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Synthesis and anticancer activity evaluation of new 2-alkylcarbonyl and 2-benzoyl-3-trifluoromethyl-quinoxaline 1,4-di-*N*-oxide derivatives

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Abstract—As a continuation of our research in quinoxaline 1,4-di-*N*-oxide and with the aim of obtaining new anticancer agents, which can improve the current chemotherapeutic treatments, new series of 2-alkylcarbonyl and 2-benzoyl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide derivatives have been synthesized and evaluated for in vitro antitumor activity against a 3-cell line panel, consisting of MCF7 (breast), NCI-H460 (lung), and SF-268 (CNS). These active compounds were then evaluated in the full panel of 60 human tumor cell lines derived from nine cancer cell types. The results have shown that, in general, anticancer activity depends on the substituents in the carbonyl group, improving in the order: ethyl < isopropyl < *tert*-butyl < phenyl-ones. Among these, the compounds **4c**, **6e**, their difluorinated analogs (**4g** and **6g**), and **5c** were the most active, with mean GI_{50} values of 1.02, 0.42, 0.52, 0.15, and 0.49 μM, respectively. All of them were also found to inhibit the growth of the all of the Leukemia cell lines studied (with 75% of the GI_{50} values less than 0.15 μM) and therefore, were selected for further evaluation for the in vivo hollow fiber assays.

1. Introduction

The quinoxaline derivatives show very interesting biological properties (antibacterial, antiviral, anticancer, antifungal, antihelmintic, insecticidal).[1,2] Oxidation of both nitrogens of the quinoxaline ring dramatically increased the diversity of certain biological properties, such as antibacterial activity,[3–6] promotion of animal growth in feed additives,[7–9] and hypoxia-selective anticancer activity.[10]

Several kinds of compounds that were activated under hypoxic conditions are at various stages of development including agents derived from 1,2,4-benzotriazine 1,4-di-*N*-oxide[11] and quinoxaline 1,4-di-*N*-oxide.[12] Our group is involved in the synthesis and biological evaluation of new agents derived from quinoxaline 1,4-di-*N*-oxide and related compounds that have proved to be efficient cytotoxicity agents for hypoxic cells in solid tumors.[12–15]

In order to learn more about the structure–activity relationships, we have explored different alkylcarbonyl and benzoyl substituents in position 2 and trifluoromethyl group in position 3 of the quinoxaline ring, together with electron-donating and electron-withdrawing groups in positions 6 and/or 7. In this paper, we report the synthesis and biological studies of 34 new compounds with cytotoxic activity in human tumor cell lines.

2. Chemistry

The aforementioned compounds were prepared according to the synthetic process illustrated in Scheme 1. The starting compounds, 5-substituted or 5,6-disubstituted benzofuroxanes, **1a**–**g**, were obtained by previously described methods.[16]

The synthesis of compounds **2**(**a**–**c**, **e**–**g**), **3a**–**g**, **4a**–**g**, **5a**–**g**, and **6a**–**g** was carried out by the classical Beirut reaction. The appropriate benzofuroxane and the corresponding 1-(alkyl/phenyl)-4,4,4-trifluoromethyl-β-dicetone were dissolved in dry chloroform in the presence of triethylamine, which acted as the catalyst. When the reaction was finished, the solvent was evaporated to dryness and a yellow crude solid or brown oil was

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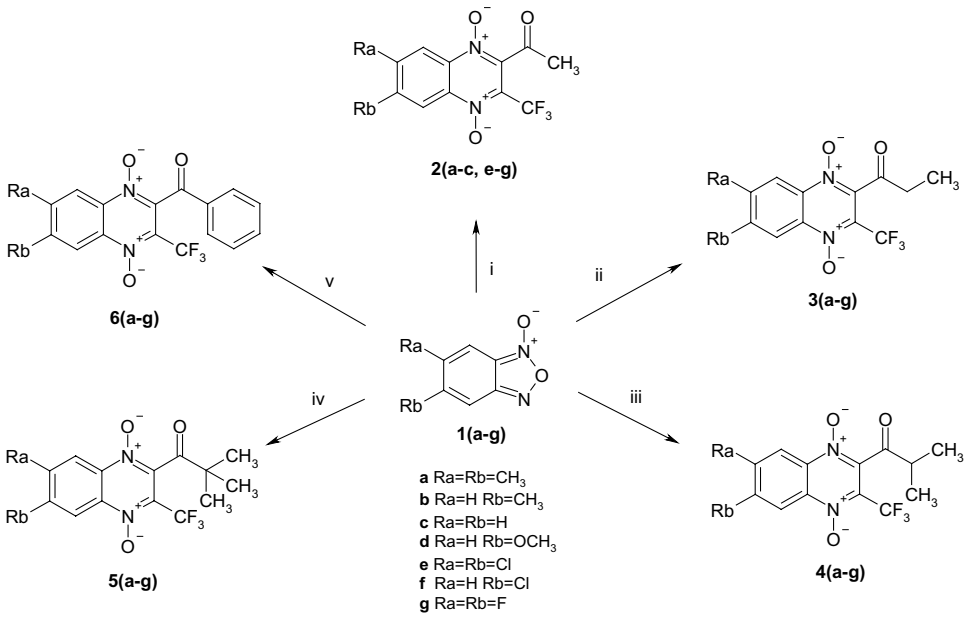

Scheme 1. Synthesis of 2-alkylcarbonyl and 2-benzoyl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide derivatives **2**(**a**–**c**, **e**–**g**), **3**(**a**–**g**), **4**(**a**–**g**), **5**(**a**–**g**) and **6**(**a**–**g**). Reagents and conditions: (i) 1,1,1-trifluoro-2,4-pentanedione, (ii) 1,1,1-trifluoro-2,4-hexanedione, (iii) 1,1,1-trifluoro-5-methyl-2,4-hexanedione, (iv) 1,1,1-trifluoro-5,5-dimethyl-2,4-hexanedione, and (v) 4,4,4-trifluoro-1-phenyl-1,3-butanedione; in dry chloroform.

obtained. Each compound was purified by either recrystallization or flash chromatography.

The formation of isomeric quinoxaline 1,4-di-*N*-oxides was observed in the case of monosubstituted benzofuroxanes. According to previous reports,[17] we have observed that 7-substituted quinoxaline 1,4-di-*N*-oxides were prevailing over the 6-isomer, or in the case of the methoxy substituent, only the 7-isomer was formed. In practice, the workup and purification allowed isolation of the 7-isomer.[18]

It is noteworthy to mention that the introduction of a CF_3 group on the Rc position, greatly increases the solubility of compounds and as a result, their ADME processes.

All of the compounds were chemically characterized by thin layer chromatography (TLC), melting point (mp), infrared (IR), and nuclear magnetic resonance (^{1}H NMR) spectra as well as elemental microanalysis.

3. Pharmacology

Compounds [**2**(**b**,**c**,**e**–**g**), **3**(**a**–**c**,**g**), **4**(**a**,**e**,**g**), **5**(**a**,**c**,**e**,**g**) and **6**(**a**,**c**,**e**,**g**)] in Table 1, were selected by the National Cancer Institute (NCI, Bethesda, USA) from among the 34 submitted (Scheme 1). Evaluation of anticancer activity was performed following the recognized[19] in vitro disease-oriented antitumor screening program.

Table 1. Primary anticancer assay (cell growth inhibitory activity) of compounds selected by the NCI

Compd	Percentage of growth inhibition			Activity
	NCI-H460 (lung)	MFC-7 (breast)	SF-268 (CNS)	
2b	18	0	16	+
2c	29	9	61	+
2e	−64	−49	−56	+
2f	0	0	0	+
2g	69	59	70	−
3a	63	98	110	−
3b	23	0	28	+
3c	36	95	84	−
3e	121	113	144	−
3g	0	0	0	+
4a	107	96	99	−
4c	0	0	0	+
4e	102	101	101	−
4g	28	100	100	+
5a	1	28	71	+
5c	0	0	0	+
5e	67	27	96	+
5g	106	106	100	−
6a	−62	−32	−86	+
6c	0	0	0	+
6e	1	78	41	+
6g	0	0	0	+

The compounds were evaluated in the 3-cell line one dose primary anticancer assay consisting of MCF7 (breast), NCI-H460 (lung), and SF-268 (CNS). The compounds, which passed the criteria set by the NCI for activity in this assay were scheduled automatically for evaluation against the full panel of 60 tumor cell lines.[20] The results of the active compounds were referred to the

Biological Evaluation Committee for Cancer Drugs (BEC/C) and compounds selected as leads from the large scale in vitro cell line screening were referred for preliminary testing in the hollow fiber-based screening. Compounds, which met the BEC/C criteria for further testing were then referred for evaluation in subcutaneous human tumor xenograft assays.

4. Results and discussion

The data of the in vitro anticancer activity is reported in Tables 1–3. The results in Table 1 suggest that most of the evaluated compounds passed the 3-cell line primary screening; moreover, their ratios increased when a phenyl group was introduced into the carbonyl position, regardless of the nature of the substituents in positions 6 and 7 on the quinoxaline ring.

Mean GI_{50}, TGI, and LC_{50} values on midpoints graph against 60 human tumor cell lines are summarized in Table 2. Having a look at the table, we can observe that most of the compounds showed an excellent anticancer activity, particularly the ones with electron-withdrawing groups in both positions 6 and 7 on the quinoxaline ring and their unsubstituted analogs. They showed mean GI_{50} values $<1\,\mu M$. Another important aspect that influenced the cytotoxic activity of the compounds is the nature of the group directly joined to the carbonyl group; increasing activity was observed in the following order: ethyl < isopropyl < *tert*-butyl < phenyl-ones. Among these compounds, **4c**, **6e**, their difluorinated derivatives (**4g** and **6g**), and **5c**, were the most active, with mean GI_{50} values of 1.02, 0.42, 0.52, 0.15, and $0.49\,\mu M$, respectively.

Throughout the entire series of products, the compounds with electron-releasing groups such as CH_3 and OCH_3 in positions 6 and 7 on the quinoxaline ring, showed notably decreasing activity. When halogen groups occupy these positions, the activity is increased, especially if two F atoms are introduced.

Table 2. $-\text{Log } GI_{50}$, $-\log$ TGI, and $-\log LC_{50}$ mean graph midpoints (MG_MID)[a] of the in vitro inhibitory activity test for compounds against 60 human tumor cells lines[b]

Compd	$-\text{Log } GI_{50}$ [μM]		$-\text{Log TGI}$	$-\text{Log } LC_{50}$
2b	5.49	3.23	4.63	4.19
2c	5.85	1.41	5.09	4.40
2e	6.24	0.57	5.76	5.20
2f	5.83	1.48	5.22	4.64
3b	ND	ND	ND	ND
3c	5.64	2.29	4.86	4.32
3g	6.18	0.66	5.59	4.91
4c	5.99	1.02	5.27	4.69
4g	6.28	0.52	5.67	5.01
5a	4.79	16.21	4.29	4.04
5c	6.31	0.49	5.78	5.35
5e	6.09	0.81	5.73	5.34
6a	5.73	1.86	5.09	4.44
6c	6.08	0.83	5.26	4.40
6e	6.38	0.42	5.97	5.54
6g	6.81	0.15	6.23	5.62

ND = No data available.

[a] (MG_MID) means graph midpoints, the average sensitivity of all of the cell lines toward the test agent.

[b] From the NCI.

In Table 3, the in vitro anticancer data recorded on subpanel cell lines for the six most active compounds is reported. Observing the activity results, an important fact can be concluded: the six most active compounds (without any exceptions), showed excellent activity against Leukemia cell lines, with 75% of the GI_{50} values studied being less than $0.15\,\mu M$. The 2-isobutyrylquinoxaline derivatives with a fluorine group in positions 6 and 7 on the ring (**4g**) and its unsubstituted analogs (**4c**), are especially active against CCRF-CEM, MOLT-4, RPMI-8226, and SR Leukemia cell lines. But the most sensitive compound on this chart is 6,7-difluoro-2-benzoyl-3-trifluoromethylquinoxaline 1,4-dioxide (**6g**), with GI_{50} values $\leqslant 0.024\,\mu M$, for all of the Leukemia cell lines. These values were 10 times better than those of its dichlorated analogs (**6e**). Furthermore, this compound (**6g**) had very good activity values against many of the cell lines studied in the rest of the charts.

The six compounds reported in Table 3 not only had very good Leukemia activity but they were also cytotoxic for other cancer types. For example, Colon cancer cell lines were most sensitive to compounds **4g** and **6e**.

Substitution of the 2-isobutyryl group (from the **4g** compound) with a 2-benzoyl group, and substitution of Cl on the positions 6 and 7 of the quinoxaline ring (from the **6e** compound) with F groups, resulted in the **6g** compound with important increasing activity.

On the other hand, most of compounds in Table 3 showed interesting results against Renal cancer, with GI_{50} values between 0.4 and $0.08\,\mu M$.

We are currently waiting for the Hollow Fiber assay results for preliminary in vivo testing; at present, we only have data for compound **6e**. This compound showed an 8 SC score and a 16 IP+SC score; 20 is the required value for further in vivo testing.

5. Conclusion

We have established a rapid and efficient protocol for the synthesis of 2-carbonyl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide derivatives. Here, we show that this class of compounds are especially active in Leukemia cell lines. The most active compound was 2-benzoyl-6,7-difluoro-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide. The presence of two F in positions 6 and 7 and the benzoyl group in position 2 on the quinoxaline ring is responsible for the increased activity. From this lead compound, more potent analogous can certainly be generated.

While the new fluorinated derivatives are being studied for biological depth, it encourages us to make an

Table 3. In vitro anticancer data recorded on subpanel cell lines for the most active compounds; A (GI_{50}), B (TGI), and C (LC_{50}) expressed in μM

Panel/cell line	3g			4c			4g			5c			6e			6g		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Leukemia																		
CCRF-CEM	0.108	0.555	>100	0.095	*	>100	0.021	>100	>100	0.141	1.11	3.58	0.127	*	*	<0.01	*	>100
HL-60 (TB)	0.638	0.655	>100	0.106	*	>100	—	—	—	0.204	1.08	4.45	0.270	0.797	3.62	<0.01	0.049	0.422
K-562	0.154	>100	>100	0.150	>100	>100	—	—	—	0.294	1.40	3.74	0.334	1.05	4.43	0.024	0.106	0.552
MOLT-4	0.143	>100	>100	—	—	—	0.382	>100	>100	0.037	0.254	0.758	0.147	0.329	0.739	0.018	0.062	0.407
RPMI-8226	0.262	0.806	7.80	0.038	0.337	>100	0.066	12.3	>100	0.023	0.246	0.768	0.119	0.463	32.0	—	—	—
SR	0.043	0.201	>100	0.038	>100	>100	—	—	—	<0.01	0.209	0.613	0.025	0.116	0.612	—	—	—
Non-small cell lung cancer																		
A549/ATCC	1.85	3.46	6.49	3.38	10.5	37.1	2.36	6.83	36.7	1.01	2.17	4.66	0.276	1.26	*	0.494	1.89	5.31
EKVX	1.67	3.97	9.45	0.383	1.62	5.67	1.45	3.08	6.55	1.17	2.72	6.30	1.03	2.20	4.69	0.240	0.630	3.81
HOP-62	1.17	2.40	4.90	2.19	3.95	7.14	0.597	1.77	4.46	1.03	2.20	4.69	0.059	0.183	0.457	0.183	0.336	0.616
HOP-92	0.327	1.38	4.25	0.171	0.708	3.95	0.904	2.30	5.48	0.238	1.28	3.58	—	—	—	0.164	0.324	0.642
NCI-H226	0.143	0.302	0.638	1.34	3.05	6.94	—	—	—	0.413	1.78	4.22	—	—	—	0.329	0.920	3.06
NCI-H23	1.17	2.73	6.35	1.66	3.28	6.49	0.306	1.38	5.76	1.17	2.39	4.89	0.165	0.346	0.725	0.020	0.050	0.206
NCI-H322M	1.41	2.88	5.90	1.72	6.96	28.4	2.01	3.88	7.52	1.46	4.37	15.7	1.84	3.34	6.07	0.536	1.80	4.33
NCI-H460	1.72	6.02	>100	0.149	0.473	*	1.59	4.92	>100	0.772	2.04	4.51	0.343	1.36	4.68	0.341	1.48	5.37
NCI-H522	0.271	0.709	>100	1.82	6.46	35.9	0.316	0.959	*	1.09	2.48	5.64	0.170	0.328	0.632	0.512	>100	>100
Colon cancer																		
COLO 205	0.466	3.16	>100	1.28	3.57	*	0.532	2.51	73.1	1.40	3.02	6.55	1.51	3.68	*	0.300	>100	>100
HCC-2998	1.45	2.90	5.80	1.45	3.07	6.49	0.326	1.37	4.93	—	—	—	—	—	—	—	—	—
HCT-116	—	—	—	2.26	*	*	0.113	0.234	*	0.166	0.473	1.67	0.172	0.316	0.580	0.016	0.034	0.069
HCT-15	1.91	>100	>100	3.09	>100	>100	0.149	0.346	*	0.753	2.03	4.50	0.197	0.412	*	0.032	0.189	*
HT29	0.228	0.492	8.25	39.1	>100	>100	0.168	0.547	>100	0.402	1.76	4.19	0.128	0.290	0.655	0.142	*	>100
KM12	1.59	3.30	6.85	0.197	0.632	3.31	1.83	4.69	19.0	0.667	1.96	4.43	0.182	0.366	0.738	0.196	0.439	0.984
SW-620	0.207	0.450	*	7.69	27.1	85.4	0.164	0.347	0.736	0.594	2.00	4.73	0.148	0.384	*	0.124	0.302	*
CNS cancer																		
SF-268	1.46	3.81	9.91	0.356	1.75	6.56	1.26	3.01	7.16	1.13	2.43	5.25	0.267	0.644	2.57	0.160	0.373	0.870
SF-295	0.618	2.01	5.27	0.261	1.26	3.91	1.01	2.28	5.14	0.368	1.53	3.91	1.48	2.80	5.29	0.427	1.58	4.67
SF-539	1.21	2.74	6.16	1.05	3.12	9.29	0.207	0.508	1.95	1.33	3.18	7.59	1.50	3.88	>100	0.172	0.319	0.549
SNB-19	1.68	5.37	>100	2.80	35.0	>100	1.75	3.44	6.74	1.32	2.87	6.25	1.35	2.74	5.57	0.193	0.533	>100
SNB-75	2.58	4.44	7.65	43.2	>100	>100	0.571	1.86	5.25	—	—	—	1.31	2.58	5.08	0.573	1.99	*
U251	1.64	4.14	>100	4.26	>100	>100	0.172	0.344	*	0.431	1.68	4.09	0.908	2.30	4.49	0.032	0.202	*
Melanoma																		
LOX IMVI	0.542	1.96	*	3.39	>100	>100	0.061	*	*	0.396	1.70	4.12	0.146	0.327	*	0.016	0.030	*
MALME-3M	0.632	2.06	5.58	0.213	0.857	3.53	0.408	1.77	6.37	1.09	2.42	5.39	1.50	3.17	6.70	0.123	0.261	0.555
M-14	0.330	0.650	2.43	1.60	3.41	*	0.232	0.830	22.0	0.637	1.87	4.32	0.370	1.56	3.95	0.108	0.227	0.477
SK-MEL-2	1.43	3.99	>100	2.01	5.58	46.9	1.25	3.12	*	1.70	4.92	>100	0.793	2.18	5.22	0.557	2.51	9.89
SK-MEL-28	1.04	2.78	7.43	—	—	—	0.591	2.01	5.32	0.792	2.08	4.70	1.62	3.11	5.96	0.264	0.855	*
SK-MEL-5	1.34	2.93	6.41	—	—	—	0.308	1.14	3.94	0.176	0.603	2.35	0.285	0.950	3.90	0.171	0.368	0.795
UACC-257	1.61	3.11	6.01	2.86	7.64	28.7	3.02	9.34	>100	1.47	2.78	5.27	1.32	2.81	5.97	0.943	2.27	5.28
UACC-62	0.251	0.653	2.35	1.59	3.17	6.34	0.777	2.04	4.64	1.02	2.18	4.67	1.04	2.41	5.58	0.254	0.741	2.67
Ovarian cancer																		
IGROV1	0.214	0.568	>100	1.04	3.47	25.9	0.393	1.92	*	0.181	0.644	2.50	0.153	0.335	0.734	0.491	2.91	>100
OVCAR-3	0.213	0.448	0.941	0.187	0.480	1.63	0.403	1.53	8.53	0.273	1.40	3.75	0.104	0.226	0.490	0.133	0.261	0.511
OVCAR-4	1.41	3.12	6.90	—	—	—	0.410	1.88	9.64	0.686	2.19	5.15	1.51	2.91	5.58	1.32	2.78	*

OVCAR-5	2.01	3.55	6.27	2.53	4.47	7.92	1.53	3.06	6.15	0.998	2.15	4.64	1.46	2.78	5.27	0.104	0.220	0.470
OVCAR-8	0.235	0.641	3.27	2.18	4.22	8.17	0.345	1.21	6.67	0.255	1.28	4.12	1.12	*	>100	0.152	0.305	0.611
SK-OV-3	2.16	4.53	9.49	2.66	>100	>100	1.95	4.17	8.89	1.67	3.07	5.64	1.59	2.93	5.41	0.410	1.64	*
Renal cancer																		
786-0	1.02	2.27	5.05	1.80	3.42	6.50	1.08	2.92	7.91	0.295	1.59	3.99	0.163	0.304	0.568	0.160	0.295	0.543
A498	—	—	—	17.4	36.0	74.5	—	—	—	0.230	1.06	3.27	—	—	—	0.424	1.79	4.54
ACHN	1.27	2.77	6.07	4.10	>100	>100	0.199	0.431	0.933	0.456	1.77	4.21	0.351	1.37	4.58	0.139	0.281	0.567
CAKI-1	0.248	0.580	2.01	0.409	1.70	4.90	0.386	1.58	6.00	0.479	1.63	4.03	—	—	—	0.145	0.277	0.526
RXF 393	1.22	3.56	13.3	0.704	2.60	*	1.31	3.19	7.79	1.24	2.49	4.99	1.06	2.39	5.37	0.201	0.432	0.929
SN12C	—	—	—	1.79	3.52	6.93	0.188	0.408	0.888	0.228	1.31	3.62	0.252	0.990	4.47	—	—	—
TK-10	0.843	2.18	5.07	0.202	0.836	3.36	0.974	2.89	8.50	0.884	2.11	4.60	0.635	2.02	4.84	0.082	0.219	0.504
UO-31	0.444	1.65	5.41	0.690	2.03	4.84	0.305	1.14	3.96	1.11	2.31	4.80	0.781	2.28	5.78	0.208	0.465	1.13
Prostate cancer																		
PC-3	1.34	2.81	5.89	0.131	0.637	*	0.191	0.467	2.25	0.377	1.67	4.08	0.242	0.904	4.05	0.225	0.487	1.19
DU-145	0.481	1.76	4.79	0.696	1.98	4.74	0.373	1.39	3.72	0.724	2.02	4.50	0.144	0.359	0.898	0.274	1.02	3.19
Breast cancer																		
MCF7	0.506	>100	>100	0.293	2.41	37.6	>100	>100	>100	0.617	1.98	4.45	0.177	0.426	1.13	0.128	0.289	0.649
NCI/ADR-RES	1.34	2.84	6.00	2.29	5.21	>100	0.291	0.922	4.59	0.571	2.36	7.73	1.70	4.42	>100	0.109	0.242	0.539
MDA-MB-231/ATCC	0.271	0.831	3.38	1.59	3.46	7.55	0.176	0.770	3.10	0.173	1.44	3.80	0.229	0.585	*	0.133	0.293	*
HS 578T	1.70	7.07	>100	14.3	37.1	95.7	1.21	4.23	>100	0.185	0.728	7.79	0.848	2.90	9.07	0.211	0.612	>100
MDA-MB-435	0.548	1.95	5.56	0.521	1.96	4.71	0.541	1.99	5.40	1.22	2.46	4.96	1.07	2.60	6.31	0.203	0.636	2.46
MDA-N	—	—	—	—	—	—	—	—	—	—	—	—	0.261	0.680	2.84	—	—	—
BT-549	1.23	2.71	5.95	0.569	2.16	6.68	0.684	2.01	4.76	—	—	—	0.666	2.27	6.08	0.238	0.720	3.60
T-47D	0.570	7.04	>100	0.543	2.60	>100	1.26	3.96	>100	1.60	3.82	9.16	—	—	—	0.379	>100	>100

(*) Below 40% growth inhibition, (—) not tested in this cell line.

ADME processes revision of this type of structures by comparing with not fluorinated compounds.

6. Experimental

6.1. Chemistry

Melting points were determined with a Mettler FP82+FP80 apparatus (Greifense, Switzerland) and have not been corrected. The ^{1}H NMR spectra were recorded on a Bruker AC-200E instrument (200 MHz) and Bruker 400 Ultrashield™ (400 MHz), using TMS as the internal standard and with DMSO-d_6 and $CDCl_3$ as the solvent; the chemical shifts are reported in ppm (δ) and coupling constant (J) values are given in Hertz (Hz). Signal multiplicities are represented by: s (singlet), d (doublet), t (triplet), c (cuadruplet), ds (double singlet), dd (double doublet), m (multiplet), and br s (broad singlet). The IR spectra were performed on a Perkin Elmer 1600 FTIR (Norwalk, CT, USA) in KBr pellets; the frequencies are expressed in cm^{-1}. Elemental microanalyses were obtained on an Elemental Analyzer (Carlo Erba 1106, Milan, Italy) from vacuum-dried samples. The analytical results for C, H, and N, were within ±0.4 of the theoretical values.

Alugram® SIL G/UV_{254} (layer: 0.2 mm) (Macherey-Nagel GmbH & Co. KG. Postfach 101352. D-52313 Düren, Germany) was used for thin layer chromatography and Silica gel 60 (0.040–0.063 mm) for Column flash Chromatography (Merck). HPLC conditions: Column Nova Pack C18 60 A 4 μm (3.9×150 mm); mobile phase: acetonitrile/water (60:40); flux: 1 mL/min.

Chemicals were purchased from E. Merck (Darmstadt, Germany), Scharlau (F.E.R.O.S.A., Barcelona, Spain), Panreac Química S.A. (Montcada i Reixac, Barcelona, Spain), Sigma-Aldrich Química, S.A., (Alcobendas, Madrid), Acros Organics (Janssen Pharmaceuticalaan 3a, 2440 Geel, België), and Lancaster (Bischheim-Strasbourg, France).

6.2. General procedure for synthesis

The corresponding 1-(alkyl/phenyl)-4,4,4-trifluoromethyl-β-dicetone (10.6 mmol) was added to a solution of the appropriate benzofuroxane (2.4 mmol) in dry chloroform (35 mL). The mixture was allowed to stand at 0 °C. Triethylamine was added drop by drop (1 mL), and the reaction mixture was stirred at room temperature in darkness for 1–5 days. After evaporating to dryness under pressure, a crude solid or a brown oil was obtained. It was then precipitated and washed by adding diethyl ether (or *n*-hexane), affording the target compound. The obtained yellow precipitate was purified by recrystallization. Flash column chromatography on silica gel (flash chromatography) was applied when necessary.

6.2.1. 2-Acetyl-6,7-dimethyl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (2a). This compound was obtained in 8% yield and then purified by recrystallization using MeOH; mp 213–214 °C; IR (KBr) ν 1730, 1340, 1171, 930 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.57 (s, 6H, $2CH_3$–Ar), 2.71 (s, 3H, $COCH_3$), 8.33 (s, 1H, H_5), 8.38 (s, 1H, H_8) ppm. Anal. ($C_{13}H_{11}F_3N_2O_3$) C, H, N; C(%): calcd: 52.00; found: 52.25. H(%): calcd: 3.66; found: 3.37. N(%): calcd: 9.33; found: 9.52. HPLC: $R_t = 2.60$ min.

6.2.2. 2-Acetyl-7-methyl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (2b). This compound was obtained in 5% yield and then purified by flash chromatography, eluting with DCM/MeOH 98:2. It was then recrystallized from MeOH; mp 137–138 °C; IR (KBr) ν 1731, 1349, 1250, 1175, 925 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.68 (s, 3H, CH_3–Ar), 2.71 (s, 3H, $COCH_3$), 7.79 (d, 1H, H_6, $J_{6-5} = 8.9$ Hz), 8.38 (s, 1H, H_8), 8.53 (d, 1H, H_5, $J_{5-6} = 9.0$ Hz) ppm. Anal. ($C_{12}H_9F_3N_2O_3$) C, H, N; C(%): calcd: 50.35; found: 50.34. H(%): calcd: 3.14; found: 3.25. N(%): calcd: 9.79; found: 9.35. HPLC: $R_t = 2.73$ min.

6.2.3. 2-Acetyl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (2c). This compound was obtained in 3% yield and then purified by flash chromatography, eluting with DCM/MeOH 98.5:1.5. It was then washed with diethyl ether; mp 175–176 °C; IR (KBr) ν 1728, 1357, 1166, 931 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.73 (s, 3H, $COCH_3$), 7.97–8.04 (m, 2H, H_6+H_7), 8.61 (dd, 1H, H_5, $J_{5-6} = 9.2$ Hz, $J_{5-7} = 1.6$ Hz), 8.66 (dd, 1H, H_8, $J_{8-7} = 6.8$ Hz, $J_{8-6} = 1.8$ Hz) ppm. Anal. ($C_{11}H_7F_3N_2O_3$) C, H, N; C(%): calcd: 48.52; found: 48.68. H(%): calcd: 2.57; found: 2.59. N(%): calcd: 10.29; found: 10.26.

6.2.4. 2-Acetyl-6,7-dichloro-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (2e). This compound was obtained in 17% yield and then purified by recrystallization using MeOH; mp 199–200 °C; IR (KBr) 1734, 1332, 1250,1164 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.60 (s, 3H, $COCH_3$), 8.70 (s, 1H, H_5), 8.75 (s, 1H, H_8) ppm. Anal. ($C_{11}H_5Cl_2F_3N_2O_3$) C, H, N; C(%): calcd: 38.71; found: 38.93. H(%): calcd: 1.47; found: 1.53. N(%): calcd: 8.21; found: 8.26. HPLC: $R_t = 8.71$ min.

6.2.5. 2-Acetyl-7-chloro-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (2f). This compound was obtained in 3% yield and then was purified by flash chromatography, eluting with DCM. It was then recrystallized from *n*-hexane/AcOEt; mp 182–183 °C; IR (KBr) ν 1729, 1354, 1262, 1182 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.71 (s, 3H, $COCH_3$), 7.90 (dd, 1H, H_6, $J_{6-5} = 7.1$ Hz, $J_{6-8} = 2.1$ Hz), 8.58 (s, 1H, H_8), 8.60 (d, 1H, H_5, $J_{5-6} = 7.0$ Hz) ppm. Anal. ($C_{11}H_6ClF_3N_2O_3$) C, H, N; C(%): calcd: 43.07; found: 42.68. H(%): calcd: 1.96; found: 1.81. N(%): calcd: 9.13; found: 9.34. HPLC: $R_t = 5.35$ min.

6.2.6. 2-Acetyl-6,7-difluoro-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (2g). This compound was obtained in 39% yield and then purified by flash chromatography,

eluting with DCM. It was then recrystallized from *n*-hexane/AcOEt; mp 180–181 °C; IR (KBr) *ν* 1732, 1347, 1251, 1101 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.61 (s, 3H, $COCH_3$), 8.58 (dd, 1H, H_5, $J_{5-6} = 7.4$ Hz), 8.64 (dd, 1H, H_8, $J_{8-7} = 7.4$ Hz) ppm. Anal. ($C_{11}H_5F_5N_2O_3$) C, H, N; C(%): calcd: 42.86; found: 43.22. H(%): calcd: 1.62; found: 1.39. N(%): calcd: 9.09; found: 8.95. HPLC: $R_t = 4.80$ min.

6.2.7. 6,7-Dimethyl-2-propionyl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (3a). This compound was obtained in 3% yield and then purified by flash chromatography, eluting with DCM/MeOH 98:2. It was then washed with diethyl ether; mp 184–185 °C; IR (KBr) *ν* 1735, 1508, 1343, 1151, 913 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.32 (t, 3H, CH_2CH_3, $J = 7.2$ Hz), 2.57 (s, 6H, $2CH_3$–Ar), 2.97 (c, 2H, CH_2CH_3), 8.32 (s, 1H, H_5), 8.66 (s, 1H, H_8) ppm. Anal. ($C_{14}H_{13}F_3N_2O_3$) C, H, N; C(%): calcd: 53.50; found: 53.60. H(%): calcd: 4.14; found: 4.23. N(%): calcd: 8.91; found: 8.55. HPLC: $R_t = 3.82$ min.

6.2.8. 7-Methyl-2-propionyl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (3b). This compound was obtained in 5% yield and then purified by flash chromatography, eluting with DCM/MeOH 99.5:0.5. It was then washed with diethyl ether; mp 153–154 °C; IR (KBr) *ν* 1734, 1349, 1249, 1165, 914 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33 (t, 3H, CH_2CH_3, $J = 7.1$ Hz), 2.68 (s, 3H, CH_3–Ar), 2.97 (c, 2H, CH_2CH_3), 7.78 (d, 1H, H_6, $J_{6-5} = 8.8$ Hz,), 8.37 (s, 1H, H_8), 8.53 (d, 1H, H_5, $J_{5-6} = 8.8$ Hz) ppm. Anal. ($C_{13}H_{11}F_3N_2O_3$) C, H, N; C(%): calcd: 52.00; found: 52.32. H(%): calcd: 3.66; found: 3.62. N(%): calcd: 9.33; found: 9.21. HPLC: $R_t = 2.82$ min.

6.2.9. 2-Propionyl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (3c). This compound was obtained in 3% yield and then purified by flash chromatography, eluting with DCM. It was then washed with diethyl ether; mp 139–140 °C; IR (KBr) *ν* 1730, 1508, 1360, 1151, 908 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33 (t, 3H, CH_2CH_3, $J = 7.2$ Hz), 2.97 (c, 2H, CH_2CH_3), 7.96–8.04 (m, 2H, H_6+H_7), 8.60 (d, 1H, H_5, $J_{5-6} = 9.0$ Hz), 8.66 (d, 1H, H_8, $J_{8-7} = 8.9$ Hz) ppm. Anal. ($C_{12}H_9F_3N_2O_3$) C, H, N; C(%): calcd: 50.35; found: 51.01. H(%): calcd: 3.15; found: 3.11. N(%): calcd: 9.79; found: 9.56. HPLC: $R_t = 2.83$ min.

6.2.10. 7-Methoxy-2-propionyl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (3d). This compound was obtained in 3% yield and then purified by recrystallization using MeOH; mp 163–164 °C; IR (KBr) *ν* 1730, 1465, 1354, 1219, 1150, 909 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33 (t, 3H, CH_2CH_3, $J = 7.2$ Hz), 2.97 (c, 2H, CH_2CH_3), 4.05 (s, 3H, OCH_3), 7.53 (dd, 1H, H_6, $J_{6-5} = 9.5$ Hz, $J_{6-8} = 2.6$ Hz), 7.84 (ds, 1H, H_8, $J_{8-6} = 2.6$ Hz), 8.53 (d, 1H, H_5, $J_{5-6} = 9.5$ Hz) ppm. Anal. ($C_{13}H_{11}F_3N_2O_4$) C, H, N; C(%): calcd: 49.36; found: 49.50. H(%): calcd: 3.48; found: 3.43. N(%): calcd: 8.86; found: 8.92. HPLC: $R_t = 2.98$ min.

6.2.11. 6,7-Dichloro-2-propionyl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (3e). This compound was obtained in 13% yield and then purified by recrystallization using MeOH; mp 202–203 °C; IR (KBr) *ν* 1734, 1339, 1252, 1172, 1146 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.15 (t, 3H, CH_2CH_3, $J = 7.1$ Hz), 2.91 (c, 2H, CH_2CH_3, $J = 7.1$ Hz), 8.69 (s, 1H, H_5), 8.76 (s, 1H, H_8) ppm. Anal. ($C_{12}H_7Cl_2F_3N_2O_3$) C, H, N; C(%): calcd: 40.56; found: 40.48. H(%): calcd: 1.97; found: 1.88. N(%): calcd: 7.88; found: 7.86. HPLC: $R_t = 5.23$ min.

6.2.12. 7-Chloro-2-propionyl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (3f). This compound was obtained in 4% yield and then purified by flash chromatography, eluting with DCM. It was then recrystallized from *n*-hexane/AcOEt; mp 134–135 °C; IR (KBr) *ν* 1733, 1348, 1267, 1164, 1044 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33 (t, 3H, CH_2CH_3, $J = 7.1$ Hz), 2.96 (c, 2H, CH_2CH_3, $J = 7.1$ Hz), 7.90 (dd, 1H, H_6, $J_{6-5} = 9.2$ Hz, $J_{6-8} = 1.9$ Hz), 8.58 (s, 1H, H_8), 8.76 (d, 1H, H_5, $J_{5-6} = 9.1$ Hz) ppm. Anal. ($C_{12}H_8ClF_3N_2O_3$) C, H, N; C(%): calcd: 44.93; found: 45.18. H(%): calcd: 2.50; found: 2.47. N(%): calcd: 8.74; found: 8.57. HPLC: $R_t = 4.02$ min.

6.2.13. 6,7-Difluoro-2-propionyl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (3g). This compound was obtained in 15% yield and then purified by flash chromatography, eluting with DCM. It was then recrystallized from MeOH; mp 192–193 °C; IR (KBr) *ν* 1731, 1349, 1248, 1209, 1137 cm^{-1}; ^1H NMR (DMSO-d_6 δ 1.15 (t, 3H, CH_2CH_3, $J = 7.1$ Hz), 2.91 (c, 2H, CH_2CH_3, $J = 7.1$ Hz), 8.57 (dd, 1H, H_5 $J_{5-6} = 7.6$), 8.63 (dd, 1H, H_8, $J_{8-7} = 7.6$ Hz) ppm. Anal. ($C_{12}H_7F_5N_2O_3$) C, H, N; C(%): calcd: 44.72; found: 44.87. H(%): calcd: 2.19; found: 2.15. N(%): calcd: 8.69; found: 8.50. HPLC: $R_t = 5.95$ min.

6.2.14. 6,7-Dimethyl-2-isobutyryl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (4a). This compound was obtained in 4% yield and then purified by flash chromatography, eluting with DCM/MeOH 99:1, and then recrystallized from *n*-hexane; mp 156–157 °C; IR (KBr) *ν* 1725, 1456, 1335, 1173, 1147, 924 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.32 (d, 6H, $CH(CH_3)_2$), 2.56 (s, 6H, $2CH_3$–Ar), 3.16 (m, 1H, $COCH(CH_3)_2$, $J = 7.0$ Hz), 8.31 (s, 1H, H_5), 8.38 (s, 1H, H_8) ppm. Anal. ($C_{15}H_{15}F_3N_2O_3$) C, H, N; C(%): calcd: 54.87; found: 55.15. H(%): calcd: 4.57; found: 4.50. N(%): calcd: 8.53; found: 8.42. HPLC: $R_t = 5.05$ min.

6.2.15. 7-Methyl-2-isobutyryl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (4b). This compound was obtained in 7% yield and then purified by flash chromatography, eluting with DCM/MeOH 99:1. It was then recrystallized from *n*-hexane; mp 122–123 °C; IR (KBr) *ν* 1725, 1457, 1352, 1247, 1180, 1151, 925 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.32 (d, 6H, $CH(CH_3)_2$), 2.67 (s, 3H, CH_3–Ar), 3.16 (m, 1H, $COCH(CH_3)_2$, $J = 7.0$ Hz), 7.78 (d, 1H, H_6, $J_{6-5} = 8.8$ Hz), 8.36 (s, 1H, H_8), 8.53 (d, 1H, H_5,

$J_{5-6} = 8.8$ Hz) ppm. Anal. ($C_{14}H_{13}F_3N_2O_3$) C, H, N; C(%): calcd: 53.50; found: 53.20. H(%): calcd: 4.14; found: 4.10. N(%): calcd: 8.91; found: 8.88. HPLC: $R_t = 4.29$ min.

6.2.16. 2-Isobutyryl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (4c). This compound was obtained in 11% yield and then purified by flash chromatography, eluting with DCM. It was then recrystallized from *n*-hexane; mp 120–121 °C; IR (KBr) ν 1722, 1447, 1358, 1250, 1161, 922 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.33 (d, 6H, $CH(CH_3)_2$), 3.18 (m, 1H, $COCH(CH_3)_2$, $J = 7.0$ Hz), 7.97–8.04 (m, 2H, H_6+H_7), 8.59 (dd, 1H, H_5, $J_{5-6} = 8.3$ Hz, $J_{5-7} = 1.7$ Hz), 8.66 (dd, 1H, H_8, $J_{8-7} = 8.2$ Hz, $J_{8-6} = 1.7$ Hz) ppm. Anal. ($C_{13}H_{11}F_3N_2O_3$) C, H, N; C(%): calcd: 52.00; found: 51.93. H(%): calcd: 3.66; found: 3.38. N(%): calcd: 9.33; found: 9.30. HPLC: $R_t = 3.56$ min.

6.2.17. 7-Methoxyl-2-isobutyryl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (4d). This compound was obtained in 12% yield and then purified by flash chromatography, eluting with DCM/MeOH 99.5:0.5. It was then washed with diethyl ether; mp 137–138 °C; IR (KBr) ν 1720, 1610, 1348, 1219, 1168, 925 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.34 (d, 6H, $CH(CH_3)_2$), 3.17 (m, 1H, $COCH(CH_3)_2$), 4.05 (s, 3H, OCH_3), 7.53 (dd, 1H, H_6, $J_{6-5} = 9.5$ Hz, $J_{6-8} = 2.7$ Hz), 7.84 (ds, 1H, H_8, $J_{8-6} = 2.7$ Hz), 8.54 (d, 1H, H_5, $J_{5-6} = 9.5$ Hz) ppm. Anal. ($C_{14}H_{13}F_3N_2O_4$) C, H, N; C(%): calcd: 50.91; found: 51.53. H(%): calcd: 3.93; found: 3.88. N(%): calcd: 8.48; found: 8.10. HPLC: $R_t = 3.91$ min.

6.2.18. 6,7-Dichloro-2-isobutyryl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (4e). This compound was obtained in 22% yield and then purified by flash chromatography, eluting with DCM. It was then recrystallized from MeOH; mp 161–162 °C; IR (KBr) ν 1723, 1400, 1331, 1249, 1156, 1047 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.33 (d, 6H, $CH(CH_3)_2$, $J = 7.0$ Hz), 3.10–3.18 (m, 1H, $COCH(CH_3)_2$), 8.69 (s, 1H, H_5), 8.74 (s, 1H, H_8) ppm. Anal. ($C_{13}H_9Cl_2F_3N_2O_3$) C, H, N; C(%): calcd: 44.28; found: 44.35. H(%): calcd: 2.44; found: 2.51. N(%): calcd: 7.59; found: 7.65. HPLC: $R_t = 8.26$ min.

6.2.19. 7-Chloro-2-isobutyryl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (4f). This compound was obtained in 4% yield and then purified by flash chromatography, eluting with DCM. It was then washed with diethyl ether; mp 120–121 °C; IR (KBr) ν 1724, 1407, 1350, 1265, 1154, 1042 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.33 (d, 6H, $CH(CH_3)_2$, $J = 7.0$ Hz), 3.10–3.19 (m, 1H, $COCH(CH_3)_2$), 7.90 (dd, 1H, H_6, $J_{6-5} = 9.2$ Hz, $J_{6-8} = 2.2$ Hz), 8.58 (s, 1H, H_8), 8.60 (d, 1H, H_5, $J_{5-6} = 9.3$ Hz) ppm. Anal. ($C_{13}H_{10}ClF_3N_2O_3$) C, H, N; C(%): calcd: 46.64; found: 46.74. H(%): calcd: 2.99; found: 2.92. N(%): calcd: 8.37; found: 8.40. HPLC: $R_t = 5.58$ min.

6.2.20. 6,7-Difluoro-2-isobutyryl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (4g). This compound was obtained in 46% yield and then purified by flash chromatography, eluting with DCM. It was then recrystallized from MeOH; mp 168–169 °C; IR (KBr) ν 1729, 1424, 1359, 1247, 1183, 1132 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.33 (d, 6H, $CH(CH_3)_2$, $J = 7.0$ Hz), 3.11–3.19 (m, 1H, $COCH(CH_3)_2$), 8.39 (dd, 1H, H_5, $J_{5-6} = 7.2$ Hz), 8.45 (dd, 1H, H_8, $J_{8-7} = 7.2$ Hz) ppm. Anal. ($C_{13}H_9F_5N_2O_3$) C, H, N; C(%): calcd: 46.43; found: 46.19. H(%): calcd: 2.68; found: 2.67. N(%): calcd: 8.33; found: 8.24. HPLC: $R_t = 4.87$ min.

6.2.21. 6,7-Dimethyl-2-(2,2-dimethylpropanoyl)-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (5a). This compound was obtained in 4% yield and then purified by flash chromatography, eluting with DCM; mp 161–162 °C; IR (KBr) ν 1718, 1451, 1333, 1177, 911 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 9H, $C(CH_3)_3$), 2.56 (s, 6H, $2CH_3$–Ar), 8.32 (s, 1H, H_5), 8.38 (s, 1H, H_8) ppm. Anal. ($C_{16}H_{17}F_3N_2O_3$) C, H, N; C(%): calcd: 56.14; found: 56.24. H(%): calcd: 4.97; found: 4.93. N(%): calcd: 8.18; found: 7.90. HPLC: $R_t = 11.82$ min.

6.2.22. 7-Methyl-2-(2,2-dimethylpropanoyl)-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (5b). This compound was obtained in 5% yield and then purified by flash chromatography, eluting with DCM; mp 127–128 °C; IR (KBr) ν 1717, 1348, 1165, 906 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9H, $C(CH_3)_3$), 2.67 (s, 3H, CH_3–Ar), 7.77 (dd, 1H, H_6, $J_{6-5} = 8.8$ Hz, $J_{6-8} = 1.7$ Hz), 8.37 (s, 1H, H_8), 8.53 (d, 1H, H_5, $J_{5-6} = 8.8$ Hz) ppm. Anal. ($C_{15}H_{15}F_3N_2O_3$) C, H, N; C(%): calcd: 54.87; found: 55.27. H(%): calcd: 4.57; found: 4.64. N(%): calcd: 8.53; found: 8.23. HPLC: $R_t = 9.85$ min.

6.2.23. 2-(2,2-Dimethylpropanoyl)-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (5c). This compound was obtained in 5% yield and then purified by flash chromatography, eluting with DCM/MeOH 99.5:0.5; mp 125–126 °C; IR (KBr) ν 1714, 1353, 1163, 909 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H, $C(CH_3)_3$), 7.97–8.03 (m, 2H, H_6+H_7), 8.59 (dd, 1H, H_5, $J_{5-6} = 8.2$ Hz, $J_{5-7} = 1.6$ Hz), 8.65 (dd, 1H, H_8, $J_{8-7} = 8.2$ Hz, $J_{8-6} = 1.6$ Hz) ppm. Anal. $C_{14}H_{13}F_3N_2O_3$) C, H, N; C(%): calcd: 53.50; found: 53.35. H(%): calcd: 4.14; found: 4.22. N(%): calcd: 8.91; found: 8.99. HPLC: $R_t = 4.78$ min.

6.2.24. 7-Methoxy-2-(2,2-dimethylpropanoyl)-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (5d). This compound was obtained in 7% yield and then purified by flash chromatography, eluting with DCM/MeOH 98.5:1.5; mp 140–141 °C; IR (KBr) ν 1716, 1348, 1218, 1164, 908 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9H, $C(CH_3)_3$), 4.00 (s, 3H, OCH_3), 7.52 (dd, 1H, H_6, $J_{6-5} = 9.5$ Hz, $J_{6-8} = 2.7$ Hz), 7.84 (ds, 1H, H_8, $J_{8-6} = 2.6$ H), 8.53 (d, 1H, H_5, $J_{5-6} = 9.5$ Hz) ppm. Anal. ($C_{15}H_{15}F_3N_2O_4$) C, H, N; C(%): calcd: 52.32; found: 52.68. H(%): calcd:

4.36; found: 4.02. N(%): calcd: 8.14; found: 7.77. HPLC: $R_t = 3.98$ min.

6.2.25. 6,7-Dichloro-2-(2,2-dimethylpropanoyl)-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (5e). This compound was obtained in 6% yield and then purified by flash chromatography, eluting with DCM. It was then recrystallized from *n*-hexane; mp 158–159 °C; IR (KBr) ν 1717, 1398, 1329, 1249, 1172, 1019 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 9H, $C(CH_3)_3$), 8.69 (s, 1H, H_5), 8.74 (s, 1H, H_8) ppm. Anal. ($C_{14}H_{11}Cl_2F_3N_2O_3$) C, H, N; C(%): calcd: 43.86; found: 43.53. H(%): calcd: 2.87; found: 2.62. N(%): calcd: 7.31; found: 7.48. HPLC: $R_t = 10.58$ min.

6.2.26. 7-Chloro-2-(2,2-dimethylpropanoyl)-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (5f). This compound was obtained in 3% yield and then purified by flash chromatography, eluting with DCM. It was then recrystallized from *n*-hexane; mp 130–131 °C; IR (KBr) ν 1709, 1405, 1340, 1266, 1164, 1020 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9H, $C(CH_3)_3$), 7.89 (dd, 1H, H_6, $J_{6-5} = 9.5$ Hz, $J_{6-8} = 2.2$ Hz), 8.58 (s, 1H, H_8), 8.59 (d, 1H, H_5, $J_{5-6} = 9.2$ Hz) ppm. Anal. ($C_{14}H_{12}ClF_3N_2O_3$) C, H, N; C(%): calcd: 48.21; found: 48.57. H(%): calcd: 3.44; found: 3.19. N(%): calcd: 8.03; found: 7.74. HPLC: $R_t = 8.16$ min.

6.2.27. 6,7-Difluoro-2-(2,2-dimethylpropanoyl)-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (5g). This compound was obtained in 8% yield and then purified by flash chromatography, eluting with DCM. It was then recrystallized from *n*-hexane; mp 151–152 °C; IR (KBr) ν 1723, 1421, 1359, 1248, 1178, 1123 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 9H, $C(CH_3)_3$), 8.39 (dd, 1H, H_5, $J_{5-6} = 7.2$ Hz), 8.59 (dd, 1H, H_8, $J_{8-7} = 7.2$ Hz) ppm. Anal. ($C_{14}H_{11}F_5N_2O_3$) C, H, N; C(%): calcd: 48.00; found: 48.38. H(%): calcd: 3.14; found: 3.31. N(%): calcd: 8.00; found: 7.83. HPLC: $R_t = 6.37$ min.

6.2.28. 2-Benzoyl-6,7-dimethyl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (6a). This compound was obtained in 14% yield and then purified by recrystallization using MeOH; mp 185–186 °C; IR (KBr) ν 1686, 1598, 1452, 1339, 1173, 902 cm^{-1}; ^{1}H NMR (DMSO-d_6 δ 2.52 (s, 3H, C_6-CH_3), 2.54 (s, 3H, C_7-CH_3), 7.58 (t, 2H, $H_{3\prime}$+$H_{5\prime}$, $J_{3\prime-4\prime} = 7.05$ Hz), 7.75 (t, 1H, $H_{4\prime}$), 8.11 (d, 2H, $H_{2\prime}$+$H_{6\prime}$), $J_{2\prime-3\prime} = 7.5$ Hz), 8.19 (s, 1H, H_5), 8.35 (s, 1H, H_8) ppm. Anal. ($C_{18}H_{13}F_3N_2O_3$) C, H, N; C(%): calcd: 59.66; found: 59.28. H(%): calcd: 3.59; found: 3.67. N(%): calcd: 7.73; found: 7.60. HPLC: $R_t = 3.96$ min.

6.2.29. 2-Benzoyl-7-methyl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (6b). This compound was obtained in 15% yield and then purified by recrystallization using *n*-hexane/AcOEt; mp 196–197 °C; IR (KBr) ν 3096, 1679, 1351, 1258, 1154, 894 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.67 (s, 3H, CH_3), 7.56 (t, 2H, $H_{3\prime}$+$H_{5\prime}$), 7.70 (t, 1H, $H_{4\prime}$, $J_{4\prime-3\prime} = 7.7$ Hz), 7.81 (d, 1H, H_6, $J_{6-5} = 8.8$ Hz), 7.90 (d, 2H, $H_{2\prime}$+$H_{6\prime}$, $J_{2\prime-3\prime} = 7.9$ Hz), 8.37 (s, 1H, H_8), 8.59 (d, 1H, H_5, $J_{5-6} = 8.8$ Hz) ppm. Anal. ($C_{17}H_{11}F_3N_2O_3$) C, H, N; C(%): calcd: 58.62; found: 58.03. H(%): calcd: 3.16; found: 3.07. N(%): calcd: 8.04; found: 7.88. HPLC: $R_t = 3.48$ min.

6.2.30. 2-Benzoyl-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (6c). This compound was obtained in 3% yield and then purified by flash chromatography, eluting with DCM/MeOH 98:2. It was then recrystallized from MeOH; mp 180–181 °C; IR (KBr) ν 1689, 1507, 1364, 1180, 904 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.60 (t, 2H, $H_{3\prime}$+$H_{5\prime}$, $J_{3\prime-4\prime} = 7.1$ Hz), 7.77 (t, 1H, $H_{4\prime}$), 8.09–8.15 (m, 4H, $H_{2\prime}$+$H_{6\prime}$+H_6+H_7), 8.41 (d, 1H, H_5, $J_{5-6} = 7.0$ Hz), 8.57 (d, 1H, H_8, $J_{8-7} = 7.0$ Hz) ppm. Anal. ($C_{16}H_9F_3N_2O_3 \cdot 1/4H_2O$) C, H, N; C(%): calcd: 56.72; found: 56.56. H(%): calcd: 2.80; found: 2.76. N(%): calcd: 8.27; found: 7.87. HPLC: $R_t = 3.44$ min.

6.2.31. 2-Benzoyl-7-methoxy-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (6d). This compound was obtained in 4% yield and then purified by flash chromatography, eluting with DCM/MeOH 98.5:1.5. It was then washed with diethyl ether; mp 197–198 °C; IR (KBr) ν 1683, 1465, 1352, 1221, 1154, 1016 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.03 (s, 3H, OCH_3), 7.54–7.58 (m, 3H, $H_{3\prime}$+$H_{5\prime}$+H_6), 7.70 (t, 1H, $H_{4\prime}$), 7.86 (ds, 1H, H_8, $J_{8-6} = 2.6$ Hz), 7.91 (d, 2H, $H_{2\prime}$+$H_{6\prime}$, $J_{2\prime-3\prime} = 8.2$ Hz), 8.60 (d, 1H, H_5, $J_{5-6} = 9.5$ Hz) ppm. Anal. ($C_{17}H_{11}F_3N_2O_3$) C, H, N; C(%): calcd: 56.04; found: 56.10. H(%): calcd: 3.02; found: 3.03. N(%): calcd: 7.69; found: 7.55. HPLC: $R_t = 3.82$ min.

6.2.32. 2-Benzoyl-6,7-dichloro-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (6e). This compound was obtained in 28% yield and then purified by recrystallization using MeOH; mp 181–182 °C; IR (KBr) ν 1685, 1333, 1251, 1175, 1015 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.61 (t, 2H, $H_{3\prime}$+$H_{5\prime}$), 7.79 (t, 1H, $H_{4\prime}$, $J_{4\prime-3\prime} = 7.2$ Hz), 8.13 (d, 2H, $H_{2\prime}$+$H_{6\prime}$, $J_{2\prime-3\prime} = 8.2$ Hz), 8.66 (s, 1H, H_5), 8.82 (s, 1H, H_8) ppm. Anal. ($C_{16}H_7Cl_2F_3N_2O_3$) C, H, N; C(%): calcd: 47.64; found: 47.76. H(%): calcd: 1.74; found: 1.76. N(%): calcd: 6.95; found: 6.42. HPLC: $R_t = 19.89$ min.

6.2.33. 2-Benzoyl-7-chloro-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (6f). This compound was obtained in 1% yield and then purified by flash chromatography, eluting with DCM. It was then recrystallized from *n*-hexane; mp 149–150 °C; IR (KBr) ν 1685, 1349, 1259, 1161, 1010 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.57 (t, 2H, $H_{3\prime}$+$H_{5\prime}$), 7.71 (t, 1H, $H_{4\prime}$, $J_{4\prime-3\prime} = 7.5$ Hz), 7.90 (d, 2H, $H_{2\prime}$+$H_{6\prime}$, $J_{2\prime-3\prime} = 8.2$ Hz), 7.93 (dd, 1H, H_6, $J_{6-5} = 9.2$ Hz, $J_{6-8} = 2.2$ Hz), 8.58 (s, 1H, H_8), 8.65 (d, 1H, H_5, $J_{5-6} = 9.2$ Hz) ppm. Anal. ($C_{16}H_8ClF_3N_2O_3$) C, H, N; C(%): calcd: 52.10; found: 52.40. H(%): calcd: 2.17; found: 2.13. N(%): calcd: 7.60; found: 7.59. HPLC: $R_t = 4.99$ min.

6.2.34. 2-Benzoyl-6,7-difluoro-3-trifluoromethylquinoxaline 1,4-di-*N*-oxide (6g). This compound was obtained in 7% yield and then purified by flash chromatography, eluting with DCM. It was then washed with diethyl ether; mp 163–164 °C; IR (KBr) ν 1694, 1347, 1244, 1185, 1115 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.61 (t, 2H, $H_{3\prime}+H_{5\prime}$), 7.79 (t, 1H, $H_{4\prime}$, $J_{4\prime-3\prime} = 7.3$ Hz), 8.13 (d, 2H, $H_{2\prime}+H_{6\prime}$, $J_{2\prime-3\prime} = 8.4$ Hz), 8.52 (dd, 1H, H_5, $J_{5-6} = 9.6$ Hz), 8.70 (dd, 1H, H_8, $J_{8-7} = 9.8$ Hz) ppm. Anal. ($C_{16}H_7F_5N_2O_3$) C, H, N; C(%): calcd: 51.89; found: 52.23. H(%): calcd: 1.89; found: 2.16. N(%): calcd: 7.56; found: 7.38. HPLC: $R_t = 4.45$ min.

6.3. Biological evaluation

6.3.1. In vitro primary anticancer assay. The compounds were evaluated in vitro against a 3-cell line panel consisting of MCF7 (breast), NCI-H460 (lung), and SF-268 (CNS). In this protocol, each cell line is inoculated and preincubated on a microtiter plate. Test agents were then added at a single concentration (100 μM) and the culture was incubated for 48 h. End-point determinations were made with alamar blue sulforhodamine B,[21] a protein-binding dye. Results for each test agent were reported as the percent of growth of the treated cells when compared to the untreated control cells. Compounds, which reduced the growth of any one of the cell lines to 32% or less (negative numbers indicate cell kill) were passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range.

6.3.2. In vitro anticancer screening against a panel of 60 human tumor cell lines. The antitumor activity of tested compounds is reported for each cell line by three parameters: $-\log GI_{50}$ value (GI_{50} = molar concentration of the compound that inhibits 50% net cell growth), $-\log TGI$ value (TGI = molar concentration of the compound leading to total inhibition of net cell growth), and $-\log LC_{50}$ value (LC_{50} = molar concentration of the compound leading to 50% net cell death). Furthermore, a meangraph midpoint (MG_MID) is calculated for each of the mentioned parameters, giving a mean activity parameter over cell lines. For calculation of the MG_MID, insensitive cell lines are included with the highest concentration tested.

6.3.3. Hollow fiber assay for preliminary in vivo testing. Each compound was tested against a standard panel of 12 human tumor cell lines including NCI-H23, NCI-H522, MDA-MB-231, MDA-MB-435, SW-620, COLO 205, LOX IMVI, UACC-62, OVCAR-3, OVCAR-5, U251, and SF-295. The cell lines were cultivated in RPMI-1640, containing 10% FBS and 2 mM glutamine. A total of 3 different tumor lines were prepared for each experiment so that each mouse would receive 3 intraperitoneal (IP) implants (1 of each tumor line) and 3 subcutaneous (SC) implants (1 of each tumor line). A value of 2 is assigned for each compound dose, which results in a 50% or greater reduction in viable cell mass. Compounds with a combined IP+SC score 20, a SC score 8 or a net cell kill of one or more cell lines are referred for further studies.[22]

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