REVIEW ON DRUGS CONTAINING STERICALLY HINDERED PHENOL - BUTYLATED HYDROXYTOLUENE ANALOGS

MADHAVI KUCHANA* ,ROHINI CHEEPURUPALLI

Institute of Pharmaceutical Technology,

Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati, Andhra Pradesh, India

kuchanamadhavi@yahoo.co.in

ABSTRACT

Butylated hydroxytoluene (BHT), a classical antioxidant used in pharmaceutical, polymer and food industry. BHT possess several pharmacological activities such as antioxidant, anti-inflammatory, antiulcer and antitumor activities. The sterically hindered phenol moiety appears to be attracting structural feature of BHT. Several compounds having BHT moiety were developed and screened for the possible biochemical and pharmacological activities. Some of the compounds were emerged as anti-inflammatory and antiarthritic drugs such as Prefelone, Tebufelone, Darbufelone and Tazofelone. In this paper the historic development, mechanism of action at the molecular level, toxicity and metabolism, other biochemical and pharmacological activities of these drugs were discussed.

Keywords: *Butylated hydroxytoluene, 2,6-Di-tert-butylphenol, Prefelone, Tebufelone, Darbufelone, Tazofelone.*

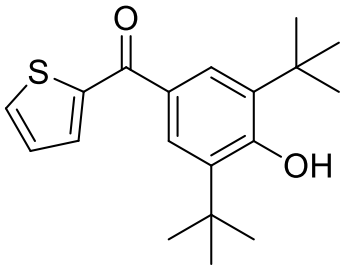
DOI: 10.31838/ecb/2022.11.12.63

INTRODUCTION

Butylated hydroxytoluene (BHT), a synthetic sterically hindered phenolic antioxidant was patented in 1947. It was initially developed as antioxidant for petroleum protection, later began its use in food industry [1-3]. Synonyms of BHT are 2,6-Di-*tert*–butyl-*p*-cresol, 2,6-Di-*tert*–butyl-*p*-methylphenol, 2,6-Di-*tert*–butyl-4-methylphenol, 2,6-Bis(1,1-dimethylethyl)-4-methylphenol, 2,6-Di-*tert*-butyl-1-hydroxy-4-methylbenzene, 3,5-Di-*tert*-butyl-4-hydroxytoluene, 4-hydroxy-3,5-di-*tert*-butyltoluene, Dibutylhydroxytoluene, Butylhydroxytoluene, Dibunol, Ionol and Tonarol [4]. BHT is a white crystalline solid, odourless and tasteless substance. It is insoluble in water and freely soluble in hydrocarbon solvents. Generally, BHT was prepared by alkylation reaction of *p*-cresol with isobutylene using sulfuric acid, alkylaluminium halide or a cation- exchange resin as a catalyst [5,6]. Alternatively, BHT has been

prepared from 2,6-di-*tert*-butylphenol by aminomethylation and subsequent hydrogenolysis [7]. The synthesis of BHT has also been reported by C4-alkylation of 2,6-di-*tert*-butylphenol with methanol using iridium catalysed hydrogen-borrowing process [8]. It was documented that BHT has natural origin and can be endogenously generated by some fresh water phytoplankton by submitting to oxidative stress condition. It was also found in oak wood and pericarp of litchi fruit [9]. BHT classified as chain breaking antioxidant, it break and slows down the autoxidation chain reaction by quenching of reactive oxygen species and acts as free radical scavenger [6, 10]. In previous years, BHT has been modified to prepare new series of antioxidants having suitable properties in both pharmaceutical and polymer industry [3, 10]. Several chalcones, benzylidene compounds, heterocyclic systems, hydrazones and quaternary ammonium compounds containing di-*tert*-butylphenol (sterically hindered phenol) were reported in the literature [11]. Recent studies indicated that several compounds containing sterically hindered phenols have various bioactivities such as antioxidant, anti-inflammatory, anticancer, antiviral and antimicrobial activities [12]. Biological and pharmacological activities of BHT and sterically hindered phenols were reported in many review articles [1, 10, 13-15], but there is no review article dedicated to the drugs having BHT moiety or di-*tert*-butylphenol such as Prifelone, Tebufelone, Darbufelone and Tazofelone. Therefore, the present review aimed to describe the historical development, mechanism of action at the molecular level, toxicity and metabolism, other biochemical and pharmacological activities of drugs containing BHT or a sterically hindered phenol moiety.

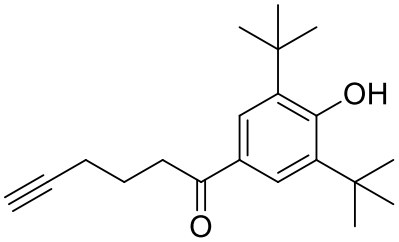
PRIFELONE



Prifelone [(3,5-di-*tert*-butyl-4-hydroxyphenyl)(thiophen-2-yl)methanone] was developed by the structural modification of H-transfer chain breaking antioxidant BHT. It was known by other synonyms Prifelonum, Prifelona, R- 830, Butafen, 3,5-Di-*tert*-butyl-4-hydroxyphenyl 2-thienyl ketone, 2,6-Di-*tert*-

butyl-4-(2′-thenoyl)phenol. The synthesis of Prifelone was carried out from 3,5-di-*tert*-butyl-4-hydroxybenzoyl chloride and thiophene. The drug has been identified as an anti-inflammatory agent with antioxidant properties. Initially, the anti-inflammatory potential of Prifelone was determined by several animal models such as carrageenan-induced rat paw edema, adjuvant-induced arthritis in rat, ultraviolet-induced erythema in guinea pig in which acidic nonsteroidal anti-inflammatory drugs are effective and localized graft vs. host reaction in rat, reversed passive cutaneous Arthrus reaction in rat, oxazolone-induced contact sensitivity in mouse in which acidic nonsteroidal anti-inflammatory drugs are not effective. The drug was shown to inhibit bovine seminal vesicle cyclooxygenase and guinea pig lung lipoxygenase. The effect of Prifelone on chemotaxis of human PMN leukocytes *in vitro* was determined and the results correlated with the *in vivo* inhibition of leukocyte accumulation in subcutaneously implanted cotton pellets in rats. The antioxidant activity of this drug was demonstrated in two *in vitro* models which include inhibition of peanut oil auto-oxidation and $FeCl_2$-induced peroxidation of liposomes [16]. Subsequently, Prifelone has been reported to be a dual inhibitor of cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) at 1 and 10 μM concentration with 96% inhibition of developing adjuvant arthritis at a dose 25 mg/kg [17]. Prifelone has structural analogy to nonsteroidal anti-inflammatory drugs (NSAIDs) especially aryl propionic acids Suprofen and Tiaprofenic acid. The difference between Prifelone and conventional acidic NSAIDs may be due to chemical uniqueness of phenolic compound with di-*tert*-butyl groups adjacent to OH group. This sterically hindered phenol appears to be more lipophilic molecule of very weak acidity having dual inhibition of COX and 5-LOX enzymes with *in vivo* anti-inflammatory activity as well as *in vitro* antioxidant effect.

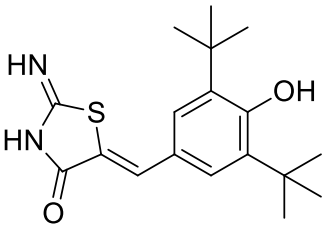
TEBUFELONE



Tebufelone [1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)hex-5-yn-1-one] is a novel member of di-*tert*-butylphenol class of nonsteroidal anti-inflammatory drug. It was patented by Procter and Gamble company in 1987. The drug was synthesized by different schematic routes, few pharmaceutical compositions were prepared and evaluated in several animal models of inflammation. In many models like carrageenan rat paw edema assay, oxazolone-induced inflamed mouse ear test, arachidonic acid – induced inflamed mouse ear test and adjuvant induced arthritis, Tebufelone showed anti-inflammatory and anti-arthritic activities due to the inhibition the cyclooxygenase and 5-lipoxygenase biosynthetic pathways [18]. Tebufelone was reported to be more effective cyclooxygenase inhibitor than indomethacin, but less potent 5-lipoxygenase inhibitor than RG-5901. The inhibition of these arachidonic acid metabolism pathways significantly responsible for the effectiveness and very low GI damage, a distinct therapeutic advantage not observed with traditional NSAIDs. Tebufelone was also reported to possess analgesic and antipyretic activity at doses much less than the standard drug aspirin [19, 20]. It has been reported that the anti-inflammatory activity of Tebufelone correlates with high tissue distribution levels than the plasma drug levels, at the examined dose levels in Sprague-Dawley rats. Due to more lipophilic nature, the drug extensively distributed to paw tissues and responsible for long duration of action after peroral and intravenous administration [21]. The absorption, bioavailability and pharmacokinetics were investigated in rats using radiolabeled Tebufelone and the pure drug Tebufelone. Comparison of experimental studies indicated that the complete absorption and bioavailability at a dose level 2mg/kg. The area under the curves (AUCs) were linearly related to dose level up to 2 mg/kg, but the AUCs appeared to increase nonproportionally between doses of 2 and 10 mg/kg indicating nonlinear pharmacokinetics [22]. In a preliminary preformulation investigation, the effects of physical/chemical properties on dissolution of Tebufelone in simulated intestinal fluid with 2% bile salts were described. Tebufelone has low aqueous solubility, but significantly increased by low levels of endogenous solubilizing agents like sodium cholate and sodium deoxycholate. The poor aqueous solubility predicted low dissolution and high lipophilicity indicated partition of drug into the lipoidal absorbing membranes of GI tract. Further, it has been mentioned that reduction of particle size alone might not be sufficient to substantially increase the rate of Tebufelone dissolution [23]. Another group of researchers reported that the enzymatic hydroxylation of *tert*-butyl group occurs in Tebufelone similar to BHT, through a primary biliary metabolic pathway in rats [24]. A Tebufelone metabolite containing dihydrodimethylbenzofuran

(DHDMBF) system was reported to possess anti-inflammatory activity equivalent to Tebufelone in rat carrageenan paw edema assay. It exhibited better inhibition of COX-2 and 5-LOX enzymes indicating the biotransformation of phenolic hydroxyl group and di-*tert*-butyl group into cyclic dihydrodimethylbenzofuran moiety retains the anti-inflammatory activity. Further, it has been reported that the structural modification of DHDMBF resulted in a series of dihydrobenzofurans as new cyclooxygenase-2/5-lipoxygenase inhibitors [25].

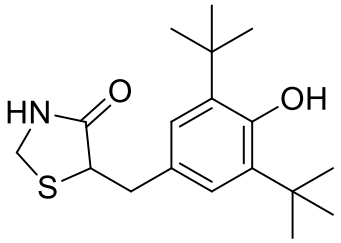
DARBUFELONE



Darbufelone [5-((Z) 3,5-di-*tert*-butyl-4-hydroxybenzylidene)-2imino-4-thiazolidinone], a novel anti-inflammatory drug having 3,5-di-*tert*-butyl-4-hydroxybenzylidene moiety at 5th position of thiazole molecular scaffold. It was synthesized in a single step by knoevenagel condensation of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde with the enolate generated from pseudothiohydantoin. The drug administered as salt of methanesulfonic acid through oral route and reported as nonulcerogenic anti-inflammatory agent [26-28]. Reports were also available for the synthesis of Darbufelone by microwave irradiation using the same reactants in ethylene glycol [29]. Darbufelone, a new member of NSAIDs acts as dual COX/5-LOX inhibitor, developed for the treatment of rheumatoid and osteoarthritis. Because of its potency, preclinical pharmacokinetic trials were carried out with doses of 1-10 mg/kg and an initial dose of 1 mg was utilized in a rising dose tolerance clinical trial [30]. A study pertaining to mechanism of action revealed that the Darbufelone potently inhibits recombinant human prostaglandin endoperoxide synthase-2 (PGHS-2) but much less effective against PGHS-1 and expressed that the PGHS-2 selectivity is consistent with nonulcerogenic nature of Darbufelone. It was reported that the Darbufelone is a non competitive inhibitor of PGHS-2 and may interact with previously unrecognized binding site on the enzyme [31]. The molecular docking studies of Darbufelone performed using Autodock-Vina software revealed the extensive hydrogen bond interaction in COX-2 cavity (COX-2 enzyme PDB ID: 6COX) and 5-LOX cavity (5-LOX enzyme PDB ID: 3O8Y) with binding energies of - 6.8 and -7.3 Kcal/mol respectively

[32]. It has been reported that the dual COX/5-LOX inhibitor Darbufelone exhibited anti-proliferative effect on human non-small cell lung cancer cell lines, *in vitro* in a time and dose dependent manner. At 20 μM concentration of Darbufelone, cell cycle arrest at G_0/G_1 phase was induced by up-regulation of p27 expression and at 60 μM concentration apoptosis was induced by activating caspase-3 and caspase-8. Inhibition of tumor growth in Lewis lung cancer mice model was also observed at a daily dose of 80 mg/kg and therefore suggested that Darbufelone treatment might represent a novel and promising approach to lung cancer chemotherapy [33]. The effectiveness of Darbufelone on proliferation, migration, invasion and apoptosis of colon cancer cells was investigated using human LoVo cells in another study. The results indicated an upregulation of p27 and downregulation of cyclin D1 as well as CDK4 leading to cell cycle arrest of LoVo cells at G_0/G_1 phase. The activation of caspase-3 and caspase-9, upregulation of Bax and downregulation of Bcl2 indicated the apoptosis of cancer cells. It has been proved that the expression of COX-2 and 5-LOX involved in regulation of cell growth, migration and invasion. Therefore, simultaneous inhibition of COX-2 and 5-LOX activity causes more effective colon cancer management [34]. It has been demonstrated that the electroactive di-*tert*-butylphenol moiety present in Darbufelone structure was responsible for the reducing properties by voltammetric techniques and density functional theory calculations [35]. Recently, Darbufelone mesylate was identified as an inhibitor of iodothyronine deiodinases. The drug was tested by single-point screening method against human deiodinase enzymes type 1, 2 and 3 at a maximum concentration of 200 μM. At 100 μM concentration it showed 74% inhibition against deiodinase type 1 and 50% inhibition or greater against deiodinase type 2 and 3 at 200 μM. Furthermore, the IC_{50} value of the Darbufelone mesylate for inhibition of deiodinase type 1 was reported to be 4.7 μM, indicating that it can adversely affect the metabolism of thyroid hormone [36].

TAZOFELONE



Tazofelone [5-[(3,5-di-*tert*-butyl-4-hydroxyphenyl)methyl]-1,3-thiazolidin-4-one], a potent antioxidant and anti-inflammatory agent, belongs to the class of di-*tert*-butylphenol. Tazofelone was

developed by Eli Lilly and Company for the treatment of inflammatory diseases [37-39]. It was prepared as similar as Darbufelone using 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde and rhodanine instead of pseudothiohydantoin. Subsequently reducing the bezylidene and thiocarbonyl moieties of intermediate (Z)-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-2-thioxothiazolidin-4-one. The product Tazofelone contains one chiral center and obtained as racemic mixture of *R* and *S* enantiomers. The pure enantiomers were separated by a kinetic resolution involving enantioselective sulfoxide formation [40-42]. Synthesis of both (*R*)- and (*S*)- Tazofelone at > 98% enantiomeric purity was also reported [43]. The crystal forms of Tazofelone were characterized by crystallographic, spectroscopic and thermal methods. The study revealed that Tazofelone has been crystallized as two polymorphic racemic compounds and as an (*S*)-(-) enantiomorph [44]. Discovery of a solid solution (mixed crystal) of the enantiomers of the chiral drug Tazofelone by seeding its recemic liquid with crystals of pure enantiomorphs was reported by another group of researchers. They further mentioned that the solid solution provides a higher-solubility alternative to the recemate for formulating and delivering the drug Tazofelone [45]. Metabolic studies of Tazofelone in human liver microsomes revealed the formation of sulfoxide and quinol metabolites. The cytochrome P450 responsible for the biotransformation of Tazofelone was found to be CYP3A [46]. Incubation of both enantiomers separately in rat, dog and human hepatic microsomes demonstrated that the (*R*)-Tazofelone was more rapidly metabolized with the formation of two diastereomeric sulfoxides as major metabolites in all three species. Total sulfoxide formation rates were measured as the two diasteromers epimerized at physiological pH. The intrinsic formation clearance (V_{max}/K_m) of sulfoxide from (R)-Tazofelone exceeded that of (*S*)-Tazofelone in all three species. The stereoselective metabolism of Tazofelone reported *in vitro* was validated by *in vivo* studies in rats and dogs dosed orally and intravenously [47].

DISCUSSION

The class of BHT analogs or di-*tert*-butylphenol derivatives has been extensively investigated to identify new dual COX/5- LOX inhibitors. The most representative molecules include Prifelone, Tebufelone, Darbufelone and Tazofelone. The sterically hindered phenol moiety not only confers antioxidant properties but also anti-inflammatory potency with low ulcerogenic potential. The ratio of anti-inflammatory efficacy to GI safety profile was found superior to the classical NSAIDs. The first member Prifelone has been identified as structural analog of Suprofen consisting di-*tert*-butylphenol

instead of aryl propionic acid. This modification was shown to be optimal for dual inhibition of COX/5-LOX enzymes *in vitro* and anti-inflammatory activity *in vivo*. Replacement of carbonyl group of Prifelone with an olefin bond resulted in styryl thiophene consisting sterically hindered phenol, exhibiting similar *in vitro* profile and a variable *in vivo* efficacy. Further, reducing the size of the *tert*-butyl group had no detrimental effect on the COX and 5-LOX inhibition, but the resulting compounds lacked the *in vivo* efficacy, possibly because of rapid phase II metabolism [48]. Replacement of thiophene ring of Prifelone with terminal alkyne1-pentyne results into another dual COX/5- LOX inhibitor Tebufelone. On comparison, the structural change leads to a drastic reduction in dose of Tebufelone. The drug exhibited excellent anti-inflammatory activity and inhibited bone resorption *in vivo* in the rat adjuvant arthritis model at a dose level of 1 to 2 mg/kg [22]. The search of similar analogs of Tebufelone having BHT moiety and other substituted alkyl groups revealed the existence of compounds CGP7930, CGP13501 and BSPP used in scientific research as positive allosteric modulators of $GABA_B$ receptors [49, 50]. Another modification of Prifelone by connecting the di-*tert*-butylphenol to pseudothiohydantoin in place of thiophene through –CH= bond generates Darbufelone. The drug Darbufelone was developed from group of compounds having the common structure of 3, 5-di-*tert*-butyl-4-hydroxybenzylidene moiety and a five or six membered ring. The examples include CI-987, KME-4, E-5110 and BF-389. Similar to Darbufelone, S-2474 [(*E*)-(5)-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-2-ethyl-1,2-isothiazolidine-1,1-dioxide] was selected as an antiarthritic drug candidate. It exerted excellent anti-inflammatory activity without any ulcerogenic effects. Its ability to inhibit both COX-2 and 5-LOX as well as production of interleukin (IL) – 1 were proved [51]. Reduction of –CH= bond and removal of 2-imino group of Darbufelone gives the structure of Tazofelone. Due to the saturation of –CH= bond, formation of two enatiomers are possible. A sterioselective metabolism of Tazofelone indicated the rapid metabolic susceptibility of *R* enantiomer. Similar BHT analogs were reported by modification of heterocyclic rings of Tazofelone and Prifelone. The resultant compounds showed potent anti-inflammatory activity, whereas BHT was found to be inactive in the tested animal model [52]. The search of other sterically hindered phenolic drugs disclosed Eldacimibe and Probucol in which di-*tert*-butylphenol linked to heteroatom nitrogen and sulfur respectively. These drugs are used in the treatment of lipid disorders. In our previous study, the molecular properties, druglikeness and bioactivity score of BHT, Prifelone, Tebufelone, Darbufelone and Tazofelone were predicted *in silico* and found that all the molecules were drug like. With few exceptions,

all the drugs were identified as moderately or highly bioactive molecules [53]. Variations in the molecular properties and bioactivity score of these drugs indicate that the structural changes within the di-*tert*-butylphenol series have major impact.

CONCLUSION

A series of compounds containing BHT moiety or di-*tert*-butylphenol were developed based on the attracting antioxidant properties. Some compounds were proved as useful drugs for the treatment of inflammation and lipid disorders. In particular, Prefelone, Tebufelone, Darbufelone and Tazofelone were used as anti-inflammatory and antiarthritic drugs. The major mechanism of action was found to be dual inhibition of COX and 5-LOX enzymes involved in arachidonic acid metabolism. The great advantage in use of these drugs over conventional NSAIDs resides on the non-ulcerogenic potential. However, none of these drugs were reached to the market due to the high toxicity and/or limited efficacy. Therefore, a continuous effort needed to develop newer analogs through modification of structural components responsible for the toxicity and poor pharmacokinetic and pharmacodynamic profile.

REFERENCES:

[1]. Babich H. (1982). Butylated hydroxytoluene (BHT): a review. *Environ Res*., 29(1):1-29.

[2]. Cai P, Fang SQ, Yang HL, Yang XL, Liu QH, Kong LY, Wang XB. (2018). Donepezil-butylated hydroxytoluene (BHT) hybrids as Anti-Alzheimer's disease agents with cholinergic, antioxidant, and neuroprotective properties. *Eur J Med Chem*., 157:161-176.

[3]. Yehye WA, Abdul Rahman N, Alhadi AA, Khaledi H, Weng NS, Ariffin A. (2012). Butylated hydroxytoluene analogs: synthesis and evaluation of their multipotent antioxidant activities. *Molecules*., 17(7):7645-7665.

[4]. Zarudii FS, Gil'mutdinov GZ, Zarudii RF, Myshkin MA, Gershanov FB, Novikov TA. (2001). 2,6-Di-tert-butyl-4-methylphenol (Dibunol, Ionol, Tonarol): a Classical Antioxidant (A Review). *Pharmaceutical Chemistry Journal. 35*:162-168.

[5]. Santacesaria E, Silvani R, Wilkinson P, Carra S. (1988). Alkylation of *p*-cresol with isobutene catalyzed by cation-exchange resins: a kinetic study. *Ind. Eng. Chem. Res*., 27(4): 541-548.

[6]. Lanigan RS, Yamarik TA. (2002). Final report on the safety assessment of BHT(1). *Int J Toxicol*., 21 Suppl 2:19-94.

[7]. Pantukh BI, Surkov VD, Logutove I. Ju. 2, 6-Di-tert-butyl-4-methylphenol (Agidol-1) production process. RU2137749C1, 1999.

[8]. Frost J, Cheong C, Donohoe T. (2016). Iridium-catalyzed C4-alkylation of 2,6-di-tert-butylphenol by using hydrogen-borrowing catalysis. *Synthesis*., 49(4):910–916.

[9]. Nieva-Echevarría B, Manzanos MJ, Goicoechea E, Guillén MD. (2015). 2,6-Di-Tert-Butyl-Hydroxytoluene and Its Metabolites in Foods. *Compr Rev Food Sci Food Saf*., 14(1):67-80.

[10]. Yehye WA, Rahman NA, Ariffin A, Hamid SBA, Alhadi AA, Kadir FA, Yaeghoobi M. (2015). Understanding the chemistry behind the antioxidant activities of butylated hydroxytoluene (BHT): A review. *Eur J Med Chem.*, 101:295-312.

[11]. Kuchana M, Bethapudi DR, Ediga RK, Sisapuram Y. (2019). Synthesis, *in-vitro* antioxidant activity and *in-silico* prediction of drug likeness properties of a novel compound: 4-(3,5-Di-*tert*-butyl-hydroxybenzylidene)-3-methylisoxazol-5(4*H*)-one. *J Appl Pharm Sci*., 9(09):105–110.

[12]. Koshelev VN, Primerova OV, Vorobyev SV, Ivanova LV. (2020). Synthesis, redox properties and antibacterial activity of hindered phenols linked to heterocycles. *Molecules*., 25(10):2370.

[13]. Malkinson AM. (1983). Review: putative mutagens and carcinogens in foods. III. Butylated hydroxytoluene (BHT). *Environ Mutagen*., 5(3):353-362.

[14]. Mankar Viraj H, Chavvi. (2020). Sterically hindered phenols as antioxidants. *Eur J Mol Clin Med*., 7(7):3481-3491.

[15]. Roginskii VA. (1982). Sterically hindered phenols – antioxidants for polyolefines. Relation of antioxidative activity to structure. Review. *Polymer Science U. S. S. R.*, 24(9):2063-2088.

[16]. Moore GG, Swingle KF. (1982). 2,6-Di-tert-butyl-4-(2'-thenoyl)phenol(R-830): a novel nonsteroidal anti-inflammatory agent with antioxidant properties. *Agents Actions*.,12(5-6):674-83.

[17]. Lazer ES, Wong HC, Possanza GJ, Graham AG, Farina PR. (1989). Antiinflammatory 2,6-di-tert-butyl-4-(2-arylethenyl)phenols. *J Med Chem*., 32(1):100-104.

[18]. Loomans ME, Matthews RS, Miller JA, Inventors. Procter and Gamble Company, Assignee. Novel anti-inflammatory agents, pharmaceutical compositions and methods for reducing inflammation. US Patent Number 4,708,966. 1987.

[19]. Powell JH, Meredith MP, Vargas R, McMahon FG, Jain AK. (1991). Antipyretic activity of tebufelone (NE-11740) in man. *Agents Actions*., 32:45-49.

[20]. Weisman SM, Doyle MJ, Wehmeyer KR, Hynd BA, Eichhold TH, Clear RM, Coggeshall CW, Kuhlenbeck DL. (1994). Effects of tebufelone (NE-11740), a new anti-inflammatory drug, on arachidonic acid metabolism. *Agents Actions.*, 41:156-163.

[21]. Doyle MJ, Eichhold TH, Loomans ME, Farmer RW, Kelm GR. (1993). Comparison of tebufelone distribution in rat blood plasma and inflamed and noninflamed tissues following peroral and intravenous administration. *J Pharm Sci*., 82(8):847-850.

[22]. Sietsema WK, Kelm GR, Deibel RM, Doyle MJ, Loomans ME, Smyth RE, Kinnet GO, Eichhold TH, Farmer RW. (1993). Absorption, bioavailability, and pharmacokinetics of tebufelone in the rat. *J Pharm Sci*., 82(6):610-612.

[23]. Kelm GR, Sakr AA. (1993). Preliminary preformulation investigation of tebufelone, a novel non-steroidal anti-inflammatory drug. *Drug Dev Ind Pharm*.,19(7):809-826.

[24]. Miller JA, Matthews RS. (1992). A short, efficient synthesis of (*tert*-butyl-hydroxylated) di-*tert*-butylphenols. *J Org Chem.*, 57(8):2514-2516.

[25]. Janusz JM, Young PA, Ridgeway JM, Scherz MW, Enzweiler K, Wu LI, Gan L, Chen J, Kellstein DE, Green SA, Tulich JL, Rosario-Jansen T, Magrisso IJ, Wehmeyer KR, Kuhlenbeck DL, Eichhold TH, and Dobson RLM. (1998). New cyclooxygenase-2/5-lipoxygenase inhibitors. 3,7-*tert*-butyl-2,3-dihydro-3,3-dimethylbenzofuran derivatives as gastrointestinal safe antiinflammatory and analgesic agents: variations at the 5 Position. *J Med Chem*., 41(18):3515-3529.

[26]. Cetenko WA, Connor DT, Sircar JC, Sorenson RJ, Unangst PC, Inventors. Warner-Lambert Company, Assignee. 3,5-Di-tertiarybutyl-4-hydroxyphenylmethylene derivatives of 2-substituted thiazolidinones, oxazolidinones, and imidazolonones as Antiinflammatory agents. US Patent Number 5,143,928, 1992.

[27]. Unangst PC, Connor DT, Cetenko WA, Sorenson RJ, Sircar JC, Wright CD, Schrier DJ, Dyer RD. (1993). Oxazole, thiazole, and imidazole derivatives of 2,6-di-tert-butylphenol as dual 5-lipoxygenase and cyclooxygenase inhibitors. *J Med Chem.*, 3(8):1729-1734.

[28]. Unangst PC, Connor DT, Cetenko WA, Sorenson RJ, . Kostlan CR, Sircar JC, Wright CD, Schrier DJ, Dyer RD. (1994). Synthesis and biological evaluation of 5-[[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]methylene]oxazoles, -thiazoles, and -imidazoles: novel dual 5-lipoxygenase and cyclooxygenase inhibitors with antiinflammatory activity. *J Med Chem.*, 37(2):322-328.

[29]. Peng T, Wang L. (2008). Synthesis of darbufelone mesylate under microwave irradiation. *Chinese Journal of Pharmaceuticals*., 8:567-568.

[30]. Andress LD, Guttendorf RJ. (1997). High-performance liquid chromatographic assay for CI-1004, a dual-inhibitor anti-inflammatory agent, in rat, rabbit, dog, monkey and human plasma. *J Chromatogr B Biomed Sci Appl.*, 698(1-2):171-179.

[31]. Johnson AR, Marletta MA, Dyer RD. (2001). Slow-binding inhibition of human prostaglandin endoperoxide synthase-2 with darbufelone, an isoform-selective antiinflammatory di-*tert*-butyl phenol. *Biochem.*, 40(25):7736-7745.

[32]. Misra S, Ghatak S, Patil N, Dandawate P Ambike V, Adsule S, Unni D, Swamy V, Padhye S. (2013). Novel dual cyclooxygenase and lipoxygenase inhibitors targeting hyaluronan-CD44v6 pathway and inducing cytotoxicity in colon cancer cells. *Bioorg Med Chem*., 21(9):2551–2559.

[33]. Ye X, Zhou W, Li Y, Sun Y, Zhang Y, Ji H, Lai Y. (2010). Darbufelone, a novel anti-inflammatory drug, induces growth inhibition of lung cancer cells both *in vitro* and *in vivo*. *Cancer Chemother Pharmacol*., 66(2):277-85.

[34]. Che XH, Chen CL, Ye XL, Weng GB, Guo XZ, Yu WY, Tao J, Chen YC, Chen X. (2016). Dual inhibition of COX-2/5-LOX blocks colon cancer proliferation, migration and invasion *in vitro*. *Oncol Rep*., 35(3):1680-1688.

[35]. Lopes de Macedo IY, Garcia LF, Menegatti R, Guimaraes FF, Liao LM, Silva de Carvalho F, Santos WTP, Verly RM, Arotiba OA, Gil ES. (2018). Electrochemical characterizations of darbufelone, a di-*tert*-butylphenol derivative, by voltammetric techniques and density functional theory calculations. *Electrochimica Acta*., 268:462-468.

[36]. Olker JH, Korte JJ, Denny JS, Hartig PC, Cardon MC, Knutsen CN, Kent PM, Christensen JP, Degitz SJ, Hornung MW. (2019). Screening the ToxCast Phase 1, Phase 2, and e1k Chemical Libraries for Inhibitors of Iodothyronine Deiodinases. *Toxicol Sci*., 168(2):430-442.

[37]. Penetta JA. Inventor. Eli Lilly and Company, Assignee. Aryl-substituted rhodanine derivaitves. US Patent Number 5,356,917, 1994.

[38]. Hansen MM, Harkness AR. (1994). Synthesis of 4-thiazolidinones from rodanines by thiocarbonyl removal. *Tetrahedron Letters*., 35(38):6971-6974.

[39]. Jack DB. (1994). Potential applications of new antioxidants. *Inpharma Wkly.*, 964:9–10.

[40]. Phillips ML, Berry DM, Panetta JA. (1992). Kinetic resolution of a racemic sulfide by enantioselective sulfoxide formation*. J Org Chem.*, 57(14):4047-4049.

[41]. Phillips ML, Panetta JA. (1997). Substrate specificity of kinetic resolution of sulfides by enantioselective sulfoxide formation. *Tetrahydron: Asymmetry*., 8(13):2109-2114.

[42]. Slattery BJ, Kjell DP, Copp JD, Ginah FO, Hansen MM, Stirm SC. (2000). The development of a manufacturable synthesis of LY213829. *Orgc Proc Res Dev.*, 4(4):295-297.

[43]. Hansen MM, Harkness AR, Khau VV, Martinelli MJ, Deeter JB. (1996). Synthesis of enantioenriched 4-thiazolidinone (−)-LY213829 by chemoselective benzylamide cleavage in the presence of a C-S bond. *Tetrahedron: Asymmetry*., 7(9):2515–2518*.*

[44]. Reutzel-Edens SM, Russell VA, Yu L. (2000). Molecular basis for the stability relationships between homochiral and racemic crystals of tazofelone: a spectroscopic, crystallographic, and thermodynamic investigation. *J Chem Soc., Perkin Trans* 2., 2(5):913–924.

[45]. Huang J, Chen S, Guzei IA, Yu L. (2006). Discovery of a solid solution of enantiomers in a racemate-forming system by seeding. *J Am Chem* Soc., 128(36):11985-11992.

[46]. Surapaneni SS, Clay MP, Spangle LA, Paschal JW, Lindstrom TD. (1997). *In vitro* biotransformation and identification of human cytochrome P450 isozyme-dependent metabolism of tazofelone. *Drug Metab Dispos*., 25(12):1383-1388.

[47]. Clay MP, Hanssen BR, Surapaneni SS, Lindstrom TD. (1999). Stereoselective metabolism of tazofelone, an anti-inflammatory bowel disease agent, in rats and dogs and in human liver microsomes. *Chirality*., 11(3):233–240.

[48]. Morphy R, Rankovic Z. (2005). Designed multiple ligands. An emerging drug discovery paradigm. *J Med Chem*., 48(21):6523-6543.

[49]. Urwyler S, Mosbacher J, Lingenhoehl K, Heid J, Hofstetter K, Froestl W, Bettler B, Kaupmann K. (2001). Positive allosteric modulation of native and recombinant gamma-aminobutyric acid(B) receptors by 2,6-Di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and its aldehyde analog CGP13501. *Mol Pharmacol*., 60(5):963-971.

[50]. Parker DA, Marino V, Ong J, Puspawati NM, Prager RH. (2008). The CGP7930 analogue 2,6-di-tert-butyl-4-(3-hydroxy-2-spiropentylpropyl)-phenol (BSPP) potentiates baclofen action at GABA(B) autoreceptors. *Clin Exp Pharmacol Physiol*., 35(9):1113-1115.

[51]. Inagaki M, Tsuri T, Jyoyama H, Ono T, Yamada K, Kobayashi M, Hori Y, Arimura A, Yasui K, Ohno K, Kakudo S, Koizumi K, Suzuki R, Kawai S, Kato M, Matsumoto S. (2000). Novel antiarthritic agents with 1,2-isothiazolidine-1,1-dioxide (gamma-sultam) skeleton: cytokine suppressive dual inhibitors of cyclooxygenase-2 and 5-lipoxygenase. *J Med Chem*., 43(10):2040-2048.

[52]. Ziakas GN, Rekka EA, Gavalas AM, Eleftheriou PT, Kourounakis PN. (2006). New analogues of butylated hydroxytoluene as anti-inflammatory and antioxidant agents. *Bioorg Med Chem*., 14(16):5616-5624.

[53]. Kuchana M, Pulavarthi M, Potthuri S, Manduri V, Jaggarapu VD. (2020). *In-silico* study of molecular properties, bioactivity and toxicity of 2-(substituted benzylidene)succinic acids and some selected anti-inflammatory drugs. *International Journal of Pharmaceutical Sciences and Drug Research*, 12(4):353-359.