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Der Pharma Chemica, 2011, 3 (4):399-412
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis and pharmacology of some novel 4(3*H*)-quinazolinone derivatives

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ABSTRACT

Several novel 4(3H)-quinazolinone derivatives incorporating four known bioactive moiety such as adamantine, piperazine, imidazolidine and thiazolidinone were synthesized by using multi-step reaction strategy. The synthesized derivatives were characterized by IR, ^{1}H-NMR, Mass and elemental analysis. Furthermore the synthesized derivatives evaluated for antibacterial, antifungal, anti-inflammatory, ulcerogenic, analgesic and acute toxicity activities.

Keywords: 4(3*H*)-Quinazolinones, Antifungal, Antibacterial, Anti-inflammatory, Ulcerogenic, Analgesic, Acute toxicity activities.

INTRODUCTION

Quinazoline-4(3*H*)-ones are versatile nitrogen pharmacophres, displaying a broad spectrum of biological and pharmalogical activities such as anti-fungal [1], anti-tumour [2], hypotensive [3-6], anti-cancer [7,8], anti-HIV [9], anti-bacterial [10] etc. Furthermore, substituted quinazoline-4(3*H*)-one derivatives play a pivotal role in the anti-inflammatory activity [11] also. Amantadine hydrochloride (1-adamantanamine hydrochloride, Symmetrel) was the first adamantane derivative introduced in medicine as effective therapy [12-14] against Asian A influenza virus. Among various substituents a growing interest in adamantyl derivatives is gaining prominence because of well known drugs like Rimantadine, Memantine, Adapalene, Adatanserin and others in clinical trials [15,16]. Since last few decades, thiazolidinones remained always point of considerable interest in the chemical synthesis due to diverse biological utility. Thiazolidin-4-

one ring system is a core structure in various pharmaceutical agents displaying a broad spectrum of biological activity profile [17-19]. Thiazolidinones explored diverse biological activities such as antimicrobial [20], anti-inflammatory [21], analgesic [22,23], antibacterial as well as antifungal [24-28], anticancer [29], anti-HIV [30]. Thiazolidin-4-ones also found in nature; thus actithiazic acid [(-)2-(5-carboxypentyl)thiazolidin-4-one] isolated from the strains of *Streptopmyces* displayed highly specific invitro activity against *Mycobacterium tuberculosis* [31,32]. On the other hand, piperazines and substituted piperazines are important pharmacophores that can be found in many marketed drugs, such as the Merck HIV protease inhibitor Crixivan [33-35] and drugs under development [36-38]. Piperazinyl linked ciprofloxacin dimers reported as potent antibacterial agents against resistant strains [39] and also explored several other biologically activity viz. insecticidal [40], anti-inflammatory [41], antibacterial [42] and antigungal [43]. It is found that bacterial infections generally result into pain and inflammation. In normal practice, two groups of drugs (chemotherapeutic, analgesic and anti-inflammatory) are prescribed simultaneously. Unfortunately, currently none of these drugs possess these three activities in a single compound. A large amount of research work and activities has been undertaken to synthesize new derivatives of quinazoline-4(3*H*)-ones having different biological activities. In continual of our research work [20,44], it is interesting to synthesize and characterize novel quinazoline-4(3*H*)-one derivatives.

MATERIALS AND METHODS

All the chemicals used for the preparation of desired derivatives, were obtained from Sisco Research Laboratories (SRL), Mumbai, India; Qualigen Fine Chemicals, Mumbai, India; E. Merck Ltd., New Delhi, India. Reference drugs ampicillin trihydrate and fluconazole were procured from Ind-Swift Pharmaceutical, Panjab, India and Macleods Pharmaceutical, Mumbai, India respectively. The melting points of the compounds were determined in open glass capillaries with the help of thermonic melting points apparatus (Campbell Electronics, Mumbai, India) and are uncorrected. The homogeneity of all the newly synthesized compounds were routinely checked by TLC on silica gel G plates and spots were located by using iodine chamber. Elemental analysis was performed in Heraeus CHN rapid analyser. The results were found within the ±0.4% of theoretical values. Infrared spectra were recorded on KBr pellets on a Perkin Elmer system 2000 FTIR spectrometer and ^{1}H- NMR spectra on Bruker DPX 200 using TMS as internal standard.

2-(Piperazinyl)methyl benzo[d][1,3]oxazin-4-one 3.

A mixture of compound 2 (0.5 mmol), piperazine (0.7 mmol) and anhydrous sodium carbonate (1.50 g) in absolute ethanol was refluxed for 12 h. The excess of amine and ethanol was removed by distillation and the residue was treated with 5 % sodium bicarbonate solution to remove acidic impurities, filtered, washed with water properly and dried. It was crystallized from ethanol (95%) to give white crystals. Yield: 84%; mp 179-181^{0}C; R_f = 0.71(cycohexane: ethyl acetate); IR (KBr, cm^{-1}): 1701 (C=O), 1610 (C…C of aromatic ring), 1570 (C=N), 1302 (C-N), 1162(C-O-C); ^{1}H NMR ($CDCl_3$) δ in ppm: 7.52-6.51(m, 4H, ArH), 2.90 (s, 4H, 2XCH_2), 2.67(s, 4H, 2X CH_2), 2.51(s, 2H,-CH_2), 1.90(s, 1H, NH). MS: $[M]^+$ at m/z 245.28. Anal.: Calcd for $C_{13}H_{15}N_3O_2$: C, 63.66; H, 6.16; N, 17.13%. Found: C, 63.72; H, 6.25; N, 17.11%.

2-[4-{(Tert-butylcarbamte)cyclohexyl}piperazinyl]methyl benzo[d][1,3]oxazin-4-one 4.
Compound 3 (0.10 mmol), N-Boc-4-aminocyclohexanone (0.25 mmol), sodium triacetoxy borohydride (0.40 mmol), acetic acid (2 drops), and anhydrous actonitrile (50 ml) were mixed together and stirred under nitrogen atmosphere until no free amine was detected by TLC (to get light yellow slurry). The reaction mixture was diluted with ethyl acetate (100 ml), washed with saturate solution of ammonium chloride, 1 N sodium hydroxide solution, water and dried over sodium sulphate. Excess of solvent was distilled off. The residue taken in ice-water, washed, dried and recrystllised with ethanol to afford compound **4**. Yield: 71%; m.p. 112-114^{0}C; R_f = 0.68(cycohexane: ethyl acetate); IR (KBr, cm^{-1}): 1705 (C=O), 1613 (C…C of aromatic ring), 1568 (C=N), 1305 (C-N), 1168(C-O-C); ^{1}H NMR ($CDCl_3$) δ in ppm : 8.10(s, 1H,NH-Boc), 7.54-6.60(m,4H,ArH), 3.56(m,1H,cyclohexane ring), 2.96 (s,4H,2XCH_2), 2.70(s,4H,2XCH_2), 2.55(s, 2H,-CH_2), 2.38(m,1H,cyclohexane ring), 1.66(s,9H,NH-Boc), 1.55-1.10(m,8H, cyclohexane ring). MS: $[M]^+$ at m/z 442.55. Anal.: Calcd for $C_{24}H_{34}N_4O_4$: C, 65.14; H, 7.74; N, 12.66%. Found: C, 65.12; H, 7.75; N, 12.51%.

2-[4-(Cyclohexylamine)piperazinyl]methyl benzo[d][1,3]oxazin-4-one 5.
Compound 4 (0.05 mmol) was taken in acetonitrile (60 ml) and stirred the reaction mixture in presence of tetrafluro acetic acid (0.5 ml) at room temperature for 1-2 hr. The resulting mixture treated with 1 N NaOH to adjust the pH to ~12. Solvent was distilled out. The obtained crude was diluted over crushed ice-water, washed, dried and recrystallised with methanol to obtain compound **5**. Yield 68%; m.p. 155-158^{0}C; R_f = 0.75 (dichloro methane: methanol); IR (KBr, cm^{-1}): 3370 (NH_2), 1710 (C=O), 1616 (C…C of aromatic ring), 1562 (C=N), 1309 (C-N), 1172 (C-O-C); ^{1}H NMR ($CDCl_3$) δ in ppm: 7.63-6.72(m, 4H, ArH), 4.95(s, 2H, NH_2), 3.50(m, 1H, cyclohexane ring), 2.94(s, 4H, 2XCH_2), 2.67 (s, 4H, 2XCH_2), 2.51(s, 2H,-CH_2), 2.32(m, 1H, cyclohexane ring), 1.58-1.11(m, 8H, cyclohexane ring). MS: $[M]^+$ at m/z 342.44. Anal.: Calcd for $C_{19}H_{26}N_4O_2$: C, 66.64; H, 7.65; N, 16.36%. Found: C, 66.62; H, 7.65; N, 16.41%.

3-(Admantan-1-yl)-2-[{4-(4-aminocyclohexyl)piperazin-1-yl}methyl]quinazolin-4(3H)one 6.
To a solution of compound **5** (0.02 mmol), amantadine (0.2 mol) was added, the mixture was heated on a free flame for 10-20 minutes in a conical flask. After the disappearance of water droplets in a conical flask it was kept at room temperature. On cooling a jelly like mass obtained which was dissolved in ethanol, refluxed and poured into ice-water. The solid thus obtained was filtered, dried and finally recrystallized from DMF-water to obtain compound **6**. Yield 66%; m.p. 213-216^{0}C; R_f = 0.61(cycohexane: ethyl acetate); IR (KBr, cm^{-1}): 3362 (NH_2), 1703 (C=O), 1611 (C…C of aromatic ring), 1556 (C=N), 1310 (C-N), 1174(C-O-C); ^{1}H NMR ($CDCl_3$) δ in ppm: 7.68-6.70(m, 4H, ArH), 5.00(s, 2H, NH_2), 3.52(m,1H,cyclohexane ring), 2.94(s,4H, 2X CH_2), 2.70 (s,4H, 2XCH_2),2.49(s, 2H,-CH_2),2.25(m, 1H, cyclohexane ring), 1.80 (m, 15H, amantadinyl ring), 1.50-1.05(m, 8H,cyclohexane ring). MS: $[M]^+$ at m/z 475.67. Anal.: Calcd for $C_{29}H_{41}N_5O$: C, 73.23; H, 8.69; N, 14.72%. Found: C, 73.32; H, 8.62; N, 14.60%.

General procedure for the synthesis of 3-[(admantan-1-yl)-2-{4-(4-arylidenamino)cyclohexyl)piperazin-1-yl}methyl]quinazolin-4(3H)-one 7(a-h). An ethanolic mixture of compound **6** (0.01 mol) and substituted benzaldehydes (0.01 mol) in the presence of few drops of glacial acid was refluxed for 6-9 hr. and poured onto crushed ice and resultant solid was recrystallized from suitable solvents to yield compounds **7a-7h**.

3-[(Admantan-1-yl)-2-{4-(4-benzylidenamino)cyclohexyl)piperazin-1-yl}methyl]quinazolin-4-(3H)-one 7a. Yield 67%; m.p. 230-232^{0}C; R_f = 0.64 (cycohexane: ethyl acetate); IR (KBr, cm^{-1}): 1704 (C=O), 1607 (C…C of aromatic ring), 1552 (C=N), 1312 (C-N); ^{1}H NMR ($CDCl_3$) δ in ppm: 7.90-6.63(m, 9H, ArH), 5.81(s,1H,CH-Ar), 3.55(m,1H,cyclohexane ring), 2.91(s, 4H, 2X CH_2), 2.67(s,4H, 2XCH_2), 2.46(s,2H,-CH_2),2.20(m,1H,cyclohexane ring), 1.78(m,15H, amantadinyl ring), 1.51-1.09(m, 8H,cyclohexane ring). MS: $[M]^+$ at m/z 563.78. Anal.: Calcd for $C_{36}H_{45}N_5O$: C, 76.69; H, 8.05; N, 12.42%. Found: C, 76.70; H, 8.10; N, 12.55%.

3-[(Admantan-1-yl)-2-{4-(4-(2-chloro)benzylidenamino)cyclohexyl)piperazin-1-yl}methyl] quinazolin-4(3H)-one 7b. Yield 56%; m.p. 199-202^{0}C; R_f = 0.67 (cycohexane: ethyl acetate); IR (KBr, cm^{-1}): 1709 (C=O), 1605 (C…C of aromatic ring), 1547 (C=N), 1309(C-N), 670(C-Cl); ^{1}H NMR ($CDCl_3$) δ in ppm: 7.96-6.70(m,8H, ArH), 5.73(s,1H,CH-Ar), 3.50(m,1H,cyclohexane ring), 2.88(s,4H,2XCH_2), 2.67 (s,4H, 2XCH_2), 2.49(s, 2H,-CH_2), 2.15(m, 1H, cyclohexane ring), 1.80 (m,15H,amantadinyl ring), 1.50-1.10(m, 8H,cyclohexane ring). MS: $[M]^+$ at m/z 598.22. Anal.: Calcd for $C_{36}H_{44}N_5OCl$: C, 72.28; H, 7.41; N, 11.71 %. Found: C, 72.30; H, 7.45; N, 11.72%.

3-[(Admantan-1-yl)-2-{4-(4-(3-chloro)benzylidenamino)cyclohexyl)piperazin-1-yl}methyl] quinazolin-4(3H)-one 7c. Yield 50%; m.p. 166-167^{0}C; R_f = 0.64 (cycohexane: ethyl acetate); IR (KBr, cm^{-1}): 1705 (C=O), 1610 (C…C of aromatic ring), 1550 (C=N), 1311 (C-N), 675 (C-Cl); ^{1}H NMR ($CDCl_3$)δ in ppm: 7.88-6.59(m, 8H, ArH), 5.70(s,1H,CH-Ar),3.47 (m,1H, cyclohexane ring), 2.93(s,4H,2XCH_2), 2.70 (s,4H, 2XCH_2), 2.51(s, 2H,-CH_2), 2.20(m,1H, cyclohexane ring), 1.90 (m,15H,amantadinyl ring),1.49-1.12(m, 8H,cyclohexane ring). MS: $[M]^+$ at m/z 598.22. Anal.: Calcd for $C_{36}H_{44}N_5OCl$: C, 72.28; H, 7.41; N, 11.71 %. Found: C, 72.32; H, 7.41; N, 11.70%.

3-[(Admantan-1-yl)-2-{4-(4-(4-chloro)benzylidenamino)cyclohexyl)piperazin-1-yl}methyl] quinazolin-4(3H)-one 7d. Yield 50%; m.p. 220-222^{0}C; R_f = 0.68 (cycohexane: ethyl acetate); IR (KBr, cm^{-1}): 1711 (C=O), 1602 (C…C of aromatic ring), 1555(C=N), 1309(C-N), 672 (C-Cl); ^{1}H NMR ($CDCl_3$) δ in ppm: 7.90-6.70(m, 8H, ArH), 5.65(s,1H,CH-Ar), 3.65(m,1H, cyclohexane ring), 2.98(s,4H,2XCH_2), 2.74 (s,4H, 2XCH_2), 2.45(s, 2H,-CH_2), 2.29(m, 1H, cyclohexane ring), 1.87 (m, 15H, amantadinyl ring), 1.50(m, 8H, cyclohexane ring). MS: $[M]^+$ at m/z 598.22. Anal.: Calcd for $C_{36}H_{44}N_5OCl$: C, 72.28; H, 7.41; N, 11.71 %. Found: C, 72.29; H, 7.44; N, 11.73%.

3-[(Admantan-1-yl)-2-{4-(4-(2-methoxy)benzylidenamino)cyclohexyl)piperazin-1-yl} methyl] quinazolin-4(3H)-one 7e. Yield 60%; m.p. 167-171^{0}C; R_f = 0.70 (cycohexane: ethyl acetate); IR (KBr, cm^{-1}): 1708 (C=O), 1603 (C…C of aromatic ring), 1547 (C=N), 1315 (C-N), 1165(C-O-C); ^{1}H NMR ($CDCl_3$) δ in ppm: 7.87-6.59(m, 8H, ArH), 5.69(s,1H,CH-Ar), 3.89(s, 3H,OCH_3), 3.54(m,1H,cyclohexane ring), 2.81(s,4H,2XCH_2), 2.72 (s,4H, 2XCH_2), 2.46(s, 2H,-CH_2), 2.16(m, 1H, cyclohexane ring), 1.89 (m, 15H, amantadinyl ring),1.53 (m, 8H,cyclohexane ring). MS: $[M]^+$ at m/z 593.80. Anal.: Calcd for $C_{37}H_{47}N_5O_2$: C, 74.84; H, 7.98; N, 11.79 %. Found: C, 74.83; H, 7.90; N, 11.80%.

3-[(Admantan-1-yl)-2-{4-(4-(3-methoxy)benzylidenamino)cyclohexyl)piperazin-1-yl} methyl] quinazolin-4(3H)-one 7f. Yield: 56%; m.p. 145-147^{0}C; R_f = 0.60 (cycohexane: ethyl acetate);IR (KBr, cm^{-1}):1706(C=O), 1605(C…C of aromatic ring), 1545(C=N), 1318(C-N), 1162 (C-O-C); ^{1}H NMR ($CDCl_3$)δ in ppm: 7.95-6.77(m, 8H, ArH), 5.73(s,1H,CH-Ar), 3.83(s,3H, OCH_3), 3.50(m,1H,cyclohexane ring), 3.00(s,4H,2XCH_2), 2.80(s,4H, 2XCH_2), 2.49(s, 2H,-CH_2), 2.22(m,1H,cyclohexane ring), 1.76(m,15H,amantadinyl ring),1.45(m,8H, cyclohexane ring). MS: $[M]^+$ at m/z 593.80. Anal.: Calcd for $C_{37}H_{47}N_5O_2$: C, 74.84; H, 7.98; N, 11.79 %. Found: C, 74.86; H, 7.93; N, 11.88%.

3-[(Admantan-1-yl)-2-{4-(4-(4-methoxy)benzylidenamino)cyclohexyl)piperazin-1-yl}-methyl] quinazolin-4(3H)-one 7g. Yield 61%; m.p. 183-186^{0}C; R_f = 0.66 (cycohexane: ethyl acetate); IR (KBr, cm^{-1}): 1710 (C=O), 1601 (C…C of aromatic ring), 1549 (C=N), 1316 (C-N), 1166 (C-O-C); ^{1}H NMR ($CDCl_3$) δ in ppm: 7.89-6.72(m, 8H, ArH), 5.71(s,1H,CH-Ar), 3.80 (s, 3H, OCH_3), 3.53(m,1H, cyclohexane ring), 2.96(s, 4H,2XCH_2), 2.76(s,4H, 2XCH_2), 2.43(s, 2H,-CH_2),2.20(m,1H,cyclohexane ring),1.79(m, 15H, amantadinyl ring),1.44(m,8H, cyclohexane ring). MS: $[M]^+$ at m/z 593.80. Anal.: Calcd. for $C_{37}H_{47}N_5O_2$: C, 74.84; H, 7.98; N, 11.79 %. Found: C, 74.85; H, 8.00; N, 11.85%.

3-[(Admantan-1-yl)-2-{4-(4-(2-hydroxy)benzylidenamino)cyclohexyl)piperazin-1-yl}-methyl]quinazolin-4(3H)-one 7h. Yield 64%; m.p. 119-122^{0}C; R_f = 0.61(cycohexane: ethyl acetate); IR (KBr, cm^{-1}): 3420(OH), 1712(C=O), 1607 (C…C of aromatic ring), 1549 (C=N), 1312(C-N); ^{1}H NMR ($CDCl_3$) δ in ppm: 10.45(s,1H,OH), 7.82-6.74(m, 8H, ArH), 5.79(s, 1H, CH-Ar),3.50(m, 1H, cyclohexane ring), 2.91(s,4H,2XCH_2), 2.72 (s,4H,2XCH_2), 2.44(s,2H,-CH_2), 2.23(m, 1H,cyclohexane ring),1.81(m, 15H amantadinyl ring), 1.40 (m, 8H,cyclohexane ring). MS: $[M]^+$ at m/z 579.77. Anal.: Calcd for $C_{36}H_{45}N_5O_2$: C, 74.58; H, 7.82; N, 12.08 %. Found: C, 74.60; H, 7.86; N, 12.06%.

General procedure for the synthesis of 3-[(admantan-1-yl)-2-[4-{4-(2-substituted phenyl-4-oxo-1,3-thiazolidinyl)cyclohexyl} piperazin-1-yl]methyl] quinazolin-4(3H)-one 8(a-h).
A solution of compound **7a-7h** (0.001 mmol) and anhydrous zinc chloride in dry benzene (50ml), thioglycolic acid (0.002 mmol) was added dropwise with stirring at ambient temperature and the reaction mixture was kept for 1 day at room temperature and then refluxed for 7-10 h. The reaction mixture was filtered. The filtrate was concentrated poured on crushed ice. The resultant solid was recrystallized from appropriate solvent to yield target compounds **8(a-h)**.

3-[(Admantan-1-yl)-2-[4-{4-(2-phenyl-4-oxo-1,3-thiazolidinyl)cyclohexyl}piperazin-1-yl] methyl] quinazolin-4(3H)-one 8a. Yield 56%; m.p. 180-182^{0}C; R_f = 0.70 (dichloro methane: methanol); IR (KBr, cm^{-1}): 1708 (C=O), 1605 (C…C of aromatic ring), 1550 (C=N), 1312 (C-N),674 (C-S-C); ^{1}H NMR ($CDCl_3$) δ in ppm: 7.88-6.65(m, 9H,ArH),5.60(s,1H,CH-Ar), 4.03 (s, 2H,CH_2-S),3.52 (m,1H,cyclohexane ring), 2.88(s, 4H, 2XCH_2), 2.63 (s,4H, 2XCH_2), 2.44(s, 2H,-CH_2),2.21(m, 1H, cyclohexane ring), 1.75 (m, 15H, amantadinyl ring), 1.52-1.10(m, 8H, cyclohexane ring). MS: $[M]^+$ at m/z 637.88. Anal.: Calcd for $C_{38}H_{47}N_5SO_2$: C, 71.55; H, 7.43; N, 10.98%. Found: C, 71.42; H, 7.44; N, 10.86%.

3-[(Admantan-1-yl)-2-[4-{4-(2-chlorophenyl-4-oxo-1,3-thiazolidinyl)cyclohexyl}piperazin-1-yl]methyl] quinazolin-4(3H)-one 8b. Yield 43%; m.p. 115-117^{0}C; R_f = 0.74 (dichloro methane :

methanol); IR (KBr, cm^{-1}): 1711 (C=O), 1608 (C…C of aromatic ring), 1550 (C=N), 1311 (C-N), 675 (C-Cl); ^1H NMR ($CDCl_3$) δ in ppm: 7.91-6.73(m, 8H, ArH), 5.56(s,1H,CH-Ar), 4.05(s,2H,CH_2-S), 3.44(m,1H, cyclohexane ring), 2.91(s,4H, 2XCH_2), 2.64 (s,4H, 2XCH_2), 2.42(s, 2H,-CH_2), 2.17(m, 1H, cyclohexane ring), 1.81 (m, 15H, amantadinyl ring), 1.45-1.08(m, 8H,cyclohexane ring). MS: $[M]^+$ at m/z 672.32. Anal.: Calcd for $C_{38}H_{46}N_5SO_2Cl$: C, 67.89; H, 6.90; N, 10.42 %. Found: C, 67.83; H, 7.00; N, 10.41%.

3-[(Admantan-1-yl)-2-[4-{4-(3-chlorophenyl-4-oxo-1,3-thiazolidinyl)cyclohexyl}piperazin-1-yl]methyl] quinazolin-4(3H)-one 8c. Yield 40%; m.p. 140-142^{0}C; R_f = 0.69 (dichloro methane: methanol); IR (KBr, cm^{-1}):1707 (C=O), 1608 (C…C of aromatic ring), 1542 (C=N), 1311 (C-N), 673 (C-Cl); ^1H NMR ($CDCl_3$) δ in ppm: 7.95-6.77(m, 8H, ArH), 5.50(s,1H,CH-Ar),3.95 (s,2H,CH_2-S), 3.40(m,1H, cyclohexane ring), 2.89(s, 4H, 2XCH_2), 2.66 (s,4H, 2XCH_2), 2.41(s, 2H,-CH_2), 2.15(m, 1H, cyclohexane ring),1.84 (m, 15H, amantadinyl ring),1.42 (m, 8H, cyclohexane ring). MS: $[M]^+$ at m/z 672.32. Anal.: Calcd for $C_{38}H_{46}N_5SO_2Cl$: C, 67.89; H, 6.90; N, 10.42 %. Found: C, 67.85; H, 7.02; N, 10.44%.

3-[(Admantan-1-yl)-2-[4-{4-(4-chlorophenyl-4-oxo-1,3-thiazolidinyl)cyclohexyl}piperazin-1-yl]methyl] quinazolin-4(3H)-one 8d. Yield 44%; m.p.129-131^{0}C; R_f = 0.76 (dichloro methane: methanol); IR (KBr, cm^{-1}):1712 (C=O), 1611 (C…C of aromatic ring), 1545 (C=N), 1308 (C-N), 716(C-S-C), 670 (C-Cl); ^1H NMR ($CDCl_3$) δ in ppm : 7.99-6.80(m, 8H, ArH), 5.52(s,1H,CH-Ar), 4.00(s,2H,CH_2-S), 3.42(m,1H, cyclohexane ring), 2.93(s,4H, 2XCH_2), 2.61(s,4H,2X CH_2), 2.44(s, 2H,-CH_2), 2.12(m, 1H,cyclohexane ring), 1.79(m,15H,amantadinyl ring), 1.45(m,8H, cyclohexane ring). MS: $[M]^+$ at m/z 672.32. Anal.: Calcd for $C_{38}H_{46}N_5SO_2Cl$: C, 67.89; H, 6.90; N, 10.42 %. Found: C, 67.83; H, 6.93; N, 10.40%.

3-[(Admantan-1-yl)-2-[4-{4-(2-methoxyphenyl-4-oxo-1,3-thiazolidinyl)cyclohexyl}piperazin -1-yl]methyl] quinazolin-4(3H)-one 8e. Yield 50%; m.p. 190-192^{0}C; R_f = 0.70 (dichloro methane: methanol); IR (KBr, cm^{-1}): 1710 (C=O), 1605 (C…C of aromatic ring), 1548 (C=N), 1310 (C-N), 1165(C-O-C), 711(C-S-C); ^1H NMR ($CDCl_3$)δ in ppm: 7.88-6.62(m, 8H, ArH), 5.55(s,1H,CH-Ar), 4.15 (s, 2H, CH_2-S), 3.70 (s, 3H, OCH_3), 3.30 (m,1H,cyclohexane ring), 2.88 (s,4H, 2XCH_2), 2.41 (s,4H, 2XCH_2), 2.18(s, 2H,-CH_2), 1.96 (m,1H,cyclohexane ring), 1.58 (m, 15H, amantadinyl ring), 1.30 (m, 8H, cyclohexane ring). MS: $[M]^+$ at m/z 667.90. Anal.: Calcd for $C_{39}H_{49}N_5SO_3$: C, 70.13; H, 7.39; N, 10.49 %. Found: C, 70.23; H, 7.30; N, 10.45%.

3-[(Admantan-1-yl)-2-[4-{4-(3-methoxyphenyl-4-oxo-1,3-thiazolidinyl)cyclohexyl} piperazin -1-yl]methyl] quinazolin-4(3H)-one 8f. Yield 48%; m.p.168-170^{0}C; R_f = 0.68 (dichloro methane: methanol); IR (KBr, cm^{-1}): 1713 (C=O), 1608 (C…C of aromatic ring), 1545 (C=N), 1313 (C-N), 1162(C-O-C), 715(C-S-C); ^1H NMR ($CDCl_3$) δ in ppm :7.90-6.62(m, 8H, ArH), 5.60(s,1H,CH-Ar), 4.12 (s, 2H, CH_2-S), 3.78(s,3H,OCH_3), 3.28 (m,1H,cyclohexane ring), 2.90(s,4H, 2XCH_2), 2.40 (s,4H, 2XCH_2), 2.20(s, 2H,-CH_2),1.90(m, 1H, cyclohexane ring), 1.51 (m, 15H, amantadinyl ring), 1.32 (m, 8H, cyclohexane ring). MS: $[M]^+$ at m/z 667.90. Anal.: Calcd for $C_{39}H_{49}N_5SO_3$: C, 70.13; H, 7.39; N, 10.49 %. Found: C, 70.20; H, 7.33; N, 10.42%.

3-[(Admantan-1-yl)-2-[4-{4-(4-methoxyphenyl-4-oxo-1,3-thiazolidinyl)cyclohexyl} piperazin -1-yl]methyl] quinazolin-4(3H)-one 8g. Yield 55%; m.p. 219-221^{0}C; R_f = 0.71 (dichloro methane: methanol); IR (KBr, cm^{-1}): 1709 (C=O), 1607 (C…C of aromatic ring), 1551

(C=N), 1311(C-N), 1165(C-O-C), 714(C-S-C); ^{1}H NMR ($CDCl_3$) δ in ppm: 7.80-6.74(m,8H, ArH), 5.34(s,1H,CH-Ar), 4.30(s, 2H, CH_2-S), 3.81 (s,3H,OCH_3), 3.34 (m, 1H,cyclohexane ring), 2.82(s,4H, 2XCH_2), 2.40 (s,4H, 2XCH_2), 2.10(s, 2H,-CH_2), 1.81 (m, 1H, cyclohexane ring), 1.40 (m,15H, amantadinyl ring), 1.12(m, 8H, cyclohexane ring). MS: $[M]^+$ at m/z 667.90. Anal.: Calcd for $C_{39}H_{49}N_5SO_3$: C, 70.13; H, 7.39; N, 10.49 %. Found: C, 70.14; H, 7.40; N, 10.50%.

3-[(Admantan-1-yl)-2-[4-{4-(4-hydroxyphenyl-4-oxo-1,3-thiazolidinyl)cyclohexyl} piperazin -1-yl]methyl] quinazolin-4(3H)-one 8h. Yield 45%; m.p. 201-203^{0}C; R_f = 0.78 (dichloro methane: methanol); IR (KBr, cm^{-1}): 3424 (OH), 1713 (C=O), 1609 (C…C of aromatic ring), 1552 (C=N), 1308 (C-N); ^{1}H NMR ($CDCl_3$) δ in ppm: 10.60(s,1H,OH), 7.85-6.70(m, 8H, ArH), 5.49(s,1H,CH-Ar), 4.21 (s, 2H, CH_2-S), 3.30 (m, 1H, cyclohexane ring), 2.88(s,4H, 2XCH_2), 2.38(s,4H, 2XCH_2), 2.12(s, 2H,-CH_2), 1.87 (m,1H,cyclohexane ring), 1.45 (m, 15H, amantadinyl ring), 1.23(m, 8H,cyclohexane ring). MS: $[M]^+$ at m/z 653.88. Anal.: Calcd for $C_{38}H_{47}N_5SO_3$: C, 69.80; H, 7.24; N, 10.71 %. Found: C, 70.00; H, 7.20; N, 10.75%

PHARMACOLOGY

Antimicrobial activity

All the newly synthesized compounds were screened for their antibacterial and antifungal activity. Microorganisms employed antibacterial studies were *Staphylococcus aureus, Escherichia coli, Klabsiella pneumoniae* and *Proteus vulgaris*. Disk diffusion method [45,46] was used for determination of the preliminary antibacterial activity. Disks measuring 6.25 mm in diameter were punched from Whatman no. 1 filter paper. Batches of 100 disks were dispensed to each screw-capped bottle and sterilized by dry heat at 140 ^{0}C for an hour. The test compounds were prepared with different concentrations using DMF. One milliliter containing 100 times the amount of chemical in each disk was added to each bottle, which contained 100 disks. Disks of each concentration were for placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37 ^{0}C for 24 h. Ampicillin trihydrate was used as a standard drug. Solvent and growth controls were kept and zones of inhibition were noted. The bacterial inhibition values of the tested compounds against the tested bacteria strains are recorded in mm (Table **I)**. On the other hand, the newly prepared compounds were screened for their in vitro antifungal activity against *Aspergillus fumigatus* (plant isolate), *Candida glabrata, Candida albacans* and *Candida krusei* in DMSO by the serial plate dilution method [47,48]. All the fungal strains were clinical isolates, identified with conventional morphological and biochemical methods. Fluconazole (antifungal) was used as reference drug. Sabouraud's agar media were prepared by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in distilled water (100 ml) and adjusting the pH to 5.7. Normal saline was used to make a suspension of the spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of the corresponding species. Agar media (20 ml) was poured into each petri dish. Excess suspension was decanted and the plates were dried by placing in an incubator at 37 ^{0}C for 1 h. Using an agar punch wells were made into each well labeled. A control was also prepared in triplicate and maintained at 37 ^{0}C for 3–4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. The fungal inhibition values of the tested compounds against the tested fungal strains are recorded in mm (Table **I)**.

Anti-inflammatory activity

Compounds **8a-h** were tested for their anti-inflammatory and analgesic activities as well as for their ulcerogenicity and acute toxicity. The experiment was performed with albino rats of Charlese Foster strain of either sex, excluding pregnant females, of 60-90 days weighing 100-120 g. Food (chaw pallet) and water was given to the animals *ad libitum*. The test compounds were dissolved in propylene glycol. Indomethacin and phenylbutazone were used as reference drugs for the comparison of anti-inflammatory, analgesic and ulcerogenic activities.

Anti-inflammatory activity against carrageenan induced rat's paw oedema: this study was done following the procedure of Winter [49]. The rats were divided into three groups (control, drug treated, and standard, drug of six animals each).A freshly prepared suspension of carrageenan (1% in 0.9% saline). 0.05 ml was injected under the planter aponeurosis of the right hind paw of each rat. Test compounds and standard drug were administered orally to the animals of drug treated groups and the standard drug group, respectively, 1 h before the carrageenan injection. The paw volume of each rat was measured before 1 and after 3 h of carrageenan treatment with the help of a plethymometer. The percent anti-inflammatory activity was calculated according to the formula given below:

$$\text{Percentage of inhibition of oedema} = (1- V_t - V_c) \text{ X } 100$$

where, V_t and V_c are the mean increase in paw volume of rats of the treated and the control group, respectively. Considering, the potentiality of compounds 5h, this one was studied in detail at three graded doses 25, 50, 100 mg/kg p.o. Results obtained were statistically analyzed (Table **II**).

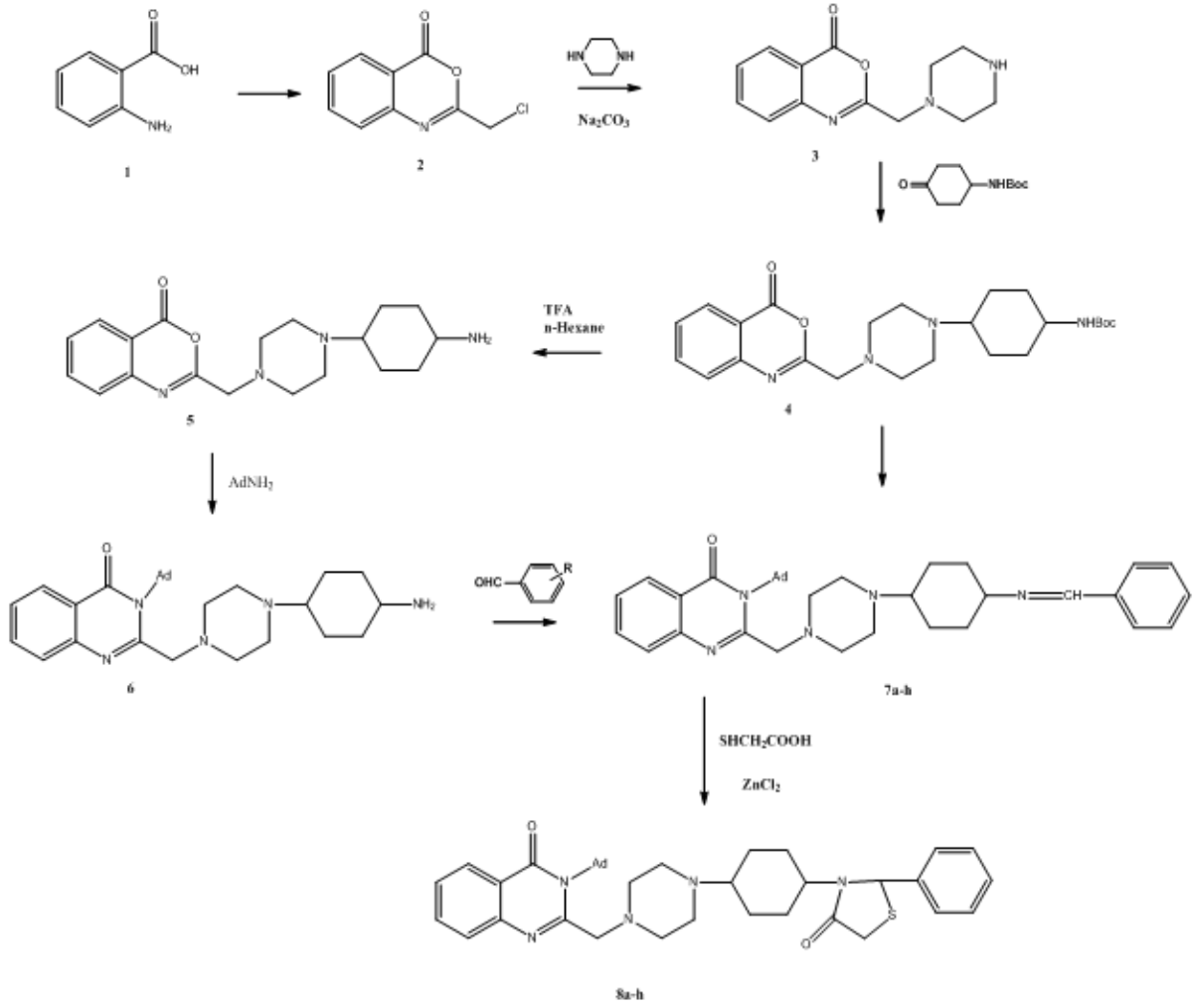
Analgesic activity

Analgesic activity was performed following the method of Berkowitz [50]. This method is based on the property of the test compound to antagonize the phenylquinone-induced pain syndrome in mice. Groups of five mice were injected intraperitonealy with 0.25 ml of a 0.02% solution of phenylquinone in ethanol (5%) 1 h after oral administration of the test compound. The number of writhes induced in each mouse was counted for 5 min (between 5 and 10 min) after injection of an irritant. The analgesic effect was expressed as percent protection in comparison to control (Table **II**).

% protection= (1- mean no. of writhes in mice of test groups/ mean number of writhes in mice of control group) X 100

Ulcerogenic activity

Ulcerogenic labilities of newly synthesized compounds were checked with method of Verma [51]. Albino rats were fasted for 24 h prior to drug administration. All animals were sacrificed 8 h after drug treatment, and their stomachs and small intestines were microscopically examined to assess the incidence of hyperemia, shedding of epithelium, petechial and frank hemorrhages and erosion or discrete ulceration with or without perforation. The presence of any one of these criteria was considered to be an evidence of ulcerogenic activity (Table **II**).



Scheme 1

Acute Toxicity activity

The test compounds were investigated for their acute toxicity (ALD_{50}) in albino mice, according to the method of Smith [52]. The test compounds were given orally at different dose levels in separate groups of animals. After 24 h of drug administration, percent mortality in each group was observed. ALD_{50} was calculated from the data obtained (Table **II**).

Cox-1 & Cox-2 activities

The compounds prepared were tested for cyclooxygenase-1 and cyclooxygenase-2 inhibitory activities. The method of Copeland [53] was followed to determine the IC_{50} values. The enzyme activity is measured using chromogenic assay based on oxidation of N, N, N, N`-tetramethyl-p-phenylenediamine (TMPD) during the reduction of prostaglandin G_2 to prostaglandin H2 by COX-1 and COX-2 enzymes. COX-1 and COX-2 enzymes used in the assay were purified from microsomal fraction. The compounds were dissolved in DMSO and stock solution was diluted to required assay concentration. The assay mixture consists of Tris-HCl. Tris-HCl buffer (pH 8.0, 100 µM), hematin (15 µM), EDTA (3 µm), enzyme (COX-1 or COX-2, 100 µM) and test compound. The mixture was pre-incubated at 25^0C for 15 min and then the reaction was initiated

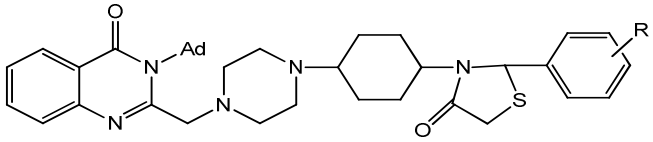
by the addition of arachidonic acid (100 µM) and TMPD (120 µM) in total volume of 1.0 ml. The enzyme activity was measured by estimating the initial velocity of TMPD oxidation for the first 25 s of the reaction following the increase in absorbance at 603 nm. IC50 values are calculated from four parameter least squares non-linear regression analysis of the log dose vs percentage inhibition plot. However, none of the compound studied here exhibited significant inhibitory activity when compared to standard inhibitors indomethacin (for COX-1) and celecoxib (for COX-2) (Table **II**).

RESULTS AND DISCUSION

Chemistry

Anthranilic acid **1**, was stirred with chloroacetyl chloride in presence of triethylamine (TEA) in n-hexane at room temperature to obtain reported 2-chloromethyl benzo[*d*][1,3]oxazin-4-one **2** [54,55]. Refluxing of compound 2 with piperazine in presence of Na_2CO_3 in absolute ethanol yielded compound **3** i.e. 2-(Piperazinyl)methyl benzo[d][1,3]oxazin-4-one, which further treated with N-Boc-4-aminocyclohexanone in anhydrous acetonitrile under inert atmosphere to afford compound 2-[4-{(Tert-butylcarbamte)cyclohexyl}piperazinyl]methyl benzo[d][1,3]oxazin-4-one **4**. Reductive alkylation of compound **3** with 4-*N*-Boc-aminocyclohexanone and sodium tri-acetoxyborohydride was carried out. 2-[4-(Cyclohexylamine)piperazinyl]methyl benzo[d] [1,3] oxazin-4-one **5** was obtained by boc deprotection of compound **4** by the use of TFA.

Table I. *In vitro* antibacterial and antifungal activity for the synthesized compounds 8(a-h)



8(a-h)

Antibacterial inhibition (mm)						Antifungal inhibition (mm)			
Comp. no.	**R**	***S. aureus***	***E. coli***	***K. pneumoniae***	***P. vulgaris***	***A. fumigatus***	***C. glabrata***	***C. albacans***	***C. krusei***
8a	C_6H_5	-	5	8	8	5	-	-	-
8b	2-Cl.C_6H_4	16	25	22	25	12	9	-	10
8c	3-Cl.C_6H_4	10	10	15	-	-	6	-	8
8d	4-Cl.C_6H_4	12	15	10	8	5	4	-	-
8e	2-OCH_3. C_6H_4	8	10	14	-	10	-	-	5
8f	3-OCH_3. C_6H_4	8	6	5	-	8	-	5	-
8g	4-OCH_3. C_6H_4	15	12	12	5	-	5	8	-
8h	2-OH. C_6H_4	10	14	10	-	9	-	-	8
Ampicillin trihydrate (std.)	16		20	20	20	-	-	-	-
Fluconazole (std.)	-		-	-	-	20	15	16	15
DMF(control)	-		-	-	-	-	-	-	-

- means no inhibition.

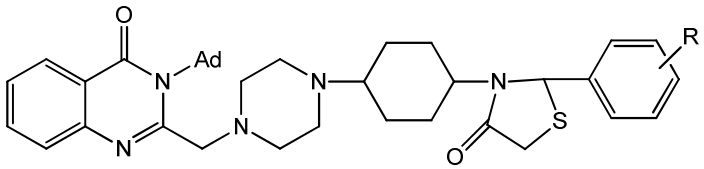
Reaction of compound 5 with amantadine yielded 3-(admantan-1-yl)-2-[{4-(4-aminocyclohexyl) piperazin-1-yl}methyl] quinazolin-4(3H)-one **6** which on reaction with aromatic aldehydes furnished 3-(admantan-1-yl)-2-[{4-(4-arylidenamino)cyclohexyl) piperazin-1-yl}methyl]quinazolin-4(3H)-one **7a-h**. Cyclisation of compound **7a-h** by means of thioglycolic

acid resulted into 3-[(Admantan-1-yl)-2-[4-{4-(2-substituted phenyl-4-oxo-1,3-thiazolidinyl)cyclohexyl} piperazin-1-yl]methyl] quinazolin-4(3H)-one **8a-h**.

Biology

The entire synthesized compounds were evaluated for antifungal and antibacterial activity against the selected panel of pathogens. Antifungal activity was performed by serial plate dilution method and disk diffusion method was adopted for antibacterial activity. Among the compounds **8(a-h)**, compound **8b** was found the most potent candidate and showed better bacterial inhibition but poor fungal inhibition in comparison to reference drugs, while remaining compounds showed milder inhibition (Table **I**).

Table II. Anti-inflammatory, analgesic, ulcerogenic and acute toxicity activity of compounds 8(a-i)



8(a-h)

Compound	R	Anti inflammatory activity		Analgesic activity		UD_{50} (mg/kg/i.p.)	IC_{50} % of inhibition		ALD_{50} (mg/kg/ i.p.)
		Dose (mg/kg /p.o.)	% inhibition of oedema	Dose (mg/kg/p.o.)	% Protection		COX-1	COX-2	
Phenylbutazone		25 50 100	26.76*** 36.80*** 64.68**	25 50 100	14.26*** 32.50*** 54.58***	66.66	-	-	800
Indomethacin		5.0 7.5 10.0	52.20*** 63.10*** 93.20***	5.0 7.5 10.0	41.30*** 59.25*** 64.33***	54.55	-	-	>800
8a	C_6H_5	50	30.55***	50	21.00**	-	37.00***	76.64***	>800
8b	2-Cl. C_6H_4	25 50 100	28.75*** 41.33*** 66.76***	25 50 100	20.50*** 35.24*** 56.93***	132.80	69.51**	92.20***	>800
8c	3-Cl. C_6H_4	50	35.12***	50	35.21***	-	38.68***	65.00***	>800
8d	4-Cl. C_6H_4	50	32.90***	50	21.18**	-	38.93***	66.45***	>800
8e	2-OCH_3. C_6H_4	50	32.07***	50	20.05**	-	39.17**	71.09***	>800
8f	3-OCH_3. C_6H_4	50	33.35***	50	22.23***	-	37.77**	65.82***	>800
8g	4-OCH_3. C_6H_4	50	30.97***	50	19.76**	-	39.29**	63.56***	>800
8h	2-OH. C_6H_4	50	31.02***	50	24.96**	-	30.20***	66.77***	>800

**P< 0.05, **P<0.01, ***P<0.001, - denotes not test*
Propylene glycol standard for control group

Furthermore, all the newly synthesized compounds of this series were tested for anti-inflammatory, analgesic, and ulcerogenic activities, at a dose of 50 mg/kg p.o. The results of anti-inflammatory activity of all the compounds are summarized in Table **II**. Only compound **8b**,

has shown the maximum percentage of anti-inflammatory activity, i.e. 41.33% at a dose of 50 mg/kg p.o. Considering, the potentiality of compounds **8b**, was studied in detail at three graded doses 25, 50, 100 mg/kg p.o. Compound **8b** exhibited better anti-inflammatory activity at all three graded doses of 25, 50 and 100 mg/kg p.o. as compared to phenylbutazone. However, compound **8b** was less active as compared to indomethacin at the three graded doses. The ulcerogenic liabilities of compound **8b** (132.80 mg/kg i.p.) are much less than that of indomethacin (54.55 mg/kg i.p.) and phenylbutazone (66.6 mg/kg i.p.).

All these compounds were also evaluated for COX-1 and COX-2 inhibitory activities. Compound **8b** exhibited 69.51% and 92.20% inhibition of action. Moreover, rest of the compounds showed moderate degree of COX-1 and COX-2 inhibitory actions suggesting that these compounds showed anti-inhibitory activity by inhibition of both COX-1 and COX-2 enzymes. ALD_{50} of all these compounds were >800 mg/kg p.o.

CONCLUSION

It is cleared from the pharmacological evaluation data that among the entire compounds, compound **8b** exhibited much potent biological spectra and 2-chloro phenyl substitution brought remarkable biological activity. It was better than the standards phenylbutazone while less active as compared to indomethacin respectively.

Acknowledgement
We are thankful for SAIF, Punjab University, India for spectral, elemental analysis and L.L.R.M. Medical College, India for biological activities.

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