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Original Article

In-vitro Antioxidant Activity of Hydroalcoholic Extract of Leaves of Colocasia Esculenta Linn

Kalariya Manisha V.*, Prajapati Rakesh P., Sheth Navin R.*****

Abstract

Context: Although *Colocasia esculenta* Linn. (Araceae) commonly known as patarveliya in Guajarati possesses several medicinal properties, a little is known about its antioxidant activity. **Objective:** The current research was designed to examine the antioxidative potential of hydroalcoholic extract of leaves of *Colocasia esculenta* (HECE) for the first time using several *in-vitro* analytical methods. **Materials and methods:** The antioxidant activity of HECE was evaluated using reducing power ability, nitric oxide scavenging assay and the estimation of total phenolic contents. **Results and Discussion:** The HECE gave an IC50 value of 45.75±0.38 µg / ml. The reducing power was investigated by Fe3+-Fe2+ transformation in the presence of extract tested using ascorbic acid as standard. The HECE showed increase in reducing ability with increase in concentration at 700nm. The total antioxidant capacity by nitric oxide scavenging method is expressed as curcumin equivalents. The content of total phenolics in HECE was found to be 49.21 ìg gallic acid equivalent / mg. **Conclusions:** Based on the above results, the higher the phenolic content, the higher the antioxidant capacity was very well observed with HECE. Hence the dried leaves of *C. esculenta* could be considered for preparation of neutraceuticals with potent antioxidant effect suitable for prevention of human disease. The results obtained in the current study indicate that HECE is a potential source of natural antioxidants.

Keywords: Antioxidant activity, *Colocasia esculenta*, Nitric oxide scavenging assay, Reducing power ability, Total phenolic contents

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Introduction

Natural products have been our single most successful source of medicines. Each plant is like a chemical factory capable of synthesizing unlimited number of highly complex and unusual chemical substances whose structures could otherwise escape the imagination forever. Although clinical trials and experiments involving whole animals are important in natural product screening but because of financial, ethical and time limitations, importance of *in-vitro* screening is gaining popularity [43]. Oxidative modifications of DNA, proteins, lipid, and small cellular molecules by reactive oxygen species (ROS) play a role in a wide range of common diseases and age-related degenerative conditions [5]. These include cardiovascular disease, inflammatory conditions, and neurodegenerative disease such as Alzheimer's, mutations, and cancer [3, 21, 37, 8, 24, 28]. Furthermore, antioxidants are also believed to play a cardinal role in the oxidative deterioration of cosmetics, foodstuffs, and pharmaceutical preparations. There is an increasing interest in natural antioxidants, namely polyphenols, present

in medicinal and dietary plants that might help prevent oxidative damage. The antioxidant activity of several plant materials has recently been described [42, 30, 39, 6, 11, 19, 27, 12] and a number of plant products, including polyphenols, flavonoids, and terpenes, exert an antioxidant action.

C. esculenta Linn. (Araceae) is widely distributed throughout India. Poi, a starchy paste produced from *C. esculenta* showed the Anti-Cancer effects on colonic adenocarcinoma cells *in vitro* [7]. Ethanolic extract of leaves showed an inhibitory effect on leukocyte migration and a reduction on the pleural exudates as well as reduction on the granuloma weight in the cotton pellet granuloma method thus showed anti-inflammatory activity [41]. Cyanoglucoside extract from *C. esculenta* showed hypoglycemic activity [18]. Arabinogalactan, dietary fiber from *C. esculenta* showed hypolipidaemic effect by decreasing hepatic production of VLDL [4]. Tarocystatin protein from *C. esculenta* showed strong antifungal activity on some ubiquitous phytopathogenic fungi [50]. Methanol and aqueous crude extract of different parts of *C. esculenta* showed antimicrobial activity against one or more species of bacteria [49]. Leaf juice is stimulant, expectorant, astringent, appetizer, and otalgia. The juice expressed from the leaf stalks are used with stalks is used with salt as an absorbent in cases of inflamed glands and buboes.

Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are commonly used in processed foods, are known to have toxic and carcinogenic effects on human health [23, 29, 31]. Therefore, screening of plant species to identify new antioxidants has become critically important in recent years. There are so far no reports related to antioxidant activity of HECE. The purpose of this study was to determine the antioxidant capacity and phenolic contents of HECE.

Methods

Plant Material

Leaves of *C. esculenta* were purchased from local market. The plant was identified and authenticated by Botanical Survey of India, Jodhpur. A voucher specimen (SU / DPS / Herb / 05) of the same has been deposited in the Department of Pharmaceutical Sciences, Saurashtra University for the future reference.

Chemicals

All chemicals were analytical grade and chemicals required for all biochemical assays were obtained from Sigma Chemicals Co., USA.

Preparation of crude extract

Leaves were dried in shade, moderately grinded by electric grinder. The powdered materials (leaves) were subjected to qualitative tests for the identification of various phytoconstituents like alkaloids, glycosides, steroids, terpenoids and flavanoids. Then the powder was macerated with hydroalcoholic solvent (ethanol: water - 50:50) for 7 days with intermittent shaking. On 8th day the macerate was filtered through muslin cloth and solvent was removed under reduced pressure and the hydroalcoholic extract was then obtained (yield-9.8% w / w). The extract was stored at 5 ºC until use.

Phytochemical screening

Preliminary phytochemical investigations conducted as per the procedures described by Kokate [26] and Trease and Evans [48].

Reducing power ability

The reducing power capacity of the extracts was assessed as described by Oyaizu [33]. The Fe2+ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. One ml of the extract (20-100 ìg / ml), 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide were incubated at 50°C for 30 min and 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged for 10 min at 3000g. About 2.5 ml of the supernatant was diluted with 2.5 ml of water and is shaken with 0.5 ml of freshly prepared 0.1% ferric chloride. The absorbance was measured at 700 nm. Ascorbic acid was used as the standard. All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

Nitric oxide scavenging method

Nitric oxide generated from sodium nitroprusside was measured by the Griess reagent by the method described by Rao [35]. Various concentration of the extract and sodium nitroprusside (10 mM) in phosphate buffer saline and 150 µl of each dose level

by dilution with methanol was incubated at room temperature for 15 min. After the incubation period, 5 ml of Griess reagent (1% sulphanilamide, 2% o-phosphoric acid, 0.1% napthyl ethylenediamine dihydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm using UV-visible spectrophotometer, shimadzu, UV-1700, Japan. The inhibition of nitric oxide generation was estimated by comparing the absorbance values of control with that of treatments. Curcumin was used as standard values are reported as mean ± SEM of three determinations.

Estimation of total phenolic content

Total soluble phenolics of the extract were determined with Folin-Ciocalteu reagent using Gallic acid as the standard [29]. An aliquot of 0.1 ml suspension of 1 mg of the extracts in water was totally transferred to a 100 ml volumetric flask and the final volume was adjusted to 25 ml by the addition of distilled water. Folin-Ciocalteu reagent (1 ml) was added to this mixture, followed by 4 ml of 20% sodium carbonate 5 min later. Subsequently, the mixture was shaken for 30 min at room temperature and the absorbance was measured at 760 nm using UV-visible spectrometer shimadzu, UV-1700, Japan. The concentration of total phenolic compounds in the extracts was determined as ìg gallic acid equivalent by using the standard gallic acid graph.

Statistical analysis

All results are expressed as mean ± S.E.M. Linear regression analysis (Origin 6.0 version) was used to calculate the IC50 values.

Results

Phytochemical investigations

Preliminary phytochemical tests of HECE show the presence of glycoside, phytosterol, phenolic compounds, saponin and flavonoids as predominant active constituent.

Reducing power ability

Antioxidants have an important role in preventing a variety of diseases and aging because they inhibit or delay the oxidation process by blocking the initiation or propagation of oxidizing chain reactions [1, 44]. The reducing ability of a compound generally depends on the presence of reductants [34], which have exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom [17]. The presence of reductants in the extracts causes the reduction of the Fe3+-ferricyanide complex to the ferrous form. Therefore, the Fe2+can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Figure 1 shows reducing capacities of HECE

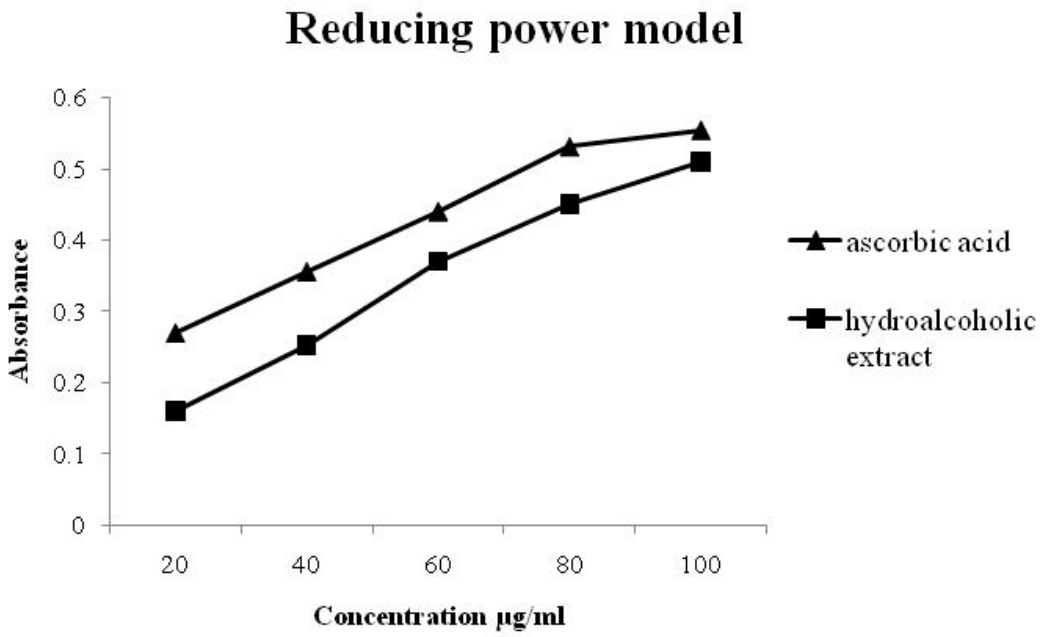

Fig.1: Antioxidant activity of different concentrations of hydroalcoholic extract and ascorbic acid in reducing power method. Each value represents mean ± SEM

compared with ascorbic acid. The reducing power of HECE and standards increased with increasing concentration of samples. The leaf extract showed the highest reducing ability. However, the activity was less than the standard, ascorbic acid.

Nitric oxide scavenging method

Active oxygen species and free radicals are involved in a variety of pathological events. In addition to ROS, nitric oxide is also implicated in inflammation, cancer and other pathological conditions. A potential determination of oxidative damage is the oxidation of tyrosine residue of protein, peroxidation of lipids, and degradation of DNA and oligonucleosomal fragments. Nitric oxide or reactive nitrogen species formed during its reaction with oxygen or with superoxide such as NO2, N2O4, N3O4, nitrate and nitrite are very reactive. These compounds alter the structure and function of many cellular 81 components. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this damage. IC50 value of leaves extract was found to be 45.75 ± 0.38 ìg / ml (Table 1).

Table 1: Comparison of IC50 values of hydroalcoholic extract with standard in nitric oxide scavenging method

Model	IC50 value of HECE (µg / ml)	IC50 value of standard (µg / ml)
Nitric oxide scavenging method	45.75 ± 0.38	28.25 ± 1.07

Total phenolic content

The amount of total phenolic compounds was investigated in HECE. Phenols are very important plant constituents because of their scavenging ability which is due to their hydroxyl groups [13]. The total amount of phenolic compounds in the plant extracts was determined as micrograms of gallic acid equivalent by using an equation that was obtained from standard gallic acid graph. HECE (1 mg) was equivalent to 49.21 ìg gallic acid.

Discussion

There are many reports that support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases [32, 38, 46]. Many synthetic antioxidant components have also shown toxic or mutagenic effects, which have shifted attention toward the naturally occurring antioxidants. Numerous plant constituents have been proved to show free radical scavenging or antioxidant activity [2, 45]. In this respect, flavonoids and other polyphenolic compounds have received greatest attention. Plant tissues contain a network of compounds that control the level of reactive oxygen species, including phenolic compounds, vitamins C and E, glutathione, and several enzymes. Phenolic compounds widely distributed in the natural plant tissues include flavonoids, tannins, hydroxycinnamate esters, and lignins [36]. Furthermore, interest in employing antioxidants from natural sources to increase the shelf life of foods is considerably enhanced by consumer preference for natural ingredients and concerns about the toxic effects of synthetic antioxidants [15, 40, 47]. *C. esculenta* seems to be a good source of plant species containing large amounts of flavonoids and phenolic compounds, so it is considered to be a promising source of natural antioxidants [25].

In the current study, the antioxidant activities of HECE were determined by using different antioxidant tests. It has been reported that reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity [51, 9]. HECE exhibited comparatively similar reducing power as ascorbic acid suggesting that it had strong electron-donating capacity.

Nitric oxide is a free radicals product in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases [22]. In the present study the nitrite produced by the incubation of solutions of sodium nitroprusside in standard phosphate buffer at 25ÚC was reduced by the HECE. This may be due to the antioxidant principles in the extract which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite.

It has also been reported that the antioxidant activity of plant materials was well correlated with the content of their phenolic compounds [14, 16, 10].

Estimation of total phenolics using the Folin-Ciocalteu reagent and gallic acid as a standard revealed that *C. esculenta* is a good source of polyphenol.

Conclusion

On the basis of the results obtained in the current study, we conclude that the HECE possesses high antioxidant activities that might be helpful in preventing or slowing the progress of various oxidative stress–related diseases. The HECE studied here could also be considered as good candidates for food preservation or functional foods, as well as for pharmaceutical and natural plant–based products. Further investigations on the isolation and identification of antioxidant components in the plants may lead to chemical entities with potential for clinical use.

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Original Article

Synthesis and Characterization of Schiff and Mannich bases of Isatin and Evaluation of their Pharmacological Activity

Mohammad Shahnaz*, Pooja Joshi, D. N. Prasad***, Dhruv Dev****, Jyoti Parkash*****

Abstract

Objective: The aim of present work is to synthesize a novel series of (Isatin) 1H-indole-2, 3-diones derivatives and evaluate its antioxidant activity. The structure of these compounds was established on basis of NMR data and IR data. **Introduction**: Isatin (1H-indole-2, 3-dione) is a synthetically versatile substrate, where it can be used for the synthesis of large variety of heterocyclic compounds, such as Indoles and quinolones. Isatin modify can considered as an important pharmacophore in the field of medicinal chemistry which can be used for conjugating it with other bioactive molecules such as antibacterial, antifungals, anticonvulsants and antiviral agents due to its potent pharmacological activity. Looking at the pharmacological potential of isatin we thought it worthwhile to synthesize and characterize some Schiff's and Mannich bases of isatin. Isatin and its derivatives undergo nucleophilic attack at position C-3. The chemo selectivity of these reactions depends on the nature of the substituents attached to the isatin nucleus, and especially of those bonded to the nitrogen atom, as well upon solvent and temperature employed. **Chemistry:** Isonitrosoacetanilide was prepared from aniline by treating aniline with chloral hydrate and hydroxylamine hydrochloride and from Isonitrosoacetanilide, indole-2, 3-dione was prepared in presence of conc. Sulphuric acid. Then various derivatives of indole-2, 3-diones were prepared by treating indole-2, 3-dione with various amines in presence of glacial acetic acid and formaldehyde to yield Schiff's and Mannich bases. The structure of synthesized compounds were confirmed by chromatographic and spectral analysis **Antioxidant activity:** The synthesized compounds were then evaluated for their Free radical scavenging activity by the DPPH assay method at 10, 20, 30, 40, 50 µg/ml concentrations of ligands & standard. Ascorbic acid was used as the standard. The data obtained were analyzed and results were expressed as mean absorbance ± standard error mean for each compound. The results of the pharmacological screening indicated that Compound ISS-2 shows more significant Free radical scavenging activity in comparison to other ligands and compounds ISS-1, ISS-5 shows moderate antioxidant activity and compounds ISS-4 and ISS-3 shows less antioxidant activity. **Conclusion:** The compounds synthesized were then characterized using various spectroscopic techniques i.e.IR, 1H-NMR. The spectroscopic studies showed spectral data confirmed the formation of new compounds. The synthesized compound possesses significant antioxidant activity.

Keywords: Isatin, antioxidant, Manichh and Schiff base, DDPH assay, ascorbic acid.

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Introduction

The term antioxidant originally was used to refer specifically to a chemical that prevented the

consumption of oxygen. In the late 19th and early 20th century, extensive study was devoted to the uses of antioxidants in important industrial processes, such as the prevention of metal corrosion, the vulcanization of rubber, and the polymerization of fuels in the fouling of internal combustion engines .An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplementation may be harmful.

Kinds of Antioxidants

Natural antioxidants:

- Tocopherols (delta>gamma>beta>alpha)
- Nordihydroguaretic Acid (NDGA)
- Sesamol
- Gossypol

Synthetic antioxidants:

- Butylated Hydroxy Anisole (BHA)
- Butylated Hydroxy Toluene (BHT)
- Propyl Gallate (PG)
- Tertiary Butyl Hydroquinone (TBHQ)

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid per oxidation. These compounds may be synthesized in the body or obtained from the diet. The different antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione or ubiquinone mostly present within cells, while others such as uric acid are more evenly distributed. Some antioxidants are only found in a few organisms and these compounds can be important in pathogens and can be virulence factors. In general, antioxidant systems either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell. However, since reactive oxygen species do have useful functions in cells, such as redox signaling, the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level.

The role of antioxidants in health

The process of aging and degenerative diseases such as cancer, cardiovascular disease, blood vessel blockage that includes hiperlipidemik, atherosclerosis, stroke and high blood pressure and disrupted his body's immune system can be caused by oxidative stress.

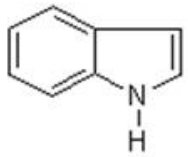
Oxidative stress is a state of balance and its total oxidant in the body. In this condition, the molecular activity of free radicals or reactive oxygen species (ROS) can cause cellular and genetic damage. Nutrient deficiencies and the existence of Xenobiotic compounds from food or too polluted environment will worsen the situation.

When the Japanese public generally or some Asian communities rarely have problems with a variety of degenerative diseases, this is due to its healthy menu of traditional rich nutrients and bioactive components. These substances have the ability as an antioxidant, which plays an important role in inhibiting the oxidation of chemical reactions, which can damage macro-molecules and can cause various health problems.

Indole and its Derivatives

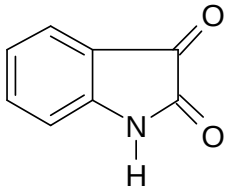
The word Indole (I) is coined from the word India, a blue dye imported from India known as Indigo. Bayer first prepared it in 1866 by zinc distillation of ox-indole. The I.U.P.A.C name of indole is 1H-benzo[b] pyrrole. Indole is a planar molecule with a conjugated system of 10 electrons. It exists in resonance form with resonance energy of 47-49 K cal / mole. It is a very weak base with pKa value 3.63. In structure a, b, and d the benzenoid 6-p system is preserved. The electrophilic attack results at 3rd position. Presence of high electron density at 3rd position has been also supported by the calculation of p electron density and by molecular orbital method.

Isatin (1H-indole-2,3-dione) (VIII) is considered as synthetically versatile molecule due to its indole-2,3-dione moiety. It was first obtained in 1841 by **Erdman**



Indole/1-H Benzopyrrole (I)

and **Laurent** as a product from the oxidation of indigo by nitric acid or chromic acid.



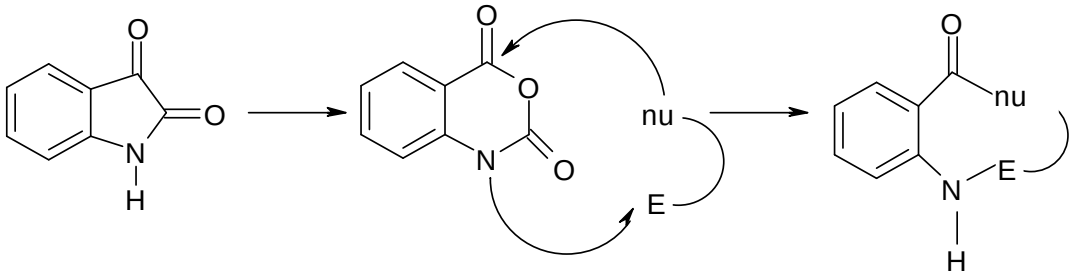
(1H-indole-2, 3-Dione) (VIII)

It forms red needles melting at 200-201°C and readily undergoes clean aromatic substitution reaction at C-5, N-alkylation via anions, and ketonic reaction at the C-3 carbonyl groups. If the 5th position is already occupied then electrophile takes the 7th position. The carbonyl group at position 2 is adjacent to the hetero atom and is stabilized by resonance. Thus it behaves as typical amide in its properties. It can be recrystallized from the hot water or ethanol. Although isatin with substituent attached to the aromatic ring are usually obtained from the corresponding functionalized anilines, they can be synthesized by electrophilic aromatic substitution too. Many synthetic methodologies have been described for the conversion of isatin to other heterocyclic systems. The chemistry of isatin can be generalized as one of the following strategies:

a) Partial or total reduction of the heterocyclic ring, leading to indoles and its derivatives.

b) Oxidation of the heterocyclic ring. For example, conversion of isatin to isatoic anhydride with subsequent conversion to other heterocyclic systems.

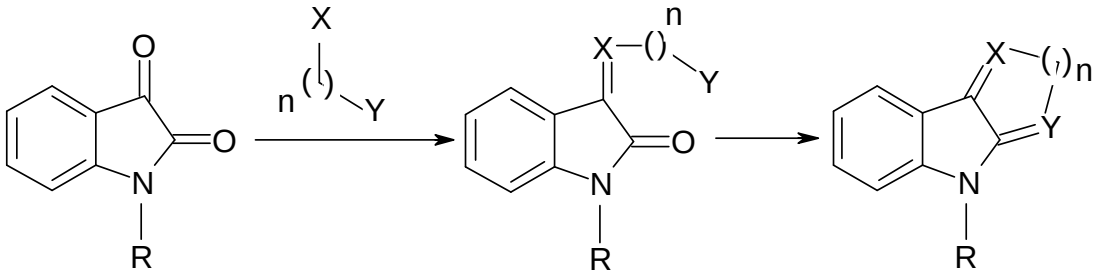
c) Nucleophilic addition at position C-3, which may be further followed by a cyclization process,



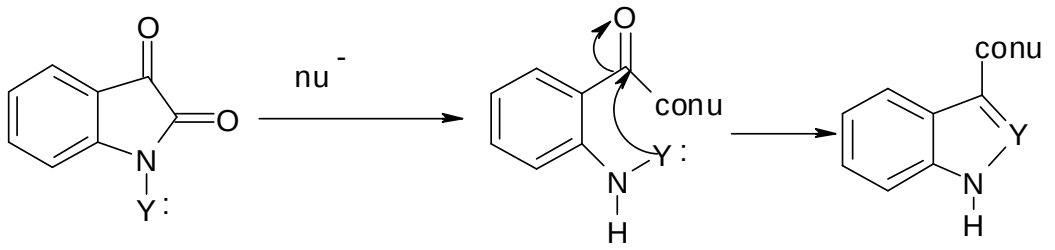
Isatoic anhydride

with or without N1-C2 bond cleavage, or by a spiro-annelation at position C-3.

d) Nucleophilic substitution at position C-2, leading to the opening of the heterocyclic ring. This



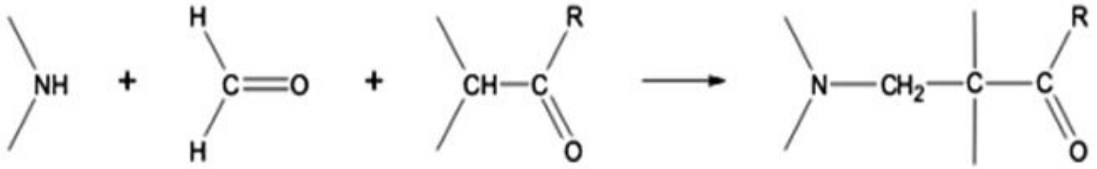
process may be followed by an intramolecular or by an intermolecular exotrig cyclization.



selectivity of these reactions depends on the nature of the nucleophile, on the nature of the substituents attached to the isatin nucleus, and especially of those bonded to the nitrogen atom, as well as upon the solvent and temperature employed. The initial products obtained can suffer further reaction in the presence of a second nucleophilic group to give cyclization products. For didactic reasons, these reactions have been sorted by the nature of the nucleophile.

Schiff Reaction

A **Schiff base** (or **Azomethine**), named after Hugo Schiff, is a functional group that contains a carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group, but not hydrogen.



an amine to a carbonyl group followed by elimination of a hydroxyl anion to the Schiff base. The Schiff base is an electrophile which reacts in step two in a second nucleophilic addition with a carbanion generated from a compound containing an acidic proton.

Material and Methods

All of the chemicals were procured from CDH, Acros and Himedia. Melting point (m.p) was recorded on Veego melting point apparatus and is uncorrected. Infra red (IR) spectra were taken using FTIR thermo Scientific; NICOLET Is10, KBR disk spectrophotometer. The 1HNMR spectra were recorded on sophisticated multinuclear FT-NMR spectrometer model Avance-II (Bruker) 400 NMR Spectrometer, using DMSO-d6 solvent. Chemical

Isatin and its derivatives can suffer nucleophilic attack at positions C-2 and/or C-3. The chemo

Schiff bases are of the general formula R1, R2, C=N-R3, where R3 is an aryl or alkyl group that makes the Schiff base a stable imine. A Schiff base derived from an aniline, where R3 is a phenyl or substituted phenyl.

Mannich Reaction

The Mannich reaction is an organic reaction and consists of an amino alkylation of an acidic proton placed next to a carbonyl functional group with formaldehyde and ammonia or any primary or secondary amine. The final product is a â-amino-carbonyl compound. Reactions between aldimines and á-methylene carbonyls are also considered Mannich reactions because these imines form between amines and aldehydes.The reaction is named after Chemist Carl Mannich. The Mannich reaction is an example of Nucleophilic addition of

shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. The homogeneity of the compounds was monitored by ascending thin-layer chromatography (TLC) on silica gel G (Rankem) and activated at 110°C for 30 min. The plates were developed by exposing to iodine vapours. All reagent and solvents were purified and dried by standard techniques.

Preparation of Indole -2,3-dione (Isatin).

Method of preparation of Isonitrosoacetanilide from aniline:-

In a 250 ml round bottom flask placed 9 gm (0.05M) of chloral hydrate and 85 ml of water. To this solution added, in order 13 gm (0.18M) of crystallized

anhydrous sodium sulfate (dried in oven), a solution of aniline (0.05 M) in 30 ml of water to which 4.3 ml (0.052M) of concentrated hydrochloric acid was added to dissolve the amine and finally, a solution of 11gram (0.158M) of hydroxylamine hydrochloride in 50 ml of water. The reaction mixture was heated to 80-900C. Vigorous boiling started in about 30-35 minutes. The reaction completed after 1-2 minutes of vigorous boiling. During the heating period, some crystal of isonitrosoacetanilide separates. On cooling the solution in running water the remainder crystallizes, was filtered under suction, and air dried.

Method of preparation of isatin from isonitroso acetanilide

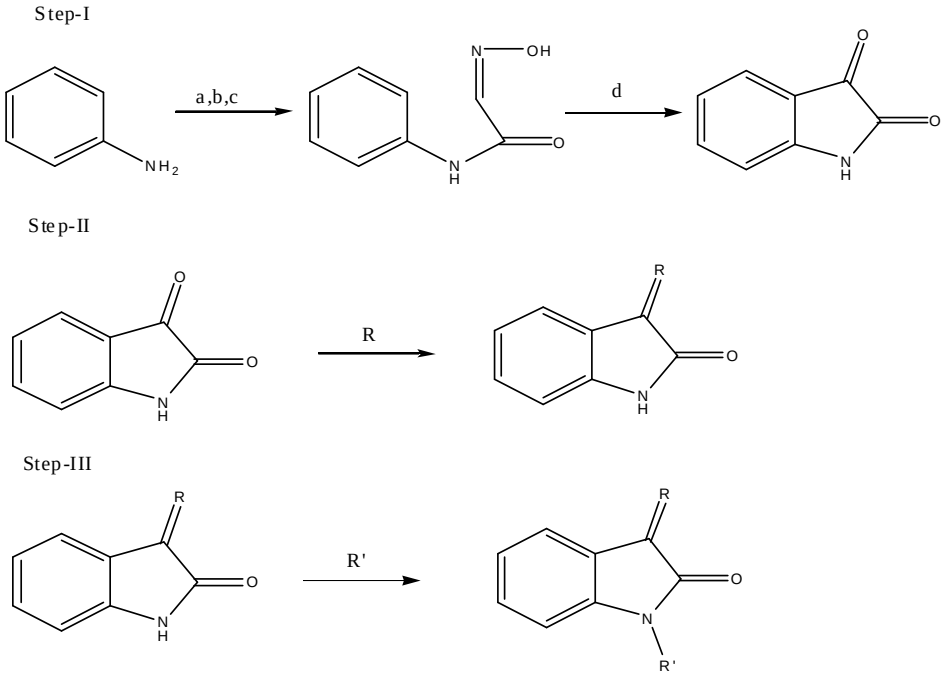
32.6 ml of concentrated sulfuric acid was warmed to 50°C in a 100 ml round bottom flask fitted with and efficient mechanical stirrer, add 7.5 gram of (0.04 M) of dry isonitroso acetanilide to such a rate that to keep the temperature 60-700C but not higher. External cooling should be applied at this stage so that the reaction can be carried out more rapidly. After the addition of the isonitrosoacetanilide compound was finished, the solution was heated to 80°C and kept at this temperature for about 10 minutes to complete the reaction. Then the reaction mixture was cooled to room temperature and poured in to 10-12 times of its volume of ice. After standing for about one and half hour the isatin was filtered under suction, washed several times with cold water to remove the sulfuric acid, and then dried in the air. It was reprecipitated by dissolving in 10 % sodium hydroxide solution followed by acidification with dil. HCl to get dark red colored product which on cooling was filtered, washed with water and dried to get pure crystals melting at 209-212 0 C.

General procedure for synthesis of novel Schiff bases of Isatin

0.01mole of Isatin was dissolved in 5 ml of ethanol. To this, was added 0.01 mole of Primary amine (4-fluoroaniline). Catalytic amount of Glacial acetic acid (1 ml) was added to the above mixture and the contents were refluxed for 10 h. The resulted solution was allowed to stand overnight and the precipitated solid was filtered, washed, dried and recrysallized from ethanol to yield the Schiff bases.

General method for the preparation of Mannich bases

0.01 mole of Schiff base was dissolved in minimum amount of hot ethanol (4ml). To this, 1 ml of 40% formaldehyde was added. To the above solution, 0.01 mole of secondary amine dissolved in minimum ethanol (1ml) was introduced. The mixture was stirred for 24 h at room temperature. The solid separated was filtered, dried and recrysallized from ethanol, to yield the Mannich bases.



Antioxidant Activity

Free radical scavenging activity by DPPH assays method.

DPPH (1, 1-diphenyl-2-picryl-hydrazil) is stable free radical. Methanol solution of DPPH is used to evaluate the antioxidant activity of several synthetic compounds. Antioxidant on interaction with DPPH, both transfer electron or hydrogen atom to DPPH, thus neutralizing its free radical character and convert it to1, 1-diphenyl-2-picryl hydrazine. The degree of discoloration indicates the scavenging activity of the drug .The change in absorbance produced at 517 nm has been used as measure of its antioxidant activity.

Chemicals used

1, 1-diphenyl-2-picryl-hydrazil (DPPH)-Sigma Ltd., Ascorbic Acid-Qualigens, Methanol-Qualigens.

Preparation of DPPH solution

It was prepared by dissolving 33 mg of DPPH in 1 lit. Of methanol just before use and kept in dark amber colored bottle to protect from sunlight.

Sample preparation

Preparation of stock solution of Isatin derivatives

It was prepared by dissolving 50 mg of **Isatin** derivatives in 100 ml of methanol.

Standard preparation

Preparation of Ascorbic Acid solution

It was prepared by dissolving 50 mg of ascorbic acid in 100 ml of methanol.

DPPH Radical scavenging assay of Compd ISS-1 and Ascorbic acid.

Compd ISS-1			Ascorbic acid		
Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition	Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition
10	1.0116±0.0014	39.6	10	0.8240±0.0015	49.3
20	0.9033±0.0004	41.2	20	0.7620±0.0022	53.2
30	0.8300±0.0005	47.8	30	0.6830±0.0002	57.9
40	0.7320±0.0005	57.2	40	0.5540±0.005	65.8
50	0.5410±0.0014	64.5	50	0.4810±0.0002	70.2

DPPH Radical scavenging assay of Compd ISS-2 and Ascorbic acid

Compd ISS- 2			Ascorbic acid		
Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition	Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition
10	1.0200±0.0006	40.7	10	0.8240±0.0015	49.3
20	0.9230±0.0005	43	20	0.7620±0.0022	53.2
30	0.8900±0.0002	47.8	30	0.6830±0.0002	57.9
40	0.7270±0.0014	50.8	40	0.5540±0.005	65.8
50	0.5133±0.0006	58.9	50	0.4810±0.0002	70.2

DPPH Radical scavenging assay of Compd ISS-3 and Ascorbic acid

Compd ISS-3			Ascorbic acid		
Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition	Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition
10	1.1233±0.0004	31	10	0.8240±0.0015	49.3
20	1.0063±0.0008	37.2	20	0.7620±0.0022	53.2
30	0.8413±0.0004	47.4	30	0.6830±0.0002	57.9
40	0.7110±0.0014	55.2	40	0.5540±0.005	65.8
50	0.5310±0.0002	65.2	50	0.4810±0.0002	70.2

DPPH Radical scavenging assay of Compd ISS-4 and Ascorbic acid

Compd ISS-4			Ascorbic acid		
Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition	Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition
10	0.9850±0.0005	36.2	10	0.8240±0.0015	49.3
20	0.9116±0.0014	43.4	20	0.7620±0.0022	53.2
30	0.8336±0.0014	48.7	30	0.6830±0.0002	57.9
40	0.7813±0.0002	55.2	40	0.5540±0.005	65.8
50	0.6516±0.0014	65.2	50	0.4810±0.0002	70.2

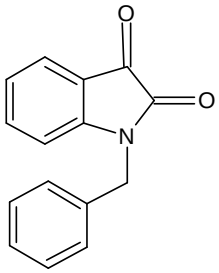
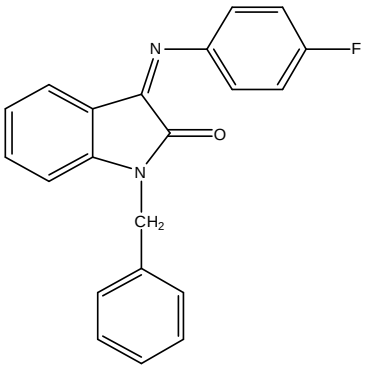
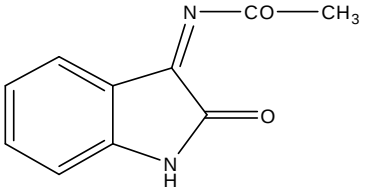
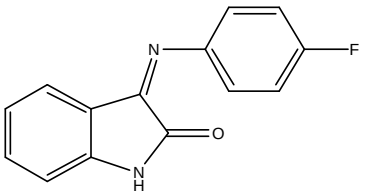
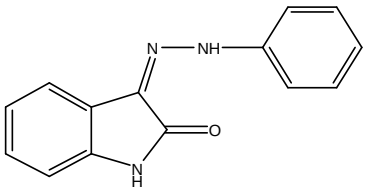
DPPH Radical scavenging assay of Compd ISS-5 and Ascorbic acid

Compd ISS-5			Ascorbic acid		
Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition	Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition
10	0.9813±0.0014	37.8	10	0.8240±0.0015	49.3
20	0.9510±0.0002	43.4	20	0.7620±0.0022	53.2
30	0.8316±0.0014	48.7	30	0.6830±0.0002	57.9
40	0.6810±0.0002	55.2	40	0.5540±0.005	65.8
50	0.5616±0.0014	65.2	50	0.4810±0.0002	70.2

Procedure

A 10, 20,30,40,50 µg / ml concentrations of ligands, and ascorbic acid were prepared. From this stock solution 1ml has been pipette out and 5ml methanol solution of DPPH was added, shaken well and the mixture was incubated at 370C for 30 min. The absorbance of all samples were measured against blank at 517 nm. The absorbance of DPPH reagent alone was taken as control (see in table 1,2,3,4,5,6). The % radical scavenging activity can be calculated following formula:

Physicochemical data for compounds

Code	Compound	M. F.	M.wt.	% Yield	M.P °C.
ISS-1		$C_{15}H_{11}NO_2$	237.25	82	156-158
1SS-2		$C_{21}H_{15}FN_2O$	330.36	84	178-180
ISS-3		$C_{10}H_6N_2O_2$	188.18	86	190-192
ISS-4		$C_{14}H_9FN_2O$	240.23	77	189-190
ISS-5		$C_{14}H_{11}N_3O$	237.26	80	198-200

Spectral Analysis Data of Synthesized Derivatives

Compound	Spectral Peaks (cm^{-1}) and Peak Characteristics
ISS-1	2922.15Ar C-H Stretching, 2851.26 Alkane C-H Stretching, 1594.94-1432.42, Ar C=C Stretching, 1739.60-C=O Stretching, 1677.27-C=O Stretching , 1349.48C-N Bending, 731.19 Oop(out of plane bending) H NMR 4.9 (2 H, N-CH_2), 6.7-7.6 (9 H, Ar-H)
ISS-2	3087.87 Ar C-H Stretching, 2839.49 Alkane C-H Stretching, 1590.98-1499.18 Ar C=C Stretching, 1612.78 -C=N Stretching, 1336.06 C-N Bending, 1728.78 -C=O Stretching, 1213.98 C-F, 754.81 Oop H NMR 4.9 (2 H, N-CH_2), 6.9-7.5 (13H, Ar-H)
ISS 3	3441.71 -N-H Stretching, 3177.70 Ar C-H Stretching, 3057.55 Alkane C-H Stretching, 1555.92-1462.74 Ar C=C Stretching, 1728.24 -C=O Stretching, 1685.47 -C=O Stretching, 1616.03 C=N Stretching 1331.92 C-N Bending, 746.79 Oop H NMR 3.3(1H,CH_3), 6.8-7.4 (4H, Ar-H)
ISS 4	3446.67 -N-H Stretching, 3063.03 Ar C-H Stretching, 2922.26 Alkane C-H Stretching, 1592.89-1467.15 Ar C=C Stretching, 1735.91 -C=O Stretching, 1611.28 -C=N Stretching, 1241.46 C-F, 1331.92 C-N Bending, 746.79 Oop H NMR 3.83(8 H, Ar-H), 8.29(1H,NH)
ISS 5	3432.16 -N-H Stretching, 3030.11 Ar C-H Stretching, 2783.50 Alkane C-H Stretching, 1494.39-1470.64 Ar C=C Stretching, 1732.10 -C=O Stretching, 1612.67 -C=N Stretching, 1348.97 C-N Bending, 753.96 Oop H NMR 6.9-7.5(9H, Ar-H), 10.9 (1H,NH), 12.8 (1H,NH)

%free radical = Absorbance of control - Absorbance of sample X 100 Scavenging activity Absorbance of control And calculated IC50 value

Results and Discussion

The synthesized compounds of Isatin (ISS-1 to ISS-5) showed diversified antioxidant activity. A series of mannish base and Schiff base of derivatives of Isatin were synthesized by mannich reaction and Schiff reaction with different secondary amines and primary amines and formaldehyde. The antioxidant activity was evaluated by the free radical scavenging activity by DPPH assay method. Comp ISS-2 showed more significant free radical scavenging activity when compared with that of standard drug i.e.

Ascorbic acid. Comp ISS-1 and Comp ISS-5 showed significant free radical scavenging activity when compared with that of standard drug i.e. Ascorbic acid. Comp ISS-3 and Comp ISS-4 showed less free radical scavenging activity when compared with that of standard drug i.e. Ascorbic acid. DPPH (1, 1-diphenyl-2-picryl-hydrazil) is stable free radical. Methanol solution of DPPH is used to evaluate the antioxidant activity of several synthetic compounds. Antioxidant on interaction with DPPH, both transfer electron on hydrogen atom to DPPH, thus neutralizing its free radical character and convert it to 1, 1-diphenyl-2-picryl hydrazine. The degree of discoloration indicates the scavenging activity of the drug.

Conclusion

The five compounds were synthesized with the standard chemicals and procedure. The compounds were characterized through their respective IR, ^{1}H NMR, UV and TLC. The compound member ISS-1 and ISS-3 show promising antioxidant activity

Acknowledgement

The author wishes to acknowledge the principal Shivalik college of Pharmacy, Nangal for providing the chemicals and Labs and SAIF, Punjab University Chandigarh for Providing IR and NMR data's.

Conflict of Interest

The author does not have any conflict of interest.

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Original Article

On-resin Intramolecular Chemoselective Oxime Bond Formation to Cyclic Peptides

Jaya T. Varkey*

Abstract

The synthesis of a peptide containing an oxime bridge performed on solid-phase is described. The strategy used takes advantage of selective acidolytic removal of Boc and acetal protecting while minimally cleaving a PAL anchor, as well as compatibility of PEG-PS resin supports with aqueous conditions for oxime formation.

Key words: cyclic peptide, oxime bond, on-resin, intramolecular bridge.

Introduction

Due to the frequency of helical secondary structures in peptide and proteins, considerable effort has been directed toward design and synthesis of different bridges stabilizing helical structures (1, 2). Synthetic helical peptides have been achieved through the incorporation of covalent or non-covalent linkages between constituent amino acid side chains. Examples include salt bridges (3), lactams (4), disulfide bridges (5), hydrophobic effects (6), metal ligation between natural (7) and unnatural amino acids (8). Usually substantial helix stabilization has been achieved when the tether was positioned between *i* and *i+4* or *i+7* residues in the peptide backbone.

Materials and Methods

All reagents and chemicals used were of analytical grade.

The present focus concentrates in the synthesis of *consolidated ligands*, which combine in the same molecule peptide sequences recognized by SH2 and SH3 domains (i.e., Pro-Val-Tyr-Glu-Asn-Val and Pro-Pro-Ala-Tyr-Pro-Pro-Pro-Pro-Val-pro, respectively), and exhibit enhanced affinities and specificities towards dual SH (32) Abelson Kinase (9, 10). For first generation consolidated ligands, binding sequences were connected by a flexible linker, e.g., Glyn. With the goal to further improve their efficacies, several second generation consolidated ligands with a more rigid linker, e.g., Alan, and optionally including an intramolecular lactam bridge “lock”, were designed (figure 1) and synthesized(11).

Probably due to lactam bridge step, the synthesis of this peptide was troublesome by the earlier attempts (12). To overcome these difficulties, we successfully performed on resin intramolecular oxime bridge, using regioselective reaction between an aldehyde and an aminoxy partner, with a model sequence from the original 32 residue structure which includes an 11 residue peptide containing an *i* to *i+7* bridge to connect the side chains of Glu and Lys(Figure 2).

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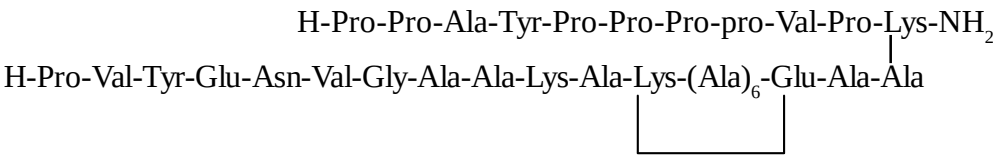


Fig. 1: consolidated ligand

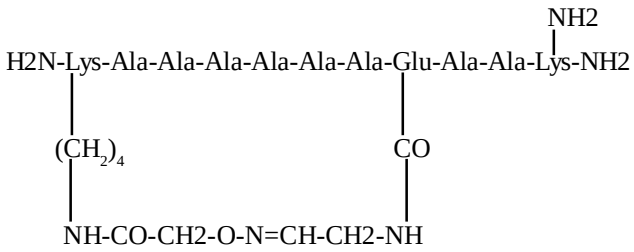
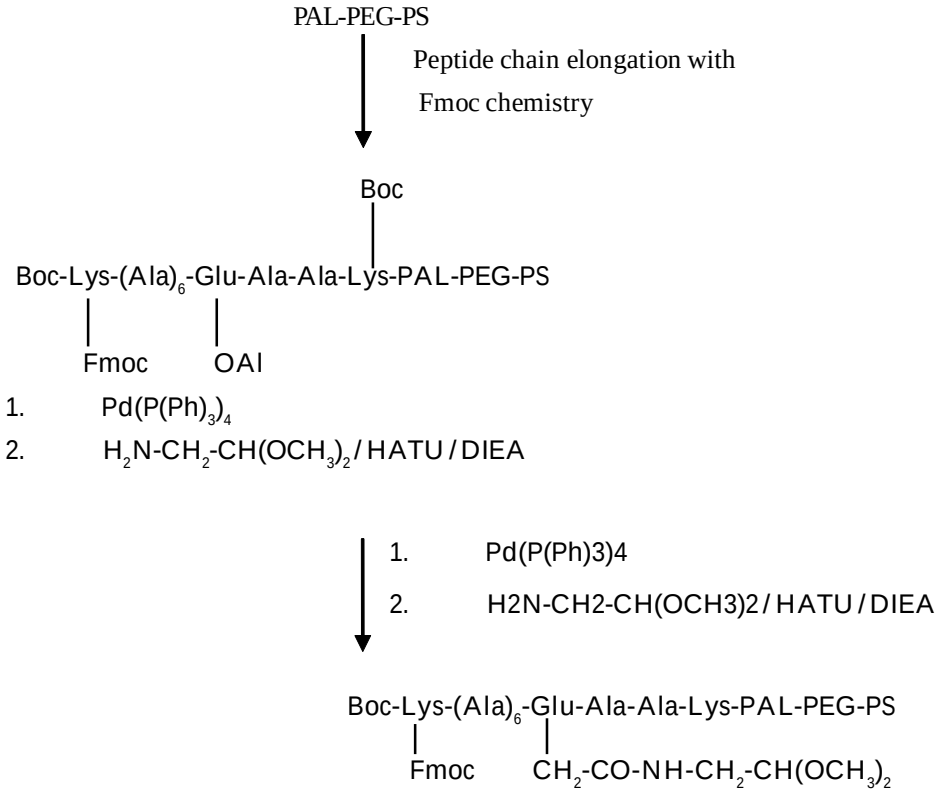
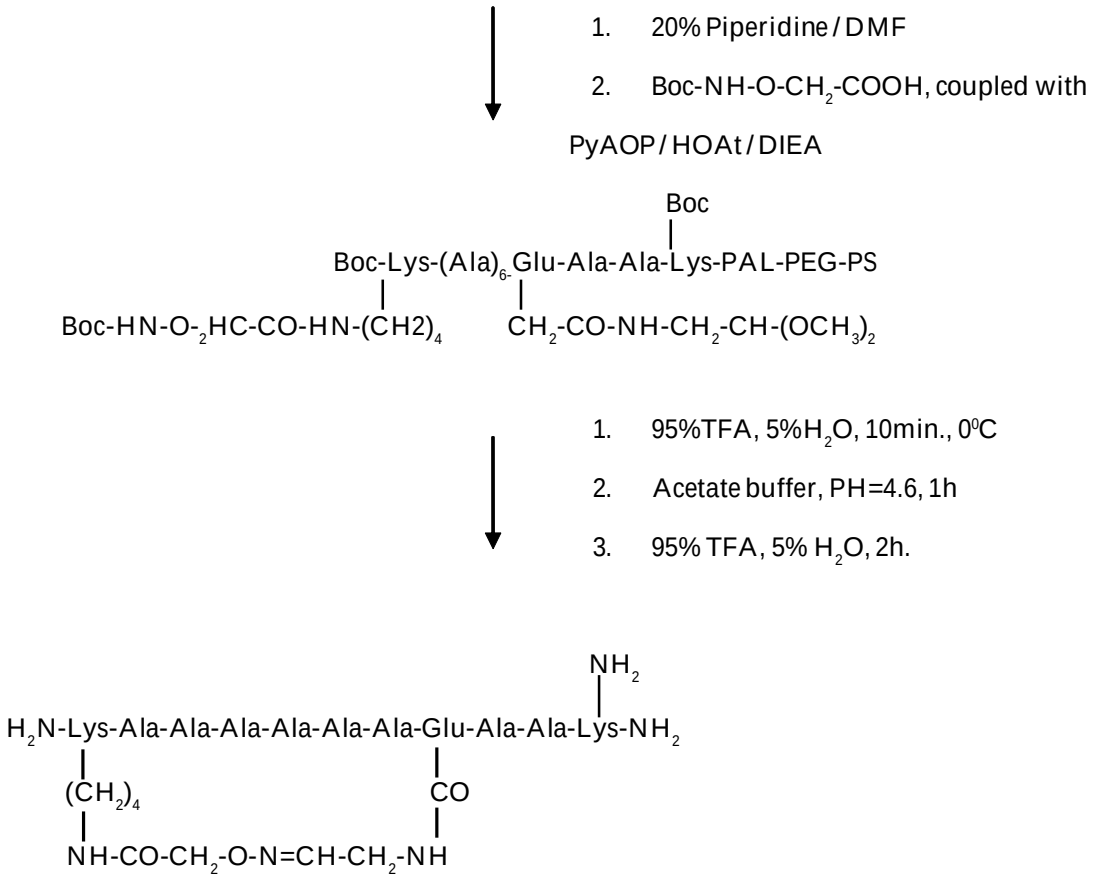


Fig. 2: 11 residue peptide with an oxime bridge

In the strategy depicted in scheme 1, we assume that 1) it is possible to optimize a TFA treatment condition which will be strong enough to remove Boc and acetal protection and thus, generate both aldehyde and aminoxy simultaneously without cleaving of peptide-PAL bond, 2)it was possible to perform on-resin intramolecular bridge by oxime bond without any acylation step.





Scheme 1: Stepwise synthesis of on-resin cyclization by oxime bond

The starting point was a PAL-PEG-PS resin (0.17 meq / g), on a Pioneer peptide synthesis system and used standard Fmoc-strategy with HBTU / HOBt / DIEA. To perform selective deprotection, Glu was introduced as Fmoc-Glu(OAl)-OH and the N-terminal lysine was introduced as Boc-Lys (Fmoc)-OH. A 4-fold molar excess of activated amino acids were used for all the couplings.

Results and Discussion

To valid our strategy, the stability of peptide-PAL bond to the TFA treatment conditions, in terms of concentration, temperature and reaction time, used for the Boc deprotection and the demasking of aldehyde was investigated. The resin stability was tested with a model tripeptide Ala-Gly-Ala synthesized using Fmoc strategy on PAL-PEG-PS resin. The conditions 95% TFA containing 5% H2O at 00C for 10 min. used to remove an acetal protection revealed that the cleavage of the peptide form the resin could be low enough (~15%). The removal of Boc group was also tested with this treatment and was found completed by comparison with the standard Boc removing conditions, i.e.; 50%TFA / DCM, 25 min., 250C.

The next steps were manually carried out in a syringe containing a fritted. The selective deprotection of the side chain glutamic acid was performed with palladium(13). The ã-COOH (1 eq.) activated by HATU / DIEA (10 eq. / 11 eq.) and treated with aminoacetaldehyde-dimethylacetal (10 eq.) to generate the masked glycinal on the side chain glutamic acid. The Nå–Fmoc group of the N-terminal lysine was removed with piperidine in DMF for 20 min. and aminooxyacetic acid (Aoa) was introduced as Boc-Aoa-OH and coupled with it. The peptide resin was treated with 95% TFA containing 5% H2O at 00C for 10 min. to free aldehyde and aminoxy fractions simultaneously. Following several water washings, 0.1 M acetate buffer at pH 4.6 was used to

form on resin bridge via an oxime bond. The peptide resin was washed with H2O and subjected to final cleavage with 95% TFA / H2O for 2h.The filterate was collected and concentrated and cold ether was added. The precipitated peptide was washed several times with ether and dried to get the crude peptide in 84% yield. It was characterized by HPLC and two peaks appeared with retention times (tR) of 10.27 and 10.81 respectively (figure 3). The identity of these HPLC peaks was confirmed by MALDI-TOF analysis, tR 10.27min., m / z 1067.65 (calcd 1067.10); tR 10.81 min., m / z 1067. 65 (calcd 1067.10), and they are likely due to syn- and anti-forms of the oxime bond (14).

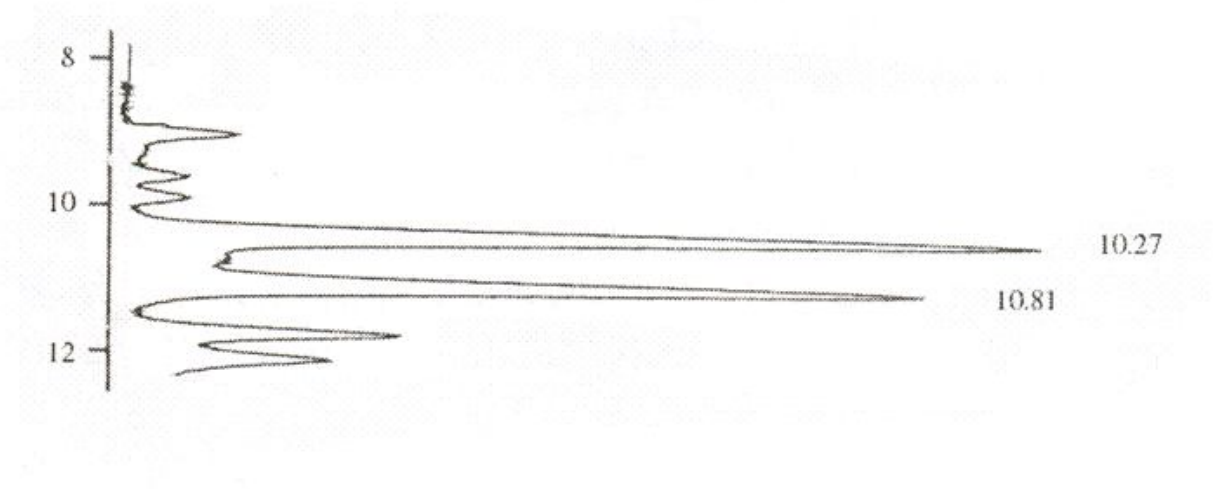


Fig. 3: Analytical HPLC profile of crude peptide. A: 0.1% TFA in water, B: 0.1% TFA in CH_3CN. Gradient: 5-20% B in 20min., Flow rate 1.2ml/min.

Conclusion

This strategy provides an elegant way to introduce an on-resin intramolecular bridge as oxime bond using an aqueous-compatible polymer support and the different stability to TFA between Boc and acetal protection on the hand and peptide-PAL bond on the other hand. The cyclization step was carried out without activation, by using a chemoselective coupling of a C-electrophile on an aldehyde and a N-nucleophile on an aminoxy. Moreover, the process demanded only commercially available products.

Abbreviations used

SH2, Src homology type 2; SH3, Src homology type 3; PAL, [5-(4-Fmoc-aminomethyl-3,5-dimethoxy phenoxy) valeric acid]; PEG-PS, polyethylene glycol-polystyrene; TFA, trifluoroacetic acid; Boc, ter-butyloxycarbonyl; OAl, allyl; DIEA, N,N-diisopropylethylamine; Fmoc, N-(9-fluorenyl)methoxycarbonyl; HATU, O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOAt, 1-hydroxy-7-azabenzotriazole; HOBt, 1-hydroxybenzotriazole; PyAOP, benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate; MALDI-TOF, matrix assisted laser desorption / ionization –time-of –flight.

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Original Article

Synthesis and Anticonvulsant Activity of Some Novel Hydrazone Derivatives

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Abstract

In the present study a series of hydrazone derivatves (III, IIIa, IV) were synthesized and characterized by their spectral data and screened for anticonvulsant properties against seizures induced by maximal electroshock (MES) and toxicity screening. The purity of the newer compounds was checked by m.p. and TLC analysis. The structures of these compounds were established on the basis of their spectral (FT-IR, ^{1}H-NMR) data analysis. These newly synthesized derivatives of phenytoin were evaluated in terms of anticonvulsant activity. Some of the investigated compounds showed significant anticonvulsant activity. Some of these may be chosen as a prototype for development of new anticonvulsants.

Keywords: Hydrazones, Anticonvulsant, Epilepsy.

Introduction

Epilepsy is a common disorder of the central nervous system (CNS). Approximately 0.4%~1% of the population worldwide suffers from this disorder. The conventional antiepileptic drugs suffer from a range of side effects. Furthermore, the convulsions of 25% of epileptics are inadequately controlled by currently available medications. During the past decade several new drugs were approved, e.g., felbamate, fosphenytoin, gabapentin, lamotrigine, vigabatrin and zonisamide. However none of the available antiepileptic drug is ideal as they can be associated with chronic and adverse side effects. Thus the search for new anticonvulsant drugs continues to be an active area of investigation in medicinal chemistry. Hydrazones and their derivatives constitute a versatile class of compounds in organic chemistry. These compounds have interesting biological properties(1),(5), such as anti-inflammatory(7), analgesic(8), anticonvulsant, antituberculosis(3),(10), antitumor, anti-HIV and antimicrobial activity(2),(6),(7),(9). Hydrazones are important compounds for drug design, as possible ligands for metal complexes, organocatalysis and also for the synthesis of heterocyclic compounds. Hydrazone has been prepared because during initial screening it has shown activity in the MES test. In view of potent anticonvulsant activity of hydrazone, we have synthesized a novel series hydrazone derivative by following reaction and evaluate them for their anticonvulsant activity.

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Materials and Methods

Chemistry

The entire chemicals used were procured from Qualigens, Himedia and C.D.H. Purity of starting

materials used for reaction was confirmed by checking their melting point or boiling point and by thin layer chromatography. Melting points were determined in open capillary tube using precision melting point apparatus and uncorrected. FT-IR (KBr) spectra were recorded on "SHIMADZU FT-IR 8400S" spectrophotometer from GLA University, Institute Of Pharmaceutical Research, Mathura (UP). 1H NMR spectra of synthesized compounds were recorded on "FTNMR AVANCE" spectrometer in DMSO using TMS as internal standard (chemical shift ä ppm) at Punjab University, Chandigarh. CHN analyzer was recorded on "ELEMENTOR" at Punjab University, Chandigarh. Physical properties of the synthesized compounds are listed in **Table 1** whereas scheme of synthesis is given in **Figure 1, 2** and **3.**

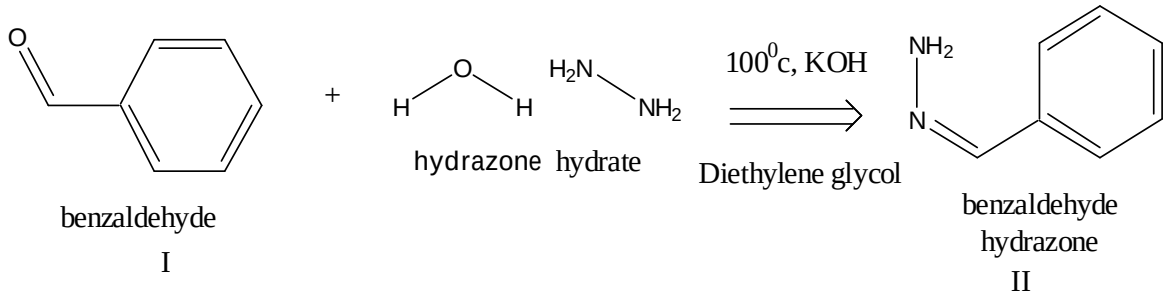


Figure 1: Synthesis of Benzaldehyde Hydrazone.

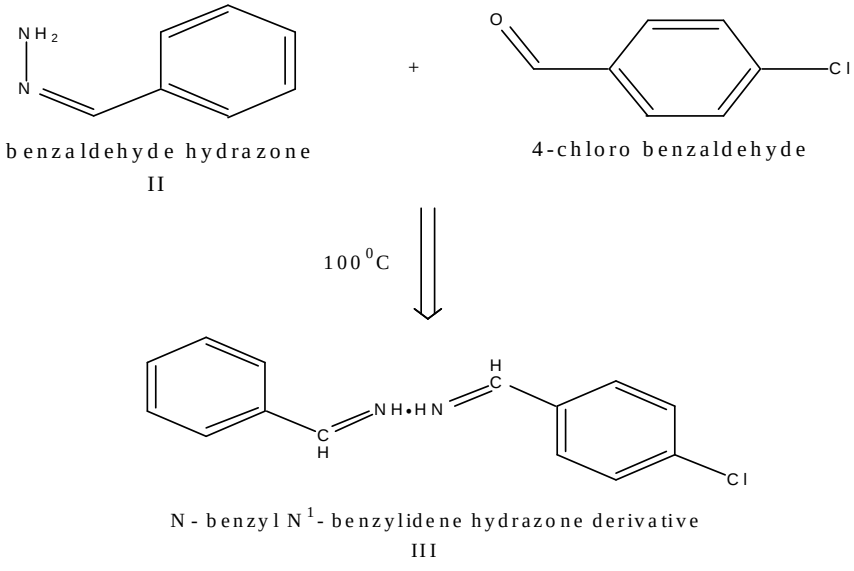
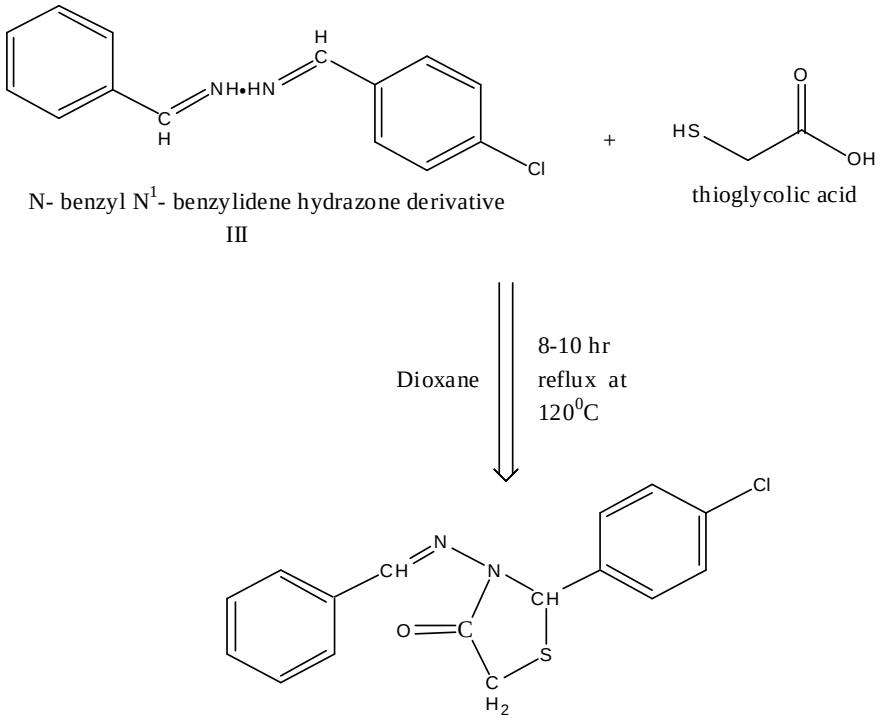


Fig. 2: Scheme 1 Synthesis of N {2 Substituted phenyl} 4 chloro benzyl hydrazone.



N- benzyl N^1- benzylidene hydrazone derivative

III

thioglycolic acid

N^1 [2 (substituted phenyl) 4-oxo -1,3-thiazolan-3-yl] 4-chloro benzyl hydrazone

IV

Fig. 3: Scheme 2 Synthesis of N1 [2 (substituted phenyl) 4-oxo-1,3-thiazolan-3-yl] 4-chloro benzyl hydrazone.

General procedure for the Synthesis of Benzaldehyde hydrazone.

Intially placed 9 g. of benzaldehyde, 60 ml. of diethylene glycol, 8 ml of 90% hydrazine hydrate and 10 g. of potassium hydroxide pellets in a 100 ml round bottom flask. After that warmed the mixture on a boiling water bath until most of the potassium hydroxide has dissolved and then refluxed for 1 hour.

General procedure for the Synthesis of N {2 Substituted phenyl}4 chloro benzyl hydrazone.(2)

In the second step a mixture of previously synthesized hydrazone (0.005 mol) and different substituted arylaldehydes (0.005 mol) was taken in absolute ethanol (15ml) as solvent in round bottom flask and refluxed for 2-3 hrs, on cooling a solid mass separated out that was filtered and then recrystalized from ethanol.

General procedure for the Synthesis of N1 [2(substituted phenyl) 4oxo,1, 3thiazolan3yl] 4-chloro benzyl hydrazone.(4)

At last in a round bottom flask the compound (0.002mol) in dioxane (50ml) was taken and thioglycolic acid (0.002mol) was added to it and the mixture was refluxed for 8-10 hrs at 120^{0}C, after that the reaction mixture was concentrated on crushed ice and neutralized with 2% sodium bicarbonate solution, the solid mass that separated out was filtered and recrystallized from ethanol.

Table 1. Physicochemical characteristics of hydrazone derivatives.

Compound	(r) substitution	% yield	Molecular formula	Molecular weight	Rf value	Melting point °c
III	Para Chloro benzaldehyde	60	$C_{14}H_{11}N_2Cl$	242.5	0.84	127
IIIa	3 methoxy 4 hydroxy benzaldehyde	72	$C_{15}H_{14}N_2O_2$	254	0.45	105
IV	4 oxo 3 thiazolanyl p chlorobenzaldehyde	74	$C_{13}H_{13}ClN_2OS$	280.5	0.88	150

Spectral Data

N[1] [2(substituted phenyl) 4oxo,1, 3thiazolan3yl] 4 chloro benzyl hydrazone. {IV}

IR (KBr, cm^{-1}): Aromatic-H at 3050 cm^{-1}, Ar-(C=C) at 1624 cm^{-1}, 1580 cm^{-1}, 1500 cm^{-1},1450 cm^{-1}, Cl-752.24 cm^{-1}, Aromatic mono substitution at 692.44 cm^{-1}, C=N at 1710 cm^{-1}; ^{1}HNMR 8.509 (3, 1H), 7.330 (4, 1H, J=6.887, J=1.406, J=1.405), 7.335 (5, 1H, J=7.410, J=6.887, J=5.446, J=0.000), 7.335 (6, 1H, J=7.412, J=6.887, J=5.453, J=0.000), Anal. Calcd. For $C_{13}H_{13}ClN_2OS$: C, 55.61,H,4.67, Cl, 12.64, N, 9.98, S, 11.40, O, 5.70;

N benzyl N[1]{4 chloro benzylidene}hydrazone. {III}

IR (KBr, cm^{-1}): Aromatic-H at 3000 cm^{-1}, Ar-(C=C) at 1614 cm^{-1}, 1563 cm^{-1}, 1519 cm^{-1},1454 cm^{-1}, C-C at 1556 cm^{1}, Cl-744 cm^{-1}, Aromatic mono substitution at 690 cm^{-1}, C=N at 1768 cm^{-1}, C=O at 1643 cm^{-1}, C-S at 731 cm^{-1}, N-H at 3321 cm^{-1}; ^{1}HNMR 8.558 (2, 1H), 8.612 (3, 1H), 7.471 (4, 1H, J=7.546, J=7.438, J=1.423, J=1.418), 7.538 (5, 1H, J=8.196, J=7.546, J=2.074, J=0.440), 7.393 (6, 1H, J=7.775, J=7.438, J=2.074), Anal. Calcd. For $C_{14}H_{11}N_2Cl$: C, 69.27, H, 4.57, Cl, 14.62, N, 11.55;

N benzyl N[1]{3 methoxy 4 hydroxy benzylidene}hydrazone. {IIIa}

IR (KBr, cm^{-1}): Aromatic-H at 3020 cm^{-1}, Ar-(C=C) at 1600 cm^{-1}, 1570 cm^{-1}, 1450 cm^{-1},1410 cm^{-1}, OH-3650 cm^{-1}, Aromatic mono substitution at 710 cm^{-1}, C=N at 1710 cm^{-1}, OCH3 (C-O) at 1090 cm^{-1}, OCH3 (C-H) at 2895-2885 cm^{-1}; ^{1}HNMR 1.407 (1, 3H), 1.407 (2, 3H), 1.407 (3, 3H), 8.502 (5, 1H), 8.605 (6, 1H), 7.284 (7, 1H, , J=7.958, J=1.420, J=1.395), 7.363 (8, 1H, J=7.958, J=7.643, J=0.548, J=0.000), 7.363 (9, 1H, J=7.958, J=7.827, J=0.728, J=0.000), 8.085 (10, 1H, J=7.643, J=1.451, J=1.395, J=0.728), 8.123 (11, 1H, J=7.827, J=1.451, J=1.420, J=0.548), 7.532 (12, 1H, J=8.427, J=1.780), 6.839 (13, 1H, J=8.427, J=1.631), 7.265 (14, 1H, J=1.780, J=1.631) ,Anal. Calcd. For $C_{14}H_{11}N_2Cl$: C, 70.84, H, 5.55, N, 11.02, O, 12.59;

Pharmacology

All the compounds were screened for their anticonvulsant activity by electroshock seizure method. Albino rats of Wistar strains, weighing 100-200g, of either sex were used. . Accommodation conditions were maintained at 200C and the number of animals were used in different experiments. Polyethylene glycol was used for dissolving the test compounds in rotarod test. The control experiments were performed with solvents alone. Four animals were used in the control test. The test compounds were administered intraperitoneally to rat, at doses of 30.100,300 mg / Kg to 1 to 4 rat. The anticovulsant activity of III, IIIa, IV has been detailed in Table No. 2.

Anticonvulsant Screening

Maximal Electroshock Seizure Test (MES)(11)

Maximal seizures were elicited by a 60Hz alternating current of 50mA intensity delivered for 0.2 seconds via corneal electrodes.. A drop of 0.9% w / v sodium chloride instilled in each eye prior to application of electrodes assured adequate electrical contact. Test solutions of all the compounds were prepared in 30% v / v polyethylene glycol 400 (PEG 400) and animals were dosed intraperitoneally 30 min prior to testing. Abolition of the hind limb tonic extension component of the seizure was defined as protection in the MES test.

Result and Discussion

The anticonvulsant activity studies following MES method revealed that, the compounds evaluated **III,**

IIIa, **IV** have found to be possessing significant anticonvulsant activity. The compounds that exhibited most potent anti-MES activity included **IIIa** which have activity comparable with phenytoin. The compounds **IV** were found to be more lipophilic having potent anticonvulsant activity. The other compounds **IIIa** were also lipophilic having same potency. The compounds **III,** are less lipophilic and are less active in MES test. The present study reveals the anticonvulsant potential of fused 4-thiazolidinone derivatives. The results indicated that electron withdrawal group in position 2 and 4 [disubstituted phenyl-4-oxo-1,3-thiazolan-3-yl]-4 chloro hydrazone(12) was essential for the activity. Thus a number of novel *N* 1-[2-(substituted phenyl)-4-oxo-1,3-thiazolan-3-yl]- 4 chloro hydrazone derivatives exhibited anticonvulsant Screening by using MES test. The compounds N benzyl N1 {4 chloro benzylidene} hydrazone. {III} were lipophilic but were less active in MES test. Some of the compounds have shown higher degree of protection and obviously may have future commitment.

Table : 2 Anticonvulsant activity (Maximal electroshock induced convulsions)

Sr no.	Treatment	Duration of tonic flexion (sec)	Duration of tonicextensor (sec)	%Protection (24h)
1	Control(propylene glycol 400)	NO	14.17 ± 0.872	16.66
2	Phenytoin(25 mg / kg)	5.83 ± 0.9804	NO ***	100
3	III(30 mg / kg)	2.9 ± 0.3	8.3 ± 0.577	33.33
4	III(100 mg / kg)	3.4 ± 0.7	7.1 ± 1.5	49.99
5	III(300 mg / kg)	3.1±0.3	6.2±0.4***	66.66
6	IIIa(30 mg / kg)	3.2±0.2	8.1±0.5***	60.0
7	IIIa(100 mg / kg)	3.5 ± 0.4	7.83 ± 0.60*	83.33
8	IIIa(300 mg / kg)	3.3±0.4	7.3±0.4***	65.34
9	IV(30 mg / kg)	2.4±0.5	8.4±0.2***	70.77
10	IV(100 mg / kg)	3.2±0.3	9.7±0.4***	80.12
11	IV(300 mg / kg)	2.3±0.2	7.6±0.3***	63.66

Values are expressed as mean ± SEM, from 6 mice. Significant at *P<0.05 and ***P<0.001 as compare to control using one way ANOVA followed by Tukey - kramer's post hoc test.

Conclusion

In this study synthesized hydrazones mimicking the effects of anti epileptic drug by reducing tonic convulsion and mortality. It also reveals that those compounds which was more lipophilic in nature tends to have better anticonvulsant effects. The present research will guide our future development of potent and selective anticonvulsant drugs. However further studies on other species of animals is recommended, and comparison with other antiepileptic drugs in different species need to perform to fill the future need of model drug.

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Original Article

Formulation and Evaluation of Orodispersible Tablets of Ondansetron Hydrochloride

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Abstract

The purpose of this research work is to formulate and evaluate the Orodispersible drug delivery system of already used therapeutic molecule to enhance bioavailability and effectiveness of the drug. Among ODT drugs, the most promising antiemetic is Ondansetron hydrochloride and it was selected for the present study. Thus the objectives of the drug work were to formulate and evaluate Orodispersible tablets of Ondansetron hydrochloride, having adequate mechanical strength, rapid disintegration and fast action. Pre-compression parameters like angle of repose, bulk density, tapped density, compressibility index & post compression parameters like wetting time, water absorption ratio, in-vitro disintegration and in-vitro dispersion time were studied. The hardness, friability and drug content of all the formulations were found to be with in the limits. The best formulation F10 have shown good disintegration time, dissolution time and dispersion time. The best promising formulation were also being found to be stable at 40°C±75%. Finally the in-vitro drug released characteristics of best formulation was compared to commercial formulation.

Keywords: Orodispersible, Ondansetron HCL, Crospovidone, Croscarmellose sodium, superdisintegrants, dispersion, disintegration, dissolution.

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Introduction

Tablets are solid preparations intended for oral administration. Many patients find it difficult to swallow tablets and hard gelatin capsules and thus not comply with prescription that results in high incidence of non-compliance and ineffective therapy[1]. Orodispersible tablets are gaining prominence as new drug delivery systems. These dosage forms dissolves or disintegrate in oral cavity within a minute without the need of water or chewing[2]. Hence the present work was aimed to formulate the orodispersible tablets of ondansetron hydrochloride, that are designed by using natural polymer (Treated agar) and synthetic polymers namely crospovidone and croscarmellose sodium. Ondansetron hydrochloride is a selective serotonin 5-HT3 receptor antagonist indicated for the prevention of nausea and vomiting. Effervescent substances like sodium bicarbonate and tartaric acid accelerates the superdisintegrant action and masks the bitter taste of ondansetron hydrochloride[3]. Faster the drug in the solution, quicker the absorption and onset of clinical effect. Some drugs are absorbed from the mouth, pharynx and oesophagus as the saliva passes down in to the stomach. In such case, bioavailability of a drug is significantly greater than those observed from conventional tablet dosage form. The advantages of mouth dissolving dosage form are increasingly being recognized in both, industry and academia[4].

Materials and Methods

Ondansetron Hydrochloride was obtained as a gift sample from Madras Pharmaceuticals Chem. Limited, Chennai. Sodium bicarbonate, talc and tartaric acid were obtained as a gift sample from SDFCL Fine Chem. Limited, Mumbai. Treated agar and DEC (corn starch: mannitol) was obtained as a gift sample from Rea Chem. Laboratory Chemical, Chennai. Croscarmellose sodium was obtained as a gift sample from Mingatai Chemicals Co. Ltd, Taiwan. Crospovidone was obtained as a gift sample from ISF Technologies, Chennai. Aspartame was obtained as a gift sample from Nutra sweet company, Mumbai. Orange 1208 was obtained as a gift sample from Firminch Ltd., Mumbai. Magnesium stearate was obtained as a gift sample from Vijlak Pharma Ltd., Hyderabad.

2.1. Preparation of Directly Compressible Excipient[5]

The directly compressible excipient (DCE) was prepared using a local variety of food grade corn starch along with mannitol in 1:1 ratio using 10% w / w starch paste for granulation.

Method

All the ingredients were powdered separately in a dry, clean porcelain mortar and passed through # 60 mesh sieve and mixed well in geometrical ratio. Granulating fluid, starch paste (10% w / w) is added to the powder mixture in small quantities, while mixing thoroughly after each addition until a coherent mass was formed. Then it was passed through # 44 mesh sieve and the wet granules were spread on a paper and dried in hot air oven at 55-60ºC. The dried granules were then passed through # 36 mesh sieve.

2.2. Preparation of treated agar[5]

Treated agar (TAG) powders were prepared by taking 10 gm agar powder in distilled water (100 ml) and stirring at 50 rpm with a three- bladed mechanical stirrer for one day. This causes water absorption and swelling. Then the liquid was poured in a large petri-dish and allowed for drying up to three days in incubator at 37±1ºC and then the mass was pulverized and sifted through # 80 mesh sieve.

2.3. Preparation of Orodispersible tablets[5]:

Orodispersible tablets of Ondansetron hydrochloride were prepared by effervescent method according to the formula. All the ingredients were passed through # 60 mesh sieves separately. The drug and directly compressible excipient were mixed by adding small portion of each at a time and blending it to get a uniform mixture and kept aside. Sodium bicarbonate and tartaric acid were pre-heated at a temperature of 80ºC for 2 h to remove absorbed / residual moisture and thoroughly mixed in a mortar to get a uniform powder and then added to the above blend. Then the other ingredients were mixed in geometrical order but magnesium stearate and purified talc were added at the last and mixed for further two minutes. The blend was compressed using 9 mm flat round punches to get tablets of 200 mg weight on 10-station rotary tablet machine. A batch of 60 tablets was prepared for all the designed formulations.

Table 1: Formulation design for Ondansetron hydrochloride tablets

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Drug	4	4	4	4	4	4	4	4	4	4
NaHCO3	18	18	18	18	18	18	18	18	18	18
Tartaric acid	18	18	18	18	18	18	18	18	18	18
Treated agar	_	10	15	20	_	_	_	_	_	_
Crospovidone	_	_	_	_	10	15	20	_	_	_
Croscarmellose sodium	_	_	_	_	_	_	_	10	15	20
Aspartame	8	8	8	8	8	8	8	8	8	8
Orange 12809	3	3	3	3	3	3	3	3	3	3
Magnesium stearate	2	2	2	2	2	2	2	2	2	2
Talc	1	1	1	1	1	1	1	1	1	1
DCE	146	136	131	126	136	131	126	136	131	126
Total weight	200	200	200	200	200	200	200	200	200	200

2.4. Evaluation of tablet

The prepared tablets were evaluated for various official and nonofficial specifications.

Pre-compression evaluation[6]:

The quality of a tablet is generally dictated by the quality of physiochemical properties of then granule blend prepared. There are many process variables which may affect the characteristics of the finished tablet. Hence the prepared granules were evaluated for the mass-volume relationship parameters like bulk density, tapped density, Angle of Repose, Compressibility index and Hausner's ratio. The results obtained are given in Table 2.

Table 2: Pre-compression evaluation of Ondansetron hydrochloride

Formulations	Parameters				
	Bulk density (gm/cm^3)	Tapped density (gm/cm^3)	Angle of repose (θ)	Carr's index (%)	Hausner's ratio
F1	0.463±0.003	0.543±0.002	34.96±0.051	14.81±0.015	1.18±0.010
F2	0.468±0.005	0.545±0.003	31.31±0.032	14.88±0.085	1.17±0.010
F3	0.471±0.013	0.569±0.006	31.08±0.091	14.30±0.135	1.18±0.010
F4	0.481±0.041	0.561±0.001	31.24±0.250	14.31±0.120	1.15±0.030
F5	0.540±0.010	0.613±0.009	30.25±0.230	11.61±0.162	1.23±0.105
F6	0.552±0.002	0.632±0.010	30.92±0.023	12.51±0.023	1.14±0.001
F7	0.568±0.001	0.663±0.001	29.51±0.022	14.53±0.250	1.12±0.038
F8	0.414±0.005	0.479±0.008	28.83±0.031	14.26±0.300	1.16±0.005
F9	0.395±0.040	0.444±0.005	28.25±0.054	13.17±0.100	1.15±0.015
F10	0.372±0.001	0.310±0.031	28.17±0.061	13.30±0.064	1.15±0.065

Post-compression evaluation:

Weight variation[7]:

Twenty tablets were taken and their weights were determined individually and collectively on a digital weighing balance. The average weight of one tablet was determined from the collective weight. Average weight was compared with the individual weight and the percentage deviation of individual tablet was calculated.

Thickness[8]:

The thickness of the tablets was determined by using vernier caliper of Electro lab model. Five tablets are randomly selected from each batch. It is expressed from mm and the average values were calculated.

Hardness [8]:

Hardness was determined by taking five tablets from each formulation and was measured by using Monsanto hardness tester. The hardness was measured in terms of kg/cm^3.

Friability[9]:

The friability of the tablet was measured using a Roche friabillator (Electro lab, India).Twenty reweighed tablets were rotated at 25 rpm for 4 rpm and dropping the tablets at a height of 6 inches at each revolution and the tablets were subjected to 100 revolutions. The tablets were then dedusted using soft muslin cloth and reweighed and the percentage of weight loss was calculated. The percentage friability of the tablets were measured as per the following formula.

Percentage friability = (Initial weight – final weight / Initial weight) ×100

Table 3: Post-compression evaluation of Ondansetron hydrochloride

Formulation code	Weight variation (mg)	Thickness (mm)	Hardness (kg/cm^2)	Friability (%)
F1	200.4±0.84	2.35±0.03	2.20±0.10	0.831±0.01
F2	200.2±1.35	2.34±0.01	2.13±0.20	0.781±0.03
F3	200.1±0.06	2.34±0.03	2.13±0.21	0.836±0.07
F4	200.3±0.94	2.32±0.01	2.30±0.17	0.747±0.08
F5	200.1±0.05	2.31±0.01	2.33±0.11	0.814±0.04
F6	200.3±0.94	2.34±0.01	2.40±0.26	0.832±0.01
F7	200.2±0.05	2.32±0.00	2.02±0.15	0.780±0.10
F8	199.9±1.10	2.33±0.01	2.16±0.11	0.907±0.08
F9	200.2±1.30	2.34±0.00	2.20±0.10	0.941±0.04
F10	199.8±1.34	2.32±0.01	2.26±0.05	0.922±0.01

Table 4: Post-compression evaluation of Ondansetron hydrochloride

Formulation code	*In vitro* Dispersion time(sec)	Wetting time(sec)	Disintegration time(sec)	Water absorption ratio	Assay (%)
F1	60.37±0.40	41.03±0.05	50.73±0.64	39.30±0.81	90.44±0.50
F2	49.16±0.15	35.83±0.73	42.26±0.35	33.06±0.51	96.54±0.48
F3	43.36±0.40	32.66±0.57	40.56±0.45	43.66±0.41	97.37±0.57
F4	40.52±0.44	30.23±0.32	36.13±0.20	33.56±0.58	100.85±0.16
F5	38.56±0.77	33.23±0.25	34.40±0.60	33.70±0.34	99.52±0.17
F6	35.26±0.28	31.63±0.30	32.18±0.23	27.96±0.95	100.48±0.22
F7	32.41±0.17	28.46±0.30	29.63±0.40	32.76±0.68	101.29±0.34
F8	30.37±0.22	30.40±0.36	25.26±0.17	31.36±0.32	102.55±0.48
F9	28.44±0.50	28.30±0.40	22.15±0.17	30.50±0.50	100.15±0.27
F10	26.45±0.41	26.26±0.30	20.27±0.40	29.50±0.30	99.96±0.06

In vitro dispersion time[10]:

In vitro dispersion time was measured by dropping a tablet in a measuring cylinder containing 10ml of simulated saliva fluid of pH 6.8. After dropping a tablet in the simulated saliva fluid, the tablet started to swell quickly, broke and followed by dispersed. Five tablets from each formulation were randomly

selected and in vitro dispersion time was performed and it was expressed in seconds.

Wetting time[11]:

Wetting time is closely related to the inner structure of the tablets and to the hydrophilicity of the excipient. A linear relationship exists between wetting time and disintegration time. Thus wetting is the important step for disintegration process to take place.

A piece of tissue paper folded double was placed in a petri plate (internal diameter is 6.5cm) containing 6ml of purified water. A tablet having a small amount of Eosin dye powder on the upper surface was placed on the tissue paper. The time required to develop a red colour on the upper surface of the tablet was recorded as the wetting time.

Disintegration time[12]:

Disintegration time was measured using disintegration test apparatus. A tablet was placed in each six tube of the basket. The basket with the bottom surface is made up of stainless - steel screen (mesh no. 10) was immersed in water maintained at 37°C as the disintegration fluid and the paddle at 100rpm as stirring element was used. The time taken for complete disintegration of the tablet with no palatable mass remaining in the apparatus was measured in seconds.

Water absorption ratio[11]:

A piece of double folded tissue paper was kept in a petri dish (internal diameter 5.5 cm) containing 6 ml of purified water. The weight of tablet before keeping in petri-dish was noted as (Wb) and after completely wetted tablet in petri plate was noted as (Wa). The wetted tablet was removed and reweighed. Water absorption ratio, R was determined according to the following equation.

$$R = 100 (Wa – Wb) / Wb$$

Where, Wb and Wa are before and after water absorption, respectively.

Drug content[13]:

Ten tablets from each batch were weighed and powdered. The required amount of the powder equivalent to 4 mg of ondansetron hydrochloride was dissolved in 100 ml of phosphate buffer pH6.8. From this solution 1 ml was taken and made up to 100 ml by using phosphate buffer pH6.8 and the solution was filtered by using whatman filter paper. The solution was analysed for drug content at 248nm using UV visible spectrophotometer.

2.5. In vitro drug release study[14]:

In vitro dissolution of the orodispersible tablets was studied in USP XXIII type-II dissolution test

Table 5: In vitro drug release data (F1-F10)

Formulation code	% Cumulative release					
	5 min	10 min	15 min	20 min	25 min	30 min
F1	56.15	62.08	62.95	64.37	65.80	66.69
F2	66.14	80.60	82.66	84.19	85.18	85.63
F3	66.98	82.94	83.92	85.46	86.45	87.45
F4	72.19	84.63	86.71	88.80	89.27	90.28
F5	67.80	81.78	84.94	87.57	88.03	90.67
F6	77.15	80.07	89.63	92.28	93.85	94.89
F7	74.47	90.26	92.26	94.50	95.00	96.59
F8	74.98	84.21	87.93	84.48	91.04	92.61
F9	76.65	89.74	91.31	92.88	94.46	95.50
F10	77.78	91.45	95.20	96.79	98.39	99.49

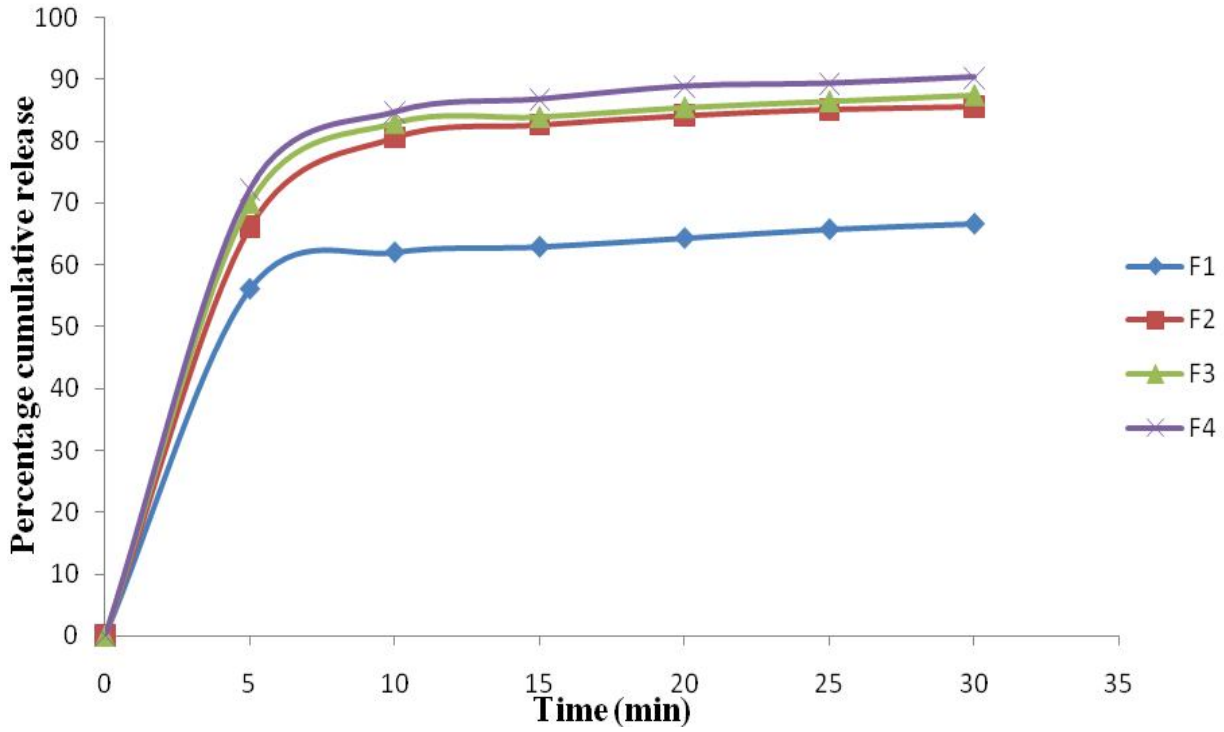

Fig. 1: In vitro drug release for F1, F2, F3 and F4

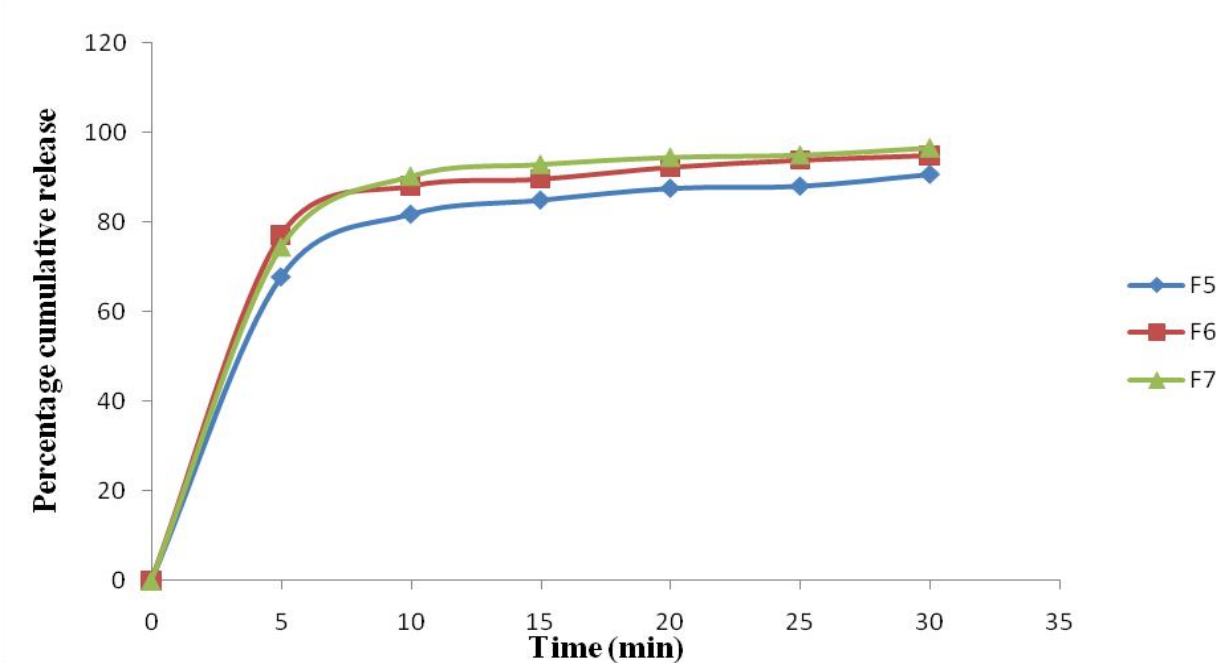

Fig. 2: In vitro drug release for F5, F6 and F7

apparatus (Electro lab, model: TDT-06N) employing a paddle stirrer at 50 rpm using 900ml of pH 6.8 phosphate buffer at 37±0.5o C as dissolution medium. One tablet was used in each test. Aliquots of dissolution medium were withdrawn at specified intervals of time and analyzed for drug content by measuring the absorbance at 248 nm. The volume withdrawn at each time interval was replaced with

fresh quantity of dissolution medium. Cumulative percent of the drug released was calculated and plotted against time. The results obtained are given in Table 5.

Dissolution Condition:

Apparatus : USP XX111 paddle apparatus 2. RPM : 50
Medium : phosphate buffer (pH 6.8)
Sampling Interval : Every 5 minute.
Sampling Volume : 5 ml.
Study Period : 30 min

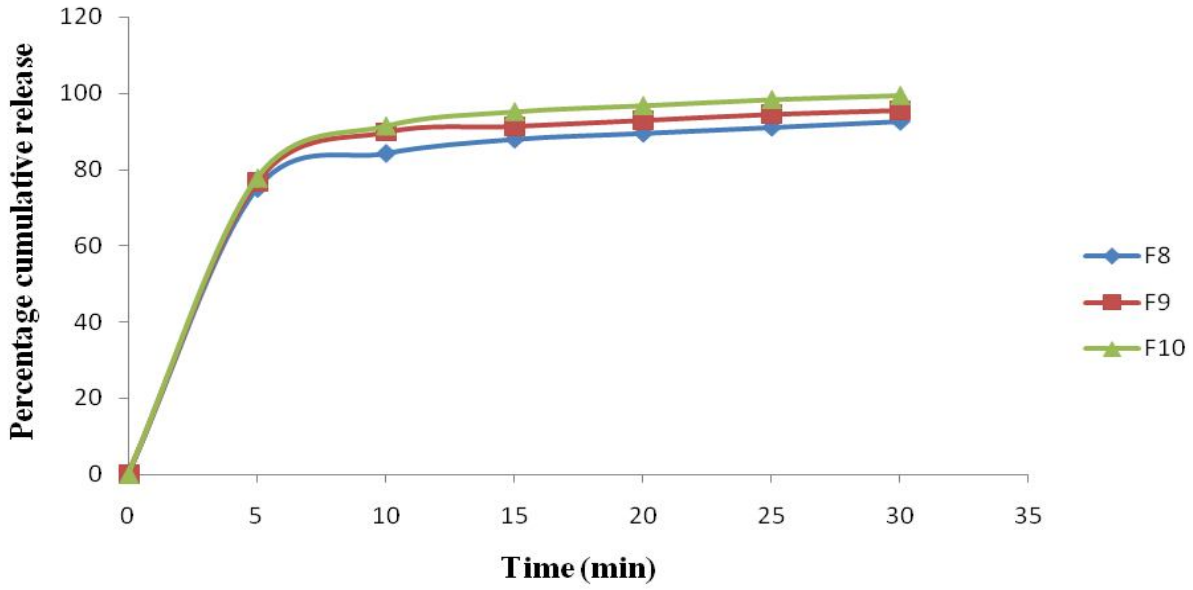

Fig. 3: In vitro drug release for F8, F9 and F10

2.6. Accelerated stability studies[15]:

The goal of a stability program is not uniquely defined, but depends on the stage of development of the product in question. At the very onset of development, it is desired to know what the inherent stability of the drug substance is and what interactions with the excipients can be expected. On the analytical side, it is usually supported by an assay procedure, which helps in developing the stability program.

The stability program varies from one dosage form to another and formulation to formulation.

Accelerated stability studies are of great interest and are attractive as which can document satisfactory results under stressed conditions time saving can be achieved.

Table 6: Stability data for formulation F10

Parameters	Time in months			
	0 (Initial)	1st month	2nd month	3rd month
Hardness (kg/cm^2)	2.93±0.02	2.92±0.01	2.89±0.01	2.86±0.01
Disintegration time (sec)	23.63±1.46	23.45±1.01	23.38±0.05	24.30±0.17
Drug content (%)	100.10±0.13	99.92±0.05	99.88±0.08	99.58±0.36
In vitro drug release (%)	99.41±0.17	99.69±0.16	99.44±0.11	99.89±0.08

Results and Discussion

Weight variation

The weight variation in all the ten formulation was found to be 199.8±1.34 to 200.4±0.84mg. Formulations were within pharmacopoeial limits with free flow of the powder blend and demonstrating the efficiency of compression of particles into tablets.

Hardness

The Hardness was maintained to be within 2.02±0.15 to 2.40±0.26 kg/cm2 as these tablets are rapidly disintegrating. No variation in the hardness was found which clearly indicates that the proper blending of the mixture for the preparation of orodispersible tablets. The prepared tablets in all the formulation possess good mechanical strength with sufficient hardness.

Thickness

Thickness of all tablets prepared in the range of 2.31±0.01 to 2.35±0.03 mm was acceptable without much variation.

Percentage friability

Percentage Friability is below 1% in all the formulation and values obtained lies between 0.747±0.08 to 0.941±0.04%. It indicated that of good mechanical resistance of the tablets.

Wetting time

The Wetting time was rapid in croscarmellose sodium followed by crospovidone, treated agar. The value lies between 26.26±0.30 to 41.03±0.05 sec. Figure 4: depicts the relation between the concentration of superdisintegrants and wetting time. It indicated that as concentration of disintegrant increases the time taken for wetting was reduced. Wetting time is used a parameter to correlate with disintegration time in oral cavity. This is an important criterion for understanding the capacity of disintegrants to swell in the presence of little amount of water.

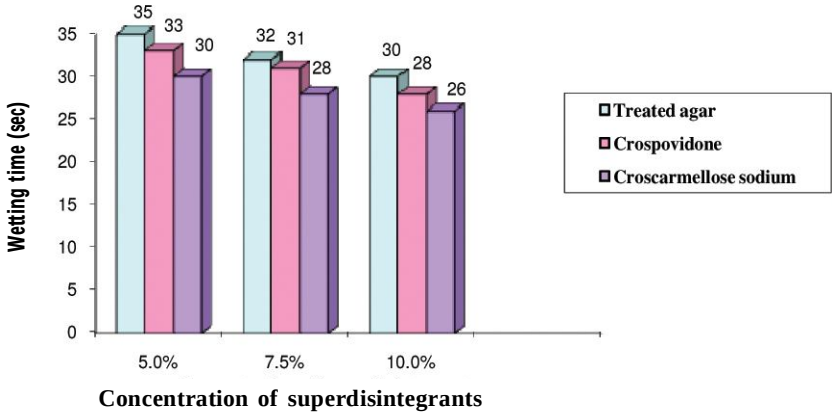

Fig. 4: Wetting time of different superdisintegrants with different concentration

Dispersion time

Further the tablets were subjected in vitro dispersion in which the time taken by the tablet to produce complete dispersion is measured. The values for all the ten formulations lie between 26.45±0.41 to 60.37±0.40 sec. The in vitro dispersion time was rapid in croscarmellose sodium followed by crospovidone and treated agar. The comparative results are shown in the following figure 5.

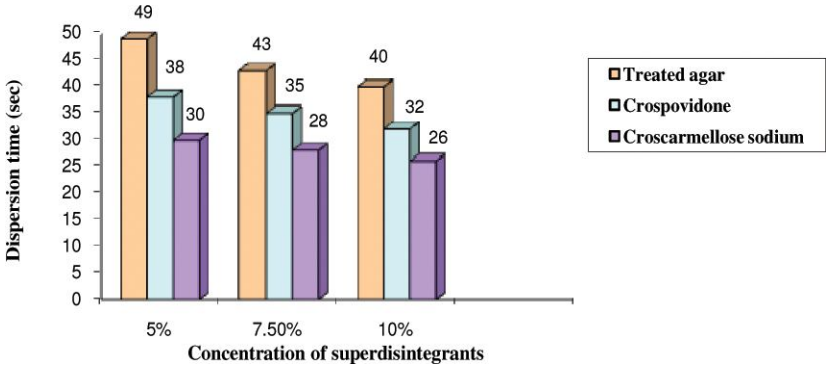

Fig. 5: Dispersion time of different superdisintegrants with different concentration

Disintegration time

The disintegration time for the entire formulations lie between 20.276±0.402 to 50.73±0.646 sec. Figure 6: depicts the disintegration behaviour of the tablets in water. This rapid disintegration of the oral dispersible tablets were due to penetration of saliva into the pores of the tablets, which leads to the swelling of super disintegrants to create enough hydrodynamic pressure for quick and complete disintegration of the tablet. Batch F10 was selected as best formulation containing croscarmellose sodium as superdisintegrant in 10% concentration. It was observed that less disintegration time of 20 sec was observed when croscarmellose sodium was used as superdisintegrant, may be due to swelling at faster rate upon contact with water and elimination of lump formation after disintegration when compared with crospovidone and treated agar.

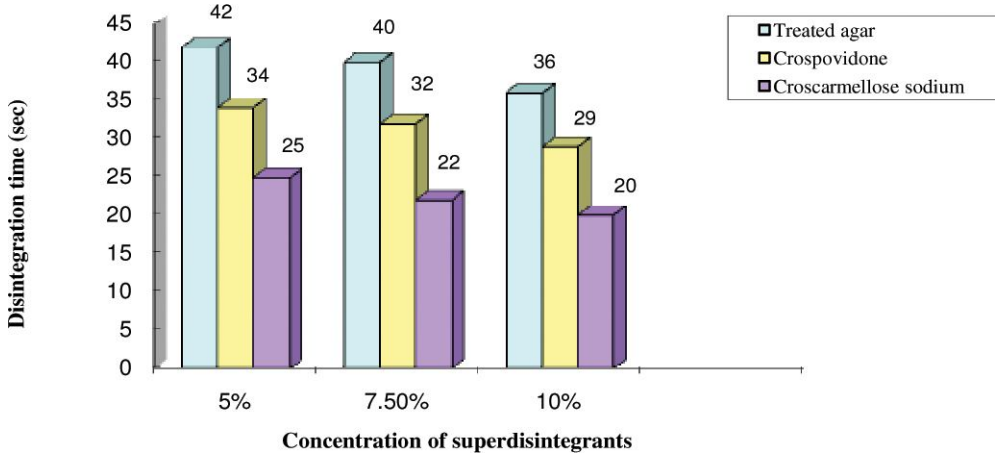

Fig. 6: Disintegration time of different superdisintegrants with different concentration

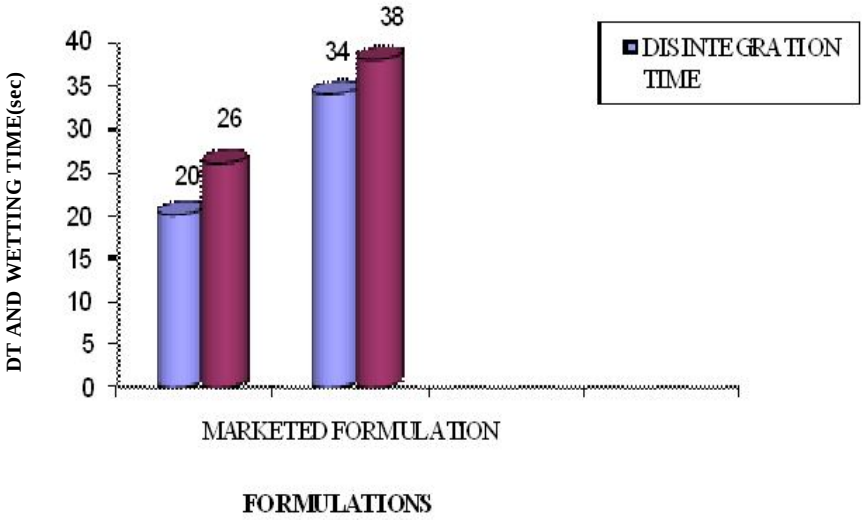

Fig. 7: Comparison of disintegration time and wetting time of best formulated tablet with marketed tablet

Finally the disintegration time of best formulation was compared with marketed formulation the results showed that formulated tablet disintegrated in 20 sec as compared to 34 sec for marketed Ondansetron tablet (ZOFER ODT). The formulation F10 was found to be the best, as this formulation showed less disintegration time and possessing good tableting properties.

The rapid drug dissolution might be due to easy breakdown of particles and rapid absorption of drug into the dissolution medium. This signifies that disintegrant concentration in 10% is suitable for the formulation of orodispersible tablets of Ondansetron hydrochloride.

The various dissolution parameter values viz., percent drug dissolved in 4 min (D4), t50% and t70% for promising formulations of 10% concentration of all the three different polymers (i.e.) F4, F7, F10 were compared with the control shown in Table 7 and the dissolution profile depicted in figure 8. This data reveals that the F10 formulation shows faster drug release compared to the commercial formulation (CF) based on t50% and t70% values in pH 6.8 Phosphate buffer. The best formulation F10 compared with marketed formulation.

Table 7: In vitro dissolution parameters in pH 6.8 phosphate buffer

Formulation code	$t_{50\%}$ (min)	$t_{70\%}$(min)	d_4(%)
F1	7.49	10.49	55.30%
F4	5.53	7.75	68.54%
F7	5.17	7.23	75.705
F10	5.02	7.03	74.16%
CF	5.72	8.01	65.77%

The Control formulation of (F1), 10% concentration of superdisintegrants for (F4, F7, F10) and commercial formulation of in vitro cumulative drug release was shown in the figure 8. The best and marketed formulation is depicted as shown in figure 9.

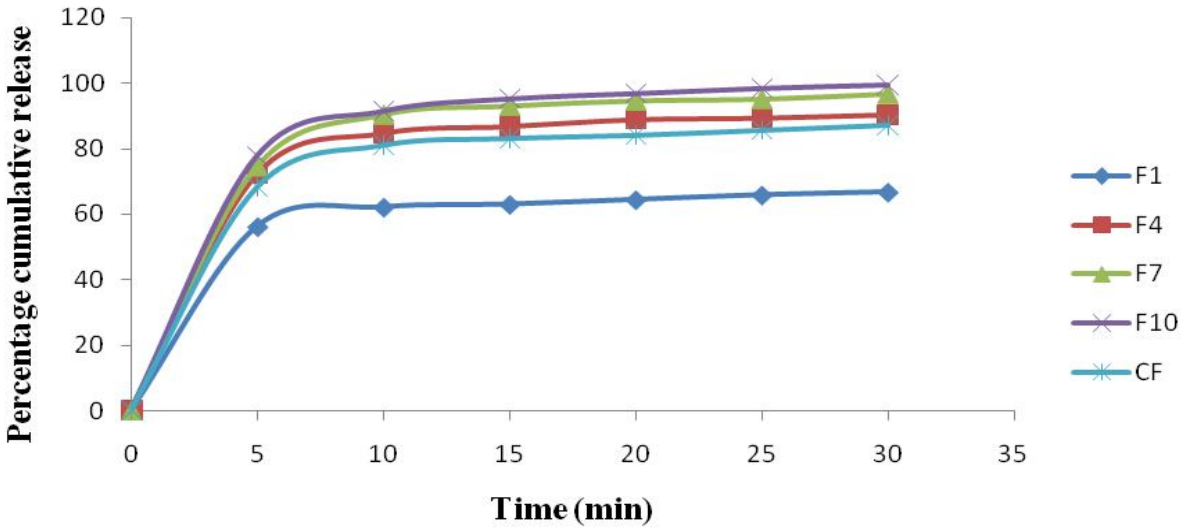

Fig. 8: Percentage cumulative drug release for F1, F4, F7, F10 and commercial formulation

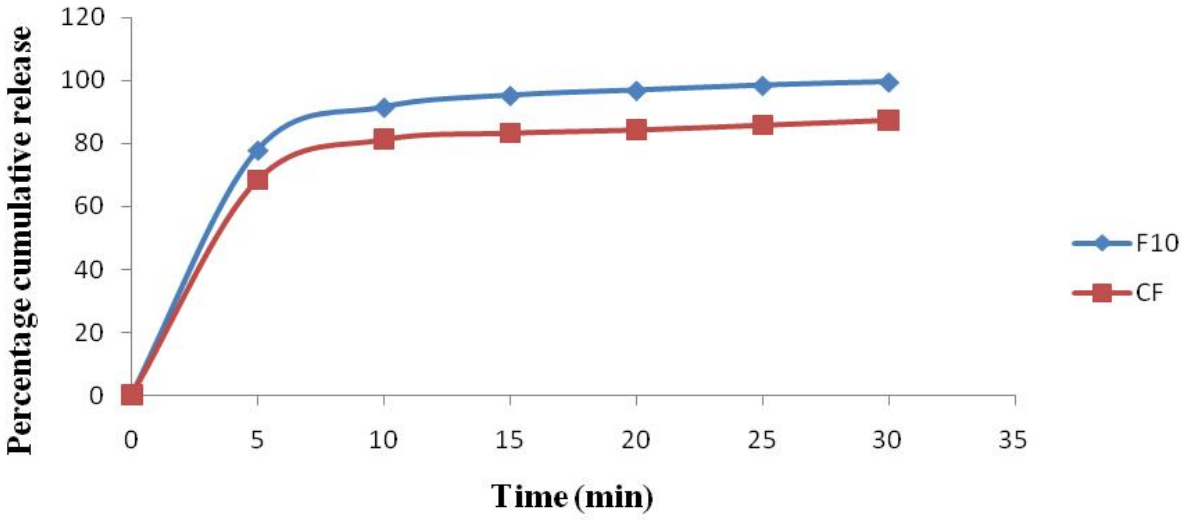

Fig. 9: Percentage cumulative drug release for F10 and marketed formulation

Conclusion

Orodispersible tablets of Ondansetron hydrochloride are prepared by direct compression method. The formulation F10 containing 10% of superdisintegrant (i.e.) Croscarmellose sodium has shown best release with 99.46% at the end of 30 min. The effervescent mixture further assists in taste masking and have pleasant mouth feel of Ondansetron hydrochloride. The tablets disintegrated rapidly in oral cavity and had acceptable hardness and friability. *In vitro* drug release from the tablets shows significantly rapid dissolution. Hence it could be concluded that the orodispersible tablets of ondansetron hydrochloride would be quite effective in emesis, providing quick onset of action without need for water for swallowing or administration.

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Review Article

Nanostructured Lipid Carriers: An Alternative to Solid Lipid Nanoparticles as Potential Second Generation Carrier for Topical Delivery of Antibiotics

Sandeep V. Nathwani*, Chetan H. Borkhataria*, Nilesh K. Patel*, Dhaval V. Patel*, Ravi A. Manek*, Kalpesh A. Patel*

Abstract

As compared to emulsion, liposomes and polymeric microparticulate systems, Nanostructured Lipid Carriers (NLC) has emerged as a novel colloidal drug carrier system which has gained a lot of popularity among researcher due to its applicability for various routes such as oral, topical and parenteral with embedded properties of site specific and controlled drug delivery with reduced side effects. Along with their advantages, some challenges such as low drug loading and drug expulsion from Solid Lipid Nanoparticles SLN during storage were needed to be addressed. These limitations were overcome in Nanostructured lipid carriers (NLC), which are second generation SLN. NLC accommodate the drug because of their highly unordered lipid structures. NLC can be administered via oral, ocular, pulmonary and intravenous routes. The present reviews correlate the types of NLC, preparation methods and characterisation of SLN and NLC. The review covers in brief the comparative study of SLN and NLC of some drugs by researchers.

Keywords: Solid lipid nanoparticles, Nanostructured lipid carriers, lipid matrix, drug carriers.

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Introduction

Solid lipid nanoparticles (SLN) are of several potential applications in drug delivery and research. Due to their unique size dependent properties, lipid nanoparticles offer possibility to develop new therapeutics. The ability to incorporate drugs into nanocarriers offers a new prototype in drug delivery that could use for drug targeting. Hence solid lipid nanoparticles hold great promise for reaching the goal of controlled and site specific drug delivery and hence attracted wide attention of researchers. SLN are the new generation of nanoparticulate active-substance vehicles and are attracting major attention as novel colloidal drug carriers for topical use. Compared with other vehicles such as creams, tinctures, and emulsions, SLN combine such advantages as controlled release, negligible skin irritation, and protection of active compounds.[1]

Although SLN have numerous advantages of controlled and targeted drug delivery increased stability of incorporated drug, there are some limitations too. During storage it was observed that drug was expelled out of SLN. The reason behind expulsion of drug was the highly ordered crystalline lipid matrix which was leaving very little space for drug molecules. To overcome the said problem nanostructured lipid carriers (NLC) were introduced. The first report on the use of SLN for oral delivery is by Speiser who termed them as nanopellets. As the science of SLN technology progressed, different methods of production for them were developed and stable formulations of SLN were discovered.[2]

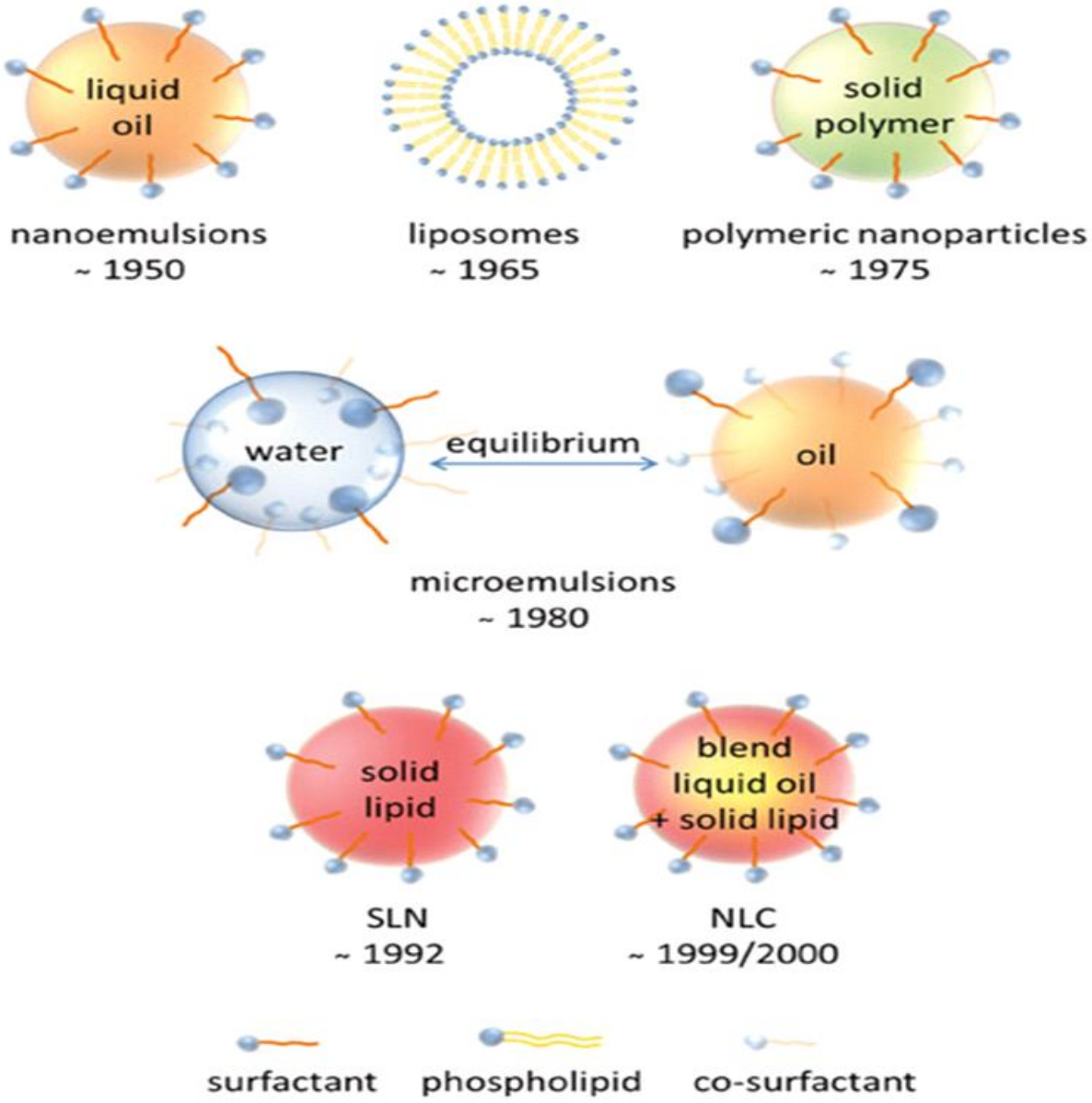

Fig. 1: Different generations of carrier dosage forms utilized for different drug delivery systems.

Earlier, the utilization of SLN for Parenteral drug delivery with focus on the definition of lipid nanoparticles and their different types such as SLN, NLC, and Lipid Drug Conjugates, their production techniques, scale-up feasibilities, stability of the incorporated drug, release and the biological and biopharmaceutical aspects have been discussed.

NLC are the second generation SLN, NLC are composed of binary mixture of solid lipid and a spatially different liquid lipid as the carrier. This consists of a lipid matrix with a special nanostructure and this nanostructure improves drug loading and firmly incorporates the drug during storage. NLC can be administered, e.g., via the oral, ocular, pulmonary and the intravenous route. NLC accommodate the drug because of their highly unordered lipid structures.[3]

Types of NLC

Type I

Solid lipids and liquid lipids (oils) are blended. The difference in the structures of the lipids and special requirements in the crystallization process lead to a highly disordered, imperfect lipid matrix

structure offering space for drug molecules and amorphous clusters of drugs. In general, drug solubility is higher in liquid lipids than in solid lipids. Based on this, particles were produced with a high content of liquid lipids (oils). During the production process, the liquid lipid particles (nanoemulsions) are cooled from the molten state to room temperature to crystallize and form solid particles.[4]

Type II

The multiple oil/fat/water, drug can be accommodated in the solid, but at increased solubility in the oily parts of the lipid matrix. At high oil concentrations a miscibility gap of the two lipids (solid lipid plus oil) occurs during the cooling phase, leading to phase separation, that means precipitation of tiny oily nanocompartments.[5]

Type III

Lipids are mixed in a way that prevents them from crystallizing. The lipid matrix is solid, but in an amorphous state.[6]

Table 1: Comparison of states between SLN and NLC along with their different subtypes.

Solid Lipid Nanoparticles	Nanostructured Lipid Carriers (Solid Lipid + Oil)
Drug With Solid Lipid Blend	Type I- Low Oil (Imperfect Matrix)
	Type II- High Oil (Multiple O/F/W Type)
	Type III-Amorphous (Noncrystalline Amorphous Nlc)

Methods of preparation for SLN and NLC

1. Homogenization Method.
   a) Hot homogenization
   b) Cold Homogenization
2. Solvent evaporation method.
3. Solvent emulsification-diffusion Method.
4. Microemulsion based method
5. Supercritical fluid method
6. Spray drying method
7. Double emulsion method
8. Precipitation technique
9. Film-ultrasound dispersion
10. High-speed homogenization followed by ultrasonication method

Hot homogenization

Hot homogenization is carried out at temperatures above the melting point of the lipid and can therefore be regarded as the homogenization of an emulsion. A preemulsion of the drug loaded lipid melt and the aqueous emulsifier phase (same temperature) is obtained by high-shear mixing device. High pressure homogenization of the pre-emulsion is carried out at temperatures above the melting point of the lipid. In general, higher temperatures result in lower particle sizes due to the decreased viscosity of the inner phase. However, high temperatures increase the degradation rate of the drug and the carrier. Increasing the homogenization pressure or the number of cycles often results in an increase of the particle size due to high kinetic energy of the particles.[7]

Cold homogenization

Cold homogenization has been developed to overcome various problems associated with hot homogenization such as temperature induced drug degradation, drug distribution into the aqueous phase during homogenization. In cold homogenization the drug containing lipid melt is cooled, the solid lipid ground to lipid microparticles and these lipid microparticles are dispersed in a cold surfactant solution yielding a presuspension. Then this pre-suspension is homogenized at or below room temperature, the gravitation force is strong enough to break the lipid microparticles directly to solid lipid nanoparticles.[8]

Solvent evaporation

SLN can also prepared by solvent evaporation method. The lipophilic material is dissolved in a

water-immiscible organic solvent (e.g. cyclohexane) that is emulsified in an aqueous phase. Upon evaporation of the solvent, nanoparticles dispersion is formed by precipitation of the lipid in the aqueous medium by giving the nanoparticles of 25 nm mean size. The solution was emulsified in an aqueous phase by high pressure homogenization. The organic solvent was removed from the emulsion by evaporation under reduced pressure (40–60 mbar).[9]

Microemulsion based method

Microemulsion based method Gasco and co-workers developed NLC preparation techniques which are based on the dilution of microemulsions.They are made by stirring an optically transparent mixture at 65-70 0 which is typically composed of a low melting fatty acid (stearic acid), an emulsifier (polysorbate 20, polysorbate 60, soy phosphatidylcholine, and sodium taurodeoxycholate), coemulsifiers (sodium monooctylphosphate) and water. The hot microemulsion is dispersed in cold water (2-3 0) under stirring. Typical volume ratios of the hot microemulsion to cold water are in the range of 1:25 to 1:50. The dilution process is critically determined by the composition of the microemulsion. According to the literature, the droplet structure is already contained in the microemulsion and therefore, no energy is required to achieve submicron particle sizes. Nanoparticles were produced only with solvents which distribute very rapidly into the aqueous phase (acetone), while larger particle sizes were obtained with more lipophilic solvents. The hydrophilic cosolvents of the microemulsion might play a similar role in the formation of lipid nanoparticles as the acetone for the formation of polymer nanoparticles. [10]

Spray drying method

It's an alternative procedure to lyophilization in order to transform an aqueous NLC dispersion into a drug product. It's a cheaper method than lyophilization. But his method can cause particle aggregation due to high temperature, shear forces and partial melting of the particle.[11]

Models for incorporation of active compounds into SLN

There are basically three different models for the incorporation of active ingredients into SLN

I. Homogeneous matrix model

II. Drug-enriched shell model

III. Drug-enriched core model

Release of active compounds from SLN

The effect of formulation parameters and production conditions on the release profile from SLN was intensively investigated by Mehnert, Müller and zur Mühlen. They investigated the release profile as a function of production temperature. It can be summarised from their findings that the release profiles were often biphasic—an initial burst release was followed by a prolonged release.

The extent of burst release could also be controlled by the amount of surfactant used in the formulation. High surfactant concentration leads to high burst release, low surfactant concentration to minimisation of the burst. This was explained by redistribution effects of the active compound between the lipid and the water phase during the heating up process and subsequently the cooling down process after production of the hot oil-in-water emulsion during the hot homogenization process. Heating the lipid / water mixture leads to an increased solubility of the drug in the water phase, the drug partitions from the melted lipid droplet to the water phase. After homogenization, the oil in water emulsion is cooled, the lipid core starts crystallizing with still a relatively high amount of active drug in the water phase. Further cooling leads to supersaturation of the drug in the water phase, the drug tries to partition back into the lipid phase; a solid core has already started forming leaving only the liquid outer shell for drug accumulation. From this it can be summarised that the higher the solubility of drug in the water phase during production, the more pronounced is the burst effect.[12]

The solubility increases with increasing production temperature and increasing surfactant concentration (the latter only when the surfactant solubilises the active compound). Consequently, little or no burst will be obtained when producing at low temperatures, low surfactant concentration or surfactant in free medium.

Conclusion

To overcome the stability and drug expulsion problems of SLN, the NLC (known as second

generation) had emerged. The highly unordered lipid matrix structured of NLC improved drug encapsulated and stability also presenting controlled and targeted drug release made them popular in nanopharmaceutical research field. NLC are attractive alternatives to micro and nanoemulsion, liposomes and

nanoparticles, but a detail study of possibility of meeting industrial needs such as process scale up, equipment qualification and validation is required. We hope in near future a number of drugs will be presented as their NLC.

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Review Article

Thiazolidinediones–Recent Advances and Biological Activities

K. Srikanth Kumar*, A. Lakshmana Rao, M.V. Basaveswara Rao*****

Abstract

Heterocyclic compounds containing sulphur, nitrogen and oxygen atoms in the core structure shows number of pharmacological and biological activities. So, various heterocyclic systems were synthesized, studied in the past decade and found to possess remarkable pharmacological activities and are employed in the treatment of commonly occurring diseases. This has been back bone for the chemists to impart interest for the synthesizing some novel derivatives. In the last few decades, the chemistry of five membered heterocyclic rings has received considerable attention owing to their synthetic and biological importance. Among that one such class of compound is thiazolidinediones. The synthesis of novel thiazolidinedione derivatives and investigation of their chemical and biological behaviour have gained much more importance in recent decades. The thiazolidinediones chemistry has been developed extensively and is still developing. Presently there are a few number of drugs used clinically which comprises thiazolidinedione moiety in association with various heterocyclic rings. The present review deals with the structural features, synthesis, mechanism of action associated with thiazolidinedione derivatives and special emphasis is given on recently reported thiazolidinedione analogues possessing various biological activities.

Keywords: Thiazolidinediones, chemistry, synthesis, biological activities.

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Introduction

Heterocyclic compounds are important components of biomolecules such as proteins, DNA, RNA, vitamins and also found in cell lining. Among the various heterocyclic compounds five membered heterocyclic systems containing sulphur, nitrogen and oxygen atoms represents one of the most active classes of compounds possessing a wide range of biological activities, including antibacterial, antifungal, anti-inflammatory, anti-cancer, etc. In earlier days drugs are obtained from the plants, animals and mineral sources, but due to lack of potency and sometimes more toxicity, there is a need for the discovery of new drugs that are less toxic and more potency is essential. Synthesis of new derivatives has been an important part and is aimed at modifying the action of drugs, particularly to reduce the side effects and to increase the drug action.

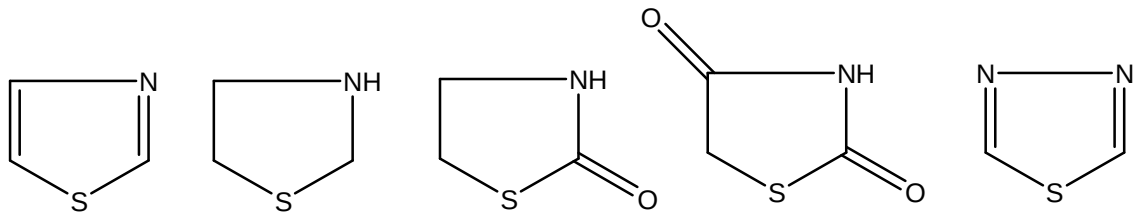
Diabetes mellitus is one of the life threatening causes found in most of the countries in the world which is due to impaired carbohydrate, protein and lipid metabolism. Thiazolidinediones (TZDs) are the novel class of hypoglycaemic agents for the treatment of NIDDM (Non-insulin dependent diabetes mellitus). Initially TZDs were identified as

antidiabetic drugs which are known to sensitize tissues to insulin. A deficient insulin secretion which translates into impaired glucose use is a characteristic feature of diabetes mellitus results in hyperglycemia[1].

Thiazolidinediones normalizes elevated blood glucose levels and is of great use in the treatment of type 2 diabetes. Thiazolidinediones having high affinity towards Peroxisome Proliferator Activated Receptor gamma type (PPARγ) receptors and acts as insulin sensitizers at PPARγ receptors. This stimulates peripheral adiposities to increase their uptake of free fatty acids, which leads to reduction in the fat stored in muscles, liver and visceral fat deposits. Thiazolidinediones improve insulin sensitivity in liver, muscle & fat tissues and thus counteract insulin resistance. Sulfonyl ureas-Metformin, common antidiabetic drug induce severe hypoglycemia and weight gain. Ciglitazone- first synthesized thiazolidinedione derivative, having antihyperglycemic activity in insulin resistant animal models, but it was withdrawn because of low potency and appearance of cataracts, anemia and oedema in animals. Troglitazone- failed to survive due to liver toxicity. Pioglitazone and Rosiglitazone- currently in clinical use. These are also having drawbacks like producing anemia, oedema and weight gain. These thiazolidinedione drugs however have been associated with hepatotoxicity[2], haematological toxicity and body weight gain problems. This situation emphasizes the need to develop new antidiabetic agents that could retain the insulin sensitizing properties of thiazolidinediones, but be safer and have better efficacy.

Chemistry of thiazolidinediones[3]

There are various biologically active molecules with five membered heterocyclic rings containing nitrogen and sulphur as hetero atoms such as thiazole(**I**), thiazolidine(**II**), thiazolidinone(**III**), thiazolidinedione(**IV**), thiadiazole(**V**) (having two nitrogens and sulphur as heteroatoms).



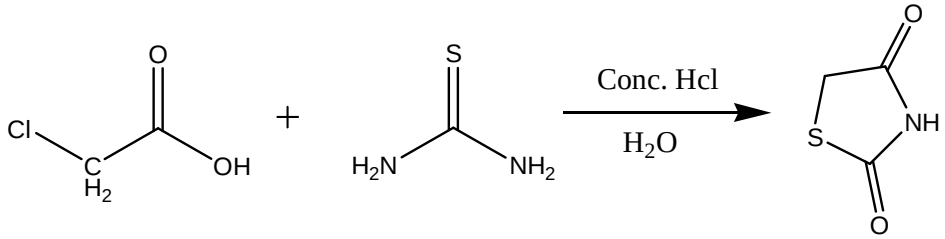
(I) **(II)** **(III)** **(IV)** **(V)**

1,3-thiazolidine-2,4-diones having thiazolidine moiety with two carbonyl groups at 2nd and 4th positions(**IV**). Substituents may be varied in the 3rd and 5th positions exhibit different biological activities. All the drugs Troglitazone, Englitazone, Ciglitazone, Pioglitazone and Rosiglitazone having a common nucleus i.e. 1,3-thiazolodine-2,4-dione is responsible for the majority of the pharmacological activities[4].

Synthesis of thiazolidinediones

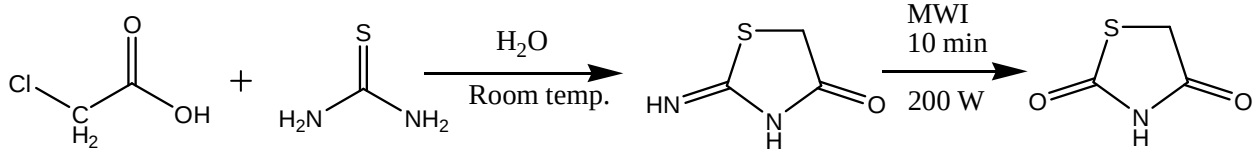
General procedure for the synthesis of 2,4-thiazolidinedione[5]:

In a 250ml three necked flask was placed a solution containing 56.4g (0.6mol) of chloroacetic acid in 60ml of water and 45.6g (0.6mol) of thiourea dissolved in water. The mixture was stirred for 15minutes to form a white precipitate, accompanied by considerable cooling. To the contents of the flask, was then added slowly 60ml of concentrated HCl from a dropping funnel. The flask was then connected with a reflux condenser and gentle heat applied to effect complete solution. Thereafter, the reaction mixture was stirred and refluxed for 8-10 hr at 100-110ºC. The mixture was cooled and product was filtered and washed with water to remove traces of hydrochloric acid. The product was purified by recrystallization from ethyl alcohol.

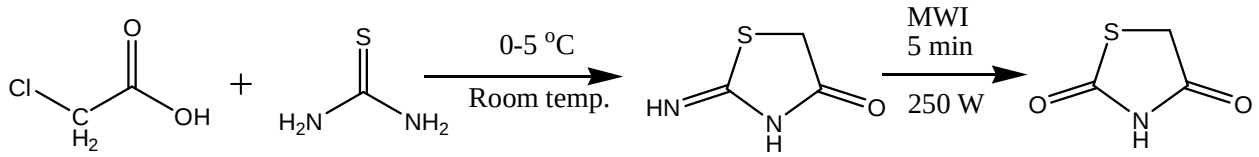


Microwave-assisted synthesis

Gaonkar SL et al[6], proposed microwave assisted synthesis: A mixture of monochloroacetic acid (1.00g, 10.58mmol) and thiourea (0.81g, 10.6mmol) in water (2ml) were introduced into microwave reaction vessel. The vessel was sealed and stirred for 1 hour at room temperature. The resulting 2-imino-thiazolidin-4-one was irradiated by 200 Watt microwave at 140°C for 10 min. The mixture was cooled to room temperature and stirred for 1 hr. The formed solid was filtered and recrystallized from hot water.



Prashantha Kumar BR et al[7], proposed microwave assisted synthesis: Microwave induced synthesis of thiazolidinedione have also been reported. Chloroacetic acid, thiourea, water are transferred into long necked vial and stirred under ice cold conditions for about 15 min to form a white precipitate of 2-imino-thiazolidine-4-one as intermediate. Irradiation with microwave is carried out at 250 Watt for 5 min. Cool the reaction mixture, followed by collection of the solid that separated by filtration and washing with water to give white crystals of thiazolidine-2,4-dione.



Mechanism of action of thiazolidinediones (TZDs)

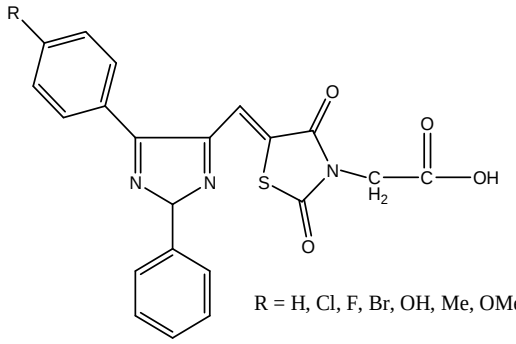
Thiazolidinediones (TZDs) are a new class of antidiabetic agents and include three compounds that have come to clinical use- Troglitazone, Rosiglitazone and Pioglitazone as well as several others that have been limited to pre-clinical study. TZDs were initially discovered by screening compounds for a hypoglycemic action in the *ob/ob* mouse[8] and subsequently they were shown to improve insulin action in a variety of obese and diabetic animal models with insulin resistance[9]. In these model systems, TZDs reduce plasma glucose and insulin levels and improve some of the abnormalities of lipid metabolism. Consistent with animal studies, clinical studies have shown that treatment of type 2 diabetic patients with TZDs can lower serum glucose and insulin levels, increase peripheral glucose uptake, and decrease triglyceride levels[10].

TZDs bind to an isoform of a nuclear receptor which is a transcription factor, after heterodimerization with the retinoid X receptor (RXR), bind to specific response elements of a number of target genes and control their transcription. There is an excellent correlation between the hypoglycemic effects of TZDs *in vivo* and their affinity for PPARγ *in vitro*, but the site of action and the molecular mechanism of TZDs still remain poorly known. Clinical studies in human have confirmed that TZDs lowered postprandial and postabsorptive glycemia and insulinemia. Glucose clamp studies have clearly shown an improvement of insulin-induced glucose utilization in skeletal muscle[11]. TZD sexert their antidiabetic effects through a mechanism that involves activation of the gamma isoform of the peroxisome proliferators activated receptor (PPARγ, a nuclear receptor. TZD-induced activation of PPARγ alters the transcription of several genes involved in glucose and lipid metabolism and energy balance, including those that code for lipoprotein lipase, fatty acid transporter protein, adipocyte fatty acid binding protein, fatty acyl-CoA synthase, malic enzyme, glucokinase and the GLUT4 glucose transporter. TZDs reduce insulin resistance in adipose tissue, muscle and the liver. However, PPARγ is predominantly expressed in adipose tissue. It is possible that the effect of TZDs on insulin resistance in muscle and liver is promoted via endocrine signalling from adipocytes. Potential signalling factors include free fatty acids (FFA) (well-

known mediators of insulin resistance linked to obesity) or adipocyte-derived tumour necrosis factor-α (TNF-α) which is over expressed in obesity and insulin resistance. Although there are still many unknowns about the mechanism of action of TZDs in type 2 diabetes, it is clear that these agents have the potential to benefit the full 'insulin resistance syndrome' associated with the disease. Therefore, TZDs may also have potential benefits on the secondary complications of type 2 diabetes, such as cardiovascular disease[12].

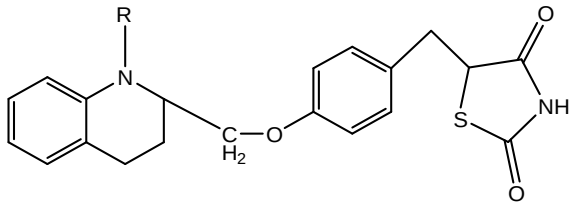
Biological activities of thiazolidinedione derivatives

Deepak KA et al[13], developed an efficient and convenient synthesis of three series of pyrazolyl-2,4-thiazolidinedione derivatives by Knoevenagel condensation. All the synthesized compounds were characterized by spectral and elemental analytical data and evaluated for their *in vitro* antifungal and antibacterial activities. Results of the antifungal activity were found to be comparable with the reference compound. On the other hand, antibacterial activity was best observed for Gram-positive bacteria only, none of the compounds showed activity against Gram-negative bacteria.



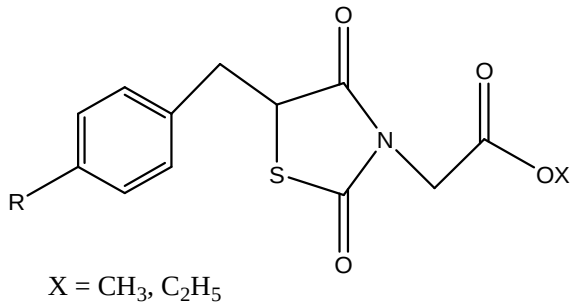
R = H, Cl, F, Br, OH, Me, OMe, NO_2

Hyesung K et al[14], a series of tetrahydroquinoline-linked thiazolidinediones was designed and synthesized and their peroxisome proliferator activated receptor-γ (PPARγ) agonistic activities were evaluated. A number of analogues were revealed to have significant PPARγ agonistic activity. Among the synthesized compounds, 5-[4-(1-Heptyl-1,2,3,4-tetrahydroquinolin-2-ylmethoxy) benzyl]thiazolidine-2,4-dione possessing *N*-heptyl moiety was found to be the most active in PPARγ transactivation assay.



R = heptyl, Methy, isopropyl, isoprenyl, n-butyl, etc

Bashir AB et al[15], synthesized 5-substituted arylidene/5-substituted benzyl-thiazolidin-2,4-dione-3-acetic acid ethyl ester and methyl ester derivatives and they were screened for their antihyperglycemic activity *in vivo* by sucrose loaded model (mice) and in vitro by PPARγ activation, found that [5-(4-hydroxy-benzyl)-2,4-dioxo-thiazolidin-3-yl]-acetic acid ethyl ester exhibited higher antihyperglycemic activity than the corresponding methyl ester.

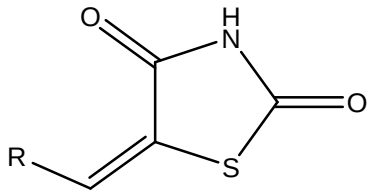


X = CH_3, C_2H_5

R = OH, Me, OMe, Cl, NH_2, CF_3, NMe_2

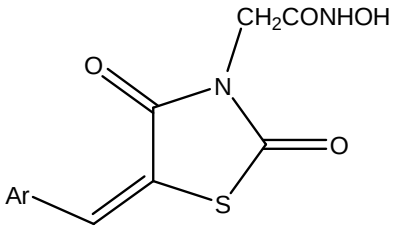
Brackman G et al[16], synthesized a series of 5-substitutedthiazolidin-2,4-dione derivatives and identified that structural resemblance between thiazolidinediones and well-known furanone type quorum sensing (QS) inhibitors such as N-acylaminofuranones and/or acyl-homoserine lactone signalling molecules which antagonized autoinducer 2 (AI-2) binding to its receptor. The synthesized thiazolodinediones affect AI-2 QS in *Vibrio harveyi*, evaluated as most active AI-2 QS inhibitors, with EC_{50} values in the low molecular range. The mechanism of inhibition was elucidated by measuring the effect on bioluminescence in series of *V. harveyi* QS mutants and by DNA-binding assays with purified LuxR protein. Results indicate that

thiazolidinediones blocked AI-2 QS in *V. harveyi* by decreasing the DNA-binding ability of LuxR.

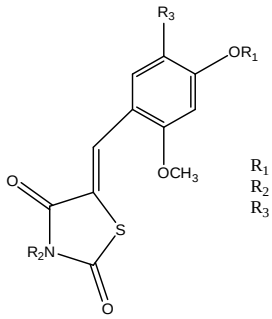


$R = C_7H_{15}, C_9H_{19}, C_{10}H_{21}, C_{11}H_{23}$

Rosanna M et al[17], synthesized (5-arylidene-2,4-dioxothiazolidin-3-yl)acetamides and analogues of N-hydroxy acetamides and they were found to be very active *in vitro* alodose ruductase inhibitors. The synthesized compounds were evaluated for their ability to inhibit the *in vitro* reduction of D,L-glyceraldehyde by partially purified aldose reductase from bovine lenses. Out of synthesized acetamides, N-hydroxy-2-[5-(4-hydroxybenzylidene)-2,4-dioxothiazolidin-3-yl] acetamide displayed an interesting micromolor IC_{50} value.

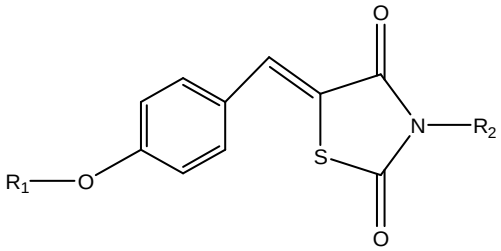


Wang Z et al[18], synthesized a series of novel 5-(substituted benzylidene)-thiazolidine-2,4-dione derivatives and they were evaluated as competitive inhibitors of protein tyrosine phosphatase 1B. Most of the synthesized compounds showed potent inhibitory effects against protein tyrosine phosphatase 1B and the compound. Compounds with allyl, prenyl, n-butyl or benzyl at 3rd position nitrogen of these series showed excellent activity.



R_1 = H, methyl, allyl, prenyl, butyl, THP
R_2 = Metthyl, prenyl, butyl, allyl, isopropyl
R_3 = Br, Allyl

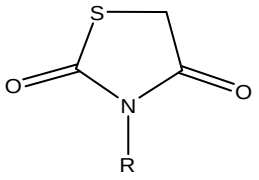
Ying Wu et al[19], synthesized a wide range of 5-(substituted)-thiazolidine-2,4-dione derivatives with different substituents on the phenyl ring and they were evaluated as 15-hydroxyprostaglandin dehydrogenase (15-PGDH) inhibitors. Compound 5-[4-{2-(thiophen-2-yl)ethoxy}benzylidene] thiazolidine-2,4-dione was identified as the most potent 15-PGDH inhibitor that was effective in nanomolar range.



R_1 = Cyclohexyl, 2-isopropoxyethyl, 2-morpholinoethyl 2-(thiophen-2-yl)ethyl, 2-(pyridin-2-yl)ethyl, etc

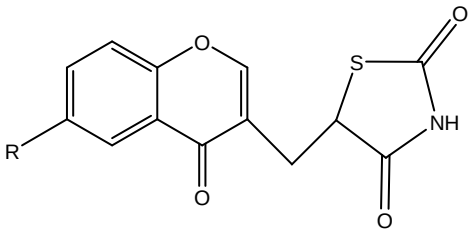
R_2 = H

Santosh LG et al[20], synthesized a series of N-substituted thiazolidine-2,4-dione derivatives by microwave irradiation method and evaluated their antibacterial activity against *S. aureus, E. coli, P. aeruginosa, K. pneumoniae,* antifungal activity against *A. fumigatus, A. flavus, P. marneffei, C. albicans.* In their study they found that 3-(4-nitrobenzyl)thiazolidine-2,4-dione and 3-(4-fluorobenzyl)thiazolidine-2,4-dione showed high potency.



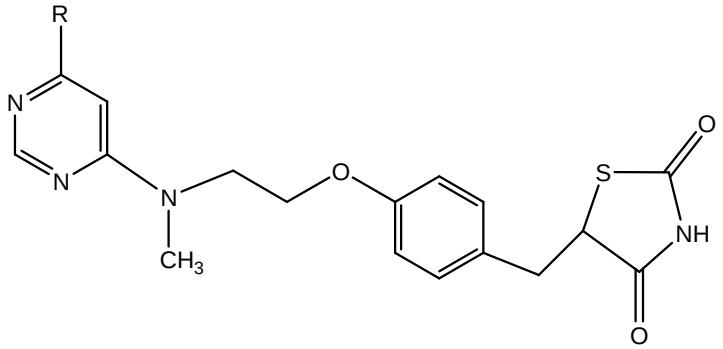
R = 4-nitrobenzyl, 4-fluorobenzyl, etc

Nazreen S et al[21], synthesized a library of conjugates of chromones and thiazolidine-2,4-dione by Knoevenagel condensation followed by reduction using hydrogen gas and Pd / C as a catalyst. In their study they found that compounds (where X = methoxy or chloro) were most effective in lowering the blood glucose level comparable to standard drug Pioglitazone. All the synthesized molecules were docked against PPARγ target showed good glide score. PPARγ gene expression was significantly increased by the compound (where X=chloro) in comparison to standard drug Pioglitazone.



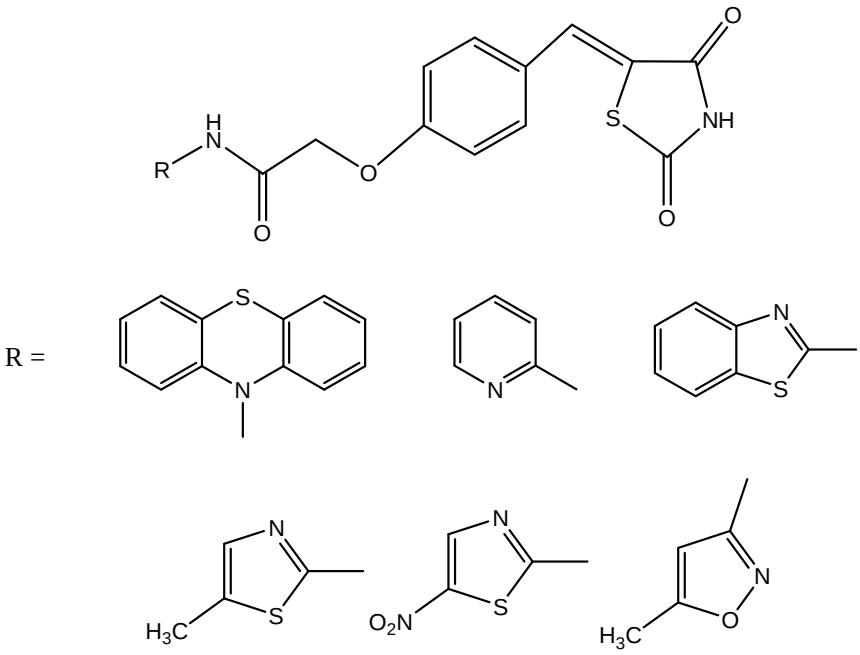
R = H, CH_3, OCH_3, Br, Cl, F

Lee HW et al[22], synthesized series of novel substituted pyrimidine derivatives having thiazolidine-2,4-dione and evaluated their hypoglycaemic and hypolipidemic activities. The synthesized compounds were evaluated for their effect on triglyceride accumulation in 3T3-L1 cells. Also, their hypoglycaemic and triglyceride lowering effects examined in mice. In their study among the synthesized analogues, 5-[4-{2-(6-[4-methoxyphenoxy]-pyrimidin-4-yl)methylamino ethoxy} benzyl] thiazolidine-2,4-dione showed the most excellent biological activity.

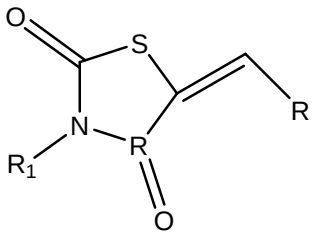


R = methoxy, isopropoxy, phenoxy, phenylamino, *p*-fluorophenoxy, *p*-methoxyphenoxy, etc

Vijay P et al[23], synthesized some novel 5-benzylidne-2,4-thiazolidinedione derivatives and all the synthesized compounds were evaluated for their antiproliferative activity *in vitro* on the 7-cell line panel consisting of HOP62(Lung cancer), PC3(Prostate cancer), MCF7(Breast cancer), HEPG2(Hepatoma), K562(Leuemia), GURAV(Oral cancer), and KB (Nasopharyngeal cancer) at 10-fold dilutions of four concentrations ranging from 10^{-4} to 10^{-7} M. Though the compounds showed varying degrees of cytotoxicity in the tested cell lines, most marked effect was observed by the compound 2-[4-{(2,4-dioxothiazolidin-5-ylidene)methyl}phenoxy]-N-[3-(trifluoromethyl) phenyl]acetamide in MCF7 (Breast cancer), K562(Leuemia) and GURAV(Oral cancer) cell lines.

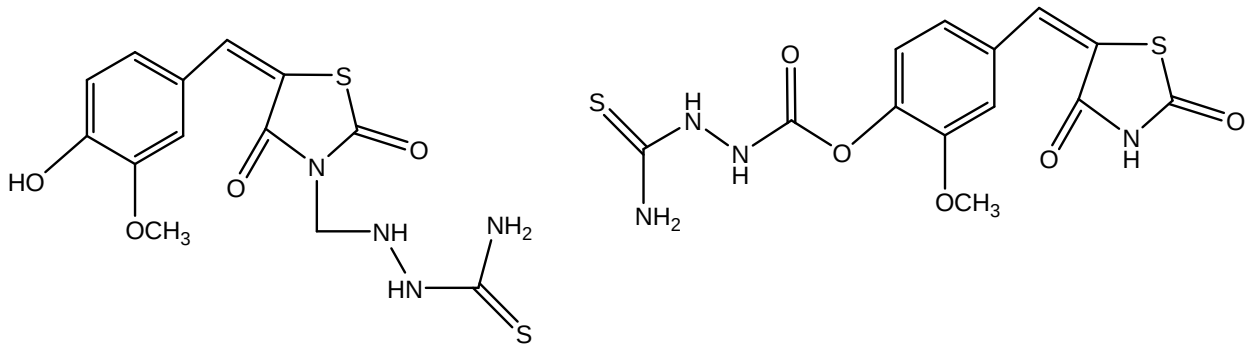


Naresh Babu C et al[24], carried out synthesis of some new 3,5-disubstituted-2,4-thiazolidinedione derivatives and the synthesized compounds were predicted for biological activities by using prediction of activity spectra for substances computerized program(*Insilico* method) based on those results the compounds were screened against *Mycobacterium tuberculosis* $H_{37}R_V$ strain in the Middlebrook 7H9 broth by MABA (Microplate Alamar Blue Assay) using Streptomycin and Pyrazinamide as standard drugs. The results revealed that among the synthesized compounds having phenyl rings with amino, aldehyde and nitro groups showed good antitubercular activity.

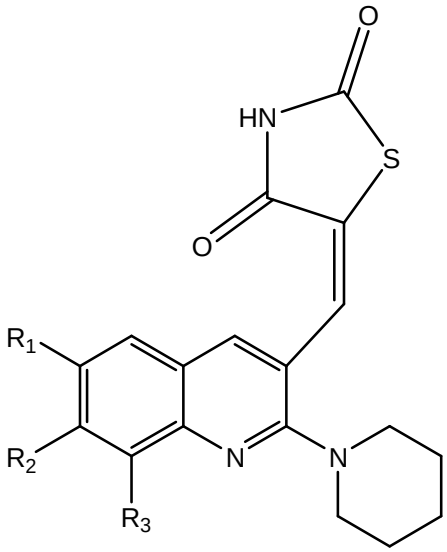


R= 4-methoxybenzyl, 4-dimethylaminobenzyl,benzyl
R_1 = 4-aminophenyl, *tert*-butyl, aminoacetyl, 2-amino-5-nitrophenyl, etc

Shital LN et al[25], synthesized a series of novel thiazolidinedione derivatives by incorporating pharmacologically significant moieties such as ester, hydrazide and substituted amine groups linked to the central phenyl ring as well as replacement of phenyl by heterocycle like substituted furan ring by employing multi-step synthetic protocols. The synthesized compounds were tested for their *in vitro* antibacterial activity against the Gram-positive bacteria such as *B. subtilis, S. aureus* and Gram-negative bacteria such as *P. aeruginosa.* The MIC values were determined by using broth dilution method in nutrient broth media. In their study found that the compounds 4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]-2-methoxyphenyl-2-carbamothioyl hydrazine carboxylate and 2-{[(5E)-5-(4-hydroxy-3-methoxybenzylidene)-2,4-dioxo-1,3-thiazolidin-3-yl] methyl}hydrazine carbothioamide containing thiosemicarbazide moiety showed good spectrum of activity.



Jyotirling RM et al[26], developed one-pot multi-component synthetic route for new quinolidinyl-2,4-thiazolidinediones using safer medium polyethylene glycol-400. They were tried different routes to develop new quinolidinyl-2,4-thiazolidinediones such as two step synthesis, conventional heating and microwave irradiation route. By comparing the different routes, microwave irradiation route produce the product with in 7-8 min giving better yields where as in conventional route it takes about 8-9 hrs.

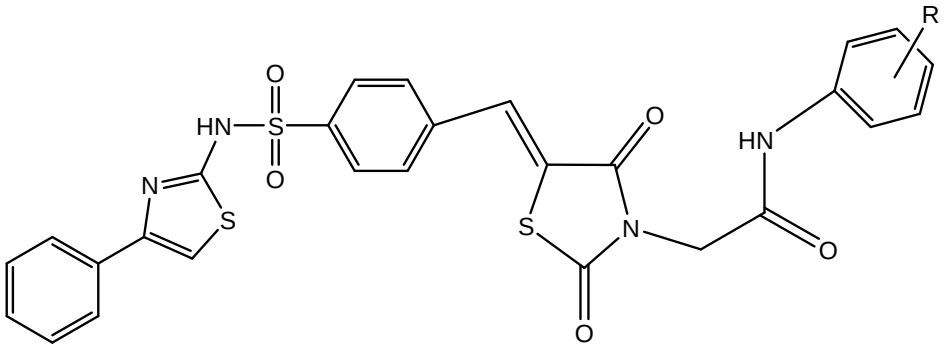


R_1 = H, CH_3, OCH_3, OC_2H_5

R_2 = H, CH_3, OCH_3

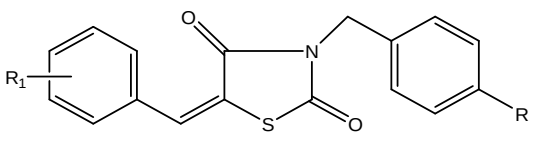
R_3 = H, C_2H_5

Krunal VJ et al[27], synthesized a new series of N-chloro aryl acetamide substituted 2,4-thiazolidinedione derivatives. The newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against *S. aureus, B. subtilis, E. coli and P. aeruginosa* using disk diffusion method. In their study revealed that few compounds showed good antibacterial activity against both Gram-positive and Gram-negative bacteria.



R = H, 3-CH_3,4-CH_3, 2-Cl, 3-Cl, 4-Cl, 3-NO_2, 4-NO_2, etc

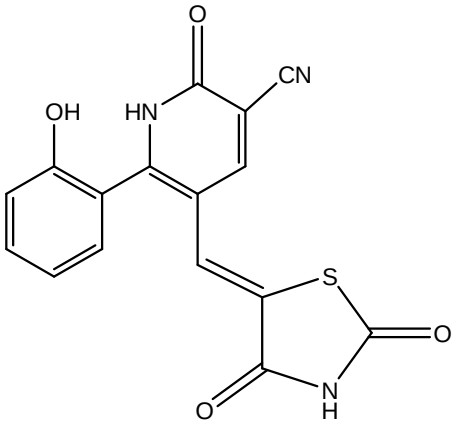
Neeru Malik et al[28], synthesized a series of N-substituted-2,4-thiazolidinedione derivatives using different substituted benzyl halides and aromatic aldehydes. The synthesized compounds were evaluated for their antimicrobial activity against the strains *B. subtilis* and *E. coli* using cup plate method. The results revealed that compounds (where R = 4-OH, 4-NMe_2, 4-hydroxy-3-methoxy) exhibit significant activity.

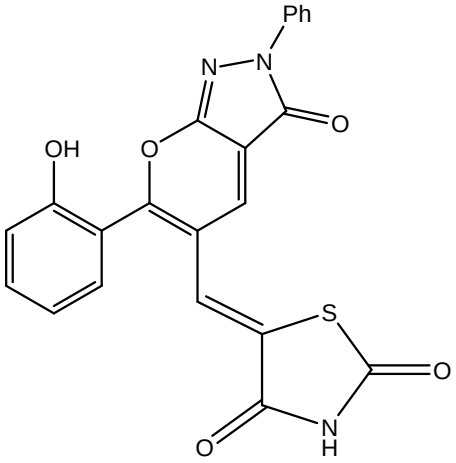


R = H R_1 = H, 4-OH, 4-Cl, 4-NMe_2, 2-OH, 4-OH-3-OCH_3

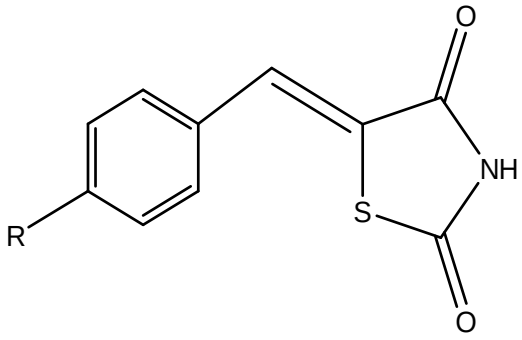
Ibrahim MA et al[29], developed a new approach for the synthesis of bioactive thiazoidine-2,4-dione derivatives and they were evaluated for their antimicrobial activity by using disc agar diffusion

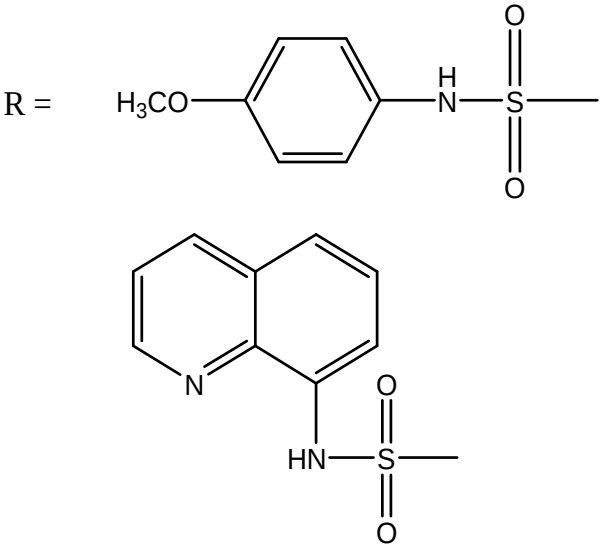
method against the organisms *S. aureus* as Gram-positive bacteria, *P. vulgaris* as Gram-negative bacteria and *C. albicans* as fungal strain. In their study they found that below given compounds showed the highest antimicrobial activity.



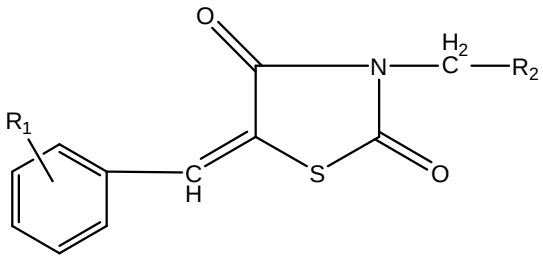


Roy A et al[30], synthesized some novel 5[4-(substituted)benzylidene]-2,4-thiazolidinedione derivatives and evaluated as oral hypoglycaemic agents by fructose loaded model. The compounds 5-{4-[(4-methoxyanilinyl)sulfonyl]benzylidene}-2,4-thiazolidinedione and 5-{4-[(8-amino quinolinyl) sulfonyl] benzylidine}-2,4-thiazolidinedione was significantly decreased the blood glucose levels in fructose induced diabetic male albino Wistar rats. Interestingly compond 5-{4-[(4-methoxyanilinyl)sulfonyl]benzylidene}-2,4-thiazolidinedione showed longer duration of action among all the synthesized compounds.



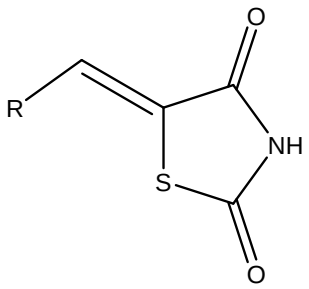


Jiwane SK et al[31], synthesized some novel 2,4-thiazolidinedione derivatives having dialkyl amine moiety at N-3 position. All the synthesized derivatives were evaluated for their antidiabetic activity in Dexamethasone induced rats. The synthesized compounds showed remarkable antidiabetic activity. The compounds where R_1 = *o*-OCH_3 / *p*-OCH_3, R_2 = diethylamino groups possess remarkable hypoglycaemic activity which indicates that the á-amino methyl group at position-3 shows different hypoglycaemic activity from that of the standard compound Rosiglitazone.



R_1 = *o*-OH, *o*-OCH_3, *p*-OCH_3

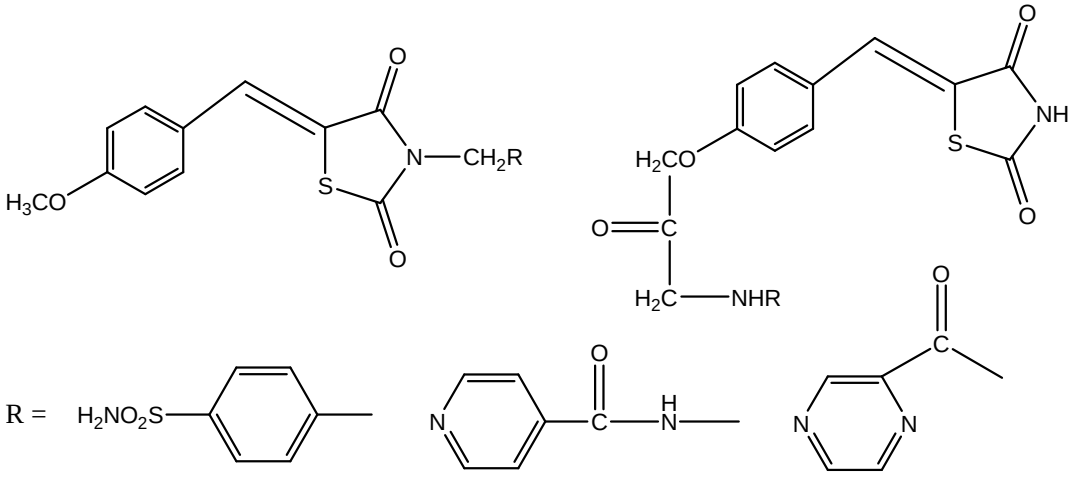
R_2 = diethylamino, diphenylamino



R = 4-chlorophenyl, 4-methoxyphenyl, 2-furyl,etc

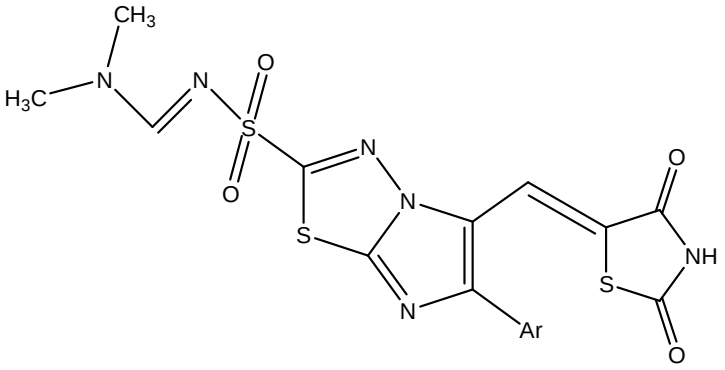
Suresh et al[32], developed a simple, rapid and environmentally benign protocol for the synthesis of 5-arylidene-2,4-thiazolidinedione derivatives via ZnO nanoparticles catalysed Knoevenagel reaction. In their study they found that the synthetic procedure does not need volatile organic solvents, catalyst ZnO nanoparticles shows an excellent catalytic activity by activating both reactants without the formation of any by-products, produces excellent yields in shorter reaction time, used catalyst can be recycled and reused many times without the reduction in catalytic potential. ZnO nanoparticles were synthesized by chemical precipitation technique using zinc acetate and absolute ethanol.

Shashikant P et al[33], synthesized a series of some novel 2,4-thiazolidinedione derivatives and evaluated for their antibacterial, antitubercular and antidiabetic activities. Antibacterial activity was carried out by cup plate agar diffusion method using nutrient agar. The compounds were tested *in vitro* for their antibacterial activity against *E. coli* and *S. aureus* using standard drug Norfloxacin. Presence of SO_2NH_2 moiety in compounds showed promising antibacterial activity. Antitubercular activity was carried out by Middlabrook 7H9 agar medium against *Mycobacterium tuberculosis* $H_{37}Rv$ strain using standard drug Streptomycin. Presence of Isoniazid and Pyrazinamide moieties in compounds shown promising antitubercular activity. Antidiabetic activity was carried out using Wistar albino rats by alloxan induced tail tipping method using standard drug Glibenclamide. Their study revealed that some compounds showed promising antidiabetic activity.



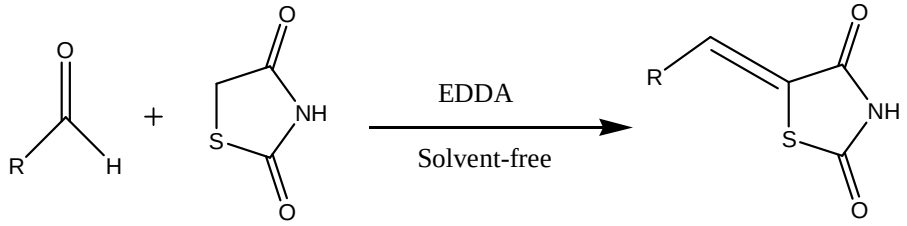
Alagawadi KR et al[34], synthesized some new 2,4-thiazolidinedione derivatives bearing imidazo[2,1-*b*][1,3,4]thiadiazole moiety. All the synthesized compounds were evaluated for their preliminary *in vitro* antibacterial and antifungal activity. Antibacterial activity and antifungal activity was

carried out against the Gram-positive *S. aureus, E. faecalis,* Gram-negative *E. coli, P. aeruginosa bacteria*; *C. albicans, A. flavus, A. niger, C. neoformans fungi.* The MIC values were determined by using two-fold serial dilution technique in Meuller-hinton broth and Sabouraud dextrose agar for the antibacterial activity and antifungal activities, respectively. In their study compounds having 6-phenyl, 6-(*p*-chlorophenyl), 6-(*p*-bromophenyl) derivatives showed very good biological activity.



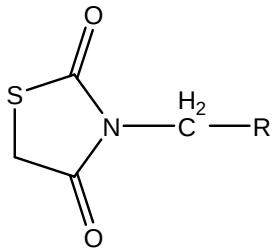
Ar = C_6H_5, 4-CH_3-C_6H_4, 4-OCH_3-C_6H_4, 4-NO_2-C_6H_4, 4-Br-C_6H_4, 4-Cl-C_6H_4

Zhang Y et al[35], developed a solvent free protocol for a simple and efficient synthesis of 5-arylidene-2,4-thiazolidinedione derivatives using ethylenediamine diacetate as catalyst. The major advantages of this method are simple experimental and work-up procedures, solvent-free reaction conditions, and small amount of catalyst, short reaction time, high yields, and utilization of an inexpensive and reusable catalyst.



R = C_6H_5, 4-CH_3-C_6H_4, 4-OCH_3-C_6H_4, 4-NO_2-C_6H_4, 4-Br-C_6H_4, 4-Cl-C_6H_4, etc

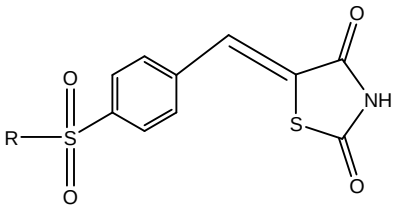
Shahnaz et al[36], synthesized some 2,4-thiazolidinedione derivative and evaluated for their antioxidant activity using free radical scavenging activity by DPPH (1,1-diphenyl-2-picryl-hydrazil) assay method and ascorbic acid was used as reference standard. A series of 2,4-thiazolidinedione derivatives was prepared by mannich reaction by reacting various secondary amines, formaldehyde with 2,4-thiazilidinedione as hydrogen active compound. Among the synthesized compounds, compounds 3-(pyrrolidin-1-ylmethyl)-thiazolidine-2,4-dione and 3-[(diethyl amino) methyl] thiazloidine-2,4-dione showed promising antioxidant activity.



R = dimethylamino, diethylamino, morpholinyl, pyrrolidinyl, piperidinyl

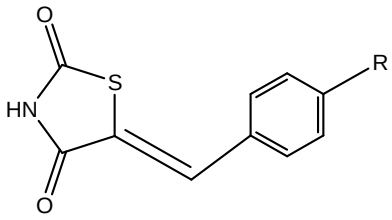
Pattan SR et al[37], synthesized new series of thiazolidinedione derivatives by reacting under

microwave irradiation. The title compounds were screened for their antidiabetic activity by Alloxan induced tail tipping method. The Albino rats of either sex weighing between 150-200 g were selected. The blood glucose level was induced and the study was carried out in six difference groups. Out of the nine synthesized compounds most of the compounds have shown significant antidiabetic activity.



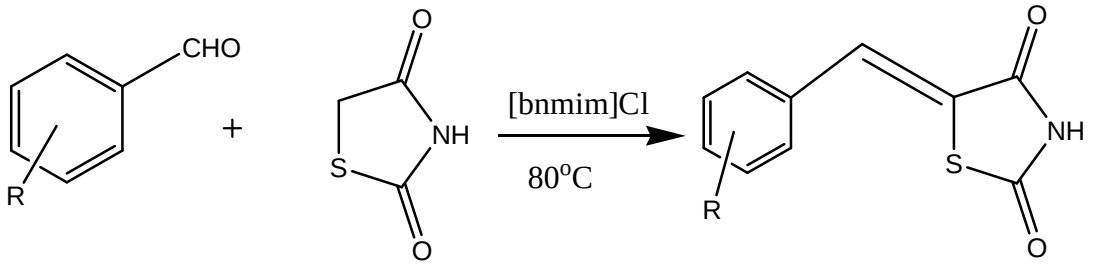
R = morpholinyl, pyrrolidinyl, piperidinyl, *p*-chloroanilino, etc

Rekha S et al[38], synthesized a series of some 5-substituted benzylidene-2,4-thiazolidinedione derivatives and evaluated for their anti-inflammatory activity. The activity was not favourable when compared to standard drug.

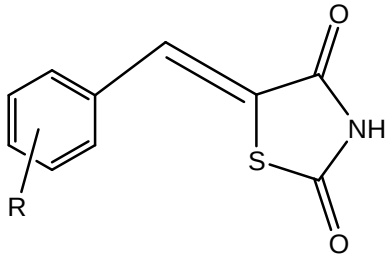


R = 4-phenoxy, 4-morpholinyl, 4-methylpiperizinyl,etc

Shelke KF et al[39], synthesized 5-arylidine-2,4-thiazolidinediones by the Knoevenagel condensation of aromatic aldehydes with 2,4-thiazolidinedione in the presence of 1-benzyl-3-methylimidazolim chloride ionic liquid at 80°C. The major advantages of this method are short reaction time, high yields and green aspects by avoiding toxic catalyst and solvent. The ionic liquid was successfully reused for four cycles without significant loss of activity.



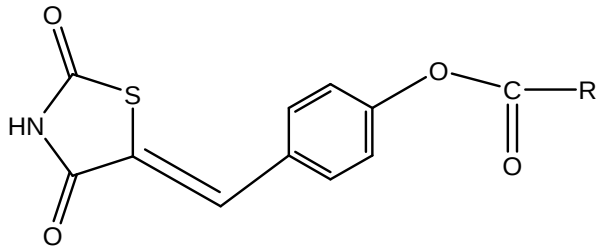
Garg A et al[40], synthesized a series of 5-substituted arylidene-2,4-thiazolidinedione derivatives and the synthesized compounds evaluated for their *in-vivo* antiinflammatory and analgesic and *in vitro* activities. Antiinflammatory activity was carried out in rats using Carragenaan-induced acute paw oedema model. Analgesic activity was carried out in Swiss albino mice of either sex by using tail flick method. Antioxidant activity was carried out by using DPPH method. In their study compound 5-(3-chlorobenzylidene)-thiazolidin-2,4-dione showed good antiinflammatory, analgesic and antioxidant activities.



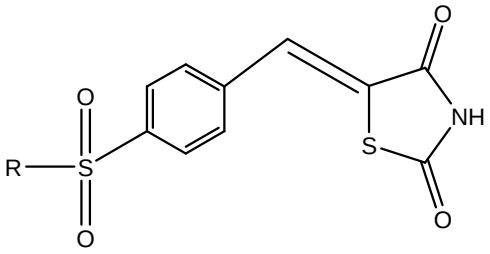
R = 4-methoxy, 3,4-dimethoxy, 3-chloro

Rekha S et al[41], synthesized the basic ring of thiazolidinedione by 1,3 dipolar cycloaddition of thiourea and monochloro acetic acid. Knoevenagel condensation with substituted aromatic aldehyde was carried out to yeild various substituted benzylidenethiazolidine-2, 4-dione. These compounds were subjected to further esterification and substitution reaction to yield target compounds.

The synthesised molecules were screened for blood glucose lowering effect *(in vivo)*. 3,5-dimethyl-pyrazole-1-carboxylic acid 4-(2,4-dioxo-thiazolidin-5-ylidenemethyl)-phenyl ester and 5-methyl-3-oxo-pyrazolidine-1-carboxylic acid 4-(2,4-dioxo-thiazolidin-5-ylidenemethyl)-phenyl ester showed blood glucose lowering effect in Dexamethasone induced insulin resistance model.

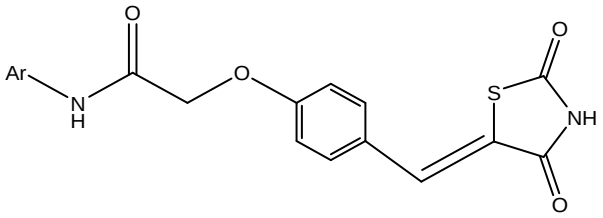


Shashikant RP et al[42], synthesized a new series of thiazolidinedione derivatives. All of the compounds were screened for antidiabetic activity on albino rats. Most of the compounds showed significant antidiabetic activity when compared with the standard drug Glibenclamide. Compounds were screened for their antidiabetic activity by Alloxan induced tail tipping method. The albino rats of either sex weighing between 150-200 g were selected. The blood glucose level was induced and the study was carried out in six difference groups. Out of nine compounds synthesized most of the compounds showed significant antidiabetic activity.



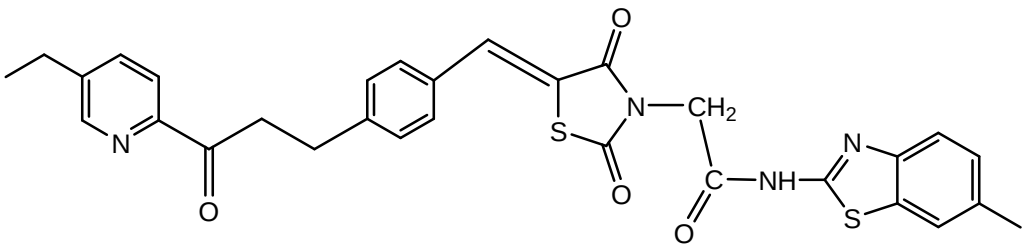
R = 4-pyridinyl, 4-morpholinyl, 4-chloroanilino, etc

Pratima AGN et al[43], synthesized twelve novel 2,4- thiazolidinedione derivatives 2-{4-[(*E*)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}-*N*-(substituted phenyl) acetamides by conventional as well as microwave-assisted method; which required very short duration, 3-5 minutes for completion of reaction and gave better yield as compared to conventional synthesis which requires 2-5 hrs refluxing. The synthesized compounds were studied for their *in vivo* hypoglycemic study by Alloxan induced hyperglycemia in Wistar albino mice model. Biological activity was expressed in terms of percent decrease in blood glucose level. Most of the synthesized compounds showed moderate hypoglycemic activity and compound 2-{4-[(*E*)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl] phenoxy}-*N*-(phenyl) acetamide has shown highest hypoglycemic activity amongst the synthesized derivatives and comparatively better activity than the standard drug, Pioglitazone.

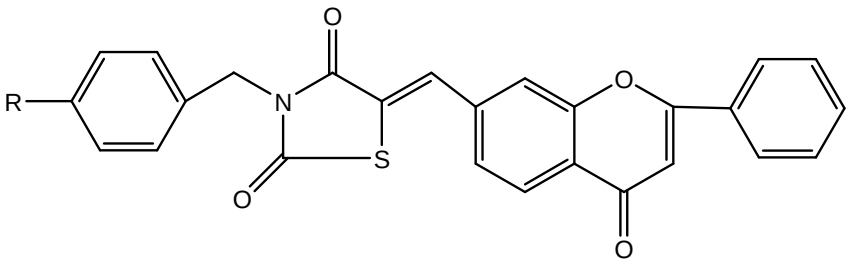


Ar = aromatic, heterocyclic, alicyclic

Navin BP et al[44], Synthesized a series of 2-(5-{4-[2-(5-ethyl-pyridin-2-yl)-ethoxy]-benzylidine}-2,4-dioxo-thiazolidin-3-yl)-*N*-heterocycle-acetamide derivatives and evaluated for antimycobacterial and antimicrobial activity. *In vitro* antimycobacterial activity was carried out against *(M. tuberculosis)* $H_{37}Rv$ strain using Lowenstein-Jensen medium and antimicrobial activity against two Gram-positive bacteria (*S. aureus*, *S. pyogenes*), two Gram-negative bacteria (*E. coli*, *P. aeruginosa*) and three fungal species (*C. albicans*, *A. niger*, *A. clavatus*) using the broth microdilution method. Their study revealed that compound (E)–2-(5-(4-(2-(5– ethylpyridin–2-yl)ethoxy)benzylidene)–2,4–dioxothiazolidin–3-yl)–N-(6-methylbenzo[*d*]thiazol-2-yl) acetamide showed very good antimycobacterial activity.

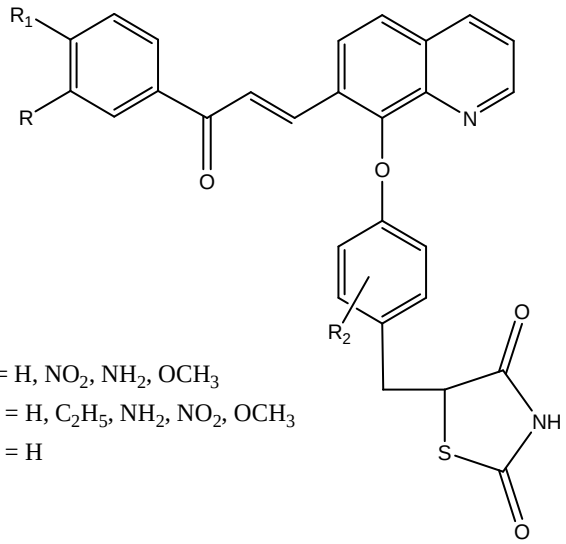


Oya Bozda G et al[45], synthesized some flavonyl thiazolidinedione derivatives. These derivatives were synthesized by Knoevenagel condensation from flavone-6-carboxaldehyde and 3-substituted-2,4-thiazolidinediones.



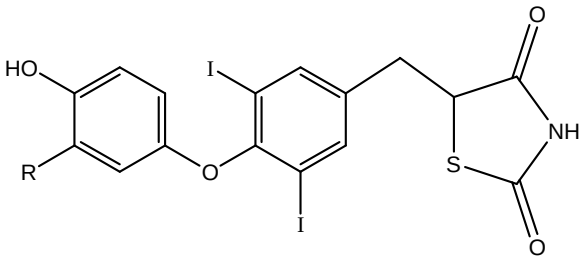
R = H, Cl, Br, F, NO_2

Srikanth L et al[46], prepared various thiazolidinedione derivatives with a quinoline ring moiety and evaluated them for antidiabetic activity. The synthesized derivatives were then screened for their hypoglycemic activity by determining their blood glucose concentration which was compared to that of the standard drug Rosiglitazone. The test was performed on rats. Derivatives 5-(4-{7-[(E)-3-(4-Nitrophenyl)-3-oxo-propenyl]-quinolin-8-yloxy}-benzyl)-thiazolidine-2,4-dione and 5-(4-{7-[(E)-3-(4-Methoxy-phenyl)-3-oxo-propenyl]-quinolin-8-yloxy}-benzyl)-thiazolidine-2,4-dione were found to be active.



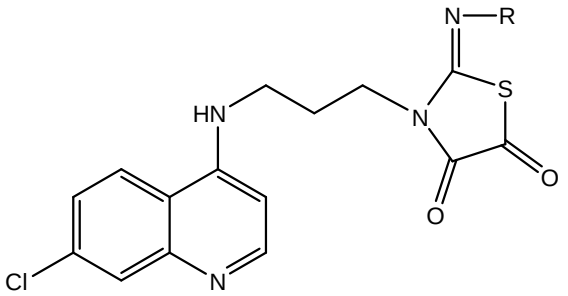
R = H, NO_2, NH_2, OCH_3
R_1 = H, C_2H_5, NH_2, NO_2, OCH_3
R_2 = H

Masayuki E et al[47], designed and synthesized several thiazolidinedione derivatives, they were evaluated as thyromimetic drugs. Among the synthesized compounds, the dihydrogenated compounds, such as 5-2-[[4-(3-*tert*-butyl-4-hydroxyphenyl)oxy-3,5-diiodophenyl]ethyl]-2,4-thiazolidinedione and its 3-isopropyl analogue, exhibited potent thyroid hormone receptor α 1 (TR α 1) activation activity.



R = tertiary butyl, isopropyl, etc

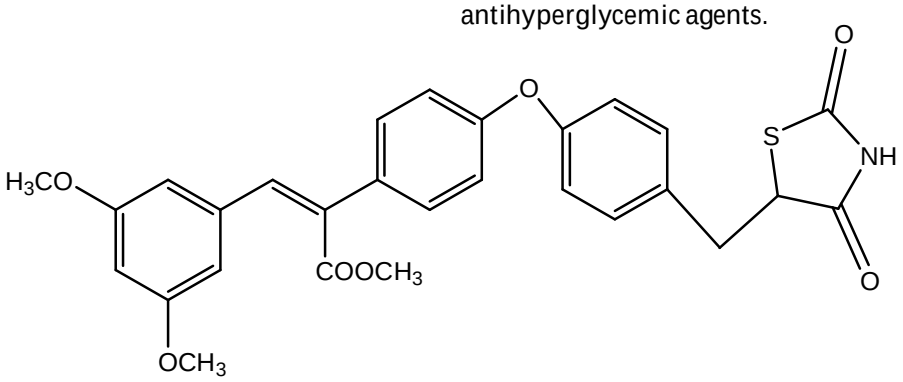
Naresh S et al[48], synthesized a series of some thiourea, thiazolidinedione and thioparabanic acid derivatives of 4-aminoquinoline were synthesized and screened for their antimalarial activity.



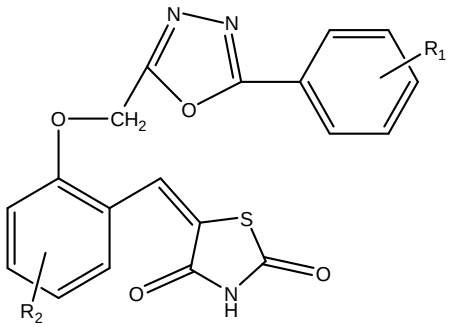
R = *o*-chlorophenyl, phenyl, butyl, allyl

Neogi P et al[49], synthesized a number of 2,4-thiazolidinedione derivatives of phenyl substituted cinnamic acid were synthesized and studied for their PPARγ agonist activity. The E-isomer of cinnamic

acid derivative showed moderate PPARγ transactivation. The corresponding Z-isomer, and double bond reduced derivative were found to be much less potent. In their study identified a new series of thiazolidine-2,4-dione substituted α-phenyl cinnamic acids with moderate PPARγ agonist activity showing strong oral glucose lowering effects in animal models of type 2 diabetes. The data suggests that the presence of the cinnamic acid double bond as well as its geometry is very important for PPAR agonism. Thus cinnamic acid based TZDs can provide lead compounds to develop new antihyperglycemic agents.



Nazreen Sd et al[50], synthesized a library of novel 1,3,4-oxadiazole and 2-4-thiazolidinedione based bis-heterocycles which exhibited significant PPARγ transactivation and blood glucose lowering effect comparable with the standard drugs Pioglitazone and Rosiglitazone. Among the synthesized compounds 5-[2-{(5-Phenyl-1,3,4-oxadiazol-2-yl)methoxy}benzylidene]thiazolidine-2,4-dione and 5-[2-[[5-{(2,4-Dichlorophenoxy)methyl}-1,3,4-oxadiazol-2-yl]methoxy] benzylidene] thiazolidine-2,4-dione did not cause body weight gain and were found to be free from hepatotoxic and cardiotoxic side effects. Compounds 5-[2-{(5-Phenyl-1,3,4-oxadiazol-2-yl)methoxy}benzylidene]thiazolidine-2,4-dione and 5-[2-[[5-{(2,4-Dichlorophenoxy)methyl}-1,3,4-oxadiazol-2-yl]methoxy] benzylidene]thiazolidine-2,4-dione increased PPARγ gene expression by 2.10 and 2.00 folds, respectively in comparison to the standard drugs Pioglitazone (1.5 fold) and Rosiglitazone (1.0 fold). Therefore those compounds considered as potential candidates for development of new antidiabetic agents.



R_1 = H, 4-Cl, 4-Br, 2-OEt R_2 = H, 3-OMe, 3-OEt, 5-Br

Conclusion

This review has highlighted the chemistry, synthesis of different derivatives of 2,4-thiazolodinediones and their biological activities. The literature reveals that 2,4-thiazolodinediones has been associated with numerous biological potential such as antidiabetic, antiprolifiretaive, antioxidant, antibacterial, antifungal, antiinflammatory, etc and by simple synthetic routes (conventional and microwave-assisted synthesis). Some of the recent studies states that thiazolidinediones possess antitubercular and antiproliferative activities may be given potent molecules in future.

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Review Article

Bilosomes-an Innovative Trend in Lipid Based Drug Delivery

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Abstract

Pharmaceutical industries are now focused towards research and development of new drug delivery technologies to fulfill the needs of the modern world. Innovations with lipid based drug delivery have become a vital component of development in dosage form. Many formulations and innovations in controlled and targeted drug delivery have been attempted for the release of drug at the right place in a safe and reproducible manner. Liposomal drug delivery systems has become successful and has led to many development in liposome based formulations like niosomes, ethosomes, transferosomes, bilosomes, etc. Bilosomes are colloidal novel drug delivery systems similar to other liposomal delivery systems but prepared by incorporating bile salts into the lipid bilayer membrane. This present article focuses on the bilosomal drug delivery systems.

Keywords: liposomal delivery systems, lipid bilayer membrane, drug delivery, niosomes, ethosomes, transferosomes, bilosomes.

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Introduction

In this modern era of drug discovery, there is a remarkable growth in the use and development of drug[1]. Pharmaceutical industries strive to meet the needs of the global demand. Novel drug delivery systems from already existing drug molecules have becomes the new scenario of the pharmaceutical world than to discover new drug moieties[2]. This can be achieved by developing a carrier system to keep the drug molecule intact and take them to the right direction to the target cells thereby exert action without altering the regular activities of the body. Thus phospholipids mediated delivery of drugs has been introduced for treatment of various diseases[3]. The therapeutic efficacies of these delivery systems are enhanced due to their specific delivery to the target organs like tissues or cells, controlled release etc.

This lipid based drug delivery systems has been used as effective carriers in delivering drugs like anticancer agent, vaccines, antigens, genetic materials, imaging agents. The principle components of these lipoidal systems are the phospholipids like phosphatidyl choline, phosphotidyl glycerols and cholesterols[4].

Advantages of vesicular drug delivery[5,6]

- Poorly soluble drugs have increased bioavailability because of the lipid covering.
- It acts as an efficient sustained release dosage form.

- Due to increase in bioavailability increase in therapeutic index and efficacy of the drug is achieved.
- Both hydrophilic and lipophilic drugs can be incorporated.
- Because of the drug being encapsulated within lipid barriers, they remain stable.
- Less toxicity, improved patient compliance and easy administration.

There are various novel lipid based carrier systems which includes ethosomes, transferosomes, niosomes, bilosomes, virosomes, emulsomes[7] etc.

This review article is aimed to give an overview about one of the lipid based carrier systems-The bilosomes.

Bilosomes-Background

They are novel innovative carriers of drug delivery which consists of bile salts incorporated into niosomal membrane. This is formulated with an aim to protect the membrane against effect of bile acids in gastrointestinal tract[8]. Further bile salts even act as penetration enhancers. Since these are produced from naturally occurring lipids, they are biocompatible, non toxic and also have high bioavailability of the drug[9].

So far there has been numerous research works carried out on different drugs using bilosomal system, few examples are

Jitender *etal* investigated the effect of bilosomal oral vaccine delivery using different blend ratios of monopalimitoyl glycerol, cholesterol, diethyl phosphate and sodium deoxycholate. His study resulted in reduction of median temperature differential change and decrease in viral cell load in influenza[10].

Another study by I.J Ayogu explained that insulin delivered with soya bean lipid extract showed better bioavailability than the normal drug administration.

Shukla *etal* showed that systemic as well as mucosal responses of antibody on oral administration of HBsAg loaded bilosomes[11].

One of the study on vaccines proved that the degradation of antigen in the GI tract and the inability to create a mucosal antibody response were solved due to the conversion of normal vaccine into bilosomal vaccine delivery.

Preparation of Bilosomes

Bilosomal drug delivery systems can be prepared by similar methods of preparations of ethosomes and other lipid based delivery systems[12].

There are 4 methods of preparation

1. Cold Method
2. Hot Method
3. Classic mechanical dispersion method
4. Classic method.

Characterisation of Bilosomes

Once the formulation of bilosomes has been completed the bilosomes should undergo characterisation studies for their shape, size, morphology, vesicle size, surface charge[13] etc.

Shape, size, morphology:

These studies were carried out by Transmission Electron Microscopy by using 0.2% phosphotungstic acid solution.

Vesicle size:

The vesicle size can be determined by zeta seizer and even the polydispersity index can also be determined by the same.

Zeta potential measurement can be performed to determine the surface charge of the bilosomes.

These are the basic characterisation studies involved in bilosomes other studies like *invitro* release study, *invitro* binding specificity, stability in simulated gastric fluid, fluorescence microscopy can be performed to determine the stability and efficiency of the product[14].

Conclusion

Vesicular drug delivery system has now changed the direction of researchers towards development of these delivery systems due to the enormous advantages being rendered by this kind. These delivery systems deliver the drugs directly to the target tissue and they are deformable too[15]. They decrease

dosing frequency and so various researches are being conducted on these dosages in the last few years.

These bilosomes can reduce toxic effects, over dosing and they have been identified to show their therapeutic efficacy from topical to genetic levels.

Thus, bilosomal drug delivery system can behave as an essential dosage form in the pharmaceutical field.

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