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Original article

Synthesis of novel 4,6-disubstituted quinazoline derivatives, their anti-inflammatory and anti-cancer activity (cytotoxic) against U937 leukemia cell lines ☆

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Abstract

In view of the link between use of NSAIDs and altered cancer incidence and a growing evidence of COX-II implication in angiogenesis, a novel series of 4,6-disubstituted quinazoline derivatives have been synthesized starting from anthranilic acid derivatives **1** through conventional methods. Initially acylation followed by cyclisation to obtain benz-oxazinones **2** which on further treatment with ammonia yielded the crucial intermediate, 2-substituted benzamide (**3**). The products were subsequently cyclised to obtain quinazolones **4**, chlorinated **5**, then hooked to various optically pure α-amino acids to have 4,6-disubstituted quinazoline derivatives **6.** All the derivatives **6** are screened for anti-inflammatory and anti-cancer activity against U937 leukemia cell lines. Some of the compounds exhibited promising anti-cancer activity with reference to standard drug Etoposide.

Keywords: Anthranilic acids; Acylation; Amination; Chlorination; Quinazoline derivatives; α-Amino acids

1. Introduction

The main target of NSAID drug action is the cyclooxygenase (COX) enzyme, which catalyses the conversion of arachidonic acid to prostaglandin precursors important in inflammatory processes. Two isozymes, which are the products of two separate genes, exist in humans: (i) COX-1, the constitutive form; and (ii) COX-2, the inducible form [1,2]. This isoenzyme is induced in response to cytokines and bacterial endotoxins [3].

A possible link between use of non-steroidal anti-inflammatory agents (NSAIDs) and altered incidence of cancer has been an area of intense investigation over the past couple of decades [4]. This led to the genesis of a substantial body of evidence supporting a role of cyclooxygenase -2 (COX-2), the enzyme implicated in the incidence of inflammatory disorders in carcinogenesis. Several studies have reported an inverse relationship for both colon [5] and breast cancers [6–8]. A number of studies have shown over expression of COX-2 in solid malignancies [9]. The effects of specific COX-2 inhibitors have been tested in animal models of angiogenesis, and celecoxib, a specific COX-2 inhibitor, has been shown to cause inhibition of the angiogenic response in fibroblast growth factor-induced rat corneal angiogenesis [10]. In addition, forced over expression of COX-2 is sufficient to induce tumorigenesis in transgenic mice [11]. This data have provided us a basis for designing molecules with a potential of anti-inflammatory and anti-tumour activities.

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In general quinazoline derivatives are known to possess remarkable anti-inflammatory activity as NOS-II [10,12], NFKB [13], TNF-α [14] IMPDH-II [15], MAPK [16], IL-6 [17], PDE-3 [18] and PDE-4 [19] inhibitors. Quinazoline derivatives are also found to show bronco-dilatory [20], anti-allergic [21] properties. Primarily non-steroidal anti-inflammatory drug (NSAID) such as acetyl salicylic acid was identified as a well established first generation drug in the treatment of pain and inflammation. Over a period phenyl butazone is considered as second generation NSAID and showed significant improvement over first generation. Subsequently proquazone and fluproquazone [22] emerged as third generation NSAIDs and are superior in safety, efficacy and are comparable with Indomethacin drug. In addition, quinazoline derivatives also have a therapeutic benefit as an anti-invasive agent with potential for activity in early and advanced solid tumors, metastatic bone disease and leukemias [23,24]. Some of the known quinazoline derivatives exhibited remarkable anti-cancer activity [25–32]. However, search is continuously on to identify a more potent lead molecule as these molecules are developing resistance over a period. Based on the importance of these molecules, our attention was attracted towards synthesis of novel quinazoline derivatives in order to find a more potent molecule. Quinazoline derivatives are synthesized mainly starting from anthranilic acid [15,33–37], benzonitrile [34] and so on with an appropriate substituent to have specific functionality and activity. In continuation of our efforts towards synthesis of potential molecules like quinazolines [33,38,39], pyrido pyrimidines [40], pyrimido[1,2-*b*]indazoles [41,42], we have synthesized several new 4,6-disubstituted quinazoline derivatives and screened for anti-inflammatory as well as anti-cancer activity against U937 leukemia cell lines. The compounds which showed promising anti-cancer activity have been identified in comparison with Etoposide, a standard drug and have been reported here for the first time.

2. Chemistry

Anthranilic acid (2-amino benzoic acid) **1** was reacted with benzoyl chloride in dry pyridine at 0–5 °C for 4 h and obtained benz-oxazinone **2** in high yields. Benz-oxazinone **2** is further treated with aq. ammonia at room temperature and resulted in respective amide derivative **3**. Compound **3** on refluxing in aq. sodium hydroxide gave cyclised product quinazolin-4-one **4**. It is further chlorinated using $POCl_3/PCl_5$ and obtained 4-chloroquinazolone **5**. In order to see the role of substituents on rate of reaction, yield of products and subsequently on activity, anthranilic acid **1** was substituted with bromine/iodine in fifth position and the sequence of reactions is carried out to obtain the respective products **5**. It is found that the rate of reaction and yield of products are independent of substituents used. The sequence of reactions are drawn in Scheme 1 and yields of products are tabulated in Table 1.

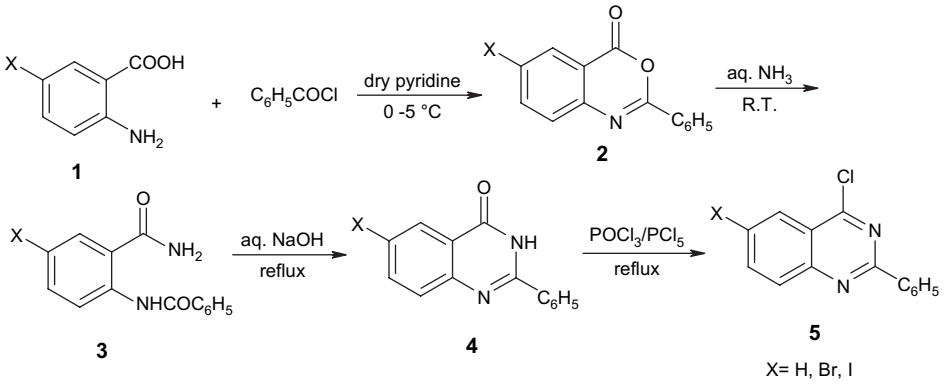
The 4-chloro quinazolines **5** are reacted with various optically pure α-amino acids such as L-methyl glycine, L-phenyl alanine, L-isoleucine, L-tryptophan and simple glycine in dimethyl sulphoxide at temperature 100 °C for 4–5 h and obtained 4,6-disubstituted amino quinazoline derivatives **6** in high yields. In order to obtain imidazo[1,2-*c*]quinazolines **7**, all the products were subjected to cyclisation under different set of conditions such as (a) glacial CH_3COOH/*p*-TSA at reflux, (b) $(CH_3CO)_2O$ at 140 °C, (c) $NaOCH_3/CH_3OH$ at reflux, (d) microwave irradiation condition on a silica gel solid support in presence of $POCl_3$ at 600 W. In all the cases no cyclised product is formed except recovering starting material. It is attributed to the stability of compounds and proton on C-4 NH is not easily polarized on to ring nitrogen due to aromaticity. The sequence of reactions are drawn in Scheme 2 and products are tabulated in Table 2.

3. Pharmacology

Quinazoline derivatives being considered as potent anti-inflammatory and anti-cancer agents, several new quinazoline derivatives **6a–6o** were synthesized and screened for anti-inflammatory and anti-cancer activities. The response of all the compounds to anti-inflammatory activity is moderate to poor, however, some of the compounds showed promising anti-cancer activity. The details of activity results are outlined below.

3.1. Anti-inflammatory activity

Test compounds **6a–6o** were screened for anti-inflammatory activity in vivo using carageenan induced rat paw edema



Scheme 1.

Table 1
Preparation of 4-chloro quinazolines **5**

Entry	Compound no.	X	M.p. (°C)	Yield (%)
1	**2a**	H	122	82
2	**2b**	Br	182	80
3	**2c**	I	199	82
4	**3a**	H	234	85
5	**3b**	Br	237	80
6	**3c**	I	248	82
7	**4a**	H	226	84
8	**4b**	Br	230	80
9	**4c**	I	241	81
10	**5a**	H	124	80
11	**5b**	Br	129	77
12	**5c**	I	135	78

model. The different test compounds were administered to the animals in the test group at the dose of 100 mg/kg and standard group with Indomethacin at the dose of 10 mg/kg as1% gum acacia suspension by oral route. Compound **6d** having L-methyl glycine on 4th position showed highest activity among all the compounds with 9.30% of inhibition against 48% of standard Indomethacin. Next highest activity is observed in the descending order of compounds **6f**, **6j**, **6i** and **6m**. In all these compounds chain length or bulkiness in amino acid on 4th position is increased and the activity is inversely proportional to the chain length. Rest of the compounds showed no activity having either bromine/iodine or no bromine/iodine on 6th position with different amino acids branch on 4th position in a given concentration. There is no clear cut trend in results to fix the optimum substituents at a specified position. The results in descending order of activity are tabulated in Table 3.

3.2. Anti-cancer activity (cytotoxic activity)

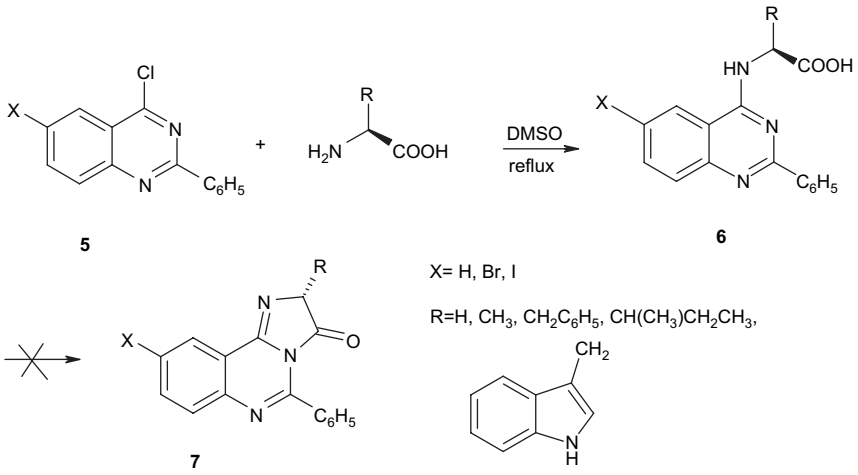
Compounds **6a–6o** were tested against U937 leukemia human lymphoma anti-cancer cell lines and showed significant decrease in cell viability with reference to concentration. Among all, compound **6i** exhibited maximum cytotoxic activity and is close to standard positive control (Etoposide). The activity is presumed to be due to the presence of iodine on 6th position with L-phenylalanine on 4th position. If iodine is replaced with bromine, the activity is drastically reduced in compound **6h**. Alternately on comparison of compounds **6k**, **6l** and **6j** with L-isoleucin on 4th position and Br, I and H on 6th position showed descending order of activity. Similar trend is followed in compounds **6n**, **6o** and **6m** with L-tryptophan and compounds **6e**, **6f** and **6d** with L-methyl glycine. In conclusion bromine on 6th position is considered to be optimum to show anti-cancer activity except in compound **6i**, which showed the highest activity with iodine on 6th position. The cytotoxic activity of test compounds with decreasing order of **6i** > **6k** > **6n** > **6o** > **6h** > **6l** > **6m** > **6e** > **6j** is tabulated in Table 4.

4. Conclusion

A series of novel quinazoline derivatives have been synthesized through a facile strategy and screened for anti-inflammatory and anti-cancer (cytotoxic) activities. Promising compounds which showed anti-cancer activity have been identified.

5. Experimental section

Melting points were recorded on Casia-Siamia (VMP-AM) melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR 240-C spectrophotometer using KBr optics. ^{1}H NMR spectra were recorded on Gemini Varian 200 MHz, Bruker AV 300 MHz and Unity 400 MHz spectrometer in DMSO-d_6 or $CDCl_3$ using TMS as an internal standard. Electron impact (EI) and chemical ionisation mass spectra were recorded on a VG 7070 H instrument at 70 eV. All reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel 60 F_{254} (mesh); spots were visualized under UV light. Merck silica gel (100–200 mesh) was used for chromatography. CHN analyses were recorded on a Vario EL analyser.



Scheme 2.

Table 2
Synthesis of 4,6-disubstituted amino quinazoline derivatives **6**

Entry	Compound no.	X	R	m.p. (°C)	Yield (%)
1	**6a**	H	H	217	81.33
2	**6b**	Br	H	245	79.76
3	**6c**	I	H	279	82.71
4	**6d**	H	CH_3	235	78.00
5	**6e**	Br	CH_3	202	76.70
6	**6f**	I	CH_3	282	78.90
7	**6g**	H	$CH_2C_6H_5$	163	77.00
8	**6h**	Br	$CH_2C_6H_5$	153	76.54
9	**6i**	I	$CH_2C_6H_5$	222	78.90
10	**6j**	H	$CH(CH_3)CH_2CH_3$	237	81.00
11	**6k**	Br	$CH(CH_3)CH_2CH_3$	203	78.86
12	**6l**	I	$CH(CH_3)CH_2CH_3$	246	80.00
13	**6m**	H	C_9H_8N	174	82.00
14	**6n**	Br	C_9H_8N	220	80.96
15	**6o**	I	C_9H_8N	272	86.59

Table 4
Cytotoxic activity of compounds **6a–6o**

Entry	Test compound	IC_{50} (μg/ml)[b]
1	Etoposide[a]	10.56 ± 0.70
2	**6i**	16.11 ± 1.12
3	**6k**	32.02 ± 1.73
4	**6n**	37.90 ± 1.10
5	**6o**	46.11 ± 1.21
6	**6h**	50.38 ± 0.92
7	**6l**	52.36 ± 0.61
8	**6m**	74.78 ± 1.80
9	**6e**	104.24 ± 1.85
10	**6j**	120.71 ± 6.09
11	**6a–6d**, **6f** and **6g**	ns

ns, Not significant.
[a] Positive control.
[b] IC_{50} is defined as the concentration, which results in a 50% decrease in cell number as compared with that of the control cultures in the absence of an inhibitor. The values represent the mean ± SE of four individual observations.

5.1. 2-Phenyl-4H-benzo[d][1,3]oxazin-4-one (**2**)

See Ref. [33].

5.2. 2-Benzamido benzamide (**3**)

See Ref. [43].

5.3. 2-Phenyl quinazolin-4(3H)-one (**4**)

See Ref. [38].

5.4. 4-Chloro-2-phenyl quinazoline (**5**)

See Ref. [38].

5.5. 2-Phenyl-4,6-disubstituted quinazoline derivatives (**6a–6o**)

5.5.1. General procedure

A stirred mixture of 4-chloro-2-phenyl 6-substituted quinazolines (4.5 mmol), optically pure α-amino acid (5 mmol) and potassium carbonate (20 mmol) in DMSO-d_6 (20 ml) was heated at 100 °C for 4–5 h. After cooling to room temperature, 1 N HCl was added till pH 4. The precipitated solid, crude product was collected by filtration. It is purified passing through a column packed with silica gel and eluents used are ethyl acetate/methanol (80:20).

Table 3
Anti-inflammatory activity of compounds **6a–6o**

Entry	Test compound	% Of inhibition
1	Indomethacin	48.0
2	**6d**	9.30
3	**6f**	6.50
4	**6j**	4.10
5	**6i**	3.70
6	**6m**	1.80
7	**6a–6c**, **6e**, **6g**, **6h**, **6k**, **6l**, **6n** and **6o**	0.0

Test compound, 100 mg/kg; Indomethacin, 10 mg/kg.

*5.5.1.1. (2-Phenyl quinazolin-4-yl amino) acetic acid (**6a**).* Yield: 0.47 g (81.33%); m.p. 217 °C; IR (KBr, cm^{-1}): 3575–3200 (OH, NH), 1720.69 (CO). ^{1}H NMR (200 MHz, DMSO-d_6): δ 4.35(2H, d, $J = 14.81$ Hz, CH_2), 7.40 (4H, m, Ar-H), 7.70 (1H, d, $J = 15$ Hz, Ar-H), 7.85 (1H, d, $J = 18.51$ Hz, Ar-H), 8.00 (1H, br., s, NH), 8.10 (1H, d, $J = 14.81$ Hz, Ar-H), 8.55 (2H, m, Ar-H); EIMS, *m/z*: 280 ($M^+ + 1$); Anal. Calcd. for $C_{16}H_{13}N_3O_2$: C, 68.81; H, 4.69; N, 15.05; Found: C, 68.56; H, 4.38; N, 14.87.

*5.5.1.2. (6-Bromo-2-phenyl quinazolin-4-yl amino) acetic acid (**6b**).* Yield: 0.42 g (79.76%); m.p. 245 °C; IR (KBr, cm^{-1}): 3200–2900 (OH, NH), 1673.92 (CO). ^{1}H NMR (200 MHz, DMSO-d_6): δ 4.35 (2H, d, $J = 13.33$ Hz, CH_2), 7.50 (3H, m, Ar-H), 7.70–7.90 (2H, m, Ar-H), 8.20 (1H, d, $J = 20$ Hz, Ar-H), 8.45 (1H, br, s, NH), 8.52 (2H, m, Ar-H); EIMS, *m/z*: 359($M^+ + 1$); Anal. Calcd. for $C_{16}H_{12}BrN_3O_2$: C, 53.65; H, 3.38; N, 11.73; Found: C, 53.30; H, 3.30; N, 11.51.

*5.5.1.3. (6-Iodo-2-phenyl quinazoline-4-yl amino) ethane peroxoic acid (**6c**).* Yield: 0.36 g (82.71%); m.p. 279 °C; IR (KBr, cm^{-1}): 3575–3200 (OH, NH), 1631(CO). ^{1}H NMR (200 MHz, DMSO-d_6): δ 4.40 (2H, d, $J = 13.79$ Hz, CH_2), 7.50–7.70 (3H, m, Ar-H), 7.90 (1H, d, $J = 13.79$ Hz, Ar-H), 8.17 (2H, d, $J = 20$ Hz, Ar-H), 8.30 (1H, d, $J = 20$ Hz, NH), 8.37–8.42 (2H, m, Ar-H); EIMS, *m/z*: 406 ($M^+ + 1$); Anal. Calcd. for $C_{16}H_{12}IN_3O_2$: C, 47.43; H, 2.99; N, 10.37; Found: C, 47.60; H, 3.25; N, 10.55.

*5.5.1.4. (2-Phenyl quinazolin-4-yl amino) propanoic acid (**6d**).* Yield: 0.40 g (78%); m.p. 235 °C; IR (KBr, cm^{-1}): 3550–3000 (OH, NH), 1669.45 (CO). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.30 (3H, d, $J = 20$ Hz, CH_3), 4.20 (1H, qui, $J = 5.56$ Hz, CH), 7.55 (4H,m, Ar-H), 7.73 (2H, m, Ar-H), 8.25 (3H, m, Ar-H), 12.30 (1H, br., s, NH); EIMS, *m/z*: 294

($M^+ + 1$); Anal. Calcd. $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33. Found: C, 69.35; H, 4.96; N, 14.01.

*5.5.1.5. (6-Bromo-2-phenyl quinazolin-4-yl amino) propanoic acid (**6e**).* Yield: 0.39 g (76.70%); m.p. 202 °C; IR (KBr, cm^{-1}): 3150–3100 (OH, NH), 1673.63 (CO). ^1H NMR (200 MHz, DMSO-d_6): δ 1.60 (3H, d, $J = 12.90$ Hz, CH_3), 4.80 (1H, qui, $J = 8.06$ Hz, CH), 7.60 (4H, m, Ar-H), 7.80 (2H, m, Ar-H), 8.35 (2H, m, Ar-H), 12.20 (1H, br., s, NH); EIMS, *m/z*: 373 ($M^+ + 1$); Anal. $C_{17}H_{14}BrN_3O_2$: C, 54.86; H, 3.79; N, 11.29; Found: C, 54.53; H, 3.53; N, 11.01.

*5.5.1.6. (6-Iodo-2-phenyl quinazolin-4-yl amino) propanoic acid (**6f**).* Yield: 0.49 g (78.90%); m.p. 282 °C; IR (KBr, cm^{-1}): 3150–3100 (OH, NH), 1673 (CO). ^1H NMR (200 MHz, DMSO-d_6): δ 1.65 (3H, d, $J = 10$ Hz, CH_3), 4.85 (1H, qui., $J = 5$ Hz, CH), 7.50 (4H, m, Ar-H), 7.70 (2H, m, Ar-H), 8.25 (2H, m, Ar-H), 12.50 (1H, br., s, NH); EIMS, *m/z*: 420 ($M^+ + 1$); Anal. Calcd. for $C_{17}H_{14}IN_3O_2$: C, 48.71; H, 3.37; N, 10.02; Found: C, 48.90; H, 3.61; N, 10.26.

*5.5.1.7. 3-Phenyl-2-(2-phenyl quinazolin-4-yl amino) propanoic acid (**6g**).* Yield: 0.40 g (77%); m.p. 163 °C; IR (KBr, cm^{-1}): 3350–3100 (OH, NH), 1635.13 (CO). ^1H NMR (200 MHz, DMSO-d_6): δ 3.40 (2H, d, $J = 6.67$ Hz, CH2), 5.00 (1H, quartet, $J = 4.45$ Hz, CH), 7.12 (1H, d, $J = 13.34$ Hz, Ar-H), 7.20 (2H, t, $J = 6.67$ Hz, Ar-H), 7.37 (2H, d, $J = 13.34$ Hz, Ar-H), 7.45 (4H, m, Ar-H), 7.70 (1H, t, $J = 6.67$ Hz, Ar-H), 7.78 (1H, d, $J = 13.33$ Hz, Ar-H), 8.27 (1H, d, $J = 13.33$ Hz, NH), 8.45 (3H, m, Ar-H); EIMS, *m/z*: 370 ($M^+ + 1$); Anal. Calcd. for $C_{23}H_{19}N_3O_2$: C, 74.78; H, 5.18; N, 11.37; Found: C, 74.93; H, 5.37; N, 11.61.

*5.5.1.8. 2-(6-Bromo-2-phenyl quinazolin-4-yl amino)-3-phenyl propanoic acid (**6h**).* Yield: 0.50 g (76.54%); m.p. 153 °C; IR (KBr, cm^{-1}): 3300–3000 (OH, NH), 1727.61 (CO). ^1H NMR (200 MHz, DMSO-d_6): δ 3.30 (2H, d, $J = 14.81$ Hz, CH_2), 4.95 (1H, quartet, $J = 4.93$ Hz, CH), 7.15–7.30 (5H, m, Ar-H), 7.45–7.60 (3H, m, Ar-H), 7.90 (2H, m, Ar-H), 8.05 (1H, br., s, NH), 8.15 (2H, m, Ar-H), 9.32 (1H, d, $J = 14.81$ Hz, Ar-H); EIMS, *m/z*: 449.3 ($M^+ + 1$); Anal. Calcd. $C_{23}H_{18}BrN_3O_2$: C, 61.62; H, 4.05; N, 9.37; Found: C, 61.84; H, 4.29; N, 9.61.

*5.5.1.9. 2-(6-Iodo-2-phenyl quinazolin-4-yl amino)-3-phenyl propanoic acid (**6i**).* Yield: 0.45 g (78.90%); m.p. 222 °C; IR (KBr, cm^{-1}): 3450–3100 (OH, NH), 1673.90 (CO). ^1H NMR (200 MHz, DMSO-d_6): δ 3.40 (2H, d, $J = 10$ Hz, CH_2), 5.10 (1H, quartet, $J = 6.67$ Hz, CH), 7.20–7.35 (2H, m, Ar-H), 7.40–7.48 (2H, m, Ar-H), 7.50–7.65 (7H, m, Ar-H), 8.25 (1H,br, s, NH), 8.48 (2H, m, ArH); EIMS, *m/z*: 496 ($M^+ + 1$); Anal. Calcd. for $C_{23}H_{18}IN_3O_2$: C, 55.77; H, 3.66; N, 8.48; Found: C, 55.50; H, 3.47; N, 8.29.

*5.5.1.10. 3-Methyl-2-(2-phenyl quinazolin-4-yl amino)pentanoic acid (**6j**).* Yield: 0.40 g (81.0%); m.p. 237 °C; IR (KBr, cm^{-1}): 3400–3000 (OH, NH), 1635.46 (CO). ^1H NMR (200 MHz, DMSO-d_6): δ 1.02 (3H, t, $J = 6.66$ Hz, CH_3), 1.10 (3H, d, $J = 6.67$ Hz, CH_3), 1.44 (1H, m, CH_2), 1.78 (1H, m, CH_2), 2.20 (1H, m, CH), 4.80 (1H, t, $J = 6.60$ Hz, CH), 7.45 (4H, m, Ar-H), 7.70–7.85 (3H, m, Ar-H), 8.37 (1H, d, $J = 13.34$ Hz, NH), 8.55 (2H, m, Ar-H); EIMS, *m/z*: 336 ($M^+ + 1$); Anal. Calcd. for $C_{20}H_{21}N_3O_2$: C, 71.62; H, 6.31; N, 12.53; Found: C, 71.75; H, 6.40; N, 12.71.

*5.5.1.11. 2-(6-Bromo-2-phenyl quinazolin-4-yl amino)-3-methyl pentanoic acid (**6k**).* Yield: 0.42 g (78.86%); m.p. 203 °C; IR (KBr, cm^{-1}): 3400–3000 (OH, NH), 1675.09 (CO). ^1H NMR (200 MHz, DMSO-d_6): δ 0.95–1.05 (3H, t, $J = 8.61$ Hz, CH_3), 1.10 (3H, d, $J = 13.79$ Hz, CH_3), 1.45 (1H, m, CH_2), 1.80 (1H, m, CH_2), 2.15 (1H, m, CH), 4.75 (1H, t, $J = 10.34$ Hz, CH), 7.48 (4H, m, Ar-H), 8.12 (1H, d, $J = 13.79$ Hz, Ar-H), 8.30 (1H, m, Ar-H), 8.60 (2H, m, Ar-H), 8.81 (1H, br., s, NH); EIMS, *m/z*: 415 ($M^+ + 1$); Anal. Calcd. for $C_{20}H_{20}BrN_3O_2$: C, 57.98; H, 4.87; N, 10.14; Found: C, 57.81; H, 4.70; N, 9.98.

*5.5.1.12. 2-(6-Iodo-2-phenylquinazolin-4-yl amino)-3-methyl pentanoic acid (**6l**).* Yield: 0.45 g (80.0%); m.p. 246 °C; IR (KBr, cm^{-1}): 3100–2965 (OH, NH), 1675 (CO). ^1H NMR (200 MHz, DMSO-d_6): δ 0.95–1.05 (3H, t, $J = 8.60$ Hz, CH_3), 1.10 (3H, d, $J = 13.78$ Hz, CH_3), 1.45 (1H, m, CH_2), 1.80 (1H, m, CH_2), 2.15 (1H, m, CH), 4.75 (1H, t, $J = 10$ Hz, CH), 7.48 (4H, m, Ar-H), 8.12 (1H, d, $J = 13.79$ Hz, Ar-H), 8.30 (1H, m, Ar-H), 8.60 (2H, m, Ar-H), 8.81 (1H, br., s, NH); EIMS, *m/z*: 415 ($M^+ + 1$); Anal. Calcd. for $C_{20}H_{20}IN_3O_2$: C, 52.07;H, 4.37; N, 9.11; Found: C, 52.31; H, 4.60; N, 9.29.

*5.5.1.13. 2-Phenyl quinazolin-4-(2-amino-3-(1H-indol-3-yl) propanoic acid) (**6m**).* Yield: 0.48 g (82.0%); m.p. 174 °C; IR (KBr, cm^{-1}): 3300–3000 (OH, NH), 1632.64 (CO). ^1H NMR (200 MHz, DMSO-d_6): δ 3.50 (2H, d, $J = 16.67$ Hz, CH_2), 5.0(1H, q, $J = 8.34$ Hz, CH), 6.95 (3H, m, Ar-H), 7.20 (2H, m, Ar-H), 7.40 (4H, m, Ar-H), 7.70 (3H, m, Ar-H), 8.45 (2H, m, Ar-H), 9.60 (1H, d, $J = 16.67$ Hz, NH), 10.65 (1H, s, NH); EIMS, *m/z*: 409 ($M^+ + 1$); Anal. Calcd. for $C_{25}H_{20}N_4O_2$: C, 73.51; H, 4.94; N, 13.72; Found: C, 73.70; H, 5.18; N, 13.91.

*5.5.1.14. 6-Bromo-2-phenyl quinazolin-4-(2-amino-3-(1H-indol-3-yl) propanoic acid) (**6n**).* Yield: 0.45 g (80.96%); m.p. 220 °C; IR (KBr, cm^{-1}): 3300–2950 (OH, NH), 1632 (CO). ^1H NMR (200 MHz, DMSO-d_6): δ 3.50 (2H, d, $J = 12$ Hz, CH_2), 5.18 (1H, q, $J = 8.0$ Hz, CH), 6.95 (3H, m, Ar-H), 7.30 (2H, m, Ar-H), 7.55 (4H, m, Ar-H), 8.0 (3H, m, Ar-H), 8.20 (1H, m, Ar-H), 9.50 (1H, d, $J = 16$ Hz, NH), 10.55 (1H, s, NH); EIMS, *m/z*: 409 ($M^+ + 1$); Anal. Calcd. $C_{25}H_{19}BrN_4O_2$: C, 61.61; H, 3.93; N, 11.50; Found: C, 61.45; H, 3.70; N, 11.32.

*5.5.1.15. 6-Iodo-2-phenyl quinazolin-4-(2-amino-3-(1H-indol-3-yl) propanoic acid (**6o**).* Yield: 0.5 g (86.59%); m.p. 272 °C; IR (KBr, cm^{-1}): 3300–2900 (OH, NH), 1632 (CO).

^{1}H NMR (200 MHz, DMSO-d_6): δ 3.55 (2H, d, $J = 12.10$ Hz, CH_2), 5.15 (1H, q, $J = 8.12$ Hz, CH), 7.0 (3H, m, Ar-H), 7.22 (2H, m, Ar-H), 7.35 (4H, m, Ar-H), 7.60 (1H, m, Ar-H), 8.40 (3H, m, Ar-H), 9.40 (1H, d, $J = 16.10$ Hz, NH), 10.50 (1H, s, NH); EIMS, *m/z*: 409 ($M^+ + 1$); Anal. Calcd. for $C_{25}H_{19}IN_4O_2$: C, 56.19; H, 3.58; N, 10.49; Found: C, 56.36; H, 3.82; N, 10.63.

5.6. Pharmacology

5.6.1. Assay method of anti-inflammatory activity in vivo

The anti-inflammatory activity of the test compounds was evaluated in Wistar rats by carrageenan induced rat paw edema model employing the method of Winter et al. (1963) [44] and Diwan et al. (1989) [45]. Male Wistar rats were used for the study. Animals were fasted overnight and were divided into control, standard and different test groups each consisting of three animals. Rats in the control group received the vehicle solution without drugs. One hour after test drugs administration, rats in all the groups were challenged with 0.1 ml of 1% carageenan in the sub plantar region of left hind paw. A zero hour paw volume was measured for the rats using digital plethysmometer (Ugo Basile, Italy) before the administration of carrageenan for all the groups. Paw volumes were again measured 3 h after the challenge of carageenan. The percent inhibition of paw volume for each rat in treated groups was calculated by comparing with mean paw volume of control group and expressed as mean (±SE) percent inhibition of paw volume for each test group.

5.6.2. Assay method of cytotoxic activity in vitro

U937 human histocytic lymphoma cell line was obtained from cell line bank of National Center for Cellular Sciences (NCCS), Pune, India. These cells were cultured in RPMI-1640 media containing 10% fetal bovine serum at 37 °C, CO_2 incubator in the presence or absence of test compounds. Cytotoxicty was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasolium bromide] assay, according to the method of Mosmann (1983) [46]. Briefly: the cells (2×10^4) were seeded in each well containing 0.1 ml of RPMI medium in 96 well plates. After 24 h different test concentrations (10–100 μg/ml) were added and cell viability was assessed after two days, 10 μl per well of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasolium bromide; 5 mg/ml; stock solution, Sigma) was added to the wells. The plates were incubated at 37 °C for additional 4 h. The medium was discarded and the formazan blue, which formed in the cells was dissolved with 100 μl of DMSO. The rate of color production was measured at 570 nm in a spectrophotometer (Spectra MAX Plus; Molecular Devices; supported by SOFTmax PRO-3.0). All experiments were conducted under the standard laboratory illumination. The percent inhibition of cell viability was determined with reference to the control values (without test compound). The data were subjected to linear regression analysis and the regression lines were plotted for the best fit. The IC_{50} (inhibition of cell viability) concentrations were calculated using the respective regression equation.

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