Full Paper

Synthesis, Antitubercular and Anticancer Activities of Substituted Furyl-quinazolin-3(4*H*)-ones

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Some novel substituted-3-{[(1*E*)-(substituted-2-furyl)-methylene]amino}quinazolin-4(3*H*)-one (**5, 6, 7**) **a – f** were synthesized by a multi-step process. These synthesized compounds are characterized by various spectroscopic techniques and evaluated for their antitubercular and anticancer activities. Biological activity indicated that some of the title compounds are potent antitubercular and anticancer agents.

Keywords: anticancer activity / antitubercular activity / furans / quinazolin-4-ones

Received: May 10, 2007; accepted: September 4, 2007

DOI 10.1002/ardp.200700096

Introduction

Substituted quinazolin-3(4*H*)-ones are among the versatile heterocyclic compounds, as they have a broad spectrum of pharmacological activities like antitubercular [1], anticancer [2 – 3], antibacterial [4], antifungal [5], anti-HIV [6], anthelmintic [7], anti-inflammatory [8], and antihypertensive activity [9]. It was also found that some of substituted furans possess potential anticancer [10], antitubercular [11], antibacterial [12], and antifungal activities [13]. Structure-activity relationship studies of the quinazolinone ring system in the literature [14 – 16] suggest that positions 2, 3, 6, and 8 are very much important for chemotherapeutic activity. Thus, it seemed of interest to combine the quinazolin-3(4*H*)-ones with substituted furans in a single molecule as compounds of this type can be expected to be biologically active and perhaps to exhibit a potential chemotherapeutic activity. This work represents a continuation of our systematic studies of quinazolin-3(4*H*)-ones as anticancer and antibacterial agents [17]. In the present contribution, we report herein the simple, novel and high yielding reactions of different furan-2-aldehydes with various 3-amino-quinazolin-4(3*H*)-ones to give substituted-3-{[(1*E*)-(substituted-2-furyl)-methylene]amino}quinazolin-4(3*H*)-one (**5, 6, 7**) **a–f**. The synthesized compounds were tested for their antitubercular and anticancer activities.

Results and discussion

Scheme 1 explains the reaction of different amino benzoic acids with benzoylchloride / acid anhydrides to yield substituted 4*H*-3,1-benzoxazin-4-ones (**1, 2**) **a – c**, which upon condensation with hydrazine hydrate produced 3-amino-substituted quinazolin-4(3*H*)-ones (**3, 4**) **a – c**. These 3-amino quinazolin-4(3*H*)-ones when treated with different furan-2-aldehydes in the presence of acid catalyst form substituted-3-{[(1*E*)-(substituted-2-furyl)methylene]amino}quinazolin-4(3*H*)-one (**5, 6, 7**) **a – f**. The infrared spectra of the 3-amino-quinazolin-4(3*H*)-ones (**3, 4**) **a – c** showed characteristic absorption bands at 3200 – 3300 cm^{-1} for amino (NH_2), which disappeared by the for-

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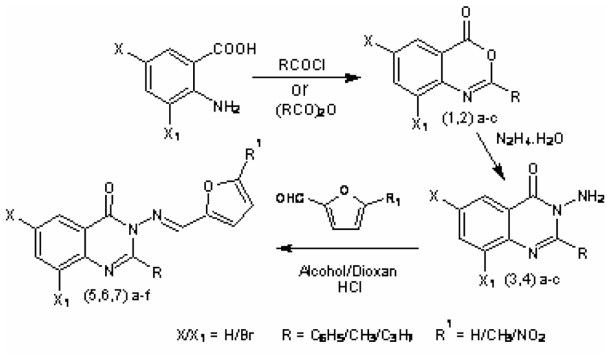
Abbreviations: Ehrlich Ascites Carcinoma (EAC); minimal inhibitory concentration (MIC); percentage increase in life span (% ILS); mean survival time (MST)



Scheme 1. Synthesis of presented compounds.

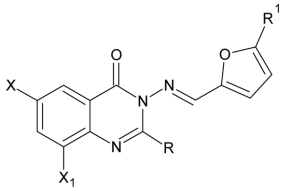
Table 1. Physical constants of the intermediates **1–4 a–c**.

Intermediates	Mp (°C)	R_f	Intermediates	Mp (°C)	R_f
1a	109	0.92	3a	172	0.38
1b	182	0.7	3b	155	0.41
1c	092	0.94	3c	235	0.63
2a	122	0.34	4a	172	0.11
2b	169	0.67	4b	225	0.33
2c	115	0.87	4c	182	0.57

mation of substituted-3-{[(1*E*)-(substituted-2-furyl)methylene]amino}quinazolin-4(3*H*)-one (**5, 6, 7**) **a–f**. Similarly, the ^{1}H-NMR spectra of title compounds (**5, 6, 7**) **a–f** showed characteristic singlets at d 8.6-9.2 due to the N=CH proton. Signals at δ 6.3–7.5 due to furyl protons established that all the 3-amino-quinazolin-4(3*H*)-ones (**3, 4**) **a–c** are converted into substituted-3-{[(1*E*)-(substituted-2-furyl)methylene]amino}quinazolin-4(3*H*)-one (**5, 6, 7**) **a–f**. Quinazolin-4-one and furan orient themselves on opposite sides of the C=N bond due to steric hindrance. Hence, all the title compounds have stable *E*-configuration at the C=N bond at position 3 of quinazolin-4-one as determined by ACD/Chemsketch software. The syntheses of compounds (**5, 6, 7**) **a–f** are mentioned in Scheme 1 and their reaction parameters are given in Table 2. The physical data for the intermediates (**1–4**) **a–c** are given in Table 1.

In-vitro antitubercular activity indicates that some of the tested compounds are promising candidates having a good activity against *M. tuberculosis* H_{37}Rv and *M. smegmatis*. Compounds **6c**, **7d**, **7f**, and **7c** showed more activity than standard ciprofloxacin. Compound **6a** was found to be equipotent to ciprofloxacin against *M. tuberculosis*.

Table 2. Physical parameters and *in-vitro* antitubercular activity of compounds **5–7 a–f.**



Compounds	X	X_1	R	R_1	Yield (%)	Mp (°C)	R_f	MIC (μg/mL) *M. tuberculosis*	MIC (μg/mL) *M. smegmatis*
5a	H	H	C_6H_5	H	50	165	0.62	> 12.5	50
5b	Br	H	C_6H_5	H	55	190	0.45	12.5	50
5c	Br	Br	C_6H_5	H	65	157	0.48	> 12.5	100
5d	H	H	C_6H_5	CH_3	70	120	0.32	> 12.5	50
5e	Br	H	C_6H_5	CH_3	45	191	0.37	> 12.5	50
5f	Br	Br	C_6H_5	CH_3	65	205	0.48	> 12.5	50
6a	Br	H	CH_3	H	50	206	0.31	1.56	12.5
6b	Br	Br	CH_3	H	60	220	0.48	3.13	12.5
6c	Br	Br	C_3H_7	H	70	195	0.71	0.2	0.78
6d	Br	H	CH_3	CH_3	50	185	0.60	> 12.5	50
6e	Br	Br	CH_3	CH_3	70	230	0.48	> 12.5	50
6f	Br	Br	C_3H_7	CH_3	75	192	0.80	> 12.5	50
7a	H	H	C_6H_5	NO_2	50	242	0.02	6.25	25
7b	Br	H	C_6H_5	NO_2	65	260	0.93	6.25	25
7c	Br	Br	C_6H_5	NO_2	75	240	0.48	0.78	6.25
7d	Br	H	CH_3	NO_2	60	197	0.30	0.78	3.13
7e	Br	Br	CH_3	NO_2	70	243	0.37	3.13	12.5
7f	Br	Br	C_3H_7	NO_2	78	205	0.42	0.78	3.13
Ciprofloxacin					–			1.56	< 0.78
Isoniazid					–			0.05	6.25

Table 3. *In-vitro* and *in-vivo* anticancer activity of compounds **5 – 7 a – f**.

Compounds	***In-vitro*** **anticancer activity Percentage cytotoxicity of drug at various concentrations on EAC cells (μg/mL)**				***In-vivo*** **anticancer activity**			
	1000	**500**	**250**	**125**	**Gain in body weight**	**Percentage decrease in body weight**	**MST[a] ± S.E.[b]**	% ILS
Control	–	–	–	–	5.46 ± 0.19	–	14.6 ± 0.21	–
5a	46.8	39.6	33.4	16.7	1.24 ± 0.087	77.28	25.0 ± 0.44	71.23***
5b	66.7	32.7	30.0	16.7	3.78 ± 0.398	30.76	18.6 ± 1.33	27.39*
5c	80.6	47.6	41.6	30.0	0.84 ± 0.0244	84.61	33.5 ± 0.76	129.45***
5d	25.4	24.5	24.5	18.0	ND	ND	ND	ND
5e	20.2	20.2	18.2	18.2	ND	ND	ND	ND
5f	18.5	18.5	17.5	18.5	ND	ND	ND	ND
6a	81.9	38	28.5	13.4	0.74 ± 0.06	86.44	36.6 ± 1.2	150.68***
6b	20.0	18.6	13.4	13.4	ND	ND	ND	ND
6c	30.9	25.0	20.0	8.3	ND	ND	ND	ND
6d	20.5	18.2	15.0	15.4	ND	ND	ND	ND
6e	19.5	18.5	18.5	10.0	ND	ND	ND	ND
6f	17.0	15.4	15.4	15.4	ND	ND	ND	ND
7a	33.3	26.2	16.3	16.3	ND	ND	ND	ND
7b	41.0	34.4	25.0	15.0	0.84 ± 0.024	84.61	24.1 ± 0.30	65.06***
7c	75.0	50.8	36.6	16.7	1.62 ± 0.1463	70.32	21.0 ± 0.36	43.83***
7d	50.0	38.4	25.0	13.4	0.92 ± 0.037	83.15	26.0 ± 0.57	78.08***
7e	13.7	13.4	13.4	13.4	ND	ND	ND	ND
7f	43.8	42.3	16.7	13.4	4.02 ± 0.037	26.37	16.0 ± 0.36	9.58*
Vincristine	86.7	69.0	46.0	43.0	0.9 ± 0.1183	83.51	32.0 ± 0.68	119.17***

[a] Mean survival time.
[b] Standard error.
[c] Not determined.
* $P < 0.05$; *** $P < 0.0001$.

Compounds **6b**, **7e**, **7a**, and **7b** were moderately active (Table 2).

In-vivo and *in-vitro* anticancer activity studies against Ehrlich Ascites Carcinoma (EAC) indicated that compounds **6a** and **5c** exhibited the highest degree of anticancer activity compared to standard vincristine (Table 3). Compound **7c** exhibited good *in-vitro* and moderate *in-vivo* anticancer activity. Compounds **5b**, **7d**, **5a**, **7f**, and **7b** had moderate anticancer activity. Other compounds had no significant anticancer activity.

Conclusions

Structure-activity relationship studies of the title compounds for antitubercular activity indicates unsubstituted furan or furan with electron-withdrawing group as in compounds **6c** ((minimal inhibitory concentrations) MIC 0.2 μg/mL), **7d** (MIC 0.78 μg/mL) seems to be more effective than furans having electron-donating group at position 3 of the quinazolinone ring as in compound **6f** (MIC > 12.5 μg/mL). An electron-withdrawing group on the aromatic portion of quinazolinone as in compounds **7c**, **f** (MIC 0.78 μg/mL) favors antitubercular activity against *M. tuberculosis* compared to non-halogen derivatives as in compound **5a** (MIC > 12.5 μg/mL) and **7a** (MIC 6.25 μg/mL). Alkyl substitutions are more effective than aryl substitution at position 2 of quinazolinone, as compound **6a** (MIC 1.56 μg/mL) is more potent than compound **5b** (MIC 12.5 μg/mL).

Structure-activity analysis of the synthesized compounds for anticancer activity indicates that the presence of a bromine at position 6 and 8 of quinazolinone as in compound **5c** (% ILS, 129.45) increases the percentage increase in life span (% ILS) as compared to non-halogen derivative compound **5a** (% ILS, 71.23). Interestingly, attachment of unsubstituted furan as in compound **6a** (% ILS, 150.68) is found to be more beneficial than nitro furan and methyl furan as in compounds **7d** (% ILS, 78.08) and **6d** (% ILS, not determined as its *in-vitro* activity was negligible), respectively. Methyl or phenyl substitution at position 2 of quinazolinone as in compounds **5a**, **5c**, **6a**, **7b**, **7c**, and **7d** prove more effective than the propyl analogues **7f** (% ILS, 9.58) and **6f** (% ILS, not determined as its *in vitro* activity was negligible).

In summary, with respect to these observations, we conclude that this series could be developed as a novel class of antitubercular and anticancer agents. However, further structural evaluation and pharmacological screening on different types of cancer cell lines is required to select the best possible potent molecule among the series.

The authors are thankful to the Indian Institute of Science, Bangalore, for the spectral analysis. The authors are also thankful to Sri. Premnath Reddy, Chairman; Acharya Institutes and Dr. Divakar Goli, Principal; Acharya and B. M. Reddy College of Pharmacy, Bangalore, for the laboratory facilities.

The authors have declared no conflict of interest.

Experimental

Chemistry

Melting points are uncorrected. IR spectra (cm^{-1}) were recorded on Shimadzu-8400 FTIR spectrophotometer (Shimadzu, Tokyo, Japan), NMR spectra on Brucker 400 MHz spectrometer (Bruker Bioscience, Billerica, MA, USA) and mass spectra on Micromass Q-tof Mass spectrometer (Micromass, Manchester, UK), Shimadzu QP 5050A and Shimadzu LCMS 2010A Mass spectrometer. Micro analysis was done using Thermo Finnigan FLASH EA 1122 CHNS analyzer (Thermo Electron Corporation, Bremen, Germany). The purity of the compounds were checked on silica gel 60 F_{254} coated aluminum TLC plates (Merck, Darmstadt, Germany) by using ethyl acetate and petroleum ether (1 : 4) as mobile phase and observed in UV light. Spectral data (IR, NMR, and mass spectra) confirmed the structures of synthesized compounds and the purity of these compounds was ascertained by microanalysis. Elemental (C, H, N) analyses indicated that the calculated and observed values were within the acceptance limits (± 0.4%). All chemicals and reagents were obtained from Lancaster (UK) and Merck (India) and were used without further purification.

*General procedure for synthesis of substituted-2-aryl-4H-3,1-benzoxazin-4-one **1a-c***

Different 2-amino benzoic acids (0.1 mol) were dissolved in 50 mL of pyridine. To this reaction mixture, benzoyl chloride (11.7 mL) was added with stirring at 27°C and stirring was continued for 20 min. Precipitates obtained were filtered, washed with petroleum ether, water, dried, and recrystallized from ethyl acetate as shining yellow crystals. IR spectra of these compounds show intense peaks at 3060 cm^{-1} (aromatic C-H stretching), 1766 cm^{-1} (C=O stretching), 1575, 1450 cm^{-1} (C=C ring stretching), 692 cm^{-1} (C-Br stretching). ^{1}H-NMR spectra of these compounds showed aromatic peaks at δ 7.5–8.5.

*General procedure for the synthesis of substituted-2-alkyl-4H-3,1-benzoxazin-4-one **2a–c***

Different 2-amino benzoic acids (0.01 mol) were refluxed with different acid anhydrides (40 mL) for 90 min. The reaction mixtures were cooled, crystals obtained were filtered, washed with water and dried. Compounds **2a–c** thus obtained were used in the next step without further purification. IR spectra of these compounds show intense peaks at 3030 (aromatic C-H stretching), 2987, 2817 cm^{-1} (CH_3 C-H stretching), 1749 cm^{-1} (C=O stretching), 1506, 1444 cm^{-1} (C=C ring stretching), 694 cm^{-1} (C-Br stretching). ^{1}H-NMR spectra of these compounds showed aromatic peaks at δ 7.5–8.5 and alkyl peaks at δ 1.0–2.48 (s, 3H, CH_3).

*General procedure for synthesis of 3-amino-2-aryl/alkylquinazolin-4(3H)-one **(3, 4) a-c***

Various benzoxazin-4-ones (**1**, **2**) **a–c** (0.01 mol) were refluxed with hydrazine hydrate (50 mL) for 3 h with occasional shaking. The reaction mixtures were cooled to room temperature. The crystals formed were filtered, washed with water, dried, and used in the next step without purification. IR spectra of these compounds show intense peaks at 3300, 3200 cm^{-1} for amino (NH_2), 3060 cm^{-1} for aromatic (C-H), 1660 cm^{-1} for carbonyl (C=O) and 1560, 1470 cm^{-1} for the aromatic ring (C=C) stretching. ^{1}H-NMR spectra of these compounds showed aromatic signals at δ 7.0–8.5, amino NH_2 signals at δ 5.0–5.5 as singlet and alkyl signals at δ 1–3.

*General procedure for synthesis of substituted-3-{[(1E)-(substituted-2-furyl)/(2-furyl)-methylene]amino}-quinazolin-4(3H)-one **(5, 6) a–f***

Different 3-amino-substituted-quinazolin-4(3*H*)-ones (**3**, **4**) **a–c** (0.006 mol) were dissolved in 50 mL of warm alcohol (dioxan for **3c**). To these, two drops of concentrated hydrochloric acid, followed by different furan-2-aldehydes (0.0062 mol) were added and refluxed for 5–6 h. On cooling, the crystals obtained were filtered, washed with petroleum ether, dried, and recrystallized from ethyl acetate to give (**5**, **6**) **a–f**.

*3-{[(1E)-2-Furylmethylene]amino}-2-phenylquinazolin-4(3H)-one **5a***

IR (KBr), ν (cm^{-1}): 3033 (aromatic C-H stretching), 1677 (C=O, stretching), 1602 (C=N stretching), 1558, 1473 (C=C ring stretching), 1355 (C-N stretching); ^{1}H-NMR, δ: 8.8–8.9 (s, 1H, N=CH), 8.2–8.26 (d, 1H, Ar-H), 7.7–7.8 (t, 1H, Ar-H), 7.4–8.1 (m, 7H, Ar-H), 7.2–7.3 (d, 1H, furan), 7.08–7.1 (d, 1H, furan), 6.7–6.78 (dd, 1H, furan); MS (m/z): 315 $[M]^+$. Anal. Calcd. (%) for $C_{19}H_{13}N_3O_2$: C, 72.37; H, 4.16; N, 13.33. Found: C, 72.65; H, 4.42; N, 13.26.

*6-Bromo-3-{[(1E)-2-furylmethylene]amino}-2-phenylquinazolin-4(3H)-one **5b***

IR (KBr), ν (cm^{-1}): 3030 (aromatic C-H stretching), 1672 (C=O, stretching), 1610 (C=N stretching), 1569, 1469 (C=C ring stretching), 1313 (C-N stretching), 686 (C-Br stretching); ^{1}H-NMR, δ: 8.8–8.9 (s, 1H, N=CH), 8.28–8.3 (s, 1H, Ar-H), 7.4–8.08 (m, 7H, Ar-H), 7.2–7.3 (d, 2H, furan), 6.7–6.78 (s, 1H, furan); MS (m/z): 394 $[M]^+$. Anal. Calcd. (%) for $C_{19}H_{12}BrN_3O_2$: C, 57.89; H, 3.07; N, 10.66. Found: C, 57.78; H, 3.42; N, 10.61

*6,8-Dibromo-3-{[(1E)-2-furylmethylene]amino}-2-phenylquinazolin-4(3H)-one **5c***

IR (KBr), ν (cm^{-1}): 3082 (aromatic C-H stretching), 1741 (C=O stretching), 1679 (C=N stretching), 1566, 1473 (C=C ring stretching), 1350 (C-N stretching), 690 (C-Br stretching); ^{1}H-NMR, δ: 8.8

(s, 1H, N=CH), 8.45 (s, 1H, Ar-H), 8.28 (s, 1H, Ar-H), 7.4-8 (m, 5H, Ar-H), 7.2 – 7.3 (d, 2H, furan), 6.7 – 6.78 (s, 1H, furan); MS (m/z): 473.6 $[M]^+$. Anal. Calcd. (%) for $C_{19}H_{11}Br_2N_3O_2$: C, 48.23; H, 2.34; N, 8.88. Found: C, 48.45; H, 2.57; N, 8.72.

3-{[(1E)-(5-Methyl-2-furyl)methylene]amino}-2-phenylquinazolin-4(3H)-one ***5d***

IR (KBr), ν (cm^{-1}): 3035 (aromatic C-H stretching), 2927, 2854 (CH_3 C-H stretching), 1753 (C=O stretching), 1681 (C=N stretching), 1562, 1473 (C=C ring stretching), 1325 (C-N stretching), 692 (C-Br stretching); ^{1}H-NMR, δ: 8.7 (s, 1H, N=CH), 8.2 – 8.3 (d, 1H, Ar-H), 7.8 – 7.9 (t, 1H, Ar-H), 7.4 – 7.8 (m, 7H, Ar-H), 7.1 – 7.2 (d, 1H, furan), 6.4 (d, 1H, furan), 2.35 (s, 3H, CH_3); MS (m/z): 329.9 $[M]^+$. Anal. Calcd. (%) for $C_{20}H_{15}N_3O_2$: C, 72.94; H, 4.59; N, 12.76. Found: C, 72.87; H, 4.82; N, 12.54.

6-Bromo-3-{[(1E)-(5-methyl-2-furyl)methylene]amino}-2-phenylquinazolin-4(3H)-one ***5e***

IR (KBr), ν (cm^{-1}): 3058 (aromatic C-H stretching), 2917, 2824 (CH_3 C-H stretching), 1679 (C=O stretching), 1641 (C=N stretching), 1556, 1444 (C=C ring stretching), 1305 (C-N stretching), 692 (C-Br stretching); ^{1}H-NMR, δ: 8.7 (s, 1H, N=CH), 8.5 – 8.6 (d, 1H, Ar-H), 8.2 (s, 1H, Ar-H), 7.4 – 8.15 (m, 6H, Ar-H), 6.8 – 7.2 (d, 1H, furan), 6.3 – 6.4 (d, 1H, furan), 2.34 (s, 3H, CH_3); MS (m/z): 409 $[M]^+$. Anal. Calcd. (%) for $C_{20}H_{14}BrN_3O_2$: C, 58.84; H, 3.46; N, 10.29. Found: C, 58.95; H, 3.54; N, 10.58.

6,8-Dibromo-3-{[(1E)-(5-methyl-2-furyl)methylene]amino}-2-phenylquinazolin-4(3H)-one ***5f***

IR (KBr), ν (cm^{-1}): 3074 (aromatic C-H stretching), 2927, 2844 (CH_3 C-H stretching), 1679 (C=O stretching), 1608 (C=N stretching), 1575, 1448 (C=C ring stretching), 1315 (C-N stretching), 694 (C-Br stretching); ^{1}H-NMR, δ: 8.68 (s, 1H, N=CH), 8.45 (s, 1H, Ar-H), 8.28 (s, 1H, Ar-H), 7.4 – 7.7 (m, 5H, Ar-H), 7.2 (d, 1H, furan), 6.4 (d, 1H, furan), 2.35 (s, 3H, CH_3); MS (m/z): 487.5 $[M]^+$. Anal. Calcd. (%) for $C_{20}H_{13}Br_2N_3O_2$: C, 49.31; H, 2.69; N, 8.63. Found: C, 49.70; H, 2.75; N, 8.91.

6-Bromo-3-{[(1E)-2-furylmethylene]amino}-2-methylquinazolin-4(3H)-one ***6a***

IR (KBr), ν (cm^{-1}): 3083 (aromatic C-H stretching), 2927, 2835 (CH_3 C-H stretching), 1672 (C=O stretching), 1620 (C=N stretching), 1596, 1469 (C=C ring stretching), 1317 (C-N stretching), 694 (C-Br stretching); ^{1}H-NMR, δ: 8.76 (s, 1H, N=CH), 8.2 (d, 1H, Ar-H), 8.1 (s, 1H, Ar-H), 7.9 – 8.1 (d, 1H, Ar-H), 7.58 – 7.62 (d, 1H, furan), 7.35 (d, 1H, furan), 6.8 (s, 1H, furan); MS (m/z): 332 $[M]^+$. Anal. Calcd. (%) for $C_{14}H_{10}BrN_3O_2$: C, 50.62; H, 3.03; N, 12.65. Found: C, 50.99; H, 2.72; N, 12.82.

6,8-Dibromo-3-{[(1E)-2-furylmethylene]amino}-2-methyl quinazolin-4(3H)-one ***6b***

IR (KBr), ν (cm^{-1}): 3083 (aromatic C-H), 2927, 2835 (CH_3 C-H), 1670 (C=O), 1616 (C=N), 1596, 1469 (C=C), 1317 (C-N), 692 (C-Br); ^{1}H-NMR, δ: 8.75 (s, 1H, N=CH), 8.35 (s, 1H, Ar-H), 8.2 (s, 1H, Ar-H), 7.9 – 8.1 (d, 1H, furan), 7.3 – 7.4 (d, 1H, furan), 6.8 (m, 1H, furan), 2.55 (s, 3H, CH_3); MS (m/z): 411.9 $[M + H]^+$. Anal. Calcd. (%) for $C_{14}H_9Br_2N_3O_2$: C, 40.91; H, 2.21; N, 10.22. Found: C, 41.29; H, 2.54; N, 10.26.

6,8-Dibromo-3-{[(1E)-2-furylmethylene]amino}-2-propylquinazolin-4(3H)-one ***6c***

IR (KBr), ν (cm^{-1}): 3086 (aromatic C-H stretching), 2981, 2939 (CH_3 C-H stretching), 1672 (C=O stretching), 1616 (C=N stretching), 1596, 1477 (C=C ring stretching), 1317 (C-N stretching), 792 (CH_2 C-H bending), 694 (C-Br stretching); ^{1}H-NMR, δ: 8.75 (s, 1H, N=CH), 8.35 (s, 1H, Ar-H), 8.2 (s, 1H, Ar-H), 8.1 (s, 1H, furan), 7.4 (d, 1H, furan), 6.8 (m, 1H, furan), 2.8 – 2.9 (dd, 4H, CH_2), 1.2 – 1.3 (t, 3H, CH_3); MS (m/z): 439.5 $[M]^+$. Anal. Calcd. (%) for $C_{16}H_{13}Br_2N_3O_2$: C, 43.76; H, 2.98; N, 9.57. Found: C, 43.51; H, 2.75; N, 9.87.

6-Bromo-2-methyl-3-{[(1E)-(5-methyl-2-furyl)methylene]amino}quinazolin-4(3H)-one 6d

IR (KBr), ν (cm^{-1}): 3062 (aromatic C-H stretching), 2923, 2852 (CH_3 C-H stretching), 1672 (C=O stretching), 1620 (C=N stretching), 1571, 1467 (C=C ring stretching), 1313 (C-N stretching), 692 (C-Br stretching); ^{1}H-NMR, δ: 8.6 (s, 1H, N=CH), 8.2 (s, 1H, Ar-H), 7.9 – 8 (d, 2H, Ar-H), 7.6 (d, 1H, furan), 6.48 (s, 1H, furan), 2.43 (s, 3H, CH_3), 2.1 (s, 3H, CH_3); MS (m/z): 346 $[M]^+$. Anal. Calcd. (%) for $C_{15}H_{12}BrN_3O_2$: C, 52.04; H, 3.49; N, 12.14. Found: C, 52.05; H, 3.54; N, 12.50.

6,8-Dibromo-2-methyl-3-{[(1E)-(5-methyl-2-furyl)methylene]amino}quinazolin-4(3H)-one ***6e***

IR (KBr), ν (cm^{-1}): 3076 (aromatic C-H), 2958, 2923 (CH_3 C-H), 1674 (C=O), 1610 (C=N), 1593, 1446 (C=C), 1309 (C-N), 694 (C-Br); ^{1}H-NMR, δ: 8.56 (s, 1H, N=CH), 8.37 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 7.28 (d, 1H, furan), 6.48 (d, 1H, furan), 2.45 (s, 3H, CH_3), 2.42 (s, 3H, CH_3); MS (m/z): 425 $[M]^+$. Anal. Calcd. (%) for $C_{15}H_{11}Br_2N_3O_2$: C, 42.38; H, 2.61; N, 9.89. Found: C, 42.47; H, 2.72; N, 10.13.

6,8-Dibromo-3-{[(1E)-(5-methyl-2-furyl)methylene]amino}-2-propylquinazolin-4(3H)-one ***6f***

IR (KBr), ν (cm^{-1}): 3083 (aromatic C-H stretching), 2981, 2933 (CH_3 C-H stretching), 1672 (C=O stretching), 1620 (C=N stretching), 1519, 1444 (C=C ring stretching), 1365 (C-N stretching), 792 (CH_2 C-H bending), 719 (C-Br stretching); ^{1}H-NMR, δ: 8.6 (s, 1H, Ar-H), 8.4 (s, 1H, N=CH), 8.2 (s, 1H, Ar-H), 7.1 (d, 1H, furan), 6.48 (d, 1H, furan), 2.8 – 2.9 (m, 4H, CH_2), 2.43 (s, 3H, CH_3), 1.2 – 1.3 (t, 3H, CH_3); MS (m/z): 453 $[M]^+$. Anal. Calcd. (%) for $C_{17}H_{15}Br_2N_3O_2$: C, 45.06; H, 3.34; N, 9.27. Found: C, 45.34; H, 3.59; N, 9.50.

General procedure for synthesis of substituted-3-{[(1E)-(5-nitro-2-furyl)methylene]amino}quinazolin-4(3H)-one ***7a – f***

(5-Nitro-2-furyl)methylene diacetate (0.0063 mol) was warmed in a mixture of 10 mL alcohol, 2 mL water, and 0.2 mL concentrated hydrochloric acid for 20 min. To this, different 3-amino-substituted-quinazolin-4(3*H*)-ones (**3**, **4**) **a – c** (0.006 mol) in 50 mL of warm alcohol (dioxan for **5c**) were added and refluxed for 5 – 6 h. On cooling, the crystals obtained were filtered, washed with petroleum ether, dried, and recrystallized from ethyl acetate to give **7a – f**.

3-{[(1E)-(5-Nitro-2-furyl)methylene]amino}-2-phenylquinazolin-4(3H)-one ***7a***

IR (KBr), ν (cm^{-1}): 3030 (aromatic C-H stretching), 1692 (C=O stretching), 1620 (C=N stretching), 1550, 1451 (C=C ring stretching), 1336 (C-NO_2 stretching); ^{1}H-NMR, δ: 9.2 (s, 1H, N=CH), 8.2 –

8.3 (d, 2H, Ar-H), 7.5 – 8 (m, 9H, Ar-H + furan); MS m/z: 361 [M + H]$^+$. Anal. Calcd. (%) for $C_{19}H_{12}N_4O_4$: C; 63.33, H; 3.36, N; 15.55. Found: C; 63.53, H; 3.57, N; 15.82.

*6-Bromo-3-{[(1E)-(5-nitro-2-furyl)methylene]amino}-2-phenylquinazolin-4(3H)-one **7b***

IR (KBr), ν (cm^{-1}): 3030 (aromatic C-H stretching), 1681 (C=O stretching), 1602 (C=N stretching), 1531, 1440 (C=C ring stretching), 1342 (C-NO_2 stretching), 692 (C-Br stretching); ^{1}H-NMR, δ: 9.15 (s, 1H, N=CH), 8.3 (s, 1H, Ar-H), 8 – 8.1 (dd, 2H, Ar-H), 7.4 – 7.8 (m, 7H, Ar-H + furan); MS m/z: 440.9 [M + H]$^+$. Anal. Calcd. (%) for $C_{19}H_{11}BrN_4O_4$: C; 51.96, H; 2.52, N; 12.76. Found: C; 52.0, H; 2.93, N; 12.44.

*6,8-Dibromo-3-{[(1E)-(5-nitro-2-furyl)methylene]amino}-2-phenylquinazolin-4(3H)-one **7c***

IR (KBr), ν (cm^{-1}): 3030 (aromatic C-H), 1681 (C=O), 1602 (C=N), 1562, 1446 (C=C), 1340 (C-NO_2), 692 (C-Br); ^{1}H-NMR, δ: 9.13 (s, 1H, N=CH), 8.23 (s, 1H, Ar-H), 7.98 – 8.03 (d, 1H, Ar-H), 7.8 – 7.9 (d, 1H, Ar-H), 7.6 – 7.7 (dd, 2H, furan), 2.65 (s, 3H, CH_3); MS (m/z): 518.9 [M + H]$^+$. Anal. Calcd. (%) for $C_{19}H_{10}Br_2N_4O_4$: C, 44.04; H, 1.95; N, 10.81. Found: C, 44.0; H, 2.30; N, 10.47.

*6-Bromo-2-methyl-3-{[(1E)-(5-nitro-2-furyl)methylene]amino}quinazolin-4(3H)-one **7d***

IR (KBr), ν (cm^{-1}): 3083 (aromatic C-H stretching), 2937, 2845 (CH_3, C-H stretching), 1674 (C=O stretching), 1614 (C=N stretching), 1533, 1496 (C=C ring stretching), 1330 (C-NO_2 stretching), 692 (C-Br stretching); ^{1}H-NMR, δ: 9.13 (s, 1H, N=CH), 8.23 (s, 1H, Ar-H), 7.98 – 8.03 (d, 1H, Ar-H), 7.8 – 7.9 (d, 1H, Ar-H), 7.6 – 7.7 (dd, 2H, furan), 2.65 (s, 3H, CH_3); MS (m/z): 377 [M]$^+$. Anal. Calcd. (%) for $C_{14}H_9BrN_4O_4$: C, 44.58; H, 2.41; N, 14.86. Found: C, 44.67; H, 2.57; N, 14.78.

*6,8-Dibromo-2-methyl-3-{[(1E)-(5-nitro-2-furyl)methylene]amino}quinazolin-4(3H)-one **7e***

IR (KBr), ν (cm^{-1}): 3080 (aromatic C-H stretching), 2922, 2852 (CH_3 C-H stretching), 1681 (C=O stretching), 1616 (C=N stretching), 1569, 1487 (C=C ring stretching), 1348 (C-NO_2 stretching), 692 (C-Br stretching); ^{1}H-NMR, δ: 9.09 (s, 1H, N=CH), 8.4 (s, 1H, Ar-H), 8.23 (s, 1H, Ar-H), 7.8 – 7.9 (d, 1H, furan), 7.6 – 7.7 (d, 1H, furan), 2.55 (s, 3H, CH_3); MS (m/z): 456 [M]$^+$, 479 [M + Na]$^+$. Anal. Calcd. (%) for $C_{14}H_8Br_2N_4O_4$: C, 36.87; H, 1.77; N, 12.29. Found: C, 36.67; H, 1.97; N, 12.52.

*6,8-Dibromo-3-{[(1E)-(5-nitro-2-furyl)methylene]amino}-2-propylquinazolin-4(3H)-one **7f***

IR (KBr), ν (cm^{-1}): 3072 (aromatic C-H stretching), 2970, 2928 (CH_3 C-H stretching), 1662 (C=O stretching), 1610 (C=N stretching), 1529, 1446 (C=C ring stretching), 1353 (C-N stretching), 759 (CH_2, C-H bending), 692 (C-Br stretching); ^{1}H-NMR, δ: 9.08 (s, 1H, N=CH), 8.4 (s, 1H, Ar-H), 8.23 (s, 1H, Ar-H), 7.85 – 7.9 (d, 1H, furan), 7.65 – 7.7 (d, 1H, furan), 2.8 – 2.9 (dd, 4H, CH_2), 1.2 – 1.3 (t, 3H, CH_3); MS (m/z): 484 [M]$^+$. Anal. Calcd. (%) for $C_{16}H_{12}Br_2N_4O_4$: C, 39.70; H, 2.50; N, 11.57. Found: C, 39.53; H, 2.34; N, 11.95.

Biological evaluation

Antitubercular activity

Compounds (**5, 6, 7**) **a – f** were evaluated for their *in-vitro* antitubercular activity by agar dilution method [18] against *M. tuberculosis* H_{37}Rv and *M. smegmatis*. The agar dilution method was performed using Middle brook 7H10 medium supplemented with Middle brook OADC medium (Hi-Media Lab., Mumbai, India). After solidification of the agar, the plates were inoculated with 0.1 mL of 10^{-4} dilutions of a McFarland 1.0 concentration of a suspension of organism. The inoculated plates were then incubated at 37°C for 4 weeks. The concentrations of the test compounds used were 0.19, 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, and 50 μg/mL. The minimum inhibitory concentration (MIC) was considered to be the lowest concentration that completely inhibited growth on agar plates, disregarding a single colony or a faint haze caused by the inoculums.

Anticancer activity

In-vitro anticancer activity of compounds (**5, 6, 7**) **a – f** against EAC cells was determined by trypan blue exclusion method [19]. The EAC cells were collected, counted, and adjusted to 10^6 cells/mL with normal saline. The drug dilutions were made with phosphate buffer saline (PBS) and were further adjusted to concentrations ranging from 125-1000 μg/mL. The drug dilutions were then added to the EAC cells and incubated at 37°C for 3 h. At the end of 3 h, the cell viability was determined and percentage cytotoxicity was calculated. The percentage cytotoxicity was calculated using the formula

$$\text{Percentage cytotoxicity} = 100 - T_c - D_c/T_c \times 100$$

where T_c = total EAC cells and D_c = dead EAC cells [17].

Compounds **5a-c**, **6a** and **7** (**b – d**, **f**) with significant *in-vitro* anticancer activity were further selected for screening *in-vivo* anticancer activity by determining different parameters like body weight analysis, mean survival time (MST) and percentage increase in life span (% ILS) [20]. The EAC cells containing 10^6 cells/0.1 mL of phosphate buffer saline were injected into the peritoneal cavity of all the animals (six swiss albino mice in each group). Treatment with test compounds (80 mg/kg body weight) and the standard vincristine (520 μg/kg body weight) was started 24 h after inoculation of cancer cells, once daily as a single dose in 0.3% CMC suspension by intraperitoneal route for 10 days. All the mice were weighed daily up to 11 days. The decrease in body weight and MST of the test and standard group animals were compared with control group. Results are shown in Table 3. Percentage decrease in the body weight was determined by using the formula, percentage decrease in body weight = ($G_c - G_t$) / $G_c \times 100$, where G_c = gain in body weight of control group, and G_t = gain in body weight of treated group. Percentage increase in life span was calculated by the formula,% ILS = (MST of treated group − MST of control group) / MST of control group 6 100. Student *t*-test was performed to ascertain the significance of the exhibited activity.

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