Symbiosis

www.symbiosisonline.org
www.symbiosisonlinepublishing.com

Research Article International Journal of Analysis in Medicinal Chemistry Open Access

Substituting tert-butyl group on Murrayanine-Chalcone Scaffold Produced Tremendously High Anti-oxidant Activity than the Individual Components

Debarshi Kar Mahapatra[1*], Ruchi S. Shivhare[2]

1Department of Pharmaceutical Chemistry, Dadasaheb Balpande College of Pharmacy, Nagpur 440037, Maharashtra, India

[2]Department of Pharmaceutical Chemistry, Kamla Nehru College of Pharmacy, Nagpur 441108, Maharashtra, India

Received: November 12, 2018; Accepted: November 13, 2018; Published: December 01, 2018

***Corresponding author:** Debarshi Kar Mahapatra, PhD. Assistant Professor, Department of Pharmaceutical Chemistry, Dadasaheb Balpande College of Pharmacy, Nagpur 440037, Maharashtra, India. E-mail: dkmbsp@gmail.com*

Abstract

Murrayanine is an active carbazole alkaloid having fairly good anti-oxidant activity. Carbazole scaffold and its hybrid molecules have been seen to exhibit potentially high radical scavenging activities. Chalcone successfully scavenges the hydroxyl, superoxide, etc. and find applications as anti-cancer, anti-inflammatory, anti-diabetic, anti-infective, and cardiovascular agents. The modern-day synthetically prepared anti-oxidants such as 2-tert-butyl-4-methoxyphenol (BHA), tert-butyl hydroquinone (TBHQ), 2, 6-di-tert-butyl-4-methylphenol (BHT) and 2, 4, 6-tri-tert-butylphenol (TBP) have a prime common structural phenomenon, i.e. tert-butyl group. Inspiring from the above facts, a hybrid molecule comprising of Murrayanine (carbazole moiety), Chalcone, and tert-butyl group was rationally designed, and it was predicted that the proposed compound will exhibit a better free-radical scavenging potential. The molecule was synthesized from Claisen-Schmidt reaction and screened for anti-oxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The tested molecule presented a tremendous radical scavenging activity as evidenced by the IC50 value of 5.94 µM. The higher activity of the novel compound may be predicted from the synergistic potentials of the carbazole, Chalcone, and tert-butyl group. The current study will positively influence, motivate, and incline millions of global researchers in developing similar therapeutically active low-molecular-weight ligands and their translated pharmaceutical products for disease prevention and further utility.

Keywords: Antioxidant; Radical; Scavenging; Murrayanine; Chalcone; tert-butyl.

Introduction

Free radicals are the chemically reactive species generated in the human body in a large amount every day under normal conditions [1]. When ionizing radiations or environmental toxins (cigarette smoking, high-oxygen atmosphere, etc.) are encountered by the individuals, an abnormally high concentration of free radicals is produced in the human body in response. The free radicals are known to precipitate various diseases such as neurological complications, cardiac abnormalities, nephritic diseases, etc [2].

Murrayanine is an active carbazole alkaloid present in the curry tree, Murraya koenigii (Family: Rutaceae). The component is having fairly good anti-oxidant activity (IC_{50} value of 7.6 µM) whereas the Schiff's base based semi-synthetic derivatives of Murrayanine have IC_{50} values of 6.5-7.3 µM [3-4].

Carbazole is a well-known heterocyclic scaffold having multifarious pharmacological activities. Numerous scientific reports on anti-oxidant activity of this scaffold are available [5]. The hybrid molecules such as carbazole-ferulic acid [6], carbazole-pyrrolidine [7], carbazole-thiazole [8], carbazole-tacrine [9], etc. have been seen to exhibit potentially high radical scavenging activities.

Chalcone is a low-molecular-weight natural scaffold with strikingly high anti-oxidant activity. The ligands successfully scavenge the hydroxyl, superoxide, etc. and find applications as anti-cancer [10], anti-inflammatory [11], anti-diabetic [12], anti-infective [13], and cardiovascular [14] agents, owing to such attributes.

The modern-day synthetically prepared anti-oxidants have a prime common structural phenomenon, i.e. tert-butyl group. Products such as 2-tert-butyl-4-methoxyphenol (BHA), tert-butyl hydroquinone (TBHQ), 2,6-di-tert-butyl-4-methylphenol (BHT) and 2,4,6-tri-tert-butylphenol (TBP) bear tert-butyl groups which are believed to play an imperative role in scavenging the free-radicals [15] (Figure 1).

Inspiring from the above facts, a hybrid molecule comprising

of Murrayanine (carbazole moiety), Chalcone, and tert-butyl group was rationally designed, with a prediction that the produced compound will exhibit a tremendous free-radical scavenging potential and play an essential role in several diseased conditions. The molecule was synthesized from Claisen-Schmidt reaction and screened for anti-oxidant activity using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay (Figure 2).

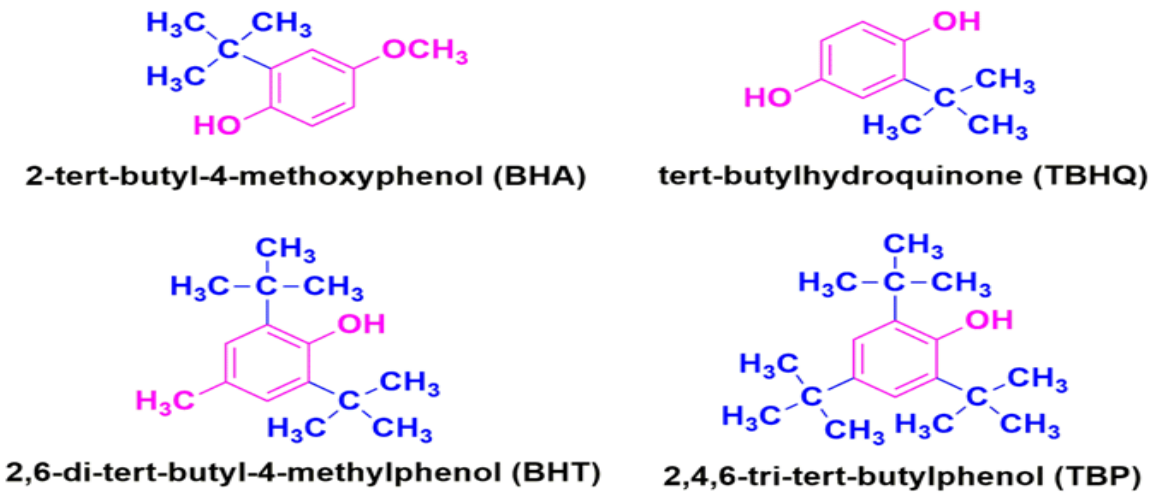


Figure 1: Examples of some common marketed synthetic anti-oxidant products with an active tert-butyl group.

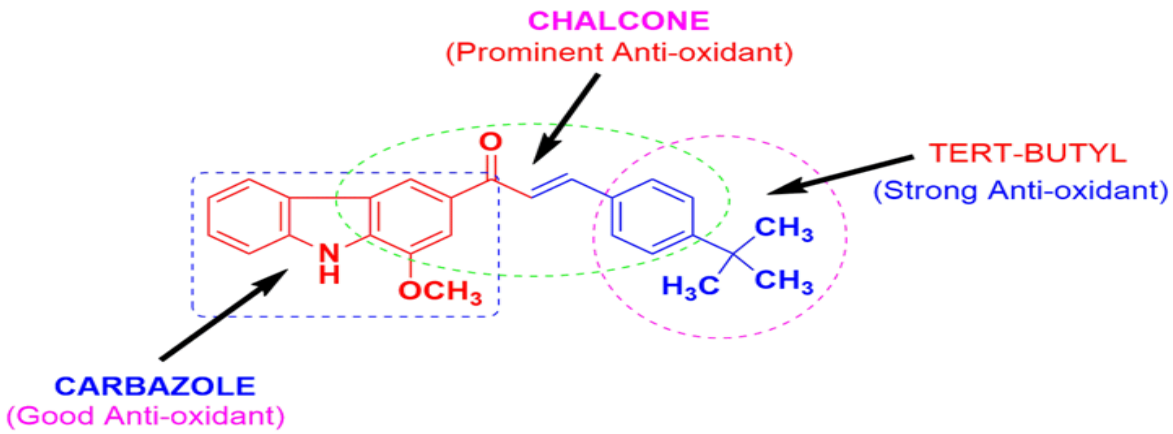


Figure 2: The rational for the designing of the novel tert-butyl containing murrayanine-chalcone compound

Materials and Methods

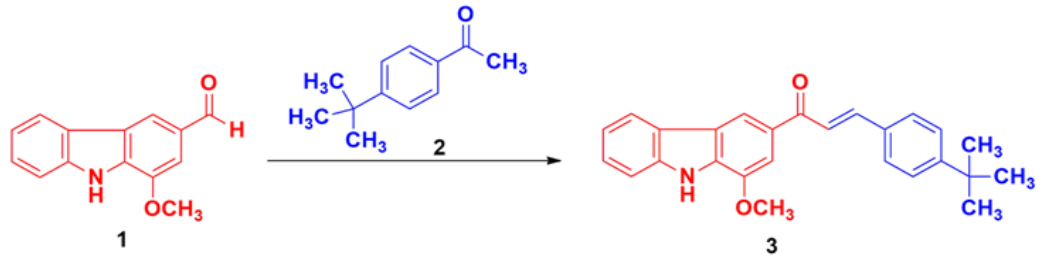
Chemicals and Instrumentation

The substrate was obtained by extraction from the *M. koenigii* powdered stem bark by n-hexane based silica gel-based column chromatography as per our previously developed method [16]. The reactant 1-(4-(tert-butyl) phenyl) ethanone (CAS Number 943-27-1) was acquired from Sigma Aldrich, Germany through a local vendor at Nagpur. The compound was characterized by Fourier-transformed Infrared Spectroscopy recorded on a Shimadzu® IR-Affinity-1 instrument, ^{1}H (proton)-NMR Spectroscopy was performed on a Bruker Avance-II instrument, Mass Spectroscopy was carried out on a MICROMASS Q-TOF instrument, and Elemental Analysis on a PerkinElmer 2400 Analyzer. The progress of the chemical reaction was monitored on Merck® pre-coated Silica gel-G TLC plates. The double-beam Shimadzu® Ultraviolet-Visible Spectrophotometer (UV-1800, Japan) with spectral characteristics (1 nm bandwidth, 10 mm

path length, and 0.3 nm wavelength accuracy), connected with a computer was employed for anti-oxidant characterization.

Synthesis of Target Compounds

The Chalcone scaffold (3) was synthesized from Claisen-Schmidt reaction where a β-hydroxyketone function was formed through an aldol condensation mechanism. The Murrayanine (1), the starting substrate having aldehydes portion was made to react with the acetyl part of the acetophenone containing reactant (2) in the presence of ethanolic NAOH solution (Scheme 1) [17].



Scheme 1: Synthesis of tert-butyl containing murrayanine-chalcone molecule.

Synthetic Protocol for (E)-3-(4-(Tert-Butyl) Phenyl)-1-(1-Methoxy-9H-Carbazol-3-Yl) Prop-2-En-1-One

0.01 M concentration of both starting substrate, Murrayanine (1) and 1-(4-(tert-butyl)phenyl)ethanone (2) were refluxed in the presence of 20 mL sodium hydroxide aqueous solution and 25 mL 90% ethanol solution. The reaction mixture was allowed to stand for the whole night and the next day, the content was poured over crushed ice containing a few drops of dilute HCl with vigorous stirring by a glass rod. The acquired product (3) was filtered completely, washed thoroughly with cold water, and recrystallized properly [18]. 62% yield; FTIR (KBr) υ (cm^{-1}): 3287 (-NH, stretching), 3073 (C-H, aromatic), 1743 (C=O), 1670 (C=C, alkene), 1592 (C=C, aromatic), 1566 (-NH, bending), 1346 (C-N), 1177 (C-O); ^{1}H NMR (δ, ppm, CDCl3): 10.19 (9, 1H), 8.17 (12, 1H), 6.7-8.3 (Aromatic, 10H), 3.88 (1, 3H), 1.31 (17, 3H). MS: M+ 383. Anal. Calcd. For C26H25NO2: C, 81.43; H, 6.57; N, 3.65. Found: C, 80.91; H, 6.14; N, 3.51.

Table 1. Anti-oxidant potential of tert-butyl bearing murrayanine-chalcone.

Compounds	IC_{50} value (μM)
3	5.94 ± 0.81**
1	7.79 ± 0.94*
Ascorbic acid	4.32 ± 0.27

n = 3; **p<0.01 with respect to standard drug

Ant-Oxidant Screening

The ability of the compound in scavenging the DPPH radical was investigated according to the given protocol. A stock solution of 1 mg/mL of the test compound was prepared initially and then 100 μg/mL of the compound was added to the methanolic DPPH solution (0.1 μM) at equal concentration. The above mixture was incubated at room temperature for half an hour and at 517 nm wavelength, the absorbance was recorded. The IC50 values of the molecules were calculated accordingly. Ascorbic acid was utilized as the positive control [19].

Statistical Treatment

The obtained anti-inflammatory data were analyzed initially by one-way ANOVA method followed by treating with Dunnett's multiple comparison tests. The P value of <0.01 was regarded as statistically considerable.

Results and Discussion

Chemistry

The spectroscopic and elemental analysis strongly supported the formation of the tert-butyl Chalcone. The transformation of the Murrayanine into murrayanine-chalcone was ascertained from the FT-IR spectra. The formation of ketonic carbonyl moiety (at 1743 cm^{-1}) from the aldehydic carbonyl moiety, which earlier appeared at 1753 cm^{-1} in the spectra confirmed the formation of the Chalcone scaffold. Moreover, the prop-2-ene-1-one component was corroborated from 1670 cm^{-1} peak in vibrational spectroscopy and 8.17 ppm in rotational spectroscopy. The presence of carbazole portion in the compound was substantiated from the FT-IR and NMR spectra. The NH stretching and bending of the carbazole were noticed at 3287 cm^{-1} and 1566 cm^{-1}, respectively. The ^{1}H-NMR spectra represented amide part from the peak at 10.19 ppm. In addition to it, C-N part of the carbazole was authenticated by FT-IR spectra at 1346 cm^{-1}. Additionally, the verification of methoxy group at carbazole moiety was performed from both FT-IR and NMR spectra. The C-O component was located from FT-IR spectra at 1177 cm^{-1} whereas the NMR spectra showed proton peak at 3.88 ppm. The tert-butyl part at B-ring was made sure from the NMR spectra at 1.31 ppm.

The appearance of beak peak corresponding to the molecular mass of the proposed structure (M+ 383) confirmed the formation of the benzylidene acetophenone scaffold. In the mass spectra, the fragment peaks in the range between 100-150 of m/z

was observed. However, no such degradation of the Chalcone into low molecular weight products was seen from the mass spectra. Furthermore, the practically estimated ratio of carbon, hydrogen, and nitrogen and its close resemblance with the theoretical values proved the formation of tert-butyl bearing murrayanine-chalcone.

Anti-oxidant activity

The anti-oxidant potential of the Chalcone is based on the ability of the compound to reduce the ferric form into ferrous form. The tested molecule presented a tremendous radical scavenging activity as evidenced by the IC_{50} value of 5.94 μM. The higher activity of the novel compound may be predicted from the synergistic potentials of the carbazole, Chalcone, and tert-butyl group. The study represented a better scavenging reduction activity than that of the parent molecule Murrayanine, which showed IC_{50} value of 7.79 μM. Therefore, the existing study rejuvenated the approach of hybridization of multiple dynamic scaffolds of similar biological activity and potency.

Conclusion

This innovative research presented a very rational approach to the successful development of potential anti-oxidant compounds, which will have a remarkable clinical perspective. The Chalcone based product obtained by hybridization of multiple dynamic scaffolds of similar biological activity and potency will positively open new avenues of applied research. From the results, it can surely be concluded that the molecule can be used for reducing the oxidative stress imposed by the reactive free radicals, thereby preventing the cancer, nephritic disorders, neurological disorders, cardiac complications, Alzheimer's disease, metabolic syndromes, etc. In addition, due to the presence of tert-butyl function which marketed anti-oxidant products do have, it may be predicted that the synthesized compound will also find application in the preservation of formulations, food products, and neutraceuticals. The current study will positively influence, motivate, and incline millions of global researchers in developing similar therapeutically active low-molecular-weight ligands and their translated pharmaceutical products for disease prevention and further utility.

Acknowledgment

Authors are highly thankful to Savitribai Phule Pune University, Pune, Maharashtra, India for providing research grants (Grant No. 13PHM000126).

Conflict Of Interest

No conflict of interest declared.

References

1. Mahapatra DK, Bharti SK. Handbook of Research on Medicinal Chemistry: Innovations and Methodologies. 1st Edition. 2017; Apple Academic Press: New Jersey.
2. Mahapatra DK, Bharti SK. Drug Design. New Delhi: Tara Publications Private Limited, 2016.
3. Shivhare RS, Mahapatra DK, Nair RR, Deshmukh SN. Schiff's base derivatives of Murrayanine demonstrated enhanced anti-oxidant activity than its parent moiety. Indian J Pharm Edu Res .2016; 50(4): 9-15. DOI: 10.5530/ijper.50.4.12
4. Mahapatra DK, Shivhare RS. Synthesizing an anti-oxidant principle 2-(((1-methoxy-9H-carbazol-3-yl) methylene) Amino) Isoindoline-1, 3-Dione from N-aminophthalimide and Murrayanine. Inventi Med Chem. 2017; 2017(4): 1-3.
5. Bashir M, Bano A, Ijaz AS, Chaudhary BA. Recent developments and biological activities of N-substituted Carbazole derivatives: A Review. Molecules. 2015; 20(8): 13496-13517. doi: 10.3390/molecules200813496
6. Fang L, Chen M, Liu Z, Fang X, Gou S, Chen L. Ferulic acid–carbazole hybrid compounds: Combination of cholinesterase inhibition, antioxidant and neuroprotection as multifunctional anti-Alzheimer agents. Bioorg Med Chem. 2016; 24(4): 886-893. doi: 10.1016/j.bmc.2016.01.010
7. Pannala P, Reddy PN, Subba Reddy BV, Misra S, Dhevendar K, Jadav SS. Synthesis, Molecular Docking, in vitro Anti proliferative and Antioxidant Activity of Novel Pyrrolidinyl-Carbazole Derivatives. Curr Org Synth. 2017; 14(8): 1172-1179. doi:10.2174/1570179414666170606120934
8. Nguyen MA, Mungara AK, Kim JA, Lee KD, Park S. Synthesis, Anticancer and Antioxidant Activity of Novel Carbazole-based Thiazole Derivatives. Phosphorus Sulfur Silicon Relat Elem. 2015; 190(2):191-199. doi: 10.1080/10426507.2014.914933
9. Thiratmatrakul S, Yenjai C, Waiwut P, Vajragupta O, Reubroycharoen P, Tohda M, Boonyarat C. Synthesis, biological evaluation and molecular modeling study of novel tacrine–carbazole hybrids as potential multifunctional agents for the treatment of Alzheimer's disease. Eur J Med Chem. 2014; 75:21-30. doi: 10.1016/j.ejmech.2014.01.020
10. Mahapatra DK, Bharti SK, Asati V. Anti-cancer Chalcones: Structural and molecular targets perspectives. Eur J Med Chem. 2015; 98:69-114. doi: 10.1016/j.ejmech.2015.05.004
11. Mahapatra DK, Bharti SK, Asati V. Chalcone derivatives: Anti-inflammatory potential and molecular targets perspectives. Curr Top Med Chem. 2017; 17(28): 3146-3169. doi: 10.2174/1568026617666170914160446
12. Mahapatra DK, Asati V, Bharti SK. Chalcones and their role in management of diabetes mellitus: Structural and pharmacological perspectives. Eur J Med Chem. 2015; 92: 839-865. doi: 10.1016/j.ejmech.2015.01.051
13. Mahapatra DK, Bharti SK, Asati V. Chalcone scaffolds as anti-infective agents: Structural and molecular target perspectives. Eur J Med Chem 2015; 101:496-524. doi: 10.1016/j.ejmech.2015.06.052
14. Mahapatra DK, Bharti SK. Therapeutic Potential of Chalcones as cardiovascular agents. Life Sci. 2016; 148: 154-172. doi: 10.1016/j.lfs.2016.02.048
15. Murakami Y, Kawata A, Katayama T, Fujisawa S. Anti-inflammatory Activity of the Artificial Antioxidants 2-Tert-butyl-4-methoxyphenol (BHA), 2, 6-Di-tert-butyl-4-methylphenol (BHT) and 2, 4, 6-Tri-tert-butylphenol (TBP), and their Various Combinations. In vivo. 2015; 29(2): 197-206.

16. Mahapatra DK, Das D, Shivhare RS, Borkar SS. Murrayanine-hydantoin and -thiohydantoin analogs as promising anti-convulsant agents: Synthesis, Characterization and Molecular Docking Studies. MOJ Bioorg Org Chem 2018; 2(2): 47-51.
17. Mahapatra DK, Dadure KM, Shivhare RS. Exploring the Site-Specific Influence of Hydroxyl group in Ring-B of Murrayanine-Chalcone on Edema Reducing Potential. MOJ Drug Design Devel Ther. 2018; 2(4): 191-193. doi: 10.15406/mojddt.2018.02.00046
18. Mahapatra DK, Shivhare RS. Anti-microbial Perspective of a Chalcone, (E)-1-(1-methoxy-9H-carbazol-3-yl)-3-(4-(methylsulfonyl) phenyl) prop-2-en-1-one: Fabrication of a Hybrid by Unification of a Natural Product with a Synthetic Component. Int J Agri Life Sci. 2018; 4(2): 236-240.
19. Kamble MA, Mahapatra DK, Dhabarde DM, Ingole AR. Pharmacognostic and pharmacological studies of Bombax ceiba thorn extract. J Pharm Pharmacog Res. 2017; 5(1): 40-54.