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ANTI-OXIDATIVE BURST, CYTOTOXICITY, AND ADME STUDIES OF THIOUREA COMPOUNDS

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ABSTRACT

This oxidative stress is linked to number of immune related disorders like hypersensitivity, autoimmune disease and inflammation. In this context, many new compounds are being synthesized and screened for the purpose of immunomodulation. In current study, we are aiming to discover the potential of thiourea derivatives as potential immunomodulators via suppressing oxidative burst mechanism in macrophages and neutrophils. The immunomodulation studies were conducted using cell based spectrophotometric techniques. Fluorescent microscopy and Predictions using web-based tools. Results suggested that among three selected thiourea derivatives comp 3 significantly suppressed the superoxide anion with 86.94 ±1.2 percent inhibition. Additionally, comp 2 and 3 were significantly involved in inhibiting the myeloperoxidase dependent pathway that produce hypochlorite anion with IC_{50} value of 45.3±0.4 and 10.4±0.2 µg/mL, respectively. Similarly, intracellular oxidative stress was suppressed by comp 2 and 3 detected by fluoresce microscopy. In addition, the comp 1 and 3 showed moderate inhibitory activity with 36.9 and 33.8% respectively. No any compound has shown cellular toxicity at the 25 µg/mL tested fibroblast cell line. Physicochemical and Pharmacokinetic Predictions of comp 2 and 3 showed high blood brain barrier (BBB) permeability along with Gastrointestinal (GI) absorption. Prediction studies revealed the lethal dose (LD50) of 1700 mg/kg and 1400 mg/kg respectively. Additionally comp 3 showed activity against pancreatic carcinoma MIA PaCa 2, Colon adeno carcinoma SW620 and Non-small cell lung carcinoma NIH838. In conclusion, new compounds have potential of being immunomodulatory agent particularly comp 3 demonstrated intriguing biological potential that could be explored further for its mechanistic studies.

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Introduction

Phagocytic NADPH oxidase, enzyme located on the membrane of immune cells like macrophages or neutrophils and become activated during the process of inflammation. Activation of this enzyme yields in the release of reactive oxygen free radicals (ROS) required to kill the invading pathogen. In addition to this another Enzyme inducible nitric oxide synthase (iNOS) is expressed in activated phagocytic cells such as macrophages and neutrophils, and it catalyzes the production of nitric oxide (NO), which is an important mediator of the immune response against pathogens. However, the excessive production of NO and other reactive oxygen species (ROS) and reactive nitrogen species (RNS) during phagocytosis can lead to destruction of macromolecules like lipids, DNA, and proteins. This is regarded as oxidative stress, and it can results to cellular dysfunction and tissue damage. ROS and RNS also behave as intracellular signaling molecules in biological processes, like cell development, differentiation, and apoptosis. However, when their levels are increased, they can initiate various pathological conditions, including inflammation, cancer, and neurodegenerative diseases [1-5].

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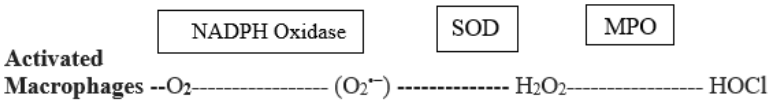


Phagocytic NADPH oxidase is activated during phagocytosis, and catalyze the production of superoxide ($\bullet O^{-2}$). Another enzyme superoxide dismutase (SOD), converts the superoxide into hydrogen peroxide H_2O_2 [6, 7]. In the presence of myeloperoxidase further chloride reacts with H_2O_2 more making more toxic by product Hypochlorous acid, HOCl. Therefore, in abnormal condition, phagocytes can become over activated and stimulate the phagocytic NADPH oxidase and inducible nitric oxide synthase iNOs, leading to excessive production of ROS and reactive nitrogen species (RNS) respectively, contribute to inflammation and carcinogenesis. Furthermore, peroxynitrite (ONOO−), which is formed by the reaction between NO and superoxide (O2•−), is a highly toxic radical that can cause mutations by inducing breaks in double-stranded DNA or modifying DNA bases. Together, the excessive production of ROS and RNS during inflammation and infection have role in the growth of various pathological conditions, involving cancer, neurodegenerative diseases, and cardiovascular disorders. Therefore, it remains imperative to regulate the production as well as elimination of ROS and RNS to maintain cellular and tissue homeostasis and prevent oxidative stress [8-10].

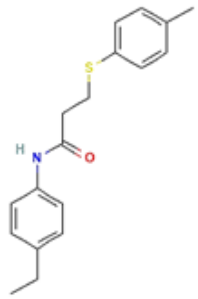
Oxidative stress is one of leading cause of inflammation and considered as potent inflammatory mediator. Antioxidants are molecules that can neutralize and scavenge free radicals, protecting cells and tissues from their damaging effects. However, when free radical generation surpasses the body's antioxidant capacity, oxidative stress can occur [11]. Inflammation is a broad category involving a lot of immune cells, enzymes and chemical mediators resulting in the beginning of a mentioned disease. In the mentioned several mediators, the free radicals can also affect the tumor microenvironment and promote tumor growth, invasion, and metastasis by modulating the immune response, angiogenesis, and extracellular matrix remodeling. Therefore, having in-depth comprehension of the role free radicals in chronic inflammation and cancer is crucial for the development of effective strategies for precaution and management of of these diseases. To identify anti-oxidative stress activity of synthetic compounds we have selected thiourea derivatives, previously reported for their wide range of biological activity. It is reported that these derivatives possess Phytotoxic, Cytotoxic, Acetylcholinesterase and Butryl cholinesterase, glucuronidase inhibitory, *α*-chymotrypsin inhibitory, urease inhibitory activity and anti-microbial activity [12-18]. We have investigated these compounds for its effect on intracellular and extracellular oxidative stress in activate mouse macrophages and whole blood phagocytes and identified it's *in vitro* cellular toxicity. Furthermore, *in silico* physicochemical and ADMET studies have done to predict the biological potential of the compounds.

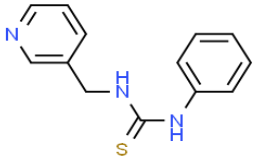
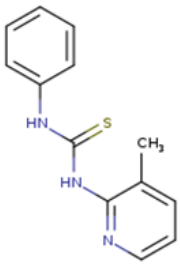
Materials and Methods

Collection of Compounds and Samples Preparation (1mg/mL Stock Solution)

Thiourea compounds were selected based on available literature for their potential biological activity and purchased from Bench chem and Sigma Aldrich. **(Table 1)** we used 1 mg/mL of stock solution by the addition of one milligram of compound in 50 µl DMSO, solubilize, if needed sonication is done till the compound solubilized completely. 950 µl phosphate buffer saline was added to get the final conc. of 1mg/mL, and stored in freezer 4°C until use.

Table 1. Selected thiourea compounds

S.no	Structure	IUPAC Names	Compound code	purchased
1		**N-(3-chlorophenyl)-N'-(3,4-dimethylphenyl)thiourea**	**Comp-1**	Benchchem C18H21NOS M.W 299.4g/mol. 95%Pure

2		**1-Phenyl-3-(3-pyridinylmethyl) thiourea**	**Comp-2**	Sigma Aldrich C13H13N3S M.W 243.3 g/mol. 95% pure
3		**N-(3-Methyl-2-pyridinyl)-N'-Phenylthiourea**	**Comp-3**	Benchem C13H13N3S M.W 243.33 g/mol. 95% pure

Determination for Oxidative Burst by Chemilluminescence Assay

Chemilluminescence assay was performed as published before [19]. Different concentration of test compounds 1 µg/mL, 10 µg/mL and 100 µg/mL were set in 96 well white flat bottom plate, incubated at 37 ºC for fifteen mins in the thermostat chamber of luminometer with 1:20 dilution of whole blood in HBSS ++. Control wells established HBSS $^{++}$ and cells without the test compounds. Following incubation luminol (7×10^{-5} M), and serum opsonized zymosan (SOZ) were added into each well except blank wells that contain HBSS $^{++}$ only to detect free radicals. Intracellular reactive oxygen detecting probe. Luminometer was used to detect oxidative burst ROS production. The level of the ROS was measured as relatively light units for fifty min using repeat scan mode.

NBT Assay for Superoxide Production

Macrophages J774 (1×10^7) cells/ml were suspended in medium, 100µl of macrophages were placed in 96-well plates in the RPMI media with FBS. Nitro blue tetrazolium assay was done as prescribed earlier [20]. Cells were and test compounds were incubated for 30 min at 37ºC, after incubation 100ul of 1mg/ml NBT solution was added and 50ul of phorbol-12- myristate-13-acetate (PMA) Diphenyleniodonium was used as a negative control after addition at a concentration of 10 µg/ml to activate the cells and after an additional 90 min incubation. Blue formazan was dissolved in DMSO and the absorbance was measured at 570 nm. Controls were set as cells in medium rather than without test compound. Percent inhibition of NBT reduction was calculated.

Determination of Intracellular Oxidative Stress

Intracellular ROS level was determined using the fluorogenic probe 2′,7′-Dichlorofluorescein (DCFH) a dye that is cell-permeable and oxidized by free radicals to highly fluorescent DCF particularly by H_2O_2. Briefly mouse macrophages J774.2 were adjusted to 5×10^4 /mL in 24 well transparent bottom plate and were activated using PMA (200 ng/mL) with or without compounds at 25 µg/mL for 30 min. Treated cells were further incubated with 20 µM DCFH for 30 mins at 37 °C. A fluorescent microscope detected fluorescence and we used Adobe Photoshop CS2 to process the images [21].

Measurement of NO in Cell Culture Supernatant

J774 macrophage cells were collected and adjusted to a concentration of 10^6 cells/ml, and 200 µl of cells were added to a 96-well microtiter plate. Lipopolysaccharide from E. coli was added at a final concentration of 30 µg/ml to activate the cells, inducing NO production. Next, test compounds were added to the Cells and plates at a concentration of 25 µg/ml were incubated at 37° C., 5% CO 2 for 24 hours. After incubation, cell culture supernatants were collected and immediately processed for nitrite accumulation studies using the Griess method. The Griess method used 50µl of 1% sulfanilamide in 2.5% phosphoric acid added to 50 µl of the cell culture supernatant, followed by 50µl of 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid. Absorbance was read at 550 nm after incubation for 10 minutes at 23°C. The micromolar concentration of sodium nitrite was calculated from a standard curve generated using sodium nitrite as the reference compound. The findings was described as means ± standard deviation (SD) of triplicate readings. This method provides a simple and reliable way to evaluate the ability of test compounds to modulate NO production in activated J774 macrophage cells, which can be useful in screening for potential anti-inflammatory or immunomodulatory agents [22].

In Vitro Cytotoxicity Assay

The MTT assay is a commonly used method to measure cell viability and proliferation. In this assay, 3T3 fibroblast cells were plated in a 96-well plate and treated with different concentrations of test compounds for forty-eight hours. After that, the cells

were washed and MTT was added to the wells. MTT is reduced by mitochondrial dehydrogenases in viable cells to form a colored formazan product. The dissolution of formazan was done in DMSO, then the absorbance was measured at 570 nm using a microplate reader. The IC50 values, which represent the concentration of the compounds required to hinder cell viability by fifty percent, were calculated. Cyclohexamide, a known inhibitor of protein synthesis, was used as a positive control to compare the effect of the test compounds on cell viability. [23] The percentage of cell viability was calculated using the formula:

$$\%\text{Cell Viability} = [\text{Total Viable cells (Unstained)} / \text{Total cells (Viable + Dead)}] \times 100. \quad (1)$$

The viable cell count was calculated as Average viable cell count per square × Dilution Factor × 10.

Physicochemical and Pharmacokinetic Predictions of Thiourea Compounds

SwissADME (http://www.swissadme.ch/index.php) and PreADMET (https://preadmet.bmdrc.kr/) predicted the physicochemical and pharmacokinetic profiles of thiourea compound. The cell-line cytotoxicity was predicted by CLC-Pred (http://way2drug.com/Cell-line/) The toxicity of the compound was identified using webtool ProTox II (http://tox.charite.de/).

Statistics

To determine the statistical significance of the results, a paired t-test was used to compare the mean values of the experimental test conditions with their respective controls. A p-value of 0.05 was regarded in order to signify a considerable difference amongst the two groups, denoted as * $p<0.05$. A p-value of less than 0.005 was regarded to signify a highly considerable difference, denoted as ** $p<0.005$.

Results and Discussion

Effect of Compounds on Superoxide Production $O_2^{\bullet-}$

Phagocytic NADPH Oxidase is activated by Phorbol Myristate Acetate PMA in mouse macrophages and initiate the protein kinase C (PKC) pathway. PKC pathway lead to the generation of superoxide anion [24]. PMA activated cells were treated exposed to 25µg/mL of compounds and effect on superoxide was detected by NBT reduction assay. Comp 3 was found to suppress the superoxide production significantly $p<0.005$ with percent inhibition of 86.94 ±1.2 %. In contrast, comp 1 and 2 moderately enhanced the superoxide generation **(Table 2)**.

Effect of Compounds on HOCl Production

During myeloperoxidase-dependent intracellular killing process of phagocytosis HOCl, hypochlorous acid is produced that can be detected with Luminol enhanced chemilluminescence technique [25]. The study demonstrated that compounds 2 and 3 have significant inhibitory activity against ROS production in whole blood cells activated with zymosan. The IC_{50} value for comp 2 was found to be less than 5.3 ± 0.4 µg/mL, indicating a strong suppressive effect on ROS. Comp 3 also showed significant inhibitory activity with an IC50 value of 10.4 ± 0.2 µg/mL, which is lower than the IC50 value of ibuprofen (43.4 ± 0.9 µg/mL). The inhibitory effect of these compounds on ROS production suggests their potential as anti-inflammatory therapeutic agents **(Table 2)**.

Table 2. Effect of Compounds on ROS, NO, and O^{-2} Production in activated macrophages and neutrophils and cytotoxicity analysis

Compounds	ROS (HOCl) IC_{50} ± SD µg/mL	Superoxide O^{-2} %inhibition ±SD	Nitric oxide (NO) %inhibition ±SD	Cytotoxicity IC_{50} ±SD µg/mL
Comp-1	>100	-29.2± 0.51	36.9±0.7	>25
Comp-2	5.3 ± 0.4**	-49.63± 0.90	-17.9±0.66	>25
Comp-3	10.4 ± 0.2 *	86.94 ±1.2**	33.8±0.80	>25
Standard drugs	Ibuprofen 43.4±0.9ug/mL	Diphenyl iodonium 92±0.94%	N^G monomethyl L arginine 64±0.05%	Cyclohexamide 0.13±0.02µg/mL

The findings are shown as the mean ± standard deviation (SD) of triplicate measurements. A paired t-test was employed in determining the statistical significance of the difference between the experimental and control groups**.** A p-value of less than 0.005 was considered to indicate a highly significant difference, denoted as ** $p<0.005$, while a p-value of less than 0.05 was considered to indicate a significant difference, denoted as * $p<0.05$.

Effect of Compound on Intracellular Oxidative Stress (H_2O_2)

Intracellular oxidative stress was also investigated using fluorescence microscopy technique and effect of comp 2 and 3, was studied on intracellular ROS using DCFH dye. Reaction of this dye with ROS, primarily hydrogen peroxide (H_2O_2), results in the production of fluorescent molecule DCF, so that DCF fluorescence can be used as a measure for intracellular ROS levels [26]. Intensity of green fluorescence is directly proportional to the production of ROS. We compare the results with positive

control in which J774.2 Mouse macrophages cells activated with PMA and negative control in which Diphenyliodonium was used to suppress ROS. Compound 2 and 3 significantly decreased the intensity of fluorescence in the cells, at 25 µg/mL we can conclude that compound 2 and 3 selectively suppress the H_2O_2 therefore they also suppress the HOCl production detected by chemilluminescence technique **(Figure 1)**.

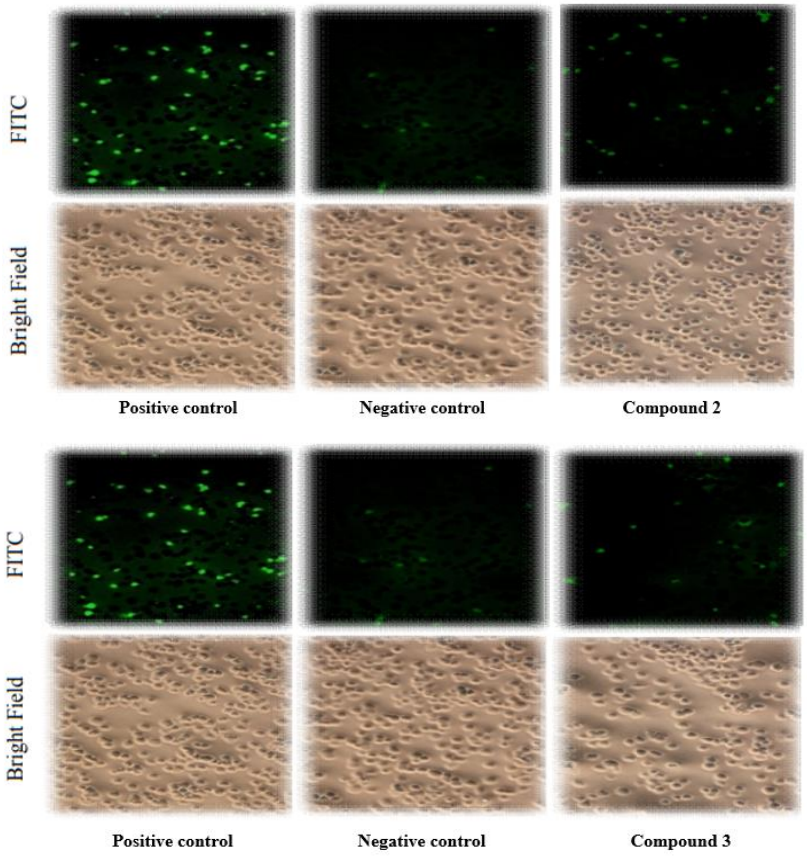


Figure 1. Effect of natural compound on intracellular oxidative stress measured with fluorescence microscopy as described in material and methods. Results are presented as images processed with adobe Photoshop.

Effect of Compounds on Nitric Oxide (NO)

The statement is accurate. Lipopolysaccharide (LPS) activate toll-like receptor 4 (TLR4) on macrophages, leading to the production of inducible nitric oxide synthase (iNOS) and subsequent production of nitric oxide (NO) [27]. In the study mentioned, the researchers investigated the effect of the test compounds on NO production in macrophages activated with LPS. The results showed that comp1 and 3 moderately suppressed NO production, with a percentage of inhibition of 36.9% and 33.8% at a concentration of 25 µg/mL, respectively. These findings suggest that the compounds may have a potential therapeutic effect on conditions that involve excessive NO production, such as cardiovascular diseases. However, further studies are needed to confirm their efficacy and safety in vivo. In contrary, comp 2 stimulated the production of nitric oxide with % stimulation of 17.9% **(Table 2)**.

Effect of Compound on Cytotoxicity

Viability of 3T3 Fibroblast cells were studied in presence of compounds at several concentrations 50, 5 and 0.5 µg/mL. Results were compared with Cyclohexamide, which is used as standard cytotoxic drug [28]. The data is presented in **Table 2**. All of the tested compound were seen to be nontoxic for fibroblast cells with IC_{50} value of >25µg/mL therefore are reliable to be further researched as compared to the Cyclohexamide with IC_{50} value of 0.13±0.07µg/mL.

Physicochemical and Pharmacokinetic Predictions of Thiourea Compounds

Compound 2 and 3 were studied for it in-silco ADMET analysis, physicochemical and toxicity effect using web based tools [29]. Prediction studies have shown that compound exhibit the significant potential of therapeutic agent with GI absorption and blood brain barrier permeability. Both compounds showed bioavailability of 55 % and does not prove to be inhibitor of major drug metabolizing enzymes except CYP2C19 and CYP3A4. Skin permeation was found to be in the range of −0.769 to −5.818 cm/s, which falls under the acceptable range. Predicted value of toxicity for both compounds showed that compounds are nontoxic to liver and possess high lethal dose (LD50) of 1700 mg/kg and 1400 mg/kg respectively, refer the **Tables 3 and 4**.

Table 3. Predicted ADMET properties of 1-Phenyl-3-(3-pyridinylmethyl) thiourea (comp2)

Physicochemical Properties	Formula C13H13N3S, Molecular weight 243.33 g/mol Num. heavy atoms17, Num. arom.12, heavy atoms Fraction Csp3 0.08, Num. rotatable bonds 5, Num. H-bond acceptors 1, Num. H-bond donors 3, Molar Refractivity73.43, TPSA 69.04 Å². Water solubility Moderately soluble.
Pharmacokinetic Properties	GI absorption = high; BBB permeant = yes; P-gp substrate = no; CYP1A2 inhibitor = yes; CYP2C19 inhibitor = yes; CYP2C9 inhibitor = no; CYP2D6 inhibitor = no; CYP3A4 inhibitor = yes; Log K_p (skin permeation) = −6.4 cm/s
Drug Likeness	Lipinski = yes, 0 violation; Ghose = yes; Veber = yes; Egan = yes; Muegge = yes; bioavailability score = 0.55
Medicinal Chemistry	PAINS = 0 alert; Brenk = 1 alert: thiocarbonyl_group ; leadlikeness = No; 1 violation: MW<250; synthetic accessibility = 2.07
Toxicity	AMES test = mutagenic; carcinogenicity in mouse= none; carcinogenicity in rats =yes; predicted LD_{50} = 1700 mg/kg; hepatotoxicity = (none) Estrogen Receptor Alpha (ER) = inactive
Cancer Cell line Prediction	None
Nontumor Cell Line Prediction	No toxicity

Table 4. Predicted ADMET properties of N-(3-Methyl-2-pyridinyl)-N'-Phenylthiourea (**comp 3**)

Physicochemical Properties	Formula C13H13N3S, Molecular weight 243.33 g/mol Num. heavy atoms17, Num. arom.12, heavy atoms Fraction Csp3 0.08, Num. rotatable bonds 4, Num. H-bond acceptors 1, Num. H-bond donors 2, Molar Refractivity75.13, TPSA 69.04 Å². Water solubility Moderately soluble.
Pharmacokinetic Properties	GI absorption = high; BBB permeant = yes; P-gp substrate = no; CYP1A2 inhibitor = yes; CYP2C19 inhibitor = yes; CYP2C9 inhibitor = no; CYP2D6 inhibitor = no; CYP3A4 inhibitor = yes; Log K_p (skin permeation) = −5.86cm/s
Drug Likeness	Lipinski = yes, 0 violation; Ghose = yes; Veber = yes; Egan = yes; Muegge = yes; bioavailability score = 0.55
Medicinal Chemistry	PAINS = 0 alert; Brenk = 1 alert: thiocarbonyl_group ; leadlikeness = No; 1 violation: MW<250; synthetic accessibility = 2.37
Toxicity	AMES test = nonmutagenic; carcinogenicity in mouse= yes; carcinogenicity in rats =no predicted LD_{50} = 1400 mg/kg; hepatotoxicity = none
Cancer Cell line Prediction	Pancreatic carcinoma MIA PaCa 2; Colon adeno carcinoma SW620; Non small cell lung carcinoma NIH838
Nontumor Cell Line Prediction	None

In current study, we have studied the effect of synthetic thiourea compounds on cascade of free radical generation during the phagocytosis process. In this context, we activated the macrophage and neutrophils with immunogens i.e PMA and LPS. We have assessed the effect of compound at an individual step to understand what enzyme might be involved in suppression of specific free radical. For instance, PMA activates the PKC pathway and activate the NADPH Oxidase enzyme in macrophages to release superoxide [30]. The comp 1 and 2 has stimulatory effect on O2•− production; However, comp 3 significantly suppress O2•−. Similarly, mouse peritoneal macrophages were activated with PMA to generate the intracellular oxygen species probe DCFHDA was used to measure the suppression of intracellular H2O2 production by fluorescent microscopy. Results were intriguing that Comp 2 and 3 suppress the H2O2 Production in terms of declines in total fluorescence. Additionally, Zymosan activated the blood phagocytes (neutrophils) and release free radicals, [31] where probe luminol measured the intensity of light, which is directly proportional to production of HOCl radical. The comp 2 and 3 significantly suppressed the HOCl suggesting their effect on myeloperoxidase enzyme that convert the H2O2 into hypochlorite. Our results clearly suggested the mechanism of action of selected compounds on the oxidative burst cascade, in which comp 2 is obvious in suppression of SOD enzyme. However, comp 3 might be a potential suppressor of PKC pathway, because it has inhibited the free radical generation at every step of the cycle i.e O2•−, H2O2, and HOCl [32]. While, comp 1 has no significant inhibitory effect on ROS at any level of respiratory burst cascade.

Nitric oxide (NO) is a highly reactive molecule that plays an important role in many physiological processes, including neurotransmission and vasodilation. However, excessive production of NO can lead to the formation of peroxynitrite, a highly toxic free radical that can cause damage to cells and tissues. Studies have shown that the suppression of NO production can be beneficial in the treatment of cardiovascular diseases such as hypertension and atherosclerosis. However, it is important to note that the mechanisms of ROS and NO production are entirely different, and compounds that suppress ROS production may not necessarily affect NO production. In the study mentioned, the compounds were found to have little to moderate effect

on NO production via the iNOS pathway, indicating that their mechanism of action is specific to ROS production. This specificity is important for the development of targeted therapies that can effectively treat specific conditions while minimizing unwanted side effects [33].

Understanding of in vitro cytotoxicity of compounds is crucial to assess their effectiveness and potential toxicity. In this experiment, the cytotoxicity of the compounds was tested on a fibroblast cell line. None of the compounds showed any toxicity to the fibroblast cells at concentrations up to 25 µg/mL. The results were compared to the control compound, Cyclohexamide. This finding suggests that the tested compounds may have a good safety profile and could be considered for further evaluation in preclinical and clinical studies. It is important to note, however, that the in vitro cytotoxicity assay may not always accurately predict the in vivo toxicity of compounds. Therefore, additional studies are necessary to fully assess the safety and efficacy of the compounds [34]. Our results also supported by prediction studies that has shown that compounds possess significant potential of therapeutic agent. According to in silico studies compounds can be absorb through gastrointestinal tract and can cross blood brain barrier. Both compounds showed bioavailability of 55 % and does not prove to be inhibitor of major drug metabolizing enzymes. Predicted value of toxicity for both compounds showed that compounds are nontoxic to liver and have high lethal dose (LD50), so could be a better candidate if utilized for in in vivo studies or clinical trial [35].

Free radicals are highly reactive molecules that can cause damage to cellular structures and molecules, leading to cell death and tissue damage. During the inflammatory process, free radicals are generated as part of the immune response, but excessive production can cause harm to the body and contribute to the development of inflammatory disorders the discovery of target-specific oxidative stress inhibitors, as mentioned in the previous statement, is a significant step in developing treatments for inflammatory disorders. By inhibiting the production of free radicals or reducing their harmful effects, these inhibitors have the potential to reduce inflammation and prevent the progression of inflammatory diseases However, as mentioned, further extensive studies are required to determine the safety and efficacy of these inhibitors in treating specific inflammatory diseases. It is important to understand their mechanism of action, potential side effects, and optimal dosing before they can be considered for clinical use [36]. Overall, the discovery of oxidative stress inhibitors is a promising development in the field of anti-inflammatory therapeutics. With further research and development, these inhibitors could potentially provide safe and effective treatments for inflammatory disorders.

Conclusion

The findings regarding the synthetic thiourea compounds and their potential as anti-inflammatory therapeutic agents are promising. The observation that the compounds showed significant suppression of the respiratory burst pathway of phagocytes and no cellular toxicity suggests that they may have the ability to reduce inflammation in the body by inhibiting enzymes involved in free radical generation. Furthermore, the fact that the compounds present less to medium influence on nitric oxide production via iNOS pathway indicates that they may be specific inhibitors of ROS generation and not have a nonspecific effect on other pathways involved in inflammation. This specificity is desirable as it reduces the risk of unwanted side effects. Overall, the findings suggest that the synthetic thiourea compounds have the potential to be developed as anti-inflammatory therapeutic agents. Further studies are needed to determine their efficacy in treating specific inflammatory diseases and to understand their mechanism of action in more detail.

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Conflict of interest: None

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Ethics statement: None

References

1. Xiong L, McCoy M, Komuro H, West XZ, Yakubenko V, Gao D, et al. Inflammation-dependent oxidative stress metabolites as a hallmark of amyotrophic lateral sclerosis. Free Radic Biol Med. 2022;178:125-33.
2. Andelova K, Bacova BS, Sykora M, Hlivak P, Barancik M, Tribulova N. Mechanisms underlying antiarrhythmic properties of cardioprotective agents impacting inflammation and oxidative stress. Int J Mol Sci. 2022;23(3):1416.
3. Hajam YA, Rani R, Ganie SY, Sheikh TA, Javaid D, Qadri SS, et al. Oxidative stress in human pathology and aging: Molecular mechanisms and perspectives. Cells. 2022;11(3):552.
4. Reth M. Hydrogen peroxide as second messenger in lymphocyte activation. Nat Immunol. 2002;3(12):1129-34.
5. Jiang F, Zhang Y, Dusting GJ. NADPH oxidase-mediated redox signaling: roles in cellular stress response, stress tolerance, and tissue repair. Pharmacol Rev. 2011;63(1):218-42.

6. Kim MY, Dong M, Dedon PC, Wogan GN. Effects of peroxynitrite dose and dose rate on DNA damage and mutation in the supF shuttle vector. Chem Res Toxicol. 2005;18(1):76-86.
7. Vejražka M, Míček R, Štípek S. Apocynin inhibits NADPH oxidase in phagocytes but stimulates ROS production in non-phagocytic cells. Biochim Biophys Acta (BBA)-Gen Subj. 2005;1722(2):143-7.
8. Szabó C, Ohshima H. DNA damage induced by peroxynitrite: subsequent biological effects. Nitric Oxide. 1997;1(5):373-85.
9. Small DM, Coombes JS, Bennett N, Johnson DW, Gobe GC. Oxidative stress, anti-oxidant therapies and chronic kidney disease. Nephrology. 2012;17(4):311-21.
10. Huong DQ, Van Bay M, Nam PC. Antioxidant activity of thiourea derivatives: An experimental and theoretical study. J Mol Liq. 2021;340:117149.
11. Perveen S, Abdul Hai SM, Khan RA, Khan KM, Afza N, Sarfaraz TB. Expeditious method for synthesis of symmetrical 1, 3-disubstituted ureas and thioureas. Synth Commun. 2005;35(12):1663-74.
12. Kirishnamaline G, Magdaline JD, Chithambarathanu T, Aruldhas D, Anuf AR. Theoretical investigation of structure, anticancer activity and molecular docking of thiourea derivatives. J Mol Struct. 2021;1225:129118.
13. Khan KM, Naz F, Taha M, Khan A, Perveen S, Choudhary MI, et al. Synthesis and in vitro urease inhibitory activity of N, N′-disubstituted thioureas. Eur J Med Chem. 2014;74:314-23.
14. Chen Z, Zhang L, Yang J, Zheng L, Hu F, Duan S, et al. Design, synthesis, and structure–activity relationship of N-Aryl-N′-(thiophen-2-yl) thiourea derivatives as novel and specific human TLR1/2 agonists for potential cancer immunotherapy. J Med Chem. 2021;64(11):7371-89.
15. Al-Ansary GH, Nasr T, Taha H, Fayad W, Mahgoub S. Biphenylurea/thiourea derivatives tagged with heteroarylsulfonamide motifs as novel VEGFR2 inhibitors; Design, synthesis and anti-angiogenic activity. Bioorg Chem. 2021;107:104640.
16. Raheel A, Badshah A, Rauf MK, Tahir MN, Khan KM, Hameed A, et al. Amino Acid Linked Bromobenzoyl Thiourea Derivatives: Syntheses, Characterization and Antimicrobial Activities. J Chem Soc Pak. 2016;38(5):959-64.
17. Soomro S, Chidrawar VR, Imran M, Alshammari HO. Immunomodulating, anti-bacterial and anti-cancer potential of Za'atar (Thymus vulgaris) and its combination with essential oil (Olive and Balsam Oil). Int J Pharm Sci. 2019;10(8):3721-6.
18. Pierzchala K, Simicic D, Sienkiewicz A, Sessa D, Mitrea S, Braissant O, et al. Central nervous system and systemic oxidative stress interplay with inflammation in a bile duct ligation rat model of type C hepatic encephalopathy. Free Radic Biol Med. 2022;178:295-307.
19. Zhang B, Peng X, Li G, Xu Y, Xia X, Wang Q. Oxidative stress is involved in Patulin induced apoptosis in HEK293 cells. Toxicon. 2015;94:1-7.
20. Kim HS, Lee JH, Moon SH, Ahn DU, Paik HD. Ovalbumin hydrolysates inhibit nitric oxide production in LPS-induced RAW 264.7 macrophages. Food Sci Anim Resour. 2020;40(2):274.
21. Strzyga-Łach P, Chrzanowska A, Podsadni K, Bielenica A. Investigation of the mechanisms of cytotoxic activity of 1, 3-disubstituted thiourea derivatives. Pharmaceuticals. 2021;14(11):1097.
22. Mohamed NA, Abd El-Ghany NA, Abdel-Aziz MM. Synthesis, characterization, anti-inflammatory and anti-Helicobacter pylori activities of novel benzophenone tetracarboxylimide benzoyl thiourea cross-linked chitosan hydrogels. Int J Biol Macromol. 2021;181:956-65.
23. Goiffon RJ. Assessing Vascular Inflammation with Bioluminescence Imaging. Washington University in St. Louis; 2015.
24. Clementi ME, Lazzarino G, Sampaolese B, Brancato A, Tringali G. DHA protects PC12 cells against oxidative stress and apoptotic signals through the activation of the NFE2L2/HO-1 axis. Int J Mol Med. 2019;43(6):2523-31.
25. Ozdemir KG, Yılmaz H, Yılmaz S. In vitro evaluation of cytotoxicity of soft lining materials on L929 cells by MTT assay. J Biomed Mater Res Part B: Appl Biomater. 2009;90(1):82-6.
26. Wenlock MC, Barton P. In silico physicochemical parameter predictions. Mol Pharm. 2013;10(4):1224-35.
27. Pattabiraman G. *Innate Apoptotic Immunity: Characterization of Macrophage Responses* (Doctoral dissertation, University of Illinois at Chicago). 2013.
28. Hirai KI, Moriguchi K, Wang GY. Human neutrophils produce free radicals from the cell-zymosan interface during phagocytosis and from the whole plasma membrane when stimulated with calcium ionophore A23187. Exp Cell Res. 1991;194(1):19-27.
29. Soomro S. Oxidative stress and inflammation. Open J Immunol. 2019;9(01):1.
30. Coleman JW. Nitric oxide in immunity and inflammation. Int Immunopharmacol. 2001;1(8):1397-406.
31. Bahuguna A, Khan I, Bajpai VK, Kang SC. MTT assay to evaluate the cytotoxic potential of a drug. Bangladesh J Pharmacol. 2017;12(2):115-8.
32. Davis AM, Riley RJ. Predictive ADMET studies, the challenges and the opportunities. Curr Opin Chem Biol. 2004;8(4):378-86.
33. Bandyopadhyay U, Das D, Banerjee RK. Reactive oxygen species: oxidative damage and pathogenesis. Curr Sci. 1999:658-66.

34. Rada B, Leto TL. Oxidative innate immune defenses by Nox/Duox family NADPH oxidases. Trends in Innate Immunity. 2008;15:164-87.
35. Deakin AM, Payne AN, Whittle BJ, Moncada S. The modulation of IL-6 and TNF-α release by nitric oxide following stimulation of J774 cells with LPS and IFN-γ. Cytokine. 1995;7(5):408-16.
36. Maeda H. The link between infection and cancer: tumor vasculature, free radicals, and drug delivery to tumors via the EPR effect. Cancer Sci. 2013;104(7):779-89.