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ANTI-INFLAMMATORY EVALUATION OF SOME NOVEL SUBSTITUTED-[1,2,4] TRIAZOLO[1,5C]QUINAZOLINONE DERIVATIVES

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Summary

Background: Quinazolinone is a compound made up of two fused six member simple aromatic rings-benezene and pyrimidine ring and have been reported to posses versatile type of biological activities such as anticancer, anticonvulsant, anti-inflammatory, antihelminthic, antimicrobial activities.

Methods: A series of novel substituted-[1,2,4]triazolo[1,5c]quinazolinone derivatives (K11-19) were synthesized by mannich reaction using formamide and different secondary amines. Structures of compounds synthesized were confirmed by FT-IR, ^{1}H-NMR and Mass spectroscopic analysis. All synthesized compounds were screened for anti-inflammatory activity. The anti-inflammatory activity was performed at concentration (100 mg/kg body mass) by rat paw oedema model. Diclofenac sodium (50 mg/kg) was used as standard.

Results: All synthesized compounds have shown anti-inflammatory activity as all has significant reduction in inflammation when compared to inflammatory control group. Compounds **K 15, K 18** and **K 19** have shown very good anti-inflammatory activity comparable to standard drug Diclofenac sodium.

Key words: [1,2,4]triazolo[1,5c]quinazolinone derivatives; anti-inflammatory activity; mannich reaction.

Introduction

Quinazolinone is the major fused six-member heterocyclic ring system and is one of the most encountered heterocyclic in medicinal chemistry and a building block for around 120 naturally occurring alkaloids.

Quinazolinone constitute an important class of medicinally important small molecules which have been reported to possess anticonvulsant [1-3], antimicrobial [4-8], anti-inflammatory [9-11], antitumor [12], anticancer [13], sedative-hypnotic [14], diuretic [15-16], antiviral [17], antihypertensive [18] activities. Several 2, 3-disubstituted Quinazolinone derivatives were synthesized and tested for different biological activities. These reports showed that aryl substitution at 2nd and 3rd position enhances biological activities.

Efforts towards the development and identification of new molecules for anti-inflammatory activities with minimal gastrointestinal ulceration side effects have gained significance in the recent past during which the quinazolinones came into the scenario.

With the revelation of exploring the diverse pharmacological nature of [1,2,4]triazolo[1,5c]quinazolinone derivatives, it was contemplated to synthesize some substituted quinazolinone derivatives by mannich reaction having general structure of figure 1 as potential anti-inflammatory agents.

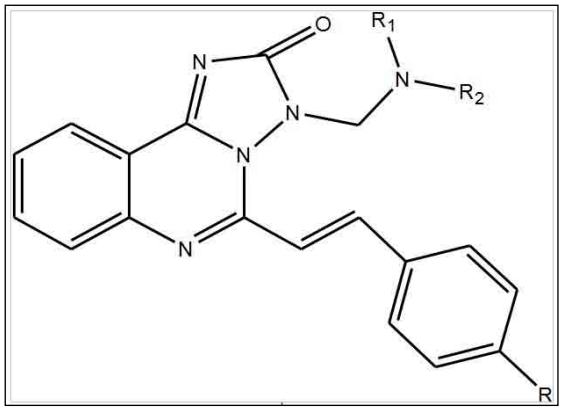

Figure 1

Methods

Experimental

Melting points were recorded in open capillaries with electric melting point apparatus and were uncorrected. IR spectra (KBr disks) were recorded using Shimadzu 8400S FTIR spectrophotometer. ^{1}H-NMR were recorded in Bruker Avance II(400 MHz) spectrophotometer in $CDCl_3$ solution and chemical shift values were reported in ppm relative to TMS(δ = 0) as internal standard. Mass spectra were recorded on a Shimazu LC-MS (2010A) spectrophotometer. TLC was performed on silica gel coated plates for monitorinf the reactions.

Synthesis of substituted [1,2,4]triazolo[1,5c]quinazolinone derivatives (K 11- K 19)

To search novel anti-inflammatory heterocyclic in the condensed quinazolinone series, representative compounds were synthesized via a suitable reaction sequences. 2-methyl-benzoxazin-4-one was prepared by reported methods [19]. Treating it with semicarbazide to produce corresponding (2-methyl-4-oxo-quinazolin-3-yl)-urea; which further heated above its melting to get 5-methyl-[1,2,4]triazolo[1,5c]quinazolin-2-one respectively. To the stirred solution of an equivalent amount of 5-methyl-[1,2,4]triazolo[1,5c]quinazolin-2-one and appropriated benzaldehyde in ethanol was added aqueous NaOH solution(10% w/v, 10ml). The title compounds could be obtained by mannich reaction using formamide and different secondary amines. Table 1 summarizes physical data of above compounds.

see Table 1

5-[2-(4-bromo-phenyl)-vinyl-3-diethylaminomethyl-[1,2,4]triazolo[1,5-c]quinazolin-2-one (K 11)

IR (cm^{-1}): 3383.86 (NH Str), 3041.10 (CH_3 Str), 1383.69 (CO Str), 1942.95 (CH_2 bending), 1593 (NH bending), 500 (Br group).

^{1}H-NMR (δ ppm): 7.9-6.7 (m, 18H Ar-H), 5.4-5.0 (2H, d, =C=CH), 3.4-3.3 (s, 2H, CH_2).

TOF MS *m/z*: 461(M^+), 269, 187, 105, 335.

5-[2-(4-bromo-phenyl)-vinyl-3-diphenylaminomethyl-[1,2,4]triazolo[1,5-c]quinazolin-2-one (K 12)

IR (cm^{-1}): 3459.93 (NH Str), 2359 (CN Str), 1590.43 (CO Str), 1020.09 (CH_2 bending), 1590 (NH bending), 590.04 (Br group).

^{1}H-NMR (δ ppm): 8.6-8.06 (m, 8H Ar-H), 3.3-3.2 (s, 2H, CH_2), 2.56-2.54 (m, 2H, CH_2).

TOF MS *m/z*: 547.3(M^+), 335.1, 168, 170, 475.

5-[2-(4-bromo-phenyl)-vinyl-3-piperazin-1-yl-[1,2,4]triazolo[1,5-c]quinazolin-2-one (K 13)

IR (cm^{-1}): 3454.01 (NH Str), 2340.82 (CN Str), 2959.5 (CH_3 Str), 1347.8 (CO Str), 1412.61 (CH_2 bending), 1593.79 (NH bending).

^{1}H-NMR (δ ppm): 7.9-6.7 (m, 18H Ar-H), 5.4-5.0 (d, 2H, -C=CH_2), 3.4-3.3 (s, 2H, CH_2).

TOF MS *m/z*: 463(M^+), 105, 269, 346, 187.

3-[(diphenylamino)-methyl]-5-[2-(4-nitro-phenyl)-vinyl-[1,2,4]triazolo[1,5-c]quinazolin-2-one (K 14)

IR (cm^{-1}): 3406 (NH Str), 3041.6 (CH_3 Str), 1706 (CN bending), 1347.8 (CO Str), 1465.61 (CH_2 bending), 1596.79 (NH bending), 1343 (NO_2 group).

^{1}H-NMR (δ ppm): 8.2-6.7 (m, 18H Ar-H), 5.6-4.9 (d, 2H, -C=CH_2), 2.64-2.0 (s, 2H, CH_2).

TOF MS *m/z*: 513(M^+), 269, 105, 335, 187.

5-[2-(4-nitro-phenyl)-vinyl-3-piperazin-1-yl-[1,2,4]triazolo[1,5-c]quinazolin-2-one (K 15)

IR (cm^{-1}): 3406 (NH Str), 2959.67 (CH_3 Str), 1254.71 (CN bending), 1347.8 (CO Str), 1254.61 (CH_2 bending), 1494.1 (NH bending), 1412 (NO_2 group).

^{1}H-NMR (δ ppm): 8.29-7.0 (m, 8H Ar-H), 6.9-6.7 (d, 2H, -C=CH_2), 3.3 (s, 2H, CH_2), 2.7-2.1 (m, 4H, -N-C).

TOF MS *m/z*: 430.6(M^+), 220, 170, 336.

3-[(diethylamino)-methyl]-5-[2-(4-nitro-phenyl)-vinyl-[1,2,4]triazolo[1,5-c]quinazolin-2-one (K 16)

IR (cm^{-1}): 3406 (NH Str), 2959.67 (CH_3 Str), 2341.25 (CN Str), 1347.8 (CO Str), 1254.71 (CH_2 bending), 1484.1 (NH bending), 1314(NO_2 group).

^{1}H-NMR (δ ppm): 8.14-7.56 (m, 8H, Ar-H), 5.6 (d, 2H, -C=CH_2), 2.40 (s, 2H, CH_2).

TOF MS *m/z*: 419(M^+), 105, 269, 187.

5-[2-(2,4-dihydroxy-phenyl)-vinyl-3-piperazin-1-yl-[1,2,4]triazolo[1,5-c]quinazolin-2-one (K 17)

IR (cm^{-1}): (3789.47 (OH Str), 2935.51 (CH_3 Str), 2341.71 (CN Str), 1097.59 (CO Str), 1412.44 (OH bending).

^{1}H-NMR (δ ppm): 8.0 (m, 7H Ar-H), 3.3 (s, 2H, CH_2), 2.5-2.1 (m, 4H, -N-C).

TOF MS *m/z*: 346(M^+), 269, 187.

3-[(diethylamino)-methyl]-5-styryl-[1,2,4]triazolo[1,5-c]quinazolin-2-one (K 18)

IR (cm^{-1}): 2935.51 (CH_3 Str), 2341.71 (CN Str), 1097.59 (CO Str).

^{1}H-NMR (δ ppm): 8.6-8.0 (s, 1H, NH), 7.9-7.0 (m, 9H, Ar-H), 3.6(2H, d, -C=CH_2).

TOF MS *m/z*: 372(M^+), 170, 182, 187.

5-[2-(4-hydroxy-phenyl)-vinyl]-3-piperazin-1-ylmethyl-[1,2,4]triazolo[1,5-c]quinazolin-2-one (K 19)

IR (cm^{-1}): (3776.09 (OH Str), 3423.88 (NH Str), 2935.51 (CH_3 Str), 2365.30 (CN Str), 1412.69 (OH bending).

^{1}H-NMR (δ ppm): 8.6-7.2 (m, 8H Ar-H), 6.9-6.8 (d, 2H, -C=CH_2), 2.7-2.3 (m, 4H,NC), 2.38-2.0 (s, 1H, NH), 2.0-1.9 (s, 2H, CH_2).

TOF MS *m/z*: 401(M^+), 281, 161, 229, 263.

Acute Toxicity Studies

The acute oral toxicity study was carried out as per OECD-423 guidelines. The synthesized compound was found to be non-toxic up to 2000 mg/kg body weight and did not cause any death and therefore 100 mg/kg dose level was selected.

Preparation of doses

The active control standard drug Diclofenac sodium and all synthesized compounds were prepared as a suspension by triturating with 1% tween 80.

Anti-inflammatory activity by Carrageenan induced hind Paw Oedema in rats

The method adopted resembles essentially that described by Winter et al[20]. Six groups of Albino rats of either sex (each comprising six animals) weighing 200 gm were deprived of food and water for 18 hours priors to experiment. The active control standard drug Diclofenac sodium and all synthesized compounds were administered i.p. to all the Rats. After 30 minutes 0.1 ml of 1% carrageenan sodium in normal saline was injected into sub plantar region of the paw of each rat. The edema volumes of the injected paw were measured at 0 minute, 30 minute, 1 hour, 2 hour, 3 hour, 6 hour, and 12 hour.

Statistical analysis

All values were expressed as mean ± SEM. The values obtained from the above parameters in case of synthesized compounds were compared with active control standard drug and controlled group by using one way ANOVA followed $p<0.001$, was considered significant.

Result and Discussion

The main aim of this work is to synthesize various substituted-[1,2,4] triazolo[1,5c]quinazolinone derivatives by mannich reaction using formamide and substituted secondary amine. The all synthesized compounds were confirmed by IR, ^{1}HNMR and mass spectra.

All synthesized compounds (K 11-19) resulted in good yields with 50-60%. The anti-inflammatory activity was performed at 100mg/kg body mass by rat paw oedema model. Diclofenac sodium (100mg/kg) was used as standard. The results were given in Table 2.

see Table 2.

All synthesized compounds have shown anti-inflammatory activity as all has significant reduction in inflammation when compared to inflammatory control group. Compounds **K 15, K 18** and **K 19** have shown very good anti-inflammatory activity comparable to standard drug Diclofenac sodium.

At 1st h compounds K 15, K 18 and K 19 showed good inhibition of inflammation when compared with other compounds. At 2nd h, all compounds have showed good inhibition of inflammation up to 12 h and shown in figure 2. These results reveals that the compounds containing piperazine and diethylamine substitution at 3rd positon of Triazolo[1,2,4]quinazolinone nucleus enhances their anti-inflammatory activities.

see Fig. 2

Acknowledgements

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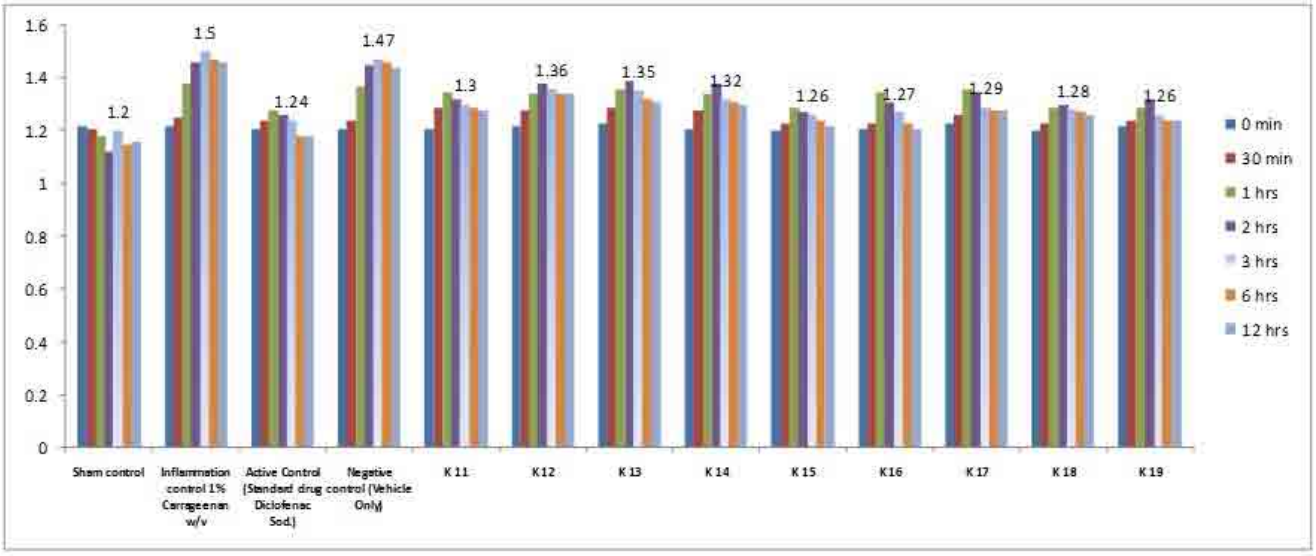


Figure 2: Anti-inflammatory activity of all synthesized compounds.

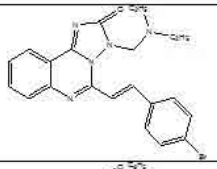
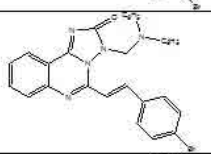
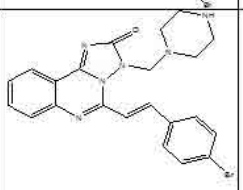
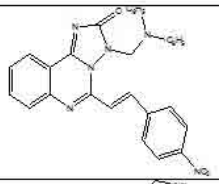
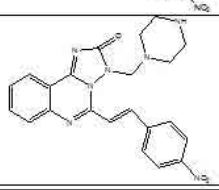
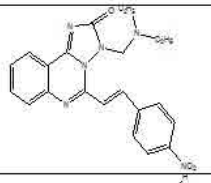
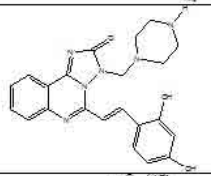
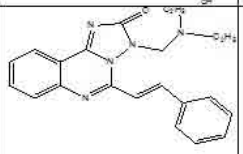
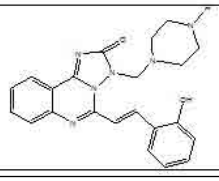
S.No.	Comp.	Structure	Mol.Formula	Mol.wt	m.p. ^{0}C	% yield
1.	K 11		$C_{22}H_{22}BrN_5O$	452.35	180	60
2.	K 12		$C_{30}H_{22}BrN_5O$	548.43	180	50
3.	K 13		$C_{22}H_{21}BrN_6O$	465.35	210	40
4.	K 14		$C_{30}H_{22}N_7O_3$	514.53	260	30
5.	K 15		$C_{22}H_{21}N_7O_3$	431.35	220	34
6.	K 16		$C_{22}H_{22}N_6O_3$	418.45	210	28
7.	K 17		$C_{22}H_{22}N_6O_3$	418.45	210	40
8.	K 18		$C_{22}H_{23}N_5O_2$	373.45	180	35
9.	K 19		$C_{22}H_{22}N_6O_2$	402.45	200	35

Table 1: Analytical data of synthesized compounds

Group	Compound	Dose mg\kg	Paw Volume (ml)						
			0 min	30 min	1 hrs	2 hrs	3 hrs	6 hrs	12 hrs
1	Sham control		1.22± 0.21	1.21± 0.23	1.18± 0.35	1.12± 0.20	1.20± 0.30	1.15± 0.28	1.16± 0.48
2	Inflammation control 1% Carrageenan w/v	0.1 ml	1.22± 0.12	1.25± 0.40	1.38± 0.28 a*	1.46± 0.30 a*	1.50± 0.36 a*	1.47± 0.38 a*	1.46± 0.42 a*
3	Active Control (Standard drug Diclofenac Sod.)	50	1.21± 0.32	1.24± 0.42	1.28± 0.30 b*	1.26± 0.38 b*	1.24± 0.38 b*	1.18±38 b*	1.18± 0.54 b*
4	Negative control (Vehicle Only)	0.5 ml	1.21± 0.23	1.24± 0.45	1.37± 0.45 a*	1.45± 0.34 a*	1.47± 0.39 a*	1.46±0.43 a*	1.44±48 a*
5	K 11	100	1.21± 0.32	1.29± 0.38	1.35± 0.38	1.32± 0.53 b*	1.30± 0.47 b*	1.29± 0.43 b*	1.28± 0.30 b*
6	K 12	100	1.22± 0.35	1.28± 0.23	1.34± 0.40	1.38± 0.25 b*	1.36± 0.52 b*	1.34± 0.30 b*	1.34± 0.50 b*
7	K 13	100	1.23± 0.35	1.29± 0.48	1.36± 0.30	1.39± 0.42 b*	1.35± 0.36 b*	1.32± 0.36 b*	1.31± 0.38 b*
8	K 14	100	1.21± 0.28	1.28± 0.50	1.34± 0.29	1.38± 0.43 b*	1.32± 0.38 b*	1.31± 0.32 b*	1.30± 0.65 b*
9	K 15	100	1.20± 0.25	1.23± 0.75	1.29± 0.48 b#	1.27± 0.38 b*	1.26± 0.39 b*	1.24± 0.28 b*	1.22± 0.43 b*
10	K 16	100	1.21± 0.21	1.23± 0.29	1.35± 0.38	1.31± 0.32 b*	1.27± 0.40 b*	1.23± 0.40 b*	1.21± 0.45 b*
11	K 17	100	1.23± 0.20	1.26± 0.65	1.36± 0.32	1.35± 0.38 b*	1.29± 0.30 b*	1.28± 0.58 b*	1.28± 0.56 b*
12	K 18	100	1.20± 0.30	1.23± 0.67	1.29± 0.36 b#	1.30± 0.32 b*	1.28± 0.42 b*	1.27± 0.50 b*	1.26± 0.76 b*
13	K 19	100	1.22± 0.32	1.24± 0.45	1.29± 0.38 b‡	1.32± 0.43 b*	1.26± 0.34 b*	1.24± 0.60 b*	1.24± 0.23 b*

Table 2: Anti-inflammatory activity of synthesized compounds:

a vs Sham Control , b vs Inflammatory Control, where * = p<0.0001; # = p<0.001; † = p<0.01; ‡ = p< 0.05