CHAPTER-VII

Synthesis of Xanthene Derivatives And

Evaluation Of Anticancer Activity

Associated With Them.
**INTRODUCTION**

Xanthene (1) is polyaromatic cyclic ether compounds which have one oxygen in the ring system. Two benzene rings are fused to tetrahydropyran which is six membered cyclic ether with five carbons and one oxygen. It is soluble alcohol and ether. Pyran ring structure is the basis of the pyranoses. Xanthene is used as a fungicide itself. But the main function is as the fundamental structure of chromophore particularly luminescent dyes. Xanthene class dyes include fluorenes, pyronins, succineins, sacchareins, rosamines, rhodamines, rhodols and fluorones. Xanthone is the xanthene ketone. It is used as an ovicide and as a larvicide. Thioxanthone is the sulfur analogue of xanthone. An sulfur atom exists on the ring system instead of an oxygen atom. Xanthene structure provides characteristic property of spectroscopic photodynamic activity. Its derivatives are widely used in biological stains, light and temperature sensitizers, photoinitiator of polymerization process, histotechnologies, photochromic and thermochromic agents and laser dyes as well as in molecular chemistry and organometallic-complexes.

Xanthene (9H-xanthene, 10H-9-oxaanthracene) (1) is a yellow organic heterocyclic compound. Its chemical formula is C$_{13}$H$_{10}$O. It is soluble in ether. Its melting point is 101-102 °C and its boiling point is 310-312 °C. Xanthene is used as a fungicide
and it is also a useful intermediate in organic synthesis. Xanthene forms the basic structure of many natural products, drugs, dyes (fluorescein, pyronin, and eosins), indicators, pesticides, antibiotics, etc.

Derivatives of xanthene are commonly referred to collectively as xanthenes, and among other uses are the basis of a class of dyes which includes fluorescein, eosins, and rhodamines. Xanthene dyes tend to be fluorescent, yellow to pink to bluish red, brilliant dyes.

Xanthene (1) forms colorless crystals and is prepared by thermal dehydration of 2,2’-dihydroxydiphenylmethane (2), its oxidation with HNO₃ leads to xanthone (3)

A number of dyes are derived from the xanthene structure (xanthene dyes) such as fluorescein (4), eosin (5) and pyronine (6).

9-Arylxanthenes (7) are formed when electron-rich aromatic aldehydes react with an excess of arynes. Initial nucleophilic attack by the carbonyl oxygen atom on the aryne generates a benzoxete which isomerises to an o-quinone methide. A [4+2] cycloaddition with the aryne completes the sequence.
The xanthene framework is also formed when salicylaldehyde reacts with alkenyl cyclohexenes in the presence of bentonite clay. Two intramolecular hetero cyclisations are involved to form xanthenes (8).

Xanthense (9) can also be formed by rearrangement for example the xanthene unit of the dibenzopyranoazepine alkaloid, clavizepine, has been obtained from a dibenzoxepinediol by a pinacol rearrangement.

**REVIEW OF LITERATURE**

George A Kraus et al ¹, reported the synthesis and antitubercular activity of tricyclic analogs of xanthenes (10).

(10)

Kelly Chibale et al ² reported the synthesis of xanthene derivatives and evaluated for their potential as trypanothione reductase (TryR) inhibitors and chloroquine (CQ) potentiating agents. Some derivatives (11) displayed inhibitory activity against TryR comparable to known tricyclic anti-depressants. On the other hand a number of derivatives increased CQ accumulation and potentiating effects in a resistant strain of *Plasmodium falciparum*
with one compound also displaying strong intrinsic antimalarial activity.

(11)

Eric Vieira et al. reported the synthesis of a new class of selectively fluorinated small molecule mGluR1 enhancers (12) with improved pharmacokinetic properties is presented. Their potential use as pharmacological tools for the study of the physiological roles mediated by mGlu1 receptors is discussed.

(12)

Thomas Troxler et al. have reported achiral β-alanine piperazine amide derivative of xanthenes (13) as highly potent and selective sst1 receptor antagonists.

(13)

Karen T Welch et al. have used virtual screening approach was used to identify new glycomimetics. The National Cancer Institute Diversity Set was docked into the carbohydrate binding site of the lectin concanavalin A (ConA). The resulting poses were analyzed and 19 molecules were tested for inhibition with an enzyme-linked lectin assay (ELLA). Eight of the 19 molecules inhibited ConA–carbohydrate binding. The two most potent
inhibitors (14) have IC$_{50}$ values that are an order of magnitude smaller than the monosaccharide methyl α-d-mannopyranoside.

Hafez HN et al ⁶ have reported The 1,3-dipolar cycloaddition of nitrile imines to 9H-thioxanthone-9-thione and 9H-xanthone-9-thione afforded novel spiro-thioxanthene-9′,2-[1,3,4]thiadiazoles and spiro-xanthene-9′,2-[1,3,4]thiadiazoles in good yields. Some of the newly synthesized compounds were tested for anti-inflammatory and analgesic activities comparable to ibuprofen. Compounds and showed significant activity compared to standard drug. The toxicity studies revealed that neither death nor other behavioral or toxicological changes were observed on rats up to a dose as high as 200 mg/kg.

Eric Vieira et al ⁷ were synthesized some heterocycles of xanthenes amides (19) as pharmacological tools for the study of the physiological roles mediated by mGlu1 receptors.

Sankar Chatterjee et al ⁸ reported potent, xanthene-derived reversible aldehyde (20a), keto-carboxamide (20b), and irreversible fluoromethyl ketone (20c) inhibitors of recombinant human calpain I.
Kent E Pryor and James J La Clair \(^9\) reported a combinatorial library of disubstituted xanthenes \((21)\) designed to resemble a known exo-protease inhibitor was screened and a new HLE inhibitor was identified.

Samo Turk et al \(^{10}\) have used the molecular docking programme eHiTS for the virtual screening of 1990 compounds from the National Cancer Institute ‘Diversity Set’ on MurD and MurF ligases enzymes. The 50 top-scoring compounds from screening on each enzyme were selected for experimental biochemical evaluation. From the approach of virtual screening and subsequent in vitro biochemical evaluation they have reported compound \((22)\) a xanthene derivative with good inhibitory activity.

Henryk Marona et al \(^{11}\) have reported series of appropriate alkanolamine and amide derivatives of xanthone \((22)\) were prepared and evaluated for anticonvulsant activity using maximal electroshock and subcutaneous pentylenetetrazole-induced seizures, and for neurotoxicity using the rotorod test on mice and rats.
Kazunari Sakagami et al \(^{12}\) describe the synthesis of \((+)-(1'R,2'R)-2-[(1'S)-1\text{-amino-1-carboxy-2-(9H-xanthen-9-yl)-ethyl}]-1\text{-fluoro cyclopropane carboxylic acid (22a)}\) a compound, that is, fluorinated at the alpha position of the carboxylic acid in the cyclopropane ring of a group II mGluRs antagonist (22a) using a previously reported stereoselective cyclopropanation reaction. The fluorinated compound exhibited almost the same affinity (IC\(_{50} = 3.49\) nM) for mGluR2 as 1 but had a superior pharmacokinetic profile.

(22a)

Yan Liu et al \(^{13}\) reported a series of novel benzoxanthones and their structurally perturbed analogs were synthesized and evaluated as \(\alpha\)-glucosidase inhibitors. These compounds exhibited strong inhibitory activities, most probably as a result of the cooperative \(\pi\)-stacking and H-bonding interactions with yeast’s \(\alpha\)-glucosidase.

Ioannis K Kostakis et al \(^{14}\) have synthesized number of new xanthenone and benzo[\(h\)]xanthenone amino derivatives and their pyrazole-fused counterparts have been designed and synthesized possessing structural analogy to the potent anticancer agent 9-methoxypyrazoloacridine. The synthesis of the compounds
proceeds through nucleophilic substitution of 1-chloro-4-nitrooxanthenone or the corresponding benzo[b]xanthenone by the appropriately substituted amine or hydrazine, reduction of the nitro group, and conversion into the suitable dialkylaminoacetamides (27, 28). This method cannot be applied for synthesis of the pyrazole-fused benzo[b]xanthenones, consequently a different, simple, and high-yielding synthetic procedure was developed for the preparation of the target molecules. In vitro cytotoxic potencies of the new derivatives toward the murine leukemia L1210 cell line, human colorectal adenocarcinoma (HT-29), and human uterine sarcoma (MES-SA and its 100-fold resistant to doxorubicin variant MES-SA/D × 5) cell lines are described and compared to those of reference drugs. The compounds exhibited significant cytotoxic activity against the tested cell lines

(27)  (28)

Sandip B Bharate et al 15 have studied the structure–activity relationships of xanthene carboxamide derivatives (29) on the CCR1 receptor binding affinity and the functional antagonist activity was described.

Stephane Moreau et al 16 reported a series of arylhydrazones derived from various 6,8-diacetoxy- or 6,8-dihydroxy-9-oxo-9H-
xanthene carboxaldehydes were synthesized and evaluated for their in vitro antifungal properties against two human pathogenic yeasts (*Candida albicans* and *C. krusei*) according to a diffusion method. The activity was strongly dependent from the position of the (1-arylhydrazinyl-2-ylidene) methyl chain in the xanthone molecular skeleton. Compounds having the nitrogen side chain in the 4-position, with a further halogen substitution on the terminal phenyl ring showed fungistatic effects.

Chung-Pu Wu *et al*[^1^] reported four new chemosensitisers against chloroquine-resistant *Plasmodium falciparum* based on the 9H-xanthene tricyclic scaffold. They designed and synthesized in an attempt to identify simplified compounds that are easily accessible from commercially available starting materials. The compounds contain a common hydrophobic tricyclic 9H-xanthene moiety and an alkyl side chain with two amino groups, one of which is a tertiary substituted terminal amine, separated by three carbons and differing only in the chemical nature of the intermediary nitrogen atom. The best chemosensitising compound has a secondary amino group, showed a response modification index of 0.36 and caused a four-fold increase in chloroquine accumulation in a resistant strain of *P. falciparum* as well as having the highest selective therapeutic index when tested against a mammalian cell line.
SCHEME-5

\[
\text{2-Naphthol} + \text{R-CHO} \rightarrow (\text{SE 1-15})
\]

i) Silicasulphuric acid
Neat 120 °C

or
Cl\(_2\)CH\(_2\)CH\(_2\)Cl/ Reflux

ii) V\(_2\)O\(_5\), Neat, 120°C
or
Cl\(_2\)CH\(_2\)CH\(_2\)Cl/ Reflux

iii) Cp\(_2\)TiCl\(_2\), Neat, 120°C

\[ R = \text{SE-1 to SE-15} \]

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General Procedure For The Synthesis Of 14H-Dibenzo [a,j] Xanthenes.

1) By Using Silica Sulfuric acid as a Catalyst:-

**Method A**: To a mixture of aldehyde (1mol) and β-naphthol (2mol), in a round bottom flask was added Silica sulfuric acid (5mol %) and the reaction mixture was stirred at 120 °C for 2-3 hrs. Cooled the reaction mixture and added methylene dichloride (20 ml) and filtered the catalyst. The solvent was evaporated to get the solid. The solid was recrystallised from 95% ethanol.

**Method B**: To a mixture of aldehyde (1mol) and β-naphthol (2mol), 1,2-Dichloro ethane(20 ml) in a round bottom flask added Silica sulfuric acid (5mol%). The reaction mixture was refluxed for 8-10 hrs. Cooled the reaction mixture and filtered the catalyst and the solvent was evaporated to get the solid. The solid was recrystalised from 95% ethanol.

2) By Using Vanadium Chloride (VCl₃) as a Catalyst:

**Method A**: To a stirred mixture of aldehyde (1mol) and β-naphthol(2mol) in a round bottom flask added VCl₃(10mol%) in small quantities with continuous stirring. The reaction mixture was heated at 120 °C for 3-4 hrs. Cooled the reaction mixture and water was added (50 ml). The solid that separated was filtered and washed with water and recrystallised from 95% ethanol.
**Method B:** To a mixture of aldehyde (1mol) and β-naphthol (2mol) in a round bottom flask containing 1,2-Dichloro ethane (20 ml) added VCl₃(10mol%) in small quantities with continuous stirring and then the reaction mixture was refluxed for 15-18hrs. Cooled the reaction mixture and water was added (50 ml). The organic layer was separated and evaporated to get the solid which was recrystallised from 95% ethanol.

**3) By Using Dicyclopentyl titinum chloride (CP₂TiCl₂) Catalyst:**

**Method A:** To a stirred mixture of aldehyde (1mol) and β-naphthol (2mol) in a round bottom flask added Cp₂TiCl₂(10mol%). The reaction mixture was heated at 120 °C for 2-4hrs. Cooled the reaction mixture and water was added (50 ml) with continuous stirring. The solid that was separated was filtered and washed with water. The obtained solid was recrystallised from 95% ethanol.

**Method B:** To the mixture of aldehyde (1mol) and β-naphthol (2mol) in a round bottom flask containing 1,2-Dichloro ethane was added Cp₂TiCl₂ (10mol%) with continuous stirring. The reaction mixture was refluxed for 10-12hrs. Cooled the reaction mixture and water was added (50 ml). The organic layer was separated and evaporated to get the solid. The obtained solid was recrystallised from 95% ethanol.
BIOLOGICAL ACTIVITY

TABLE-16: Anticancer Data of Synthesized Compounds against cell line MCF-7

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<th>Compound code</th>
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Result and Discussions

The synthesized compounds are tested for anticancer activity by MMT assay based mitochondrial reduction of yellow MMT tetrazolium dye to a highly colored blue formazan product against MCF-7 (Breast cancer cell lines) by using DMEM (Dulbecco's Modified Eagles Medium).

The synthesized derivatives SE-1, SE-2, SE-6, SE-10, SE-12, and SE-14 were screened for cytotoxicity. Compounds SE-10 and SE-14 exhibited potent activity against the cell line MCF-7. The substitution of phenyl ring in the molecule (SC-10) has exhibited promising anticancer activity against the cell line MCF-7. The detailed analysis of the activity of the compounds revealed that instead of phenyl if any other moiety is substituted or any substitution in the phenyl ring itself causes gradual decrease in the activity.
REFERENCES


8. Sankar Chatterjee, Mohamed Iqbal, James C Kauer, John P Mallamo, Shobha Senadhi, Satish Mallya, Donna Bozyczko-


