Synthesis, characterization, anticholinesterase action and in silico studies of xanthone derivatives

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Abstract

The objective of the present work was to synthesize xanthone derivatives and perform the *in silico* ADMET studies and evaluate anti-cholinesterase activity (*in vitro*) of the compounds. Xanthone was synthesized from salicylic acid and resorcinol followed by derivatization to yield reactive epoxy intermediate which finally reacted with amine to produce the desired xanthone derivatives. The anti-cholinesterase activity of the synthesized compounds was studied by modified Ellman's method and the percent inhibition and IC_{50} was calculated. The spectral characterization of the synthesized derivatives revealed the presence of aromatic protons, NH protons and the protons of methyl and hydroxy in all the compounds. The FTIR spectrum exhibited the stretching vibrations due to O-H, C-H, C-C, C=C, C-O and C-N in the compounds. The synthesized derivatives were able to inhibit AChE activity dose dependently form 8.17 to 68.13%. The IC_{50} was calculated from inhibition percentage and was found to be 17.53 µg/mL, 11.15 µg/mL, 6.58 µg/mL, 14.96 µg/mL and 9.41 µg/mL for RX_1 to RX_5 respectively. The anti-cholinesterase action of the compounds followed the electronegativity order and the compounds activity was in order $RX_3>RX_5>RX_2>RX_4>RX_1$.

Keywords

Xanthone, anti-cholinesterase, Alzheimer's disease, Lipinski rule, ADMET study

Introduction

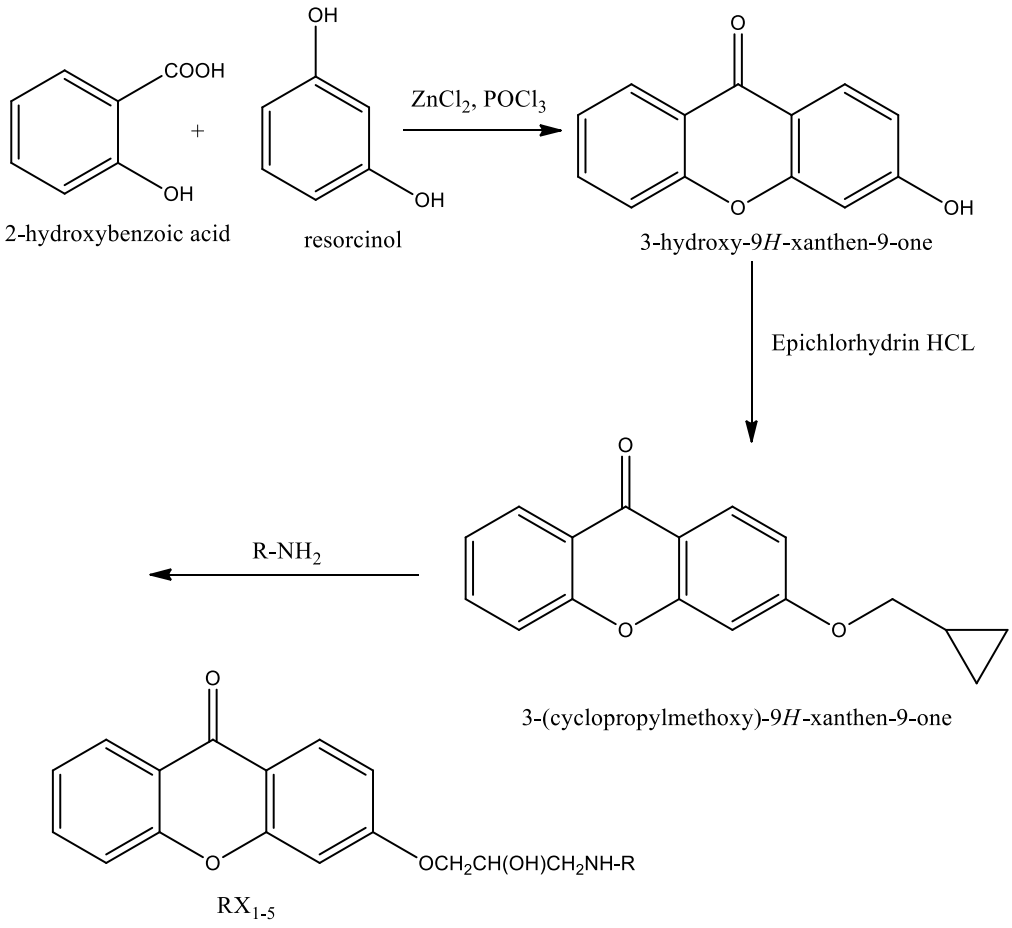
Alzheimer's disease (AD) is a progressive neurodegenerative disorder, and the most predominant cause of dementia in the elderly, associated with gradual memory loss, cognitive deficit and behavioral abnormalities[1,2]. Reduced cholinergic activity and oxidative stress has been recognized as major contributing factor in the pathogenesis of AD[3,4]. The loss of cholinergic neurons and the associated decrease in levels of acetylcholine (ACh) has been found to correlate well with the cognitive impairment seen in AD patients. Therefore, inhibition of acetylcholinesterase (AChE) as well as butyrylcholinesterase (BChE), which are involved

in the breakdown of acetylcholine, has been the main strategy followed for the treatment of AD.

Xanthones are natural polyphenolic compounds that are widely produced by higher plants, fungi, and lichens as secondary metabolites[5]. Their interesting structural scaffolds and significant biological activities[6-10] have prompted many groups to isolate and modify xanthones for the development of new drug candidates. In the present work we have attempted to design new xanthone based compounds that would be able to present good anticholinesterase activities.

Material and Methods

All reagents and chemical were of analytical or synthetic grade and were used as obtained. TLC was performed using precoated silica gel TLC plates and melting point were assessed by open capillary method. The scheme for synthesis of the target xanthone derivatives was designed by modification of synthetic strategies reported by Qin et al[1] and Mishra et al[12].



R-NH$_2$
Aniline, 4-chloroaniline, 4-nitronaniline
4-Bromoaniline, 4-Fluoroaniline

Scheme 1. Synthetic pathway

Synthesis of 3-hydroxy-9H-xanthen-9-one

The synthesis of 3-hydroxyxanthone was conducted by mixing the salicylic acid (0.01mol) with resorcinol (0.01 mol) in the presence of zinc chloride and phosphorus oxychloride under reflux. The reaction mixture was poured into ice water, filtered, and subsequently washed with water

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Synthesis of 3-(cyclopropylmethoxy)-9H-xanthen-9-one

3-Hydroxyxanthone (0.1 mol) was added to epichlorhydrin (0.1 mol) in the presence of 15 mL of 10% alcoholic potassium hydroxide. The reaction mixture was refluxed until the completion of the reaction. On cooling, the product separated which was filtered, dried and recrystallized from ethanol.

General method for synthesis of aminopropyloxy derivatives, RX_{1-5}

To a solution of 3-(cyclopropylmethoxy)-9H-xanthen-9-one (0.01 mol) in 20 mL of alcoholic potassium hydroxide was added appropriate aromatic amine (0.01 mol). The reaction mixture was refluxed until the completion of the reaction. On cooling, the product separated which was filtered, dried and recrystallized from ethanol to give the corresponding final product. The solvent system used for monitoring the reaction progress by TLC was Chloroform-Acetone in the ratio 4:6.

Evaluation of Anti-cholinesterase Activity

Preparation of test solutions

The synthesized compounds were individually dissolved in required volume of dimethylsulfoxide (DMSO) and diluted using phosphate buffer solution (PBS) (pH = 7.8) for the final range of concentrations (2-10 µM).

Test procedure[13,14]

A common chromogenic agent, Ellman's reagent was used for AChE catalyzed hydrolysis. In fresh 96-well microplates, each well was filled with 40 µL of 50 mM Tris-HCl buffer pH 8.0, 20 µL of test sample solution, 100 µL of Ellman's reagent (prepared by dissolving 18.5 mg reagent in 10 mL of buffer), and 20 µL of AChE solution (prepared by dissolving 0.26 U/mL enzyme in 15 mL buffer). The contents were mixed and incubated at 25°C for 15 min. The absorbance was measured at 412 nm. Now, 20 µL of 15 mM acetyl thiocholine (ACTh) (prepared by dissolving in water) was added as a substrate to the wells and the plate was incubated for 20 min at 37°C. Blank determination was done without test samples to obtain 100% AChE activity. The absorbance was measured at 412 nm and the percentage AChE inhibition was calculated using formula:

%AChE inhibition = $(A_{blank}-A_{sample})/A_{blank}$ *100

***In silico* ADMET study**

The SMILES notation of the designed compounds was submitted to SwissADME as well as pkCSM online tools for the ADMET analysis, the prediction of physicochemical parameters and the drug-likeness using the Lipinski rule of five.

Results and Discussion

Five xanthone derivatives (**RX_{1-5}**) were synthesize. All the compounds were soluble in chloroform and DMSO whereas insoluble in water and methanol. The ^{1}H-NMR and FTIR spectra of the synthesized compound was observed for the vibrations of functional groups in the FTIR spectra and protons in the ^{1}H-NMR spectra.

3-(2-hydroxy-3-(phenylamino) propoxy)-9H-xanthen-9-one, RX_1

Yield: 67%; M.P: 201-203°C; Mass: 361.39; ^{1}H-NMR (δ ppm): 6.58-8.32 (aromatic protons), 3.20-4.19 (methyl/methylene protons), 4.40 (NH proton), 3.97 (OH proton); FTIR (cm^{-1}): 3619.87 (O-H), 2888.32 (C-H), 1327.08-1225.82 (C-C, aromatic), 850.64 (C-N)

3-(3-((4-chlorophenyl) amino)-2-hydroxypropoxy)-9H-xanthen-9-one, RX_2

Yield: 71%; M.P: 161-163°C; Mass: 395.84; ^{1}H-NMR (δ ppm): 6.50-8.32 (aromatic protons), 3.20-4.19 (methyl/methylene protons), 4.76 (NH proton), 3.97 (OH proton); FTIR (cm^{-1}): 3050.55 (C-H), 1327.08-1225.82 (C-C, aromatic), 850.64 (C-N)

3-(2-hydroxy-3-((4-nitrophenyl) amino) propoxy)-9H-xanthen-9-one, RX_3

Yield: 66%; M.P: 213-215°C; Mass: 406.39; ^{1}H-NMR (δ ppm): 6.68-8.32 (aromatic protons), 3.20-4.19 (methyl/methylene protons), 5.26 (NH proton), 3.97 (OH proton); FTIR (cm^{-1}): 3017.39 (C-H), 1399.26-1253.77 (C-C, aromatic), 821.51 (C-N)

3-(3-((4-bromophenyl) amino)-2-hydroxypropoxy)-9H-xanthen-9-one, RX_4

Yield: 64%; M.P: 199-201°C; Mass: 440.29; ^{1}H-NMR (δ ppm): 6.57-8.32 (aromatic protons), 3.20-4.19 (methyl/methylene protons), 4.80 (NH proton), 3.97 (OH proton); FTIR (cm^{-1}): 3029.11 (C-H), 1325.36-1221.91 (C-C, aromatic), 867.04 (C-N)

3-(3-((4-fluorophenyl) amino)-2-hydroxypropoxy)-9H-xanthen-9-one, RX_5

Yield: 68%; M.P: 209-211°C; Mass: 379.38; ^{1}H-NMR (δ ppm): 6.71-8.32 (aromatic protons), 3.20-4.19 (methyl/methylene protons), 4.80 (NH proton), 3.97 (OH proton); FTIR (cm^{-1}): 2972.32 (C-H), 1412.08-1296.02 (C-C, aromatic), 851.13 (C-N)

The synthesis of xanthone from salicylic acid and resorcinol followed a Lewis acid catazlyzed cyclization. The nucleophilic attack of the hydroxyl group of the xanthone to the electron deficient chloride of the epiclorhydrin resulted in nucleophilic substitution resulting in the formation of the reactive epoxy intermediate. The epoxy intermediate underwent substitution reaction with amine group resulting the formation of the desired products. The spectral characterization of the synthesized derivatives revealed the presence of aromatic protons, NH protons and the protons of methyl and hydroxy in all the compounds. The FTIR spectrum exhibited the stretching vibrations due to O-H, C-H, C-C, C=C, C-O and C-N in the compounds.

Anticholinesterase activity

The anticholinesterase activity was evaluated by Ellman's method and the results of inhibition of cholinesterase activity was calculated from the absorbance of blank and the test solutions (Table 1).

Table 1. %Inhibition of cholinesterase action

	% AChE inhibition				
Code	**2 mM**	**4 mM**	**6 mM**	**8 mM**	**10 mM**
RX_1	8.17	15.51	18.86	24.10	30.39
RX_2	18.02	24.10	31.23	38.99	46.12
RX_3	25.36	37.73	45.07	58.28	68.13
RX_4	14.46	19.70	25.15	30.39	36.68
RX_5	21.59	28.93	36.89	43.81	52.83

The synthesized derivatives were able to inhibit AChE activity dose dependently from 8.17 to 68.13%. The IC_{50} was calculated from inhibition percentage and was found to be 17.53 µg/mL, 11.15 µg/mL, 6.58 µg/mL, 14.96 µg/mL and 9.41 µg/mL for RX_1 to RX_5 respectively.

The inhibition of cholinesterase enzyme prevents the breakdown of acetylcholine into its component's choline and acetyl CoA. The compounds were able to inhibit cholinesterase enzyme in a dose dependent manner. The results show that the presence of strong electron withdrawing group in the compound was beneficial for anticholinesterase action (RX_3). Non-substituted compound (RX_1) was least effective in inhibition of cholinesterase action. The anti-cholinesterase action of the compounds followed the electronegativity order and the compounds activity was in order $RX_3>RX_5>RX_2>RX_4>RX_1$.

***In silico* ADMET properties of synthesized naphthyridine derivatives**

The SMILES of the synthesized naphthyridine derivatives was generated using chemdraw ultra 12.0 software. The physicochemical properties of the compounds predicted *in silico* by

SwissADME are presented in Table 2 and the ADMET properties predicted by pkCSM are presented in Table 3.

Table 2. Physicochemical properties of RX_{1-5}

Compound Code	HBD	HBA	Log P	NRB	PSA	MR	Log S
RX_1	2	4	4.00	6	71.70	106.08	-4.75
RX_2	2	4	4.62	6	71.70	111.09	-5.34
RX_3	2	6	3.82	7	117.52	114.91	-4.80
RX_4	2	4	4.69	6	71.70	113.78	-5.66
RX_5	2	5	4.10	6	71.70	106.04	-4.91

Table 3. ADMET of RX_{1-5}

ADMET Parameters	RX_1	RX_2	RX_3	RX_4	RX_5
Absorption					
Water solubility (log mol/L)	-4.888	-5.123	-4.961	-5.161	-4.989
Caco2 permeability (log Papp in 10^{-6} cm/s)	0.873	0.713	-0.37	0.705	0.994
Intestinal absorption (human) (% Absorbed)	93.224	91.879	93.317	91.812	92.78
Skin Permeability (log Kp)	-2.739	-2.739	-2.737	-2.739	-2.746
Distribution					
VDss (human) (log L/kg)	-0.183	-0.123	-0.408	-0.106	-0.285
CNS permeability (log PS)	-2.168	-2.055	-2.393	-2.032	-2.211
Metabolism					
CYP2D6 substrate	No	No	No	No	No
CYP3A4 substrate	Yes	Yes	Yes	Yes	Yes
Excretion					
Total Clearance (log ml/min/kg)	0.361	0.029	0.378	0.007	0.252
Renal OCT2 substrate	No	No	No	No	No
Toxicity					
AMES toxicity	Yes	Yes	Yes	Yes	No
Hepatotoxicity	Yes	Yes	Yes	Yes	Yes
Oral Rat Acute Toxicity (LD50) (mol/kg)	1.888	1.894	2.981	1.897	1.852

Physicochemical property is an important parameter of a molecule that influences efficacy, safety or metabolism which could be predicted by using Lipinski's rule of five, Veber's rule or Muegge's rule. We have used the Lipinski's rule that defines an orally active drug, which confirms to the number of hydrogen bonds acceptor (HBA) ≤ 10, hydrogen bonds donor (HBD)

≤ 5, molecular weight (MW) < 500 Da and Log P (the logarithm of octanol water partition coefficient) ≤ 5. The physicochemical properties include molecular weight, number of the rotatable bonds (NRB), HBA, HBD, molar refractivity (MR, in m3.mol-1) and polar surface area (PSA, in Å). The other two significant determinant are lipophilicity and solubility that are monitored for favorable drug development.

Table 3 reveals that all compounds meet every single criterion of Lipinski's rule of five and thus fully obey the rule. Consequently, all the investigated compounds present a good drug-likeness profile, since they are predicted to be easily absorbed and have good permeability and bioavailability.

Conclusion

Due to its planar structure, the xanthone derivatives have been extensively studied for exploring their diverse biological activities. In this work, xanthone derivatives were synthesized and evaluated for anticholinesterase action. Compound RX_3 and RX_5 were able to produce anticholinesterase action on less than 10 µg/mL concentration.

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