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Research Article

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Design, synthesis and discovery potent of novel anticancer agents based on the coumarin scaffold

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ABSTRACT

*Several effective anticancer therapeutic drugs containing coumarin nucleus targeting carbonic anhydrase enzyme. Thus, some coumarin derivatives **1-22** were prepared. The structures of these compounds established on the basis of IR, ^{1}HNMR, ^{13}CNMR and MS data. Moreover, the optimization geometries for compounds **1-22** were discussed using DFT theory with B3LYP\6-311G base set. The molecular docking simulations into the active site of COX-2 were performed, and showed that, some compounds (**7,8, 11,13,19b** and **20)** suitable inhibitor against CAII, and can used as anti-cancer drugs. These compounds (**7,8, 11,13,19b** and **20)** were evaluated against Ehrlich solid carcinoma (EAC) tumor model in Swiss albino mice on dose 50µg . The activity was assessed using survival time and average increase in body weight, which showed that, administration of derivatives (**7,8, 11,13,19b** and **20)** were effective in reducing solid tumor mass EAC cells. In silico, The ADMET profiles showed that, these compounds are good oral bioavalability, and the most active compounds 6 and 10 are CNS active agents without marked health effects observed for rodent.*

Key words: anticancer agent, coumarin, NMR Spectra

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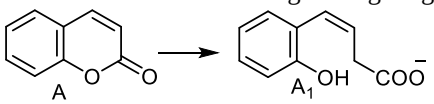
1. INTRODUCTION

Cancer is a major public health[1], these diseases produced from a single cell begins to divide uncontrollably forming a tumour, which known as metastasis [2-4]. The chemotherapy agent is primary strategy for treatment of tumour cell, which depending on poisoned cancer cell [5]. The coumarin derivatives is a novel class inhibitors of the carbonic anhydrase (CA)[6-8], the CA is a metalloenzymes family, and involved in the catalysis of an important physiological reaction: the hydration of CO_2 to bicarbonate and a proton [9-12]. Reduction supplies of bicarbonate radical is the suggested mechanism action of Sulfonamide CA inhibitors (CAIs) [8], through interaction with the Zn metal ion of enzyme. Recently [13,14], coumarin derivatives acting as CAIs against many isoforms CA I–CA XV, by the hydrolyzed coumarins to 2-hydroxy-cinnamic acids (equation 1), which bind with unexpected way with CA [15]. This postulation, was confirmed with X-ray crystallography of enzyme-inhibitor adducts, and kinetic methods[6-8]. From above facts, synthesis of novel coumarin derivatives acting as CAIs[16-18], are attractive target to development effective cancer therapeutic drugs. So, continuation of our work[17-29], was aimed to synthesized coumarin derivatives as anticancer agent targeting CA.



equation1. Formation of 2-hydroxycinnamic acids A1 by the CA-mediated hydrolysis of coumarins A.

2. EXPERIMENTAL SECTION

Melting points were taken on a Griffin melting point apparatus and are uncorrected. Thin layer chromatography (R_f) for analytical purposes was carried out on silica gel and developed. Benzidine, ninhydrin, and hydroxamate tests used for detection reactions. The IR spectra of the compounds were recorded on a Perkin–Elmer spectrophotometer model 1430 as potassium bromide pellets and frequencies are reported in cm^{-1}. The NMR spectra were observed on a Varian Genini-300 MHz spectrometer and chemical shifts (δ) are in ppm. The mass spectra were recorded on a mass spectrometer HP model MS–QPL000EX (Shimadzu) at 70 eV. Elemental analyses (C,H,N) were carried out at the Microanalytical Centre of Cairo University, Giza, Egypt.

Swiss albino mice of 5 weeks to 7 weeks old, weighing 18-25 g were collected from the National Cancer Institute (Cairo, Egypt) (NCIE), Cairo. The mice were kept in iron cages with sawdust and straw bedding that was changed once a week regularly. Standard mouse diet (recommended and prepared by the (NCIE) and water were given in adequate amounts.

EAC cells were obtained from a line of EAC cells was obtained from the Cancer Biology Department of the National Cancer Institute (Cairo, Egypt). Body weight was balance by using Sartorious (500.0) g AC-DC CHARGER MODEL:MW79.

Tumour volume was measured by VERNIER CALIPER 150X0.02 MM/6 XL/1000 and viable cell count was determined in a neubauer counting chamber.

2.1. 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetyl chloride(2):

The starting material (**2**) was prepared as mentioned by Dey*et al* [30].

2.2. 4-(2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetamido)benzoic acid(3)

The 4-aminobenzoic acid (*1.36gm.* 0.01mol)was dissolved in 1N-NaOH(2ml), followed by portion-wise addition of acid chloride (**2;** 2.38 g, 0.01 mol) in acetone (10 ml) during 1/2hat 10°C, the stirring was continued for additional 3h., the acetone was removed under reduced pressure, water was added and acidified with 1 N HCl to pH =5. The separated solid was filtered off and recrystallized from ethanol to give compound (**3**) as white crystal;yields=85%;R_f=0.55 ($CHCl_3$/MeOH=3/1); mp.125-127 °C; IR (KBr cm^{-1}) ν; 2950 cm^{-1} (broad band OH and NH); 2906 cm^{-1} (CH- ali); (1705, 1660) cm^{-1} (C=O).; ^{1}H NMR (300 MHz, Chloroform) δ= 12.10 (s, 1H, OH-carboxylic), 9.84(s,1H,OH-arm), 8.17-6.30 (m,8H,CH-arm.) 5.57(s,H,NH), 3.54(s,2H,CH_2-ali.); Anal./Calcd. for **$C_{18}H_{13}NO_6$**: C (63.7%), H (3.83%), N (4.12%). Found: C (63.7); H (3.86); N, (4.13).

2.3. 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetylisothiocyanate(5)

A mixture of compound (**2**, 2.38 g., 0.01 mol) and NH_4NCS(7.6g, 0.01mole) was stirred for 30 min., the NH_4Cl formed was filtered off, the filtrate containing compound (**5**) was used in other experiment without further purification.

2.4. 4-(2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetamido)benzoyl chloride(6)

The free acid (**3**; 3.39 g., 0.01mol) in acetone (10 ml) was refluxed with thionyl chloride (2 ml) for 30 min. the deeply colored solution obtained of acid chloride (**6**) was used directly in further experiments.

2.5. 4-(2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetamido)-N-(4-sulfamoylphenyl)benzamide(7).

The acid chloride solution (**6,** 0.01 mole) added portionwise to sulfanilamide (1.36 g.; 0.01mol) in acetone (10 ml) during 30 mins., the reaction mixture was continued stirring for additional 3 hrs. at room temperature. After evaporation of the solvent, the resulting brown crystals were filtered off and recrystallized from benzene to give compound (**7**) as yellow crystals; in yield 60 %; R_f = 0.96($CHCl_3$/MeOH=3/1); M.P. = 175-77Cº; IR (KBr cm^{-1}) ν; 3378 (broad band OH and NH), 2861cm^{-1} (CH-ali), 1706 cm^{-1} (C=O), 1620 cm^{-1} (CONH), 1312,1094 cm^{-1} (SO_2NH).; MS (m/z, %) **493** (0.29);; Anal./Calcd. for **$C_{24}H_{19}N_3O_7S$**: C (58.40%), H (3.85%), N (8.51%). Found: C (58.41); H (3.88); N, (8.51).

2.6. Methyl-4-(2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetamido)–benzoate(8)

The thionyl chloride (2 ml) was added in dropwise to a stirred cold solution (5 °C) of compound (**3**,3.39 g., 0.01 mol) in absolute methanol (30 ml.) during 30 mins. The stirring was continued for additional 3 hrs., the mixture was left for 24 hrs. at room temperature. After removing the solvent under reduced pressure, another portion of abs. methanol (10 ml.) was added and re-evaporated. The solid obtained was recrystallized from benzene to give compound (**8**) as brownish red crystals. Yield: (75%); R_f = 0.63($CHCl_3$/MeOH=3/1);M.P.=180-182°C; IR (KBr cm^{-1}) ν; 3397(broad band, OH and NH) 2960 cm^{-1} (CH ali), 1705 cm^{-1} (C=O), 1615cm^{-1} (CONH amide).;^{1}H NMR (300 MHz, Chloroform) δ= 9.54 (s,1H,OH-arm), 8.06-6.25(m,8H,CH-arm.) 5.46 (s,1H,NH), 3.92(s, 3H,CH_3-OCH_3),

3.58(s,2H,CH_2-ali.);MS (m/z, %) **353** (0.22);; Anal./Calcd. for **$C_{19}H_{15}O_6N$**: C (64.57%), H (3.96%), N (4.24%). Found: C (64.59); H (3.96); N, (4.28).

2.7. N-(4-(hydrazinecarbonyl)phenyl)-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetamide(9).

The compound (**8**) was reacted with hydrazine hydrate in boiling absolute ethanol for 1/2h. The crude product (**9**)was filtered off , washed with water and recrystallized from benzene to give compound (**9**) in 85% yield.;R_f = 0.61($CHCl_3$/MeOH=3/1); M.P.=230-232°C; IR (KBr cm^{-1}) ν; 3398 cm^{-1}(broad band, OH, NH_2 and NH) 2961cm^{-1}(CH ali), 1619cm^{-1}(CONH amide).;^{13}C NMR (300 MHz, Chloroform) δ= 171.86-162.64 (3C, C=O), 161.81 -104.44 (14C,C-arm.), 38.16 (1C, C-CH ali.);MS (m/z, %) **353** (012); Anal./Calcd. for **$C_{18}H_{15}O_5N_3$**: C (61.17%), H (4.24%), N (11.89%). Found: C (61.19); H (4.28); N, (11.89).

2.8. N-(4-(2-formylhydrazinecarbonyl)phenyl)-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetamide(10).

The hydrazaide derivative (**9**;3.53 g**.,**0.01 mol) was heated under reflux with formic acid (0.01 mole) for 10hrs. The solid obtained after cooling was filtered off and recrystallized from benzene to give compound (**10**) as brown crystals Yield: (65 %); R_f = 0.83($CHCl_3$/MeOH=3/1); M.P.=125-27°C; IR (KBr cm^{-1}) ν; 3408 cm^{-1} (broad band, OH and NH) ,2984cm-1 (CH-aliphatic), 1628cm^{-1} (CONH amide).;^{1}H NMR (300 MHz, Chloroform) δ= 9.89(s, 2H,NHNH), 9,27(s, 1H,CHO), 8.24 (s,1H,OH-arm), 8.01-6.21(m,8H,CH-arm.) 5.43 (s,H,NHCO), 3.47(s,2H,CH_2-ali.); Anal./Calcd. for **$C_{19}H_{15}O_3N_6$**: C (59.82%), H (3.93%), N (11.02%). Found: C (59.84); H (3.96); N, (11.02).

2.9. ***(2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetyl)-4-aminobenzoyl acetylhydrazide (11):***

Compound (**9**, 3.53g 0.01 mole) was refluxed with gl. acetic acid (20 ml.) for 3hrs., The solid obtained (**11**) after cooling was filtered off and recrystallized from benzene to give compound **(11)** as brown crystals ,Yield: (70 %); R_f = 0.80($CHCl_3$/MeOH=3/1); M.P.=213-215°C; IR (KBr cm^{-1}) ν; 3402 cm^{-1} (broad band, OH and NH), 2959cm^{-1} (CH-aliphatic), 1714 cm^{-1} (C=O), 1617cm^{-1}(CONH amide);^{1}H NMR (300 MHz, Chloroform) δ= 10.59(s, 2H,NHNH), 9.36 (s, 1H, OH-arm), 8.02-6.20 (m, 8H, CH-arm.) 5.43 (s, H, NHCO), 3.47(s,2H,CH_2-ali.), 2.06(s,3H,CH_3); MS (m/z, %) **395** (67.96); Anal./Calcd. for **$C_{20}H_{17}O_3N_6$**: C (60.74%), H (4.30%), N (10.62%). Found: C (60.76); H (4.33); N, (10.63).

2.10. 2-(7-acetoxy-2-oxo-2H-chromen-4-yl)acetyl)-4-aminobenzoylacetyl hydrazide (12):

The hydrazide derivatives (**9**, 3.53g 0.01; mol) was heated with acetic anhydride (20 ml.) with fused sodium acetate for 3hs. The solid obtained (**12**) after cooling was filtered off, washed with pet. ether (60/80) and recrystallized from abs. ethanol to give compound **(12)** , as brown crystals; Yield: 70 %; R_f =0.82($CHCl_3$/MeOH=3/1); M.P.=193-95°C; IR (KBr cm^{-1}) ν; 3412 cm^{-1} (NH), 2962cm^{-1} (CH-ali.), 1767 cm^{-1} (C=O), 1617cm^{-1} (CONH amide), 1617cm^{-1}(CONH amide); MS (m/z, %) **437** (5.94); Anal./Calcd. for **$C_{22}H_{19}O_3N_7$**: C (60.39%), H (4.34%), N (9.60%). Found: C (60.41); H (4.38); N, (9.61).

2.11. 2-(7-acetoxy-2-oxo-2H-chromen-4-yl)acetyl)-4-aminobenzoylhydrazonyl- thiourea (13):

A mixture of compound (**9**; 3.53 g, 0.01mol) and ammonium thiocyanate (0.76 g, 0.01mmol) in conc. HCl acid (20 ml) was heated under reflux for 5hs. The reaction mixture was concentrated, and left to cool. The separated solid (**13**)was filtered off, washed with water, dried and recrystallized from ethanol to give compound (**13**) as brownish red crystals; Yield: 60%; R_f = 0.78($CHCl_3$/MeOH=3/1); M.P.=308-10°C, ; IR (KBr cm^{-1}) ν; 3423cm^{-1} (broad band, OH, NH_2 and NH) ,2950 cm^{-1} (CH-ali), 1698 cm^{-1} (C=O), 1610 (CONH amide);^{1}H NMR (300 MHz, Chloroform) δ= 11.63 (s,2H,NH_2), 9.82 (s,1H,OH-arm), 8.81(s,H, NH, CSNH), 7.93-6.21 (m,8H,CH-arm.) 5.49 (s,H,NH,NHCO), 3.62(s,2H,CH_2-ali.); MS (m/z, %) **395** (67.96); Anal./Calcd. for $C_{19}H_{15}N_3O_5S$: C (57.42%), H (3.77%), N (10.57%). Found: C (57.42); H (3.80); N, (10.57).

2.12. 2-(7-acetoxy-2-oxo-2H-chromen-4-yl)acetyl)-4-amino benzoyl -1,3,4-oxadiazol-2-yl)-5- amino acids(14):

Compound (**9**; 3.53 g, 0.01mol) was grinded with p-aminobenzoic acid (1.36g, 0.01 mole), then dissolved in conc.H_2SO_4 (10 ml.). The reaction mixture was stirred for 6hrs. at 80°C. The reaction mixture was cooled, poured into crushed ice, and neutralized with solid sodium carbonate at PH=7. The crude product (**14**) formed after standing for 1hrs, was filtered off, washed with water, dried and recrystallised from ethanol to give compound (**14**) as white crystal; Yield: 55%; R_f = 0.49 ($CHCl_3$/MeOH=3/1); M.P.=305-307°C; 3460cm^{-1} (broad band, OH, NH_2 and NH), 2957 cm^{-1} (CH-aliphatic), 1623 cm^{-1} (CONH amide); ^{1}H NMR (300 MHz, Chloroform) δ= 8.59 (s,1H,OH-arm.), 7.69-6.29(m,8H,CH-arm.) 5.44 (s,2H,NH2), 5.32(s, 1H,NH) 3.52 (s,2H,CH2-ali.); Anal./Calcd. for $C_{25}H_{18}N_4O_5$: C (66.06%), H (3.96%), N (12.33%). Found: C (66.08); H (3.99); N, (12.33).

2.13. 2-(7-acetoxy-2-oxo-2H-chromen-4-yl)acetyl)-4-amino-benzoyl -1,3, 4-oxadiazol (17):

The alc.KOH was added to compound (**9**, 0.5 g, 0.01mol) in ethanol (20 ml.). Carbon disulfide (0.01 mol) was added to the reaction mixture and heated for 8hrs. The reaction mixture was removed under reduced pressure; the residual salt was treated with water and filtered off. The filtrate was neutralized to pH= 6 using dil. HCl, the separated product(**14**) was filtered, washed with water, and dried as brown crystal of compound (**14**) was crystallized by ethanol in Yield 75 %); R_f = 0.84($CHCl_3$/MeOH=3/1); M.P.=165-67°C; IR (KBr cm^{-1}) ν; 3412 cm^{-1} (broad band, OH and NH), 2958 cm^{-1} (CH-ali.), 1619cm^{-1}(CONH amide); ^{1}H NMR (300 MHz, Chloroform) δ= 9.59 (s, 1H, OH), 7.80-6.43(m,9H,(1H,NH-oxadiazol) + (8H,Ar-H.) 5.44 (s,H,NH), 3.61(s,2H,CH_2-ali.).; Anal./Calcd. for $C_{19}H_{13}N_3O_5S$: C (57.71%), H (3.29%), N (10.63%). Found: C (57.72); H (3.31); N, (10.63).

2.14. 2-(7-acetoxy-2-oxo-2H-chromen-4-yl)acetyl) -4-aminobenzoyl hydrazonyl aryls (18a-c):

The compound (**9**, 3.53 g.,0.01mol) in gl. acetic acid (10 ml.) was heated with aromatic aldehydes (0.01 mole), the reaction mixtures were heated under reflux for 6-8 hrs. The solid products (**18a-c**) obtained after cooling, collected by filtration and recrystallized from ethanol to give compounds (**18a-c**),

1.14.1. N-(4-(2-(2,4-dihydroxybenzylidene)hydrazine-1-carbonyl)phenyl)-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetamide (18a)

Brown crystal; Yield 65 %; R_f = 0.84($CHCl_3$/MeOH=3/1); M.P.=165-67°C; IR (KBr cm^{-1}) ν; 3407,3196 cm^{-1} (broad band, OH and NH) , 2983 cm^{-1} (CH-ali), 1719 cm^{-1} (C=O); MS (m/z, %) **473** (18.36); Anal./Calcd. for $C_{25}H_{19}N_3O_7$: C (63.40 %), H (4.01%), N (8.87%). Found: C (63.42); H (4.05); N, (8.88).

1.14.2. N-(4-(2-(4-(dimethylamino)benzylidene)hydrazine-1-carbonyl)phenyl)-2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetamide (18b)

Red crystal; Yield 65 %; R_f = 0.84($CHCl_3$/MeOH=3/1); M.P.=165-67°C; IR (KBr cm^{-1}) ν; 3383 cm^{-1} (broad band, OH and NH), 2955 cm^{-1} (CH-ali), 1705 cm^{-1} (C=O), 1617 cm^{-1} (CONH amide); MS (m/z, %) **484** (11.42); Anal./Calcd. for **$C_{27}H_{24}N_4O_5$**:C(66.91%), H (4.95%), N (11.56%). Found: C (66.93); H (4.99); N, (11.56).

1.14.3. 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)-N-(4-(2-(4-methoxybenzylidene)hydrazine-1-carbonyl)phenyl) acetamide (18c)

Red crystal; Yield 65 %; R_f = 0.84($CHCl_3$/MeOH=3/1); M.P.=165-67°C; IR (KBr cm^{-1}) ν; 3351 cm^{-1} (broad band, OH and NH) ,2902 cm^{-1} (CH_2-ali), (1730) cm^{-1} (C=O), 1629 cm^{-1} (CONH amide);); ^{1}H NMR (300 MHz, Chloroform) 10.91 (s,1H,NHN), 9.75 (s,1H,OH), 8.39-6.30(m,12H,Ar-H.), 5.52 (s,H,NH), ,3,77(s,3H,CH_3-OCH_3) , 3.52(s,2H,CH2-ali.).; MS (m/z, %) 471 (16.02); Anal./Calcd. for **$C_{26}H_{21}N_3O_6$**: C (66.22%), H (4.45%), N (8.91%). Found: C (66.24); H (4.49); N, (8.91).

2.15. Synthesisof4-(2-(4-(4-acetyl-5-(aryl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenylamino)-2-oxoethyl)-2-oxo-2H-chromen-7-ylacetate (19a,b):

Compounds (**18 a,b**; 0.01mol) were heated under reflux with acetic anhydride (25ml) for 15hrs, cooling, the excess of acetic anhydride was decomposed with water (10ml), the reaction mixture was stirred for 30 mins. The separated solids were filtered off, washed with water, dried and recrystallized from ethanol to give compounds **(19a,b)** respectively.

2.15.1 4-(2-((4-(4-acetyl-5-(2,4-dihydroxyphenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yl acetate (19a)

Red crystal; Yield 65 %; R_f = 0.92($CHCl_3$/MeOH=3/1); M.P.=154-56°C; IR (KBr cm^{-1}) ν; 3423 cm^{-1} (OH and NH), 2928 cm^{-1} (CH-ali), 1767, cm^{-1} (C=O), 1617 cm^{-1}(CONH amide); ^{1}H NMR (300 MHz, Chloroform) 9.47(s,1H,NH), 8.13,8.06 (s,2H,OH.), 7.53-6.24 (m,12H,Ar-H.), 3.54 (s,2H,CH_2-ali.), 2.24, 2.03 (s,6H,2CH_3); MS (m/z, %) 471 (16.02); Anal./Calcd. for **$C_{29}H_{23}N_3O_9$**: C (62.46%), H (4.12%), N (7.53%). Found: C (62.48); H (4.16); N, (7.54).

2.15.2 4-(2-((4-(4-acetyl-5-(4-(dimethylamino)phenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yl acetate (19b).

Red crystal; Yield 65 %; R_f = 0.96($CHCl_3$/MeOH=3/1); M.P.=195-97°C; IR (KBr cm^{-1}) ν; 3419 cm^{-1} (NH), 2962 cm^{-1} (CH-ali), 1768 cm^{-1} (C=O), 1617cm^{-1} (CONH amide)(; ^{1}H NMR (300 MHz, Chloroform) 9.48 (s,1H,NH),7.61-6.49 (m,12H,Ar-H.), 3.54(s,2H,CH_2-ali.) 2.24, (s,6H,2CH_3),; MS (m/z, %) 568 (10.68); Anal./Calcd. for **$C_{31}H_{28}N_4O_7$**: C (65.46%), H (9.85%), N (8.91%). Found: C (65.48); H (4.96); N, (9.85).

2.16. 4-(3-(2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetyl) thioureido)benzoic acid (20)

The pABA (1.36g; 0.01 mol) in acetone (10ml.) was added portionwise, over compound (**6;**261g**,**0.01 mol) in acetone (10 ml) over 30 mins. at 20-25 °C. The reaction mixture was additionally stirred for 6hs., poured onto ice. The solid obtained **(20)** was filtered off and recrystallized from ethanol to give compound (**20**) as white crystal; Yield: (85 %); R_f = 0.90($CHCl_3$/MeOH=3/1); M.P.=243-45°C.; ν; 3385 cm^{-1}(broad band, OH and NH), 2992 cm^{1} (CH-ali), 2080 cm^{-1}(NCS) ,1700 cm^{-1} (C=O), 1617 cm^{-1}(CONH amide).; ^{1}H NMR (300 MHz, Chloroform) 12.08(s,1H,OH-carboxylic), 10.33(s,1H,NHCS), 9.46 (s,1H,OH), 8.13-6.11 (m,8H,Ar-H.), 5.41(s,1H,NHCO), 3.15 (s,2H,CH_2-ali.); Anal./Calcd. for $C_{19}H_{14}N_2O_6S$: C (57.27%), H (3.51%), N (7.03%). Found: C (57.28); H (3.54); N, (7.03).

2.17. Methyl 4-(3-(2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetyl) thioureido) benzoate (21)

The acid (**20,** 3.98 g**.,** 0.01 mol) was allowed to react with thionyl chloride (0.01 mol) in presence of methanol, using the technique described in the preparation of compounds (**8**) to give compound (**21**) which was recrystallized from ethanol, as brown crystal; Yield: 3.50 g (85 %); R_f = 0.89 ($CHCl_3$/MeOH=3/1); M.P.=195-97°C; ν; 3428cm^{-1} (broad band, OH and NH), 2963 cm^{-1} (CH-ali), (1622) cm^{-1} (CONH amide); ^{1}H NMR (300 MHz, Chloroform) 10.34(s,1H,NHCS) 9.45 (s,1H,OH), 7.99-6.11 (m,8H,Ar-H.), 5.40(s,1H,NHCO), 3.92(s,3H, CH_3), 3.16 (s,2H, CH_2-ali.); MS (m/z, %) 412 (16.18%) Anal./Calcd. for $C_{20}H_{16}N_2O_6S$: C (58.24%), H (3.88%), N (6.79%). Found: C (58.28); H (3.91); N, (6.79).

2.18. N-((4-((hydrazinyloxy)carbonyl)phenyl)carbamothioyl)-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetamide (22)

A hydrazine hydrate (1.08 ml, 0.01mol.) refluxed with compound (**21**) in ethanol (20 ml) for 30 mins., then left overnight at room temperature. The separated solid was filtered off, washed with methanol and light pet.ether (60/80°C) and recrystallized from benzene. To give white crystal of (**22**) Yield: 3.70 g (90 %); R_f = 0.81 ($CHCl_3$/MeOH=3/1); M.P.=220-222°C; ν; 3404cm^{-1} (broad band, OH ,and NH,NH_2) ,2960 cm^{-1} (CH-ali), 1622 cm^{-1} (CONH amide); ^{13}C NMR (300 MHz, Chloroform) 181.77(C, C=S), 172.63, 167.44, 162.64, (3C, C=O), 161.82-104.44 (14C, C-arm.), 38.04(1C,C-CHali.); MS (m/z, %) 412 (32%) Anal./Calcd. for $C_{19}H_{16}N_4O_6S$: C (55.32%), H (3.88%), N (13.58%). Found: C (55.33); H (3.91); N, (13.58).

2.19. Pharmacological activity:

1.19.1. Experimental Design:

Swiss albino mice were divided into 26 groups (n=5). All the groups were injected with Ehrlich Ascites Carcinoma (EAC) cells (0.2 ml of 2×10^6 cells/mouse) intraperitoneally and intramuscularly in the thigh of each recipient mouse except the normal group, This was taken as day zero, the normal saline and tween (0.2 ml /mouse/day) was administered to normal and EAC control groups, the compounds (**11,12,15 and 20**) and standard drug Dose (50 µg/kg/day) were administered in groups (4,5) respectively for 9 days intraperitoneally, after the administration of last dose followed by 24 hrs. Fasting 3 mice form each group was sacrificed for the study of antitumor activity.

2.19.2.1 Drug Preparation: Preparation and Administration of Doses:

Solution of tested compounds (**11,12,15 and**)were prepared in normal saline. The required volume was emulsified in distilled water by using Twin 80 (0.5% of the total volume). The emulsion was prepared in such a way that the required daily dose was contained in 0.2mL of the emulsion. 0.2mL of this emulsion was administered to each mouse intraperitoneally, daily from day 0 to 9.

1.19.2. Tumor Transplantation :

Ehrlich's Ascites Carcinoma was maintained by serial transplantation from tumor bearing Swiss Albino mice. EAC cells were obtained from donor mice (Swiss albino) of 18–20 g body weight and suspended in sterile isotonic saline. A fixed number of viable cells usually (2×10^6 cells/20 g body weight) were injected intramuscularly in the thigh of each recipient mouse[36].

1.19.3. Body Weight:

Animals were weighted on every other day throughout the period of the experiment.

1.19.4. Solid Tumor Volume and Tumor Growth Inhibition:

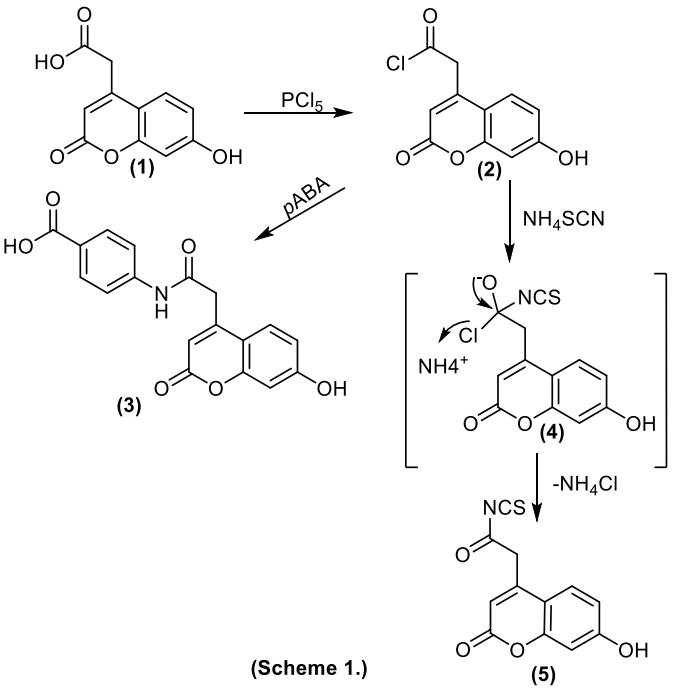
Antitumor effects for the different treatments were evaluated by tumor growth inhibition. Tumors were measured individually using a caliper. Tumor volume was determined by the following equation[37]:

Tumor Volume = (Width^2 x length)/2

The percent tumor growth inhibition was calculated on day13 by comparing the average values of treated groups that of tumor bearing control group. Tumor growth in saline. Treated control animals was taken to be 100%[37].

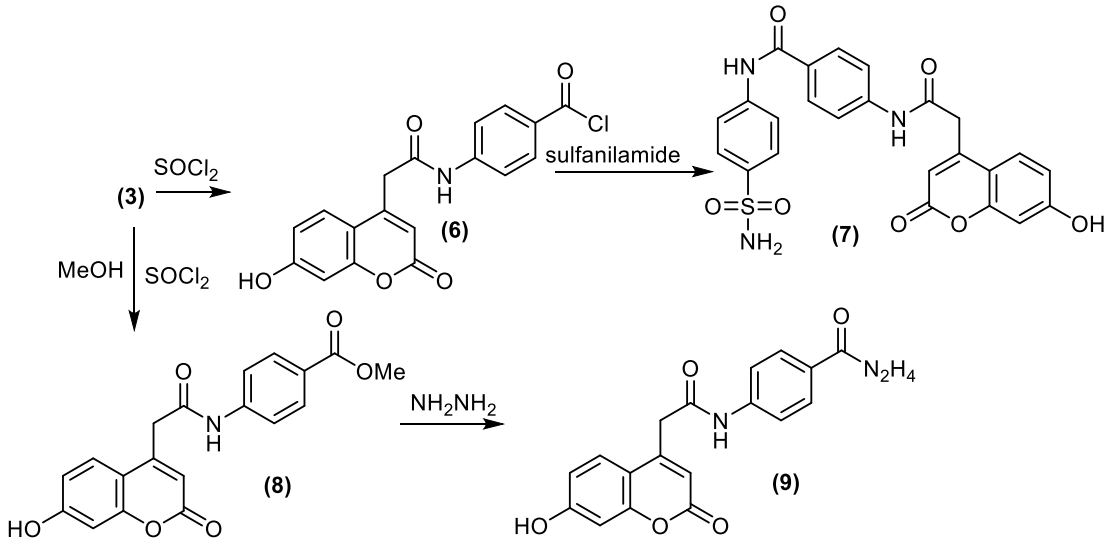
3. RESULTS AND DISCUSSION

The synthetic routes to obtain the target compounds **1-22** were depicted in (Schemes 1-6). The starting material **2** was prepared as mentioned by elhenawy *et al* [30] (Scheme **1**), which reacted with *4-amino benzoic acid and* gave the acid derivative **3**. The presence COOH proton(δH=12.10 ppm) for ^{1}HNMR data, characterized the structure. The isothiocyanate **5** was prepared by the reaction of acid chloride **2** with ammonium thiocyanate via intermediate **4** (Scheme **1**).



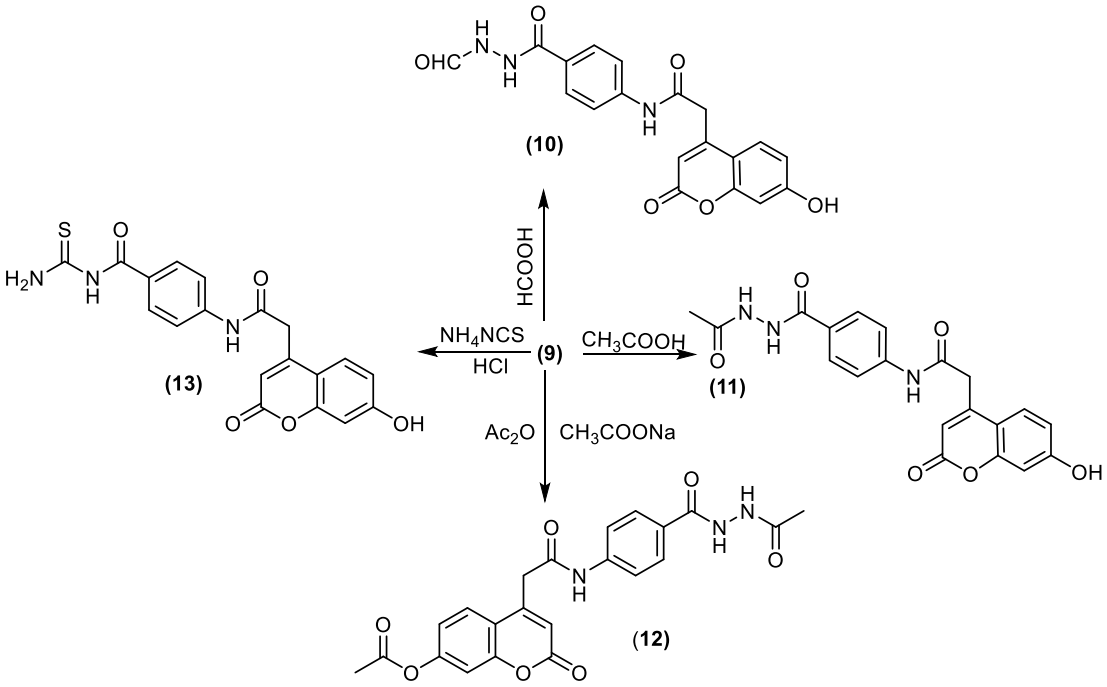
(Scheme 1.)

The free acid derivative **3** undergoing *SN*^{2} mechanism and give the corresponding acid chloride **6**. The sulphanilamide was reacted with the acid chloride **6**, to give the sulfanilamide derivative **7**, which supported by IR data, due to the appearance of characteristic sulfonamide bands at (1312,1094 cm^{-1}). Disappearance of characteristic peak (δH=12.10) for COOH proton of compound **3**, it is evidence for methylated of The compound **3** to corresponding methyl ester derivative **8**. The compound **8**was hydrazonlized to corresponding hydrazide derivative **9**, and confirmed with ^{13}CNMR, which displayed peaks at 171.8, 162.64) for CO protons **9 (Scheme 2)**.



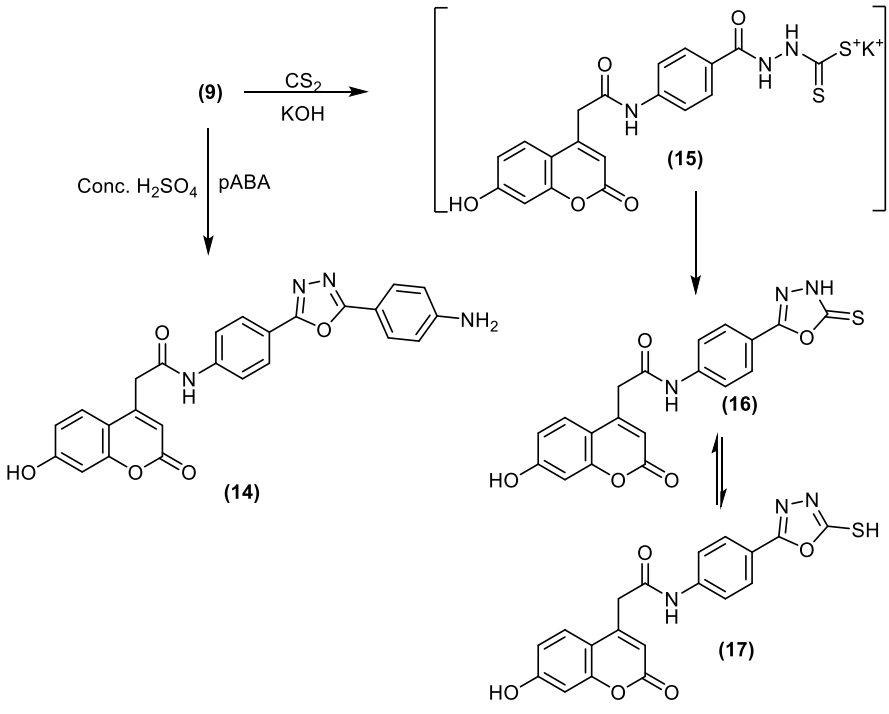
(Scheme **2.)**

N-formyl derivative **10** was obtained by formylation of hydrazide derivative **9**, which was acylated with acetic acid to give N-(4-(2-acetylhydrazinecarbonyl)phenyl)-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetamide **10** in good yield. When hydrazide **9** was acylated with acetic anhydride gave 4-(2-(4-(2-acetylhydrazinecarbonyl)phenylamino)-2-oxoethyl)-2-oxo-2H-chromen-7-yl acetate **11** in 70% yield. The compound **9** was reacted with NH_4SCN in catalytic amount of HCl to give thiourea derivative in 60% yield(**Scheme 3**).



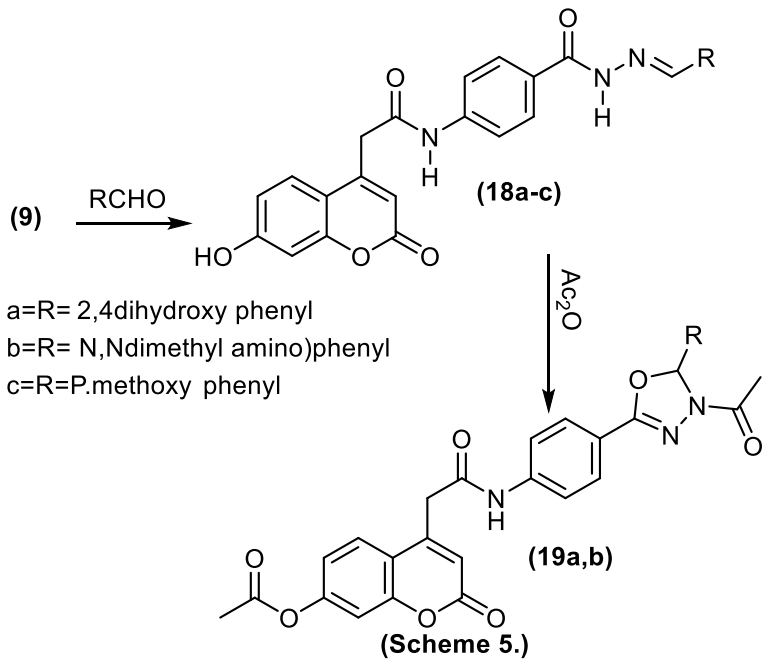
(Scheme3.)

Compound **9** was cyclized to oxadizole derivative **14,** through reaction with pABA in Conc. H_2SO_4. The CS_2 was reacted with compound **9**, and led to formation thion **16** or thiol **17** derivatives via salt formation intermediate **15,** the disappearance peak of thiol group in spectral data led to preferred the formation of thion derivative **16** in 75 % yield **(Scheme 4) .**



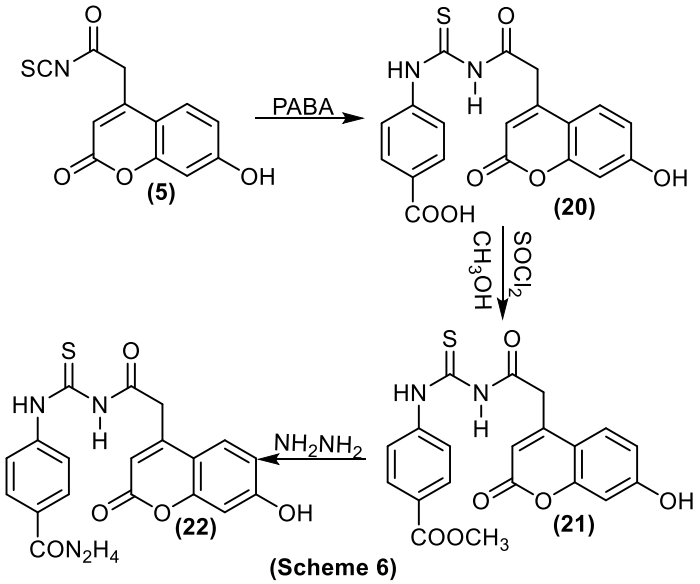
(Scheme 4.)

The compound **9** was condensed with different aromatic aldehydes, and afforded the corresponding Schiff¨s bases **18a-c,** which undergoing cyclization reaction and led to formation oxadizole derivatives **19a,b.** The disappearance of CH=N peak of ^{1}HNMR spectrum for Schiff¨s bases **18a-c,** the appearance of C-O-C band 1132-1160 cm^{-1} at IR spectrum of **19a,b** , and mass spectrum which exhibited molecular ion peaks corresponding to molecular formula of compounds confirmed its structures **19a,b** (Scheme 5).



(Scheme 5.)

Thiourido derivative **20** was prepared by coupling of compound **5** with pABA, the Thiourido free acid **20** was methylated with thionyl chloride via formation acid chloride intermediate to give methyl ester derivative **21**, the disappearance of peak (δH=12.08) of COOH proton, which confirmed the proposed molecular structure **21**. The methy ester derivative **21** was hydrazonolysis and exhibited of hydrazide derivative **22** in good yield 90%, the molecular ion peak at (412 m/z) corresponding to the molecular formula of its compound (**Scheme 6**).



(Scheme 6)

3.1. Pharmacological activity:

3.1.1. Ehrlich Tumor (Solid):

The antitumor activity of tested compounds (**7**,**8, 11,13,19b** and **20)** were assayed by observation with various parameters like Body weight of animals, tumour volume, tumour growth inhibition and the histochemical examination of apoptosis/ necrosis in tumour tissue[31-36] .

3.1.2. The body weights

The average weight loss of tested compounds (**7**,**8, 11,13,19b** and **20**) were observed compared with untreated (table 1). The data showed that, the compounds (**19b**) showed higher average weight loss (21.4±0.12) upon reference drug (DOX) mice group (21.85±0.25), the compound (**8**) exhibited significant average weight loss (22.5±0.25), the rest tested members have lower observed average weight change between untreated groups (Figure 1).

Fig.1 : solid tumor volume in mice before (left) and after (right) treatment of compound 12

3.1.3. Solid Tumor Volume and Tumor Growth Inhibition:

The average tumor volume calculated on day 13 of tested compounds (**11, 12, 15** and **20**) in compared with untreated control (table 3, Fig. 135), the all members showed decrease average tumor volume in decreasing order **12<20** in percent range (**~11-82**)%.

Table 1: Average Weights and Tumor Inhibition of Ehrlich Solid Tumor after Treatment compounds (11, 12, 15, 20, 26 and 27)

Treatment	Body weight of animal on days (g)			day 13			
	day (0)	**Day 5**	**day 9**	**Avg. body wt.(g)**	**Avg. tumor volume**	**%Tumor growth inhibition**	**Mortality**
Normal	20.2 ±0.25^{e}	21.6 ±0.25^{ed}	22.8 ±0.25^{b}	23.2 ±0.16^{a}	-	-	0/5
Untreated	20.9 ±0.25^{e}	21.4 ±0.25^{e}	22.2 ±0.25^{c}	23. ±0.07^{ed}	1.8 ±0.09^{a}	-	0/5
11	22.7 ±0.25^{c}	24.2 ±0.12^{a}	24.5 ±0.25^{a}	25.2 ±0.25^{a}	1.3 ±0.09^{bc}	23.9	0/5
12	22.5 ±0.25^{c}	22.8 ±0.12^{c}	22.5 ±0.25^{c}	-	0.9 ±0.09^{d}	47.7	1/5
15	22.6 ±0.25^{c}	23.0 ±0.25^{b}	23.7 ±0.25^{b}	23.7 ±0.16^{c}	1.5 ±0.09^{ab}	15.9	2/5
20	22.4 ±0.25^{c}	23.5 ±0.12^{b}	23.3 ±0.25^{b}	24.6 ±0.16^{b}	1.1 ±0.09^{cd}	38.1	0/5

Values are expressed as Mean ± SEM and, $p \leq 0.05$ indicates the level of statistical significance as compared with control. Treatments show highly significant deference at pr>0.0001, the letters (a-f) represents statistically significant

3.2. Structure activity relationship:

The antitumor activity of tested compounds (**7**,**8, 11,13,19b** and **20)** were examined in vivo of mice, using the following parameters, body weight of animals, tumour volume, and tumour growth inhibition. The above data showed that, the member containing thiourea *p*ABA free acid **20** moiety showed highest inhibition potency **(83%),** the members have *p*ABA methyl ester moiety and thiourea fragment **8** and **13** showed moderate inhibition potency (47 and 38 %), respectively, the other members showed lower inhibition potency.

3.2.1. ADMET Profile:

Oral bioavailability was considered to play an important role in the development of bioactive molecules as therapeutic agents. Many potential therapeutic agents fail to reach the clinic, because of their ADMET Factors. Therefore, a computational study for prediction of ADMET properties of the molecules was performed for testing compounds (**7**,**8, 11,13,19b** and **20)**, by the determination of topological polar surface area (TPSA), a calculated percent absorption (%ABS) [38], and Lipinski rules [39], In addition, the total polar surface area (TPSA) is another key property linked to drug bioavailability, the passively absorbed molecules with (TPSA>140) have low oral bioavailability [40]. All calculated descriptors were performed using MOE Package [41], and their results were disclosed in (Table 2). Our results revealed that, the CLogP (factor of the lipophilicity [42] was less than 5.0, hydrogen bond acceptors between (2-8), hydrogen bond donors between (1-5), these data show these compounds fulfill Lipinski's rule. Also, the absorption percent in ranged between (~ 66-99%). The HOMO and LUMO of a molecule play important roles in intermolecular interactions [43], through the interaction between the HOMO of the drug with the LUMO of the receptor and vise versa. The interactions were stabilized inversely with energy gap between the interacting

orbitals. Increasing HOMO energy and decreasing LUMO energy in the drug molecule lead to enhancement stabilizing interactions, and hence, binding to the receptor.

Table 2: Pharmacokinetic parameters important for good oral bioavailability of compounds (**7**,**8, 11,13,19b** and **20**):

CPD	HBD	HBA	LogP	V	TPSA	%ABS	Log S	ΔE
3	2	7	2.63	0	101.93	73.83	-4.67	8.39
7	4	9	1.64	0	133.83	62.83	-4.74	8.32
12	3	10	1.88	0	139.89	60.73	-5.45	8.29
13	5	10	3.90	0	157.55	86.73	-7.96	7.09
19b	3	8	2.52	0	146.05	69.14	-6.40	8.25
20	5	10	2.24	1	164.89	98.53	-6.30	8.53

***TPSA**: Polar surface area **(A°2)**,%**ABS**: Absorption percentage, **Vol**: Volume (A3), **HBA**: Number of hydrogen bond acceptor, **HBD**: Number of hydrogen bond donor, **V**: Number of violation from Lipinski's rule of five., **Log P**: Calculated lipophilicity., **Log S**: Solubility parameter, Δ**E**: Energy Gaps(eV).*

3.2.2. Prediction of blood–brain barrier permeability

Blood–brain barrier (BBB) permeability, is a one of the most important challenges in the pharmacology of CNS active drugs. Many drugs have limited usage and fail to pass the clinical trials, due to failure penetration of CNS . In silico (Table 3), the pharmakinetic parameters were calculated for most active compound **20**, using ADME-T algorthim, and defined human intestinal absorption (HIA) model [44,45], which predicted that, the compounds should be able to transported across the intestinal epithelium, which probably have high affinity binding to the plasma proteins, and may be passed through the blood-brain barrier, and it is necessary for ability drug transported throughout the body**.** In general, these data (table 3) suggested that, no marked health effects observed for rodent toxicity profiles, among the most active compounds **20**, its compounds are a good ability transport against ***BBB***, good activity for CNS can be used as a good oral bioavalabilty.

Table3: The prediction of blood brain barrier most active compounds **8** and **20.**

ADME-Tox	20
LogBBB (Blood-brain barrier.)	0.038
PPB% (Plasma protein binding)	96.67
LD50 rat/mouse(mg kg^{-1}, oral)	500/590
LD50 rat/mouse(mg kg^{-1}, intraperitoneal)	310/360
LD50 mouse(mg kg^{-1}, intravenous)	30
LD50 mouse(mg kg^{-1}, subcutaneous)	360
Ames test (genotoxicity, %)	0.36
Prob. of blood effect	0.45
Prob. of cardiovascular System	0.86
Prob. of gastrointestinal System	0.93
Prob. of kidney effect	0.80
Prob. of liver effect	0.32
Prob. of lung effect	0.54

4. Conclusion:

The present work aims to synthesis some novel coumarine derivatives. The synthesized compounds were characterized by different spectral data (IR, 1HNMR). The antitumor activity of tested compounds (**7**,**8, 11,13,19b** and **20)** were examined in vivo against Ehrlich solid carcinoma (EAC) tumor model in Swiss albino mice on dose 50µg , using the following parameters, body weight of animals, tumour volume, and tumour growth inhibition. The data showed that, introducing thiourea free acid fragment **20** enhancements antitumor activity than reference drug (DOX). The ADMET profiles in silico showed that, these compound are good oral bioavalability, and the most active compounds 6 and 10 are CNS active agents without marked health effects observed for rodent toxicity. On the light of these data, we think that, these compounds may be used in the future as lead anticonvulsant candidates as they resemble in their structure of lamotrigine the known anticonvulsant drug.

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