination: RT=7.89 min, purity 98.65%, 85:15 $CH_3OH/H_2O$.

***5-(4-iodophenyl)-3H-1,2-dithiole-3-thione*** (**Compound 59**). A similar method for compound **3** was employed to get compound **59** exploiting corresponding **g2** as intermediate to afford a maroon solid. Yield: 33.1%; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.935-7.902 (m, 2H), 7.844 (s, 1H), 7.705-7.672 (m, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 215.497, 172.524, 138.408, 135.845, 130.602, 128.738, 100.143; MS-EI m/z: 336 ($M^+$, 100), 271 (86.01), 101 (28.62), 144 (21.86), 120 (18.89), 75 (18.37), 145 (18.12), 337 (15.14), 69 (14.81), 104 (13.46), 338 (13.21), 89 (12.79), 228 (12.22), 93 (11.47), 272 (10.74), 74 (10.05); Purity determination: RT=8.25 min, purity 98.38%, 80:20 $CH_3OH/H_2O$.

## 4.2. Biology

### 4.2.1 Materials

Dulbecco's modified Eagle's medium (DMEM), dimethyl sulfoxide (DMSO), 6-hydroxydopamine (6-OHDA), glutathione reductase (GR) from yeast, 2',7'-dichlorfluorescein diacetate (DCFH-DA), N-acetyl-Asp-Glu-Val-Asp-*p*-nitroanilide (Ac-DEVD-pNA), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), 2,6-dichlorophenol-indophenol (DCPIP), Hoechst 33342, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Hemin Bovine, streptomycin, and penicillin were bought from Sigma-Aldrich (St. Louis, USA). NADPH was obtained from Roche (Mannheim, Germany). Bovine serum albumin (BSA), sodium orthovanadate ($Na_3VO_4$), and phenylmethylsulfonyl fluoride (PMSF) were purchased from Beyotime (Nantong, China). Fetal bovine serum (FBS) and Ham's F12 nutrient medium were from HyClone. Primary antibodies against Nrf2, Trx1, NQO1, actin, and lamin were purchased from Sangon Biotech (Shanghai, China). Primary antibody for TrxR1 was from Santa Cruz Biotechnology (Santa Cruz, CA). Primary antibody against HO-1 was provided by Proteintech (Chicago, IL). Horseradish peroxidase-conjugated secondary antibodies were provided by Santa Cruz

825 Biotechnology. The recombinant *Escherichia coli* Trx was expressed and purified as described in our previous
826 publication.[68] The recombinant protein of rat TrxR1 as a generous gift was provided by Prof. Arne Holmgren
827 (Karolinska Institute). GeneTran III transfection reagent was supplied by Biomiga (CA, USA).The sh*RNA* plasmids
828 interfering the functional sequences of the rat *Nrf2* gene (sh*Nrf2*) and non-targeting sh*RNA* (sh*NT*) as the control
829 were supplied by Gene Pharma Co, Ltd (Shanghai, China). All the other chemicals and reagents were of analytical
830 grade.

831 ## 4.2.2 Cell Cultures

832 PC12 cell line, the rat pheochromocytoma cells possessing neuronal characteristics to a great extent, has been well
833 established and widely applied for studies related to PD. The cell line was purchased from Shanghai Institute of
834 Biochemistry and Cell Biology, Chinese Academy of Sciences. N2a cell line, mouse neuroblastoma N2a cells, is a
835 cell line usually exploited in researches with respect to chemical neurobiology and bio-neurochemistry. N2a cell
836 line was a gift from Prof. Min Chang (Lanzhou University). The N2a cells were cultured in a mixture of DMEM
837 and F12 (1:1) media using standard cell culture conditions as described by our previous publications[28, 31-37] and the
838 PC12 cells we employed in this studies are in low level differentiation.

839 ## 4.2.3 MTT Assay and Lactate Dehydrogenase (LDH) Release Assay

840 PC12 cells ($1 \times 10^4$ cells/well) were cultured for 24 h, followed by addition of indicated concentrations of
841 compounds **10** and **11** for incubation another 24 h. Afterwards the cell viability was measured by MTT assay
842 according to the procedures described in our previous publications.[28, 31-37] The method of evaluation of the survival
843 of N2a cells after compounds treatment only was the same as described above. For the purpose of searching for an
844 optimal concentration of $H_2O_2$ employing for establishment of a PD model based on N2a cells, four concentrations
845 of $H_2O_2$ (100 μM, 200 μM, 400 μM, 800 μM) was tested, and 200 μM was found to be suitable for the follow-up
846 experiment. In order to evaluate the protective effect of compounds **10** and **11**, PC12 cells ($1 \times 10^4$ cells/well) were

grown in 96-well plates for 24 h followed by treatment with various concentrations of **10** and **11** for further 24 h, and then the cells were exposed to the new medium filled of $H_2O_2$ (500 μM) or 6-OHDA (200 μM) in place of the original culture medium for another 12 h. At the time, the MTT assay was conducted to determine the cell viability. The procedure of evaluation of the viability of N2a cells after compounds protection from $H_2O_2$ (200 μM)-induced damage was the same as described above.

For the LDH release assay, PC12 cells ($2 \times 10^5$ cells/well) were bred in 12-well plates for 24 h followed by treatment with indicated concentrations of **10** and **11** for another 24 h, and then the cells were cultured in the new medium filled of $H_2O_2$ (500 μM) or 6-OHDA (200 μM) in place of the original culture medium for further 12 h. Afterwards the activity of LDH, leaking from the damaged cells, was measured in accordance with the method described previously.[28, 31-37] The procedure of determination of the released LDH in N2a cell culture medium was the same as described above.

### 4.2.4 Measurement of the Level of Intracellular ROS

The cells treated with compounds **10** and **11** were exposed to $H_2O_2$ (500 μM) or 6-OHDA (200 μM) for 12 h. Subsequently, DCFH-DA (10 μM) was added after the original medium was discarded. Then it was maintained for 30 min in dark. Finally, the cells were visualized and imaged with the aid of Floid cell imaging station (Life Technology). The detailed experimental procedures can be found in our other papers[32-33].

### 4.2.5 Determination of Caspase-3 Activity, Hoechst 33342 Staining, and Annexin V/PI double staining assay

The cells ($1 \times 10^6$ cells/well) were seeded into 60-mm dishes for 24 h, and then were treated with different concentrations of compounds **10** and **11** for another 24 h. Then the cells were exposed to $H_2O_2$ (500 μM) or 6-OHDA (200 μM) for further culture 12 h, after which time the cells were harvested, washed with ice-cold PBS, and lysed with RIPA buffer. The total protein contents were quantified using Bradford method. Ac-DEVD-pNA

was added to the protein extracts, which was further incubated for 4 h. Then the absorbance at 405 nm was measured as a reflection of caspase activity of each group. The detailed procedures were based on our previous publications.[68-69]

For the Hoechst 33342 staining assay, 5 μg/ml Hoechst 33342 was added to the treated PC12 cells for staining 30 min in dark. Then positive fluorescence microscope (Leica DM4000B) was employed to visualize and photograph the cells.

For Annexin V/PI double staining assay, PC12 cells were incubated with indicated concentrations of **10** or **11** for 24 h followed by $H_2O_2$ (500 μM) or 6-OHDA (200 μM) treatment for 12 h, and then the population of live cells, apoptotic cells and dead cells was identified by staining the cells with fluorescein 5-isothiocyanate (FITC)-conjugated Annexin V and PI in the light of the instructions from the supplier (Zoman Biotech, Beijing, China) on a FACS-Canto flow cytometer (BD Biosciences, San Jose, CA, USA). The data were analyzed with the aid of the CellQuest software (BD Biosciences).

### 4.2.6 Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

After the cells ($1 \times 10^6$ cells/well) were seeded in 60 nm dishes for 24 h, they were treated with 20 μM compounds for the indicated time. Then the cells were collected and total RNA were prepared using the RNAiso plus RNA extraction kit (TaKaRa, Dalian, China) in the light of operating instructions. cDNA were obtained from the extracted RNA with the help of reverse transcription kit Primescript RT (TaKaRa, Dalian, China). Afterwards the qRT-PCR analysis was performed on the Mx3005P RT-PCR system (Agilent Technologies) with the help of Power SYBR Green PCR Master Mix (TaKaRa, Dalian, China). The sequences of each primer is as follows: *GCLC* forward 5'-caaggacaagaacacaccatct-3' and reverse 5'-cagcactcaaagccataacaat-3'; *GCLM* forward 5'-ggcacaggtaaaacccaatagt-3' and reverse 5'-ttcaatgtcagggatgctttct-3'; *HO-1* forward 5'-gccctggaagaggagatagag-3' and reverse 5'-tagtgctgtgtggctggtgt-3'; *TrxR1* forward 5'-actgctcaatccacaaacagc-3' and reverse

5'-ccacggtctctaagccaatagt-3'; *NQO1* forward 5'-tcaccactctactttgctccaa-3' and reverse 5'-ttttctgctcctcttgaacctc-3'; *Trx1* forward 5'-ccttctttcattccctctgtgac-3' and reverse 5'-cccaacctttgaccctttttat-3'; *GAPDH* forward 5'-cagtgccagcctcgtctcat-3' and reverse 5'-aggggccatccacagtcttc-3'; *Nrf2* forward 5'-gccttcctctgctgccattagtc-3' and reverse 5'-tgccttcagtgtgcttctggttg-3'. The expression level of each target gene was normalized by employing the *GAPDH* expression as an internal standard.

### 4.2.7 Western Blot Analysis

Different proteins fractions were prepared by the methods described by our group.[32-33] The expression level of target proteins were detected by Western blots analysis.

### 4.2.8 Measurement of Intracellular Total Glutathione and Determination of TrxR, Trx, and NQO1 Activities

After treatment of PC12 cells with compounds **10** and **11** for 24 h, the cells were harvested, and the cell fractions were in preparation using the KPE buffer (0.1% Triton X-100 and 0.6% sulfosalicyclic acid in 0.1 M potassium phosphate buffer with 5 mM EDTA disodium salt, pH 7.5). The level of intracellular total GSH was assessed following our published procedure.[28, 31-37]

For the determination of activities of TrxR, Trx and NQO1, the PC12 cells were collected and washed with ice-cold PBS after treatment of **10** and **11** for 24 h, and then the cells were lysed with RIPA buffer on the ice followed by the preparation of various cell extracts. The protein contents were determined by the Bradford method. Evaluation of activities of TrxR, Trx and NQO1 was in accordance with the methods described in our published procedures[28, 31-37].

### 4.2.9 HO-1 Activity Assays

The assay was essentially described in our published method.[70] Briefly, after cells were treated with compounds **10** and **11** for 24 h, they were collected and lysed in buffer A (0.25 M sucrose in 20 mM Tris-HCl, pH 7.4, 1 mM

913 $Na_3VO_4$, and 1 mM PMSF) and the protein contents were determined by the Bradford method. Crude extracts
914 containing biliverdin reductase was prepared from bovine liver. Afterwards, a mixture of 50 μg total protein from
915 cells lysate, 1 mM NADPH, 25 μM hemin, and crude biliverdin reductase extracts in a final volume of 100 μL was
916 vortexed several times and incubated for 20 min at 37 $^oC$ in dark. Then 1 volume of chloroform was added to
917 terminate the enzymatic reaction, after a simple processing absorbance at 464 nm of chloroform phase was
918 measured. The negative control samples were just the same as other samples instead of replacing the NADPH with
919 an equal volume of buffer A. The more detailed experimental protocol can be found in our published procedures.[70]

920 ### 4.2.10 Generation of Nrf2 Knockdown Cells

921 The procedure was essentially described in our published work.[31, 33, 35] The short hairpin RNA sh*Nrf2-842*
922 selectively targeting mice *Nrf2* gene was used for *Nrf2*-knockdown assays, while a scrambled non-targeting
923 sequence sh*NT* was applied to generate the non-target PC12 cells as a control with the aid of GeneTran III
924 transfection reagent according to the instructions of the manufacturer. The stably transfected PC12-sh*Nrf2* cells and
925 PC12-sh*NT* cells were generated by using G418 (0.5 mg/mL) for selection. *Nrf2*-Knockdown efficiency was
926 examined by Western blots analysis.

927 ## 4.3 Statistical Analysis

928 Data are presented as mean ± standard deviation (SD). Statistical differences between two groups are evaluated
929 using Student's *t*-test, and one-way analysis of variance (ANOVA) was used to analyze differences among multiple
930 groups. $P < 0.05$ was used as the criterion for statistical significance.

931

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## Supporting Information

Cytotoxicity of compounds **10** and **11** to N2a cells, and protection of N2a cells against the $H_2O_2$-induced injury by compounds **10** and **11**.

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