ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 8, pp. 13171-13177, August, 2016

International Journal of Recent Scientific Research

Research Article

DOCKING AND INVITRO ANTICANCER STUDIES OF SOME NOVEL SYNTHESIZED 4-METHYL BENZOCOUMARIN 8-(N-SUBSTITUTED) SULFONAMIDES

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ARTICLE INFO

Article History:
Received 17th May, 2016
Received in revised form 12th June, 2016
Accepted 04th July, 2016
Published online 28th August, 2016

Key Words:
Benzocoumarin, MCF-7, COX-2.

ABSTRACT

A series of 4-methyl benzocoumarin 8-(N-substituted) sulfonamides were designed, synthesized and evaluated for their *in silo* COX-2 inhibition and *in-vitro* anticancer activity. Compounds **7, 10, 14, 15, 18** have shown growth inhibition against MCF -7 cell lines. Docking simulations performed on COX-2 indicated compound **18** with hydrophobic key interactions that may govern enzyme-inhibitor binding.

INTRODUCTION

Cancer, a diverse disease characterized by uncontrolled cell proliferation, lack of cell differentiation, and loss of contact inhibition, which confers upon the tumor cell a capability to invade local tissue and metastasize. In some types of cancers such as breast, throats, etc. are associated with chronic inflammation and pain. Currently, there is a huge scientific and commercial interest in the discovery of potent, safe and selective drugs in the treatment of cancer with inflammation.

Nonsteroidal anti-inflammatory drugs (NSAIDS) are the most widely used therapeutics worldwide for the treatment of inflammation, pain, fever, and for the prevention of thrombosis. These drugs are inhibitors for substrates COX-1 and COX-2, specifically for COX-2 as the COX-2 expression is significantly up-regulated in humans under various acute chronic inflammatory conditions. It is also well documented that COX-2 is overexpressed in numerous human cancer such as colorectal, gastric, and breast cancer (Yasojima *et al*, 2001; Herschamn HR 1996; Vane JR *et al,* 1998). Recognition of the importance of COX-2 in inflammation and carcinogenesis has promoted the synthesis of various COX-2 selective inhibitors over the last two decades. The close structural similarities of both COX isoforms represent a formidable challenge for the development of selective COX-2 inhibitors

Benzocoumarins have evoked good importance due to their biological properties and characteristic conjugated molecular architecture. Many of them display important pharmacological effects, including analgesic (Adami E *et al*,1959), anti-arthritis (Chiarino D *et al*, 1988), anti-inflammatory (Luchini AC *et al*, 2008), anti-pyretic(Stern P *et al,* 1957), anti-bacterial (Chohzan ZH *et al,* 2006), anti-viral (Kirkiacharian BS *et al*, 2008), anti-cancer (Velaso-Velazzaquez MA *et al,* 2003), anti-coagulants (Shapiro S *et al*,1943;Butsch WL *et al,* 1942) and certain cardiac conditions (Hintz KK *et al*, 2003)

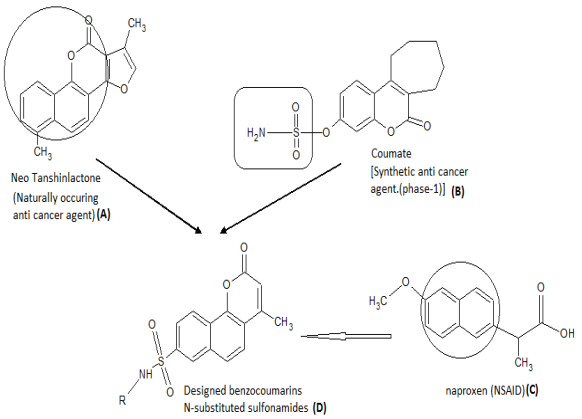

Figure 1 Design of benzocoumarins N-substituted sulfonamides

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In earlier papers (Koneni V.Sashidhara *et al,* 2010; Selvam C *et al*, 2005), different authors have reported the synthesis of Benzocoumarins from natural coumarins (figure-1) which are used in the treatment of inflammation and cancer. As a part of our program to design benzocoumarins as specific inhibitors of COX-2 and as anticancer agents in the hope that these molecules may be further explored as powerful and novel lead compounds.

MATERIALS AND METHODS

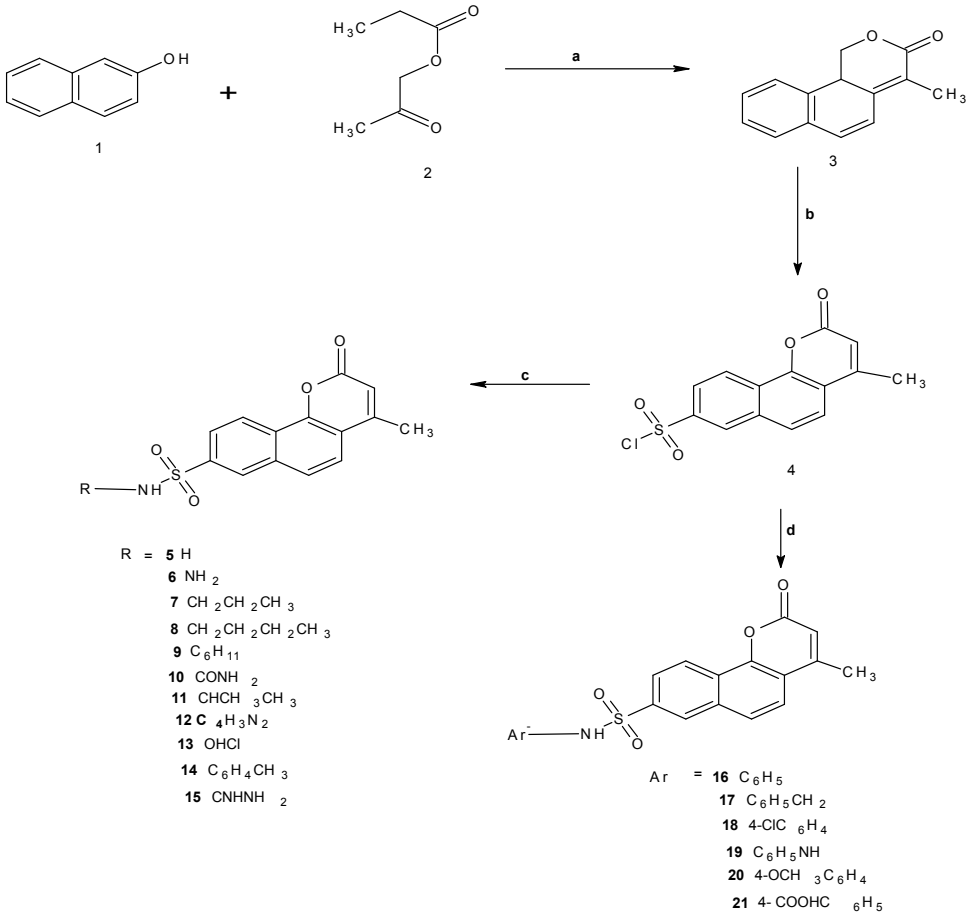
The chemicals used for the synthesis were supplied by LOBA chemicals. The purity of the compounds was checked on thin layer chromatography (TLC) plates (Silica Gel G) using the solvent systems ethyl acetate: hexane (1:1). The spots were located under UV light (254 and 365 nm). Melting points were determined on GallenKamp (MFB-600) melting point apparatus and were uncorrected. The IR spectra of the compounds were recorded on a shimadzu FTIR-8300 spectrometer as KBr disk. The 1H-NMR and 13C-NMR spectra (solvent CD3OD) were recorded on Bruker 400 MHz spectrophotometer using TMS as an internal standard. The (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliuum bromide (MTT) were purchased from Sigma-Aldrich Co, (St Louis, MO, USA), cancerous cell lines such as HeLa-B75 (cervix cancer), MCH-7 (Breast cancer) were obtained from the National Centre for Cell Science, Pune (Maharashtra, India). All other reagents and solvents used were obtained from commercial sources and were of analytical grade.

General synthesis (3-21)

Chlorosulphonic acid was added slowly to 4-methyl benzocoumarins synthesized by Pechmann condensation of equimoles of 1-naphthol and ethyl acetoacetate under acidic conditions. The reaction mixture was allowed to cool and the product was precipitated in crushed ice, filtered and dried. The dried 4-methyl benzocoumarins- 8-sulfonylchloride was treated with various amines and refluxed for 30 min .The reaction mixture was allowed to cool the product obtained was filtered, recrystallized from ethanol. The synthesis of 4-methyl benzocoumarins8-(N-substituted) sulphonamides followed the general reaction pathway outlined in scheme 1

4-Methyl-2h-Benzo [H] Chromen-2-One (3)

Reactiontime:10min;%yield:40%;R_f:0.566(ethylacetate:hexane 1:1);M.P(^{0}c):150155^{0}C;IR(KBr,V_{max},Cm^{-1}):3734(-NH),3433(-OH),3087(-C=C-),2812(CH_3),1665(C=O),1244(C-O),1210(C-O-C),1600(C=C-aromatic), 1384(assymetricSO_2)1132(SymmetricSO_2).;^{1}H NMR(400MHZ,MeOD) 2.4(CH_3(M)),7.38,7.35,7.68,8.08,7.26,7.21(CHprotons1.71(S),^{1} ^{3}CNMR(400MHZ,MeOD):21.2(CH_3(S)),126.8,126.2,127.5,134.1,126,121.7,121.1,122.6,124.8,154.8,152.8,112.5,160.(basic ring carbons)



Scheme1 Synthesis of 4-methyl benzocoumarin (3) ,4-methyl benzocoumarin 8-sulphonyl chloride (4) and 4-methyl benzocoumarin 8-(N-substituted) sulphonamides(5-21).

4-Methyl-2-OXO-2H-Benzo [H] Chromene-8-Sulfonyl Chloride (4)

Reaction time:10min; %yield: 45% ;R_f: 0.6(ethyl acetate:hexane1:1); M.P:1400C ;IR (KBr,V_{max}, Cm^{-1}):3733(NH),3423(-OH),3085(-C=C-), 2810(CH_3),1669(C=O),1238(C-O),1208(C-O-C),1600(C=C Aromatic),1383(assymetricSO_2),1132(SymmetricSO_2).;^{1}HNMR (400MHZ,MeOD): 0.9,1.71,2.46 (CH_3(T)),7.24,7.47,8.02,7.23,7.20 (CH-protons), ;^{13}C NMR (400MHZ,MeOD): 34,21.2,24.6(CH_3(T) ,136.3,126.8,134,124,120,120.5,122.4,123.7,154.3,152.8,112.5, 160.9(basic ring carbons)

4-Methyl-2-OXO-2H-Benzo [H] Chromene-8-Sulphonamide (5)

Reaction time:10min; %yield: 37.06%;R_f:0.7(ethyl acetate:hexane1:1); M.P(^{0}C) 160^{0}C ;IR (KBr,V_{max}, Cm^{-1}): 2954cm^{-1} (CH stretching),1707cm^{-1}(C=O),3737cm^{-1}(NH stretching),1077cm^{-1}(C-O-C),1370cm^{-1}(SO_2 asymmetric),1162cm^{-1}(SO_2 symmetric),. ;^{1}H NMR(400MHZ,MeOD): 1.71(CH_3(S)),8.05,8.35,8.36,7.26,7.21,(CHprotons),2(NH_2);^{13} CNMR(400MHZ,MeOD):21.2(CH_3(S)),125,125.5,134.4,129.2 ,122,121.122.6,124.8,154.8,152.8,112.5,160.9,(basic ring carbons)

4-Methyl-2-OXO-2H-Benzo[H]Chromene-8-Sulphonamide(6)

Reaction time:10min; %yield: 12.336% ;R_f:0.59(ethyl acetate:hexane1:1); M.P(^{0}c):154-156^{0}C;IR (KBr,V_{max},Cm^{-1}):2891cm^{-1}(CH-stretching),1710cm^{-1}(C=O),3740cm^{-1}(NH),1372cm^{-1}(SO_2 asymmetric),1170cm-^{1}(SO_2symmetric);^{1}HNMR (400MHZ,MeOD):1.71(CH_3(S)),8.05,8.35, 8.36 ,7.26,7.21,(CHprotons),2(NH,(s)),2(NH(S));^{13}CNMR(400MHZ ,MeOD):21.2(CH_3(S)),125,125.5,134.4,129.2,122,121.1,122.6, 124.8,154.8,152.8,112.5,160.9,(basic ring carbons),137(NH-side chain carbons)

4-Metyhl-2-OXO-N-Propyl-2H-Benzo [H] Chromene-8-Sulphonamide (7)

Reaction time:10min; %yield:32.326 ;R_f:0.66 (ethyl acetate:hexane1:1); M.P(^{0}c):159-164;IR (KBr,V_{max},Cm^{-1}):2982cm^{-1}(CH-stretching),3736cm^{-1}(NH),1709cm^{-1}(C=O),1079cm^{-1}(C-O-C),1370cm^{-1}(SO_2 asymmetric), 1168cm^{-1}(SO_2symmetric) ;^{1}H NMR(400MHZ,MeOD): 0.96(CH_3(S)),3.16,1.59 (CH_2-protons), 2 (NH_2,(s)),8.05,8.35,8.36,7.26,7.21(CH protons) ;^{13}C NMR(125MHZ,$CDCl_3$):21.2(CH_3(S)),112.5,152.8,124.8,160.9 ,154.6,19.2,134.4,121.1,122.6,125.5,125,122.0(basic ring carbons),137,45,22.3,11.2(side chain)

N-Butyl-4-Methyl-2-OXO-2H Benzo [H] Chromene-8-Sulphonamide (8)

Reaction time:10min; %yield:40.06;R_f:0.58(ethyl acetate:hexane1:1); M.P(^{0}c):158-162 ;IR (KBr,V_{max}, Cm^{-1}):1557cm^{-1}(CH- aromatic stretching),3739cm^{-1}(NH),1709cm^{-1}(C=O),1081cm^{-1}(C-O-C),1372cm^{-1}(SO_2 asymmetric),1171cm^{-1}(SO_2symmetric) ;^{1}H NMR(400MHZ,MeOD): 1.71,0.96(CH_3(D)),3.16,1.55,1.33 (CH_2-protons), 2(NH_2,(s)), 3.16,1.55,1.33,0.96(side chain protons),;^{13}CNMR(125MHZ,MeOD):21.2,13.8(CH_3,(D)),112.5 ,152.8,124.8,160.9,154.6,19.2,134.4,121.1,122.6,125.5,125,122 .0 (basic ring carbons),137,42.5,31.4,19.9,13.8(side ring carbons)

N-Cyclohexyl-4-Methly-2-OXO-2HBENZO[H]Chromene-8-Sulphonamide(9)

Reaction time:10min; %yield:26.415;R_f:0.58 (ethyl acetate:hexane1:1); M.P(^{0}c):160-165;IR (KBr,V_{max}, Cm^{-1}): 1506cm^{-1}(CH- aromatic stretching),3736cm^{-1}(NH),1711cm^{-1}(C=O),1079cm^{-1}(C-O-C),1371cm^{-1}(SO_2 asymmetric),1170cm^{-1}(SO_2symmetric) ;^{1}H NMR(400MHZ,MeOD): 1.71(CH_3(S)),1.49,1.39,1.46,1.43,1.49,1.39,1.78,1.53(CH_2protons),7.21,7.26,8.35,8.36,8.05(basicringprotonsprotons);^{13}CNMR(125MHZ,MeOD):21.2(CH_3(S)),112.5,152.8,124.8,160.9,154.6,19.2,134.4,121.1,122.6,125.5,125,122.0 (basic ring carbons);137,32.9,42.7,32.9,22.9,28,22.9,(side ring carbons).

{4-Methyl-2-OXO-2H-Benzo [H] Chromene-8-Sulphonyl} UREA (10)

Reaction time:10min; %yield: 34.36 ;R_f:0.61 (ethyl acetate:hexane1:1); M.P(^{0}c):158-164;IR (KBr,V_{max}, Cm^{-1}): 1551cm^{-1}(CH- aromatic stretching),3860cm^{-1}(NH),1708cm^{-1}(C=O),1077cm^{-1}(C-O-C),1369cm^{-1}(SO_2 asymmetric),1167cm^{-1}(SO_2symmetric). ;^{1}H NMR(400MHZ,MeOD): 1.71(CH_3(S)),8.05,8.35,8.36,7.26,7.21,(CHprotons),6(NH_2,(s)) ,6(NH(S));^{13}CNMR(125MHZ,MeOD):21.2(CH_3(S)),112.5,152 .8,124.8,160.9,154.6,19.2,134.4,121.1,122.6,125.5,125,122.0 (basic ring carbons),137,161(Side chain carbons).

4-Methyl-2-OXO-N-(PROPAN-2-YL)-2HBENZO [H]Chromene-8- Sulphonamide (11)

Reaction time:10min; %yield:33.35;R_f:0.67(ethyl acetate:hexane1:1); M.P(^{0}c):159-164;IR (KBr,V_{max}, Cm^{-1}):1506cm^{-1}(CH- aromatic stretching) , 3735cm^{-1}(NH),1710cm^{-1}(C=O),1077cm^{-1}(C-O-C),1370cm^{-1}(SO_2 asymmetric),1142cm^{-1}(SO_2symmetric). ;^{1}H NMR(400MHZ,MeOD): 1.71,1.05,1.05(CH_3(T)), 8.05,8.35,8.36,7.26,7.21,2.97(CH-protons), ,2(NH(S)) ;^{13}C NMR (125MHZ,MeOD):21.2,22.4,22.4(CH_3(S)),112.54,152.8,124.8, 160.9,154.6,19.2,134.4,121.1,122.6,125.5,125,122.0 (basic ring carbons),.

4-Methyl-2-OXO-N-(Pyrimidin-2-YL)-2H-Benzo[H]Chromene-8-Sulphonamide (12)

Reaction time:10min; %yield:35.23 ;R_f:0.60(ethyl acetate:hexane1:1); M.P(^{0}c):160-165 ;IR (KBr,V_{max}, Cm^{-1}): 1603cm^{-1}(C=N -stretching),3738cm^{-1}(NH),1709cm^{-1}(C=O),1079cm^{-1}(C-O-C),1371cm^{-1}(SO_2 asymmetric),1170cm^{-1}(SO_2symmetric) ;^{1}H NMR(400MHZ,MeOD): 1.71(CH_3(S)) 8.05,8.35,8.36,7.26,7.21,2.97,6.58,8.38(CH_2-protons), ,4(NH(S)) ;^{13}C NMR (125MHZ,MeOD):21.2(CH_3(S)),112.54,152.8,124.8,160.9,154 .6,19.2,134.4,121.1,122.6,125.5,125,122.0 (basic ring carbons).;169.3,157.9,110.3,157.9 (side ring carbons).

4-Methyl-2-OXO-2H-Benzo[H]Chromene-8-Sulphonamido Hypochlorite:(13)

Reaction time:10min; %yield:44.24;R_f:0.61(ethyl acetate:hexane1:1); M.P(^{0}c):158-164;IR (KBr,V_{max}, Cm^{-1}): 1557cm^{-1}(CH- aromatic stretching),3738cm^{-1}(NH),1709cm^{-1}

^{1}(C=O),1078cm^{-1}(C-O-C), 1371cm^{-1}(SO_2asymmetric), 1169cm^{1}(SO_2symmetric).; ^{1}HNMR(400MHZ,MeOD): 1.71(CH_3(S)),8.05,8.35,8.36,7.26,7.21(CH_2-protons), 2(NH(S)); ^{13}CNMR(125MHZ,MeOD): 21.2(CH_3(S)),112.54,152.8,124.8,160.9,154.6,19.2,134.4,121.1 ,122.6,125.5,125,122.0 (basic ring carbons).137(side chain carbons)

4-Methyln-(2-Methylphenyl)-2-OXO-2H-Benzo[H]Chromene-8-Sulphonamide :(14)

Reaction time:10min; %yield: 23.43 ;R_f:0.65(ethyl acetate:hexane1:1); M.P(^{0}c):158-165;IR (KBr,V_{max}, Cm^{-1}):3061cm^{-1}(CH-stretching),3744cm^{-1}(NH),1711cm^{-1}(C=O),1081cm^{-1}(C-O-C),1373cm^{-1}(SO_2asymmetric),1172cm^{-1}(SO_2symmetric).;^{1}HNMR(400MHZ,MeOD): 1.71, 2.35 ,(CH_3(D)), 8.05,8.35,8.36,7.26,7.21 6.50,6.82,6.34,(CH-protons), 4(NH(S));^{13}C NMR (125MHZ ,MeOD):21.2,15.2(CH_3(S)),112.54,152.8,124.8,160.9,154.6,19 .2,134.4,121.1,122.6,125.5,125,122.0 (basic ring carbons).;137.0,116.2,126.6,118.7,129.9,129.0,136.3,15.2(Side chain carbons)

1-{Methyl-2-OXO-2H-BENZO [H] Chromene-8-Sulphonyl} Guanidine:(15)

Reaction time:10min; %yield:28.39;R_f:0.59 (ethyl acetate:hexane1:1); M.P(^{0}c):154-160;IR (KBr,V_{max}, Cm^{-1}):2955cm^{-1}(CH-stretching),3739cm^{-1}(NH),1710cm^{-1}(C=O),1079cm^{-1}(C-O-C),1372cm^{-1}(SO_2asymmetric),1169cm^{-1}(SO_2symmetric),1604cm^{-1}(C=N).;^{1}H NMR (400MHZ ,MeOD):1.71(CH_3(S)),8.05,8.35,8.36,7.26,7.21,(CHprotons),2(NH(S)),2(NH(s));^{13}CNMR(125MHZ,MeOD):21.2(CH_3(S)),11 2.54,152.8,124.8,160.9,154.6,19.2,134.4,121.1,122.6,125.5,125 ,122.0 (basic ring carbons) ,137.0,163(Side chain carbons)

4-Methyl-2-OXO-N-Phenyl-2H-Benzo [H] Chromene-8-Sulphonamide (16)

Reaction time:10min; %yield: 38.8;R_f:0.62 (ethyl acetate:hexane1:1); M.P(^{0}c):162-165;IR (KBr,V_{max}, Cm^{-1}):1555cm^{-1}(CH- aromatic stretching),3740cm^{-1}(NH),1711cm^{-1}(C=O),1079cm^{-1}(C-O-C),1372cm^{-1}(SO_2 asymmetric),1171cm^{-1}(SO_2symmetric). ;^{1}H NMR(400MHZ,MeOD): 1.71(CH_3(S)),8.05,8.35,8.36,7.26,7.21,6.46,7.01,6.62(CH-protons), 4(NH(S));^{13}C NMR (125MHZ,MeOD):21.2(CH_3(S)),112.54,152.8,124.8,160.9,154 .6,19.2,134.4,121.1,122.6,125.5,125,122. (basic ring carbons),; 137,116.3,129.6,118.8,129.6,116.3,137.8(side ring carbons).

N-Benzyl-4-Methyl-2-OXO-2H-Benzo [H] Chromene-8-Sulphonamide (17)

Reaction time:10min; %yield: 30.05;R_f:0.616(ethyl acetate:hexane1:1); M.P(^{0}c):160-164;IR (KBr,V_{max}, Cm^{-1}):3010cm^{-1}(CH-stretching),3740cm^{-1}(NH),1712cm^{-1}(C=O),1079cm^{-1}(C-O-C),1373cm^{-1}(SO_2 asymmetric),1171cm^{-1}(SO_2symmetric). ;^{1}H NMR(400MHZ,MeOD): 1.71(CH_3(S)),7.21,8.05,8.35,8.36,7.26,7.21,7.07,7.14,7.06,7.06 ,7.14(CH-protons), 2(NH(S));^{13}C NMR(125MHZ,MeOD):21.2,(CH_3(S)),112.54,152.8,124.8,160 .9,154.6,129.2,134.4,121.1,122.6,125.5,125,122. (basic ring carbons),137.0,46.2,141.7,127.0,128.6,126.8,128.6(side chain carbons)

N-(4-Chlorophenyl)-4-Methyl-2-OXO-2H-Benzo[H]Chromene-8-Sulphonamide (18)

Reaction time:10min; %yield: 34;R_f:0.59 (ethyl acetate:hexane1:1); M.P(^{0}c):154-160;IR (KBr,V_{max}, Cm^{-1}): 3393cm^{-1}(CH-stretching),3745cm^{-1}(NH),1709cm^{-1}(C=O),1079cm^{-1}(C-O-C),1372cm^{-1}(SO_2asymmetric),1170cm^{-1}(SO_2symmetric),564cm^{-1}(C-Cl). ;^{1}H NMR(400MHZ, MeOD): 1.71(CH_3(S)),8.05, 8.35,8.36,7.26,7.21, 7.02,7.02,6.40,6.40(CH-protons), 4(NH(S)); ^{13}CNMR(125MHZ,MeOD):21.2(CH_3(S)),112.54,152.8,124.8,1 60.9,154.6,129.2,134.4,121.1,122.6,125.5,125,122(basicringcar bons),137.0,135.9,117.7,129.7,124.3,129.7,117.7(side chain carb ons)

4-Methyl-2-OXO-N^{1}-Phenyl-2H-BENZO[H]Chromene-8-Sulphonohydrazine (19)

Reaction time:10min; %yield:34;R_f:0.58 (ethyl acetate:hexane1:1); M.P(^{0}c):158-162;IR (KBr,V_{max}, Cm^{-1}):1555cm^{-1}(CH- aromatic stretching), 3744cm^{-1}(NH), 1709cm^{-1}(C=O), 1078cm^{-1}(C-O-C),1371cm^{-1}(SO_2 asymmetric),1170cm^{-1}(SO_2symmetric). ;^{1}H NMR(400MHZ,MeOD): 1.71(CH_3(S)),8.05,8.35,8.36,7.26,7.21,6.66,7.18,6.71,7.18,6.66 (CHprotons),2(NH(S));4(aromaticCNH);^{13}CNMR(125MHZ,M eOD):21.2(CH_3(S)),112.54,152.8,124.8,160.9,154.6,129.2,134. 4,121.1,122.6,125.5,125,122 (basic ring carbons),;137.0,151.0,113.2,129.3,119.2,129.3,113.2(side ring carbons).

N-(4-Methoxyphenyl)-4-Methyl-2-OXO-2H-Benzo[H]Chromene-8-Sulphonamide:(20)

Reaction time:10min; %yield: 40;R_f:0.619 (ethyl acetate:hexane1:1); M.P(^{0}c):158-164;IR (KBr,V_{max}, Cm^{-1}): 1508cm^{-1}(CH- aromatic stretching),3739cm^{-1}(NH),1707cm^{-1}(C=O),1078cm^{-1}(C-O-C),1371cm^{-1}(SO_2 asymmetric),1169cm^{-1}(SO_2symmetric).;^{1}H NMR(400MHZ,MeOD): 1.71,3.73(CH_3(D)),8.05,8.35,8.36,7.26,7.21,6.35,6.52,6.52,6.3 5,(CH-protons), ;4(NH(S)), ;^{13}C NMR(125MHZ,MeOD):21.2,55.9(CH_3(S)),112.54,152.8,124.8 ,160.9,154.6,129.2,134.4,121.1,122.6,125.5,125,122 (basic ring carbons);137, 117.2,115.1,150.9,55.9,115.1,117.2(side ring carbons)

4-{4-Methyl-2-OXO-2H-Benzo [H] Chromene 8-Sulfonamido} Benzoic Acid (21)

Reaction time:10min; %yield: 40;R_f:0.603 (ethyl acetate:hexane1:1); M.P(^{0}c):160-165;IR (KBr,V_{max}, Cm^{-1}): 3393cm^{-1}(CH-stretching),3744cm^{-1}(NH), 3861cm^{-1}(NH_2),1711cm^{-1}(C=O), 1079cm^{-1}(C-O-C),1372cm^{-1}(SO_2 asymmetric),1169cm^{-1}(SO_2symmetric).;^{1}H NMR(400MHZ, MeOD): 1.71(CH_3(S)),8.05,8.35,8.36,7.26,7.21,6.67,7.88,7.88,6.67(CH -protons), 11.0(OH (s)) ,. 4(NH $_2$(S));^{13}C NMR (125MHZ,MeOD): 21.2(CH_3(S)), 112.54,152.8, 124.8,160.9,154.6, 129.2,134.4,121.1,122.6,125.5,125, 122(basic ring carbons), 137.0,116.2,131.1 ,120.3, 169.3, 131.116.2,143 (side ring carbons).

Cytotoxicity bioassay

Cytotoxicity bioassay was carried out against human cancer cell lines MCF-7(breast adenocarcinoma) HELA(cervical

carcinoma) obtained from the National Centre for Cell Science, Pune(Maharashtra, India). The adherent cells were trypsinized according to protocol and were resuspended in fresh medium after centrifugation. The cell suspension was mixed thoroughly by pipetting several times to get a uniform single cell suspension. Different dilutions of drug solutions were made in media with final DMSO concentration in the well to be less than 1%.100µl of cell suspension was transferred aseptically to each well of a 96 well plate and to it 100µl of 1% media/ drug solution (in quadruplicate) in media was added. The plate was then incubated at 37^{o}C for 72 hours in CO_2 incubator. After 72 hours of incubation, 20µl of MTT was added to each well. The plate was again incubated for 2 hours. 80µl of lysis buffer was added to each well the plate was wrapped in aluminium foil to prevent the oxidation of the dye and the plate was placed on a shaker for overnight. The absorbance was recorded on the ELISA reader at 562nm wavelength. The absorbance of the test was compared with that of DMSO control to get the %inhibition. The graph was plotted with concentration (µg/ml) and % inhibition in an XY scatter. The cytotoxic effects of compounds were calculated as percentage inhibition in cell growth as per the formula [100-(absorbance of compound treated cells/absorbance of the untreated cell)] *100. Determination of 50%inhibitory concentration (IC_{50}) was based on dose response curves. The dose response of each cell line was established by determining the number of viable cells after 72hrs of continuous treatment against 5 different concentration of each compound (1-100µM range). Table 1 shows the GI_{50} values, the concentration of each compound required to inhibit the growth of each tumor cell line by 50%, determine for each compound in the series.

Table 1 Anti Cancer Activity (Gi_{50}µm)

Compounds	MCF-7	HELA
3	30.12	35.42
4	37.72	40.13
5	31.41	34.45
6	41.94	36.62
7	2.74	5.06
8	50.71	44.59
9	43.35	59.87
10	2.26	5.08
11	40.28	47.28
12	52.52	33.59
13	45.76	38.92
14	2.79	6.26
15	2.31	8.45
16	39.19	71.54
17	61.44	28.64
18	2.20	5.06
19	57.04	97.25
20	55.16	51.37
21	58.42	60.18
Cisplatin	2.67	5.47

Docking

Further to understand the anti-inflammatory property of benzocoumarins and *in silico* docking study was performed.

The crystals structure of COX-2 (3NT1) (Thuresson, E.D *et al*, 2001) complexed with naproxen was used for docking. The complex was prepared for docking studies by adding hydrogen atoms, removing water and co-crystallized inhibitors and refined by using the protein preparation tool implemented in GLIDE programme of Schrodinger 2013software installed on HPZ 600 workstation**. GLIDE**, an automated docking program, was used to dock these compounds into the active site, of COX-2 enzyme and the most stable conformation based on the best scoring function was selected. All the calculations were performed using GLIDE. The results are represented in Table 2.

RESULTS AND DISCUSSION

4-methyl benzocoumarins-8-(N-substituted) sulphonamides were synthesized by following three step synthetic scheme (scheme-1) .the step 1 of three steps was accomplished using microwaves as microwave method of synthesis is more efficient and faster.the remaining two steps by a conventional method.

Nineteen 4-methyl benzocoumarins-8-(N-substituted) sulphonamides (5-21) were synthesized by treating 4-methyl benzocoumarins- 8-sulfonylchloride(4) with various amines. the reactions were completed in 30 minutes. The progress of the reaction was carried out by TLC. The sulphonamides synthesized exhibited fluore scence under UV light .Rf value of sulfonamides obtained by using solvent system ethylacetate and hexane in the ratio 4:1. The structures and the behavior of sulfonamides were established by elemental analysis and by their corresponding IR, HNMR,C13 NMR spectral studies.

Out of nineteen compounds evaluated five compounds 7, 10, 14, 15, 18 shows GI_{50} values 2.74, 2.26, 2.79, 2.31, 2.20 which were comparable with cisplatin. It is interesting to reveal that though all compounds were screened against MCF-7 and HELA cancer cell lines, these derivatives exhibited selective and significant inhibition in MCF-7 cell lines. A significantly higher activity of 10, 15, 18was observed as compared to cisplatin. Particularly compound 18 induced significant loss of cell viability and had lowest GI_{50}.

The results of *Invitro* anticancer study revealed that synthesized benzocoumarins have different responses of growth inhibition against cancer cell lines, the possible mechanism of biological activity by benzocoumarins has been reported that substituent's at C2 ,C4 of the heterocyclic ring of benzocoumarins induce apoptosis as these molecules are fluorescent (due to extended conjugation) (Li L *et al*, 2003), this property is responsible to enhance the anti-cancer activity due to photosensitization and known microtubule inhibitor

Table 2 G-Scores of Compound-18, Naproxen and Their Isomers

ligand	GScore	LipophilicEvdW	PhobEn	HBond	Sitemap	LowMW	RotPenal	Electro
18	-9.88	-6.42	-2.7	-0.15	-0.4	-0.17	0.16	-0.2
18(1)	-9.59	-6.37	-2.7	-	0.4	-0.17	0.16	-0.11
18(2)	-8.48	-5.63	-1.94	-	0.4	-0.4	0.15	-0.26
NPS	-9.73	-5.29	-1.04	-2.15	-	-0.5	0.14	-0.89
NPS-2	-9.34	-5.31	-1.01	-1.94	-	-0.5	0.14	-0.73
NPS-3	-9.19	-5.63	-1.29	-1.77	-	-0.5	0.28	-0.63
NPS-4	-9.07	-5.36	-1.35	-1.79	-	-0.5	0.28	-0.35

with antimitotic activity (Liu M *et al*, 2001; Sabzevari O. *et al*,2004; LeeY. S *et al*,2006 ; Rozmer Z *et al*,2006 ; Mayur Y.C *et al*,2009; Solomon V.R *et al*, 2009; Song H.Y *et al*,2009; Belluti, F *et al*, 2010)

Compound 18 could dock into the active site of COX-2 successfully with G –SCORE of -9.88. It formed one hydrogen bond with TYR385 and one hydrophobic bond with TYR355. The bonding distance between 2C=0 of compound 18 and OH of TYR385 is 2.325A° (Figure -2). The molecule cyclic rings are surrounded by the active site amino acid residues ARG120, TYR355, MET113, SER530, and TYR115.

TYR385 is responsible for the abstraction of 13-pro-s-hydrogen from arachidonic acid due to this probable reason compound 18 shows selective inhibition towards COX-2. While it was noted that COX-2 has an active site volume 417A^3, determined using site- map module programmer. It is also important to note that the mode of binding of compound 18 is due to its difference in the active site volumes shown Figure-2. This selectivity issue had to be further supported by in future anti-inflammatory experimental work.

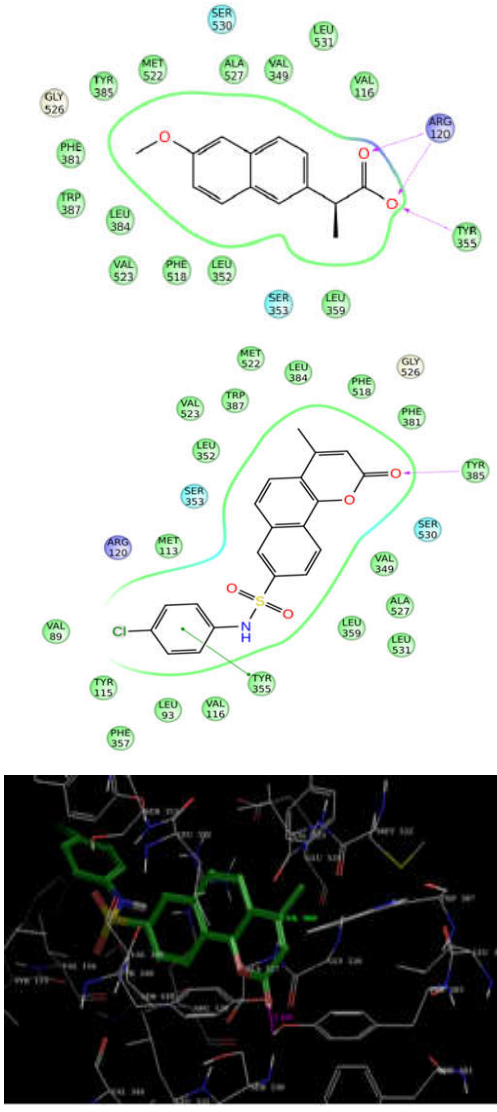


Figure 2 Difference in the active site volumes of compound 18, standard naproxen and hydrogen Bond between 2C=O of Compound 18 and Hydrogen of TYR 385.

CONCLUSION

The present study revealed a synthesis of nineteen benzocoumarin derivatives, with pronounced growth inhibition against MCF-7 and HELA cancer cell lines. Two compounds exhibited greater anti-cancer activity than cisplatin. One compound showed in silico COX-2 inhibition. The findings of the study inferred that the dual functioning of compound 18 as a COX-2 inhibitor and anti-cancer agent, render it as a lead molecule for the further development of new agents in the treatment of cancer associated with inflammation.

Acknowledgements

The authors are grateful to KVSR Siddhartha College of Pharmaceutical Sciences for their support and to Department of Biotechnology K L U University for access to CADD programme.

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How to cite this article:

KNVChenchuLakshmi *et al*.2016, Docking and Invitro Anticancer Studies of Some Novel Synthesized 4-Methyl Benzocoumarin 8-(N-Substituted) Sulfonamides. *Int J Recent Sci Res.* 7(8), pp. 13171-13177.