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European Journal of Medicinal Chemistry 39 (2004) 249–255
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Original article

Tetrazolo[1,5-*a*]quinoline as a potential promising new scaffold for the synthesis of novel anti-inflammatory and antibacterial agents

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Received 21 July 2003; received in revised form 16 December 2003; accepted 17 December 2003

Abstract

Three series of tetrazolo[1,5-*a*]quinoline derivatives have been synthesized. The first series was synthesized starting by the condensation of tetrazolo[1,5-*a*]quinoline-4-carboxaldehyde **2** with substituted thiosemicarbazides, followed by cyclization of the resulting thiosemicarbazones **3** with malonic acid in the presence of acetyl chloride to give pyrimidyl derivatives **4a–c.** The second series was prepared by the condensation of the latter compounds **4a–c** with the selected aromatic aldehydes to afford the arylidene derivatives **5a–f.** The third series **7a–c** was synthesized by condensation of tetrazolo[1,5-*a*]quinoline-4-carboxaldehyde **2** with the appropriate acetophenone, followed by cyclocondensation of the formed α,β-unsaturated ketones with thiourea. The newly synthesized compounds were evaluated for their anti-inflammatory and antimicrobial activities. Four compounds were proved to be as active as indomethacin in animal models of inflammation.

Keywords: Tetrazolo[1,5-*a*]quinoline; Pyrimidyl; Anti-inflammatory; Antimicrobial; Ulcerogenic; Acute toxicity

1. Introduction

Identification of novel compounds which treat effectively both infectious and inflammatory states, and which lack side effects associated with current therapies remains a major challenge in biomedical research. The use of several drugs to treat inflammatory conditions associated with infection is a problem, especially in case of patients with impaired liver or kidney functions or to avoid drug–drug interaction. In addition, from the pharmacoeconomic cost-effective stand-point, and seeking better patient compliance, a dual anti-inflammatory–antimicrobial agent with minimum adverse effects and high safety margin is highly desirable. We have established a programme to find out agents that have a dual effect as anti-inflammatory–antimicrobial agents [1–4]. Compounds containing quinoline [5–7], pyrimidine [8,9] or tetrazole [10–14] functionality have been reported to exhibit anti-inflammatory activity. In addition, the antimicrobial activities of these functionalities are well documented [15–20]. This initiated constructing compounds containing both the quinoline and tetrazole ring systems in the same matrix to serve as a new scaffold for the synthesis of anti-inflammatory–antimicrobial agents. It was also designed to make hybrid compounds between the pyrimidine moiety and the tetrazolo[1,5-*a*]quinoline nucleus. The pyrimidine derivatives were attached to the 4-position of tetrazolo[1,5-*a*]quinoline nucleus either directly or through iminomethyl bridge in order to investigate the effect of such molecular variation on the anti-inflammatory–antimicrobial activities. It should be pointed out that, in addition to the targeted anti-inflammatory and antibacterial activities, the ulcerogenic and acute toxicity profiles of the newly synthesized compounds were also examined. The results revealed that some compounds showed promising activities.

2. Chemistry

Reactions outlined in Fig. 1 were adopted to synthesize the desired compounds. The key intermediate tetrazolo[1,5-*a*]quinoline-4-carboxaldehyde **2** was prepared by the reaction of 2-chloroquinoline-3-carboxaldehyde **1** [20] with sodium azide in DMSO/AcOH mixture. A study of azido-tetrazolo isomerization in substituted tetrazolo[1,5-*c*]

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doi:10.1016/j.ejmech.2003.12.005

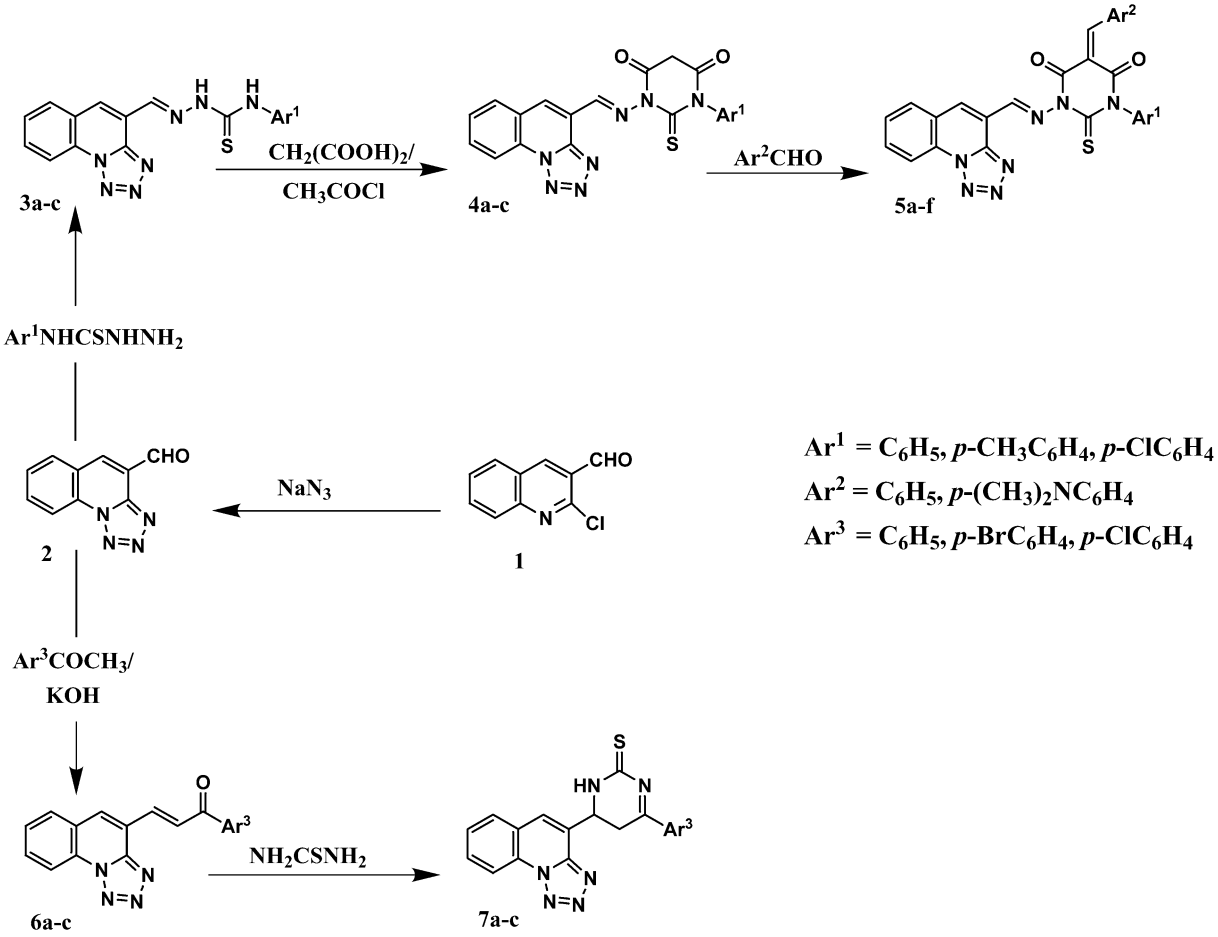

Fig. 1. Synthesis of the intermediate and target compounds.

quinazolines is reported in the literature [14,21]. We were not able to find any vibration band of the azido group of 2-azidoquinoline-3-carboxaldehyde in the IR spectrum of **2.** This observation showed that the tetrazole ring in **2** is relatively stable. Condensation of **2** with the selected substituted thiosemicarbazides afforded the corresponding thiosemicarbazones **3a–c** (Yield% = 81–85). The latter underwent cyclization with malonic acid in the presence of acetyl chloride [22] to give the pyrimidine derivatives **4a–c** (Yield% = 76–79). Condensation of **4a–c** with the appropriate aromatic aldehyde gave rise to arylidene derivatives **5a–f** (Yield% = 54–78). On the other hand, the pyrimidine derivatives **7a–c** (Yield% = 60–75) were synthesized by the cyclocondensation of α,β-unsaturated ketones **6a–c** with thiourea [8]. Compounds **6a–c** (Yield% = 82–87), in their turn, were prepared by the reaction of tetrazolo[1,5-*a*]quinoline-4-carboxaldehyde **2** with the proper acetophenone in presence of KOH.

3. Results and discussion

3.1. Anti-inflammatory activity

3.1.1. Cotton pellet-induced granuloma bioassay

The anti-inflammatory activity of the synthesized compounds **4c, 5a–f** and **7a–c** was evaluated applying the cotton pellet granuloma bioassay in rats [23] using indomethacin as a reference standard. The ED_{50} values were determined for each compound (Table 3).

In general, all the test compounds significantly inhibit granuloma formation. The arylidene derivatives **5a–f** (ED_{50} values range 8.51–11.94 μmol) are more active than their precursors **4a–c** (ED_{50} values range 12.46–16.61 μmol). Introduction of the pyrimidine moiety directly (**7a–c**) or through iminomethyl bridge (**5a–f**) to the 4-position of tetrazolo[1,5-*a*]quinoline did not affect the anti-inflammatory profile. It could safely be concluded that compounds **5d, 5e, 7a** and **7b** (ED_{50} values range 8.50–9.84 μmol) have anti-inflammatory activity comparable to that of indomethacin (ED_{50} value 9.28 μmol).

3.1.2. Carrageenan-induced rat paw edema

Compounds **5d, 5e, 7a** and **7b,** that showed anti-inflammatory activity comparable to that of indomethacin in the cotton pellet-induced granuloma bioassay, were further evaluated for their in vivo systemic effect using the carrageenan-induced paw edema bioassay in rats [24]. The results (recorded in Table 4) revealed that compounds **5d, 5e, 7a** and **7b** exhibit systemic anti-inflammatory activity (% protection 73.86, 70.45, 72.72 and 77.27, respectively) comparable to that of indomethacin (% protection 75.00).

Table 1
Physical and analytical data of compounds **3–5**

Compound number	Ar^1	Ar^2	Molecular formula (molecular weight)	Yield (%)	m.p. °C
3a	C_6H_5	–	$C_{17}H_{13}N_7S$ (347.40)	81	243–245
3b	*p*-CH_3–C_6H_4	–	$C_{18}H_{15}N_7S$ (361.42)	87	267–268
3c	*p*-Cl–C_6H_4	–	$C_{17}H_{12}ClN_7S$ (381.84)	85	242–243
4a	C_6H_5	–	$C_{20}H_{13}N_7O_2S$ (415.42)	79	204–205
4b	*p*-CH_3–C_6H_4	–	$C_{21}H_{15}N_7O_2S$ (429.10)	76	226–227
4c	*p*-Cl–C_6H_4	–	$C_{20}H_{12}ClN_7O_2S$ (449.87)	78	233–234
5a	C_6H_5	C_6H_5	$C_{27}H_{17}N_7O_2S$ (503.53)	58	222–223
5b	*p*-CH_3–C_6H_4	C_6H_5	$C_{28}H_{19}N_7O_2S$ (517.56)	60	156–157
5c	*p*-Cl–C_6H_4	C_6H_5	$C_{27}H_{16}ClN_7O_2S$ (537.98)	54	216–217
5d	C_6H_5	*p*-$(CH_3)_2NC_6H_4$	$C_{29}H_{22}N_8O_2S$ (546.60)	67	181–182
5e	*p*-CH_3–C_6H_4	*p*-$(CH_3)_2NC_6H_4$	$C_{30}H_{24}N_8O_2S$ (560.63)	73	172–173
5f	*p*-Cl–C_6H_4	*p*-$(CH_3)_2NC_6H_4$	$C_{29}H_{21}ClN_8O_2S$ (581.04)	78	189–190

Table 2
Physical and analytical data of compounds **6–7**

Compound number	Ar^3	Molecular formula (molecular weight)	Yield (%)	m.p. °C
6a	C_6H_5	$C_{18}H_{12}N_4O$ (300.31)	87	236–237
6b	*p*-Br–C_6H_4	$C_{18}H_{11}BrN_4S$ (379.21)	82	218–219
6c	*p*-Cl–C_6H_4	$C_{18}H_{11}ClN_4O$ (334.76)	83	256–257
7a	C_6H_5	$C_{19}H_{14}N_6S$ (358.42)	75	238–239
7b	*p*-Br–C_6H_4	$C_{19}H_{13}BrN_6S$ (437.31)	62	164–165
7c	*p*-Cl–C_6H_4	$C_{19}H_{13}ClN_6S$ (392.86)	60	198–199

Table 3
The anti-inflammatory activity (ED_{50}, μmol), ulcerogenic effects and toxicity of the test compounds

Test compound	ED_{50} (μmol)	(%) Ulceration	Toxicity (mg/kg)
Control	–	0.0	–
Indomethacin	9.28	100	–
4a	16.61	NT [a]	NT
4b	16.37	NT	NT
4c	12.46	NT	NT
5a	10.63	NT	NT
5b	11.94	NT	NT
5c	13.24	NT	NT
5d	8.51	20	>300
5e	9.84	0.0	>300
5f	11.52	NT	NT
7a	9.52	10	>300
7b	8.50	10	>300
7c	11.00	10	NT

[a] NT, not tested.

Table 4
Effect of compounds **5a, 9a, 9b, 10b** and **12a** on carregeenan-induced rat paw edema (ml), % protection and activity relative to indomethacin

Test compound	Increase in paw edema (ml) ± S.E.M. [a,b]	(%) Protection	Activity relative to indomethacin
Control	0.88 ± 0.027	0.0	0.0
Indomethacin	0.22 ± 0.025	75.00	100
5d	0.23 ± 0.021	73.85	98.48
5e	0.26 ± 0.031	70.45	93.93
7a	0.24 ± 0.012	72.72	96.96
7b	0.20 ± 0.023	77.27	103.02

[a] S.E.M. denotes the standard error of the mean.
[b] All data are significantly different from control ($P < 0.001$).

3.2. Ulcerogenic effects

Compounds **5d, 5e, 7a** and **7b** that exhibited potent anti-inflammatory activity in the pre-mentioned animal models were evaluated for their ulcerogenic potential in rats [25]. All the active compounds revealed a superior GI safety profiles (0–20% ulceration) in the population of the test animals at the oral doses of 30 μmol/kg per day, when compared with indomethacin, the reference standard drug, which was found to cause 100% ulceration under the same experimental conditions (Table 3).

Gross observation of the isolated rat stomachs showed a normal stomach texture for compound **5e** (0% ulceration), whereas the others showed slight hyperemia (10–20% ulceration).

3.3. Acute toxicity

The active compounds **5d, 5e, 7a** and **7b** were further evaluated for their oral acute toxicity in male mice using a literature method [9,26]. The results (Table 3) indicated that the tested compounds proved to be non-toxic and well tolerated by the experimental animals up to 300 mg/kg, although no mortality was recorded at 500 mg/kg. Moreover, these compounds were tested for their toxicity through parenteral route [2]. The results revealed that all the test compounds were non-toxic up to 100 mg/kg.

Table 5
Minimal inhibitory concentrations (MICs, μg/ml) of test compounds

Test compound	*E. coli* ATCC: 25922	*S. aureus* ATCC: 19433	*C. albicans*
4a	>200	>200	>200
4b	50	>200	>200
4c	>200	>200	>200
5a	200	100	>200
5b	100	50	>200
5c	50	>200	200
5d	200	>200	>200
5e	50	>200	>200
5f	50	100	>200
7a	50	12.5	>200
7b	50	>200	>200
7c	50	25	100
Ampicillin	25	12.5	–
Clotrimazole	–	–	12.5

3.4. Antimicrobial activity

The designed compounds **4a–c, 5a–f** and **7a–c** have been evaluated for their antimicrobial activity. The microdilution susceptibility test in Müller-Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) were used for the determination of antibacterial and antifungal activities [27]. The minimal inhibitory concentration (MIC) listed in (Table 5) showed that all the test compounds have no antifungal activity as compared with clotrimazole (Canesten®, Bayer). The activity of compounds **4b, 5c, 5e, 5f, 7a, 7b** and **7c** was 50% of that of ampicillin against *E. coli.* Compounds **7a** and **7c** showed activity against *S. aureus* comparable or half fold of that of ampicillin, respectively.

4. Conclusion

It can be safely concluded that compounds **5d, 5e, 7a** and **7b** proved to be the most active anti-inflammatory and antibacterial agents in the present study with no or minimum ulcerogenic effect and good safety margine. Therefore, such compounds would represent a fruitful matrix for the development of a new class of dual non-acidic anti-inflammatory–antimicrobial agents using tetrazolo[1,5-*a*]quinoline as a promising scaffold, that would deserve further investigation and derivatization.

5. Experimental protocols

5.1. Chemistry

All chemicals were purchased from E. Merck, Fluka AG and Aldrich companies. Melting points were determined in open glass capillaries using a Thomas capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on 470-Shimadzu infrared spectrophotometer using the KBr disc technique. ^{1}H NMR spectra were recorded on Jeol-400 MHz spectrometer (DMSO-d_6), and the chemical shifts are given in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard. Splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet. Elemental analyses were performed on Perkin-Elmer 2400 elemental analyzer, and the found values were within ±0.4% of the theoretical values. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254 nm for few seconds.

5.1.1. Tetrazolo[1,5-a]quinoline-4-carboxaldehyde 2

To a solution of 2-chloroquinoline-3-carboxaldehyde **1** (4 g, 20 mmol) in dimethyl sulphoxide (200 ml), a solution of sodium azide (2 g, 30 mmol) in water (10 ml) was added portionwise. The reaction mixture was stirred at 40 °C for 3 h. Stirring was continued for further 5 days at ambient temperature. The white precipitate formed was filtered, washed with water and crystallized from dimethylformamide. Yield: 3.2 g (78%); m.p. 234–236 °C. IR (cm^{-1}): 1700 (C=O), 1568 (C=C). ^{1}H-NMR: δ 7.98 (dd, J_1 = 7.32, J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.17 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.67 (d, J = 8.8 Hz, 1H, tetrazoloquin C_6-H), 8.75 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H), 9.02 (s, 1H, tetrazoloquin C_5-H), 10.43 (s, 1H, C*H*=O). Analysis calculated for $C_{10}H_6N_4O$ (198.18).

5.1.2. 4-Substituted thiocarbamoylhydrazonomethyltetrazolo[1,5-a]quinolines 3a–c

To a solution of aldehyde **2** (1 g, 10 mmol) in dimethylformamide (25 ml), was added an equivalent amount of N^4-substituted thiosemicarbazide. The reaction mixture was heated under reflux for 4 h, partially concentrated and cooled. The separated solid product was filtered, dried and crystallized from dimethylformamide (Table 1). IR (cm^{-1}): 3300–3288, 3210–3202 (NH), 1640–1635 (C=N), 1528–1523, 1335–1310, 1159–1155 and 906–894 (NCS amide I, II, III and IV bands respectively). ^{1}H-NMR of compound **3a:** δ 7.26 (t, J = 7.4 Hz, 1H, phenyl C_4-H), 7.52 (d, J = 7.94 Hz, 2 H, phenyl $C_{2,6}$-H), 7.61 (dd, J_1 = 7.4, J_2 = 7.94 Hz, 2 H, phenyl $C_{3,5}$-H), 7.88 (dd, J_1 = 7.32, J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.12 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.31 (d, J = 8.8 Hz, 1H, tetrazoloquin C_6-H), 8.62 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H), 8.74 (s, 1H, tetrazoloquin C_5-H), 9.02 (s, 1H, C*H*=N), 10.32 (s, 1H, N^4-H, D_2O exchangeable), 12.36 (s, 1H, N^2-H, D_2O exchangeable). ^{1}H-NMR of compound **3b:** δ 2.33 (s, 3H, CH_3), 7.23 (d, J = 8.08 Hz, 2H, phenyl $C_{2,6}$-H), 7.53 (d, J = 8.08 Hz, 2H, phenyl $C_{3,5}$-H), 7.86 (dd, J_1 = 7.32, J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.09 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.21 (d, J = 8.8 Hz, 1H, tetrazoloquin C_6-H), 8.64 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H), 8.70 (s, 1H, tetrazoloquin C_5-H), 9.04 (s, 1H, C*H*=N), 10.36 (s, 1H, N^4-H, D_2O exchangeable), 12.34 (s, 1H, N^2-H,

D_2O exchangeable). ^{1}H-NMR of compound **3c:** δ 7.26 (d, J = 8.17 Hz, 2H, phenyl $C_{2,6}$-H),7.48 (d, J = 8.17 Hz, 2H, phenyl $C_{3,5}$-H), 7.84 (dd, J_1 = 7.32, J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.11 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.26 (d, J = 8.8 Hz, 1H, tetrazoloquin C_6-H), 8.68 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H), 8.73 (s, 1H, tetrazoloquin C_5-H), 9.10 (s, 1H, C*H*=N), 10.38 (s, 1H, N^4-H, D_2O exchangeable), 12.35 (s, 1H, N^2-H, D_2O exchangeable).

*5.1.3. 4-(3-Aryl-4,6-dioxo-2-thioxohexahydropyrimidin-1-yl)-imino-methyltetrazolo[1,5-*a*]quinolines* ***4a–c***

To the appropriate **3a–c** (15 mmol), malonic acid (2.08 g, 20 mmol) and acetyl chloride (10 ml) were added. The reaction mixture was heated for 5 h at 50–55 °C on a water bath, cooled, then poured into ice cold water (50 ml). The separated solid product was filtered, washed with water, dried and crystallized from dimethylformamide (Table 1). IR (cm^{-1}): 1698–1690 (C=O), 1630–1625 (C=N), 1530–1522, 1334–1312, 1155–1150 and 910–895 (NCS amide I, II, III and IV bands respectively). ^{1}H-NMR of compound **4a:** δ 3.14 (s, 2H, pyrimid C_5-H), 7.28 (t, J = 7.4 Hz, 1H, phenyl C_4-H), 7.53 (d, J = 7.94 Hz, 2 H, phenyl $C_{2,6}$-H), 7.66 (dd, J_1 = 7.4, J_2 = 7.94 Hz, 2 H, phenyl $C_{3,5}$-H), 7.87 (dd, J_1 = 7.32, J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.10 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.33 (d, J = 8.8 Hz, 1H, tetrazoloquin C_6-H), 8.65 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H), 8.72 (s, 1H, tetrazoloquin C_5-H), 9.04 (s, 1H, C*H*=N). ^{1}H-NMR of compound **4b:** δ 2.33 (s, 3H, CH_3), 3.17 (s, 2H, pyrimid C_5-H), 7.25 (d, J = 8.08 Hz, 2H, phenyl $C_{2,6}$-H), 7.54 (d, J = 8.08 Hz, 2H, phenyl $C_{3,5}$-H), 7.84 (dd, J_1 = 7.32, J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.11 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.20 (d, J = 8.8 Hz, 1H, tetrazoloquin C_6-H), 8.66 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H), 8.72 (s, 1H, tetrazoloquin C_5-H), 9.07 (s, 1H, C*H*=N). ^{1}H-NMR of compound **4c:** δ 3.15 (s, 2H, pyrimid C_5-H), 7.25 (d, J = 8.17 Hz, 2H, phenyl $C_{2,6}$-H), 7.44 (d, J = 8.17 Hz, 2H, phenyl $C_{3,5}$-H), 7.82 (dd, J_1 = 7.32, J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.13 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.27 (d, J = 8.8 Hz, 1H, tetrazoloquin C_6-H), 8.65 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H), 8.72 (s, 1H, tetrazoloquin C_5-H), 9.08 (s, 1H, C*H*=N).

*5.1.4. 4-(3-Aryl-5-arylidene-4,6-dioxo-2-thioxohexahydro-pyrimidin-1-yl)-iminomethyl-tetrazolo[1,5-*a*]quinolines* ***5a–f***

To a solution of the selected **4a–c** (1 mmol) in glacial acetic acid (20 ml) the proper aromatic aldehyde (1 mmol) and anhydrous sodium acetate (0.098 g, 1 mmol) were added. The reaction mixture was heated under reflux for 6 h, cooled, then poured into ice cold water (50 ml). The separated solid was crystallized from ethanol (Table 1). IR (cm^{-1}): 1695–1690 (C=O), 1630–1625 (C=N), 1532–1525, 1336–1315, 1157–1154 and 904–896 (NCS amide I, II, III and IV bands respectively). ^{1}H-NMR of compound **5a:** δ 7.24–7.69 (m, 10H, phenyl-H), 7.85 (dd, J_1 = 7.32, J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.11 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.31 (m, 2H, C*H*=C and tetrazoloquin C_6-H), 8.62 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H), 8.73 (s, 1H, tetrazoloquin C_5-H), 9.06 (s, 1H, C*H*=N). ^{1}H-NMR of compound **5b:** δ 2.34 (s, 3H, CH_3), 7.25–7.78 (m, 9H, phenyl-H), 7.83 (dd, J_1 = 7.32, J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.09 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.22 (d, J = 8.8 Hz, 1H, tetrazoloquin C_6-H), 8.27 (s, 1H, C*H*=C), 8.63 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H), 8.70 (s, 1H, tetrazoloquin C_5-H), 9.08 (s, 1H, C*H*=N). ^{1}H-NMR of compound **5c:** δ 7.22–7.76 (m, 9H, phenyl-H), 7.81 (dd, J_1 = 7.32, J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.12 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.28 (m, 2H, C*H*=C and tetrazoloquin C_6-H), 8.63 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H), 8.71 (s, 1H, tetrazoloquin C_5-H), 9.10 (s, 1H, C*H*=N). ^{1}H-NMR of compound **5d:** δ 2.82 (s, 6H, $2CH_3$), 6.73 (d, J =7.7 Hz, 2H, aminophenyl $C_{2,6}$-H), 7.46–7.73 (m, 7H, phenyl-H), 7.83 (dd, J_1 = 7.32, J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.09 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.30 (m, 2H, C*H*=C and tetrazoloquin C_6-H), 8.61 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H), 8.71 (s, 1H, tetrazoloquin C_5-H), 9.05 (s, 1H, C*H*=N). ^{1}H-NMR of compound **5e:** δ 2.34 (s, 3H, CH_3), 2.81 (s, 6H, $2CH_3$), 6.71 (d, J =7.7 Hz, 2H, aminophenyl $C_{2,6}$-H), 7.39–7.74 (m, 6 H, phenyl-H), 7.82 (dd, J_1 = 7.32, J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.10 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.21 (d, J = 8.8 Hz, 1H, tetrazoloquin C_6-H), 8.27 (s, 1H, C*H*=C), 8.61 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H), 8.72 (s, 1H, tetrazoloquin C_5-H), 9.05 (s, 1H, C*H*=N). ^{1}H-NMR of compound **5f:** (δ), 2.81 (s, 6H, $2CH_3$), 6.71 (d, J = 7.7 Hz, 2H, aminophenyl $C_{2,6}$-H), 7.46–7.76 (m, 6H, phenyl-H), 7.81 (dd, J_1 = 7.32, J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.12 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.28 (m, 2H, C*H*=C and tetrazoloquin C_6-H), 8.63 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H), 8.71 (s, 1H, tetrazoloquin C_5-H), 9.10 (s, 1H, C*H*=N).

*5.1.5. 4-(3-Aryl-3-oxopropenyl)-tetrazolo[1,5-*a*]quinolines* ***6a–c***

To a well-stirred solution of the appropriate acetophenone (10 mmol) in alcoholic potassium hydroxide (2%, 25 ml), was added gradually a solution of aldehyde **2** (1.98 g, 10 mmol) in dimethylformamide (10 ml). Stirring was continued for 24 h at room temperature. The separated solid product was filtered, washed with water, dried and crystallized from dimethylformamide (Table 2). IR (cm^{-1}): 1665–1660 (C=O), 1630–1625 (C=N), 1575–1570 (C=C). ^{1}H-NMR of compound **6a:** δ 7.43–7.92 (m, 6H, CH=C*H*CO, phenyl-H), 7.82 (dd, J_1 = 7.32, J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.12 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.15 (s, 1H, tetrazoloquin C_5-H), 8.32 (m, 2H, C*H*=CHCO and tetrazoloquin C_6-H), 8.59 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H). ^{1}H-NMR of compound **6b:** δ 7.51–7.93 (m, 5H, CH=C*H*CO, phenyl-H), 7.80 (dd, J_1 = 7.32,

J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.13 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.12 (s, 1H, tetrazoloquin C_5-H), 8.33 (m, 2H, C*H*=CHCO and tetrazoloquin C_6-H), 8.58 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H). ^{1}H-NMR of compound **6c:** δ 7.46–7.91 (m, 5H, CH=C*H*CO, phenyl-H), 7.81 (dd, J_1 = 7.32, J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.11 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.14 (s, 1H, tetrazoloquin C_5-H), 8.32 (m, 2H, C*H*=CHCO and tetrazoloquin C_6-H), 8.59 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H).

*5.1.6. 4-(4-Aryl-2-thioxo-1,2,5,6-tetrahydropyrimidin-6-yl)-tetrazolo[1,5-*a*]quinolines **7a–c***

To a mixture of the selected **6a–c** (1 mmol) in dry dimethylformamide (30 ml), concentrated sulphuric acid (0.5 ml) and thiourea (0.076, 1 mmol) were added. The mixture was heated under reflux for 6 h. The reaction mixture was allowed to cool, poured into ice cold water, dried and crystallized from acetonitrile (Table 2). IR (cm^{-1}): 3400–3390 (NH), 1635–1628 (C=N), 1526–1522, 1333–1312, 1158–1152 and 902–898 (NCS amide I, II, III and IV bands, respectively). ^{1}H-NMR of compound **7a:** δ 2.08–2.49 (m, 2H, pyrimid C_5-H), 3.87–4.04 (m, 1H, pyrimid C_6-H), 7.34–7.65 (m, 6H, NH, phenyl-H), 7.80 (dd, J_1 = 7.32, J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.13 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.16 (s, 1H, tetrazoloquin C_5-H), 8.33 (d, J = 8.8 Hz, 1H, tetrazoloquin C_6-H), 8.61 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H). ^{1}H-NMR of compound **7b:** δ 2.06–2.47 (m, 2H, pyrimid C_5-H), 3.91–4.06 (m, 1H, pyrimid C_6-H), 7.44–7.55 (m, 5H, NH, phenyl-H), 7.81 (dd, J_1 = 7.32, J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.11 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.15 (s, 1H, tetrazoloquin C_5-H), 8.34 (d, J = 8.8 Hz, 1H, tetrazoloquin C_6-H), 8.60 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H). **7c:** δ 2.02–2.46 (m, 2H, pyrimid C_5-H), 3.93–4.11 (m, 1H, pyrimid C_6-H), 7.33–7.61 (m, 5H, NH, phenyl-H), 7.82 (dd, J_1 = 7.32, J_2 = 8.08 Hz, 1H, tetrazoloquin C_8-H), 8.10 (dd, J_1 = 7.32, J_2 = 8.8 Hz, 1H, tetrazoloquin C_7-H), 8.13 (s, 1H, tetrazoloquin C_5-H), 8.32 (d, J = 8.8 Hz, 1H, tetrazoloquin C_6-H), 8.62 (d, J = 8.08 Hz, 1H, tetrazoloquin C_9-H).

5.2. Anti-inflammatory activity

5.2.1. Cotton pellet-induced granuloma assay

Adult male Sprague–Dawley rats (120–140 g) were used. They were acclimated 1 week prior to use and allowed unlimited access to standard rat chow and water. Prior to the start of experiment, the animals were randomly divided into groups (six rats each). Cotton pellets (35 ± 1 mg) cut from dental rolls were impregnated with 0.2 ml (containing 10 μmol) of a solution of the test compound in chloroform and the solvent was allowed to evaporate. Each cotton pellet was subsequently injected with 0.2 ml of an aqueous solution of antibiotics (1 mg penicillin G and 1.3 mg dihydrostreptomycin/ml). Two pellets were implanted subcutaneously, one in each axilla of the rat, under mild general anesthesia. One group of animals received the standard reference indomethacin and the antibiotics at the same dose level. Pellets containing only the antibiotics were similarly implanted in the control rats. Seven days later, the animals were sacrificed and the two cotton pellets, with adhering granulomas, were removed, dried for 48 h at 60 °C and weighed. The increment in dry weight (difference between the initial and final weights) was taken as a measure of granuloma ± S.E. This was calculated for each group and the percentage reduction in dry weight of granuloma from control value was also calculated [23]. The ED_{50} values were determined through dose-response curves, using doses of 4, 7, 10 and 15 μmol for each compound.

5.2.2. Carrageenan-induced rat paw edema

Male albino rats weighing 120–150 g were used throughout the work. They were kept in the animal house under standard conditions of light and temperature with free access to food and water. The animals were randomly divided into groups of six rats each. The paw edema was induced by subplantar injection of 50 μl of 2% carrageenan solution in saline (0.9%). Indomethacin and the test compounds were dissolved in DMSO and were injected subcutaneously in a dose of 10 μmol/kg body weight, 1 h prior to carrageenan injection. DMSO was injected to the control group. The volume of paw edema (ml) was determined by means of water plethysmometer immediately after injection of carrageenan and 4 h later. The increase in paw volume between time 0 and +4 h was measured [24]. The percentage protection against inflammation was calculated as follows:

$$V_c - V_d/V_c \times 100$$

where V_c is the increase in paw volume in the absence of test compound (control) and V_d is the increase of paw volume after injection of the test compound. Data were expressed as the mean ± S.E.M. Significant difference between the control and the treated groups was performed using Student's t-test and P values. The differences in results were considered significant when $P < 0.001$. The anti-inflammatory activity of the test compounds relative to that of indomethacin was also calculated. The anti-inflammatory activity of the test compounds relative to that of indomethacin was also determined.

5.3. Ulcerogenic effects

Compounds **5d, 5e, 7a** and **7b** that exhibited potent anti-inflammatory activity in the pre-mentioned animal models were evaluated for their ulcerogenic potential in rats [25]. Indomethacin was used as reference standard. Male albino rats (100–120 g) were fasted for 12 h prior to the administration of the compounds. Water was given ad libitum. The animals were divided into groups, each of six animals. Control group received 1% gum acacia orally. Other groups received indomethacin or the test compounds orally in two equal doses at 0 and 12 h for three successive days at a dose of 30 μmol/kg per day. Animals were sacrificed by diethyl

ether 6 h after the last dose and the stomach was removed. An opening at the greater curvature was made and the stomach was cleaned by washing with cooled saline and inspected with a 3× magnifying lens for any evidence of hyperemia, haemorrhage, definite haemorrhagic erosion or ulcer. An arbitrary scale was used to calculate the ulcer index which indicates the severity of the stomach lesions (Table 1). The percentage ulceration for each group was calculated as follows:

$$\% \text{ ulceration} = \frac{\text{number of animals bearing ulcer in a group}}{\text{total number of animals in the same group}} \times 100$$

5.4. Acute toxicity

The same active compounds were further investigated for their approximate LD_{50} in male mice [9,26]. Eight groups of mice each consisting of six animals were used. The compounds were given orally in doses of 1, 10, 100, 200, 250, 500 mg/kg, respectively. Twenty-four hours later, the % mortality in each group and for each compound was recorded.

Moreover, these compounds were tested for their toxicity through parenteral route [2]. Groups of mice each consisting of six animals were used. The compounds or their vehicle, propylene glycol (control) were given by intraperitoneal injection in doses of 10, 25, 50, 75, 100 mg/kg, respectively. Survival was followed up to 7 days.

5.5. In vitro antimicrobial activity

The microdilution susceptibility test in Müller-Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) were used for the determination of antibacterial and antifungal activity [27]. The utilized test organisms were: *Escherichia coli* (*E. coli*) ATCC 25922 as an example of Gram-negative bacteria, *Staphylococcus aureus* (*S. aureus*) ATCC 19433 as an example of Gram-positive bacteria and *Candida albicans* (*C. albicans*) as yeast-like fungus. Ampicillin trihydrate and clotrimazole were used as standards antibacterial and antifungal agents, respectively. Solutions of the test compounds, ampicillin trihydrate and clotrimazole were prepared in DMSO at concentration of 1600 μg/ml. The twofold dilution of the compounds were prepared (800, 400, ..., 6.25 μg/ml). The microorganism suspensions at 10^6 CFU/ml (Colony Forming Unit/ml) concentration were inoculated to the corresponding wells. Plates were incubated at 36 °C for 24 h to 48 h. The incubation chamber was kept sufficiently humid. At the end of the incubation period, the minimal inhibitory concentrations (MIC) were determined as the lowest concentration that completely inhibit visible growth of the microorganism as detected by the unaided eye. Controls for the DMSO microorganisms and media microorganisms were also done.

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